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Effect of Potassium Fertilization on Improving Some Physiological and Vegetative Traits of Barley under Different Irrigation Levels

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Abstract: A field experiment was during winter seasons 2013-2014 and 2018-2019, to identify the potassium fertilization contribution in reducing the harmful effect of water stress and improving some physiological and vegetative parameters of barley crop. The experiment was in split-plot design arrangement according in randomized complete block design with three replications. Furthermore, the main plots included irrigation treatments at different levels, depletion 50% of available water (control) and 70 and 40% of the control treatment, while the potassium levels of 0, 90, and 180 kg K.ha^{-1} were represented the subplots. There were no significant differences between the depletion 50% of available water (control) 70% of the control treatment in the plant height, leaf area, chlorophyll index, and relative water content, thus providing 30% of the total irrigation needs. The irrigation treatment 40% of the control treatment gave the highest average of proline content (of 3.304 and 3.011 µM.g^{-1} fresh weight for both seasons respectively). The potassium fertilization effected significantly most of the studied traits, The fertilization at the level of 180 kg K.ha^{-1} gave the highest average of plant height, flag leaf area , the chlorophyll index in the second season. Besides, the relative water content in the first season, from the previous results, it is possible to irrigate at a rate of 70% of the irrigation need (depletion 50% of available water) and adopt fertilization at the level of 180 kg K.ha^{-1} to reduce the harmful effect of water stress.

Keywords: Water stress, Potassium, Barley

The importance of cereal crops for humans, especially wheat and barley crops, their availability in the required quantities has become an urgent necessity to achieve food security. Therefore, best area specific agronomic practice for the growth of these crops and productivity and improving their quality in essential. Furthermore, need to understand the interaction factors in soil and water management, where the water is one of the main elements in plant growth and productivity. Thus, water availability in the appropriate amount in the root zone requires attention as the large areas affected by drought and the limited water sources, as well as the changes taking place on climate (Fahad 2017). The lack of water reduces the rate of nutrient absorption by the roots and their transmission to vegetative parts (Waseem et al 2011). Earlier studies indicated that water stress causes a reduction in vegetative and physiological traits of the barley crop. Jalilian et al (2014) observed that irrigation after depletion 20% of field capacity gave the highest average of plant height, leaf area, and chlorophyll index compared to the irrigation treatment after depletion 80% of the field capacity. Al-Temimi et al (2013) and Chéour et al (2014) observed that water stress reduced the chlorophyll content, leaf area and plant height of barley and wheat crop. Jain (2011) mentioned that the lack of irrigation water leads to the destruction of the

main chlorophyll pigment for the carbon fixation process. The process of adding potassium fertilizer is one of the important means that help the plant to withstand water stress for its important role in regulating the work of stomata, which have importance in water use efficiency. Baque et al (2006) explained that potassium might contribute to maintaining turgor the cell and thus improving the affected plant growth process because of water stress. Waseem et al (2011) also indicated that potassium plays a role in stimulating the plant to absorb nitrogen and phosphorus to ensure the food balance process. Consequently, this study aimed to understand potassium's ability to help plants withstand water stress through their physiological roles.

MATERIAL AND METHODS

Field experiment was carried out during the two winter seasons 2013-2014 and 2018-2019 at field crops department in Abu Ghraib for the first season, and in the research station of the field crops department at the Faculty of Agricultural Engineering Sciences for the second season. The main research goal was to identify the role of potassium fertilization in improving the vegetative and physiological traits of the barley crop (Var IPA 99) under different irrigation levels. The experiment was implemented using a split-plot

design arrangement according to randomized complete block design with three replications. The main plots included irrigation treatments at different levels, which are; depletion 40,50 and 70 percent of available water while the potassium levels were 0, 90, and 180 kg K.ha^{-1} represented the sub-plots. The experimental unit area was 2 * 2 m included ten lines with a distance of 20 cm between one line and another. The seeds seeding was 120 kg.ha^{-1}, and the experiment was fertilized according to the recommendations of urea fertilizer (46% N) by 200 kg N.ha^{-1} and triple superphosphate (46% P_2O_5) by 200 kg.ha^{-1} (P_2O_5). Potassium sulfate (41% K) was added as a source of potassium fertilizer in three equal batches at the tillering, elongation, and booting stage. Random samples were taken from the field soil at a depth of (0-30) cm before planting, where the analysis was carried out of some chemical and physical soil properties (Table 1). The irrigation operation was carried out with flexible pipes tied to a pump, and the depth of the water to be added was calculated to compensate for the moisture depletion according to the (Allen et al 1998) equation.

$d = (\Theta_{fc} - \Theta_w) \times D$

Where:

D: Added water depth (mm).

Θ_{fc}: Volumetric moisture at field capacity (cm^{3}cm^{-3}).

Θ_w: Volumetric moisture before irrigation (cm^{3}cm^{-3}).

D: Effective root depth (cm).

The characteristics of plant height (cm), the flag leaf area (cm^2), leaf dry weight (leaf.mg^{-1}), specific leaf weight (gm.cm^{-2}) = dry weight of the flag leaf / flag leaf area, chlorophyll index (Spad), and proline content (µM.g^{-1} fresh weight), relative water content (%) were studied.

$d = (\Theta_{fc} - \Theta_w) \times D$

RESULTS AND DISCUSSION

Plant height (cm): The plant height decreased due to the effect of irrigation treatments. The irrigation treatment of depletion 50% of available water gave the maximum height for both seasons (100.56 and 99.56 cm, respectively). However, it did not differ significantly from irrigation treatment 70% of the depletion, while the plant height for irrigation treatment 40% of the control treatment by 17.79 and 22.77% for the two seasons respectively. Water shortages may lead to low division and elongation of cells due to the decrease in the water stress of plant cells (Mumtaz et al. 2014), Earlier study indicated that the water stress caused a decrease in the plant height (Al-Temimi et al 2013, Hashim and Ahmed 2017). The potassium had a positive effect on this trait, as fertilization treatment 180 kg K.ha^{-1} gave the highest average plant height of 99.89 and 99.22 with an increase of 21.17 and 23.16% compared to the non-additional treatment. This reason may be due to the role of potassium in the increasing division of cell expansion and elongation due to the increase in capacity and efficiency of carbon fixation, as well as its role in improving the work of the growth regulators that are directly involved in plant growth. The effect of irrigation treatments and potassium levels was significant on plant height. The irrigation treatment combination of depletion 50% of available water (control) which fertilized at the level of 180 kg K.ha^{-1} gave the highest average for the trait by 109.33 and 113.33 cm for the two seasons respectively. However, it did not differ significantly from the irrigation treatment combination 70% of the control treatment at the same level of fertilizer in the first season. Finally, the lowest average for this trait of the irrigation treatment combination 40% of the control treatment and non-additional treatment recorded 77.67 and 73.33 cm for the two seasons respectively (Table 4).

Flag leaf area (cm^2): The flag leaf area decreased due to the effect of irrigation treatments The irrigation treatment of depletion 50% of available water (control) recorded the highest means of flag leaf area in both seasons (21.74 and 21.42 cm^2). However, it did not differ significantly from the irrigation treatment 70% of the control treatment; while the flag leaf area for irrigation treatment of 40% of the control treatment by 23.41 and 21.47% for the two seasons, respectively. The may be due to the low relative water content (Table 5) which led to a decrease in the water stress-causing reduction in the size of cells and their ability to elongate and expand. This finding is consistent with (Mansouri and Radhouan 2015, Hashim and Ahmed 2017) which they indicated that the lack of available water in the soil has led to a reduction in the leaf area. The potassium had a positive effect on this trait, as fertilization treatment 180 kg K.ha^{-1} gave the highest means of flag leaf area (22.85 and 22.44 cm^2) with an increase of 37.65 and 31.38% compared to the non-additional treatment. This is due to its role in most vital activities within the plant that are related to growth and division processes, as well as its role in maintaining leaf water stress, turgor potential, and high relative water content (Table 5). Pettigrew (2008) also indicated that a low level of potassium reduces the expansion of the leaf area and reduces its size. The effect of irrigation treatments and potassium levels was significant on flag leaf area. The irrigation treatment 70% of the control treatment (depletion 50% of the available water) and the level of potassium 180 kg K.ha^{-1} was re 25.70 cm^2 which did not differ significantly from the combination of irrigation treatment (control) at the same level of fertilizer (24.09 cm^2). The combination of irrigation treatment 40% of the non-additional treatment recorded the lowest mean of this trait was 14.11 cm^2 in the second season only (Table 4). Finally, it was observed that the addition of

potassium at the level of 90 and 180 kg K.ha^{-1} and the irrigation level 40% of the control treatment increased the flag leaf area compared to the same irrigation treatment without addition of potassium. This shows the role of potassium in reducing the negative effects of water shortage because

plants that supplied with potassium lose less water due to the . closer the stomata (Abu Dahi and El-Younes 1988).
Dry weight for flag leaf (mg): There was non-significant difference in this trait for the effect of irrigation treatments and potassium levels for both seasons (Tables 2, 3). The

Table 1. Chemical and physical properties of the experiment soil before planting

Property		Value		Unit
		First location	Second location	
pH		7.8	7.6	
Organic matter		3.1	5.2	g.kg^{-1}
Available nitrogen		47.00	25.00	mg.kg^{-1}
Available phosphorus		17.00	10.32	
Available potassium		276.00	160.00	
Bulk density		1.25	1.41	g.cm^3
Soil texture	Sand	120	225	gm.kg^{-1}
	Silt	489	447	
	Clay	392	328	
Volumetric moisture content (33kpa)		0.30	0.26	cm^3.cm^{-3}
Volumetric moisture content (1500kpa)		0.15	0.14	cm^3.cm^{-3}
Available water		0.15	0.12	cm^3.cm^{-3}

Table 2. Effect of irrigation treatments on the plant height, the flag leaf area, the dry and specific weight of the flag leaf of barley crop for the two seasons

Irrigation treatments	Cultivation season	Plant height (cm)	Flag leaf area (leaf cm^2)	Dry weight of flag leaf (mg leaf^{-1})	Leaf specific weight (g cm^2)
Depletion 50% of available water (control)	First season	100.56	21.74	0.072	0.00347
	Second season	99.56	21.42	0.307	0.01310
70% of the control treatment	First season	97.00	21.06	0.080	0.00407
	Second season	97.22	21.65	0.147	0.00750
40% of the control treatment	First season	82.67	16.65	0.067	0.00411
	Second season	76.89	16.82	0.070	0.0042
LSD (p=0.05)	First season	7.61	3.55	N.S	N.S
	Second season	4.85	2.95	N.S	N.S

Table 3. Effect of Potassium levels on the plant height, the flag leaf area, the dry and specific weight of the flag leaf of barley crop for the two seasons

Potassium level	Cultivation season	Plant height (cm)	Flag leaf area (leaf cm^2)	Dry weight of flag leaf (mg leaf^{-1})	Leaf specific weight (g cm^2)
Non-additional treatment	First season	82.44	16.60	0.074	0.00457
	Second season	80.56	17.08	0.153	0.00850
90 kg ha^{-1}	First season	97.89	19.99	0.076	0.00384
	Second season	93.89	20.38	0.083	0.00410
180 kg ha^{-1}	First season	99.89	22.85	0.070	0.00325
	Second season	99.22	22.44	0.286	0.01230
LSD (p=0.05)	First season	3.93	2.65	N.S	N.S
	Second season	4.11	1.48	N.S	N.S

interaction between the two treatments in the second season showed significant effect (Table 4). The irrigation treatment of depletion 50% of the available water (control) and 180 kg K ha^{-1} of potassium fertilizer gave the highest value (0.744 mg leaf^{-1}). This was due to the availability of suitable moisture and potassium increases the activity of the carbon fixation process and increases the production of dry matter.

Specific weight for flag leaf (g.cm^2): There was non-significant difference in this trait for the effect of irrigation treatments and potassium levels for both seasons (Tables 2 and 3). The interaction between the two treatments in the second season only showed significant effect, the irrigation treatment of depletion 50% of the available water (control) and 180 kg K.ha^{-1} of potassium fertilizer gave the highest value (0.0307 g.cm^2).

Chlorophyll Index (SPAD): The chlorophyll index decreased due to the effect of irrigation treatments The irrigation treatment of depletion 50% of available water

(control) and treatment of 70% of the control treatment recorded the highest means of chlorophyll index (45.16, 46.19, 46.59 and 44.94 SPAD) in both seasons respectively, while the irrigation treatment 40% of the control treatment recorded the lowest means for this trait (39.17 and 39.48 SPAD), with a decrease by 15.29 and 17.73% compared to the control treatment, and 18.94, 13.83% compared to the treatment 70% of the control treatment. The decrease may be due to the decrease in the relative water content that was associated with a decrease in the leaf area (Tables 2, 5). Besides, a decrease in the quantities of irrigation water, which led to a decrease in the chlorophyll index due to the inhibition of photosynthesis in the leaves as a result of the closure of the stomata, which negatively affected the growth of chloroplasts and then reduced the chlorophyll pigment. It may also be due to the plastid membranes damage due to water stress because of the formation of free radicals such as a root of Superoxide and hydrogen peroxide that lead to

Table 4. Effect of interaction between irrigation levels and potassium fertilization on the plant height, the flag leaf area, the dry and specific weight of the flag leaf for barley crop for the two seasons

Irrigation treatment	Potassium level	Plant height (cm)	Flag leaf area (leaf cm^2)	Dry weight of flag leaf (mg leaf^{-1})	Leaf specific weight (g cm^2)
First season					
Depletion 50% of available water (control treatment)	Non-additional treatment	87.33	17.88	0.079	0.0044
	90 kg ha^{-1}	105.00	20.49	0.068	0.00327
	180 kg ha^{-1}	109.33	26.86	0.070	0.00267
70% of the control treatment	Non additional reatment	82.33	18.29	0.082	0.00483
	90 kg ha^{-1}	100.33	20.85	0.090	0.00439
	180 kg ha^{-1}	108.33	24.03	0.068	0.00298
40% of the control treatment	Control (0)	77.67	13.63	0.060	0.00440
	90 kg ha^{-1}	88.33	18.64	0.071	0.00385
	180 kg ha^{-1}	82.00	17.67	0.072	0.00408
LSD (p=0.05)		8.23	N.S	N.S	N.S
Second season					
depletion 50% of available water (control)	Non-additional treatment	83.33	19.36	0.086	0.0044
	90 kg ha^{-1}	102.00	21.50	0.091	0.0307
	180 kg ha^{-1}	113.33	24.09	0.744	0.0043
70% of the control treatment	Control (0)	83.00	17.76	0.314	0.0043
	90 kg ha^{-1}	103.33	20.80	0.088	0.0166
	180 kg ha^{-1}	105.33	25.70	0.038	0.0015
40% of the control treatment	Control (0)	75.33	14.11	0.061	0.0038
	90 kg ha^{-1}	76.33	18.83	0.071	0.0043
	180 kg ha^{-1}	79.00	17.53	0.078	0.0045
LSD (p=0.05)		6.77	3.16	0.430	0.0193

oxidation of pigments (Anjum et al 2011). The potassium had a significantly effect on this trait in the second season, as fertilization treatment 90 kg K.ha^{-1} gave the highest mean of chlorophyll index (45.45 SPAD) with an increase of 12.42% compared to the non-additional treatment. Potassium leads to delaying the protein catabolism and then delaying the leaf aging and continuing leaves activity in the photosynthesis process. In addition to that its role in the process of adenosine triphosphate ATP metabolism that is reflected in the process of photosynthesis and the transfer of its products to the plant's need (Abu Dahi and Al-Yunus 1988). The effect of irrigation treatments and potassium levels was significant on chlorophyll index in the first season only. The combination of irrigation of 70% of the control treatment (depletion 50% of the available water) and the fertilizer level 180 kg.ha^{-1} gave the highest mean (49.41 SPAD), which did not differ significantly from the combination of the same irrigation treatment with the level of fertilization 90 kg.ha^{-1}, while the irrigation 40% of the control treatment and non-additional treatment recorded the lowest mean (35.46 SPAD). It can be inferred that although the availability of moisture affects the behavior of nutrients and increases their movement within the plant, which reflected in increase the chlorophyll index. Nevertheless, at the same time, potassium works to reduce the negative effects of water shortage by regulating osmotic potential, closing stomata and less water loss by an increase in the chlorophyll index in the plants leaves that treated with irrigation level 40% of the control treatment.

Proline content of flag leaf (µM.g^{-1} fresh weight): The proline content of leaf increased with increasing water stress (Table 5). The irrigation 40% of the control treatment (depletion of 50% of the available water) produced the highest mean of 3.304 and 3.011 µM.g^{-1} fresh weight for the two seasons, respectively with an increase by 133.53 and 164.90% over the control treatment. Water stress increases the proteolytic enzymes such as proteinase, which contributes to an increase in the accumulation of the amino acid proline, which may reduce the osmotic potential of plant tissue, which helps to increase the ability of the plant to absorb water from the soil (Szabados and Savoure 2010). Chachar et al (2016) and Hashem (2017) observed an increase in the proline content in the flag leaf of wheat plants subjected to water stress. The addition of potassium has led to an decrease in the flag leaf content of proline (Table 6). The

Table 5. Effect of irrigation treatments on the chlorophyll index, proline content and the relative water of flag leaf for barley crop for the two seasons

Irrigation treatments	Cultivation season	Chlorophyll index (Spad)	Proline content µ (M g^{-1} fresh weight)	Relative water content of flag Leaf (%)
Depletion 50% of available water (control)	First season	45.16	1.427	79.51
	Second season	46.19	1.362	80.33
70% of the control treatment	First season	46.59	1.541	79.08
	Second season	44.94	1.396	80.46
40% of the control treatment	First season	39.17	3.304	65.35
	Second season	39.48	3.011	68.03
LSD (p=0.05)	First season	4.61	0.140	2.38
	Second season	1.57	0.102	3.80

Table 6. Effect of potassium levels on the chlorophyll index, proline content and the relative water of flag leaf for barley crop for the two seasons

Potassium levels	Cultivation season	Chlorophyll index (Spad)	Proline content µ (M g^{-1} fresh weight)	Relative water content of flag Leaf (%)
Non-additional treatment	First season	40.78	2.469	71.43
	Second season	40.43	2.126	72.03
90 kg ha^{-1}	First season	44.17	1.947	73.73
	Second season	45.45	1.824	79.89
180 kg ha^{-1}	First season	45.97	1.857	78.78
	Second season	44.72	1.819	76.90
LSD (p=0.05)	First season	n.s	0.235	2.18
	Second season	2.61	0.239	1.85

Shatha Abdul Hassan Ahmed

fertilizer level 180 kg K.ha⁻¹ gave the lowest average (1.857 and 1.819 µM.g⁻¹ fresh weight) compared to the non-additional treatment which gave the highest mean (2.469 and 2.126 µM.g⁻¹ fresh weight). The interaction between irrigation treatments and potassium levels had non-significant effect on the flag leaf content of proline for two seasons respectively (Table 7).

Relative water content in the flag leaf (%): The irrigation treatment of depletion 50% of the available water (control) and the treatment 70% of the control treatment gave the highest means of relative water content of 79.51 and 80.33% for the control treatment and 79.08 and 80.46% for the irrigation treatment 70% of the control treatment with a significant difference (Table 5). The irrigation treatment 40% of the control treatment gave the lowest means with a decreasing percentage of 17.81 and 15.31% over the control treatment and 17.36 and 15.45% over the irrigation treatment 70% of the control treatment. The reason for the decrease in the relative water content may be attributed to the lack of water absorption by the plant under conditions of water stress due to the decrease in the soil water potential. The loss of water from the leaves due to the high temperature and low relative humidity which was reflected in the water condition of the plant (Zareian et al 2014). Potassium levels had significantly affected in this trait (Table 6). The fertilization treatment at the level of 180 and 90 kg K.ha⁻¹ achieved the highest mean of relative water content (78.78 and 79.89%) compared to control treatment which gave the lowest mean (71.43 and 72.03%) for both seasons respectively. It may be attributed that the addition of potassium led to the regulation of leaf water potential under water stress conditions by creating a tendency for the movement of water from wood toward the leaf, which increased the relative water content in the leaf (Pier and Berkowitz 1987). The interaction between the two treatments in the first season only showed significant effect (Table 7). The irrigation treatment of depletion 50% of the available water (control) and 180 kg K.ha⁻¹ of potassium fertilizer gave the highest value (81.96%) with non-significant

Table 7. Effect of interaction between irrigation levels and Potassium fertilization on the chlorophyll index, proline content and the relative water of flag leaf for barley crop for the two seasons

Irrigations treatments	Potassium levels	Chlorophyll index (Spad)	Proline content µ (M g⁻¹ fresh weight)	Relative water content of flag Leaf (%)
First season				
Depletion 50% of available water (control)	Non-additional treatment	39.54	1.623	77.55
	90 kg ha⁻¹	48.54	1.310	79.01
	180 kg ha⁻¹	47.41	1.347	81.96
70% of the control treatment	Control (0)	41.85	1.810	76.32
	90 kg ha⁻¹	48.51	1.390	79.13
	180 kg ha⁻¹	49.41	1.423	81.80
40% of the control treatment	Control (0)	35.46	3.973	60.42
	90 kg ha⁻¹	40.94	3.070	63.06
	180 kg ha⁻¹	41.11	2.870	72.58
LSD (p=0.05)		7 93	N.S	3.51
Second season				
Depletion 50% of available water (control)	Non-additional treatment	41.04	1.343	75.75
	90 kg ha⁻¹	50.42	1.310	81.74
	180 kg ha⁻¹	47.10	1.433	83.50
70% of the control treatment	Control (0)	41.49	1.533	77.08
	90 kg ha⁻¹	46.16	1.337	81.38
	180 kg ha⁻¹	47.15	1.317	82.92
40% of the control treatment	Control (0)	38.75	3.500	63.26
	90 kg ha⁻¹	39.77	2.827	67.57
	180 kg ha⁻¹	39.91	2.707	73.26
LSD (p=0.05)		N.S	N.S	N.S

difference from the irrigation treatment 70% of the control treatment at the same level of fertilizer. The irrigation treatment 40% of the control treatment with the non-additional treatment gave the lowest value for this trait (60.42%). The lack of water hinders the growth of roots and thus becomes unable to transport water and nutrients to the vegetative part, and this deficiency leads to plant stress then the water content of tissues is reduced (Hashem 2017).

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Effect of Nutrient Solution and Corncob Extract on Growth and Yield of Strawberry and NPK Content in Leaves under Hydroponic System

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Abstract: The study was carried out in Anbar Governorate - Heet District (70 km west of Ramadi, the center of the governorate), to find out the effect of the best level of corncobextract, which is H0 (without addition), H1 (0.5 g L^{-1}), H2 (0.75 g L^{-1}) and H3 (1 g L^{-1}), with three levels of the nutrient solution (60, 80 and 100%) and symbolized by them (F1, F2, and F3 respectively).The addition of corncob extract at 0.5 gL^{-1} (H1) was significantly superior and gave the maximum vegetative dry weight (10.61 g), root dry weight (4.57 g) and fruit yield (185.9 g plant^{-1}). The concentration of nitrogen and phosphorous in leaves was 1.92 and 0.37% in treatments H1 (0.5 g L^{-1}) and H2 (0.75 g L^{-1}) respectively. The adding the nutrient solution at 100% (F3) was significantly superior and recorded the highest means of vegetative dry weight (9.33 g) and the root dry weight (4.92 g), while the adding of nutrient solution at the level of 80% (F2) achieved the highest of fruit yield (158.3 g plant^{-1}) and the highest concentration of nitrogen and phosphorous in leaves (1.91 and 0.36%) respectively.

Keywords: Hydroponics, Strawberry, Nutrient solution, Organic solutions

Hydroponics is one of the soilless farming techniques used on a limited scale, as succeeded to a large extent in overcoming soil defects, problems and diseases, and it is one of the successful alternative methods to increase production (Kang et al 2020). The nutrient solution is the backbone of soilless agriculture, and is responsible for supplying the plant with nutrients necessary for plant growth instead of the soil. The preparation of nutrient solutions depends on the quality of the waste water, the level of different elements, pH, electrical conductivity (EC) and osmotic pressure (Lee et al 2016). Different concentrations of corncob extract can be added to the nutrient solution to reduce the use of mineral solutions and replace with organic solutions. Corncob extract acts as a bio-stimulant and plays an important role in plant growth, increasing the dry weight of buds and roots, plant tolerance to stress, as well as enhancing the stability of the cell membrane and its preservation, water absorption under osmotic pressure, potassium absorption, synthesis of proteins and hormones and elongation of root cells, in addition to help the plant to resist salt stress and improve plant growth under salt stress (Canellas et al 2015).

MATERIAL AND METHODS

The study was carried out in Anbar Governorate - Heet District (70 km west of Ramadi, the center of the governorate). An aerobic decomposition process was carried out for corncob waste and preparing the greenhouse and experiment materials, after which the seedlings were planted on October 15, 2020 and the study continued until May1, 2021.The experiment included the study of two factors, the first factor was adding of corncob extract at three levels: H1 (0.5 g L^{-1}), H2 (0.75 g L^{-1}) and H3 (1 g L^{-1}) in addition to H0 (without adding), and the second factor was adding the nutrient solution at three levels: F1 (60%), F2 (80%) and F3 (100%).

Decomposition of corncob waste: Sixty kg of crushed corncob were weighed and placed on pieces of polyethylene as a pile to conduct the aerobic decomposition process. The was moistened with continuous stirring until the entire pile was moistened and then 1% soil was added from planted land with alfalfa plant for the purpose of adding some microorganisms to assist in the process of organic decomposition. Urea fertilizer was added to the pile (1%), and then re-moistened and stirred with re-covering. The phosphorous was added to the pile, and the wetting and stirring continued every 3-5 days up to three months, after which the decomposing organic waste was brushed to dry and then the organic solution was extracted.

Extraction of corncob waste: After the process of decomposition of corncob is completed, it was dried under the sun for two days. The solution was extracting at 1:10ratio (1 kg of corncob extract: 10 liters KOH 0.01N) and the solution was shaken in the vibrator for 24 hours and then was filtered by using filter paper and the solution was kept in container.

Preparation of the nutrient solution: The 1.5 liters of nutrient solution A and 1.5 liters of solution B were taken according to the fertilizer recommendation for strawberry (Morgan 2006), and then each 1 liter of solution was diluted with 100 liters of deionized water (RO).

Studied Traits

Vegetative dry weight (g plant^{-1}): Strawberry plants were taken from hydroponic tubes, the roots were separated from the leaves, the leaves were placed in paper bags and aerobically dried and weighted to measurement the vegetative dry weight (Al-Sahhaf 1989).

Root dry weight (g plant^{-1}): The roots were aerobically dried and weighed to measure the dry weight of the rootstock (Al-Sahhaf 1989).

Fruit yield (g plant^{-1}): The fruit yield was calculated by multiplying the number of fruits per plant by the weight of fruit.

NPK contents in leaves (%): Nitrogen was measured in strawberry leaves by a semi-microkjeldal method (AOAC 1980), phosphorous by a spectrophotometer type Ultraspectronic at a wavelength of 620 nm, while potassium a flame photometer type PGI 2000 Automatic flame photometer.

Statistical analysis: Statistical analysis was done using the SAS statistical program version 9 (SAS and Guide 2003).

RESULTS AND DISCUSSION

Vegetative dry weight (g plant^{-1}): The adding of corncob extract to strawberry plants grown by hydroponics had a significant effect on vegetative dry weight (Table 1). The adding of corncob at 0.5 g L^{-1}(H1) had the highest mean of 10.61 g plant^{-1} compared with 1 g L^{-1} (H3) (4.44 g plant^{-1}). The increase may be attributed to the role of adding corncob extract in increasing the permeability of the cell membrane of the roots and absorption of elements, or it contains of two or more carboxyl groups arranged in a way that enables the formation of a chelating complex which is necessary to regulate the bioavailability of ions, and the most important feature of these extracts lies in their high ability to bind un-dissolved ions, oxides and hydroxides, and release them slowly and continuously to plants when needed which lead to activating plant enzymes and then increasing vegetative dry weight (Fathy et al 2010). The results showed that the vegetative dry weight significantly increased when using the full fertilizer recommendation of nutrient solution (F3 = 100%) which gave 9.33 g plant^{-1} compared with 60% (F1) and 80% (F2) of the fertilizer recommendation which gave 7.85 and 7.92 g plant^{-1} respectively. The interaction between adding the levels of corncob and the levels of the nutrient solution had significant effect, H0F3 combination gave the highest (12.54 g plant^{-1}), while e H3F2 combination gave the lowest (3.93 g plant^{-1}).

Root dry weight (g plant^{-1}): The adding of corncob extract had a significant effect on root dry weight (Table 1). The adding of corncob at 0.5 g L^{-1}(H1) recorded maximum dry root weight (4.57 g plant^{-1} but with non-significant difference with H0 (control treatment) (4.53 g plant^{-1}). The adding of corncob at 1 g L^{-1} (H3) had the lowest (3.94 g plant^{-1}). This may be attributed to the fact that the availability of nutrients in the nutrient solution enriched the plant from increasing the root dry weight and is reinforced by the fact that the yield traits were not negatively affected with the low dry weight of the root. The root dry weight significantly increased when using the full fertilizer recommendation of nutrient solution (F3 = 100%) which gave ٤.92 g plant^{-1} compared with 60% (F1) and 80% (F2) of the fertilizer recommendation (3.73 and 4.15 g plant^{-1}) respectively. The interaction between adding the levels of corncob and the levels of the nutrient solution had significant effect, H0F3 combination gave the highest value (6.21 g plant^{-1}), whilethe H3F1 combination gave the lowest (2.66 g plant^{-1}). The plant depends on the absorption of nutrients on the root system, and the increase of the root dry weight means an increase absorption of plant to nutrients

Table 1. Effect of different levels of corncob extract and nutrient solution on the vegetative dry weight and root dry weight (g plant^{-1})

Corncob extract (H)	Vegetative dry weight			Mean(H)	Root dry weight			Mean(H)
	Nutrient solution (F)				Nutrient solution (F)			
	F$_1$	F$_2$	F$_3$		F$_1$	F$_2$	F$_3$	
H$_0$	8.80	7.44	12.54	9.59	4.28	3.11	6.21	4.53
H$_1$	12.02	9.21	10.62	10.61	4.09	4.74	4.87	4.57
H$_2$	5.91	11.09	9.47	8.83	3.87	3.19	5.00	4.02
H$_3$	4.68	3.93	4.70	4.44	2.66	5.58	3.59	3.94
Mean (F)	7.85	7.92	9.33		3.73	4.15	4.92	
LSD (p=0.05)	0.32= H	0.28= F	0.56= H×F		H=0.22	F=0.19	H×F=0.39	

and then an increase in the yield, but there was relative decrease in the weight of the roots when adding corncob extract, but it did not negatively affect the yield but on the contrary, it increased it. The reason of the plant is not affected by the increase in the root dry weight may be attributed to the cultivation method. In hydroponics, the nutrient solution helps in transferring the nutrients to the plant. Hydroponics is considered to be the best for supplying the nutrient needs of plants, as nutrients are supplied directly to the roots through their contact with them. The hydroponics has proven to be superior and sustainable for the cultivation of various cropsas a result of controlling environmental conditions. So, it has turned into a very effective method for producing high-quality and high-yield fruits (Nunes et al 2021).

Fruit yield (g plant^{-1}): The adding of corncob extract had a significant effect on fruit yield (Table 2). The adding of corncob at 0.5 g L^{-1}(H1) recorded highest mean of 185.9 g plant^{-1}while at 1 g L^{-1}(H3) lowest (86.3 g plant^{-1}). The fruit yield significantly increased when using 80% of fertilizer recommendation of nutrient solution (F2) which gave 158.3 g plant^{-1}compared with 60% (F1) and 100% (F3) of the fertilizer recommendation which gave 130.0 and 155.1 g plant^{-1} respectively. The interaction between adding the levels of corncob and the levels of the nutrient solution had significant effect. H1F3 combination gave the highest value (204.4 g

plant^{-1}), whereas the H3F1 combination gave the lowest (54.7 g plant^{-1}).The increase may be due to the content of nutrients, the increase of phosphorus may be lead to an increase the size and yield of the fruits, as well as potassium improves the floral and fruit production and fruit quality. Hence, use of corncob extract at a level of 0.5 g L^{-1} had a positive effect in increasing the plant yield even with the reduction of the fertilizer recommendation 80% (F2). This may be attributed to the role of cornflower extract in increasing the effectiveness of growth-stimulating hormones (Ameri et al 2012).

NPK content in leaves (%): The adding of corncob extract had a significant effect on leaves content of N and P (Table 1, 2) while corncob extract levels hadn't significant effect on leaves content of K (Table 3). The adding of corncob at 0.75 g L^{-1}(H2) had the highest mean of N and P in leaf (1.92 and 0.37%) respectively with non-significant difference with 0.5 g L^{-1} (H1) which gave 1.92 and 0.36% respectively while the adding of corncob at 1 g L^{-1}(H3) had the lowest of 1.89 and 0.34% respectively, with non-significant difference with H0. The N and P significantly in leaf increased with 80% of full fertilizer recommendation of nutrient solution (F2) which gave ١,٩١and 0.36% respectively compared with 60% of the fertilizer recommendation which resulting in 1.89 and 0.35% respectively. The levels of nutrient solution had no significant

Table 2. Effect of different levels of corncob extract and nutrient solution on the fruit yield (g plant^{-1}) and leaves content of N (%)

Corncob extract (H)	Fruit yield (g plant^{-1})			Mean(H)	N content in leaves (%)			Mean(H)
	Nutrient solution (F)				Nutrient solution (F)			
	F_1	F_2	F_3		F_1	F_2	F_3	
H_0	180.3	143.5	166.7	163.5	1.87	1.88	1.92	1.89
H_1	165.7	187.6	204.4	185.9	1.93	1.96	1.88	1.92
H_2	119.4	201.8	145.2	155.5	1.89	1.94	1.94	1.92
H_3	54.7	100.2	104.1	86.3	1.89	1.89	1.90	1.89
Mean (F)	130.0	158.3	155.1		1.89	1.91	1.91	
LSD (p=0.05)	1.89= H	1.64= F	3.28= H×F		H=0.007	F=0.006	H×F=0.01	

Table 3. Effect of different levels of corncob extract and nutrient solution on P and K contents in leaves (%)

Corncob extract (H)	Leaves content P			Mean(H)	Leaves content K			Mean(H)
	Nutrient solution (F)				Nutrient solution (F)			
	F_1	F_2	F_3		F_1	F_2	F_3	
H_0	0.33	0.35	0.34	0.34	1.74	1.73	1.79	1.75
H_1	0.37	0.38	0.35	0.36	1.84	1.88	1.81	1.84
H_2	0.35	0.38	0.37	0.37	1.45	1.81	1.85	1.71
H_3	0.34	0.33	0.34	0.34	1.76	1.75	1.76	1.76
Mean (F)	0.35	0.36	0.35		1.70	1.79	1.80	
LSD (p=0.05)	0.006= H	0.005= F	0.01= H×F		H=0.16	F=0.14	H×F=0.28	

effect K content in leaf .The interaction between adding the levels of corncob and the levels of the nutrient solution had significant effect.H2F2and H2F3 combinations gave the highest value of N (1.94%), while the H0F1 combination gave the lowest (1.87%). P months in leaf indicated that H1F2 and H2F2 combinations gave the highest of P in leaves (0.38%) respectively, while the H0F1 combination gave the lowest (0.33%). However, the interaction between two factors did not have any significant effect content of K in leaves. The earlier study indicated that corncob extract plays a prominent role as an organic stimulating substance, and is involved in many biological processes that lead to an increase the plant growth, and stimulate the growth of roots, enable plants to better absorb of water and nutrients, and increase root respiration and the formation of root hairs that increase plants absorb nitrogen, which is included in most of the plant's physiological activities and the formation of proteins, amino acids and nucleic acids (DNA and RNA) (Eyheraguibel et al 2008).

CONCLUSION

The addition of corncob extract at 0.5 or 0.75 g L^{-1} improved the growth indicators (vegetative and root dry weight), fruit yield and leaves content of major nutrients even with the use of 80% of the fertilizer recommendation of nutrient solution.

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Assessment of Desertification in Some Parts of Arid Land in Iraq using Soil Quality Indicator

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Abstract: This study was conducted to assess the degradation of arid and semi-arid lands located between longitudes 43.9071-44.5750 E and latitudes 34.9960 - 36.0755 N and occupies an area of 360.000 ha. Thirty- six samples were taken from the surface layers of soil in the study area, air arid for physical and chemical analysis. The state of desertification was assessed using the Mediterranean Model of Desertification and Land Use (MEDALUS) project. Arc Map 10.4.1 was used to analyze and prepare the layers of soil quality map. The results showed that the soil quality indicator for all soils of the study area falls within the second class (moderate quality).

Keywords: Desertification, Medalus, Arid land, Soil degradation, GIS

Desertification/ land degradation, in the context of assessment, is land degradation in arid, semi-arid and dry sub-humid areas resulting from adverse human impact. Degradation implies reduction of the resource potential by one or a combination of processes acting on the land, including water and wind erosion, sedimentation and siltation, long-term reduction in the level of diversity in natural vegetation, crop yields, soil salinization and sodification (Helldén 2003). Desertification is the process of land degradation resulting from the interactions between physical, biological, political, social, economic and cultural factors with climatic changes. Using MEDALUS model in Iraq showed that during,1985- 2012, the 75.55-96.26% belong to sensitivity Critical class 3.74-20.71% % to Fragile class, and 0.0-3.74% to potential class., respectively (Al-Rawi and Al-Juraysi 2014). Awda (2015) studied the spatial analysis of desertification phenomenon using remote sensing and geographic information system (GIS) technology Northwest Wasit Province, Iraq, the result showed high and intense desertification

Jasem et al (2016) studied the classification of some soils from province of Kirkuk and statement extent of their sensitivity to desertification and indicated that soil quality index of soil series of the studied location was within the class very low for series 441CCW whereas the series 432CCW, 431CCW was within the class low, whereas the series 433CCW, 451CCW were within the class moderate. The lower series sensitivity is the series 451CCW. Plakaing et al (2020) using MEDALUS model in Upper Lamcchiengkrai watershed, Thailand, pointed out that the main causes of desertification in the high- resolution part are due to the climate factor and vegetation cover, while the medium-resolution part is affected by human activity factor and soil factor. Due to the exposure of large agricultural areas in Kirkuk-Iraq to the risk of desertification, this study aims to evaluate the state of desertification in this region.

MATERIAL AND METHODS

Location: The study area includes arid and semi-arid lands located at longitude 43.9071 - 44.5750 E and latitudes 34.9960 - 36.0755 N, with an area of 360.000 ha, which includes parts of Kirkuk and Erbil governorates in Iraq (Fig. 1).

Climate and vegetation: The mean annual air temperature is around 20°C. The coldest month is January, but the average temperature dose not drops below 5C° annually. July and August are the hottest months where the average temperature exceeds 40°C. Mean annual perception ranged200-500)mm.year^{-1} from November to April. The types of grasses and natural herbs that are used as natural pastures and branched shrubs represent the basic components of vegetation cover, as well as cereal crops that are always grown in this area,

Desertification assessment according to MEDALUS project (Kosmas et al 1999): The assessment involves four indicators for soil, climate, vegetation and management quality which were calculated providing a measure of the inherent quality of the physical environment and the man induced stress of desertification (Table 1).

Soil quality index(SOI): SOI was calculated as the product of the above attributes, namely soil texture, parent material, rock fragment, soil depth, slope grade, organic matter, electrical conductivity, Calcium carbonate content, and
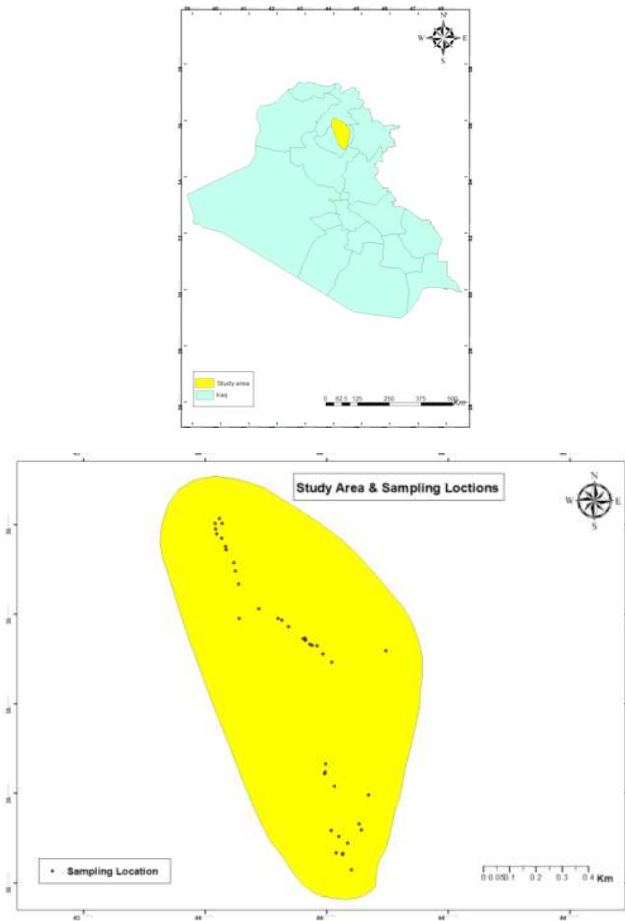
Fig. 1. Location of the study area

drainage conditions as the following algorithm. The soil quality is then defined using Table 2.

SQI = (texture × parent material × rock fragment × depth × slope × drainage × O.M% × Ec × CaCO$_3$ × pH)$^{1/10}$ …(1)

RESULTS AND DISCUSSION

Soil quality criteria: The soil texture was ranged between class 1 (good), class 2 (moderate) and class 3 (poor) with an area of 142457, 315668 and 114 ha. Which covered 31.09, 68.89 and 0.02%, respectively (Table 3, Fig. 3, 4). In general, soil texture has a medium to poor risk on desertification. As the results showed that soil texture in general were mostly silty clay to silty clay loam , clay loam, loam, and silty clay which leads to the risk of erosion, in particular wind erosion, as well as its effect on the soil water holding capacity, which is an important factor in the impact on desertification due to its effect on the vegetation cover and soil aggregation that affects desertification. The severe class of soil degradation dominated the areas was characterized by sandy soil texture (Wijitkosum and Yolpramote 2013). The sandy texture of the soil resulted in a low water holding capacity. For this reason,

soil texture is a key factor affecting the desertification risk of the area (Wijitkosum et al 2013). The index of parent material of all the soils of the study area within class 2 (moderate), because the parent material is limestone or loses deposits, which is rich in carbonate minerals and it is susceptible to erosion over time, which is dangerous in desertification. The rock fragment index reached the most dangerous level, because index for all soils was generally within class 3 (bare to slightly stony) with an area of 100 %. This causes suitable conditions for the acquisition of both water and wind erosion in the absence of rough surfaces to protect the soil from erosion. Soil slope index is class 1 (very gentle to flat).

The effect of water erosion in the gentle to flatlands is almost non- existent. In addition, the water holding capacity is in larger quantities which help to alleviate the runoff, erosion and desertification. The soil depth index was classified as a class 1(deep). Soil depth is linked to water availability. A deep soil can assure water reserves and can then provide a good condition for vegetation development and growth (Lamqadem et al 2018). This results in increasing vegetation, which in turn reduces the surface runoff and water erosion, as well as rough surface formation that impairs wind erosion. Soil drainage classes were in class 1 (good). The slow process of water infiltration increases the probability of surface runoff during the rainfall, this leads to increase the risk of soil erosion, even if it is average, this did not happened in the study area. Study area contains different amounts of the organic matter, which was divided into class 2(good) and class 3 (moderate).The area of the class 2 was 172897 ha. with a rate of 37.73% thus succeeded the class 3 with area of 285342 ha with a rate of 62.27% of the total study area. This indicate that organic matter has played an important role in reducing the risk of desertification. The presence of organic matter helps to increase the growth of plants, especially herbs, which helps to increase vegetation. In addition to that the accumulation of organic matter helps to enhance of soil aggregation, all these help to increase the soil resistance to erosion. Calcium carbonate in the soil study area is within the class 3 (poor). The soil reaction index (pH) was within class 3 (moderate) and class 4 (high) with an area of 121782 and 336457 which covered 26.58% and 73.42%, respectively. The soil in the study area is inclined to alkaline, and this in turn affects the readiness of most nutrients for the plant, thus reducing the percentage of vegetation cover that leads to desertification. The electrical conductivity (EC) for all the area was in class 1 (low). There was no effect of salinity in the events of the desertification process. Finally the results showed that the soil quality indicator (SQI) is within class 2 (Moderate) (Fig. 4). This means that all study area are in moderate dangerous of desertification.

Table 1. Quality index according to Medalus method soil (Kosmas et al 1999)

| Indicator	Class	Description	Texture	Index
Texture	1	Good	L, SCL, SL, LS,CL	1
	2	Moderate	SC, SiL, SiCL	1.2
	3	Poor	Si, C, SiC	1.6
	4	Very poor	S (sandy)	2
Parent material	1	Good	Shale, schist, basic, ultra basic, Conglomerates	1
	2	Moderate	Limestone, marble, granite, Rhyolite, Ignibrite, gneiss, siltstone, sandstone.	1.7
	3	Poor	Marl*, Pyroclastics	2
Slope	1	Very gentle to flat	<6	1
	2	Gentle	6-18	1.2
	3	Steep	18-35	1.5
	4	Very steep	>35	2
Soil depth	1	Deep	>75	1
	2	Moderate	75-30	2
	3	Shallow	15-30	3
	4	Very shallow	<15	4
Rock fragment	1	Very stone	>60	1
	2	Stony	20-60	1.3
	3	Bare to slightly stony	<20	2
Organic matter (OM)	1	Very good	>3	
	2	Good	2-3	
	3	Moderate	2-1	
	4	Poor	1-0.5	
	5	Very poor	0.5<	
Indicator	Class	Description	Texture	Index
Electrical conductivity (EC)	1	Very low	>4	1
	2	Low	4-8	1.2
	3	Moderate	8-16	1.4
	4	Almost high	16-32	1.6
	5	High	32-64	1.8
	6	Very high	<64	2
$CaCO_3$ content (%)	1	High quality	<2.5	1
	2	Moderate quality	2.5-5	1.5
	3	Low quality	> 5	2
pH	1	Very low	< 5.5	1
	2	Low	5.5 – 6.5	1.2
	3	Moderate	6.5 – 7..5	1.5
	4	High	7.4 – 8.4	1.7
	5	Very high	> 8.4	2
Drainage	1	Well drained		1
	2	Imperfectly drained		1.2
	3	Poorly drained		2
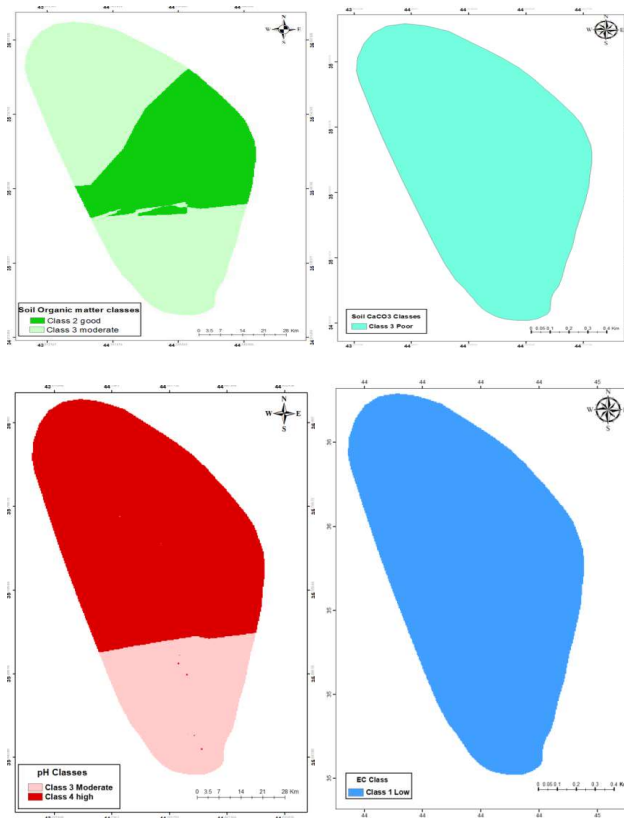
Fig. 3. Soil chemical characteristics based on soil quality index (SQI)
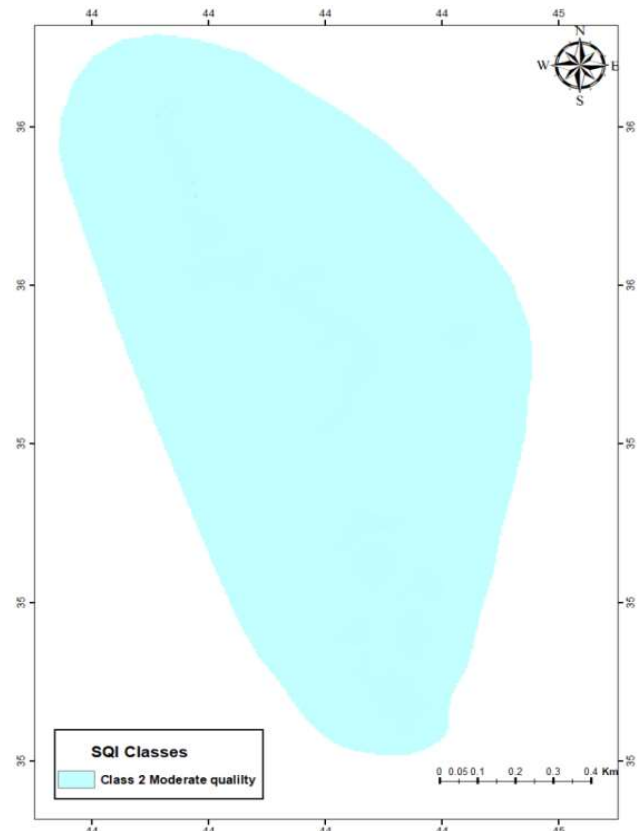
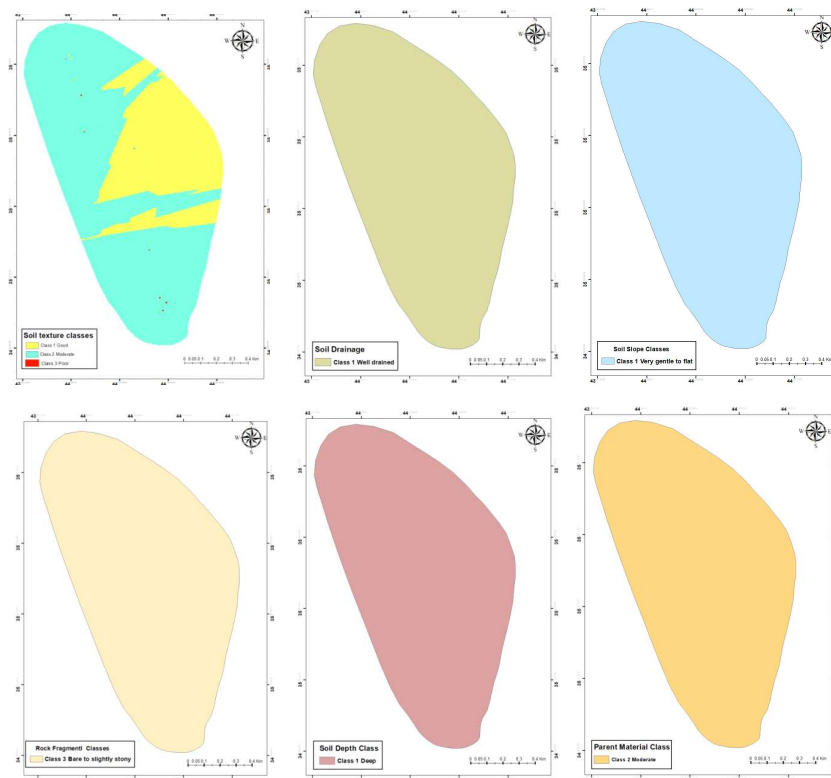**Fig. 5.** Soil quality indicator (SQI) for study area

Fig. 4. Soil physical characteristics by soil quality index (SQI)

Table 2. Quality indicator according to Medalus structure of range and weight index soil method

Indicator	Class	Description	Range
Soil quality indicators	1	High quality	<1.13
	2	Moderate quality	1.13-1.45
	3	Low quality	>1.46

Table 3. Some physical and chemical properties of soil samples

Locations		Particle size distribution (g kg^{-1})			Texture	Bulk density (Mg.m^{-3})	EC dS.m^{-1}	Ph 1:2.5	O.M. gm.Kg^{-1}	CaCO$_3$ gm.Kg^{-1}
E	N	Sand	Silt	Clay						
44.2183	35.7069	291.2	428	280.8	Clay Loam	1.47	0.35	7.6	13	265.5
44.3631	35.155	79.2	408	512.8	Clay	1.63	0.38	7.4	16.6	220.5
44.385	35.1386	131.2	456	412.8	Silty Clay	1.69	0.37	7.4	13.8	162
44.3733	35.1119	171.2	396	432.8	Clay	1.6	0.47	7.2	16	189
44.4144	35.1867	363.2	364	272.8	Clay Loam	1.41	0.31	7.1	12.4	252
44.4203	35.1719	159.4	472	368.6	Silty Clay Loam	1.33	2.38	7.5	11	301.6
44.3728	35.1086	159.8	560	280.2	Silty Clay Loam	1.46	1.18	7.9	15.3	261.3
44.3947	35.0694	307	524	168	Silty Loam	1.54	0.61	7.8	21.3	243.3
44.4389	35.2608	347.2	384	268.8	Loam	1.42	0.32	7.9	18.8	229.7
44.3514	35.2833	147.2	480	372.8	Silty Clay Loam	1.48	2.39	7.2	12.8	274.3
44.3556	35.1133	359.2	420	220.8	Loam	1.62	2.36	7.2	12.4	333.8
44.33	35.3406	459.2	432	108.8	Loam	1.67	2.3	7.5	12.7	286.5
44.3272	35.3156	199.2	392	408.8	Clay	1.49	0.83	7.5	25.5	286.2
44.3286	35.3194	459.2	252	288.8	Silty Clay Loa	1.52	1.27	7.5	11.7	317.6
44.4828	35.6292	315.2	335.2	349.6	Loam	1.56	0.24	7.7	17.6	153
44.3075	35.6414	139.2	492	368.8	Silty Clay Loam	1.6	0.54	7.8	26.3	225.2
44.2772	35.6614	219.2	508	272.8	Silty Clay Loam	1.67	0.25	7.6	14.8	279
44.2183	35.7069	167.2	452	380.8	Clay	1.6	0.44	7.5	18.7	222.7
44.2083	35.7117	95.2	464	440.8	Silty Clay Loam	1.59	0.37	7.3	15.3	274.5
44.2778	35.6553	139.6	602.8	257.6	Silty Clay	1.43	0.3	7.6	18.9	193.5
44.3447	35.6	111.2	548	340.8	Silty Loam	1.7	0.49	7.5	14.5	355.5
44.3233	35.62	259.2	560	180.8	Silt Loam	1.65	0.33	7.3	20.1	187
44.2944	35.6431	219.2	560	220.8	Silt Loam	1.41	0.5	7.3	12.4	288.3
44.2719	35.6597	192.2	560	240.8	Silt Loam	1.57	0.43	7.4	23.1	247.7
44.2344	35.6906	193	520	208.8	Silty Clay Loam	1.22	0.72	7.3	25.1	293
44.29	35.6461	259.2	560	180.8	Silt Loam	1.48	0.44	7.4	22.7	277
44.0994	35.8328	135.2	440	424.8	Clay	1.62	0.26	7.7	10.3	364.5
44.0953	35.8531	99.2	444	456.8	Silty Clay	1.65	0.3	7.7	13.5	337.5
44.075	35.8953	203.2	500	296.8	Silty Clay	1.56	0.34	6.9	17.8	276.7
44.0667	35.9542	143.2	376	480.8	Clay Loam	1.65	0.33	7.6	17.2	317.5
44.0475	35.9536	187.2	496	316.8	Clay	1.58	0.3	7.7	18.5	315
44.05	35.9406	131.2	572	296.8	Silty Clay Loam	1.68	0.3	7.7	17.2	292.5
44.0528	35.9269	139.2	560	300.8	Silty Clay Loam	1.65	0.25	7.9	14.8	346.5
44.0647	35.9167	139.2	520	340.8	Silty Clay Loam	1.58	0.29	7.7	13.9	288
44.0764	35.8864	279.2	440	280.8	Clay Loam	1.56	0.29	7.7	13.4	319.5
44.1081	35.7986	191.2	488	320.8	Silty Clay Loam	1.67	0.28	7.9	14.4	243

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Growth and Yield of Mung Bean Genotypes (*Vigna radiata* L.) in Response to Foliar Nutrient of Potassium and Spraying of Kinetin

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Abstract: A field experiment was carried out during the autumn season of 2019 in the Al-Khalidiya area - Al-Habbaniyah district - Anbar Governoratein order to study the response of growth and yield of three mung bean genotypes (Local green, Indian green VC6089A10 and Indian black GOLDSTAR) to foliar nutrient of potassium at three concentrations (0, 400 and 800 mg K L^{-1}) and spraying of kinetin at three concentrations (0, 100 and 200 mg L^{-1}). The foliar nutrient of potassium at 800 mg KL^{-1} gave the highest means of number of leaves, plant dry weight, weight of 100 seeds and plant seed yield, whereasthe foliar nutrient of potassium at 400 mg KL^{-1} achieved the highest efficiency of catalase enzyme. The spraying of kinetin at 200 mg L^{-1} achieved the highest means of number of leaves, number of seeds per pod, weight of 100 seeds and plant seed yield, while the spraying of kinetin at 100 mg L^{-1} gave the highest means of plant dry weight and activity of catalase enzyme. The local green genotype was superior in the number of leaves, plant dry weight and number of seeds per pod, while the VC6089A10 genotype was superior in the activity of catalase enzyme, weight of 100 seeds and plant seed yield.

Keywords: Fabaceae, Antioxidant, Nitrate reductase, Enzyme

Mung bean (*Vigna radiata* L.) is one of the important leguminous crops that are used in human nutrition as well as animal feed because its seeds contain protein at a rate 19-29%. It is also considered one of the crops that suit the Iraqi environment, in addition to its short growth period. This crop suffers from several problems that lead to a significant decrease in its productivity due to high temperatures during the flowering period, which leads to a high percentage of drop the flowering and pods, in addition to the failure of the ovaries to produce seeds. The use of some nutrients, especially potassium, as a spraying on the vegetative parts leads an important role such as expanding cells, controlling the process of opening and closing of stomata, activating more than 80 enzymes in addition to its role in increasing the leaves content of chlorophyll and proteins and increasing the fertility of flowers and seed production (Issa 1990), as well as its effective role in increasing the photosynthesis efficiency and the transfer of its products to their places of need in the plant and regulation of biological and physiological processes within the plant which directly affects the growth and development of the plant (Hawkesford et al 2012, Hasanuzzaman et al 2018). Plant growth regulators, including kinetin, work to regulate the growth of the crop and stimulating the physiological activities within the plant to increase the yield and its components. Kinetin stimulates the growth of lateral buds, modulates the apical dominance which leads to stimulate the plant to increase reproductive buds and then increasing seed yield, as well as kinetin has a

vital role in regulating growth by activating cell division and biological effectiveness in differentiation of tissues into buds, and it also works to encourage the growth of shoots. Otherwise, the mung bean genotypes differ in their response to potassium and kinetin. Therefore, the aim of this study to determinate the response of growth and yield of mung bean genotypes to foliar nutrient of potassium and spraying of kinetin.

MATERIAL AND METHODS

A field experiment was carried out during the autumn season of 2019 in the Al-Khalidiya area - Al-Habbaniyah district - Anbar Governorate in order to study the response of growth and yield of three mung bean genotypes (local green, Indian green VC6089A10 and Indian Black GOLDSTAR) to foliar nutrient of potassium at three concentrations (0, 400 and 800 mg K L^{-1}) and spraying of kinetin at three concentrations (0, 100 and 200 mg L^{-1}).Randomized complete block design (RCBD) arranged according to split plots used at three replicates, where the main plots included the genotypes of the mung bean, the subplots included kinetin concentrations, while the sub-sub-plots included potassium concentrations. Potassium sulfate (50% K) was used as a source of potassium fertilizer. Potassium and kinetin concentrations were sprayed in two hold, the first before the flowering stage, the second at the flowering stage, whereas the control treatment was sprayed with distilled water only. The plants were sprayed before sunset using a 2-

liter sprayer, bubbler drops were added as a diffuser to reduce the surface tension of the water, ensure complete wetness of the leaves and increase the efficiency of the spray solution (Abu Dhahi et al 2001). Soil management were carried out, and then the experiment land was divided into 36 experimental units, the area of each experimental unit was 7.5 m^2(3 m long × 2.5 m width) which contained 5 lines, 50 cm apart and 25 cm between hills to obtain a plant density of 80,000 plants.ha^{-1}. The seeds of genotypes were sown on the 27 July 2019 at a depth of 2-3 cm by 2-3 seeds per hill, irrigated and then the plants were thinned to one plant per hill.The experiment was fertilized with triple super phosphate (46%P_2O_5) at a 75 kg $P_2O_5ha^{-1}$ before planting, and nitrogen fertilizer as urea (46% N) at a of 40 kg N ha^{-1}at two doses, the first after seeds germination and the second at the beginning of the flowering stage .

Studied Traits

1. Activity of CAT enzyme (unit / ml protein): The enzyme activity was estimated according to the Aeib method (1984). This method uses the absorbance of hydrogen peroxide by using a UV-Spectrophotometer at a wavelength of 240 nm for a solution of 30 m/mol of hydrogen peroxide and 50 m /mol of phosphate buffer solution pH = 7 and the change was followed up for 60 seconds, the control was prepared in the same way but without adding the sample.

2. Number of leaves per plant: All leaves per ten plants were calculated for each experimental unit and then their average was extracted.

3. Plant dry weight (g / plant): It was calculated by harvesting ten plants and placing them in perforated paper bags, dried in an electric oven for 48 hours at 65-70 Cº and weighted by using a sensitive scale and the dry weight of the leaves of those plants added to obtain the average total dry weight of the plant.

4. Number of seeds per pod: 30 pods were randomly taken from each experimental unitand the number of its seeds was calculated and then divided by the number of its pods.

5. Weight of 100 seeds (g): It was calculated after mixing the seeds of the harvested plants, 100 seeds were taken randomly and then weighed by using a sensitive scale.

6. Plant seed yield (g):It was calculated by adding the seeds of the ten harvested plants for each experimental unit, then weighed and their average was extracted.

Statistical analysis: Statistical analysis was conducted by using the GneStat statistical program. The significant differences between the means were tested by using the least significant difference (LSD) test at the level of probability 0.05.

RESULTS AND DISCUSSION

Activity of CAT enzyme: The catalase enzyme (CAT) was significantly increased when foliar nutrient with potassium at 400 mg K L^{-1} (31.8 unit / ml protein) compared with 800 mg K L^{-1} and control treatment which recorded the lowest (19.4 unit / ml protein) (Table 1). The an increasing of CAT enzyme activity as result the spraying of potassium may be due to the role of the mineral elements, including potassium, in activating of many enzymes, as well as entering into the composition of the mineral part of the enzyme (the non-protein part) which will positively reflect on the enzyme's performance and effectiveness (Issa 1990). The spraying of kinetin had a significant effect on the CAT enzyme activity, the spraying of kinetin at 100 and 200 mg L^{-1} gave the highest means (27.7 and 27.4 unit / ml protein) respectively compared with control treatment which gave the lowest (23.1 unit / ml protein). The CAT enzyme was more effective in Indiangreen VC6089A10 genotype with a significant increase of 60.9 and 37.6% than the CAT enzyme activity in the local green and Indian black GOLDSTAR genotypes. The difference in CAT enzyme activity among mung bean genotypes could be to their genetical components and their response to the environmental conditions, as well as the difference among them in the leaves content of nutrients. The interaction between potassium concentrations and mung bean genotypes had significant effect on the CAT enzyme activity, the Indiangreen VC6089A10 genotype with spraying of potassium at 400 mg L^{-1}had the highest value (38.6 unit / ml protein) with non-significant difference with same genotypes with spraying of potassium at 800 mg L^{-1}(37.5 unit / ml protein) while the local green genotype with non-spraying of potassium had the lowest value (17.1 unit / ml protein).The interaction between kinetin concentrations and mung bean genotypes had significant effect on the CAT enzyme activity, the Indian green VC6089A10 genotype and spraying of kinetin at 200 mg L^{-1}had the highest value (37.5 unit / ml protein) with non-significant difference with same genotypes and spraying of kinetin at 100 mg L^{-1}(35.2 unit / ml protein) whereas the Indian black GOLDSTAR genotype with non-spraying of kinetin had the lowest value (17.0 unit / ml protein).The interaction between three factors had significant effect on the CAT enzyme activity, the Indiangreen VC6089A10 genotype with spraying of potassium at 800 mg $L^{-1}$and spraying of kinetin at 100 and 200 mg L^{-1}had the highest values (48.4 and 47.9unit / ml protein) compared with local green genotype with non-spraying of potassium and spraying the kinetin at 200 mg L^{-1} which had the lowest value (9.7unit / ml protein) (Table 2).

Number of leaves per plant: The number of leaves was significantly increased when foliar nutrient with potassium at 400 and 800 mg K L^{-1} (30.99 and 31.11 leaf / plant) respectively compared with control treatment which gave the

Table 1. Effect of the foliar nutrition of potassium and the kinetin on activity of CAT enzyme of mung genotype

Cultivars (V)	Kinetin H (mg/l)	Potassium K (mg/l)			V × H
		0	400	800	
V1	0	35.4	29.9	16.2	27.2
	100	14.7	42.5	48.4	35.2
	200	21.1	43.6	47.9	37.5
V2	0	27.8	30.8	16.4	25.0
	100	13.5	23.4	22.9	20.0
	200	9.7	20.3	21.1	17.1
V3	0	15.1	19.4	16.4	17.0
	100	20.4	37.3	26.9	28.1
	200	17.3	38.5	26.9	27.6
Mean K		19.4	31.8	26.9	
L.S.D (0.05)			K = 1.5		3.3
			V×H×K =4.8		
V			V × K		Mean V
		0	400	800	
V1		23.7	38.6	37.5	33.3
V2		17.2	24.9	20.1	20.7
V3		17.6	31.7	23.2	24.2
L.S.D (0.05)			2.7		2.3
H			H × K		Mean H
		0	400	800	
0		26.1	26.7	16.3	23.1
100		16.2	34.4	32.6	27.7
200		16.1	34.2	31.9	27.4
L.S.D (0.05)			2.8		2.1

Table 2. Effect of the foliar nutrition of potassium and the kinetin on number of leaves per plant of mung genotype

Cultivars (V)	Kinetin H (mg/l)	Potassium K (mg/l)			V × H
		0	400	800	
V1	0	14.07	14.63	12.53	13.74
	100	12.70	9.27	14.33	12.10
	200	12.73	15.90	14.70	14.44
V2	0	49.37	45.80	35.87	43.68
	100	39.97	43.30	44.47	42.58
	200	38.23	47.57	45.53	43.78
V3	0	30.17	25.90	31.83	29.30
	100	32.70	37.37	32.37	34.14
	200	31.50	39.13	48.37	39.67
Mean K		29.05	30.99	31.11	
L.S.D (0.05)			K = 1.38		2.991
			V×H×K =4.40		
V			V × K		Mean V
		0	400	800	
V1		13.17	13.27	13.86	13.43
V2		42.52	45.56	41.96	43.34
V3		31.46	34.13	37.52	34.37
L.S.D (0.05)			2.42		1.865
H			H × K		Mean H
		0	400	800	
0		31.20	28.78	26.74	28.91
100		28.46	29.98	30.39	29.61
200		27.50	34.20	36.20	32.63
L.S.D (0.05)			2.64		1.911

lowest (29.05 leaf / plant) (Table 3). The spraying of kinetin had a significant effect on the number of leaves, the spraying of kinetin at 200 mg L^{-1} gave the highest mean (32.63leaf / plant) compared with control treatment which gave the lowest (28.91 leaf / plant). The number of leaves per plant was more in local green genotype (43.34 leaf / plant) with a significant increase of 26.10 and 222.71% than the number of leaves in the Indian greenVC6089A10 and Indian black GOLDSTAR genotypes which had the lowest (34.37 and 13.43 leaf / plant, respectively). The difference in the number of leaves among mung bean genotypes could be to their genetical components and their response to the environmental conditions, or may be due to the superiority of the local green genotype in the number of branches per plant. These results are in agreement with Al-Khafaji (2015). The interaction between potassium and kinetin concentrations had significant effect on the number of leaves, the spraying of potassium at 800 mg L^{-1} with spraying the kinetin at 200 mg L^{-1} had the highest value (36.20 leaf / plant) with non-significant

difference with the spraying of potassium at 400 mg L^{-1} with spraying the kinetin at 200 mg L^{-1}while the spraying of potassium at 400 mg L^{-1}with non-spraying of kinetin had the lowest value (26.74 leaf / plant).The interaction between potassium concentrations and mung bean genotypes had significant effect on the number of leaves, the local green genotype with spraying of potassium at 400 mg L^{-1}had the highest value (45.56 leaf / plant) compared with other combinations which the Indian green VC6089A10 genotype with non-spraying of potassium had the lowest value (13.17leaf / plant). The interaction between kinetin concentrations and mung bean genotypes had significant effect on the number of leaves, the local green genotype under all kinetin concentrations had the highest value of the number of leaves compared with the Indian greenVC6089A10 genotype under all kinetin concentrations which had the lowest. The interaction between three factors had significant effect on the number of leaves, the local green genotype with non-spraying of potassium and kinetin had the

Table 3. Effect of the foliar nutrition of potassium and the kinetin on plant dry weight of mung genotype

Cultivars (V)	Kinetin H (mg/l)	Potassium K (mg/l)			V × H
		0	400	800	
V1	0	33.80	33.93	34.50	34.08
	100	36.30	31.13	50.43	39.29
	200	35.80	30.37	35.97	34.04
V2	0	37.83	44.27	46.10	42.73
	100	46.83	44.00	50.27	47.03
	200	42.03	50.80	52.17	48.33
V3	0	15.53	17.40	18.80	17.24
	100	17.87	17.33	20.27	18.49
	200	18.27	20.70	22.67	20.54
Mean K		31.59	32.21	36.80	
L.S.D (0.05)			K = 1.31		1.695
			V×H×K =3.55		
V			V × K		Mean V
		0	400	800	
V1		35.30	31.81	40.30	35.80
V2		42.23	46.36	49.51	46.03
V3		17.22	18.48	20.58	18.76
L.S.D (0.05)			2.42		0.136
H			H × K		Mean H
		0	400	800	
0		29.30	31.87	33.13	31.35
100		33.06	30.82	40.32	34.94
200		17.22	33.96	36.93	34.31
L.S.D (0.05)			2.146		1.197

highest value (49.37leaf / plant) compared with Indian green genotype with spraying of potassium at 400 mg L^{-1} and kinetin at 200 mg L^{-1} which had the lowest value (9.27leaf / plant) (Table 3).

Plant dry weight: The plant dry weight was significantly increased when foliar nutrient with potassium at800 mg K L^{-1} (36.80 g / plant) compared with control treatment which gave the lowest (31.59 g / plant) (Table 4). The reason of increasing may be due to the role of potassium in delaying the aging of leaves (Issa 1990), and the activation of more than 80 enzymes which leads to an increase in the products of photosynthesis and then increase the dry matter in the plant (Farhan 2012 , Hashem 2018).

The spraying of kinetin had a significant effect on the plant dry weight, the spraying of kinetin at 100 and 200 mg L^{-1} gave the highest means (34.94 and 34.31 g / plant respectively) compared with control treatment which gave the lowest (32.35g / plant).The reason of increasing may be attributed to the role of kinetin in prolonging the vitality of the leave

which leads to an increase the efficiency of the photosynthesis process and the accumulation of its products in the plant as a dry matter (Mohammed and Al-Younis 1991). These results are in agreement with Sohair et al (2006). The plant dry weight was more in local green genotype (46.03 g / plant) with a significant increase of 28.58 and 145.36% than plant dry weight of the Indian greenVC6089A10 and Indian black GOLDSTAR genotypes which had the lowest (35.80 and 18.76g / plant, respectively).This superiority may be due to the superiority of the same genotype in the number of leaves per plant (Table 3). These results are in agreement with Rehman et al (2009), and Al-Dabbagh and Al-Dulaimi (2017).The interaction between potassium and kinetin concentrations had significant effect on the plant dry weight, the spraying of potassium at 800 mg L^{-1} with spraying the kinetin at 100 mg L^{-1}had the highest value (40.32g / plant) compared with non-spraying of potassium with spraying of kinetin at 200 mg L^{-1} which had the lowest of (0.22g / plant. The interaction between potassium concentrations and

Table 4. Effect of the foliar nutrition of potassium and the kinetin on number of seeds per pod of mung genotype

Cultivars (V)	Kinetin H (mg/l)	Potassium K (mg/l)			V × H
		0	400	800	
V1	0	8.60	8.33	8.16	8.37
	100	7.66	8.20	8.90	8.26
	200	9.56	9.50	9.43	9.50
V2	0	10.53	19.86	1.43	10.94
	100	11.16	11.86	11.20	11.41
	200	11.23	11.33	10.97	11.17
V3	0	6.43	6.46	6.46	6.46
	100	6.56	6.53	6.53	6.54
	200	6.46	6.56	6.57	6.53
Mean K		8.69	8.85	8.85	
L.S.D (0.05)			K = NS		0.380
			V×H×K = NS		
V			V × K		Mean V
		0	400	800	
V1		8.61	8.68	8.83	8.71
V2		10.98	11.35	11.20	11.18
V3		6.49	6.52	6.52	6.51
L.S.D (0.05)			NS		0.352
H			H × K		Mean H
		0	400	800	
0		8.52	8.56	8.68	8.59
100		8.46	8.86	8.89	8.74
200		9.09	9.13	8.99	9.07
L.S.D (0.05)			NS		0.181

mung bean genotypes had significant effect on the plant dry weight, the localgreen genotype with spraying of potassium at 800 mg L^{-1}had the highest of 49.51g / plant) compared with other combinations which the Indian black GOLDSTAR genotype with non-spraying of potassium had the lowest of 17.22g / plant. The interaction between kinetin concentrations and mung bean genotypes had significant effect on the plant dry weight,the local green genotype with spraying of kinetin at 100 and 200 mg L^{-1} had the highest value (47.03 and 48.33 g / plant) compared with the Indian blackGOLDSTAR genotype with non-spraying of kinetin which had the lowest (17.24 g / plant). The interaction between three factors had significant effect on the plant dry weight, the local green genotype with spraying of potassium at 800 mg L^{-1}and kinetin at 200 mg L^{-1}had the highest value (52.17g / plant) compared with Indian black genotype with non-spraying of potassium and kinetin (15.53g / plant).

Number of seeds per pod: The number of seeds was non-significantly affected when foliar nutrient with potassium,

while the spraying of kinetin had a significant effect on the number of seeds (Table 5). The spraying of kinetin at 200 mg L^{-1} gave the highest mean (9.07 seed / pod) compared with control treatment (8.59seed / pod).The reason of increasing when spraying kinetin may be due to its effect on increasing the number of leaves per plant and plant dry weight (Tables 3 , 4), as well as increasing the CAT enzyme activity (Table 2), and its impact on preparing the pods with their requirements of photosynthesis products and increasing the pod fertility and then increasing the number of seeds per pod. Issa (1990) indicated that the plant can hold seeds that can be processed only with the products of photosynthesis. The number of seeds was more in local green genotype (11.18 seed / pod) compared with Indian black GOLDSTAR genotype which had the lowest (6.51 seed / pod).The increasing of number of leaves per plant in the local green genotype led to an increase the efficiency of light interception and absorption by the plant and then an increase the of photosynthesis products that are transmitted to the pods to increase the

Table 5. Effect of foliar nutrition of potassium and the kinetin on of mung genotype

Cultivars (V)	Kinetin H (mg/l)	Potassium K (mg/l)			V × H
		0	400	800	
V1	0	6.93	7.40	6.40	6.91
	100	7.33	7.47	7.10	7.30
	200	7.20	6.80	7.13	7.04
V2	0	3.26	3.67	3.43	3.45
	100	3.50	3.40	3.83	3.57
	200	3.57	3.53	3.60	3.57
V3	0	5.27	5.17	5.47	5.30
	100	4.73	5.53	5.47	4.91
	200	4.60	5.47	5.60	5.22
Mean K		5.16	5.27	5.34	
L.S.D (0.05)			K = 0.13		0.177
			V×H×K = 0.36		
V			V × K		Mean V
		0	400	800	
V1		7.16	7.22	6.88	7.09
V2		3.44	3.53	3.62	3.53
V3		4.87	5.06	5.51	5.17
L.S.D (0.05)			NS		0.109
H			H × K		Mean H
		0	400	800	
0		5.16	5.41	5.10	5.22
100		5.19	5.13	5.47	5.26
200		5.12	5.26	5.44	5.28
L.S.D (0.05)			0.214		NS

percentage of fertility and then increase the number of seeds per pod (Singh et al 2014, Khan et al 2016, Abdullah, 2019). The interaction between kinetin concentrations and mung bean genotypes had significant effect on the number of seeds per pod, the local green genotype with spraying of kinetin at 100 and 200 mg L^{-1} had the highest value (11.41 and 11.17 seed / pod, respectively) compared with the Indian black GOLDSTAR genotype with non-spraying of kinetin which had the lowest (6.46 seed / pod), whereas the interaction between potassium and kinetin concentrations, potassium concentrations and mung bean genotypes as well as the interaction between three factors had non-significant effect on the number of seeds per pod.

Weight of 100 seeds: The weight of 100 seeds was significantly increased when foliar nutrient with potassium at 800 mg K L^{-1} (5.34 g) with non-significant difference with 400 mg K L^{-1} (5.27 g) while the control treatment gave the lowest (5.16g) (Table 6). The reason of increasing could be attributed to the superiority of plants sprayed with potassium in the dry

weight (Table 4), which was positively reflected on the processing of seeds with their requirements of photosynthesis products to increase their fullness and increase their weight. These results are in agreement with Al-Dulaimi and Al-Fahdawi (2015), Al-Fahdawi (2016), Shafeek et al (2018) and Shawer (2019). The spraying of kinetin had non-significant effect on the 100 seeds weight. Indiangreen VC6089A10 genotype gave the highest mean (7.09 g) with a significant increase of 100.80% than the weight of 100 seeds in the local green genotype which gave the lowest (3.53 g). The superiority of the Indian green VC6089A10 genotype in the weight of 100 seeds may be due to its high efficiency in transferring the products of the photosynthesis process from sources to the sinks (seeds) to an increase their fullness and weight. Other researchers found a significant difference among mung bean genotypes in 100 seeds weight (Hussain et al 2011, Al-Muhammadi 2012, Al-Khafaji 2015, Buriro et al 2015, Al-Dabbagh and Al-Dulaimi 2017). The interaction between potassium and kinetin concentrations had significant effect on the 100 seeds weight,

Table 6. Effect of the foliar nutrition of potassium and the kinetin on seed yield of mung genotype

Cultivars (V)	Kinetin H (mg/l)	Potassium K (mg/l)			V × H
		0	400	800	
V1	0	13.80	18.10	18.30	16.73
	100	15.60	18.43	18.37	17.47
	200	17.00	19.50	18.77	18.42
V2	0	13.07	11.13	11.27	11.82
	100	9.83	11.23	13.40	11.49
	200	10.30	13.43	13.77	12.50
V3	0	8.60	8.37	7.70	8.22
	100	9.70	11.47	12.93	11.37
	200	8.47	11.27	13.60	11.11
Mean K		11.82	13.66	14.23	
L.S.D (0.05)		K = NS			0.776
		V×H×K = NS			
V		V × K			Mean V
		0	400	800	
V1		15.47	18.68	18.48	17.54
V2		11.07	11.93	12.81	11.94
V3		8.92	10.37	11.41	10.23
L.S.D (0.05)		NS			1.143
H		H × K			Mean H
		0	400	800	
0		11.82	12.53	12.42	12.26
100		11.71	13.71	14.90	13.44
200		11.92	14.73	15.38	14.01
L.S.D (0.05)		1.768			NS

the spraying of potassium at 800 mg L^{-1} with spraying the kinetin at 100 mg L^{-1}had the highest value (5.47g) compared with non-spraying of potassium and spraying of kinetin at 200 mg L^{-1}which had the lowest value (5.12 g).The interaction between potassium concentrations and mung bean genotypes had significant effect on the 100 seeds weight, the Indiangreen VC6089A10genotype with spraying of potassium at 400 mg L^{-1}had the highest value (7.22 g) compared with local green with non-spraying of potassium which had the lowest value (3.44 g).The interaction between kinetin concentrations and mung bean genotypes had significant effect on the 100 seeds weight, the Indian green VC6089A10 genotype with sprayingof kinetin at 100 mg L^{-1}had the highest value (7.30 g) compared with the localgreengenotype with non-spraying of kinetin which had the lowest value (3.45 g). The interaction between three factors had significant effect on the 100 seeds weight, the IndiangreenVC6089A10 genotype with spraying of potassium at 400 mg L^{-1}and kinetin at 100 mg L^{-1}had the highest value (7.47 g) compared with local green genotype with non-spraying of potassium and kinetin which had the lowest value (3.26 g).

Seed yield: The concentrations of potassium and kinetin had non-significant effect on the plant seed yield, while the mung bean genotypes were significantly difference in this traits (Table 7). Indian green VC6089A10 genotype gave the highest mean (17.54 g) with a significant increase of 46.90 and 71.50% than the plant seed yield in the local green and Indian black GOLDSTAR genotypes which gave the lowest (11.94and 10.23 g ,respectively).The reason of the superiority of the Indian green VC6089A10 genotype in this trait could be due to its superiority in the weight of 100 seeds (Table 6) as well as the CAT enzyme activity (Table 2). Abdel-Baky et al (2019) and Al-Jubouri and Al-Jubouri (2019) also indicated that mung bean genotypes were significantly different in seed yield. The interaction between potassium and kinetin concentrations had significant effect on the plant seed yield, the spraying of potassium at 800 mg L^{-1} with spraying the kinetin at 200 mg L^{-1}had the highest value (15.38 g) compared with non-spraying of potassium and spraying of kinetin at 100 mg L^{-1} which had the lowest value (11.71 g).The interaction between kinetin concentrations and mung bean genotypes had significant effect on the plant seed yield, the Indian green VC6089A10 genotype with spraying of kinetin at 100 and 200 mg L^{-1} had the highest value (17.47 and 18.42 g respectively) compared with the Indian black GOLDSTAR genotype with non-spraying of kinetin which had the lowest value (8.22 g). The interaction between potassium concentrations and mung bean genotypes and the interaction between three factors had non-significant effect on the plant seed yield (Table 7).

CONCLUSIONS

This study confirms the positive role that potassium and kinetin plays, in improving the growth and yield characteristics of the mung bean crop, an increase in the activity of CAT enzyme. The genotypes differ in their response to growth factors and study factors, especially the green Indian genotype, which showed an increase in the activity of CAT enzyme, seed yield and its components.

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Scaling of Field Estimated Unsaturated Hydraulic Conductivity for a Stratified Soil

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Abstract: A field experiment was carried out to estimate and scaled the unsaturated water conductivity in an agricultural field south of Baghdad governorate. The instantaneous profile method Kipm was used to calculate the unsaturated water conductivity from the change in water content and water suction as a function of time and soil depth during the drainage cycle. The Van Genuchten (1980) model was matched to the moisture description curve data as a function of depth. Darcy's law was the theoretical basis for calculating Kipm then The Davidson et al (1969) model was selected and used as a function that was matched to the water conductivity values estimated by the Kipm method describing the soil hydraulic conductivity as a function of the water content only. The factors of the function Davidson model which is an exponential function were estimated in three ways after converting it to straight-line functions: Discrete model, Parallel line model, and Single Curve model. The first method (Discrete model) matched the Davidson model with Kipm and water content gave high correlation coefficient values (R^2) reaching 0.9448 with root mean square error (RMSE) reaching 0.3259. It resulted from the different slope (b) and different intercept (α) for each depth. The second method (Parallel line model) to match the Davidson model with Kipm and water content gave high correlation values (R^2) reaching 0.9032 with root mean square error (RMSE) reaching 0.4152. It resulted from one slope (b) for all depths and a different intercept (α) for each depth. The third method (Single Curve model) to match the Davidson model with Kipm and water content gave high correlation values (R^2) of 0.9968. It resulted from one slope (b) and one intercept (α) for all depths. This study aims to reduce the dispersion and heterogeneity of the data due to the heterogeneity in the inherited characteristics of the soil and makes it easier to describe this facilitating its adoption in ready-made programs.

Keywords: Unsaturated soil, Instantaneous profile method (Kipm), Hydraulic conductivity, Scaling methods

Saturated hydraulic conductivity (Ks) is an important parameter in hydrology, but getting representative Ks values, particularly in nature is difficult due to spatial and temporal heterogeneity and field measurement challenges (Soulis et al 2020). Unsaturated soil is defined as that part of the land or section of the soil in which the water content is less than the porosity of the soil or when the (matric suction) of water carries a negative value and is less than the free water potential the importance of studying the unsaturated water conductivity of the soil which is a function of the porous medium comes from its importance in the hydrologic cycle in nature and is represented by the layer of soil sandwiched between surface water and groundwater and is the carrier and interacting medium between them. It determines the amount of water that is transferred into the soil after rain and how much water it will run on the surface of the soil which causes floods and water erosion that most of the rainwater enters the soil and is absorbed by the plant or evaporates directly into the atmosphere and part of it seeps out by deep penetration to join the underground water storage, surface water and groundwater which may contain many pollutants. It is important to know how long it will take for these pollutants

to reach the surface layer of the soil or groundwater (Selker et al 1999) and thus reach the environment and the plants on which we feed. In addition to that unsaturated soil provides the water necessary for plant growth, it also provides oxygen and nutrients that the plant needs to continue its physiological functions and continue its growth, and productivity. In addition, knowing that unsaturated water conductivity significantly impacts the spread and growth of plant roots, biological diversity within the soil, salinization of land, and desertification. This has a major role in managing the land through its impact on agricultural operations such as plowing, the use of modern mechanization, and the date of sowing of economic crops (Dirksen 2000). Guber (2007) observed most biological and chemical processes in addition to water and dissolved nutrient absorption and gas exchange occur in unsaturated soil so the biological layer is defined as the unsaturated soil layer that lies between the field capacity and the permanent wilting point. Unsaturated water conductivity is defined by Klute (1965) as the ability of an unsaturated porous medium to conduct a soil solution and it is a variable and unstable property that varies from one soil to the next based on total porosity and pore size distribution as

well as the physical, chemical and biological properties of the soil. Hille (2004) defined it as the ratio of flow to water potential gradient.

In simple terms scaling is a series of processes for determining a scale's elements that link one system's characteristics to the comparable qualities of another system Dimensional analysis, investigative analysis, and functional analysis of the physical parameters of the soil are all strategies for scaling in soil physics (Reichardt et al 2010). Black et al (1969) suggested a fixed regression model in data scaled and assumed that the regression in water content is constant with depth during drainage. Davidson et al (1969) added to the idea of a regression linear equation. Sisson et al (1980) used several functions as a function of time and depth of soil volume to estimate water conductivity and volumetric moisture content during water subsidence and also, use a variety of approaches to conducting data matching and scaling. Reichardt et al (1972) were able to achieve remarkable success in scaling even with natural porous mediums such as soil which has a wide range of textures and compositions, they assumed that soil is a similar porous media and that each soil is different. It has its scaling factor: the soil is a similar porous media and each soil is different. Its scaling factor indicates the factors of difference which can be estimated to perform the scaling process between two soil systems and establish a mathematical model for the process.

MATERIAL AND METHODS

A field experiment was conducted in the Al- Rashid district south of Baghdad. The field soil was classified as sedimentary with a clay loam texture of (0-0.55 m layer) and Silty clay texture from 0.55 to more than 0.9 m layers, classified at the series level as DM97 (Al-Agidi 1976)and Typic Torrifluevents at sub group level Soil Survey manual (2004). The experiment site is located at 33°04' 28" latitude north and 44° 29' 41" longitude eastward at an altitude of 29 m above sea level.

Instantaneous profile method (Kipm) According to the method suggested by Ogata and Richards (1957)

To use the Instantaneous Profile method a field plot with dimensions (4×4 m) was selected and surrounded by ridges approximately 0.2 m height and a basic preliminary leveling of the soil surface within the plot area was made to provide an even and consistent distribution of floodwater in all sections of the plot. Drilling was done vertically in the plot center to a depth of 120 cm and a diameter of 0.5×8 m. The hole was moistened by immersing it in water and left for a second day to allow soil moisture sensors of the type (5ET) to be installed within the hole in the center of the plot. The data logger device type (Em50.ECH2O) was used to connect the sensors. The

sensors are then put in the soil trough at depths of 25, 50, 75, and 100 cm below the soil surface. The soil was gradually returned to the pit after adding each layer to conserve the density of the soil as much as possible. The water was added to the inside of the plot while maintaining a stable column of water 5 cm above the soil surface until reaching a state of balance in the moisture content after that the supply of water to the plot was stopped and after the water disappeared from the soil surface completely, the plot was covered by two layers of Nylon (polyethylene) to prevent evaporation from the soil surface and to ensure that the movement of water only occurs in the vertical direction downwards, A light layer of soft soil was placed over the nylon cover to reduce the effect of direct sunlight on the surface. The time for the disappearance of water from the soil surface was considered the zero time for the beginning of the drainage cycle which continued for 25 days. Then the soil cover was lifted and two samples of soil were taken from each depth from the area near the sensors site to estimate the physical and chemical properties in the laboratory (Table 1).

Theory and Statistical Analyses

First step (Internal drainage): Determining the unsaturated water conductivity during the drainage cycle as a function of time and soil depth. The Buckingham and Darcy 1907 equation is the theoretical basis for calculating unsaturated water conductivity.

$$q = - Ki \quad (1)$$

Where: q is the flow ($cm^2.cm^2.s^{-1}$), K is hydraulic conductivity ($cm. day^{-1}$) and is the water matric potential gradient (-cm) and when e measure water content at specific time intervals as a function of soil depth, the depth of the water stored in the soil it will be as a function of time and depth of the soil and is calculated by the following equation:

$$d(z,t) = \int_0^z \theta dz \quad (2)$$

where: d is a stored water depth as a function of depth and measurement time and (θ) is the volumetric water content measured at each depth. In the case of homogeneous soils θ represents the average water content for the entire depth (Libardi et al 1980). The stored water depth can be calculated directly from equation (2). However, in the case of heterogeneous soils (Layered soil) as in our case, the study must take into account the change in water content and water potential as a function of time and soil depth for the layer of the soil profile (Salim and Salih 2008).

The depth of the water stored in the soil volume from its surface (z = 0) to the upper limit (Z_{n-1}) of the soil layer for which the water conductivity is to be calculated is given by the following equation:

$$d_1(z_i,t_i)\Big|_{i=0}^{n-1} = \sum_{i=0}^{n-1} \int_{z_i}^{Z_{n-1}} \theta_i \, dz_i \quad (3)$$

As for the depth of the water stored in the layer between levels (n-1) and (n), it is given by the following equation:

$$d_2(z_i, t_i) = \sum_{i=n-1}^{n} \int_{z_{n-1}}^{z_n} \theta_i \, dz_i \qquad (4)$$

The flux is determined by the following equation in the absence of evaporation from the soil surface. According to Olsson and Rose (1978):

Where: $q = \int_0^Z \int_{t_1}^{t_2} \frac{\partial \theta}{\partial t} \, dz \qquad (5)$

q is the flow during the time between the measurement times t_1 and t_2. The time rate of change in the depth of water stored in the soil volume from its surface to the upper border of the layer, plus half the time rate of change in the depth of water stored in that layer between two successive measurement times, was used to describe the flow of a soil layer.

The time rate of change in the depth of the water stored in the soil profile above the layer between the two levels (n_1) and (n-1) is given by the following equation:

$$q_1 = \frac{d_1|_{t1} - d_1|_{t2}}{t_2 - t_1} \qquad (6)$$

As for the time rate of change in the water depth stored for this layer, it is given by the equation:

$$q_2 = \frac{d_2|_{t1} - d_2|_{t2}}{t_2 - t_1} \qquad (7)$$

Therefore, the flow rate (q) of the layer is given by the equation: $q = q_1 + \frac{1}{2}q_2 \qquad (8)$

The matric suction corresponding to each water content measured during the study was calculated using the factors of the moisture description curves obtained by matching the van Genuchten and Mualem (1980) equation which is the relationship between the matric suction and volumetric water content when applying different stresses to undisturbed samples soil for a range of 0 To 50 kPa, and by using a device Tempe pressure cell and by using a pressure plate apparatus device on disturbed soil samples in the range of 100 to -1500 kPa.

The following equation was used to generate moisture description curves:

$$\psi(\theta) = \frac{\left[\left(\frac{\theta - \theta_r}{\theta_m - \theta_r} \right)^{-1/m} - 1 \right]^{1/n}}{\alpha} \qquad (9)$$

θ_r represents the residual moisture content, θ_s the moisture content at saturation, (Ψ) the applied tension

(α,n,m,) the output constants of the (RETC) program. The gradient in water matric suction for a soil layer was calculated in a similar way to that used (Salim and Masood 2020). The gradient in the water potential of a soil layer was defined as the rate of the gradient in the water potential at two successive measurement times. Therefore, the average gradient in the water potential of the layer between the two levels is given by the following equation:

$$\overline{\frac{\partial \psi}{\partial z}}\bigg|_{Z_{n-1}}^{Z_n} = \frac{1}{2} \left[\frac{\partial \psi_T}{\partial z}\bigg|_{t_1} + \frac{\partial \psi_T}{\partial z}\bigg|_{t_2} \right] \qquad (10)$$

The unsaturated water conductivity was calculated from the product of dividing equation (8) by equation (10) to represent the value of the unsaturated water conductivity measured by the instantaneous soil profile method (Kipm).

Second step: Scaling of unsaturated hydraulic conductivity data. The theoretical basis was adopted by scaled unsaturated water conductivity data K θ calculated by the Kipm method by using three methods (Salim 1986, Sisson 1980, and Sisson et al 1988).

1-Discrete model: Davidson equation for the unsaturated water conductivity data for the layers of instantaneous soil volume was used to estimate the matching parameter. The spatial variance of the water conductivity can be expressed as a function of depth and water content) according to the following Warrick 1977:

$$k(\theta, z) = g(z) \, \hat{k}(\theta) \qquad (11)$$

k (θ, z) is the spatially variable water conductivity and g = g (z) is a scaling factor expressed as a function of depth (z).

$$\log[k(\theta, z)] = \log kg(z) + \log[\hat{k}(\theta)] \qquad (12)$$

Nominal hydraulic conductivity is a function representing the water conductivity it only depends on the water content. and when taking the logarithm of both sides of the equation (11) then it is a linear equation to solve a problem (12) first choose a function that connects the relationship between water conductivity and water content. Which is an equation by the Davidson equation that characterize water conductivity as a function of volumetric water content as follows

$$K(\theta) = K_m \exp\alpha(\theta - \theta_m) \qquad (13)$$

θ represents the volumetric water content, θr is the residual water content, and Km is the water conductivity when (θm= θ) and (α,m) position factors. Sisson and Salem (1988) demonstrated how to calculate the constants of Davidson models by converting them to linear equations by taking the natural logarithm of both sides of the equations

$$\ln\{K(z, \theta)\} = \ln\{K_m\} + \alpha(\theta - \theta_m) \qquad (14)$$

$$\ln\{K(z, \theta)\} = \alpha + b\theta \qquad (15)$$

Km, θm, θr, n, and α are factors that can change with depth and need to be estimated from scaling analysis. The values of n and α are used in linear regression analysis to match (Kipm) for each depth and values of θr and θm were estimated the matching factors were obtained using the statistical program (Statistical Analysis System SAS).

2. DAV models; These were matched based on Parallel line model, and using Dummy Variables as mentioned in Draper and Smith (1981) which results in matching lines with one slope for all depths in this case the regression equation for the Davidson model is as follows:

$$\ln(K) = \ln K_{m25} V_{25} + \ln K_{m50} V_{50} + ... + \ln K_{m100} V_{100} .... + b*(\theta_{25} - \theta_m) \ V_{25} + ...$$
$$.... + b*(\theta_{100} - \theta_m) \ V_{100} \qquad (16)$$

Since: (b) Represent the slope and its constant for all depths, four Intercept(α) represent $\ln K_m$, and (V_n) are position factors (Dummy variables) that take two values, either 0 or 1, (θ_n) The measured values of volumetric water content at each time and depth θ_m water content for each layer of soil volume.

3. Single curve model: In this method, the average values obtained from the second method are taken and the standard depth principle is used to obtain a single nominal hydraulic conductivity curve.

RESULTS AND DISCUSSION

Figure (2) shows the curves of the soil moisture description for layers [0-25, 25-50, 50-75, and 75-100 cm] for the Al-Rasheed soil site which was obtained experimentally in the laboratory for soil samples and determined soil moisture description curves Relationship between structural suction (Ψ) and volumetric water content (θ) and matching function factors of Van Genuchten (1980) (equation 9). The figure shows the matching function factors (lines) correlation coefficient (R^2) and measured values (scatter) for the four layers. There were wide differences in the water content between the layers at high suction (from 0 saturation to 1 bar), while there are few differences in the water content between the layers at low suction (less than 1 bar). The reason for this is that the structure of the soil and the large pores determine θ(Ψ) at high suction or the percentage of large pores at this suction while the specific porosity controls

Table 1. Physical and chemical properties of the studied depths of the soil profile in the Al-Rasheed site

Feature	Depth (cm)			
	(0-25)	(25-50)	(50-75)	(75-100)
Sand (g.kg^{-1})	256.0	254.0	168.4	182.0
Silt (g.kg^{-1})	412.4	394.4	422.8	405.2
Clay (g.kg^{-1})	331.6	351.6	408.8	412.8
Soil texture	Clay Loam		Silty Clay	
Bulk Density (m.g.m^{-3})	1.296	1.367	1.436	
True Density (m.g.m^{-3})	2.618	2.632	2.634	2.650
Porosity (%)	50.4	48	45.4	40.2
O.M (%)	1.2	0.6	0.4	0.1
EC 1:1	1.75	2.4	3.5	4.1
pH	7.0	7.1	7.2	7.3
CaCO^3(%)	25	28	30	29
Gypsum (%)	0.9	0.7	0.4	0.1
CEC (meq^{-1}.100 gm Soil)	16.0	14.6	15.2	22.1
Ca^{++} (mmol+. L^{-1})	5.7	7.4	9.1	11.2
Na$^+$ (mmol+. L^{-1})	8.8	11.2	15.9	20.4
K$^+$ (mmol+. L^{-1})	0.82	0.75	0.89	0.85
Mg^{++} (mmol+. L^{-1})	2.2	4.6	7.6	8.6
HCO$_3$⁻ (mmol+. L^{-1})	2.3	2.1	1.8	1.9
Cl⁻ (mmol+. L^{-1})	12.4	14.1	11.9	9.8
So$_4$⁻⁻ (mmol+. L^{-1})	2.8	7.8	19.8	29.4
SAR (mmol+. L^{-1})$^{0.5}$	3.1	2.9	3.9	4.6

θ(Ψ) at low suction. The decrease in the values of the volumetric water content of the measured samples from the values of the samples estimated by the model at the suction varied from 0 to 10 kPa, at the two depths [50-75 and 75 -100 cm] . This is due to the difference in the value of the water content at saturation (θ_s) for the values estimated by a model Van Genuchten (1980) from the value of the volumetric water content at saturation for the soil samples, The Figure shows that the highest water content at saturation was (0.5611 cm3 cm3, and at a depth of 75-100cm. This is attributed to the high percentage of the clay and silt) at this depth which amounted to 81.8% .The figure shows a decrease in the water content at depth 0-25 cm from the rest of the depths at the same suction value. The reason for this is due to the high percentage of sand at this layer, as well as to the lower bulk density than the rest of the layers (Table 1) and the depth 25-50 cm retained a high-water content at near a saturation suction due to the upper layers of soil retaining quantities of water higher than the lower at low suction close to a saturation value. The discrepancy in the values of the criteria of the matching function obtained from the (RETC) program ($\theta_r, \theta_s, \alpha, n, m$) for the studied depths (Table 2). The studied soil characteristics in the Al-Rasheed site have stratigraphic variation and the soil is heterogeneous in its water properties, θs reaching 0.4728, 0.5344, 0.4458, 0.5611 cm3 cm-3 for the four layers respectively, at an applied suction from 0.1 to 1500 kPa.

The Figure 2 shows the change in the measured volumetric water content versus the change in suction which ranged from 0.1046 cm^3 cm^{-3} to 0.4689 cm^3 cm^{-3} and from 0.1088 cm^3 cm^{-3} to 0.5289 cm^3 cm^{-3} and from 0.1062 cm^3 cm^{-3} to 0. 4501 cm^3 cm^{-3} and from 0.1056 cm^3 cm^{-3} to 0.5681 cm^3 cm^{-3} for depths [0- 25, 25-50, 50-75, 75-100 cm respectively] when shedding suction from 10 to 1500 kPa.

RETC program used in matching the Van Genuchten (1980) model represents the remaining water content at the permanent wilting point corresponding to the tensile value 1500 kPa, from 0.1088 to 0.1046 for depths of 25-50 cm and 0-25 cm, respectively. The values of criteria ranged from -120

to -12 -cm. H_2O, for the layers 75-100 cm and 0- 25 cm. The decrease in the value of (α) for the layer 75 -100 cm compared to the upper layers may be because this layer has a higher bulk density and higher clay content, which affects the distribution of soil pore volumes and the lower value of air entry (negative value). This is consistent with Rawls et al (2019). The values of (n) ranged from 1.324 to 1.4797 for the layers 0-25 cm and 25-50 cm, respectively. The value of (n) represents the slope of the moisture description curve at each depth. This criterion is affected by the distribution of soil pore volumes. The curve of the moisture description of the soil volume of the Al-Rasheed site indicated increase in the volumetric water content of the second and fourth layers and this appears to be related to the decrease in the values of (n) at these two layers. The variation in the different values of the parameter (θ_r, θ_s, α, n, m) for each depth from the rest of the
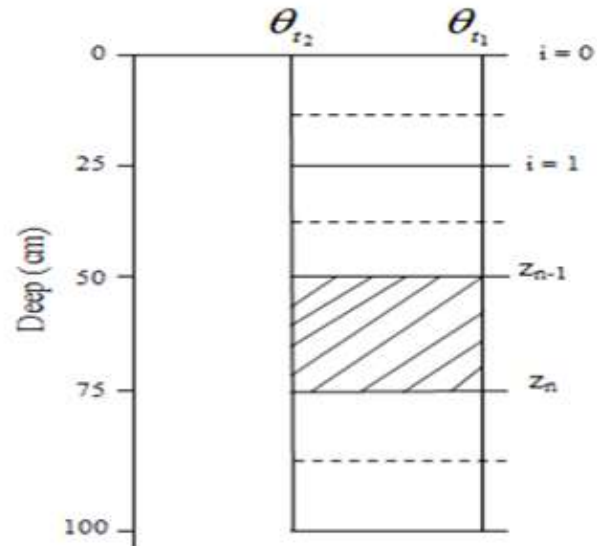

Fig. 1. Theoretical basis for calculating the flow of soil layers using the instantaneous soil profile method

Table 2. Values of the Van Genuchten model parameter used in matching and estimating the unsaturated water conductivity

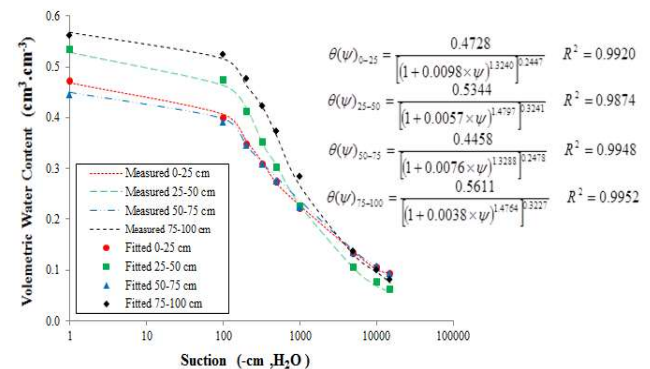| Parameter	Depth (cm)			
	25	50	75	100
θs	0.4728	0.5344	0.4458	0.5611
θ_r	0.1046	0.1088	0.1062	0.1056
α	0.0098	0.0057	0.0076	0.0038
n	1.3240	1.4797	1.3288	1.4764
m	0.2447	0.3241	0.2478	0.3227
R^2	0.9920	0.9874	0.9948	0.9952

Fig. 2. Soil water retention curves show four layers in the field of study

depths explains the variation in the form of the moisture description curve for each of the studied layers in the soil of Al-Rasheed district and that refers to the effect of the difference in morphological characteristics such as structure, texture, porosity and bulk density with the curves of the moisture description of the studied layers in this research.

Discrete line method: Figure 3 shows the results of matching the first method of scaling for the Davidson model represented by (lines) to the Kipm data (scatter) as a function of the water content of the layers [0-25, 25-50, 50-75, 75-100 cm]. Table (3) shows the estimated values of the parameter function equation (15). The regression (b) values ranged from 32.4503 to 57.1697, the intercept v (α) ranged from -1.3917 to 1.8601, the correlation coefficient was 0.9448 and the square root of the mean squared error was 0.3259. The high correlation coefficient value indicates that the Davidson model showed a 94.48 % variance in the measured values for unsaturated water conductivity.

This method requires the use of (8) factors to describe the unsaturated water conductivity the figure shows that the highest value of the unsaturated water conductivity occurred at a depth of 50-75 cm, with a value of 1.9 cm^3 day^{-1} at the beginning of the puncture stage, and 0.024 cm^3 day^{-1} at end of the drainage cycle the reason. This can be attributed to the high percentage of sand separated at this depth and to the difference in texture in this layer from the layers that precede it and the unsaturated water conductivity of the depth 0-25 cm reached 0.1663) cm. day^{-1} at the beginning of the drainage cycle with a value of 0.069 cm. day^{-1} after the drainage period ends. The may be attributed to the fact that the highest sand content occurred in this layer reaching 25.6% at the beginning of the drainage stage, the water begins to move rapidly down from the upper layers under the influence of gravity and the flow increases downward until the water reaches the layer 25-50 which is a layer saturated with water under which is a layer that differs in texture and constitutes an obstacle to the penetration of water through it as well as its bulk density High compared to the rest of the layers the water conductivity of the layer 25-50 cm with value of 0.16 cm day^{-1} at the beginning of the drainage cycle and 0.07 cm. day^{-1} at the end of the drainage cycle. The figure shows that the layer 75-100 cm recorded a decrease in the unsaturated water conductivity values reaching 1.3 cm. day^{-1} at the beginning of the drainage cycle and 0.11 cm. day^{-1} after the drainage cycle ended. This is due to the high percentage of fine soil separations (clay and silt) and the higher bulk density in this layer than the rest of the layers, and this is consistent with Feride and Coskun (2008) which states that the water conductivity decreases with the increase in the ratio of the separations (clay and silt).

Parallel line method: Figures 4 show the unsaturated water conductivity values obtained by the Kipm method (scatter) and the Parallel line Method values (lines) obtained using the Davidson model. This method requires matching lines of equal slope representing the slope of all depths with an intersection point those changes with depth. The figure shows the four functions of the Davidson models.

The parallel lines were drawn using the Dummy Variables method using Draper and Smith 1981 method, and the correlation coefficient (R2) was 0.9032 and the root means square error (RMSE) was 0.2152, and the intercept values were 0.4624, 0.9141, -0.0921, 0.8239 for different depths [0-25, 25-50, 50-75, 75-100 cm, respectively], and the value of the slope (b) for depth from 0-100 cm was 43.1456. The main objective of using Parallel line method is to reduce the amount of dispersion in the measured data values of the unsaturated water conductivity using the Kipm method for each depth without significant impact on the real values measured in the field by scaling the data for each depth relative to one common regression represents the regression of the entire profile in preparation for conducting the scaled
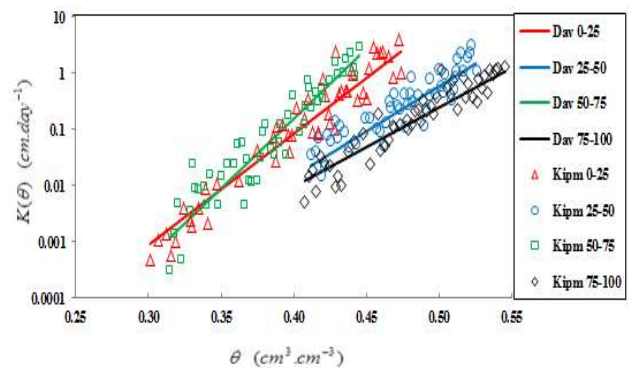

Fig. 3. Hydraulic conductivity-volumetric water content relations for all depths (cm). Solid lines are given by (15) and by assuming the Davidson model
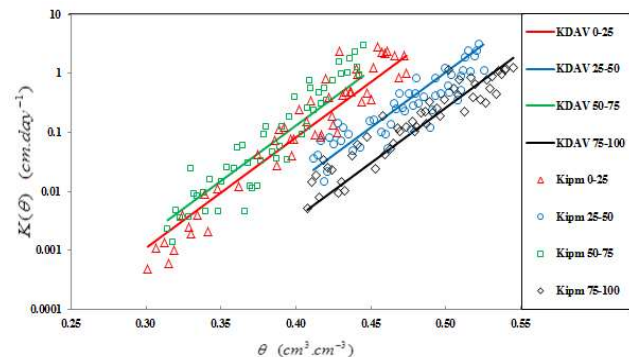

Fig. 4. Hydraulic conductivity-volumetric water content relations for all depths (cm). Solid lines are given by 16 and by assuming the Davidson model

process using the second method of scale (Nominal Hydraulic Conductivity).

Single curve method: Sisson (1987) formulated the term Nominal Hydraulic Conductivity for the curves resulting from the second method of scaling and collecting them in one curve, where the section (α) is equal to the average of the intercept values (Table 4). The use of a matching factor for the volumetric water content under the hypothesis of the reference depth, and the (solid line) represents the nominal hydraulic conductivity for all depths with a slope equal to the Parallel line Method and with a segment equal to the (intercept) rate, and used in the second method Parallel line Method. It represents the relationship between (Kipm) and volumetric water content, under the principle of Reference depth theory obtained from a simple calculation process based on calculating the average of the values for the studied depths. Figure (5) shows the Nominal Hydraulic Conductivity function using the Davidson model which was obtained using the Dummy Variables method and the outputs of the SAS program.

The relationship in the second method of scaling (Parallel line) gave four parallel lines with one regression value, in

Davidson et al.(1969) Model (Single Curve)

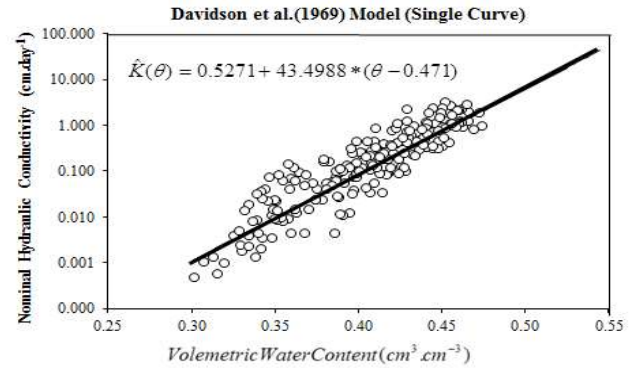$$\hat{K}(\theta) = 0.5271 + 43.4988 * (\theta - 0.471)$$

Fig. 5. Nominal hydraulic conductivity based on the Davidson model (solid line) resulted from horizontally shifting the lines shown in figure (4) to the line from 100 -cm depth. The scatter represented the Scaled θ m^3.m^{-3}

which each regression line represents the matching model used for a specific depth of the ridge and spread around each of these lines the unsaturated water conductivity values measured by the instantaneous profile method (Kipm) and represented by scatter. This data dispersion occurs due to the variation in the inherited traits in each of the four layers in which the change in the volumetric moisture content with depth during the time was monitored. In the third method of scaling, this dispersion was treated as a result of the effect of variation in the layers of the volume by adopting one regression value and one intersection point corresponding to the rate of change in the averages of the volumetric water content data at each of the studied depths. The main objective and benefit of using the Single Curve Method was to find the water content at any point inside the soil volume relative to the total regression line of the model function used in the Silem (1986) scale.

Table 3. Values of the Davidson model parameter used in matching and estimating the unsaturated water conductivity

Depth (cm)	Davidson et al 1969 Model			
	Intercept	Slope	R^2	RMSE
0-25	1.8601	45.5422	0.9448	0.3259
25-50	-0.7095	36.8927		
50-75	3.5827	57.1697		
75-100	-1.3917	32.4503		

Table 4. Regression (b) and intercept (α) values used in scaled data according to Davidson models, the correlation coefficient, and the root means square error (RMSE) for the Davidson model

Depth (cm)	Davidson et al 1969 Model			
	Intercept	Slope	R^2	RMSE
0-25	0.46248	43.1456	0.9032	0.2152
25-50	0.91410			
50-75	-0.09210			
75-100	0.82398			

Table 5. Values of the matching factors used in the model and the correlation coefficient

Depth (cm)	Davidson et al 1969 Model		
	Intercept	Slope	R^2
0-100	0.52712	43.4948	0.9968

CONCLUSION

The time rate of change in the depth of the stored water was the highest at the beginning of the drainage cycle and increased with increasing depth The results of calculating the unsaturated water conductivity according to the Kipm method gave excellent and encouraging results when implemented in the site, confirming its effectiveness and importance in being a measurement reference for all other laboratory and field methods. When match parallel lines the variance ratio may decrease but adopting the Scaling theory using one scaling factor reduces the number of factors needed to describe the data. and having a single function to describe all the data reduces the dispersion and heterogeneity of the data as a result of the heterogeneity in the inherited characteristics of the soil.

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Investigation on Presence of Zearalenone in Infertile Individuals and Effect on Immunological Parameters

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Abstract: The study detects the concentration of Zearalenone in blood of individuals with infertility and investigates the relationship between the ZEA and infertility in the Province of Al- Najaf Al Ashraf city using Enzyme- linked Immunosorbent Assay (ELISA). In addition, the study assesses the relationship between Zearalenone concentration and IL-6 levels in individuals with infertility, also using ELISA. Current study results show that Zearalenone was detected in 75% of individuals with infertility assessed for the presence of ZEA in their serum. The 56% of the ZEA-carrying infertility group were male and 44% were female. In addition, the highest concentration of zearalenone detected with ELISA infertility patients (18.49 ng/ml) and the lowest concentration (5.74 ng / ml) with a significant difference with the control group (4.892 ng/ml). ZEA toxicity leads to a marked decrease in Interleukin-6 levels in infertility individuals. The results of the study have revealed that the concentrations of (IL 6) in the total number of patients and the control group have a significant decrease (3.38 and3.79 ng/ml respectively).

Keywords: Infertility, ZEA zearalenone, IL6 Interleukin 6, semen azoospermia

Infertility is characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. Common causes of infertility using standard fertility investigations, include semen analysis, assessment of ovulation and tubal patency test. Zearalenone (ZEA) or F-2, one of the secondary types of toxic metabolism acts as estrogen receptor, is produced by fusarium. Aiko 2015) Cereals are main staple foods in diets worldwide. The presence of toxins in many cereals can occur in human food sources like corn, maize, barley and oats infected in the field or exported as food products such as flour or canned bad storage (Andared et al 2017, Marin et al 2019, Mariana 2020). ZEA is found in high concentration in domestic animals such as in cows, chickens, pigs, rabbits and even fish, or in animal products such as milk, meat (Sanzani 2016, Degen et al 2017). Different concentrations of ZEA can cause different toxicity effects. ZEA is metabolized in the liver, which can induce liver and kidney damage resulting in increased aspartate, amino transferase, and alkaline phosphatase activity Gao et al (2018). Low concentrations of ZEA stimulate cell growth, mainly in estrogen- dependent tumor cells, as a result of estrogen effects Gratz 2017). immunotoxicity damage to lymphoid organs, leading to atrophy of the thymus, and subsequently a decrease in multiple immune cells by inhibiting cell growth .and promoting apoptosis .Hueza et al 2014. ZEA toxicity affects both male and female reproductive systems, as reported in different animal models and these effects can be transferred throughplacenta to cause embryonic toxicity

(Zhen et al 2016 and Bielas et al 2017). ZEA induced-reproductive toxicity damage can be mediated by different mechanisms, either by acting as estrogenic receptor, (Ya. et al 2018)or by causing DNA damage and cell apoptosis which were detected in the testicular cells by ZEA exposure disrupting spermatogenesis, or by affecting counts, motility, activity and grad of sperm. Many genetic ways are known to play vital roles in spermatogenesis and sperm functions and sperm morphology (Alshannaq and Yu 2017). ZEA enters the nucleus and binds to estrogen response elements, triggers target gene transcription and related protein synthesis, disrupts normal endocrine function, and leads to endocrine toxicity. ZEA is metabolized by hydroxylation-reduction reaction, mainly occurring in the liver, which is reduced to α-zearalenol and β-zearalenol. These two zearalenols can be further reduced to metabolites; conjugation reaction metabolite, such as zearalenone-14-glucoside (Z14G) and zearalenone-16 glucoside (Z16G) (Rogowska et al 2019). This study is to know effect of ZEA on infertility patients and interloken 6 (IL6).

MATERIAL AND METHODS

This study was conducted in the laboratories of the Department Biology, University of Kufa. Standard Zearalenone ZEA obtained from the Ela science (USA). Sixty samples of individuals with infertility and thirty samples from individuals without infertility as controls were collected from Fertility Centers. Five ml of blood was withdrawn from each individual and placed in gel tube at room temperature for 10

minutes. After that, blood is separated by centrifugation at 3,000RPM for 5 minutes .and then the serum was collected in sterile tubes determining concentration of (ZEA) and IL-6. The samples were taken from both gender (male and female) and installed information for each patient in data sheet included all the ages from 19-< 43year. All reagents, standard solutions and samples are prepared as instructed. All reagents are brought to room temperature before use. The assay is performed at room temperature. The number of strips required for the assay determined. The strips are inserted the frames for use. The unused strips stored at 2-8°C. 3.A 50µl standard is added to standard well. Antibody was not e added to standard well because the standard solution contains biotinylated antibody. The 40µl sample is added to sample wells and then 10µl anti-ZEN antibody is added to sample wells, then ٥٠µl streptavidin-HRP is added to the sample wells and standard wells (not blank control well) mixed well. The plate is covered with a sealer. Incubation was for 60 minutes at 37°C.

The sealer is removed and the plate is washed 5 times with wash buffer. Wells are soaked with at least 0.35ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. 50 µl substrate solution A is added to each well and then a 50 µl substrate solution B is added to each well. The plate covered with a new sealer is incubated for 10 minutes at 37°C in the dark. The 50µl stop Solution is added to each well, the blue color will change into yellow immediately. The optical density (OD value) of each well is determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.**RESULTS AND DISCUSSION**5

Detection of Zearalenone with infertility patients (Serum): In individuals with infertility, Zearalenone was recognized after the examinations of the serum samples by using by Elisa (Enzyme-linked-Immunosorbent assay). The Zearalenone was detected in 75% of individuals with infertility, and in 25% without ZEA in infertility individuals (Table 3-1). Ali (2001) found high concentration of mycotoxin ZEA in human body effects on the many physiological important in human body and in reproductive system. The high concentration of ZEA detected in human blood is attributed to the high toxin concentrations of contaminated food or animal products consumed or due to direct contamination of the hands with toxins on infected-fruits infected with toxin, which could be due to injury of plants in the field, bad storage, or transports conditions (Nariana 2020).

Detection of in fertility in patients (Gender): Males are more affected by infection than females, as the rate of infection in males was 56 % while in female 44 %. The results imply that toxic injury in humans has its effect according to the concentration or dose of the toxin, low, medium, and high. The percentage of toxicity in male is higher than that in female because of the exposure of male to the external environment, working in places that sell animals and their products and fruits, or by consumption of food outside/during work hygienic control Shuo (2020). The significant increase with highest and lowest concentration of Zearalenone in male was 18.495 and 5.077 ng/ml while in female were 13.45 and 5.44 ng/ml (Table 3). The infertility significantly increased to 9.44 and 7.76 ng/ml respectively compared with the control group (3.42 and 3.03ng/ml). The variation depends on age (19-26, 27-34 and 3<43 old years being 10.062, 7.677 and 5.99ng/ml respectively) compared with the control group (4.546, 3.245 and 2.951 ng/m) (Figs. 3-1 to 3-3). The study concluded that the toxin caused harmful effects on the organs and reproductive cells of females and males, but with different mechanisms. In men, the effect of the Zearalenone ZEA was clear in the process of spermatogenesis, where the effect was in low concentrations and for long cumulative periods, where the Zearalenone ZEA concentrations showed a high percentage as a result of the accumulation of the toxin inside the body for long periods. This effect may be due to the effect of the ZEA on the acrosome and on the mitochondria, which are responsible for the energy and movement of the sperm (Pereira 2014). In women, the study relied on toxic concentrations and the diagnosis of the specialized doctor through colored rays and ultrasound of the patient and patient information in the fertility center. The toxic effect was clear through high toxic concentrations for short periods, as the effect on ovarian development was the emergence of the phenomenon of early puberty, a defect in the uterus, female hormones, and on the cycle, Menstruation is clear and also the appearance of ovarian cysts. The poison may also have a role in the failure of the assisted insemination processes (IUI-IVF) themselves, as well as the occurrence of abortion of the fetus after the success of the operation (Zhang 2016).

ZEA with infertility patient's according to age: There was significant increase in the concentrations of (ZEA) in the total number patients suffering from infertility disease with ZEA and the control group (1.569 and 0.3096 ng/ml respectively). Concentration of Zearalenone in male and female individuals with infertility was significantly increased to 2.984 ng/ml as compared with the control group (0.531ng/ml). In addition, variation depends on ages (19-26, 27-34 and 35-<43 old years was 10.05, 7.67 and 5.99 ng/ml respectively) compared with the control group (4.54, 3.24 and (2.95 ng/m) (Figs. 1, 2, 3 and Table 1). The results of this age group 19-26

showed highest concentrations of the toxin, (10.245 to 17.231 ng/ml). The number of women in this age group was more than men and the toxic concentrations were higher than the other groups. The study attributed this to the fact that the sufferers did not review the infertility centers from after puberty until after marriage one or two years after pregnancy, so we see the cumulative concentration of the poison was

high and the effect of the poison on the organs were strong a defective in this category. In the second age group (27-34), the toxin concentrations in this category were less than that in the previous category in terms of concentrations and effect, and the effect was clear in the results of semen on movement, number, quantity of fluid and viscosity. Pregnancy, but natural causes and taking them for hormonal

Table 1. Concentration of ZEA in blood according to gender

Male	Female
18.495	13.455
17.108	12.639
12.865	12.033
12.012	11.082
10.89	10.455
10.702	10.129
9.823	9.739
9.478	9.495
8.421	8.512
8.222	8.474
8.113	8.235
8.055	7.804
8.004	7.364
7.811	7.268
7.717	7.069

Fig. 3. Concentration of ZEA (ng/ml) infertility patients and control age group

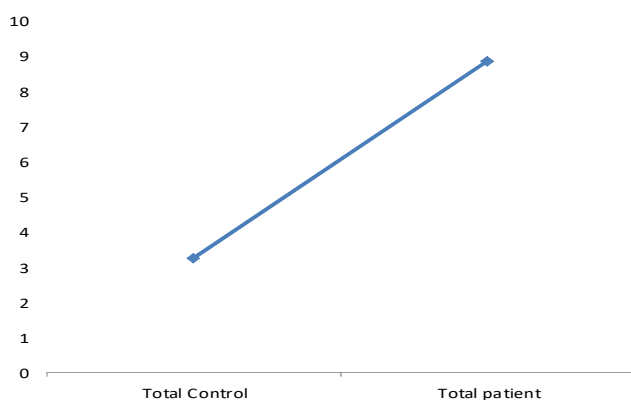

Fig. 1. Concentration of ZEA (Ng/Ml)

Fig. 4. Concentration of IL6 (ng /l) in total number of infertility individuals and control group

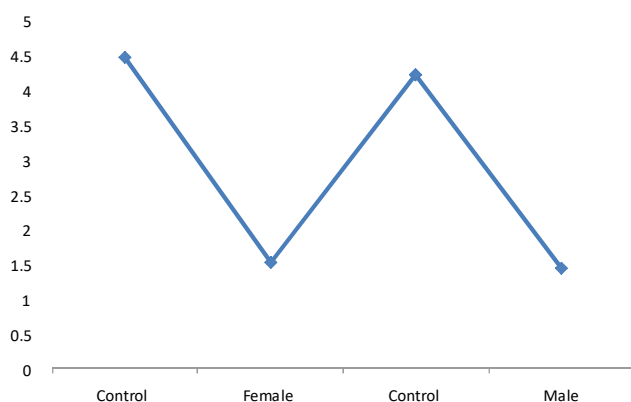**Fig. 2.** Concentration of Zea (Ng/Ml) in male and female of patients and control group

Fig. 5. Concentration of IL6 (ng /l) in male and female infertility individuals and control group
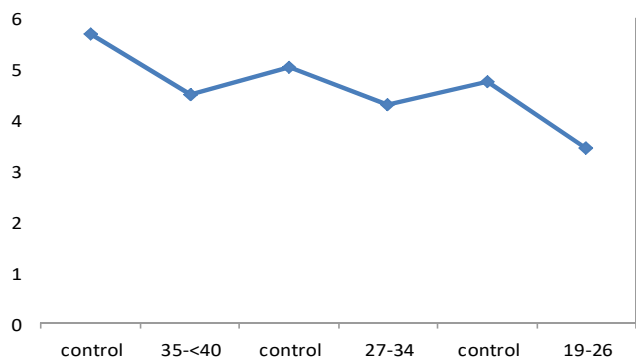
Fig. 6. Concentration of IL6 (Ng/Ml) infertility patients and control age group

treatments may have a negative impact on the toxic concentrations and their effect on the hormones inside the body, or the glands work again properly. Pregnancy has been proven in patients with primary and secondary infertility after taking treatments regularly. The emergence of polycystic ovaries in most patients of this category, a condition that is difficult to treat with surgical intervention or treatment for a long time and has a significant impact on irregular menstruation and non-incidence of pregnancy.

Interleukin 6 (IL6) concentration in serum: The results of the study states that the concentrations of IL6 in the total number of infertility individuals and the control group have a significant decrease (1.876 and 5.446 ng/l, respectively). The concentration of IL6 in male and female individuals with infertility was significantly decreased to 1.447 and 1.522 ng/l, respectively compared to the control group (4.229 and 4.468 ng/l respectively). It also revealed a variation depending on age ranges (19-26), (27-34) and (35-< 43) years old in patients suffering from infertility patient compared with the control group (3.45 , 4.29 and 4.48ng/l) in the total number patients suffering from infertility patient and the control group (5.67 , 5.02 and 4.75 ng/ml) (Figures 4, 5,6). These results agree with previous studies of Cai et al (2018).In physiological conditions, the testicular cells and somatic cells such as Sertoli and Leydig and the cells surrounding the seminiferous tubules produce large amounts of interleukin-6 (IL6), which is involved in the formation of sperm, the maturation of the seminal fluid and in the functioning of the glands. The study demonstrated the effect of zearalenone ZEA on the destruction of the cells of the testis, seminiferous tubules, semen and its components, and this leads to a defect in the work and production of these cells. The decrease in the level of (IL6) in infertile patients was also observed by Yang (2018). The study also found the effect of the Zearalenone ZEA on anther organs like liver and kidneys. The study found that as estrogen, did not stimulate the immune system to increase IL6 production, despite the toxin affecting the organs responsible for it. The up-regulated the expression of inflammatory cytokines Interleukin, and decrease of IL6. The important role of IL6 in body immunity showed by `the production of proteins that lead to elevation of body temperature and activated phagocytes secrete cytokines and chemokines that attract and active the innate immune responses.

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Determination of Amino Acid Content in Leaves of Mung bean (*Vigna radiate* L.) Plant Treated with Bio-Fertilizer

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Abstract:A field experiment was conducted during the summer agricultural season 2019 at University of Al-Qadisiyah in order to determine and estimate the quantity and quality of amino acids in the leaves of two cultivars of Mung bean plant *Vigna radiate* L.(Iranian cultivar and Iraqi cultivar) treated with bacterial inoculum or bio-fertilizer (specialized rhizobium bacteria on mung bean plant *Rhizobium leguminosarum* and *Bacillus sp.* bacteria). The vaccine was obtained from the Agricultural Research Department , Al-Zafaraniya. Baghdad, Iraq. A factorial experiment was carried out using the completely randomized design, the first factor included the inoculated rhizobia and bacillus bacteria and a mixture of bacteria and a treatment without the inoculation and the other factor includes cultivars (Iraqi variety and Iran genotype). The results indicated that the use of bacteria increased the amount of amino acids for the two cultivars, while the treatment of the fertilizer mixture with the Iraqi variety excelled in obtaining the largest amount or concentration of acids in the plant leaves, with largest concentration for proline acid, which was 499.22 micrograms/dry weight, followed by leucine and lysine, which recorded concentrations of 498.21 and 389.21 µg per dry weight .

Keywords: Biofertilizer, Cultivars ,Amino acids, Mung bean

Mung bean (*Vigna radiate* L.) is a leguminous summer annual, herbaceous, branching, with a length of 25-125 cm. It is grown in Iraq for many purposes, including obtaining seeds of high nutritional value for human nutrition because seeds contain a high percentage of protein, ranging from 19-29%, consisting of amino acids, including lysine (Avenido and Haltori 2001, Al-Fahdawi 2004, Salih et al 2017). It may be used as green fodder for animals or as green manure to improve soil properties (Al-Saadoun and Abdullah 2015). Biological fertilizers are considered one of the most important and most recent technologies used in agriculture, with the aim of reducing chemical fertilizers, which leads to reducing environmental pollution and reducing production costs. It also improves the agricultural production of leguminous crops rich in proteins, including mash and increase the yield in terms of quality and quantity and reduces the incidence of fungal and nematode diseases. This leads to the production of healthy and safe food for humans and animals, as it contains a high amount of amino acids, which are the basic unit for the formation of proteins (Egamberidyva 2007, Mkhaimer 2008). These acids have a benefit in the plant body as well as a benefit in the field of industry as an addition to animal feed. The lysine, methionine and threonine are among the most used acids in the production of feed, and also used as flavouring in the food industry , glutamic acid is one of best taste enhancers (Robiquet and Vaughelin 2005, AL-Swaf 2011). The soil in the rhizosphere plays an important role in promoting plant growth. The most important organisms used in this field are *Bacillus bacteria*. It improve the physical properties of soil, including porosity (Jabbar 2018) and increase the readiness of unfavourable phosphorous to triple calcium phosphate (Sharma et al 2013). It also acts as an antibiotic and promoter of plant growth (Acuna et al 2011, Atiya et al 2018). Rhizobia is one of the most

important bacterial organisms used as biofertilizer, as it is originally present in Iraqi soil and is inactive and relatively few in number (Jamal and Al-January 2006).   So it is necessary to add the appropriate bacteria as fertilizer for the purpose of improving production in quantity and quality. Abbas et al (2004) explained that the protein amount of the mung bean plant increased significantly when inoculated with root nodules bacteria as compared to non-inoculated plants. Al-Burki (2020) confirmed that the use of specialized rhizobia on bean plant led to an increase in the concentration of tryptophan in plant leaves compared to non-inoculated plants. The use of bacteria as a suitable bio-fertilizer and effect on the quality of amino acids in the leaves of the plant as an indicator.

MATERIAL AND METHODS

A field experiment was carried out in clay loam soil for cultivating the mung bean crop during the summer agricultural season at University of Al-Qadisiyah in order to identify and estimate the amino acids in the leaves of two cultivars of mung bean plant treated with bio-fertilizer. A factorial experiment (4×2) was carried out in a completely randomized design with three replications. The averages were compared using genestate program (Al-Sahoki and Whib 1990). The experimental land was prepared from ploughing and clouding, and field was divided into meadows, the length of each meadow was 2 m and the distance between one meadow and another was 50 cm. The distance from plant to plant was 25 cm after soaking them with bacteria for half an hour using 10% gum arabic by the carrier method. After the germination process the plants were reduced to one plant. Soil samples were taken randomly before planting at a depth of 0-30 cm and were mixed homogeneously and

analyzed in University of Baghdad for the purpose of obtaining their physical and chemical characteristics (Table 1) (Naseem et al 2019). The amount of amino acids in plant leaves was estimated according to the method of Scriver et al (2001) using an amino acid analyser.

RESULTS AND DISCUSSION

The effect of the interaction of bio-fertilizer with the variety on the content of the marrow plant leaves of amino acids was observed. The concentration or quantity of amino acids in the leaves of the plant treated with Rhizobia and Basil bacteria (inoculated) compared to the non-treated (non-inoculated) the control. The interaction between the two factors (fertilizer + variety) had a significant effect on the amount of amino acids (Table 2). The interaction of the Iraqi variety with the mixture of Rhizobia and Basil bacteria achieved the highest quantity of amino acids(389.21 micrograms), lucein acid recorded in 498.21 micrograms, and glycine acid was 261.21 microns, while arginine and glutamine recorded concentration of 6235.44 and 271.33 each, respectively. The treatment of the Iranian variety without bacteria or fertilizer recorded the lowest rate or

concentration of amino acids, which was 96, 39, 18 2, 36, 59.48, 79.69 and 10759 micrograms. Dry weight of each of the acids, respectively lysine, leucine, glycine, arginine and glutamine, and the percentage of increase in the amount of acids was 23.21, 21.43, 39.32, 42.41 and 50.86%, when compared with the control treatment, respectively.

The proline acid recorded highest value when treating the mixture of bacteria (Rhizobia + Bacillus) with the Iraqi variety, and was 499.22 micrograms compared to the control treatment. 11, 197.34, 195.33 and 179.40, respectively, and due to an increase as follows: 21.65, 30.67, 20.53, 31.12 and 30.95%, the increase in amino acids in plant leaves. It is considered as an indicator of an increase in the amount of protein in the plant and thus an increase in the quality of the plant. The increase in acids when treating the fertilizer with rhizobia and bacillus is due to the role of the rhizobia bacteria in the process of fixing atmospheric nitrogen and increasing the nitrogen element in the plant, which is included in the construction of amino acids, which is the basic unit for building proteins (Lordianis et al 2013). It may be due to the availability of

Table 1. Physical and chemical characteristics of the field experiment soil

pH	E.C. (ds.m^{-1})	Organic matter (%)	N (mg.kg^{-1})	P (mg.kg^{-1})	K (mm ch.L^{-1})	Clay (%)	Silt (%)	Sand (%)	Soil texture
7.28	3.54	1.10	90.2	38. 3	72.7	72.76	18.84	8.4	Clay loam

Table 2. Effect of acids in mung bean plants of bio fertilizer and variety interaction on leaf content of amino

Variety	Fertilizer	Glutamine	Arginine	Glycine	Lucien	Lysine
Iraqi	Co	179.85	165.32	187.48	410.26	315.89
	B	256.48	200.36	210.29	465.58	345.69
	R	*268.44	*233.47	*256.29	*497.15	*386.32
	B + R	**271.33	**235.44	**261.21	**498.21	**389.21
Iranian	Co	107.59	79.69	59.48	182.36	96.35
	B	123.33	88.24	72.18	210.39	112.48
	R	147.89	123.24	110.39	296.28	175.46
	B + R	149.77	125.44	120.31	296.55	181.36
LSD (p=0.05)		18.2	15.5	31.3	67.6	75.5

Table 3. Effect of bio-fertilizer and variety interaction on leaf content of amino acids in mung bean plants

Variety	Fertilizer	Phenylalanine	Serine	Tryptophan	Methionine	Proline
Iraqi	C	148.79	136.99	148.96	215.89	410.36
	B	178.58	156.32	160.35	253.02	474.11
	R	*193	*174.13	*195.22	*278.20	*497.17
	B + R	**197.34	**179.40	**195.33	**282.11	**499.22
Iranian	Co	88.29	58.49	49.87	88.47	211.36
	B1	97.41	66.38	51.29	97.98	235.48
	R2	117.46	91.20	83.29	136.98	312.29
	B + R	120.71	93.41	90	139.81	320.31
LSD (p=0.05)		13.7	18.9	22.6	33.4	66.22

phosphorous in the soil by the activity of the basillus bacteria, which increases the readiness of the element and is one of the elements necessary for the growth of leguminous crops, as the lack of this element in the soil reduces the size of the root nodes of the plant and thus reduces the amount of nitrogen installed. These microorganisms also share with plant growth a number of positive interactions in the rhizosphere, whether it is Bacillus bacteria or rhizobia and plants (Morel et al 2012, Guttman et al 2014). It supports the plant with defensive means and runs the necessary nutrients for the plant (Vivance and Badri 2009). The Iraqi variety outperformed the Iranian variety by giving it the largest amount of amino acids, and this is due to its genetic structure as well as the suitability of the Iraqi environment to it.

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Effect of Ultraviolet (UV) Light and Some Chemical Stimuli in Growth Qualities and Parthenolide Concentration in Leaves of *Tanacetum parthenium* L. Grown In-vitro

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Abstract: The research was carried out College of Agricultural Engineering Sciences for the period from February 2015 to August 2016, to study the effect of treatment with ultraviolet (UV) rays and some chemical stimuli on growth characteristics and concentration of parthenolide in leaves. The experiment was carried out according to a complete random design. The seeds were exposed to ultraviolet (UV. B) rays for different periods of time 5,10,15,20 minutes, Ultraviolet (UV) treatment for 15 minutes was the best and it was approved in the experiment. Then the plants were grown in MS medium prepared with different concentrations of salicylic acid (SA) (0, 0.5, 1, 2, 3, 4 mg. L^{-1}) and methyl Jasmonite (Me) (0, 2, 4, 6, 8 mg. L^{-1}), salicylic acid (SA) 3 mg. L^{-1} A significant increase in the vegetative growth indicators of plants exposed to UV, which included the number and length of vegetative branches, chlorophyll content of leaves, fresh and dry weight, and the highest concentration of Parthenolide in leaves, as it recorded 6,100 branches. And 1.865 cm and 13.32 mg.100 g fresh weight and 0.869 g and 0.096 g and mg. g-150.99 measurements methyl Jasmonite (MeJa) after preparing them for MS food medium with different concentrations, led to the superiority of treating the prepared medium with 4 mg.L1 by giving it the highest concentration of Parthenolide (17.16 mg. L^{-1}) for plants exposed to UV As well as the significant increase in the vegetative growth indicators of plants exposed to UV, which included the length and number of vegetative branches, the content of leaves of chlorophyll, and the fresh and dry weight (1,950 cm, 6,300 branches. respectively, while high concentrations of like Jasmonite (MeJa) led to plant growth inhibition.

Keywords**:** Salicylic acid**,** Methyl Jasmonite**,** Parthenolide

Plant tissue technology has gained great importance in some developed countries to increase the production of secondary metabolic compounds in medicinal and aromatic plants, which are sources for drug production as it helps the rapid production of these materials and compounds without being restricted by natural factors such as geographical location, seasonal changes or environmental stresses (Zhou and Wu 2006, Ahsan et al 2013), and the researchers concluded that the substance that is added in low concentrations to the living cell system reduce or increase the biosynthesis of a particular compound is known as elicitor, and these stimuli can be divided into biotic and non-biotic stimuli. (abiotic depending on its nature or to internal or external stimuli depending on its source (Park 2008). Salicylic acid (SA) is a growth regulator it has an explicit impact in many plant processes such as growth, flower induction (Al-Khafaji and Al-Jubouri 2016). It is one of the most important chemical catalysts influencing the stimulation and production of secondary compounds. The stimulating effect that is characterized by salicylic acid SA is due to its role as a key to signaling and the emergence of secondary compounds in the biological pathway, and as a mechanism for plant defense against itself (Blokhina et al 2003 and El-

Tayeb 2005). Jasmonite, one of the new endogenous growth regulators that participates in the defense system of plants (Browse 2009), it may be classified as a plant hormone because affects physiological activities with very low concentrations and shows its effect outside its building areas (Rohwer and Erwin 2008). Jasmonate defense against environmental changes, as it serves as the master switch in the plant's response to several biotic and abiotic stresses (Wasternack 2007) and plays a role in plant physiological processes, including carbon metabolism, flowering and senescence (Choi and Choing 2003 and Zhou et al 2013).

MATERIAL AND METHODS

This research was carried out at University of Baghdad. The seeds were irradiated and placed in a desiccator containing 75% glycerol solution and 25% water at a temperature of 25°C for 4 days, with the aim of obtaining a moisture content of 11% (Conger et al 1977) after which were exposed to ultraviolet rays with extended periods of time (0, 5, 10, 15, 20 minutes) by means of the ray-generating device (Shaukat et al 2013). The intensity of the light emitted was measured by means of a Lux-meter, where the average intensity of illumination was 300 lux (at the surface of the

exposed seeds). The comparison treatment included all conditions except for exposure to radiation. The seeds exposed to ultraviolet (UV-B) rays after sterilization were sown on MS food medium prepared with 30 g.L 1-sucrose and 7 g. Liter 1- Akar. The cultures were incubated in the growth room at a temperature of 25 0 C ± 2 for 2 days in the dark, after 16 hours of light and 8 hours of darkness to stimulate germination for a period of 4 weeks (Castillon and Cornish 2000). Vegetative branches with a length of 1 cm obtained from the best treatment with ultraviolet rays for a duration (0, 15 minutes) were evaluated for best type and concentration. The SA was tested at concentrations 0, 0.5, 1, 2, 3,4 mg. L^{-1} on plant growth and stimulating the production of the active substance. The effect of (MeJa), which was obtained from Duchefa Biochemie Company, was added to the MS food medium. The growths were planted on the stimulus medium with 10 replications for each concentration and for each experiment separately for a period of 4 weeks. The cultures were incubated in the growth room at a temperature of 25 °C ± 2 and the intensity of illumination 1000 lux for 16 hours / day and darkness 8 hours / day.

RESULTS AND DISCUSSION

Effect of irradiation and salicylic acid and the interaction between them on the number of branches length and chlorophyll concentration (mg.100g^{-1} wet weight) given in Table 1. The addition of salicylic acid (SA) to the MS food media caused a significant increase in the number and length of vegetative branches chlorophyll (1.645 cm, 5.200 and 11.11 mg.g^{-1} wet weight) in comparison to control (0.768 cm, 1.750 branches, 4.653 mg. g^{-1}). Treatment with ultraviolet (UV1) rays led to an increase in the average length and number of vegetative branches and chlorophyll concentration (1.306 cm, 4.133 branches, 8.133 mg.g^{-1} wet weight, respectively), compared to the non-irradiated treatment (UV0), (0.933 cm, 2.933 branch, 6.037 mg.g-1 fresh weight). The interaction treatments, SA5UV1 recorded highest length and number of vegetative branches and chlorophyll concentration, (1.865 cm, 6.100 branches, 13.321 mg.g^{-1} wet weight) compared with the non-irradiated measurement treatment, (0.615 cm, 1.400 branches, 4.569 mg.g^{-1} wet weight respectively).

Effect of irradiation and salicylic acid and the interaction between them on the fresh and dry weight (g) and the concentration of parthenolide mg.g^{-1} given in Table 2. The average fresh and dry weight and the concentration of parthenolide were significantly affected by the difference in the concentrations of salicylic acid (SA) added to the MS food medium (0.707 g, 0.082 g, 36.17 mg.g^{-1}, respectively), while the lowest averages of fresh weight (0.039 g), dry weight

(0.021 g) and parthenolide concentration rate (1.690 mg.l^{-1}) were recorded in control. The irradiation treatment (UV$_1$) was significantly superior with the highest fresh and dry weight and the concentration of parthenolide (0.315 g, 0.055 g, 20.03 respectively), while the lowest were in the comparison treatment not exposed to irradiation (0.168 g, 0.032 g, 8.068 mlg.l^{-1}). The interaction treatments led to a significant increase in the above-mentioned traits, as the SA5 UV$_1$ treatment was characterized by a significant increase in the average fresh and dry weight and parthenolide concentration (0.869 g, 0.096 g, 50.99 mg) than in control treatment not irradiated (SA1UV0) (0.019 g, 0.017   and 1.380 mg.g^{-1}, respectively).

Effect of irradiation with methyl Jasmonite (MeJa) on the number and length of branches (cm) and chlorophyll concentration of *Tanacetum parthenium* L. indicate that the average length and number of vegetative branches and the concentration of chlorophyll were affected by adding different

Table 1. Effect of irradiation (UV) and salicylic acid on length, number of branches and chlorophyll concentration of *Tanacetum parthenium* L.

Measured attributes treatment	Soft weight (G)	Dry weight (G)	Parthenolide concentration (mg g^{-1})
SA$_1$	0.039	0.021	1.690
SA$_2$	0.058	0.026	3.109
SA$_3$	0.088	0.035	4.926
SA$_4$	0.123	0.037	7.827
SA$_5$	0.707	0.082	36.17
SA$_6$	0.433	0.061	30.57
LSD (p=0.05)	0.016	0.0014	0.595
UV$_0$	0.168	0.032	8.068
UV$_1$	0.315	0.055	20.03
LSD (p=0.05)	0.009	0.008	0.344
SA$_1$ UV$_0$	0.019	0.017	1.380
SA$_2$ UV$_0$	0.042	0.018	3.182
SA$_3$ UV$_0$	0.063	0.021	3.740
SA$_4$ UV$_0$	0.086	0.030	5.212
SA$_5$ UV$_0$	0.544	0.069	21.35
SA$_6$ UV$_0$	0.252	0.039	13.54
SA$_1$ UV$_1$	0.058	0.025	1.999
SA$_2$ UV$_1$	0.074	0.034	3.036
SA$_3$ UV$_1$	0.114	0.049	6.111
SA$_4$ UV$_1$	0.160	0.044	10.44
SA$_5$ UV$_1$	0.869	0.096	50.99
SA$_6$ UV$_1$	0.615	0.083	47.60
LSD (p=0.05)	0.023	0.002	0.842

concentrations of methyl Jasmonite (MeJa) to the MS food medium with branch length (1.613 cm), average number of vegetative branches (5.350) and chlorophyll concentration (11.44 mg.g.1-fresh weight) (Table 3) while the values for the above-mentioned indicators in MJ0 were 0.773 cm, 3.150 branches, 7.868 mg.g.1-wet weight, respectively .The irradiation treatment (UV1) was significantly superior to the increase in the length and number of vegetative branches and the concentration of chlorophyll (1,320 cm, 3,960, 8.984 mlg. g^{-1}) compared to the non-irradiated treatment (UV0), which recorded the lowest of 0.969 cm, 2,880, 6,821 mlg The significant effect of the interaction treatments was observed and the irradiation treatment (MJ2UV1) with a concentration of 4 mlg .L^{-1}, was significantly superior by achieving the highest average length of vegetative branches (1.950 cm), number of vegetative branches (6,300) and chlorophyll concentration (12.22 mg. g^{-1} wet weight) compared with the lowest values (1.00 cm, 1.700, 3.556 mg. g^{-1} wet weight respectively) when treated (MJ4UV0) not exposed to UV rays.

The effect of irradiation and methyl Jasmonite (MeJa) on the average fresh and dry weight (g) and the concentration of parthenolide given in Table 4. The different concentrations of methyl Jasmonite (MeJa) added to the MS food medium significantly affected the average fresh and dry weight and the concentration of parthenolide and in MJ2 highest rate of fresh weight (0.337 g) and dry weight (0.051 g) and the concentration of parthenolide (12.79 mg .g^{-1}) as observed and the lowest values was in MJ4)\ (0.030 g, 0.027 g, 0.775 mg. g^{-1} respectively). The irradiation had significant effect on the above measured traits, as the UV1 irradiation treatment significantly increased the fresh and dry weight rate and parthenolide concentration, which amounted to 0.232 g, 0.047 g, 8.353 mg.g-1, respectively, compared to its non-irradiated counterpart. UV0), which recorded the lowest average of fresh and dry weight and parthenolide concentration (0.143 g, 0.025 g, 4.459 mg. g^{-1} respectively). The highest rate of fresh and dry weight and the highest concentration of parthenolide was in interaction treatment MJ2UV1 (0.416 g, 0.072 g, 17.16 mlg. L-1 respectively)

Table 2. Effect of irradiation (UV) and salicylic acid on the average fresh and dry weight (g) and parthenolide of leafs *Tanacetum parthenium* L.

Measured attributes treatment	Soft weight (G)	Dry weight (G)	Parthenolide concentration (mg g^{-1})
SA$_1$	0.039	0.021	1.690
SA$_2$	0.058	0.026	3.109
SA$_3$	0.088	0.035	4.926
SA$_4$	0.123	0.037	7.827
SA$_5$	0.707	0.082	36.17
SA$_6$	0.433	0.061	30.57
LSD (p=0.05)	0.016	0.0014	0.595
UV$_0$	0.168	0.032	8.068
UV$_1$	0.315	0.055	20.03
LSD (p=0.05)	0.009	0.008	0.344
SA$_1$ UV$_0$	0.019	0.017	1.380
SA$_2$ UV$_0$	0.042	0.018	3.182
SA$_3$ UV$_0$	0.063	0.021	3.740
SA$_4$ UV$_0$	0.086	0.030	5.212
SA$_5$ UV$_0$	0.544	0.069	21.35
SA$_6$ UV$_0$	0.252	0.039	13.54
SA$_1$ UV$_1$	0.058	0.025	1.999
SA$_2$ UV$_1$	0.074	0.034	3.036
SA$_3$ UV$_1$	0.114	0.049	6.111
SA$_4$ UV$_1$	0.160	0.044	10.44
SA$_5$ UV$_1$	0.869	0.096	50.99
SA$_6$ UV$_1$	0.615	0.083	47.60
LSD (p=0.05)	0.023	0.002	0.842

Table 3. Effect of irradiation(UV) and methyl Jasmonite (MeJa) length and number of branches and chlorophyll concentration of *Tanacetum parthenium* L.

Treatment	Length of the vegetative branches (cm)	Number of vegetative branches	Chlorophyll mg 100 g^{-1} fresh weight
MJ$_0$	0.773	3.150	7.868
MJ$_1$	1.013	4.000	9.706
MJ$_2$	1.613	5.350	11.44
MJ$_3$	1.125	2.650	5.899
MJ$_4$	1.200	1.950	4.594
LSD (p=0.05)	0.174	0.530	0.551
UV$_0$	0.969	2.880	6.821
UV$_1$	1.320	3.960	8.984
LSD (p=0.05)	0.110	0.335	0.349
MJ$_0$ UV$_0$	0.620	2.600	6.675
MJ$_1$ UV$_0$	0.900	3.500	8.323
MJ$_2$ UV$_0$	1.275	4.400	10.65
MJ$_3$ UV$_0$	1.050	2.200	4.890
MJ$_4$ UV$_0$	1.000	1.700	3.556
MJ$_0$ UV$_1$	0.925	3.700	9.061
MJ$_1$ UV$_1$	1.125	4.500	11.08
MJ$_2$ UV$_1$	1.950	6.300	12.22
MJ$_3$ UV$_1$	1.250	3.100	6.907
MJ$_4$ UV$_1$	1.350	2.200	5.632
LSD (p=0.05)	0.246	0.750	0.780

compared to the lowest in MJ4UV0 not exposed to irradiation (0.013 g, 0.020 g, 0.387 mlg. g^{-1}).

The growth regulators (SA and MeJa) have an effective role in improving the vegetative and physiological growth indicators in the tissue culture of the media equipped with them. (El-Bassiouny et al 2014, Talaat et al 2014) and this is reflected in an increase in the number of side branches (Table 3) and the external addition of salicylic acid leads to a positive effect on the entry and transfer of ions as well as in physiological processes (Khan et al 2003) . This may be due to the synergistic effect produced by some phenolic compounds, and since salicylic acid is a widely distributed phenolic compound in plants and directly contributes to the regulation of physiological processes with other growth regulators such as IAA, which have a role in the process of cell division, enlargement and elongation (Hayet and Ahmed 2007) and therefore this led to an increase in the length of the branches (Table 2). Alam (2012) also observed an increase in the length of the plant (*Catharanthus roseus* L.) . Saupe (2007) referred to the active role played by growth organizations in increasing the number of branches within a short period. As for its role in improving physiological indicators, salicylic acid had a role in increasing the content of chlorophyll in leaves (Table 2), perhaps due to the activation of the formation of chlorophyll pigment by stimulating the formation of carna sheets and the development of chloroplasts, as well as its inhibition of the enzyme chlorophyllase and increasing the efficiency of carbonation (Singh and Singla 2008). Or, the reason may be attributed to the role of active salicylic acid in increasing the carbonic pigments and auxiliary hormones, which is a means of protection by affecting the reactions of the second photosystem, and as a result it prevents the decline in the efficiency of photochemical energy and thus prevents the release of tissue and provides protection for chloroplasts and other cellular structures, as It stimulates the production of enzymes responsible for building carbon-representing pigments and inhibiting the activity of catabolic enzymes (Gunes et al 2007). It also contributes in production of IAA and ABA growth regulators and stimulating the production of a number of multiple amines and osmotic regulators, including glycine, and stimulating it to the formation of krana and the development of chloroplasts (Hayet and Ahmed). Treatment with high concentrations of salicylic acid (above 200 mg.L^{-1}) negatively affects the fresh weight due to its influence on cell growth and vitality. Salicylic acid also increases secondary compounds (Table 2) by stimulating gene expression and regulating signal transduction during gene expression (Morris et al 2000) as well as its role in stimulating the activity of ADS proteins PAD4, ED5, and ICS

Table 4. Effect of irradiation (UV) and methyl Jasmonite (MeJa) on fresh and dry weight and Parthenolide concentration of *Tanacetum parthenium* L.

Ttreatment	Soft weight (G)	Dry weight (G)	Parthenolide concentration (mg g^{-1})
MJ$_0$	0.169	0.031	3.808
MJ$_1$	0.282	0.038	10.33
MJ$_2$	0.337	0.051	12.79
MJ$_3$	0.119	0.033	4.318
MJ$_4$	0.030	0.027	0.775
LSD (p=0.05)	0.006	0.0014	0.451
UV$_0$	0.143	0.025	4.459
UV$_1$	0.232	0.047	8.353
LSD (p=0.05)	.004	0.009	0.285
MJ$_0$ UV$_0$	0.110	0.021	3.123
MJ$_1$ UV$_0$	0.226	0.027	5.836
MJ$_2$ UV$_0$	0.258	0.030	8.430
MJ$_3$ UV$_0$	0.107	0.029	4.518
MJ$_4$ UV$_0$	0.013	0.020	0.387
MJ$_0$ UV$_1$	0.228	0.042	4.493
MJ$_1$ UV$_1$	0.339	0.050	14.82
MJ$_2$ UV$_1$	0.416	0.072	17.16
MJ$_3$ UV$_1$	0.131	0.038	4.118
MJ$_4$ UV$_1$	0.048	0.035	1.162
LSD (p=0.05)	0.0093	0.0020	0.638

which are important in the phenyl propanoic pathway (Metraux 2001, Shohael 2006). Differences in the concentration of secondary compounds may be due to genetic or external factors represented by light, temperature, humidity, type of plant part and stage of plant growth, as well as other factors such as the physiological state of the plant before extraction. As for methyl Jasmonite (MeJa) and its relationship to plant growth and development, growth indicators showed a response when adding methyl Jasmonite to food media. The jasmonic acid has ability to act as binary regulators in development, growth, and protection, as its effect appears on growth characteristics at low concentrations of methyl jasmonic added to the food medium under stress conditions, which leads to improving vegetative growth such as increasing plant height and increasing fresh weight (Table 4). It also increases the chlorophyll content of the leaves (Table 3) by encouraging the gene expression of chlorophyll building and thus improving the carbon metabolism process (Wasternat and Hause 2002) or may be due to the role of methyl Jasmonite in increasing the effectiveness of the plant's defense system under the influence of stress represented by the two systems,

enzymatic and non-enzymatic (Rahimi et al., 2013) and these results agreed with when adding low concentrations of methyl Jasmonite (MeJa) to artichoke seedlings, as it showed a significant response in increasing plant height and fresh weight of the shoot, while it was conversely, at high concentrations of this acid, caused significant decrease in the above traits.

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Numerical Taxonomic Cultivars Species of Genus *Morus* L. (Moraceae) Cultivated in North of Iraq

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Abstract: The present research deals with the numerical taxonomy cultivars species of the genus *Morus* L. which cultivated in the north of Iraq, including the cultivars of the species *M. alba* L. ('Beautiful Day', 'Big White', 'Rease', 'Greece', Pearl', 'Border Sweet', 'Pendula') and the cultivar 'Kokuso Korean'of the *M. latifolia* Poir, 'Amarah' cultivar of the *M. rubra* L.*,* 'shami' cultivar of the *M. nigra* L. and the two cultivars ' king white' and 'dwarf' of *M. macroura Miq*, and 'tice', 'and Wellington' of cultivar *M. hybrid*, using the morphological, anatomical and spectral characteristics of which 40 characteristics were used to show the relations of similarity and difference as well as the dendogram for cultivars of the species. Twelve morphological characteristic were chosen to draw polygonal shapes for all cultivars studies. The results of the polygonal shapes and the dendograms supported and confirmed the morphological, anatomical and spectral characteristics of the studied cultivars species of the genus *Morus* L. The extent of similarity between these cultivars, and the highest level of similarity between the two cultivars 'Greece' and ' Pearl' of *M. alba* L. and the lowest level of similarity of the cultivars 'Shami' of the *M. nigra* L.*,* and 'Greece' and 'Pendule' of the *M. alba* L.. This evidence of the wide variations shown by the species' cultivars among themselves for this genus.

Keywords: Numerical, Taxonomy, *Morus* L., Cultivars

The recent taxonomic studies used computers to show the degree of interdependence and convergence between the taxonomic units (OUTS), which led to the establishment of a new branch in taxonomy, i.e. numerical taxonomy, which is the numerical evaluation of the total similarity between the groups with the aid of computers, and then arranging them in the form of clusters according to the similarity (Stuessy 2009). The numerical taxonomy program is implemented through many methods developed to solve classification problems (Pavlinov 2020). Numerical taxonomy seeks to study the distinction among cultivars with the help of computational means through programs, and then classifying them into various taxonomies based on their similarity (Das et al 2018). Many studied described numerical taxonomy as being highly efficient in plants classification and boundary drawing between them even in the difficult cases (Ogie-odia et al 2019).

The mulberry genus *Morus L.* belongs to *Moraceae* family, the rank of Rosales and it grows in different soils in the shape of trees or perennial shrubs. This plant is unilocular or bilocular and it blooms well in acidic soils with a pH range (6.2-7) and grows at (18-30°C). It is genetically complex and has a high potential for variation, adaptation and wide spread in different environments (Wani et al 2012, Wani et al 2017, Choudhary et al 2018). The scientific name (*Morus*), is derived from the word 'mora' which means (delay) in Latin

based on the condition of delayed and slow growth of buds (Jan and Ahmad, 2021). An alternative interpretation is that it comes from the word 'mor' or (black), which refers to the colour of the black fruit (Orwa et al 2009).

Numerical taxonomy was used to find the degree of similarity and to determine the degree of kinship between the taxa by several researchers (Bello et al 2013, Al-Joboury 2017, Al-Juary et al 2018, Igbari et al 2019, Al-Zahrani 2021, Wang et al 2022). Rahman et al (2013) experienced the importance of numerical taxonomy when studying the similarity between 11 species of the genus (*Senna mill*) which grows in Bangladesh, using 32 morphological characteristics, cluster analysis revealed that they were divided into three main groups and that the results of the study are identical with the cytological and anatomical studies. When studying the genus *Jatraopha* (*Euphorbiaceae*) using numerical taxonomy Kolawole et al (2016) proved that it is more accurate in diagnosing the species and finding the degree of genetic kinship among them and provided a greater distinction along the spectrum of the taxonomic differences between the species of the genus which contributed to more information about the level of relationship within the species of the studied genus.

Yaradua et al (2018) reported that the numerical taxonomy's strength lies in determining the similarity of the

species, rather than relying on a small number of plant characteristics, when using numerical taxonomy methods to diagnose and distinguish 7 species of the genus (*Crotalarial* L.) which grows in Nigeria on the basis of quantitative and qualitative morphological characteristics.

Cu (2019) indicated that numerical taxonomy provided valuable data and a basic reference for the classification of the genus (*Hemerocallis* spp.) using the characteristics of the flower through the colour scale when studying the numerical order of 183 cultivars. Salman et al (2021) used the numerical classification to clarify the taxonomic identity of the cultivars species of the genus *Ficus* L. using 36 morphological characters and the results showed that the qualitative traits are significantly better in distinguishing the cultivars and their species. Patel et al (2022) shed light on the numerical classification of 30 species belonging to 26 genera in the Asteraceae family using 52 morphological traits and found that numerical analysis is important in finding taxonomic relationships in that family. The present study aims to numerically diagnose the cultivars of the genus (*Morus* L.) species using the morphological, anatomical and spectral characteristics

MATERIAL AND METHODS

In this research, computational methods were used to find the similarities and differences between the cultivars of species of the genus (*Morus* L.), which were collected from the Nineveh and Dohuk horticulture stations and fields and nurseries, and which were diagnosed by the Ministry of Agriculture. All cultivars of the species of this genus were used as operational taxonomic units (OTUS) based on morphological, anatomical, pollen grains, spectroscopic, chemical and genetic aspects. In this study, 12 phenotypes were selected for preparing polygonal diagrams (Table 1) and analysed numerically (Table 2). The 39 characteristics were selected for numerical taxonomy to compare 14 taxonomic units numerically by Sokal and Sneath (1973) (Table 3) after selecting the cultivar as an operational taxonomic unit OTU and assigning a symbol for each of the morphological, anatomical and spectral characteristics and their details by using the numbers 1,2,3,4,5,6,7. Then, data were arranged for the details of the characteristics of the cultivars under study after encoding them with the previous numbers (Table 4). The encoded data was analysed using the *SPSS20* program and resulted in a dendogram shows similarity relationships (kinship) as well as difference between the studied cultivars within clusters after finding the similarity and difference matrix between the cultivars under study which are studied for the first time in this way.

Table 1. Morphological characteristics selected for drawing polygonal shapes of the studied species cultivars the genus of (*Morus* L.)

No. symbol	Characteristics	Details	Characters states
A	Habit growth stem	Erect	1
		Semi erect	2
		Spreading	3
		Dropping	4
B	Bud shape	Round	1
		Elongate round	2
		Acute triangle	3
		Elongate triangle	4
C	Blade leaf shape	Cordate	1
		Wide ovate	2
		Ovate	3
		Narrow ovate	4
D	Leaf bse	Truncate	1
		Abtuse	2
		Cordate	3
		Oblique	4
E	Blade leaf nature	Entire	1
		Lobed	2
		Heterophyllous	3
F	Sexual catikin	Gynoecious (♀)	1
		Androgyno mocious (♂+♀)	2
		Polygamous (+♂+♀♂)	3
G	Stigma type	Erect	1
		Semi erect	2
		Divaricate	3
		Twisted	4
H	Stigma nature	Papillate	1
		Pubescent	2
I	Fruiting stalk (peduncle)	Sessile	1
		Necked	2
J	Fruit colour	White	1
		White-light violet	2
		Violet	3
		Dark violet	4
		Red-Black	5
		Greenish yellow	6
K	Seed surface ornamentation (SEM)	Reticulate	1
		Muricate	2
		Foreate undulate	3
		Polygonal	4
		Ruminate	5
L	Flowering period	Early flowering	1
		Optimum	2
		Late flowering	3

RESULTS AND DISCUSSION

Numerical taxonomy was used 12 specific phenotypes were selected (Table 1) and analysed numerically (Table 2) to draw polygonal shapes (Fig. 1) and the polygonal diagrams showed clear contrast among the cultivars of the species of genus in degrees of similarity through the selected characteristics, as the cultivars 'Shami' of the species (*M. nigra* L.) and 'King White' of the species (*M. macroura* Miq) were distinguished by the shape of their patterns, which confirms their difference in the selected morphological characteristics from the rest of the cultivars. As for the cultivar of the species (*M. alba* L.) , were the most similar to each other compared to the rest of the other studied cultivars of species, and this may be attributed to the fact that belong to the same species. This was supported by the similarity matrix (Table 5) as the similarity rate was 31.5% between 'Shami' of (*M. nigra* L.) and the rest of the cultivars. As for 'King White' of *M. macroura* Miq; it recorded a similarity rate of (31.9%) compared to the rest of the other cultivars. The highest similarity was 50.2% between the cultivar 'Beautiful Day' *M. alba* L. and cultivars of the other species. The cultivars 'Greece and 'Pearl', were distinguished by the highest similarity between them (75%) and may be that they belong to the same species. (*M. alba* L.). The lowest level of similarity was 20% between the cultivar 'Shami' (*M. nigra* L.) and the two cultivars 'Greece' and 'Pendula' (*M. alba* L.)*,* indicating the wide variances between the species.

The cultivars of the studied genus' species were also divided into two main groups in the dendogram (Fig. 2) based on the numerical taxonomy of the morphological, anatomical
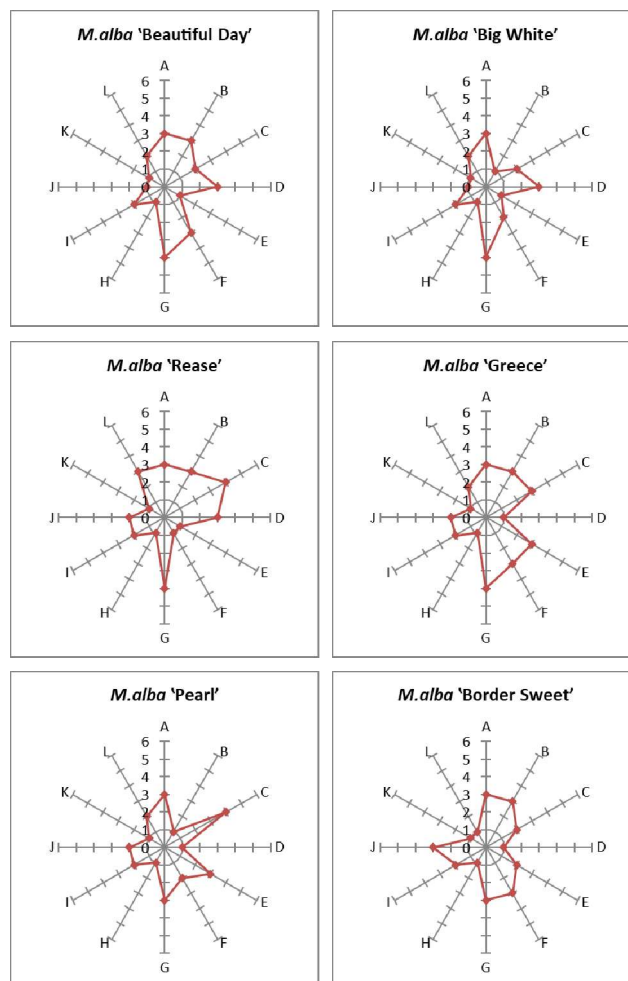
Fig. 1-a: Polygonal diagrams of cultivars of the genus (*Morus* L.) species

Table 2. Matrix of morphological characteristics selected for drawing polygonal shapes of the studied cultivars species of the genus *Morus* L. for 12 morphological traits listed in Table 1

| Species	Cultivars	A	B	C	D	E	F	G	H	I	J	K	L
M.alba	'Beautiful Day'	3	3	2	3	1	2	4	1	2	1	1	2
	'Big White'	3	1	2	3	1	3	4	1	2	1	1	2
	'Rease'	3	3	4	3	1	1	4	1	2	2	1	3
	'Greece'	3	3	3	1	3	3	4	1	2	2	1	2
	'Pearl'	3	3	4	1	3	2	3	1	2	2	1	2
	'Border Sweet'	3	1	1	1	2	3	3	1	2	3	1	1
	'Pendula'	4	2	4	3	3	1	1	1	2	5	1	2
M.latifolia	'Kokuso Korean'	3	2	1	4	3	2	4	1	2	4	1	2
M.rubra	'Amarah'	3	4	4	2	3	1	2	1	2	5	5	1
M.nigra	'Shami'	3	4	1	3	1	1	2	2	1	5	2	3
M.macroueae	'King White'	3	4	3	3	1	1	3	1	2	6	5	2
	'Dwarf'	1	4	2	1	2	1	1	2	2	5	3	2
M.hybrid	'Tice'	2	3	2	3	1	1	2	1	2	5	4	2
	'Wellington'	3	2	4	1	3	1	4	1	2	5	1	2

Table 3. List of characters scored for cluster analysis (dendrogram) for the cultivars species of the genus *Morus* L.

Characteristics	Details	Characters states
Habit growth stem	Erect	1
	Semi erect	2
	Spreading	3
	Dripping	4
Bark colour	Gray	1
	Blackish gray	2
	Grayish brown	3
	Blackish brown	4
Bud shape	Round	1
	Elongate round	2
	Acute triangle	3
	Elongate triangle	4
Bud attachment	Adhering to branch	1
	Slanting outward	2
	Tilting to oneside	3
Accessory buds	Absent	1
	Present	2
Bud colour	Light brown	1
	Brown	2
	Reddish brown	3
	Dark brown	4
	Dark red	5
Blade leaf shape	Cordate	1
	Wide ovate	2
	Ovate	3
	Narrow ovate	4
Blade leaf apex	Obtuse	1
	Acute	2
	Acuminate	3
	Caudate	4
Blade leaf base	Truncate	1
	Abtuse	2
	Cordate	3
	Oblique	4
Blade leaf marg	Crenate	1
	Dentate	2
	Serrate	3
Blade leaf nature	Entire	1
	Lobed	2
	Heterophyllous	3
Blade leaf colour	Light green	1
	Green	2
	Dark green	3
	Yellowish green	4

Cont...

Table 3. List of characters scored for cluster analysis (dendrogram) for the cultivars species of the genus *Morus* L.

Characteristics	Details	Characters states
Seual catikin	Gynoecious (♀)	1
	Androgyno mocious (♂+♀)	2
	Polygamous (+♂+♀♂)	3
Indumentum of catikin stalk	Puberulent	1
	Villous	2
	Pilose	3
	Pilose-glabrous	4
Stigma type	Erect	1
	Semierect	2
	Divaricate	3
	Twisted	4
Stigma nature	Papillate	1
	Pubescent	2
Style	Absent	1
	Present	2
Fruiting stalk (peduncle)	Sessile	1
	Necked	2
Fruit shape	Semispherical-elliptic	1
	Ovoid	2
	Elongate ovoid	3
	Elliptic	4
	Clyindric	5
Fruit colour	White	1
	White-light violet	2
	Violet	3
	Dark violet	4
	Red-black	5
	Greenish yellow	6
Number fruitlet / on fruit	Small rang between (22.3-34.4)	1
	Medium between (40.5-66.8)	2
	Large between (127)	3
Fruit size	Small range size between (1-1.33)cm^3	1
	Medium range size between (1.5-2cm^3)	2
	Large range size between (2.35-3.22cm^3)	3
See shape	Elongate ovoid	1
	Ovoid-sub ovoid	2
	Sub spherical	3
	Sub spherical-sub ovoid	4

Cont...

Table 3. List of characters scored for cluster analysis (dendrogram) for the cultivars species of the genus *Morus* L.

Characteristics	Details	Characters states
Seed colour	Light yellow	1
	Light brown	2
	Yellowish brown	3
	Dark brown	4
	Blackish brown	5
Seed surface ornamentation (SEX)	Reticulate	1
	Muricate	2
	Foreate undulate	3
	Polygonal	4
	Ruminate	5
Number sees of fruit	Few rang (1.5-4.2) seed / fruit	1
	Middle rang (10-29.5) seed / fruit	2
	A lot rang (39.2-59) seed / fruit	3
Flowering period	Early flowering	1
	Optimum flowering	2
	Late flowering	3
Ordinary epidermal cells shape of leaf (Adaxial surface)	Polygonal	1
	Elongated polygonal	2
Ordinary epidermal cells shape of leaf (Abaxial surface)	Polygonal	1
	Elongated polygonal	2
Leat venation	Brochidodromous	1
	Craspedromous	2
Petiole shape	Oval non-winged	1
	Oval winged	2
	Oblate oval winged	3
	Renal winged	4
Spong cells tissue nature	Compact	1
	Loose	2
Shape farc vesselin midrib	Crescent	1
	Deep crescent	2
	U	3
Number of midrib	One bandle	1
	Two bandle	2
	Three bandle	3
Vessels of midrib	Small vessel rang between (18.80-24.4) □m	1
	medium vessel rang between (25.68-30.25) □m	2
	Large vessel rang between (30.4-35.4) □m	3

Cont...

Table 3. List of characters scored for cluster analysis (dendrogram) for the cultivars species of the genus *Morus* L.

Characteristics	Details	Characters states
Prismatic crystals	Absent	1
	Present	2
Types the cystolith crystals in a daxial surface of blade leaf	*Type A	1
	Type B	2
	Type C	3
	Type A,B	4
	Type A,C	5
	Type A,B,D	6
	Type A,C,D	7
Horn gland hairs on baxial surface blade leaf	Absent	1
	Present	2
Values of chlorophyll in alcoholic extraction	664-650 nm	1
	468-458 nm	2
	428 nm	3
	414 nm	4

*Type AB,C,D: the idioblast without projection, a small projection, medium projection, and big projection
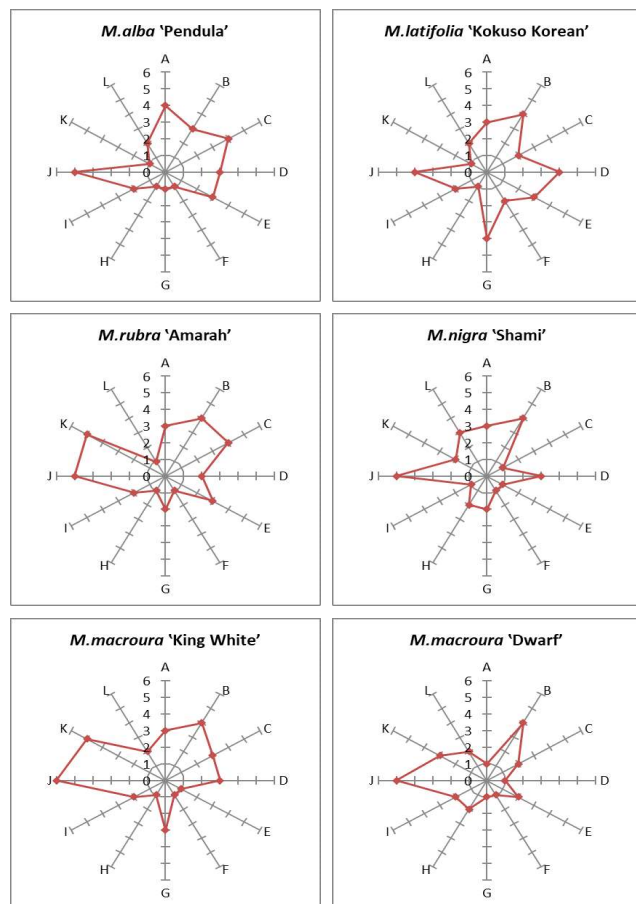
Fig 1-b: Polygonal diagrams of cultivars of the genus (*Morus L.*) species

Table 4. Data matrix of morphological, anatomic and spectral characteristics used in drawing dendograms of cultivars of the genus' (*Morus* L.) species used in the study for 39 characters presented in Table (3)

Species	Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
M. alba	'Beautiful Day'	3	1	3	2	1	1	2	2	3	2	1	2	3	1	4	1	1	2	4	1	2	2	1	2	1	2	2	1	2	1	3	1	1	1	2	2	1	2	1
	'Big White'	3	1	1	2	2	2	2	3	3	2	1	3	2	2	4	1	2	2	3	1	2	2	1	2	1	3	2	1	2	2	2	1	2	1	3	1	5	2	1
	'Rease'	3	1	3	2	1	1	4	2	3	3	3	1	1	1	4	1	2	2	4	2	2	3	2	3	1	3	3	1	2	1	3	1	3	1	3	2	4	2	1
	'Greece'	3	1	3	1	2	1	3	2	1	3	1	3	3	1	4	1	2	2	2	2	1	1	2	2	1	2	2	1	2	1	3	1	2	1	1	2	1	2	1
	'Pearl'	3	1	1	1	2	1	4	2	1	2	3	1	3	2	4	1	2	2	2	2	1	1	1	2	1	2	2	2	2	1	2	1	2	1	2	2	1	2	1
	'Border Sweet'	3	1	3	1	1	1	2	3	1	2	2	3	3	2	3	1	1	2	4	3	1	2	1	4	1	2	1	2	2	2	2	2	3	1	3	2	7	2	1
	'Pendula'	4	1	3	1	2	1	4	2	3	2	3	1	1	1	1	1	2	2	2	5	1	1	2	2	1	2	2	1	2	2	2	1	1	1	1	1	1	2	1
M. latifolia	'Kokuso Korean'	3	1	4	2	2	3	2	2	4	2	3	3	3	1	4	1	2	2	3	4	2	2	1	2	1	3	2	2	2	2	1	1	2	2	3	2	6	2	1
M. rubra	'Amarah'	3	3	4	1	1	5	4	4	2	1	3	2	1	1	2	1	2	2	4	5	2	2	4	3	5	1	1	2	1	1	2	1	1	3	2	2	3	1	1
M. nigra	'Shami'	3	4	4	2	1	4	1	3	3	2	1	3	1	2	2	2	1	1	1	5	1	2	4	5	2	3	3	2	1	1	1	1	2	1	3	2	3	2	2
M. macroura	'King White'	3	2	4	3	1	4	3	1	3	3	1	4	1	3	3	1	2	2	5	6	3	2	2	1	1	1	2	2	2	1	2	2	2	1	1	2	1	2	1
	'Dwarf'	1	4	4	3	1	4	2	4	1	3	2	3	1	1	1	2	2	2	4	5	2	3	3	1	3	1	1	2	1	1	1	2	3	1	3	1	1	2	2
M. hybrid	'Tice'	2	2	3	2	2	1	2	3	3	3	1	1	4	4	2	1	2	2	4	5	2	3	4	5	4	1	2	1	2	4	4	1	3	1	2	2	5	2	1
	'Wellington'	3	3	2	3	1	1	4	2	1	2	3	2	1	1	4	1	1	2	3	5	2	1	1	4	1	3	2	1	2	1	2	2	3	1	1	2	1	2	1

Table 5. Similarity matrix for selected morphological, anatomical and spectral characteristics of cultivars species of the genus (*Morus* L.)

Plant species	Cultivars	'Beautiful Day'	'Big White'	'Rease'	'Greece'	'Pearl'	'Border Sweet'	'Pendula'	'Kokuso Korean'	'Amarah'	'Shami'	'King White'	'Dwarf'	'Tice'	'Wellington'
M. alba	'Beautiful Day'	1													
	'Big White'	0.600	1												
	'Rease'	0.650	0.525	1											
	'Greece'	0.625	0.450	0.600	1										
	'Pearl'	0.550	0.550	0.450	0.750	1									
	'Border Sweet'	0.600	0.450	0.525	0.500	0.550	1								
	'Pendula'	0.550	0.500	0.500	0.700	0.675	0.425	1							
M. latifolia	'Kokuso Korean'	0.525	0.650	0.475	0.500	0.500	0.450	0.425	1						
M. rubra	'Amarah'	0.375	0.225	0.375	0.275	0.475	0.350	0.325	0325	1					
M. nigra	'Shami'	0.325	0.350	0.350	0.200	0.275	0.350	0.200	0.350	0.325	1				
M. macroueae	'King White'	0.375	0.350	0.400	0.425	0.400	0.375	0.350	0.325	0.375	0.350	1			
	'Dwarf'	0.225	0.250	0.350	0.250	0.225	0.350	0.275	0.300	0.325	0.425	0.475	1		
M. hybrid	'Tice'	0.525	0.425	0.575	0.475	0.425	0.325	0.475	0.325	0.375	0.350	0.425	0.325	1	
	'Wellington'	0.600	0.450	0.550	0.550	0.525	0.550	0.550	0.450	0.400	0.250	0.425	0.375	0.425	1

See Table 4 for details

Table 6. Difference matrix for selected morphological, anatomical and spectral characteristics of cultivars of the genus (*Morus* L.) species

Plant species	Cultivars	'Beautiful Day'	'Big White'	'Rease'	'Greece'	'Pearl'	'Border Sweet'	'Pendula'	'Kokuso Korean'	'Amarah'	'Shami'	'King White'	'Dwarf'	'Tice'	'Wellington'
M. alba	'Beautiful Day'	0													
	'Big White'	0.400	0												
	'Rease'	0.350	0.475	0											
	'Greece'	0.375	0.550	0.400	0										
	'Pearl'	0.450	0.450	0.550	0.250	0									
	'Border Sweet'	0.400	0.550	0.475	0.500	0.450	0								
	'Pendula'	0.450	0.500	0.500	0.300	0.325	0.575	0							
M. latifolia	'Kokuso Korean'	0.475	0.350	0.525	0.500	0.500	0.550	0.575	0						
M. rubra	'Amarah'	0.625	0.775	0.625	0.725	0.525	0.650	0.675	0.675	0					
M. nigra	'Shami'	0.675	0.650	0.650	0.800	0.725	0.650	0.800	0.650	0.675	0				
M. macroueae	'King White'	0.625	0.650	0.600	0.575	0.600	0.625	0.650	0.675	0.625	0.650	0			
	'Dwarf'	0.775	0.750	0.650	0.750	0.775	0.650	0.725	0.700	0.675	0.575	0.525	0		
M. hybrid	'Tice'	0.475	0.575	0.425	0.525	0.575	0.675	0.525	0.675	0.625	0.650	0.575	0.675	0	
	'Wellington'	0.400	0.550	0.450	0.450	0.475	0.450	0.450	0.550	0.600	0.750	0.575	0.625	0.575	0
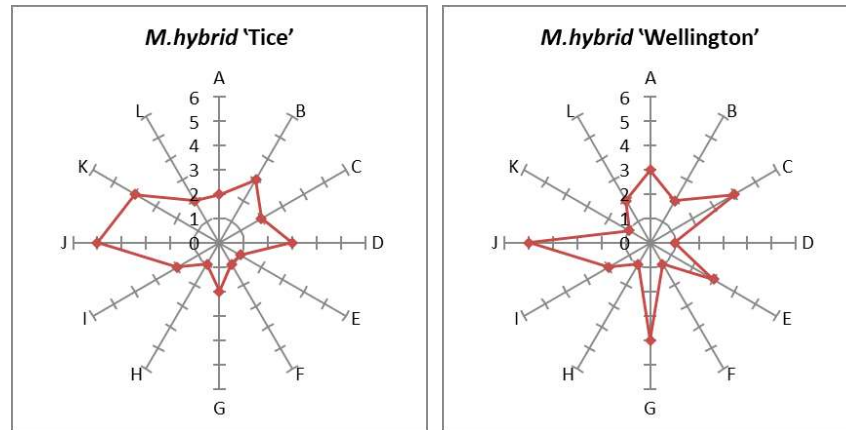
Fig. 1-c: Polygonal diagrams of cultivars of the genus (*Morus* L.) species
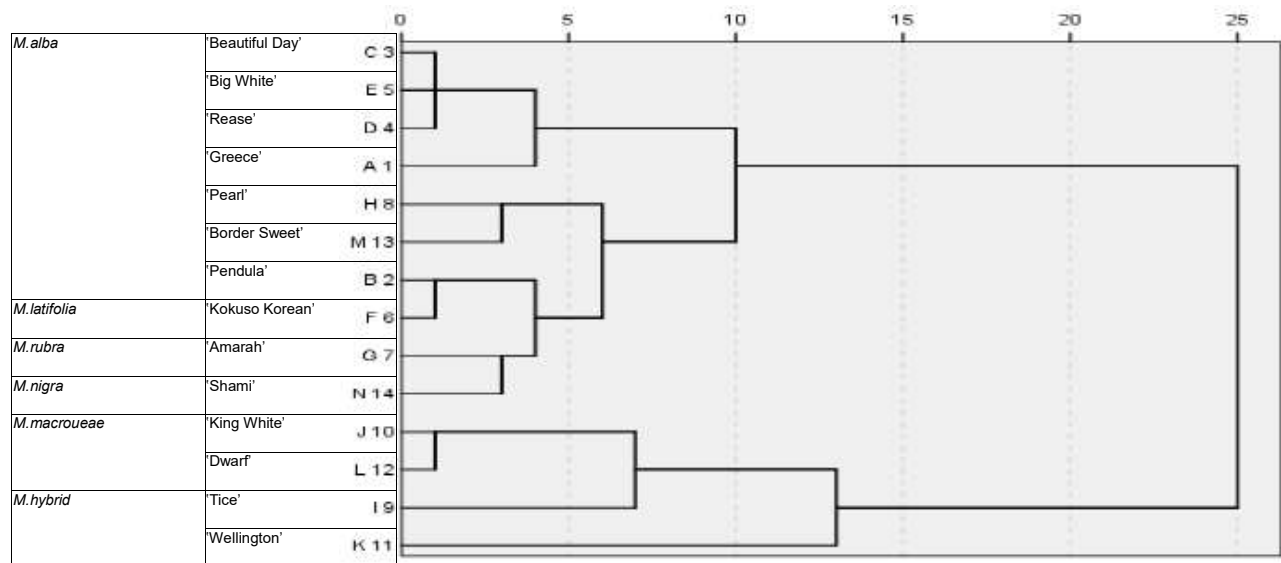
Fig. 2. Dendrogram for the cultivars of the genus (*Morus* L.) species based on the selected morphological, anatomical and spectral characteristics

and spectral characteristics, The first main group included the cultivars 'Beautiful Day', 'Rease', 'Greece' and 'Pearl' and the second included 'Big White', 'Border Sweet', and 'Pendula' f (*M. alba* L.), and 'Tice' and 'Wellington' (*M. hybrid*), and the cultivar 'Kokuso Korean' (*M. latifolia* Poir). The second main group also included two subgroups; the first was represented by the cultivars 'Shami' of the species (*M. nigra* L.), 'Dwarf' of the species (*M. maeroura* Miq) and 'Amarah' of the species (*M. rubra* L.). The second was marked by the cultivar 'King White' of the species (*M. macroura* Miq). These results were consistent with the study of: (AL-Jowary et al (2018) on Pine; Ogie-odia et al (2019) on the species of the family Euphorbiceae, and Bona (2020) on *Lepidium* L., Soliman et al (2021) on *Ficus* L.

CONCLUSION

It is possible to identify the cultivars species belongs of the genus *Morus* L. numerically and polygonal shapes. Dendrogram supported and confirmed the morphological, anatomical and spectral characteristic of cultivars species. Cultivars of the species under study have highest level of similarity (75%) between the two cultivars 'Greece and ' Pearl' of *M. alba* L. and the lowest level of similarity (20%) of the cultivars 'Shami' (*M. nigra* L.) and 'Greece' and 'Pendule' (*M. alba* L.) and this is evidence of the wide variations shown by the species' cultivars among themselves for genus *Morus* L.

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Effect of Spraying with Gibberellic Acid on Growth, Flowering and Chemical Content of Bougainvillea (*Bougainvillea glabra)*

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Abstract: The experiment was conducted at- University of Kirkuk. during September 1,2020 to May 1, 2021 to study the effect of spraying with gibberellic acid at three concentrations (0, 150 and 300) mg L^{-1} on the growth and flowering of *Bougainvillea glabra*. The spraying with gibberellic acid at a concentration of 300 mg. L^{-1} increased plant height by 27.88%, stem diameter 23.16%, leaf area 13.42 cm2, carbohydrates 16.81%, phosphorous content of leaves 0.215%, number of days required for flowering 13.03 days, number of bracts 89.44. Plant^{-1}, bract diameter 28.41 mm, bract weight 0.054 gm, flowering period 23.33 days, and anthocyanin pigment concentration 0.882 mg.l^{-1}, while the concentration 150 mg. L^{-1} increased in the number of leaves by 62.65% compared to the comparison treatment.

Keywords: Bougainvillea, Gibberellic, *Bougainvillea glabra*

The experiment was conducted at- University of Kirkuk. during September 1,2020 to May 1, 2021 to study the effect of spraying with gibberellic acid at three concentrations (0, 150 and 300) mg L^{-1} on the growth and flowering of *Bougainvillea glabra*. The spraying with gibberellic acid at a concentration of 300 mg. L^{-1} increased plant height by 27.88%, stem diameter 23.16%, leaf area 13.42 cm2, carbohydrates 16.81%, phosphorous content of leaves 0.215%, number of days required for flowering 13.03 days, number of bracts 89.44. Plant^{-1}, bract diameter 28.41 mm, bract weight 0.054 gm, flowering period 23.33 days, and anthocyanin pigment concentration 0.882 mg.l^{-1}, while the concentration 150 mg. L^{-1} increased in the number of leaves by 62.65% compared to the comparison treatment

Bougainvillea spp. belongs to the family Nyctaginaceae, which includes 33 genus and 29 species. The days of the year and the color of these leaves vary according to the type and variety. Gibberellin acid (GA3) is one of the plant growth regulators that has stimulating effects in most plants and the response to it is visible, such as increasing the length of branches resulting from cell division and its elongation, as well as the unique effect in directing the plant towards flowering (Hassan et al 2011). Gibberellin acid plays an important role in encouraging the process of cell elongation and stimulating the formation and development of flowers by directing its metabolic products to the developing flowers and the formation of important enzymes during germination, as well as directing growth in the vertical direction and increasing the size of leaves and root system and works to improve the size of flowers and makes them with excellent specifications (Verma 2003). Chandra Sekhar et al (2020) in a observed that spraying gibberellic acid on *Jasminum sambac* L. at f 75 mg.L^{1}, which led to the maximum height of the plant and number of flowers for each plant. . Yaquby et al (2020) also observed gibberellic acid at concentrations of 300 and 200 mg/L-1, increased the characteristics of stem diameter, stem length, flower length and number of branches for each plant, while concentration 200 showed a significant superiority in flower diameter characteristic. El-sayad et al (2017) showed in a study the effect of spraying with gibberellic acid on Bougainvillea spp. plant at a concentration of 1500 mg.L^{-1}, which led to a significant improvement in all vegetative and flowering traits, as well as the total carbohydrate content of leaves and an increase in the percentage of phosphorous. Saifuddin et al (2009) in an experiment on Bougainvillea spp. at a concentration of 100 mg.L^{-1}, it increased the bract size, number of bract and bract weight. Due to the importance of the bougainvillea plant as one of the flowering climbers, the research aims to study the effect of gibberellic acid on the growth and prolongation of the flowering period in the bougainvillea plant, and to study the use of the bougainvillea plant as flowering anvils.

MATERIAL AND METHODS

The experiment was conducted at University of Kirkuk, on bougainvillea seedlings for from September 1, 2020 to May 1, 2021 at a longitude of 44.394416o E, latitude of 35.524969o N and an altitude of 398 m above sea level for a site.

The seedlings were obtained from one of the nurseries in the city of Kirkuk at the age of 1.5-2 years. The plants were grown in pots with a diameter of 30 cm. The soil mixture used consisting of 2 part of soil is mixed and 1 bitmoss, was prepared and nylon was placed under the pot in order to preserve the solutions and materials that are added to plants as well as to prevent and reduce the growth of the bush. A sample of this mixture was taken after mixing and before planting for the purpose of laboratory analysis (Table 1).

The experiment included spraying with gibberellic acid at three concentrations: 0, 150, and 300 mg. L^{-1}. the seedlings were sprayed a week after planting and the plants were sprayed in the early morning and using the hand sprinkler until complete wetness on 9/8/2020 and narrowed a drop of shampoo solution in the spray bottle to break the surface tension and to facilitate the process of sticking and spreading on the surface of the paper, the comparison treatment was sprayed with distilled water only, and the spraying process was repeated three times monthly interval.

Observations

Vegetative growth characteristics: percentage increase in plant height diameter - increase number of leaves, leaf area ($cm^2 plant^{-1}$).

Chemical characteristics: The chlorophyll content in leaves was measured using a field device (chlorophyll content meter) of the type CCM-200 plus, which measures chlorophyll content index in CCI unit (Chlorophyll content meter index) (Biber 2007). Carbohydrates were estimated according to the method of Joslyn (1970). The phosphorous was estimated Sparks (1966).

Characteristics of flowering growth: The number of days required for flowering (day), the number of flowering bracts (bract. $flower^{-1}$), dry weight of the bracts (gm), diameter of the flowers (mm), dry weight of the flowers (gm), length of

Table 1. Physical and chemical properties of the medium used in the research before planting

Parameter	Value	Units
Nitrogin (N)	0.236	%
Phosphorous (P)	54.2	ppm
Potassium (K)	20.32	ppm
Organic Matter (om)	2.632	%
EC	0.123	$ds.m^{-1}$
Ph	7.30	Ph
Soil texture	Sandy mixture	
Clay	8	%
Silt	12	%
Sand	80	%

The soil was analyzed in the soil laboratory of the Kirkuk Agriculture Directorate

the flowering period (day) - anthocyanin (mg. g^{-1} dry weight) dye was estimated by taking 0.01gm of dried and ground bracts , 20 ml of acidified ethanol (HCL 1.5, ethyl alcohol 9.5) and added to 25ml of roasted ethanol. The optical absorption was measured using a Spectrophotometer at The wavelength is 535 nanometers.

Statistical anlysis: The experiment was designed with a RCBD design and the data were analyzed statistically using the SAS (2002) program.

RESULTS AND DISCUSSION

The with gibberellic acid at 300 mg.$L^{-1}$of increased plant height, leaf area, phosphorous and carbohydrate content of leaves (27.88%, 13.42 cm^2 $plant^{-1}$, 0.215%, 16.81%). The spraying with gibberellic acid at 150 mg.L^{-1} significantly increased stem diameter and number of leaves (24.14 and 62.65%) while there were no significant differences between the levels of gibberellic acid spray in the chlorophyll content of leaves. However , spraying with gibberellic acid at a concentration of 150 mg.L^{-1} recorded an increase in the chlorophyll content of leaves(48.85 CCI) as compared to the comparison treatment which amounted to (39.15 CCI).

The spraying with gibberellic acid caused a significant increase in most vegetative growth characteristics, as it increased the percentage of plant height, which was positively reflected on the percentage increase in stem diameter and number of leaves, which was reflected in an increase in leaf area. Encouraging vegetative growth increase the division and elongation of cells in the apical meristem in addition to the increase in the content of chlorophyll in leaves. Perhaps the reason for this increase is the role of gibberellic acid in increasing the number of cells as a result of the increase in cell division and elongation and the increase in cell length, which is reflected in the increase in plant growth and leaves area (Mayoli 2009). The reason may also be due to the role of gibberellic in stimulating enzymes that weaken cell walls and thus cells elongation (Taheri Shiva et al 2014). The increase in plant growth may be due to the role of gibberellic acid in improving the efficiency of the photosynthesis currency, which is reflected in the improvement of the chlorophyll content of the leaves, which was positively reflected on the increase in photosynthesis and thus improving the growth characteristics as a result of the accumulation of growth materials (Janowska and Stancki 2013 and Sardoei et al 2014).

The superiority of spraying with gibberellic acid at concentrations of 300 and 150 mg. L^{-1} affected flowering growth, the number of days required for flowering, the number of bracts, diameter of the bract, weight of the bract , length of the flowering period and concentration of

Table 2. Effect of spraying with gibberellic acid on vegetative growth characteristics and chemical content of Bougainvillea

Gibberellic acid (mg. L^{-1})	Increase in plant height (%)	Increase in stem diameter (%)	Increase in the number of leave (%)	Leaf area (cm^2 plant^{-1})	Chlorophyll content guide in the leaves (CCI)	Total carbohydrates in leaves (%)	Phosphorous content of the leaves (%)
Control	10.73 b	11.71 b	23.52 b	8.14 c	39.15 a	12.57 b	0.149 b
150 (mg. L^{-1})	25.36 a	24.14 a	62.65 a	11.38 b	48.85 a	16.66 a	0.169 ab
300 (mg. L^{-1})	27.88 a	23.16 a	55.18 ab	13.42 a	49.36 a	16.81 a	0.215 a

Table 3. Effect of spraying with gibberellic acid on the flowering growth characteristics of Bougainvillea

Gibberellic acid (mg. L^{-1})	Number of days required for flowering (day)	Number of flower bracts (bract. flower^{-1})	Bracts dry weight (gm)	Bract diameter (mm)	Flower diameter (mm)	Dry weight of flowers (gm)	Length of the period of flowering (day)	Anthocyanins (mg. g^{-1} dry weight)
Control	23.10 a	50.22 b	0.041 b	23.11 b	8.30 a	0.021 a	11.77 c	0.555 b
150 (mg. L^{-1})	16.03 b	73.11 a	0.053 a	26.84 a	8.09 a	0.024 a	19.00 b	0.628 ab
300 (mg. L^{-1})	13.03 b	89.44 a	0.054 a	28.41 a	8.41 a	0.027 a	23.33 a	0.882 a

anthocyanin pigment, which were 13.03 and 16.03 days, 89.44 and 73.11 bract. Plant^{-1},28.41 and 26.83 mm, 0.054 and 0.054 g, 23.33 and 19.00 days, 0.886 and 0.628 mg.g.g^{-1} dry weight, respectively, while the lowest was recorded in comparison treatment, which amounted to 23.10 days, 50.22 bracts.plant^{-1},23.11 mm, 0.041 g , 11.77 days, 0.555 mg, g^{-1} dry weight, respectively, while there were no significant differences between the levels of gibberellic acid spray in the characteristics on flower diameter and flower weight

These results indicate that there is a positive relationship between the development of vegetative growth and flowering growth The reason is due to the increased absorption of water and mineral elements, as well as the increase in chlorophyll production as a result of the increase in the outputs of photosynthesis, which was reflected in stimulating floral growth and an increase in the number of bracts and the number of flowers due to the continuous supply of organic energy compounds that stimulate growth. In addition, gibberellins work to receive the products of the photosynthesis process from carbohydrates and transfer them to the regions of the developing peaks to increase the occurrence of cell division (Taiz and Zeiger 2010). The increase in the number of bracts, their diameter and weight of the flower may be due to the increase in the stimulating substances for growth (internal) such as gibberellins, and in contrast to reduce the growth-inhibiting substances such as abscisic acid ABA, where the gibberellin increases the occurrence of apical division of the apical meristems, which increases the height of the plant and thus increases the number, bracts and diameter flower weight and diameter. This is attributed to the role of gibberellins in the formation of secondary growth centers within the flower stems through division, in addition to its controlling role in directing and transferring nutrients from the leaves to the florets and bracts

(Taiz and Zeiger 2010). In addition, gibberellin works to increase the life of the flower and cannabis due to the resistance to aging of the plant, and increase the tissue resistance to ethylene in natural accumulation. In addition improve chlorophyll in the plant by increasing the durability of the membrane for chloroplasts and thus its positive role in increasing the vitality of flowers and maintaining age of the cannabis (Taheri Shiva et al 2014 and Hamidimoghadam et al 2014). There was also increase in the concentration of anthocyanins pigment in the cannabinoids. This is attributed to the role of gibberellic acid in promoting the absorption of ions and opening and closing stomata (Popova 1997). It also activates the formation of carotenoids and other pigments through its role in the formation of carna plates and the development of chloroplasts (Singh 2008)

CONCLUSION

The response of bougainvillea plant to spraying with gibberellic acid was observed on vegetative and flowering growth characteristics. The best results were obtained when spraying with gibberellic acid at 300 mg.L^{-1}.

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Assessment and Monitoring of Wheat Growth Stage (*Triticum aestivum* L.) using NDVI

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Abstract: A study was conducted in Tikrit district to study growth stages of wheat crop and relationship to the NDVI index using remote sensing data. Five wheat fields (type abaa) were selected, which differ in agronomic practices such as irrigation (axis and fixed sprinkler irrigation and dripping irrigation), fertilization, topography of the site, physical and chemical properties of the soil and fertility. Thirty-eight plant samples were collected at three growth stages (S1, S2, S3). The nutrients (nitrogen, phosphorous and potassium) and chlorophyll content were estimated in conjunction with spectral readings of the NDVI index of the satellite Landsat-8, on the four growth stages. The results revealed the variation in nitrogen, phosphorous, potassium and chlorophyll concentrations among the fields of the study area. There was increase in spectral index with the progression of the growth stage, as the best spectral stage was at the visible one acquired on the 2nd April. The results also showed a positive regression relationship between the values of NDVI and the concentrations of chlorophyll, N, P and K in the wheat crop for the spectral stage (S3), So the values of the coefficient of determination reached 0.81, 0.87, 0.60 and 0.63, respectively.

Keywords: NDVI, Wheat growth stage, Landsat 8, NPK, Chlorophyll

The wheat crop *Triticum aestivum* L. is one of the main grain crops in Iraq and the world, as it comes at the forefront of crops in terms of cultivation area and production. Although the increase in productivity per unit area doubled at the end of the last century, but this does not meet the increasing human need because of the continuous increase in the population, In Iraq climatic conditions, soil and water, farmers' failure of following the scientific and extension methods led to a negative impact on growth and productivity. Gypsum soils constitute large areas of Iraq, estimated at more than 20%, or approximately 88 thousand square kilometers, where high percentages of gypsum affect the chemical and physical properties of soils due to its dissolution observing the growth stages of wheat throughout the growing season is of vital importance irrigation, fertilization and plant health. Satellite remote sensing offers several options in observing crop growth, especially in low-data areas, and biophysical variables retrieved from remote sensing data can be coupled with leaf area index Wang et al (2016). Puri and Linesh 2(017) observed that the leaves of the plant in ideal conditions give the highest reflectivity to the near infrared rays and the lowest reflectance to the red rays, but if the plant is aged or the plant is exposed to diseases and pests or lack of water and key nutrients or because of salts, then the reflectivity of the near infrared rays decreases and approaches the reflectivity of the red rays, else in the event of plant death, all visible and invisible rays are equal. By tracking the spectral behavior of the vegetative spectral indices during the growth period of the wheat crop, these indicators were low at the beginning of the plant's life, and gradually rise to reach the period of the greatest vegetative growth, after that their values return to decline with the age of the plant and entering the stage of aging and finally to the harvest. The reflectance in the red and blue spectral field recorded the lowest values affected by the increase in chlorophyll concentration Pradhan et al (2014) find that when the plant reaches the aging stage, the values of the vegetative spectral indices decrease again. The NDVI index was positively correlated with the biomass and nitrogen content of the wheat crop in the flowering stage and was validated by laboratory (Vian et al 2018). Abd-Alwahab (2020) estimated in his study that wheat crop productivity using remote sensing technique for an optional region in central Iraq, the researcher used NDVI values from Landsat8 and Sentinel-2B satellite images in order to observe the vegetative growth of wheat crop and the results of NDVI values showed good vegetation cover in general And good growth of the crop in many farms. These affected fields gave the lowest NDVI values. He pointed out that this technique is effective for detecting the change between the different stages of growth and estimating the yield, as the results were close to the amount of yield. Since the remote sensing techniques are important in managing and estimating the state of agricultural crops, the most important of which is wheat crop and growth stages observing, and because of the lack of studies available in the country, the study aims to assess the stages of wheat crop growth in gypsum soils using the normalized difference vegetation index (NDVI).

MATERIAL AND METHODS

Study area: The study area is located within Salah al-Din Governorate in the Tikrit district between longitudes 30 43 43 and 0 31 43 east and latitudes (0 39 34) and (30 28 34) north (Fig. 1). The study area suffers from the presence of gypsum in the soil in different proportions per depth, and the mixed and sandy textures. It is also characterized by its poor fertility, low cationic exchange capacity, low water retention, hardness when exposed to drought, and its light texture. And in terms of topography, it is generally flat to slightly undulating.

Field data: The study sites were selected based on different agricultural methods and inputs adopted by the farmers, such as fertilizer additions, irrigation method, plowing system, planting date and variety. Therefore, the study included the selection of five different sites and irrigated according to the pivotal and fixed sprinkler irrigation system, and one field irrigated by the tourist irrigation system. Where sites A, D and E are irrigated with center pivot irrigation system, site B is irrigated by fixed sprinkler irrigation system, and site C is irrigated by tourist irrigation system (Table 1).

Sampling and dry weight measurement: 10 plants (stem + leaves) were taken in the initial spectral growth stage (S1) dated (1/13/2021) as well as in growth stage (S2) on March 2, 2021 and (stem + leaves + spike) linear spectral growth (S3)

on April 3, 2021. These were washed with normal water and then distilled to remove .The soils was dried in an electric oven at a temperature of 65-70C0 for 48 hours and left until reaching the state of constant weight, Thereafter grinded to and was placed in plastic box until the required analysis is performed (Alhameer 2015).

Preparation of botanicals: 0.2 g of dried and ground plants were taken and placed in a glass volumetric flask (Pyrex) and 3 ml of concentrated sulfuric The 3 ml of concentrated sulfuric acid is added to it and left until the next day. After the color turns black, 1 ml of the concentrated acid mixture is added to it in a ratio of 3:1 as follows: 98 % concentrated sulfuric acid H2SO4 + 96% concentrated pyro chloric acid HClO4, and placed on a thermal plate for the purpose of heating to complete the digestion process until the color of the solution became clear (colorless), which is evidence of the completion of the digestion process, then the samples were cooled and transferred quantitatively with filtration with special paper to volumetric flask with a capacity of 50 cm³ and complete the volume to the mark with distilled water (Al-Mashhadani 2021).

Nitrogen: The concentration of nitrogen was determined by distillation after adding sodium hydroxide (10 M) (Al-Zobaie 2021).

Phosphorous: Measurement of phosphorous
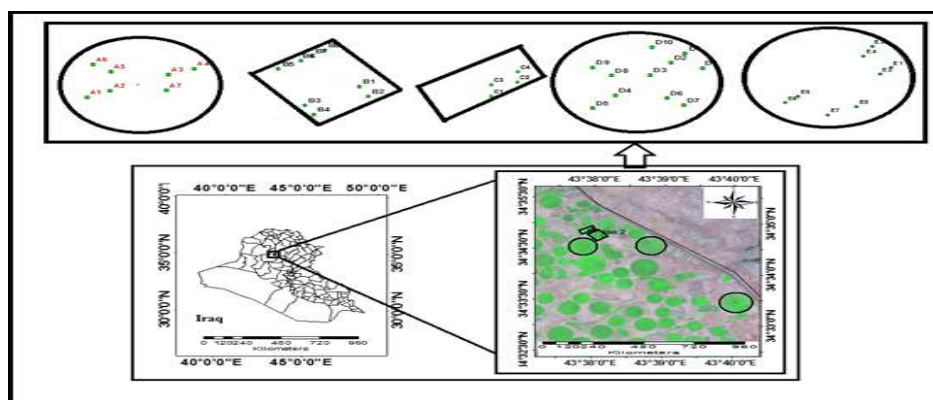
Fig. 1. The location of the study area in Iraq's map

Table 1. Data and information related to the fields of the study area

Field Symbol	Coordinates		Field area acres	No. of points pinned into the field	Irrigation system	Agric. Date in 2020	Seed class	Fertilizer added Kg. H-1	
	X	Y						DAP (NH₄)2HPO₄	UREA (NH₂)₂CO
A	375782	3826812	132.33	7	Axial spray	16/11	Ebaa99	100 with seed	--
B	374834	3827213	37.19	8	Fixed spray	15/11		155 with seed	--
C	374541	3827426	19.89	4	Immersion	22/11		155 with seed	--
D	374471	3826872	118.19	11	Axial spray	14/11		200 with seed	100 25/2/2021
E	377974	3824832	162.47	8	Axial spray	15/11		100 with seed	--

measurement using ammonium molybdate and ascorbic acid after modifying the used professional with the use of paraterphene dye as a proof and then measuring it with a spectrophotometer at a wavelength of 882 nm (Al-Zobaie 2021).

Total potassium: It was measured with a flame apparatus (flame pressure meter) Al-Zobaie 2021.

Measurement of chlorophyll (SPAD): The measurement of chlorophyll in leaves during the spectral growth stages of the wheat crop (S1, S2 and S3) was done by selecting the mature and fully dilated flag leaf that is at the peak of its physiological activity plants by using a chlorophyll measuring device, a chlorophyll meter of the type SPAD-502 Plus and measured in SPAD units (Islam et al 2014).

Satellite images used in the study: The growth of the wheat crop was monitored by satellite visuals captured by the American satellite Landsat-8 for the Sensor (OLA) Operational Land Imager, throughout the growth period of the wheat crop in order to keep pace with the plant growth, 8 within Path and Row (36, 169) on November 92020 before planting, then the download of the visuals are repeated every 16 days until the full maturity of the crop. The total number of videos that were downloaded during the growth period of the wheat crop was 12 satellite images. However, some of the visuals were cloudy and it was difficult to monitor and track the crop through them (Table 2).

Digital processing of satellite visuals: The digital processing of satellite visuals was carried out through mathematical algorithms within the program environment ERDAS Ver.15; numerical values from the range 0-65536 are converted to spectral reflectance values within the range 0-1 depending on the location of the USGS 2021. Satellite visuals of the Landsat generations differ in the DN values, starting with the MSS scanner, which includes 64 color levels (26 bit) and the TM 0-256 color level (28 bit) and currently the development process has reached 0-65536 digital level (216 bit). This wide discrepancy makes difficult to compare the spectral reflectivity values, which are relied upon in the process of calculating the spectral indices, and therefore it is necessary to convert the digital values into spectral reflectivity values ranging from 0-1 for all satellite visuals, as

Table 2. Growth stages of wheat crop for obtaining plant samples and calculating the NDVI index

The stage	Symbol	Germination date	
Initial slow growth stage	S1	6/12/2020	5/2/2021
Exponential growth phase	S2	6/2/2021	5/3/2021
Linear growth stage	S3	6/3/2021	5/4/2021
Final slow growth stage	S4	6/4/2021	Harvest

they are available in the ENVI program environment through the application of FLAASH and the ERDAS program Integrated steps ready to be dealt with. Equation (1 and 2) shows a part of the digital processing mechanism, which includes the date and location of the visible and spectral bands.

$$P'_\lambda = Mp \times Qcal + Ap \qquad (1)$$

P'λ=TOA Planetary Spectral Reflectance.

Mp=Reflectance multiplicative scaling factor for the band (Metadata file)

Qcal. = Pixel value in DN for band 2 to b7.

Ap= is the additive rescaling factor (Metadata file).

$$P_\lambda = \frac{p'\lambda}{\sin(\theta i)} \quad (2)$$

Pλ=TOA planetary reflectance.

Sin (θ) = Local sun elevation angle; the scene center sun elevation angle in degrees is provided in the metadata File.

The mechanism of calculating the NDVI using the program ERDAS Ver.15 by matching the satellite visuals, and this is done by choosing Raster a side window will appear and through it would be selected Spectral and then select Layer stack, a window will appear through which we call the satellite visual, after calling each package or channel by specify the command (add) and so on until calling all spectral packets or channels, then select all and through (output) and choose the name, location and file extension, which are in the image format, being the best format that the program deals with to store the satellite image, and then we execute by selecting the command OK. The satellite visual is called again so that we can calculate the spectral indices available in the program environment, as the work is by selecting Raster and from it choose Unsupe and from this go to Indices and through the main window that appears will call the satellite visual and through the window and finally select NDVI (Fig. 2). Arithmetic equation for the NDVI-Norma listed Difference Vegetation Index

$$NDVI = \frac{(B5 - B4)}{(B5 + B4)} \qquad (3)$$

Since:

NDVI: Normalized Difference Vegetation Index. NIR (B5): reflectivity in the near infrared wave. RED (B4): reflectivity in the visible red wave. This evidence has been adopted in many studies to monitor the growth of the wheat crop, including Vian et al (2018) (Fig. 2).

RESULTS AND DISCUSSION

NDVI values among the five fields ranged between 0.13 - 0.32. The NDVI index of field A ranged from 0.14-0.16, field B from 0.22-0.32, C from 0.15-0.20 and D from 0.17-0.23 and field E from 0.13-0.20. The values of spectral index are low at

S1 of the plant, and this is due to the fact that the plant at this stage has low living mass and leaf, and therefore the reflection in the near infrared field is low in addition to the high reflectance. In the red spectral field as a result of the low concentration of chlorophyll and the effect of soil reflection due to the fact that the plant did not cover the entire earth at this stage of the plant life, which leads to a decrease in the values of the spectral index at this stage. Ibrahim 2014 also indicated overlapping effect of soil inversion in early plant life which leads to lower values of vegetative spectral indices. In this stage as the extreme climatic conditions, where rainfall and low temperatures low, are not ideal for plant growth, and plant goes through a state of stunted growth. This is considered spectrally unimportant which is consistent with Ibrahim (2014) and Agrees with Alhameer (2015). As for the exponential growth stage (S2), NDVI values ranged between 0.24-0.45. The values of NDVI of field A ranged from 0.29-

0.36, B from 0.31-0.42, C from 0.32-0.40, D from 0.37-0.45 and E from 0.24-0.42. The plant growth rate is continuous and changes relatively at close intervals in time, with its influence on external and internal factors (climate, disease and insect infestations or soil properties). The stage of linear growth (S3) led to the continuation of the increase in the values of the spectral index for this stage, as the values of the NDVI ranged from 0.39-0.72. The NDVI values of field A ranged from 0.46-0.61, B from 0.48-0.68, C from 0.56-0.63, D from 0.62-0.70 and field E from 0.39-0.72. It was noticed during field visits and laboratory analyzes that this linear growth stage (S3) was the best growth stage for wheat crop in terms of live mass, leaf area and dry weight, and this is consistent with Ibrahim (2014.) Thus, can find that the spectral index values reached the highest values as a result of the high reflectance, which reached its highest value in the near infrared spectral field, affected by the height of the live
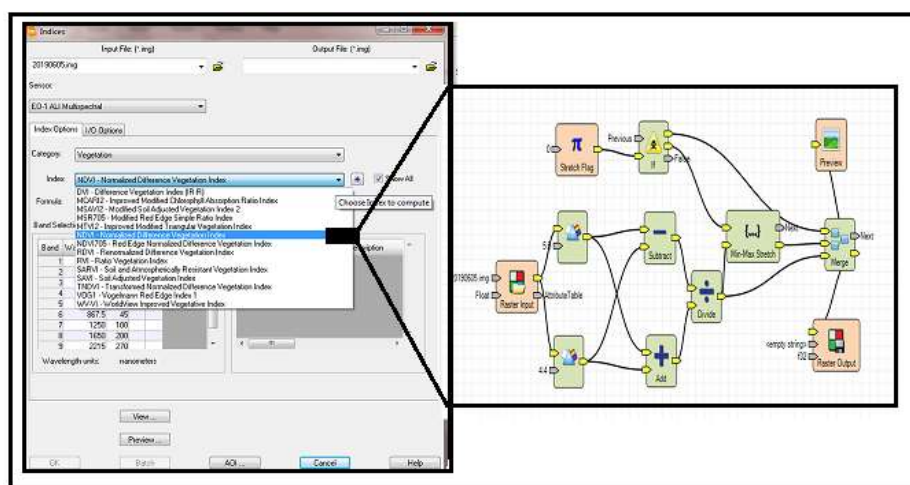
Fig. 2. ERDAS program in calculating the NDVI

Table 3. Landsat-8 visual information and spectral phases for the agricultural season 2020-2021

No.	Weather condition	Visual history	Spectral stages of wheat growth
1	Partly cloudy	9/11/2020	Before planting
2	Partly cloudy	25/11/2020	Before germination
*3	Clear	11/12/2020	S1
4	Partly cloudy	27/12/2020	S1
**5	Clear	12/1/2021	S1
6	Cloudy	28/1/2021	S1
*7	Clear	13/2/2021	S2
**8	Clear	1/3/2021	S2
9	Partly cloudy	17/3/2021	S3
**10	Clear	2/4/2021	S3
11	Cloudy	18/4/2021	S4
*12	Clear	4/5/2021	S4

* Visuals that were used in the spectral growth profile. ** The visuals that correspond to the modeling of the plant samples

Assessment and Monitoring of Wheat Growth Stage 65

mass, leaf area and dry weight, while the reflection in the red and blue spectral field recorded the lowest values affected by the high concentration of chlorophyll, and this agrees with to Pradhan et al (2014) where when the plant reaches its greatest growth stage, the spectral index values reached the highest values.

At at this stage field (D) continues to outperform the values of all spectral indicators over the rest of the fields within the study area, due to nitrogen fertilization in the last part of the second spectral stage (S2), whose results were reflected in this stage, as 100 kg of urea fertilizer were added. Al-Budairi (2018) indicated, that the largest amount of nitrogen fertilizer added in the second batch of one of the fields gave the highest values of NDVI compared to other fields. The addition of nitrogen fertilizer to the field (D) led to an increase in the activity of the root and vegetative group, and as a result this led to an increase in the absorbed amount of nitrogen, phosphorous and potassium and an increase in the concentration of chlorophyll in the leaves of the plant, thus increasing the health of the plant, which led to an increase in the spectral index values of the field (D) compared to e values of the spectral indices for the rest of the fields. Alshujairy (2017) also showed the vegetative spectral indices a relationship with the health of the plant and its optimal absorption of nutrients. The higher the nutrient content of the plant, the higher its spectral index values.

As for the final slow growth stage (S4) significant decrease in the values of spectral indices for this stage and for all points in the five fields within the study area. The NDVI index values ranged from 0.14- 0.24. Its values ranged of field A from 0.16-0.21, B from 0.14-0.18, C from 0.18-0.20, D from 0.17-0.18 and E from 0.14-0.24. When the plant reaches this stage, values of the spectral indexes decrease again, affected by the decrease in the reflection in the near infrared spectral field, which is due to the decrease in the plant's living mass and leaf area. Al-Khalid et al (2017) indicated that the spectral index values decreased when the plant reached the aging stage due to the low reflectivity of the near-infrared rays due to the decrease in the plant biomass, as well as the high reflectivity of the red rays due to the low concentration of chlorophyll in the plant at this stage. There was a discrepancy in the concentration of chlorophyll in the first spectral stage (S1), as the values ranged from 32-45.5 SPAD of all points in the study area, where the concentration of chlorophyll in the wheat crop of field A in this stage ranged from 32 -44.2 SPAD and field D from 38.9-44.8 SPAD. The nitrogen concentration in general ranged between 2.08-4.38%, as the nitrogen concentration in the wheat crop of field A for this stage ranged from 2.08-3.70%, B from 2.56-4.38%, C from 2.76-3.61%, C from 2.76-3.61%, D from 2.92-4.33% and E from 2.82-3.75 %. There was a discrepancy in the phosphorous concentration in the wheat crop, as the

Table 4. Standards of descriptive statistics for the NDVI index at the growth stages of the wheat crop

Parameters	NDVI							
	S1	S2	S3	S4	S1	S2	S3	S4
	A				B			
Min	0.14	0.29	0.46	0.16	0.22	0.31	0.48	0.14
Max	0.16	0.36	0.61	0.21	0.32	0.42	0.68	0.18
Mean	0.15	0.32	0.55	0.18	0.26	0.36	0.57	0.16
Stdev	0.01	0.03	0.05	0.02	0.04	0.04	0.07	0.01
CV (%)	5.94	7.99	9.62	9.39	13.62	11.38	12.78	9.04
	C				D			
Min	0.15	0.32	0.56	0.18	0.17	0.37	0.62	0.17
Max	0.20	0.40	0.63	0.20	0.23	0.45	0.70	0.18
Mean	0.18	0.36	0.60	0.19	0.20	0.42	0.67	0.18
Stdev	0.02	0.04	0.03	0.01	0.02	0.02	0.02	0.01
CV(%)	13.60	10.14	5.27	6.08	10.58	5.37	3.49	2.98
	E				General mean			
Min	0.13	0.24	0.39	0.14	0.1	0.2	0.4	0.1
Max	0.20	0.42	0.72	0.24	0.3	0.5	0.7	0.2
Mean	0.16	0.33	0.56	0.18	0.2	0.4	0.6	0.2
Stdev	0.03	0.06	0.11	0.04	0.0	0.1	0.1	0.0
CV (%)	18.29	16.96	18.92	21.00	24.5	14.4	13.4	12.0

concentrations ranged between 0.12-1.19% for all points in the study area, as the phosphorous concentration in the wheat crop of field A at this stage ranged from 0.14-0.29% , B from 0.16 -1.19% , C from 0.19-0.21%, D from 0.14-0.31%, and E from 0.15-0.39 %. The potassium concentration in the wheat crop ranged between 0.93-3.23%, while the lowest potassium concentration in field A ranged between 0.93-3.06 %. Therefore, chlorophyll and NPK elements in plants are relatively high at some points within the same field, and this discrepancy may be attributed to the heterogeneity of the single field in its physical and chemical characteristics, as well as agronomic processes followed, the distribution of

Table 5. NPK nutrients concentration and chlorophyll content of wheat growth stages

Parameters	Chlorophyll			N%			P%			K%		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
A												
Min	32.0	38.6	42.2	2.1	1.5	0.9	0.1	0.2	0.1	0.9	1.7	0.9
Max	44.2	47.2	51.4	3.7	2.4	1.3	0.3	0.3	0.2	3.1	2.8	1.3
Mean	39.5	43.2	45.5	3.1	2.1	1.1	0.2	0.2	0.1	1.8	2.3	1.1
Stdev	3.8	2.8	3.5	0.6	0.3	0.1	0.1	0.1	0.0	0.8	0.5	0.1
CV(%)	9.7	6.5	7.7	18.9	12.7	12.1	30.4	25.9	15.3	43.1	21.0	13.3
B												
Min	40.2	42.0	43.5	2.6	1.8	0.9	0.2	0.2	0.1	2.1	1.9	0.9
Max	45.5	47.5	51.3	4.4	2.6	1.4	1.2	0.3	0.3	3.0	3.2	1.4
Mean	42.7	44.3	47.2	3.7	2.1	1.1	0.4	0.3	0.2	2.6	2.5	1.1
Stdev	2.0	2.3	2.6	0.6	0.3	0.2	0.4	0.0	0.1	0.3	0.4	0.2
CV(%)	4.7	5.2	5.5	15.8	12.7	16.6	95.7	14.5	35.9	12.4	17.8	16.5
C												
Min	38.0	43.7	45.1	2.8	2.1	1.1	0.2	0.2	0.1	1.2	2.0	1.0
Max	40.4	46.4	48.4	3.6	2.3	1.3	0.2	0.4	0.3	2.8	3.1	1.2
Mean	39.1	45.5	46.9	3.2	2.2	1.2	0.2	0.3	0.2	1.9	2.6	1.1
Stdev	1.2	1.3	1.6	0.4	0.1	0.1	0.0	0.1	0.1	0.8	0.5	0.1
CV(%)	3.0	2.8	3.4	12.8	3.0	8.2	5.8	29.8	52.4	40.3	18.1	6.5
D												
Min	38.9	44.8	47.0	2.6	2.3	1.0	0.1	0.3	0.2	1.4	1.7	0.9
Max	44.8	50.9	53.1	4.3	3.6	2.0	0.3	0.3	0.3	3.2	3.7	1.5
Mean	41.4	47.0	49.3	3.5	2.9	1.5	0.2	0.3	0.2	2.0	2.8	1.2
Stdev	2.0	1.6	1.8	0.5	0.4	0.2	0.0	0.0	0.0	0.5	0.6	0.2
CV(%)	4.9	3.3	3.7	14.0	14.5	15.5	24.8	10.6	15.5	23.8	20.3	14.2
E												
Min	37.2	40.1	40.2	2.8	1.6	0.7	0.2	0.2	0.1	1.1	1.7	0.8
Max	41.6	48.1	51.4	3.8	2.8	1.6	0.4	0.4	0.2	2.6	3.0	1.3
Mean	38.9	44.3	45.0	3.3	1.9	1.1	0.3	0.3	0.2	1.8	2.5	1.0
Stdev	1.6	3.0	3.4	0.4	0.4	0.3	0.1	0.0	0.0	0.5	0.4	0.1
CV(%)	4.1	6.9	7.7	13.2	19.2	25.2	32.1	13.7	23.6	31.1	16.8	13.3
General mean												
Min	32.0	38.6	40.2	2.1	1.5	0.7	0.1	0.2	0.1	0.9	1.7	0.8
Max	45.5	50.9	53.1	4.4	3.6	2.0	1.2	0.4	0.3	3.2	3.7	1.5
Mean	40.6	45.0	47.0	3.4	2.3	1.2	0.3	0.3	0.2	2.0	2.6	1.1
Stdev	2.7	2.6	3.1	0.5	0.5	0.3	0.2	0.1	0.1	0.6	0.5	0.2
CV(%)	6.6	5.8	6.6	15.8	22.7	21.0	80.0	18.0	30.0	30.4	19.6	15.3
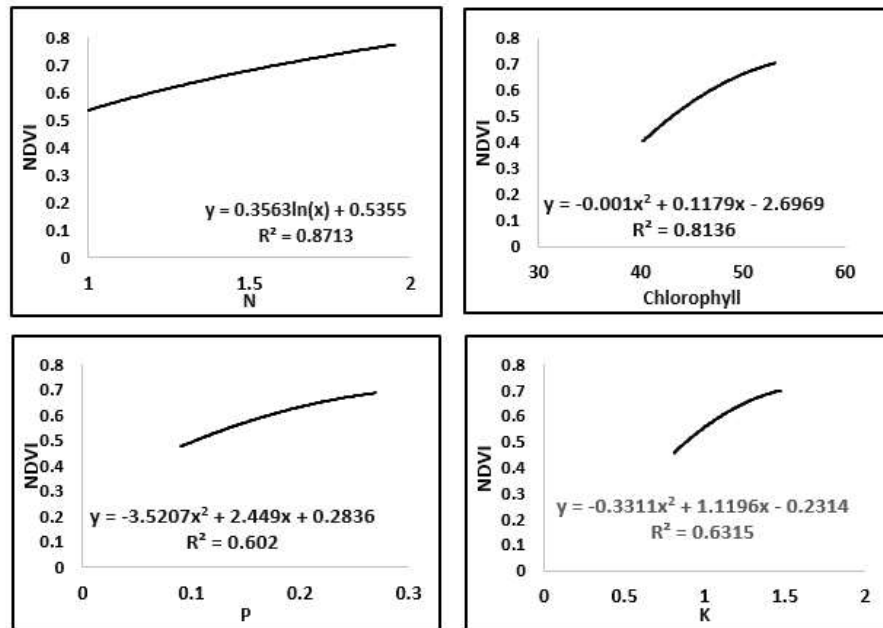
Fig. 3. Relationship of the regression between the concentration of NPK and chlorophyll with NDVI at the third stage

fertilizer quantities, and the nature and condition of the surface, as the topographical heterogeneity of the field may affect the condition and moisture content and nutrients as well as plant density within a single field. But if the discrepancy at the level of fields within the study site, there is a superiority of some fields compared to other fields, as concentrations of chlorophyll and NPK reached the highest in fields (B) and (D), and this may be attributed to the superiority of field (B) in the values of chlorophyll and NPK concentrations.

NPK for this stage to the irrigation system, as the fixed sprinkler irrigation system for this field enables the farmers to complete the irrigation process for the whole field within a few hours, while the fields with the pivot sprinkler irrigation system located within the study area needed seven days to complete the first irrigation, and the field (C), which depends on the tourist irrigation system, the decrease in the values of chlorophyll and NPK concentrations from fields (B) and (D) at this stage can be attributed to the delay in the sowing date for the rest of the studied fields, as the difference between this and the other fields was six days to complete the seeding process. As for field (D), its superiority in chlorophyll and NPK concentrations can be attributed to the addition of diammonium phosphate (DAP) fertilizer to at sowing , which was the highest compared to the rest of the fields, in addition to the good amount of phosphorous ready in the soil for this field, which led to increased growth and revitalization The

root system of the plant, thus increasing the absorption of macro and micro nutrients, including nitrogen and magnesium, which have a major role in increasing the concentration of chlorophyll in the plant, and this is in agreement with Al-Mashhadani ٢٠٢١(.

As for the exponential growth stage (S2), the chlorophyll content of field A ranged from 38.6-47.2 SPAD, B from 42-47.5 SPAD, C from 43.7-46.4 SPAD, D from 44.8-50.9 SPAD, and E from 40.1-48.1 SPAD. It was observed that the concentration of chlorophyll in wheat leaves increased for all points in the study area and this may be due to the expansion of the root system of the plant and the efficiency of obtaining the necessary nutrients as well as the moisture content as well as the increase in the leaf area, which has a role in photosynthesis as well as climate elements such as humidity and moderate temperature, as during this stage the plant can increase the processes of development and growth of the vegetative system and thus increase the leaf area, and this is offset by an increase in the process of photosynthesis and thus a high concentration of chlorophyll which is consistent with Alhameer (2015). The lowest values of nitrogen concentration in plants were in field (A), (1.94 %), while the highest values of nitrogen concentration in plants were at field D (2.96%). This was observed that points which excelled in chlorophyll concentrations in plants also outperformed in nitrogen concentrations in plants, and this is due to the fact that nitrogen is one of the basic components of chlorophyll.

Alhameer (2015) also found that there is a very close relationship between chlorophyll and nitrogen concentration in plants because nitrogen represents a structural element in chlorophyll and protein molecules, and thus affects the construction of chloroplasts and the accumulation of chlorophyll in them. Phosphorous concentration ranged between 0.15-0.35% for all points in the study area, as the highest values of phosphorous concentration were in field D (0.29 %). The superiority of field D in the concentration of phosphorous in the wheat crop may also be attributed to the fertilizer addition (DAP) to this field, as it was the highest compared to the rest of the studied fields, reaching 200 kg ha^{-1}. The potassium concentration in the wheat crop at this stage, ranged from 1.67-3.67% for all points in the study area, as the lowest values of potassium concentration were found in field A (2.26%), while the highest potassium concentration was in field D (2.81 %). Although potassium fertilizers were not added to all fields in the study area, the presence of potassium in medium concentrations may be due to the addition of phosphate fertilizers, and this is consistent with Al-Budairi (2018).

The stage of linear growth (S3), showed high concentration of chlorophyll in wheat and reaching its peak, as the chlorophyll values ranged between 40.20-53.10 SPAD for each study area.. The results of the study indicate the superiority of field D in the chlorophyll for this spectral stage. The 100 kg.ha^{-1} of urea fertilizer was added to the field (D). Nitrogen is an essential component of chlorophyll in plants and this is confirmed by Guendouz et al (2014). The nitrogen concentration in the wheat crop of field A ranged from 0.91-1.28 % at a rate of 1.08 % and field D ranged from 0.99-1.95 % at a rate of 1.45%.

There was a discrepancy in the phosphorous concentration in the wheat crop, as the values ranged from 0.09-0.27 % for all points in the study area, as the average phosphorous concentration in the wheat crop ranged among 0.14% 0.16, 0.17, 0.22 and 0.15%, respectively. Therefore, the numerical decrease in the values of NPK concentrations of elements, but this decrease does not mean a decrease in the total plant content of elements, but rather is the result of the dilution effect of the concentration of the element in the general dry matter, meaning that the concentration of the element will be distributed over a greater amount of dry matter when the rate of plant growth increases, As the plant content of the element is a product of multiplying the concentration of the element by the weight of the dry matter of the plant and this is consistent with Wang et al (2017). The coefficient of determination (R^2) between the values of NDVI and the concentrations of chlorophyll, N, P and K in the wheat crop for the spectral phase S3 was 0.81, 0.87, 0.60 and 0.63,

respectively (Fig. 3). When studying the regression relationship between the spectral indices and the concentration of chlorophyll, N, P and K at this spectral stage, the results showed that the highest value of the coefficient of determination (R^2) between the spectral indices and the concentration of chlorophyll, nitrogen, phosphorous and potassium was with the NDVI index, and these results were in agreement with Al-Budairi (2018). This stage was characterized by acceleration in the physiological activity represented by an increase in the accumulation rate of dry matter. It is also characterized by a stable growth rate and an increase in leaf area and chlorophyll concentration. This is consistent with Ibrahim (2014) and Alhameer (2015).

In general, that the spectral evidence reached the highest values at this stage, affected by the height of the plant's living mass, leaf area and dry weight. The reflection recorded the highest value in the near infrared spectral field, as well as the reflection in the red and blue spectral field recorded the lowest values affected by the high concentration of chlorophyll, which reached its concentration The yield of wheat is highest at this stage of plant life and this is in agreement with several studies by Alhameer (2015.) The high values of the coefficient of determination between the chlorophyll concentration and the spectral indices values at this stage may be attributed to the similar behavior of the spectral indices and the chlorophyll concentration during the wheat growth stages, as in this spectral phase of the plant's age, the spectral indices and the chlorophyll concentration in the plant reach the highest values.

The superiority of coefficient of determination (R^2) is due to the addition of nitrogen fertilizer to field (D) only among the five studied fields, which in turn increased the nitrogen concentration in the wheat crop for this field and this is consistent with Al-Zobaie (2021) therefore the spectral index values were high for tin field without Other fields, which led to a rise in the values of the coefficient of determination (R^2) between the spectral index and the nitrogen concentration at this stage. The a rise in the values of the spectral indexes of the wheat crop in the field D was observed after the addition of nitrogen to this field and this is consistent with Alhameer (2015) and the high values of the coefficient of determination between the spectral index and the concentrations of phosphorus and potassium in the wheat crop may be attributed to the nitrogenous addition of the field D, as the nitrogen fertilization had an important role in increasing the absorption of phosphorous and potassium by the plant and this is consistent with Al-Zobaie (2021). There were differences in the spectral index values of the points distributed within the five studied fields. The spectral index were found to be relatively higher for the points within the field

D than in the other fields, which led to higher values of the coefficient of determination (R^2) among the indexes.

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Effect of Treating Potato with Jasmonic and Tannic Acid on Some Biological Aspects of Green Peach Aphid

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Abstract: A laboratory study was conducted to find out the effect of treating of two potato cultivars (synergy and florice) with different concentrations of jasmonic and tannic acid on some biological aspects of the of green peach aphid *Myzus persicae*. There was significant difference in the development period of the different stages of the *M. persicae*, the highest development period of the first nymph (3.000 days) when treated with jasmonic acid at a 1000 ppm for both cultivars. This was observed that the development period of the second, third and fourth nymph increased with the increase the concentration used of jasmonic and tannic acid. The highest period before reproduction was when treated with jasmonic acid at a 1000 ppm (3.66 days) on the synergy variety. There was an inverse relationship between the concentration used and period of reproduction, period after reproduction, number of nymphs and virgin female productivity, as it decreased with the increase the concentration used for both jasmonic and tannic acid concentrations. The results showed that the longest generation period was 17.666 and 14.666 days for both cultivars respectively when treated with jasmonic acid at a 1000 ppm, respectively. The average insect longevity decreased in the different treatments compared with control treatment.

Keywords: *S. tuberosum, M. persicae,* Induced resistance, Jasmonic acid, Tannic acid

Potato (*Solanum tuberosum* L.), belongs to the Solanaceae family and is one of the most important major vegetable crops in the world. It ranks fourth after corn, wheat and rice for its adoption in nutrition in many countries. Potatoes are grown all over the world (FAO 2020). Alaphilippe et al (2016) indicated that the potato is one of the crops with a high nutritional value because it contains a high percentage of carbohydrates, proteins, fiber, vitamins, essential amino acids and other nutrients beneficial to humans. Potato are exposed to many insect pests during all phases of crop growth and can cause yield losses ranging from 30-70% (Mujica and Kroschel 2013, Kroschel and Schoub 2013), including *Myzus persicae* (Hemiptera: Aphididae), which is one of the important insects and has a wide family range, spread in most agricultural areas in the world (Merfield et al 2019). The insect causes direct damage to the crop by feeding and absorbing plant juices, in addition to its great role in transmitting many economically important viruses, as it transmits more than 70 types of plant viruses (Geary et al 2016). The use of chemical pesticides is accompanied by a number of determinants, topped by the negative effects on humans, biodiversity and the environment, as well as the development of insect resistance (Lamichhane et al 2017). The search for alternative pest control strategies has begun, including the use of environmentally friendly materials or stimulating the

mechanisms of plant resistance against pests by treating the plant with some substances capable of stimulating plant defenses against various pests, such as jasmonic and tannic acid (Al-Khazraji et al 2016, Li et al 2018, Al-Khazraji and Shaher 2020). Due to the importance of the green peach aphid (*Myzus persicae*) that infects the potato crop and due to the lack of studies on the effect of jasmonic and tannic acid in enhancing the resistance of potato plants against insects in Iraq, so this study aimed to know the effect of treating potato with different concentrations of jasmonic and tannic acid on the life aspects of *M. persicae*.

MATERIAL AND METHODS

One of the open fields was selected in the College of Agricultural Engineering Sciences , University of Baghdad in the season of 2020-2021. The area of the field reached 300 m^2, and it was prepared by carrying out all the agricultural operations according to the approved recommendations for cultivation. The field was divided into four rows, the length of the row is 50 m, while the distance between them 1 m. A factorial experiment according to randomized complete block design with three replications. The field experiment included three factors, the first factor included two cultivars of potato (synergy and florice), the second factor included two sprayed substance (jasmonic and tannic acid) while the third factor included three sprayed concentrations (250, 500 and 1000

ppm) as well as control treatment (spraying of distilled water only). Potato tubers were planted on 17 September. 2020, at a rate of one tuber per hill, with a distance of 20 cm between them. The spraying process was carried out on 4 November. 2020 by using a small sprinkler with a capacity of 2 liters, and repeated spraying two weeks after the first treatment.

In order to study the effect of treating potato plants with different concentrations of jasmonic and tannic acid on some biological aspects of *M. persicae* in the laboratory, the leaves from each experiment unit were brought and placed in petri dishes with a diameter of 9 cm, and one adult insect was transferred by a soft brush to a petri dish. The petri dish was covered by a piece of thermal nylon perforated from the top with soft holes to ensure ventilation, then the petri dishes were transferred to the incubator, after the birth of the nymphs, one nymph was left in each dish. The petri dishes were transferred to an incubator at a 25 ± 2 C° and a relative humidity of 50-60%. Follow-up and examination were carried out on a continuous basis and the leaves were replaced whenever necessary until reaching the adult's turn and then the natural death of the adult, and the following was calculated:

Number of nymph instars: It was determined by calculating the number of moultings that the nymph passes through before reaching the adult stage.

Period of the nymph's cycle: It was calculated based on the number of days that the nymph passed from its birth until its adult stage. The period of each instar was calculated per day on the basis of the period between each two moultings, except for the first nymph instar, it was calculated from the birth of the nymph until its moulting.

Productivity of one female: It is the total number of nymphs produced by the female until its death. The average number of nymphs produced per day has been calculated.

Period of reproduction: It is the period from the birth of the first nymph to the production of the last nymph.

Period before reproduction: It is the period from the end of the fourth nymph instar and its moulting to the birth of the first nymph.

Period after reproduction: It is the period from the birth of the last nymph to the death of the insect.

Generation length: It is the period from the birth of the first nymph and after it becomes an adult and the first nymph is born.

Insect longevity: It is the period from the birth of the insect until its natural death.

RESULTS AND DISCUSSION

Development of Nymphs and Adults of *M. persicae*

Nymph stage: There was a significant difference among studied treatments (1, 2, 3, 4 and 5) in the growth period of the nymph of *M. persicae*, the insects which feed on synergy cultivar, spraying of jasmonic acid at a 1000 ppm recorded the longest growth period of the four nymph's instar and the period of the total nymph stage (3.000, 3.666, 4.000, 3.333 and 13.999 days) respectively, while the insects which feed on florice cultivar spraying of tannic acid at a 250 ppm recorded the shortest growth period of the four nymph's instar and the period of the total nymph's stage (2.000, 2.000, 1.666, 2.000 and 7.666 days, respectively), without significant difference with synergy cultivar and reached in control treatment (1.666, 1.666, 1.333, 1.666 and 6.331 days, respectively). The reason of the difference in development period may be due to the effect of jasmonic and tannic acid and the contents of the two potato's cultivars of chemical compounds on the *M. persicae* insect, as they play an important role in inducing the resistance of the potato plant by making the leaves of the plant unpalatable to the insect, which leads to a lack of nutrition and thus leads to a delay the molting process and the transition to the other phase. Cooper and Goggin (2005) reported that the application of jasmonic acid at a 1.5 mM to tomato leads to stimulating plant resistance and thus leads to a decrease in *Macrosiphum euphorbiae* nutrition, growth and reproduction. Ma et al (2019) show that the spraying of tannic acid on *Aphis gossypii* increased development period.

Adult Stage

Period before reproduction, period of reproduction and period after reproduction: There was a significant difference among studied treatments (Table 6) in the period before reproduction, the synergy cultivar when spraying of jasmonic acid at a 1000 ppm gave the longest period before reproduction (3.666 days), while the florice cultivar, control treatment gave the shortest period before reproduction (1.333 days) without significant difference with control treatment of synergy cultivar (2.000 days).

There was a significant difference among studied treatments in the period of reproduction (Tables 5). The control treatment of florice cultivar recorded the longest period of reproduction (18.660 days), whereas the synergy cultivar when spraying of jasmonic acid at a 1000 ppm recorded the shortest period of reproduction (7.000 days) without significant difference with synergy cultivar and spraying of tannic acid at a 1000 ppm (8.000 days). Regarding the period after reproduction reveal that there was a significant difference among studied treatments in this trait (Table (6). The control treatment of florice cultivar achieved the longest period after reproduction (2.517 days), whereas the synergy cultivar when spraying of jasmonic acid at a 1000 ppm achieved the shortest period after reproduction (1.000

days) without significant difference with synergy cultivar with other concentrations of jasmonic and tannic acid in addition to florice with tannic acid at a 1000 and 500 ppm.

Number of nymphs produced and virgin female productivity: The results in There was a significant

Table 1. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the growth period of the first nymph instar of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Jasmonic acid	1000	3.000	3.000
	500	2.667	2.333
	250	2.333	2.000
Tannic acid	1000	2.333	2.333
	500	2.333	2.000
	250	2.000	2.000
Control	0	1.666	1.666
LSD (p=0 .05)		1.270	

Table 2. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the growth period of the second nymph instar of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Jasmonic acid	1000	3.666	3.000
	500	3.000	2.666
	250	2.666	2.333
Tannic acid	1000	2.666	2.333
	500	2.666	2.000
	250	2.333	1.666
Control	0	2.000	1.666
LSD (p=0 .05)		1.224	

Table 3. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the growth period of the third nymph instar of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Jasmonic acid	1000	4.000	3.333
	500	3.333	2.666
	250	3.000	2.666
Tannic acid	1000	3.333	3.000
	500	3.000	2.333
	250	2.666	2.000
Control	0	1.666	1.333
LSD (p=0 .05)		1.224	

difference among studied treatments in the number of nymphs produced and virgin female productivity (Table 7). The control treatment of florice cultivar achieved the highest means of these traits (3.885 femaleday^{-1}and 72.494 females), while the synergy cultivar when spraying of jasmonic acid at a 1000 ppm achieved the lowest means (1.500 female day^{-1} and 10.500 females) without significant difference with synergy cultivar with other concentrations of jasmonic and tannic acid in addition to florice with concentrations of jasmonic acid in number of nymphs produced as well as without significant difference with synergy cultivar when spraying of tannic acid at a 1000 ppm in the virgin female productivity.

Generation length and insect longevity: There was a significant difference among studied treatments (Table 8) in the generation length, the synergy cultivar when spraying of jasmonic acid at a 1000 ppm gave the longest generation length (17.666 days), while the control treatment of florice cultivar gave the shortest generation length (7.664 days) without significant difference with control treatment of

Table 4. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the growth period of the fourth nymph instar of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Jasmonic acid	1000	3.333	2.666
	500	3.000	2.333
	250	2.666	2.000
Tannic acid	1000	2.666	2.666
	500	2.333	2.000
	250	2.333	2.000
Control	0	2.000	1.666
LSD (p=0 .05)		1.270	

Table 5. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the period development of the total nymph stage of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Jasmonic acid	1000	13.999	11.999
	500	11.998	9.999
	250	10.663	8.999
Tannic acid	1000	10.998	10.332
	500	10.332	8.333
	250	9.330	7.666
Control	0	7.333	6.331
LSD (p=0 .05)		1.708	

synergy cultivar and florice cultivar when spraying of tannic acid at a 250 ppm (9.333 days). Regarding the insect longevity there was a significant difference among treatments (Table 8) in this trait. The control treatment of florice cultivar achieved the longest insect longevity (28.840 days) without significant difference with control treatment of synergy cultivar (27.663 days), whereas the florice cultivar when spraying of tannic acid at a 250 ppm achieved the shortest insect longevity (22.000 days) without significant difference with florice cultivar when spraying of tannic acid at a 500 ppm (22.330 days), florice cultivar with spraying of jasmonic acid at a 250 and 500 ppm (23.330 and 23.670 days respectively)and synergy cultivar with all concentrations of tannic acid (23.330, 23.665 and 23.663 days respectively).

Table 6. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the period before reproduction, period of reproduction and period after reproduction of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Period before reproduction			
Jasmonic acid	1000	3.666	2.667
	500	3.000	2.333
	250	2.667	2.000
Tannic acid	1000	3.000	2.333
	500	2.667	2.000
	250	2.667	1.667
Control	0	2.000	1.333
LSD (p=0.05)		1.270	
Period of reproduction			
Jasmonic acid	1000	7.000	9.000
	500	9.000	9.666
	250	9.660	10.333
Tannic acid	1000	8.000	9.666
	500	9.333	10.000
	250	10.000	10.666
Control	0	16.000	18.660
LSD (p-0.05)		1.798	
Period after reproduction			
Jasmonic acid	1000	1.000	1.000
	500	1.333	1.667
	250	1.333	1.990
Tannic acid	1000	1.333	1.660
	500	1.333	1.990
	250	1.666	2.000
Control	0	2.333	2.517
LSD (p=0.05)		0.675	

Table 7. Effect of spraying of potato's cultivars with jasmonic and tannic acid on the number of nymphs produced and virgin female productivity of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Number of nymphs produced			
Jasmonic acid	1000	1.500	1.750
	500	2.037	2.366
	250	2.133	2.566
Tannic acid	1000	1.731	2.429
	500	2.220	2.693
	250	2.330	2.722
Control	0	3.000	3.885
LSD (p=0 .05)		1.179	
Virgin female productivity			
Jasmonic acid	1000	10.500	15.750
	500	18.333	22.952
	250	20.604	26.536
Tannic acid	1000	13.848	23.464
	500	20.719	26.930
	250	23.300	29.016
Control	0	48.000	72.494
LSD (p=0 .05)		3.116	

Table 8. Effect of spraying of potato's cultivars jasmonic and tannic acid on the generation length and insect longevity of *M. persicae*

Treatment	Concentration (ppm)	Cultivars	
		Synergy	Florice
Generation length			
Jasmonic acid	1000	17.666	14.666
	500	14.999	12.333
	250	13.330	10.999
Tannic acid	1000	13.998	12.665
	500	12.999	10.333
	250	11.997	9.333
Control	0	9.333	7.664
LSD (p=0 .05)		2.292	
Insect longevity			
Jasmonic acid	1000	25.670	24.666
	500	25.330	23.670
	250	24.330	23.330
Tannic acid	1000	23.330	24.000
	500	23.665	22.330
	250	23.663	22.000
Control	0	27.663	28.840
LSD (p= 0.05)		1.741	

There is a difference in the biological aspects of the *M. persicae* and this may be due to the role of jasmonic and tannic acid in inducing the resistance of potato plants against *M. persicae*, as well as could be due to the difference in some secondary metabolic compounds present in these cultivars. Thaler (1999) showed that the tomato treated with jasmonic acid increased the production of proteinase inhibitors (PIs) and polyphenol oxidases and decreased the food preference of insects. Karban and Baldwin (1997) indicated that the jasmonic acid is responsible for the production of specific secondary receptors that act as an insect repellent, and these secondary receptors are responsible for reducing the survival and reproduction of *N. lugens*. Bruce et al (2003) demonstrated that the spraying of jasmonic acid reduced aphids on wheat. Li et al (2018) reported that the tannic acid has an important role in phytochemical defense against insects due to its toxic and anti-nutritional effect and has an important role in activating PIs that combine with digestive enzymes to form complex proteins which impede digestion in insects. The production of PIs is a defensive strategy in some plants to avoid insects and was observed that insects exposed to PIs have a low rate of growth and development with an increase in mortality rate, as PIs affect the digestion of food inside the bodies of insects, which leads to reducing or inhibiting the necessary protein synthesis for growth, development and reproduction of insects (Ellis et al 2002, Wasternack and Hause 2013, Merino-Cabrera et al 2018). Ma et al (2019) explained the effect of tannic acid on the cotton aphid *Aphis gossypii*, as the treatments led to a higher percentage of insect mortality and also caused an increase in the development period of the treated individuals and a decrease in the rate of reproduction.

CONCLUSION

There is an increase in the period of growth of the nymph and a decrease in the productivity of the adults of *M. persicae* with increasing the concentrations of the jasmonic and tannic acid. The spraying of jasmonic acid at a 1000 ppm is superior compared with the other concentrations.

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Evaluation of Hardness in Drinking Water in Medhatiyah City, Iraq

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Abstract: This study aimed to estimate hardness in drinking water of Almedhatiya cityby comparing the total hardness, calcium and magnesium hardness with permissible standard of drinking water. This was to indicate if there was removal of hardness ions during water treatment for drinking purpose. The samples were collected from five locations, three drinking water stations without using reverse osmosis, while two of purified water with reverse osmosis. The results showed total hardness, calcium and magnesium hardness in all study samples was within standard of WHO and Iraqi drinking water except calcium hardness in sample number SH and SA were the average concentrations of calcium were above Iraqi drinking water standard.

Keywords: Hardness, Diseases, Diabetes, Drinking water

Natural water can have hardness in different levels depending on the rocks that river water runs and can take many of its properties from components of these rocks. Rivers in Iraq have hard to very hard water specially water of Euphrates (Hassan et al 2008, Al-Heety et al 2011).Hardness of drinking water means appeared variety of metallic cations dissolved and multiple valent in water mainly calcium and magnesium in less forms Al, Ba, Fe, Mn, St and Zn (McGowan 2000). This hardness can form precipitations of insoluble elements and salts in hardness water. Total hardness produces by cations derived naturally from sedimentary rocks. Calcium ions are common elements in natural water followed by magnesium ions and are important due to low concentration in human diet as a reason of feeding habitat and their abundant in sources of water (Ong et al 2009). Humans need calcium and magnesium and if they cannot have enough then many health problems like hypertension, kidney stones, obesity, colorectal cancer, stroke, insulin resistance, coronary artery disease and osteoporosis (WHO 2009) while in excessive concentration can caused problems in unhealthy kidney, hyper-calcaemia in some people, increasing in metabolic alkalinity and reducing in absorption of many essential elements inside intestines (Akram 2018). Magnesium is second most important ion in intercellular fluid and very important to cellular enzymatic activity, low concentration of this ion can cause hypertension, endothelial dysfunction, decrease in insulin sensitivity, diabetes 2, arrhythmia, eclampsia and coronary heart disease (Jianget al 2016), while excessive concentration can cause diarrhea and renal insufficiency (Stevanovic et al 2017 Langan (2009) referred that exposure to water hardness can cause eczema. This study was aimed to know the concentration of some hardness ions in water that used for drinking purpose that collected from five stations.

MATERIAL AND METHODS

Samples collection: Samples were collected at monthly interval from five locations in Almedhatiya city from January - December, 2018 (Table 1),

1-R- Alhilla river south of Alhussain district for comparing purpose

2-SH- Alhussain station for drinking water

3-SA- Alameer station collection that provide center city and Aljameeya city with drinking water

4-RO- Market of Alhussain district where the drinking water treated with reverse osmosis technique and sold in bottle.

5-NRO-Market of Alhussain district where the drinking water treated with reverse osmosis technique and sold in unpackaged.

Measuring of total hardness, calcium and magnesium hardness: Total hardness of water measured in Environmental College, Al-kufa University as per standard method of American Public Health Association (APHA 2003; 2005). The calcium hardness used meroxide as indicator. The magnesium hardness was calculated by using multiple equations that depending on concentration of total and calcium hardness.

Data analysis: The data was analyzed for differentiation between samples and among months and also finding correlation coefficient.

RESULTS AND DISCUSSION

The maximum total hardness was 461 mg/l in October in

SH and low was 133 mg/l in mostly study months in sample RO (Table 2) and these values comparable sample R and lower values indicate the removal of hardness in treatments. The total hardness values within standard limiting of WHO (2011) and drinking water Iraqi standard (2001). This means the total hardness was in permissible limited of drinking water, which that could be due to the removal of some ions that caused total hardness from water in stations of drinking water comparing to river sample R. The bottle water and unpackage water can be used for drinking as mentioned in studies on drinking water stations of Hassan and Mahmood (2018) and Alnasrawi et al (2019). But, there was some human illness with use the water from stations in drinking purpose as in mention in some studies (Sengupta (2013). Some studies pointed that bottle water and unpackage water treated with reverse osmosis are unfit to use with infants (Kožíšek (2003, Poursafa et al 2014).

The high value of calcium hardness was (75 mg/l in February and April in SA and low was 62 mg/l in all months in sample RO (Table 3) and these values were comparable to sample number R. The lower values, means there was removal of calcium ions from water through treatments. Results showed a significant variation and according to WHO

the calcium hardness was in permissible limited in some study samples. Jazza and Al-saad (2016) observed that in some treatment stations of drinking water to remove some ions that caused hardness due to calcium. But according to drinking water Iraqi standard the average concentrations in sample SH and SA were above limiting standard. Saleh and Hussain (2017) also recorded increase in concentration of calcium in stations above Iraqi limiting standard to high demand on drinking water and delay in washed filters that can caused decreasing in removing calcium salts from water, or in some cases filtration basins cannot removed dissolved calcium (JICA 2015), Some studies pointed out that some drinking water stations were unable to remove dissolved ions when it found in high loaded in source of water (Salih and Al-azzawi 2016). (Stevanovic et al 2017), or in pipes system (Swietlik et al 2012). Positive correlation coefficients was observed between total hardness and calcium hardness appeared in study samples (Table 5, Fig. 1).

Table (4) magnesium hardness in some water types that used for drinking purpose. The high magnesium hardness was (139 mg/l) in July in SH and SA and low value was (60 mg/l) in mostly all months in sample number RO and these values compare to sample R was lower which means also there was removing from magnesium ions in treatment process. According to WHO and drinking water Iraqi standard the magnesium hardness was in permissible limit (Table 4), Salih and Al-azzawi (2016) showed decreased in magnesium hardness after treatment in drinking water station in spite of higher hardness in river source.

The bottle water and unpackage water that treated with reverse osmosis according to WHO and Iraqi standard limit were suitable for drinking used, and these results matched to

Table 1. Locations of the monitoring stations

Stations/months	Latitude	Longitude
Alhilla river south of Alhussain (R)	32.401090	44.686653
Alhussain station (SH)	32.388784	44.679692
Alameer station (SA)	32.399252	44.671093
Market of Alhussain (RO)	32.395752	44.679637
Market of Alhussain (NRO)	32.397226	44.672028

Table 2. Total hardness (mg/l) in 5 locations in Almedhatiya city

Stations/months	1	2	3	4	5	6	7	8	9	10	11	12
Alhilla river south of Alhussain (R)	742	746	754	769	772	781	799	804	771	769	752	748
Alhussain station (SH)	347	371	435	442	442	439	435	433	443	461	435	383
Alameer station (SA)	377	374	447	446	445	439	433	434	443	453	439	383
Market of Alhussain (RO)	133	133	133	136	136	135	135	134	133	133	133	133
Market of Alhussain (NRO)	139	140	141	142	140	141	142	142	140	140	140	141

Table 3. Calcium Ca (mg/l) in 5 locations in Almedhatiya city

Stations/months	1	2	3	4	5	6	7	8	9	10	11	12
Alhilla river south of Alhussain (R)	410	409	413	421	417	421	417	407	402	399	381	396
Alhussain station (SH)	170	171	168	162	160	161	169	164	159	153	147	159
Alameer station (SA)	174	175	174	175	165	162	172	169	166	157	154	164
Market of Alhussain (RO)	62	62	62	62	62	62	62	62	62	62	62	62
Market of Alhussain (NRO)	66	65	68	67	67	67	68	68	68	68	68	68

Table 4. Magnesium Mg (mg/l) in 5 locations in Almedhatiya city

Stations/months	1	2	3	4	5	6	7	8	9	10	11	12
Alhilla river south of Alhussain (R)	263	262	269	272	273	271	273	265	271	271	261	267
Alhussain station (SH)	130	133	134	135	134	136	139	136	131	129	122	128
Alameer station (SA)	132	134	135	137	137	137	139	138	132	133	125	129
Market of Alhussain (RO)	60	60	60	61	61	61	61	61	60	60	60	60
Market of Alhussain (NRO)	61	61	61	63	63	64	63	62	61	62	62	62

Table 5. Total hardness, Ca* and Mg** (mg/l) of water in Almedhatiya city

Stations/tests	Total hardness	Ca	Mg
Alhilla river south of Alhussain (R)	263	262	269
Alhussain station (SH)	130	133	134
Alameer station (SA)	132	134	135
Market of Alhussain (RO)	60	60	60
Market of Alhussain (NRO)	61	61	61
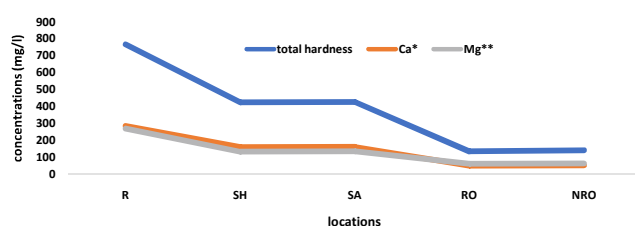
Fig. 1. Correlation coefficient between total hardness, Ca* and Mg** (mg/l) in 5 location in Almedhatiya city

some study of AL-Dulaimi and younes (2017). In another study magnesium ions in any concentration could be unsuitable for few children and some chronic disease states and t can lead to problems in health in infants and children after continuously of water with magnesium (WHO 2013, APHA 2012). Catling e al(2005) pointed to the important to find calcium and magnesium in drinking water when the food lack to these ions. The results showed positive correlation coefficients between total hardness and magnesium hardness and between calcium hardness and magnesium hardness in study samples (Table 5, Fig 1).

CONCLUSIONS

Total hardness in drinking water stations and water bottle and unpackage water that treated with reverse osmosis could be suitable for drinking purpose. Calcium hardness in drinking water stations is unsuitable for drinking purpose according to Iraqi standard limiting, but water bottle and unpackage water that treated with reverse osmosis were suitable for drinking purpose. Magnesium hardness in drinking water stations and water bottle and unpackage water that treated with reverse osmosis were e suitable for drinking purpose.

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Effect of Date Palm Pollen on Puberty and Sexual Maturity in Bull's Cows in Iraq

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Abstract: The current study was conducted to investigate the effect of adding date palm pollen (DPP) to accelerate puberty and sexual maturity in the bulls. Ten crossbred Holstein bulls' 6-month-olds were divided randomly into two treatment groups: T1- bulls were fed basal ration plus date palm pollen (DPP), 2 g/head/ day and T2- bulls were fed basal ration only. There was a significant effect for T1 in acceleration of puberty and sexual maturity as compared to T2. Bulls reached puberty and sexual maturity age at 8.5 and 9.5 month in T1 and 13.0 and 18.0 month in T2 respectively. There was significant differences between T1 and T2 in FSH, LH, estrogen and testosterone concentrations at the age of puberty, as well as in FSH, LH, estrogen and testosterone concentrations at age of sexual maturity. There was a significant effect differences between T1 and T2 in body weight at puberty age and on maturity age. The study also results showed a significantdifferences in between T1 and T2 in BCS at maturity age.

Keywords: Date palm pollen, Bulls, Puberty, Maturity, Hormones

Date palm pollen is one of the most important by-products of date palm trees (El-Neweshy et al 2013) and used as a treatment remedy for male infertility (Mohamed et al 2018). DPP has nutritional properties that enable to fight infections and increase immunity (Elberry et al 2011). Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are the major glycoprotein hormones that were synthesized and secreted by the pituitary (Pirahanchi et al 2022). DPP was used as an additive nutrient because it contains nutrients distinct in its chemical composition including fatty acids, proteins, carbohydrates, vitamins, and minerals (Araújo de Oliveira et al 2021). LH hormone helps in the development of the corpus luteum and the production of progesterone and testosterone which increases male sexual desire (Fortes et al 2012), while the FSH hormone plays a role in the growth and development of testis in animals, and stimulates sperm production in testis and sertoli cells (Cannarella et al 2018). Testosterone was responsible for the development of primary sexual characteristics, which include testicular descent, sperm formation, enlargement of the penis and testicles, and increased sexual desire (Li et al 2019). Puberty in bulls is the first sperm enter the epididymis. The onset of puberty was dependent on many factors like age, species, genotype, body weight, growth rate, and energy status (Anjum and Saghar 2014). There is no information on the specific weight of mature, the percent of herd average weight is well accepted and close to 400-500 kg in beef cattle, or using maturity index which consists age, nutrition, and birth weight (Olsen et al 2020). Body condition scores (BCS) are used as subjective estimates of the amount of subcutaneous fat on a cow (Kumar et al 2020, Khairunnisa et al 2021). Therefore, the aim of this study was to investigate the effect of adding date palm pollen on advanced of puberty and sexual maturity in bulls.

MATERIAL AND METHODS

Animals and diets: This study was conducted at Al-Najaf Al-Ashraf Governorate using 10 Holstein crossbred bulls 6-months-old from 1 August 2020 to 1 April 2021which was approved by Bioethics Committee at University of Kufa for using animal in experiments. Bulls were divided randomly into two treatment groups: T1- bulls were fed basal ration plus date palm pollen (DPP) at 2 g/head/ day, and T2- bulls were fed basal ration only. All bulls were fed on basal diet contained 49% straw, 25% green forage, 25% concentrate, 1% salt and vitamins. Fresh water and minerals block were available throughout the experimental period.

Blood collection and analysis: Blood samples were collected from the jugular vein of all bulls at three times: at the first day of study (prepubertal period), at puberty (pubertal period) and at maturity age to determine effect of adding of DPP on FSH, LH, estrogen and testosterone concentrations. After blood collection and centrifugation, blood samples were transported under cooling to the laboratory. The FSH, LH, testosterone and estrogen concentrations were estimated by mini-vides instrument using min-VIDAS® fertility kit (BioMerieux-France).

Body weight and body condition score: Bulls were weighted at same three times of blood collection. Body

condition score is performed before feeding and drinking of water.

Statistical analysis: Data were analyzed statistically using SAS (2012)

RESULTS AND DISCUSSION

There was no significant different between T1 and T2 at the first day on all sextual hormones concentrations in the plasma of bulls (Table 1). At age of puberty, there were significant difference between T1 and T2 on FSH (5.0, 1.19 ng/mL), LH (15.97, 8.81ng/mL), estrogen (8.39, 4.26 pg/mL) and testosterone (1.67, 0.42 ng/mL) concentrations in the plasma of bulls. Similarly, significant difference between T1 and T2 on FSH (11.93, 2.76ng/mL), LH (29.70, 20.76ng/mL), estrogen (11.52, 5.44pg/mL) and testosterone (3.19, 0.78ng/mL) at age of sexual maturity (Table 3). This could be due that DPP leads to improve the growing of pituitary gland and contain gonadotropin-like compounds which increase the concentration of FSH, LH and testosterone, these hormones play a major role in growing of testis, controlling sperm formation and improving male fertility, which led to advance male puberty and sexual maturity (Abedi et al 2013 and Mehraban et al 2014).

Significant difference between T1 and T2 on body weight at first day, but there was a significant difference between T1 and T2 on body weight at puberty age) and in maturity age (Table 4). The DPP rich in minerals like chlorine and sodium increase appetite and thus weight gain (Pei et al 2016). DPP also contains zinc which is needed for hormone production, vitamin synthesis, energy production, enzyme activity and other physiological processes related to reproduction, growth and health (Hassan 2011).

There was no significant different between T1 and T2 on BCS at first day at puberty age, but there was a significant different (P ≤0.05) between T1 and T2 on BCS at maturity age (4.70and 3.65months) between T1 and T2, respectively (Table 5). This may be due that DPP rich in antioxidants, which work to improve scoring. Antioxidants activity improves growth cells, immunity, body weight, body condition score, stress of heat and fertility, which leads to improve health of heifers and finally enhancement of sexual maturity (Hassan 2011).

There was significant effect on advance of puberty age (3.5, -1.0 months) and maturity age (8.5, 0.00 months) between T1 in T2, respectively (Table 6) and significant effect (on advanced of puberty and maturity age 8.5 and 9.5 months

Table 1. Concentrations of hormones in blood plasma of bulls at first day of study

Treatments	Means ± S.D.			
	Follicle stimulating hormone (ng/mL)	Luteinizing hormone (ng/mL)	Estrogen (pg/mL)	Testosterone (ng/mL)
T1	0.44± 0.09	8.92± 1.4	3.65± 0.38	0.32± 0.048
T2	0.4±0.04	8.27± 1.1	3.76± 0.41	0.29± 0.043
Significance	N.S	N.S	N.S	N.S

Table 2. Concentrations of hormones in blood plasma of bulls received DPP at age of puberty

Treatments	Means ± S.D.			
	Follicle stimulating hormone (ng/mL)	Luteinizing hormone (ng/mL)	Estrogen (pg/mL)	Testosterone (ng/mL)
T1	5.75 ± 0.99 a	15.97± 1.66 a	8.39± 1.10a	1.67± 0.08 a
T2	1.19 ± 0.18 b	8.81± 0.95 b	4.26± 0.62b	0.42± 0.03 b
Significance	**	*	*	*

*,** = Level of significance (P ≤0.05) and (P ≤0.01)

Table 3. Concentrations of hormones in blood plasma of bulls received DPP at age of maturity

Treatments	Means ± S.D.			
	Follicle stimulating hormone (ng/mL)	Luteinizing hormone (ng/mL)	Estrogen (pg/mL)	Testosterone (ng/mL)
T1	11.93 ± 2.14 a	29.70± 2.11 a	11.52± 1.64 a	3.19± 0.91 a
T2	2.76 ± 0.33 b	20.76± 2.01 b	5.44± 0.82 b	0.78± 0.04 b
Significance	**	*	*	*

*,** = Level of significance (P ≤0.05) and (P ≤0.01)

Table 4. Body weight (kg) of bulls received DPP at first day of study, puberty and maturity

Treatments	Means ± S.D.		
	1st day	Puberty	Maturity
T1	105.5 ±6.86 a	155± 3.30 a	212.5± 2.11 a
T2	99.5 ±5.22 a	139.5± 3.17 b	196± 2.42 b
Significance	N.S	*	*

N.S= Non significant ; * = Level of significance (P ≤0.05)

Table 5. Body condition sore of bulls received DPP at first day of study, puberty and maturity (months)

Treatments	Means ± S.D.		
	1st day	Puberty	Maturity
T1	1.00 ±0.0 a	3.25± 1.30 a	4.70± 0.50 a
T2	1.00 ±0.0 a	3± 0.17 a	3.65± 0.22 a
Significance	N.S	N.S	*

N.S= Non significant ; * = Level of significance (P ≤0.05)

Table 6. Advance of puberty and sexual maturity (months) in bulls received DPP

Treatments	Means ± S.D.			
	Puberty	Maturity	Puberty advanced	Maturity advanced
T1	8.5 ±2.80 a	9.5± 0.30 b	3.5± 0.11 a	8.5 ± 1.10 a
T2	13.0 ±3.11 a	18.0± 2.17 a	-1.0± 0.02 b	0.00 ±0.00 b
Significant	*	**	**	**

* = Level of significance (P ≤0.05), ** = Level of significance (P ≤0.01)

between T1 and T2 reached 13.0 and 18.0 months. This may be due to the DPP led to accelerate puberty and sexual maturity, because DPP rich in carbohydrates, proteins, saponins, alkaloids, minerals. Furthermore, DPP rich in antioxidants so it was suitable as a regular component in the human and animal diet (Al-Samarai et al 2018).

The high concentration of carbohydrates and minerals like zinc and magnesium in the ration leads to precocious puberty and sexual maturity. Furthermore, the iron is necessary for energy metabolism and respiration, and as part of the enzymes involved in the synthesis of collagen and some neurotransmitters and increases immunity, improves the health of calves and reduces anemia, which leads to increased body acquisition and leads to early puberty and sexual maturity (Al-Fada and Abu Ayyana 2012). The biofeedback episode causes the hypothalamus and thyroid gland to release a series of youth-promoting hormones such as growth hormone, estrogen and testosterone, as well as hormones that stimulate the adrenal gland and thyroid gland (Li et al 2019). This leads to precocious puberty and sexual maturity and increase in the level of hormones in the blood plasma with a high value of BCS and body weight are all factors that advance the age of sexual maturity and puberty of cows. The environmental factors, feeding management

and the sexual composition of animals affect these dates. The effect of breed type on lifespan at reaching bulls has been demonstrated in several studies comparing dairy and beef bulls, and within breeds early versus late maturity (Vanvanhossou et al 2018).

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Effect of Using Different Levels of Chia Seeds in Broiler Diets on Productive Performance

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Abstract: This study was conducted in the poultry fields of the College of Agricultural Engineering Sciences / University of Baghdad with the aim the effect of using different levels of Chia Seeds (*Saliva hispanica)* in broiler diets on production performance. 250 unsexed broilers of one-day age breed (Ross 308) with a starting weight of (40) g were used. Chicks were randomly distributed to five treatments, 50 birds for each treatment, and one treatment included five replicates (10 birds/replicate). The experimental treatments were T_1, T_2, T_3, T_4 and T_5 with an addition average of (0, 2, 4, 6, 8%) on the respectively, the birds were also fed three diets: the starter, growth and final diet, and the results showed a significant improvement (P<0.05) in the body weight average and the food conversion ratio for all treatments of using chia seeds (T_2, T_3, T_4, T_5) compared to the control treatment T_1. significant increase (P<0.05) in the average of weight gain of treatments T_4 and T_5 compared to treatment T_1 and T_2, and a significant improvement of the feed consumed for each of T_1, T_4 and T_5.

Keywords: Chia seeds, Broiler diets, Productive performance

Chia seeds have been approved by *Salvia hispanica* L. In recent years, because they are oily and rich in omega-3 fatty acids, it has been classified as one of the most nutritionally important oilseeds. Chia is grown for seed production and is marketed as a functional food. However, the leaves have potential commercial benefit due to their composition (Bushway 1981) and content of active compounds of nutritional value, antioxidants and antimicrobials (Elshafie et al 2018). Peiretti 2010 indicated the possibility of using chia seeds and whole chia plants as feed for animals, including poultry. Dietary proteins, fibers, and bioactive components such as tocopherol and phenolic compounds (Capoitain et al 2012) and that the active compound in chia seeds is the essential oil that consists of ketones, ketones, alpha-a thujone, and beta-thujone-ß. And other effective compounds, such as borneol, cineol, cornsole, fumaric and acids, such as Cornsolic acid, tannic acid, nicotinic acid, and flavonoids, such as: flavones and glycosides, estrogenic compounds, and a group of B vitamins, such as: folic acid, thiamine, pyridoxine and riboflavin. A high source of vitamin. It is a source of vitamin C and a number of important minerals such as iron, potassium, calcium, copper, magnesium, zinc and manganese, as it is possible to use and add chia seeds in feeding poultry because it contains nutrients of high nutritional value and for its vital effects as it is anti-bacterial and anti-fungal in addition to its anti-fungal activities Natural oxidation and its role in improving the health and productivity of poultry (Nijveldt et al 2001). In view of the lack of studies on

the use of chia seeds in the diets of poultry in general, especially broilers in Iraq, the aim of this study is to use several levels of chia seeds in broiler diets and their impact on productive performance.

MATERIAL AND METHODS

The experiment was conducted in the poultry field of the Department of Animal Production, College of Agriculture, University of Baghdad / Abu Ghraib for the period from 6/10/2020 to 11/16/2020 to study the effect of using different levels of chia seeds in broiler diets on production performance *Saliva hispanica*. Using 250 Ross 308 chicks, one-day old, with an average initial weight of 40 g, which were prepared from the hatchery of the Iraqi National Association. The chicks were randomly distributed at the age of one day into five treatments, 50 chicks per treatment. Each treatment included five replicates of 10 chicks for each replicate, and the replicates were distributed homogeneously, with (25) replicates, each replicate equipped with a 3-liter ground plastic manhole and a circular plate for feed during the first week and replace the circular dishes with a cylindrical hanging feeder. it used continuous lighting for 24 hours, The temperature was controlled by using the electric heater to obtain a temperature of 35 degrees Celsius until the end of the first week, and then lowered two degrees Celsius weekly to reach a temperature of 24 degrees Celsius. I underwent a preventive program that provided the sugar solution (50 g sugar / liter of water) for 12 hours as a source of energy and

used. The health preventive program, as the prevailing and approved vaccinations of chickens against Newcastle, Kamburu and IB diseases were conducted. All chicks were fed ad-libitum free feeding during the duration of the experiment, as a starter diet was used for the period from 1-11 days of chick life, followed by the growth ration from 12-22 days, and then followed by the final diet for the period from 23-42 days. Tables show 1,2,3 Percentage of feed ingredients used in the study and their chemical composition. The treatments of the experiment were as follows: -

1. The first treatment T_1: the control treatment without the use of chia seeds.
2. The second treatment T_2: the basic diet with the use of 2% of chia seeds.
3. The third treatment T_3: the basic diet with the use of 4% of chia seeds.
4. Fourth treatment T_4: the basic diet with the use of 6% of chia seeds.

5. Fifth treatment T_5 : The basic diet with the use of 8% of chia seeds.

The diets were prepared by crushing and mixing the components of the feed according to the required proportions, and chia seeds were added according to their proportions in the various treatments' diets. Note that the preparation of rations was done weekly in order to preserve the chia seeds and vitamins from spoilage. As shown in Tables 1, 2, 3, respectively.

Statistical analysis: An experiment was used to study the effect of a single factor that was applied with a complete random design (CRD) to study the effect of treatment and duration on different traits. The significant differences between the means were compared with the Duncan (1955) polynomial test, and SPSS was used in the statistical analysis of the data according to the following mathematical model: $Y_{ij} = \mu + T_i + e_{ij}$

The body was weighed weekly until the sixth week and

Table 1. Percentages and the calculated chemical analysis of the components of the starter diets from 1-11 days used in the study

Components	Starter treatments				
	T_1	T_2	T_3	T_4	T_5
Yellow corn	42	45	47	47	42
Wheat	14.3	10.3	6.7	5.5	10
Soybean meal*	33	33	32.6	32.8	31.3
Chia seeds	-	2	4	6	8
Protein Concentrate**	5	5	5	5	5
fat	3	2	2	1	1
Dicalcium phosphate	0.7	0.7	0.7	0.7	0.7
Limestone	1.2	1.2	1.2	1.2	1.2
Salt	0.3	0.3	0.3	0.3	0.3
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated Chemical Analysis***					
represented energy	3033.71	3016.61	3058.73	3033.37	3066.87
% protein	23.05	23.2	23.11	23.42	23.14
% fat	5.53	5.2	5.82	5.47	6.03
% fiber	2.75	3.37	3.96	4.60	5.23
Methionine + cysteine%	1.13	1.14	1.15	1.17	1.17
% lysine	1.52	1.53	1.53	1.56	1.53
Calcium%	0.98	0.99	1	1.01	1.02
% available phosphorous	0.48	0.50	0.51	0.53	0.54

* The soybean meal used is from an Argentine source, the percentage of crude protein is 48%, and 2440 kcal / kg is a representative energy.** The protein concentrate used is animal produced by a Dutch company (imported) Brocon contains 40% crude protein, 2107 calories / kg protein represented energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 4.68% phosphorous, 3.85% lysine methionine, 4.12%, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine. It contains a mixture of rare vitamins and minerals that provide the bird's need for these elements.*** 1994 NRC Calculation of chemical composition based on*** The total energy of chia seeds is 485 kilocalories/kg represented energy

collectively with an electronic scale sensitive to the nearest gram and according to the average weight through the following law: The sum of the weights of birds (g) on the number of birds. Weight gain (g) equals the weight of the live body at the end of the week (g) minus the weight of the living body at the beginning of the week (g). The average feed consumption was calculated through the equation that. The amount of feed consumed at the end of each week was calculated by the weight of the remaining feed in the feed at the end of the week, and it was subtracted from the feed provided at the beginning of the week, and the feed consumed weekly was extracted. According to the weekly feed conversion ratio until the end of the experiment, As follows: the amount of feed consumed (g) on weight gain (g).

RESULTS AND DISCUSSION

The results in Table (4) indicate the effect of adding different levels of chia seeds to the diet of broilers on the average live body weight (1-6 weeks), where it was noted that there were no significant differences between the experimental treatments in the first week of the birds' age. While there was a significant excelled (P<0.05) for the treatments T_2, T_3 and T_5 in the second week of age compared to the control treatment T_1, as T_5 and added to it (8% of chia seeds) reached the highest average live body weight (728.90 g), while it did not differ. Significantly T_4 with all experiment treatments. As for the third week, it appeared that the two treatments T_2 and T_5 were significantly (P<0.05) excelled on the control treatment T_1 and T_3, and the last two treatments did not differ significantly from T_4, which in turn did not differ significantly from T_2 and T_5. In the fourth week, the significantly excelled (P<0.05) was clear for the birds of treatments T_2 and T_5 compared to treatments T_1, T_4 and T_3 did not differ with all treatments of the experiment. At the fifth week of life, there was a significant (P<0.05) improvement in the body weight for the birds of the two treatments T_2 and T_3 supplemented with (2 and 4% chia seeds) compared to treatment T_1, while they did not differ significantly from T_4 and

Table 2. Percentages and calculated chemical analysis of the components of 12-22-day growth diet used in the study

Components	Growth treatments				
	T_1	T_2	T_3	T_4	T_5
Yellow corn	51	51	48	50.2	50.7
Wheat	10	9	10	9	7
Soybean meal*	28	27.5	27	25.8	25.8
Chia seeds	-	2	4	6	8
Fat	4	3.5	3	2	1.5
Protein Concentrate**	5	5	5	5	5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
Limestone	1.14	1.14	1.14	1.14	1.14
Salt	0.1	0.1	0.1	0.1	0.1
Methionine	0.13	0.13	0.13	0.13	0.13
Lysine	0.13	0.13	0.13	0.13	0.13
Total	100	100	100	100	100
Calculated Chemical Analysis***					
Represented energy (Kcal/kg feed)	3169.05	3177.85	3182.05	3168.97	3175.52
% protein	20.93	20.92	20.98	20.74	20.90
% fat	6.72	6.84	6.94	6.61	6.73
% fiber	2.62	3.23	3.88	4.50	5.12
Methionine + cysteine%	0.96	0.97	0.97	0.98	0.99
% lysine	1.29	1.29	1.29	1.27	1.29
Calcium%	0.84	0.90	0.92	0.93	0.94
% available phosphorous	0.44	0.45	0.97	0.48	0.50

* The soybean meal used is from an Argentine source, the percentage of crude protein is 48% and 2440 Kilo calories / kg represented energy.** The protein concentrate used is animal product from a Dutch company (imported) Brocon contains 40% crude protein, 2107 calories / kg protein represented energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 4.68% phosphorous, 3.85% lysine methionine, 4.12%, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine. It contains a mixture of rare vitamins and minerals that provide the bird's need for these elements.1994 NRC Calculation of chemical composition based on*** The total energy of chia seeds is 485 kilocalories/kg represented energy.

T_5, which were not significantly different from T_1, In the last week of the experiment, the sixth week, a significant increase (P<0.05) in the average body weight of T_3 and T_4 appeared, compared to treatments T_1 and T_2, which differed significantly between them, as T_1 recorded the lowest average live body weight (2632.40 g) and did not differ. T_5 is significant for T_2 as well as for T_3 and T_4.

Table (5) shows a significant improvement (P<0.05) for

Table 3. Percentages and the calculated chemical analysis of the components of the final diets from 23-42 days used in the study

Components	Final treatments				
	T_1	T_2	T_3	T_4	T_5
Yellow corn	54.64	54.14	54.14	53.14	51.14
Wheat	10	10	10	10	10
Soybean meal*	24	23	22	22	22
Chia seeds**	-	2	4	6	8
Fat	4.5	4	3	2	2
Protein Concentrate***	5	5	5	5	5
Diphosphate Calcium	0.4	0.4	0.4	0.4	0.4
Limestone	1.1	1.1	1.1	1.1	1.1
Salt	0.1	0.1	0.1	0.1	0.1
Methionine	0.13	0.13	0.13	0.13	0.13
Lysine	0.13	0.13	0.13	0.13	0.13
Total	100	100	100	100	100
Calculated Chemical Analysis****					
Represented energy (Kcal/kg feed)	3238.39	3249.44	3232.24	3205.94	3236.14
% protein	19.31	19.14	19.01	19.28	19.46
% fat	7.32	7.44	7.09	6.71	7.29
% fiber	2.55	3.17	3.8	4.45	5.07
Methionine + cysteine%	0.91	0.92	0.92	0.94	0.95
% lysine	1.18	1.17	1.16	1.17	1.19
Calcium%	0.84	0.85	0.83	0.88	0.89
% available phosphorous	0.42	0.43	0.44	0.46	0.48

* The soybean meal used is from an Argentine source, the percentage of crude protein is 48%, and 2440 kcal / kg is a representative energy. ** The total energy of chia seeds is 485 kilocalories/kg represented energy.*** The protein concentrate used is animal produced by a Dutch company (imported) Brocon contains 40% crude protein, 2107 calories / kg protein represented energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 4.68% phosphorous, 3.85% lysine Methionine, 4.12% methionine, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine It contains a mixture of rare vitamins and minerals that provide the bird's need of these elements.****NRC 1994

Table 4. Effect of using different levels of Chia seeds on the average live body weight (g) (mean ± standard error) for broilers during the weeks of the experiment

Period (week)	Treatments **					Sg.
	T_1	T_2	T_3	T_4	T_5	
1	130.10±1.83	146.50±2.43	140.10±1.22	139.60±2.57	137.30±2.92	N.S
2	283.20±3.70[b]	713.20±6.44[a]	684.00±24.12[a]	699.40±20.29[ab]	728.90±9.67[a]	*
3	669.00±13.58[b]	713.20±6.44[a]	684.00±24.12[ab]	699.40±20.29[ab]	728.90±9.67[a]	*
4	1211.90±21.09[b]	1268.50±15.44[a]	1232.60±11.93[ab]	1213.70±35.92[b]	1271.20±22.61[a]	*
5	1875.80±26.18[b]	1969.50±21.05[a]	1966.40±28.79[a]	1946.30±38.16[ab]	1928.80±19.51[ab]	*
6	2632.40±43.23[c]	2723.30±65.29[b]	2760.30±52.04[a]	2773.30±35.73 [a]	2744.00±18.37[ab]	*

* The different letters within the same column indicate the presence of significant differences at the level of significance (P<0.05).N.S means that there are no significant differences between the transactions.** Treatments T_1, T_2, T_3, T_4 and T_5 to which chia seeds are added in the following proportions (0, 2, 4, 6, 8%)respectively

treatment T_2 compared to control treatment T_1 and T_5, while treatments T_3 and T_4 did not differ significantly with treatments T_2 and T_5 in the first week of age. In the second week, a significant (P<0.05) was observed for all experimental treatments compared to the control treatment T_1, and a significant (P<0.05) increase in the average of weight gain was observed for birds of the two treatments T_1 and T_5 in the third week of the experiment compared to treatment T_2, T_3, T_4.While no significant differences were recorded during the fourth week of bird age. In the fifth week, it was found that treatments T_3 and T_4 were significantly (P<0.05) excelled compared to treatments T_1, T_2 and T_5. At the sixth and last week of the birds' age, a significant increase (P<0.05) was observed for the birds of the two treatments T_4 and T_5, compared to the treatments T_1 and T_2, while T_3 did not differ significantly with all the treatments of the experiment. As for the total weight gain rate, it was significantly (P<0.05) superior to both T_3 and T_4 compared to treatments T_1 and T_2, and it did not differ significantly from T_5, which in turn did not differ significantly from T_2, as it was noted through the table that T_2 and T_5 differed significantly. Compared to the control

treatment T_1, which recorded the lowest overall increase rate (1-6).

Table (6) indicates that there were no significant differences between the experimental treatments for the first, third and fourth weeks. In the second week, there was a significant (P<0.05) excelled for the birds of the experimental treatments in the average of feed consumption (T_2, T_3, T_4, T_5). At the fifth week of age, there was a significant (P<0.05) increase for the birds of the treatments T_1, T_3 and T_4 in feed consumption compared to the treatment T_5, which recorded the lowest feed consumption but did not differ significantly from T_2, which did not differ significantly from T_1, T_3 and T_4. In the sixth week of the age of the birds, it was found that there was a significant and clear decrease in feed consumption for both birds of T_2 and T_3 compared to the control treatment, which recorded the highest average of feed consumption. T_4 and T_5 did not differ significantly from T_1 and also from T_2 and T_3, As for the percentage of cumulative feed consumption, it was found that the control treatment T1 improved significantly (P<0.05) compared to the two treatments T_2 and T_3, while the two treatments T_4 and T_5 did not differ

Table 5. Effect of using different levels of chia seeds on the average weekly total weight gain (gm) (mean ± standard error) for broilers in the weeks of the experiment

Period (week)	Treatments **					Sg.
	T_1	T_2	T_3	T_4	T_5	
1	88.90±2.21[c]	105.40±2.07[a]	99.30±1.23[ab]	99.60±2.42[ab]	95.60±3.39[bc]	*
2	153.10±3.12[b]	204.80±3.39[a]	203.00±7.77[a]	199.60±4.92[a]	200.10±7.42[a]	*
3	385.80±15.44[a]	361.90±3.56[ab]	340.90±22.38[b]	360.20±14.96[b]	391.50±7.90[a]	*
4	542.90±24.86	555.30±10.10	548.60±14.49	514.30±20.56	542.30±14.84	N.S
5	663.90±25.67[b]	701.00±7.43[ab]	733.80±20.86[a]	732.60±15.75[a]	657.60±13.41[b]	*
6	756.60±23.01[b]	753.80±44.46[b]	793.90±24.31[ab]	827.00±26.46[a]	815.20±15.52[a]	*
6-1	2591.20±43.49[c]	2682.20±65.06[b]	2719.50±52.03[a]	2733.30±35.67[a]	2702.30±18.47[ab]	*

* The different letters within the same column indicate the presence of significant differences at the level of significance (P<0.05).N.S means that there are no significant differences between the transactions.** Treatments T_1, T_2, T_3, T_4 and T_5 to which chia seeds are added in the following proportions (0, 2, 4, 6, 8%), respectively

Table 6. Effect of using different levels of chia seeds on the average weekly and total feed consumption (g) (mean ± standard error) for broilers in the weeks of the experiment

Period (week)	Treatments **					Sg.
	T_1	T_2	T_3	T_4	T_5	
1	106.80±1.29	104.10±0.76	102.50±1.17	111.00±7.07	106.30±1.53	N.S
2	164.90±5.33[b]	229.80±8.16[a]	226.00±8.49[a]	215.20±7.77[a]	221.80±9.21[a]	*
3	541.40±13.23	527.00±9.32	489.80±25.41	500.70±15.51	522.60±11.12	N.S
4	832.02±19.94	857.30±11.83	791.60±36.21	808.70±22.82	859.90±13.82	N.S
5	1163.20±12.18 [a]	1095.20±26.27[ab]	1115.04±32.17[a]	1131.56±25.99[a]	1042.64±10.92[b]	*
6	1491.10±27.53 [a]	1276.10±73.70[b]	1318.82±38.65[b]	1400.14±44.49[ab]	1381.30±4.10[ab]	*
6-1	4299.42±36.17 [a]	4089.50±102.79[b]	4043.76±60.49[b]	4167.30±57.22[ab]	4134.54±19.05[ab]	*

significantly with the rest of the other experimental treatments. The data in Table (7) indicates a significant improvement (P<0.05) in the feed conversion ratio for the two treatments T_2 and T_3 compared to treatment T_1, but it did not differ significantly from T_4 and T_5 and all treatments did not differ significantly from T_1 during the first week of the experiment, and the results indicated To the absence of any sperm differences in the second week. In the third week, a significant (P<0.05) improvement was observed for treatment T_5 compared to treatment T_2, as T_5 did not differ significantly (P<0.05) from T_1, T_3, and T_4, and T_2 did not differ (P<0.05) from T_1 and T_4. T_3 and T_4, and both treatment T_1, T_4 and T_5 recorded a significant (P<0.05) improvement in the fourth week of the experiment compared to treatment T_3, which did not differ significantly from T_2 and this treatment also differed from T_1 and T_4 and T_5

It was noted in Table (7) that there was a significant improvement (P<0.05) in the feed conversion ratio of the experimental treatments in which Chia seeds were used in different proportions in the fifth and sixth weeks of the experiment compared to the control treatment T_1, Which recorded the highest food conversion ratio during these two weeks, and this professional improvement (P<0.05) was reflected for the experimental treatments (T_2, T_3, T_4, T_5) on the total food conversion ratio compared to the control treatment. The productive performance of broilers is affected by the level and type of nutrients included in the composition of the diet, in addition to other factors related to bird breeding and management, so the most important and influential factor in the productive performance of broilers for this.

The reason for the improvement in the average body weight and weight gain may be due to the amino acid template of chia seeds, which contains a high percentage of amino acids of high biological value (De Falco et al 2018).These acids belong to the main component of proteins that contribute to increasing the building of physical muscles

and increasing the rate of body weight and weight gain, including cinnamic acid, which improves and supports the mucous membrane of the small intestine and improves the digestion and absorption of the essential amino acids found in weighty chia seeds (Kennedy et al 2010).The significantly excelled in the productive performance of chia seeds with a high content may be due to the oil-rich in unsaturated essential fatty acids of the omega 3 and 6 and saturated essential oils. Oils rich in sources, including valenolic acid, with the highest percentage of fatty acids in chia seed oil (Amato et al 2015) have a significant role in weight gain by increasing the efficiency of digestion and absorption in the intestines and then benefiting from food. The improvement in production performance may be due to the fact that chia seeds contain effective compounds and high percentages such as polyphenols, is flavones and tocopherols, which are natural antioxidants that increase the effectiveness of antioxidant enzymes such as glutathione peroxidase and catalase. Protein digestion and thus leads to an increase in body weight and an improvement in the productive performance of birds (Azhar et al 2012) and that these active compounds have a role in improving the secretions of the gastrointestinal tract, reducing the number of pathogenic bacteria, and increasing the number of beneficial bacteria in the intestine, which was observed in this study in the form (1), which improves surfaces, absorption and utilization of nutrients inside the intestine, which is reflected in strengthening immunity and improving the condition Health for the bird and thus increase the body rate, weight gain, and food conversion ratio , This may also be the reason for the significant sensitivity in the net percentage in the chia seed treatments, where the improvement of the health of the intestines and the increase in the absorption efficiency of the biomolecules increases the clearance rate of the carcass due to its association with body weight and the improvement of the characteristics of the carcass (Salazar et al 2009). We

Table 7. Effect of using different levels of chia seeds on the average weekly and total food conversion ratio (g of feed/g of weight gain) (mean ± standard error) for broilers in the weeks of the experiment

Period (week)	Treatments **					Sg.
	T_1	T_2	T_3	T_4	T_5	
1	1.20±0.03 [a]	0.99±0.02 [b]	1.03±0.01 [b]	1.12±0.09 [ab]	1.12±0.03 [ab]	*
2	1.08±0.01	1.12±0.03	1.12±0.03	1.08±0.02	1.11±0.03	N.S
3	1.41±0.03 [ab]	1.46±0.03 [a]	1.44±0.03 [ab]	1.39±0.03 [ab]	1.34±0.04 [b]	*
4	1.34±0.04 [b]	1.54±0.02 [ab]	1.44±0.05 [a]	1.58±0.03 [b]	1.59±0.03 [b]	*
5	1.76±0.08 [a]	1.56±0.02 [b]	1.52±0.01 [b]	1.54±0.01 [b]	1.59±0.02 [b]	*
6	1.98±0.06 [a]	1.69±0.01 [b]	1.66±0.02 [b]	1.69±0.01 [b]	1.70±0.03 [b]	*
6-1	1.66±0.03 [a]	1.52±0.01 [b]	1.49±0.01 [b]	1.52±0.02 [b]	1.53±0.01 [b]	*

conclude from this study that the use of chia seeds in broiler diets has a positive effect in improving production characteristics and raising production efficiency.

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Comparative in Vitro Study of Antibacterial Activity between Colicin E1 or Enterocin A1 with Classical Antibiotics against Non-O157 Shiga Toxin-Producing *Escherichia coli*

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Abstract: The present study was designed to investigate infection by non-O157 Shiga toxin-producing *Escherichia coli* (STEC), which is considered highly potentially pathogenic for humans and animals. The samples were obtained from children under five years old with severe diarrhea and from dogs suffering from hemorrhagic diarrhea. Isolation and detection of bacteria from diarrheal samples were carried out using traditional and selective media, followed by biochemical tests to confirm the diagnosis of non-O157 STEC. In vitro study including minimum inhibitory concentration (MIC) was done to compare effect of colicin E1 as an antibacterial agent with most effective antibiotics by measuring the zone of inhibition on agar and showed that colicin E1 effect demonstrated the highest activity in inhibition of non-O157 STEC growth than enterocine A1 and enrofloxacin.

Keywords: Shiga toxin-producing, *Escherichia coli*, Bacteriocins, Colicin E1, Enterocine A1

The most common STEC serotypes causing infection in humans were *E. coli* O157:H7 (STEC O157), but many non-O157 STEC serotypes have been associated with serious illness and major outbreaks (Rounds et al 2012). At least 150 non-O157 *E. coli* strains were known to cause infection and multiple incidents, which have been emerged as major agent of food-borne infection. *E. coli* O157:H7 became nationally relevant in 1994, while non-O157 STEC infections were not reported until 2000 (Wasilenko et al 2012, Jandhyala et al 2013, Wasilenko et al 2014, Beutin et al 2015). These six non-O157 serogroups were still the most common types nationally, according to the Centers for Disease Control and Prevention (CDC) (Blankenship et al 2021). The human and animal strains of STEC with the same serotype are identical in relation to the presence of recognized virulence-associated factors (Mannan et al 2021). STEC was differentiated from non-pathogenic *E. coli* strains isolated from the intestinal flora of healthy humans and animals by developing one or more variants of Shiga toxin (*Stx*), encoded by the *stx1* and *stx2* genes. The presence of *stx2* was already associated with more serious illnesses. In addition to the development of *Stx*, STEC had other potential virulence factors, such as intimin, an outer membrane protein encoded by the *eae* gene that facilitates intense adhesion to intestinal epithelial cells (Oporto et al 2019). Diarrheal diseases are a major problem in third-world countries and are responsible for the deaths of millions of people and animals

each year. It can be either acute or chronic (Alaa and Ikram 2019). Probiotics were widely considered to be the microorganisms that promote health, as established by the World Gastroenterology Organization (WGO). Probiotics were described by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as "live microorganisms that provide a health benefit to the host when administered in sufficient quantities" (Hill et al 2014). The functionality of probiotics can be mediated by bacteriocins through distinct mechanisms. By competitive inhibition of resident microbiota, bacteriocins may facilitate the colonization of the host. Pathogens may also be specifically inhibited by them. Finally, bacteriocins may act as signaling peptides, either by quorum sensing host immune system cells to signal other bacteria and thus modulating the host immune system (Egan et al 2017). Bacteriocins are bacterially produced peptides that are active against other bacteria and against which the producer has a specific immunity mechanism (Kumariya et al 2019, Hernández-González et al 2021). For pathogens, bacteriocins can be highly selective and used for their control. Bacteriocins influence various basic functions of the living cells in a wide variety of their chemical structures (transcription, translation, replication, and cell wall biosynthesis). Most of them act by forming membrane channels or pores that impair the energy potential of sensitive cells (Oscáriz and Pisabarro 2001).

There are few differences between bacteriocins and

antibiotics in terms of host cell immunity, mechanism of target cell resistance or tolerance, mode of action, toxicity, and side effect mechanism. Colicins are known to be a family of bacterial antibiotic proteins developed by and active against *Escherichia coli* and closely related bacterial species to provide a competitive advantage over competitors in the same ecological niche. Numerous strains of the *Enterobacteriaceae* family, including *Escherichia* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella*, and *Proteus* species, have subsequently been found to produce several forms of colicin. Colicin activity was seen to be dependent on binding to a particular receptor on the surface of the susceptible strains (Yang et al., 2014). Enterocins are actually bacteriocins formed by several members of the genus *Enterococcus*. Enterocins have become desirable as natural food preservation additives in recent years. They present broad-spectrum activity against many of the gram-positive bacteria and foodborne pathogens (Almeida et al 2011, Salvucci et al 2012). Enterocins have the cytoplasmic membrane as their primary target, as most bacteriocins do. They form pores, thereby depleting the transmembrane potential and/or the pH gradient and leading to the leakage of important intracellular molecules (Johnson et al 2018).

MATERIAL AND METHODS

Study samples: Totally, 50 stool sample from children under 5years old of both genders suffering from severe diarrhea. They were collected from various private clinics for children in Baghdad. Additionally, 50 fecal samples were taken from dogs, male and female, of different ages and breeds suffering from severe diarrhea (antibiotics were not previously administered yet) and collected via recto-anal mucosal swabs from different veterinary clinics.

Diagnostic assays: Commercially available media and biochemical tests have been used for the isolation and identification of non-O157 STEC EMB, SMAC, and CHROM O157 agar. The determination of the minimum inhibitory concentration (MIC) was done according to (Babu et al., 2011). First preparation of bacterial culture by inoculated into Tryptone soy broth, and incubated 24hours at 37°C, then the inoculum streaked onto Tryptone soy agar plate and incubated overnight. The inoculums were prepared throughout by removing 3–5 colonies (of similar appearance) from the culture plate with a sterile loop and transferring them to a saline tube. Then, the concentration of bacteria was measured to be 1.5×10^8 CUF/ml by comparing the tube with the turbidity standard of 0.5 McFarland and adjusting the density of the test suspension to that of the standard by adding more bacteria or more sterile saline. Muller Hinton agar plates were inoculated by dipping a sterile swab into the

inoculum, removed excess inoculum by press and rotate the swab firmly against the side of the tube above the level of the liquid, the swab was streaked all over the surface of the medium. The inoculum was left to dry for a few minutes at room temperature with the lid closed, wells were punched in the Muller Hinton agar plates and each well filled with 20µl of different concentration of colicin (5000, 2500, 1250, 625, 312.5, 156.25, 78.125 µg/ml), enterocin A1 (5000, 2500, 1250, 625, 312.5, 156.25 µg/ml), and enrofloxacin (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.0975, 0.0487, 0.0343, 0.0121 µg/ml respectively), The well containing distilled water alone set as a control. The plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones around the wells displaying inhibitory activity were measured using a ruler in millimeter units to obtain the results.

Statistical analysis: To determine the effect of different factors on the research parameters, the Statistical Analysis System- SAS (2012) program was utilized.

RESULTS AND DISCUSSION

Bacterial isolates were identified by modified and selective media to isolate serotype pathogens, allow the appearance of these bacteria, reduce the performance of other microbes, and supply more positive samples. The percentage of *E. coli* non-O157 isolates by using culture methods and biochemical tests. Non-O157 gave bluish-green color colonies, while O157 STEC gave dark purple to magenta color colonies in this media, Fig (Table 1, Fig. 1). Results showed the importance of CHROM agar media in the diagnosis of *E. coli* O157STEC 33.33% and other non-O157 STEC 33.33%. Chromogenic agar has a sensitivity of greater than 85%. It is less costly than other methods and can be easily substituted into a laboratory's by replacing the current O157-specific plates. However, chromogenic agar can be inhibitory to some STEC serotypes (Chui et al., 2018). The *E. coli* O157: H7 have been distinguished from *E. coli* non-O157 with substrates particularly recognized with β-D-galactosidase as well as β-D-glucuronidase. All *E. coli* strains produced B-D-galactosidase, while β-D- glucuronidase was almost produced by all other *E. coli* strains except for STEC O157:H7 (Hussein et al 2008).

The current study found a low prevalence of non-O157 STEC in dogs (6%) in Baghdad city. The prevalence of non-O157 STEC in children in Baghdad revealed positive results (2%) of children as non-O157 STEC, but we isolated 3 positive samples of STEC in children under 5 years with watery and not bloody diarrhea. The current study showed low prevalence of non-O157 STEC in dogs. Priya et al (2017) also observed that hemorrhagic gastroenteritis (HGE) varies

from mild to extreme forms and is generally encountered in puppies (6.4%) that were positive for *Escherichia coli* shiga toxin. Other epidemiological studies have shown that newborn puppies have a higher incidence of infections. This feature do not agree with what (Vally et al 2012) where record 0.07 over 100000 people and 0.49 over 100000 children under 5 years that were positive to STEC in HUS. This disagreement may be due to the affinity of *E.coli* coli for intestinal tissues rather than urinary tissues. Particularly immunocompromised children are increasingly exposed to foodborne pathogens such as diarrheagenic *E. coli* (Falup-Pecurariu et al 2019).

The various concentrations of colicin E1 and the mean inhibition zone obtained from each concentration stock solution of colicin E1 gave 18.15 mm. The concentration was halved and decreased in each of the subsequent to seventh concentrations, (Table 2). The seventh concentration resemble the MIC of colicin E1, which significantly differ from concentration the sixth . The seventh concentration was chosen as the MIC of colicin E1 because it represents the minimum concentration that inhibits *E. coli* non-O157 STEC in vitro (Fig. 2).

The different concentration, gave an inhibitory zone of 8.65 mm and ended with the fifth concentration of 156.25 µg/ml that gave 3.15 mm inhibition zone (Table 3), which present the MIC because to its minimum inhibition potential against *E.coli* non-O157 STEC and significant difference from the third concentration (Fig. 3). The MIC for enrofloxacin begin with stock solution, end with the MIC, which was at the concentration of 0.195µg/ml and represent an inhibitory zone of 3.15 mm (Table 4, Fig. 4). This concentration had the sequence as the ninth concentration and has significant
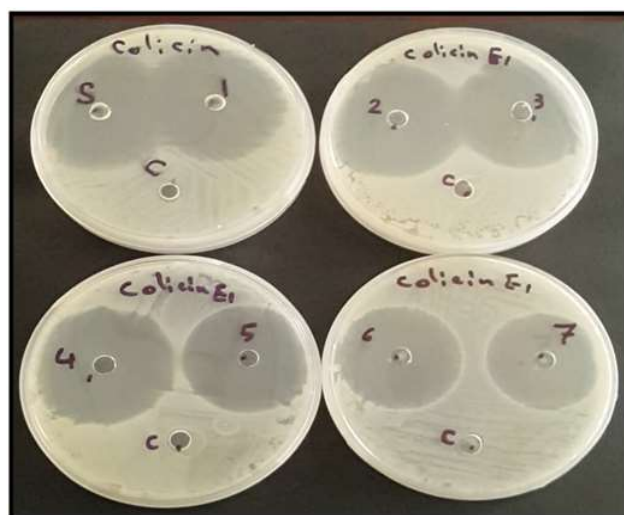
Fig. 2. Zone of inhibition of colicin E1 against non-O157

Fig. 3. Zone of inhibition of Enterocin A1 against non-O157 STEC

Table 2. In vitro antimicrobial resistance and minimum inhibitory concentration (MIC) for colicin E1 in different concentration on non-O157 STEC

No. of dilution	Concentration (µg/ml)	Zone of inhibition (mm)
Stock	5000	18.15 ±0.15 [A]
1	2500	17.65 ±0.12 [B]
2	1250	17.15 ±0.12 [B]
3	625	16.65 ±0.09 [C]
4	312.5	16.15 ±0.11 [C]
5	156.25	15.15 ±0.10 [D]
6	78.125	14.15 ±0.08 [E]
7	39.0625	13.15 ±0.07 [F]
--	Control (D.W)	0.00 ±0.00 [G]

Means having with the different letters in same column differed significantly *(P≤0.05)
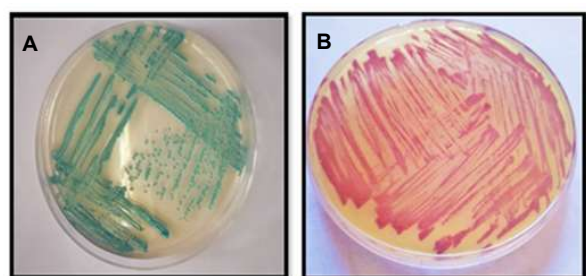
Fig. 1. *STEC* on CHROM O157 agar
(A): Non-O157, (B): O157:H7

Table 1. Percentage of non-O157 isolates by using culture methods and biochemical tests

Source of sample	Total NO.	EMB agar		SMAC agar		CHROM agar O157		Total
		No	(%)	No	(%)	No	(%)	(%)
Children samples	50	11	22	3	27.27	1	33.33	2.00
Animal samples	50	19	38	9	47.36	3	33.33	6.00

Table 3. In vitro antimicrobial resistance and minimum inhibitory concentration (MIC) for enterocin E1in different concentration on non-O157 STEC

No. of dilution	Concentration (µg/ml)	Zone of inhibition (mm)
Stock	5000	8.65 ±0.07 A
1	2500	7.15 ±0.06 B
2	1250	6.65 ±0.06BC
3	625	6.15 ±0.06 C
4	312.5	3.65 ±0.04 D
5	156.25	3.15 ±0.04D
--	Control (D.W)	0.00 ±0.00E

Means having with the different letters in same column differed significantly
* (P≤0.05)

Table 4. In vitro antimicrobial resistance and minimum inhibitory concentration (MIC) for enrofloxacin in different concentration on non-O157 STEC

No. of dilution	Concentration (µg/ml)	Zone of inhibition (mm)
Stock	100	16.15 ±0.11 A
1	50	15.65 ±0.11 A
2	25	13.65 ±0.09 B
3	12.5	12.15 ±0.10 C
4	6.25	10.65 ±0.09 D
5	3.125	8.15 ±0.08 E
6	1.56	7.65 ±0.08 E
7	0.78	5.65 ±0.06 F
8	0.39	4.15 ±0.05 G
9	0.195	3.15 ±0.05 H
--	Control (D.W)	0.00 ±0.00 I

Means having with the different letters in same column differed significantly
* (P≤0.05)

Fig. 4. Zone of inhibition of enrofloxacin against non-O157 STEC

differences from the eighth concentration (P≤0.05).

Bacteriocins may be used as medicinal or preventive supplements that control pathogens because they are harmless compounds. The bacteriocins used in our invitro study show clear action, represented by their wide inhibitory zone. This result is in high agreement with that of (Fathizadeh et al., 2020), who found that enterocin A and colicin E1 had a lot of antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *E. faecalis*. All of these results point to the possibility that enterocin A-colicin E1 could open the way for the potential production of an effective antibacterial agent. The results of the current study's minimum inhibitory concentration revealed significant variations between the bacteriocins and enrofloxacin. The mode of action and chemical structure against non-O157 STEC may be the cause of these differences in levels. When compared to bacteriocins, enrofloxacin had the lowest MIC for non-O157 STEC cultures. Similar, trend was observed by an agreed-upon result generally with observation of Hassan (2014). Gharban and Yousif (2020) also observed the high ability of colicin E1 to limit wide range of gram negative and gram positive pathogens. This fact may be related to colicins ability in foods and agriculture to prevent sensitive diarrheagenic *E. coli* strains (Askria and Ghanbarpour 2019). Colicins are bacteriocins developed by *E. coli* that destroy non-host *E. coli* strains mostly by forming pores within the inner membrane, preventing cell wall synthesis, and even degrading nucleic acids (Jin et al 2018). In general, bacteriocins are also multifunctional, ribosomally formed proteinaceous substances with strong antimicrobial activity in certain concentrations (Negash and Tsehai 2020).

CONCLUSIONS

In this study, the evolution and ecology of bacteriocins have resulted in the identification of a promising alternative to classical antibiotic use. The presence of a unique pattern or common patterns of colicin sensitivity in strains within a diarrheal *E. coli* clone may aid in devising subtyping schemes useful in the epidemiological tracking of outbreaks and sporadic cases of diarrheal disease. Sensitivity to colicins may also be a useful tool for biological control of pathogenic *E. coli* strains. Bacteriocin can solve some of the most challenging problems of multidrug-resistant pathogens. We recommend confirming health benefits in humans and animals regarding a certain amount of probiotics needed to minimize the clinical effects.

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Evaluating the Performance Efficiency of Abu Ghraib Dairy Plant According to Production Energy and Production Criteria

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Abstract: The study aimed to evaluate the performance efficiency of Abu Ghraib factory according to the standards of production capacity and production standards (2016-2018) in order to identify the most important obstacles that the industry suffers from, in addition to detecting the deviations that occurs, in order to find the solutions for those obstacles. The results revealed that plant dairy suffers due to lack of government support, production and a weak administration in exploiting its available resources, in addition to the existence of job slack that increases the production costs. The study recommended that it is necessary to provide the government support, as well as the use of administrative cadres that have the ability to manage the project more efficiently with the development of training programs that work to raise the efficiency of workers.

Keywords: Production costs, Production capacity, Dairy plant

The dairy industry is one of the important industries that will push the wheel of development towards progress and is one of the important transformative industries, and that interest in this industry leads to the development of both the agricultural and industrial sectors, providing food security to the country and dispensing with importing many products from foreign markets. One of the most important and largest dairy factories in Iraq is the Abu Ghraib dairy factory affiliated to the General Company for Food Products, and despite the importance of this factory in achieving economic development and meeting consumers 'needs, it has faced many difficulties and obstacles because of the exceptional circumstances that the country is going through. Al-Dulaimi and Saleem (2019) conducted a study during the period 2010-2016 and identified many obstacles and problems facing the industry represented by the scarcity of capital required for the development process for this industry and company's economic indicators showed during the study the rates of utilization, operation, implementation and exploitation were low for most. Al-Kuraiti and Al-Fatlawi (2019) conducted an economic study to evaluate the performance of the Al-Ali field for the production and breeding of chicken meat in Karbala Governorate during the period 2013-2017. In the field observed that the percentage of dead birds was between 22-30%. This study was aimed to evaluate the performance efficiency of the Abu Ghraib dairy plant, in order to identify performance and to identify the weaknesses and strengths points.

MATERIAL AND METHODS

Research hypothesis: The Abi Gharib factory of the General Company for Food Products suffers from a decrease in the level of performance efficiency and that the process of evaluating the efficiency of performance through which it is possible to identify and detect the deviations and the causes that led to those deviations in order to be able to identify the weaknesses and deviations and strengthen the strength points.

Research limits: This includes spatial boundaries of the Abu Ghraib dairy factory in Baghdad governorate and duration of the study period (2016-2018).

Research methodology: To fulfill the research requirements, the study focused on two main approaches:

Descriptive approach: It was involved in the study by reviewing the literature that dealt with the evaluation process as well as the dairy industry through books, magazines and letters.

Analytical approach: The analysis was based on the data and information obtained by the Abu Ghraib dairy Lab.

Concept of performance efficiency assessment (theoretical framework): The evaluation process is carried out by the organization for the purpose of comparing the target performance with the actual performance, and then the organization works to identify the weaknesses and strengths in performance and their reasons in order to ascertain the amount of the performance contribution to ensuring the continuity of the organization (Abu Qahf 2002). The evaluation and analysis of the performance of workers in their

work and observation of their behavior during work, in order to judge the extent of their success and efficiency in their current work, as well as to judge the possibility of growth and progress of the individual in the future, and his ability to assume greater responsibilities or promoting him to a higher position or another Abdel-Baqi 2002.

RESULTS AND DISCUSSION

Economic analysis: Results revealed that the factory is producing weak actual production compared to the planned production, and that the weakness of this production is due to many factors, which are the lack of government support for the plant, the weakness of management in exploiting its available resources, as well as the high prices of raw materials used in the production process (Table 1). Despite the decrease in the design capacity during the study period there was increase in the actual capacity during the same period (Table 2). The utilization of the design capacity was weak due to the weakness of the actual production capacity. The operating ratio, it is low during the study period due to the decrease in the planned energy compared to the designed capacity. The implementation rate was acceptable to medium, because the actual capacity has reached half of

what the planned capacity has reached. The utilization rate was good during the study period because the available energy is good compared to the design energy.

The productivity of the worker was in a state of fluctuation during the study period due to the fluctuation of the number of workers during the same period (Table 3). The capital was low during the study period because the actual production was low compared to the invested capital and wage productivity during the study period was less productive because the total salaries and wages were much higher than the actual production. The price of raw materials was higher than the productivity of capital and wage productivity, but it was also low because most of the plant's dependence on imported raw materials, which led to higher cost.

The total productivity of the dairy plant was very low and that its highest value was in 2018 (Table 4). This low productivity can be due to production factors which is much higher than the value of actual production. the rate of utilization of the design energy during the study period was at 14.64, 19.31 and 25.90% respectively, because the actual capacity of the plant was much less than the design capacity and this indicates the weakness of the dairy plant management in the production process. In addition to the low

Table 1. Total production capacities of the dairy plant in Abu Ghraib (2016-2018) (one thousand IQD)

Years	Designed energy	Available energy	Planned energy	Actual energy at actual prices
2016	51380600	38356000	14943500	7525837
2017	46493500	35541200	15774000	8979256
2018	36402750	27930700	14440250	9430237

Source: The State Company for Food Products, Abu Ghraib Dairy Factory, Planning Department

Table 2. Production capacities and the rates of utilization, operation, implementation and exploitation (2016-2018) (one thousand IQD)

Years	Designed energy	Available energy	Planned energy	Actual energy	Utilization %	Operation %	Implementation %	Exploitation %
2016	51380600	38356000	14943500	7525837	14.64	29.083	50.36	74.65
2017	46493500	35541200	15774000	8979256	19.31	33.92	56.92	76.44
2018	27930700	36402750	14440250	9430237	25.90	39.66	65.30	76.72

Column 1, 2, 3 and 4 reference: The General Company for Food Products, Abu Ghraib Dairy Factory, Planning Department. Column 5, 6, 7 and 8 was calculated by the researcher

Table 3. Total production values and their indicators of the Abu Ghraib Dairy (2016-2018) (IQD)

Year	Actual production	Total number of employees	Total invested capital	Total salaries and wages	Value of raw materials	Worker productivity	Capital productivity	Wage productivity	Productivity of raw materials
2016	7525837	2197	6729386	13009051	370732.5	3425.50	1.118	0.578	20.29
2017	8979256	1897	7521347	11916295	363553	4733.39	1.193	0.753	24.69
2018	9430237	2561	7521347	11245867	347827	3682.24	1.253	0.838	27.11

Column 1, 2, 3, 4 and 5, reference: The General Company for Food Products, Abu Ghraib Dairy Factory, Finance Department. Column 6, 7, 8 and 9 was calculated by the researcher

Table 4. Total production values of Abu Ghraib Dairy (2016-2018) (IQD)

Year	Total actual production	Value of production factors	Total production
2016	7525837	20111366.5	0.37
2017	8979256	19803092	0.45
2018	9430237	19117602	0.49

Column 1 reference: The General Company for Food Products, Abu Ghraib Dairy Factory, Planning Department. Column 3 was calculated by the researcher

employment rate during the study period, reaching 29.083, 33.92 and 39.66%, respectively, because the planning production capacity is less than the design production capacity, and this indicates the weakness of the dairy plant management in the extent of its use of the design energy.

The implementation rate during the study period was acceptable to medium at the level of production desired by the plant administration, as it achieved 50.36, 56.92 and 65.30%, respectively. The actual production during the school years was on the rise, but the production value was not at the high level. The low exploitation of the working and administrative staff was due to the redundant labor and the decrease in the productivity investigations, as well as the low use of raw materials in the factory due to the high value compared to the low actual production. The decrease in the utilization of human resources was during the study period. Wage productivity has also declined, in addition to the decline in capital productivity during the school years due to its high cost compared to the low actual production. The decline in the value of total production during the study period due to the high value of production factors compared to the total value of the actual low production.

CONCLUSIONS

The factory suffers from a weakness in the actual production, in addition to the weakness of the administration in exploiting the available resources. The factory suffers from functional slack, which led to a significant increase in costs and the inability of the dairy plant to achieve profits. The study recommended that it is necessary to provide the government support to by providing funds and monitoring the disbursement of those funds for achieving the production goals. Additionally, it is important to optimize the employment slack in the factory for the purpose of increasing production by opening new production lines and reopening production lines that were suspended due to faults that occurred previously in equipment and machines, and developing training programs that work to increase the productive efficiency of the worker with the purchase of modern equipment and machines.

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Fortification of Meat Burger with Protein Isolate Extracted from Local Pumpkin Seeds

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Abstract: Protein isolate was achieved from local peeled non soaked pumpkins seeds by using petroleum ether with protein percentage of 53.15%. Protein isolate was used in manufacturing meat burger with two substitution10 and 20%. The shrinkage percentage for burger diameter was decreased from 25.5 to 16.6%, with 10% substitution with water holding capacity (WHC) of 54.52%. Sensitive evaluation for these samples showed that the burger with 10% substitution was similar to the control.

Keywords: Pumpkin seeds, Protein isolate, Substituted burger

Pumpkins belong to the genus Cucurbita, from Cucurbitaceae family, which consists of about 130 genera and 800 species. The popularity of pumpkin in many systems of traditional medication has led researchers turning their attention to this crop (Aktaş et al 2018). Bioactive compounds in pumpkin seeds exhibit promising activities such as anthelmintic, antidiabetic, antidepressant, antioxidant, antitumor and cytoprotective (Dotto and Chacha 2020). Pumpkin seeds are a rich source of iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorous, unsaturated fatty acids, tocopherols and carotenoids (Syed *etal* 2019). There are several types of pumpkin grown around the world such as *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita muschata*, is used as food in the preparation of jam and candy or as fodder (Lovatto et al 2020, Mishra et al 2019).

Pumpkin has attracted increasing attention from scientists due to its nutritional profile (Batool et al 2022). Pumpkin seed protein isolate is rich in antioxidants and effective in reducing the harmful effects of malnutrition**.** Many plants nutrients have many effects on several chronic and deadly diseases (Rasheed et al 2022). The use of plant proteins, especially from grains and oilseeds, has increased dramatically over the past decades as they have been used as a substitute for animal proteins in human nutrition, functional factors, and bioactive ingredients in food, cosmetics and pharmaceutical products. Usually plant proteins are used as a protein isolate or protein concentrate, but their use may be limited due to some inappropriate functional properties such as low solubility, and to expand their field of use there are a number of alternative techniques to improve the functional properties of proteins by increasing the nutritional value and bioactivity. Plant proteins can be modified using chemicals, enzymatic modifications, or physical treatments using high temperature, pressure and ultrasound (Živanović et al 2011, Ozuna and León-Galván 2017). The objective of this study was to find the best substitution percentage of pumpkin seeds protein isolate in meat burger in order to reduce costs.

MATERIAL AND METHODS

Thelocal pumpkin seeds were used that were obtained from local markets. Pumpkin seeds were cleaned, washed with distilled water, and dried with air at room temperature, and a certain amount of seeds were soaked with 1/5 distilled water (seeds /water) for 8 hours, and then they were also dried with air at room temperature (Int.Net4). Then was ground with a laboratory mill, sieved with a 200-mesh sieve, then preserved in polyethylene bags at the refrigerator temperature until use.

Preparation of defatted pumpkin seed powder: The powder was prepared according to Rezig et al (2013) by treating ground pumpkin seed powder soaked, not soaked, and peeled (pulp only) with two types of solvents separately, Petroleum ether 40-60°C and a mixture of chloroform / methanol in a ratio (3: 1 volume /volume). The ratio of the seed powder / solvent used was 1:10 (weight/volume) with continuous stirring by the magnetic stirrer for a period of 24 hours, then the solvent was separated using a central centrifuge and the sediment was taken and dried at room temperature, and then stored in an airtight bottle at the refrigerator temperature until usage.

A protein measurement test was performed for all the defatted samples (soaked, not soaked, peeled * pulp only *) in order to select the best solvent and form for use later in the study, using the standard Microkjeldal method (AOAC 2008) and the protein percentage was extracted by multiplying the percentage of nitrogen in the sample by a conversion factor of 6.25.

Preparation of protein isolate: The protein isolate of defatted pumpkin seed powder (peeled) was prepared according to the method mentioned by Rezig et al (2013), and stored until use.

Manufacture of burger: Meat burger was made according to the method described by Abdul Rahim (2010), using pure ground beef 85%, 1.5, 2, (2-5)% salt, spices (black pepper, crushed garlic) and filler, and cold water for the kneading, and the substitution was (0%, 10%, 20%) with protein isolate, which are named (A, B, and C) respectively, mixed well and formed with a weight of 100 g per each, kept in the refrigerator for 4 hours, until it was grilled and had some physical tests done.

Physical Characteristics of Burger during the Manufacturing Stages

Measurement of diameter reduction: The aforementioned method was adopted by Dosh et al (2016) in measuring the percentage of shrinkage by diameter, and diameter was measured using vernier and in three specific locations in the Barker model with four replications per treatment, and the percentage of shrinkage per diameter was calculated:

$$\text{Diameter reduction (\%)} = \frac{R1 - R2}{R1} \times 100$$

R_1, R_2 = Diameter of burger sample before and after cooking.

Water Holding Capacity WHC: The water holding capacity of the protein isolate before adding it to the burger was estimated according to the method described by Dosh et al (2016) with some modification, as one mg of the sample was placed in a pre-weighed 10 ml test tube, then 1 ml of distilled water was added and stirred by a vortex magnetic stirrer for 10 minutes at room temperature. The centrifugation was carried out at a speed of 5000g for 10 minutes at room temperature, and the holding capacity of water was calculated:

$$\text{WHC} = \frac{W1 - W2}{W0}$$

W_0 = Dry sample weight.

W_1 = Weight of the tube + weight of the precipitate after adding the water.

W_2 = Weigh the tube + the dry sample before adding water.

The water holding capacity of the burger sample (A, B, C) was estimated according to the method mentioned by Dosh *etal* (2016), as mentioned above.

Sensory evaluation: The sensory evaluation of the burger sample was carried out by 10 people specialized in food processing. The method mentioned by Warner et al (2021) in estimating the degrees of perceptual appreciation of the product was followed after grilling process. The characteristics included color, flavor, juiciness, freshness and the degree of general acceptance (Table 1).

Statistical analysis: Statistical Analysis System -SAS (2012) was used in analyzing the data.

RESULTS AND DISCUSSION

Defatted pumpkin seed powder: The percentage of protein in defatted pumpkin seeds when using petroleum ether solvent was 37.12 and 34.12%, respectively in unsoaked and soaked seeds. The protein ratio chloroform/methanol mixture (1:3 volume/ volume was 33.25 and 32.15% respectively. In peeled defatted not soaked seeds using petroleum ether outperformed the rest of the test sample in terms of protein content, (53.15%) and on this basis this type of seed was used in the preparation of protein isolate later (Table 2). Apostol et al (2018) indicated that the percentage of protein in defatted peeled pumpkin seeds (*Cucurbita maxima*) using petroleum ether was 42.75%.

Preparation of protein isolate: In peeled pumpkin seeds after defatted, every 100 grams of dried peeled seeds gives approximately 59 grams dry weight of defatted seed powder,(Fig1) and this weight of the powder when used to prepare the protein isolate gives approximately (18 grams dry weight, 63.87 percent)

Physical Tests of the substituted Burger

Water holding capacity (WHC) of protein isolate: The water holdingcapacity of the protein isolate was 4 mg/ml. Muhamyankaka et al (2013) indicated that the water absorption capacity of the defatted pumpkin seeds of *Cucurbita moschata* was 1.72 mg/ml, while the water holding capacity of the decomposers increased and this may be due to polar groups such as –COOH and –NH2 increased during enzymatic hydrolysis. Among the four hydrolysates used, protamex hydrolysates had the highest water preservation capacity (2.24 mg/ml). In alcalase hydrolysates, there were less water preserving, (1.68 mg/ml). This is due to the difference in the water holdingcapacity of the proteinisolated from the defatted seeds, as the proteinisolate increases the protein concentration, as well as to the difference in the variety used. Lovatto et al (2020) mentioned that water holdingcapacity of defatted pumpkin seeds was 1.99 g water/g product.

Diameter and water holding capacity (WHC) of the burger after grilling: The diameter of the burger after grilling in control sample (A) was decreased (25.5%), while the

decrease in the diameter for B, C samples was 10 and 20% (Table 3) when meat was substituted with protein isolate 16.6%. The highest water carrying capacity of sample B, was 54.52% compared to the control sample (A) and sample (C), which was 28.0 and 23.2%, respectively. The increase in water absorption capacity depends on the large ability of the protein isolate to swell and open and then expose the sites of a new correlation, as for the decrease in the (C) sample, it may be due to the protein reaching the saturation point, and this was reflected in the characteristics of the sensory evaluation mentioned later. Kamani et al (2019), observed that substituting meat in chicken sausage with vegetable proteins, indicated that the use of plant proteins as a substitute for meat led to a significant change in the shrinkage of samples. There was as no shrinkage in the sample of the sausage without chicken, and this indicates the ability of the soy protein isolate. Dosh et al (2016), in preparing chicken burger by partial replacement of chicken meat with mushroom powder showed that the modification sample with 15% substitution was the best in terms of change in diameter compared to the control sample and had the

highest reduction in diameter, for the control sample (18.04%), while this was decreased in the replacement sample to 10 and 15% and decline in diameter was 8.38 and 5.95%,, respectively. The replacement ratios had a

Table 2. Determination of protein in defatted pumpkin seeds soaked, unsoaked and peeled

Seed treatment	Solvent	Protein (%)
Whole soaked	Chloroform / Methanol(1:3v/v)	32.15
Whole unsoaked	Chloroform / Methanol(1:3v/v)	33.25
Whole soaked	Petroleum ether (40-60° C)	34.12
Whole unsoaked	Petroleum ether (40-60° C)	37.12
Peeled unsoaked	Petroleum ether (40-60° C)	53.15

Table 3. Measurement of shrinkage in diameter and water holding capacity WHC for burger samples after grilling

Sample	Shrinkage in diameter (%)	WHC (%)
A	25.5	28%
B	16.6	54.52%
C	16.6	23.2%

Table 1. Scores of the evaluation questionnaire for the perceptual characteristics of burger

Scores	Color	Flavor	Juiciness	Freshness	General acceptance
7	Very acceptable	Strong flavor	Very juicy	Too mushy	Very acceptable
6	Acceptable	Medium flavor	Juicy	mushy	Acceptable
5	A little tolerable	Little flavor	A little juicy	Slightly tender	A little tolerable
4	Center	flavorless	Center	Center	Center
3	A little unacceptable	A little unacceptable flavor	Little dehydration	Slightly tough	A little unacceptable
2	Unacceptable	Medium unacceptable flavor	dry	Solid	Unacceptable
1	Very unacceptable	Very unacceptable flavor	very dry	Very solid	Very unacceptable

Tenderness can be determined after five chewing and a feeling of chewing strength.
Juiciness can be recognized after the first chew and the amount of moisture in the mouth after differentiating moisture from saliva.
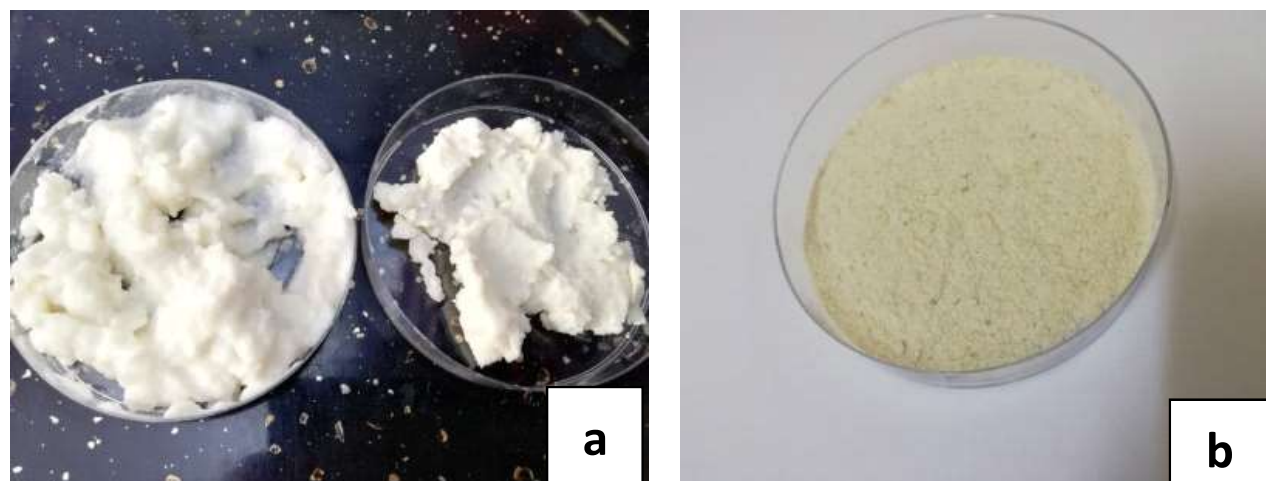
Fig. 1 a: The protein isolate before drying b: the protein isolate after drying

Table 4. Organoleptic evaluation of burger meat substituted with protein isolate from pumpkin seeds (10, 20%)

Sample	Color	Flavor	Juiciness	Tenderness	General acceptance	Total
A	5.7	6.2	5.2	5.5	5.5	28.1
B	6.0	5.8	5.5	5.3	5.5	28.1
C	5.3	5.4	4.2	4.5	4.6	24.0
LSD (p=0.05)	0.6	0.7	0.8	0.9	0.8	3.1

significant effect in increasing the water holding capacity to 30.13, 52.25 and 58.03% in the control sample and the replacement sample 10 and 15%, respectively.

Sensory Evaluation of Burger Sample After Grilling

Color: There was a significant difference in color between the B sample (6.0) compared to the C sample (5.3), while there was no significant differences between the B sample compared with the control sample A, and this indicates the proximity of the color characteristics in the two samples (Table 4).

Flavor: There was no significant difference in flavor for the control sample A (6.2) with sample B (5.8), while there was significant superiority for the control sample A compared with sample C (5.4). This indicates the proximity and acceptance of sample B in terms of flavor compared to sample C, which did not outperform significantly. The samples were slightly spicy due to the quality of the black pepper, which affected the flavor acceptance.

Juiciness: The sample B had a significant superiority in juiciness (5.5) over the sample C (4.2), and there was no significant superiority of sample B over the control sample A (5.2), and sample A was significantly superior to the sample C. This explains the reflection of the increased water holding capacity in sample B.

Tenderness: There were no significant differences in the softness between the control sample A (5.5) compared with the replacement sample B (5.3). This indicates the proximity of the softness of the control sample with sample B, while both sample A and B were superior to the replacement sample C (4.5).

General acceptance: The values of the control sample A and the 10% substitution sample B equaled the general acceptance (5.5), while the values of these two sample significantly exceeded the 20% replacement sample C (4.6). This indicates the acceptance of the replacement sample B.

Total: The values of the control sample A and the substitution sample 10% B were equal in the final total (28.1), and thus significantly outperformed the 20% replacement sample C (24.0).

Sample B is the best sample, with a ratio of replacing 10% of the meat with the protein isolate from pumpkin seeds and closer to the control sample. The used plant protein in the experiment is a by-product after extracting oils from pumpkin seeds and does not require a high cost for hydrolysis by enzymes.

Kamani et al (2019) observed that substituting meat in chicken sausage with vegetable proteins was significantly superior in taste, and there was no significant difference in terms of color, smell, and general acceptance between chicken sausage, free and reduced chicken sausage and the proportion of chicken. The last two types have better texture and appearance in general compared to the chicken sausage sample. Dosh et al (2016) indicated that preparing a chicken Burger by partial replacement of chicken meat with mushroom powder and in ratio (0, 10, 15%) improved the juiciness and freshness characteristics as the values increased by increasing the substitution rate compared to the control sample and this increase were significant. Flavor scores and general acceptability increased significantly for the replacement sample.

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Detection of MRSA Virulence Factor from Clinical Isolates by PCR Assay

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Abstract: *Staphylococus aureus* can be found as a commensal on skin and nasal flora or it may cause local and invasive infections. *S. aureus* has many virulence factors. The aims of this study were to detect mecA, fnbA and pvl genes in clinical isolates of methicillin resistant Staphylococcus aureus (MRSA). To investigate the methicillin resistance and frequency of various virulence factors in *S. aureus* isolates (blood, wound swab, urine, abscesses, vaginal swab and throat swab) . Samples collected from patients admitted to Ramadi Teaching Hospital and Ramadi Maternity & Children Teaching Hospital. Identification of *S. aureus* depends on the morphology and cultural features on the blood agar, mannitol salt agar, coagulase and catalase test, and API Staph kit (biomerieux, France). Antibiotic susceptibility tests were conducted, and the methicillin resistance was determined. The *mecA, fnA* and *pvl* staphylococcal toxin genes were examined by polymerase chain reaction (PCR). *S. aureus* was isolated in 54 clinical isolates. In total, 54 (100%) *S. aureus* isolates were methicillin-resistant. *S. aureus* isolates had a high prevalence of mecA, fnA and pvl genes (100, 87.04 and 66.7%, respectively). In this study, a large number of virulence factors were examined in *S. aureus* blood, wound, urine, abscess, vaginal swab and throat isolates respectively, and the data obtained from this study can be used for monitoring the prevalence of virulence genes in *S. aureus* strains isolated from above positions.

Keywords: Methicillin resistance, *S. aureus*, mecA and virulence factors

Staphylococcus aureus is present on the skin and nasal flora as a commensal and can cause local, serious and invasive infections such as bacteremia or pneumonia (Sakr et al 2018). The anterior nostrils are the most common region for staphylococcal colonization; longitudinal studies have shown that around 50 percent of individuals are *S. aureus* nasal carriers (van Belkum et al 2009). The nasal colonization has been described as an important risk factor for the development of community-acquired and nosocomial infections *S. aureus* (Rebollo-Perez et al 2011). The ability of *S. aureus* to acquire genes for antibiotic resistance is important. Isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) have subsequently been identified worldwide in hospital and community settings. The increasing prevalence of MRSA in the acquired hospital (HA-MRSA) and the acquired population (CA-MRSA) is threatening. All MRSA (carrying mecA gene) species are immune to beta-lactam antibiotics, by definition. Additionally, other resistance determinants can be derived from MRSA isolates. Some research, including healthy individuals, have shown that the prevalence of (*S. aureus*) and MRSA detection has increased (Sabbagh et al 2019).

S. aureus has various virulence factors associated with and secreted by cells that facilitate cellular adhesion, invasion, bacterial replication, and immune response deficiency. Panton-Vlentine Leukocidin (PVL), and fibronectin binding protein A (fnA) are some of the virulence factors (Shettigar and Murali, 2020). PVL is a toxin commonly associated with complex infections of the skin and soft tissue, diffuse cellulitis, necrotizing pneumonia, and osteomyelitis (Nhan et al 2011). Fibronectin binding protein A (FnbA) has been shown to contribute significantly to tissue colonization in various pathological conditions and infections linked to indoor medical devices (Mirzaee et al 2015). This study aims to investigate the methicillin resistance and rates of the mecA gene, fibronectin binding proteins A (fnA) and Panton-Valentine Leukocidin gene (PVL) in *S. aureus* clinical isolates from patients admitted to Ramadi Teaching Hospital and Ramadi Maternity and Children Teaching Hospital.

MATERIAL AND METHODS

Isolation and identification of MRSA: A total of 54 clinical specimens from blood, wound, urine, abscesses, vaginal swab and throat swab were collected from patients admitted to Ramadi Teaching Hospital and Ramadi Maternity & Children Teaching Hospital. Identification of *S. aureus* was done on basis of e morphology and cultural features on the blood agar, mannitol salt agar, coagulase and catalase test and API Staph kit (biomerieux, France) (BioMérieux 2014).

Maintenance of bacterial isolates: Three approaches were used for the preservation of bacterial isolates (Vandepitte et al 2003) as follows:

Short time (Week): The nutrient agar plates were sub-cultured by pure colonies for reserved a short period. The petri dishes were firmly covered with para-film. Incubation period for 18 hours at 37C°, after this period the new growth was kept at 4 °C.

Medium time (3 months): For a moderate period, the target isolates were grown on slant nutrient agar tubes for 18 hrs at 37ºC and kept at 4ºC. The slant tubes were firmly covered with para film.

Long time (6 months): The pure colonies were inoculated in the brain heart infusion broth tubes and incubation for 18 hours at 37 ºC with shaking. After the incubation period was added 20% from the filtered diluted glycerol to this new growth with the final concentration. This was done by adding 20 ml of purified glycerol to 80 ml brain heart infusion broth and distributed to 5 ml into each screw-capped bottle or white tube with full loop from exponential growth of microorganisms and left for 30 minutes at room temperature. Then kept in deep freezer at (-20 to -70ºC).

Preparation of culture media: Preparation of culture media was done according to Collee et al (1996).

Mannitol salt agar, Muller-Hinton agar, brain –heart infusion broth: These were ready as commended by the manufacturing company and autoclaved at 121ºC for 15 minutes.

Blood agar base: The medium was ready by adding sterile blood to sterilized blood agar base that has been liquefied and cooled to 75ºC, 50ºC respectively. The concentration of blood was 5 to 10%.

Identification Tests

Gram stain (Becerra et al 2016): The first step in identification of microorganism is determining cell morphology such as shape, size, arrangement of cells and their reaction with the stain. All bacteria isolates are observed under light microscope, smears are prepared from fresh culture and stained by gram stain.

Catalase tests: One to two drops from hydrogen peroxide solution (3%) and fewer colony from bacterial growth that were mixed on a glass slide. If air bubbles formation was considered as positive result (Ganamani et al 2017).

Coagulase test (Sagar 2018): Coagulase test is used to distinguish the enzyme coagulase *Staphylococcus aureus* (positive) from *S. epidermis* and *S. saprophyticus* (negative) which does not generate the enzyme coagulase. i.e Coagulase Negative *Staphylococcus* (CONS) . Coagulase is an enzyme-like protein which converts fibrinogen to fibrin and causes plasma to clot. *S. aureus* develops two coagulase forms: bonded and free.

Bound coagulase: Clumping factor binds to the bacterial cell wall and directly interacts with fibrinogen. This results in an alternation of fibrinogen to precipitate onto the staphylococcal cell, allowing the cells to clump under a plasma-mixed bacterial suspension. This does not require coagulase-reacting factor.

Free coagulase: Activation of plasma coagulase-reacting factor (CRF), a modified or derived thrombin molecule, to a coagulase-CRF complex requires activation. In exchange, this complex reacts to the fibrin clot by creating fibrinogen.

Procedure and Coagulase Test Types

Slide TEST (to detect bound coagulase)

1. Place on either end of a slide a drop of physiological saline, or on two different slides.

2. In each drop, emulsify a portion of the isolated colony with the loop, straight wire or wodden handle, to create two thick suspensions.

3. Add one of the suspensions to a drop of human or rabbit plasma, and blend gently.

4. Look for the species to clump within 10 sec.

5. No plasma is applied to the second suspension to separate some of the organism's granular appearance from true coagulase clumping.

Tube test (to detect free coagulase)

1. In physiological saline, dilute plasma 1 in 10 (mix 0.2 ml of plasma with 1.8 ml of saline).

2. Take 3 small test tubes with T (Test), P (Positive Control) and N (Negative Control) labels. Test is broth culture 18-24 hrs, Positive control is 18-24 hrs Sterile broth is *S. aureus* broth culture and Negative regulation.

3. Pipette 0.5 ml of plasma diluted inside each tube.

4. To the tube labeled "T" add 5 drops (0.1 ml) of the test organisms, 5 drops of *S. aureus* culture on the "P" tube and 5 drops of sterile broth on the tube labeled "N ».

5. Incubate the three tubes at 35-37 degree centigrade after mixing.

6. After 1 hour examine for clotting. If no coagulation has occurred, examine for up to 6 hours at intervals of 30 minutes.

Mannitol fermented: Mannitol salt agar plate was prepared as stated above A single colony was lined on mannitol salt agar and incubated for 24-48 hours at 37 C°. As a fermentation result, when the indicator (phenol red) is altered from pink to yellow color, because the medium pH is decreased and converts to acidic. That was verified as mannitol fermented isolates (Lade et al 2019).

API Staph. system (Abidemi 2018): This identification process was done according to the supplier protocol (Bio-merieux, France) as below:

A. Preparation of the inoculum: The pure colonies with 18-20 hours was suspended in staph. Suspension solution that provided by API kit (biomerieux, France) or in 5 ml of sterile normal saline for reach to turbidity equivalent to (0.5

McFarland standard).

B. Preparation of the strip: It was done by distributing of 5 ml of distilled water on to strip box fissures to create a moist environment.

C. Inoculation of the strip: By using a clean syringe, the inoculum suspension was distributed on wholly micro ampules of the strip. For avoidance of bubbles development at the tube lowest, the strip was fixed with slanted location and the tip of the needle was located at the side of the higher portion of the micro tube. Then wholly micro ampules was filled exclude the two micro ampules which contain [hydrolysis of arginine and production of urease] exams. In both previous micro ampules, the mineral oil was added to the upper tube after filling of the tube bottom by the inoculum. Lastly, the strip box was enclosed and incubated for 18-24 hr at 37 c°.

D. Reading the strip: Previously recording results, specific substances (which was provided via API Staph kit) were added to three test micro ampules comprised nitrate reduction (NIT), alkaline phosphates (PAL) and acetyl-methyl carbinol production (Voges proskauer VP) (Table 1).

When 10 minutes of the reagents adding, the results were verified depending on the supplier guidance: these results were verified as positive or negative according to their colors (show chapter three) and changed into numbers. Every strip is divided into seven groups, each group comprises three examinations. The positive tests were numbered (1 or 2 or 4) but the negative test was numbered (0). Next that, the summation of 3 sub units (numbers) of each group was calculated. To finish, a result of each strip signified seven numbers. To recognize genus and species of bacteria, these seven numbers were likened with the code number of an analytical profile index that provided by the Biomeriux company.

Molecular Identification

Genomic DNA extraction: Genomic DNA was extracted and purified as the following process:

Preparation of bacterial cells: The bacterial isolates were grown on blood agar. Then, a pure colony from overnight bacterial culture was inoculated in 5 ml of brain heart infusion broth and incubated aerobically at 37C° for 18 hr. The bacterial cells after incubation were precipitated by transported each cultured broth to a 1.5 ml eppendroff tube and centrifugation at 14500 rpm for 2 min. The supernatant was disposed, the pellet was washed with sterile deionized distilled water and dried. The last pellet was kept in deep freezer (-20 ºC).

Procedure of extraction and purification of DNA: Genomic DNA was extracted from the culture of bacterial isolates from different sources by using promega kits

extraction, purification depending on the instruction of a manufacturing company (promega, USA).

Valuation of DNA concentration and purity: Nano drop spectrophotometer was used for valuation of DNA concentration and purity. Via putting 1 µl of the elution buffer to Nanodrop device as a blanked and waiting for 20 second, then the pedestal was washed. After that, putting 1 µl of the extracted DNA specimen to the pedestal of the Nano drop device and the data of the DNA concentration and purity was collected and saved. The basic principle in the calculation of DNA concentration in ng/µl, and purity identified by calculation of the ratio of absorbance (optical density) at wavelength 260/280 nm. The measurement of the DNA purity which should range between (1.8-2.0) (Desjardins and Conklin 2010; Simbolo et al 2013).

Detection Genes by Polymerase Chain Reaction (PCR)

Primer and solutions preparation:

Stock solutions of primers (100 pmol.µl-1): According to the information of primers manufacture (GENEWIZ), each lypholized primers (forward and reverse) were dissolved in a

Table 1. API Staph reagents were used in this study

Test	Reagents	Amount
VP	VP1 and VP2	One drop of each
NIT	NIT1 and NIT2	One drop of each
PAL	ZYM A and ZYM B	One drop of each

Table 2. Oligonucleotides primers used in this study and their data

Primers	Primers' Sequences (5'→3')	Tm (C°)	Product size (bp)
fnA	F: AAATTGGGAGCAGCATCAGT	58	643
	R: GCAGCTGAATTCCCATTTTC	58	
Pvl	F: GTCGTTAGGAATAATCACTCC	53	423
	R: CCTGTTGATGGACCACTATTAA	54.3	
mecA	R: ACTGCTATCCACCCTCAAAC	60	163
	F: CTGGTGAAGTTGTAATCTGG	58	

* F: Forward sequences, R: Reverse sequences

Table 3. Component, concentration and amount in a mixture of PCR

Component	Final concentration	Amount for a single tube
Master mix	-	12.5 µl
Forward primers	0.5µM	0.5*6 µl
Reverse primers	0.5 µM	0.5*6 µl
Deionized water (ddH2O)	-	3.5µl
DNA template	60 ng	3 µl
Total V.		25 µl

DNAse - RNAse free deionized distilled water (ddH$_2$O) with a described volumes in this information to give a final concentration of (100 pmol/µl) (as stock solution), they were mixed by vortex and stored at -20 C until use.

Working solution of primers (10 pmol/µl): Each working primer solution was prepared by mixing of 10 µl of stock solution (100 pmol/µl) with 90 µl of DNAse - RNAse free deionized distilled water (ddH$_2$O) to reach a final concentration 10 pM, mixed well and kept in -20oC. They were mixed by vortex to homogenize before use.

Preparation of master mix for PCR reaction: For each isolate, a total of 25 µl mixture was prepared by mixing of lypholized master mix, template DNA, forward primer, revers ward primer and DNAase - RNAase free deionised distilled water with the subsequent volume (Table 3).

Tris base ,acetic acid and EDTA buffer (1X-TAE): TAE buffer (CARL ROTH) was diluted from (10x) to (1x) by mixing 100 ml of stock TAE-10x with 900 ml of sterile deionized distilled water, then kept at 4°C until used in DNA gel electrophoresis (Abel-Santos et al 2003).

Agarose gel (1% and 1.5 %): To prepare of agarose gel (1 and 1.5)% W/V, 1.0 and 1.5 gm respectively from agarose powder were suspended in each 100 ml of 1X-TAE buffer. Then, this solution was homogenized by heating with microwave. After cooling, 10 µl of rade safe dye was added to the clear agarose solution. Then, the agarose solution was placed in the specific tank "which previously prepared" with setting of wells-forming comb at a 1 mm from the bottom. Immediately, the formed bubbles were removed by using micro pipette. The gel was allowed for 30 minutes to solidification, then the comb was removed with carefully (Sanderson et al 2014).

Primers design: The sequences of *mecA, pvl and fnbA* genes were gotten from GenBank / NCBI, then, primers design by using MP primer program. The recommended sequences of primers by program were established with preceding used primers that designated in the testified studies by Abdelhady et al 2014. Lastly, the designated oligonucleotides of revers and forward primers were manufactured by GENEWIZ Genomic Company, USA (Table 2).

Polymerase chain reaction (PCR) technique: For amplification the six genes (*mecA, pvl and fnbA*), Polymerase Chain Reactions (uniplex-PCR) were applied using thermal cycler apparatus (TECHNE, TC-3OOO, Bibby scientific Ltd, USA). The master mix: NEB / England was used for reactions with primers (Table 3).

Molecular diagnostic of *S. aureus* by Uniplex-PCR amplification of thermo stable (*mecA, pvl and fnbA*): These genes were augmented using the PCR-PreMix "which were distributed in a thin wall sterile micro ependroff tube ". The master mix contents were described in Table 3. The two specific primer (revers and forward) of genes (Table 2) were used for this reaction. Master mix was equipped in a total volume of 25 microliters, collected of master mix (NEB England), primer solution, deionized water, and template DNA. The amounts of these contents were described in Table 3. By using the ExiSpin device (centrifuge with vortex) was mixed the micro ependroff tubes, were place in the thermal cycler device with the programme (Table 4). Annealing temperatures were adjusted for each primer pair by the use of melting curve analysis and by post-PCR agarose gel electrophoresis for the products obtained. The identities of all PCR products were established by sequencing and the amplification competence for each primer set was determined by a PCR analyse in order to estimate of target amplification. In our study was showed many of annealing temperature for each primer set such as mecA (53C°), pvl (55 C°) and fnbA (49 C°). Lastly, the end products were electrophoresed with 1.5% agarose gel.

Detection of genomic DNA bands by electrophoresis with 1.5 % agarose gel: To detect the genomic DNA bands were used the agarose gel electrophoresis technique . Agarose gel (1.5%) was prepared as described technique in section (E). After remove the comb, the freshly agarose gel was transferred to electrophoresis container and engulfed with 1X-TAE buffer upto equal with the gel surface. By use the micropipette was mixed ten microliters of DNA with two microliters of loading dye. The combination (DNA and loading dye) of each specimen was loaded to well gel and the agarose gel was flooded with 1X-TAE buffer until the optimum level (Sanderson et al 2014). The ladder marker

Table 4. The thermal cyclic condition for amplification of genes

Gene	Cycle	Initial denaturation	Denaturation	Annealing	Extension	Extension final	Hold	Product
mecA	35	94°C, 5 min	94°C, 1 min	53°C, 1min	72°C, 30 sec	72°C , 10 min	∞	163
Pvl	35	94°C, 5 min	94°C, 1 min	55°C, 1min	72°C, 30 sec	72°C , 10 min	∞	423
fnA	35	94°C, 5 min	94°C, 1 min	49°C, 1min	72°C, 30 sec	72°C , 10 min	∞	643

(100-2000 bp) was loaded in first well for contrast and determination of bands sizes. The tank was covered with its lid and the cathode pole was linked with the edge containing the wells. Then, the electrical current was matched for one hour at 70 volts. After that, a gel was transferred on to UV transilominater device for detect the DNA bands by ultra violet ray at 350 nm. Lastly, the observable bands were photographed (Lee et al 2012).

Statistical analysis: Chi square test was used for determination of association of genes with infections sources and relationship of these genes with biofilm production ability and with MRSA strains. Moreover, relationship between biofilm production ability and methicillin resistance ability (Shafer and Zhang 2012).

RESULTS AND DISCUSSION

Isolation of *Staphylococcus aureus*: Forty five *S. aureus* confines were isolated from numerous clinical specimens (Table (5)).

High incidence of *Staphylococcus aureus* in blood and abscesses samples was observed as compared to other sources. It is interpreted that blood and abscesses tissues represent a perfect medium for growth bacteria and the frequent use of antibiotics, especially penicillins and cephalosporins of the third generation. Which have a negative effect on other bacterial genera, and encourage colonization of the genus *S. aureus*. The antibiotics may destroy the wild cells of the genus susceptible to antibiotics and prevent the multiple antigen-resistant mutant cells. The inability of the defense mechanisms of a patient's body is allowed to lose these mutated cells as a result of the patient's fluid and immune proteins loss during sepsis and wounding. In addition to the lack of vital cells and the lack of pumping blood to wound, as a result, these multiply mutated cells predominate to cause infection (Gomes et al 2017, Menetrey et al 2019).

In the case of urinary tract, a high concentration of

antibiotics reaches to the infection site and does not allow the breakout of amutant cells. They have most competitive ability to infect more than the wild type of the genus *Staphylococci*. The scientific studies are indicating the difference in the isolation rates of these bacteria may be due to the difference in the seasons of sample collection, their number, and the source of isolation (Vigil and Hickling 2016).

Haque et al (2018) confirmed the existence of a consensus between the ability of *S. aureus* on the antibiotics resistance and detergents. These disinfectants may become contaminating agents and transmitters of these bacteria are among those hospitals patient's, particularly operating theaters, and surgical lounges. Besides it has an ability of growing at room temperature, low nutritional requirements, inefficiency of environmental and pharmacological factors in controlling this bacterium and its unique ability to replicate in a wide range of environmental conditions. This state led to weakens the patient's resistance, in addition to the fact that some patients do not benefit from antibiotics.

MRSA has become a health problem Compounded, MRSA isolates are prevalent in the hospital environment on a large scale, and cause a different infection to incoming patients. Some factors are predisposed to infection such as age, hospitalization, and other diseases such as diabetes, cancer and eating broad-spectrum antibiotics for a long time and excessively increase the risk of exposure to these bacteria (Gajadacs 2019).

Morphology of Staphylococcus *aureus*

Microscopic investigation: Forty-five isolates that made

Table 5. Sources of the confined *S. aureus* in this consider

Specimens	Number of *S. aureus* isolates (%)
Blood	14 (25.92%)
Abscesses	12 (22.22%)
Wound	10 (18.52%)
Urine	8 (14.81%)
Vaginal swab	5 (9.26%)
Throat swab	5 (9.26%)
Total	54 (100%)
Chi-Square	16.640
P value	0.005

Table 6. Measurement of the DNA fold concentrations of the studied bacterial isolates, measured (nanograms /µl)

Blood	Wound	Urine	Abscess	V. swab	Throat swab
1.8	1.74	2.0	1.72	1.8	2.0
2.0	1.7	1.7	1.8	1.7	1.9
1.72	1.81	1.77	1.84	1.7	1.82
1.88	1.92	1.8	1.73	1.76	1.71
1.72	2.0	1.91	2.0	-	1.73
2.0	1.7	1.9	1.7	-	-
1.9	1.8	-	1.8	-	-
1.77	2.0	-	1.85	-	-
1.81	1.9	-	1.82	-	-
1.92	-	-	1.75	-	-
1.9	-	-	1.7	-	-
1.7	-	-	2.0	-	-
1.8	-	-	-	-	-
1.79	-	-	-	-	-

gram stain positive come about. The cells showed up in microscopic analysis with circular shapes, purple color and organized into irregular clusters in single, pairs and grape-like form. Procurement of purple color occurs due to high-rate peptidoglycan cells that have soaked in these cells with crystal violets and the non-appearance of the outer membrane (Hui et al 2020).

Morphology of *Staph. aureus* on different media: Isolates revealed pattern of biochemical reactions typical for *Staph. aureus*.

Blood agar media: After incubation period at 37 °C, *S. aureus* grew with round, opaque, shining appearance, white to creamy colored, convex surface, smooth, rising and large colonies (Fig. 1). The clear zones around colonies were showed around some isolates, whereas they were not showed around other (Kobayashi et al 2015). As a result, 72% of isolates gave beta hemolysis was detected, 28% of isolates gave no zone (gamma hemolysis).

Mannitol salt agar: *S. aaureus* colony appear on mannitol salt agar as circular, glistening, yellow to buff and small colonies. All specimens were changed the medium color from pink to yellow color. The medium has a high salt concentration (7.5%) as the selected factor for *Staphylococcus spp.* (Lade et al 2019), that reason of small colonies where the medium in this circumstance is serious and miserly in differentiate with wealth of nutrients in blood agar. The colored colonies happen due to creating of staphyloxanthin by *S. aureus*. On the other hand, the medium color alters happened as a result to mannitol fermentation by *S. aureus*, where the acidic products of this way will reduce the pH that affected on the medium pointer (phenol red) and caused yellowness in it, moreover (Amit et al 2016) detailed that *S. aureus* can turn this media to yellow. On the other hand, the golden pigmentation of *S. aureus* colonies is caused by the nearness of carotenoids that ensure

microscopic organisms against oxidants delivered by the immunity (Liu et al 2020).

Biochemical Tests

Catalase test: All of these isolates were positive for catalase testing since they were able to produce an enzyme Catalase, which parts hydrogen peroxide H_2O_2 which is a powerful oxidizer to oxygen and water H_2O. The bubbles are shaped when the substance is included to the colonies of these *staphylococcus* spp (Ganamani et al 2017).

Coagulase test: All of these isolates were appeared a positive results for both slide coagulase test and tube coagulase test. The plasma is altered to clot with the coagulase enzyme that formed by *S. aureus,* bound and free coagulase are the two types of this enzyme that identified by slide and tube method respectively (Sagar 2018).

Diagnosis by API Staph system: Documentation was made by using the analytic profile index with the API Staph system. The pattern of the reactions is obtained and coded to numerical profile. All of these isolates were diagnosed as *S. aureus* by API Staph system (Abidemi 2018).

Molecular Approaches

Extraction, concentration and purity valuation and bands detection of genomic DNA: The microbiological process criterion. The development of molecular detection methods based on DNA amplification had been stimulated by the discovery of *S. aurues* (MRSA). In this analysis, a total of 54 bacteria identifying strong producers of biofilms by recognizing and defining PCR virulence genes. The extraction strategy utilizing Promega kit was fruitful for extraction of the genomic DNA from all *S. aureus* isolates in this think about. The comes about of the nanodrop spectrophotometer demonstrated tall amount and quality of the extricated DNA. The concentration extended from 52.8 to 739.6 ng/µl, while the purity (OD 260nm / OD 280nm) ranged between 1.78-2.0 (Table 6).

Table 7. Distribution of MRSA gene among 54 *S. aureus* isolates from different sources

Isolates sources	Molecular detections					
	mecA		pvl		fnA	
	+	-	+	-	+	-
Blood (14)	14	0	9	5	12	2
Wound (10)	10	0	7	3	8	2
Urine (8)	8	0	6	2	7	1
Abscess (12)	12	0	8	4	10	2
Vaginal s. (5)	5	0	3	2	5	0
Throat (5)	5	0	3	2	5	0
Sum +ve	54		36		47	
+ve %	100%		66.7%		87.04%	

Fig. 1. Positive catalase test in *S. aureus*

Fig. 2. Positive and negative coagulase test by *MRSA*

Fig. 3. API staph. system, A: show negative result and B: show positive result
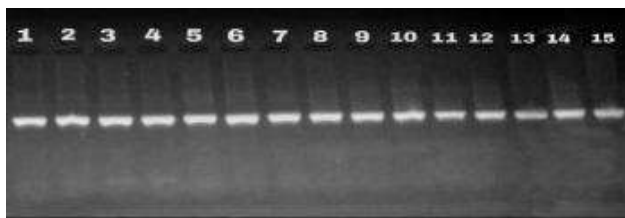
Fig. 4. Genomic DNA bands of some isolates [agarose 1.5 %, TAE buffer (1X), 1 hr,70 V and stained with Red safe stain]

Fig. 5. 1.5% agarose gel electrophoresis of *mecA* gene with end PCR product size (163 bp) of some tested isolates. [1.5-2 h, 70 V]; M(bp): DNA sizes marker
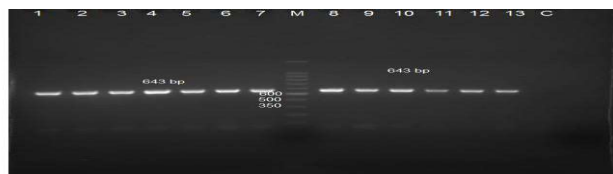
Fig. 6. 1.5% agarose gel electrophoresis of *fnA* gene with end PCR product size (643 bp) of some tested isolates. [1.5-2 h, 70 V]; M(bp): DNA sizes marker

Fig. 7. 1.5% agarose gel electrophoresis of *PVL* gene with end PCR product size (423 bp) of some tested isolates. [1.5-2 h, 70 V]; M(bp): DNA sizes marker

An effective PCR method is depends on a victory of DNA extraction with high immaculateness and concentration (Chloe et al 2020). The virtue of DNA (A260/A280) must run between 1.8 and 2.0; the proportion with esteem higher than 2.0 implies that the DNA test is contaminated with RNA, the proportion with esteem less than 1.7 proposes that the DNA test is sullied with proteins (Al-Talib et al 2013; Theyab and Abed 2018). For 25 µl PCR response, the DNA concentration must be higher than 30 ng of microbial genomic DNA (de Bruin and Birnboim 2016; Theyab and Abed 2018).

The 54 of *S. aureus* confines was DNA extraction and PCR test for recognition of (mecA, pvl and fnA). Each quality was meant by a single band within the identical locale of the DNA step. The *S. aureus* qualities of this ponder were appeared positive comes about in 100% of confines. In this study all serovars *S. aureus* was intensified to PCR test.

Polymerase chain reactions (PCR): Distribution of all detected genes in this study were detailed in Table 7.

Molecular diagnosis of MRSA by detection of thermo stable mecA gene using uniplex-PCR: All bacterial isolates (100%) sheltered *mecA* gene with end product size equivalent to 163 bp (Table 2, Fig. 7).

The molecular based identification of *S. aureus* is PCR, required to amplify of species-specific targets (Kanagarajah et al 2017). PCR based on mecA gene is considered the gold standard method for detection of MRSA (Ahmed et al 2014). The larger part of investigation in this field recommended that mecA gene that's display in all MRSA strains and is known to encode penicillin binding protein 2a (PBP2a), which contains a low tropism to all β-lactam anti-microbials, is the corner stone capable for creating MRSA marvel (Alfouzan et al 2019). Beta-lactam resistance is credited for the most part to transformations within the mecA quality, but other genetic components may too be considered for the clarification of the mechanism of resistance (Roch et al 2019). Subsequently, the discovery of mecA gene in all isolates give high precision in confinement of *S. aureus* and affirms its distinguishing proof of this study.

Detection of fibronectin binding protein A gene (fnA) using uniplex-PCR technique: Higher percentage of isolates (87.04%) contained the fnA quality with last PCR item size (643) bp (Table 2 and Fig. 8) (groups of this quality was identified in uniplex PCR in figure 3-28). The FnBP A is exceptionally important protein for *S. aureus* colonization. They intercede official with fibronectin, fibrinogen and elastin (Burke et al 2011). This protein offer assistance the colonization of this organism to epithelial and endothelial cells (Massey et al 2020). More distant more, the fnA is bind to platelet and contact with α5β1 integrin by bridge arrangement, that offices the avoidance from safe reaction

by sneaking of organism from blood stream in to organs (Liang et al 2016). The fnA is more essential than fnB for invivo and invitro contamination in *S. aureus* (Kwiecinski et al 2019). From over past studies, the multifunction of FnBP deciphers the tall conveyance of fnA in all segregates without special case of any contamination source, that increases the pathogenicity of tried confines in this study. On the other hand, the predominance of fnA can increase the bacterial resistance to antibiotic operators, the FnBP is required for cell-cell official and biofilm formation in *S. aureus* (McCourt et al 2014).

Detection of PVL genes by using PCR technique: The panton -valentine leukocid*in* (PVL) gene was presented in isolates (66.7%) (table 2) with PCR product sizes 423bp (Fig. 9).

In the late 1990s, the first PVL positive MRSA was noticed and these strains have been spread worldwide in recent years. The role of PVL in boosting *S. aureus* virulence and its pathogenicity is being discussed. Panton-Valentine leukocidin rises the pathogenicity of *S. aureus* by necrosis, quickening apoptosis and damage of polymorphonuclear and mononuclear cells, thereby contributing to mortality and morbidity. In general, PVL is used as a marker for MRSA acquired by the population, responsible for deep dermal infections like soft tissue (Amit et al 2018).

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Antimicrobial Activity of Iraqia *Artemisia herba-alba* against sar A gene Expression in MRSA

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a human pathogen responsible for significant morbidity and mortality worldwide. The pathogen has achieved high priority in the World Health Organization (WHO) list of multidrug-resistant (MDR) pathogens. Emerging MDR strains *Staphylococcus aureus* is clinically difficult due to failure in traditional antibiotic therapy. Biofilm formation is one of the underlying mechanisms behind the resistance of antibiotics. One of the fundamental mechanisms behind the resistance of antibiotics is biofilm formation. Therefore, attenuating the development of biofilms has become an alternative technique for managing recurrent infections. The current research is to focus on *Artemisia herba-alba* (volatile oil) and antivirulence ability against MRSA and clinical isolates. Volatile oil can offer a new strategy to tackle multidrug-resistant bacteria. The present study discusses the impact as antimicrobial spectrum .and combined effect of volatile oil with Ciprofloxacin and Augmentin against methicillin resistance *Staphylococcus aureus* (MRSA). Transcriptional analysis revealed the down-regulation of regulator *sarA* and *sarA* mediated virulence genes upon volatile oil treatment, is well correlated with results of phenotypic assays. Thus, the results of the present study revealed the *sarA* mediated antibioflim and antivirulence potential of volatile oil against MRSA.

Keywords: *A. herba-alba*, Volatile oil, MRSA, sarA, MDR, WHO, Phenotypic

Bacterial infections are treated globally and even avoided by antibiotics. In developing such antibiotic resistance, the widespread and excessive use of antibiotics causes selective pressure on the bacterial population. Consequently, the production of MDR reduces the potential of antibiotics against bacteria (Qu et al 2019) Antimicrobial resistance is a significant problem for global public health, according to the WHO (WHO 2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the MDR pathogens of great concern and has been identified by WHO 2017 as a high priority MDR pathogen. Most MRSA infections were associated with hospitalized patients in earlier days, and thus MRSA strains isolated from hospital settings are referred to as MRSA associated with healthcare (HA-MRSA). MRSA incidence has increased over the course of time and also in patients not previously hospitalized, MRSA strains have been identified and are referred to as community-associated MRSA (CA-MRSA) (Turner et al 2019). In general, infections of the skin and soft tissue, bacteremia, infectious endocarditis, osteomyelitis, and pneumonia are known to cause MRSA. In addition, MRSA is primarily active in infections of indwelling catheters, prosthetic devices and related implant infections (Lee et al 2018, Wu et al 2019).

The ability to generate biofilm on biotic and abiotic surfaces, which is the primary cause of its resistance and survival, is one of the main and fundamental features of MRSA (Bowler et al 2020). The biofilm of MRSA is made of self-produced polymeric extracellular substances comprising polysaccharides, proteins and eDNA. Biofilm protects MRSA from the innate immune response of the host and serves as a barrier to antibiotic entry (Rita et al 2019). With several cell surfaces and virulence factors secreted, MRSA is armored. Cell surface virulence factors such as staphylococcal protein A (SpA), fibronectin-binding proteins (FnBPs), collagen-binding protein (Cna) and clumping factors are primarily involved in adherence and persistence and include capsular polysaccharides, staphyloxanthin and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (ClfA and ClfB). Secreted virulence factors, like staphylococcal enterotoxins, alpha-hemolysin, leukocidin and virulence enzymes such as lipases, nucleases, proteases, aureolysin, hyaluronidase, and staphylokinase, are frequently correlated with tissue invasion and disease progression (Selvaraj et al 2019, Lima et al 2020). Global regulatory systems such as accessory gene regulator (agr), staphylococcal accessory element (sae) and even staphylococcal accessory regulator A are significantly mediated by such virulence factor secretion and biofilm formation (sarA) (Banghui and Baolin 2020). A sarA protein binding to DNA encoded by the sar locus has been reported to play an important role in regulating the expression of MRSA virulence factors. By binding to the intergenic space

between P2 and P3 agr regulon promoters, SarA modulates the expression of the virulence gene. Although the crosstalk between agr and sarA is well known, by an independent mechanism, sarA regulates the expression of many essential adhesion genes. Therefore, as a master regulator of biofilm and virulence genes, sarA currently stands as a drug development therapeutic target. Furthermore, sarA inhibitors are well known to inhibit *S.aureus* biofilm formation (Selvaraj et al 2019, Banghui and Baolin 2020).

The antibiotic therapeutic approach is an alternative method for overcoming resistance to antibiotics. Instead of destroying the bacteria, the aim of novel therapy is to inhibit the production of biofilm and virulence factors and therefore exclude the selection pressure on bacteria, thus preventing resistance growth (Abd El-Hamid et al 2020). The objective of the current study is to classify the antibiofilm and antivirulence agent against MRSA from natural resources. *Artemisia herba-alba* was used against MRSA in the present study because of its antibiofilm ability, among which volatile oil showed high antibiofilm activity. Anti-diabetic, leishmanicidal, antibacterial and antifungal effects have been identified in investigations into the medicinal properties of *A. herba-alba* extracts (ez zoubi et al 2018).

MATERIAL AND METHODS

Collection and identification of isolates: One hundred and fifty isolates of the *S. aureus* (MRSA) used in this study were collected from blood culture , urine culture, wound swabs ,abscesses, throat and vaginal swabs from patients admitted to Ramadi Teaching Hospital and Ramadi Maternity & Children Teaching Hospital. The specimens were taken through the period from January, 2019 to June, 2019. Identification of *S. aureus* was based on the morphology and cultural features on the blood agar, mannitol salt agar, coagulase and catalase test, MRSA screen kit(Denka Seiken, Japan) and Vitik2 (biomerieux, France) .

Media preparation: Mannitol salt agar plate was prepared according to the manufacture company. A single colony was lined on mannitol salt agar and incubated for 24-48 hours at 37°C. As a fermentation result, when the indicator (phenol red) is altered from pink to yellow color, because the medium pH is decreased and converts to acidic. That was verified as mannitol fermented isolates (Lade et al 2019). Blood agar base was prepared by adding sterile blood to sterilized blood agar base that has been liquefied and cooled to 50ºC. The concentration of blood was 5 to 10%.

Bacterial standardization: The tube which contained bacterial suspension was associated with the turbidity standard and the concentration of the test suspension was attuned visually to that of the standard by addition more microorganisms or sterile saline. Furthermore, for every test suspension, fine adjustment of turbidity was achieved until the optical density value for the test suspension agreed to optical density for 0.5 McFarland standard solution (Andrews 2009).

Identification Tests

A- Gram stain (Becerra et al 2016): The first step in identification of microorganism isdetermine cell morphology such as shape, size, arrangement of cells and their reaction with the stain. All bacteria isolates are observed under light microscope, smears are prepared from fresh culture and stained by gram stain.

B- Catalase tests: One to two drops from hydrogen peroxide solution (3%) and fewer colony from bacterial growth were mixed on a glass slide. If air bubbles formation was considered as positive result (Ganamani et al 2017).

C- Coagulase test (Sagar 2018): Coagulase test is used to distinguish the enzyme coagulase *S. aureus* (positive) from *S. epidermis* and *S. saprophyticus* (negative) which does not generate the enzyme coagulase. i.e Coagulase Negative *Staphylococcus* (CONS) .

D- Latex agglutination process: This process was used according to Alipour et al (2014).

E- VITEK2 compact system for MRSA (Isolation and identification): This test was utilized to classify different bunches of species as takes after: GN (Gram Negative) aging and non-fermenting bacilli, GP(Gram Positive) cocci and non-spore forming bacilli, yeasts and yeast-like species, GP spore forming bacilli (Biomerieux 2014).

Biofilm Production

A- Congo red agar test:

1- Microbial strain suspension was vaccinated on a specifically prepared solid medium (BHI) supplemented with 5 % sucrose and red stain in Congo.

2- Congo's red agar medium contains of 37 gm / L brain heart infusion, 50 gm/L sucrose, 10 gm/L agar and 0.8 gm/L red congo stain.

3- Congo red stain was arranged as a concentrated watery solution and autoclaved at 121 C for 15 min. independently from other medium constituent and was at that point added when the agar had cooled to 55°C.

4-Petri dishes were vaccinated and incubated aerobically at 37°C for 1-2 days .

5- If black colonies with a dry crystalline consistency appear were indicated a positive result. Weak sludge makers more often than not remained pink in spite of the fact that intermittent darkening at the middle of colonies was observed.

6- A darkening of the colonies with the nonappearance of a

dry crystalline colonial morphology shown an uncertain result (Oliveira and Cunha 2010).

B- Value adhesion by tube method

1- About loop occupied with each isolate from the petri dish and vaccinated into a glass test tube which contain of brain heart infusion broth and incubated at 37 C for overnight.

2-The test tubes were discarded of BHI then the glass tubes were stained by the 0.1% safranin solution, by distilled water washed the tubes for three times and dried.

3- The presence of visible film lined on the wall and bottom of the tube is considered a positive result. A negative result when nonappearance of a film or the exclusive observation of a stained ring at the liquid air border.

4-Based on biofilm formation, the positive results were recorded as weak,(+), moderate (++) and strong (+++) (Nivedita et al 2012).

C- Micro-titter plate method

For a biofilm production assay was used the micro-titter plate method according to (Yousefi et al 2016).

1- Preparation of the inoculum (Turbidity standard): In 5 ml of sterile nutrient broth, A few drops from over-night broth cultures were suspended. The turbidity of these suspension was compared with 0.5 Mc- Farland standard (which was described in section Bacterial standardization) to obtain 1.5 x 10^8 CFU for each ml.

2- Preparation of the 96-well micro plate (ELISA): By utilizing micropipette, the diluted cultures of tested confines were disseminated within the micro wells (200 μl in each well, three duplicates of each isolate). 200 μl of sterile nutrient broth were added in 6 wells as a negative control. Then, the plate was covered with lid and it was incubated aerobically at 37 ºC for 18 hours. Later this period, the bacterial cultures were removed from all wells. They were carefully washed twice with sterile Phosphate buffer solution (PBS) for remove non-adhered bacterial cells. The plate was inverted on filter paper for drying. Add 200 μl of methanol in each well for 10 min for fix the adhered bacterial cells (forming biofilm cells). Then, the washing process was repeated carefully at room temperature for methanol removed. After that, 200 μl from the crystal violet stain (0.1 % w/v) was added to each well and left the plate for 15 minutes at room temperature. The execs stain was washed by PBS for three times and was perfectly dried. The 200 μl of the ethanol (95 % v/v) was added to each well at room temperature for 10 min. then, the microtiter-plate was transported to ELISA reader for the results reading with optical density (OD) at 630 nm. This step was used to detect the formed biofilm. After reading of the results were calculated the mean from the three replicates for each isolate. Then, the results of microtiter-plate reader were classified as none (OD ≤ ODc), weak (ODc < OD ≤ 2×ODc),

moderate (2×ODc < OD ≤ 4×ODc) and strong (OD > 4×ODc) biofilm formation.

Artemisia herba-alba Plant

A- Collection and identification of plant sample: *Artemisia herba-alba* aerial parts (leaves and stems) were collected in 2019 from plants originated from different localities in 18 kilo domains of Ramadi. Aerial parts of the studied specie was identified taxonomically from Herbarium, Center of Desert Studies, University of Anbar.

B- Extraction of volatile oils: The herbal raw materials were washed and air-dried at room temperature (15-20°C) for two weeks. Then, the leaves were separated from the other parts and used for the analyses. The air-dried leaves of the studied plants were used to extracted volatile oils using steam-distillation, a Clevenger-type apparatus,100g of clean air-dried leaves **(***Artemisia herba- alba***)** put with 500 ml of DW in (1000 ml) round flask. The lower and higher parts, were associated to a heating blanket and a condenser. The extraction method was directed for a period of 2.5 hours while the solution left boiling. The water vapour obtained in the flask leaves the plant, loaded to the condenser with volatile oil, in which it is condensed. After condensation, the product is separated by decantation from water. Then stored at 4°C in densely locked vials until tested and analysed (Amor et al 2019). After the extraction of volatile oil from the *Artemisia herba-alba* plant. Gas chromatography-mass spectrometry (GC-MS) gas chromatography was used in laboratories of the Ministry of Science and Technology, Department of Environment and Water/Ecology Research Centre, to determine the chemical composition of volatile oil.

Antimicrobial Activity of Volatile Oils

A- Disk dissemination strategies: The disk dissemination strategies of Volatile Oils(*Artemisia herba-alba*) to MRSA were observed based on several dilution from stock solution of Volatile Oil .The antimicrobial action volatile oil decided by the adjusted Kirby Bauer of diffusion strategy on Muller-Hinton Agar (MHA) plates (Dorota and Tichaczek-Goska 2013). A bacterial culture was organized by spreading 100μl of bacterial suspension with cell thickness 1.5×108 CFU/mL of each test organism in the plated Muller Hinton agar . The plates were kept for 5 minutes to permit culture assimilation (Balouiri et al 2016) . These were sterilized by autoclave, then immersed with volatile oil solution with diverse concentration of (0.5, 0.25, 0.125, 0.0625 and 0.03125%) for one hour and after that left to dry totally, and after that the bacterial suspension was placed on Muller Hinton agar plate within the same disk dissemination strategy. The Petri dishes were left for 3-5 minutes for suspension was absorbance, and after that the sterilized filter paper disk was settled as immersed with volatile oil solution. The plates were incubated at 37°C

for 18-24 hrs, then calculating the inhibition zone (mm) around the volatile oil disk (Amor et al 2019).

B- Minimum inhibitor concentration (MIC): Minimum, inhibitory concentration (MIC) of the volatile oils and antibiotics solutions were estimated by Resazurin, Microtitre-plate Assay (REMA) with minor alterations. In aseptic situations, the volume of 100 µl of Mueller-Hinton Broth (MHB) was added to all wells of microtitre-plates. Then 100 µl of test material (1% volatile oil) transported into the first row of the 96 well plates. Sequential dilutions were done by pipetting 100 µl of the material test from the first row to the other rows in consecutively declining concentrations (1/2,1/4,1/8,1/16,1/32,1/64,1/128 and 1/256). To each well were added 10 µl of bacterial suspension which containing 1.5×10^8 CFU/ml. The microtiter plates were enveloped loosely with para-film and incubated for 18-24 h at 35±2°C (Manuel and Abdulrahman, 2017). After this, to each well was added 10 µl of resazurin solution (Alamar blue), and the plate was re-incubated for 4 hours for the observation of colour alteration. The results were examined visually by observing the changes in the colour of resazurin, alterations from purple to pink or colourless existence d as positive. The lowermost concentration with no alteration of resazurin colour was taken as the MIC value (Kolarević et al 2016).

Synergism between volatile oils and antibiotics: Augmentin and Ciprofloxacin in combination with Volatile Oil used to study impact of gene expression of *S. aureus* (MRSA) by using serial dilutions.

Estimation combination effects between antibiotics and volatile oils by checkerboard method: The potential occurrence of synergy interaction between the volatile oil and antibiotics was verified by the checkerboard technique in 96 well microplates (Langeveld et al 2014). The checkerboard examine was designed such that the two antimicrobials to be tested in the checkerboard experimental were serially diluted against each other in a cross manner, and achieved on two standard 96-well plates. This technique, the first antimicrobial-volatile oil is serially diluted vertically, and the second antimicrobial-antibiotic is serially diluted horizontally. The test was designed such that the determined MIC from the REMA test for volatile oils (Mutambuze 2014). To make the microtiter-plates for the checkerboard test, in aseptic situations, all the wells of microtiter-plates were filled with 100 µl of Muller-Hinton broth (MHB). 100 µl of antibiotic solution was added to the first column, thus 100 µl moving to next wells (horizontally) and discarding the last 100 µl transfer. In the second microtitre-plate, 100 µl of (1%) the material test (volatile oil) added to the first row and 100 µl transferring to next wells (vertically) and discarding the last 100 µl to get a serial concentration (1/2 to 1/128). Then antibiotic

dilutions are added to the second microtiter-plate into each respective concentration horizontally. 10 µl was taken from bacterial suspension (1.5×10^8 CFU/ml) and then added to each well. The microtiter-plate also comprises two controls, (A) a column with MHB only, (B) a column with MHB and bacterial suspension. They were not allowed to be dehydrated and the microtiter plates were enveloped loosely with para-film and incubated at 37°C for 24-48 h. After this step, to each well was added 10 µl of resazurin solution (Alamar blue), and the plate was re-incubated for 2 hours for the observation of colour alteration. After 2 hours, the well with no colour change was considered as the point at which the MIC of the two antimicrobials in combination intersect (Martin 2010, Mutambuze 2014). Then we determined the Fractional Inhibitory Concentration (FIC) of each antimicrobial in combination and then used this value to determine the Fractional Inhibitory Concentration Index (FICI). The FICI is most often used to describe or to define drug interactions.

For two antimicrobials A and B acting alone and in combination, the FIC is given as:

FIC (compound) = MIC (compound in the presence of antibiotic) MIC (compound alone)

$$\text{FIC (A)} = \frac{\text{MIC of A in the presence of antibiotic}}{\text{MIC of A alone}}$$

$$\text{FIC (B)} = \frac{\text{MIC of B in the presence of A}}{\text{MIC of B alone}}$$

A: Volatile oil. B: Antibiotic.
The ∑ FIC (FICI) is therefore given by: FICI= FIC (A) + FIC (B)

A FICI value of ≤ 0.5 is indicative of a synergistic influence. FICI values >0.5-4 are indicative of an additive effect (no drug interaction).

Molecular Identification

Genomic DNA extraction: Genomic DNA was extracted and purified as the following process:

A-Preparation of bacterial cells: The bacterial isolates were grown on blood agar. Then, a pure colony from overnight bacterial culture was inoculated in 5 ml of brain heart infusion broth and incubated aerobically at 37C°for 18 hr. The bacterial cells after incubations were precipitated by transported each cultured broth to a 1.5 ml eppendroff tube and centrifugation at 14500 rpm for 2 min. The supernatant was disposed; the pellet was washed with sterile deionized distilled water and dried. The last pellet was kept in deep freezer (-20ºC).

B-Procedure of Extraction and purification of DNA: Genomic DNA was extracted from the culture of bacterial isolates from different sources by using promega kits extraction, purification depending on the instruction of a manufacturing company (Promega, USA).

C- Valuation of DNA concentration and purity: Nano drop spectrophotometer was used for valuation of DNA concentration and purity. Via putting 1 µl of the elution buffer to Nanodrop device as a blanked and waiting for 20 second, then the pedestal was washed. After that, 1 µl of the extracted DNA was added to the pedestal of the Nano drop device and the data of the DNA concentration and purity was collected. The basic principle in the calculation of DNA concentration in ng/µl, and purity identified by calculation of the ratio of absorbance (optical density) at wavelength 260/280 nm. The measurement of the DNA purity which should range between 1.8-2.0 (Desjardins and Conklin 2010, Simbolo et al 2013).

Detection genes by polymerase chain reaction (PCR): This was done according to the information of primers manufacture (GENEWIZ).

Quantitative -PCR Programs: It regulates only three MRSA isolates and three isolates without treatment were selected to demonstrate the effect of volatile oils alone, volatile oil combination with ciprofloxacin and Augmentin on sarA and 16S rRNA gene expression

Statistical analysis: All data were analyzed by test of Chi-square (Cross tabulation) or Mann-Whitney by Microsoft Excel ver 2016.

RESULTS AND DISCUSSION

Isolation and Identification of *Staphylococcus aureus*: A total of 150 clinical specimens have been taken from blood, wound, urine, abscesses, vaginal swab and throat (Table 3). The isolates obtained from blood agar and mannitol agar were identified by coagulase and catalase test, MRSA screen kit (Denka Seiken, Japan) and Vitik2 (Biomerieux, France). *Staphylococcus* aureus appeared on blood agar as shining appearance, white to creamy colored, convex surface, smooth, rising and large colonies. The clear zones around

Table 3. Sources of *S. aureus*

Specimens	Number of S. *aureus* isolates (%)
Blood	39 (26.0%)
Wound	30 (20.0%)
Urine	26 (17.33%)
Abscesses	24 (16.0%)
Vaginal swab	18 (12.0%)
Throat swab	13 (8.67%)
Total	150 (100%)
Chi-Square	16.640
P value	0.005

Table 1. Oligonucleotides primers used

Primers	Primers' Sequences (5'→3')	Tm (C°)	Product size(bp)
Cap5	F: ATGAGGATAGCGATTGAAAA	56	518
	R: CGCTTCTTAATCACTTTTGC	54	
Cap8	F: ATCGAAGAACATATCCAAGG	56	834
	R :TTCATCACCAATACCTTTTA	52	
mecC	R: TAAGCAATAATGACTACC	48	304
	F: ACTGCTATCCACCCTCAAAC	52	
sarA	R: TCTTGTTAATGCACAACAACGTA	53.5	Real time
	F: TGTTTGCTTCAGTGATTCGTTT	52.5	
16S rRNA	R: ACTGGGCCTGTACAACTTCCGA	59.99	Real time
	F: GCTGCTGTCGATATTGGGGCTT	59.99	

* F: Forward sequences, R: Reverse sequences

Table 2. Thermal cyclic condition for amplification of genes

Gene	Cycle	Initial denaturation	Denaturation	Annealing	Extension	Extension final	Hold	Product
mecC	35	95°C, 2 min	95°C, 30 sec	51°C, 30 sec	72°C, 1 min	72°C , 5 min	4°C ∞	304
Cap5	35	95°C, 2 min	95°C, 30 sec	44°C, 30 sec	72°C, 1 min	72°C , 5 min	4°C ∞	518
Cap8	35	95°C, 2 min	95°C, 30 sec	48°C, 30 sec	72°C, 1 min	72°C , 5 min	4°C ∞	834

colonies were showed around some isolates, whereas they were not showed around other (Kobayashi et al 2015). As a result, 72% of isolates gave beta hemolysis and 28% of isolates gave no zone (gamma hemolysis). They appeared on mannitol agar as circular, glistening, yellow to buff and small colonies. All specimens were changed the medium color from pink to yellow color.

Biofilm formation of *MRSA* isolates: Biofilm formation is known to be an actual reason of most *Staphylococcus aureus* related with the infection of the biomedical system. Since biofilm provides the source for infections or are correlated to antimicrobial resistance and chronic infections, it is also of clinical interest that biofilm produces *Staphylococci* isolated from many other clinical samples. Therefore, a dependable and simple process is required for their analysis (Ramakrishna et al 2014). Via three in-vitro screening procedures, our study tested 150 clinical MRSA isolates for their ability to form a biofilm.

A-Congo Red Agar method: 150 of MRSA isolates are tested for biofilm production by Congo red agar method. The present study showed that black colonies with dry crystalline consistency were formed strong biofilm. The Congo red stain was used directly to identify exopolysaccharide production, which was an essential criteria for the formation of biofilms (Neeli et al 2016). The result in the present study, by Congo red agar method in blood 33.33% , wound 30.0%, urine 34.62% , abscess 33.33%, vaginal swab 33.33% and throat swab 30.77% (isolates of MRSA formed biofilm, while eest isolates did not biofilm produced (Fig. 1). There are not significant differences among these groups with (P-value > 0.21). These results are agreed with (Rania et al 2018).

B-Tube method: 150 isolates of *MRSA* isolates were screened for biofilm production by tube method. The result values of biofilm formation depended on bacterial adherence on glass tube. In the present study in blood 38.46%, wound 30.0%, urine 34.62% ,abscess 33.33%, vaginal swab 27.78% and throat swab 32.1% isolates formed strong biofilm formation, while the result of blood (38.46%), wound (40.0%), urine (42.31%) , abscess (41.67%) , vaginal swab (38.89%) and throat swab (38.46%) isolates were moderate biofilm formation, and the rest isolates were non- producer biofilm (Fig. 2).
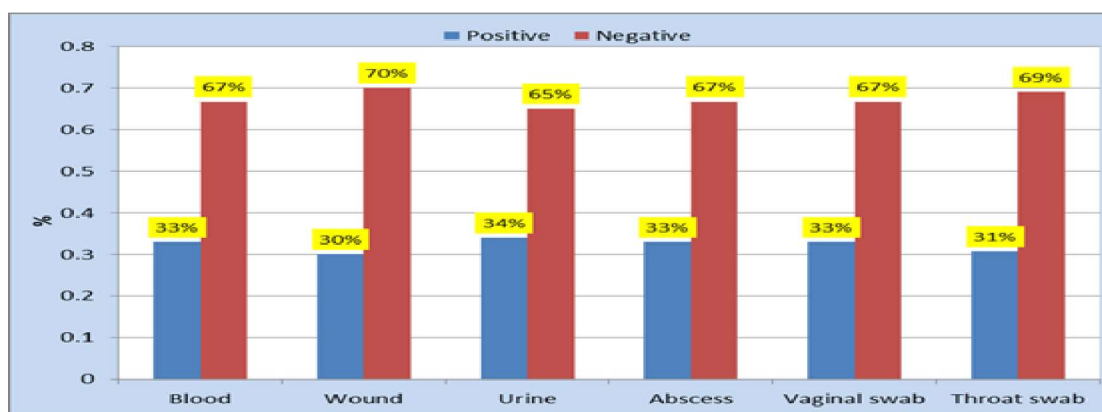
Fig. 1. Percent of biofilm formation MRSA by Congo red agar method according to the specimen's source
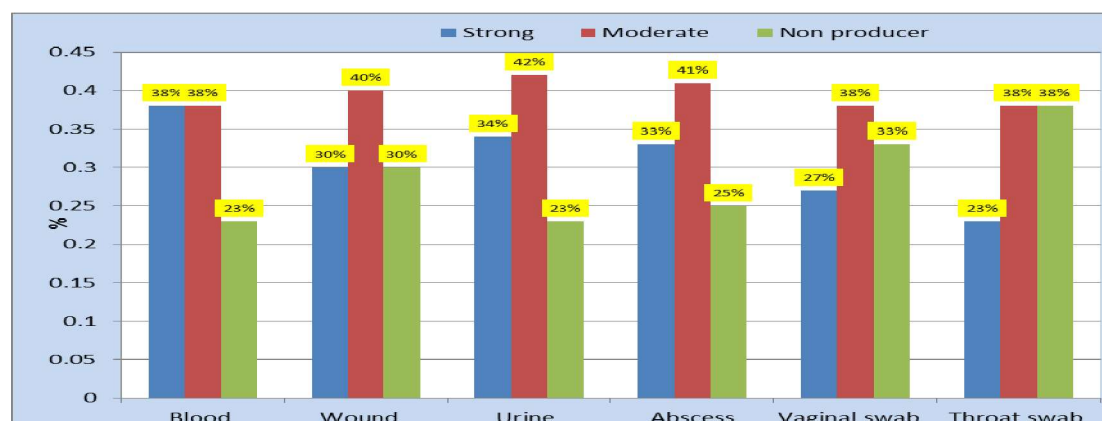
Fig. 2. Percent the biofilm formation of MRSA by tube method

C-Ability of strains of *Staphylococcus aureus* to develop bioflim on Micro-titter plate: *Staphylococcus aureus* strains have been tested on the surface of polystyrene for biofilm formation. At 630 nm, 150 strains were biofilm makers based on absorbance values and were considered to be weak, moderate and strong biofilm makers. Almost (36% of strains were strong makers, whereas over 44.67 and 19.33%) of strains were moderate and weak makers, respectively. The most elevated rate of strong biofilm makers was among the strains isolates from blood (35.9%), wound (33.3%), urine (30.8%), abscess (50%), vaginal swab (27.8%), and throat (38.5%) (Fig. 3).

The contrast of biofilm biomass (absorbance at 630 nm) of six bunches of strains confined from blood, wound, urine, abscess, vaginal swab and throat swab revealed that strains confined from abscess delivered significantly more biofilm than strains isolated from urine and vaginal swab but distinction among biofilm biomass of strains from throat, blood and from wound were not statistically significant. No statistically significant distinction in biofilm generation was observed between strains isolated from blood and wound. Whereas, the capacity of biofilm shaping by the strains isolated from vaginal swab was y

lower comparing to strains isolated from throat (Fig. 3).

The majority of the 150 MRSA strains examined (44.67%) made moderate biofilms. The 36 % of the MRSA strains produced strong biofilms, while 19.33 % were weak biofilm producers. A statistical test evaluation of biofilm biomass (absorbance at 630 nm) showed that MRSA strains had significantly higher biofilm formation ability (Fig. 3). The standard gold technique is the micro-titter plate method, so it was considered a standard method for interpreting our findings (Manandhar et al 2018). When MTP was carried out, biofilm generating *Staphylococcal* isolates were 80.67% (36% strong producers and 44.67% moderate producers). As it was used to cut off values, a technique provided the finest discrimination among solid, moderate and poor biofilm. The findings of research indicated higher values than those of other researchers (Snehali et al 2014, Rania et al 2018).

The isolates of a strong biofilm producer will be used in study. The findings indicate a high percentage of biofilm development isolated from blood culture specimens in *Staphylococci* (35.9 %). On the other hand, in 2018, Rania et al discovered the synthesis of biofilms in staphylococcal isolates from blood culture specimens (43.3 %).
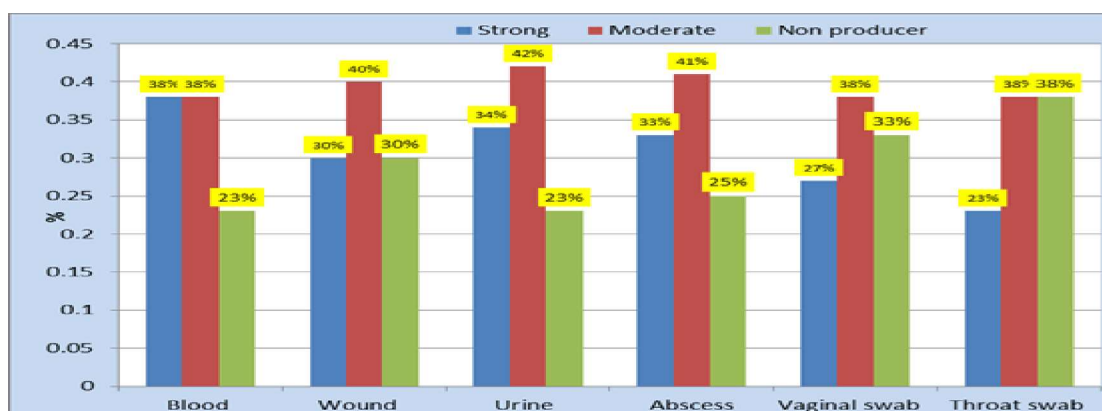
Fig. 3. Biofilm formation of MRSA by micro-titer plate (%)
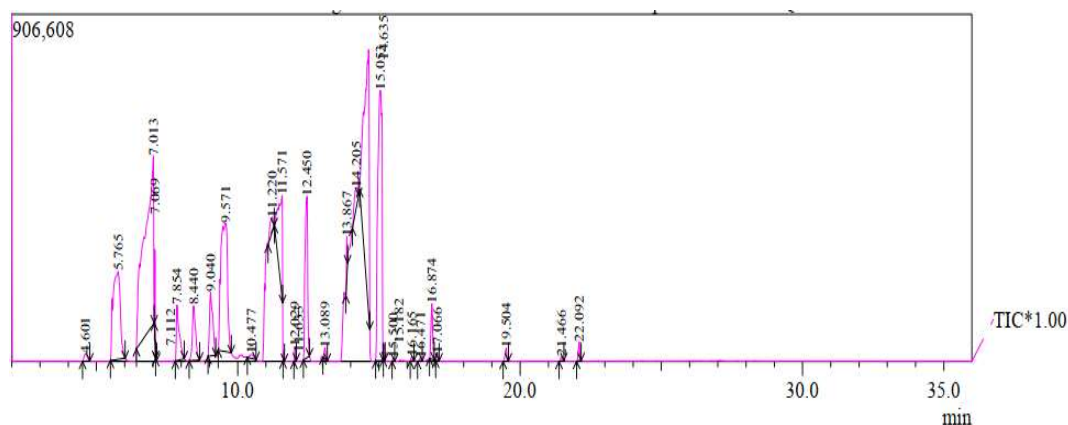
Fig. 4. GC-mass analysis of *Artemisia heba-alba* volatile oil

Antimicrobial Activity of *A. herba-alba Asso* (Volatile Oils) against *MRSA*

Extraction and investigation of volatile oils: The volatile oils were investigation by Gas chromatography-mass for estimation the active chemical compounds in this oil.

The aerial parts of *Artemisia herba alba* resulted in pale yellow oils. GC analysis of the Volatile oil from *A. herba-alba* showed Alpha-Thujene, Beta-pinen, Sabinene, Beta-Ocimene, Beta-Cymene, Alpha-Terpinene, Yomogi alcohol, Limonene, Carvone, Alpha-Amorphene, Myrcenol and Palmitic acid are the main components , similar to Paolini et al (2010) and Chaieb et al (2018). The antimicrobial activity of *A. herba-alba* volatile oils was tested at different concentrations against MRSA.

Disk Dissemination Strategies

A- Estimation of volatile oil activity: Volatile oil (*A. herba-alba*) was tested for antibacterial activity against pathogenic MRSA. Different concentrations (50, 25, 12.5, 6.25 and 3.125%) of Volatile Oils were loaded on the sterile disks. The inhibition zones included, 15.7 mm at 0.5%, 14.4 1 mm at 0.25%, 8.8 mm at 0.125%, 4.2 mm at 0.0625% and 0 at 0.03125% concentration of Volatile oil for *MRSA* (Table 5, Fig. 5).

Table 4. Chemical composition of volatile oils of *Artemisia herba-alba*

Compound	RT	Area (%)
Alpha-Amorphene	13.867	0.40
Alpha-Terpinene	7.112	0.09
Alpha-Thujene	4.601	0.33
Beta-Cymene	7.069	0.59
Beta-Ocimene	5.765	8.83
Beta-pinen	5.765	8.83
Carvone	9.040	2.84
Limonene	9.040	2.84
Myrcenol	16.874	0.08
Palmitic acid	19.504	0.31
Sabinene	5.765	8.83
Yomogi alcohol	7.854	2.26

RT: Retention tme

Table 5. Influence of volatile oil with various concentrations on growth of MRSA

Concentration of volatile oil (%)	Mean ± SD (mm) of inhibition zone of MRSA
0.5	15.7 ± 1.3
0.25	14.4 ± 1.1
0.125	8.8 ± 0.4
0.0625	4.2 ± 0.6
0.03125	0 ± 0.0

The results of the current study agree with many studies that have proven the effectiveness of wormwood extract as an antimicrobial. Its ability to inhibit bacterial growth, and the inhibition values vary depending on the type of extract, concentration, the studied plant part and t habitat of the plant. Mohamed et al (2015) also showed high extract inhibitory efficacy towards pathological isolates of *S. aureus* from different positions. The inhibitory activity is due to the nature of the substances contained in the *A. herba-alba*, as it contains volatile oils that are considered anti-bacterial substances, which are characterized by their ability to penetrate into the bacterial cell and interfere with the DNA and work to inhibit the enzymes and transport proteins present in the cell membrane. The effectiveness of the materials found in Volatile oils can form a complex with the cell wall and to break down the cell membrane of bacteria.

B- Valuation of volatile oil activity in combination with Augmentin: The synergistic effects of disc diffusion method to evaluation of volatile oil and Augmentin region inhibition by using different concentrations against MRSA was observed. The combination of volatile oil with Augmentin showed MRSA inhibition zone being maximum at 0.5% (Table 6, Fig. 6).

The combinations of Augmentin and volatile Oil have tended to inhibit the formation of biofilm actively to varying degrees.
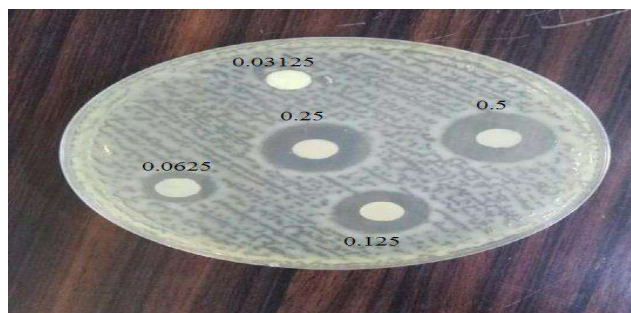
Fig. 5. Effect of different conncetration from Volitale oil on MRSA growth in nutrient agar
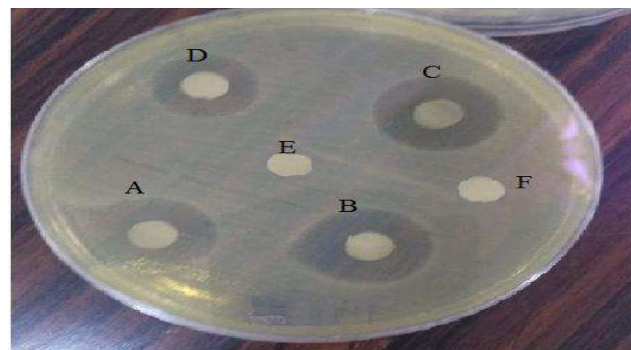
Fig. 6. Combination between volatile oil and Augmentin by disk diffusion method

At lower antibiotic concentrations, an important anti-biofilm effect can be achieved. Various action mechanisms are demonstrated by volatile oil and Augmentin. The synergism may have been caused by a binding reaction between Augmentin and Volatile oil, as Augmentin molecules exhibit groups like hydroxyl and amino groups that can easily react with volatile oil and will assist as a carrier of antibiotics (Ez zoubi et al 2018). In the presence of Volatile oil and the antibacterial activities of Augmentin were increased against gram positive and gram-negative bacteria (Faiq et al 2020).

Determination of minimum inhibitor concentration by micro-titter plate with Resazurin dye: Resazurin is an oxidation-reduction indicator used, especially in various cytotoxicity assays, for the evaluation of cell growth. When reduced to resorufin by oxidoreductases inside viable cells, it is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent. Resorufin is further reduced to hydro resorufin (uncoloured and non-fluorescent) (McNicholl et al 2006). A direct comparison of the MIC determination of the use of antibiotics (Ciprofloxicin and Augmentin) and volatile oils (*A. herba-alba*) has shown the efficacy of this resazurin assay.

A- *A. herba-alba* against *MRSA*: In the present study that measured of antimicrobial effect of Volatile Oil (*A. herba-alba*). MIC was estimated by micro-dilution method against *MRSA* according to (Manandhar et al 2018). The (MIC) of the Volatile oil showed that the MIC value was 1/16 against *MRSA* are shown in Table 7 and Figure 7.

The results showed that the Volatile Oil of the *A. hera-alba* has a high efficacy in inhibiting the growth of *S. aureus* bacteria, as the dilution factor was 1/16 % inhibited the reproduction of *S. aureus* . This result indicate the *A. herba-alba* is able to effectively penetrate the matrix, a protective "shield" around biofilms, and kill persisted cells as well as disrupting established biofilms.

The MIC values showed that the possible activity against MRSA was high in volatile oil (*A. herba-alba*). Other researchers have reported comparable results using *A. herba-alba* against gram positive bacteria (Rania et al 2018). The change of colour was then visually examined. Any color changes were reported as positive, from blue to pink or colorless. As the MIC value for *Staphylococcus aureus* was reported, the lowest concentration at which colour change occurred was taken. It may be linked to the effect of (*A. herba-alba*) causing this lipophilic small molecular weight molecule to penetrate and destabilize the MRSA cytoplasmic membrane leading to nutrient and energy depletion and inhibitory for enteric bacteria growth, but showed neither external membrane-disintegrating activity nor intracellular ATP depletion (Md Shahadat et al 2018).

B- Ciprofloxacin and Augmentin against *MRSA*: MRSA biofilms produced in a 12-well microtiter plate and 2-well control for 48 h at 37°C were treated with different concentrations of Ciprofloxacin and Augmentin for 24 h. All biofilm biomass was quantified using Resazurin dye that stains all components of biofilms (Lu et al 2014). Up to 0.0625 µg/ml Ciprofloxacin and 32 µg/ml Augmentin MICs of antibiotics were unable to disrupt MRSA biofilms. While, 0.125 µg/ml ciprofloxacin and 64 µg/ml Augmentin MICs of antibiotics removed 50% of biofilm biomass, then 0.25 µg/ml Ciprofloxacin and 128 µg/ml Augmentin MICs of antibiotics completely disassembled the biofilm. These findings suggest that antibiotics are capable of effectively penetrating the matrix, a defensive "shield" around biofilms, destroying persistent cells and disrupting existing biofilms.

Table 6. Combination between volatile oil and augmentin by disk diffusion method

Con. of volatile oil (%)	Con. of Augmentin	Zone diameter (Mean ± SD)
0.5	256	21.2 ± 1.0
0.25	128	19.7 ± 0.9
0.125	64	14.3 ± 0.9
0.0625	32	9.6 ± 0.6
0.03125	16	0 ± 0.0
	Control	

Table 7. Volatile oil (*A. herba-alba*) serial dilution used and MIC calculated to MRSA in present study

Antimicrobial	Bacterial type	Protocol of serial dilution
A. herba-alba	MRSA	1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and 1/256

Fig. 7. MIC of *A. herba-alba* in *MRSA*, with MIC=1/16

Fig. 8. MIC of Ciprofloxacin in MRSA, with MIC=0.25 µg/ml

Fig. 9. MIC of Augmentin in MRSA with MIC=128 µg/ml

Estimation of *A. herba-alba* activity in combination with antibiotics (Ciprofloxacin and Augmentin) by checkerboard method: The reduction in the antimicrobial MIC can be caused by the addition of volatile oils to antibiotics and the maximum effect has been observed with Ciprofloxacin and Augmentin (Hatem et al 2018). It is important to find methods to enhance the efficacy of antibiotics**.** Improving the diffusion of antibiotics through bacterial membranes and/or hindering efflux pumps in gram-positive bacteria as a general resistance mechanism (Limaverde et al 2017). In order to study whether volatile oils (*Artemisia herba-alba*) and other antibiotics in combination generate higher inhibition by synergistic, additive or antagonistic interaction and enhance antibiotic activity against gram positive bacteria, checkerboard studies were carried out. Checkerboard analyses of gram-positive bacteria revealed only synergistic profiles when the combination of volatile oils with selected antibiotics was not antagonistic at the measured concentrations, and additive effects were observed. Synergistic effects between the combination of *Artemisia herba-alba* oil and (Ciprofloxacin and Augmentin) against MRSA were observed. The MIC values for the volatile oils, antibiotics and the FIC index values for their combinations are shown in Table 8. The MICs for the individual antimicrobials were used in the formulae to calculate the FIC indices. *A. herba-alba* oil with Ciprofloxacin and Augmentin showed synergistic effects against *MRSA*. Combination therapy is an effective technique for pathogens, as synergistic. Interactions can theoretically improve

effectiveness, decrease toxicity, recover more rapidly, prevent resistance from developing, and have a wider variety of activities than monotherapy regimens (Salwa et al 2020). Plant Volatile oils have many biological functions, including antihypertensive, antidiabetic, antiviral, antibacterial, antioxidant and non-phytotoxic compounds (Ali et al 2019). In Libya, this plant is historically used by indigenous people as an anthelmintic by drinking after boiling the aerial parts in water and is often used after surgery to avoid wound infection by smelling the plant after heating it in olive oil (Salwa et al 2020).

Molecular Approaches

Extraction, concentration and purity valuation and bands detection of genomic DNA: The development of molecular detection methods based on DNA amplification had been stimulated by the discovery of *S. aurues* (MRSA). In this analysis, a total of 54 bacteria identified as strong producers of biofilms by recognizing and defining PCR virulence genes. The extraction strategy utilizing Promega kit was fruitful for extraction of the genomic DNA from all *S. aureus* isolates. The nanodrop spectrophotometer demonstrated amount and quality of the extricated DNA. The concentration varied from 52.8 ng/µl to 739.6 ng/µl, while the purity (OD 260nm / OD 280nm) ranged between 1.78-2.0**.** The effective PCR method depends on of DNA extraction with high immaculateness and concentration (Dibbern et al 2015, Chloe et al 2020). The virtue of DNA (A260/A280) must run between 1.8 and 2.0; the proportion with esteem higher than 2.0 implies that the DNA test is contaminated with RNA

Table 8. Influence of the combination of antibiotics and volatile oils on growth of MRSA

Bacterial name	Antibiotic name	FIC Antib.	FIC Vol. oil	FICI (∑FIC)	Interpretation
MRSA- 47	CIP	0.125	0.0156	0.141	Synergistic
MRSA- 47	AMC	0.32	0.0313	0.351	Synergistic

Table 9. Distribution of MRSA gene among 54 *S. aureus* isolates from different sources

Isolates sources	Molecular detections											
	mecA		mecC		cap5		cap8		pvl		fnA	
	+	-	+	-	+	-	+	-	+	-	+	-
Blood (14)	14	0	14	0	11	3	12	2	9	5	12	2
Wound (10)	10	0	10	0	9	1	8	2	7	3	8	2
Urine (8)	8	0	8	0	7	1	7	1	6	2	7	1
Abscess (12)	12	0	12	0	11	1	12	0	8	4	10	2
Vaginal s. (5)	5	0	5	0	4	1	4	1	3	2	5	0
Throat (5)	5	0	5	0	4	1	4	1	3	2	5	0
Sum +ve	54		54		46		47		36		47	
+ve %	100		100		85.18		87.04		66.7		87.04	

and the proportion with esteem less than 1.7 proposes that
the DNA test is sullied with proteins (Al-Talib et al 2013,
Theyab and Abed 2018). For 25 µl PCR response, the DNA
concentration must be higher than 30 ng of microbial
genomic DNA (de Bruin and Birnboim 201, Theyab and Abed
2018). The 54 of *S. aureus* confines was DNA extraction and
PCR test for recognition of (mecA, mecC, cap5, cap8, pvl and
fnA). Each quality was meant by a single band within the
identical locale of the DNA step. The *S. aureus* qualities of
this ponder were appeared positive comes about in 54
(100%) of confines.

Polymerase chain reactions (PCR):   Distribution of all
detected genes in this study are presented in Table 9.

Detection of *mecC* gene using uniplex-PCR technique:
The *mecC* gene was presented in all isolates (100%)
(Table 9) with PCR product sizes 304bp (Table 1, Fig. 12).
One major issue is where molecular discovery of mecC is
utilized to recognize or affirm MRSA. Research facilities
utilizing this approach, most frequently PCR, will ought to
consider joining widespread mec gene primers able to
intensify both mecA and mecC or the addition of mecC-
specific primers. This last-mentioned alternative has the
good thing about separating mecC MRSA, in this manner
encouraging their reconnaissance and the separation of
strains for assist characterization. Different adjusted PCR
measures have been created to distinguish and/or separate
mecC MRSA, and many commercial PCR-based assays are
being, or have been, modified to include *mecC* detection
(Stegger 2011, Becker 2013). Commercial slide
agglutination tests for mecA-encoded PBP2a will to
misidentify mecC MRSA as being methicillin-susceptible.
These tests may be altered in due course to distinguish mecC
MRSA, but right now the utilize of commercial slide
agglutination tests alone will create false-negative comes
about for these strains. Strains found to be phenotypically
safe but mecA and/or PBP2a-negative are possibly mecC
MRSA, and mecC. PCR would be justified to affirm this.

**Detection of cap5 and cap8 genes by using PCR
technique:** *S. aureus* strains have developed several forms
of virulence factors; capsular polysaccharides (CP) forms 5

and 8 are the prevalent types of CP among isolates of *S.
aureus* (Tarek et al 2020). A PCR method for the detection of
capsular *S. aureus* isolates has been established. The PCR
method was used to analyze all strains used in the current
analysis. Therefore, all *S. aureus* isolates were characterized
in our sample by the detection of unique genes for cap5 and
cap8. The PCR method allowed 100% of strains to be
genotyped, and all strains carried both cap5 (85.18% of
cases) and cap8 (87.04% of cases) (Table 9) with PCR
product sizes 518 bp (cap5) (Fig. 13) and 834 bp (cap8) (Fig.
14). The findings showed that cap8 was more prevalent than
cap5; this result was consistent with another study Tarek *et
al.,*2020 that recorded a higher percentage of cap8 than cap5
in isolates *S. aureus* (87.04 and 85.18 %, respectively).
Another research in Nigeria showed that 94 and 6 % of *S.
aureus* isolates were present with cap8 and cap5
respectively (Ayepola et al 2015).

Real Time-qPCR: Total RNA was reverse-transcribed to
complementary DNA (cDNA) using the GRS cDNA Synthesis
Kit (Grisp) for the quantification of 16S rRNA by qPCR.
Specifically, by using random primers, 100 ng of total RNA
was reversely transcribed into 10 µL of reaction volume.
Samples were incubated for 5 min at 65 °C, for 60 min at 37
°C, and for 10 min at 70°C. Primers, 16S rRNA specific,
household gene, and *S. aureus* virulence-related gene. The
qPCR reaction was carried out by mixing together 5 µL of the
SYBR Xpert Fast SYBR (Grisp) master mix, 2 µL of 1:100
dilute cDNA, 0.5 µL of forward and reverse primes (5 µM) and
water up to 10 µL of total volume. No reverse transcriptase
(NRT) and no template control (NTC) were included to verify
the reaction mixtures were DNA and other contaminants-
free. By the dilution method, the efficiency of the primers
used was measured (França et al 2011). A CFX 96 (Bio-Rad)
with the following cycle parameters was used to conduct the
qPCR run: 95 ° C for 3 minutes, 40 cycles for 5 s at 95°C and
60°C for 20 s. Melting curves for unspecific products or
primer dimer formation were analyzed by qPCR products. To
measure the 16S rRNA of *S. aureus* and the ref mRNA in
each sample, the qPCR was applied independently. By using
the delta Ct method ($2^{\Delta Ct}$), a variant of the Livak method,

Table 10. MRSA isolate gene expression values of *sarA* gene

Bacterial isolate	Anti-bacterial	sarA	16SrRNA	ΔC_T	$\Delta\Delta C_T$	Fold change
MRSA	Before treat	16.86	15.73	-1.13	0	1.000
	Cip-treat	21.14	22.40	1.126	2.39	0.191
	V. oil-treat	16.27	16.55	0.28	1.41	0.376
	V. oil and Augmentin	9.68	10.84	1.16	2.29	0.204

Before treat: isolates *MRSA* before treated with any antibacterial agent. Cip-treat: isolates *MRSA* after administration with Ciprofloxicin. V. oil-treat: isolates *MRSA*
after Volatile oil is treated. V. oil and Augmentin: isolate *MRSA* after treated with Volatile oil and Augmentin in combination. *sarA :* Target gene. *16SrRNA*:
Housekeeping gene

where $\Delta C_T = Ct_{\text{target gene}} - Ct_{\text{reference}}$, the normalized gene expression was determined. The relative fold increase of the *sarA* gene was calculated using 16S rRNA as the reference gene by applying the Pfaffl equation (Pfaffl 2001).

Extraction and quality of RNA: RNA was extracted from MRSA isolates before and after treatment with subMIC of Ciprofloxacin, Volatile oil (*A. herba-alba*) alone and combination Volatile oil with Augmentin. All sample RNA was extracted with GENEzol TriRNA purification kit. The concentration ranging between 106-218 ng/µl and the purity fluctuated from 0.94 to 1.82.

Ciprofloxacin and *A. herba-alba* volatile oil) effects, with combination Volatile oil and Augmentin on gene expression of *sarA* in *MRAS* isolates: The findings
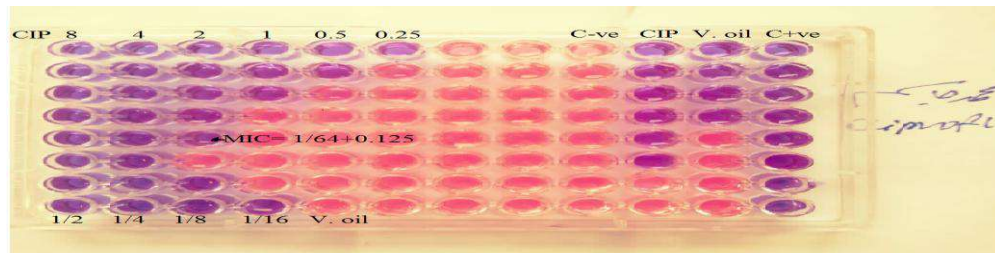
Fig. 10. Checkerboard method used to achieve the fractional inhibitory concentration index (FIC index) of MRSA= 0.141, to evaluate combinations of volatile oil- Ciprofloxacin , MIC for Ciprofloxacin (0.125) + volatile oil (0.0156)
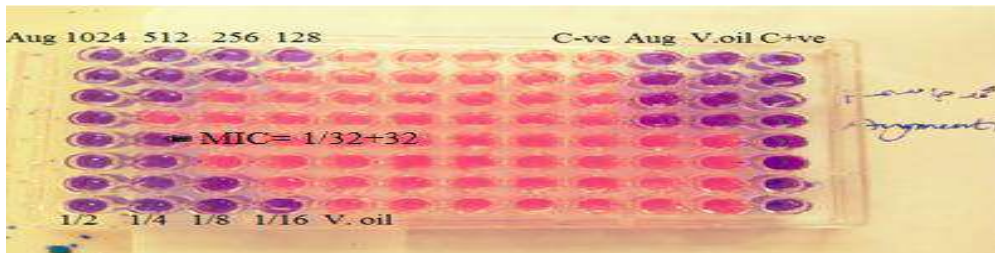
Fig. 11. Checkerboard method used to achieve the fractional inhibitory concentration index (FIC index) of MRSA = 0.351, to evaluate combinations of volatile oil with Augmentin, MIC for Augmentin (0.32) + volatile oil (0.0313)
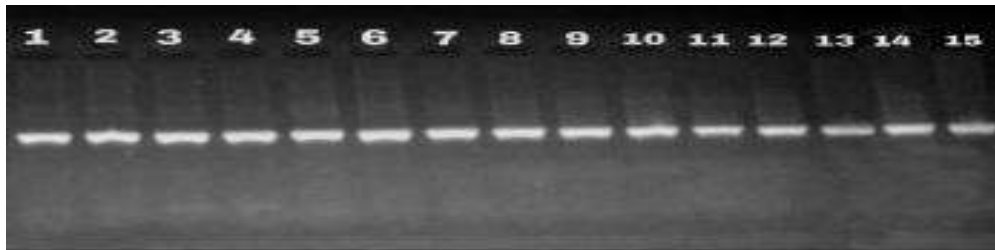
Fig. 12. Genomic DNA bands of some isolates [agarose 1.5 %, TAE buffer (1X), 1 hr,70 V and stained with Red safe stain
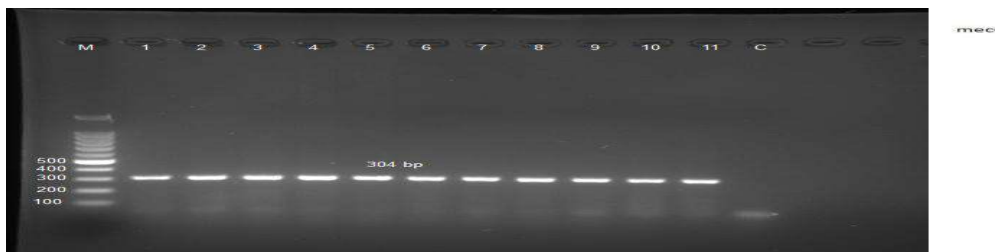
Fig. 13. 1.5% agarose gel electrophoresis of *mecC* gene with end PCR product size (304 bp) of some tested isolates. [1.5-2 h, 70 V]; M(bp): DNA sizes marker

showed a down-regulation of the sarA expression in *MRSA* isolates after treatment with Ciprofloxacin at sub-MIC(0.25 µg / ml), in sub-MIC volatile oil (1/16 µg / ml), in sub MIC volatile oil (1/16 µg / ml), in sub-MIC volatile oil combination with Augmentin (1/16 + 256 µg / ml). The results of this study showed a difference in the degree of expression of Ciprofloxacin, Volatile Oil Alone and the combination of volatile oil and Augmentin stress compared with the regulation of *sarA* in *MRSA* isolates after treatment.

Estimation gene expression of *sarA* gene of *MRSA*: The expression level (fold change) of the *sarA* MRSA gene ranged from 0.191 to 0.204. These findings showed that the level of sarA gene expression in MRSA isolate decreased compared to control isolate compared to Ciprofloxacin, Volatile oil alone and the combination of volatile oil with Augmentin at sub-MIC. These findings showed that, compared to control isolate, Ciprofloxacin, volatile oil alone and the combination of volatile oil with Augmentin at sub-MIC decreased the level of *sarA* gene expression in MRSA isolate and the amplification curves of 16SrRNA (Housekeeping) gene Ct sample values, amplification curves of *sarA* gene samples showed that the results of *sarA*

gene expression of MRSA isolate prior to sub-MIC therapy of all therapies were 1.000 (Table 10). e *MRSA* isolate gene expression after treatment with Volatile Oil sub-MIC was 0.376, Ciprofloxacin 0.191 and volatile oil combination with Augmentin 0.204.

Based on CIP sub-MIC study, biofilm formation was inhibited by inhibiting *sarA* gene expression, resulting in decreased release of extracellular polysaccharides (Grundstad et al 2019). By affecting gene expression at concentrations of Sub-MIC values, the bactericidal effects of certain antibiotics may be the result of physiological microorganisms. The diffusion into biofilms of ciprofloxacin is based on its charge and that of the biofilm. Ciprofloxacin is positively charged at neutral pH and can penetrate easily into a biofilm with improved activity (Adire et al 2015). The sub-MIC of ciprofloxacin was reduced by inhibiting biofilm growth and microorganism virulence (Gupta 2015). Ciprofloxacin resistance may be due to mutations in the IV genes of DNA gyrase or topoisomerase (Lindren et al 2003). Amplified expression of transporters which avoid the antibiotic from reaching critical concentrations within the cell is also a recognized resistance mechanism. The third mechanism is
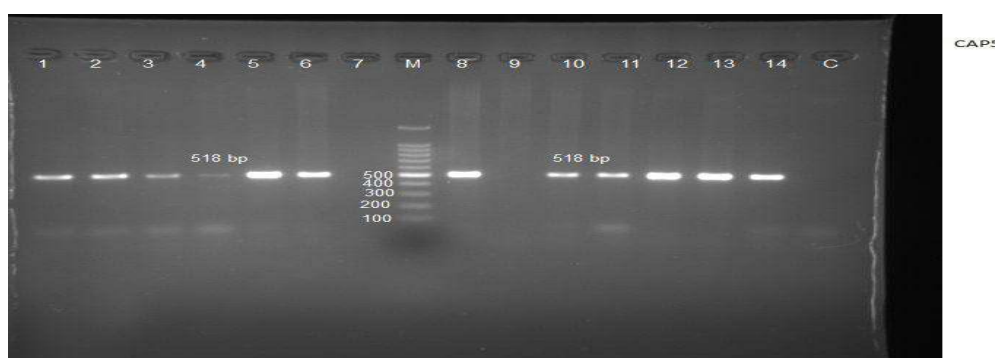
Fig. 14. 1.5% agarose gel electrophoresis of *cap5* gene with end PCR product size (518 bp) of some tested isolates. [1.5-2 h, 70 V]; M(bp): DNA sizes marker

Fig. 15. 1.5% agarose gel electrophoresis of *cap8* gene with end PCR product size (834 bp) of some tested isolates. [1.5-2 h, 70 V]; M(bp): DNA sizes marker

plasmid mediated and produces greater efflux pumps (Jacoby et al 2014).

The volatile oil compound was further tested for its ability to inhibit the development of biofilms against clinical isolates and isogenic (*sarA*). The *sarA* had shown a significantly higher biofilm expression while when treated with Volatile oil the expression was decreased. The result suggested that the Volatile oil affects the biofilm on the negative regulating *sarA*-specific interaction of Volatile oil to down-regulate *sarA* targeted genes expression that establishes biofilm. *SarA* strains treated with Volatile Oil did not cause the formation of biofilms. These findings indicated that by opposing the effects of *sarA*, volatile oil acts and most likely inhibited the binding of *sarA* to the DNA (Arya and Princy 2013), thereby influencing the processes of quorum sensing. Thus, in suppressing the process of cell attachment, proliferation and invasion, the *sarA* selective inhibitor, Volatile oil was very effective. In staphylococcal pathogenesis, including biofilm formation, proliferation and evasion, expression of the various genes including RNAIII, *fnbA* and *hld* plays an imperative role (Arya et al 2015).

In the *S. aureus* culture supplemented with volatile oil, the transcription of RNAIII was significantly reduced and thus modified the temporal expression of various virulence factors. The absence of adherence and biofilm formation in clinical isolates treated with *sarA* and Volatile oil has also indicated that activation of fibronectin-binding proteins promotes adherence to the surface or host epithelial cells is directly regulated by *sarA* (Arya et al 2015). This was substantiated remarkably by the RT-qPCR data where the down-regulation of the gene was observed after treatment during the exponential process, demonstrating Volatile oil interference with *sarA* to demonstrate its transcriptional level response to its target genes. As predicted, the transcript expression was associated with the clinical isolates reduced biofilm formation activity.

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Screening, Identification and Purification of Klebocins LHMAS produced by *Klebsiella pneumoniae* Isolated from Different Source of Iraqi Patient

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Abstract: Klebocins (Bacteriocins) are ribosomal synthesized natural antimicrobial peptides. These peptides are excreted by most bacteria for killing other bacteria. They vary from classical antibiotics in having lethal action against only bacteria which are closely connected to the producing strains. The action of Klebicin includes several steps for killing the sensitive bacteria. Antimicrobial peptides bacteriocins have attracted considerable interest as agents that may subvert many of the problems related to biomaterial-associated infections (BAI). They display antimicrobial activity against bacteria resistant to antibiotics and residing within biofilms. The present study aimed at characterization of purified klebocin isolated from local clinical isolate of *Klebsiella pneumoniae* as well as studying antibacterial effectiveness therefore 40 isolates of *K. pneumoniae* were collected from different hospitals in Baghdad city. The isolates were distributed according to their clinical sources as follows: 16 isolates from urine, 11 isolates from wounds and burns swabs, 4 isolates from Ear swabs, 5 blood and 4 sputum isolates of different ages for patients and males and females for a period of two months from October to December 2019. Klebocin LHMSA prepared in Tryptic Soy broth medium as a crude preparation then extraction by ammonium sulphate and purified by ion exchange and gel filtration chromatography.

Klebsiella pneumoniae is a Gram-negative belongs to the family *Enterobacteriaceae*, is widely distributed in the gastrointestinal, urinary, and respiratory tracts of healthy people. It cause opportunistic infections mainly nosocomial infections, it is a common hospital-acquired pathogen causing severe respiratory infections such as pneumonia. *K. pneumoniae* have different virulence factors which give the bacteria the ability to invade the host, such as capsular polysaccharide, lipopolysaccharide, serum resistance, siderophore production, fimbriae and other factors the production of different kind toxin, enzyme and protein like urease and bacteriocin (Li et al 2014). Bacteriocins are antibacterial proteins produced by bacteria. They are found in almost every bacterial species examined to date, and within a species tens or even hundreds of different kinds of bacteriocins are produced (Hassan et al 2012). They also differ from traditional antibiotics in having a relatively narrow spectrum of action and being lethal only for bacteria which are closely related to the producing strains. As a type of bacteriocins, klebocins have a narrow spectrum of action and are lethal only for bacteria which are closely related to the producing strains (homologous activity) (Chavan et al 2005). On the other hand, it was reported that the antimicrobial spectrum of klebocins of *Klebsiella pneumoniae* was broad and was not limited by the frames of the genus and family (Brooks et al 2004).

Due to the common occurrence of *Klebsiella spp*. and high virulence that cause in the absence of accurate and early detection them, severe damage may lead to the death of the patient. Treatment of *Klebsiella* infections is complicated (Van Giau et al 2019). However, antibiotic resistance properties are the major factor in its pathogenicity that it resists for wide spectrum of antibiotics and specially β-lactam antibiotics.

This is due to the prevalence of infections acquired in hospital which led to the orientation of the research on alternative therapies (Dubey et al 2013). Antimicrobials resistance is the significant topic of this era and there is need to discover alternatives to conventional antibiotics in order to counter the pathogens as well as for protection human health. Therefore, the sole alternative to controller the infection is represented by immunomodulation with an aim to growth the immune potential of the body (Verma et al 2014).

MATERIAL AND METHODS

Isolation of *K. pneumoniae*: All *K. pneumoniae* samples isolates from different source from males and female patients with ages ranging from 10 - 65 years old were collected under aseptic condition in sterile capped containers and transmitted immediately to the laboratory for four months and classified based on their biochemical properties, initially

diagnosed by the phenotypic and microscopic characteristics as well as by biochemical tests of the vitek 2E compat system. All strains were maintained in the same media containing 20% (w/v) glycerol at 80 °C.

Indicator bacteria isolates: Twenty isolates of S. *aureus* and *E. coli* obtained from Biology Department, College of Science, University of Mustansiriyah were identified by Vitek 2 (VITEK 2 Compact 30-bioM-erieux-USA) according to the manufacturer's instruction.

Detection of *K. pneumoniae* bacteriocin: The cultures of *Escherichia coli* and *S. aureus* were grown in nutrient broth for 24 h. 0.5 McFarland Standard was prepared to have the cell concentration of 1×10^8 at wavelength 600 nm. To prepare 0.5 McFarland Standard, 995 ml of 1 % sulphuric acid was mixed with 5 ml of 1% barium chloride and a turbid solution was obtained due to formation of barium sulphate precipitate. For screening Klebocin production by *K. pneumoniae* isolates used method the well-diffusion method (Montville et al 1993)

Agar well diffusion method: Agar well diffusion method procedure was used for screening of isolates for bacteriocin production (Montville et al 1993).For this, Mueller-Hinton (MH) agar plates were prepared and 0.1 ml cultures of *E. coli* and *S. aureus* have which were prepared in accordance with 0.5 McFarland standards were spreader on MH agar plates. The plates were incubated for 20 min at 37 °C. Wells on MH agar plates were made with sterilized borer. The BHI broth culture supernatant of each isolate was obtained by centrifuging the culture and the pH of the supernatant fluids was then adjusted to pH 6.5- 7 with 5N NaOH or 5N HCl to rule out any inhibition by the production of organic acids. Wells were then filled with neutralized supernatant (50-100 μl). The plates were then incubated at 37°C for 24 hrs. After incubation, plates were observed for inhibition zones.

Klebocin LHMSA activity assay: The antibacterial activity of the bacteriocin was determined by the well diffusion method (Mahdi 2017).

Preparation of crude *K. pneumoniae* bacteriocin: *K. pneumoniae* no. 26 was used to produce crude Klebocin LHMSA. Crude Klebocin LHMSA extract (CEE) was heated at 80°C/10 min before starting purification to denaturant proteases and any heat-labile proteins as investigated by Powell et al (2007).

Purification of *K. pneumoniae* bacteriocin: *K. pneumoniae* bacteriocin extracted in Tryptic Soy broth medium as a crude preparation then extra proteins precipitated using ammonium sulfate in two steps and after centrifugation in 10000 x g, 30 min at 4°C. The precipitate was dissolved in 20 mM phosphate buffer then diafilt- eration finally, the protein powder obtained by freeze drying was

purified with ion exchange column (DEAE cellulose column) and sephadex G-100 (Sigma-USA), gel filtration chromatography (Mahdi et al 2019). Concentrated protein at each step was determined by Bradford method.

Antibacterial activity of crud, semi purified and purified klebocin against *E coli* and *S. aureus:* The agar well diffusion method was used to detect antibacterial activity of purified klebocin LHMAS against indicator bacteria, 10 isolates of *S. aureus* and 10 isolates of gram negative bacteria *E coli* at concentration (32 mg/ml) (Lewus et al 1991).

RESULTS AND DISCUSSION

Bacterial isolates: Forty isolates of *K.pneumoniae* from the total samples 95 were collected from different hospitals in Baghdad city. The isolates of *K. pneumoniae* were collected from different clinical samples 40.01% isolates from urine, 27. 5% isolates from burns swab, 4(10.07 %) isolates from ear swabs, 5 (12.5%) isolates from blood and 10.01% isolates from sputum (Fig. 1).

Detection of *K. pneumoniae* bacteriocin production: The study showed the differences inhibitory spectra of *K pneumoniae* isolates by the agar well diffusion method in modified trypton soya medium containing mitomycin c, only 17 isolates of *K pneumoniae* showed antibacterial activity against indicator bacteria, 10 isolates of *S. aureus* and 10 isolates of Gram negative bacteria *E coli* with inhibition zone diameters between (5 and 30) mm (Table 1, 2). The results above show that some bacterial isolates of *K pneumoniae* were able to inhibit almost all the indicator bacteria, while others were active against only few isolates. This suggest that detection of production of klebocin (Mahdi et al 2018) in broth was very important as previously reported for some
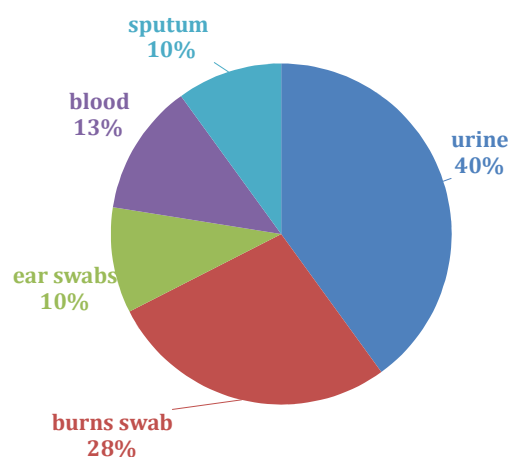
Fig. 1. *Klebsiella pneumoniae* isolates from different clinical samples

other bacteriocins such as curvatc in LHM enrerocin GLHM and Salivaricin LHM (Mahdi et al 2019, Mahdi et al 2020). Klebocin was produced from *K. pneumoniae* (no. 1) in Tryptic Soy broth medium incubation for 18 hours at 37°C at 50cycle/min in shaker incubator in order to obtain suitable aeration and prevent primary metabolites from aggregating. Then Mitomycin C was added as an inducer (mutagenic agent) according to its specific action to induce SOS response in the bacterial cells to express Klebocin, the produced bacteriocin is called Klebocin LHMAS.

These peptides are excreted by most bacteria for killing other bacteria (Elayaraja et al 2014). They vary from classical antibiotics in having lethal action against only bacteria which are closely linked to the producing strains (Riley and Wertz 2002). The action of Klebicin includes several steps for killing the sensitive bacteria. These steps starting with binding of klebocin to specific receptors, which are outer membrane proteins utilized for the entry of particular nutrients then transport through the outer membrane and pass through the periplasm by either the Tol or TonB systems (Lloubès et al 2001). Klebicins would reach their target and their action would do either by forming a voltage-dependent channel into the inner membrane or by utilizing their endonuclease activity on DNA, rRNA or tRNA (Kareem et al 2019). Epidemiological investigations on *Klebsiella's* wild colonization referred to the production of specific proteins that named Klebocins, which had antimicrobial effect (Hammami et al 2007). Klebocins were also chromosomally encoded(Foutus et al 2008) and Kebocins (Bacteriocins) are ribosomal synthesized natural antimicrobial peptides.

Purification of Klebocins LHMAS: To purify Klebocins LHMAS produced by *K. pneumoniae* no.1 under the optimum conditions, after incubation period of induction, the Klebocin was released into Tryptic Soy broth medium and should be

Table 1. Detection of bacteriocin production by agar well diffusion method diameters of inhibition zones of klebocin LHMAS against *S. aureus*

K. pneumoniae LHMAS	S. aureus 1	S. aureus 2	S. aureus 3	S. aureus 4	S. aureus 5	S. aureus 6	S. aureus 7	S. aureus 8	S. aureus 9	S. aureus 10	SUM	Medium ± SD	
K. pneumoniae LHMAS1	0	0	0	0	14	0	11	0	0	0	25	2.5	5.3
K. pneumoniae LHMAS2	0	0	0	0	10	0	0	0	0	0	10	1	3.2
K. pneumoniae LHMAS3	0	0	20	0	0	0	0	0	0	0	20	2	6.3
K. pneumoniae LHMAS4	0	0	23	0	15	0	10	0	0	0	48	A 4.8	8.3
K. pneumoniae LHMAS6	0	0	0	0	0	11	0	0	0	0	11	1.1	3.5
K. pneumoniae LHMAS7	0	0	0	15	0	25	0	0	0	0	40	B 4	8.8
K. pneumoniae LHMAS25	5	0	5	5	0	5	5	0	0	0	25	E 2.5	2.6
K. pneumoniae LHMAS26	0	0	0	0	0	0	5	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS28	7	0	0	0	0	25	0	0	0	0	32	D 3.2	8.0
K. pneumoniae LHMAS29	0	0	0	0	0	0	5	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS30	0	30	0	0	0	0	0	0	0	0	30	3	9.5
K. pneumoniae LHMAS32	5	0	0	0	0	0	0	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS35	0	0	0	0	5	0	0	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS36	0	5	13	0	5	0	0	10	0	0	33	C 3.3	4.8
K. pneumoniae LHMAS37	0	0	0	0	5	0	0	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS39	0	5	13	0	5	0	0	0	0	0	23	F 2.3	4.3
P-value						0.01							

extracted depending on biological assay because using chemical assay would affect its activity (Chhibber and Vadehra 1986). Klebocin was separated by centrifugation with cooling centrifuge at 4°C to keep Klebocin from deterioration according to its proteinaceous configuration (Malik et al 2003). Chemical way as ammonium sulfate might affect Klebocin activity and it considered as a chaotropic agent would result in the incensement of the chaos (Entropy) in water and therefore it would increase hydrophobic interaction of the Klebocin and make it less stable which might interfere with the further filtering process (Cavard 2002), these assays were used for separation done by other researchers only for chemical characterization of Klebocin ignoring its antimicrobial effect after purification (Buffenmyer et al 1976). Firstly, the crude extracellular klebocin was subjected to ammonium sulfate precipitation with 25-50% saturation ratio. This ratio gave specific activity of 1000 Unit/mg proteins. This result indicated that there was an increase in the specific activity compared with that of the crude extract (16.6 unit/mg proteins). Three purification steps the Klebocins LHMAS specific activity (1000 U/mg protein) of ammonium sulfate for Klebocins LHMAS activities used ion exchange chromatography to separate immunity protein which bounded to the column, while the active protein appears in the wash step. This step resulted in partial purification of Klebocin from associated proteins that bounded to the gel.

Purification by DEAE- cellulose chromatography resulted in further increase in Klebocins LHMAS activity. The specific activity of Klebocins LHMAS recorded 3.426 AU/mg protein with 0.068 purification folds and 206 % yield. The second peak (eluted at 0.5 of NaCl) hadn't Klebocins LHMSA activity thus it was neglected.

Gel filtration chromatography technique was the final step

Table 2. Detection of bacteriocin production by agar well diffusion method diameters of inhibition zones of klebocin LHMAS against *E. coli*

K. pneumoniae LHMAS	E. coli 2	E. coli 3	E. coli 4	E. coli 5	E. coli 6	E. coli 7	E. coli 8	E. coli 9	E. coli 10	SUM	Medium ± SD	
K. pneumoniae LHMAS1	20	20	30	11	17	0	23	0	0	121	A 12.1	11.4
K. pneumoniae LHMAS2	0	0	0	0	0	0	18	0	0	18	1.8	5.7
K. pneumoniae LHMAS3	0	0	18	0	0	0	0	0	0	18	1.8	5.7
K. pneumoniae LHMAS4	17	0	21	15	0	0	0	0	0	53	B 5.3	8.7
K. pneumoniae LHMAS6	0	20	0	0	0	0	0	0	0	20	2	6.3
K. pneumoniae LHMAS7	0	16	15	17	0	0	0	0	0	48	C 4.8	7.7
K. pneumoniae LHMAS25	5		5	5		5	5	0	0	25	F 3.1	2.6
K. pneumoniae LHMAS26	0	0	0	0	0	0	5	0	0	5	0.5	1.6
K. pneumoniae LHMAS28	7	0	0	0	0	25	0	0	0	32	E 3.2	8.0
K. pneumoniae LHMAS29	0	0	0	0	0	0	5	0	0	5	0.5	1.6
K. pneumoniae LHMAS30	0	30	0	0	0	0		0	0	30	D 3.3	10.0
K. pneumoniae LHMAS32	5	0	0	0	0	0	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS35	0	0	0	0	5	0	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS36	0	5	13	0	5	0	0	0	10	33	D 3.3	4.8
K. pneumoniae LHMAS37	0	0	0	0	5	0	0	0	0	5	0.5	1.6
K. pneumoniae LHMAS39	0	5	13	0	5	0	0	0	0	23	2.3	4.3
K. pneumoniae LHMAS40	0	0	0	0	6	0	0	0	10	16	1.6	3.5

in the purification of Klebocins LHMAS produced by local isolate *K. pneumoniae* no.1. After purification by ion exchange purification step high final specific activity of purified Klebocins LHMSA reached. The maximum activity of Klebocins LHMAS was observed in the fractions (19-36). The specific activity for these fractions was 5.705 AU/mg protein with 0.051 purification folds and 343% yield (Fig. 2). Enterocin U36 (Kandela, 2006) and bacteriocin NEF42 (Al-Bayati 2008) used gel filtration in the purification of bacteriocin produced from Enterococcus faecalis. Purification of bacteriocin using gel filtration was reported for some bacteriocins such Bifidoadocin (Mahdi et al 2018). A good resolution of different sizes of proteins could be obtained by using this technique, if some criteria follow such as volume of matrix to volume of samples, low flow rate, appropriate column diameter with high length, quality of sample application, and absence of any denaturizing agents in elution buffer (Ersson et al 2011).

Antibacterial activity of crude, partial purified and purified Klebocins LHMAS against *S. aureus* and *E coli* in vitro: Agar well diffusion method was used to detect inhibition effect crude, partial purified and purified klebocin LHMAS at concentration 32Mg/ml against indicator bacteria, 10 isolates of S. *aureus* and 10 isolates of *E coli*. The crude, partial purified and purified klebocin LHMAS possess significant antibacterial activity against all pathogenic isolates contrast with control, and the antibacterial activity of purified klebocin LHMAS was significantly higher than partial purified and crude klebocin LHMAS against all isolates (Fig. 3). S. *aureus* was the most bacterial isolates affected by klebocin LHMAS followed by *E coli*. Mahdi et al (2019) showed that curvatcin LHM from *Lb curvatus* was active

Fig. 3. Antibacterial activity of klebocin LHMAS against pathogenic bacteria (1: Purified klebocin, 2: Partial purified, 3: Crude and 0: Control)

against Gram positive *streptococcus mutants* isolated from mouth. The klebocins action is homologous activity because of harmful action on limited spectrum of bacteria which are closely related to the producing isolates (Kareem et al 2019), and they have a broad antimicrobial spectrum that not limited by the genus and family (Sharga and Turianitsa 1993). Klebocins are divided into two types, A and B, according to cross resistance (Heng et al 2007). Their action on the aim cell, causing pores in the outer membrane of the target cell making ionic channels in it. The other endonucleases klebocins hydrolyze the nucleic acid of the target cell in the cytoplasm.

CONCLUSION

A antimicrobial peptides produced by *K. penumoniae* showed remains active in different conditions and able to inhibit the important pathogens such as*, E. coli* and *Staphylococcus aureus.* Agar well diffusion methods is considered one of the most efficient methods used to investigate the production of Klebocins LHMAS.

Fig. 2. Gel filtration chromatography of purified Klebocins LHMAS

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Efficacy of Paromomycin-loaded Chitosan Nanoparticles as Therapeutic Agent Against *Giardia lamblia*

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Abstract: This study was carried out to evaluate the effect of Paromomycin loaded Chitosan nanoparticles against *Giardia lamblia* infected mice. Chitosan NPs were prepared according to sol-gel methods with some modifications and characterization by AFM, FTIR. After inoculating mice with *Giardia* cysts, treatments were given for four days. The highest percentage of reduction in the *Giardia* cyst count were in the group received Paromomycin loaded Chitosan nanoparticles (100%), while Chitosan NPs was less effective in reducing cysts. The results indicated that many histological alterations were enormously reduced in all groups after treatment, while pathological changes in the intestinal were mild in group after the treatment with Paromomycin loaded Chitosan nanoparticles.

Keywords: *Giardia*, Paromomycin, Chitosan nanoparticles, Mice, Treatment, Efficacy

Giardia lamblia, also known as *Giardia intestinalis*, is a flagellated parasitic microorganism that colonizes and reproduces in the small intestine, causing Giardiasis, affecting hundreds of millions of people, mostly in developing countries. It has been included in the Neglected Diseases Initiative of the WHO. Infection occurs by ingestion of water or food contaminated with the environmental-resistant and infectious cyst stage or by person-to-person contact or person-to-animal contact with cyst-positive feces .After ingestion, the trophozoite stage hatches from the cyst and actively replicates and colonizes the small intestine and the cycle completes once trophozoites differentiate into cysts and are shed in feces (Lalle et al 2018). Waterborne transmission is the main route of spread of Giardiasis, but Food and Agriculture Organization of the United Nations (FAO)/WHO has also ranked *Giardia* as the 11th most important food borne parasite globally (Efstratiou et al 2017). Cysts are spread through drinking water, food, and person-to-person contact (Adam et al 2011).The clinical symptoms of Giardiasis include diarrhea, abdominal pain, malabsorption, and weight loss (Thomas et al 2014). No effective and approved human vaccine against Giardiasis is available and pharmacotherapy is the only available option to treat Giardiasis. Treatment of Giardiasis relies on antimicrobial drug therapy, most commonly with 5-nitroheterocyclic drugs, particularly metronidazole and more recently, nitazoxanide (Granados et al 2012). For the treatment of Giardiasis, oral dosage for adults, adolescents, and children of 30 mg/kg/day for at least 5 days are applied. It is commonly recommended for treatment of Giardiasis, at least during the first trimester of pregnancy because of its perceived safety in that setting (Lalle 2010).Chitosan (CS) is a polysaccharide (partially or fully deacetylated chitin), which has been used in the medical field during the last two decades. It is an important material for the nanoparticles preparation as it is biodegradable and nontoxic (Yien et al 2012). Many studies have been done on Chitosan nanoparticles in treatment of *Giardia lamblia* infection (Said et al 2012), Chitosan NPs increased the effect of ivermectin as anti-filarial drug (Ali et al 2013). Spiramycin loading Chitosan nanoparticles increased its antiparasitic effect on acute *T. gondii* infection (Hanaa et al 2018).

MATERIAL AND METHODS

This study was carried out on 25 mouse from white Swiss mice (males and females) were obtained from the Iraqi center for cancer research and an average age between 4-6 weeks with weight of 18-22gm. The animals placed in plastic cages and fed a private provender were obtained from the same place and provided sterile water for drinking by special bottles with the provision of temperature and proper ventilation. Samples of stool were collected from patients infected with diarrhea from AL- Karama Hospital.

Isolation of *Giardia* Cysts from faeces of Infected Human: Depending on Sheffield and Bjorvatan method of purifying the cyst with some modification. The stool sample was diluted by 1-10 mL of distilled water. The sample was filtered by a nylon cloth, and then took about 4-5mL of the filtered sample and centrifugation 1800 cyclesminutes^{-1} for 5 minutes. The precipitate was diluted 1-10 mL of distilled water and centrifugation 1800 cyclesminutes^{-1} for 5 minutes. Put 4 ml of distilled water to precipitate. This method was restored 3 times. The precipitate was hung in 4 mL distilled

water at 4C until use. Cysts were counted with blood cell count slide (Haemocytometer) to obtain 5000 cell mL^{-1}.

Drug

Paromomycin: Paromomycin was purchased from Pfizer pharma pfe GmbH 250 mg, Germany. Paromomycin was orally given to mice in a dose of 250 mg kg body^{-1} weight once daily. The administration doses of drugs started from the fourth day post – infection (Grosset et al 2011).

Chitosan: Nanoparticles were prepared by chemical method and using the Sol-Gel Method with some modifications (Ghadi et al 2014). Chitosan was dissolved into deionized distilled water and placed in the ultrasonic path for 30 minutes. The pH was adjusted to 12 by NaoH (1N), and placed on the rotor with a magnetic stirrer for 60 minutes at room temperature. Then the pH was adjusted to 4 by HCL (1N), and also placed on the rotor with a magnetic stirrer for 60 minutes at room temperature. Then the pH was adjusted to 7 with HCL (1N), and placed on the rotor with a magnetic stirrer for 60 minutes at room temperature. Capsules of Paromomycin (Humatin) were diluted in saline then put in ultrasonic bath for 30 minutes. Add solution of chitosan nanoparticles to Paromomycin solution molar ratio (1:1) and was stirrer an hour at room temperature.

Characterization of Chitosan Nanoparticles

Atomic Force Microscope: This characterization was used to assess the surface topography of Chitosan nanoparticles by angstrom advanced Inc. SPM-AA300. U.S, use the AFM communication mode. Five drops of Chitosan nanoparticles solution has been added at cover slide. Three-dimensional structure (topography) of the sample surface at a high resolution can be imagined by the reaction between the probe and the forces of the sample. This is accomplished by a raster scan of the location sample with respect to the tip and by recording the height of the probe that corresponds to a constant between the probe – sample interaction. To alter the properties of the sample in a controlled manner, the forces between the tip and the sample can be manipulated. Examples of this include atomic manipulation, local stimulation of cells and scanning probe lithography (Yang 2014).

Fourier Transform Infrared Spectroscopy: After sample homogenization with KBr , the FT-IR spectra of CS nanoparticles, PM, CS-PM nanoparticles were analyzed, under a vacuum at 100 ° C for 48hours. Potassium bromide (AR-grade) was dried and then mixed 100 mg of KBr with 1 mg of sample separately for KBr pellet preparation. Data was collected between 500-4000 wave number/cm. In Shimadzu-IR affinity-I spectrophotometer, Uv spectra were recorded. The spectra are plotted as intensity versus number of waves (Augustine et al 2005).

Experimental design: Twenty-five of healthy mice were grouped into 5 groups, each group contained 5 mice. The first (4) groups were inoculated orally by micropipette with (0.1) ml of prepared inoculum of *Giardia* and their feces were examined daily to confirm the presence of this parasite in the stool. The last group (5) were not infected, kept as a normal control group to compare between healthy tissues and infected tissues.

Group 1: Animals were given (0.1) ml of prepared inoculum of *Giardia* orally and considered as a positive control group.

Group 2: Animals were given Paromomycin (250) mgkg^{-1} orally for 4 consecutive days, as a single dose per day (Grosset et al 2011).

Group 3: Animals were given Chitosan Nanoparticles (50) mgkg^{-1} orally for 4 consecutive days, as a single dose per day (Said et al 2012).

Group 4: Animals were given Paromomycin Loaded-Chitosan Nanoparticles (50) mgkg^{-1} orally for 4 consecutive days, as a single dose per day.

Group 5: Animals were given (0.1) ml of normal saline orally. This group was considered as a negative control group.

Histopathological examination: Histological samples of small intestine were collected from treated and untreated animals after they were sacrificed at the end of the experiment and were kept in sterile containers filled with formalin (10%).

Statistical analysis: The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. The treatment efficacy in this study was measured based on the reduction in cyst excretion for each treatment group compared to the control group and on differences in cumulative cyst excretion. The reduction in cyst excretion was calculated using method of (Etewa et al 2018):

$$\text{Reduction} = \frac{(\text{Mean of infected control} - \text{mean of treated groups})}{\text{Mean of infected control}} \times 100$$

RESULTS AND DISCUSSION

Characterization of Chitosan Nanoparticles

Atomic force microscope (AFM): This was used to know the surface morphology and to determine topography. AFM gives a two and three dimensional image of the surface of nanoparticles at an atomic level (Fadhil et al 2015). The average particle diameter was calculated in nanoscale size. The Chitosan NPs prepared by using sol-gel were studied using (AFM) (Table 1). Surface analysis (AFM) requires good attention because of factors that effect on results such as pollutions (Fig. 2).

Fourier transform infrared spectroscopy (FTIR): FT-IR

measurements were recorded to identify the major functional groups to examine their possible involvement in the production and capping of CSNPs. The range of synthesized CSNPs by using infrared spectrum was 400-4000cm-1 wave number which identifies the chemical bonds as well as functional group in the compound. Figure 2 displays the FTIR spectra sequence for Chitosan NPs, Paromomycin and Chitosan NPs-Paromomycin. In the IR spectrum of paromomycin can observe the stretching of (N-H) and (O-H) groups at 3425.34-3122.54 cm^{-1} and the stretching of C-H group at region 2920.03-2852.52 cm^{-1}. In addition, the absorption band at 1631.67cm^{-1} due to amines compounds groups. Characteristic band at near region 1537.16-1525.59 cm^{-1} due to stretching of N-O group. In band 1112.85 cm^{-1} observe stretch of (C-O) group due to alcohol compound. The peak at 619.11 cm-1is due to the presence of oxygen double bond and is due to metal oxide (Khan et al 2011). The FTIR spectrum of Chitosan NPs clearly shows three bands. The first bands are observed around at 3421.48-3309.62 cm^{-1}, corresponding to the stretch movement of hydroxyl group (O-H) bonded. The second bands are about 1747.39-1649.02 cm^{-1}, referring to (C=O) compound type dimer. The third bands are observed around at 1541.02-1512.09 cm^{-1} related to stretch movement of (N-O) group. The last bands
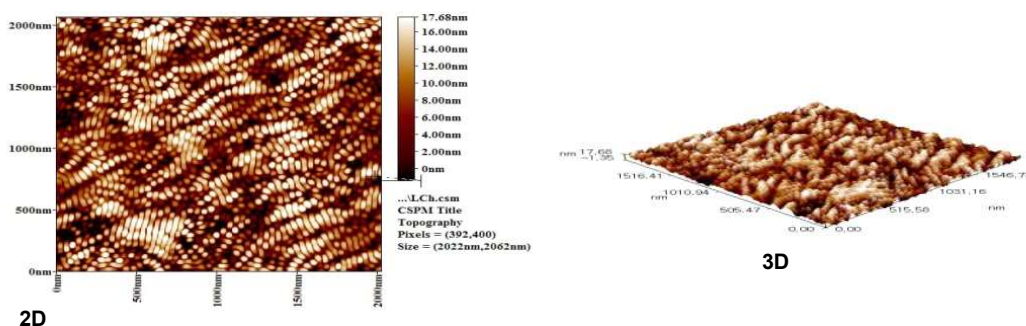
Fig. 1. Atomic force microscopy of Chitosan NP synthesized using sol-gel methods illustrate 2D and 3D topological
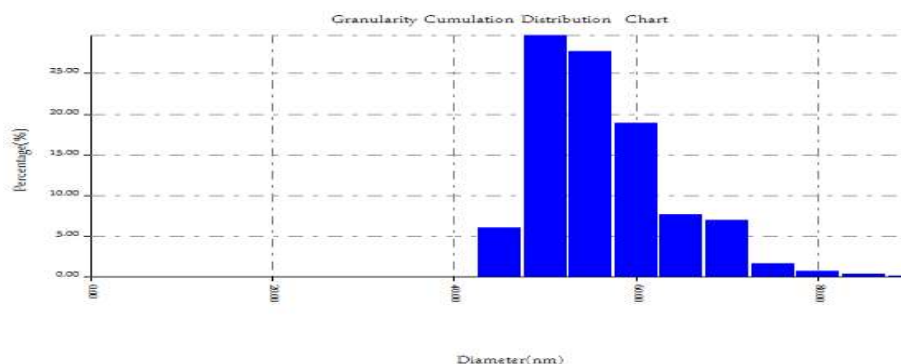
Fig. 2. Average size of Chitosan nanoparticles by AFM

Table .1. Estimation size of chitosan nanoparticles

Samplelch					Code. Sample Code			
Line No .lineno					Grain No.530			
Instrument, CSPM					Date:2020-08-09			
Avg. Diameter.53.91 nm					<=10% Diameter.45.00 nm			
<=50% Diameter.50.00 nm					<=90% Diameter.65.00 nm			
Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
45.00	6.04	6.04	65.00	7.74	90.00	85.00	0.38	99.81
50.00	29.62	35.66	70.00	6.98	96.98	90.00	0.19	100.00
55.00	27.74	63.40	75.00	1.70	98.68			
60.00	18.87	82.26	80.00	0.75	99.43			

are corresponding to the stretch movement of group (C-O) as Aliphatic ether compound (Ali et al 2013). In the IR spectrum of CSNPs-PM (Fig. 3 and 4) the peak at 3436.91 cm^{-1} correspond to (O-H) stretching of alcohol compound and the absorption band of (C=C) stretching of the alkene bond can be observe at 1639.38-1629.74 cm^{-1} and (C-H) bending at

1400.22 cm^{-1}. The peak seen at 1137.92 cm^{-1} were the aliphatic and ether groups.

Parasitological results of faecal examination: In all studied infected groups, mice began to shed cysts with their faeces after four day post infection. Several symptoms were seen, a lose hair coat and ascites with slow motion. Table 2

Table 2. Mean number of cysts count in all studied groups

Group	Mean ± SE				LSD value
	Day 1	Day 2	Day 3	Day 4	
Normal control	0.00 ±0.00 Ad	0.00 ±0.00 Ad	0.00 ±0.00 Ad	0.00 ±0.00 Ac	0.00 NS
Infected non treated	920.00 ±86.02Aa	1000.00 ±90.82Aa	1056.00 ±98.72Aa	1110.00 ±104.16Aa	285.39 NS
Infected treated with paromomycin	650.00 ±58.50Abc	374.00 ±25.02Bbc	182.00 ±16.85Cc	63.60 ±4.35 Dc	98.88 **
Infected treated with ChitosanNPs	779.00 ±26.47Aab	504.00 ±39.06Bb	384.00 ±19.39Cb	280.00 ±16.73Db	80.48 **
Infected treated with Chitosan NPs+paromomycin	574.00 ±22.49Ac	146.60 ±3.67Bc	32.00 ±3.74Cd	0.00 ±0.00Cc	34.62 **
LSD value	144.70 **	134.65 **	134.67 **	139.30 **	---

Means having with the different small letters in same column and big letters in same row differed significantly. ** (P≤0.01).

a-Paromomycin
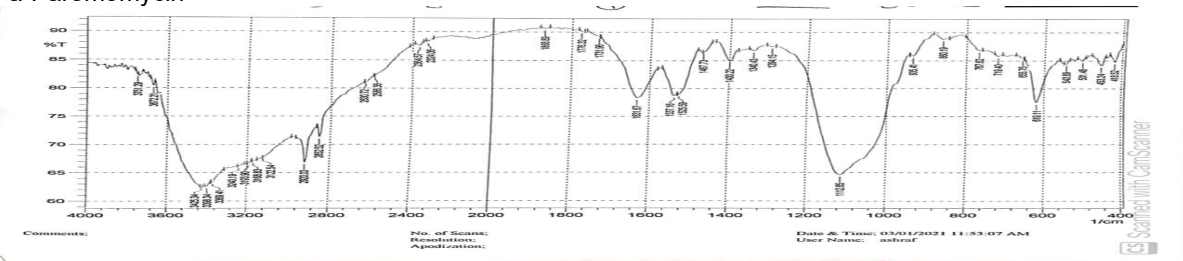
b-Chitosan NPs
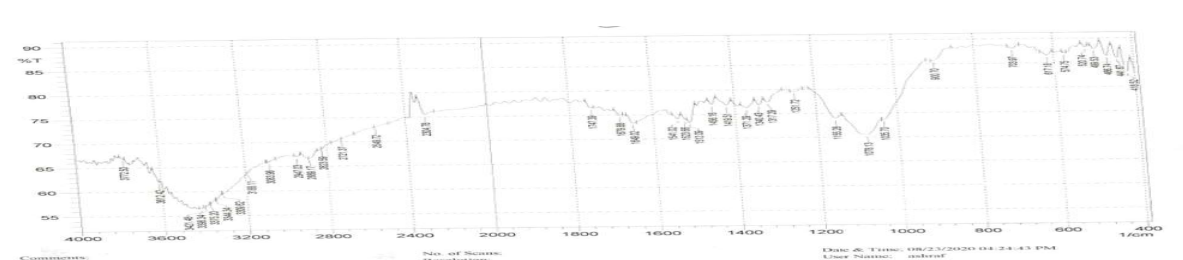
c- Chitosan NPs loaded Paromomycin
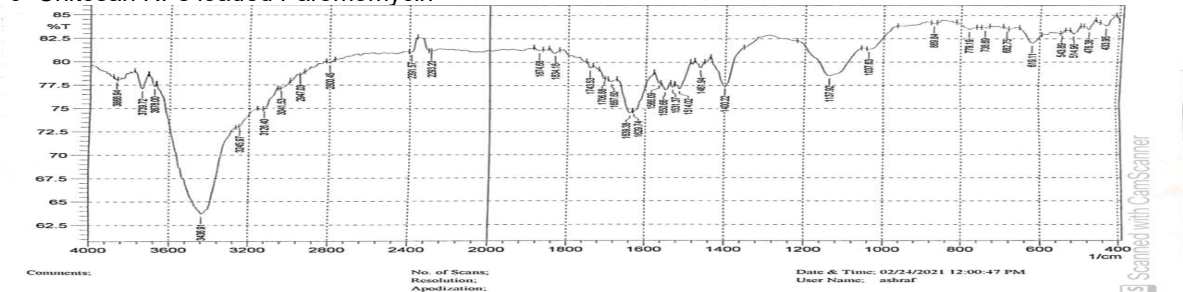
Fig. 2. FT-IR spectra of a- Paromomycin b-Chitosan NPs , c- Chitosan NPs loaded Paromomycin

show the mean number of Giardia cyst in the feces of infected groups of mice in the four days after treatment. Statistical analysis showed significant differences between all treated groups as compared to infected control group. The percent reduction in number of Giardia cyst of treated groups are demonstrated in Table 3.

In the present study, Paromomycin was administered orally in 250 mgkgday^{-1} for four days for treatment of *G. lamblia* , which showed the better effect of paromomycin against Giardia lamblia cyst, the percentage of reduction in number of cyst excretion was 94%. Geurden et al (2006) suggested the efficiency of paromomycin in calves give the therapeutic efficiency of 99% at the day 4 after treatment. Albay et al (2011) reported the efficacy of different doses of paromomycin against *Giardia lamblia* in lambs showing reduction of cyst excretion as 96.6% at the day 4 post treatment .Chitosan in the form of nanoparticles as a carrier for antiparasitic agent to increase their efficacy and decrease the toxicity. The present study indicated that Chitosan in nanoform was least effective of cyst excretion of *Giardia lamblia* by 74%. Said et al (2012) showed a decrease in the number of *Giardia* cyst in treated infected rats as 68.2% on treatment with Chitosan NPs, however reduction of *Giardia* cyst in treated infected was 44.2% on treatment with Chitosan alone. Elgendy et al (2021) observed 63.6% reduction in Chitosan NPs of *Giardia* cyst count in stool. In the current work, Chitosan NPs combined paromomycin, had better effect than alone of decreasing cyst excretion . This combination may enhance the bioavailability and prolong the retention time of paromomycin and maintain its effect on *Giardia lamblia*. Paromomycin is poorly absorbed in the gut and by association with nanoparticles it will increase absorption to intestine(Geurden at el 2006) and the Chitosan nanoparticles is good choice because it has attractive characteristics for drug delivery and formulated nanoparticulate form proved to be effective. Its cationic character and its solubility in aqueous medium have been reported as important properties for the success of this polysaccharide (Grenha et al 2010). However, ability to adhere to mucosal surfaces is considered as the most attractive property (Dasneves et al 2011), leading to prolongation of its presence at drug absorption sites and increasing the drug permeation (Andrade et al 2011). Capacity of Chitosan to augment permeation of macromolecules epithelial through transient opening of epithelial tight junctions has been demonstrated. The polymer is characterized by two mandatory requisites for drug delivery applications which are its biocompatibility and low toxicity (Andrade et al 2011). Elgendy et al (2021) conducted on *Giardia lamblia* that evaluate the efficacy of

loading metronidazole on Chitosan nanoparticles showed the high percentages of reduction in the *Giardia* cyst counts were in group that received loaded drug by (94%).In study Esfandiari et al (2019) used Paromomycin-loaded mannosylated Chitosan nanoparticles against leishmaniasis which affected both stages of the parasite, especially the amastigote. Afzal et al (2019) observed Mannosylated thiolated Paromomycin-loaded   PLGA nanoparticles for visceral leishmaniasis. Hanaa et al (2018) investigated the Spiramycin-loaded Chitosan nanoparticles treatment on acute toxoplasmosis and noticed that there was significantly decrease in the mortality rate of infected mice with RH strain.

Histopathological Study

Small Intestine of normal mice control group: There was normal appearance of intestinal villus, epithelial crypts and tunica as well as normal   appearance of intestinal epithelial cells, lamina propria and muscularis (Fig. 3)

Small Intestine of infected group: Sections of duodenum in infected group showed enteritis which characterized by marked thickening and fusion of duodenal villi that associated with infiltration of mononuclear leukocytes and crypt abscess. The magnified sections revealed figures of epithelial sloughing, with marked hyperplasia of the epithelium and there were crescent-shape trophozoites overlying the mucosa (Fig. 4).

Several degrees of inflammatory changes were seen in the group infected with the *Giardia lamblia* in comparison to section of control group. The duodenal was found to be the site with the heaviest burden in mice group infected with *Giardia lamblia*. Mallakh (2015) found the same effect of *G. lamblia* on small intestine.

Infected group treated with paromomycin: Examination of intestinal sections from group treated with Paromomycin showed mild thickening of villi with normal appearance and cytoarchetecture of villi that revealed marked increase population of goblet cells within villus epithelium and epithelial crypts. On the other hand, the basal part of epithelial crypts revealed hyper activities of Paneth cells (Fig. 5).

The group treated with Paromomycin showed partial improvement of moderate to mild thickening of villi with increase of goblet cell and Paneth cells comparing with infected untreated group this is due to the poor absorbance after oral dosing (Geurden et al 2006). This leads to a weak ability of healing the infected tissues. Augustine et al (2005) also observed the same results.

Infected Group Treated with chitosan nanoparticle: This revealed normal appearance of duodenal mucosa, normal thickness of duodenal villi, normal cytoarchetecture of villus cells and basal part of epithelial crypts revealed hyper activities of Paneth cells (Fig. 6).

Fig. 3. Section of duodenum (normal control group) showing normal appearance of intestinal villi (V), epithelial crypts (Asterisk) and tunica muscularis (M)- H and E stain 100x
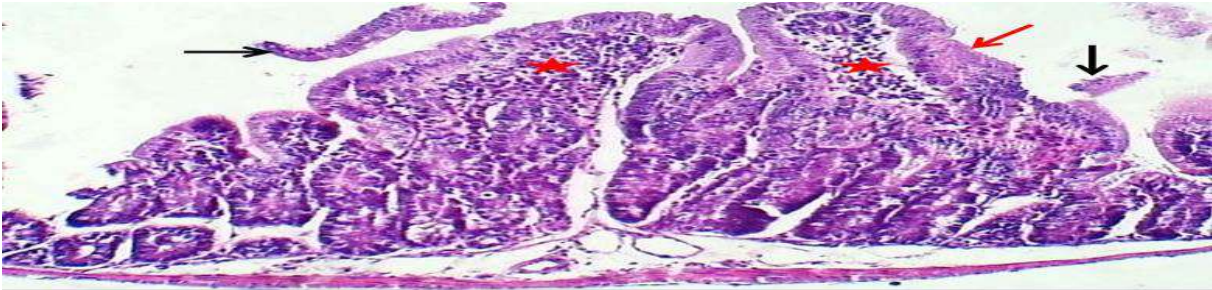
Fig. 4. Section of duodenum (infected group) shows enteritis characterized by epithelial sloughing (Black arrows), mucous degeneration (Red arrow) and thickening and fusion of villi associated with infiltration of mononuclear leukocytes (Asterisks)-H and E stain.40x
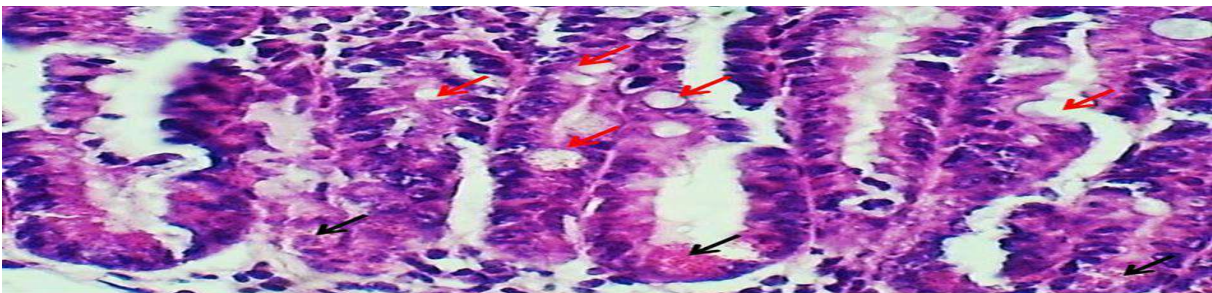
Fig. 5. Section of duodenum (paromomycin group) shows normal appearance with increase of goblet cells (Red arrows) and hyper activity of Paneth cells (Black arrows).-H and E stain.400x

Fig.6. Section of duodenum (chitosan nanoparticles group) shows normal appearance of villi (V) and crypts (asterisks) cytoarchitecture- H and E stain.100x

Table 3. Percentage reduction in number of *Giardia* cysts

Groups	Day 1	Day 2	Day 3	Day 4
Infected treated with Paromomycin	29	62	82	94
Infected treated with Chitosan NPs	15	49	63	74
Infected treated with Chitosan NPs + Paromomycin	37	85	96	100
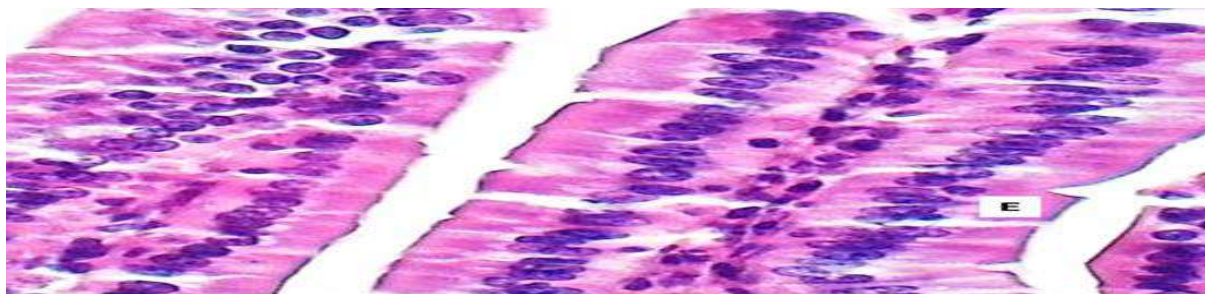
Fig. 7. section of duodenum (Chitosan nanoparticles+ Paromomycin) shows normal appearance of villi epithelium (E) and core cytoarchetecture.-H and E stain.400x
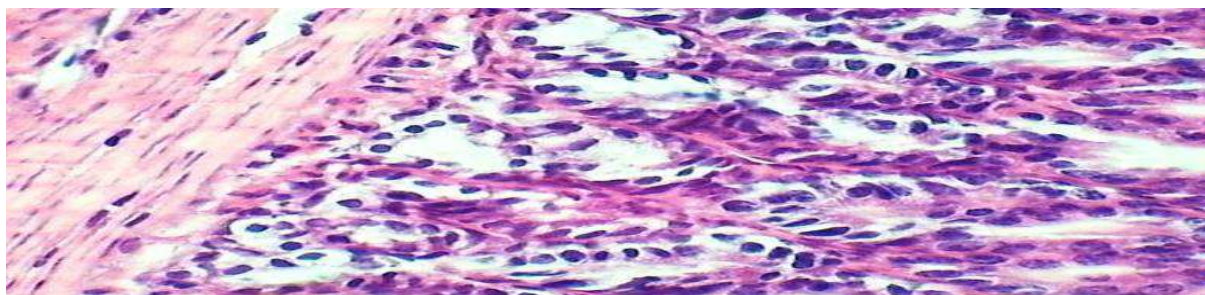
Fig. 8. Section of duodenum (Chitosan nanoparticles+Paromomycin) shows normal appearance of epithelial crypts cytoarchitecture- H and E stain.400x

Intestinal section of mice treated with Chitosan nanoparticles showed high degree of healing of mucosa and normal thickness of duodenal villi, normal cytoarchetecture of villus cells. Yousef et al (2020) also reported that Chitosan nanoparticles has a therapeutic effect against *Giardia* infection in hamsters with best recovery in intestinal pathology. Ahmed et al (2016) reported that Chitosan NPs showed significant effect against local GIT diseases and intestinal disinfection. However, Wardani et al(2018) found that chitosan nanoparticles improved GIT mucosal epithelial cells necrosis caused by toxic agents. HuYl et al (2011) reported that Chitosan nanoparticles produced cellular oxidative stress by increasing reactive oxygen compounds associated with cytotoxicity to various cells including mucosal epithelium.

Infected group treated with Chitosan Nanoparticles+ Paromomycin: The Figure 7 and 8 showed normal appearance of duodenal mucosa, normal thickness of duodenal villi, normal cytoarchitecture of villus cells and the basal part of epithelial crypts revealed hyper activities of Paneth cells.

In the current study, histopathological finding of sections from infected group and treated with Chitosan NPs loaded with Paromomycin seemed to ameliorate on intestine tissue and that was evidenced by partial improvement of histopathological changes more than those of treated with Paromomycin ,that showed re-shape of normal duodenal

mucosa, normal thickness of duodenal villi, and normal cytoarchetecture of villus cells. Albay et al (2011) recorded therapeutic effect of Chitosan Nanoparticles and Metronidazole in treatment of experimentally Giardiasis infected hamsters, showed loading of Metronidazole on Chitosan nanoparticles enhanced therapeutic effect of both Chitosan NPs as well as Metronidazole. The assessment of Spiramycin-loaded Chitosan nanoparticles treatment on acute and chronic toxoplasmosis in mice noticed that Spiramycin loaded Chitosan NPs significantly decreased the mortality rate of infected mice with both strains (Etewa et al 2018). Moreover, loading of bee venom on Chitosan NPs enhanced its efficacy against amoebiasis (Aber et al 2017).

CONCLUSIONS

The combined therapy gave best results than single and was detected in the group of mice treated with Paromomycin combined with Chitosan NPs, reduction rate was 100%. Histopathological examination showed marked healing of intestinal mucosa after treatment with Paromomycin combined with Chitosan NPs and Chitosan NPs alone. Loading Paromomycin on Chitosan nanoparticles enhanced therapeutic effect of both Chitosan NPs as well as Paromomycin.

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Production and Effect of Killer Toxins by *Saccharomyces cervisiae* on *Pythium sp.* in Vitro

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Abstract: The study was achieved by collecting 12 isolates of yeasts obtained from different sources including soil, leaves, flowers, bark and fruits of some plants, in addition to dry yeast available in the local market with different brands. Diagnostic results depending on morphological, cultural and biochemical tests showed that only five isolates belong to *Saccharomyces cerevisiae*, indicated by S1, S2, S3, S4 and S5. The antagonistic activity between *S. cerevisiae* isolates showed the ability of S2, S3, S4 and S5 isolates to produce the killer toxin against S1 isolate with inhibition zones diameter 25.0, 20.5, 16.0 and 18 0 mm, respectively. The sensitive isolate didn't achieve any inhibitory effects against other isolates (S2, S3, S4 and S5), was was diagnosed as a sensitive isolate. Antagonistic ability between *S. cerevisiae* isolates and *Pythium sp.* by dual culture technique, the yeast isolates showed ability to inhibit the growth of 31.06%. The crude toxins of T2, T3 and T5 isolated from *S. cerevisiae* isolates (S2, S3 and S5) respectively, showed the toxins have high Inhibitory activity against S1 isolate, where the rates of the inhibition zones (13.50, 16.50 and 15.00 mm, respectively) formed when it grown on the yeast extract peptone Dextrose-Methylene Blue medium, with a significant difference. The crude toxins T2, T3 and T5 showed a disparity in the impact of inhibiting the pathogen *Pythium sp.* T2 toxin showed the maximum inhibition (46.36%), while T3 toxin indicated the lowest inhibition percentage (27.27%). The estimated proteins of crude toxin solutions T2, T3 and T5 in quantities of 0.130, 0.093 and 0.081 mg / ml, respectively, showed a varied inhibition activity of toxin against *Pythium sp.* A positive relationship between the amount of protein and the inhibition activity was observed. The crude toxins purified in three stages that included: precipitation with 80% ammonium sulfate, gel filtration chromatography using a Sephacryl 6B column, and electrophoresis using Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). The results also showed that the molecular weights of the pure toxins ranged between 18 and 22 kDa.

Keywords: *S. cerevisiae*, Killer toxin, Killer activity, *Pythium* sp.

The killer toxins were first discovered in *Saccharomyces cerevisiae* when described the secretion of molecules by certain isolates of *S. cerevisiae* inhibited the growth of other strains of yeast (Bevan and Makower 1963). They classified *S. cerevisiae* strains into one of three phenotypes: killer, sensitive and neutral, when killer and sensitive cells are grown together in the same culture medium, a large proportion of sensitive cells are killed, while neutral cells do not have the ability to kill sensitive cells and are not killed by the effect of killer cells. The killer factor releasing from killer cells that causes dead of sensitive cells was called the killer factor. Wood and Bevan (1968) identified secreted molecules as proteins, which were called "killer factors" or "killer toxins" or "killer proteins", also called zymosins and mycosins, and the producing strain was called "killer yeast" (Acun 2003). Yeast killer toxins are proteins or glycoproteins with low molecular weight (El-Banna et al 2011, Buzdar et al 2011). *S. cervisiae* produce killer toxins K1, K2 and K28. Killer toxin K1 has a molecular weight of 19 kDa and consists of two subunits: α (molecular weight 9.5 kDa) and β (molecular weight 9.0 kDa). The killer toxin K2 has a molecular weight of 21.5 kDa and it consist of two subunits α and β, and the killer toxin k28 consists of the two subunits α and β and has a molecular weight of (10.5 and 11) kDa, respectively (Magliani et al 1997, Dignard et al 1991).

Pythium species are ubiquitous in soil and in water, distributed worldwide, and with very diverse host ranges. They include some of the most important and destructive plant pathogens, causing losses of seeds, pre-emergence and post-emergence damping-off, rots of seedlings, roots, or basal stalks, decays of fruits and vegetables during cultivation, storage, transit or at the market, and serious damages of a wide variety of crops (Abdelzaher et al 1997). Nowadays, a great attention focusses on the possibility of using the natural and safe agents for control phytopathogenic fungi. *Saccharomyces cerevisiae* became in the last few decades a positive alternative to chemical fertilizers safely used for human, animal and environment (Omran 2000). Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi (Whipps 2001). So, application of yeasts as biocontrol agents acts as a new trend against different pathogens. Potential use of killer yeasts as biocontrol agents of soil-borne fungal plant pathogens such as *Botrytis cinerea* and *Fusarium* sp.,

Rhizoctonia solani and *Pythium aphanidermatum* were recent investigation (Issa and Saleh 2012). The aims of this study were production and purification of killer toxins produced by *S. cerevisiae* and investigate the killer activity of toxins against *Pythium* sp.

MATERIAL AND METHODS

Yeast isolates: A diagnosed isolate of *S. cerevisiae* was obtained from the College of Agriculture - University of Baghdad. *S. cerevisiae* was isolated from soil and leaves of plants according to Rosa-Margi et al *(*2011). *S. cerevisiae* isolated from fruits of some plants including apples, dates, grapes, pears (Chanchaichaovivat et al 2007). Three isolates of dry yeast available in the local markets were collected (Altunsa, Instant yeast and Mil Brand). *S. cerevisiae* isolates identification based on their microscopic (Ellis 1994) and culture characteristics (Kurtzman and Fell 1998) and biochemical tests (Barnett et al 1990).

Culture media: YEPD broth (1.0% yeast extract, 2.0%peptone, 2.0% glucose, 0.1M citrate-phosphate buffer pH 5.0) was used for killer toxin production. YEPD-MB agar (YEPD containing 0.01% methylene blue and 2.0% agar) was used for killer phenotype determination.

Isolation of pathogenic fungus *Pythium sp*.: *Pythium sp*. isolated from the soil by the Soil dilution plating method mentioned in Tojo (2017). *Pyhtium sp*. identification based on their microscopic and cultural characteristics (Watanabe 2002).

Interaction between *S. cerevisiae* isolates: The killer yeasts detected on YEPD agar 10 ml of YEPD broth inoculated with the tested yeast isolates, and the tubes incubated for 24 hours at 26 ± 2°C in shaker incubator at 120 rpm. 1.0 ml of the yeast suspension was added to a 50 ml Erlenmeyer flask containing 20 ml of YEPD-MB, mixed well, and poured into Petri plates. 6 mm discs from yeast cultures grown on YEPD for 48h were placed on the surface of YEPD-MB medium following incubation at 26 ± 2°C for 3 days. The isolates were considered mycocinogenic (killer toxin producers) when an inhibition halo and blue zone were produced around the isolate, indicating cell death of the sensitive strain (Soares and Sato 1999, Dabhole Joishy 2005, Rosa-Margi 2010).

Antagonistic activity between *S. cerevisiae* isolates and *Pythium sp.*: The potential of *S. cerevisiae* isolates against *Pythium sp.* evaluated using a Dual Culture Technique (Ghosh et al 2013). Six mm diameter of mycelial colony from the margin of actively growing colony of *Pythium* sp. placed on one end of Petri plate and yeast suspension was streaked onto the opposite end of the same plate containing 25 ml of sterilized PDA medium. PDA plates inoculating only with 6mm diameter of mycelial colony from *Pythium* sp. culture was employed as control. The cultures were incubated at 26 ± 2 C until Plate fullness in control. Each treatment was performed with three replicates. The degrees of antagonism were calculated by the formula:

$$I = (R1 - R2)/R1 \times 100$$

Where, R1=Radial growth *Pythium sp*. in control plate. R2= Radial growth of *Pythium sp.* in dual culture plate interacting with yeast.

I=Percent of Inhibition of Radial growth *of Pythium sp*

Production of crude killer toxin: The killer toxins were produces by *S. cerevisiae* isolates following the method of Dabhole and Joishy (2005) and Soares and Sato (2000).

Evaluation of crude toxin killer activity against sensitive yeast: Killer activity (KA) of crude toxins against sensitive yeast (S1) were quantified by following the well test method (Dabhole and Joishy 2005, Soares and Sato 2000).

Evaluation of crude toxin killer activity against *Pythium sp.*: The method mentioned in in Liu et al (201 was followed to evaluate Killer activity (KA) of crude toxins against *Pythium sp.*

Estimation of protein concentration: Bradford (1976) method was used to estimate proteins in crude toxin solutions

Purification of killer toxin: Ammonium sulfate (NH4)2SO4 saturation (20-80%) used to concentration of crude extract for killer toxin of yeast, the precipitate after centrifugation at 5000 rpm for 30min in 4°C, was dissolved in 10ml of Tris-HCL buffer 0.1M, pH 7.4 and dialysis against same buffer for 24h at 4°C. 10 ml killer toxin solution that loaded on a Sephacryl 6B column (2×20cm) which equilibrated and collected separate fractions by Tris-HCL buffer 0.1M, pH 7.4 at a flow rate of 3ml\fraction. The active fractions which collected were keep at freezing until use (Aziz et al 2014).

Sodium dodecyl sulfate-polyacrylamide gel)SDS) : Purity of toxins and their molecular weights were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) as described by Hames (1998).

Statistical analysis: The statistical analysis software Genstat 12th was used for analysis of data.

RESULTS AND DISCUSSION

Isolation and identification of *S. cerevisiae* isolates: Eight isolates of yeasts were also isolated from different sources, including soil, leaves, flowers, bark and fruits of some plants. In addition, three isolates of dry bread yeast available in the local markets (Altunsa, Instant yeast and Mil Brand) (Table1).

Morphological, cultural and biochemical tests applied to identify isolated yeasts revealed five isolates were belong to

S. cerevisiae according to the findings of Barnett et al *(*1983) and Kurtzman and Fell (1998). The morphological, cultural and biochemical data of the investigated isolates are described and represented in Table 2.

Identification of *Pythium* sp.: *Pythium sp.* isolated from soil and identification based on morphological and cultural characteristics. White cottony growth appeared When the fungus grown on PSA. Microscopic observations revealed aseptate hyphae and intercalary sporangia as well as the vesicle and zoospores. These characteristics are identical with Watanabe 2002 (Fig. 1).

Interaction between *S. cerevisiae* isolates: *S. cerevisiae*

Table 1. *S. cerevisiae* isolates and their sources

Isolate number	Isolation symbol	Isolation source	Plant scientific name
1	S1	College of Agriculture	-
2	S2	Oleander leaves	*Nerium sp.*
3	S3	Altunsa	-
4	S4	Instant yeast	-
5	S5	Mil Brand	-
6	S6	Rose flowers	*Rosa sp.*
7	S7	Apple fruit	*Malus sp.*
8	S8	Date fruits	*Phoenix sp.*
9	S9	Grape fruits	*Vitis sp.*
10	S10	Soil	-
11	S11	Sidr leaves	*Ziziphus sp.*
12	S12	Oleander flowers	*Nerium sp.*

Table 2. Morphological, cultural and biochemical characteristics of *S. cerevisiae* isolates

Characteristics		*S. cerevisiae* isolates				
		S1	S2	S3	S4	S5
Surface		Smooth	Smooth	Smooth	Smooth	Smooth
Margin		Circular	Circular	Circular	Circular	Circular
Color		Creamy white	Creamy white	Creamy white	Creamy white	Creamy white
Cells		Spherical/ oval	Spherical/ oval	Spherical/ oval	Spherical/ oval	Spherical/oval
Urea hydrolysis		-	-	-	-	-
Acid production		+	+	+	+	+
Ester production		+	+	+	+	+
Carbon source fermentation	Glucose	+	+	+	+	+
	Fructose	+	+	+	+	+
	Sucrose	+	+	+	+	+
	Maltose	+	+	+	+	+
	Galactose	+	+	+	+	+
	Raffinose	+	+	+	+	+
	Lactose	-	-	-	-	-
Carbon source assimilation	Glucose	+	+	+	+	+
	Fructose	+	+	+	+	+
	Sucrose	+	+	+	+	+
	Maltose	+	+	+	+	+
	Galactose	+	+	+	+	+
	Raffinose	+	+	+	+	+
	Lactose	-	-	-	-	-

+ = Positive result, - = Negative result

isolates S1, S2, S3 and S4 showed killer activity against S1 with inhibition zones were 25.0, 20.5, 16.0 and 18 .0 mm, respectively (Table 3). However, S1 isolate showed sensitivity to the killer toxin produced by the isolates S2, S3 and S4, therefore it was considered as a sensitive isolate. The killer-sensitive reaction depends on the type of sensitive and killer strains and conditions (Maturano et al 2012). The results were agreement with the findings of Bajaj et al 2013, Melvydas et al 2016, Fredericks et al 2021.

Antagonistic activity between *S. cerevisiae* isolates and *Pythium* sp.: The co-cultured *Pythium sp.* in PSA petri plates with each *S. cerevisiae* isolates, indicated that all yeast isolates inhibited mycelial growth of *Pythium sp.* as compared with control treatment (Fig. 2). The S2, S3 and S5 the most inhibitions (33.33, 32.94 and 29.77% respectively). S4 isolate was the least effective (Table 4). These results were agreement with the findings of Abdalmoohsin et al (2019). Several studies have shown that *S. cerevisiae* inhibited the growth of *Colletotrichum gleosporioides, Fusarium oxysporum, Botrytis cinerea, Penicillium spp., Rhizopus stolonifer, Colletotrichum* spp., and *Aspergillus*

Table 3. Inhibition zones diameters caused by resistance/susceptibility interactions between *S. cerevisiae* isolates

S. cerevisiae isolates	Inhibition zones diameters(mm)				
	S1	S2	S3	S4	S5
S1	-	0.0c	0.0c	0.0c	0.0c
S2	25.0a	-	0.0c	0.0c	4.75c
S3	20.5a	3.0c	-	0.0c	0.0c
S4	16.0b	4.5c	2.75c	-	3.0c
S5	18.0b	0.0c	0.0c	0.0c	-

Different letters within each column means values are significantly different at *p* ≤ 0.05

niger (El-Nady 2008, Liu et al 2018, Shalaby and Zhang et al 2020).

Evaluation of crude toxin killer activity against sensitive yeast: There was significant effect of crude killer toxins T2, T3 and T5 against sensitive isolate (S1) when grown on YEPD-MB medium by the well test method (Table 5). The

Table 4. Percentage inhibition of *Pythium sp.* by *S. cerevisiae* isolates

S. cerevisiae isolates	Percent inhibition of *Pythium* sp
S2	33.33 a
S3	32.94 a
S4	28.23 ab
S5	29.77 b

Same letters within each column means values are not significantly different at *p* ≤ 0.05
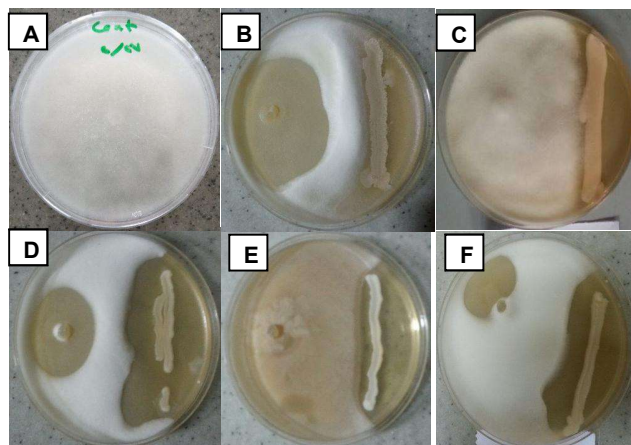
Fig. 2. inhibition of *Pythium sp.* by *S. cerevisiae* isolates S2, S3, S4 and S5 respectively. A= control treatment, B= *S1+Pythium*, C= *S2+Pythium*, D= *S3+Pythium*, E= *S4+Pythium* , F= *S5+Pythium*

Fig. 1. Intercalary sporangia (⬆), vesicle (⬜) and zoospores (⬛) of *Pythium sp*

inhibition zones were 13.50, 16.50, 15.00 and 0.0 mm respectively in T1, T2, T3 and control with significant differences as compared control. Some strains of *S. cerevisiae* have the ability to produce killer toxins, or mycocins, that are killer to other susceptible yeasts (Bajaj and Singh 2017). These results agree with Rosa-Magri (2011) and Fredericks et al (2020).

Evaluation of crude toxin killer activity against *Pythium* sp.: There was variation in the efficiency of crude toxins T2, T3 and T5 in inhibiting the pathogenic fungus *Pythium sp*. in petri plates. The treatment of pathogenic fungi with T2 toxin achieved the highest percentage of inhibition, 50.47% followed by inhibition percentages 40.39 and 39.75% in T3 and T5, respectively (Table 6). The killer toxins extracted from some types of yeasts, such as *S. cerevisiae*, responsible for the biological control of plant pathogenic fungi (Walker et al 1995). Santos et al (2004) indicated that the killer toxins extracted from *Pichia membranifaciens* achieved high inhibition activity against Botrytis *cinerea*, so it was recommended that it could be used as one of the biological control factors of the fungus *Botrytis cinerea*. The killer toxins

differ according to the genera, species and strains of producing yeast, and these differences are due to the difference in the structural genes that encode toxin, processing and maturity, size, composition and molecular weight of the killer toxins, as well as the difference in killing mechanism (Marquina et al 2002).

Estimation of protein: The largest protein concentration was in T2 solution (0.130 mg/ml), followed by T3 solution (0.093 mg/ml), while the lowest protein concentration was in T5 solution (0.081 mg/mL).

Purification of killer toxins: Ammonium sulfate $(NH_4)_2SO_4$ saturation (20-80%) was used to precipitate killer toxin proteins produced from *S. cerevisiae* isolates. The 80% of $(NH_4)_2SO_4$ precipitated most of the proteins in the crude toxins which were 0.2, 0.1 and 0.2 mg / ml in T2, T3 and T5. The precipitation of the crude toxin proteins using salting-out method, led to an increase in the killer toxin activity against *Pythium sp*. so this step is one of the important steps that must be performed before starting the subsequent purification steps because of its ability to remove large amounts of water and some proteins from the crude extract (Janson and Ryden 2011, Aziz et al 2014). Proteins precipitation is due to the equation of charges on the surface of the protein caused by the external salting of protein phenomenon, as the salt ions pull the water molecules from the protein and the proteins separate when the charges of the salt ions are equal with the charges of the protein molecules, which leads to a decrease in the solubility of the protein and then its precipitation (Sattayasia 2012). The results are in agreement with Soares and Sato (2000) and Alsoufi and (Aziz 2017). Purification of killer toxin through Sephacryl 6-B column (2×20cm). Tris-HCL buffer 0.1M, pH 7.4 at a flow rate

Table 5. The diameter of inhibition zones of crude killer toxins on sensitive yeast (S1)

Crude killer toxin	Inhibition zone diameter (mm)
T2	13.50a
T3	16.50a
T5	15.00a
Control	0.0b

Different letters within each column means values are significantly different at $p \leq 0.05$

Table 6. Purification of T2, T3 and T5 killer toxins

Killer toxin	Step	Volume (ml)	Percent inhibition	Protein (mg/ml)
T2	Crude toxin	100	50.47 (45.29)	0.130
	20-80% $(NH_4)_2SO_4$	30	59.43 (50.42)	0.2
	Sephacryl 6-B	21	66.35 (54.57)	0.099
T3	Crude toxin	100	40.39 (39.47)	0.093
	20-80% $(NH_4)_2SO_4$	30	47.37 (43.51)	0.1
	Sharyl 6-B	21	54.86 (47.81)	0.078
T5	Crude toxin	100	39.75 (39.11)	0.081
	20-80% $(NH_4)_2SO_4$	30	45.50 (42.42)	0.2
	Sephacryl 6-B	21	50.27 (45.17)	0.070

() = angular transformation

of 0.5 ml/minute (Fig. 3a, b, c). show one peak of the toxins fractions that recovered from gel filtration column after measuring the absorbance at the 280 nm and collected to give percent inhibition for *Pythium* sp. 66.35, 54.86 and 50.27% respectively, with protein concentration 0.099, 0.07 and 0.070 mg/ml in T2, T3 and T5 respectively (Table 6).
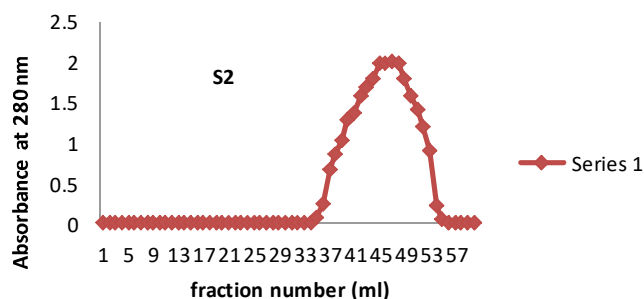
Fig. 3. Purification of T2 killer toxin through Sephacryl 6-B column (2×20cm). Tris-HCL buffer 0.1M, pH 7.4 at a flow rate of 0.5 ml/minute
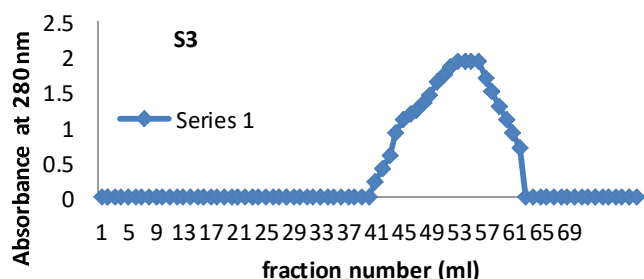
Fig. 4. Purification of T3 killer toxin through Sephacryl 6-B column (2×20cm). Tris-HCL buffer 0.1M, pH 7.4 at a flow rate of 0.5 ml/minute
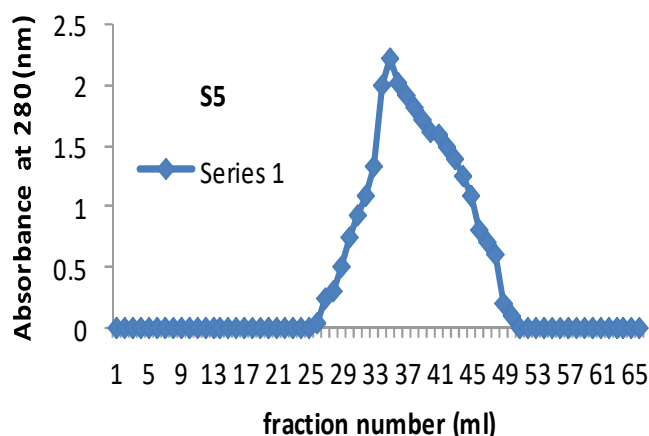
Fig. 5. Purification of T5 killer toxin through Sephacryl 6-B column (2×20cm). Tris-HCL buffer 0.1M, pH 7.4 at a flow rate of 0.5 ml/minute
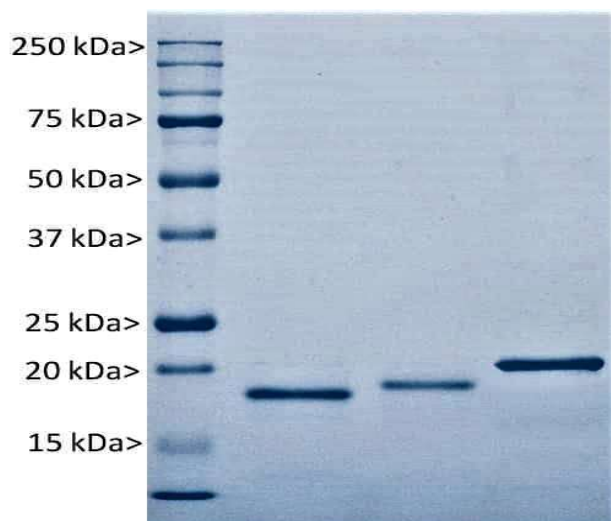
Fig. 6. SDS Polyacrylamide gel electrophoresis of killer toxins T2, T3 and T5 produced by *S. cerevisiae* isolates

Sodium dodecyl sulfate- Polyacrylamide gel (SDS-PAGE): The results of the analysis of purified toxins on SDS PAGE shown one protein band for each T2, T3 and T5 with molecular weights ranging between 18 and 22 kDa compared with Ladders (Fig. 6). Orentaite et al (2016) reported that killer toxins K1, K2, k28 and Klus produced by *S. cerevisiae* are proteins with low molecular weights ranging between 19-21.5 kDa. Sato and Soares (2000) determined the molecular structure of the killer toxin protein purified from *S. cerevisiae* Y500-4L using SDS-PAGE technique observed that a single protein bundle appeared with a molecular weight of about 18-20 kDa. Gier et al (2020) also reported that the molecular weight of the killer toxin K1 is 18 kDa.

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Antibiotic Resistance to Bacterial Neonatal Sepsis

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Abstract: Neonatal sepsis is a global disease that poses a management challenge for neonatal care groups. The goal of this study is to determine the bacteria that lead to the development of sepsis in the newborn period so that early therapy can be used to reduce the incidence of sepsis as well as determine the antibiotic sensitivity. Common clinical variables (sociodemographic characteristics) studied were bacteriological profiles and antibiotic susceptibility testing. The sepsis was confirmed in 31.8% neonates admitted to the special care baby unit with clinical signs and symptoms. All bacterial isolates were identified using traditional methods (cultural, morphological, and biochemical characteristics), and VITEK-2 compact system was used to confirm the results. Only 60 percent samples revealed bacterial growth from isolates of a different genus, 40 percent had no bacterial growth. The 71.6 % samples were EOS, and rest LOS. The highest isolates was of *Staphylococcus epidermidis* (43.3%), followed by *Klebsiella pneumoniae.* (21.7%). *Staphylococcus aureus*, 16.7%), *Pseudomonas aeruginosa.* 13.3%) and *Escherichia coli* 5%). Gram-negative bacteria showed high resistance (100%) to Aztreonam, Ceftazidime, Piperacillin, Ceftriaxone and Gentamicin. Gram-positive bacteria in this study showed 100 percent resistance to Penicillin, Cefoxitin, Vancomycin, Nirofuranation.

Keywords: Neonatal sepsis, Bacteria, Antibiotic susceptibility, Risk factor

Neonatal sepsis is characterized as any systemic bacterial infection confirmed by a positive blood culture in the first month of life (Fanaroff et al 2007). Despite advancements in hygiene, the advent of new and effective antimicrobial agents for treatment, and advanced diagnostic measures, neonatal septicemia remains one of the leading causes of mortality and morbidity. Infections affect up to 10% of babies in their first month of life, accounting for 30-50% of total neonatal deaths in developing countries (Al-Saady et al 2018). These neonatal deaths are mostly the result of infection, birth asphyxia, and the effects of premature birth with a low birth weight (Movahedian et al 2006). The risk factors related to neonatal bacterial sepsis are complex and they include the interaction of maternal-fetal colonization, trans- placental immunity and physical and cellular defense mechanisms of the neonate (Jumah et al 2007). Neonatal sepsis may be classified as either early-onset or late-onset. Early onset neonatal sepsis (EOS), which occurs during the first 72 hours of life, continues to be a major cause of illness and death in very low birth weight (Stoll et al 2005), Microorganisms acquired from the mother before or during birth (vertically transmitted and prenatally acquired) cause EOS; hence, microorganisms from the maternal genital tract can play an important role in early infection (Kerur et al 2006). EOS is linked to the mother's acquisition of microorganisms. Organisms that colonize the mother's genitourinary tract (GUT) may cause trans placental infection or ascending infection from the cervix, the neonate acquires the microorganisms as it passes through the colonized birth canal at delivery (Klinger et al 2009). EOS is often associated with respiratory distress and pneumonia (Rajaratnam et al 2010). Gram-negative species are the most common pathogens associated with EOS (Salman et al 2013). GBS, *E. coli,* and coagulase-negative bacteria are the most frequently associated microorganisms with an early-onset infection with *Staphylococcus aureus, Haemo philus influenzae* and *Listeria monocytogenes*, (Klinger et al 2009).

Gram-negative pathogens have been identified as the causative organisms in VLBW infants with EOS more frequently. Late onset sepsis (LOS) present during 7-28 days of age. In LOS, the infection is either nosocomial (hospital-acquired) or community-acquired, and neonates usually present with septicemia, pneumonia, or meningitis. Gram-positive bacteria are the most often isolated pathogens in VLBW infants diagnosed with LOS (Carolin et al 2012), Gram-negative bacilli that cause neonatal LOS are primarily *E. coli, Klebsiella spp., Enterobacter* spp., and *Pseudomonas spp*. Fungi, especially *Candida* spp., have been identified as one of the major pathogens of LOS in some areas (Leal et al 2012).

MATERIAL AND METHODS

Study design: This is case-control study conducted in the Department of Pediatric in Al-Batool Teaching Hospital, Diyal

Governorate, Iraq for 6 month the period first week of November, 2020 to 30th April, 2021.

Samples culture: Blood samples drawn from newborns were placed in the Bacteriology Alert Device and incubated for different periods. The positive samples were dealt with according to the traditional isolation and diagnosis methods, which included blood agar and MacConkey agar. The isolates were purified by selective media which included mannitol salt agar, EMB agar and pseudo agar by streaking method. The agar plates were incubated for 24 h at 37°C (Jameel et al 2020, Hameed et al 2021). Then, biochemical tests and diagnostic tests were performed for the bacteria under study. The positive isolates were confirmed using VITEC system.

Bacterial isolates: The total clinical isolates were 5 isolates of a different genus of bacteria from patients with septicemia which include *Staphylococcus epidermidis, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*. All samples were entered into the Back Alert system to ensure that they were positive for bacterial growth and were incubated for various times. All isolates were diagnosed based on using conventional isolation and diagnosis methods and biochemical and bacteriological tests after cultured in the cultures media, these isolates gave a positive results for these tests. The positive isolates were confirmed using VITEK 2 compact system.

Antibiotics susceptibility test: The sensitivity test procedure was done according to (CLSI 2020) as the following steps: -

1. Mueller-Hinton agar plates were used for the use of rapidly growing species in the Kirby- Bauer method. The solvent was sterile in the plates and had a depth of around 4 mm.

2. Pure culture has been used as inoculum; 2-4 related colonies have been selected and transferred to around 5ml of standard sterile saline. To get an average number equal to $1.5x\,10^8$ CFU/ml, the turbidity of microbial suspension was compared with the turbidity of the McFarland Standard 0.5.

3. The sterile cotton swab was immersed into the standard inoculum, streaking was performed 3 times on the entire agar surface of the plate with the swab, rotary the plate between each line at 50 degrees. The inoculum had been allowed to dry with a lid in place for 5-10 minutes and after that, the antibiotics mentioned in the table (3-4) were distributed on the plates.

4. The plates were subsequently incubated at 37°C and analyzed 18-24 h. Inhibition zones were measured, and the zones' diameters were reported to the nearest millimeter.

RESULTS AND DISCUSSION

Bacterial isolated from neonates with different types of sepsis: From blood samples, the highest isolates was *Staphylococcus epidermidis* 43.3%, followed by *Klebsiella pneumoniae* (21.7%). *Staphylococcus aureus* 10/60 (16.7%), *Pseudomonas aeruginosa* 8/60 (13.3%) and *Escherichia coli* (5%), (Fig. 1). There were differences in gender with all bacterial isolates but there are significant differences in gender with *Escherichia coli* infected. Neonates with EOS 0-7 days of age forming the highest rate (71.6%) as compared with Neonates with LOS 7-30 days of age (28.3%).

S. epidermidis was the most common Gram-positive bacteria isolate (43.3 %), Shrestha et al (2010), also showed that the *S. epidermidis* was a major isolate, and disagree with Sharma et al (2013) in India showed that *S. aureus* was the most prevalent. The *Klebsiella pneumoniae* was the most common cause sepsis in Gram-negative bacteria isolate (21.7%). Gyawali et al (2013) and Shrestha et al (2012) also showed that *K. pneumoniae* isolate accounts the highest causes infection in neonates. Aftab et al (2007) in a study of the Pakistani observed *E. coli* was the most common organism, followed by *Klebsiella*. The result differed with current study. Gram-positive bacteria was more common than the Gram-negative bacteria, this results was agreement with Naderi-Nasab et al (2007), in NICUs of Mashhad, Iran, and disagreement with Shrestha et al (2007), which showed the majority of the isolate were gram-negative bacteria. The early onset sepsis was highest than late onset sepsis for newborns. The 42 % of all hospitalized neonates with sepsis in the NICU were EOS (Aftab et al 2006). The occurrence of EOS appears to be influenced by antibiotic resistance in common pathogens, the effectiveness of interventions used, and whether or not accurate information about the burden of sepsis and its effects is available.

Antibiotic Susceptibility of Bacterial Isolates

S. aureus: The current study's findings suggested that 20 percent of the 10 isolates of *Staphylococcus aureus* were resistant to Ciprofloxacin, and sensitivity was 80% (Fig. 3). The sensitive to Vancomycin Gentamicin and Amikacin was 20 and 50 and 90 percent .

The results of the current study showed that *S. aureus* isolates were resistant to vancomycin by 80%. Hanna (2008) showed all *S. aureus* strains were susceptible to Ceftriaxone and Vancomycin. The sensitivity to ciprofloxacin, gentamicin, and amikacin was 80, 50 and 90%, respectively. Marwah et al (2015) also found that the majority of *S. aureus* isolates from neonatal sepsis were cephalosporin-resistant but sensitive to aminoglycosides (Fig. ٢).

S. epidermidis: The 26 isolates of *S. epidermidis* tested, 73.10 percent of them were resistant to Ciprofloxacin (Fig. 4). The Ofloxacin and Gentamicin resistance was 53.80 and

76.90 percent. Isolates of *S. epidermidis* bacteria showed resistance to ciprofloxacin, gentamycin and ofloxacin . Abdul-Rahman (2019) also showed resistance against ciprofloxacin, gentamycin and ofloxacin was 100% . Antibiotic resistance is difficult to compare between the countries because neonatal sepsis epidemiology is highly variable as seen in some countries (Kosovski et al 2019). The most common cause of neonatal sepsis is *S. aureus* and methicillin-resistant strains *S. aureus* (MRSA), which are the common bacteria have three aminoglycoside-mutating enzymes that are encoded by plasmids. These enzymes act by inhibiting antibiotics such as gentamicin, as well as causing resistance to this group due to a shift in the S30 subunit to which the antibiotics were attached, while Chloramphenicol inhibits protein synthesis since inhibitory antibiotic due to the fact that it binds to the S30 subunit, Enzymatic inhibitions are the most well-known public resistance mechanism in bacteria. This mechanism is based on a variety of techniques for altering the structure of antimicrobial agents, including hydrolysis, which is a type of reaction that occurs primarily with beta lactam agents (Bhullar et al 2012).The explanation for the continuous rise in antibiotic resistance may be due to widespread use of these antibiotics by humans, which leads to the emergence of new strains with high resistance to antibiotics (Llarrull et al 2009).

The current study showed that *Pseudomonas aeruginosa* isolates were sensitive to ceftazidime. Sami (2018) found that all Gram-negative bacteria were resistant to Ceftazidime (77.4%), but all *Pseudomonas aeruginosa* isolates were sensitive to Ceftazidime (100%), It showed resistance to Ticarcillin by 62.50%. Köksal et al (2001) from India, that showed Gram-negative bacteria were resistance to Amoxicillin, Ticarcillin, Cefotaxime, Ceftazidime, Ceftriaxone, and Gentamicin, and only with 57.14 % of *E. coli* sensitive to Gentamicin. These results are consistent with the current study. The variation in resistance may be due to sample sources, as well as environmental and test conditions. Based on this studies and   previous studies, it was observed that *Pseudomonas aeruginosa* resist to antibiotics due to its ability to alter membrane permeability represents a variety of mechanisms and manufactures wide-narrow beta-lactamase enzymes and biofilm formation, as well as own R-resistance plasmids that carry various antibiotic resistance genes (Hong et al 2016) (Fig. 4).

Escherichia coli: *Escherichia coli* isolates showed resistance to Cefotaxime by 33.30 % and there was no Meropenem resistance. Gentamicin resistance was by 33.3 % and there was no resistance to Amikacin.

The isolates of *Escherichia coli* showed sensitive to Meropenem, Cefotaxime and Gentamicin with high

sensitivity to Amikacin. Lebea and Davies (2017) showed all isolated of *E. coli* strains were sensitive to Meropenem and Cefotaxime, with high sensitivity to Amikacin (65%) and Piperacillin (92.8%) from South Africa. These results
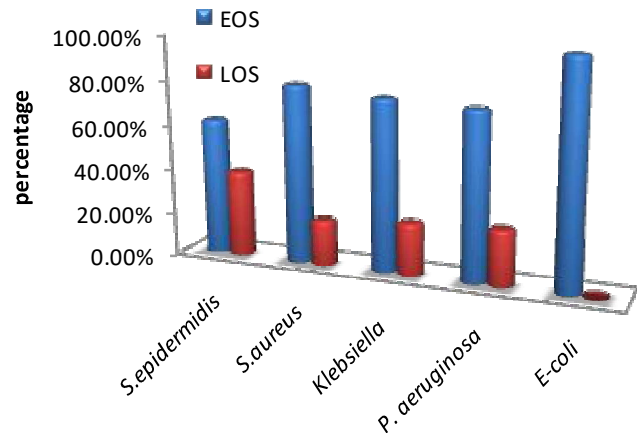
Fig. 1. Bacterial isolated from neonates with different types of sepsis which include (EOS: Early Onset Sepsis, LOS: Late Onset Sepsis)
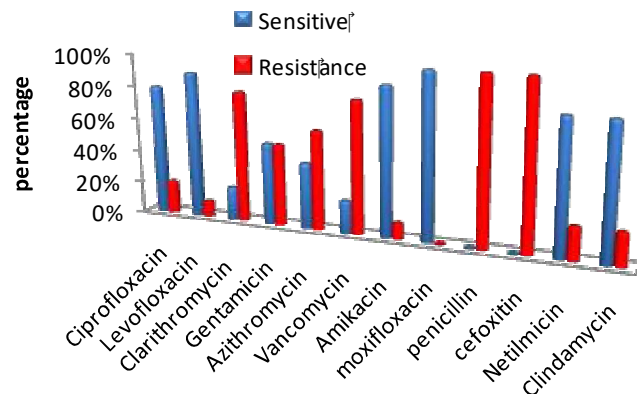
Fig. 2. Percentages of antibiotic resistance and sensitivity of *S. aureus* isolates
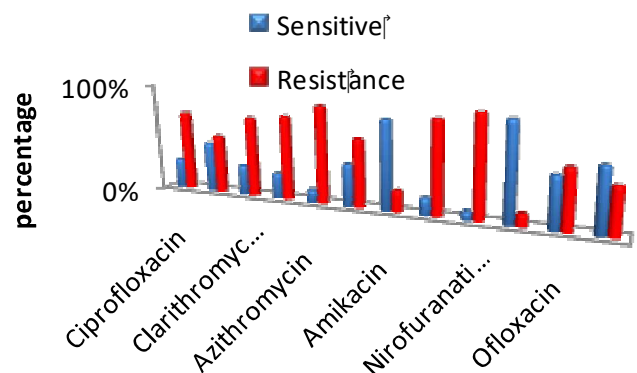
Fig. 3. Percentages of antibiotic resistance and sensitive of *S. epidermidis* isolates

disagree to with El-Din (2015) from Egypt where most Gram-positive bacteria isolated were 100% resistant to Penicillin and Cefoxitin, but 100% sensitive to Moxifloxacin. Results of antimicrobial sensitivity tests for all isolated Gram-negative
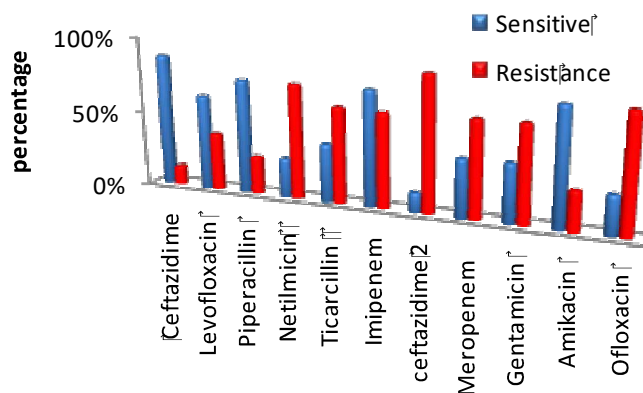
Fig. 4. Antibiotic resistance and sensitive of *Pseudomonas aeruginosa* isolates
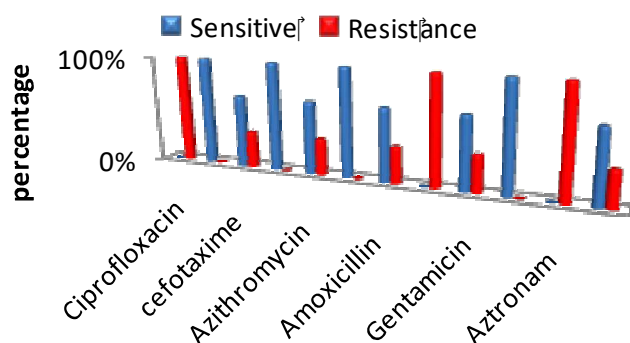
Fig. 5. Antibiotic resistance and sensitive of *Escherichia coli* isolate
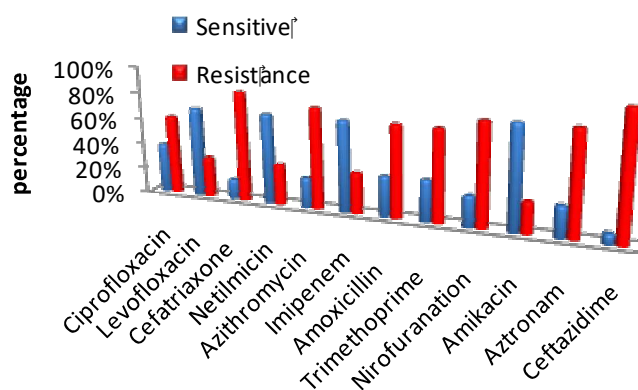
Fig. 6. Percentages of antibiotic resistance and sensitive of *Klebsiella Pneumonia* isolates

bacteria against certain antibiotics showed variable sensitivity patterns in antibiotic resistance which is now a worldwide problem. Studies of multidrug-resistant bacteria that cause neonatal sepsis in developing nations are increasing, especially in NICU (Aletayeb et al 2011). There was no total resistance to all antimicrobials displayed by any isolated bacteria in this study. *E. coli* isolates may have several antibiotic-resistant mechanisms, such as the ability to form biofilms. *E. coli* produces beta-lactamase enzymes, which are an effective way for bacteria to avoid beta-lactam antibiotics. B- Lactamase enzymes are encoded by genes found in the majority of Gram-negative bacteria genera (Chuma et al 2013) (Figure 5). The antibiotic susceptibility trend varies in different studies as well as at different times in the same hospitals (Lebea and Davies 2017). Furthermore, the emphasis in Baqubah city in the last ten years has been on antibiotic resistance, as evidenced by the current study and other studies (Khder 2008, Aziz et al 2014, Bakir and Ali 2016, Abdulrahman et al 2018, Jassim et al 2021). The bacterial resistance to antibiotics has been increasing in recent years. This is a real crisis and there are many explanations for it, including, many antibiotic prescriptions are written in clinical settings without first investigating the infectious germ and without conducting an antibiotic sensitivity test. The patient does not take or follow the entire prescription exactly as prescribed and the patient does not take or follow the entire prescription exactly as directed, and if feel better, they stop to take the antibiotic before the infection is fully eradicated, than the bacteria are more likely to develop drug resistance. Many medicines are available on the market (pharmacy) that are poor quality, poor hygiene and sanitation, and are stored at high or low temperatures (Salah 2017, Motib et al 2017).

Klebsiella pneumonia

Klebsiella Pneumoniae isolates showed resistance to Amikacin (23.10 %). Ceftriaxone (84.60 %) and Ceftazidime (92.30%).

The isolates of *Klebsiella pneumoniae* showed resistance to ceftazidime and cefatriaxone and these results are in agreement with Köksal et al (2001) from India which showed that Gram-negative bacteria were resistant to Ceftazidime, Cefatriaxone, Ticarcillin, and showed sensitive to Amikacin by 76.90% and resistance by 23.10%. Farshed and Emamghoraishi (2010) showed that most Gram positive and Gram negative bacteria were totally sensitive to Amikacin. The ability of the Enterobacteriaceae family to produce beta-lactamase enzymes and its resistance to beta-lactamase group is attributed to its ability to produce beta-lactamase enzymes and its resistance by several mechanisms, including reducing antibiotic permeability into the cell,

analyzing antibiotics with beta-lactamase enzyme and reducing affinity to the enzyme. Penicillin-Binding Proteins are proteins that bind to penicillin (Latteef et al 2017, Motib et al 2020). The bacterial resistance to the group of quinolones anti-quinolone resistance in *Klebsiella* spp, is mediated by flow pumps, which cause multiple antibiotic resistance. The widespread use of this antibiotic has resulted in a high rate of bacterial resistance as shown in Figure 6.

CONCLUSION

This study showed the high prevalence of *S. epidermidis* as Gram-positive bacteria and *K. pneumoniae* as Gram-negative bacteria among suspected neonatal cases. Overall isolates showed maximum sensitivity towards aminoglycosides and quinolones, emergence of antibiotic resistance among bacterial isolates from neonatal sepsis is a major cause for treatment failure, higher morbidity and mortality. Proper antibiotic guidelines and its effective implementation could be milestone for revolution in the field of antibiotic resistance control. The epidemiology of neonatal sepsis, causative risk factors and antibiotic resistance pattern of pathogens may be used to develop guidelines for management of neonatal sepsis.

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Role of *OprD* Gene to Imipenem Resistance in Multi-Drug Resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Isolated from Medical and Clinical Waste Samples

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Abstract: Study was undertaken to highlight the role of the outer membrane porin (OprD) in resistance to imipenem (IPM). In the presence of the sub- minimum inhibitory concentration (sub-MIC) of imipenem, *OprD* gene expression in multi-drug resistance (MDR), and imipenem-resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, were determined and compared with that in the absence of it. In *Pseudomonas aeruginosa* isolates, *OprD* gene expression reduced after treatment with the sub- minimum inhibitory concentration of imipenem and the reduction was statistically significant. Treatment with the sub- minimum inhibitory concentration of imipenem plus the sub-minimum inhibitory concentration of ethanol extract of pumpkin seeds slightly increases average of folding. In *Acinetobacter baumannii* isolates, *OprD* gene expression increased after treatment with the sub- minimum inhibitory concentration of imipenem and the increase was statistically significant. The study indicate that *OprD* gene plays a key role in imipenem resistance in *Pseudomonas aeruginosa* by reducing expression, unlike its role in *Acinetobacter baumanni*. Ethanol extract of pumpkin seeds can have a role in increasing of *OprD* gene expression in *Pseudomonas aeruginosa* as attempt to solve the problem of resistance created from reduced expression.

Keywords: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, Multi-drug resistance, Imipenem, *OprD* gene, Pumpkin seeds

Pseudomonas genus is the main one of the family Pseudomonadaceae, which includes a heterogeneous group of Gram-negative, rod shaped, and polarly flagellated bacteria (Clarke 1990). It shows 332 species (Parte et al 2020). Most of these species are not pathogens in animals and plants and a few species, such as *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, and *Pseudomonas asiatica* infect humans (Tohya et al 2020). *Acinetobacter* genus belongs to phylum Proteobacteria, class Gamma proteobacteria, order Pseudomonadales, family Moraxellaceae (Berman 2019). Based on DNA-DNA hybridization studies, more than 40 different *Acinetobacter* genomic species (genospecies) have been identified (Erdem and Leber 2018).

The non-fermentative Gram negative bacilli *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have emerged as serious of particular concern. They are among the most common and serious multi-drug resistant pathogens documented along with *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae* and *Enterobacter* spp.(Motbainor et al 2020). Multidrug-resistant *P. aeruginosa* and *A. baumannii* cause severe human nosocomial infections and are more difficult to treat than methicillin-resistant *Staphylococcus aureus* (MRSA) (Lee et al 2013). Earlier findings elsewhere in the world reported that both of these pathogens commonly possess inherent resistance to antimicrobial agents through reduced or lack of expression of outer membrane proteins, up-regulation of efflux pumps, enzymatic inactivation and biofilm formation (Matar 2018). Beside the cell wall's lipopolysaccharide, another mechanism confers reduced permeability involves outer membrane porins. Because porins mediate the passive diffusion of antibiotics across the outer membrane are closely associated with antibiotic resistance (Admassie 2018, Choi and Lee 2019). OprD is a small, specialized outer membrane porin protein allowing for selective permeation of basic amino acids, small peptides, and carbapenem antibiotics (Angeletti et al 2018). In *P. aeruginosa* decreased expression of OprD, *oprD* mutations, and loss of OprD mainly contribute to carbapenem resistance (Liu et al 2018). The expression of OprD is strongly suppressed by two-component regulators, including CopRS, CzcRS, ParRS, and efflux pump regulator MexT and induced when basic amino acids (arginine, histidine, glutamate, or alanine) serve as the sole source of carbon (Novoa-Aponte et al 2020, Caille et al 2007, Ochs et al 1999a).

The amino acid conservation at structural domains between *A. baumannii* and *P. aeruginosa* OprD porins indicate its proposed function in *A. baumannii*. However, Catel-Ferreira et al (2012) concluded that the *A. baumannii*

OprD-like protein is not involved in specific antibiotic diffusion and would consequently not contribute to carbapenem resistance. On the other hand, Luo et al (2011) summarized that OprD-like protein and CarO may play an important role in the regulation of imipenem resistance in *A. baumannii*. They suggested that the overexpression of the efflux pump in conjunction with downregulation of CarO and OprD-like protein may contribute to imipenem resistance. The pumpkin is the one of the famous edible plants that is utilized as the cure of many disorders due to the occurrence of many edible components and phytochemicals. Pumpkin with oily seeds belongs to the *Cucurbitaceae* family. Although there are various varieties grown throughout the world, the most commercially important species are *Cucurbita pepo* (most common), *C. moschata*, *C. maxima*, *C. stilbo*, and *C. mixta*. Pumpkin seeds are utilized for the cure of different diseases as an herbal remedies separately or in combination with medicines. Cell culture studies have indicated promising antimicrobial activity of pumpkin seed extract on various strains of microorganism. The seed extract has effective antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* (Montesano et al 2018, Syed et al 2019, Dotto and Chacha 2020).

MATERIAL AND METHODS

Samples collection: A total of 394 (300 medical and 94 clinical waste) samples were collected during February 2020- October 2020 from hospitals in Baghdad, Iraq.

DNA extraction Genomic DNA was isolated from bacterial growth according to the protocol of Wizard® Genomic DNA Purification Kit (Promega Corporation 2019). Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted quantity of flour dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected.

Polymerase chain reaction (PCR) cycle: The DNA of isolates was targeted for 16S rRNA, *gyrB*, and *bla* $_{OXA-51}$ genes using primers listed in Table 1. A reaction mixture (20 µl) contained 3 µl of DNA, 1 µl of each primer, 10 µl of Master Mix, and 5 µl of nuclease free water. The experiment was sustained according to the program (Table 2). The PCR products were analyzed using 1.5% agarose gel electrophoresis and the ethidium bromide stained bands in gel were visualized using Gel imaging system.

Antibiotic susceptibility test: Susceptibility to antibiotics was determined by Vitek 2 system. The Vitek 2 system automatically measures a turbidity signal for each test well containing an antibiotic, every 15 minutes for up to 18 hours. These data are used to generate growth curves and by comparing with a control, the minimum inhibitory concentration (MIC) of each antibiotic is estimated (Saegeman et al 2005). MIC results (µg/ml) were translated into clinical categories (Susceptible, Intermediate, and Resistant) by comparing with breakpoints for susceptibility category determination recommended by the clinical and laboratory standards institute (CLSI) guidelines.

Phenotypic Detection of MBL Activity: Combined disk test

Table 2. PCR program

Steps	°C	m:s	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing		00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Table 1. Primer sequences

Genes	Primer sequence (5'-3')	Size (bp)	Annealing Temp °C	References
P. aeruginosa 16S rRNA-F 16S rRNA-R	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	956	58	Altaai et al 2014
gyrB-F *gyrB*-R	AAGTACGAAGGCGGTCTGAA GTTGTTGGTGAAGCAGAGCA	171	55	Hassan and Abdullah 2018
OprD-F *OprD*-R	GCGCATCTCCAAGACCATG GCCACGCGATTTGACGGAG	193	55	Cai et al 2016
A. baumanni 16S rRNA-F 16S rRNA-R	TTTAAGCGAGGAGGAGG ATTCTACCATCCTCTCCC	242	55	Vanbroekhoven et al 2004
bla OXA-51-F *bla OXA-51*-R	CTATGGTAATGATCTTGCTCGTG TGGTGGTTGCCTTATGGTG	104	65	Dou et al 2017
OprD-F *OprD*-R	ATCGTAAGCTGAACCAT CGTT TCATTTCTGCGGCAATAATTT	305	60	Catel-Ferreira et al 2012

was performed as described by Yong et al (2002). Briefly, an overnight culture of an isolate was diluted with peptone water to 105 CFU/mL and spread on Mueller-Hinton (MH) agar plate using cotton swab. Two IPM disks were placed on the surface of the agar at a distance of 4-5 cm from each other. 5 µL of 750 µg/mL Ethylenediaminetetraacetic acid (EDTA) solution was then added to one of the IPM disks. The inhibition zones displayed around the IPM and the IPM-EDTA disks were compared after 14 to 16 hours of incubation at 37 ºC. The difference of ≥7 mm between the inhibition zone diameter of the IPM-EDTA disk and that of IPM only disk was considered to be a positive for the presence of MBLs. The procedure was repeated twice to ensure the reproducibility of results.

Seeds extract: Pumpkin seeds were brought from market then washed, dried at room temperature, taking into consideration that Exposure to sunlight must be avoided to prevent the loss of active components (Thakare 2004), and grinded to get powder. Soxhlet ethanol extraction was used to get extracted plant material (Redfern et al 2014). Briefly, in this method, finely ground sample is loaded into the porous cellulose thimble, which is placed inside the Soxhlet extractor. Following this, the solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 16 hours. Once the process has finished, the ethanol should be evaporated using a rotary evaporator.

Minimum inhibitory concentration (MIC) determination: MIC was performed on seed extract and IPM. The MIC was determined by serial dilution, as described by Mogana et al (2020) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Stock solutions of the seed extract and IPM were prepared by dissolving dry plant extract in dimethylsulphoxide (DMSO) and IPM in phosphate buffer (Kowalska-Krochmal and Dudek-Wicher 2021) to a final concentration. The serial dilutions from the stock solution were made using Mueller–Hinton broth in 96-well microplates. The bacterial suspension was prepared and adjusted to the McFarland standard required by the test (0.5McF) using DensiCHEK Plus Meter. From this suspension, 1 µl was inoculated into each well. A sterility control well and a growth control well were also studied for each strain. The microtiter plates were incubated at 37 °C, 24 hours. After incubation, the microtiter plates were scanned with GloMax® Discover Microplate Reader on 600n. MIC values were determined at least in duplicate and repeated to confirm activity.

***OprD* Expression:** Total RNA was isolated from strains using the TRIzol Reagent (Thermo Scientific, USA) according to the standard protocol. *OprD* was amplfied using the GoTaq® 1-Step RT-qPCR System Kit (Promega, USA) according to the manufacturer's instructions. The relative expression of *oprD* was calculated by the $2^{-\Delta\Delta ct}$ method. 16S rRNA and *RpsL* genes were used as the internal control.

Statistical Analysis: The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters.

RESULTS AND DISCUSSION

The 16S rRNA, *gyrB*, and *bla*$_{OXA-51}$ genes confirmed that 12 (3.4%) and 16 (4.5%) isolates were *P. aeruginosa* and *A. baumannii* respectively (Fig. 1 and Fig. 2). 8 (66.7%) and 11 (68.8%) of *P. aeruginosa* and *A. baumannii* isolates respectively were positive for *OprD* gene (Fig. 3).

Since the 16S rRNA gene is present in all bacteria and subject to different evolutionary rates depending on the gene region considered. It has historically been used for classification of isolates (Winand et al 2020). Studies have shown that the sequences of *gyrB* gene of *P. aeruginosa* and other species of *Pseudomonas* showed a higher divergence than 16S rRNA genes. The reason is that the molecular evolution rate of *gyrB* gene is higher than that of 16S rRNA gene and it is rarely transmitted horizontally. Therefore, the *gyrB* gene is a more appropriate target than 16S rRNA gene for the identification of the *pseudomonas* species (Aghamollaei et al 2015). On the other hand, basic local alignment search tool (BLAST) results for *gyrB* primers showed that the *gyrB* primer sequences designed by Qin et al (2003) are 100% identical to the *gyrB* gene from *Pseudomonas composti*. This bacterium is a novel species that was isolated from compost and reported by Gibello et al (2011). The *gyrB* gene fragment of this strain has a 99% identity to *P. aeruginosa*, and thus, it is possible to obtain false-positive results with *gyrB* uniplex PCR. *A. baumannii* species identification may be confirmed by detecting the presence of the *bla*$_{OXA-51}$ gene because it naturally occurs in the *A. baumannii* chromosome (Kobs et al 2016). The chromosomally encoded *bla*$_{OXA-51}$ gene 104bp was detected in all isolates (100%) supporting those of other studies demonstrating that the detection of the *bla*$_{OXA-51}$ gene can be used as an additional tool for identifying *A. baumannii* at the species level together with other methods.The frequency of *OprD* gene among *P. aeruginosa* isolates reported in this study is low when compared with other studies: 90% and 91% *OprD* positive isolates reported by Nmema et al (2019)
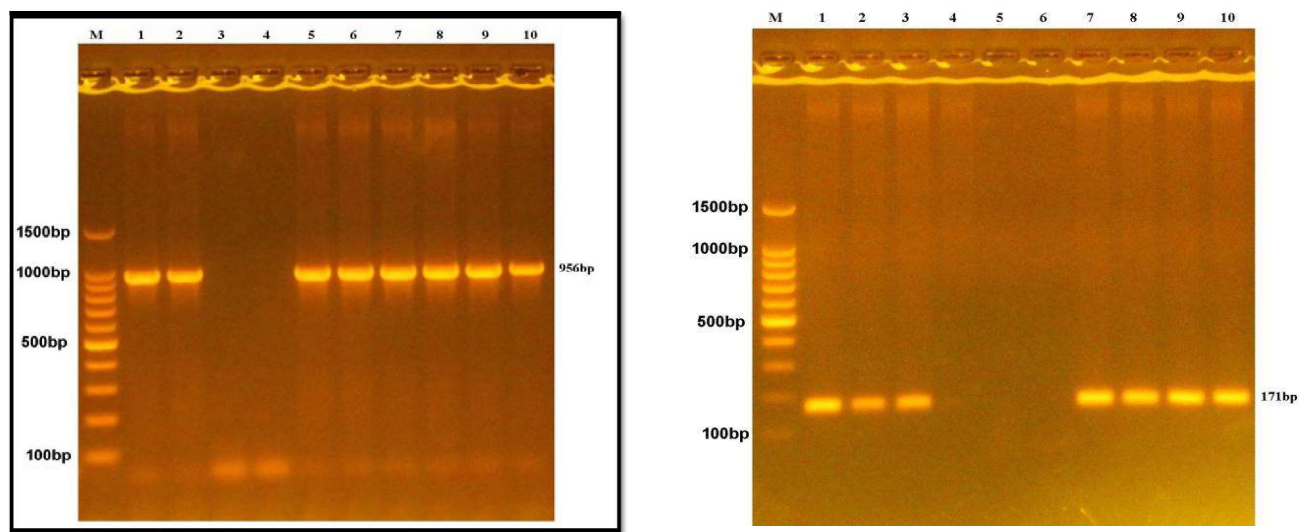
Fig. 1. Amplification of 16SrRNA and *gyrB* genes of *Pseudomonas aeruginosa*
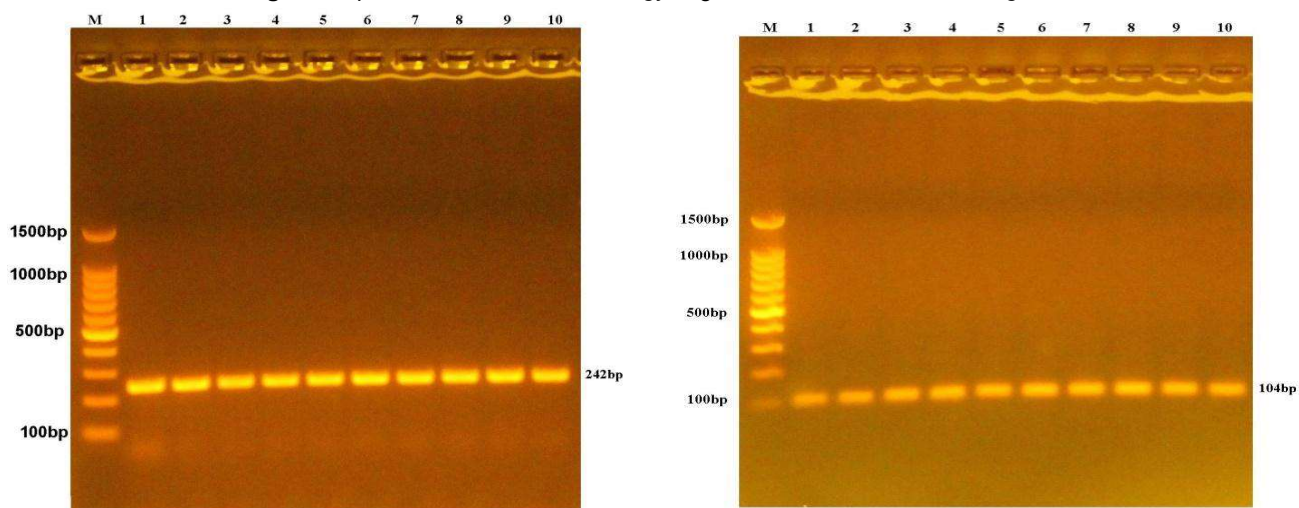
Fig. 2. Amplification of 16SrRNA and *bla* OXA-51 genes of *Acinetobacter baumannii*
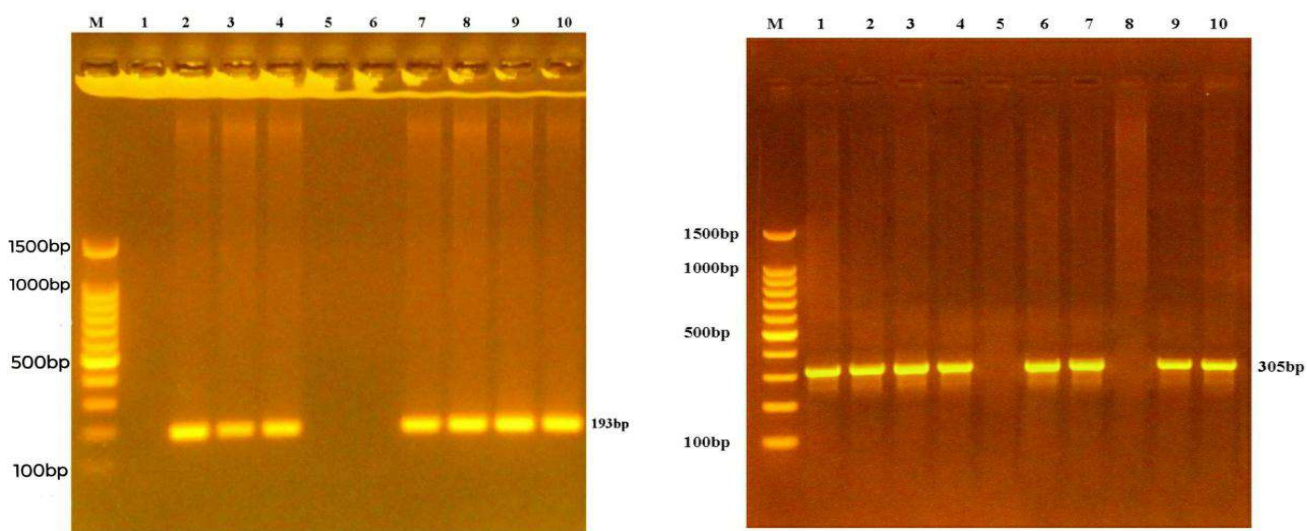
Fig. 3. Amplification of *OprD* gene of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

and Sadredinamin et al (2017) respectively. The frequency of *OprD* gene among *A. baumannii* isolates observed is low when compared with other studies. Noori et al (2019) observed that the frequency of *OprD* gene among *A. baumannii* strains was 98%.

Multidrug resistant *P. aeruginosa* and *A. baumannii* isolates: The 75% isolates of *P. aeruginosa* were resistant to at least one antibiotic agent in three of the major antibiotic classes namely, Beta-lactams, aminoglycosides, fluoroquinolones, and polymyxins. Thus, they were considered multidrug resistant (MDR) isolates. The rising frequency of infections caused by MDR-PA is a big problem for physicians all over the world. The resistant strains are associated with a nine-fold higher rate of secondary bacteremia, three-fold higher rate of mortality, two-fold increase in length of hospital stay, and a considerable increase in healthcare costs (Ali et al 2015). Furthermore, the emergence of MDR-PA strains in recent years leading to severe infections with high mortality rates due to very limited treatment options has prompted the World Health Organization (WHO) in 2017 to rate MDR Gram-negative bacteria including *P. aeruginosa* as serious global threats to human health. This further highlighting the need for novel antibiotics-independent treatment strategies (Heimesaat et al 2019). All confirmed *A. baumannii* isolates (100%) revealed resistance to at least one antibiotic agent in three of the major antibiotic classes namely, beta-lactams, aminoglycosides, fluoroquinolones, tetracyclines, polymyxins, and Sulfonamides. Thus, they were considered MDR isolates. MDR-AB infection could significantly prolong the hospital stay, increase economic costs, and increase mortality (Huang et al 2018). Several retrospective studies conducted in the United States of America show a significant positive association between mortality and colonization with MDR-AB (Ashuthosh et al 2020).

Metallo-beta-lactamase (MBL) producing *P. aeruginosa* and *A. baumanni* isolates: Based on the combined disk test (CDT), 50% of *P. aeruginosa* isolate were identified as MBL producers. The findings are similar to Wang and Wang (2020) in China. On the other hand it was high when compared with other previous studies: 20 & 28% MBL producing *P. aeruginosa* strains reported by Choudhary et al (2019) in India and Azeez and Bakr (2019) in Kurdistan Region, Iraq respectively. In present study was low when compared with 83.33 and 81.8% MBL producing *P. aeruginosa* strains as observed by Hussein and Shamkhi (2018) in Wasit, Iraq and Mohanam and Menon (2017) in India, respectively. *A. baumannii*, 15 (93.8%) isolates were MBL producers and only one isolate (6.6%) was MBL negative. The findings are in agreement with 91.1 and 93.2%

MBL positive isolates of *A. baumannii* reported by previous study of by Ridha et al (2019) and Al-Ouqaili et al (2016), respectively.

***OprD* expression:** The *P. aeruginosa* experiment of the quantitative RT-PCR reaction was completed by using 5 isolates (4 MDR, MBLs-negative, & IPM-resistant isolates and one MDR, MBLs-positive, & IPM-sensitive isolate). The quantitative changes in the mRNA expression levels are shown in (Table 3). Before treating with IPM, the range of Ct value was 14.88-18.63 and after treatment it became 18.40-27.80 (Table 3). The difference between averages before and after treatment with IPM was statistically significant. The mechanisms of resistance to carbapenems in *P. aeruginosa* include the outer membrane protein (OprD) loss or reduced expression (down-regulation of transcription), acquired Amber class B metallo-beta-lactamase (MBLs) production, and over expression activity of the efflux pumps MexAB-OprM. The resistant to IPM is confined to the OprD and MBLs (Codjoe and Donkor 2018, Silva et al 2020). The one of the key factor for consideration with respect to carbapenem (IPM) resistance in *P. aeruginosa*, both from a therapeutic standpoint and from an epidemiological standpoint, is whether this is mediated by transferable MBLs or by chromosomal *OprD* porin gene.

The present study revealed that all MDR, MBLs-negative, and IPM-resistant isolates which were our target for measuring *OprD* gene expression showed reduced expression after treatment with sub-MIC of IPM. Oliver (2017) stated that in all cases of carbapenemase negative *P. aeruginosa* strains, resistance resulted from *OprD* mutation, either through the creation of premature termination codons, insertions, deletions or through interruption of the gene due to insertion sequence. The one isolate (isolate number =7) revealed increasing in transcriptional level of *OprD* by 23.125 fold compared to control. This isolate was sensitive to IPM despite its production of MBLs which confirms the important role of *OprD* porin gene in resistance to IPM in *P. aeruginosa*. The increased expression of this isolate may be a result of mutation or reduced expression in genes that down regulate OprD gene expression such as *mexT* gene which located upstream of the *mexEF-oprN* efflux operon. When MexT is overexpressed it increases the expression level of the mexEF-oprN efflux while decreases the expression level of OprD (Ochs 1999b). This is positive news, on the one hand because the risk of horizontal spread of the resistance mechanism is much lower, and on the other hand because, as production of MBLs, is not involved, the new combinations of β-lactams plus inhibitors of β-lactamase, ceftazidime–avibactam and ceftolozane–tazobactam could show activity in these strains. After treatment with

combination of sub-MIC of IPM plus sub-MIC of ethanol extract of pumpkin seeds, average of folding slightly increases (statistically nonsignificant) comparing to that of samples treated with IPM alone. The possible explanation for this is that Pumpkin seeds have one of the highest concentrations of arginine. Evaluation of amino acid concentrations in pumpkin seeds revealed that arginine (1.70-23.10 g/100g), glutamic, Leucine and aspartic acids are the most considerable residues, but methionine is the least (Dowidar et al 2020, Dotto and Chacha 2020). The

Table 3. Cycle threshold and folding values of *OprD* and *rpsL* genes for *Pseudomonas aeruginosa* isolates

Groups	Isolate number	Mean of Ct values of *rpsL* gene	Mean of Ct values of *OprD* gene	ΔCt	ΔΔCt	Folding	Average of folding
Control (Untreated)	2	6.93	14.88	7.96	0.00	1	1.00 ±0.00 a
	8	6.19	18.03	11.84	0.00	1	
	11	3.71	16.71	12.99	0.00	1	
	17	5.50	18.63	13.13	0.00	1	
	7	3.33	20.72	17.39	0.00	1	
Treated with sub-MIC of IPM	2	5.08	20.00	14.92	6.96	0.008	0.099 ±0.02 b
	8	5.00	27.80	22.80	10.96	0.001	
	11	4.39	26.87	22.48	9.49	0.001	
	17	3.90	18.40	14.5	1.37	0.386	
	7	3.71	16.57	12.86	-4.53	23.125	
Treated with sub-MIC of IPM and extract	2	2.48	17.29	14.81	6.85	0.009	0.168 ±0.06 b
	8	6.93	27.11	20.18	8.34	0.003	
	11	4.74	19.42	14.68	1.69	0.309	
	17	3.97	18.61	14.64	1.51	0.351	
	7	3.77	17.51	13.74	-3.65	12.550	
LSD value	--	--	--	--	--	--	0.371 **

Means having with the different letters in same column differed significantly. ** (P≤0.01).
IPM: imipenem, isolate number 7 was excluded from statistical analysis

Table 4. Cycle threshold and folding values of *OprD* and 16SrRNA genes for *Acinetobacter baumannii* isolates

Groups	Isolate number	Mean of Ct values of 16SrRNA gene	Mean of Ct values of *OprD* gene	ΔCt	ΔΔCt	Folding	Average of folding
Control (Untreated)	2	16.486	25.599	9.113	0.000	1.000	1.00 ±0.00 b
	5	13.212	23.717	10.505	0.000	1.000	
	6	13.193	23.059	9.866	0.000	1.000	
	12	11.173	24.487	13.314	0.000	1.000	
	14	12.237	23.524	11.287	0.000	1.000	
Treated with sub-MIC 1 of IPM	2	18.351	26.727	8.376	-0.737	1.667	3.40 ±0.08 a
	5	17.604	25.281	7.677	-2.828	7.101	
	6	14.913	25.539	10.626	0.76	0.591	
	12	14.051	24.891	10.84	-2.474	5.556	
	14	14.023	24.228	10.205	-1.082	2.117	
Treated with sub-MIC 2 of IPM	2	18.381	23.797	5.416	-3.697	12.969	4.01 ±0.13 a
	5	14.630	24.430	9.8	-0.705	1.630	
	6	11.322	26.679	15.357	5.491	0.022	
	12	14.652	26.934	12.282	-1.032	2.045	
	14	13.522	23.036	9.514	-1.773	3.417	
LSD value	--	--	--	--	--	--	0.7023 **

Means having with the different letters in same column differed significantly. ** (P≤0.01).

expression of OprD is strongly induced when basic amino acids (arginine, histidine, glutamate, or alanine) serve as the sole source of carbon. The arginine-mediated induction depends on the Arginine-responsive regulator (ArgR) which can bind to the operator region of *oprD* and upregulate OprD expression (Ochs 1999a).

With regard to *A. baumannii*, the experiment of the quantitative RT-PCR reaction was completed by using 5 isolates (4 MDR, MBLs-positive, & IPM-resistant isolates and one MDR, MBLs-negative, & IPM-sensitive isolate). The quantitative changes in the mRNA expression levels are shown in (Table 4). After treatment with sub-MICs of IPM, average of folding increased by 3.40 & 4.01 fold compared to control. There was a significant difference between averages of folding before and after treatment with sub-MIC 1, while there wasn't between those of treatments with sub-MICs. This indicates that the *A. baumannii* OprD-like protein is not contributed to carbapenem (IPM) resistance in this species. Catel-Ferreira et al (2012) concluded that the *A. baumannii* OprD-like protein is not involved in specific antibiotic diffusion and would consequently not contribute to carbapenem resistance. This conclusion was confirmed by Smani and Pachón (2013) who stated that the *A. baumannii* OprD-like protein is not related to permeability to carbapenems. This channel may have functions similar to those of the Pseudomonas OprQ protein, offering specific binding sites for iron and magnesium ions and allowing *A. baumannii* to adapt to stress conditions. Nucleo et al (2009) concluded that exposure to sub inhibitory concentrations of IPM can stimulate biofilm formation and induce iron uptake in a pathogenic strain of *A. baumannii*, with potential implications on antibiotic susceptibility and ability to persist in the human host. Cabral et al (2011) indicated the implication of OprD-like protein in *A. baumannii* biofilm formation.

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Vitamin D Receptor (VDR) Gene Polymorphism Rs 1544410 and Relation to Hypocalcemia in Iraqi Pediatrics Beta Thalassemia Major

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Abstract: In Iraq, the β-thalassemia with calcium and vitamin D deficiency are a real problem. So, this study was conducted to investigate vitamin D receptor (VDR) gene polymorphism and relation to hypocalcemia in Iraqi Pediatrics beta thalassemia major . Blood samples were collected from 50 patients suffering from B-TM with hypocalcemia as work group and 25 patients suffering from B-TM with normal calcium as positive control and from 25 of apparently healthy volunteers as a negative control with age (3-8) years. Serum level of vitamin D3 was measured using enzyme-linked sorbent assay (ELISA) technique, calcium and alkaline phosphatase levels were determined by Beckman Coulter AU480 . To assess relationship of VDR gene single-nucleotide polymorphism (SNP) C/A (Rs 1544410) effected on vitamin D levels with hypocalcemia in pediatrics with B-TM, DNA was extracted from peripheral blood, and genotyped for VDR C/A SNP by high resolution method (HRM) RT PCR. The work group recorded the lowest weight (11.47 kg) relative to age of 5.69 year. The results of serological and biochemical study showed that significantly lower serum calcium (1.721 mg/dL) along with lower serum vitamin D3 (23.53 ng/ml), elevated ALP level (186.06 U/L.) with significant differences compared to control groups. The patients with at least one copy of (A) allele had a higher risk for hypocalcemia with OR (1.318) as well as there was association between the existence of the A allele whether in heterozygous (AC) or homozygous (AA) genotypes) and the development of hypocalcemia. This can be concluded from study that the incidence of VDR (C/ A) SNP plus (A) allele could be related to hypocalcemia in B-TM Iraq pediatrics.

Keywords: Vitamin D receptor, Gene polymorphism Rs 1544410, Hypocalcemia

Thalassemia is one of the most common genetic diseases especially, group β-Thalassemia major (B-TM) form a serious health problem in which children are in need of regular blood transfusions from a very young age to survive (Elalfy et al 2014) and is considered as the most important cause of mortality in patients (Mustafa et al 2020). World health organization (WHO) estimates that almost 270 million people are carriers of the syndrome (De Sanctis et al 2017). According to the Iraqi Ministry of Health (2019), there are about 22,000 people with thalassemia The importance of the disease comes from the fact that it is difficult to treat, the only curative treatment for patient is steam cell transplantation which has limited availability due to a lack of donors (Nameq et al 2020). Hypocalcemia is a condition in which there are lower-than-average levels of calcium in the blood is linked to most cases of magnesium or vitamin D deficiency. Vitamin D deficiency is commonly identified in beta thalassemia major patients, related to iron accumulation. Vitamin D mediates its action upon binding to vitamin D receptor (VDR), a classical nuclear receptor (Jwaid and Gata 2020). In Iraq, in spite of the β-thalassemia is considered a real problem due to the lack of medicines (Underland et al 2020), but was observed that the studies dealing with thalassemia major associated with hypocalcemia in children were rare or limited. In the present study examined the effect of VDR gene polymorphism on hypocalcemia.

MATERIAL AND METHODS

Blood samples were collected from 50 patients (29 female and 21 male) suffered from beta thalassemia major with hypocalcemia, the age of the was ranged between 3-8 years with mean age 5.69 year) who attended the genetic hematology center that located in Feminine and Children Ibn Al-Baladi hospital in Baghdad from the beginning October 2020 to February 2021. Data privacy was protected according to the protocol Helsinki Declaration (1964) and was agreed with the medical ethics committee. Written acquainted approval was collected from patients and their parents for each participant. Control individuals were divided into two groups; the first included 25 samples were taken from children with beta thalassemia major with normal value of calcium as positive control from genetic hematology center that located in Feminine and Children Ibn Al-Baladi hospital. The second groups included 25 samples were taken from apparently healthy children as negative control and have been chosen depending on two criteria: First the children had normal vitamin D and calcium levels, second with age 2 -10 years

Sample collection: Five milliliter of venous blood were obtained by a sterile venipuncture under optimal condition, and divided into 2 parts: first part (2ml) was put into an ethylene diamine tetra-acetic acid (EDTA) tube and kept at (-

20 C°(for DNA extraction and subsequent VDR gene polymorphism). The second part of blood sample (3ml) put in gel tube for two hours, then centrifuged at 5000 rpm for 5 minutes. The serum was collected and kept at (-20 C°) for biochemical tests and serological analysis by Enzyme-Linked Immunosorbent Assay (Elisa).

Biochemical test: Serum levels of calcium and alkaline phosphtase (ALP), were measured by fully automated Coulter AU480 chemistry analyzer in Ibn Albaladi hospital's laboratory according to Mohammed (2018)

Determination of Vitamin D3 Titer: Titer determination of vit D3 was done by using ELISA technique according to Norman and Anthony (1998).

Detection Vitamin D receptor (VDR) gene polymorphism (Rs1544410): Total genomic DNA extracted from whole frozen blood was done according to Shi et al (2017). By using commercial genomic DNA purification kit (Transgene Biotech/ China) according to Mohammed et al (2016). Concentration and purity of the DNA were estimated by Qubit device (Thermo Fisher/ USA). DNA was analyzed by gel electrophoresis using 1% agarose gel and DNA Red safe dye, at voltage 50 v/cm for 45min. Primers supplied by Macrogen/ Korea are listed with their sequences in Table 1.

Solis Bio Dyne was employed to perform qPCR-HRM. The cycling protocol was programed for the following optimized cycles and according to the thermal profile shown in Table 2. To detect allelic differences, triplicate synthetic controls were analyzed by qPCR-HRM, and normalized melting curves (NMC) and differential curves (DC) were obtained using the HRM tool included in the integrated software.

Statistical analysis: SAS (2018) program was used to detect the effect of different factors in study parameters. Least significant difference –LSD test was used to significant comparison between means. Chi-square test was used to significant comparison between percentage (0.05 and 0.01 probability). Odds ratios (ORs) with a 95% confidence interval (CI) was used to evaluate the potential associations between genetic variants.

RESULTS AND DISCUSSION

The distribution of thalassemia major with calcium deficiency patients was 42% in males and 58% females, as well as for thalassemia major with no calcium deficiency patients was 44% males and 56% females with significant difference (Table 3). These findings agreed with the study of Jwaid and Gata (2020). The frequency of thalassemia major was in female was more than male. However current results were disagreed with the study of Al-Ali and Faraj (2016) demonstrated that males were significantly more affected than females.

Regarding to association between Beta Thalassemia Major with age and body weight results found a significant difference between the three groups included in the study in terms of weight relative with ages, where the work group recorded the lowest weight (11.47 kg) relative to age of 5.69 year, followed by the positive control group (14.95 kg relative to age 6.83 year), while the negative control had normal weights (18.17 kg in age 5.83 year) (Table 4).

Weights differences are due to these children were relatively anorexic and suffer from severe anemia. These results are almost identical with study of Rathaur et al (2020) where 77% of thalassemic children were underweight because of failure in growth. Moiz et al (2018) noticed that all thalassemic children were malnourished and the obesity was absent. Sherief et al (2014) observed that beta thalassemia major patients had significant lower weight as compared to

Table 1. Primers of BsmI SNP (Rs1544410) of VDR gene (C /A)

Sequence (5'-3')	Product size(bp)	Reference
5'-GCAAGAAACCTCAAATAACAGG-3'	121	Lv et al 2016
5'- ATTCTGAGGAACTAGATAAGCAGG-3'		

Table 2. Program of qPCR-HRM HRM

Step	Temperature	Duration	Cycles
Enzyme activation	95ºC	5 min	1
Denature	95ºC	15 sec	40
Annealing	60ºC	15 sec	
Extension	72ºC	30 sec	
HRM	65-95	0.2sec for 1 degree	

Table 3. Distribution of patients with thalassemia major according to gender

Gender	Patients	Negative control	Positive control
Male	21 (42%)	19 (76.00%)	11 (44%)
Female	29 (58%)	6 (24.00%)	14 (56%)
Total	50	25	25
P-value	0.0094 **	0.0001 **	0.0097 **

** (P≤0.01)

Table 4. Association between beta thalassemia major with age and body weight (Mean ± SE)

Group	Age (Year)	Weight (kg)
Work group	5.69 ±0.22	11.47 ±0.32 c
Negative control	5.83 ±0.61	18.17 ±1.05 a
Positive control	6.83 ±0.51	14.95 ±1.01 b
LSD value	1.1873 NS	1.930 **
P-value	0.1188	0.0001

Means have different letters in same column mean significantly different
** (P≤0.01)

controls because of trace elements and vitamins deficiency. Nevertheless, study given by Kattamis et al (1970) which included several groups, one of the groups had normal weights for the patients, while the second group had a low weight of the children. This study proves that the growth of thalassemic children during the first decade largely depends upon the maintenance of fairly high Hb levels.

The results of estimation of Vit. D, Ca and ALP levels in serum of work and control groups shows that the level of ALP was elevated (186.06 U/L) while level of vitamin D (23.53 ng/ml) and calcium (1.721 mg/dL) was low in work group when compared with control groups (positive and negative) with high significant differences (P<0.01). Nevertheless, all other values were normal (Table 5).

The present results agreed with study of Waheeb et al (2019) who showed that the deficiency of hydroxylase vitamin D (25 OH D) was common in thalassemia children along with low level of calcium. Alhoseiny et al (2015) observed highly statistically significant difference between patients and controls regarded to ALP vit and D serum calcium levels, which were significantly high in ALP level, besides significantly low in vit D and serum calcium in thalassemic patients comparing with controls. Singh et al (2012) also observed that thalassemia patients had low serum calcium and vit D levels with elevated of serum ALP levels. Chatterton et al (2003) demonstrated that the vit D deficiency, osteocalcin, and rickets in thalassemia patients as a result of defective 25 hydroxylation of vitamin D due to iron overload and subsequent liver dysfunction. Other mechanisms lead to disturbed calcium, ALP and vitamin D homeostasis include decreased intake, impaired absorption, and reduced synthesis of vitamin D (Pollak et al 2000). Tantawy et al (2008) found that 75 % of their beta thalassemic patients had a low calcium level which was probably caused by osteomalacia evidenced by elevated bone ALP presumably resulting from deficient calcium intake. In contrary to the current results the study of El-Edel et al (2010) found normal levels of 25 (OH) D3 in their thalassemic patients. Salama et al (2006) showed that there is no alteration in calcium level in β-thalassemia major patients as compared with controls. This dissimilarity in findings might be due to variation in nutritional status in study groups as mentioned by Mahachoklertwattana et al (2003).

The results of the effect of gender on Vit. D, Ca and ALP levels in thalassemia major patients, indicated that there was no significant difference for vitamin D and calcium between males and females (Table 6), but there was elevated in alkaline phosphatase level (211.88 U/L)) in females when compared with males (149.36 U/L)) with significant difference.

These differences may be caused by osteomalacia and osteoporosis which were more common in patient with beta thalassemia major specialty in (Gaudio et al 2019), because females have thinner and lighter bone compared with males (Alswat 2017). Waheeb et al (2019) observed significant difference between male and female in ALP value when studied biochemical were performed in 50 patient's beta thalassemia ,but it disagreed with Singh et al (2012) where significant difference in ALP value in male more than female with P value (0.168) was observed when detected 25-hydroxyvitamin D deficiency and effect of vitamin D receptor gene polymorphisms on bone mineral density in thalassemia patients of North India . Turan et al (2011) suggested that no significant difference in ALP level between female and male until puberty, addition to there was no significant difference regarding to vitamin D and calcium between male and female when they investigated. Waheeb et al (2019) when studied vitamin D3 according to the genders there were no significant difference (15.6 ng/ml) in female and (15.5ng/ml) male as for calcium also there was no difference between gender (1.85) in female (1.89) in male. The result showed that DNA purity was good and ranged between 1.7- 1.9. and concentration of acceptable ranged from 40-60μg/m areThe results of gel electrophoresis showed sharp bands of chromosomal DNA (Fig.1).

The results of the relationship between VDR gene polymorphisms and beta thalassemia major patients, show that among 50 patients of beta thalassemia major with calcium deficiency as work group, 25 patients of beta thalassemia major with normal calcium level as positive control group, and 25 children apparently healthy as a

Table 5. Estimation of Vit. D, Ca and ALP levels in serum of work and control groups (Mean ± SE)

Group	Vit. D (ng/ml)	Ca (mg/dL)	ALP (U/L)
Patients	23.53 ±0.52C	1.721 ±0.02B	186.06 ±16.22A
Negative control	71.63 ±0.70A	2.504 ±0.02A	70.70 ±2.87B
Positive control	64.52 ±1.43B	2.491 ±0.02A	80.50 ±3.32B
LSD value	2.595 **	0.097 **	63.938 **
P-value	0.0001	0.0001	0.0001

Means have different letters in same column mean significantly different ** (P≤0.01)

Table 6. Effect of gender on Vit. D, Ca and ALP levels in thalassemia major patients (Mean ± SE)

Gender	Vit. D (ng/ml)	Ca (mg/dL)	ALP(U/L)
Male	23.15 ±0.84	1.742 ±0.04	149.36 ±23.22
Female	23.80 ±0.67	1.707 ±0.03	211.88 ±21.24
P-value	0.552 NS	0.472 NS	0.050 *

* (P≤0.05), NS: Non-significantly

Table 7. Genotype and allele frequency of VDR Rs 1544410 gene in work and control groups

Genotype	Work group (50)	Negative control (25)	Positive control (25)	Chi-Square (χ^2)	P-value	O.R (C.I)
CC: Wild	28 (56 %)	17 (68%)	19 (76%)	7.95 **	0.0076	1.00 (Reference)
AC: Hetero	17 (34%)	1 (4%)	1 (4%)	9.026 **	0.0037	1.318 (0.72-2.75)
AA: Mutant	5 (10 %)	7 (28%)	5 (20%)	6.33 **	0.0097	0.882 (0.47-1.65)
Allele		Frequency		--	--	--
C	0.73	0.70	0.78	--	--	
A	0.27	0.30	0.22	--	--	

** ($P \leq 0.01$)
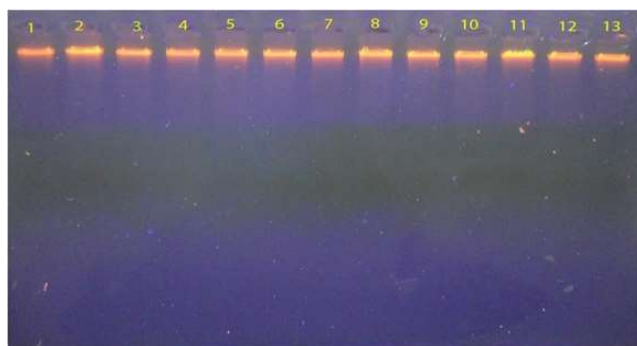
Fig. 1. DNA bands on 1% agarose gel at 100 Volts for 45 min. Lane 1-13 Genomic DNA extracted from blood samples of work group

negative control. The distribution of VDR Rs 1544410 C/A alleles genotype was the CC wild genotype 28 (56%) in work group, 19 (76%) in positive control group, and 17 (68%) in negative control, while CA genotype was 34% in work group, and 4% in both of positive and negative control groups, whilst the AA genotype was 10% in work group, 20% in positive control, and 28% in negative control with high significant difference and frequency of C allele was 0.73 in work group, 0.78 in positive control, and 0.70 in negative control, as well as frequency of A allele was 0.27 in work group, 0.22 in positive control, and 0.30 in negative control (Table 7). The results of the present study indicated that there was risk factor in work group compared to control group with hetero genotype AC have O.R 1.3 this indicated that VDR Rs (1544410) gene was related to hypocalcemia in pediatric with beta thalassemia major. Sakamoto et al (2021) also found the VDR (*Bsm*I) genotype interacted with the calcium intake and be related to a lower bone mass under conditions of low calcium in study on relationship between vitamin D receptor gene polymorphisms *Bsm*I, *Taq*I, *Apa*I, and *Fok*I and calcium intake on bone mass in young Japanese women. Also was agreement with Gaffney-Stomberg et al (2017) who found the relation between calcium and vitamin D with SNP Rs (1544410) when they studied association between single gene polymorphisms and bone biomarkers and response to calcium and vitamin D supplementation in young adults undergoing military training. Elhoseiny et al (2015) also found patients with beta thalassemia major BsmI genotypes were associated with lower calcium and lower vitamin D3 level in they studied Vitamin D receptor VDR gene polymorphisms (FokI, BsmI) and their relation to vitamin D status in pediatrics beta thalassemia major. These result as well as was disagreement with Ferrari et al (1998) as no relation between VDR Rs (1544410) and vitamin D status with calcium metabolism was observed Al-Ghafari et al (2019) also observed *VDR* polymorphisms (Rs1544410) did not affect on levels of vitamin D_3, and calcium when they studied relationship between serum vitamin D and calcium levels and vitamin D receptor gene polymorphisms in colorectal cancer.

The results of relationship between VDR gene Genotypes and Vit. D, Ca and ALP levels in work group observed no significant changes in Vit. D, Ca and ALP association with VDR gene genotypes in work group who suffered from beta thalassemia major with calcium deficiency (Table 8). The genotype AA showed a highest level of ALP (211.10 U/L) among the other genotype groups, also the genotype CC had highest levels of Vit. D (24.73 ng/ml) and Ca (1.760 mg/dL) among the other genotype groups, moreover the genotype AC showed a lowest levels of ALP (135.06 U/L)), Vit. D (21.80 ng/ml)) and Ca (1.692 mg/dL)) among other

Table 8. Relationship between Genotype of VDR Rs 1544410 gene and Vit. D, Ca and ALP in work group (Mean ± SE)

Genotype	Vit. D (ng/ml)	Ca (mg/dL)	ALP(U/L)
AA	24.12 ±0.56	1.727 ±0.03	211.10 ±21.57
AC	21.80 ±1.23	1.692 ±0.05	135.06 ±20.10
CC	24.73 ±1.31	1.760 ±0.06	178.40 ±62.54
LSD value	3.174 NS	0.148 NS	98.598 NS
P-value	0.108	0.684	0.117

NS: Non-significantly

genotype groups. The results show non-significant between genotyping for Ca, Vit D and ALP. Schuch et al (2013) suggested that deficiency in Vit D and Ca caused by independent of location, age and socioeconomic or cultural levels, and is mainly related to inadequate sun exposure aggravated by inadequate consumption of foods containing this vitamin not by gene polymorphism, when studied relationship between vitamin D receptor gene polymorphisms and components of metabolic syndrome . ALP elevated in level may be because of correlate with rate of bone growth in children special in this age (Corathers 2006).

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Screening, Optimization and Partial Purification for Tannase Produced from Some Gram-Negative Bacterial Isolates from Different Hospitals in Baghdad City

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Abstract: In this study, one hundred isolates of gram negative bacteria have been collected from several hospitals located in Baghdad city, these isolates were 33 *Escherichia coli,* 32 *Pseudomonas aeruginosa,* 27*Klebsiella pneumoniae,* 3*Shigella dysenteriae and Enterobacter cloacae* and two isolates for *Salmonella typhi*. The screening for detecting tannase production was performed by semi-quantitative and quantitative analysis. The results revealed that out of all obtained isolates, 60% were tannase producers by semi-quantitative method while by quantitative screening method only 50 percent isolates revealed specific activity ranged from 28.34 to 38.25 U/mg and the maximum specific activity was for *P.aeruginosa*W16, which was selected as best producer isolates. The tannase activity reached its maximum level when this isolate was cultivated under the optimal conditions, which is consisted of using tannic acid broth medium supplemented with 1% pomegranate peel and 1% beef extract at pH 6 and incubated at 37°C for 48 hour. The tannase had been partial purified by using ammonium sulfate and the best percentage ratio was 80% and activity and specific activity was 64.3 and 189.11U/mg, respectively.

Keywords: Gram negative bacteria, Optimization, Purification, Tannase

Gram-negative bacteria (GNB) are among the most significant public health problems in the world due to their high resistance to antibiotics (Muslim et al 2021). These microorganisms have great clinical importance in hospitals because they often require patients to be in the intensive care unit (ICU), and are at high risk of morbidity and mortality (Hormozi et al 2018). The myriad of disease-causing general/species in humans, including urinary tract infections, pneumonia, diarrhea, meningitis, sepsis, endotoxic shock, and many others. The general/species that frequently affect humans are *Escherichia* sp*., Proteus* sp*., Enterobacter* sp*., Pseudomonas* sp., Klebsiella sp*., Citrobacter* sp*., Yersinia* sp*., Shigella* sp*.* and *Salmonella* sp*., (*Oliveira and Reygaert, 2021).

The tannin acyl-hydrolase (EC 3.1.1.20) belonging to the superfamily of esterase catalyzes hydrolysis of ester and depside bonds in hydrolysable tannin by releasing as gallic acid and glucose (Aharwar and Parihar 2021). It is known as membrane bound enzyme and it also secrete extracellular and can be from various sources such as animals, plants and microorganisms but it is produced in large amount by microorganisms bacteria, fungi and yeast (Thiyonila et al 2020). The aim of this research was to detect the tannin lytic activity in some gram-negative bacteria, besides to optimization production conditions and partial purification of tannase.

MATERIAL AND METHODS

Collection of bacterial isolates: One hundred clinical of gram negative bacterial isolateswere collected from different hospitals at Baghdad city. These bacterial isolates obtained from different clinical specimens including: urine, stool and blood samples as well as wound and ear swabs. After collection, all bacterial isolates were identified depending on cultural characteristics, microscopical examination, biochemical tests and VITEK 2 compact system.

Detection of Tannase Production

Semi-quantitative analysis: All bacterial isolates were inoculated to tannic acid agar medium. Plates incubated at 37°C for 48 hrs., an appearance of a greenish brown zone around the colonies refer to tannase production and then the diameters of clear zones around the colonies were measured (Muslim *et al* 2015).

Quantitative analysis: The chosen bacterial isolates were inoculated to tannic acid broth medium and incubated at 37°C for 48 hrs. after centrifugation at 8000 rpm for 20 min. The resulting supernatant was used as the crude extract to establish the tannase activity (Muslim et al 2015).

Assay of tannase activity: Ultra violet spectrophotometric method was used for detecting tannase activity. This method was based on changing of ultra violet absorption. The enzyme activity was determined by the hydrolysis of the ester bonds of tannic acid. The assay method performed by adding

0.5 ml of crude enzyme to 2 ml of 0.35% (w/v) tannic acid dissolved in acetate buffer solution. 20µl of the reaction mixture was withdrawn from the total system and 2 ml of ethanol solution was used to stop enzyme reaction. Absorbance was noted as t1 at 310 nm immediately after adding ethanol and as t2 after 10 min of incubation at 37°C (Brahmbhatt and Modi 2015). Tannase activity was calculated from the following formula:

$$\text{Enzyme activity(U/ml)} = \frac{114 \times \text{Change in absorbance}}{\text{Difference in time}(t2 - t1)}$$

Determination of protein concentration: Protein concentration was measured by the method of Bradfored (1976) with Bovine serum albumin (BSA).

Determination the Optimal Conditions for Tannase Production

Effect of nitrogen sources: Tannic acid broth medium was used to detect the effect of different nitrogen sources on tannase production that carried out by using organic and inorganic nitrogen sources including (yeast extract, beef extract, tryptone, ammonium chloride and ammonium nitrate). These compounds were tested at 1gm /100ml.

Effect of nitrogen source concentration : Many concentrations of the best nitrogen sources including 0.5, 1, 1.5, 2, 2.5 and 3.0 gm/100ml were used for detection the best concentration chosen of nitrogen source for tannase production.

Effect of carbon sources: The tannic acid was replaced with various tannin-rich agro-residue wastes such as pomegranate peel, celery seeds, berries fruits, grape seeds, palm pollen, tamarind seeds and olive leaf at 1gm /100 ml as carbon sources for tannase production.

Effect of carbon source concentrations: Many concentrations of the best carbon source including 0.5, 1, 1.5, 2 and 2.5 gm/100 ml were used for detection the best used concentration as carbon source for tannase production.

Effect of pH: In order to determine the optimal pH for crude tannase activity, pH of the culture medium was adjusted to different values range (2, 3, 4, 5, 6, 7, 8, and 9).

Effect of temperature: Optimal temperature for crude tannase activity was determined by incubation the culture medium at different temperatures (20, 30, 35, 37, 40 and 45°C).

Effect of incubation period: Effect of incubation period for tannase production was studied for different period of incubation (24, 48 and 72hrs.).

Effect of inoculum size: The best inoculum size was achieved by inoculating the production medium, individually with different inoculum size (0.5, 1, 1.5, 2 and 2.5 %).

Extraction and partial purification of tannase: The tannase extraction and partial purification was carried out according to (Brahmbhatt and Modi, 2015). The selected isolate was grown in the best culture medium with optimal conditions for tannase production. The culture was centrifuged at 8000 rpm for 20 min (under cooling), the obtained supernatant (crude extract) was subjected to ammonium sulfate precipitation at different concentrations (20-90%) saturations. All samples were left overnight at 4°C, and then the precipitates were collected by centrifugation at 1000 rpm for 15 min and dissolved in acetate buffer. The tannase activity, protein concentration and the specific activity were measured. The obtained ammonium sulfate precipitate (in solution) was introduced into a dialysis tube with 3000 kDa cutoff against acetate buffer overnight. The obtained tannase enzyme preparation was kept at 4°C.

RESULTS AND DISCUSSION

Collection of bacterial isolates: The results showed that most collected isolates were obtained from UTI infections (42) followed by wound infections (24), stool (180, blood and ear infections (9 and 7).

Most bacterial isolates were belonged to the *Escherichia coli* and *Pseudomonas aeruginosa* 33 and 32 followed by *Klebsiella pneumoniae* (27). The other isolates included *Shigella dysenteriae* and *Enterobacter cloacae* (3) and two isolates for *Salmonella typhi*. The ability of bacterial isolates for tannase production was examined by using semi-quantitative and quantitative methods.

Semi quantitative method: There were strong, moderate and weak producer for tannase that surrounded with visible dark green zone in different levels (Fig. 2A1, 2, 3). The negative isolates had not shown hydrolysis dark zone (Fig. 2A4).

Depending on the diameter of dark green zones, only 60% isolates had the ability to produce tannase. Most of tannase producers were *Pseudomonas aeruginosa* (74.97%) isolates with the diameter of dark zoon ranged between 14.4-29.5 mm followed by *E.coli* (60.60%) and *K. pneumoniae* (59.24%) with diameter of dark zone ranged
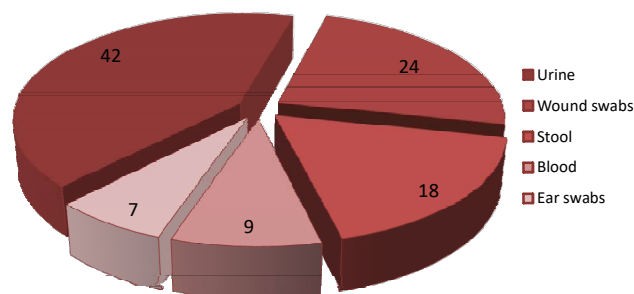
Table 1. Screening of tannase producing isolates

between 13-25.5mm and 9-25mm, respectively (Table 2). *Shigella dysenteriae, Salmonella typhi* and *Enterobacter cloacae* isolates had not any ability to produce of tannase. *Pseudomonas aeruginosa* W16 was the strongest producer for tannase with highest diameter (29.5mm) and intensity dark green zone (Figure 2B). The differences in tannase production by bacterial isolates may due to differences in the sources of these isolates or the variation in the gene expression for synthesis of tannase (Sheela et al 2016).

B. Quantitative screening for tannase production: According to semi-quantitative method there were 60
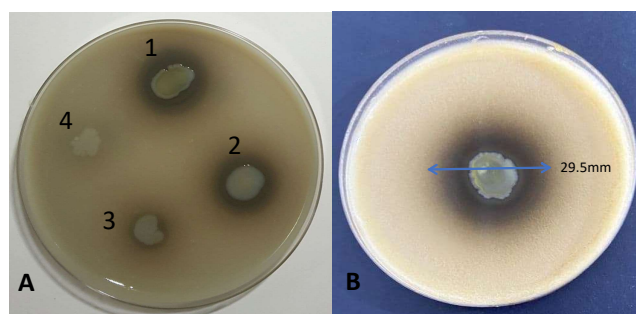
Fig. 2. (A) 1: strong producer, 2: moderate producer, 3: weak producer, 4: non-producer (B) *P. aeruginosa* W16 producer isolate on tannic acid agar medium

bacterial isolates including P. *aeruginosa, E. coli* and *K. pneumoniae* isolates had an ability to produce tannase enzyme but in quantitative method only 23 isolates out of these 60 isolates that gave larger hydrolysis dark green zone. These 23 isolates revealed different levels in tannase production with specific activities ranged from 28.34 to 38.25 U/mg of with maximum tannase activity equals to 15.3 U/ml and 38.25 U/mg of specific activity by *P. aeruginosa* W16 (Fig. 3). Abdal et al (2020a) observed that tannase produced from *K. pneumoniae* which isolated from UTI clinical samples, that gave a higher yield of tannase and it chosen based on hydrolytic zone formation around the colonies, Abdal et al (2020b) reported that *K. pneumoniae* gave highest activity level of tannase activity of 40U/ml and specific activity of 86.95U/mg. Muslim et al (2017) found that *Serratia marcescens* had an ability to produce tannase in different levels.

Optimization of Growth Conditions for Tannase Production

Effect of nitrogen sources: The effect of different nitrogen sources on tannase production was carried out using organic and inorganic nitrogen sources including yeast extract, beef extract, tryptone, ammonium chloride and ammonium nitrate. The beef extract proved to be the best for tannase production from *P.aeruginosa* with specific

Table 1. Distribution of bacterial isolates according to source of isolations

Bacterial isolates	Source of isolation					
	Wound swabs	Stool	Urine	Blood	Ear swabs	Total
Escherichia coli	5	8	14	4	2	33
Klebsiella pneumonia	3	2	18	3	1	27
Pseudomonas aeruginosa	16	2	8	2	4	32
Shigelladysenteriae	0	3	0	0	0	3
Salmonella typhi	0	2	0	0	0	2
Enterobacter cloacae	0	1	2	0	0	3
Total	24	18	42	9	7	100

Table 2. Numbers and percentages of selected tannase producer bacterial isolates

Clinical isolates	No. and percentage of tannase producer isolates		
	P. aeruginosa	*E.coli*	*K.pneumoniae*
Urine	3 (9.37%)	6 (18.18%)	8 (29.62%)
Wound swabs	13 (40.6%)	5 (15.15%)	3 (11.11%)
Blood	2 (6.25%)	4 (12.12%)	3 (11.11%)
Stool	0	3 (9.09%)	1 (3.7%)
Ear swabs	6 (18.75%)	2 (6.06%)	1 (3.7%)
Total	24 (74.79%)	20 (60.60%)	16(59.24%)
Total isolates	32 (100%)	33 (100%)	27 (100%)

activity reached to 44.5 U/mg protein, followed by yeast extract which was the second nitrogen source suitable for the tannase production with specific activity of 33.7 U/mg protein. In contrast, ammonium chloride and ammonium nitrate represented the poor nitrogen sources for tannase production (Fig. 4). Nitrogen source is major nutrient after carbon that is essential for the growth of microorganisms in larger amounts. Nitrogen is an essential part in protein, nucleotides, enzymes and a cofactor which plays a vital role in the metabolism (Suribabu et al 2016). Kulkarni et al (2012) reported similar results and found that the beef extract yielded the highest tannase activity.

Effect of carbon sources: Seven different natural carbon sources were used for determining the optimal carbon source for tannase production by *P. aeuroginosa* W16. Among seven various tannin-rich agro-residue wastes, the pomegranate peel was the best carbon source and gave higher tannase specific activity 76U/mg. The remaining media gave tannase

specific activity ranged between (49-68U/ml). The olive leaf revealed lower tannase specific activity 18U/mg (Fig. 5). Muslim et al (2015) showed that the tannase productivity and activity was increased when the cultivation of *Erwinia carotovora* in the culture medium containing pomegranate peels extract in comparison with traditionally used substrates like tannic acid and other saccharides.
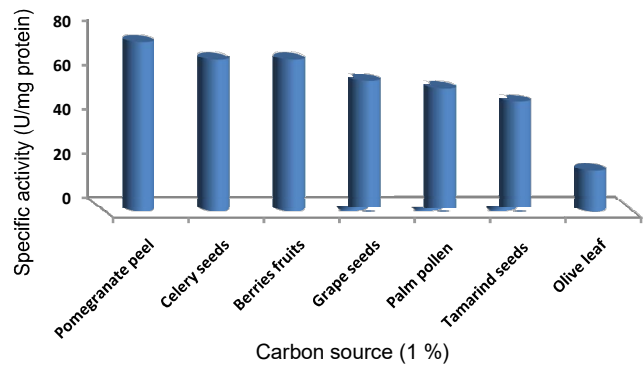
Fig. 5. Tannase produced by *P. aeruginosa* W16 at different carbon sources
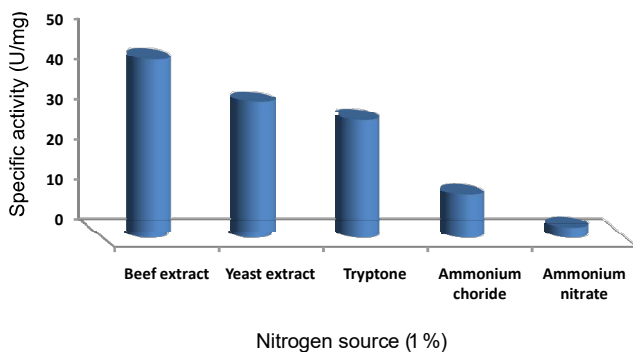
Fig. 4. Tannase production by *P. aeruginosa* W16 at different nitrogen sources
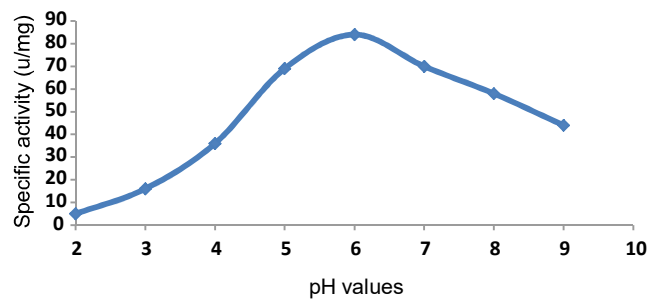
Fig. 6. Tannase production by *P. aeruginose* W16 at different pHs
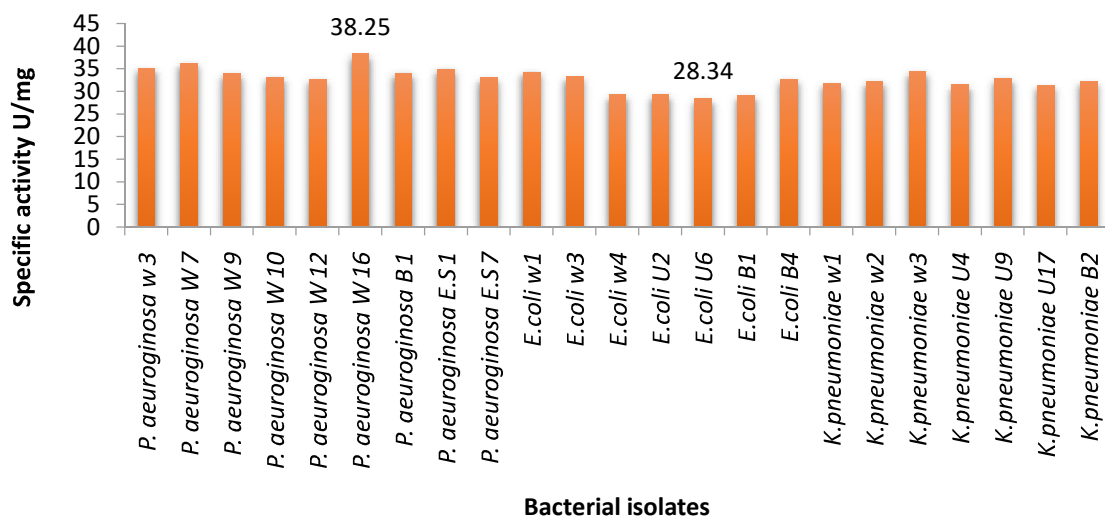
Fig. 3. Specific activities for selected bacterial isolates in quantitative method by using tannic acid broth

Effect of pH: The productivity of tannase was variable at different pHs. The optimal pH for tannase production by *P.aeruginosa*W16 was at 6 with tannase specific activity 84.6U/ mg than other pH values that led to reduce the specific activity of 5.2 and 44.9 U/mg when the pH of the medium was 2 and 9, respectively (Fig. 6). Changes in the pH or acidity of the environment can take place that would alter or totally inhibit the enzyme from catalyzing a reaction. (Shanmugam et al 2009).

Effect of incubation temperature: Different incubation temperatures (20-45ºC) were tested to determine the optimum one for tannase production by *P.aeruginosa* W16. Maximum production of tannase was reached at 37ºC. The specific activity was 84.1 U/mg protein at this temperature. However, other temperatures led to decrease the specific activity (Fig. 7). Higher temperatures caused thermal denaturation of metabolic pathway which increased the

maintenance energy requirement of cellular growth and leading to poorer metabolites production (Jana et al 2014).

Effect of different incubation periods: *P. aeruginosa* W16 after 24 hrs. of incubation gave 68 U/mg of tannase specific activity and the specific activity increased with increasing the
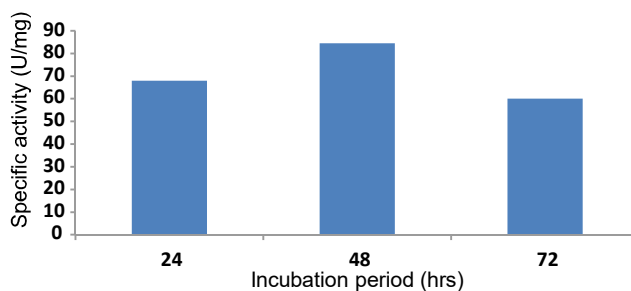
Fig. 8. Tannase production by *P. aeruginosa W*16 at different incubation periods
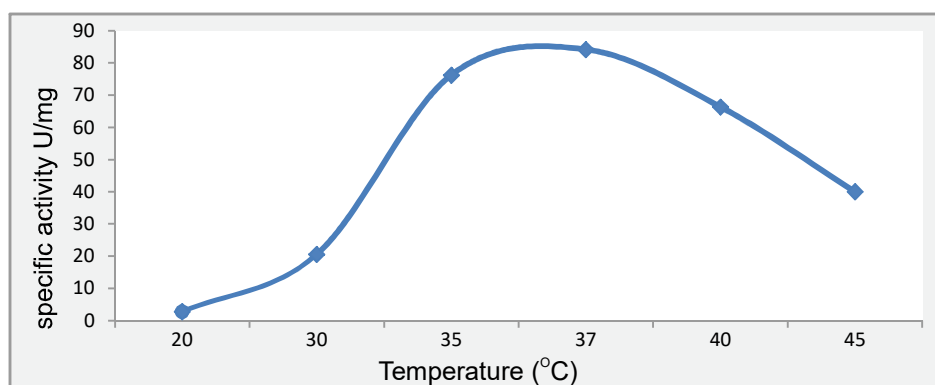
Fig. 7. Effect of incubation temperature on tannase production

Table 3. Purification steps of tannase from *pseudomonas aeuroginosa* W16

Purification step	Volume (ml)	Tannase activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude extract	100	33.93	0.39	87	3393	1	100
Ammonium sulphate	35	64.3	0.34	189.11	2250	2.17	66.3
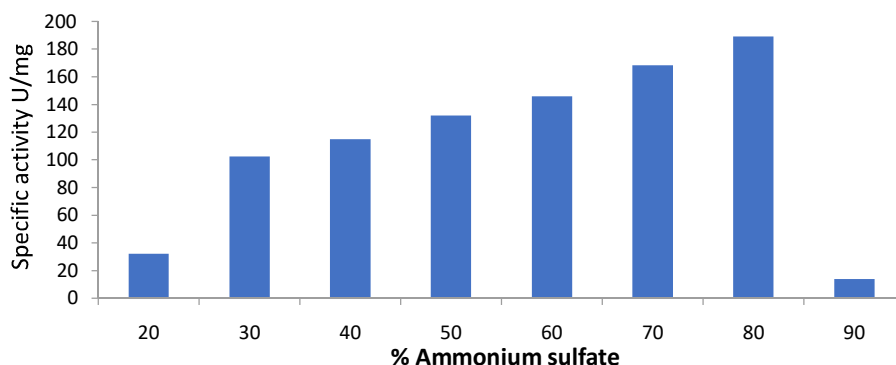
Fig. 9 Precipitation of tannase in different ammonium sulfate saturation percentage

incubation time and reached to the maximum value (84.5U/mg) after 48hrs. of incubation. After 48h tannase activity started to decline (Fig. 8). Hidayathulla et al (2018) used partial purification of tannase from *A. nidulans* and *K. pneumonia*, respectively and maximum tannase activity at the saturation ratio 80% ammonium sulfate.

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Polymorphism (rs1761667 G> A) of CD36 Gene and Association with Cardiovascular Disease in Iraqi Patients

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Abstract: Cluster of differentiation 36 (CD36) gene is involved in lipid metabolism and polymorphisms in the CD36 gene is related to cardiovascular disease risk factors. Furthermore, variations in it may play a role in the development of hypertension or coronary artery disease (CAD). The study was designed to look into the possible link between the polymorphism rs1761667 (G > A) of CD36 gene and hypertension or CAD in a group of Iraqi patients. 160 samples were examined for Iraqi patients suffering from various medical conditions. The PCR-RFLP method was used to detect the genotype of the CD36 rs1761667 (G > A) polymorphism. The CD36 rs1761667 (AA) genotype was significantly different among the four study groups. The allelic frequency variance of the (A) allele differed across all research groups in this investigation, with the highest ratio observed in the (CAD) and (L.LIPI) groups (A = 0.51). The results showed a relative relationship between the CD36 rs1761667 polymorphism and susceptibility to hypertension and CAD in Iraqi patients.

Cardiovascular disease (CVD) is one of the most important threats to human life around the world, and includes subcategories such as coronary artery disease (CAD), acute coronary syndrome (ACS) and ischemic cardiomyopathy. CAD is a multifactorial disorder which develops and progresses as a result of both environmental and genetic factors. Various risk factors are involved in the development of CAD, including, atherosclerosis, hypertension, smoking, lifestyle, high fat diet, non-exercise and diabetes mellitus (DM) (Momeni-Moghaddam et al 2019). The human Cluster Determinant 36 (CD36) gene belongs to the Human Consensus Coding Sequence set of genes (CCDS). The gene is found on the long arm of chromosome 7 at band 21.11 (7q 21.11) in humans (Ramos-Jiménez et al., 2022). Multiple ligands are attached to this receptor and CD36 participates in many biological processes (Zhang et al 2014). link between CD36 gene polymorphisms and obesity on the one hand, and CD36 gene polymorphisms, hypertension, and CVDs on the other. SNP rs1761667 (G > A) is found in the 5′ intron, flanking exon 1A.The CD36 receptor is involved in lipid metabolism and has a high affinity for long chain fatty acids (FAs). The (A) allele of rs1761667 reduces CD36 expression and is associated with higher fat identification taste thresholds (i.e., reduced oral sensitivity to fat). Furthermore, clinical studies have revealed that SNPs in this gene (such as rs1761667, rs10499859, rs3173798, and rs1049673) can affect lipid metabolism, cardiovascular risk, essential hypertension, insulin resistance, familial type 2 diabetes mellitus (T2DM),

and body mass index (BMI) (Momeni-Moghaddam et al 2019). CD36 polymorphisms are thought to increase the risk of hypertension and coronary artery disease (CAD) due to CD36's activities in regulating lipid metabolism, atherosclerosis, and blood pressure (Boghdady et al 2016).

MATERIAL AND METHODS

Subjects: The study was conducted from December 2020 to July 2021, in the laboratories of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Ibn Al-Bitar Specialized Center for Cardiac Surgery, and the Iraqi Center for Cardiac Surgery in the City of Medicine for the recorded groups as shown below. 160 blood samples were collected from men and women between the ages of (25-83), 40 samples of which were for patients diagnosed with high blood pressure and had high blood lipids, 40 samples were collected from people diagnosed with coronary heart disease and 40 samples It was collected from people who were diagnosed with high blood pressure, but who had a normal or sub-normal lipid level in the blood, and 40 samples were collected for what appeared to be healthy people as a control group.

Blood sample collection: Five milliliters of whole blood under sterile conditions were obtained by intravenous puncture using a disposable syringe. Whole blood was divided into sterile EDTA and simple tubes used for biochemistry and genetic testing. EDTA tubes for human DNA isolation. Serum was obtained by placing blood samples in a clean, dry normal plastic tube and allowing it to

coagulate at 37°C for 30 minutes before centrifugation. Tubes are centrifuged at 6000 rpm for 5 min for biochemistry tests. The blood and serum sample was placed in a cool box and then transferred to the laboratory to be kept at -20 ° C and processed within 24 hours. Using commercially available kits, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured. The body weight was estimated by (kg). During supine rest, blood pressure was also taken from the left arm with a mercury sphygmomanometer at 10-minute intervals. The following criteria were used to determine the presence of hypertension: a systolic blood pressure (SBP) of ≥140mmHg, a diastolic blood pressure (DBP) of ≥ 90mm Hg, and the use of antihypertensive medication.

Genotyping: DNA extraction was performed from whole blood according to Kit manufacturer's instructions and protocol (Sahan and Aziz 2018). A Nano-drop spectrophotometer (NAS-99/Taiwan) was used to determine DNA concentration and purity. It was then frozen at -70 °C to be used in next investigations. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the genotypes of the CD36-rs1761667 polymorphism. PCR was used to detect

the SNP, and the following primers were used: 5′-CAAGGTCTGGTATCCACCTGTT − 3′ (forward), 5′-ATGAAGCTTCCCGCCTTAGAA − 3′ (reverse). Applied Biosystems PCR device was used for DNA amplification. The PCR conditions were as follows: initial denaturation at 94 °C for 30 seconds, followed by 35 cycles of amplification consisting of denaturation at 94 °C, annealing at 60 °C, extension at 72 °C (each 30 seconds), and final extension at 72 °C for 7 minutes. At 37 °C for 6 hours, PCR products (10 µl) were digested with (*HhaI*) restriction endonuclease (Sib Enzyme/Russia), and fragments were separated by agarose gel electrophoresis (1.5 % agarose) dyed with a green star dye. Following that, three genotypes of CD36-rs1761667 were discovered using ultraviolet light: GG (161 and 264 bp), GA (161, 245, and 425 bp), and AA (425 bp).

Statistical analysis: The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors and their relationship with CD36 genotypes and the impact of the same factors on the rs1761667 (G > A).

RESULTS AND DISCUSSION

Demographics analysis: There were highly statistically significant differences in all of the parameters between the four groups at except for body weight, HDL and the distribution of males and females in the group of blood pressure patients who had a normal lipid level and the control group non-significant differences (Table 1 and 2).

Genotype and allele frequency distribution of CD36 rs1761667: The comparison of the genotypes of the CD36 gene for the groups participating in the study that the genotype GG was higher in the control group (37.50%) with highly significant differences than in the three patient groups, where it was recorded in the hypertensive with hyperlipidemia patients group (30.00%), in coronary artery

Table 1. Distribution of sample study according to sex in difference groups

Group	Male (%)	Female (%)	P-value
HST + H. lipid	40	60	0.0089 **
CAD	60	40	0.0089 **
HST + L. lipid	45	55	0.071 NS
Control	55	(45	0.071 NS
P-value	0.0092 **	0.0092 **	---

**: Highly Significant (P≤0.01), NS: Non-significant, P-value: probability value

Table 2. Comparison between different groups in all parameters of study (Mean ± SE)

Group	HST+ H. lipid	CAD	HST+ L. lipid	Control	LSD value	P-value
Parameters						
Age (year)	59.12 ±1.71	57.17±1.19	59.15±1.52	48.67±2.53	5.051 **	0.0001
B.W. (kg)	82.82 ±2.47	87.37±2.42	85.10±3.23	78.02 ±2.52	NS	0.0890
D.B.P (mm/hg)	152.82±2.50	131.25±2.21	158.15±3.44	120.25±0.43	6.732 **	0.0001
S.B.P (mm/hg)	94.00 ±1.23	84.75±1.07	93.25 ±1.49	80.02 ±0.66	3.225 **	0.0001
S.Ch (mg/dl)	217.70±6.02	172.97±7.87	159.35±4.32	151.02±4.47	16.35 **	0.0001
S.TG (mg/dl)	198.25±11.7	171.62±10.35	127.60±6.01	109.07±5.07	23.95 **	0.0001
HDL (mg/dl)	40.36 ±1.54	38.48 ±1.28	38.40 ±1.28	39.47 ±1.05	3.643 NS	0.680
LDL (mg/dl)	136.05±6.63	101.95±7.65	93.76 ±3.86	90.78 ±3.73	16.019 **	0.0001
VLDL (mg/dl)	39.63	34.29 ±2.07	25.62 ±1.17	21.66±09.99	4.770 **	0.0001

**: Highly Significant (P≤0.01), NS: Non-significant, P-value: probability value

Table 3. Genotype and Allele frequency of CD36 gene/ rs1761667 G>A in Patients and control groups

CD36 rs1761667 G<A	HTS+H.LIP. (%)	CAD (%)	HTS+L.LIP. (%)	Control (%)	P-value	Chi-Square (χ^2)
Genotype						
GG	30.00	25.00	17.50	37.50	0.0084	8.023 **
GA	52.50	47.50	62.50	47.50	0.0397	5.061 **
AA	17.50	27.50	20.00	15.00	0.0498	4.988 *
Allele		Frequency			---	---
G	0.56	0.49	0.49	0.61	---	---
A	0.44	0.51	0.51	0.29	---	---

*: Significant (P≤0.05), **: Highly Significant (P≤0.01), P-value: probability value

patients (25.00%) and a group of hypertensive with normal lipid level patients(17.50%). The genotype GA was recorded highly in the group of hypertensive with normal lipid levels patients (62.50%) than in coronary artery patients and a control group scored the same value (47.50%) and the group of hypertensive with hyperlipidemia patients scored (52.50%) with highly significant differences. The AA genotype was recorded at (17.50%) in the group of hypertensive with hyperlipidemia, (27.50%) in coronary artery patients, which is the largest value recorded and (20.00%) in the group of hypertensive patients with normal lipids and (15.00%) in the control group, achieving a significant difference. The frequency of alleles (A) varied across all groups, with the highest percentage observed in the (CAD) and (L.LIPI) groups (A = 0.51) (Table 3). These investigations were conducted on many people and in various regions of the world but no research on patients with CAD and hypertension in an Iraqi community has been conducted. The current investigation studies the relationship between the CD36 rs1761667 gene polymorphism and susceptibility to CAD and hypertension in a sample of the Iraqi population. The research on CD36 has revealed that this receptor is important in various diseases such as CVD, hypertension, and metabolic syndrome. (Roth et al 2020). Molecular studies have highlighted the critical function of CD36 in hypertension (Tao Xiang et al 2022). The capillary endothelium in the renal medulla and other kidney areas expresses CD36 in caveolae of endothelial cells, CD36 is also known to colocalize with endothelial nitric oxide synthase (eNOS) and is a factor in eNOS activation by fatty acids. It is plausible to assume that Cd36 mutations may have an impact on the control of BP through nitric oxide-related pathways in the kidney given that decreased nitric oxide activity in the renal medulla has been linked to the etiology of hypertension (Weihrauch Dorothee et al 2021). However, under a recessive inheritance model, there was an increased risk of hypertension, whereas A allele carriers of the rs1761667 SNP had a protective impact on hypertension.

Many studies have highlighted the function of CD36 in macrophages as a modulator of ox-LDL uptake, indicating that it plays a unique role in the development of atherosclerotic plaque and CAD (Zhang Yun et al 2022). In contrast to prior research, CAD patients and those with a combination of CAD and hypertension in this investigation exhibited a reduced risk in CD36 rs1761667 SNP A allele carriers. CD36 binds to fatty acids and is implicated in lipid metabolism (Li Yunxia et al 2022). Previous research has revealed that there is no significant difference in TG between CHD and CAD patients with various rs1761667 genotypes (Navarro-Rios et al 2022).

In this research, the GA genotype, the only substantial rise in TG was seen in the CAD and HTN groups and believed the type of medications used and the length of time they were taken are thought to be plausible reasons for these differences. It's worth noting that, even though CD36's function in a variety of illnesses has been established, this isn't the first study to look into CD36's genetic variation in CAD and hypertension susceptibility. When evaluating the current findings, keep in mind that selecting the hypertension group that has hyperlipidemia was challenging and time-consuming, and hypertensive individuals also were included in the study.

CONCLUSIONS

The current investigation provided the first indication that the CD36 rs1761667 gene polymorphism may be a risk factor for hypertension and CAD in Iraqi patients. It should be highlighted that the findings will need to be confirmed in future, more conclusive and prospective research using a diverse range of ethnicities and a gene expression and functional assay.

ABBREVIATION

CD36:cluster of differentiation 36 gene; ACS: Acute coronary syndrome; BMI: Body mass index; CAD: coronary artery disease HST: hypertension; CHD: Coronary heart

disease; Ctrl: Control; CVD: Cardiovascular disease; DBP: Diastolic blood pressure; DM: Diabetes mellitus; eNOS: Endothelial nitric oxide synthase; FA: Fatty acid; HDL-C: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein; LDL-C: Low-density Lipoprotein cholesterol; NO: Nitric oxide; ox-LDL: Oxidized LDL; PCR: Polymerase chain reaction; PCR-RFLP: Polymerase Chain Reaction Restriction Fragment length polymorphism; SBP: Systolic Blood Pressure; SNP: Single Nucleotide Polymorphism; 2DM: Type 2 Diabetes Mellitus; T.Ch: Total cholesterol; TG: Triglycerides.

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Effect of Vitamin B1 on Performance of Local Female Rabbits Exposed to Lead Poisoning

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Abstract: This study aimed to assess the effects of thiamin on lead acetate poisoning in female rabbits on body weight and lymphocyte DNA damage. Twenty-eight local female rabbits were divided into four groups equally: first G1 group received orally once a day, G2 received lead acetate 5 mg/kg B.W. orally, once every 48 hours (control), G3 group rabbits dosed similarly with the G2 group and with 75 mg/day/animal of vitamin B1 orally, G4 group animals were dosed with lead acetate 5 mg/kg B.W and with 150 mg/day/animal of vitamin B1, orally. The animals received lead acetate have a significant decrease in body weight gain. The high DNA damage of lymphocytes, was observed. The improvement was observed in the groups that received vitamin B1, especially the G4 group recorded better results at the level of vitamin 150 mg. The values studied were close to the control group G1 and no significant differences between them in studied parameters. In conclusion, supplementation of vitamin B1 with lead could be beneficial in mitigation of the harmful effects caused by lead in body weight change and DNA fragmentation.

Keywords: DNA damage, Lymphocyte, Parameter, Rabbits, Vitamin B1

Lead is one of the heavy metals that having a harmful impact in environment. In recent years, developing countries experience a significant increase in lead pollution due to increasing different sources of emissions that containing lead to become a future threat of public health humans and animals (Manisalidis et al 2020). When these pollutants reach the body and all body organs, it causes highly poisonous, even at low doses. It is widely accepted lead has harmful to humans and animals (Savchenko et al 2015, Karimfar et al 2016). Moreover, consuming a high amount of lead has resulted in poor performance, poisoning, and death in living things (Burki 2012). Lead toxicity is closely related to accumulation in various tissues and its interference with the bio-elements that hamper in several physiological processes (Berrahal et al 2007, Rabiu et al 2019). However, the introduction of lead has been associated primarily with the growth of industry (Rubin et al 2008).

The highest concentrations of lead in blood, liver, kidney, and bones can be enter to the body through the ingestion, inhalation, and skin, varies from individuals to individuals and depends on the chemical form of lead and type of exposure. Majority road of lead enters the body through the gastrointestinal tract via contaminated food and water. Then absorption enters the bloodstream and is deposited in the bones and internal organs (Savchenko et al 2015). A lot of reports indicate to the accumulation of lead in the body cells is subjected to induce oxidative state to lose biometric of the cell membrane (ATSDR 2007). In some studies in diverse animals have shown that lead causes oxidative stress by generation reactive oxygen species and has been evidenced that excess generation of reactive oxygen species give rise to reducing the antioxidant the defence system of cells by means of depleting glutathione inhibiting sulfhydryl-dependent enzymes (Shields et al 2021).

Lead can cause harm at extremely low quantities; yet, rabbits with no obvious clinical indications of poisoning have been shown to have low levels of blood lead contamination (Walter et al 2017). Genotoxic reactions mechanisms may entail direct DNA damage altering chromatin stability or interactions with repair systems (Valverde et al 2002). The goal of this study was to assess the therapeutic effects of vitamin B1 (thiamine) on certain health measures as well as DNA damage in local female rabbits.

MATERIAL AND METHODS

Experimental animals: Twenty eight local rabbits were randomly selected at the animal House University of Baghdad. Rabbits were placed in square cages 2 × 1.5 m³ and positioned in for ten days for a purpose of adaption to the new environment with temperature 22-25˚C, humidity 50-60%. Rabbits were divided and equally into four groups that contain seven rabbits, first group control negative (G1), 2nd group control positive (G2) were given 5 mg/kg body weight lead acetate dissolved in 2 ml distilled water, once every 48

hours, in 3rd and 4th groups (G3 and G4) given lead acetate in the same dosage in G2 above with 75 and 150 mg/ head vitamin B1 respectively. Each animal in their experiment had daily dosage for two months. All chemical materials that used in this study including lead acetate and thiamine vitamin B1 produced by lead acetate by sigma- Aldrich , Germany and vitamin B1 by HiMedia , India .

Lead acetate trihydrate and vitamin B1 (thiamine) were prepared according to the dose in this experiment. The dissolved powder with distilled water after weighing in sensitive balance and each chemical material and the administration orally by intubated using. Parameters include determination of body weight change, the initial body weight of all animals was recorded, at the starting of the experiment and weekly weight was obtained until the finish of the experiment. Blood samples withdrawn by cardiac puncture technique, lymphocyte extraction from EDTA whole blood sample with the use of the described method according to Boyum (1968). Comet assay (single cell gel electrophoresis assay) provides a simple and effective method for detect DNA damage in cells. The principle of the assay is based upon the ability of denatured cleaved DNA fragments to migrate out of the cell under the influence of an electric potential, while undamaged supercoiled DNA remains within the confines of the cell membrane when a current is applied. Evaluation of the DNA "comet" tail shape and migration pattern allows for the assessment of DNA damage (Olive et al 1990, De Boeck et al 2000).

Statistical analysis: Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1), (Al-Gharban 2016).

RESULTS AND DISCUSSION

Impact of lead acetate with thiamin on the body weight in local female rabbits: The body weight gains decreased significantly in animal group G2 with progress trials, that's probably a little feed intake and increase the state of catabolism, through exposure to lead acetate and related to lack of balance in the metabolism resulting from the zinc dependent enzyme changes (Al-Chalabi et al 2014). In addition to the antioxidant enzymes decrease as glutathione reductase possibly leads to the decrease of animal appetite due to oxidative stress (Saxena et al 2008, Murad and Al-Okaily 2019).

Klaassen (2001) describes the long exposure to lead, which results in a decrease in body weight, which might be explained by anorexia caused by heavy metal intake. Vitamin B1 supplementation ranges from 75 to 150 mg each animal per day. However, there were no significant changes in weight increase between the 75 and 150 mg vitamin B1 treated groups. The high level of vitamin B1 150 mg/animal results in good growth when compared with control G1and

Table 1. Ameliorative effect of vitamin B1 on reducing impact of lead acetate on body weight of female rabbits

Group	Week				
	0	1st	2nd	3rd	4th
G1	1649.60±129.42 Ba	1660.00 ± 129.39 AB	1673.20 ± 129.17 ABab	1718.00 ± 130.27 ABa	1742.80 ± 148.52 Aa
G2	1659.40 ± 154.13 Aa	1634.20 ± 113.14 Aa	1612.20 ± 91.98 Ab	1608.00± 90.74 Ab	1603 ± 83.45 Ab
G3	1620.60 ± 169.48 Ba	1638.40 ± 170.71 ABa	1647 ± 167.57 ABab	1710.00± 164.20 a	1714.40 ± 161.9 2 Aa
G4	1643.00±130.74 Ca	1700.00 ± 166.94 BCa	1700.20 ± 123.29 Ba	1785.20 ± 138.54 ABa	1800.60 ± 141.60 Aa

Means with a different small letter in the same column are significantly different (P<0.05), Means with a different capital letter in the same row are significantly different (P<0.05). G1 control negative, G2 control positive (receive lead acetate 5 mg/kg of body weight every 48 hr), G3 (given 5 mg/Kg of BW every 48 hr lead acetate and 75 mg /head vitamin B1 daily orally), G4 (given 5 mg/kg of BW every 48 hr lead acetate and 150 mg/head vitamin B1 daily orally)

Table 2. Ameliorative effect of vitamin B1 on reducing impact lead acetate on DNA of local female rabbits

Parameters	Group				
	G1	G2	G3	G4	LSD
Comet length	30.45 ± 2.91 B	84.85 ± 7.31 A	82.14 ± 10.21 A	38.66 ± 3.66 B	17.502
Comet area	3570.30 ± 37.12 D	15710.86 ± 227.90 A	7450 ± 417.99 B	5010.46 ± 52.92 C	610.79
Head diameter	27.55 ± 3.13 B	84.07 ± 7.63 A	79.21 ± 10.33 A	33.80 ± 4.36 B	18.245
Tail length	4.90 ± 1.02 C	20.78 ± 0.57 A	12.92 ± 1.25 B	6.86 ± 1.95 C	3.7122
Tail area	17.00 ± 9.04 B	33.60 ± 1.35 A	27.75 ± 25.83 A	21.8 ± 11.35 B	17.152
% DNA in tail	3.9 ± 0.37	5.3 ± 0.96	4.4 ± 6.41	4.2 ± 1.26	5.719 NS

Means with a different letter in the same row are significantly different (P<0.05)

G3 (75 mg/animal) groups, which could be the vitamin B1 act as limiting factor for the limited impact of lead acetate and improved the body weight. These results corroborated with the findings of Wang et al (2007). In local rabbits the vit. B1 is one of the main factors for increased utilization efficiency in monogastric animals and reduce the impact of lead acetate toxicity. Although the specific mechanism of thiamine in reducing lead toxicity has yet to be determined,. The exposure of sulfhydryl groups in thiamine molecules allows for a variety of thiamine and lead complex combinations (Kim et al 1992). Thiamine aids in removing of lead from the body, and inhibits lead deposition in specific tissues by reducing lead absorption from the gastrointestinal system (Radostits et al 2007, Wang et al 2007).

Impact of lead acetate with thiamin on DNA damage in local female rabbits: The genetic material inside the cell, deoxyribose nucleic acid, is exposed to harm due to the interplay between DNA structure and pattern. Because lead acetate poisoning caused serious damage to DNA as a result of its interaction with DNA, the fragmentation in 2nd group T2 documented significant differences in DNA of the test cells as compared to other experimental groups, which is because exposure to lead acetate poisoning caused serious damage to DNA as a result of its interaction with DNA (Table 3, Fig. 1).

Jadoon and Malik (2017) observed that heavy metal is a genotoxic chemical that is the primary cause of genetic material destruction lead acetate has a direct effect on DNA causing damage and apoptosis in the tissue of female rabbits (Ahmed et al 2012).

He et al (2007) and Meda et al (2019) demonstrated that increasing fragmentation in the nuclear DNA by lead exposure caused which could be explained by an excessive intracellular generation of ROS that can induce genetic cellular damage leading to impairment of cellular functions and involved in gene mutations. This may be the effects of activation of the caspases and endonucleases chromosomal tautomer's enzymes and chromatin becomes cross-linked and increase breaks strands of DNA (García-Ferreyra 2015, Haouas et al 2015). Further, various kinds of cells have differing tolerances to lead's genotoxic effects. This difference might be due to the existence of proteins in erythrocytes, such as metallothionein, which sequestered lead into a nonbioavailable form (thiols producing an increase in trace metals) shielding the person from metal poisoning (Hasan et al 2017). The mechanisms behind these genotoxic reactions might include indirect DNA damage that affects chromatin stability (Ahmed et al 2012), or by interfering with healing mechanisms (Restrepo et al 2000).
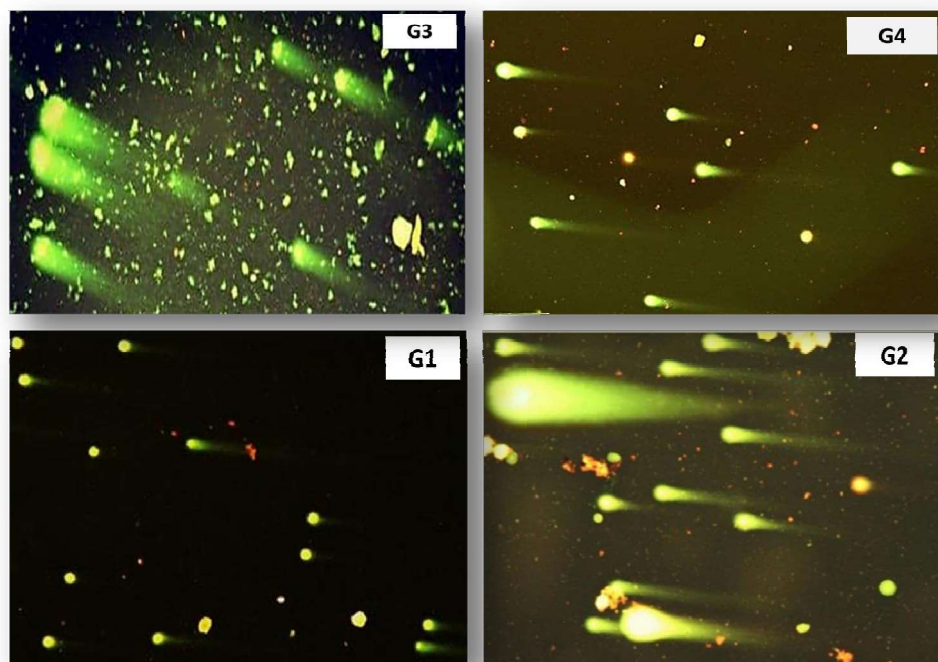
Fig. 1. Samples of DNA damage in G1: Normal DNA in rabbits lymphocytes cell, G2: Showed scoring categories for comet assay long tail and highly DNA damage, G3: Showed scoring categories for comet assay medium length tail and medium DNA damage, G4: Showed scoring categories for comet assay short tail and low DNA damage.

Lead has the capacity to alter the precision with which DNA is synthesized (Karimfar et al 2016). In vitro, another study found that administering lead acetate to cells increased their proliferative capacity (Iwakura et al 2014).

The presence of an increase in cell population in various organs/tissues of rats suggests that lead can cause tumor development. In comparison to the 2nd group, the 3rd and 4th groups had considerably less DNA damage, thus the result of the 4th group was very near to the control group G1. This might be attributed to the impact of vitamin B1 dose on reducing DNA damage through repair damage of DNA (Owain and Yousif 2018). They emphasized the importance of vitamin B1 in decreasing DNA damage by comparing the high rate of DNA repair following treatment with thiamine or Ca-EDTA to lead controls.

Thiamin was recognized as a preventive agent for lead exposure due to its significant role as an endogenous SH-containing molecule (Shah and Jain 2016). The creation of complexes between thiamine and lead, followed by excretion, is thought to be the mechanism by which thiamine counteracts lead poisoning. Thiamine has also been shown to protect rats' liver and kidneys against lead-induced LPO (Shah and Jain 2016). It may directly scavenge O_2 and OH, affecting the physiological response to oxidative stress (Jung and Kim 2003). Thiamine, according to Gliszczyńska-Świgło (2006) may serve as a powerful antioxidant by scavenging free radicals. Nourian et al (2019) discovered that co-administering vitamin C and thiamine was an efficient method for lowering lead levels in tissues and reversing unfavorable biochemical changes.

CONCLUSION

Heavy metals exposure is an important source of DNA damage in animals. lead acetate is one of the heavy metals that causes damage to the DNA .Vitamin B1 may have an important role in lead acetate associated toxicity and stop the DNA damage .In addition to the high dosage of vitamin B1 contributes significantly to reduce the harmful effect of lead acetate through its antioxidant properties, on the experimental parameters studied in the rabbits.

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Influence of HbA1c Levels on C-reactive Protein, Vitamin D and Cortisol levels in Patients with Type 2 Diabetes mellitus

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Abstract: Type 2 diabetes mellitus is a chronic disease with widespread and have serious long-term complications. A strong relationship was determined between glycated hemoglobin (HbA1c) levels and some serological parameters in patients with type 2 Diabetes mellitus. The study designed to evaluate the association between levels of HbA1c and CRP, Vitamin D, Cortisol in Type 2 diabetes mellitus patients. Diabetic subjects were divided into two groups according to the HbA1c, group (I) include 35 patients with HbA1c <7%, and group (II) include 45 patients with HbA1c >7%, In addition to third group (Controls) include 20 apparently healthy individuals. In group I and II patients, the serum cortisol levels were increased significantly with increasing HbA1c levels and there was significant increase in CRP levels in group II patients compared with other groups. However, there was no significant difference in vitamin D level between studied groups. In group (II) a strong positive correlation was observed between CRP levels and HbA1c. The results show a significant relation between HbA1c levels and levels of CRP and Cortisol. However, further large-scale, prospective studies are needed to confirm these results.

Keywords: Diabetes mellitus, C-Reactive Protein (CRP), Glycated hemoglobin (HbA1c), Vitamin D, Cortisol

The Type 2 diabetes is a heterogeneous metabolic disorder characterized by chronic hyperglycemia (Ivers 2019). It differs from the first type in terms of resistance to the action of insulin as well as a reduction of insulin secretion and insulin receptors located in the cell membranes of various tissues of the body do not respond properly to insulin (Aljumaily et al 2019), which is accompanied by high levels of insulin in the blood. The level of blood glucose can be reduced at this stage by means and drugs that increase the effectiveness of insulin and reduction the production of glucose by the liver. The efficiency of insulin secretion from the pancreas decreases as the disease progresses and insulin injections are required. There are many factors that can determine the cause and mechanism of type 2 diabetes, including the concentration of visceral fat in the abdominal area, which leads to insulin resistance. 55% of patients with type 2 diabetes suffer from obesity. Hemoglobin (A1c) is formed from glucose bonding with various amino groups including valine and lysine for both the (Alpha) chain and the two (Beta) chains of normal adult hemoglobin (Hemoglobin A), where the rate of HbA1c formation is directly proportional to the concentration of blood glucose. Glycated hemoglobin is a good test that shows the average amount of sugar in the blood during the past three months (Kuzuya 2002) and this test is not used to diagnose diabetes, but it is the best way to know the extent to which the diabetic patient controls the level of sugar in the blood, and therefore it gives the treating doctor Important information that may determine the amount of insulin the patient needs or the type of diet to maintain a good blood sugar level. In patients with diabetes, hyperglycemia causes excess glucose to bind with free amino acids in the circulation or tissue proteins, and this non-enzymatic reaction results in advanced glycosylation (Mantovani et al 2008). Accumulating evidence indicates that inflammation may Show a mediating role in the pathogenesis of type 2 diabetes, CRP is one of the greatest reactants in the acute phase of inflammation after tissue damage caused by inflammation leading to activation of the complement pathway due to the inflammatory response. High levels of CRP and poor glycemic control lead to major cardiovascular disease and lead to an increased risk of future symptoms of diabetes (Tahir et al 2020).

Vitamin D is a unique 'vitamin' that can be obtained through the diet and is also produced endogenously in response to exposure to ultraviolet (B-UV) sunlight. In addition to its classical function in bone mineralization and skeletal muscle, has functions elsewhere in the body and is associated with type 2 diabetes through multiple mechanisms. Vitamin D may help in reducing the risk of type 2 diabetes by reducing insulin resistance, reduce inflammation, enhance insulin secretion and β-cell survival (Pittas 2012). Cortisol plays a major role in many conditions that are related to complications of diabetes, including the synthesis of hepatic glucose, which directly affects blood

glucose, as well as lipolysis, which encourages the release of free fatty acids and thus the construction of triglycerides in fatty tissues, in addition to that cortisol can directly decreased insulin sensitivity and secretion by activating glucocorticoid receptors on pancreatic beta cells.

MATERIAL AND METHODS

The 80 blood samples were collected from patients with type 2 diabetes, and 20 blood samples were collected from normal healthy people with non-diabetes or chronic diseases in Ibn Al-Haytham laboratories for pathological and hormone analyzes Iraq, Kirkuk for the period from September 2020 to February 2021. Patients ages ranged from 35 to 65 years, and patient samples were divided according to HbA1c levels, as they were distributed as follows:

Group (I): (35) patients with controlled diabetes (HbA1c < 7.0).

Group (II): (45) non-controlled diabetes patients (HbA1c > 7.0).

Group (III)(Control): (20) control samples that included healthy people.

Demographic characteristics were collected from participants and laboratory results including HbA1c, CRP, vitamin D, and evening cortisol levels of the subjects. HbA1c analyses were carried out in tubes containing ethylenediaminetetraacetic acid K2 (EDTA). HbA1c values and serum CRP levels of participants were measured by Fluorescence-based Lateral Flow Immunoassay (ichroma II) system (Boditech, Seoul, Korea). Vitamin D and cortisol levels were analyzed on a mini Vidas device system (Enzyme-Linked Fluorescent Assay of (Biomeriuex, Marcy-

l'Etoile, France). Daily quality control was carried out with commercial quality control materials to ensure the precision and accuracy of measurements in our laboratory.

Statistical analysis: Statistical analysis to be performed using the statistical package for the social science (SPSS) and t-test was used to measure the difference in means between two groups.

RESULTS AND DISCUSSION

The current study showed that patients suffer from T2DM, the proportion of males was 41.25% (in group I 45.71%, and group II 37.8%). The proportion of women was 58.75% (in group I 54.28%, and group II 62.2. The laboratory data show that were no differences among the three groups according to the numbers of participants (Table 2). In the present study, the mean HbA1c value in group (II) was 8.87%, in group (I) 6.23±%, and in the control group 4.78). The mean HbA1c % value was significantly higher in non-controlled diabetic patients compared with patients group (I) and the control group (C). While there were significantly increased in CRP and cortisol in (I) and (II) groups compared with control group. Simultaneously with an increase in glycated hemoglobin. The prevalence of hypovitaminosis (vitamin D) was 83.7 % and the vitamin D sufficiency was 16.3 % in patients with T2DM. There were no significant differences in vitamin D levels among groups and there was numerical increased in control group compared with diabetic patient.

The correlations between HbA1c and other parameters (CRP, Vitamin D, and Cortisol) are presented in Table 3. In group (I) controlled patients with T2DM, CRP, vitamin D, and cortisol did not show a significant correlation with HbA1c. In

Table 1. Diabetic patients' data of groups (I), and (II)

Parameters	Group I (HbA1c <7%)			Group II (HbA1c >7%)		
Number	35			45		
Age	(35-45) 20%	(45-55) 57.14 %	(55-65) 28.85 %	(35-45) 24%	(45-55) 36 %	(55-65) 40 %
Gender	Male 45.71%		Female 54.28%	Male 37.8%		Female 62.2%
Body Mass Index	Perfect 37.1%		Increase 62.9%	Perfect 22.2%		Increase 77.8%
Complications (kidney disease, heart disease, Blood pressure)	Blood pressure 17.1%	Heart disease 14.2%	Kidney disease 3 %	Blood pressure 24.4%	Heart disease 20 %	Kidney disease 6.6%
Patients suffering from mental disorders (anxiety, depression, lack of sleep, and rapid irritability)		51 %			60 %	
Smokers		5.7 %			20 %	
Diabetes years	(1-3) 42.8%	(3-7) 40 %	(> 7) 17.2%	(1-3) 42.2%	(3-7) 40 %	(> 7) 17.8%

group (II) non-controlled patients with T2DM, a significant increase in CRP levels were observed with worsening glycemic status. Cortisol did not show significant correlations. There was a negative association in levels of Vitamin D and HbA1c in group I (r= -0.192) and group II (r= -0.128). In group (I and II). CRP levels were inversely associated with Vitamin D.

A positive correlation was observed between HbA1c and highly sensitive C-reactive protein that associated with rapid development of cardiovascular diseases in patients with type 2 diabetes (Sarinnapakorn and Wanicagool 2013). Some studies also confirmed that adequate levels of vitamin D in people with type 2 diabetes helped to significantly improve the proportion of glycated hemoglobin (Randhawa et al 2017). The results of our study showed that proportion of HbA1c and CRP levels were inversely associated with Vitamin D. some studies show that dyslipidemia is often associated with elevated levels of inflammatory markers (TNF, IL-6, and hs-CRP) and is closely related with metabolic diseases including obesity, type 2 diabetes, and cardiovascular disease (Mahdi and Abdulla 2020), This inflammation result's from metabolic disorder and called metabolic inflammation (Hotamisligil 2017).

The studies indicated that there are no significant differences between healthy people and diabetics in vitamin D levels. there are nearly a billion people around the world classified as having insufficient levels of vitamin D. The deficiency in our vitamin D level is due to environmental conditions where temperatures are high most of the time. People stay indoors and exposure to sunlight is reduced, in addition to the nature of nutrition in Iraq, as most canned foodstuffs such as milk and yogurt are not sufficiently fortified with vitamin D. In addition to the fact that seafood rich in vitamin D is very few, which is a cause for concern because of its association with many chronic diseases and negative health outcomes (Mirzavandi 2020, Alkady 2017). In the study, which was conducted on patients with type 2 diabetes vitamin D was inversely associated with C-reactive protein

(CRP), and the association was significant (and similar trend was observed in present study results agreed (r = -0.295) . When a person is exposed to anxiety, it stimulates the secretion of cortisol. Anxiety is one of the most common problems facing diabetics and causes health complications and complications in the long-time, and it is one of the most common mental illnesses. Anxiety is characterized by excessive and persistent worry that can reduce the ability to perform normal activities in life (Andrews 2018). The general anxiety rate is higher in diabetic patients than in healthy people (Perrin 2017) and diabetic patients are considered more susceptible to oxidative stress, heart disease, and anxiety disorders (Brown et al 2020). Studies had shown that tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) are increased in the case of anxiety due to inflammation and neuroimmune-pathways, and anxiety can weaken the immune system, while some studies show that

Table 3. Correlation between HbA1c (%) and other variables in the group (I), and (II)

Group (I)	HbA1c	
	p	r
CRP	0.866	-0.030
Vitamin D	0.269	-0.192
CORT	0.169	0.265
Group (II)	HbA1c	
	p	r
CRP	<0.01**	0.448
Vitamin D	0.404	-0.128
CORT	0.120	0.453
Group (I)	Vitamin D	
	p	r
CRP	0.866	-0.258
Group (II)	Vitamin D	
	p	r
CRP	0.049*	-0.295

Table 2. Laboratory data of different group (Mean±SD)

Parameters	Group (I) HbA1c ≤ 7	Group (II) HbA1c >7	Control Non-diabetic	P-value
Number	35	45	20	---
HbA1c %	6.234±0.663b	8.878±1.598a	4.787±0.537c	≤0.01**
Vitamin D (ng/ml)	18.27±2.94a	16.39±2.93a	19.75±3.59a	0.221
CRP (mg/L)	8.72±1.58b	16.94±1.90a	6.06±1.75b	≤0.01**
Cortisol (ng/ml)	215.3±23.1b	359.0±22.7a	121.3±26.0c	≤0.01**

-     Different letters indicate a significant difference (a b c)
-     Similar letters indicate no significant differences
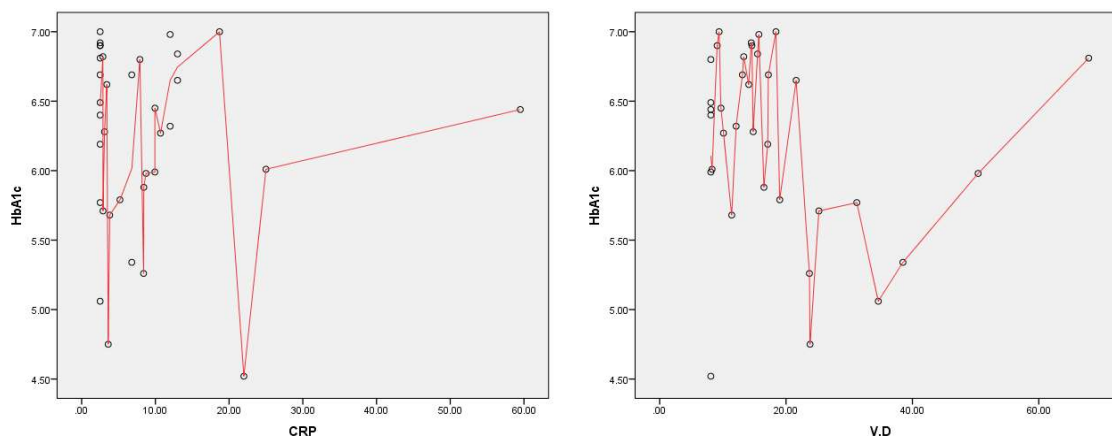
Fig. 1. Correlations between HbA1c and CRP, and Vitamin D in Group (I) (HbA1c < 7%)
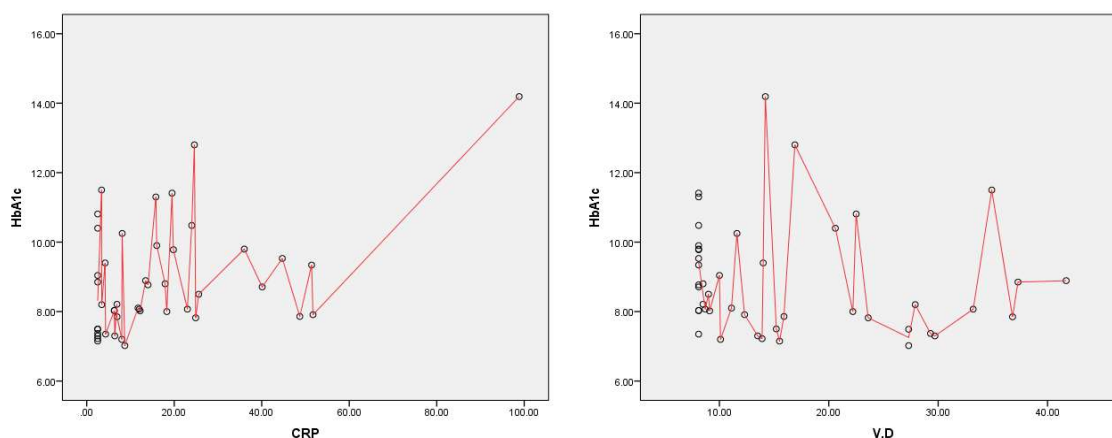
Fig. 2. Correlations between HbA1c and CRP, and Vitamin D in Group (II) (HbA1c > 7%)

diabetic patients have higher levels of inflammatory markers such as IL-1, IL-6, TNF-α, CRP) (Luis-Rodriguez 2012, Abbas 2019), and evidence suggests that both depression and anxiety stimulate inflammation in patients with diabetes (Hajebrahimi et al 2016). These findings in the diabetic groups are consistent with these studies.

CONCLUSION

There is a close, positively association between HbA1c% and inflammation especially CRP. Vitamin D is inversely related to glycated hemoglobin. In addition to controlling diabetes, Inflammation levels must be controlled. Further large-scale, long-term, prospective studies in different regions of the world are required to get a better understand of the influence HbA1c on the other bio-variables in diabetic cases.

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Efficacy of Selenium on Lung Cancer Cell Line

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Abstract: This study shed light on the effect of selenium on the A549 cancer line and with various tests. The first test included the effect of selenium on cell growth of the A549 lung cancer cell line and the normal cell WRL68 cell line. The inhibitory activity direct the cell towards programmed death through the MTT cytotoxicity assay through its effect on the A549 cancer cell line, and antitumor activity which was directly proportional to the increase in concentration. The selenium has an inhibitory effect activity in the cells of the cancer cell line A549, where the highest value of inhibition of cell growth was 59.06% at concentrations of 400 μg / ml and the lowest value of inhibition was 11.92% at concentrations of 25 μg / ml compared to normal cells. IC50 was 88.89 for cancer cells and for normal cells were 191.3. The effects that occur on some high-content screening cell markers (HCS), including cell permeability, cell count, nuclear intensity, mitochondrial membrane potential (MMP) and Cytochrome C was examined. Selenium has a clear effect on the cellular parameters at high concentrations and effect decreases in the lower concentrations. The results showed that there was significant effect at the concentrations 100 and 200 μg/ml in all the cellular indicators but its effect on the lowest concentrations was weak.

Keywords: Selenium, A549 cell line, Lung cancer

Cancer is mainly caused by genomic instability, including changes in tumor suppressor genes and oncogenes that lead to the expression of abnormal proteins involved in stimulating cell proliferation and survival (Erenpreisa and Errag 2007). More than 100 types of cancer have been identified (Qi et al 2010). Tumors arise from cells in which mutations that disrupt normal control of cellular proliferation and genetic integrity accumulate. Control disorder can be caused by genetic mutation of genes that control growth, viral infection, increased stimulation by growth factors or a combination of these factors (Weinberg 1994, Glickman et al 2004). Lung cancer is the uncontrolled growth of abnormal cells in one or both lungs (American Lung Association 2010) due to smoking of cigarettes and other tobacco products to 80 to 90% of all lung cancers (Alberg et al 2013) and other causes of lung cancer are air polluting chemicals such as benzene and formaldehyde (Asomaning et al 2008)

Studies have proven that selenium is among a group of elements whose deficiency in food causes a number of diseases, and has an impact on human and animal health through its direct relationship with the immune system. (Kieliszek and Blazejak 2013, El-Ramady et al 2016, Kieliszek 2019) and also plays an essential role in a number of vital processes, as it is essential for cell growth and development, such as the production and growth of new skin cells, strengthening the immune system, memory, strengthening nervous system functions, and hormonal activity. for men (Hendrickx et al 2013, Lipinski 2015). The main donors of selenium in the diet are cereals, bread, legumes, fish and soybeans. Sunflower seeds, liver, eggs, milk and milk products, red and white meat, and some types of Brazil nuts in particular (Rayman et al 2008, Sunde 2012, Ullah et al 2018). The risk of prostate cancer is reduced by 22% and bladder cancer is reduced by 22% (Dennert et al 2011). Selenium compounds have gained significant attention due to Its promising chemotherapeutic potential is well established that selenium at higher doses can easily be converted into peroxide and thus exerts its potential anti-cancer properties. However, the biological activity of selenium compounds and the mechanism underlying these effects is highly dependent on their species and specific metabolic pathways of cells and tissues (Fernandes and Gandin 2015). It has been shown that the anticancer effect of selenium depends on its chemical structure, dose quantity, as well as the type of organ in which it occurs tumor (Venza et al 2015). The purpose of the study is to know the inhibitory activity of selenium on the A549 cell line of lung cancer.

MATERIAL AND METHODS

The selenium used in the study was sourced from organic Seleno-L-methionine (SeMet) from Sigma Corporation (St. Louis, MO, USA). Solutions and media used for cell line were prepared according to (Freshney 2010) by dissolving 2.2 g of $NaHCO_3$ in 1000ml distilled water. The solution was sterilized by autoclaving and kept at 4ºC until use. Trypsin solution was prepared by dissolving 1 g of trypsin powder in 100ml PBS

and sterilized by filtration using Millipore's filter (0.22μm). The solution was dispensed into 10ml aliquots and stored at -20ºC. Phosphate buffer saline (PBS) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.15 g NaH_2PO_4 and 0.2g Na_2HPO_4 in 900ml of distilled water, pH was adjusted to 7.2. The solution was sterilized by autoclaving and stored at 4ºC until use.

Cell Lines

A549 cell line: Adenocarcinoma human cells (A549) were derived from human alveolar cell carcinoma (Giard et al 1973) and share main characteristics with human primary alveolar epithelial cells which were widely studied as models of lung cancer (Wang et al 2009). This cell line obtained from Center of Biotechnological.

WRL 68 cell line: The human hepatic cell line WRL 68 exhibits morphology similar to hepatocytes and hepatic primary cultures. Cells secrete albumin and alpha-feto protein and express liver specific enzymes such as alanine amino transferase (Asita and Salehhuddin 2013).

Cell line maintenance (Freshney 2010): When the cells in the vessel formed confluent monolayer, the following protocol was performed:

A. The growth medium was aspirated and the cell sheet washed with PBS.

B. Two to three ml trypsin / EDTA solution was added to the cell. The vessel was turned over to cover the monolayer completely with gentle rocking. The vessel allowed incubation at 37°C for 1 to 2 minutes, until the cells were detached from the vessel.

C. Fresh complete RPMI medium (15-20 ml) was added and cells were dispersed from the wedding surface into growth medium by pipetting.

D. Cells were redistributed at required concentration into culture vessels, flasks or plates whatever needed and incubated at 37°C in 5% CO_2 incubator.

MTT protocol: Tumor cells (1×10^4 – 1×10^6 cells/ml) were grown in 96 flat well micro-titer plates, in a final volume of 200ml complete culture medium per each well. The microplate was covered by sterilized parafilm and shacked gently. The plates were incubated at 37°C, 5% CO_2 for 24 hrs. After incubation, the medium was removed and two-fold serial dilutions of the desired compound (25, 50, 100, 200 and 400 mg/ml) were added to the wells. Triplicates were used per each concentration as well as the controls (cells treated with serum free medium). Plates were incubated at 37°C, 5% CO_2 for selected exposure time (24 hrs). After exposure, 10ml of the MTT solution was added to each well. Plates were further incubated at 37°C, 5% CO_2 for 4 hrs. The media were carefully removed and 100ml of solubilization solution was added per each well for 5 min. The absorbance was determined by using an ELISA reader at a wavelength of 575 nm.

High content screening (HCS) test for cytotoxicity assessment: This test was carried out according to method (AL-SAFFAR et al 2017). This test included the measurement of and to monitor the changes that occur in the nuclei of cells and the permeability of cell membranes and the release of cytochrome from Mitochondria. This test was conducted at University of Malaya. Department of Pharmacology, New Drug Investigation Center in Malaysia.

RESULTS AND DISCUSSION

Cytotoxicity assay (MTT): The cancer cell line (A549) and normal line WRL68 were exposed to concentrations of (400-25 μg/ml) selenium for 24 hours at 37°C. The extent of the toxicological effect was also assessed by extracting the percentage rate of growth inhibition compared to the control (100% growth). Used a specialized incubator for this. The MTT cytotoxicity assay was selected for the purpose of determining the effect of selenium on the A549 cell line after using a range of concentrations of each compound on these cells. The results showed that selenium had varying inhibitory activity on the A549 cancer cell line when treated with selenium concentrations. WRL68, but at a lower rate than its effect on cancer cells, as it reached the highest inhibition value 59.06% at concentration 200 μg compared to 32.08% effect on normal cells and the lowest inhibition value was 11.92% at concentration 25 μg for cancer cells, while the lowest inhibition value for normal cells was 4.59% at concentration 25 μg/ml (Table 1). The percentage of inhibition of cancer cells increased with increasing concentration, as the IC50 for cancer cells reached 88.89 μg (the concentration that kills half the cells) and IC50 for normal cells reached 191.3 μg. The higher the concentration, the higher the rate of cell death (Fig. 1).

Effect of selenium on the A549 cancer line using high content screening: The selenium has a clear effect on the cytotoxicity of the cells of the cancer line A549 represented by the numbers of live cells after treatment with selenium at all concentrations. 0.01) for all cytological indicators compared with the control treatment (Table 2, Fig. 2). The results also showed that the concentration of 50 μg/ml had a significant effect on the cytotoxicity of the cells.

The study indicate that survival rate of the cancer cells under study decreased with the increase in selenium concentration and there is an inverse relationship between the concentration and the survival rate of cancer cells alive. MIL had a highly significant effect on the permeability of cancer cells, while the rest of the concentrations did not show any effect compared to the control treatment. On the other
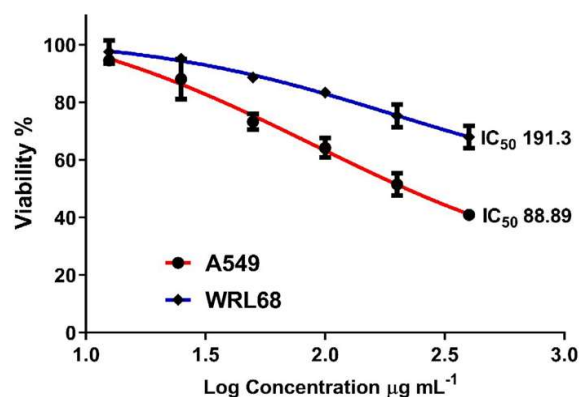
Fig. 1. Effect of selenium particles on each of the A549 cell line of normal cell lung cancer type WRL68
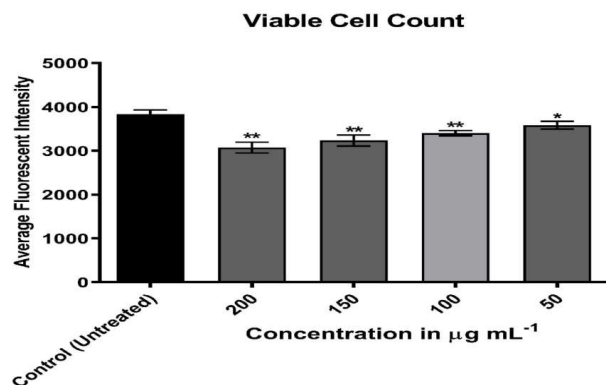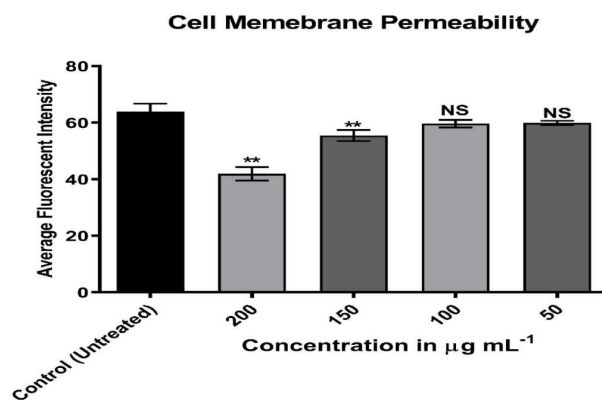

Fig. 2. Effect of different concentrations of selenium on the number of live cells of the A549 cancer line using high screening content technology and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C
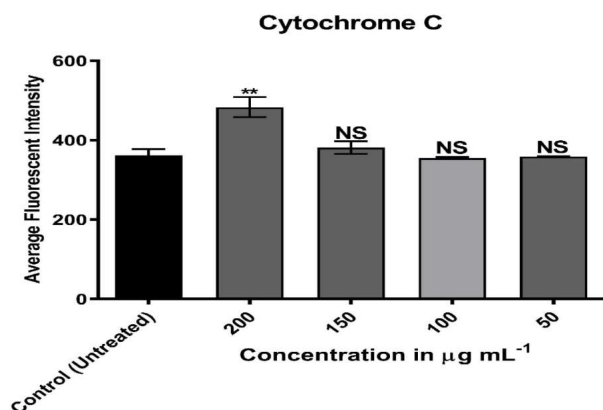
Table 1. Percentage of inhibition of tumor cells for the cancer cell line (A549) and the normal cell line WRL68 using selenium

Concentration (μg/ml)	Inhibition A549	Inhibition WRL-68
25	11.92	4.94
50	26.77	11.38
100	35.88	16.63
200	48.53	24.77
400	59.06	32.08

hand, selenium had a significant effect on mitochondrial membrane permeability at concentration 100 and a highly significant effect at concentrations 150, 200 μg/ml compared with the control treatment and when following up on the effect of selenium on mitochondrial metabolic pathways Inside the cell by observing the changes that occur in the permeability of

Fig. 3. Effect of different concentrations of selenium on the permeability of cells of the cancer line A549 using high screening content technology and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C
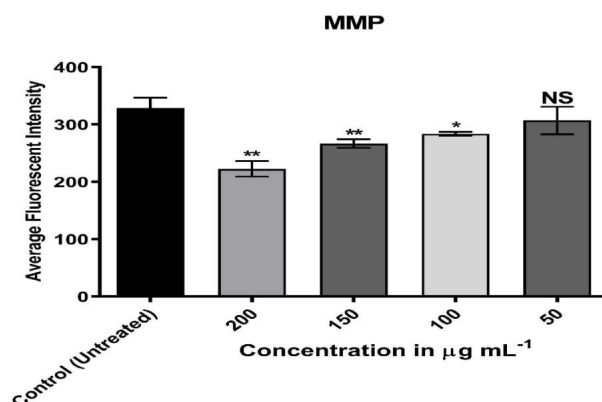
Fig. 4. Effect of different concentrations of selenium on the mitochondrial membrane permeability of cells of the cancer line A549 using high screening content technique and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C

Table 2. Cytometric indicators used to detect selenium activity on cells of the A549 cell line using the HCS assay

Viability	Cell membrane permeability	Nuclear intensity	Mitochondrial membrane potential	Cytochrome	Concentrations (μg \ ml)
3832	63.90	327.2	328.6	362.3	Control
3583	59.92	320.3	307.0	358.5	50
3402	59.67	329.4	283.9	354.9	100l
3235	55.48	340.0	266.8	381.2	150l
3074	41.92	425.9	222.7	483.0	200l

Cytochrome C

Fig. 5. Effect of different concentrations of selenium on the cytochrome C of cells of the cancer line A549 using High Screening Content HSC technology and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C

Total Nuclear Intensity
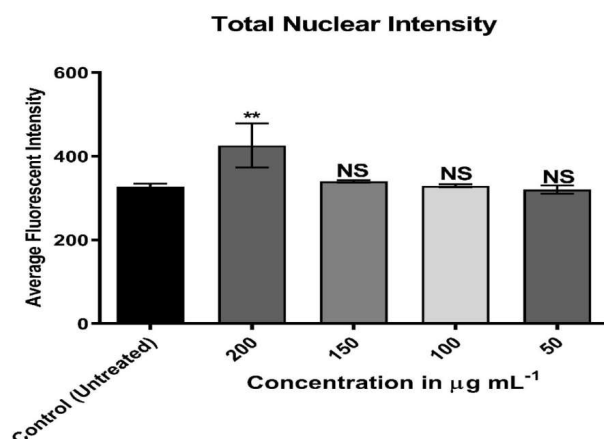
Fig. 6. Effect of different concentrations of selenium on the nuclear density of cells of the cancer line A549 using High Screening Content HSC technology and comparing it with cells untreated with selenium for 24 hours at a temperature of 37°C

the mitochondrial membranes and measuring the level of cytochrome C release from the mitochondria inside the cell (Table 2, Figure 4). It was observed that there was an increase in the level of cytochrome C at the concentration of 200 µg / ml of selenium (Table 2, Figure 4). Selenium has an effect on the nuclear density of cancer cells at a concentration of 200 µg/ml (Table 2, Figure 6). These studies indicate that high concentrations of µg/ml of selenium significantly affected all cellular parameters of the A549 cancer cell line when exposed to selenium for 24 hours and at a temperature of 37°C, which leads to the induction of cells to enter the process of programmed death, apoptosis.

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Evaluation of Efficacy of Selenium on Lung Cancer Cell Line

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Abstract: This study shed light on the effect of selenium on the A549 cancer line with various tests. The first test included the effect of selenium on cell growth of the A549 lung cancer cell line and the normal cell WRL68 cell line. which was showed inhibitory activity and direct the cell towards programmed death through the MTT cytotoxicity assay and through effect on the A549 cancer cell line, The antitumor activity was directly proportional to the increase in concentration, and selenium has an inhibitory effect activity in the cells of the cancer cell line A549, where the highest value of inhibition of cell growth was 59.06% at concentrations of 400 µg / ml and the lowest inhibition was 11.92% at concentrations of 25 µg / ml compared to normal cells. IC50 reached 88.89 for cancer cells and for normal cells, IC50 was 191.3. The examining the effects that occur on some high-content screening cell markers (HCS), including cell permeability, cell count, nuclear intensity, mitochondrial membrane potential (MMP) and Cytochrome C through the study, it was observed that selenium has a clear effect on the cellular parameters at high concentrations and effect decreases in the lower concentrations. There was significantat the concentrations 200 and 100 µg/ml in all the cellular indicators but its effect on the lowest concentrations was weak.

Keywords: Selenium, A549 cell line, Lung cancer

Cancer is a disease mainly caused by genomic instability, including changes in tumor suppressor genes and oncogenes that lead to the expression of abnormal proteins involved in stimulating cell proliferation and survival (Erenpreisa and Errag 2007). More than 100 types of cancer have been identified (Qi et al 2010). Tumors arise from cells in which mutations that disrupt normal control of cellular proliferation and genetic integrity accumulate. Control disorder can be caused by genetic mutation of genes that control growth, viral infection, increased stimulation by growth factors or a combination of these factors (Weinberg 1994, Glickman et al 2004). Lung cancer is the uncontrolled growth of abnormal cells in one or both lungs (American Lung Association 2010). The smoking of cigarettes and other tobacco products causes 80 to 90% of all lung cancers (Alberg et al 2013) and other causes of lung cancer are air polluting chemicals such as benzene and formaldehyde (Asomaning et al 2008)

Selenium is one of the most important and very necessary microelements for the proper functioning of humans (El-Ramady et al 2016). Studies have proven that selenium is among a group of elements whose deficiency in food causes a number of diseases, and has an impact on human and animal health through its direct relationship with the immune system (Kieliszek and Blazejak 2013, Kieliszek 2019), and also plays an essential role in a number of vital processes, as it is essential for cell growth and development, such as the production and growth of new skin cells, strengthening the immune system, memory, strengthening nervous system functions, and hormonal activity for men (Hendrickx et al 2013, Lipinski 2015)

The main donors of selenium in the diet are cereals, bread, legumes, fish and soybeans. Sunflower seeds, liver, eggs, milk and milk products, red and white meat, and some types of Brazil nuts in particular are also a rich source of selenium (Rayman et al 2008, Sunde 2012, Ullah et al 2018). The risk of developing cancer is 31% and the risk of death is 45%.The risk of prostate cancer is reduced by 22% and bladder cancer is reduced by 22% (Dennert et al 2011).Selenium compounds have gained significant attention due to Its promising chemotherapeutic potential is well established that selenium at higher doses can easily be converted into peroxide and thus exerts its potential anti-cancer properties. However, the biological activity of selenium compounds and the mechanism underlying these effects is highly dependent on their species and specific metabolic pathways of cells and tissues (Fernandes and Gandin 2015). It has been shown that the anticancer effect of selenium depends on its chemical structure, dose quantity, as well as the type of organ in which it occurs tumor (Venza et al 2015). The purpose of the study is to understand inhibitory activity of selenium on the A549 cell line of lung cancer.

MATERIAL AND METHODS

The selenium used in the study was sourced from organic Seleno-L-methionine (SeMet) from Sigma Corporation (St.

Louis, MO, USA). The Solutions and media used for cell line were prepared according to Freshney (2010). Sodium bicarbonate solution was prepared by dissolving 2.2 g of NaHCO$_3$ in 1000ml distilled water. The solution was sterilized by autoclaving and kept at 4ºC until use. Trypsin solution was prepared by dissolving 1 g of trypsin powder in 100ml PBS and sterilized by filtration using Millipore's filter (0.22μm). The solution was dispensed into 10ml aliquots and stored at -20ºC. Phosphate buffer saline (PBS) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.15 g NaH$_2$PO$_4$ and 0.2g Na$_2$HPO$_4$ in 900ml of distilled water, pH was adjusted to 7.2. The solution was sterilized by autoclaving and stored at 4ºC until use.

2-4 cell lines:

A549 cell line: Adenocarcinomic Human Cells (A549) were derived from human alveolar cell carcinoma (Giard et al 1973) and share main characteristics with human primary alveolar epithelial cells which were widely studied as models of lung cancer (Wang et al 2009). This cell line obtained from Center of Biotechnological

WRL 68 cell line: The human hepatic cell line WRL 68 exhibits morphology similar to hepatocytes and hepatic primary cultures. Cells have been shown to secrete albumin and alpha-feto protein and express liver specific enzymes such as alanine amino transferase (Asita and Salehhuddin 2013).

Cell line maintenance (Freshney 2010): When the cells in the vessel formed confluent monolayer, the following protocol was performed:

A- The growth medium was aspirated and the cell sheet washed with PBS.

B- Two to three ml trypsin/EDTA solution was added to the cell. The vessel was turned over to cover the monolayer completely with gentle rocking. The vessel allowed incubation at 37°C for 1 to 2 minutes, until the cells were detached from the vessel.

C- Fresh complete RPMI medium (15-20 ml) was added and cells were dispersed from the wedding surface into growth medium by pipetting.

D- Cells were redistributed at required concentration into culture vessels, flasks or plates whatever needed and incubated at 37°C in 5% CO$_2$incubator.

2-4 MTT protocol: Tumor cells ($1 \times 10^4 - 1 \times 10^6$ cells/ml) were grown in 96 flat well micro-titer plates, in a final volume of 200 complete culture medium per each well. The microplate was covered by sterilized parafilm and shacked gently. The plates were incubated at 37°C, 5% CO$_2$for 24 hrs. After incubation, the medium was removed and two fold serial dilutions of the desired compound (25, 50, 100, 200 and 400 g/ml) were added to the wells. Triplicates were used per each

concentration as well as the controls (cells treated with serum free medium). Plates were incubated at 37°C, 5% CO$_2$ for selected exposure time (24 hrs).After exposure, 10 ml of the MTT solution was added to each well. Plates were further incubated at 37°C, 5% CO$_2$ for 4 hrs.

The media were carefully removed and 100 l of solubilization solution was added per each well for 5 min. The absorbance was determined by using an ELISA reader at a wavelength of 575 nm.

High content screening (HCS) test for cytotoxicity assessment: This test was carried out according to method (AL-SAFFAR et al 2017). This test included the measurement of and to monitor the changes that occur in the nuclei of cells and the permeability of cell membranes and the release of cytochrome from Mitochondria. This test was conducted at Department of Pharmacology, New Drug Investigation Center University of Malaya in Malaysia.

RESULTS AND DISCUSSION

Cytotoxicity assay (MTT): The cancer cell line (A549) and normal line WRL68 were exposed to concentrations of 400-25 μg/ml selenium for 24 hours at 37°C. The extent of the toxicological effect was also assessed by extracting the percentage rate of growth inhibition compared to the control (100% growth). Used a specialized incubator for this. The MTT cytotoxicity assay was selected for the purpose of determining the effect of selenium on the A549 cell line after using a range of concentrations of each compound on these cells. The selenium had varying inhibitory activity on the A549 cancer cell line when treated with selenium concentrations. WRL68, but at a lower rate than its effect on cancer cells, as it reached the highest inhibition value 59.06% at concentration 200 μg compared to 32.08% effect on normal cells and the lowest inhibition value was 11.92% at concentration 25 μg for cancer cells, while the lowest inhibition value for normal cells was 4.59% at concentration 25 μg/ml (Table 1). The percentage of inhibition of cancer cells increased with increasing concentration, as the IC50 for cancer cells reached 88.89 μg (the concentration that kills half the cells) and IC50 for normal cells reached to 191.3 μg. The higher the concentration, the higher the rate of cell death (Fig. 1).

Effect of selenium on the A549 cancer line using high content screening (HCS): The selenium has a clear effect on the cytotoxicity of the cells of the cancer line A549 represented by the numbers of live cells after treatment with selenium at all concentrations for all cytological indicators compared with the control treatment (Table 2, Fig. 2). The results also showed that the concentration of 50 μg/ml had a significant effect on the cytotoxicity of the cells.

The survival rate of the cancer cells under study
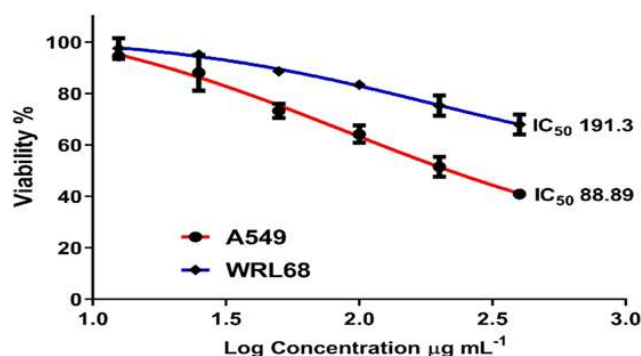
Fig. 1. Effect of selenium particles on each of the A549 cell line of normal cell lung cancer type WRL68
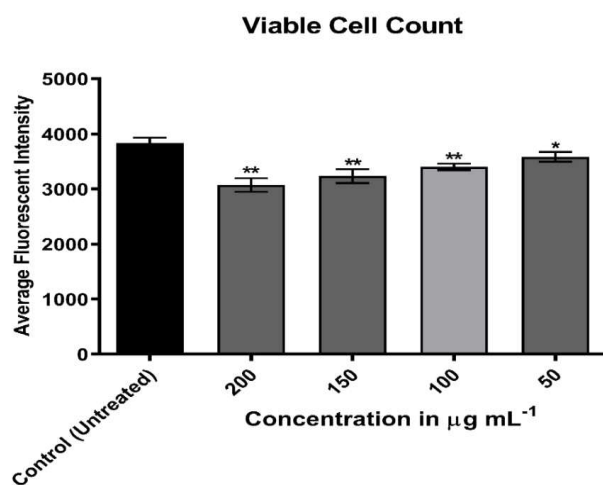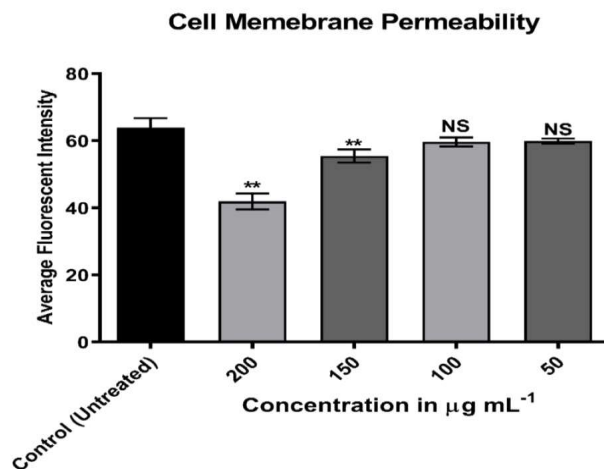

Fig. 2. Effect of different concentrations of selenium on the number of live cells of the A549 cancer line using High Screening Content HSC technology and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C
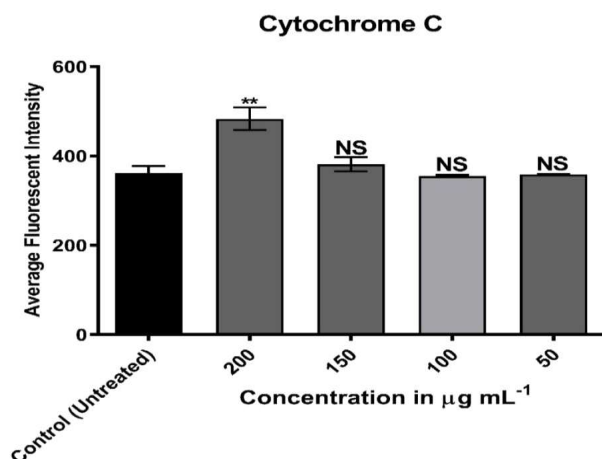
Table 1. Inhibition of tumor cells for the cancer cell line (A549) and the normal cell line WRL68 using selenium (Per cent)

Concentration (µg/ml)	A549	WRL-68
25	11.92	4.94
50	26.77	11.38
100	35.88	16.63
200	48.53	24.77
400	59.06	32.08

decreased with the increase in selenium concentration and there is an inverse relationship between the concentration and the survival rate of cancer cells alive. MIL had a highly significant effect on the permeability of cancer cells, while the

Fig. 3. Effect of different concentrations of selenium on the permeability of cells of the cancer line A549 using High Screening Content HSC technology and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C
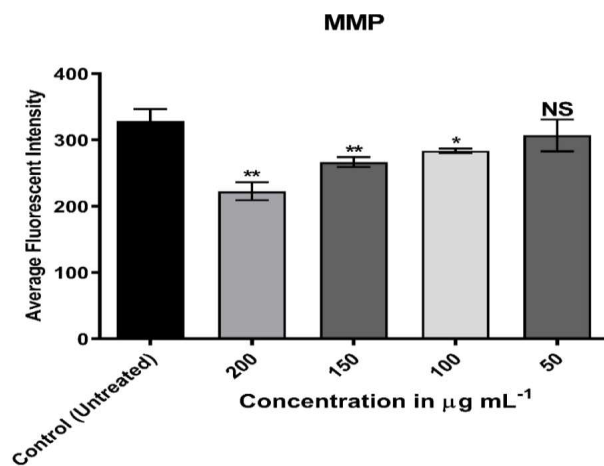
Fig. 4. Effect of different concentrations of selenium on the mitochondrial membrane permeability of cells of the cancer line A549 using High Screening Content HSC technique and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C

Table 2. Cytometric indicators used to detect selenium activity on cells of the A549 cell line using the HCS assay

Concentrations	Cytochrome C	Mitochondrial membrane potential	Nuclear intensity	Cell membrane permeability	Viability
Control	362.3	328.6	327.2	63.90	3832
50 µg\ml	358.5	307.0	320.3	59.92	3583
100 µg\ml	354.9	283.9	329.4	59.67	3402
150 µg\ml	381.2	266.8	340.0	55.48	3235
200 µg\ml	483.0	222.7	425.9	41.92	3074

Cytochrome C

Fig. 5. Effect of different concentrations of selenium on the cytochrome C of cells of the cancer line A549 using High Screening Content HSC technology and comparing it with cells not treated with selenium for 24 hours at a temperature of 37°C

Total Nuclear Intensity
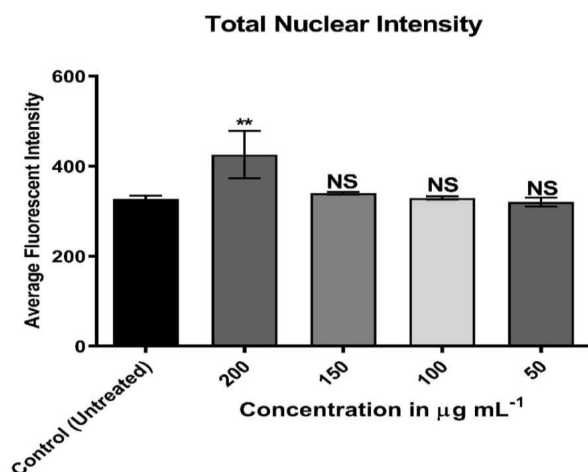
Fig. 6. Effect of different concentrations of selenium on the nuclear density of cells of the cancer line A549 using High Screening Content HSC technology and comparing it with cells untreated with selenium for 24 hours at a temperature of 37°C

rest of the concentrations did not show any effect compared to the control treatment. On the other hand, from Table (2) and Figure (4) showed that selenium had a significant effect on mitochondrial membrane permeability at concentration 100 and a highly significant effect at concentrations 150, 200 μg/ml compared with the control treatment and when following up on the effect of selenium on mitochondrial metabolic pathways Inside the cell by observing the changes that occur in the permeability of the mitochondrial membranes and measuring the level of cytochrome C release from the mitochondria inside the cell (Table 2, Fig. 5).

There was an increase in the level of cytochrome C at the concentration of 200 μg / ml of selenium. Selenium has an effect on the nuclear density of cancer cells (Table 2, Fig. 6) at a concentration of 200 μg/ml. From the above, can conclude that high concentrations of μg/ml of selenium significantly affected all cellular parameters of the A549 cancer cell line when exposed to selenium for 24 hours and at a temperature of 37°C, which leads to the induction of cells to enter the process of programmed death, apoptosis.

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Study on Presence of Microorganisms in Hospital Environment

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Abstract: Swabs were taken from the departments of Burns Hospital, Medical City, Baghdad city from surfaces, floors, patients and the air for the purpose of evaluating the level of microbiological contamination represented by bacteria and fungi inside the hospital. This study was performed from September 2020 to August 2021. The samples were collected in six units in a public hospital in the city of Baghdad from Operations Hall No1, Operations Hall No. 2, Consulting Pharmacy, Emergency Room, and Reception Room. The presence of microbiological contamination (bacteria and fungi) in the hospital air indicated the presence of fungal contamination in all departments were studied, while bacterial contamination in the hospital air was noted within the studied hospital departments. As for the microbiological contamination (bacteria and fungi) on the surfaces inside the hospital departments, there were noticeable differences in the results. The presence of contamination was in the departments of Consulting pharmacy, Emergency Room, and Reception Room, and the absence of contamination was in Operations Hall No1, and Operations Hall No2. In conclusion, the study proved the presence of microbiological contamination in hospital air and surfaces, which poses a risk of microbiological contamination of patients in burn halls.

Keywords: Microorganisms, Hospital environment, Potential related risk

Bioaerosols are particles suspended in the air of biological origin, e.g., bacteria, viruses, fungi, toxins, and plant debris (Haig et al 2016). Microorganisms could affect air quality, ecosystem, and human health. The indoor environment could be responsible for spreading airborne infectious and pathogenic microbial agents (Guan et al 2015). Microbial agents could be transmitted through droplets and airborne means. Hospital infections are a major health concern and airborne microorganisms are potential agents of hospital infection. A variety of microorganisms are found in hospital air. Air quality in hospitals is becoming a growing concern. It requires special care to protect patients from infections. In addition, the hospital can be considered a dynamic environment affected by seasons (Ghosh et al 2015), weather, ventilation systems, and humidity intrusion. Human activities, the presence of visitors, and cleaning can also affect the air quality inside a hospital (Jung et al 2015). These factors may be associated with microbial growth conditions, which may cause serious infections. Although air pollution varies qualitatively and quantitatively over time, from institution to institution and within the same institution depending on the patient, hospital units, techniques used, and nature of care (El Rhazi et al 2007). Air control in health facilities is important. Indeed, it is important to know the types of bacteria present in the suspension in the air sample to assess the initial situation within the hospital and the effectiveness of corrective measures. In addition, microbiological monitoring of the air is an imperative topic for the prevention of nosocomial infections. After a burn injury, most patients suffer significant consequences from bacterial and fungal infections. It is the most common cause of disease control and mortality. As a result of the rupture of the cutaneous barrier by a burn injury, local and systemic immune responses will occur, involving complications of microorganism infection. The two most common infections of burn injuries are *Pseudomonas aeruginosa* and S*taphylococcus aureus*, which are difficult to treat. It was discovered that Gram-negative bacteria are the most common invasive infectious bacteria due to the presence of significant virulence factors and antimicrobial resistance (Clark et al 2003).

MATERIAL AND METHODS

Study area: The samples were collected in six units in a public hospital in the city of Baghdad.: Operations Hall no. 1, and 2, consulting pharmacy, Emergency room, and Reception room. Sampling locations were selected based on the hospital unit's most critical and representative sites.

Sampling procedures: Air samples were taken from 5 randomly selected wards of the hospital, and bacterial population ns were made by passive air sampling technique i.e., the settle plate method using 9 cm diameter Petri dishes. In each ward, five Petri dishes were exposed for 30-minute sampling at the height which approximated to human breathing zone was 1 m above the floor and at the center of the room. To minimize the dilution of air contaminants, openings like doors

and windows were closed including the mechanical ventilators during sampling. In addition, the movement of people during sampling was restricted to avoid air disturbance. Bacteria were collected on Nutrient agar, Mannitol salt agar, MacConkey agar, Sabouraud dextrose agar and Blood agar.

Air samples: Both quantitative and qualitative analyses were conducted. The quantitative analysis was mainly conducted to determine bacterial load or the number of bacteria in the indoor air. To determine the load, exposed culture media/ air samples were taken to the laboratory and incubated at 37 °C for 24 h. After a 24 h incubation period, bacterial load was enumerated as colony forming units (CFU) and CFU/m3 were determined $N=5a*10^4 (bt)^{-1}$ (Ortu 2005 (where N=microbial CFU/m3 of indoor air; a = number of colonies per Petri dish; b = dish surface (cm2); and t = exposure time (minutes).

Surface samples: Each swab was immersed in liquid nutrient broth (BHI) and incubated at 37 ± 1 °C for 24 h. Growth was observed, and swabs were cultured in Nutrient agar, Mannitol salt agar, McConkey agar, Sapporo dextrose agar and Blood agar. Marked distinct colonies were isolated and purified by sub-culturing in fresh medium incubated at 37 ± 1 °C for 18–24 h to obtain pure strain isolates (Meunier et al 2005). With the modification of this method, swabs were immediately placed in the nutrient solution (BHI) to obtain the true number of bacteria present on the surface.

RESULTS AND DISCUSSION

The fungal contamination in operations Hall No1was with *Aspergillus niger, A. parasiticus, Trichoderma* (445.597cfu/m³). In Operations Hall no2 with *Aspergillus niger, A.parasiticus (*602.883cfu/m³) and in Consulting Pharmacy with *Chrysomelidae* (576.655 cfu/m³)*,* 417.800 cfu/m³ with *Aspergillus niger, A. parasiticus,* in reception room (524.232cfu/m³) *Aspergillus niger and Trichoderma* (Table 1). Bacterial contamination was unnoticeable in Operations Hall 1 and 2 while contamination was observed with *Bacillus, and Staphylococcus* in Consulting Pharmacy (419.386 cfu/m³), Emergency Room (366.972 cfu/m³) and Reception Room (445.597 cfu/m³).

The presence of fungal contamination appeared at 5000cfu/m³ in Operations hall no1 *with AsP. niger, Penicillium, Cladosporium, and Trichoderma. In* Operations hall no2 23000cfu/m³ with *AsP. Niger, Asp. Parasiticus.* In Consulting Pharmacy 6000 cfu/m³ with *AsP. Niger. Cladosporium penicillium, Chrysomelida.* In Emergency Room 2000 cfu/m³ with *Chrysomelidae, AsP. niger Yest*, 13000 cfu/m³ *AsP. niger Chrysomelidae, Alternaria, Trichoderma.* The presence of unnoticeable bacterial contaminationin Operations Hall No 1and Operations Hall No2 while contamination was observed inconsulting pharmacy (63500) cfu/m³with *Bacillus, Staphylococcus*, in the Emergency Room (5500) cfu/m³ with *Bacillus, Staphylococcus*, in Reception Room (66312.5) cfu/m³ with *Bacillus, Staphylococcus*.

Exposure to certain pathogenic microorganisms increases the risk of nosocomial infections. Such infections are a major public health concern due to the length of patients' stay and the cost of hospital care. Microbiological monitoring of the environment in healthcare facilities allows for highlighting the impact of saprophytic bacteria on hospital infections despite cleaning protocols and ventilation systems in place. Air is a ubiquitous vector inside the hospital. In the study, bacterial and fungal loads are present indifferent sites analyzed within the studied unit. 100% of the tests were positive for the Consulting Pharmacy. Ortu (2005) reported
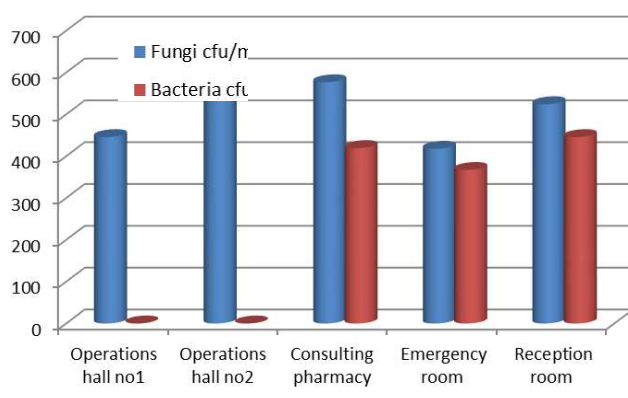
Fig. 1. Fungi and bacteria analysis of air sample

Table 1. Microbiological analysis of air sample

Type	Fungi cfu/m³	Isolate identified	Bacteria cfu/m³	Isolate identified
Operations Hall No1	445.597	*AsP. niger Asp. parasiticus Trichoderma*	Nil	Nil
Operations Hall No2	602.883	*AsP. niger Asp. parasiticus*	Nil	Nil
Consulting Pharmacy	576.655	*Chrysomelidae*	419.386	*Bacillus, Staphylococcus*
Emergency Room	417.800	*AsP. niger Asp. parasiticus Cladosporium*	366.972	*Bacillus, Staphylococcus*
Reception Room	524.232	*AsP. niger Trichoderma*	445.597	*Bacillus, Staphylococcus*

Table 2. Microbiological analysis of the surface sample

Type	Fungi cfu/m³	Isolate identified	Bacteria cfu/m³	Isolate identified
Operations Hall No1	5000	*As P. niger, penicillium, Cladosporium, Trichoderma*	Nil	Nil
Operations Hall No2	23000	*AsP. niger Asp. Parasiticus, Yest*	Nil	Nil
Consulting Pharmacy	6000	*As P. niger Cladosporium pencillium Chrysomelidae*	63500	*Bacillus, Staphylococcus*
Emergency Room	2000	*Chrysomelidae, As P. niger Yest,*	5500	*Bacillus, Staphylococcus*
Reception Room	13000	*As P. niger Chrysomelidae, Alternaria Trichoderma*	66312.5	*Bacillus, Staphylococcus*
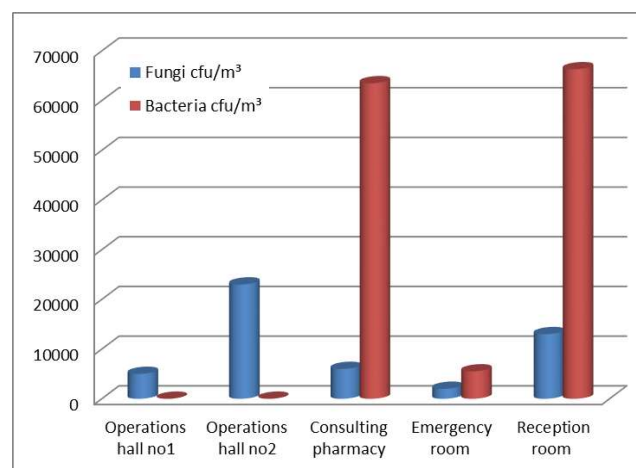
Fig. 2. Fungi and bacteria of surface sample

that the presence of certain pathogenic microorganisms in the air of the hospital, especially in the operating could be the cause of severe postoperative infections. The bacterial and fungal load found in these units' air could cause an increased risk of infection in hospitalized patients (Adjide et al, 2010). In the study also bacteria and fungi are present in different locations. The fungi included *Aspergillus niger, Aspergillus parasiticus, Trichoderma, Chrysomelidae, Cladosporium, and Alternaria.* In hospitals, the air is largely colonized by bacteria belonging to pathogenic species. Haig et al (2016) also observed high bacterial load in hospital units' air systems. These types of germs in hospitals are generally linked to several types of infections. Skin infections are common, but bacteria can infect distant organs through the bloodstream. They are mainly involved in lung, bone, heart, and blood infections.

Regular and careful monitoring is necessary to assess air control's effectiveness and detect the irregular introduction of airborne particles through patients, visitors, and/or medical staff. Furthermore, microbiological survey data should be used to establish clearly defined guidelines for air quality, especially in controlled environments in hospitals. Compliance with good hygiene practices can also reduce hospital infections and their increasing resistance to antibiotics, which has always been a global concern (Berrada et al 2015).

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Use of Beetle Larvae as A Source of Protein in Broiler Diets and Effect on Carcass and Some Physiological Characteristics

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Abstract: This study was conducted in Animal Production Department, College of Agricultural Engineering Sciences, University of Baghdad, October-November, 2019. In order to study the use of *Tenebrio molitor* larvae powder (TML) powder not supplementing with amino acids was used as a source of protein in broiler diets to study the effect Carcass and some physiological characteristics. In this experiment, 180 chicks of strain 308 Ross were used. First treatment, T_1 (control) - contained the imported commercial protein concentrate by 5%. The second treatment, T_2, T_3 and T_4- completely replacement the TML at 2.5, 5 7.5% instead of the commercial protein concentrate. There were no significant differences between T_1 and T_2, T_3, and T_4 in the relative weight of the edible internal organs, as well as the relative weight of the spleen and the fabricius gland. However, the comparison treatment was significantly superior in the relative weight of the breast cut, as for the relative weight of the cuts (thighs and wings). The treatments T_2 and T_3 were significantly superior over the treatments T_1 and T_4. Moreover, there were no significant differences in the concentration of glucose and protein between the treatments, as for the bacterial count, a significant increase in the number of harmful bacteria *E. coli* was observed in the gut of T_2 and T_3 birds compared with the treatment T_1 and T_4. There were no significant differences in the number of beneficial bacteria *L. bacillus* between all treatments.

Keywords: Beetle larvae, Protein, Broiler diets, Physiological characteristics

The natural increase in the world population to about 9 billion people in 2050 has led to an increase in the consumption of foods of animal origin and an increase in the demand for protein to feed animals (Alexandratos and Bruinsma 2012). The animal production exploits 70% of arable land, in addition to pressure from the world's growing population on those limited agricultural resources. Therefore, urgent care is required, considering the change in climate and the scarcity of animal feed. Besides, these animal feeds are the main protein source in feeding poultry and livestock, as the process of providing them has become a big problem that must receive a great deal of attention. Since the cost of these feeds is high along with their scarcity, especially soybeans - peanuts - and fishmeal, have become major obstacles in the commercial poultry industry and production (Adeniji 2007). Therefore, the great challenge of great importance will be to identify alternative sources rich in energy, protein, and other foodstuffs to be a viable alternative to traditional sources in order to avoid these obstacles. Several studies have shown that insects can become the primary and alternative components in animal feeds (Van Huis 2013, Henry et al 2015) due to their high protein content and quality (Makkar et al 2014). However, the powder of larvae or insects, whether powder or living organisms, can be used as the main component in diets due to their protein and

energy content in addition to many nutrients, and thus they represent an important food source for animals Among these Yellow Worms type *Tenebrio molitor*, has the ability to provide proteins - fats - energy produced from organic waste, which can be exploited for feeding (Zheng et al 2013). Ramos-Elorduy et al (2002) used powdered beetle larvae in broiler diets as a nutritional supplement in proportions 0, 5, 10% *Tenebrio molitor* larvae (TML). The results indicated that there were no significant differences in weight gain, the rate of feed consumption, and the efficiency of feed conversion, and observed no negative effects on the growth performance of broiler chickens.

MATERIAL AND METHODS

First stage (the larvae breeding stage): A 2 kg of fresh yellow worm larvae, called (mealworm), was purchased in the local market in Baghdad. The beetle *Tenebrio molitor* was reared at home and provided with the supplies and a suitable place to live in special drawers with a depth of 2-3 cm of oats as a food medium. Besides, a suitable medium for insects to lay eggs, and provided with potato chips of the unhealthy and cheap type in the market, carrot pens and apples that could not be used by humans (Fig. 1).

The newly hatched larvae turned gradually and continuously to white after 10 days, indicating maturity, as

they began to turn into cocoons It gradually began to turn into a brown beetle within 7 days, There after adults mate and lay eggs under the layer of oats. These mature larvae were collected and dried with hot air blowing device under an appropriate temperature of about 41.7^{O}C. Then they were exposed to sunlight for four days and then crushed finely to obtain larvae powder.

Chemical analysis: The *chemical analysis* of the larvae powder was performed at the University of Putra, Malaysia (Table 1)

Experimental details: The experiment was conducted in

Animal Production Department. College of Agricultural Engineering Sciences, University of Baghdad for a period of 42 days from October 12, 2019 to November 22, 2019. 180 chicks of broiler Ross 308 were used with an average initial weight of 40.8 g obtained from a local hatchery (Al-Shukur hatchery) Baghdad-Abu Ghraib (Iraq). It was distributed randomly into 4 treatments by 45 birds for each treatment $_{T1}$ (control) - contained the imported commercial protein concentrate by 5% T$_2$ T3 T4 completely replacement the TML at 2.5,5,7 %. Raw feed materials were prepared from the local market to form four diets according to the above-mentioned treatments, each according to its percentage in the diet. The milling was in the fodder manufacturing plant in the poultry field of the College of Agricultural Engineering Sciences, University of Baghdad (Abu Ghraib). The mixing was done manually in three stages according to the Breeding Manual for Broilers Ross 308 (2019) as shown in Table 2.

Blood sample collection: Blood samples were collected by 6 birds from each treatment, 3 males and 3 females, for a total of 24 samples, randomly, as they were collected from the

Table 1. Chemical analysis of TML used in the study

Food component	Percent
Crude protein	65.23
Methionine	1.95
Lysine	1.86
Calcium	0.008
Phosphorous	0.004
Metabolism Energy (kcal / kg)	5981.93

Table 2. Composition of diets provided to experimental birds

Item	Start diet (1-10) d				Growth diet (11-24) d				Production diet (25-42) d			
	T$_1$	T$_2$	T$_3$	T$_4$	T$_1$	T$_2$	T$_3$	T$_4$	T$_1$	T$_2$	T$_3$	T$_4$
Corn	47.7	39.1	28	21.7	45.7	38.4	42	36.9	58.6	55	59	57.3
Soybean meal	33	32.8	28.5	25	28.9	29.2	25.9	21.9	26.2	26.6	23.5	19.7
Wheat	10	22	33.2	36.5	15.2	24.5	23.4	27	4.1	10.2	8.5	10
Protein concentration[1]	5	-	-	-	5	-	-	-	5	-	-	-
Larvae powder2	-	2.5	5	7.5	-	2.5	5	7.5	-	2.5	5	7.5
Fat	2	0.6	0.1	-	3.2	2.1	0.4	-	4.1	2.7	1	0.1
Limestone	1.1	1.5	1.1	1.2	1.1	1	1	1	1.2	1	1	1
Dicalcium phosphate	0.7	1	1.6	1.6	0.5	1.8	1.8	1.8	0.4	1.6	1.6	1.6
Salt	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.2
Vitamins &mineral	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Bran	-	-	2	4	-	-	-	3.4	-	-	-	2.4
Barley	-	-	-	2	-	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100	100	100	100	100
Chemical composition (%)												
Crude protein	23	23	23	23.1	21.5	21.5	21.5	21.5	20.00	20.00	20.00	20.07
Met	0.50	0.37	0.39	0.42	0.48	0.35	0.38	0.40	0.47	0.34	0.37	0.40
Lys	1.32	1.18	1.12	1.08	1.21	1.0	1.04	0.98	1.13	1.00	0.96	0.91
Ca	0.92	0.9	0.9	0.9	0.87	0.87	0.87	0.86	0.878	0.82	0.81	0.81
P	0.47	0.4	0.4	0.4	0.42	0.42	0.43	0.43	0.41	0.40	0.40	0.40
Kcal/kg	3000.5	3000.1	3003.33	3029.19	3103.7	3101.8	3104.1	3105.75	3204.65	3202.3	3204.1	3201.07

T1. Wafi protein concentrate, each kg contains:40% crude protein, 2157kcal metabolism energy, 3.85% lysine, 3.7% methionine, 4.12% methionine+cysteine, 5% fat, 5% Ca, 4.68Ph, 2.4% Na,2.26%fibre.
T2. Larvae powder TML contains: 65.23%crud protein, 5981.93% kcal metabolism energy, 1.95% methionine,1.86% lysine,0.008% Ca,0.004%Ph.
T3. Chemical analysis of composition the diets.
T4. Chemical analysis of the components of the diets according to (NRC 1994).

wing vein of the bird at the end of the experiment. 1 gm was taken from the contents of the birds' intestines (duodenum, jejunum, ileal) according to the treatments, by four birds from each treatment at the end of the experiment. Petri is empty, then the nutrient medium (akar) is added to it in the autoclave after cooling to 45°C, and then the sample is mixed by moving the dish in the mentioned medium in order to obtain the divided bacterial cells that can be seen with the naked eye, meaning that (colonies) were formed in order to be counted The number of bacterial cells in the mentioned medium Gracias and McKillip (2004).

RESULTS AND DISCUSSION

Carcass Characteristics

Dressing percentage: The dressing percentage in T_1 was significantly superior as compared to the treatments T_2, T_3, and but was at par with T_4 (Table 3). The average body weight and average carcass weight of a control treatment T_1 was higher over the treatments T_2 and T_3, respectively. However, it did differ significantly with the treatment T_4.

Relative weight of the edible and immunological internal organs: There are no significant differences between the treatment T_1 and T_2, T_3, and T_4 in the percentage of edible and immune internal organs (Table 4). Biasato et al (2017 b) also conserved complete replacement of larvae powder in its different percentages of broiler females did not affect edible and immunological internal organs. The results differ with Bovera et al (2015 a) in the weight of the spleen Broilers fed with the alternative protein from TML5.57 g compared to 4.40 g in the broilers that were fed soybean meal depended on

complete replacement and effect on growth performance and carcass characteristics.

Relative weight of the major and secondary cuts: The control T_1 was significantly superior in the percentage of chest cut over T_2, T_3, and T_4, where the two treatments T_3 and T_4 did not differ between them, which were significantly superior over the treatment T_2. However, T_2 and T_3 were significantly superior to the control treatment T_1, and t T_4 for the thigh cut percentage, which did not differ between them (Table 5). In the secondary cuts, there was no significant difference between the trial treatments in the relative weight of back cut, with a significant superiority in T_2 in the wing cut percentage over the control T_1 and T_4. Moreover, it did not differ significantly with T_3, while the treatments T_1, T_3, and T_4 did not differ between them. Neck relative weight, T_2 was significantly superior over T_1, T_3 and T_4, and T_3 and T_4 did not differ with each other while they were significantly superior to the control T_1.

Glucose and protein concentration: There was no significant difference in the glucose concentration and the total protein concentration between the control and the treatments T_2, T_3, and T_4 in the blood plasma (Table 6), Biasato et al (2017a) also indicated that there were no significant differences in blood and serum characteristics of male broilers in the experiment.

The control treatment T1 and the total replacement treatment T4 increased the numbers of harmful bacteria, which did not differ between them (Table 7). The increase in the harmful bacteria in the intestines of the broilers of the two treatments T2 and T3 may be due to the decrease in the

Table 3. Effect of TML larval powder application on dressing percentage (Mean ± standard error)

Treatments	Average live weight (g)	Average carcass weight (g)	Dressing percentage %
T_1	2456.00 ± 79.91 A	1812.00±70.00A	73.7±0.54AB
T_2	1684.67±128.58C	1126.00±77.78C	66.03±0.82C
T_3	2125.33±62.84B	1460.00±60.01B	68.69±1.01BC
T_4	2327.33±99.76AB	1646.67±73.72AB	70.75±0.21B
Significance level	0.01	0.01	0.01

Table 4. Effect of TML larvae powder application on the relative weight of edible and immunological internal organs (Mean ± standard error)

Treatments	Relative weight (%)				
	Heart	Gizzard	Liver	Spleen	Fabricius gland
T_1	0.59±0.14	2.32±0.14	2.58±0.19	0.13±0.02	0.04±0.00
T_2	0.62±0.06	2.30±0.16	3.10±0.18	0.12±0.02	0.06±0.01
T_3	0.58±0.01	2.30±0.19	2.68±0.13	0.09±0.01	0.06±0.02
T_4	0.64±0.01	2.17±0.05	3.23±0.36	0.12±0.01	0.05±0.00
Significance level	N.S	N.S	N.S	N.S	N.S

Table 5. Effect of TML larvae powder application on the relative weight of major and secondary carcass cuts (Mean ± standard error)

Treatments	Relative weight (%)				
	Major cuts		Secondary cuts		
	Breast	Thigh	Back	Wings	Neck
T_1	37.02±0.36A	27.78±0.51B	19.77±0.60	9.70±0.44B	5.58±0.10C
T_2	28.45±0.56C	31.35±0.20A	21.08±1.24	11.47±0.10A	7.64±0.05A
T_3	30.78±1.49B	30.79±0.94A	21.03±0.44	10.62±0.35AB	6.40±0.40B
T_4	32.01±1.23B	28.56±0.69B	21.68±1.34	10.33±0.07B	6.81±0.17B
Significance level	0.01	0.05	N.S	0.01	0.01

Table 6. Effect of TML larvae powder application on glucose concentration and total protein concentration in blood plasma (Mean ± standard error)

Treatments	Glucose concentration (mg/100ml)	Total protein concentration (g/100ml)
T_1	210.25±8.40	3.15±0.11
T_2	225.50±8.38	2.87±0.03
T_3	222.75±3.82	3.35±0.38
T_4	217.25±1.89	3.10±0.16
Significance level	N.S	N.S

Table 7. Effect of using TML larvae powder on the numbers of harmful and beneficial microorganisms in the gut of experimental birds (Mean ± standard error)

Treatments	*Escherichia coli*	*L. bacillus*
T_1	10.33±0.33B	9.00±0.58
T_2	13.67±0.33A	10.00±0.58
T_3	13.33±0.33A	11.00±1.53
T_4	11.00±1.00B	10.00±0.58

percentage of beetle larvae powder, which is the protein concentrate that has been replaced by the commercial protein concentrate and because it is also It has a low level of essential amino acids that the bird needs in building different body tissues (Aviagen 2009) as they perform vital metabolic functions such as blood plasma proteins, enzymes, hormones, and antibodies, each with a specific role in the body (Pond et al 1995), The usefulness of the feed provided to poultry depends on the adequate content of the essential amino acids needed by the bird as well as the digestibility of the protein (Scanes et al 2004. Which led to increase in the numbers of harmful bacteria compared to the beneficial bacteria in the treated broilers. The percentage of beetle larvae powder in the fourth treatment T4 increased to 7.5%, an increase in the percentage of proteins and their content of amino acids led to an improvement in the functional performance of the various organs of the body in the treatment broilers and was significantly superior over T1 control treatment in its percentage in the harmful bacteria. The results of our study did not agree with what Biasato et al (2016) which showed no significant differences at the level in gut morphology among the control broiler group as compared to a group of broilers whose diet included beetle larvae powder at a rate of 7.5% (TML) with no negative effects on the health of the birds of this group, which matched with the results of the same researcher (Biasato et al 2017b) on female broilers. On intestinal morphology and histological results the results of study indicated an improvement in body weight and carcass characteristics when the percentage of beetle larvae powder increased when total replacement was treated by 7.5% TML and an improvement in the health of these birds. The treatment did not record any fatalities, and the results of study did not agree with the researcher (Bovera et al 2015a) whose results indicated that there were significant differences in gut morphology and a significant in length and weight of the intestine, ileum, and cecum in favor of the group fed beetle larvae powder (TML) compared to the soybean meal (SBM) group.

CONCLUSIONS

The use of raw beetle larvae powder not supplementing with amino acids type TML as the main source of protein which was a complete replacement with different percentages in the experimental treatments (2.5, 5 and 7.5%). Instead of the imported commercial protein, concentrate in the control treatment T_1 with a percentage of 5 % of broiler meat did not have a significant effect on the product characteristics and carcass characteristics, especially in the final body weight and dressing percentage, which are the most important indicator of economic return.

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Evaluation of Economic Performance of Al-Hamdaniya Farm for Poultry Breeding and Fattening the Production

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Abstract: This research aimed to assess the performance of broilers breeders in Al- Hamdaniya farm for the production year 2018. The research was based on the hypothesis that Al- Hamdaniya farm for breeding broilers is one of the economically important fields which benefit owners with a rewarding and encouraging economic return to expand their production and to enter into other productive projects. To achieve the goal, many economic assessment indicators were used, and data were collected through numerous visits and interviews with fam management during the production season. The rate of return on investment was (37%). The criterion of one chickens revenue was 115.993 thousand dinars. The study concluded that there is a lack of support and price subsidy by the relevant institutions, especially regarding the policy of protecting the local product. This had its impact on the profits of the owner of the farm on the one hand, and the reluctance of capital owners to invest in poultry projects on the other hand. Thus recommend the need to provide support by the state, and to pay attention to the quality of production to the extent that it becomes competitive with its foreign counterpart.

Keywords: Al- Hamdaniya farm, Poultry products, Performance assessment

Livestock farming is one of the important economic activities in the agricultural sector and a basic pillar for achieving agricultural and economic development. One of these activities is the production of poultry, which is the most notable among the important activities, and white meat and eggs have an economic and rewarding return compared to other productive projects (Jaafar et al 2020) as they are distinguished by the rapid turnover of capital invested in them, and the continuous demand for red and white meat and eggs as necessary source of protein in human food. These products are raw materials for other industrial activities, so animal production became the basic base for other sectors such as industry and commerce, in addition to the use of wastes from animal production activities as an organic natural fertilizer for plant production. The poultry production is one of the important livestock projects (Farhan et al 2020). Hudhud et al (2015) conducted an economic study of poultry meat production in Sulaymaniyah governorate, and the descriptive and quantitative statistical method was adopted to describe and analyze the data using some statistical parameters such as growth rates and multiple regression model in estimating the cost function. The research aimed to show the current situation of poultry meat production in Sulaymaniyah governorate through studying the most important factors affecting production, studying the structure of production costs, estimating the function of production costs and the optimal and profit maximizing volume. Al-

Obaidi (2014) conducted a study entitled assessing performance efficiency criteria in broilers production projects in *Nineveh Governorate*. The researcher adopted the analytical method in his approach, by collecting data and information through a questionnaire form and using criteria for evaluating the performance efficiency of agricultural projects and other technical criteria to reach the desired results. The study aimed to evaluate the efficiency of the performance of the Raiber project to produce broiler meat using various economic evaluation criteria and indicators, and to estimate the food conversion factor and its impact on the feasibility of investment in the farms of broiler meat production. The study concluded that broilers production farms are economically feasible, i.e. they achieve profitability more than many other investments, and that food conversion rate was consistent with international standards. Therefore, the study recommended that capital owners invest their money in the fields of broilers production. The research problem is based on the fact that Al-Hamdaniya farm faces great challenges represented by the lack of supportive policies from the state towards protecting the local product that it produces, which gave the foreign product superiority over it, and thus the price fluctuation and gives a bad reflection to other investors about the investment conditions in this field. The importance of broiler breeding projects indicate that these are economically rewarding because they are characterized by a rapid turnover of capital due to their

short breeding period, and also that the establishment of these projects does not need large areas compared to other livestock projects. The research aims to assess the performance of Al- Hamdaniya farm and to find out whether this farm achieves a rewarding economic profit margin that encourages others to enter into such projects.

MATERIAL AND METHODS

The data and information obtained from the questionnaire after personal interviews with the management of Al-Hamdaniya farm for the production of broilers, using some indicators of the economic assessment of agricultural projects, as well as the adoption of the descriptive economic approach that was based on some theoretical studies and previous research that dealt with this topic.

Descriptive and economic analysis of the farm: The farm is located in Al- Hamdaniya district in Nineveh governorate, about 40 km from Mosul city centre. Its area is about (27500)

square meters. It contains a number of halls for breeding, yet, the capacity of the farm is not fully utilized. One hall, of 720 square meters i.e. 60 meters in length and 12 meters in width, was rehabilitated, and its production capacity reaches up to 10500 chickens. A desert-type cooling system is used at the farm, namely air extractors, and the heating system used is incubators that operate by gas. The source of water is an artesian well equipped with delivery pipes, and watering is done through water distribution pools, farm is equipped with an automatic feeder, and there is an incinerator outside the farm. During the 2018 productive season, 6 productive batches were introduced, each lasted for 40-45 days during the breeding period then it is marketed. Varieties which are bred are foreign eggs varieties entered into local hatcheries and after hatching and when the chicks reach 2 days old, they are entered into raising period, and then when the chicks reach the normal marketing weight, the final product is sold live to some retailors.

RESULTS AND DISCUSSION

Fixed costs: These costs represent about 6.6% of the gross production cost of the farm. The annual depreciation premium for fixed assets was calculated on the basis of the productive life that was 15 years for machinery, equipment and instruments, and 20 years for roofed buildings and halls (Table 1-3).

Variable costs: Variable costs constituted about 93.4% of the total costs of Al- Hamdaniya farm. Chicks costs were 18.8% of the total variable costs, whereas labour costs were about 4.1% in addition to other costs (Table 4).

Total costs: The total costs include the fixed costs that are represented in the annual depreciation premiums for buildings, machines, and instruments, the interest on invested capital, administrative and family work. The

Table 1. Items of fixed costs for Al-Hamdaniya poultry field

Item	Total cost (Dinar)
Construction of a hall	35000000
Cooling system	2775000
Heating system	2400000
Artesian well	5000000
Automatic feeder	3600000
Large water distribution pools	360000
Small manual water distribution pools	250000
Diesel generators /2	9600000
Electrical wires	3000000
Water pipes+ water tank	1000000
Total	62985000

Values are calculated based on the questionnaire form

Table 2. Annual depreciation premiums

Item	Total cost (Dinar)	Economic life (Year)	Annual premiums (Dinar)
Construction of a hall	35000000	20	1750000
Cooling system	2775000	15	185000
Heating system	2400000	15	160000
Artesian well	5000000	15	333,34
Automatic feeder	3600000	15	240000
Large water distribution pools	360000	15	36000
Small manual water distribution pools	250000	15	25000
Diesel generators /2	9600000	15	640000
Electrical wires	3000000	10	200000
Water pipes+ water tank	1000000	10	66,67
Total	62985000	145	3236400

Values are calculated based on the questionnaire form *** Annual depreciation premium= total costs/ economic life

variable costs that represent the requirements of the production process in which the total production costs are considered (Table 5).

Total incomes: The major revenues from sales of live marketed broilers, and the gross and total number of marketing during one production cycle was 9555 chickens as

Table 3. Other items of fixed costs for Al-Hamdaniya poultry field

Item	Total cost (Dinar)
Depreciations	3236400
Interest on the capital	5038800
Administrative work	7200000
Total	15475200

Table 4. Types of variable costs of the farm

Item	Cost of one batch (Dinar)	Total cost/ year (Dinar)
Chicks cost	6930000	41580000
Medicines and medical supervision	2400000	14400000
Hall furnishing	350000	2100000
Labour wages	1500000	9000000
Electricity fees	300000	1800000
Cleaning and preparing costs of the hall	200000	1200000
Fodder cost	20160000	120960000
Fuel (Gasoil) and gas costs	2750000	24900000
Other costs (Food+ maintenance…etc.)	1000000	6000000
Total		221940000

Table 5. Total annual costs

Item	Value (Dinar)
Fixed costs	15475200
Variable costs	221940000
Total costs	237415200

Values are calculated based on the questionnaire form

Table 6. Total incomes during this cycle and production year

Item	Details
Average number of marketed Chickens	9555 chickens
Average weight of one chicken	2250 dinars
Total marketed weight (One marketing cycle)	21498750 kg live weight
Average sale price	2000 kg/live weight
Sales incomes (One production cycle)	42997500 dinars
Secondary income (One production cycle)	500000 dinars
Total incomes (Production cycle)	43497500 dinars
Total incomes (Annual)	260985000 dinars

Values are calculated based on the questionnaire form

Table 7. Economic assessment of the farm

Criteria	Value
Return on invested Dinar	1.9 Dinar
Net farm profit	23569800 Dinar
Average return on investment	37 %
Gross added value	39045000 Dinars
Net added value	35808600 Dinars
Period for capital recovery	3 years and 3 months
Gross economic surplus	16845000 Dinars
Net economic surplus	13608600 Dinars
Return on farm work= Return on farm management	18531000 Dinars
Average food conversion	1.628 g
Cost of 1kg of live meat	1720 Dinars/kg

Values are calculated based on the questionnaire form

an average during the 2018 production season after subtracting the fatalities which (9%) of chicks which entered the breeding field.

Economic assessment of the farm: The process of project assessment has become an important activity in the management of any project. Many project owners, decision-makers, and project financing agencies emphasize the importance of the benefit that assessment studies of their fields provide for them (Al-Banna 2011). Each Dinar invested achieves a return of 1.9 Dinars, which indicate higher the efficiency of the farm, and the net profit of the farm. The average return on investment reached 37%. The improvement in efficiency of the farm depends on improving the rate of return of the invested Dinar. As for the criterion of the capital recovery period, it was 3.3 This means that the farm needs a period of two years and three months to recover its invested capital in the farm, and the returns from agricultural work represent the same returns of the farm management because there is no work for family members on this farm. The food conversion rate was 1.628 kilograms, and this is within global standards that range between (1.6 - 1.8) kilograms; any increase over this percentage increases the amount of fodder consumed, which leads to a reduction in profit for the project. Finally, the cost of one kilogram of live meat reached 1720 dinars and this is considered acceptable and suitable to the prices to achieve acceptable profit margin for the investor.

CONCLUSION

In Al-Hamdaniya, the lack of supportive and back up policies for the local product, and its protection from foreign product competition, and consequently there was reluctance of investors to enter this industry and invest in this activity. The economic assessment indicators and criteria that they

are rewarding, and achieve an acceptable profit margin, and that the efficiency of food conversion is within the specified global standards, which is reflected on the percentage of profits achieved by the farm because the conversion efficiency measures the cost of the most important and largest part of the costs in these projects. The high rate of mortality is mostly due to the poor quality of the medicines and vaccines used. There is need to provide material and moral support by the responsible authorities, and enhancing confidence for investors to urge them to return to operating their farms again.

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Effect of Feed Diluting by Residue of Canning Factories on Productive Traits of Broiler

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Abstract: This study aimed to compare the effect of diluting the feed provided to broilers with the residues of canning factories (date, tomato, grape pomace and their mixture) on the productive characteristics. This experiment was conducted in the poultry field of the College of Agriculture and Marshes, University of Thi Qar. In the experiment, 240 unsexed Ross-308 broiler chicks were used, one day old, and an average starting weight of 42 g. Chicks were fed on a standardized diet which is the initiator diet for the period of 1-7 days, then the chicks were randomly distributed to five experimental treatments with 48 chicks per treatment. The experiment diets were used from the beginning of the second week until the end of the third week (14 days) as T1: control diet without addition, T2: dilute the control diet with 20% date pomace, T3: dilute the control diet with 20% tomato pomace, T4: dilute the control diet with 20% grape pomace, T5: dilute the control diet with 20 % homogeneous and equal mixture of the three types of pomaces. At the end of the experiment, there were no significant differences but a compensatory growth of birds in productive traits (live weight rate, weight gain rate, feed consumption rate Total food conversion efficiency, mortality ratio) with an arithmetic improvement in all production traits in favor of the two treatments (T5, T3) and there was a significant improvement in the economic efficiency of the diet in favor of all dilution treatments compared to the control.

Keywords: Feed diluting, Residue factories, Productive traits, Broiler

The continuous selection of modern breeds of broilers led to an acceleration of the growth rate, an improvement in the efficiency of food conversion and an increase in the amount of body fat (Sahraei and Shariatmadari 2007, Xu et al 2017). But the immune response of birds was negatively affected by this rapid growth due to the negative genetic correlation between growth speed and immune response (Al-Khatib and Clore 2013, Ahmed and Butris 2014). It was also associated with an increase in metabolic disorders such as ascites, sudden death syndrome (SDS) and skeletal problems (Dawkins and Layton 2012, Karume et al 2012, Alttai et al 2017). All of this led to an increase in the mortality rate, especially in the first three weeks of the birds' lifespan (Jahanpour et al 2015, Xu et al 2017). Despite all the efforts made, the cost of feeding remained 70-75% of the production cost, which is the highest cost in the commercial broiler production project (Tawfiq 2009, Al-Kassar 2012), as the raw materials included in the composition of the diets, such as corn and soybeans, are imported from other countries at high prices, and need to have feed alternatives with low economic costs, provided that these materials meet the basic requirements for the growth of the bird) of the diet must address eliminating the damages of the speed of growth and increasing of body fat percentage, reducing metabolic disorders and reducing the cost of nutrition (Al-Khafaji 2018). Researchers have resorted to using food rationing in several

ways, including the method of reducing feed with food industry residues such as pomace (Taleb et al 2003, Al-Zuhairi 2019) and providing the appropriate conditions to boost growth in the advanced period of the broiler's life to compensate for the growth that the birds missed during the rationing (Ahmed and Butris 2014, Xu et al 2017). The materials that can be used to feed poultry is factory waste from vegetables and fruits, which are produced in large quantities as by-products of manufacturing processes (Kumanda et al 2019). It provides a physiological benefit to birds as preventing or delaying the onset of diseases and is considered one of the effective means to increase their ability to improve performance and disease resistance (Ognik et al 2016).

MATERIAL AND METHODS

This study was conducted in University of Thi Qar, for the period from November 8, 2020 to December 12, 2020. The 240 unsexed one-day-old chicks of the strain (ROSS-308) were used, with an average initial weight of 42 grams, from Al-Anwar hatchery in Babil, Al-Kifl and were well cared for in the field according to the Ross-308 breeding guide. The raw materials included in the experiment, the pomace, which is the remaining substance after the squeezing of dates, tomatoes and grapes. The date pomace was obtained from the molasses factories (date juice) from the Al-Najaf

Governorate, as well as the tomato pomace was brought from the tomato paste plants, and grape pomace was collected from the juice shops in Thi Qar Governorate. These materials were dried by spreading them in a light layer of direct sun for 7 days and were turned three times a day until the humidity reached less than 10%. Then the materials were crushed in a grinder until its granules became the size of the starter pellet itself. They were sifted with a sieve with a diameter of 2-3 mm and kept in closed bags and transferred to the poultry field, where the starter ration was diluted with crushed pomace. The 20 kg of pomace (date pomace, tomato pomace, grape pomace or their mixture) were added to the treatments, bringing the total to 100 kg for the treatments. T1: control diet without addition. T2: dilute the control diet with 20% date pomace, T3: dilute the control diet with 20% tomato pomace, T4: dilute the control diet with 20% grape pomace, T5: dilute the control diet with 20 %. Homogeneous and equal mixture of the three types. The diluted feed was provided to the birds for the second and third weeks of the experiment, then all the birds were fed on a growth diet for a period of 35-22 days. The nutrients, they were calculated for the contents of 80 kg of feed and 20 kg of pomace for each of the energy, protein, fiber and the rest of the nutrients (Table 1 and 2).

Live weight: The chicks were weighed weekly until the end of the experiment, day 35, according to the equation of Al-Zubaidi (1986):

Average live body weight = total weights of birds / total number of birds.

Weekly weight gain rate: This was calculated according to Al-Zubaidi (1986).

Weight gain (gm) = live body weight at the end of each week - live body weight at the beginning of the week.

Feed consumed: This was according to Al-Zubaidi (1986).

Amount of feed consumed weekly (gm) = the amount of feed provided at the beginning of the week - the amount of feed remaining at the end of the week.

Feed conversion efficiency: This was calculated for each week using the following equation (Al-Zubaidi 1986).

Food conversion factor = Average amount of feed consumed per week/ Weekly weight gain.

Mortality: The mortality was recorded daily during the study period and converted into percentages.

Mortality (%)= (Number of dead chicks during the experiment period)/ total number of chicks at the beginning of the experiment) ×100

Economic efficiency: This was calculated as Naji (2006):

Economic efficiency = Cost of the forage (feed consumed (dinar)/ live weight (Kg) x the efficiency of food conversion.

Statistical analysis: The Complete Random Design (CRD) was used to study the effect of different treatments on the studied traits and to compare the significant differences between the means using Duncan's test (1955) by using the statistical program SPSS (2012).

RESULTS AND DISCUSSION

Live body weight and weekly weight gain: The effect of diluted feed with pomace (dates, tomatoes, grapes and their mixture) in the starter period (7-21 days) on live body weight and weekly weight gain indicated that control treatment superior in the second and third weeks (Dilution period). The average body weight decreased in these two weeks as a result of reducing the feed and is consistent with Al-Gharawi et al (2018) and Kumanda et al (2019). The decrease in the average weight after reducing the feeding of broilers with bean husks and grape pomace, respectively, and explained the reason for the decrease in weight and weight gain due to the high percentage of cellulosic fibers in the diluted feed, which affects the ratios of the rest of the nutrients such as energy and protein in the ration, so weight and gain decrease. Teimori et al (2005) also confirmed that increasing fiber in broiler feed leads to a decrease in the basic metabolic rate, so the soluble part of fiber with water forms a gelatinous substance that reduces the speed of food passage in the

Table 1. Chemical analysis of pomace (grapes, tomatoes, dates, mixture)

Ingredients	Grapes pomace	Tomato pomace	Date pomace	Mixture
Humidity	3.16	8	9	6.72
Dry matter	96.84	92	91	93.28
Crude protein	13.22	20.73	6	13.32
Crude fibers	27.31	30.94	24	27.42
Raw fat	0.6	1.53	0.95	1.03
Soluble carbohydrates	56.31	35.1	53.60	48.37
Ash	2.56	3.3	5.53	3.82
Energy kcal/kgm	2433.5	2416	2333	2394.17

alimentary canal and enhances the sense of satiety in birds. The present results did not agree with Ayhan and Aktan (2004) and Gonit et al (2007) as did not notice significant differences in the average live weight or weight gain when they used similar materials to dilute feed for broilers. In the fourth week of rearing, the chicks weights in all the treatments improved, and in the diluted treatments began to converge with the weights of the control treatment and in the fifth week, the significant differences between the treatments disappeared. Al-Jiashi (2018) observed that diluting with date seed powder by 20% for the period 21-28 reduces the differences in the treatments with the control and was as a result of providing the free ration where the birds began to eat larger quantities of feed, which was reflected in the body weight and weight gain. The earlier worker also observed an improvement in live weight due to the presence of multiple phenols, antioxidants, oxidation and lycopene in the feed materials, which supported the health status by reducing the oxidative stress to which the bird is exposed to free feeding,

and after the end of the feed rationing and the consumption of standard diets reflected increase in weight (Zhan et al 2007), Pop et al (2015), Dorri et al (2012), Wan et al (2021). Al-Fayyad (2010) and Khudair and Ibrahim (2010) explained the absence of significant differences after the early feed diluting into a compensatory growth for the diluted treatments, and confirmed that broilers can compensate for the growth missed in later periods when the appropriate conditions are provided during the period not less than two weeks.

The results indicated that there were no significant differences in the first week, but in the second and third week was noted that the control treatment was superior in feed consumption (Table 5) and this is consistent with Teimori et al (2005) who explained the decrease in feed due to the high percentage of fiber in the diluted feed, which slows down the speed of food passage in the alimentary tract of the bird because of the decrease in the basic metabolic rate due to the soluble part of the fiber with water forms a gel which enhances the feeling of satiety in birds. It also agrees with

Table 2. Composition of the diets used in the experiment during the stages of initiator and growth and the calculated chemical analysis

Feed material	Starter diet (1-21) days (control)	Initiator diets for dilutive treatments (7-21) days				Growth diet (22-35)days for all treatments
	T1	T2	T3	T4	T5	
Maize	43.5	34.8	34.8	34.8	34.8	47.4
wheat	17	13.6	13.6	13.6	13.6	17
soybean	28	22.4	22.4	22.4	22.4	26
Protein concentrate	10	8	8	8	8	6
Vegetable oil	0.5	0.4	0.4	0.4	0.4	2.6
Limestone	0.7	0.56	0.56	0.56	0.56	0.7
Salt	0.3	0.24	0.24	0.24	0.24	0.3
Date pomace	-	20	-		-	-
Tomato pomace	-		20	-	-	-
Grape pomace	-		-	20	-	-
Mixture	-		-	-	20	-
Computed chemical analysis						
Total	100	100	100	100	100	100
Crude protein %	22.44	19.15	22.10	20.6	20.61	20.31
Metabolizable energy kcal/kg	2966.5	2839.8	2856.4	2859.9	2852	3154
The ratio of energy to protein	132.16	148.24	129.23	138.83	138.32	155.24
Crude fiber	4.06	8.2396	9.44	8.71	8.79	3.91
Calcium	1.34	1.26	1.27	1.16	1.23	1.08
Phosphorous %	0.71	0.70	0.69	0.60	0.70	0.59
Methionine%	0.55	0.62	0.62	0.51	0.63	0.41
Lysine %	1.31	1.11	1.67	1.09	1.13	1.11
Methionine +Cysteine %	1.00	0.91	0.91	0.90	0.80	0.85

The equations were used to calculate the productive characteristics of broilers

ALGharawi et al (2018) and Kumanda et al (2019) pointed to the high percentage of fibers and cellulosic materials in the diluted feed, which leads to a volume increase in the intake of feed (physical satiety condition) which leads to eating less feed, which affects the ratios of the rest of the basic nutrients such as energy and protein. Mahata et al (2013) also observed a decrease in the feed consumed in broilers when fed a mixture of pomace fruits and vegetables due to the presence of tannins (a bitter astringent that is unpalatable to birds) (Ahmed 2011) and did not agree with Ayhan and Aktan (2004) and Dorri et al (2012) when used similar materials and did not notice significant differences in feed consumption

during the initiator period. In the fourth week, significant increase was observed in the feed consumption rate in all dilution treatments, and this agrees with ALGharawi et al (2018) when used the dilution of bean husks in feeding, and they noticed a significant increase in feed consumption after the end of the food rationing period, explained that the free ration prompted the birds to eat larger quantities of feed as a result of the hunger that the birds suffered during the period of food rationing and begin to compensate for what birds suffered from a deficiency in the main nutrients during the period of food rationing. As for the fifth week and the cumulative feed consumption (1-35 days) there are no

Table 3. Effect of diluting the feed with pomace (dates, grapes, tomatoes and their mixture) during the starter period on the average weekly live body weight (gm) (Mean ± standard error)

Treatments	Age (weeks)				
	1	2	3	4	5
T1	201.47±1.39	476.29±2.66 a	1055.21±9.82 a	1699.79±6.62 a	2367.33±17.95
T2	202.41±0.53	453.30±5.20 bc	975.09 b±6.93	1651. 9±2.46 bc	2358.22±13.28
T3	200.30±1.141	460.0±01.15 b	990.00±4.62 b	1665.77±4.22 bc	2373.85±3.90
T4	202.09±1.09	446.27 c±6.36	969.27±10.39 b	1643.67±3.93 c	2355.88±6.93
T5	200.98±0.79	457.13 bc±1.04	982.27±6.93 b	1673.00 b±12.70	2378.04±16.74
Significance	N.S	*	*	*	N.S

Table 4. Effect of diluting the feed in the starter period with pomace (dates, tomatoes, grapes and their mixture) on the average weekly weight gain (gm) (Mean± standard error)

Treatments	Age (weeks)					Cumulative weight gain rate (1-35) days
	1	2	3	4	5	
T1	159.47±1.39	274.82 ±3.52a	578.92±7.21 a	644.58 ±3.92 b	667.54 ±11.60 b	2325.33±17.95
T2	160.41±0.53	250.89 ±5.56 bc	521.79 ±1.74 b	676.81 ±8.03 a	706.32±14.23 a	2316.22±13.28
T3	158.30±1.42	259.70±0.53b	530.00 ±4.16 b	675.77 ±1.36 a	708.08±2.58 a	2331.85±3.90
T4	160.09±1.09	244.18 ±6.02 c	523.00 ±4.04 b	674.40 ±7.88 a	712.21±5.39 a	2313.88±6.93
T5	158.98±0.79	256.15 ±1.63 bc	525.14 ±6.45 b	690.73 ±5.78 a	705.04±4.04 a	2336.04± 16.74
Significance	N.S	*	*	*	*	N.S

Average weekly feed consumption

Table 5. The effect of diluting the feed in the starter period with pomace (dates, tomatoes, grapes and their mixture) on the weekly feed consumption rate (gm) (Mean± standard error)

Treatments	Age (weeks)					Cumulative weight gain rate (1-35) days
	1	2	3	4	5	
T1	174.17±0.72	382.92 ±1.10 a	844.07±6.87 a	971.29±1.33 b	1195±4.04	3567.45±12.58
T2	173.67±0.88	360.96± 1.02 c	793.97 ±1.73 bc	995.63±2.36 a	1212.08 ±7.23	3536.31±2.86
T3	172.42±0.39	371.51 ±1.26 b	804.20 ±4.05 b	1001.33±10.91 a	1205.92± 11.61	3555.38±20.28
T4	172.81±0.43	355.11 ±1.49 b	790.21 ±6.75 c	1014.67±3.93 a	1216.57±8.44	3549.37±15.09
T5	173.65±0.81	367.93 b±0.64	792.07 bc±2.79	1005.17a±6.98	1211.63±7.63	3550.45±15.19
Significance	N.S	*	*	*	N.S	N.S

Table 6. Effect of feeding dilution in the starter period with pomace (dates, tomatoes, grapes and their mixture) on the feed conversion factor (gm of feed/gm of weight gain) (Mean± standard error)

Treatments	Age (weeks)					Cumulative weight gain rate (1-35) days
	1	2	3	4	5	
T1	1.09±0.006	1.39±0.019	1.46±0.007 b	1.51a±0.011	1.78±0.025 a	1.53±0.007
T2	1.08±0.011	1.44±0.04	1.52±0.008a	1.47±0.015ab	1.72±0.025 b	1.53±0.008
T3	1.09±0.005	1.43±0.005	1.52±0.019a	1.48±0.018 ab	1.70±0.02 b	1.52±0.01
T4	1.07±0.009	1.45±0.039	1.51±0.011a	1.49 ±0.019 ab	1.71±0.01 b	1.53±0.002
T5	1.09±0.003	1.44±0.006	1.51±0.017a	1.46±0.003 b	1.72±0.002 b	1.52±0.004
Significance	N.S	N.S	*	*	*	N.S

significant differences and these results are consistent with Khudair and Ibrahim (2010) and Aditya et al (2018).

Feed conversion factor: The effect of diluting the feed during the starter period with pomace on the feed conversion factor indicate no significant differences in the first week of the experiment. In the second week also, there are no significant differences in the food conversion factor between the four treatments and control treatment, but the value of the food conversion factor has decreased arithmetically in the dilution treatments. Hady (2018) and Rayes (2019) observed dried tomato pomace and dried grape pomace during the initiator period and did not notice significant differences in the feed conversion factor. In third week there are significant differences in the feed conversion factor between the dilution and control treatments to the superiority of the control treatment over the rest of the treatments. Lira et al (2010) and Kumanda et al (2019) noticed the deterioration of the efficiency of food conversion during the period of rationing the feed and explained this due to the deterioration of the digestion and absorption processes as a result of the high percentage of fibers, which reduced the rest of the nutrients, so the live weight decreased and this was reflected in the weight gain and then the efficiency of food conversion.

Mortality and economic efficiency of the ration: There were no significant differences between all dilution and control treatments in the percentage of mortality (Table 7). The rearing period 35 days recorded a small mortality rate for several reasons, including choosing chicks of good breed and a good hatcher, and following scientific and practical methods for good management and attention to health and administrative matters, especially the feed materials that were used in the process of diluting according to the proportions determined in the research plan. There were no deaths in three treatments from the experiment T2, T3, T4 may be to slow the growth rate in the first weeks of the experiment as a result of reducing the feed for the period 7-21 days which one of its benefits is to improve the immune status of the bird, as there is a negative genetic correlation between

Table 7. Effect of feeding dilution with pomace (dates, tomatoes, grapes and their mixture) on the mortality rate and the economic efficiency of the ration (Mean± standard error)

Treatments	Mortality	Economic efficiency of the ration
T1	4.17±2.09	1617.69±13.56 a
T2	0.0±0.0	1498.18±14.59 b
T3	0.0±0.0	1492.89 b±17.05
T4	2.08±2.08	1513.31 b±4.02
T5	0.0±0.0	1484.768.14 b±8.14
Significance	N.S	*

the speed of growth and the immune response of the birds and this was confirmed by earlier researchers (Saleh et al 2005, AL-Betawi 2005, Abdul and Al-Qaisi 2013) and there were no significant differences in the mortality rate when using dried tomato and dried grape pomace crushed, respectively, in broiler diets. There was significant improvement in the economic cost of the diet in all treatments compared to the control (Table 7) due to a decrease in the amount of feed consumed as a result of shortening 20% of the consumed feed for two weeks and replacing with 20% of dried pomace. This was consistent with Yitbarek, (2013) used tomato pomace. Attia and Al-harthi (2015) and Al-Jiashi, (2018) used the date residues and observed significant improvement in the economic characteristics of the treatments compared to the control.

CONCLUSION

It is possible to use the residues of pomace canning factories (dates, tomatoes, dates and their mix) to dilute the broiler feed for 7-21 days at 20% of the control feed without affecting the productive qualities. Mixing of factory residues from pomace (dates, tomatoes, grapes) in equal proportions led to an arithmetical improvement in the productive characteristics and some of the carcass cuts and the health

status of the bird compared. Reducing the cost of the bush in all mitigating transactions because the price of the pomace is very high.

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Effect of Replacing Red Sorghum with Yellow Corn in Broiler Diets on Productive Performance, Immunological and Microbial Traits

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Abstract: The study was conducted in Department of Animal Production / University of Baghdad in the old site (Abu Ghraib) dueing 2020. The study aims to investigate the effect of partially and completely substituting crude and fermented red sorghum with the yellow corn in broiler diets on the productive performance and some characteristics of carcasses and immunological and microbial traits. In the experiment, 240 broiler chicks of strain 308 (Ross) were used that were sexed at one day old and distributed among the treatments by five treatments. Each treatment contained 48 chicks with three replicates for each treatment, each replicate contained 11 males and 5 females to ensure homogeneity of the replicates in terms of sex, with a general initial weight of 39.47 g. The chicks were fed on three diets according to the breeding guide (ROSS) 2019: the starter diet from age (0 - 10 days), grower diet from (11 - 24 days) and the finisher diet from (25 - 42 days). The study included five treatments as the first treatment (T_1) represents the comparison treatment that contains yellow corn with a percentage of 48%. The second treatment (T_2) is a partial replacement of crude red sorghum instead of yellow corn (and at a percentage of 24% (crude red sorghum + 24% yellow corn). Furthermore, the third treatment (T_3) represents the complete replacement of crude red sorghum instead of yellow corn at a percentage of 48%. The fourth treatment (T_4) was partially replacing the fermented red sorghum instead of yellow corn (24% fermented red sorghum + 24% yellow corn). Finally, the fifth treatment (T_5) treatment of the complete replacement of fermented red sorghum instead of yellow corn (48%). There were no significant differences in all characteristics of productive performance (live body weight, accumulative weight gain, feed consumption. Similarly, the cumulative feed conversion rate (0-42 days old) for treatments T_2, T_4, and T_5 compared to the control treatment$_1$. Thr complete replacement treatment for crude red sorghum T_3 recorded a significant decrease in the live body weight, weight gain, and total food conversion compared with the control treatment T_1 and the rest of the replacement treatments. The four replacement treatments also recorded a significant decrease in the number of harmful bacteria *E.coli* compared with the control treatment while the treatment T_5 and T_4 were significantly superior in the numbers of beneficial bacteria compared with T_1, T_2, and T_3.

Keywords: Broiler, Red sorghum, Fermentation, Iraqi probiotic

The search for alternative feed sources to the traditional sources adopted in poultry feeding is still of interest to many researchers and workers in the field of the poultry industry and poultry feeding. Besides, the imported feed materials represented by grains and protein concentrates constitute large numbers of the diet cost. The shortage of grain and feed materials, as well as the competition between humans and animals, leads to a significant increase in grain prices, which negatively affects the bird industry by increasing the cost of manufacturing feed (Sharif et al 2012). Therefore, it is necessary to move towards finding the available alternatives that can it replace the main sources of grains such as maize and wheat, as well as the use of these alternatives when these main sources radiate in the local markets. Therefore, an integrated nutritional evaluation of non-traditional fodder alternatives must be conducted to partially or completely replace the main sources as a beneficial and feasible replacement. Several studies have indicated the possibility of using red sorghum in the diets of broilers and laying hens because red sorghum has positive and encouraging properties for its introduction into the diets. In comparison, it is characterized by a high percentage of metabolizable energy as it reaches (2871.9 kcal/ kg) and crude protein (10.2). It is also rich in some essential amino acids, such as methionine and lysine, which is similar to what is found in yellow corn (Nyamambi et al 2007). As well as containing phenolic acids and flavonoid compounds (Seitz 2004), it also contains a percentage of β-carotene, which is a natural antioxidant that has a role in improving the performance of birds. Despite these advantages, red sorghum crops such as maize and wheat contain phytic acid and tannin, which are anti-nutritional that affect the performance of chickens. The percentage of these antibiotics varies according to the different varieties and the place of cultivation, but there are techniques adopted to reduce these inhibitors, and one of these methods is the adoption of the fermentation process. Studies have indicated that the fermentation process leads to the reduction of anti-nutritional, especially tannin and phytic acid, as well as increasing the activity of the trypsin and phytase enzyme. In view of the limited studies on the

replacement of crude and fermented red sorghum instead of yellow corn in the broiler diet. The study aims to identify the effect of the fermentation process with the probiotic in improving the nutritional value of red sorghum (Dicko et al 2005). Moreover, comparing the use of crude and fermented red sorghum with yellow corn in terms of its effect on the productive performance of broilers. Furthermore, determining the best level for the use of red sorghum in broiler diets and identifying its impact on the tissue characteristics of the intestine and the numbers of beneficial and harmful microorganisms. . The main aim was to study the effect of partial and complete replacement of crude and fermented red sorghum instead of yellow corn in broiler diets on productive performance and some immunological, histological and microbial characteristics.

MATERIAL AND METHODS

This experiment was conducted Department of Animal Production University of Baghdad / (Abu Ghraib) for the period from November 1, 2020 to December 12, 2020

Fermentation method: The fermentation process was carried out in the poultry fieldand , the red sorghum was also procured from local markets in Abu Ghraib, after verifying that it was clean and free of impurities, foreign materials, and insects. The fermentation process was carried out by adding 50% water to the red sorghum, then probiotic (0.5%) and mixed well, and then it was filled in polyethylene bags. Taking into account the good pressure when packing the bags to discharge the air from the bags as much as possible, then it was injected with a quantity of CO_2 to provide an anaerobic environment for microorganisms. The bags closed tightly after the air was discharged and placed in a dark room at a temperature of 37°C for 72 hours. Then the red sorghum was emptied from the bags and spread on a clean floor for about seven days in order to dry them, with constant stirring to ensure that it was completely dry, then it was collected and ready to be mixed with the ingredients of the diet.

Chick distribution and management: In this study, 240 one-day-old broiler chicks (ROSS) were used, which were procured from the hatchery of Al-Shukr Company, in Abu Ghraib. They were sexed in the field at the age of one day and distributed into five treatments with three replicates for each treatment. Thus, each treatment included 16 chicks per replicate Each replicate contained 11 males and 5 females with an average initial weight of 39.47 g. However, the treatments included were:

T_1: Comparison treatment contained 48% yellow corn.

T_2: A partial replacement of crude red sorghum instead of yellow corn, at a percentage of (24% crude sorghum + 24% yellow corn).

T_3: A complete replacement of crude red sorghum instead of yellow corn, at a percentage of 48%.

T_4: Treatment of partial replacement of fermented red sorghum with the probiotic instead of yellow corn (24% of fermented red sorghum + 24% of yellow corn).

T_5: Treatment of complete replacement of fermented red sorghum with the probiotic instead of yellow corn (48%).

Experimental diets: Birds are reared for 42 days, during which three diets are provided according to the Breeding Guide 308 ROSS (0-10 days starter diet) (11-24 days grower diet) (25-42 days finisher diet) (Tables 1, 2, and 3).

Studied traits

Productive traits

Bodyweight (g/bird): The birds were weighed at intervals (stages) with an electronic scale of 30 kg for each replicate, and the total weight was divided by the number of replicate chicks to extract the average live body weight for each chick, according to the equation listed below (Al-Zubaidi 1986):

$$\text{Average live body weight (g)} = \frac{\text{Birds total weights (g) per replicate}}{\text{Total bird number per replicate}}$$

Weight Gain (WG) (g/bird)

WG = body weight at the end of the period (g) – body weight at the beginning of the period (g)

Feed Consumption FC (g/bird)

$$FC = \frac{\text{Feed provided at the period beginning (g) –}}{\text{the remaining feed at the end of the period (g)}}$$
$$\frac{}{\text{The number of birds per replicate}}$$

The amount of feed consumed during the period was calculated by weighing the remaining feed in the feeders for each replicate at the end of the period and subtracting it from the amount of feed provided at the beginning of the period.

Feed conversion rate (g feed/g weight gain)

$$\text{Feed conversion rate} = \frac{\text{Amount of feed consumed in duration (bird/g)}}{\text{Average weight gain in duration (bird/g)}}$$

Mortality rate: No mortality occurred between treatments during the study period.

Microbial count for the number of bacteria in the small intestine *E. coli, Lato bacillus*: One g of the duodenum, jejunum and ileum contents (2 birds/replicate) were taken at the end of the experiment and under sterile conditions, from which a series of decimal dilutions were made (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) using Buffer Peptone Water (PBS) by Macro Pipits and was placed in the incubator for two hours at a temperature of 37°C. The purpose of adding peptone water is to dilute the sample and preserve its vitality and then transferred to the solid culture media for the purpose of its calculation (Noveir and Halkman 2000) and then the microorganisms were estimated as follows:

***E. coli* bacteria in the ileum:** 0.1 ml of each decimal dilution was withdrawn into two Petri dishes (Duplicate) containing solid culture media (eosin methylin blue) and cultured in an l-shaped glass stick in a circular motion and placed in the incubator at 37°C for 24 hours. Then, the isolated bacterial cells were examined in the laboratory to observe their shape, the way they aggregate, and their response to Gram stain (Scoter et al 2000). The developing colonies were calculated using the Sc6 Colony Counter Stuart and the number was extracted by multiplying the number of colonies by the dilution inverse / g of the intestine sample (logarithmic cycle). ***Lactobacilli* in the ileum:** 0.1 ml of each decimal dilution

Table 1. Chemical composition of the diet ingredients (starter diet 0-10 days)

Material	T_1	T_2	T_3	T_4	T_5
Yellow corn	48	24	—	24	—
Red sorghum	—	24	48	24	48
Soybean meal 48% protein	33	32.9	32.9	32.3	31.6
Wheat	9.7	10.7	11.6	11.4	13.1
Protein Concentrate	5	5	5	5	5
Oil	2	1.1	0.2	1	—
Stone	1.1	1.1	1.1	1.1	1.1
DCP	0.8	0.8	0.8	0.8	0.8
Salt	0.2	0.2	0.2	0.2	0.2
Vitamins and minerals	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100
Calculated chemical analysis					
Crude protein	23.0	23.0	23.01	23.06	23.09
Metabolisable energy	3001.1	3003.6	3005.4	3005.23	3009.2
Methionine	0.507	0.5	0.5	0.51	0.51
Lysine	1.32	1.3	1.3	1.3	1.3
Calcium	0.94	0.942	0.938	0.941	0.935

Table 2. Chemical composition of the diet ingredients (Grower diet from 11-24 days)

Material	T_1	T_2	T_3	T_4	T_5
Yellow corn	48	24	—	24	—
Red sorghum	—	24	48	24	48
Soybean meal 48% protein	29.1	29.1	29	28.3	27.6
Wheat	12.8	13,7	14.8	14.7	16.4
Protein Concentrate	5	5	5	5	5
Oil	3.1	2.2	1.2	2	1
Stone	1.1	1.1	1.1	1.1	1.1
DCP	0.5	0.5	0.5	0.5	0.5
Salt	0.2	0.2	0.2	0.2	0.2
Vitamins and minerals	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100
Calculated chemical analysis					
Crude protein	21.5	21.52	21.5	21.52	21.5
Metabolisable energy	3101.75	3103.55	3100.15	3100.59	3104.63
Methionine	0.485	0.46	0.4355	0.4885	0.49
Lysine	1.218	1.187	1.154	1.189	1.162
Calcium	0.87	0.868	0.863	0.8665	0.860

was taken into two Petri dishes (duplicate) containing MRS (Deman Regose Sharp Broth) containing 1% calcium carbonate by L-shaped glass sticks, cultured on the solid medium in a circular motion and incubated at 37°C for 24 hours under anaerobic conditions, the isolated bacterial cells were examined microscopically to observe their shape, way of aggregation and response to gram stain (Scoter et al 2000).

The developing colonies were calculated using the Sc6 Colony Counter Stuart, and the number was extracted by multiplying the number of colonies by the dilution inverse / g of the intestine sample (logarithmic cycle).

Statistical analysis: This was conducted according to the completely random design using Duncan (1955) new multiple-range test and the statistical program (SAS) was used in the statistical analysis.

RESULTS AND DISCUSSION
Productive Traits
Live body weight: There were significant differences between all treatments in the trait of live body weight at the first age stage (0-10 days), as the comparison treatment T_1 was significantly superiorto) overall replacement treatments (Table 4). It was followed by treatment T_2 (partial replacement of crude red sorghum instead of yellow corn). The significant superiority of treatment T_1 continued through the second age

Table 3. Chemical composition of the diet ingredients (the finisher diet 24-42 days)

Material	T_1	T_2	T_3	T_4	T_5
Yellow corn	48	24	—	24	—
Red sorghum	—	24	48	24	48
Soybean meal 48% protein	25,5	25.5	25.3	24.7	24
Wheat	15	16	17.2	17	18,7
Protein Concentrate	5	5	5	5	5
Oil	4.5	3.5	2.5	3.3	2.3
Stone	1.1	1.1	1.1	1.1	1.1
DCP	0.5	0.5	0.5	0.5	0.5
Salt	0.2	0.2	0.2	0.2	0.2
Vitamins and minerals	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100
Calculated chemical analysis					
Crude protein	20	20.06	20	20.05	20.08
Metabolisable energy	3208.5	3204.47	3201.75	3201.51	3205.55
Methionine	0.464	0.44	0.414	0.4678	0.470
Lysine	1.1186	1.088	1.052	1.0897	1.0627
Calcium	0.86	0.859	0.854	0.857	0.852

1. Wafi protein concentrate of Dutch origin: Energy 2125 kcal / kg, crude protein 40%, calcium 5%, available phosphorous 3.85%, methionine 2.85%, lysine 3.8%, methionine + cysteine 3.29%
2. According to the chemical composition of the feed materials, based on what was mentioned in the NRC (1994)

Table 4. Effect of partial and complete replacement of crude and fermented red sorghum instead of yellow corn in diets on the average live body weight (g) of broilers (Mean + standard error)

Treatments	Average live body weight (g)		
	Weight at 10 days old	Weight at 24 days old	Weight at 42 days old
T_1	261.15 ± 1.99 A	A 1124.56 ± 14.50	A 2964.02 ± 90.92
T_2	B 246.60 ± 2.15	B 986.38 ± 12.89	A 2886.84 ± 132.07
T_3	C 228.08 ± 6.88	B 949.25 ± 30.25	B 2722.75 ± 49.98
T_4	D 213.05 ± 2.38	B 991.36 ± 5.55	A 2898.33 ± 41.01
T_5	C 233.98 ± 3.21	B 974.79 ± 47.27	A 2934.50 ± 37.86
Significance level	**	**	**

N.S: There are no significant differences between the averages of the treatments, ** Significant difference (P<0.01)

stage of (11-24) days as compared to overall replacement treatments. In comparison, not all of the replacement treatments differed significantly between them, but at the age of 42 days, positive results were observed in favor of the partial and complete replacement treatments, except for the treatment T_3, which included completely replacing for the crude red sorghum instead of the yellow corn. However, the bird's weight of these treatment did not differ significantly from the control treatment T_1 except for a significant decrease for treatment T_3 compared with control treatment T_1 and the rest of the other replacement treatments. The reason for this decrease may be due to the high percentage of crude red sorghum in this treatment, and this increase is offset by an increase in the percentage of antibiotics such as phytic acid and tannin acid because of their negative effects on domestic birds.

Weight gain: There were significant differences between the different replacement treatments and the comparison treatment at the age stage from 0-10 days. Treatment T_1 was significantly superior to all the replacement treatments of crude and fermented red sorghum and this superiority continued for the comparison treatment T_1 even in the second age stage from 11-24 days, while there were no significant difference between all the replacement treatments.

Conversely, a significant decrease was observed in the weight gain of treatment T_3 compared with the comparison treatment and the rest of the replacement treatments in weight gain during the period from 25-42 days as well as the cumulative period from 0-42 days. In contrast, the replacement treatments T_2, T_4, and T_5 did not show a significant difference compared to the comparison treatment T_1 in this period.

Feed consumption: There were no significant differences between all the experimental treatments and in the period from 0-10 days and 25-42 days, while there were significant differences in the treatments in the feed consumption rate during the period from 11-24 days (Table 6). The treatment T_3 recorded a significant increase (compared with the replacement treatments (T_2, T_4, T_5), while it did not differ significantly from the comparison treatment T_1. There were no significant differences in the cumulative feed consumption rate (0-42 days) between all the replacement and comparison treatments. These results give an idea of the high percentage of crude and fermented red sorghum to 48% that does not change the palatability of birds for feed and has no effect on increasing or decreasing feed consumption.

Food conversion: There were significant differences between the replacement treatments of crude and fermented

Table 5. Effect of partial and complete replacement of crude and fermented red sorghum instead of yellow corn on the weight gain (g) for broilers (Mean ± standard error)

Treatments	Weight gain (g)			
	0-10 days	11-24 days	25-42 days	0- 42 cumulative
T_1	A 221.68 ± 1.99	A 863.40 ±12.81	A1839.46±81.92	A 2924.55 ± 90.92
T_2	B 207.13 ± 2.15	B 739.78 ±15.05	A 1900.45 ±121.30	A 2847.37 ±132.07
T_3	C 188.61 ± 6.88	B 721.17 ±25.21	B 1773.50 ± 20.19	B2683.28 ± 49.98
T_4	D 173.58 ± 3.38	B 778.31 ±2.68	A 1906.97 ± 42.99	A 2858.86 ± 41.01
T_5	C 194.51±3.21	B 740.81 ±50.45	A 1959.71 ± 62.87	A 2495.03± 37.86
Significance level	**	**	**	**

N.S: There are no significant differences between the averages of the treatments, ** Significant difference (P<0.01)

Table 6. Effect of partial and complete replacement of crude and fermented red sorghum instead of yellow corn in diets on the feed consumption (g/bird) for broilers (Mean ± standard error)

Treatments	Weight gain (g)			
	0-10 days	11-24 days	25-42 days	0- 42 cumulative
T_1	A 221.66 ± 2.14	AB 1329.49 ± 39.70	A 3177.10 ± 160.73	A 4728.26 ± 174.23
T_2	A 216.50 ± 4.50	D 1211.77 ± 4.53	A 3211.45 ± 32.99	A 4639.72 ± 34.57
T_3	A 209.33 ± 9.64	A 1378.89 ± 24.49	A 3271.00 ± 115.17	A 4859.22 ± 94.93
T_4	A 215.79 ± 9.96	DC 1237.30 ± 5.76	A 3214.38 ± 32.85	A 4667.47 ± 38.00
T_5	A 210.84 ± 8.49	BC 1302.43 ± 13.42	A 3341.87 ± 95.42	A 4855.14 ± 91.36
Significance level	N.S	**	N.S	N.S

N.S: There are no significant differences between the averages of the treatments, ** Significant difference (P<0.01)

red sorghum and the comparison treatment in the first and second age stages (0-10 days) and (11-24 days) (Table 7). In the stage of 0-10 days there was a significant deterioration in the feed conversion for each of the treatments T_3 and T_4 compared with the comparison treatment T_1. Besides, the two treatments T_2 and T_5 did not show any significant differences compared to the comparison treatment T_1. The comparison treatment T_1 recorded a significant improvement in the feed conversion overboth treatments T_3 and T_5, while treatment T_2 and T_4 did not differ significantly from the treatment T_1 in the second stage (11-24 days. There was significant deterioration of the treatment T_3 compared with the treatment T_1 as well compared with the rest of the other replacement treatments. This effect was evident at the cumulative period of (0-42 days) for the treatment T_3 compared with the treatment T_1 and the rest of the other replacement treatments (T_2, T_4, T_5). The reason for this deterioration and the productive performance of the third treatment birds (containing crude red sorghum at a rate of 48% and free of yellow corn) may be due to the high rate of use and the nutritional inhibitors it contains, such as phytic acid and tannin as in (Table 3). Besides that, these inhibitors work to form complexes with each of the nutrients, especially the mineral elements (Konietzny and Greiner 2002). As well as the formation of complexes with proteins as a result of their association and thus hinder the digestion of proteins by digestive enzymes and then affect their absorption and utilization (Ravindran et al 1995). These inhibitors also reduce the digestion of carbohydrates through interaction with them and the formation of carbohydrate complexes, thus inhibiting the shelf life of the amylase enzyme through the formation of a complex with calcium, which negatively affects the performance of birds (Selle et al 2000). These inhibitors also work on the sedimentation of minerals within the alimentary canal, and this affects the fat metabolism (Vohra and Satyanavayan 2002), and then these substances reduce the productive performance when it is increased in bird diets.

The productive performance of broilers, especially the cumulative period, indicate no significant differences in body weight, weight gain rate, and feed consumption rate. In addition to the feed conversion for each of the treatments T_2, T_4, T_5 compared with the treatment T_1. Although there are no significant differences, it may be considered a positive result from using crude red sorghum at a rate of 24% and fermented red sorghum at a rate of 24% and 48% in broiler diets, which did not negatively affect the performance of broilers. Perhaps this may be due to the nutritional value of red sorghum and content of nutrients s. In addition, the coefficient of digestion and facilitation of these nutrients may be high, which reflected positively on the metabolism and benefit of these elements significantly. Otherwise, perhaps the role of the fermentation process of red sorghum may be due to the Iraqi probiotic, as the fermentation process has a positive role in improving the nutritional value for red sorghum, as shown in Table (3) for the chemical analysis of crude and fermented sorghum. There was improvement in most nutrients, and it also reduced the proportion of antibiotics, especially phytic acid and tannin. Numerous studies have indicated that the fermentation process plays a major role in improving the quality characteristics of the food, especially the fermentation using Lactobacillus. Besides that, the activity of this bacteria in the feed material causes clear changes in its specific characteristics and increases its readiness for nutrients. This is reflected positively on the performance of birds as a result of an increase in the proportion of digestion and absorption of nutrients in birds. The fermentation process increases the activity of bacteria and yeasts used in the process, as the activity of bacteria increases the decomposition of materials, especially cellulose, and increases the facilitation of nutrients that are related, such as phosphorous and also, increases the activity of the phytase enzyme as a result of the effectiveness of beneficial microorganisms used in fermentation (Al-Ashch and Davanjak 1995). In general, the fermentation process increases the level of digestive enzymes such as protease,

Table 7. Effect of partial and complete replacement of crude and fermented red sorghum instead of yellow corn in diets in the feed conversion (g/feed/g weight gain) for broilers (Mean ± standard error)

Treatments	Weight gain (g)			
	0-10 days	11-24 days	25-42 days	0- 42 cumulative
T_1	C 0.99 ± 0.00	C 1.54 ± 0.06	B1.73 ± 0.17	B1.62 ± 0.11
T_2	BC 1.04 ± 0.03	BC 1.63 ± 0.03	B1.69 ± 0.08	B1.63 ± 0.06
T_3	B 1.10 ± 0.02	A 1.91 ± 0.03	A 1.84 ± 0.06	A 1.81 ± 0.04
T_4	A 1.24 ± 0.03	BC 1.58 ± 0.00	B1.68 ± 0.05	B1.63 ± 0.03
T_5	BC 1.08 ± 0.03	AB 1.76 ± 0.10	B1.70 ± 0.05	B1.67 ± 0.01
Significance level	**	**	**	

N.S: There are no significant differences between the averages of the treatments, ** Significant difference (P<0.01)

Table 8. Effect of partial and complete replacement of crude and fermented red sorghum on the microbial count of beneficial and harmful bacteria for broilers (Mean ± standard error)

Treatments	Microbial count of beneficial and harmful bacteria	
	E. Coli	*Lactobacilli*
T$_1$	46.87 ± 26.31A	61.75 ± 16.12C
T$_2$	2.18 ± 2.05B	86.87 ± 21.89C
T$_3$	0.18 ± 0.13B	84.87 ± 33.24C
T$_4$	0.56 ± 0.22B	323.25 ± 72.22B
T$_5$	1.18 ± 0.47B	539.68 ± 81.88A
Significance level	**	**

*N.S: There are no significant differences between the averages of the treatments.
** Significant difference (P<0.01).

amylase, lipase, phytase, and catalase, and then the complex nutrients such as carbohydrates, fats and proteins are decomposed into simpler units that are easy to digest in birds. This, in turn, increases the flavor, palatability, and acidity of the feed material, which positively affects the performance of birds. In addition, the fermentation process increases beneficial microorganisms in the fermented feed material, and this in turn positively affects when birds eat such feed. This effect was evident in the birds of treatments T$_4$ and T$_5$, as percentage of beneficial microorganisms in them increased, as shown in Table (8). The positive action may be due to red sorghum containing good proportions of β-carotene, as these substances play an important role as antioxidants (natural antioxidants) and have a role in free radicals scavenging and removing toxicity from the organism (Balakam 2010), as these substances perpetuate raw materials needed for cell growth.

Numbers of beneficial and harmful bacteria in the intestines: There was significant decrease in the numbers of *E. coli* in the intestines of birds in favor of the four replacement treatments T$_2$, T$_3$, T$_4$, and T$_5$, compared with the treatment T$_1$. However, the four replacement treatments did not differ significantly between them. There was a significant increase in the number of beneficial microorganisms of *Lactobacilli* for each of the treatment. T$_5$, which significantly

superior overall treatments followed by treatment T$_4$, which was superior over the comparison treatment and the crude red sorghum treatments, while the replacement treatments of the crude red sorghum T$_2$ and T$_3$ did not differ significantly over the comparison treatment.

CONCLUSIONS

The fermentation process of red corn with the Iraqi probiotic plays an important role in improving the nutritional value of this substance, especially by reducing the inhibitors present in it such as phytic acid and tannin. It is possible to replace partially raw red corn and partially or fully fermented corn in the place of yellow corn in broiler diets without negative effects on production performance. The use of raw and fermented red corn in broiler diets has improved the lengths of villi in the fasting duodenum, as well as the number of beneficial microorganisms in the intestine.

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Clinical and Molecular Detection of *Mycoplasma haemocanis* by using Real-time PCR in Dogs of South Provinces of Iraq

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Abstract: Present study aims to detect and quantify the *Mycoplasma haemocanis* in dogs using various diagnostic methods study included examination one hundred and twenty-five dogs 100 animals that showed different clinical signs in the southern governorates (Basrah, Dhi-Qar, Maysan and Al-Muthanna / Iraq) and twenty-five as control in both sexes and different age groups. Blood samples studies on anticoagulation for smear, complete blood count and PCR technique showed various clinical signs using different test at first blood smears stained with the Gemsia stain to detection of the *Mycoplasma haemocanis*. The diagnosis was confirmed using real-time polymerase chain reaction dogs in present study showed many clinical signs partial or complete loss of appetite 96%, pale of the mucus membranes 54.5%, congestion of the mucous membranes 18.1%, rapid and difficult respiration 51.5%, lethargy 24.2%, weight loss 78.7% and the presence of ticks on the animal's body 100%. *Mycoplasma haemocanis* is observed in blood smears (72%) parasitizing the red blood cell wall clustered separately or in the form of single chains .The polymerase chain test confirmed that 45.8% of the cases examined were positive for the test, and the results recorded that the highest infection rate (42.4%) in age of 5-10 years compared to other age groups**.** The present study recorded that the infection rate in males (38.7%) was higher than in females (27.4%).

Keywords: *Mycoplasma haemocanis*, Clinical signs, Real Time PCR, Iraq

Hemotropic mycoplasmas are characterized as cell wall-deficient bacteria that are found in a variety of feline, canine, bovine, ovine and wild animal hosts (Sashida et al 2013, Aquino et al 2014, Hampel et al 2014). The spherical, rod shaped, or ring-shaped microorganisms formerly named as *Haemobartonella* are uncultivable in culture until today. They attach to the outside surface of red blood cells and grow upon them (Messick 2004). *Hemotrophic mycoplasmas* are pleomorphic, epicellular, gram negative bacteria that are present on the surface of erythrocytes.The brown dog ticks *Rhipicephalus sanguineus* usually live in the crack and crevice of kennels that seriously infest dogs are threat in dogs' health These ticks transmit *M. haemocanis* to dogs during the process of blood sucking in dogs, three hemoplasma species have been identified: *Mycoplasma haemocanis, Candidatus M. haematoparvum,* and Candidatus *M. haemominutum* (Sykes et al 2005, Zhuang et al 2009, Roura et al 2010, Obara et al 2011). *Mycoplasma haemocanis*, a dominant canine *hemitropic Mycoplasma*, infects erythrocyte and usually leads to asymptomatic manifestation, but sometimescauses subclinical symptoms in dogs such as fever, anemia, anorexia, lethargy, weight loss and thrombocytopenia in more severe cases acute hemolytic anemia maybe occurs and frequently recovers. However, in some dogs this may cause death (Messick 2004, Kemming et

al 2004b, Chalker 2005). Animals' present severe anemia to chronic infections without clinical manifestation chronically infected animals is usually asymptomatic. However, the infection tends to become noticeable in splenectomized dogs immunosuppressed, stressed, with concomitant diseases such as babesiosis, parvovirus, demodicosis and distemper, make the significant *Hemotrophic Mycoplasma*infection (Messick Harley 2015). There was no scientific document explaining the registration of *Mycoplasma haemocanis* in the Iraq southern governorates (DhiQar, Basra, Muthana, Maysan) in dogs, so current study conducted to identification present of *Mycoplasma haemocanis* in dogs with documented clinical signs and emphasized infection by molecular technique.

MATERIAL AND METHODS

Animals of study: Present study conducted to include one hundred of dog (49) males and females (51) aged between one and ten years in different breed the dogs suspected infected with *Mycoplasma haemocanis* from different southern Iraqi province (Basra, Dhi-Qar, Maysan and Al-Muthanna) during August to November 2020.

Clinical examination: All doges which suspected infected with *Mycoplasma haemocanis*exhibition to through physical examination include body temperature, heart and respiratory

rate, abnormality signs which recorded in special clinical card.

Samples collection: One hundred blood samples were collected from dogs cephalic vein, 10 ml of blood sample put in EDTA tube for blood smear, and PCR techniquesblood smear Giemsa staining of blood smears according to the standard procedure (Coles 1986) for identification of RBC infected with *Mycoplasma haemocanis* was used to diagnose infection with *Mycoplasma haemocanis*.

Real-Time PCR (qPCR): The 16Sribosmal RNA gene of Mycoplasma haemocanis was directly detected using the qPCR technique, which was carried out according to the method described previously (Wengi 2008).

Primers: The Real Time PCR primer for direct detection *Mycoplasma haemocanis*were designed in this study using NCBI Genbank sequence database (MN294708.1) and Primer 3 plus primer design online software and these primers were synthesized by (Scientific Researcher Co. Ltd, Iraq), (Table 1).

Statistical analysis: SPSS was used. For analysis of data.

RESULTS AND DISCUSSION

Clinically infected animals showed different clinical manifestations which include partial or complete loss of appetite (96%), anemia which manifested with pale mucous membranes (54.5%) and congestion mucous membranes (18.1%) which detected on conjunctival, nictitating membrane also icteric mucous membranes rapid and difficult respiration, (51.5%), In addition other animals were suffering from, lethargy (24.2%) and weight loss (78.7%) the presence of ticks on the animal's body (100%), (Table 2). The dogs

suspected infected with, *M. haemocanis*appears coccoid or rod shape, it might find individually or in chains on the erythrocyte cell wall (Fig. 1).

Result of Real Time PCR (qPCR)

Figures show the amplification curve of a segment of the *Mycoplasma haemocanis* 16S rRNA gene produced by real-time PCR (Figs. 2-4). Result of *Mycoplasma haemocanis* according age of animal indicate that animals at age of 1-3 years 8 (24.2%) positive, and in 3-5 years 11(33.3%) and the high infected rate in animals at age of 5-10 years old 14 (42.4%) with significant different by PCR (Table 3). Result of *Mycoplasma haemocanis* according to gender show that high infection was with *Mycoplasma haemocanis* in male (38.7%) than in female (27.4%) with significant different

There was no scientific document explaining the registration of *Mycoplasma haemocanis* in the Iraq southern governorates (Dhi-Qar, Basra, Maysan and Al- Muthana) in dogs, however clinical infection with *Mycoplasma haemocanis* was documented in dogs or other animals in Mosul (Arsalan 2005, Al-Badrani and Raaima 2012). The infection appear in animals imported from European countries.. Clinical appearance of Hemoplasma occurs in most domesticated animals such as dogs, cats, pigs, buffalo, sheep, llamas, and cows and latent haemoplasma may also affect deer, mules, and goats, where the organisms predominantly appear (Jarad and Alsaad 2016, Constable et al 2017). The organism has been criminalized in the rickettsial parasite (*Mycoplasma*) of the erythrocyte cell membrane in mammals, causing clinical disease, fever and hemolytic disease in various animals (Sudan et al 2012). The clinical signs that appeared on dogs infected with
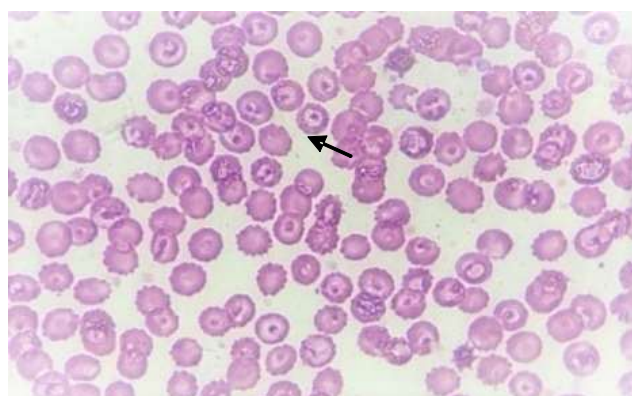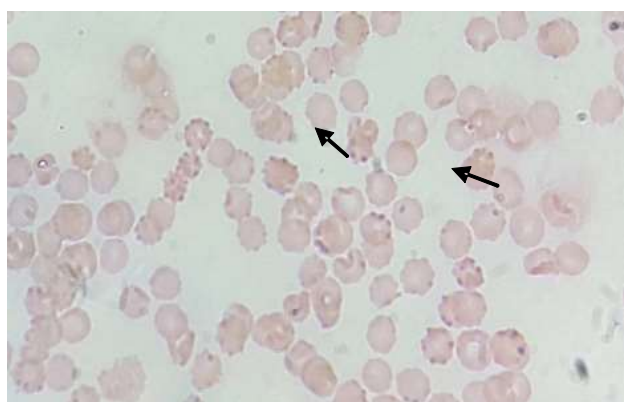
Fig. 1. Infected erythrocytes *M. haemocanis* with high parasitemia, Giemsa stain ×1000

Table 1. Primers used in the present study

Primers		Sequence (5'-3')	Product size
16S ribosomal RNA gene qPCR primer	F	TTTGGTTAAGTCCCGCAACG	143bp
	R	TCGCCATTGTAGCACGTTTG	

Mycoplasma haemocanis in the governorates of southern Iraq are variable and non-specific, such as loss of appetite, lethargy, weight loss, depression, fever, pallor of the mucous membrane, the presence of ticks in suspicious dogs and weakness, and this corresponds to (Kemming et al 2004, Shrivyazdi et al 2014, Yu and Huang 2016, Abed and Alsaad 2017, Al-Abedi et al 2020, Jarad and Abed, 2020) signs that are mentioned by others (Hoelzle 2008). The presences of pale mucus membranes will exhibit the development of anemia and reduction of blood parameters was due to destruction and removal of parasitized erythrocytes by the reticulo-endothelial system, whereas icteric mucus membranes which were also seen reflected the progressive anemia (Tagawa et al 2010, Sahey 2016, Jabbar and Amery 2020, Hasan and Al-Amery 2020). In a study on the spread of *M .haemocanis* and their geographical distribution in the governorates of southern Iraq, indicate positive among 72 suspicious dogs that were analyzed. 45.8% were positive for *M. haemocanis* by real-time polymerase chain reaction. In the current study, *Mycoplasma haemocanis* was diagnosed with the advent of molecular diagnostic techniques, such as conventional PCR and Real time PCR (high sensitivity and specificity).. It is widely used and has known success in both acute and chronic infected animals the same result obtained by Messick (2004),,Hoelzle (2008) and Gharban (2021). The current work used PCR and Real-time (qPCR) for molecular diagnostics of Mycoplasma. The prevalence of *M. haemocanis* using molecular technique in France, Spain, Switzerland, Tanzania, Trinidad and Tobago, the United States and Greece was 3.3, ,14.3 , 0.9 , 19,, 4.9,, 0.6 and 5.6% ,respectively (Roura et al 2010, Barker et al 2010, Compton et al 2012).   In present study of the diagnosis of *Mycoplasma haemocanis* examined by (PCR) was 45.8%. The infection rate was in males (38.7%) higher in females (27.4%). Earlier researchers have obtained similar trend (Barker et al 2010, Mohammad et al 2022).

Among the factors associated with variables such as age, and type of sex, race, frequency of brushing, and sedative type were statistical correlations with *Mycoplasma* spp. however Mohammad et al (2022) noted in its study that among the variables analyzed during anamnesis was a link to *Mycoplasma* spp. With the presence of sex, variants among in relation to sex, the percentage of infection is higher in male dogs and with respect to the age group there was a higher rate of infection in adult dogs according to (Messick 2004). Animals can be susceptible to acute infection and the age, not as a sole factor, but as long as with the disease which concomitant or immunosuppressed regarding the concomitant factors,

infection with *Mycoplasma* spp*.* There is a significant association sex was introduced as male dogs twice the likelihood of infection in females (Sasaki and Collaborators 2008).

The highest incidence rate   because they may be at greater risk of exposure to *R.sanguineus* fleas (Kemming et al 2004, Novacco et al 2010) and ticks (Dantas Torres 2008)
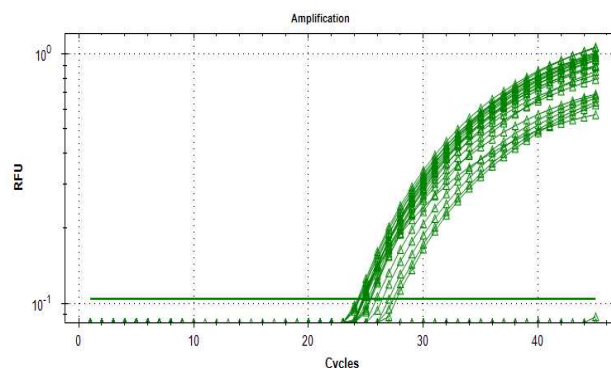
Fig. 2. Real Time PCR amplification plot for 16SrRNA gene in *Mycoplasma haemocanis* positive samples
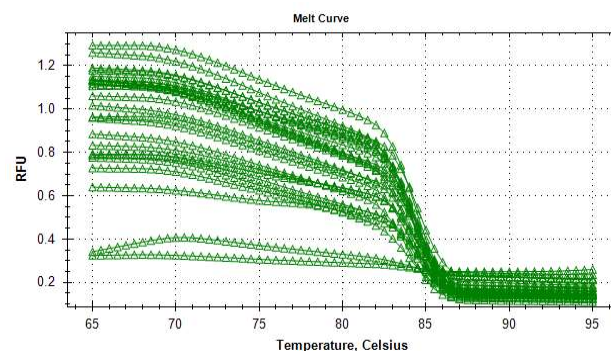
Fig. 3. Real Time PCR melting curve for 16SrRNA gene in *Mycoplasma haemocanis* positive samples
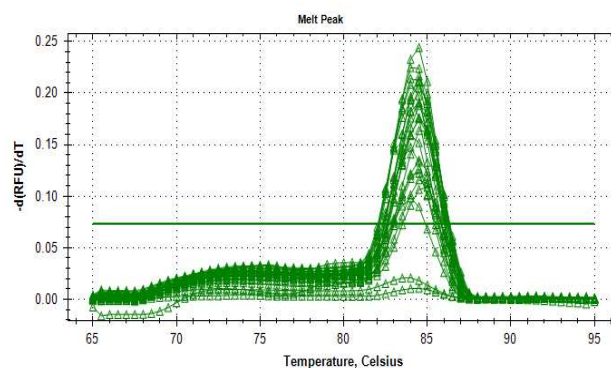
Fig. 4. Real Time PCR specifity by melting peak for 16SrRNA gene in *Mycoplasma haemocanis* positive samples

Table 2. Clinical signs of infected dogs with *Mycoplasma haemocanis*

Clinical signs	Infected dogs	
	No.	%
Partial or complete loss of appetite	32	96
Congestion mucous membranes	6	18.1
Pale mucous membranes	18	54.5
Rapid and difficult respiration	17	51.5
Weight loss	26	78.7
Lethargy	8	24.2
Presence of ticks on the animal's body	33	100

Table 3. Percentage of infection according to age group

Age group	No.	Infection rate
1-3 years	8	24.2%
3-5 years	11	33.3%
5-10 years	14	42.4%
Total	33	45.8%

Table 4. Infection with *Mycoplasma haemocanis* according to gender

Sex	Total no.	Positive	
		No.	%
Female	51	14	27.4%
Male	49	19	38.7%
Total	100	33	45.8%

due to association between age and infection was also observed adults animals having an infection rate ten times higher than that of puppies (Baker et al 2010, Tennant et al 2011). Neither gender nor age factors had a significant effect on the incidence of infection mentioned by some researchers (Novacco et al 2010, Hassiri et al 2016). The Swiss and French studies found no association between age or gender (Kenny et al 2004, Wengi et al 2008). Mulwa and Kitaa (2018),observed that 49.3% of males and 47.9% of female dogs were affected by hemoplasmosis, which is an indication of a lack of sexual orientation for this infection, and this result is also consistent with an observation also noted by Valle et al (2014).

CONCLUSION

Mycoplasma haemocanis agents have been identified in dogs from the southern governorates (Basrah, Maysan, Dhi-Qar and Al- Muthanna) for the first time using various diagnostic methods particularly the fact that male and adult dogs are more susceptible to infection which conceder

important factor in selective breeding more research into the disease in other domesticated animal species is needed lik cat.

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Isolation, Characterization and Antibiotic Susceptibility of *Serratia marcesens* from Human and Birds in Iraq

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Abstract: This study was conducted to isolate *Serratia marcescens* from birds for the first time in the field of veterinary medicine in Iraq to test their susceptibility to antibiotics. *S. marcescens* is an aerobic, Gram-negative bacillus first identified in 1819. Samples were collected from people raising birds in different areas of Baghdad, Iraq. One hundred fifty human samples of *S. marcescens* were collected from the eye swab, nasal cavity, wounds, burns, catheters, urine, and blood and 150 bird samples were isolate. Samples were striped on McConkey and blood agar, and suspect colonies were grown on nutrient agar media and DNase Test Agar. Determination of isolates was made according to colony morphology, Gram staining, and conventional biochemistry interactions and analysis profile Vitek® 2 compact. *S. marcescens* isolates were maximum from urine was (17.5%) followed by burns (9.09%) and wound (2.85%). The most urine isolates were from females 4 (57.1%) than males 3 (42.85%). Wound and burn isolates were found in males only of samples from people raising birds in Baghdad. In birds, the percentage of *S. marcescens* was 2.67% which isolated two isolates from tracheal swabs from ornamental birds and pigeons in Baghdad and two isolates from wound swabs from local chickens in Baghdad and Kut governorate. *S. marcescens* from humans had different antibacterial sensitivity profiles, all isolates were susceptible (100% to Azithromycin, Ciprofloxacin, and Meropenem, in contrast, 70% against Ceftazidime, 60% to Amoxicillin. Birds *S. marcescens* isolates were absolute 100% resistant to Amoxicillin, Ceftazidime, and Trimethoprim.

Keywords: *Serratia marcescens*, Test agar, Azithromycin, Ciprofloxacin, Meropenem, Ceftazidime

Serratia species are a gram-negative family that is an opportunistic pathogen classified in the ethnic group *Klebsiella* and the large family *Enterobacteriaceae*. It`s also widespread in the environment (Donnenberg et al 2010), and is characterized as a motile gram-negative bacillus relating to the family of *Enterobacteriaceae* that is lactose fermenting, catalase-positive, and produces an extracellular DNase. It can survive and grow under anaerobic conditions with very low quantity of organic material. *Serratia* bacteria emit extracellular enzymes such as AmpC type β-lactamases, elastase, gelatinase, lecithinase, alkaline protease, and caseinase, all of which increase bacterial resistance to antimicrobial therapies. Communication occurs through channels within the biofilm - a type of cell signaling known as quorum sensing. It is also known that drug resistance in *S. marcescens* is significantly more prevalent than in other *Enterobacteriaceae*. Furthermore, the transfer of R-factors between different strains of these bacteria is possible (Iguchi et al 2014, Ray et al 2017). *Serratia spp.* is widely distributed in the environment. It can be found in water and soil and in association with plants, insects, humans, and other animals (Iguchi et al 2014). *S. marcescens* is commonly involved in hospital-acquired infections (HAIs), particularly catheter-associated bacteremia, urinary tract infections, and wound infections (Hertle 2005). It is an important opportunistic pathogen that causes a wide range of diseases and clinical presentations with high morbidity. *S. marcescens* is usually growing in bathrooms. *Serratia* genus often produces a red pigment known as prodigiosin. It should be mentioned that not all pathogenic strains of *Serratia* produce this red coloration (Van et al 2007).

This study aims to provide information on the prevalence of *S. marcescens* strains from people raising birds in different areas of Baghdad city and from birds raised in bird shops and houses through the following parameters: Isolation and identification of *S. marcescens* from different samples collected from humans and birds and detection through an antimicrobial susceptibility test.

MATERIAL AND METHODS

Study samples: This study was approved by the Ethical Research Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq as well as the Ministry of Higher Education and Scientific Research. *Serratia marcescens* was isolated from human and birds' sources in Baghdad city -Iraq from the beginning of October 2020 until the end of February 2021. Human samples from sterilized (eye swab, nasal, wound, burn, catheter, urine, and blood) and were secured from people raising birds, and bird samples were obtained from pigeon birds, local chicken and

various ornamental birds raised in bird shops and in houses.

Diagnostic assays: Samples were drawn on MacConkey agar and blood agar and incubated at 37°C for 24 h. Cultures were confirmed on Nutrient agar media and DNase Test Agar. They were identified by colony, morphology, and Gram stain, biochemical reaction using motility assay, catalase assay, Voges proskier, citrate calling, gelatin hydrolysis, indole, oxidase assay, urease assay, methyl red, and TSR agar. These results confirmed that these isolates belong to *S. marcescens* and as described by Donnenberg (2010). They were also confirmed by biochemical tests using the Vitek® 2 compact system (BioMérieux, France). Mueller-Hinton agar was placed into 100 mm or 150 mm Petri dishes at a depth of 4 mm. The pH of the agar was between 7.2 and 7.4. Bacterial inoculation was prepared by diluting a broth culture to match a 0.5 McFarland turbidity standard, which are about 150 million cells / ml. The minimum inhibitory concentration (MIC) for each antimicrobial agent was determined using the BSAC agar dilution method. Antimicrobials included β -lactams (penicillin: (Amoxicillin 25 µg); cephalosporins: (Ceftazidime 30 µg), and aminoglycosides: (Amikacin 10 µg), fluoroquinolones: (Ciprofloxacin 5 µg), Carbapenem: (Meropenem 10 µg), Sulfonamides: (Trimethoprim 10 µg) and Macrolide: (Azithromycin 15 µg). The distribution of MIC values was compared with those from principles of Clinical and Laboratory Standards Institute (CLSI).

Statistical analyses: This Statistical analysis system (SAS), program was used to detect difference factors in the parameters employed in the current study. Chi-square test was also applied to compare significances between percentages in this study.

RESULTS AND DISCUSSION

S. marcescens was present in 4.67% samples distributed as 150 humans and 150 birds. Isolates from humans comprised 6.67% and from birds 2.67% (Table 1).

The bacterial isolates seeded on MacConkey and blood agar plates in aerobic conditions. On MacConkey agar is characterized by the production of small pale pink smooth round colonies (late lactose fermenter) (Fig. 1), with a production of red pigment when grown at 25°C (Fig. 2). While in blood agar, *S. marcescens* colonies showed hemolytic activity visible as clear zones on human and sheep blood agar plates (Fig. 3). On MacConkey agar *S. marcescens* is a late lactose fermenter as in (Fig. 1). It may show red prodigiosin production, especially if the plate was incubated at 25°C as in (Fig. 2). Prodigiosin is considered one of the secondary metabolites produced by S. marcescens isolates (Streling et al 2018). To clone the *S. marcescens* hemolysis identified on blood agar, blood agar plates were examined for hemolytic activity on humans and sheep, *S. marcescens* isolates were identified based on the hemolysis-positive clones, as shown in (Fig. 3). The *ShlA* is the only hemolysin that has been reported in *S. marcescens* (Hertle and Schwarz 2004). Serratia marcescens showed hemolytic activity visible as clear zones on human and sheep blood agar plates. The zones were larger for bacteria grown at 30°C than at 37°C. Also, the bacterium was showed contact-dependent hemolytic activity on human RBC, which was also

Table 1. Distribution of *S. marcescens* isolates in human and bird samples

Sample	Total No.	Positive isolates	
		No.	%
Human	150	10	6.67
Bird	150	4	2.67
Total	300	14	4.67

Fig. 1. Isolates of *S. marcescens* on MacConkey agar medium production of small pale pink smooth round colonies

Fig. 2. Isolates of *S. marcescens* on MacConkey agar medium production of red pigment when grown at 25°C
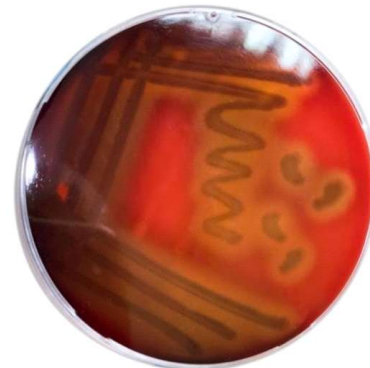
Fig. 3. Isolates of *S. marcescens* on blood agar medium producing hemolytic activity when grown at 37°C

greater for bacteria grown at 30°C than at 37°C (Shimuta et al 2009).

The zones were larger for bacteria grown at 30°C than at 37°C. On DNase agar with indicator Toluidine Blue O (TBO). *S. marcescens* isolates DNase-positive colonies appear with rose-pink halos on a blue background as shown in (Fig. 4, 5). Culturing *S. marcescens* on the test of DNase agar medium was performed to determine the ability of an organism to hydrolyze DNA. DNase agar is a differential medium that tests the ability of an organism to produce an exo-enzyme, called deoxyribonuclease. The Toluidine Blue O (TBO) is a color-changing pigment that changes color when complicated with other substances. When TBO complexes with polymerized DNA (non-immune medium), a royal blue result is obtained; when DNA has been hydrolyzed, TBO complexes with oligonucleotides or mononucleotides, resulting in a change in the dye structure and absorption spectrum, yielding a light pink color as shown in (Fig. 4 ,5) (Lior and Patel 1987).

The isolates were prodigiosin pigment producers on Nutrient agar medium with an optimum temperature of production at 25°C (Fig. 6, 7, 8). Eleven of the studied isolates were prodigiosin pigment producers on Nutrient agar medium with an optimum temperature of production at 25°C as showed in (Fig. 6 and 7). Kim et al (1999), mentioned three of study isolates were non-pigmented, the compound responsible for red pigmentation production and prodigiosin secretion to the culture medium as in (Fig. 8). Glucose may inhibit prodigiosin production due to catabolic repression or by lowering the medium pH during growth and fermentation (Sole et al 1997), or *S. marcescens* strains, that belong to non-pigmented groups such as A5 and A8 are frequently isolated from human specimens. Swarming colonies of *S. marcescens* grown cells were inoculated in a thin streak across the surface of Nutrient agar medium and incubated overnight at 25°C.

Swarming colony of *S. marcescens* grown cells were inoculated initially in a thin streak across the surface of the Nutrient agar medium and were incubated at 25°C overnight (Fig. 9 and 10). After incubation at 37°C for 24 hours, study showed the growth of *S. marcescens* on Luria-Bertani (LB) agar medium as the cultured colonies appeared circular, small, and devoid of color (Fig. 11). When grown on soft agar (0.7-0.8%) and a rich medium, cells of *S. marcescens* as in (Fig. 9, 10) and other Gram-negative bacteria were elongate, produce more flagella, and move over the surface of the agar in a coordinated manner. Serrawettin, a lipopeptide, appears to be important as a wetting agent (Harshey 1994). The Luria-Bertani agar medium could not be considered as a differential medium for *S. marcescens* as in (Fig. 11). This medium was

developed for phage and *Shigella* growth studies and LB later became the preferred method for the growth of *Escherichia coli* and other types of the *Enterobacteriaceae* (Bertani 2004). In addition to its use of the growth surface when attempting to analyze colony morphology, this culture medium did not aid in bacterial selectivity during this study and was not effective for reliance on *S. marcescens*.

Serratia marcescens, microscopically, is as gram-negative, straight rods with parallel sides and rounded ends. *S. marcescens* showed the results of tests gave positive results for motility the catalase, Voges proskier, summon citrate, and gelatin hydrolysis while giving negative results for indole and oxidase. The urease test and methyl red are a variable, some isolates were positive and others were negative for the test. TSR agar gives alkaline - acid (pink-yellow) on the slant and the bottom does not appear gas bubbles and no H2S production (Table 2). These results are confirmed that these isolates belong to *S. marcescens* by Identification of isolates was dependent on the VITEK2 compact system the result of this study was 99% probability
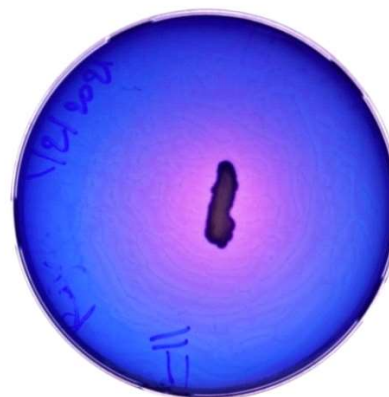
Fig. 4. *S. marcescens* on DNase agar medium producing bright pink color when grown at 37°C in human
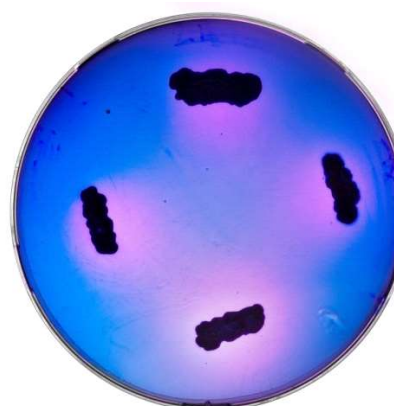
Fig. 5. *S. marcescens* on DNase agar medium producing bright pink color when grown at 37°C in bird
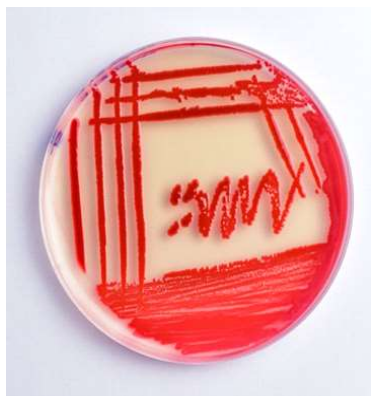
Fig. 6. Isolates of *S. marcescens* on Nutrient agar medium producing red pigment when grown at 25°C

Fig 7 Isolates of *S. marcescens* on Nutrient agar medium producing red pigment when grown at 25°C

Fig. 8. Isolates of *S. marcescens* on Nutrient agar medium producing non-pigment when grown at 25°C

Fig. 9. Swarming colony of *S. marcescens* grown in thin streak across surface of the Nutrient agar medium and incubated at 25°C overnight

Fig. 10. Swarming colony of *S. marcescens* grown in thin streak across surface of the Nutrient agar medium and incubated at 25°C overnight

Fig. 11. Isolates of *S. marcescens* on Luria-Bertani agar medium appeared colorless colonies when grown at 25°C

Fig. 12. Antibiotic susceptibility testing of human *S. marcescens* isolates Mueller Hinton agar. (1) Amoxicillin 25 µg, (2) Azithromycin 15 µg, (3) Amikacin 10 µg, (4) Ciprofloxacin 5 µg, (5) Meropenem 10 µg, (6) Ceftazidime 30 µg, and (7) Trimethoprime 10 µg

Fig. 13. Antibiotic susceptibility testing of birds *S. marcescens* isolates Mueller Hinton agar. (1) Amoxicillin 25 µg, (2) Azithromycin 15 µg, (3) Amikacin 10 µg, (4) Ciprofloxacin 5 µg, (5) Meropenem 10 µg, (6) Ceftazidime 30 µg and (7) Trimethoprim 10 µg

S. marcescens. These results of biochemical identification IMIVIC tests as shown in (Table 2) confirmed that these isolates belong to *S. marcescens* as described by Donnenberg (2010).

One hundred and three shows positive culture out of 150 samples. A total of (6.67 %) positive isolates of *S. marcescens* were isolated, from urine was (17.5%) followed by burns (9.09%) The most urine isolates were from females (57.1%) than males (42.85%). Wound and burn isolates were found in males only of samples of people raising birds in Baghdad/Al-Karkh and Al-Rusafa as in (Table 3, 4). *S. marcescens* is now recognized as an opportunistic pathogen in humans and has the potential to spread epidemically, causing nosocomial infections in hospitalized patients. Infections caused by *S. marcescens* include cystitis, arthritis, eye infections, respiratory tract infections, UTIs, septicemia, meningitis, and wound infections (Cristina et al 2019). Munim et al (2013) isolated 77 % of *S. marcescens* from human urine samples and Anfal et al (2011) 57.14 % of UTIs. In present

study *S. marcescens* was higher than reported 2.2% of UTIs (Al-Jebouri and Mdish 2013). The majority of UTIs in this study were of female, which may be because women are more likely than men to have UTIs because the urethra in females is much shorter and closer to the anus than in males, and lack the bactericidal properties of the prostate. Secretions are substances that are secreted (Al-Jubouri 1989, Al-Jubouri and Madish 2013). *S. marcescens* was 9.09 and 2.85% from burns and wounds Muslim et al (2017) *S. marcescens* was from wounds to extent of 23.8% (Anfal et al 20115% (Thikra et al 2007). and 11% (Posluszny et al 2011). The difference in percentages may be due to the number and size of samples.

In this study, *S. marcescens* isolates were collected in 150 samples of pigeons, local chickens, and various ornamental birds a raised in bird stores and houses. Bird samples were collected from wound and tracheal swab of different areas in Baghdad, Kut, and Diyala governorates. A total of four positive samples (2.67 %) were isolated from

Table 2. Biochemical test for *S. marcescens* isolates

Test	Result status
Indole	-
Methyl red	V
Oxidase	-
Catalase	+
Vogesprosker	+
Simmons citrate	+
TSR test	K/A, - H2S, - Gas
Motility	+
Urease	V
Gelatin hydrolysis	+

Negative (-), Positive (+), Variable (V)

Table 4. Total sample, number and percentage of *S. marcescens* from all positive culture samples according to gender

Type of samples	No. of *S. marcescens*	No. of positive (%)	
		Male	Female
Eye swab	0	0(0%)	0 (0%)
Nasal swab	0	0 (0%)	0 (0%)
Wound swab	1	1 (100%)	0 (0%)
Burn swab	2	2 (100%)	0 (0%)
Catheter swab	0	0 (0%)	0 (0%)
Urine sample	7	3 (42.85)	4 (57.1)
Blood samples	0	0 (0%)	0 (0%)
Total	10		

Table 3. Biochemical tests and culture methods were used to obtain some human isolates of *S. marcescens*

Type of swab samples	Baghdad City / Al-Karkh		City of Baghdad / Al-Rusafa		Total
	Total number	Positive	Total number	Positive	
Eye	18 (28.57%)	0 (0%)	5 (5.75%)	0 (0%)	23
Nose	12 (19.05%)	0 (0%)	5 (5.75%)	0 (0%)	17
Wound	5 (7.94%)	0 (0%)	30 (34.48%)	1 (3.33%)	35
Burn	5 (7.94%)	1 (20%)	6 (6.90%)	1 (16.67%)	11
Catheter	5 (7.94%)	0 (0%)	7 (8.05%)	0 (0%)	12
Urine	15 (23.81%)	3 (20%)	25 (28.74%)	4 (16%)	40
Blood	3 (4.76%)	0 (0%)	9 (10.34%)	0 (0%)	12
Total	63	4 (6.35%)	87	6 (6.90%)	150
x^2	8.69 **	7.52 **	8.96 **	6.77 **	---

** (P≤0.01)

tracheal and wound swabs in 150 samples from different ornamental birds, pigeons, and local chickens raised in shops and houses in different areas of Baghdad, Kut, and Diyala. The two positive samples were isolated from tracheal in Baghdad from ornamental birds and pigeons, while the other two positive samples were isolated from wound swabs from local chickens in Baghdad and Kut governorate as in (Table 5).

The isolates of *S. marcescens* from human showed that all isolate were susceptible 100% to Azithromycin, Ciprofloxacin, Meropenem with low susceptibility to other antibiotics, in contrast, resistant 70% against Ceftazidime, Amoxicillin 60%, susceptible were reported in Amikacin and Trimethoprim 80% (Table 6, Fig. 12). The results agree with the study of earlier researchers (Asmaa et al 2017, Elsherbiny et al 2018, Sahar et al 2020, Omololu-Aso and Awoderu 2021) . The present studies are similar These results are unlike the results described by other authors, which reported the existence of carbapenemase-producing *Serratia* which encoding genes confer broad-spectrum antimicrobial resistance (Silva et al., 2015). *S. marcescens* is naturally resistant to Penicillin, macrolides, and first-

generation cephalosporin and, 92% of the strains are resistant to cefotaxime, but 99% are still susceptible to ceftazidime. Extended spectrum *β* -lactamases are produced and most *S. marcescens* strains (Mlynarczyk et al 2007).

The isolates of *S. marcescens* from birds showed that all isolate were susceptible (100%) to (Azithromycin, Ciprofloxacin, Meropenem), in contrast, absolute resistant (100%) against (Ceftazidime, Amoxicillin, and Trimethoprim). Increase in susceptibility was observed in Amikacin (75%), (Table 7, Fig. 13). The results in this study agree with Roya et al (2017). *S. marcescens* is still mostly susceptible to antibiotics, yet many human clinical isolates now demonstrate multiple antimicrobial resistances to critically important antibiotics. Most animals were given antibiotics, in particular amoxicillin or amoxicillin/clavulanic acid. In line with the prudent use of antibiotics, it is of utmost importance to distinguish infections (Keck et al 2020). Tekiner and Özpinar (2016) observed that involving multidrug resistance (MDR) bacteria in foods are still very scarce and need to be further investigated, and in dairy food, specifically, these MDR bacteria can originate from contaminated milk (bovine

Table 5. Total sample of *S. marcescens* isolates in various ornamental birds, pigeon and local chicken

Geographical locations	Various ornamental birds				Pigeon bird				Local chicken				Total
	Wound swab		Tracheal swab		Wound swab		Tracheal swab		Wound swab		Tracheal swab		
	Total No.	Positive	Total No.	Positive	Total No.	Positive	Total No.	Positive	Total No.	Positive	Total No.	Positive	
Baghdad	5	0	11	1 (9.09%)	3	0 (0%)	21	1 (4.76%)	24	1 (4.17)	23	0 (0%)	87
Kut	0	0 (0%)	0	0 (0%)	0	0 (0%)	3	0 (0%)	9	1 (11.1%)	20	0 (0%)	32
Diyala	2	0 (0%)	5	0 (0%)	0	0 (0%)	0	0 (0%)	6	0 (0%)	18	0 (0%)	31
Total	7	0 (0%)	16	1 (6.25%)	3	0 (0%)	24	1 (4.17%)	39	2 (5.13%)	61	0 (0%)	150
x^2	0.00 NS		4.05 *		0.00 NS		0.832 NS		4.93 *		0.00 NS		---

* (P≤0.015)

Table 6. Antibiotic susceptibility test for human to *S. marcescens* isolates

Antibiotic	Concentration (µg / disc)	Diameter of inhibition zones (mm)			Number of isolations			p-value
		Susceptible	Intermediate	Resistant	Susceptible (%)	Intermediate (%)	Resistant (%)	
Amoxicillin 25 µg	AM	≥18	14-17	≤13	2 (20%)	2 (20%)	6 (60%)	0.001 **
Azithromycin 15 µg	AZM	≥32	__	≤12	10 (100%)	0 (0%)	0 (0%)	0.001 **
Amikacin 10 µg	AK	≥17	15-16	≤14	8 (80%)	2 (20%)	0 (0%)	0.001 **
Ciprofloxacin 5 µg	CIP	≥40	22-25	≤21	10 (100%)	0 (0%)	0 (0%)	0.001 **
Meropenem 10 µg	MEM	≥40	20-22	≤19	10 (100%)	0 (0%)	0 (0%)	0.001 **
Ceftazidime 30 µg	CAZ	≥21	18-20	≤5	2 (20%)	1 (10%)	7 (70%)	0.001 **
Trimethoprim 10 µg	TMP	≥30	11-15	≤10	8 (80%)	2 (20%)	0 (0%)	0.001 **
p-value	-		-	-	0.001 **	0.039 *	0.001 **	---

* (P≤0.05), ** (P≤0.01)

Table 7. Antibiotic susceptibility test for birds to *S. marcescens* isolates

Antibiotic	Concentration (µg / disc)	Diameter of inhibition zones (mm)			Number of isolations			p-value
		Susceptible	Intermediate	Resistant	Susceptible (%)	Intermediate (%)	Resistant (%)	
Amoxicillin 25 µg	AM	≥18	14-17	≤6	0 (0%)	0 (0%)	4 (100%)	0.001 **
Azithromycin 15 µg	AZM	≥22	__	≤12	4 (100%)	0 (0%)	0 (0%)	0.001 **
Amikacin 10 µg	AK	≥17	15-16	≤14	3 (75%)	1 (25%)	0 (0%)	0.007 **
Ciprofloxacin 5 µg	CIP	≥35	22-25	≤21	4 (100%)	0 (0%)	0 (0%)	0.001 **
Meropenem 10 µg	MEM	≥40	20-22	≤19	4 (100%)	0 (0%)	0 (0%)	0.001 **
Ceftazidime 30 µg	CAZ	≥21	18-20	≤5	0 (0%)	0 (0%)	4 (100%)	0.001 **
Trimethoprim 10 µg	TMP	≥16	11-15	≤5	0 (0%)	0 (0%)	4 (100%)	0.001 **
P-value	-	-	-	-	0.001 **	0.502 NS	0.001 **	---

** (P≤0.01)

mastitis cases, for example), pasteurization problems, or even from post pasteurization. Other studies observed that many clinical isolates of *S. marcescens* were highly resistant to current antibiotics, and they attributed this resistance to R-plasmid that was transferable from *Serratia spp.* to *E. coli* recipients. That the decline in *S. marcescens* susceptibility might be in part due to conjugational transfer of resistance between various bacterial species (Thikra et al 2007).

CONCLUSIONS

S. marcescens was highly resistant to Ceftazidime, Amoxicillin and Trimethoprim, and MDR in birds would lead to a problem in healthcare in human and animals.

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Frequencies of Some Virulence Genes and Relationships to Antibiotic Resistance of MDR-*Pseudomonas aeruginosa* Isolated from Burn Clinical Samples

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Abstract: One hundred and twenty clinical samples which including *Pseudomonas aeruginosa, Proteus , Klebsiella , Ecoli , Staphylococcus , Streptococcus* and no growth were collected from different hospitals and private clinics in Baghdad province from 15th September 2020 to 15th December 2020. Antibiotic susceptibility test for the 50 isolates of *Pseudomonas aeruginosa*, were tested by disc diffusion method using 15 antibiotics and the percentage of resistance was maximum in Erythromycin (98%) and minimum mipenem (14%). *Pseudomonas* is still the most common source of infection in fire burn victims, followed by *S. aureus*. The antibiotic susceptibility test revealed that the most active compound against *P. aeruginosa* was Imipenem followed by Amikacin. The clinical *Pseudomonas* isolates in Iraq havea significant percentage of virulence associated genes and a high frequency of multidrug resistance . There was relationship between multidrug-resistant isolates and presence of MexR Operon.

Keywords: *Pseudomonas aeruginosa*, Virulence genes, Antimicrobial resistance

Nosocomial infections, often known as healthcare associated infections, occur in hospitals and healthcare settings. The patient gets exposed to germs from a variety of sources in the environment. Health care workers and other infected patients, as well as hospital garbage, serve as sources of infection (Khan et al 2017). Burns and lung infection in individuals with cystic fibrosis are two significant clinical problems produced by *Pseudomonas aeruginosa* and are the greatest cause of death in these individuals with a fatality rate of around 50%. It has several virulence components, such as las B elastase, a zinc metalloprotease encoded by the las B gene, that contribute to bacterial invasion and toxicity (Faraji et al 2016, Kany et al 2018). The neuraminidase gene (nan1) aids bacterial adhesion to epithelial cells (Lillehoj et al 2015). Exoenzyme S, which is encoded by the exo, helps *P. aeruginosa* to avoid phagocytosis and, as a result, kills the host cell (Saleeb et al 2017). Exotoxin A, which is expressed by the toxA gene, inhibits host cell protein synthesis (Aminia et al 2016).

MATERIAL AND METHODS

A total of 120 burn swab specimens were collected for this study from and private clinics. Swabs injected in 15 ml brain heart broth and incubated for overnight at 37°C. All isolated were identified depending on this morphological, biochemical test, RT-PCR and selective media (Cetrimide agar media). All specimens were grown on Cetrimide agar and aerobically incubated overnight at 37°C.

Biochemical tests positive oxidase, triple sugar iron (TSI) agar reaction of alkaline over no transition, growth at 42° C, and development of bright - blue to blue - green diffusible pigment on Mueller - Hinton agar were conducted. All media had been prepared according to the instructions of the manufacturing company Sterilization of culture media and solutions were achieved by autoclaving at 121°C⁄15 minutes (Brown and Smith 2017). After sterilization, 20 percent sterile urea solution was added to the urea agar , and 5 percent fresh human blood was added to the blood agar base., then media poured on petri dish or plane tubes, and incubated at 37°C for 24 hours to ensure their sterility. Storage of sterile media in the refrigerator to prevent dehydration (Cappuccino and Welsh 2018). The pH was adjusted to 7.0 and sterilized by autoclaving (Brown and Smith 2017).

Antimicrobial susceptibility testing: The disc diffusion method was used to assess antibiotic sensitivity of isolates. Briefly, 20 mL Mueller Hinton agar was produced aseptically and distributed into a Petri dish, where it solidified. The Mueller Hilton agar was seeded with a standardized 0.5 McFarland inoculum size of test bacteria (106 CFU/mL).The antibiotic disc was placed aseptically on the Mueller Hinton agar surface and allowed to prediffusion for 30 minutes.The experiment was carried out in triplicate, with a control disc containing no antibiotics. After a 24hour incubation period at 37°C, the inhibitory zone diameters were assessed. (Angus et al 2017)

DNA extraction and PCR analysis of bacterial isolates: Bacterial genomic DNAs were extracted using the Wizard Genomic DNA Purification Kit according to the manufacturer's protocols. The DNAs were stored at -20 ℃ until used for RT-PCR reaction.

RESULTS AND DISCUSSION

The biochemical tests for *P. aeruginosa* characteristics revealed that all isolates were oxidase and catalase positive. All isolates were had given negative result for indole, methyl red- Voges Proskauer and urease test .Citrate assimilation and motility positive. *P. aeruginosa* was most frequently isolate (41.6%), followed by *Staphylococcus aureus* (23.33 %), *Klebsiella* spp (10.83 %), *Proteus* spp. (10 ,*Escherichia coli* (10%) and *Streptococcus* (4.17 %). The fifteen isolates were tested for susceptibility using different antibiotics according to the CLSI, 2017 guidelines (Imipenem, Ciprofloxacin, Norfloxacin, Ampicillin, Streptomycin, Aztreonam, Levofloxacin, Gentamicin, Amikacin, Tobramycin, Piperacillin, Chloramphenicol, Tetracycline, Erythromycinand Trimethoprim). *Pseudomonas aeruginosa* isolates showed varying levels of resistance to antibiotic and

the percentage of resistant for Tobramycin was 48,14, 98, 52,30,30 32 and 96.67 Imipenem, Erythromycin. Ciprofloxacin, Gentamicin, Streptomycin, Amikacin. Piperacillin and Aztreonam. (96.67%) and the 33% were negative (Fig. 1). The toxA gene is encoded to exotoxin, detection toxA gene by RT-PCR revealed positive percentage were (76.67%) and (23.33%) were RT-PCR negative (Fig. 2). The 76.67% isolates of *P. aeruginosa* were RT-PCR positive for exoS gene and 23.33% RT-PCR negative (Fig. 3). The 63.33% isolates of *P. aeruginosa* were RT-PCR positive for Hemolysin phospholipase gene and 63.67% RT-PCR negative (Fig. 4). The 66.67% isolates of *P. aeruginosa* were RT-PCR positive for MexR Operon and 33.33% RT-PCR negative (Fig. 5).

The most common cause of infection in fire burn patients in our setting was *Pseudomonas*, followed by *S. aureus*. Imipenem was most effective antibiotic against *Pseudomonas aeruginosa* in the antibiotic susceptibility test, followed by Amikacin. The percentage of Imepenem resistant patients in this study was 14 percent and in South Korea where the percentage was 35.8 percent (Wi et al 2017).

The result of exotoxin, RT-PCR detection of the tox A
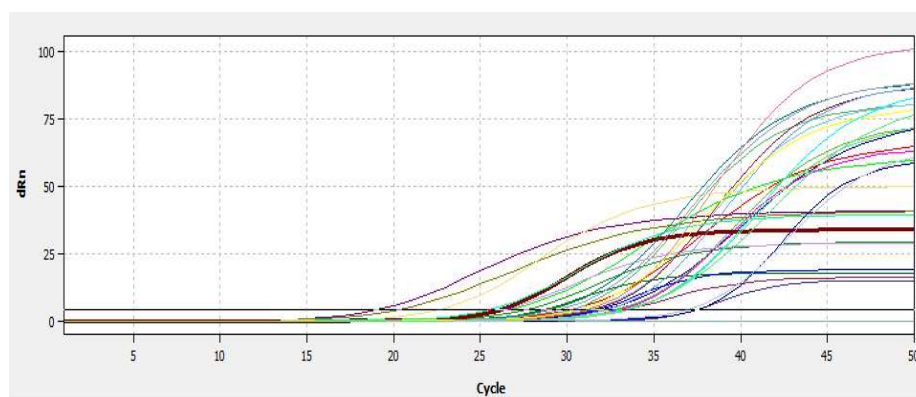
Fig. 1. Identification of *Pseudomonas eurogenosa* by Real-Time
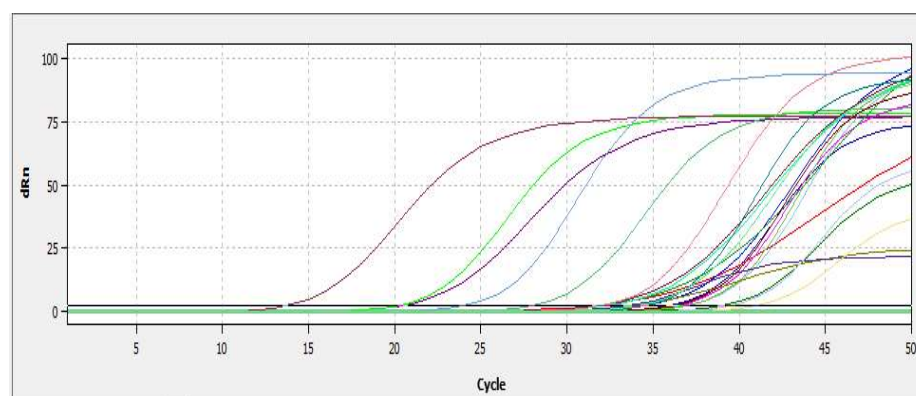
Fig. 2. Identification of Exo A gene by Real-Time PCR
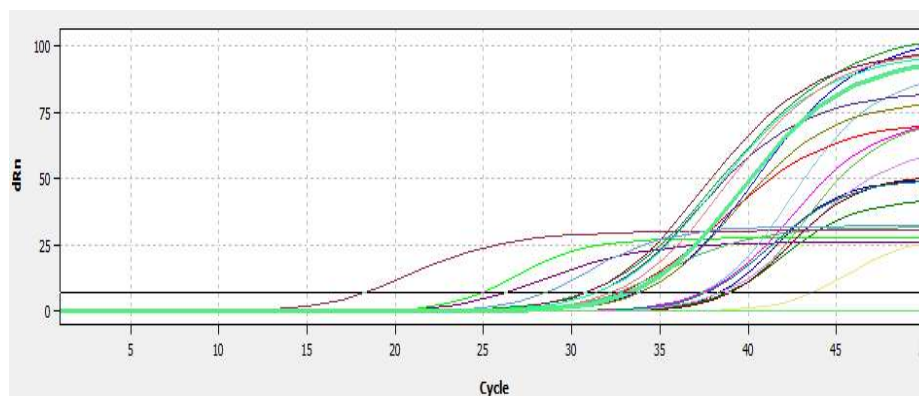
Fig. 3. Identification of Exo S gene by Real-Time PCR
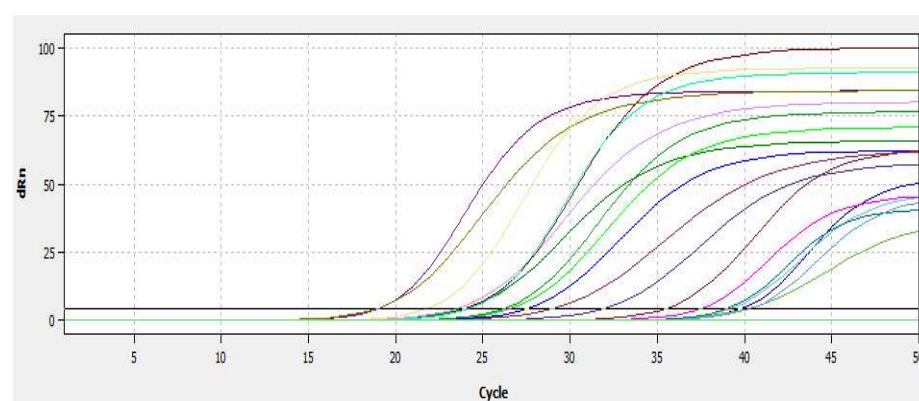
Fig. 4. Identification of Hemolysin Phospholipase gene by Real-Time PCR
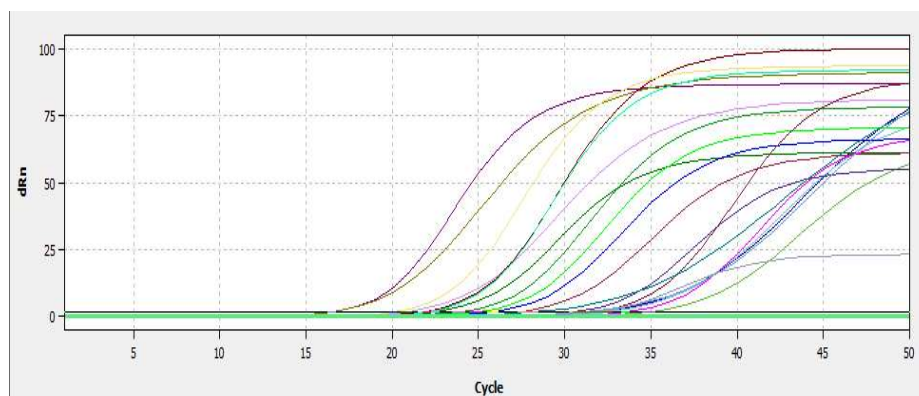
Fig. 5. Identification of Mex R gene by Real-Time PCR

gene revealed a positive percentage of 23 (76.67%). There was a link between the presence of MexR Operon and multidrug-resistant isolates. *Pseudomonas aeruginosa* isolates showed varying levels of resistance to each antibiotic and the percentage of resistance was 14% for Imipenem and 98% for Erythromycin. Ciprofloxacin and Gentamicin showed 52%.

CONCLUSION

Molecular diagnostic techniques(RT-PCR) are widely suggested for quick diagnosis of nosocomial infections, which are a major source of morbidity and mortality in hospitals. To stop the transmission of nosocomial diseases, all healthcare staff should be educated on infection control on a regular basis. Sterilization in hospitals should be at the

highest level possible, especially in the burn unit. *Pseudomonas aeruginosa* isolates from the hospital environment should be tested for virulence genes.

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Detection of Human Papillomavirus in Breast Cancer Patients in Kirkuk City

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Abstract: Breast cancer is the most frequent cancer among women, and also causes the greatest number of cancer-related death among women all over the world. The involvement of viruses in certain breast tumors and cell lines has been described in different studies. Viruses, such as specific types of human papillomaviruses (HPV) emerged as major causal factors of some human cancers. Human papillomaviruses are present in more than 99% of cervical cancer cases. The aim of the present study was to identify the role of human papilloma virus in the development of breast cancer. The present study was carried out in Kirkuk city from of 10th September 2019 to 15th of March 2020. The number of breast cancer women under study were 130 women whose ages were between 25-75 years old. The control group matched to the breast cancer patients studied, included 20 women diagnosed as having benign breast lesions with between 25-75 years. These women presented to Kirkuk Oncology Center and Azadi Teaching Hospital. The highest rate of women with breast cancer and HPV infection was within the age group 40-49 year (36.4 %), only one women with benign breast lesion was infected with HPV and she was within the age group 50-59 year. The infection with HPV was higher rate in women with breast cancer than those with benign breast lesion (33.8% and 5% respectively). The highest rate of women with breast cancer who were infected with HPV genotype 16 (43.2%), only one women with benign breast lesion was infected with HPV, the genotype was 16 (5.3%). The difference in the level of IL-19 between women with breast cancer and HPV infection and those without infection was statistically non-significant. Regarding women with benign breast lesion, non-significant difference was in IL-19 was between those with HPV infection and those without infection.

Keywords: Breast cancer, HPV, IL-19

Breast cancer is the most prevalent cancer in women, and it is also the main cause of cancer-related death in women worldwide. It is associated for about 15% of all cancer deaths (WHO 2018). Following the discovery of an elevated risk of breast cancer in women with a positive family history of breast cancer, the cases are divided into hereditary (family) and sporadic instances. Obesity and other risk factors are linked to the development of breast cancer (Badve 2016)

Viruses have been implicated in the development of certain breast cancers and cell lines, according to various researches. Viruses, such as some forms of human papillomaviruses (HPV), have been identified as important causes of human cancer. It has been suggested that the presence of HPV-16 or HPV-18 in the breast is linked to the development of a malignant phenotype. (Traylen 2016) Human papilloma virus is associated with malignant and benign lesions in the anogenital tract of both males and females. (Araujo et al 2016) Human papillomavirus (HPV) is in more than 99 % of cervical cancer cases and is one of the leading causes of penile, vulvar, vaginal, and anal carcinomas, as well as a growing number of head and neck squamous cell carcinomas (Yan et al 2016). IL-19 is a cytokine that belongs to the IL-10 family, which also contains IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26. Monocytes, macrophages, B cells, endothelium and epithelial cells, as well as immune and non-immune cells, produce this cytokine (Sabat, 2010). Interleukin (IL)-19 has been demonstrated to cause mice monocytes to produce tumor necrosis factor (TNF)- and IL-6, while increasing IL-10 and decreasing TNF-in humans (Jordan et al 2005) .

MATERIAL AND METHODS

A cross sectional study was carried out in Kirkuk city from of 10th September 2019 to 15th of March 2020. The number of breast cancer women under study were 130 women whose ages were between 25-75 years old. These patients admitted to Kirkuk Oncology Center and Azadi Teaching Hospital. An interview was carried out with these patients using questionnaire form designed by the investigator. The control group who were matched to the breast cancer patients studied, included 20 women diagnosed as having benign breast lesions and their ages were between 25-75 years old from Kirkuk Oncology Center and Azadi Teaching Hospital. Blood samples were taken from breast cancer and benign breast lesions patients and were examined by immunological methods, enzyme linked immuno sorbent assay for detection

interleukin-19 (IL-19). The blood samples were examined by molecular (byReal time PCR which include amplification DNA of human papilloma virus based on the specific primers. Seven and half ml of blood from 150 women was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study. Blood samples were placed into two sterile test tubes, in one of them 2.5 ml of blood was put in test tube containing anticoagulant ethylene diamine tetra acetic acid (EDTA) and used for DNA extraction of HPV. The second part of sample was 5ml placed in plain tubes left for 30 minutes at 37 °C then were centrifuged at 3000 round per minute (rpm) for 15 minutes then the clot was removed and the remain re- centrifuged at 3000 rpm for 10 min and the obtained sera were then aspirated using automatic micropipette and transferred into two clean test tubes, for serological tests. Label was fixed on each test tube which then stored in deep freeze at -20°C for late serological testing for determination the level of IL-19 by using ELISA technique. The 50 fresh breast tissue samples were collected from the same women undergoing breast surgery at Azadi Teaching General Hospital, (30 samples from breast cancer patient and 20 samples from benign breast lesion patients). Samples were placed into tubes containing DNA shield at concentration of 300µl stored at -20°C for DNA extraction of HPV. For DNA extraction, kit from Zymo/USA Company, for molecular detection of HPV by Real Time PCR using AmpliSens *HPV* HCR genotype-FRT PCR kit. Detection of IL-19 was done by using ELISA kits.

Statistical analysis: Computerized statistically analysis was performed using 11 statistic program. Comparison was carried out using; Chi-square (X2), T-Test probability and F-ratio (P value).

RESULTS AND DISCUSSION

A total of 150 breast cancer women and benign breast lesion women were examined, their age ranged between 25-75 years old were investigated for prevalence of HPV and IL-19. The rate of breast cancer women with HPV was higher than that of the benign breast lesion women (33.8% and 5% respectively) and was statistically highly significant (Table 1). The sample distribution according to the age showed that the highest rate of women with breast cancer were within the age group 40-49 (33.1%) followed by those within the age group 50-59 (30.8%), then 60-69 years 36(27.7%), while the highest rate of women with benign breast lesions were within the age group 50-59 years (55%) followed by those within the age group 60-69 years (25%). The women with breast cancer and those with benign breast lesion indicated no significant difference (Table 2). The highest rate of women with breast cancer and HPV infection was within the age

group 40-49 year (36.4 %), followed by those within the age group 50-59 year and 60-69 year (27.3% for each group). Only one women with benign breast lesion was infected with HPV and she was within the age group 50-59 year (Table 3).

The present study showed that highest rate of women with breast cancer was from rural areas and highest rate of women with benign breast lesion was from urban areas. The

Table 1. Distribution of HPV infection in the study groups

HPV infection	Breast cancer		Benign breast lesion	
	No.	%	No.	%
Positive	44	33.80	1	5
Negative	86	66.20	19	95
Total	130	100	20	100
X^2	13.56		16.2	
P value	0.00026		0.000057	
X^2		6.686		
P value		0.009		

Table 2. Distribution of the study groups according to age

Age (Year)	Breast cancer		Benign breast lesion	
	No.	%	No.	%
≤39 years	6	4.6	2	10
40-49 years	43	33.1	2	10
50-59 years	40	30.8	11	55
60-69 years	36	27.7	5	25
≥70 years	5	3.8	0	0
Total	130	100.0	20	100
X^2	54.846		10.8	
P value	0.0003		0.013	
X^2		7.82		
P value		0.098		

Table 3. Distribution of study groups with HPV infection according to age

Age (Year)	Breast cancer			
	Positive HPV		Negative HPV	
	No.	%	No.	%
≤ 39	3	6.80	3	3.50
40-49	16	36.40	27	31.40
50-59	12	27.30	28	32.60
60-69	12	27.30	24	27.90
≥70	1	2.30	4	4.70
Total	44	100	86	100
X^2		1.613		
P value		0.8		

difference was statistically highly significant between the two groups of study (Table 4). The highest rate of women with breast cancer who were infected with HPV had the genotype 16 (43.2%), followed by genotype 18 (29.5%), genotype 52(11.4%), genotype 56 (9.1%) and combination of genotype 16 and 18 (6.8%) (Fig. 1). Only one women with benign breast lesion was infected with HPV, the genotype 16 (5.3%). The difference in the level of IL-19 between women with breast cancer and HPV infection and those without infection was statistically non-significant .Regarding women with benign breast lesion, non-significant difference was between level of IL-19 between those with HPV infection and those without infection (Table 5).

Breast cancer is the most prevalent cancer in women, and it is also the leading cause of cancer-related death in women worldwide. It is responsible for about 15% of all cancer deaths (WHO 2016). The current study discovered that women with breast cancer had a greater rate of HPV infection than those with benign breast lesions.. HPV and breast cancer have been linked in several studies, with prevalence rates ranging from 4 to 86%**. (Sigarood et al 2012, Van Der and Arens 2015, Akil et al 2014). However, other studies have not detected HPV in breast cancer women. (Akhter et al 2014, Hedau et al 2014, Hachana et al 2010).   Many research agreed with the results of the current study: Sulaiman, (2017) and AL-Mansour et al (2012) proved a significant relation between HPV and breast cancer in Iraq. , in Australia, HPV was detected in 23% (Heng et al 2012), in Brazil 24.8%, (Damin et al 2004), ran 25.9% (Sigarood et al 2012), Japan 21% (Khan et al, 2008) and USA 35% (Van Der 2015). In this study, the age group 40-49 had the highest rate of breast cancer followed by those within the age group 50-59 and 60-69 years. Sulaiman, (2017) and Aswed (2018)
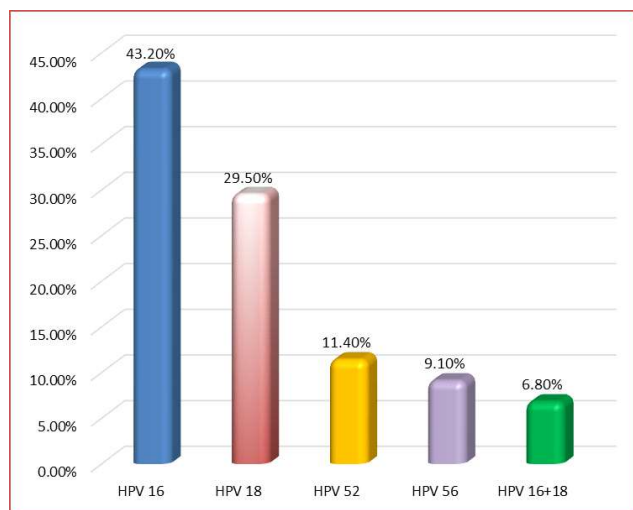
Fig. 1. Frequency of HPV genotype among women with breast cancer

Table 4. Distribution of the study groups according to residence

Residence	Breast cancer		Benign breast lesion	
	No.	%	No.	%
Rural	71	54.60	3	15
Urban	59	45.40	17	85
Total	130	100	20	100
X²	1.108		9.8	
P value	0.29		0.002	
X²		10.883		
P value		0.001		

Table 5. Relation of HPV infection with IL-19 among study groups (Mean±SD)

Study group	HPV state		P value
	Positive	Negative	
Breast cancer	41.91±16.19	39.52±26.24	> 0.05
Benign breast lesion	39.1± 0.00	64.91±18.7p	>0.05

showed the age group 40-49 years represent the highest rate of women with breast cancer. Another study revealed that approximately 81% of breast cancers occurred in women over the age of 50 year (Jemal et al 2011).

Women in the 40-49 year age group had the highest rate of breast cancer and HPV infection, followed by those in the 50-59 year and 60-69 year age groups. The distinction was not statistically significant. According to a few published papers, the mean age of breast cancer in Iranian women was between 47.95 to 54.6 years. (Shahidsales 2014, Noghabaei 2005) In contrast, according to research in 2008 for Syrian women, the age range was 26 to 66 years (Akil et al 2014). Also, in another study, the age range of HPV positive patients were from 33 to 73 years with a viral prevalence of 11.8% (Mofrad et al 2020) but in a study in Venezuela the age range was 51 to 60 years with a viral prevalence of 41.67%. (Jemal et al 2011) In Turkey, the analysis conducted to determine whether the HPV-DNA positivity was different according to the age of individuals included in the study revealed that HPV-DNA positivity was detected in 1 of 36 (2.8%) patients aged 50 or less and in 15.9% patients aged 50 or older (Saeed et al 2018). The present study revealed that the highest rate of women with breast cancer was from rural areas 54.60%. Abbas (2019) reported that women from rural areas had the greatest risk of breast cancer. Breast cancer was shown to be the leading cause of morbidity and mortality among Indian women in a study on breast cancer epidemiology. Breast cancer development was also influenced by activities among urban and rural women. (Shresstha et al 2017).

The genotype 16 was shown to be the most common in women with breast cancer who were infected with HPV, followed by genotype 18, genotype 52, and genotype 56. Ghaffari et al (2018) stated that the most common HPV genotypes were 16, 18, 35, and 31. In Asia, 20% of high-risk HPV in breast tumors are from Japanese women and HPV-16 was the most frequent HPV genotype that was integrated into the host genome with a low viral load. (Khan et al 2008). In Mexico, HPV was in 29.4% of women with breast cancer, being HPV-16 the most prevalent genotype. (Cantu et al 2009). In addition, the most commonly detected HPV types in breast cancer tissues in the UK population were HPV 39, 18 and 45, whereas the most commonly detected HPV types in the Qatari population were HPV 16, 35, and 58. (Salman et al 2017) According to Iranian study, the HPV genotypes 16 and 18 with the accumulated prevalence of 53.34% in breast cancer patients were the most predominant. (Sigarood et al, 2012). HPV prevalence in women with breast cancer were 41.6 % in Venezuela and 40 % in Mexico (Fernandes et al 2012, Herrera et al 2013). The difference in IL-19 levels between women with breast cancer and HPV infection and those without infection was statistically non-significant in this investigation. Interleukin-19 is found within the IL-10 family gene cluster: IL-10, -19, -20, -22, -24, -26, -28, and -29 (Dumoutier et al 2001, Commins and Borish 2008) Interleukin-19 functions as a monomer and is structurally similar to IL-10 (Zdanov 2004). Interleukin-19 is produced largely by macrophages, but also by B cells and non-immune cells such as epithelial cells, endothelial cells, skin keratinocytes, and fetal membranes to a lesser level (Menton et al 2006). IL-19 has been shown to have proinflammatory properties in numerous studies (Gallagher 2010). In contrast, IL-19 has an anti-inflammatory property identified in inflammatory bowel disease and vascular inflammatory diseases (Azuma et al 2010, Cuneo et al 2010) The presence of interleukin-19 mRNA in Epstein-Barr virus-transformed lymphocytes suggests that viral infection can cause IL-19 production in some cells (Wolk et al 2005).

CONCLUSIONS

The study concluded that HPV may play a role in the development of breast cancer and may be an etiology or it may induce immunosuppression that enhance the development of breast cancer. The highest rate of infection with HPV in women with breast cancer that those with benign breast lesion. The relation was statistically highly significant, also the highest rate of women with breast cancer who were infected with HPV had the genotype 16 .There was highest rate of women with breast cancer within the age group 40-49year ,while the highest rate of women with benign breast lesions were within the age group 50-59 years.

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Antibacterial Activity of Silver Nano Particles against *Klebsiella pneumonia* Isolated from Urinary Tract Infections

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Abstract: One hundred urine specimens collected from AL- Yarmouk Hospitals between October and December 2020. All the isolates were cultured on macConkey agar medium. Forty-two isolates were shown to be *K. pneumoniae* after performing further biochemical tests and microscopic examination then were identified by VITEK2 compact system, which were chosen as dominant isolates to completed study. All isolates were run for antibiotic susceptibility test, ciprofloxacin was shown to be the most effective antibiotic in the study against the bacterial isolates. The bacterial isolates were screened for biofilm production by using Congo-red agar method and Micro-titerplate assay. The results showed 15 (35.7%) separates as strong, 17 (40.5%) separates as moderate, 7 (16.7%) separates as weak, 3 (7.1%) separates as non-biofilm makers. The isolates that were ciprofloxacin resistant were tested to determine the minimum inhibitory concentration of ciprofloxacin against it by microtiter plate methodand MIC was 128 µg/ml. The green synthesis of silver nanoparticles from *A. vera* show first indicator of nanoparticles formation was the color change from milky color to dark Gray. Result of optimization of the synthesis variables showed that optimum conditions of the green synthesis include: 1M silver ion concentration, reaction time of 24 hr, and 100oC as plant extraction temperature. AgNPs was characterized with UV–Vis Spectral Analysis, Atomic Force Microscopy (AFM) that showed the average size of AgNPs was 31 nm, Also Field Emission Scanning Electron Microscopy (FE-SEM) was used to examine these nanoparticles. The result of toxic effect of sliver nanoparticle by agar diffusion methods showed irregular inhibition zone that the higher inhibition was 30 mm against *K. pneumoniae.* The minimal inhibitory concentration of sliver nanoparticles was determined, the results showed that the MIC was 0.125 mg/ml.Treatment effect of silver nanoparticles on planktonic and mature *K. pneumonia* biofilm showed that the MIC concentration 0.5 mg/ml is needed to treatment mature biofilm is higher than MIC concentration 0.125 mg/ml is needed to prevent biofilm formation.

Keywords: Antibacterial activity, Silver nano particles, *Klebsiella pneumonia*

Urinary tract infections, including community-acquired and healthcare-associated infections, are among the most frequent bacterial infections in humans. It is the second most frequent healthcare-associated infection and the most prevalent nonsurgical nosocomial infection in postoperative patients (Gastmeier 1998). UTI is a major factor of antibiotic consumption globally since it is the second most prevalent reason for empirical antibiotic treatment (Rané and Dasgupta 2013). Urine tract infection is one of the most common diseases caused by *K. pneumoniae* after *Escherichia coli* (Chiu et al 2013, Guo et al 2016). *K. pneumoniae* bacteria possess many virulence factors including production of polysaccharide, capsule, serum resistance, production of iron siderophore, production of enterotoxin and urea and thus this bacterium with high virulent in addition to developing its resistance to many antibiotics (Dubey et al 2013). *K. pneumoniae* is one of the most important genera of the Enterobacteraceae familyand is characterized by being Gram-negative bacilli, nonmotile, and lactose fermented, non spore forming, appear under the microscope are made up of thick edges, as the edges are curved outward and have rounded ends and have a capsule that increases its pathogenicity (Bensen 2001, Braun et al 2004). One major factor contributing to antibiotics resistance

is the ability of the *K. pneumoniae* to form biofilms on biotic and abiotic surfaces including catheters and other, clinically this issue is very importance due to the association between biofilm formation and the resistance to antibiotic treatment (Chen et al 2014). Biofilm is an organized collection of bacteria that live inside an extracellular polymeric matrix that they generate and that is permanently adhered to fetish or living surface and will not be removed unless promptly rinsed. These adhering cells get encased in a slimy extracellular matrix made up of extracellular polymeric substances (EPSs). The EPS components are generally a polymeric agglomeration of extracellular polysaccharides, proteins, lipids, and DNA produced by the cells inside the biofilm. Because they contain a three-dimensional structure and depict a microorganism's communal lifestyle, they have been referred to as "Cities for microbes" (López et al 2010). Silver nanoparticles (AgNPs) have been applied for medical purposes since ancient times due to their bactericidal properties, and their highly reactive surfaces imply their potential role in antimicrobial applications (Salem et al 2015). AgNPs' microbial killing activity could be mediated by a variety of antimicrobial processes, including attachment to the bacterial membrane and bacterial cell wall, penetration

into the cell and destruction of macromolecules and intracellular organelles, and generation of oxidative stress (Dakal et al 2016).

MATERIAL AND METHODS

Collection and isolation of specimens: Between October 2020 and December 2020, one hundred urine specimens were collected from patients and maintained in sterile containers at AL- Yarmuk hospital in Baghdad. Pink mucoid colonies on MacConkey agar plates were reinoculated on another MacConkey agar plate and cultured for 24 hours at 37°C. Colonies formed as a result of mucoid lactose fermentation, were purified, and utilized for identification tests.

Identification

Macroscopic observations (Cultural characteristics): Morphological features of colonies on culture medium were observed, as well as lactose fermentation on MacConkey agar, consistency, colony elevation and diameter, odor, non-hemolysis on blood agar, and other characteristics (Atlas et al 1995)

Microscopic examination: Gram stain was used to identify bacterial slide smears' reaction to stain, cell morphology, and cell arrangement. A tiny quantity of bacterial suspension was collected on a glass slide by combining a colony of tested bacteria with a drop of distilled water, allowing it to dry in the air, then heating it to fix it to the glass, making a thin smear, gram staining and examining it with a light microscope at x100 magnification (Atlas et al 1995)

Biochemical tests of *Klebsiella* spp.: Biochemical tests were performed for further identification of *Klebsiella* spp. at the levels of species and subspecies following Bergey's Manual of Systematic Bacteriology, 2nd edition (Atlas et al 1995, Macfaddin 2000, Garrity 2005).

Antibiotic susceptibility testing: The antibiotic sensitivity test for 6 various antibiotic was performed according to Kirby-Bauer disc diffusion method as well-defined by the World Health Organization (WHO 2003).

Determination of minimal inhibitory concentration of ciprofloxacin: Micro-titer plates of 96 well method was used in this study (Wiegand et al 2008).

Synthesis of silver nanoparticles (AgNPs) by green method: At room temperature (25-30) C, 5 ml of A. vera extract was mixed to 45 ml of 1 mM AgNo3 for the production of the Silver Nanoparticles. The color shift after 24 hours indicates the production of silver nanoparticles (Kumar et al 2016).

Characterization of synthesized silver nanoparticle: The reaction of silver nitrate solution with aloin derivative extracted from *Aloe vera* as the capping and reducing agent

was optically measured using shimadzu UV-Visible spectrophotometer. Shape and size of the synthesized silver nanoparticles were studied by using Atomic Force Microscope, Field Emission Scanning Electron Microscope.

Antibacterial activity of AgNPs: To determine if the produced AgNPs are toxic to bacteria, the agar dilution method was used, with a McFarland tube filled with No. 0.5 turbidity standard solution. Petri dishes were grown with diluted bacterial species in culture media in the presence of 100 µL of AgNPs suspension at varying concentrations (1,2,4,8 and16 mg/ml) in wells for 24 hours at 37 0C. It was compared to Petri dishes grown with bacterial species that did not contain AgNPs to determine their antibacterial impact (Padil et al 2013).

RESULTS AND DISCUSSION

Sample collection, isolation, identification, cultural characteristics and biochemical tests: All urine samples were inoculated by sterile loop immediately on blood agar plate and MacConkey agar plate for 24 hrs at 37°C for primary identification. Many types of bacterial isolate appeared on MacConkey agar. The positive result was identified by depending on the colony morphology such as (colour, shape, texture and size) and identified according to gram stain, lactose or non-lactose fermenter on MacConkey agar plate and according to standard biochemical tests such as motility, IMVIC, oxidase, catalase and TSI. The morphological examination of colonies of *Klebsiella* isolates that grown on MacConkey agar medium showed large, round, pink and mucoid lactose fermenting colonies. Out of one hundred specimens, the genus *Klebsiella* formed 40% (42 isolates) in which 60.0% other gram-negative bacteria. All of these isolates identified as K. pneumoniae.

Antibiotic susceptibility: All isolates were evaluated for antimicrobial susceptibility by the Kirby-Bauer's Discs Diffusion Method recommended by the clinical and laboratory standards institute guidelines (CLSI 2020). Susceptibility was tested to all isolates of *K. pneumoniae*. toward 6 antibiotics: to ciprofloxacin, trimethoprim, cefotaxime gentamicin and amoxicillin-clavulanic acid (augmentin) and erythromycin. This study concluded that 96% of clinical isolates of *K. pneumoniae* were resistance to trimethoprim and amoxicillin-clavulanic acid (augmentin) and 90% were resistance to cefotaxime, whereas 63% of isolates were resistance to gentamicin and erythromycin. In this study the ciprofloxacin was the most active antibiotic on *K. pneumoniae* were only 36% of clinical isolates of *K. pneumoniae* were resistance to ciprofloxacin. The result showed that all the isolates were multi-resistance for antibiotics. The trimethoprim and augmentin had the highest resistance followed, cefotaxime, gentamicin and

erythromycin but was sensitive to ciproofloxacin. Due this reason the increase in the bacterial resistance of some bacteria to antibiotics is a serious problem and a limitation to the future usage and effectiveness of these agents. The result was similar to Mohammad and Ismail (2020).

Determination of minimal inhibitory concentration of ciprofloxacin: The isolates which were ciprofloxacin resistant was tested to determine the ciprofloxacin's minimum inhibitory concentration by using the micro-titer plates of 96 well method.   The minimal inhibitory concentration of ciprofloxacin against the isolate was 128 µg/ml (Fig. 1). Gram-negative bacilli, particularly *K. pneumoniae*, are major problematic organisms that have the ability to resist different types of antibiotics, including fluoroquinolones (Thabit et al 2020).

Synthesis of silver nanoparticles (AgNPs) by green method: The result revealed that aqueous extracts (red color) of *A. vera* acts as a good reducing and stabilizing agent to formation silver nanoparticles (Hashoosh et al 2014). The first indicator of nanoparticles formation was the color change from milky color to dark Gray (Fig. 2) and overnight was the time required for the formation of silver nanoparticles at room temperature.

Characterization of Silver Nanoparticles

UV–Vis spectral analysis: Development of silver nanoparticles by *A. vera* extract at optimum conditions of green synthesis process was studied via UV-Vis spectroscopy of the reaction solution. Appearing the sharp peaks at visible region is an evidence for the formation of silver nanoparticles naturally with particle diameters of 10-30 nm (Vilchis-Nestor et al 2008). Silver nanoparticles synthesized at optimum conditions exhibit surface plasmon resonance spectra at 460 nm with brownish-yellow color. Okafor et al (2013) also reported similar spectrae.

Atomic force microscope (AFM): Atomic force microscope (AFM) was used to determine the surface morphology and topography of samples synthesis of silver and also to detecting the average diameter of samples. AFM provides a two- and three-dimensional imaging of a nanoparticle's surface at the atomic level (Fadhil and Hadi 2015). The results obtained in this study showed the average particle size of silver synthesized at these conditions is 31 nm (Fig. 5).

Antibacterial activity of AgNPs: In order to estimate that prepared AgNPs are having toxic effect against bacteria Agar dilution method was used. Petri dishes were cultured with diluted bacterial species in culture medium in the presence of 100 µL of AgNPs suspension with First, to know if the prepared AgNPs are having toxic effect against bacteria Agar dilution method was used, Petri dishes were cultured with diluted bacterial species in culture medium in the presence
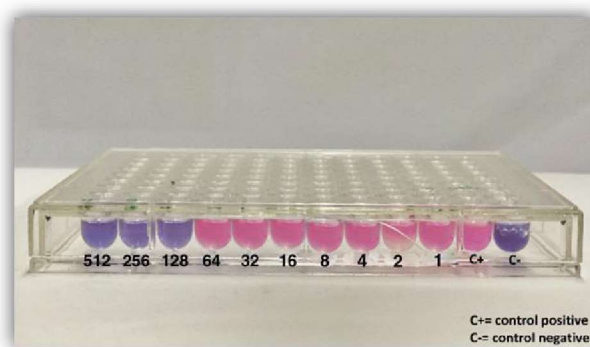
Fig. 1. The MIC of ciprofloxacin (128 µg/ml)
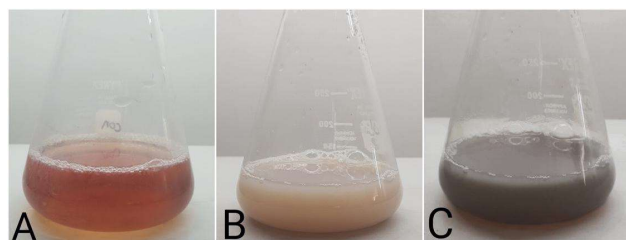
Fig. 2. Formation of silver nanoparticles, A) *A. vera* Aqueous extracts B) color change immediately after added silver nitrate to Aqueous extracts C) color Change after 24 hr indicates the formation of silver nanoparticles
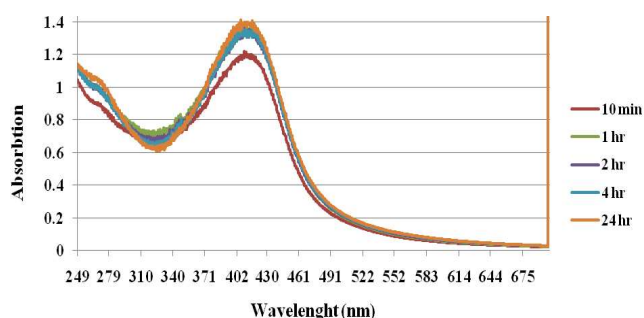
Fig. 3. UV-Vis spectra responsible to the development of silver nanoparticles by *A. vera* extract at optimum conditions
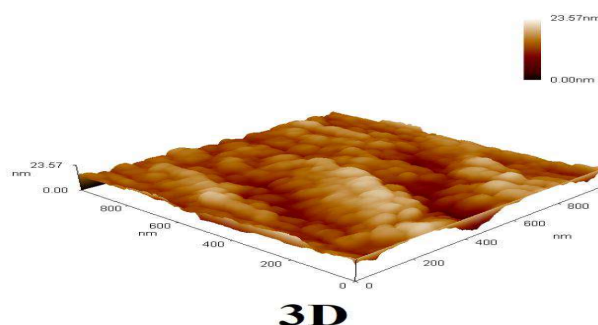
Fig. 4. Atomic force microscopy of AgNps 3D topological
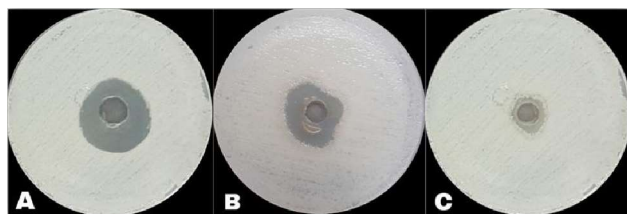
Fig. 6. Inhibition Zone of AgNPs by agar-well diffusion testing A) 16 mg/mlconcentration B) 8 mg/mlconcentrations C) 2 mg/mlconcentrations

of 100 µL of AgNPs suspension with different concentrations (1, 2, 4, 8 and 16 1 mg/ml) in wells for 24 h incubation period at 37 0C. The result showed that the higher inhibition was 30 mm against *K. pneumoniae* Figure 6. But the inhibition shows irregular zone that is due to different diffusion of nanoparticles through the agar media. This result lined with Owaid et al (2018). Exposure of the membrane to silver nanoparticles' reduces the membrane components (proteins, polysaccharides, lipid, phospholipid, nucleic acid), causing the membrane to gradually break down (Cheng et al 2014, Haider et al 2018). Gram negative biofilm bacteria was more susceptible to SNPsand thought that SNPs cause pits in gram negative cell wall leading to increase permeability of cell membrane and inhibit respiratory chain lead to kill treated bacterial (Siegel et al 2007, Paredes et al 2014). Marini et al (2007) proposed that Ag+ ions interact with the thiol groups in bacteria proteins, affecting the replication of DNA.

CONCLUSIONS

Most of the *K. pneumonia* bacteria isolates were resistant to a wide range of tested antibiotics and The *A. vera* were good reducing and capping agent for green silver nanoparticles synthesis. Silver ion concentration, synthesis reaction time, temperature of plant extraction had a significant effect on the silver nanoparticles size. The biosynthesized AgNPs was more effective than the most common antibiotics.

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Histopathological Study of Brain by Different Doses of Topiramate Intake in Male Rats

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Abstract: The current study was designed to scrutinized the detrimental effects of increasing intake of topiramate on brain of laboratory rats. Thirty two of male rats were randomly divided into two main groups. The first group control group was not treated with topiramate but received distilled water and feed, the main second group was divided into three subgroups which received a different doses of topiramate (20, 40 and 80 mg/kg body weight /day) for two months respectively. Samples of brains of all treated groups were kept for preparation of histological sections. The present results indicated that topiramate caused many histopathological changes in brain resulted from treatment at different doses. These included hemorrhage in brain tissue, vacuolation of neurons, congestion of blood vessels, aggregation of neutrophils around congested blood vessels, perivascular edema, proliferation of astrocytes in white matter of cerebellum and appearance of pair nuclei arrangement surrounded by clear space. Varied histological damages were occurred in two other groups which high doses of topiramate such as aggregation of microglial cells, severe hemorrhage and congestion, occurrence of vascular prominent duct with aggregation of inflammatory cells in the lumen, degeneration of neurons by dark blue stained and infiltration of neutrophils in blood vessels.

Keywords: Topiramate, Brain, Histopathological changes, Rat

Topiramate is one of common drugs used to treatment epilepsy, prevent headache of migraine and generalized seizures (Gursahani and Gupta, 2012) and in addition is used for neuropathy and amelioration of behavioral impairments (Owona et al 2019). The psychiatrists were e topiramate for treating other diseases as disorder of anxiety, attention deficit hyperactivity disorder (ADHD) and nociceptive pain (Rus et al 2013). Thes known brand is Topamax which available as tablets for administration oral as (25 mg, 50 mg, 100 mg and 200 mg). Topamax has efficacy included anticonvulsant, analgesic and mood stabilizing properties that used in adult and children (Kholin et al 2013).

Although large therapeutic role of Topamax also have adverse effects as kidney failure, body weight alterations, disturbances of liver architecture and hepatitis. The symptoms of Topamax intake include dizziness, drowsiness, nausea, diarrhea, appetite losing and kidney stones (Khivsara et al 2017). The important mechanisms of Topiramate have role in antiepileptic efficacy by blocking of sodium channels, transmission of GABA-ergic, blockage kainite receptors and motivation of potassium channels (Herrero et al 2002). The current study was designed to observe the negative impacts of Topiramate in brain of male rats.

Laboratory animals: Twenty four of male rats (*Rattus norvegicus*) were used in this study weighting 200-230 grams and aged 10-13 weeks from animal house of college of education for pure sciences, University of Thi-Qar. Rats were maintained at temperature 20±4°C and 12:12 hours light: dark cycle.

Experimental design: Male rats were used in current experiment which divided into two mains groups as:

First group: (Control group) (n=8): Rats were treated with distilled water and feed.

Second group: (Treated group): This group divided into three subgroups (n=8).

Group A, B, C: Rats were treated with Topiramate (20, 40 and 60 mg /kg body weight/ day for two month .

Histopathological examination: Brains were eliminated from the skulls of all treated groups. The method of Bancroft and Gamble (2008) was used to prepared of histological sections. This method included fixation of samples by putting in 10% formalin for one day, washed by tap water and dehydration through ascending concentrations of alcohol. Brains were cleared in xylene for 15 minutes, after that embedded in paraffin wax. Histological sections at 6 microns thickness were prepared and stained with hematoxylin and eosin then examined by light microscope to estimate the damages in brains.

RESULTS AND DISCUSSION

Many histological changes were observed in brains of rats which treated by topiramate drug at 20, 40 g and 80 mg. These changes included severe hemorrhage in brain tissue,
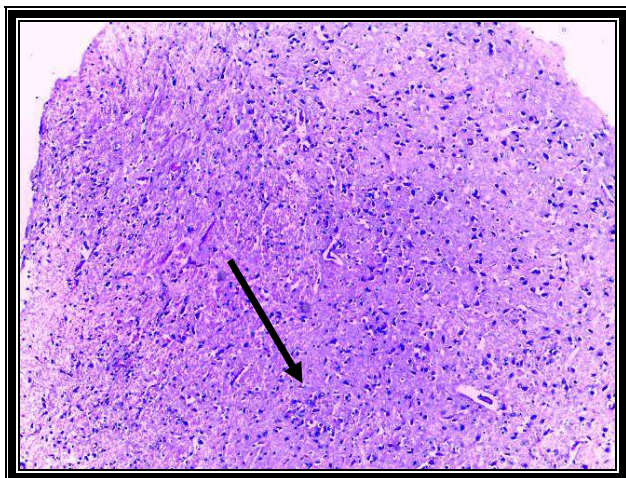
Fig. 1. Section of brain of control group showing normal structure of tissue (H&E) (100 X)
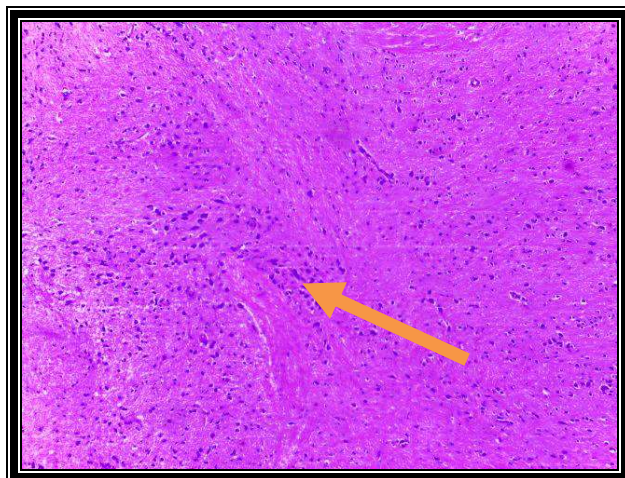
Fig. 2. Section of brain of control group showing normal appearance of perkengi cells (H&E) (100 X)
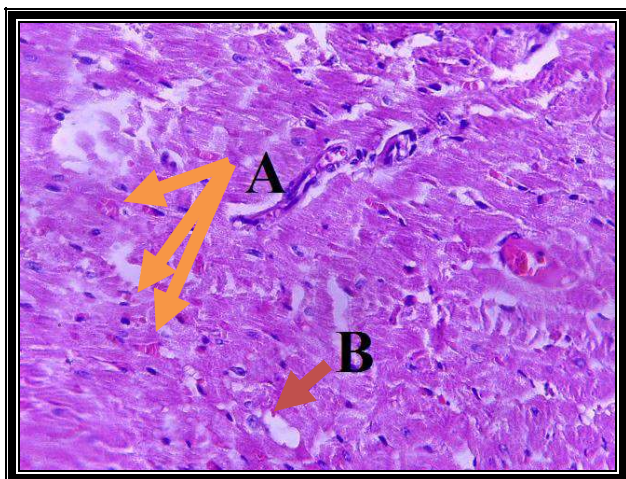
Fig. 3. Section of brain in treated group (20 mg/kg/day) showing hemorrhage (A) vacuolation of neurons (B) (H&E) (100 X)
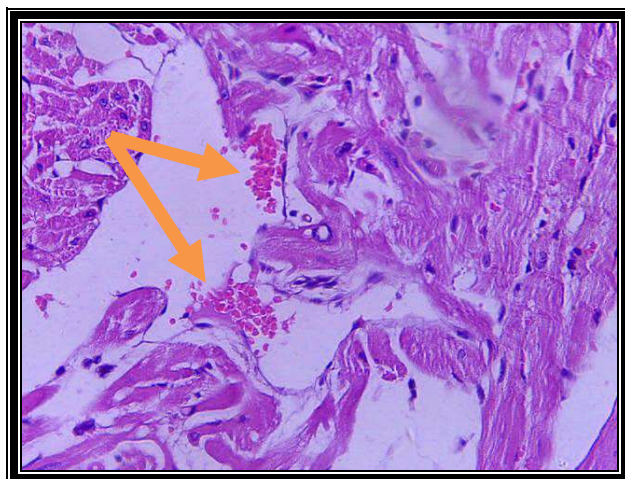
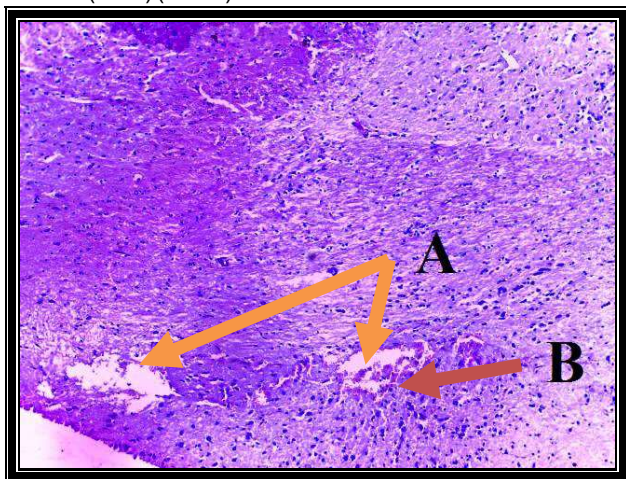**Fig. 4.** Section in brain of treated group (20 mg/kg/day) showing congestion of blood vessel (H&E) (100 X

Fig. 5. Section of brain of treated group treated group (20 mg/kg/day) showing congestion of blood vessel (A) aggregation of neutrophils around congested blood vessel (B) (H&E) (100 X)
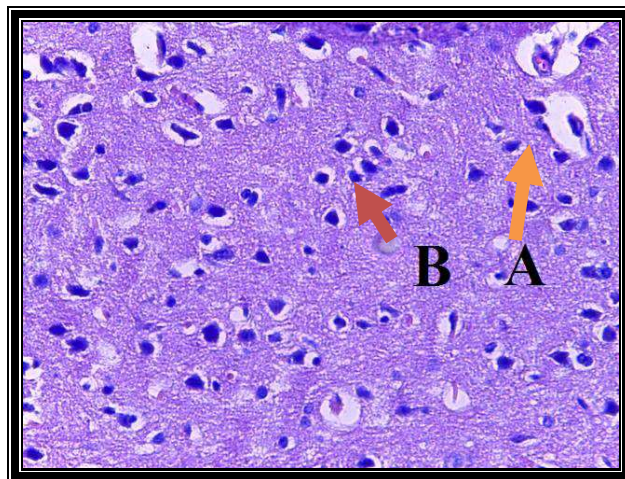
Fig. 6. Section of brain of treated group (20 mg/kg/day) showing perivascular edema (A) proliferation of astrocytes in white matter of cerebellum (B) (H&E) (400 X)
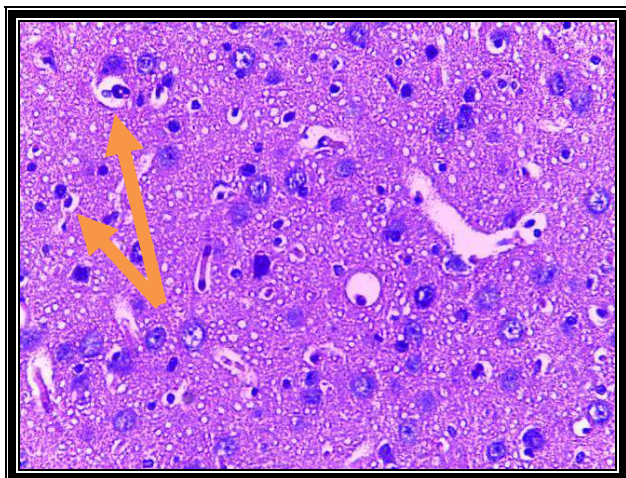
Fig. 7. Section of brain of treated group (20 mg/kg/day) showing pair of nuclei arrangement surrounded by clear space (H&E) (400 X)
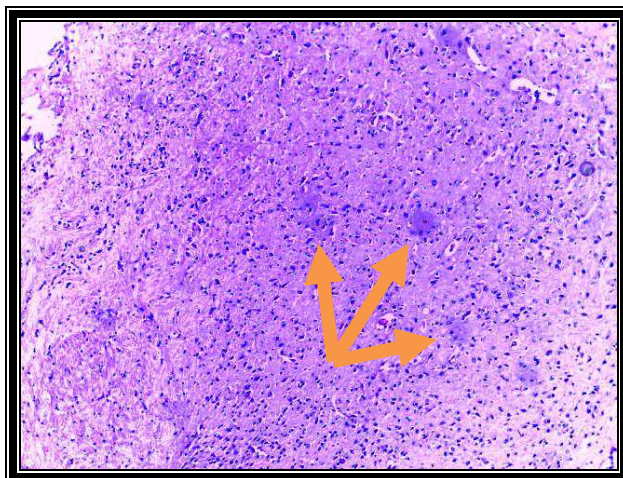
Fig. 8. Section of brain of treated group (20 mg/kg/day) showing perivascular edema (H&E) (100 X)
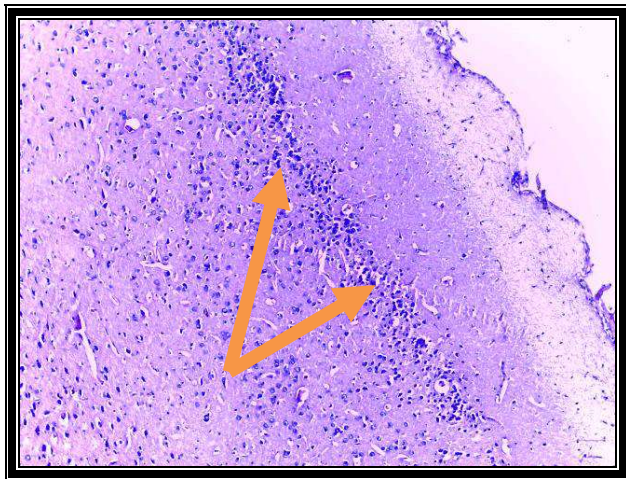
Fig. 9. Section of brain of treated group treated group (40 mg/kg/day) showing aggregation of microglial cells (H&E) (100 X)
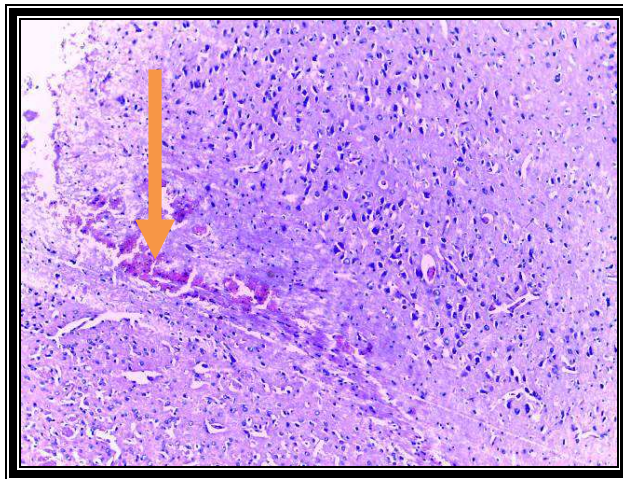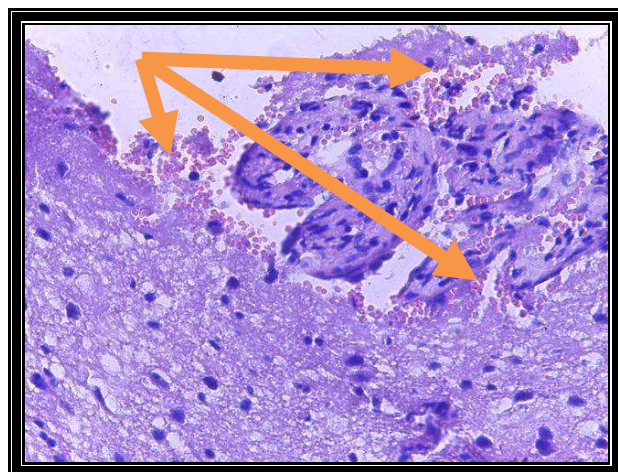

Fig. 10. Section of brain of treated group (40 mg/kg/day) showing hemorrhage in brain tissue (H&E) (100 X)
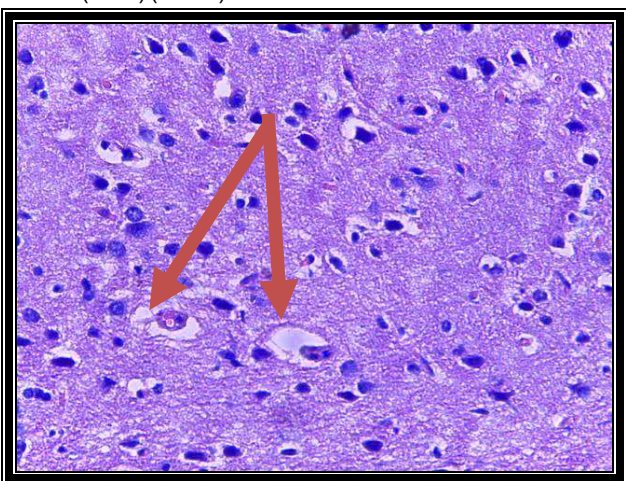
Fig. 11. Section of brain of treated group treated group (40 mg/kg/day) showing vacuolation of neurons (H&E) (400 X)
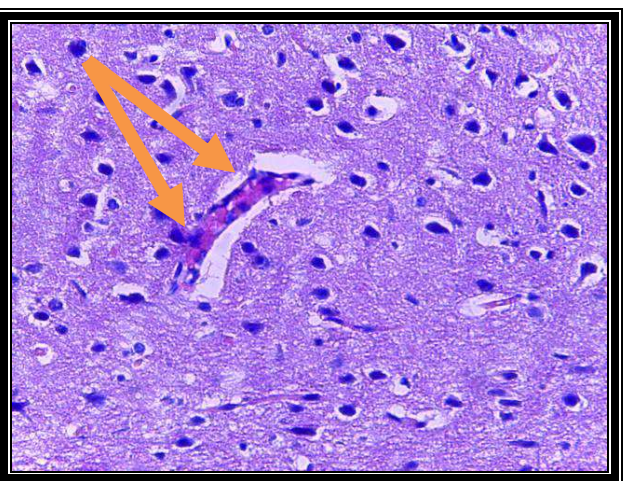
Fig. 12. Section of brain of treated group treated group (40 mg/kg/day) showing vascular prominent duct with aggregation of inflammatory cells in the lumen (H&E) (400 X)
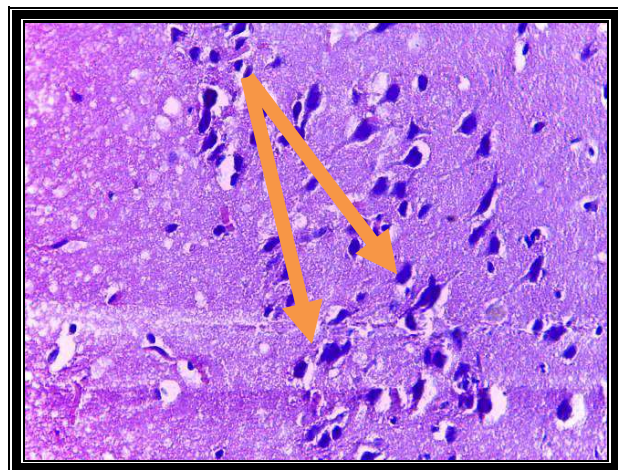
Fig. 13. Section of brain of treated group treated group (40 mg/kg/day) showing degeneration of neurons which stained in dark blue (H&E) (400 X)
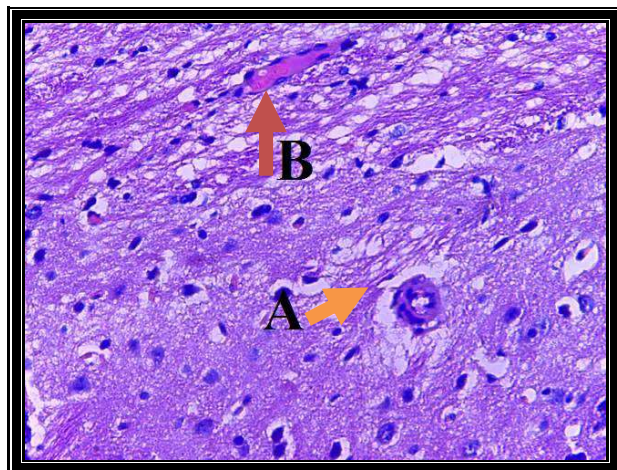
Fig. 14. Section of brain of treated group treated group (40 mg/kg/day) showing perineuronal edema (A) congestion (B) (H&E) (400 X)
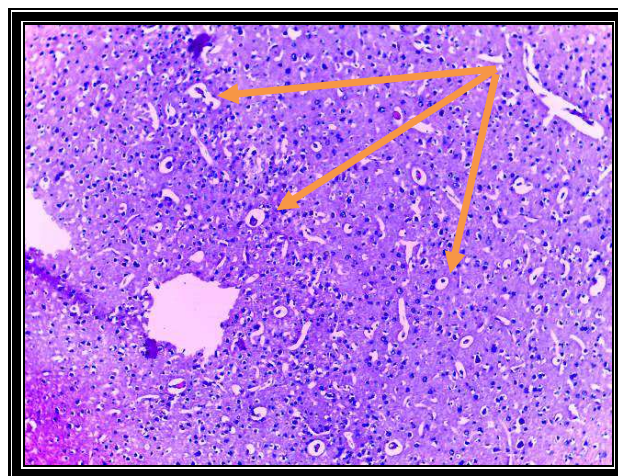
Fig. 15. Section of brain of treated group treated group (80 mg/kg/day) showing different stages of neurons vacuolation (H&E) (100 X)
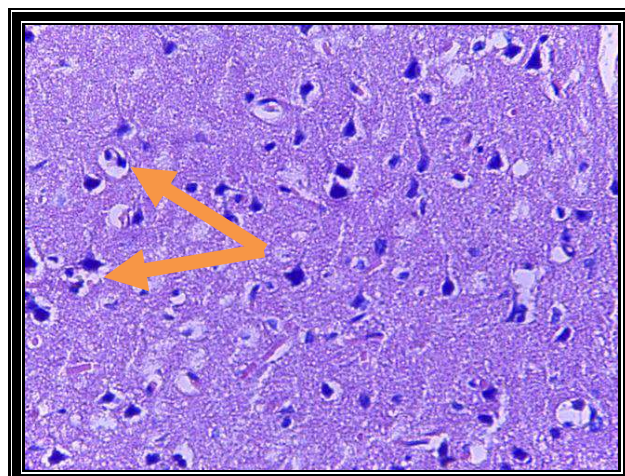
Fig. 16. Section of brain of treated group treated group (80 mg/kg/day) showing arrangement of pair nuclei surrounded by clear space (H&E) (400 X)
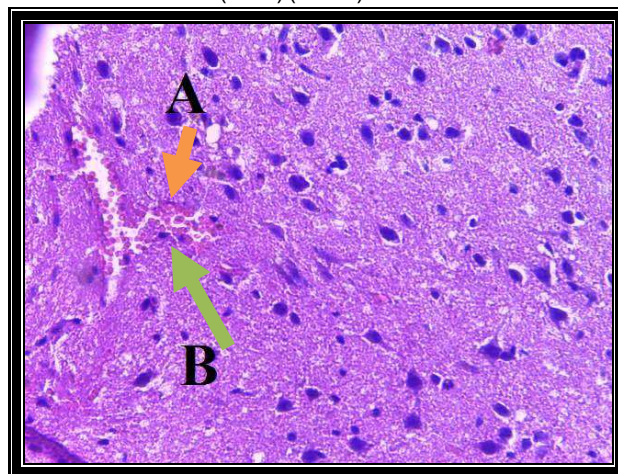
Fig. 17. Section of brain of treated group treated group (80 mg/kg/day) showing congestion clearly (A) presence of neutrophils in lumen of blood vessel (H&E) (400 X)
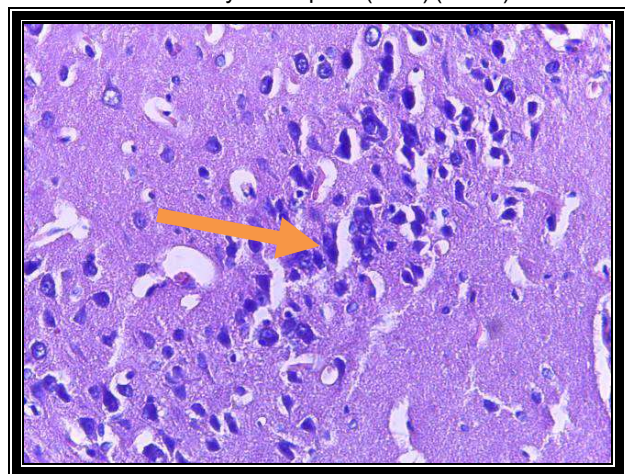
Fig. 18. Section of brain of treated group treated group (80 mg/kg/day) showing aggregation of inflammatory cells (H&E) (400 X)

Fig. 19. Section of brain of treated group treated group (80 mg/kg/day) showing sever hemorrhage (H&E) (400 X)

Fig. 20. Section of brain of treated group treated group (80 mg/kg/day) showing sever congestion of blood vessel with infiltration of neutrophils in the lumen pia mater (H&E) (400 X)

vacuolation of neurons, congestion of blood vessels, aggregation of neutrophils around congested blood vessels, perivascular edema, proliferation of astrocytes in white matter of cerebellum, appearance of pair nuclei arrangement surrounded by clear space, aggregation of microglial cells, occurrence of vascular prominent duct with aggregation of inflammatory cells in the lumen, degeneration of neurons by dark blue stained and infiltration of neutrophils in blood vessels and in piamater (Plate 3 to 20). These damages may be associated to high doses of topiramate which causes defect of blood brain barrier and injury in the blood vessels causing leakage of fluids from blood vessels to the brain resulting edema. Salameh et al (2016) observed that topiramate prevent blood brain barrier disruption in diabetic mice (in suitable dose), Zahroon (2009) recorded fluids fleeing and perivascular edema in nervous tissue.

These severe damages related to administration of topiramate drug which cause oxidative stress, oxidative stress causes generation of reactive oxygen species (ROS) that affected DNA and RNA of neurons subsequently ROS responsible for nerval damage. Maertens et al (1995) revealed to long term exposure to antiepileptic drugs led to oxidative stress induction. Agarwal et al (2011) reported that topiramate increase oxidative stress in PTZ- kindled mice. Cantuti et al (2000) also stated dysfunction, neurodegenerative disturbances and morphological abnormalities in brain by oxidative stress. Pathological lesions were observed in the present results after topiramate intake due to effect of free radicals which induce lipid peroxidation by blitzing polyunsaturated sites in biological membranes causing dysfunction of cells. Agarwal et al (2011) and Gupta et al (2003) measured lipid peroxidation

parameters as malondialdehyde (MDA), increased MDA indicate to oxidative damage. Rauca et al (1999) mentioned elevated oxidative stress in brains of kindled animals. Pavone and Cardile (2003) showed accentuation of oxidative stress in astrocytes of rats by topiramate treatment.

Histological examination of brains exposed to topiramate manifested inflammation and aggregation of neutrophils and may be linked to administration of topiramate that causes heigh nitric oxide resulted from oxidative stress. Cardile et al (2001) exhibited excessive of nitric oxide and production of ROS were occurred by topiramate .h Xiaodong et al (2012) also reported that nitric oxide causes inflammation states in different body tissues. The neuronal damages were observed may be belong to decrease of glutathione level in serum of topiramate treated groups. The decline of glutathione concentration caused by interaction with free radicals. Agarwal et al (2011) found diminution of glutathione level by topiramate exposure.

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Detection of *Helicobacter pylori* Infection in Women with Recurrent Spontaneous Abortion

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Abstract: To determine the relationship between *Helicobacter pylori* infection and recurrent spontaneous abortion seventy women patients who undergo spontaneous abortion (17-38) years were investigated during 2019-2020 and compared with forty apparently healthy women (20 from pregnant female and 20 from non-pregnant female). All subjects were tested for anti-*H.pylori* IgA and anti- *H. pylori* IgG antibodies by Enzyme linked Immunosorbent Assay (*ELISA*). There were non-significant difference in the concentration of anti-*H. pylori* IgA Ab(8.44 U/ml) compared to control groups pregnant (8.90 U/ml) and non-pregnant (7.27 U/ml) There were significant difference in the concentration of anti-*H. pylori* IgG Ab (14.79 U/ml) compared to control groups pregnant (13.38 U /ml) and non-pregnant (8.89 U/ml). The percentage of *H. Pylori* IgA, IgG antibodies was 27 and 64%, respectively. There was significant difference between control and test groups. The result indicates that the infection with *H. pylori* may play a role in the pathogenesis of recurrent spontaneous abortion.

Keywords: Spontaneous abortion, *Helicobacter pylori*, IgA, IgG

Helicobacter pylori are common microaerophilic bacteria inhabit the mucous layer of the human stomach affecting about half of the world's population (Mitchell 2001, Tonkic et al 2012, Malfertheiner et al 2017). Infection with this microaerobic gram-negative bacterium has been identified as an etiological factor in the development of peptic ulcer disease. *H. Pylori* infection has been strongly associated with the development of gastric neoplasia, including gastric adenocarcinomas and gastric mucosa-associated lymphoid tissue lymphomas (Versalovi 2003). About 80 percent of bacterium-infected individuals are a symptom (Konturek 2003*). H. Pylori* infection is associated with increased gastrin release from the antrum and that evidence indicates that cytokines play an important role in the pathogenesis of peptic ulcer and gastritis associated with a pylori. Additionally, the Infection with *H. pylori* impairs the secretion of bicarbonate. This results in an increase in duodenal acid load in person with *H. pylori* infection (Marshall 2002).

Recent meta-analysis showed that the average global prevalence estimate was 44.3%, with the prevalence as high as 89.7% in Nigeria and low 8.9% in Yemen (Zamani et al 2018). Abid and Aboud (2020) showed relationship between infertility and *Helicobacter pylori* infection. There was a significant difference in the detection of DNA of *H. pylori* between patients and control groups. Linciers et al (1999) found an increase in levels of IgM (marker of recently acquired infection) in pregnant women compared with non-pregnant women, suggesting that pregnancy itself may increase susceptibility to infection with *H. pylori.*

MATERIAL AND METHODS

Patients' samples: The study selected of seventy patients suffering from recurrent spontaneous abortion during first, second and third trimester from different capitals in Iraq during the period October 1, 2019-January 1, 2020. The age of the total patients was ranged from 17-39 years. The interviews were performed for each patient.

Control samples: Forty samples of healthy women; 20 from pregnant female and 20 from non-pregnant were kept as a control group of same ages and sex.

Blood samples collection: Blood samples (5ml) were collected by disposable syringe into gel tubes and stand at room temperature until the coagulant was form. Then the samples were centrifuged at 3000 rpm for 5 minutes. Serum samples were dispended separated on a four Ependroff tubes. All samples were marked by the name, day and numbering and stored at (-20°C) until carried out to immunological examinations.

Immunological tests for Diagnosis *Helicobacter pylori* infection by enzyme link immunosorbent assay (ELISA): All the studied patient group and apparently healthy individuals (Control group) were submitted to estimate the anti-*H. pylori* antibodies IgA and IgG antibodies by using *ELISA* test (Germany) according to protocol of the kit .

Statistical analysis: The Statistical Analysis System- SAS (2012) program was used to detect difference in study parameters.

RESULTS AND DISCUSSION

There was no significant difference in concentration of IgA *H. pylori* (8.44 U/ml) compared to pregnant and non-pregnant (8.90 and 7.27 U/ml) (Fig. 1). There was significant difference in concentration of IgG Ab *H. pylori* (14.7 U/ml) compared to pregnant and non-pregnant (13.38 and 8.89 U/ml) (Fig. 2). The differences in percentage distribution of IgA *H. pylori* 19 (27.14) in aborted women compared to pregnant and non-pregnant groups (40.00) were significant (Table 1). There were significant differences in percentage distribution of IgG *H. pylori* (64.29) in aborted women compared to pregnant and non-pregnant groups (60.00 and 50.00) (Table 2).

The findings agreed with many others studies. Shalabi et al (2015) observed 66.7% of group 1 (Aborted cases) were *H. pylori* seropositive compared to 8% in controls. Ibrahim (2020) found a highly significant difference in *H. pylori* IgG and IgA between women with a history of abortion. In Iran Hajishafiha et al (2011) observed that Women infected with Cag-A positive strains were more likely to have EPL and had no association between EPL and maternal age. These cytokines can cause systemic inflammation that may affect the integrity of the fetoplacental unit and threaten the well-being of the fetus (Heimonen et al 2008). The poor oral hygiene also associated with a history of miscarriage,

Table 1. Distribution of *H. pylori* IgA in different groups

Group	Positive No. (%)	Negative No. (%)	P-value
Abortion	(19) 27.14%	(51)72.86%	** 0.0001
Control : Pregnant	(8) 40.00%	(12) 60.00%	** 0.0084
Control : Non-pregnant	(8) 40.00%	(12) 60.00%	** 0.0084
P-value	*0.0319	*0.0319	---

(P≤0.01)**; (P≤0.01)*

Table 2. Distribution of *H. pylori* IgG results in different groups

Group	Positive No. (%)	Negative No. (%)	P-value
Abortion	(45) 64.29%	(25) 35.71%	** 0.0016
Control : Pregnant	(12) 60.00%	(8) 40.00%	** 0.0084
Control: Non-pregnant	(10) 50.00%	(10) 50.00%	1.00 NS
P-value	*0.02074	*0.02074	---

(P≤0.01)**; (P≤0.01)*

probably due to systemic inflammatory response to oral bacterial infection. (Heimonen et al 2008). It may be assumed that a parallel mechanism underpins the EPL in women infected with CagA-positive *H. pylori* strains. The study revealed that infection with *H. pylori* may play a role in reproduction disorder such as recurrent spontaneous abortion.

Figure 1: Comparison between abortion and control groups in conc. of IgA
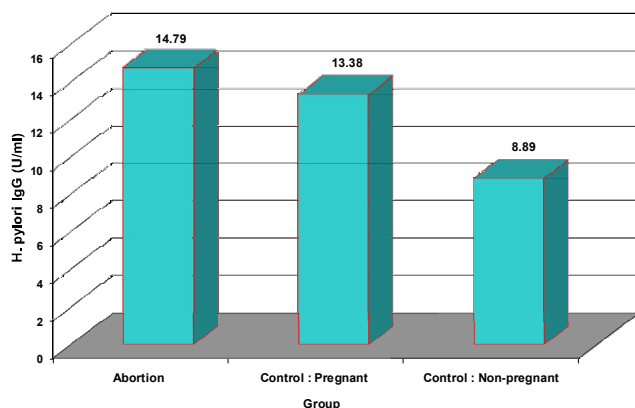
Figure 2: Comparison between abortion and control groups in conc. of IgG

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Spectrophotometric Kinetic Methods for Determination of Sulphdimidine Sodium in Pure Form and Pharmaceutical Preparations by using Cloud Point Extraction

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Abstract: Sensitive and simple kinetic methods were developed to obtain sodium sulphdimidine in pure form for pharmaceutical preparations. This methods are based on direct reaction (di azo coupling with cloud point extraction) of Sulphdimidine sodium with salicylic acid by sodium nitrite to form the dye and then measured by UV-VISBLE at λ= 473 nm. The diazonium salt product was studied kinetically by fixed time and initial rate (at 30 minutes) methods and the optimization parameters were studied and fixed. The calibration curve for drug determination was linear in the concentration ranges (1-12 μg.ml^{-1}) for two methods the initial rate and the fixed time methods at 30 min. The two methods were applied effectively for the determination of Sulphdimidine Sodium in pharmaceutical.

Keywords: Sulphdimidine sodium, Salicylic acid, Cloud point extraction, Diazonium salt, Kinetic spectrophotometry, Fixed method, Initial rate

Sulphdimidine sodium is the name given to 2, 4, 6-(Aminobenzensulfonamido-dimethylpyrimidine) a formula molecule $NaS_2OH_4N_{14}H_{13}C$ (Fig. 1). The molecular weight is 300.312g mol-1, it is a crystalline white, soluble in water, more soluble in alcohol (Esraa Amer Kadhim 2018). This drug are one of Sulpha drugs, that the greater groups of pharmacologically energetic material, this drug used in veterinary medication to day (Alsamarrai MSA-RKF 2016). Their sulpha drugs discovery in 1935, suggested the start of a new era in bacterial diseases and a number of protozoan toxicities (Saadiyah and Amal 2012). Sodium sulphadimidine (SDMS) is one of the greatest antibiotics broadly used in animals. The antibiotics are used to treat disease by inhibiting their growth or destroying pathogenic microorganisms at concentration low enough to avoid" damage to the host (Darweesh 2017). This drugs contain some chemical substances that are produced by microorganisms and by chemical synthesis which have very low concentrations are destroy or partially inhibit microorganisms. Antibiotics have wide spread of bacterial disease (Saadiyah and Kdhim 2018).

MATERIAL AND METHODS

In the binging prepare diazonium salt in ice bath by dissolved 0.1 gm of (SDMD) in 5 ml distilled water, then added 0.37 ml of(1M) HCl in volumetric flask 100 ml after 5 minute added 0.025 gm of $NaNO_2$ and take this solution 5 min. in ice bath. Afterward complete the volumetric flask in distilled water that will be prepared diazonium salt have concentration 1000 ppm, then transfer 10 ml of this solution to volumetric flask 100 ml and complete to the mark by distilled water which prepare 100 ppm of diazonium salt. After that added 1 ml of each parameters sulfamic acid (10%), Salicylic acid (100ppm), triton x-114 (10%) and NaOH (0.5 M) in a volumetric flask 10 ml then complete the volume by distilled water .Then this complex was lived 10 min. 60^0 c in water bath. The tubes transferred to centrifuge at 20 min and separated by using centrifugation. Test tube transfer in ice bath to rising viscosity micelles for layer 1min., then became easily separated (Esraa Amer Kadhim et al 2020). The sediments separated, dissolved by 1ml of ethanol then measured at 453 (Fig. 2).

RESULTS AND DISCUSSION

Stoichiometry: The stoichiometry were comparison relationship between sodium sulphadimidine with salicylic acid, was recognized by the method logarithmic (Saadiyah and Esraa 2019), used two set. The primary set, the sodium sulphadimidine varied concentration at the same time as the concentration of salicylic acid keeping a constant (7.24×10^{-4} M). The second set were the opposite of the first set, when the concentration of salicylic acid was mixed custody a regular concentration of sodium sulphadimidine (0.003672 Molary).

A plan (a): the log molar [SDMS] and (b) alog [salicylic acid] give directly line. The slopes where have volume 0.971 , 1.495 correspondingly (Fig. 3,4).

The base of the obtained molar reactivity and subsequent

of reaction on the results above was shown in scheme 1.

Quantization SDMS after the optimization conditions investigational outline on top of result in a fake - first arrange with esteem to concentration anywhere salicylic acid was at lower 15 point of concentration SDMS. Though, rate of proportional were straight to SDMS conc. in an equation pseudo - first order as follow:

Rate= $k^{'}$[SDMS] .... (1)

Where $k^{'}$ is the rate constant of pseudos - first order.

Many of experiment were approved out to get hold of
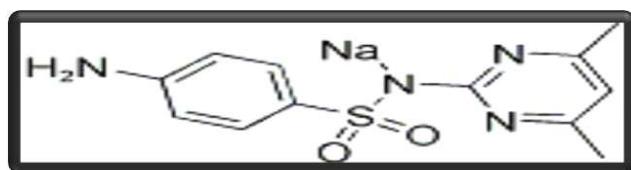
Fig. 1. Chemical structure of Sulphdimidine Sodium
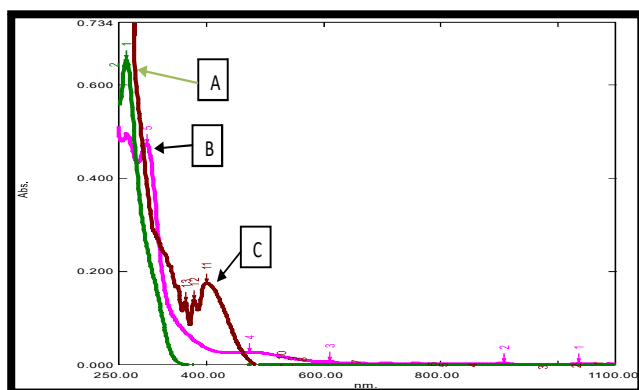
Fig. 2. Absorbance spectra of color species against reagent blank (C) and reagent blank
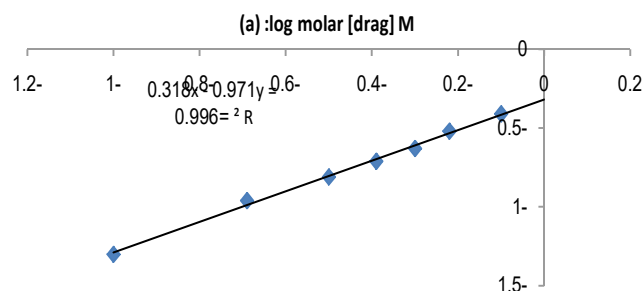
Fig. 3. Plot of the log absorbance [Sulphadimidine sodium]
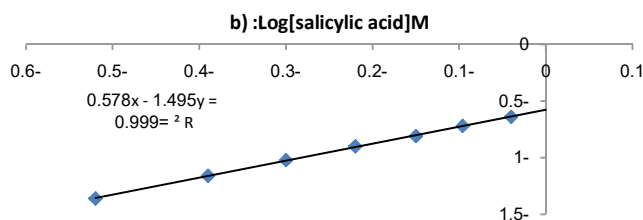
Fig. 4. Plot of the log absorbance [Salicylic acid]

SDMS concentrate from the records of the rate accept to equation (1). Fixed time methods, initial rate (Saadiyah and Sahar 2012) were chosen and tried the largest part appropriate analytical methods were chosen and taken explanation the correlation coefficient (r^2), the intercept sensitivity and applicability.

Initial rate method: The rates initial of reactions be determined by measure the slopes of the initial of the absorbance time curve for the first 10 minutes (Fig. 5). Furthermore, log analysis of the rate reaction (R= log $\Delta A/\Delta t$) were plot beside log [drug].

The reaction rate be found too reliant on Sodium sulphadimidine concentrate the reactions rates were follow at (30°C) by a range of conc. of Sodium sulphadimidine in the series of 1-7 µg ml^{-1} keep the reagent. The rate of reaction:

Rate = $k^{'}$[SDMS]^n ——————— (2)

Anywhere n is the reactions orders in addition to $k^{'}$ was the order pseudo of the rate constant. The reaction rate can be found by changeable time method (Domagk and Prize 2017) (different initial method rate) (Shatha 2017) as the $\Delta A/\Delta t$, wherever it was time in minutes with A wave absorbance.
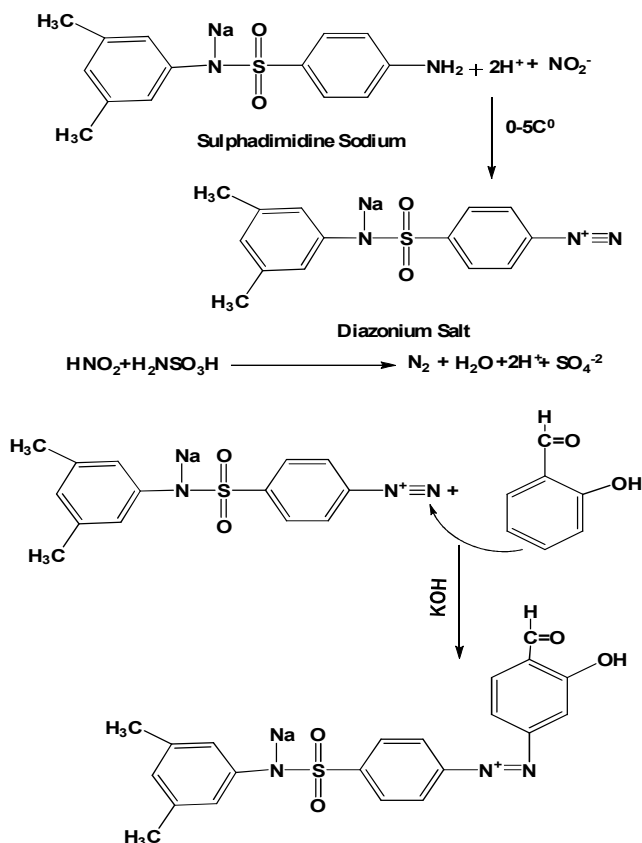
Scheme 1: The propose of mechanism for sequence reaction between Sulphadimidine sodium and Salicylic acid to form a yellow

Pleasing log of concentration with rates(equation (3) is distorted into:

Logarithm (rate) = log $\Delta A/\Delta t$ = log k$^{\backslash}$+ n log [SDMS] ⸺ (3)

Regression of log (rate) against log [SDMS] gave the equation:

Log (rate) = 3.212 + 1.017 logartime C

wherever (r =0.9601).

k = 3712.6 minite $^{-1}$ = 61 second $^{-1}$ and the reactions were first orders (n = 1.017) with high opinion to Sodium sulphadimidine concentrate .

Rate method constant: The most excellent way to find a customary K` value of this reactions by draw line log (A) against time of SDMS with the series of concentration 1-7 µg.ml$^{-1}$ ($3.32\times10^{-9}$-$2.33\times10^{-8}$ M) (Fig. 13). The obtained of first rate K` constant identical to dissimilar SDMS concentrate (Saadiyah and Sana 2015). The values of K` be calculate by the slops of curve as shown as in Table (1).

Regression of [SDMS] against K` given the following equation:

K$^{\backslash}$=0.467[SDMS]-3.971

Where r^2 = 0.985as shown as in Figure (7).

Fixed-time method: Fixed times of (5-60) min the absorbance of calibration graphs in opposition to initial concentration of SDMS be determined at preset times through equations regression (Table 2).

It is apparent that the absorbance increase with time and the value of r^2 were accepted with the obtain preset time at 30 minute. After optimizing of the reaction conditions to the determination of SDMS in pure at the fixed time method was applied the range (10-1 µg.ml^{-1}) (Fig. 8). The of values analytical for the calibration curve were shown in Table 3.

Precision and accuracy: The precision and accuracy of SDMS were determination in 3 singular concentrations
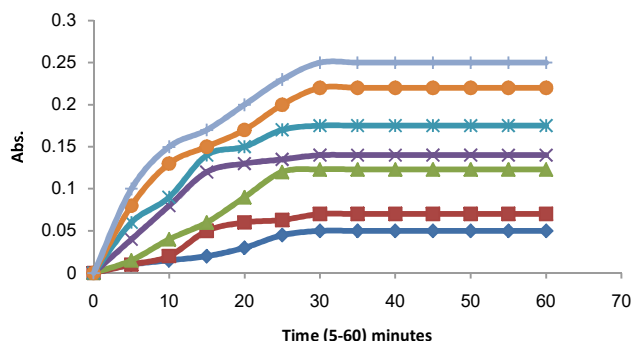

Fig. 5. Graph of the reaction on sodium sulphadimidine (1-7 µg.ml^{-1}) (1)3.32×10^{-9}M, (2)6.65×10^{-9}M, (3)9.98×10^{-9}M, (4)1.33×10^{-8}M, (5)1.66×10^{-8}M, (6) 1.99×10^{-8}M and (7) 2.33×10^{-8}M, these absorbance were measured at 453 nm against reagent blank

Table 1. K values intended from of log A slops against t

Drug in µg.ml^{-1}	Equation	K` / min^{-1}
1	Logartime A = 0.0484 t – 2.892	-0.1117
2	Logartime A = 0.0330 t – 1.940	-0.7643
3	Logartime A = 0.0271 t – 1.716	-0.0626
4	Logartime A = 0.0201 t – 1.393	-0.0465
5	Logartime A = 0.0180 t – 1.311	-0.0417
6	Logartime A = 0.0122 t – 1.084	-0.0283

Table 2. Regression equations to SDMS at different time with variety concentration (3.32×10^{-9}-2.33×10^{-8} Molarty) at temperature room

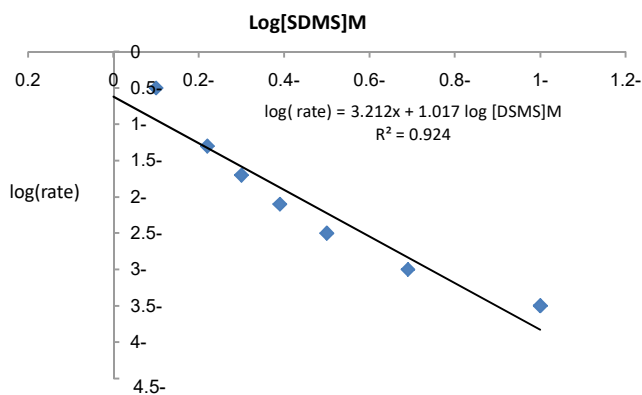| Time (min)	Equation regression	r^2
60	y = 0.003x + 0.085	0.9917
50	y = 0.003x + 0.085	0.9917
40	y = 0.003x + 0.085	0.9917
35	y = 0.003x + 0.085	0.9917
30	y = 0.003x + 0.085	0.9917
25	y = 0.001x + 0.059	0.991
20	y = 0.001x + 0.029	0.987
15	y = 0.02x + 0.022	0.984
10	y = 0.012x + 0.019	0.980
5	y = 0.001x + 0.006	0.970

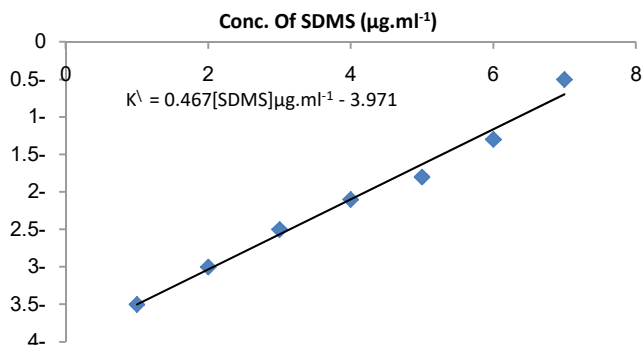**Fig. 6.** Effect of variation of log [SDMS] on log (rate)

Fig. 7. Plot of constant K` (min^{-1}) against conc. of Sulphdimidine sodium (µg.ml^{-1}

(Shatha 2017) (Tables 4 and 5).

Interferences: Study the optimum experimental conditions to test the efficiency and selectivity of this method to pharmaceutical preparations, such as starch, talc, lactose is shown in Table 6.

Pharmaceutical applications: The fixed-time method and

Table 3. Analytical values for the calibration curve at the present time

Parameter	Value
Correlation coefficient, r	0.9989
Correlation of determination , r^2	0.9980
Beers low limits ($\mu g.ml^{-1}$)	1-10
Regressions equations	y=0.139x+0.023
Slop b, ($ml.\mu g^{-1}$)	0.139
a , Intercept	0.023
Standard deviation of the residuals, $S_{y/x}$	0.010674
Standard deviation of the slop, S_b	0.00076
Standard deviation of the intercept, S_a	0.00469
Molar absorptivity $\mathcal{E}$ ($l.mol^{-1}.cm^{-1}$)	9.970×10^3
S ($\mu g.cm^{-2}$) Sandell's sensitivity	0.042
LOD ($\mu g.ml^{-1}$) Limit of detection	0.0259
LOQ ($\mu g.ml^{-1}$) Limit of quantification	0.029563

Table 4. Accuracy and precision of the initial-rate method

Conc. of SDMS/ ppm	Mean*	RSD (%)	E_{rel} (%)	Recovery (%)
9	9.048	1.008	0.5333	100.5333
6	5.872	1.8277	0.395	99.605
3	2.976	2.3892	-0.8	99.2

*Mean of five

Table 5. Precision and Accuracy of the fixed-time method

Conc. of SDMS/ ppm	Mean*	RSD (%)	E_{rel} (%)	Recovery (%)
9	8.9999	0.5409	-1.111	98.9988
6	6.1343	1.235	2.2	102.2383
3	2.9843	3.4057	-0.5066	99.4933

*Mean of five

Table 6. Determination of 4 μg ml^{-1} of SDMS in the presence of excipients

Interference	Absorbance	E_{rel} (%)	Recovery (%)
Starch	0.175	9.603	109.606
Talc	0.160	1.513	100.515
Glucose	0.159	3.270	102.2727
Tri methyprine	0.160	2.97	101.78
Lactose	0.161	3.88	102.7878
Without interference	0.157	0.756	100.4545

Table 7. Application of the proposed method of SDMS by the initial-rate method

Conc. of SDMS/ ppm	Mean*	RSD (%)	E_{rel} (%)	Recovery (%)
9	8.958	0.3336	-0.3555	98.6444
6	5.952	0.5622	-0.08	99.2
3	3	1.1925	0	100

Table 8. Application of SDMS by the fixed-time method

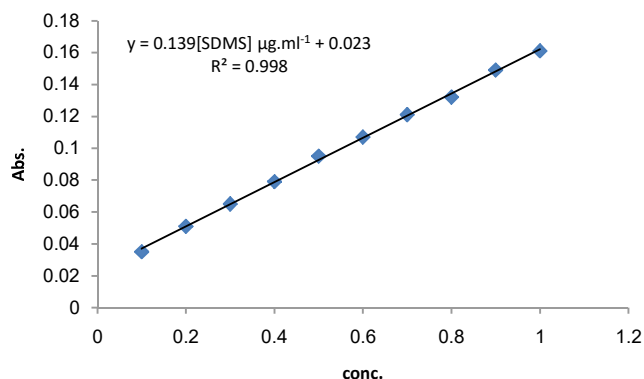| Quantity of SDMS	Found*	RSD (%)	E_{rel} (%)	Recovery (%)
9	9.12118	1.3890	1.3465	101.3464
6	5.0605	1.9514	1.2116	101.2116
3	2.9090	4.3554	-0.03033	96.9668

Fig. 8. Calibration curve of paracetamol at 25 min

initial-rate method were be appropriate to the determination of SDMS in pharmaceutical drugs preparation (Esraa et al 2020) .The projected method practical on (Sulphdimidine sodium 32.3% injection Egypt) which have (32.3 gm each one 100gm (Table 7).

CONCLUSION

The projected methods shown good quality sensitivity. The related selectivity allow analysis without providing steps and separation, and suitable to the many of procedures proposed chromatographic. Methods were valuable as they were comparing with method colorimetric in have top sensitivity. This method canister be used as option method to report for the determination of SDMS in pharmaceutical preparations with the pure form.

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Microbial Community of Soil Cultivated with Corn Intercropped with Cowpea under different Phosphorus Fertilizer Treatments

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Abstract: A field experiment was carried out in Al-Jazirah Althanea region , Shatt Al-Arab district , Basra Province on silty clay soil to study the effect of the intercropping system on microbial community in soil under different treatments of phosphorus . The experiment was designed as a factorial experiment under complete randomized block design. The treatments included phosphate fertilizer type (concentrated superphosphate and diammonium phosphate), the level of phosphorus fertilizer (0, 25 and 50 kg P ha^{-1}) and the cropping system (sole corn and intercropping of corn and cowpea). Phosphorus fertilizers were added as side dressing at the time of sowing. Corn and cowpea seeds were sowing on August 5, 2019. Soil samples were taken from the rhizosphere zone and the zone away from the rhizosphere (bulk soil) of corn plant, at the flowering stage of the cowpea, the number of bacteria, fungi, and actinomycetes were estimated. The number of bacteria, fungi and actinomycetes were increased with increasing level of phosphorus at both the rhizosphere soil and bulk soil. Diammonium phosphate recorded the higher number of bacteria and actinomycetes, while the type of fertilizer did not affect the number of fungi in both the rhizosphere soil and bulk soil. Higher values of bacteria and actinomycetes were obtained in intercropping system as compared of sole corn system, while the results were reversed in terms for fungi number. The results of the study also indicated an increase in the number of all organisms in the rhizosphere soil compared to their numbers in the bulk soil.

Keywords: Diammonium phosphate, Bacteria, Fungi, Corn -cowpea, Intercropping

Phosphate fertilizers are of several types, some of which consist of phosphorus only, and some of them consist of two nutrients. Most of the compound fertilizers contain. Many studies indicated that diammonium phosphate is more efficient than the concentrated superphosphate in processing with available phosphorous in the soil due to its physical characteristics such as the increase in the diffusion coefficient of the fertilizer and its low ability to aggregate in addition to the high solubility which reaches 100% (Khan et al 2010). Measuring the numbers and activity of microorganisms in the soil is an indicator for nutrients availability including phosphorus, which is one of the important nutrient for formation of nucleic acids, phospholipids and protoplasmic compounds, which are very important for the organisms to analyze the organic matter in the soil. Phosphorus content reaches 0.5-1% of the fungi mycelium dry weight and 1-3% of the dry weight of actinomycetes (Liu et al 2012). Likewise, microbial communities and their activity are affected by the cropping system used. Presence of two different crops in such place produces different types of root exudates, which changes the composition of the biological community in the rhizosphere and changes the activity and competition of the neighborhoods (Xu et al 2018). Qin et al (2017) stated that the intercropping system leads to an increase in root secretions and root residues, which provides greater energy sources that encourage the reproduction of organisms and increase their numbers.

MATERIAL AND METHODS

Location and treatments: A field experiment was carried out during 2019 in the AL-Jazeera Althanea region (30°, 36 '15 "N ; 47° 47' 26" E 3m above sea level) at Shatt Al-Arab district, which is 7.5 km from the center of Basra province, in silty clay soil classified as Typic torrifluvents (Al-Atabb 2008) to test the effect of the intercropping system of corn and cowpea and phosphorus fertilization on the numbers of microorganisms in the soil . Before planting, soil samples were taken from 0-30 cm depth and initial properties were estimated according to standard methods mentioned (Black 1965, Page et al 1982).

Field preparation and implementation of treatments: The field was plowed twice, smoothing, and leveling, then divided into three equal blocks. Each block represents a replicate and each replicate contains 18 experimental units that represent the overlap of the three study factors. The size of the experimental unit for sole corn was 4 m length x 2.7 m width contains 3 rows at 90 cm leaving a distance of 45 cm side borders. As for the experimental unit of corn and cowpea, it was 4 m long x 3.6 m wide contains 3 rows of corn

at 90 cm and 4 rows cowpea of interspersed corn rows leaving a distance of 45 cm as side borders. Plant to plant distance within row for corn and cowpea were kept as 40 and 25 cm, respectively. Corn (variety Furat) and cowpea (variety Ramshorn) were used. Sowing was done on 5 August 2019 at 3-4 seeds in each hole for both plants and after 20 days of planting the plants were thinned out and one plant was left farm operations were carried out for the crop whenever required all over the growing season . River water was used for irrigation for all experimental units. The Table 2 represents the number of lines and plant density for each treatment.

Table 1. Soil characteristics of soil before planting

Property		Quantity	Unit
pH		8.2	----------
EC		12.30	dS m^{-1}
CEC		13.10	Cmol (+) kg^{-1} soil
Total carbonate minerals		81.60	g kg^{-1}
Organic matter		8.03	g kg^{-1}
Ca^{++}	Dissolved cations	37.50	mmol L^{-1}
Mg^{++}		18.03	mmol L^{-1}
K^{+}		3.12	mmol L^{-1}
Na^{+}		1.50	mmol L^{-1}
CO$_3^{=}$	Dissolved anions	0.00	mmol L^{-1}
HCO$_3^{-}$		4.33	mmol L^{-1}
SO$_4^{=}$		20.08	mmol L^{-1}
Cl^{-}		90.60	mmol L^{-1}
N available		57.30	mg kg^{-1}
P available		30.80	mg kg^{-1}
K available		171.65	mg kg^{-1}
The total number of bacteria		2.2×10^5	CFU g^{-1} soil
The total number of fungi		4.00×10^3	CFU g^{-1} soil
The total number of actinomycetes		7.43×10^5	CFU g^{-1} soil
Sand		42.70	g kg^{-1}
Silt		550.50	g kg^{-1}
Clay		406.80	g kg^{-1}
Texture		Silty clay	

Three factors were used in the experiment were cropping system includes sole crop of corn and intercropping of corn with cowpea, type of phosphorus fertilizer includes concentrated superphosphate fertilizer (20.21% P) and diammonium phosphate fertilizer (21% P) and phosphorus fertilizer levels includes 0, 25 and 50 kg P ha^{-1}

Phosphorus fertilizer as concentrated superphosphate or diammonium phosphate were added close to corn rows at one dose at the time of sowing. Potassium sulfate fertilizer (43% K) was added at level of 83 kg K ha^{-1} in two doses, the first equivalent to 15% of the level was added immediately before planting and the rest was added at the stage of flowering near to corn rows. Nitrogen was applied at a level of 180 kg N ha^{-1} through urea (46% N) by two equal doses, the first at planting time and the other at the stage of flowering near to corn rows. The nitrogen was added through diammonium phosphate was subtracted from the nitrogen dose and remaining nitrogen was added through urea.

Estimating the number of microorganisms in soil: Soil microbial populations (bacteria, fungi, and actinomycetes) were counted separately in the rhizosphere and the zone far from the rhizosphere (bulk soil) of the maize plant when the cowpea entered the flowering stage after storing the samples in the refrigerator until the analysis was carried out . Two plants were taken from each experimental unit and their roots were extracted and cleaned from the surrounding soil, shake the plant by hand several times and collect the soil around the root with a small brush and combined to one composite sample. The (dilution method) was used in which 1 g of soil was added to 9 ml sterile distilled water and shaken well in attest tube and dilution series to 10^{-6} were prepared . One ml of the 10^{-5} and 10^{-6} dilutions were spread on (yeast beef – extract peptone media) to determine the bacteria, 1 ml of the 10^{-3} and 10^{-4} dilutions were spread on (potato Dextrose Agar) to determine fungi population, and 1 ml of 10^{-5} and 10^{-6} dilutions were spread on starch casein agar to determine the actinomycetes population (Njeage et al 2017). The number of colonies was calculated after incubation for 7 days at 28°..

Statistical analysis: The separate data of rhizosphere soil or bulk soil were subjected to analysis of variance using GenStat Procedure Library Replacement PL 18.2 program to obtain the means. The data of rhizosphere soil and bulk soil were compared using t-test analysis (Al-Rawi and Khalaf Allah 2000).

RESULTS AND DISCUSSION

Number of bacteria: There was an significant increase in the number of bacteria at rhizosphere soil comparted bulk soil with increasing the level of phosphorus respectively

Table 2. Number of lines and plant density according to the cropping system

Cropping system	No. of lines		Planting distances (m)		Plant density (Plant ha^{-1})		Total plant density (Plant ha^{-1})
	Corn	Cowpea	Corn	Cowpea	Corn	Cowpea	
Sole corn	3	0	0.90	-------	27777	0	27777
Corn+Cow pea	3	4	0.90	0.90	20833	44444	65277

(Table 3). These results were similar to those of AL- Bahrani (2015). Phosphorus enhances the biological activity in the soil and increases the number of microorganisms. Liu et al (2012) indicated that soils in which carbon and nitrogen availability are high, are more sensitive to the addition of phosphorus with respect to number and activity of microbes. They also stated that phosphorus is an important factor for plant growth and this growth increases the amount of carbon secreted to microorganisms in the soil , which leads to an increase in their numbers.

Diammonium phosphate fertilizer was superior to the concentrated superphosphate fertilizer. The values were increasing at the rhizosphere soil comparison for bulk soil. The is due to the high solubility of diammonium phosphate and thus the increase in the source of nutrients (P and N), which is reflected in the increase of the number of microorganisms, especially bacteria. In addition to the neutral effect of this fertilizer, which reduces the phosphorus degradation and increases its availability for microorganisms and that the addition of nitrogen with phosphorus is synchronized by the same fertilizer which can have a complementary effect in increasing the number of bacteria or increasing plant growth, which improves the growth of soil microorganisms. The diammonium phosphate is superior to the concentrated superphosphate at all phosphorous levels, and this superiority was significant only in the rhizosphere soil. The intercropping was superior to the single cultivation with at the rhizosphere soil comparison at bulk soil. Xu et al (2018) also found that presence of two crops together produce different types of root exudates than they are in a single crop, which changes the composition of the biological community in the rhizosphere and changes the effectiveness and numbers of neighborhoods and the competition between them. The superiority of intercropping over single cultivation at most levels and types of phosphate fertilizers was observed and the differences were significant in the

rhizosphere soil .This indicates the superiority of intercropping under different conditions of phosphate treatments including the treatment of non-addition of fertilizer. The highest value at rhizosphere soil was values by 20 - 96%. The highest value at bulk soil was surpassing the rest values by 8-10%. The highest differences between the two cropping system was at 50 kg P ha^{-1}which indicate efficiency of intercropping is more at the high level of phosphorus, and perhaps this is due to the improvement of plant growth at this level.

There was a significant increase in the number of bacteria in the rhizosphere soil of the maize plant compared to the bulk soil (Fig. 1). This may be explained by the fact that the root exudates in the rhizosphere lead to develop a good environment for the growth of organisms and increasing their numbers, including bacteria (Qin et al 2017). Tang et al (2015) also observed obtained bacteria in the rhizosphere soil compared to the bulk soil. The large differences may arise in the number of neighborhoods between one site and another in the soil a few centimeters apart, which is the result of variation in terms of moisture content, organic matter or pH.

Al- Hammadi (2014) confirmed that the number of bacteria in the rhizosphere of beans and barley increased by 108 times and 200 times, respectively, compared to their numbers in the bulk soil indicating that the concentration of microorganisms is on the newly growing root hairs that represent the root secretions of sugars, amino acids and salts , and these root hairs rot easily, resulting in addition of organic matter to the soil, a source of many materials that the organisms feed . In addition both roots and microorganisms consume O_2 and produce CO_2 by respiration and decomposition of organic matter, which produces carbonic acid, which controls the soil pH and availability of an important elements to the reproduction of organisms. When calculating the R/S reached 14 indicating that the number of

Table 3. Soil bacteria number (x10^5 CFU g^{-1} soil) of different P fertilizer treatments under sole and intercropping system of corn

(kg P ha^{-1})	P source	Rhizosphere soil			Bulk soil		
		Sole corn	Corn+cowpea	Average	Sole+corn	Corn+cowpea	Average
0	CSP	77.44a	82.27a	79.85a	5.98a	6.32a	6.15a
	DAP	77.44a	82.27a	79.85a	5.98a	6.32a	6.15a
25	CSP	78.60a	102.66b	90.63b	6.45a	6.52a	6.48a
	DAP	125.88c	107.66bc	121.77cd	7.41a	7.46a	7.44a
50	CSP	106.21b	126.33c	116.27c	8.73a	9.66a	9.19a
	DAP	106.60b	151.77d	129.18d	10.79a	9.84a	10.32a
Average		95.36A	110.49B		7.56A	7.69A	

CSP: Concentrated superphosphate; DAP: Diammonium phosphate. Values followed by different letters are significantly different among treatments with P< 0.05

bacteria has been clearly affected by the rhizosphere, and numbers have doubled compared to the bulk soil, and this percentage may be appropriate in the root zone of a non-leguminous plant.

Number of fungi: The number of fungi increased by increasing the levels of phosphorus at the rhizosphere soil comparison at bulk soil for levels of 0, 25 and 50 kg P ha^{-1} (Table 4). These results are similar to Liu et al(2012) and Tang et al (2015). This may be attributed to the ability of fungi to compete and grow in acidic media after addition of phosphate fertilizers with an acidic effect and phosphorous represents 0.5 - 1% of the dry weight for fungi mycelium.

No significant differences between the two types fertilizers were observed in the rhizosphere soil and the bulk soil. Sole cropping of corn significantly increased the number of fungi as compared to intercropping of corn with cowpea , and this result was contrary to the results of the bacteria (Table 3). Increasing the numbers of bacteria and actinomycetes at intercropping system leads to a decrease in the number of fungi and improved of the ratio of bacteria: fungi. Increasing the number of bacteria is important to increase the microbial activity and improve the chance of eliminating pathogens Yang et al (2016). This result is may be in favor of the intercropping system, because fungi are among the organisms that can cause many diseases for plants (Brussaard et al 2007).

The number of fungi of concentrated superphosphate treatments was higher than the numbers of fungi of diammonium phosphate treatments at the level of 50 kg P ha^{-1} (Table 4) while the fungi number of diammonium phosphate treatments was higher than the number of fungi of concentrated superphosphate treatments at the level 25 kg P ha^{-1}. It is observed that the treatment included the addition of superphosphate fertilizer at a level of 50 kg P ha^{-1} surpassed the rest treatments , with an increase ranging between 5 -

47% at the rhizosphere soil and ranged between 14-209% at bulk soil . The lowest values were in control treatment (no phosphate fertilizer was added). The superiority of sole cropping over intercropping in most phosphate fertilizer treatments, but without significant effect on both the rhizosphere soil and the bulk soil.

It was showed that there is a significant increase in the number of fungi in the rhizosphere soil compared to the bulk soil respectively this is due to the diversity of root exudates and their interaction with each other leads to the creation of a rich environment for biodiversity and an increase in the number of microbes in the rhizosphere. These results are in agreement with Yan et al (2008), Qin et al (2017) and Xu et al (2018). Many studies indicate that the effect of the rhizosphere on fungi and actinomycetes is very small compared to the numbers of bacteria, as the roots do not cause the quantitative change of fungi, but only encourage the growth of certain species at the expense of others. In addition to that the dominant species in the rhizosphere differ according to the type of plant, plant age and the soil type. It is clear when calculating the R/S ratio as it reached within 2, while the bacteria had about 14 (Fig. 1). Al-Hammadi (2014) obtained similar results as the number of fungi increased in the rhizosphere of barley or beans by about 10 times as compared with the bulk soil. It concluded that the root exudates may help germinate the dormant stages of many fungi that remain dormant due to their inability to compete with other microorganisms.

Number of actinomycetes: Number of actinomycetes in rhizosphere soil and bulk soil were increased significantly with increasing phosphorus levels (Table 5). for the rhizosphere soil for the bulk soil at levels of 0, 25 and 50 kg P ha^{-1}. These results are in agreement with Tang et al (2015) However, Mander et al (2012) found that increasing the level of phosphorous reduces the numbers of actinobacteria while

Table 4. Soil fungi number (x 10^3 CFU soil) of different P fertilizer treatments under sole and intercropped system of corn

(kg P ha^{-1})	P source	Rhizosphere soil			Bulk soil		
		Sole corn	Corn+cowpea	Average	Sole+corn	Corn+cowpea	Average
0	CSP	13.66a	10.33a	11.99a	4.77a	4.11a	4.44a
	DAP	13.66a	10.33a	11.99a	4.77a	4.11a	4.44a
25	CSP	14.22a	13.33a	13.77ab	8.77a	8.99a	8.88b
	DAP	15.66a	15.66a	15.66b	10.55a	9.33a	9.94b
50	CSP	18.44a	16.38a	17.66c	13.66a	13.77a	13.71d
	DAP	17.10a	16.55a	16.82bc	12.66a	11.33a	11.99c
Average		15.45B	13.84A		9.19A	8.60A	

See Table 3 for details

increases their numbers at phosphorus deficiency. Liu et al (2012) indicated that the exploitation of microorganisms of available soil carbon as a source of energy depends on the availability of nutrients, and phosphorus is one of the most specific elements in that and thus in the development of microbes numbers and activity Phosphorus constitutes 1-3% of the dry weight of actinomycetes cells form of nucleic acids, phospholipids, monoester phosphate or multiple phosphates. The fertilizer type had significant effect on the number of actinomycetes, as the diammonium phosphate was superior to the concentrated superphosphate at the rhizosphere soil comparison at bulk soil, respectively. These results were similar with the results of the bacteria (Table 3), which were attributed to the high solubility of the diammonium phosphate and the abundance of nutrients (N and P) together, and this is reflected in the increase in the number of organisms in the soil, including actinomycetes.

The significant higher value of actinomycetes was recorded when corn cultivated with cowpea as compared with sole corn (Table 5). These results were similar to the results for bacteria (Table 3), but it differed from the results for fungi (Table 4). These results are in agreement with Tang et al (2015) and Qin et al (2017) and this to the fact that the presence of two different crops in the same place leads to increased exudates of roots and residues of roots, which provide greater energy sources and encourages the reproduction and increase of their numbers . The number of actinomycetes increased by increasing the levels of addition of phosphate fertilizer for both sources and in the rhizosphere soil and bulk soil (Table 5). This result shows the superiority of the diammonium phosphate at all levels due to the presence of two elements in the fertilizer component added at the same time in addition to the neutral reaction for this fertilizer, which does not allow to lose a large percentage of phosphorus. Intercropping system recorded higher values of actinomycetes number at all fertilization treatments in the
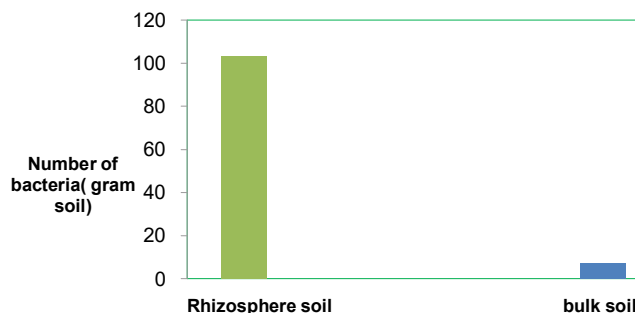

Fig. 1. Number of bacteria (10^5 CFU g^{-1}soil) in the rhizosphere and bulk soils of maize plant
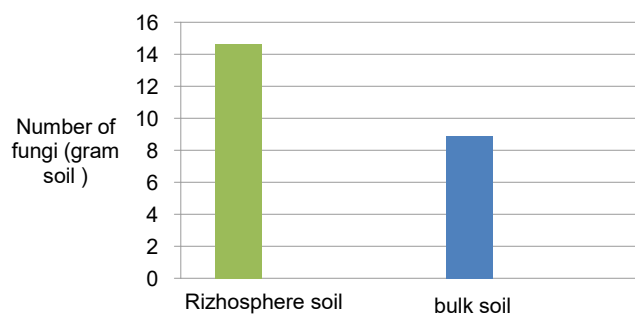

Fig. 2. Number of fungi (x 10^3 CFU g^{-1} soil) in the rhizosphere soil and bulk soil of the maize plant
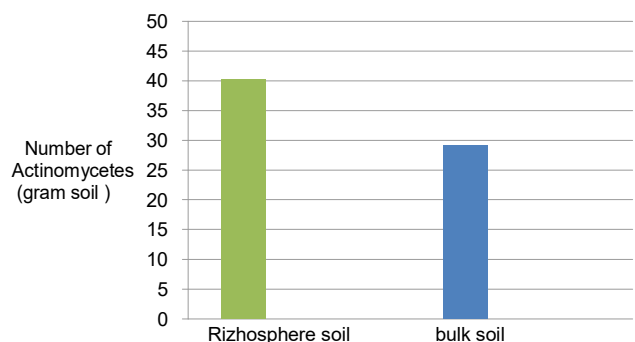

Fig. 3. Number of actinomycetes (10^5 CFU g^{-1} soil) in the rhizosphere soil and bulk soil of the maize plant

Table 5. Soil actinomycetes r (x 10^3 CFU g^{-1} soil) of different P fertilizer treatments under sole and intercropped system of corn

(kg P ha^{-1})	P source	Rhizosphere soil			Bulk soil		
		Sole corn	Corn+cowpea	Average	Sole+corn	Corn+cowpea	Average
0	CSP	24.00a	31.99a	27.99a	20.99a	20.99a	20.99a
	DAP	24.00a	31.99a	27.99a	20.99a	20.99a	20.99a
25	CSP	35.88a	46.33a	41.10b	28.00a	28.55a	28.27b
	DAP	44.60a	51.33a	47.96c	32.66a	32.55a	32.60bc
50	CSP	40.11a	48.22a	44.16bc	35.00a	32.99a	33.00cd
	DAP	47.33a	58.33a	52.83d	38.99a	36.33a	37.66d
Average		35.98A	44.70B		29.44A	28.73A	

See Table 3 for details

rhizosphere soil, while there were no clear differences between the two cropping systems at bulk soil, and this confirms the effective role of root exudates in controlling living numbers and improving this role by the presence of two different plants in the quantity and type of secretions. There were greater numbers of actinomycetes in the rhizosphere soil compared with bulk soil with significant differences (Fig. 3). This is due to the root exudates in the rhizosphere encourage the growth of organisms and increase theirnumbers . Al Hammadi (2014) and Tang et al (2015) observed an increase in the number of actinomycetes in the root zone compared to the bulk soil. The roots is little compared to bacteria, as the R/S value ranges between 1/2 - 1/3, but under certain conditions, such as those available around the roots of old plants this percentage may increase. Al-Hammadi (2014) also obtained an increase in the number of actinomycetes in the rhizosphere of beans and barley at the last stages of growth due to the increase of difficult decomposed substances such as peptides, organic acids, amino acids, biotin, inositol and choline, which are needed in their growth after the end of the easy decomposed substances. Calculating the R/S value for the present study, it was 1.4, which is less than that of bacteria and fungi.

CONCLUSIONS

The addition of diammonium phosphate fertilizer is more suitable for the soil under study compared to concentrated superphosphate fertilizer concerning of increasing the biological activity of the soil. To confirm this, experiments can be conducted on other soils from the region. The use of cowpeas as a support crop for corn led to the improvement of the micro-phylogenetic condition of the soil and increased the number of microbes, especially in the rhizosphere compared to the cultivation of sole corn, which resulted in increasing the availability of nutrients in the root zone and improving plant growth.

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Role of Potassium Fertilizers Source and Bio-fertilization on Nitrogen, Phosphorus and Potassium Availability and Productivity of Mungbean Grown under Water Stress

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Abstract: An experiment was carried out throw autumn of 2020 at Baghdad University, to study the effect of potassium fertilizer sources and bio-fertilization on soil potassium availability and carried water stress. The experiment included three main factors, the first one contains three levels of water stress symbolizing W_1, W_2, W_3, respectively. The second factor was adding two sources of potassium fertilizers (K_0, K_1, K_2 respectively), and the third factor was by adding biofertilizer (*Bacillus mucilagnosus*) with and without adding, symbolizes it (B_0, B_1) sequentially, it was designed with using split split plot design and randomized complete block design . Water stress treatment resulted in depletion of 60% from water availability and gave the highest value compared with other traits concentrations at harvest. Potassium hydrogen sulfate fertilizer superiority and an additional level (50 kg h^{-1}) gave the highest value compared with other traits.3- Biofertilizer superior and gave the highest value compared with other traits, and concentrations at harvest.4- Tripartite overlap superiority treat W_2 K_2 B_1 in total potassium, available nitrogen, available phosphorus, available potassium concentrations at harvest.

Keywords: Potassium hydrogen sulfate fertilizer, *Bacillus mucilagnosus*

Moisture stress is one of the environmental stresses facing agricultural expansion all over the world, especially in arid and semi-arid areas, especially with climate change in recent decades, which reduces food production at a time when the population increases (Song et al 2010). This issue draws attention to the fact that water sources will become scarcer in the future, which calls for taking care of these sources and not wasting them in order to obtain the highest productivity with the least amount of water and thus high-water productivity. Potassium plays in improving the ability of crops to withstand water stresses and will have an important role in increasing the tolerance of crops to environmental stresses, including water stress and the possibility of production even under moisture stress (Cakmak 2005, Amtmann et al 2008). Potassium has several roles to play to resist environmental stresses, including improving the transfer of photosynthesis products, regulating the mechanism of opening and closing stomata, improving the efficiency of water use, and strengthening the root deepening force (Romheld and Kirkby 2010). Biological fertilization is one of the modern fertilization technologies that, by integrating it with organic and mineral fertilization, can obtain good productivity and a safe environment (Ali et al 2014). Biofertilization, through the use of some plant growth stimulating organisms (PGPR), provides some nutrients and contributes to the decomposition of organic matter, nutrient

cycling, and protects the plant from some biological and environmental stresses, thus improving productivity in quality and quantity (Ali et al 2014). There are some types of bacteria that can contribute to improving the readiness of potassium to the roots of the plant, including *Bacillus mucilaginosus* (Saiyad et al 2015 Meena et al 2015 Bhattacharya et al 2016 Al-shammari et al 2018 and Alzuhayri 2019).

MATERIAL AND METHODS

An experiment was carried out in autumn of 2020 at Baghdad University using split split plot design in randomized complete block design and three repeaters. The whole plot (water stress), for irrigation factors (40, 60, 80% from water availability), while sub plot (potassium fertilizer) represented potassium levels (0, 50 and 100 kg h^{-1}) using potassium hydrogen sulfate and potassium sulfate symbolizes (K0, K1, K2 respectively), and sub sub plot (biofertilizer) with the addition of biofertilizer (*Bacillus mucilagnosus*) with adding and not adding (B_0, B_1). The land was plowed and divided into plots (3*3 m) and lines in the plots at a distance 60 cm between line 25 cm among plants with density of 66666 plant h^{-1}). There was left space between plots and repeaters to avoid leakage of water between factors. Soil samples from A horizon (0-30 cm) were taken from the different sites in the field, dried, grounded to pass a 2 mm sieve, and analyzed for some physical and chemical soil properties. The results of

the analyses are listed in Table 1. Nitrogen and phosphorous were applied later on at a rate of (40 kg^N h^{-1}, 75 kg^P h^{-1}) in the form of diammonium phosphate.

The bacterial inoculate loaded on peat moss was received from the Agricultural Research Office, Ministry of Science and Technology. The seeds were sterilized with a solution of Sodium hypochlorite 1% (NaClO) and washed with distilled water three times (Hafez 2001). Putting gum arabic and then adding the loaded bio-fertilizer. Potassium was added after two weeks of germination. Irrigation was carried out by plastic tubes connected to pump and meter is installed on the tube to measure the water passing through the tube in liters. Equal amounts of water were added to all plots when planting and to the limits of field capacity. The gravimetric humidity was measured and then transformed into the depth of irrigation water according to the humidity levels used in the experiment. Irrigation is carried out when 40, 60, and 80% of the water availability is lost. The gravimetric method was used by sampling by an auger to find out the moisture content in the soil for the purpose of irrigation according to the treatments.

RESULTS AND DISCUSSION

Total potassium concentration in the soil at the harvest stage (%) indicates that W_2 was significantly, superior with highest average total potassium concentration in the soil (2.07 %) and lower average was 1.72% at W_1 with an increase rate of 20.93%. It was also superior to potassium hydrogen sulfate fertilizer (K_2) significantly over the potassium sulfate fertilizer and gave the highest total potassium concentration in the soil reaching 2.22% compared to the lowest average of 1.55% when potassium is not added (K_0) with an increase in the amount 43.22%.

Bacillus mucilaginosus: The average concentration of total potassium in the soil, was in B_1 was significantly superior in increasing the concentration with highest of 2.03% compared to the lowest value in B_0, which gave a value of 1.76% with an increase in the amount 15.34%. As for the interaction of the level of water availability with potassium sources there were significant differences in the interaction, and the highest average concentration of potassium was 2.38% in treatment W_2K_2 and the lowest average was 1.45% with W_1K_0 with an increase in the amount 64.13%. The interaction between the level of water availability and biofertilization significantly

affected the total potassium concentration in the soil. The highest average concentration of 2.26% was in W_2B_1 with an increase of 43.03% from treatment W_1B_0 (1.58%). The interaction between potassium sources and biofertilization was significant in increasing the total potassium concentration in the soil. The highest mean potassium concentration was 2.33% in treatment K_2B_1 and the lowest average for this was 1.44% in K_0B_0 an increase by 61.80%. The tripartite overlap between the level of water availability, the source of potassium, and the biofertilization had a significant effect on the total potassium concentration in the soil, as it was the highest mean was in $W_2K_2B_1$ (2.44%) and the lowest average was 1.33% in $W_1K_0B_0$ an increase by 83.45%.

Available nitrogen concentration in the soil at harvest (mg kg^{-1}): The W_2 was significantly superior with highest average nitrogen concentration in the soil (59.82 mg kg^{-1}) and was lower average W_1 (47.02 mg kg^{-1} at level) with an increase of 27.22%. It is also superior to potassium hydrogen sulfate fertilizer (K_2) significantly over the potassium sulfate fertilizer, as it gave the highest nitrogen concentration in the soil reaching 62.66 mg kg^{-1} compared to the lowest average of 50.16 mg kg^{-1} when potassium is not added (K_0) with an increase of 24.92%. The results in the also showed the significant effect of biofertilization on the bacteria *Bacillus mucilaginosus*. The highest average concentration of nitrogen the soil was in B_1 and was significantly superior in increasing the concentration (58.03mg kg^{-1}) compared to the lowest in B_0 (52.28 mg kg^{-1} w) with an increase in the amount by 10.99%. There were significant differences in the interaction, and the highest average concentration of nitrogen was 72.00 mg kg^{-1} inW_2K_2 (Table 3) and the lowest average was 45.16 mg kg^{-1} in W_1K_0 an increase by 59.43%. The interaction between the level of water availability and biofertilization significantly affected the nitrogen concentration in the soil. The highest average concentration of 67.44 mg kg^{-1} in W_2B_1 an increase in the amount of 47.08% from treatment W_1B_0 (45.85 mg kg^{-1}). The interaction between potassium sources and biofertilization was significant in increasing the nitrogen concentration in the soil. The highest mean nitrogen concentration was 64.22 mg kg^{-1} in K_2B_1)and the lowest average was 49.22 in K_0B_0 an increase of 30.47%. The tripartite overlap between the level of water availability, the source of potassium, and the biofertilization had a

Table 1. The results of the analyses

Soluble cations and anions (Mmole L^{-1})							Soil texture	O.M (%)	Available (Mmole L^{-1} soil)			EC (dS.m^{-1})	pH
CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Na^+	Mg^{2+}	Ca^{2+}			K	P	N		
Nil	1.5	20.14	Nill	3.2	5.0	9.12	C.L	6.8	89.14	3.45	2 8	1.0	7.64

Table 2. Effect of potassium fertilizer source and biofertilizer under water stress conditions on total potassium concentration at harvest stage (%)

Potassium levels	BIO	W1 40% of water availability	W2 60% of water availability	W3 80% of water availability	K*B
0	B0	1.33	1.49	1.51	1.44
	B1	1.58	1.93	1.51	1.67
K1	B0	1.45	1.84	1.97	1.75
	B1	1.83	2.43	2.08	2.11
K2	B0	1.96	2.33	2.03	2.10
	B1	2.18	2.44	2.39	2.33
LSD 0.05			0.05		0.54
					K
K*W		1.45	1.71	1.51	1.55
		1.64	2.13	2.02	1.93
		2.07	2.38	2.21	2.22
LSD 0.05			0.43		0.23
					B
B*W		1.58	1.88	1.83	1.76
		1.86	2.26	1.99	2.03
LSD 0.05			0.03		0.23
W		1.72	2.07	1.91	
LSD 0.05			0.03		

Table 3. Effect of potassium fertilizer source and biofertilizer under water stress conditions on available nitrogen concentration at harvest stage (mg kg^{-1})

Potassium levels	BIO	W1 40% of water availability	W2 60% of water availability	W3 80% of water availability	K*B
0	B0	43.33	59.00	45.33	49.22
	B1	47.00	57.67	48.67	51.11
K1	B0	44.89	47.00	47.67	46.52
	B1	46.33	67.67	62.33	58.78
K2	B0	49.33	67.00	67.00	61.11
	B1	51.00	77.00	64.67	64.22
LSD 0.05			0.43		0.68
					K
K*W		45.16	58.33	47.00	50.16
		45.61	57.33	55.00	52.64
		50.16	72.00	65.83	62.66
LSD 0.05			0.20		0.88
					B
B*W		45.85	57.66	53.33	52.28
		48.11	67.44	58.55	58.03
LSD 0.05			0.37		0.16
W		47.02	59.82	55.94	
LSD 0.05			0.76		

significant effect on nitrogen concentration in the soil, highest was 77.00 mg kg^{-1} in$W_2K_2B_1$) and the lowest average was 43.33 mg kg^{-1} in $W_1K_0B_0$ an increase by 77.70%.

Available phosphorus concentration in the soil at harvest (mg kg^{-1}): The W_2 was with significantly highest average phosphorus concentration in the soil (25.8 mg kg^{-1}) and lower average was 23.6 mg kg^{-1} in W_1 With an increase of 9.32%. It is also superior to potassium hydrogen sulfate fertilizer (K_2) significantly over the potassium sulfate fertilizer, withe highest phosphorus concentration in the soil reaching 27.9 mg kg^{-1} as compared to the lowest average of 21.4 mg kg^{-1} in K_0 with an increase of 30.37%. The results effect of biofertilization on the bacteria *Bacillus mucilaginosus* presented in Table 4. The highest average concentration of phosphorus in the soil was in B_1 significantly superior in increasing the concentration and gave the highest value of 27.4 mg kg^{-1} compared to the lowest value in B_0, which gave a value of 22.2 mg kg^{-1} with an increase of 23.42%. As for the interaction of the level of water availability with potassium sources, showed significant differences with highest average concentration of phosphorus (29.7 mg kg^{-1}) in W_2K_2 and the lowest average was 21.2 mg kg^{-1} in W_1K_0 an increase of 40.09 %. The interaction between the level of water availability and biofertilization significantly affected the

phosphorus concentration in the soil. The highest average concentration was 28.4 mg kg^{-1} in W_2B_1 an increase of 31.48% from W_1B_0 with the lowest of 21.6 mg kg^{-1}. The potassium sources and biofertilization was had significant effect in increasing the phosphorus concentration in the soil. The highest mean phosphorus concentration was 31.7 mg kg^{-1} in K_2B_1 and the lowest was 20.9 mg kg^{-1} in treatment K_0B_0 with an increase by 51.67%. The tripartite overlap between the level of water availability, the source of potassium, and the biofertilization had a significant effect on phosphorus concentration in the soil land was the highest was 34.6 mg kg^{-1} in $W_2K_2B_1$ and the lowest was 19.9 mg kg^{-1} in $W_1K_0B_0$ with an increase of 73.86%.

Available potassium concentration in the soil at harvest (mg kg^{-1}): The W2 was significantly superior with highest average of 143.3 mg kg^{-1} while was with lower average of 132.5 mg kg^{-1} at level W_1 (Table 5) with an increase of 8.15%. The potassium hydrogen sulfate fertilizer K_2 was significantly superior over the potassium sulfate fertilizer with highest potassium concentration in the soil reaching 160.0 mg kg^{-1} compared to the lowest average of 114.3 mg kg^{-1} when potassium is not added (K_0) with an increase in the amount 39.98%. The effect of biofertilization on the bacteria *Bacillus mucilaginosus* indicate highest average concentration of

Table 4. Effect of potassium fertilizer source and biofertilizer under water stress conditions on available phosphorus concentration after harvest stage (mg kg^{-1})

Potassium levels	BIO	W1 40% of water availability	W2 60% of water availability	W3 80% of water availability	K*B
0	B0	19.9	22.7	20.2	20.9
	B1	22.6	20.0	23.3	21.9
K1	B0	20.6	22.4	21.6	21.4
	B1	27.2	30.7	26.6	28.1
K2	B0	24.3	24.9	23.3	24.1
	B1	27.2	34.6	33.3	31.7
LSD 0.05			0.3		0.8
					K
K*W		21.2	21.3	21.7	21.4
		23.9	26.5	24.1	24.8
		25.7	29.7	28.3	27.9
LSD 0.05			0.6		0.9
					B
B*W		21.6	23.3	21.7	22.2
		25.6	28.4	27.7	27.4
LSD 0.05			0.4		0.3
W		23.6	25.8	24.8	
LSD 0.05			0.5		

potassium the soil, in treatment B₁ and was significantly superior in increasing the concentration and gave the highest value of 147.9 mg kg⁻¹ compared to the lowest value in B₀ (130.7 mg kg⁻¹) with an increase of 13.15%. As for the interaction of water availability with potassium sources, there were significant differences with highest average concentration of potassium of 165.4 mg kg⁻¹ in $W_2K_2$and and the lowest average was 111.0 mg kg⁻¹ in W_1K_0 an increase in the amount 49.00%. The interaction between the level of water availability and biofertilization significantly affected the potassium concentration in the soiland the highest average concentration of 154.3 mg kg⁻¹ was in W_2B_1 an increase in the amount of 24.42% from treatment W_1B_0 with lowest value 124.0 mg kg⁻¹. The interaction between potassium sources and biofertilization was significant in increasing the potassium concentration in the soil, The highest mean potassium concentration was 169.0 mg kg⁻¹ in K_2B_1 and the lowest average of 99.9 in K_0B_0 an increase of 69.1%. The tripartite overlap between the level of water availability, the source of potassium, and the biofertilization had a significant effect on potassium concentration in the soil, as it was the highest (177.1 mg kg⁻¹) in $W_2K_2B_1$ and the lowest average of 99.1 mg kg⁻¹ in $W_1K_0B_0$ with increase of 78.7%.

An increase in the concentration of total potassium is observed in the role of biological fertilization, by producing organic acids that increase the acidity of the rhizosphere soil and complex compounds are formed, thus turning the mineral potassium into a soluble form (Uroz et al 2009). This is due to the role of biofertilization in the decomposition of organic matter and fixation of atmospheric nitrogen, causing an increase in the readiness of atmospheric nitrogen (Hassan 2011). The water stress reduced the concentration of available nitrogen during the growth stages. AL-Fuhaymi and Abdullatif (2018) observed highest value of nitrogen after harvesting maize was 68.33 at 75% water stress, nitrogen remained in a large amount in the soil because the water-stressed plant cannot fully absorb the element, potassium replaces the NH₄ ion installed on the surfaces of colloids and releases the ammonium ion and soil nitrogen increases. The increase in the concentration of available phosphorous in the soil was observed during the growth stages. The increase is also attributed to the role of fertilizer addition, especially potassium hydrogen sulfate, for its role in increasing the readiness of phosphorus. Acidity-producing fertilizers reduce the degree of reaction of the radical ocean, which affects the readiness of elements, including phosphorus, where the most appropriate reaction degree for the readiness of phosphorus is 6.2-6.8 (Ali 2015). Water stress also affected the decrease in the concentration of available phosphorous. Al-Tamimi (2012) showed that the

Table 5. Effect of potassium fertilizer source and biofertilizer under water stress conditions on available potassium concentration at harvest stage (mg kg⁻¹)

Potassium levels	BIO	W1 40% of water availability	W2 60% of water availability	W3 80% of water availability	K*B
0	B0	99.1	100.5	100.3	99.9
	B1	122.9	132.9	130.1	128.6
K1	B0	133.9	142.9	147.1	141.3
	B1	143.9	153.1	141.3	146.1
K2	B0	139.1	153.8	160.1	151.0
	B1	161.9	177.1	168.1	169.0
LSD 0.05			0.5		0.9
					K
K*W		111.0	116.7	115.2	114.3
		128.9	148.0	144.2	140.3
		150.5	165.4	164.1	160.0
LSD 0.05			0.8		0.5
					B
B*W		124.0	132.4	135.8	130.7
		142.9	154.3	146.5	147.9
LSD 0.05			0.3		0.2
W		132.5	143.3	141.1	
LSD 0.05			0.7		

absorption of nutrients (N-P-K) decreases with increasing moisture stress, which in turn leads to a decrease in the regularity of photosynthesis and cell division and an increase in dry matter accumulation due to stress. There is also an increase in the concentration of available potassium in the soil during the growth stages. This is due to the role of biological fertilization in dissolving mineral potassium through the production of organic acids, chelation of minerals, and transforming it into potassium ready for absorption by the plant (Bahadur 2014). The increase is also attributed to the role of fertilizer addition, especially potassium hydrogen sulfate, which works because of its acidic property to reduce the pH of the soil, which increases the liberated potassium and decreases the exchange of aluminum, which determines from potassium on the surfaces of seasoning of the metal and this is consistent with(Havlin et al 2019). Water stress also affected the decrease in the concentration of available potassium during the growth stages as a result of the decrease in the mobility of potassium (Marschner 2012).

Plant height (cm): W_3 was significantly superior with highest average plant height in the soil, (74.26 cm). The minimum was 71.81 cm at W_1 with an increase of 3.43%. It was also superior to potassium hydrogen sulfate fertilizer (K_2) significantly over the potassium sulfate fertilizer, as it gave the highest plant height of 75.21 cm compared to the lowest average of 70.88 cm when potassium is not added (K_0) with an increase in the amount 6.10%. The effect of biofertilization with the bacteria *Bacillus mucilaginosus* indicate highest average of plant height and treatment B_1 was significantly superior in increasing the concentration and gave the highest value of 74.42 cm compared to the lowest in B_0, which gave a value of 72.16 cm with an increase in the amount 3.13%. There were significant differences in the interaction, and the highest average plant height was 76.92 cm inW_2K_2 and the lowest was 70.23 cm (Table 6) in W_1K_0 an increase of 9.52%. The interaction between the level of water availability and biofertilization significantly affected the plant height. The highest average of 75.37 cm in W_2B_1 an increase in the amount of 7.13% from treatment W_2B_0 which gave the lowest of 70.35 cm. The interaction between potassium sources and biofertilization, was significant in increasing the plant height. The maximum plant height was 76.48 cm in K_2B_1 and the lowest was 70.17 cm when the treatment in K_0B_0 an increase in the amount 8.99%. The Tripartite overlap between the level of water availability, the source of potassium, and the biofertilization had a significant effect on plant height, as it was the highest mean for this treatment 78.09 cm in $W_2K_2B_1$ and the lowest average was 68.60 cm in $W_1K_0B_0$ an increase in the amount 13.83%. Effect of potassium concentration in

Table 6. Effect of potassium fertilizer source and fertilization in conditions of moisture stress conditions on plant height (cm)

Potassium levels	BIO	W1 40% of water availability	W2 60% of water availability	W3 80% of water availability	K*B
0	B0	68.60	71.83	70.10	70.17
	B1	71.87	72.10	70.80	71.59
K1	B0	71.83	71.90	73.40	72.38
	B1	74.40	75.93	75.27	75.20
K2	B0	70.63	75.75	75.47	73.28
	B1	73.67	78.09	77.67	76.48
LSD 0.05			1.06		0.59
					K
K*W		70.23	71.96	70.45	70.88
		72.89	73.91	74.33	73.71
		72.15	76.92	76.57	75.21
LSD 0.05			0.50		0.76
					B
B*W		70.35	73.16	72.99	72.16
		73.31	75.37	74.58	74.42
LSD 0.05			0.55		0.07
W		71.81	74.26	73.78	
LSD 0.05			0.15		

increasing plant height is attributed to the role of biological fertilization in increasing potassium concentration in growth stages. The biological fertilization influence the rate of plant growth through the secretion of stimulants that stimulate root growth and increase the readiness of nutrients (Kalequzaman and Hossain 2008). The effect of water stress in reducing the height of the plant is a result of the decrease in the water potential of the plant cells, causing the lack of division and elongation of plant cells when the water is ready for the plant (Mehrvarz et al 2013).

Total yield (Mg h^{-1}): The W_3 was significantly superior and gave the highest average total yield of 2.288 Mg h^{-1} and was lower in W_2 average of 1.918 Mg h^{-1} was in W_2 with an increase of 19.29%. The potassium hydrogen sulfate fertilizer (K_2) was significantly superior over the potassium sulfate fertilizer, as it gave the highest total yield reaching 2.131 Mg h^{-1} compared to the lowest average of 1.786 Mg h^{-1} when potassium is not added (K_0) with an increase by 19.31%. The results in the same table also show the effect of biofertilization on the bacteria *Bacillus mucilaginosus* The highest average total yield, as the treatment B_1 was significantly superior in increasing the concentration and gave the highest value of 2.097 Mg h^{-1} compared to the lowest value of the parameter B_0, which gave a value of 1.894 Mg h^{-1} with an increase in the amount 10.71%. As for the interaction of the level of water

availability with potassium sources there were significant differences in the interaction, and the highest average total yield was 2.247 Mg h^{-1} in W_2K_2 and the lowest average for this trait was 1.558 Mg h^{-1} in W_2K_0 an increase of 44.22%. The interaction between the level of water availability and biofertilization significantly affected total yield being highest average concentration of 2.237 Mg h^{-1} in W_2B_1 an increase of 31.20% from treatment W_1B_0 which gave the lowest value 1.705 Mg h^{-1}. As for the interaction between potassium sources and biofertilization, it was significant in increasing total yield. The highest mean total yield was 2.218 Mg h^{-1} in K_2B_1 and the lowest average for this trait was 1.643 Mg h^{-1} in K_1B_0 an increase of 34.996%. The tripartite overlap between the level of water availability, the source of potassium, and the biofertilization had a significant effect on total yield, and highest mean was 2.557 Mg h^{-1} in $W_2K_2B_1$ and the lowest average for this trait was 1.417 Mg h^{-1} in $W_1K_0B_1$ an increase of 80.451%. The increase in the total plant yield is attributed to the role of potassium-dissolving bacteria in increasing the weight of seeds through the production of a good amount of cytokinin (Biswas et al 2018). The fertilizer addition of potassium, especially acidic potassium sulfate, led to an increase in the weight of the pods and one hundred grains. The yield of one plant and the total yield of the plant increases the efficiency of the manufactured valley due to the increase

Table 7. Effect of potassium fertilizer source and biofertilizer under moisture stress conditions on the total yield (Mg h^{-1})

Potassium levels	BIO	W1 40% of water availability	W2 60% of water availability	W3 80% of water availability	K*B
0	B0	1.417	1.433	2.081	1.643
	B1	1.669	1.964	2.124	1.929
K1	B0	1.811	2.028	2.148	1.995
	B1	2.091	2.191	2.155	2.129
K2	B0	1.886	1.937	2.315	2.046
	B1	2.193	2.557	1.904	2.218
LSD 0.05			0.807		0.524
					K
K*W		1558	1.698	2.102	1.786
		1.951	2.109	2.151	2.062
		2.039	2.247	2.109	2.131
LSD 0.05			0.674		0.462
					B
B*W		1.705	1.799	2.181	1.894
		1.994	2.237	2.062	2.097
LSD 0.05			0.348		0.235
W		1.918	2.018	2.288	
LSD 0.05			0.263		

in the efficiency of the photosynthesis process and the transfer of manufactured materials in the leaves to their places of sadness in the grains, which is reflected positively on pill weight (Ebrahimi et al 2011). The effect of moisture stress in reducing the weight of one hundred grains, the yield of one plant, and the total yield of the plant, moisture stress works on the formation of seeds and forces the plant to complete its life cycle and shorten the growth stages within a short period of time and form small wrinkled atrophic seeds due to the speed of growth and the speed of seed filling (Nasri et al 2012).

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Effect of Vermicompost on Survival of Bacterial Biofertilizer in Soil and Role of Interaction with Phosphate Fertilizer on Availability of Soil Nitrogen and Phosphorous

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Abstract: This study included two experiments one was a laboratory experiment that carried out in the Microbiology Laboratory, Department of Field Crops, College of Agriculture, University of Wasit with vermicompost and two bacteria isolates (*Bacillus megaterium* and *Azotobacter chroococcum*) were with a completely randomized design. The aim of this experiment was to evaluate the effect of incubation of these two bacterial isolates with or without vermicompost in the soil on its survival during three incubation periods (one day, 14 day, and 30 days). Then, a serial dilutions method was used to count the numbers of bacteria after culturing its on the nutrient agar medium for each period time. The results showed that both bacteria isolate in their numbers in the presence of vermicompost, the number of *Bacillus megaterium* reached to 10^6 * 199.3 CFUg^{-1}soil. *Azotobacter chroococcum* reached to 10^6 * 152.4 CFUg^{-1} soil, compared to the presence of soil alone in which the bacterial numbers decreased. During the different periods of time, increased in the presence of vermicompost and ranged between (10^6 * 160 - 10^6 * 252.7) and between (106 * 133 - 106 * 188.7CFU g^{-1}) of soil during one day - 30 days for *Bacillus megaterium* and *Azotobacer chroococcum*, respectively, compared with it indensity when in the presence of soil only, in which the bacterial density decreased with time due to the lack of energy and food sources. The second experiment was carried at the field of the station of Agricultural Research and Experiment of Wasit Governorate , Agriculture Directorate in 2019-2020 season, The aim of this experiment was to study the effect of three types of fertilizers and the interaction between them on the availability of nitrogen and phosphorous. This experiment included 27 treatments that consisted from the interaction of three factors, the first is biofertilizer with three levels (without addition and a combination consisting of *Bacillus megaterium* + *Glomus mosseae* and the second combination consisting of *Glomus mosseae*+ *Azotobacter chroococcum*+ *Bacillus megaterium* + *Azosprillum brasilense*). The second factor was vermicompost, with three levels (without adding, 3 and 6 Mg h^{-1}), while third factor was the mineral phosphate fertilizer that added in the form of triple superphosphate at three levels (0, 40 and 80%) of the fertilizer recommendation. The treatments were distributed in a factorial experiment with the randomized block design with three replicates. The results showed that the addition of Biofertelizer, vermicompost or mineral fertilizers, each separately, had a significant and positive effect on the availablity of nitrogen and phosphorous in the soil compared with the control treatment. In the case of interaction between the biofertilizer added in the form of a complete cambination with 6 Mg h^{-1} of vermicompost and gave higher averages of available soil nitrogen and phosphorous compared with the control treatment. The triple interaction between biofertilizer in the form of a complete combination with 6 Mgs h^{-1} of vermicompost and 80% of the fertilizer recommendation achieved more significant increase in the available nitrogen and phosphorous than in the case of adding fertilizers singly or in a binary interaction or with. The control treatment that gave the lowest concentrations.

Keywords: Biofertilizer, *Bacillus megaterium*, *Azotobacter chroococcum*, Earthworms

Soil is the appropriate environment that promotes the growth of microorganisms, whether beneficial or harmful bacteria or fungi. It is abundantly located within the root zone, as it is estimated that every 1 cm^3 contains millions of diverse microbes that are important for the existence of the vegetation cover in terms of being responsible for providing it with the nutrients it needs, whether they are major or minor (Pathma et al 2011b). Earthworms are responsible for the natural tillage of the soil in which they are located affects the microbial community and the chemical properties of the soil, as it works to break down large masses in the soil and break up plant residues. Thus, increasing the abundance of organic matter available for decomposition by microorganisms and has a role in promoting the growth of beneficial organisms,

suppressing pathogens and promoting plant growth by increasing its enrichment with nutrients and the recycling that these worms carry out through several natural biological reactions to decompose organic matter (Maboeta and Van Rensburg 2003).

Biofertilizers have become one of the most important types of fertilizers, as they provide the nutrients needed by the plant, such as nitrogen, which is biologically fixed in a symbiotic or free form, as well as phosphorous. These biofertilizers increase availability of nutrients for to the plant through dissolution processes in addition to being cheap in price and do not cause any pollution compared to mineral fertilizers. These microorganisms used in biofertilization include *Azotobacter chroococcum and Bacillus*

smegaterium. These bacteria are heterotrophic and need source of energy and food through the availability of organic matter that they decompose and benefit from first and recycle the elements to the soil. The excessive use of chemical fertilizers has become responsible for a lot of environmental damage to soil health in terms of affecting microbial activity, the lack of nutrients availability and the increase of pollutants. Therefore, modern technologies have been used to reduce these damages, which are environmentally friendly and economically cheap fertilizers, which are biofertilizers, that consist of microorganisms added to soil and contribute to increasing the availability of nutrients. The second type are organic fertilizers. One of the most important and common in recent years is vermicompost, which is rich in nutrients and increases the activity of microorganisms that decompose organic matter, and thus it is considered as a complementary fertilizer to mineral fertilizers. Therefore, the study aimed to evaluate effect of vermicompost on the survival of *Bacillus megaterium* and *Azotobacter chroococcum* in soil and evaluation of the importance of biofertilizer and vermicompost under different levels of mineral fertilization in the availability of some nutrients in the soil.

MATERIAL AND METHODS

Laboratory experiment: Soil samples were collected from the rhizosphere of different crops from five agricultural fields in Wasit Governorate, and *Azotobacter chroococcum* and *Bacillus megaterium* were isolated and diagnosed in the Microbiology Laboratory, Department of Field Crops, College of Agriculture /Wasit University according to standard methods (Baron 2001). Several vermicompost was obtained from a private farm in Salah El-Din Governorate. Then, the incubation process for bacteria in the soil was carried out with the presence and absence of this organic fertilizer, as follows:

*Five g of agricultural field soil was placed in 100 ml volumetric flasks and sterilized by autoclave for an hour at a temperature of 121°C and a pressure of 15 pounds ang-2 for three consecutive days, A broth culture was prepared for *Bacillus megaterium* and *Azotobacter chrococcum* isolates, 1 ml of broth culture each bacterial isolate was added separately and with three replicates, The experiment included the following treatments:

1. Three glass flasks, each containing 5 gm of soil only, inoculated with 1 ml of *Azotobacter chrococcum* isolate inoculum.

2. Three glass flasks, each containing 5 gm of soil only, and inoculated with 1 ml of *Bacillus megaterium* isolate.

3. Three glass flasks, each containing 5 gm of soil, to which the equivalent of 10 Mg h^{-1} of vermicompost was added and inoculated with 1 ml of *Azotobacter chrococcum* inoculum.

4. Three glass flasks, each containing 5 gm of soil, to which the equivalent of 10 Mgs h^{-1} of vermicompost was added and inoculated with 1 ml of *Bacillus megaterium*

Field experiment: Field experiment was carried out during the 2019-2020 agricultural season at the Agricultural Research and Experiment Station of the Wasit Governorate, Agriculture Directorate a project for a horticultural development station Silty clay soil. Soil samples were taken from different locations of the field at a depth of 0-30 cm and mixed to determine some physical, chemical and fertility characteristics before planting (Table 1).

The soil was prepared for cultivation by plowing, smoothing and leveling, then preparing the furrow, then the field was divided into experimental units with an area of 6 m^2 (length 2 m and width 3 m). Each experimental unit in each block included three furrows, the width of each strand was 60 cm, and the distance between the two ridges was 60 cm. Then a drip irrigation system was installed and the distance between one drip and another is 40 cm, which is the same distance between one plant and another. 18 holes were prepared in each experimental unit planted with 18 plants. Vermicompost fertilizer was added to some of the holes according to the treatments in three levels: (V0, V1 and V2), which are equal to (0, 3 and 6) Mg h^{-1}. The biofertilizer was also added in three levels (B0, B1 and B2), which means (no addition (B0), the first combination (B1) consisting of *B. megaterium* +*G. mosseae* and the second combination (B2) consisting of *A. chroococcum* + *A. brasilense* + *B. megaterium* + *G. mosseae*. The triple superphosphate fertilizer was added (S0, S1, and S2), equivalent to (0, 40 and 80%) of the approved fertilizer recommendation (Ali 2012). Vital inoculums were prepared from the above-mentioned

Table 1. Chemical, physical and fertility properties of the study soil before planting

Soil properties		Value
$_{1:1}$Ec (ds m^{-1})		3.1
$_{1:1}$pH		7.36
CaCo$_3$	mg kg^{-1}	28.21
O.M		8.5
Available N		77
Available P	mg kg^{-1}	10.5
Available K		122
(C.F.U gm^{-1} Soil)		10^7×25.5
Sand		124
Silt		440
Clay		436
Texture		Silty Clay

bacterial isolates in a broth medium, then the roots of Cauliflower seedlings were immersed in these broth for 15 minutes and in the presence of 20% gum arabic, while triple superphosphate was added by making an incision to the side of the plant at a distance of 5 cm and a depth of 5 cm and in two batches during planting and the second at the beginning of the formation of flower discs (Fares and Abbas 2009). The planting took place on October 15, 2019 and harvested on 27 February, 2020. At the end of the experiment measered according to Bremner (1965) and available-made phosphorous using a spectrophotometer (Page et al 1982).

RESULTS AND DISCUSSION

Laboratory experiment: Some chemical and fertility properties of vermicompost in Table 2 and fertilizer contains nitrogen and phosphorous. Thus, it is a good source for supplying organisms and plants with these necessary elements in proportion to the needs of these organisms to carry out their vital activities, at rates of (3.498, 1.73 and 1.78%), respectively. There was decrease in the electrical conductivity, which reached 1.5 ds m^{-1}, and on the C/N Ratio, gave avalue of to 10.16. These results came in agreement with Al-Mamouri (2020).

There were significant differences in the number of bacteria for both genes, with or without vermicompost. The presence of vermicompost led to an increase in the population density to $10^6 * 175.9$ CFU g^{-1} soil. The number decreased to $10^6 * 134.8$ CFU g^{-1} soil in the event that vermicompost was not added to the soil. The incubation time significantly affected the number of bacteria. It was observed that a continuous increase in the number of bacterial cells with the progression of the time period until the arrival of the largest population density after 30 days of incubation amounted to $10^6 * 171.4$ CFU g^{-1} soil. The population density after 24 hours of incubation reached ($140.1 * 10^6$) CFU g^{-1} soil.

The results also showed a significant interaction between the two genes of bacteria and the addtion of vermicompost, as the total number of both bacterial genes increased by adding vermicompost, compared to the control soil and the

Table 2. Chemical and fertility properties of vermicompost

Property	Value	Unit
EC	1.5	ds m^{-1}
N total	3.49	%
P	1.73	%
K	1.78	%
Total carbon	35.54	%
C/N	10.16	

number of *Azotobacter chroococcum* increased from ($10^6*119.2$ to $10^6*152.4$) CFU g^{-1} soil. The increase in the number of *Bacillus megaterium* ranged from ($10^6 * 150.3$ to $10^6 * 199.3$ CFU g^{-1} soil. The interaction between the vermicompost and the time period showed a significant superiority of the soil medium with vermicompost when the incubation time was increased to 30 days in the number of cells ($10^6 * 220.76$ CFU g^{-1})soil compared with the soil medium not inoculated with vermicompost which recorded ($10^6*148.5$) CFU g^{-1} after 14 days. The bilateral interaction between the genes of bacteria and the time period, observed that the number of colonies increased significantly for both genes with the increase in the incubation time.

The triple interaction between the two genes of bacteria and the presence or absence of vermicompost during the time periods, indicated that two genes of bacteria *Azotobacter chroococcum* and *Bacillus megaterium* increase in its population density with the time period of 14 days and then decrease after 30 days with soil that not treated with vermicompost. The response of organisms with soil inoculated with vermicompost differed during the time periods, as the increase in the density of organisms continued positively until it reached its maximum density after 30 days of inoculation for both genes above which gave $188*10^6$ and $252.7* 10^6$ CFU g^{-1}, respectively.

The increase in the density of organisms in the presence of vermicompost more than the numbers in the case of control soil may be because organic fertilizer is a type of fertilizer that contains earthworms which works to add an organic fertilizer rich in humus, nitrogen, phosphorous and potassium. Therefore, the presence of these substances is considered as sources of energy for the activity of living organism, which in turn consume what they need first and then begin to decompose the organic matter in which there are living, and vice versa (Hanc and Drslova 2016). The presence of vermicompost may reduce soil pH and salinity and increases the availability of nutrients necessary for the plant and microbial activity (Lim et al 2015). The increase in numbers in the presence of vermicompost with the progression of time may be due to the organic matter needing sufficient time for decomposition, because this fertilizer increases the soil's retention of moisture content, which is necessary for microorganisms, but slows down the decomposition process. Thus, the presence of organic matter means a continuous energy source, but the number has decreased in the case of incubation with the soil only after 14 days have passed, which means that the sources of energy that it needs in its activity and density have decreased. The presence of vermicompost provides a source of energy and encouraging conditions for bacterial

growth, on the contrary in soils not inoculated with vermicompost, in which the density of bacteria increased in the early stages (14 days) and then began to decline with the consumption of the necessary elements (Piya et al 2018). The increase in the activity of *Azotobacter chroococcum* and *Bacillus megagaterium* with the addition of vermicompost indicates the possibility of increasing the efficiency of biofertilizers by loading the biofertilizers with vermicompost and keeping them active and effective as long as possible (Sekar and Karmegam 2010).

Field Experiment

Available nitrogen mg N kg^{-1} soil: The addition of biofertilizer, vermicompost or mineral fertilizers, each separately, had a significant effect on the available soil nitrogen (Table 4). The highest value was recorded with the treatment of the complete combination of bio-fertilizer and 6 Mg h^{-1} of vermicompost and 80% of the fertilizer recommendation that gave 54.66, 51.80 and 53.53 mg N kg^{-1} soil, respectively. There were significant differences for the bilateral interaction between bio-fertilizer and vermicompost, as the treatment of adding bio-fertilizer in the form of a complete combination with 6 Mg ha^{-1} of vermicompost gave the highest concentration of available nitrogen in the soil which was 56.09 mg N kg^{-1} soil.

The statistical analysis of the triple interaction between the averages of the bio-organic and vermicomposting treatments and mineral fertilizers, showed a significant difference in the concentration rates of available nitrogen in the soil. The treatment of adding biofertilizer in the form of a combination of *Glomus mosseae*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Azosprillum brasilense* with 6 Mg h^{-1} of vermicompost with 80% of the fertilizer recommendation was superior then other treatments and gave the highest rate of 59.67 mg N kg^{-1} soil, followed by the treatment of using the same combination with 3 Mg h^{-1} of vermicompost and 80% of the fertilizer recommendation, which gave an average of 58.93 mg N kg^{-1} soil compared with the control treatment, which gave the lowest rates and reached 40.33 mg N kg^{-1} soil.

Available phosphorous mg P kg^{-1} soil: The results of the statistical analysis in showed that the addition of biofertilizer, vermicompost or mineral fertilizers alone had a significant effect on the soil phosphorous (Table 5). The addition of biofertilizer in the form of a complete combination gave the highest average of available phosphorous concentration of 26.257 mg P kg^{-1} in soil, while adding organic fertilizer at a level of 6 Mg h^{-1} gave an average of available phosphorous 23.798 mg P kg^{-1} soil. The addition of phosphate mineral fertilizer with a level of 80% of the fertilizer recommendation record the highest average of available phosphorous and 21,883 mg P kg^{-1} of soil compared with the control treatment, which recorded the lowest average. It was found through the results of the statistical analysis that the bilateral interaction between the bioertilizer and the vermicompost was significant, as the addition of the biofertilizer in the form of a complete combination with 6 Mg ha^{-1} of vermicompost gave

Table 3. Population density of *Azotobacter chroococcum* and *Bacillus megaterium* after different time periods * (10^{6}) CFU g^{-1} soil

Incubation	Bacteria	Time			Incubation*Bacteria
		After 1 day	After 14 day	After 30 day	
Soil	Azotobacter	127.3	136.3	94	119.2
	Bacillus	140	160.7	150.3	150.3
Soil+ vermicompost	Azotobacter	133	135.7	188.7	152.4
	Bacillus	160	185.3	252.7	199.3
LSD$_{0.05}$			25.4		14.6
Average for time		140.1	154.5	171.4	
LSD$_{0.05}$ time			12.7		
				Time * Incubation	Soil
Soil		133.7	148.5	122.2	134.8
Soil with vermicompost		146.5	160.5	220.7	175.9
LSD$_{0.05}$ soil*time			17.9		10.4
				Bacteria	Bacteria
Azotobacter		130.2	136	141.3	135.8
Bacillus		150	173	201.5	174.8
LSD$_{0.05\ bacteria}$ * time			17.9		10.4

the highest average of available phosphorous in the soil, which to 31,717 mg P kg^{-1} compared to soil with the treatment of not addition of the two fertilizers, which gave the lowest average of 15,333 mg P kg^{-1} of soil. The result of the triple interaction between biofertilizer, vermicompost and mineral fertilizer, there were significant effects on the amount of available phosphorus in the soil, as the addition of biofertilizer in the form of a combination consisting of *Glomus mosseae*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Azosprillum brasilense* with 6 Mg h^{-1} and 80% of The fertilizer recommendation was superior to the control treatment, which gave the lowest rate, and the rates were 33.1 and 14.050 mg P kg^{-1} soil.

The biofertilization in the form of a complete combination of nitrogen and phosphate fertilizers led to a significant increase in the soil available nitrogen (Table 4, 5). This is because this combination contains bacteria that stimulating root growth and nitrogen fixation by the *Azospirillum brasilense*, which perform the process of nitrogen fixation in an associative manner with the plant roots and its nitrogenase enzyme increase the rate of nitrogen fixation.

This leads to an increase in the available nitrogen in the soil, and this result were agreed with Dubey et al (2020) and Al Mamouri (2020). The presence of *Azotobacter chroococcum*, which freely fixes nitrogen in the soil, as well as the amount of nitrogen that is released in the form of ammonia after death and decomposition of Azotobacter and the production of plant hormones and growth regulators contributes to the high nitrogen values in the field soil inoculated with this bacterium and a significant increase in the concentration of IAA acid. This is because it is a bacterium that stimulates plant growth, and adding it in interaction with phosphate-dissolving bacteria leads to an increase in the amount of available nitrogen in the soil, and this was confirmed by (Ramandeep et al 2018).

As for the increase in available phosphorous in the soil as a result of the organic acids produced by *Bacillus megaterium*, it contributes to reducing pH of interaction of the soil or the culture medium in which these isolates grow. Its role is not limited to representing phosphorous in its body only, but rather increases the amount of it dissolved in the medium in which it is located, and this is confirmed by (Ekin

Table 4. Effect of adding bio-fertilizer, vermicompost and mineral fertilizer on the amount of available nitrogen, mg N kg^{-1}soil

Bio fertilizer	Vermicompost	P fertilizer			Bio fertilizer *
		S0	S1	S2	
B0	V0	40.33	41.43	42.67	41.48
	V1	42.67	44.33	46.67	44.56
	V2	43.33	47.33	51.67	47.44
B1	V0	48	51.67	55.83	51.83
	V1	45.83	52.54	54.1	50.82
	V2	47	52.63	55.93	51.86
B2	V0	49.37	53.53	56.33	53.08
	V1	49.6	55.9	58.93	54.81
	V2	52.4	56.2	59.67	56.09
LSD$_{0.05}$			3.64		2.1
	P	46.5	50.62	53.53	
LSD$_{0.05}$			1.21		
		p fertilizer * bio fertilizer			
B0		42.11	44.37	47	44.49
B1		46.94	52.28	55.29	51.5
B2		50.46	55.21	58.31	54.66
LSD$_{0.05}$			N.S		1.21
		p fertilizer * Vermicompost			
V0		45.9	48.88	51.61	48.8
V1		46.03	50.92	53.23	50.06
V2		47.58	52.06	55.76	51.8
LSD$_{0.05}$			N.S		1.21

2019). Vermicompost is an organic fertilizer that activates micro-organisms and improves soil properties such as structure soil and its ability to hold water, thus increasing the availability and facilitating the necessary elements, especially nitrogen, phosphorous and potassium (Devi and Khwairakpam 2020). The concentration of available nitrogen increased in the treatment of mineral fertilizer, and this may be because the added amount of urea led to the availability of nitrogen for absorption by the plant. Thus, the positive role of mineral fertilizers in increasing the amount of available nitrogen in the soil becomes clear (Yoda 2018). The same results were noted phosphorous, when adding highest level of phosphorous in the form of triple superphosphate this led to an increase in the available quantity in the soil solution. The same case for potassium, higher adding quantity means the higher available quantity in the solution (Abu Dahi and Younis 1988). The bilateral interaction between biofertilizer and vermicompost affected the availability of nutrients in the soil because this interaction increases the process of nitrogen fixation through the enzymatic activity of microorganisms added as biofertilizer, such as the nitrogenase enzyme, which is an important factor in the fixation process and because vermicompost provides the appropriate environment for these bacteria. Among these conditions suitable for these environments are the appropriate pH, suitable humidity, and an energy source, and this confirms what was stated (Gopinathan et al 2014). The addition of biofertilizer, vermicompost and mineral fertilizers in the form of a triple overlap led to an increase in the availability of nutrients, including nitrogen, and the use of biofertilizer contributes to a better degree in increasing the readiness of this element. This interaction also led to an increase in the concentration of available phosphorous in the soil due to the role of microorganisms added in the form of a bio-fertilizer consisting of phosphate-dissolving bacteria and mycorrhizae interfering with vermicompost and mineral fertilizer. As these bacteria are provided with the appropriate conditions for them in the presence of available elements

Table 5. Effect of adding biofertilizer, vermicompost and mineral fertilizer on the amount of available phosphorous, mg P kg^{-1} soil after picking

Bio fertilizer	Vermicompost	p fertilizer			Bio fertilizer* Vermicompost
		S0	S1	S2	
B0	V0	14.05	15.75	16.2	15.333
	V1	16.8	17.55	17.85	17.4
	V2	16.767	18.1	18.9	17.922
B1	V0	18.133	19.6	19.8	19.178
	V1	19.8	20.5	21.15	20.483
	V2	21.35	21.567	22.35	21.756
B2	V0	22.45	23.167	23.267	22.961
	V1	23.65	24.3	24.333	24.094
	V2	30.55	31.5	33.1	31.717
requmindition fertilizer100%		17.33			
LSD$_{0.05}$		1.425			0.823
	P	20.394	21.337	21.883	
LSD$_{0.05}$		0.475			
p fertilizer * bio fertilizer					
B0		15.872	17.133	17.65	16.885
B1		19.761	20.556	21.1	20.472
B2		25.55	26.322	26.9	26.257
LSD$_{0.05}$		N.S			0.475
p fertilizer * Vermicompost					
V0		18.211	19.506	19.756	19.157
V1		20.083	20.783	21.111	20.659
V2		22.889	23.722	24.783	23.798
LSD$_{0.05}$		N.S			0.475

added from the mineral fertilizer and the organic fertilizer, their activity will increase in reducing the pH of the medium through the production of organic acids after the decomposition of the organic fertilizer. As well as being a source of energy for microorganisms and contributes to improving the properties of soil fertility in addition to the nutrients provided by mineral fertilizer, and this was confirmed by (Al-Mamouri 2020, Abdullah 2020).

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Effect of Cultivars, 2,4-D Herbicide and Sorghum Extract On Weeds Associated with Corn (*Zea mays* L.)

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Abstract: A field experiment was carried out in the experimental field of the Field Crops Sciences Department, College of Agricultural Engineering Sciences, University of Baghdad, Jadriya located within 33° north latitude and 44° east longitude during the 2019 autumn season. The main research aims were to find out the performance of three varieties of Maize (*Zea mays* L.) under the effect of weed herbicides and sorghum extract and its effect on the growth of companion weeds, growth characteristics yield, and its components. The experiment was carried out using a split-plot design arrangement according to randomized complete block design (RCBD) with three replicates, the main factor included three varieties of maize, which are Fajr1, Baghdad3, and Maha. Furthermore, the second factor included weed control treatments which are: spraying 2,4-D herbicide with full recommendation (1.50 lha^{-1}) when the plants reached a height of 25 cm (T_1), spraying sorghum extract with full recommendation 15 l.ha^{-1} at a concentration of 5% (T_2), spraying 2,4-D herbicide with half recommendation (0.75 l.ha^{-1}) , sorghum extract (T_3), spraying 2,4-D herbicide with half recommendation and sorghum extract with half recommendation (T_4) as well as weedy free treatment (T_5) and with weed (T_6). The experiment results showed that cultivar Fajr1 achieved the lowest average number of companion weeds after 60 days of control and gave an average of 52.17 weed.m^{-2}, which led to reduce the weed dry weight by 107.55 g.m^{-2} and gave the highest number of grains/ear 287.18 grain.ear^{-1} which reflected positively on its superiority in the grain yield 7.12 Mg.ha^{-1}, while the cultivar Maha gave the highest average of weight 500 grains by 77.33 g. Moreover, the treatment T_4 reduced the companion weed density of corn after 60 days of control and gave an average of 42.22 weed.m^{-2}, respectively, as it reduced the weed dry weight to 103.11 g.m^{-2}. Besides, significantly superior in number of grains/ear (279.73 grain.ear^{-1}) and the grain yield (6.98 Mg.ha^{-1}). The treatment T_3 gave the highest average of weight 500 grains by 77.89 g, without significant difference from the treatment T_1. Finally, the effect of interaction between the two study factors was significant in most of the studied characteristics. Fajr1 with treatment T_5 achieved the best results in weed control, followed by the same cultivar with treatment T_4. The interactions did not differ significantly in the characteristics of number of grains/ear and grain yield, while the cultivar Maha with treatment T_1 achieved the highest average of weight 1000 grains without significant difference from the same cultivar with the treatment T_3.

Keywords: Cultivars, Sorghum extract, Corn, 2,4-D herbicide

Corn (*Zea mays* L.) is an important cereal crop due to its protein, carbohydrates, and oil content, as well as its vitamin content (Barnes 2007). Despite the importance of this crop, its cultivation in Iraq faces a significant decrease in the cultivated area and the low productivity compared to global production. Although, it has a wide environmental range and adapts to different environmental conditions average yield of the crop in Iraq reached 1133.8 kg per 55837 dunums area (Ministry of Planning 2018). However, the decrease in productivity is due to the lake of follow-up scientific methods and modern agricultural, field practices in improving the growth and development of the crop, and reducing the harmful impact of the companion weeds. Possibly, the use of the genetic nature of maize varieties in weed competition is one of the steps that can achieve the desired object. Therefore, understanding and identifying the performance of varieties under conditions of weed competition and their control is lead to know their behavior and response to this

condition, which enables genetic potential through the simultaneous growth. Besides, their morphology with the growth and competition of the weed and the effect of this on the characteristics of vegetative growth, yield, and its components play key role (Watson et al 2006, Harker and Blackshaw 2009). The increase in weed density leads to a decrease in the efficiency of the vital activities of the crops and thus a decrease in their productivity due to the weed competition for the requirements for growth. Farmers are therefore using chemical herbicides, including the 2,4-D herbicide in the weed control. Therefore, the attention has focused on reducing the dependency on the use of herbicides by finding alternative techniques for weed management that include many strategies, including Allelopathic management that can be used as an alternative to weed herbicides (Kong et al 2008). Several researchers have indicated that some field crops have an allelopathic effort that affects companion weed growth, such as sorghum

(Cheema et al 2002, Alsaadawi 2007). Sorghum is one of the crops that is characterized by the phenomenon of allelopathy due to its effect on some crops and weed, and water extract and the residues of the shoot and root of sorghum caused an inhibiting effect on the germination and growth of different weed and crops. The Sorgoleone is one of the allelopathic compounds that the sorghum crop secreted into the environment, which works to inhibit the release of oxygen in the photosynthesis for the leaves of some crop. This compound when secreted from the sorghum roots is relatively non-toxic, but it converted into quinone when it oxidized and then becomes a strong inhibitor of weed growth (Einhellig et al 1993). Accordingly, this becomes evident that followed alternative methods of control by using the allelopathic phenomenon of some crop residues, including sorghum or water extracts will decrease weed damage and reduce environmental and health pollution as a result of using chemical control. Therefore, this study aims to know the performance of varieties of corn under the effect of 2,4-D herbicide and sorghum extract and its effect on the growth of the companion weeds and yield, and its components.

MATERIAL AND METHODS

A field experiment was carried out in the experimental field of the Field Crop Sciences Department, College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriya located within 33º north latitude and 44-east longitude during the 2019 autumn season in a silty clay loam soil. To identify the performance of three varieties of corn under the effect of weed herbicides and sorghum extract and its effect on the growth of companion weeds, yield, and its components. The experiment was carried out using a split-plot design arrangement according to the RCBD with three replications. The main factor included three varieties of corn, which are Fajr1, Baghdad3, and Maha, while the second factor included weed control treatments, which are:

T_1. Spraying (2,4-D) herbicide with full recommendation (1.50 l.ha^{-1}) when the plants reached a height of 25 cm.

T_2. Spraying sorghum extract with full recommendation 15 l.ha^{-1} at a concentration of 5%.

T_3. Spraying (2,4-D) herbicide with half recommendation (0.75 l.ha^{-1}) and sorghum extract with full recommendation

T_4. Spraying (2,4-D) herbicide with half recommendation and sorghum extract with half recommendation).

T_5. Weedy free.

T_6. Weedy.

Agronomic operations were carried out, including plowing, harrowing, and leveling, and the experimental land was divided into experimental units with an area of 9 m^2, with dimensions of 3x3 m, and each experimental unit included four furrows, the distance between them is 75 cm. The experiment land was fertilized with urea fertilizer (46% N) in 400 kg.ha^{-1} in two batches, the first after 20 days of emergence and the second after one month from the first, where the fertilizer diammonium phosphate (DAP) was added at a rate of 320 kg.ha^{-1} in one batch at sowing. Furthermore, maize varieties were planted on July 15, 2019, at a depth of 5 cm, with a distance of 25 cm between one plant and another to reach a plant density of 53,333 plant ha^{-1}. The corn stem borer (*Sesamia cretica* L.) was controlled with a 10% granular diazinon insecticide at 6 kg ha^{-1} and in two batches, the first batch after 20 days of emergence and the second after 15 days from the first. The types of weeds are summarized as shown in Table 1.

Preparation of sorghum extract: A 250 g of the dry sample for sorghum leaves was taken and added 700 ml of distilled water to it and the solution was kept at a temperature of 100°C, after that it was placed for half an hour on a horizontal vibrator at medium speed. Then, the solution was left to settle for an hour, and then the solution was filtered with three layers of gauze cloth to remove solid obstacles in solution (Harborne 1984).

Studied characteristics: Weed species and their density (plant.m^{-2}): weed species (broad and narrow) and their density were recorded after 60 days of control by calculating the number of weeds in one square meter per experimental unit (Al-Chalabi and Al-Majidi 2001).

Control percentage (%): The control percentage was estimated after 60 days of planting, using the following equation (Ciba-Giegy 1975):

$$\text{Control percentage}\,(\%) = \frac{\text{No. of weed in weedy treatement} - \text{No. of weed in the control treatment}}{\text{No. of weed in weedy treatment}} \times 100$$

Weed dry weight (g.m^{-2}): The dry weight of the broad and narrow weed was calculated at harvest, as the weed was cut at the level of the soil surface and collected with perforated paper bags and then placed in the oven at 70°C until the weight was constant.

Inhibition percentage (%): The inhibition percentage was estimated at harvest using the following equation (Al-Chalabi 2003):

$$= \frac{\text{Weed dry weight in weedy treatement} - \text{Weed dry weight in the control treatment}}{\text{Weed dry weight in the weedy treatment}} \times 100$$

The number of seeds/ear: The seeds of five ears harvested randomly from the two middle furrows for each experimental unit were separated and the average number of seeds was obtained.

Weigh 500 grains (g): After separating the grains of five ears, which were harvested randomly from the two middle furrows for each experimental unit, 500 grains were counted and weighed using a sensitive balance.

Grain yield (ton.ha^{-1}): Ears of five plants were harvested from the two middle lines, then they were separated manually, and the grains were weighed, and then the average weight of the grains/plant was calculated. The yield was obtained by multiplying the average weight of a single plant seed x the plant density used.

Statistical analysis: The data were analyzed statistically for all the studied characteristics according GenStat program, and simple correlation coefficient.

RESULTS AND DISCUSSION

Number of weeds, dry weights, and inhibition percentages: There was significant effect of the treatments of weed control and cultivar on the average weed density after 60 days of control (Table 2). The variety Fajr-1 gave the lowest mean (52.17 weed m^{-2}) with a significant difference from variety Baghdad-3 (60.89 weed m^{-2}) and Maha (70.33 weed m^{-2}). This may be due to variation in the varieties under study, especially the Fajr-1 variety, which was able, according to its genetic nature, to reduce the weed density associated with its plants which was positively reflected on the decrease in weed density.   These results are in agreement with the findings of Al-Khazali 2015, Al-Barzanji 2017, Al-Qaisi and Al-Heti 2017 where corn cultivars differ genetically in their ability to compete with the accompanying weed during the growing season.

The Treatment T4 achieved the lowest mean weed density after 60 days of control, which was 42.22 weed m^{-2}, with a significant difference from treatments T3, T2 and T1, which achieved averages of 56.11, 66.33 and 87.56 weed m^{-2}, respectively. The treatment T6, which achieved the highest

mean for the characteristic, which was 115.56 weed m^{-2}. This this agrees with the findings of early researchers (Cheema et al 2003, Cheema et al 2004 and Mahmood et al 2015) and these studies   indicated the significant superiority of weed control treatments associated with corn over the weed treatment . The spraying 2,4-D herbicide alone (T1) and spraying sorghum extract alone (T2) were less effective than the two treatments of spraying the herbicides and the extract together (T3 and T4), which may be attributed to the role of integration between the herbicides and the extract by affecting the growth of the weed associated with corn crop and then reducing its density after 60 days of control. These was significant effect of the interaction between corn cultivars and control treatments on the average weed density after 60 days of control. The Fajr-1 cultivar with treatment T4 achieved the lowest mean of 31.00 weed m^{-2}, which may be attributed to the integrative effectiveness between Varieties, herbicides and extract by affecting the growth of the weed accompanying the crop, while the Maha cultivar with the weedy treatment (T6) achieved the highest average of 124.00 weed m^{-2}, which may be due to the weak competitiveness of Maha plants in the resistance of the accompanying weed, In T5 average weed density after 60 days of control was 0.00 m^{-2} weed for the yellow corn cultivars under study respectively. There is a significant effect of the control treatments of the weed and cultivars on the percentage of the weed control after 60 days of control. The Fajr-1 gave the highest control of 50.94%, with a significant difference from other two cultivars. Maha variety, which gave the lowest control rate of 43.17%. Treatment T4 achieved the highest percentage of weed control after 60 days of control, (63.58%) with a significant difference from treatments T3, T2 and T1, which achieved 51.79, 42.94 and 24.43%, respectively, and treatment T6, which achieved the lowest control rate of 0.00%, The results indicate integrative

Table 1. Species of companion weed in the experiment

English name	Scientific name	Family name	Type	Life cycle
Purslane	*Portulaca oleracea* L.	Partulacaceae	Broad leaves	Annual
White goosefoot	*Chenopodium album* L.	Chenopodiaceae	Broad leaves	Annual
Wild beet	*Beta vulgaris* L.	Chenopodiaceae	Broad leaves	Annual
Smeller Bind Weed	*Convulus arrensis* L.	Convolvulacea	Broad leaves	Perennial
Common sowthistle	*Sonchus oleraceus* L.	Compositae	Broad leaves	Perennial
Milk thisth	*Silybum marianum* L.	compositae	Broad leaves	Perennial
Rough pigweed	*Amaranthus retroflexus* L.	Amaranlhaceae	Broad leaves	Annual
Dwarf mallow	*Malva parviflora* L.	Malvaceae	Broad leaves	Annual
Johnson grass	*Sorghum halpense* L.	Poaceae	Narrow leaves	Perennial
Cogon grass	*Imperata cylindrica* L.	Poaceae	Narrow leaves	Perennial
Purple pain-grass	*Echinochloa colonum* (L.) Link	Poaceae	Narrow leaves	Annual

effectiveness of spray the herbicide and the extract by affecting the growth of the weed associated with the yellow corn crop by achieving lowest weed density after 60 days of control compared to the treatment of spraying the herbicide 2,4-D (T1) or the treatment of spraying the sorghum extract (T2).

The interaction effect between corn cultivars and the control treatments was significant in the percentage of weed control after 60 days of control (Table 2), as Fajr-1 cultivar with treatment T4 achieved the highest control percentage of 70.97%, with a significant difference from other combinations, especially the weed treatment (T6). The three cultivars achieved a control rate of 0.00% which may be due to the presence of the weed without control and then increasing its numbers after 60 days of control. On other hand, the treatment T5 gave the 100.00% control after 60 days of control. There is a significant effect of the treatments of weed control and cultivars on the average dry weight of the

Table 2. Effect of Cultivars, 2,4D and sorghum extract on associated weed of corn

Characteristics/treatments		Number of weeds m^{-2} after 60 day of control	Control percentage after 60 day of control	Weed dry weight (g.m^{-2})	Inhibition percentage (%)
Fajr1		52.17	50.94	107.55	43.82
Baghdad3		60.89	47.40	122.99	42.43
Maha		70.33	43.17	137.26	40.29
L.S.D 0.05		2.88	1.16	5.27	1.30
T$_1$		87.56	24.43	163.65	22.71
T$_2$		66.33	42.94	135.37	36.24
T$_3$		56.11	51.79	121.87	42.47
T$_4$		42.22	63.85	103.11	51.38
T$_5$		0.00	100.00	0.00	10.00
T$_6$		115.56	0.00	211.58	0.00
L.S.D 0.05		2.36	1.05	3.10	1.16
Fajr1	T$_1$	76.67	28.09	146.29	23.56
	T$_2$	56.33	47.23	117.72	38.51
	T$_3$	42.67	59.36	98.86	48.38
	T$_4$	31.00	70.97	91.3	52.46
	T$_5$	0.00	100.00	0.00	100.00
	T$_6$	106.67	0.00	191.42	0.00
Baghdad3	T$_1$	88.33	23.90	164.57	22.93
	T$_2$	61.00	47.46	132.01	38.24
	T$_3$	57.67	50.31	125.05	41.49
	T$_4$	43.33	62.71	102.81	51.90
	T$_5$	0.00	100.00	0.00	100.00
	T$_6$	116.00	0.00	213.48	0.00
Maha	T$_1$	97.67	21.28	18.10	21.64
	T$_2$	81.67	34.14	156.39	31.97
	T$_3$	67.33	45.71	141.72	38.34
	T$_4$	52.33	57.89	115.50	49.77
	T$_5$	0.00	100.00	0.00	100.00
	T$_6$	124.00	0.00	229.83	0.00
L.S.D 0.05		4.09	1.81	5.37	2.02

T$_1$. Spraying (2,4-D) herbicide with full recommendation (1.50 l.ha^{-1}) when the plants reached a height of 25 cm
T$_2$. Spraying sorghum extract with full recommendation 15 l.ha^{-1} at a concentration of 5%.
T$_3$. Spraying (2,4-D) herbicide with half recommendation (0.75 l.ha^{-1}) and sorghum extract with full recommendation .
T$_4$. Praying (2,4-D) herbicide with half recommendation and sorghum extract with half recommendation.
T$_5$. Weed free
T$_6$. with Weeds

weed after 90 days of control. The variety Fajr-1 recorded the lowest average of 107.55 gm m-2, with a significant difference from the experimental plots planted with the cultivar Baghdad-3, which recorded 122.99 gm m^{-2}, and the cultivar Maha, which recorded the highest mean of 137.26 gm m^{-2}. The decrease in the dry weight of the weeds associated with the yellow corn cultivar Fajr-1 may be due to the decrease in the number of weeds accompanying this cultivar and the increase in the percentage of control after 60 days of control , which effectively contributed to reducing the dry weight of the weed after 90 days of control compared to the two cultivars, Al-Barzanji (2017) and Al-Qaisi and Al-Heti (2017) indicated that the decrease in weed numbers associated with the yellow corn cultivars led to a decrease in their dry weight. Treatment T4 achieved the lowest mean dry weight of the weed after 90 days of control, which was 103.11 gm m^{-2}, with a significant difference from treatments T3, T2 and T1 (121.87, 135.37 and 163.65 gm m^{-2} respectively) and treatment T6, (211.58 g m^{-2}). Cheema et al (2004) and Mahmood et al (2015) also indicated that weed control treatments were significantly superior to the overgrown treatment in the dry weight of the weed accompanying corn. The spraying the 2,4-D alone (T1) and the treatment of spraying sorghum extract alone (T2) differed from spraying the herbicide and the extract together (T3 and T4), which may be attributed to the synergistic role between the herbicide and the extract in influencing the growth of the weed accompanying the crop corn and then reduce weed numbers and increase the percentage of control after 60 days of control (Table 2), and then reduce the dry weight of the weed after 90 days of control. There is a significant effect of the interaction between corn cultivars and the control treatments on the average dry weight of the weed after 90 days of control. The cultivar Fajr-1 with treatment T4 gave the lowest mean of 91.03 gm m^{-2}, while the cultivar Maha with the weed treatment (T6) showed the highest average (229.83 g m^{-2}) which confirms the weak competitiveness of Maha plants in the resistance of the accompanying weed and the resulting increase in the number of weeds and their dry weight. There was significant effect of the control treatments of the weed and cultivars on the inhibition rate after 90 days of control. The experimental plates planted with the variety Fajr-1 gave the highest inhibition rate of 43.82%, with a significant difference from the experimental plates planted with the other two cultivars, especially the Maha cultivar, which gave the lowest inhibition rate of 40.29%. For the weed after 60 days of control (Table 2).

The treatment T4 achieved the highest inhibition rate after 90 days of control, which was 51.38%, with a significant difference from treatments T3, T2 and T1, which achieved 42.27, 36.24 and 22.71%, respectively, and treatment T6, achieved the lowest control rate of 0.00%, and this shows the synergistic role of spraying aquatic extract For sorghum with half the quantity with half the recommended dose of 2,4-D (T4) herbicide to influence the growth of the weed accompanying the corn crop by reducing the dry weight of the weed after 90 days of control (Table 2) as compared to the treatment of spraying the herbicide 2,4- D (T1) or sorghum extract (T2) spray treatment. The interaction effect between corn cultivars and the control treatments was significant in the percentage of weed control after 90 days of control (Table 2). The Fajr-1 cultivar with treatment T4 achieved the highest inhibition of 52.46%, with a significant difference from other combinations, especially the weed treatment (T6) in three cultivars that achieved an inhibition rate of 0.00% sequentially, which may be due to weed without control and then increasing its numbers and dry weight after 90 days of control. The T5 treatment gave an 100.00% inhibition rate in the dry weight of the weed after 90 days.

Yield and Yield Contributory Components

Number of grains/ear: There was a significant effect of the different weed control treatments and the varieties on the number of grains/ear of corn. Fajr1 achieved the highest average of 287.18 grain.ear^{-1} with a significant difference compared to the varieties Baghdad3 and Maha, which achieved 270.15 and 249.63 grain.ear^{-1}, respectively. The reason for difference in this characteristic may be due to number of grains in the ear is one of the characteristics of high heritability (Mahmoud 2019). The varietal difference in the number of grains in the ear may be due to the nature of the genetic material for each variety (Al-Khazali 2015). This result is consistent with findings of earlier researchers (Al-Hadithi 2011, Al-Fahdawi 2010 , Ramadan and Kazem 2013, Al-Dulaimi and Al-Hadithi 2015 , Abdel-Azim and Ahmad 2017) which they indicated that the varieties of corn differ among themselves in the number of grains in ear. Additionally, the treatment T_5 gave the highest average number of grains in the ear (282.26 grains/ear) and did not significantly differ from treatment T_4 (279.73 grains/ear) with a significant difference from treatments T_3, T_1 and T_2, which achieved an average of 270.80, 264.49 and 262.23 grains/ear, respectively. The reason for the significant superiority of weed control treatments over the weedy treatment in this characteristic may be attributed to the role of these treatments in reducing weed competition by eliminating the weed or reducing its growth and density. The effect of increase the number of leaves and leaf area and index and the accumulation of dry matter and improving the correlation between the source and the sink, which reflected positively on increasing the development of florets in the ear

rows, and thus the increase in the number of grains/ear (Al-Khazali 2015). Mahmood et al (2015) findings indicated that the weed control treatments were significantly superior to the number of grain.ear[-1] compared with the weedy treatment. The interaction between the two study factors, effect was significant on the average number of grains/ear (Table 3). However, the variety Fajr1 with treatment T_5 achieved the highest average for the characteristic (303.57 grain.ear[-1]) and without significant difference from the same variety with treatment (T_4) that achieved 299.03 grain.ear[-1], while the variety Maha with the treatment T_6 achieved the lowest average for the characteristic (235.70 grain.ear[-1]).

Weight of 500 grains (g): There were significant differences between the varieties of corn in average weight of 500 grains (Table 3). Variety Maha gave the highest average of 77.33 g, followed by Baghdad3 and Maha, which gave 70.56 g, while the variety Fajr1 recorded lowest average of 67.44 g. The increase in weight of 500 grains in variety Maha is due to the small number of grain per ear[-1], which led to the grain obtaining a greater amount of metabolic materials and then increasing its weight. The difference in this characteristic may be due to the nature of the genetic material of each variety and response to environmental conditions, which led to the varieties efficiency difference in converting the

Table 3. Effect of cultivars, 2,4-D and sorghum extract on yield and yield components of corn

Characteristics/Treatments		Number of seeds ear[-1]	Weigh 500 grains (g)	Grain yield (ton.ha^{-1})
Fajr1		287.18	67.44	7.12
Baghdad3		270.15	70.56	6.15
Maha		249.63	77.33	4.86
L.S.D 0.05		3.95	1.99	0.56
T_1		264.49	76.78	5.38
T_2		262.23	77.89	5.81
T_3		270.80	73.33	6.20
T_4		279.73	69.89	6.98
T_5		282.26	68.11	7.36
T_6		254.41	67.67	4.53
L.S.D 0.05		3.13	1.39	0.42
Fajr1	T_1	284.77	72.0	6.22
	T_2	277.17	74.33	6.92
	T_3	289.90	68.33	7.61
	T_4	299.03	66.00	8.20
	T_5	303.57	63.33	8.66
	T_6	268.63	60.67	5.12
Baghdad3	T_1	266.23	74.00	5.30
	T_2	263.70	76.67	5.98
	T_3	271.07	70.67	6.50
	T_4	279.67	69.33	7.16
	T_5	281.33	67.67	7.37
	T_6	258.90	65.00	4.56
Maha	T_1	242.47	84.33	4.61
	T_2	245.83	82.67	4.52
	T_3	251.43	81.00	4.49
	T_4	260.50	74.33	5.57
	T_5	261.87	73.33	6.05
	T_6	235.70	68.33	3.92
L.S.D 0.05		5.43	2.40	0.73

See Table 2 for details

products of the photosynthesis process to the reproductive parts. This result is consistent with the earlier researchers (Aziz and Muhammad 2012, Ramadan and Kazem 2013, Al-Khazali 2015, Al-Ani and Al-Obaidi 2017 and Mahmoud 2019). Treatment T_2 was significantly superior and gave the highest average weight of 500 grains (77.89 g) and it did not differ significantly from treatment T_1 (76.78 g) with a significant difference from treatments T_3. T_4 and T_3 gave an average of 69.89 and 68.11 g, respectively without significant difference between them. The T_6 achieved the lowest average of 67.67 g without significant difference from T_5. The reason for the decrease in the grain weight in the two treatments (T_4 and T_5) may be attributed to the highest average number of grains/ear. As whenever the grains/ear increase, the competition between them for the metabolic compounds of photosynthesis increases, which leads to the distribution of these products over a greater number of grains, and thus their weight decreases. The lowest average weight of 500 grains for (T_6) treatment may be due to the weed competition in crop during the growing season on the basic growth requirements such as water and nutrients and also the competition for the light, which contributed to reducing plant growth and the efficiency of physiological processes that take place within its tissues especially photosynthesis. Cheema et al (2004) and Mahmood et al (2015) indicated that the weedy treatments significantly reduced the number of grains/ear and the grain weight. The effect of interaction between the two study factors was significant (Table 3). The variety Maha with treatment achieved the highest average of 84.33 g and did not differ significantly from the same variety with treatment T_2 (82.67 g) while the Fajr1 with the treatment T_6 achieved the lowest average (60.67 g). However, it was evident that the three varieties achieved the lowest results in the treatment T_6, which confirms the role of weed in reducing the efficiency of physiological processes. Besides, the chemical reactions that were carried out inside the plant tissues, resulted in a reduction in photosynthesis products and reducing the efficiency of their transport and distribution to the sinks, thus reducing the number of grains/ear and the grain weight.

Grain yield (Mg.ha^{-1}): There was a significant effect of the different weed control treatments and varieties of corn in the grain yield (Table 3). Fajr1 achieved the highest average grain yield of 7.12 Mg.ha^{-1}, with an increase of 15.77% over the variety of Baghdad3 and 46.50% over the variety Maha, which achieved the lowest average for the characteristic (4.86 Mg.ha^{-1}). The superiority of Fajr1 in this characteristic may be attributed to its superiority in most of the growth characteristics such as plant height, the number of leaves, leaf area, which resulted in increasing the number of ear/plant

and the number of grains/ear as well as, the positive effect of that in increasing the grain yield compared to the other two varieties. This result is consistent with earlier researchers (Aziz and Muhammad 2012, Al-Fahdawi 2010, Ramadan and Kazem 2013, Al-Khazali 2015, Al-Dulaimi and Al-Hadithi 2015 and Mahmoud 2019) where findings indicated that the varieties of corn differ among themselves in grain yield. T_5 achieved the highest average grain yield of 7.36 Mg.ha^{-1}, but it did not differ significantly from T_4, which achieved 6.98 Mg.ha^{-1} with a significant difference from the treatments T_3, T_1 and T_2, which achieved 6.20, 5.81 and 5.38 Mg.ha^{-1} respectively. The treatment T_6 achieved the lowest average of 4.53 Mg.ha^{-1}. The reason for the significant superiority of weed control treatments over the treatment (T_6) in this characteristic may be attributed to the fact that the role of these treatments in reducing weed competition by eliminating or reducing the weed growth and density. In addition to reducing its dry weight and its effect on improving the vegetative growth for the plant which resulted in increasing the dry matter accumulation and improving the source correlation to the sink, which was positively reflected in increasing the number of ear/plant and the number of grains/ear, and then the grain yield increase. Cheema et al (2004) and Mahmood et al (2015) findings indicated that the weed control treatments in corn were significantly superior in the grain yield compared to the weedy treatment. The interaction between the two study factor was a significant on average grain yield (Table 3). The Fajr1 with treatment T_5 achieved the highest average for the characteristic reached 8.66 Mg.ha^{-1} without a significant difference from the same variety with the treatment T_4 that achieved 8.20 Mg.ha^{-1}, while variety Maha with treatment T_6 achieved the lowest average for the characteristic reached 3.92 Mg.ha^{-1}. The three varieties achieved the lowest results in the treatment T_6 which confirms the weed negative effect in reducing the efficiency of genetic energy for variety on the exploitation of surrounding environmental conditions by reducing the effectiveness of the physiological processes. Besides, the biochemical reactions carried out inside the plant tissues reduces the products of photosynthesis and its transfer to the reproductive parts of the plant, which led to a decrease in the grain yield, which represents the outcome of the plant's vital activities.

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Effect of Nano Fertilizer NPK Fertigation and Foliar Application of Nano Zinc and Boron on NPK, Zn and B on Vegetative Part of Potato (*Solanum tuberosum* L.)

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Abstract: Field experiment was carried out on potato crop (*Solanum tuberosum L*), Boren variety, during spring season of 2019-2020 at University of Baghdad, Jadria region, on silty clay soil to study effect of Nano and traditional NPK compound fertilizers (balanced 20:20:20 fertilizer) and spraying Nano and traditional (zinc and boron) fertilizers on growth, qualitative characteristics and potato yield. The fertilization was of two types of NPK (traditional and Nano) fertilizers beside the control treatment and the second factor includes spraying of (Nano Zinc+Nano boron) and spraying (traditional Zinc+traditional boron), beside the control treatment (water only) and were applied according to complete random sectors. The traditional NPK fertilizer Tron (20:20:20) was added according to the full fertilizer recommendation 300 kg h^{-1} as well as for the Nano fertilizer (20:20:20). Addition of the Nano NPK fertilizer was at 5, 10, 15, 20 and 100% of the traditional fertilizer recommendation and was added in three doses, the first dose was 30% after completion of germination (30 days of planting), the second dose was 40% after 20 days of the first dose and the third dose at a rate of 30% after 15 days of the second dose addition. The traditional and Nano Zinc and boron fertilizers were sprayed on the plant, and their concentrations were 40 mg B L^{-1} and 100 mgZn L^{-1}. They were sprayed at three stages of potato growth 50, 60, and 70 days from planting according to concentration traditional Zinc fertilizer 20% Zn (0.5 g L^{-1}), and traditional boron 11%B (0.363 g L^{-1}), Nano-Zinc 19%Zn (0.526 g L^{-1}) and Nano-boron 9%B (0.444 g L^{-1}). All types of the added fertilizers recorded a significant increase in various parameters compared with the control treatment. The 5% Nano NPK treatment recorded the superiority in the percentage of nitrogen in the vegetative part in the flowering stage without significant differences with the 100% Nano NPK treatment and traditional fertilizers. The treatment of 10% fertilizer surpassed the concentration of Zn in the vegetative part without significant differences with the treatment of 100% Nano NPK fertilizer. The treatment of fertilizer addition of 20% NPK also outperformed the concentration of boron in the vegetative part in the flowering stage and without a significant difference with the addition of 100% NPK of traditional fertilizer and the treatment was superior. Fertilizer: 100% NPK of conventional fertilizer in K, P concentration in the vegetative part in flowering stage without significant differences with treatment of 100% Nano NPK.

Keywords: Nano fertilizer, NPK, Fertigation, Foliar application, Nano zinc, *Solanum tuberosum* L.

Despite the importance of adding nutrients to plant growth and development, the constant addition of traditional chemical fertilizers and their overuse to compensate for the lack of soil nutrients leads to environmental pollution in addition to the high costs of these fertilizers (Walpola and Yoon 2012). Due to negative effects of the ill-advised use of chemical fertilizers cause soil pollution and increasing the salinity of soils in arid and semi-arid regions, as most elements of added fertilizers deteriorate their readiness due to many factors such as washing, adsorption and sedimentation. Thus it is necessary to reduce the loss of nutrients in fertilization, and to increase the productivity of crops. It was necessary to use modern fertilizers as an alternative to traditional fertilizers and use them to provide the necessary nutrients for plant growth and increase productivity while maintaining the soil in good condition and environmentally clean (Miransari 2011). From these environmentally friendly and highly effective fertilizers called

Nano fertilizers or Nano-nutrients have emerged that have effective properties to accelerate crop growth and release nutrients upon demand, controlling the release of nutrients that regulate plant growth and enhance plant activity (DeRosa et al 2010, Nair et al 2010, Morales-Díaz et al 2017).

In the past few years, possibility of using nanotechnology to improve the efficiency of fertilizer use and development of Nano fertilizers can be more soluble, effective and faster to sorbtion in plant tissues than conventional fertilizers were explored (Naderi, Danesh-Shahraki 2013, Rameshaiah 2015). This study aims to find out the effect of conventional and nanoparticle NPK (20:20:20) fertilizer and spraying of Nano-boron and zinc on the content of N, P, K, Zn, B in the vegetative part of potato plants.

MATERIAL AND METHODS

Field experiment was carried out during the spring season at University of Baghdad in the Jadriya region in

mixed alluvial soils classified at a level under the great groups Typic Torrifluvent according to Soil Survey Staff (Table 1).

The field was plowed by a flat plow and graded by disc harrows, was divided into 63 experimental units with dimensions of 2.25 m* 3 m and with three moorings, with an area of 6.75 m^2 and with three replications, leaving distance of 1 m between the repeaters and a distance of 0.75 m between one and another and a distance of 0.25 m between plants and a distance of 0.75 m between treatments and a distance of 1.5 m between sectors to ensure that fertilizers are not transferred between treatments. The spraying treatments were control (spraying with water only), zinc spraying with boron nanoparticles (100Zn + 40B) mgL^{-1}, and zinc spraying treatment with traditional boron (100Zn + 40B) mgL^{-1}. As for the fertilizing factors for NPK, fertilizer was added as traditional (20:20:20) (N: P: K) at a rate of 300 kg ha^{-1}. Nano NPK composited (20:20:20)(NPK) was added at different levels in rate equal to 100% of the recommendation used for traditional NPK fertilizer (300 kg ha^{-1}) and as 5% of the traditional fertilizer recommendation (15 Kg ha^{-1}), 10% of the recommendation for traditional fertilizer (30 kg ha^{-1}), 15% of the recommendation for traditional fertilizer (45 kg ha^{-1}), and 20% of the recommendation for traditional fertilizer (60 kg ha^{-1}). All of the traditional and Nano NPK fertilizers were

Table 1. Chemical and physical characteristics of the studied soil

Property	Value		Unit
pH	7.76		---------
EC	2.30		ds.m-1
CEC	17.29		cmol kg^{-1}
Organic matter	1.56		gmKg^{-1}
Soluble cations	Ca	12.72	mmol L^{-1}
	Mg	7.5	
	K	0.25	
	Na	2.11	
Soluble anions	Bicarbonate	0.73	
	Sulfates	12.82	
	Chlorides	10.12	
Available ions	N	57	mg Kg^{-1} soil
	P	22.3	
	K	210	
	B	0.43	
	Zn	1.8	
Soil particles	Sand	348	mg Kg^{-1} soil
	Silt	464	
	Clay	188	
Texture		SiL	

added to soil by using drip irrigation system. The NPK fertilizers were added at levels 5, 10, 15, 20 and 100% of the traditional fertilizer recommendation. The Nano and traditional fertilizers of Zinc and boron were added by spraying on the plant.

Potato tubers (*Solanum tuberosum* L.) of Purin variety was sown on February 1, 2019, at depth 8-10 cm, with a rate of 36 tubers for the experimental unit, i.e. a distance of 25 cm between each tuber. The Nano chelated fertilizers were brought from the KHAZRA company for the production of fertilizers from the Islamic Republic of Iran for the balanced Nano composition 20-20-20 NPK, the nanostructured zinc fertilizer 19% Zn and the Nano boron 9% B. In the flowering stage, the vegetative parts of the plants were washed with a stream of tap water, then with distilled water, dried, and then placed in perforated paper bags and placed in an oven at 65 C^0 for 24 hours, then milled and sifted with a sieve with a diameter of 1 mm holes. Samples of the plants powder was digested by acid for digestion and the N, P, K, Zn, and B were determined.

RESULTS AND DISCUSSION

Vegetative part at flowering stage (%): All treatments significantly increased the percentage of nitrogen in the vegetative part at the flowering stage compared to the control (2.29). The highest values recorded in 100%-NPK treatment of the Nano and traditional type followed by the Nano treatments at (10, 15, 5 and 20%) levels without significant differences. The treating the potato crop with the treatment of macronutrients from its Nano and traditional sources, and the difference in their combinations may cause a significant increase in the percentage of nitrogen in the vegetative part. Spraying with Nano and traditional boron and zinc fertilizer indicated non-significant differences compared to the control treatment (3.39%). The spraying with Nano zinc and Nano boron recorded highest average (3.55%). Interaction of the addition factors of NPK fertilizer and the spraying treatments of zinc and boron nanoparticles, all showed significant differences with the control treatment (2.11%), where the highest value of the rate was reached in the addition of NPK 100% nanoparticles and zinc and boron nanoparticles which amounted to (3.89%). The fertilizers with a low level of addition have given results without a significant difference from the levels of large addition, and this prompts to focus on low additives to prevent the waste of large quantities of fertilizers as well as reduce soil pollution, that is concern for optimal fertilizer management in terms of following the fertilizer recommendations set out on the envelope of the fertilizer.

Phosphorus concentration in the vegetative part at

flowering stage (%): All of the treatments three were significant increases in the percentage of phosphorus in the vegetative part compared to the control treatment (0.24%), and the highest was recorded when treating the fertilizer addition of traditional and Nano fertilizers 100% (NPK) without significant differences between them and followed by a significant difference for each of the treatments (Table 3). Fertilizer addition is 20%, 15% and 10% without a significant difference between them, followed by significant difference in the addition treatment of 5%, as their rates reached 0.40, 0.39, 0.37, 0.35, 0.35 and 0.32% with an increase of 16.5, 62.50 and 54.17, 45.83, 45.83 and 33.33%, respectively. These results agree with the results obtained by Banjare et al (2014) when treating the potato crop with the triple fertilizer treatment (NPK), where it increased in the percentage of phosphorus and with an increase in the percentage of traditional fertilizer (NPK) over all other fertilization treatments. This was attributed to the large addition of traditional fertilizer compared with the low levels of addition of the nanoparticles. The spraying with zinc and Nano and traditional boron fertilizer and showed significant differences compared with the control treatment, and the highest value was in the treatment of adding traditional zinc and boron (0.36%). As for the effect of the interaction of the NPK addition factors and the spraying treatments, the highest value of the rate was in the treatment of adding the traditional fertilizer NPK 100% and the zinc and boron nanoparticles were sprayed (0.42%).

Potassium concentration in the vegetative part in the flowering stage%: All of the treatments increased the percentage of potassium in the vegetative part in the flowering stage, with significant differences compared to the control treatment (2.05%), and the highest value was recorded for its fertilizer addition factor of Nano scale and traditional fertilizers 100% without significant differences between them. Their rates reached (3.54 and 3.45%), followed by a significant difference in the treatment of fertilization with nanoparticle 5%, reaching (3.23%), followed

Table 2. Effect of fertilizers addition and spraying and their interaction on N concentrations (%) in the vegetative part at the flowering stage

NPK fertilizers ground addition	Spraying			Mean of ground addition
	Without spraying Zn + B	Traditional Zn + B	Nano Zn + B	
Control	2.11	2.35	2.41	2.29
Traditional	3.81	3.86	3.79	3.82
100% Nano	3.8**8**	3.77	3.89	3.85
5% Nano	3.33	3.56	3.66	3.52
10% Nano	3.59	3.65	3.70	3.65
15% Nano	3.58	3.64	3.71	3.64
20% Nano	3.41	2.66	3.69	3.25
Spraying mean	3.39	3.36	3.55	
LSD (p=0.05)	A: 37	B: 24	AB: 0.65	

Table 3. Effect of fertilizers addition and spraying and interaction on P concentration (%) in the vegetative part at the flowering stage

NPK fertilizers ground addition	Spraying			Mean of ground addition
	Without spraying Zn + B	Traditional Zn + B	Nano Zn + B	
Control	0.20	0.25	0.26	0.24
Traditional	0.38	0.41	0.42	0.40
100% Nano	0.37	0.39	0.40	0.39
5% Nano	0.30	0.33	0.33	0.32
10% Nano	0.34	0.38	0.34	0.35
15% Nano	0.37	0.35	0.34	0.35
20% Nano	0.34	0.38	0.38	0.37
Spraying mean	0.33	0.36	0.35	
LSD (p=0.05)	A: 0.01*	B: 0.02*	AB: 0.03*	

* (P≤0.05)

by a significant difference of 10%, reaching (3.08%), and followed by a significant difference between (15% and 20%) and without a significant difference between them. It reached (2.89 and 2.88%) with an increase of 72.68, 68.29, 57.56, 50.24, 40.98 and 40.49%, respectively (Table 4).

Spraying with zinc and boron Nano and traditional fertilizers, all the treatments recorded significant differences with the control treatment (2.89%), and the highest e recorded for the treatment of adding Nano composite NPK was 100%, reaching (3.09%). The interaction of NPK addition treatments, all the treatments recorded significant differences with the control treatment (1.93%). The highest rate was recorded in the treatment of adding NPK 100% Nano-zinc and boron nanoparticles with (3.67%). The increase in K% in the vegetative part may be due to the flowering phase. One of the main factors affecting the method of addition is the final concentration of fertilizers that reach the plant. In practical application, much less concentration (well below the minimum required concentration) reaches the target site due to failure of washing, erosion, runoff, evaporation, hydrolysis, stabilization, and microbiology representation in soil.

The absorption, transfer, or transformation and accumulation of nanoparticles in plants is a very important case related to the type of plant, the stage of growth and the nanoparticle nutrition, and the nanoparticles have a high ability to penetrate and enter the various plant tissues, especially the additive to the shoots (Zuverza-Mena et al 2017).

Concentration of zinc in the vegetative part in the flowering stage: All of the treatments increased the percentage of zinc in the vegetative part, with significant differences compared to the control treatment (57.37 mg Zn kg^{-1} dry matter), and the highest value was recorded when the fertilizer addition of NPK was 15%, as its value reached (62.53 mg Zn kg^{-1} dry matter), followed by the two treatments with NPK 100% and 10% Nano fertilizer fertilization, without significant differences between them, and their rates were (62.29 and 61.84 mg Zn kg^{-1} dry matter), followed by a significant difference of 100% traditional fertilizer treatment of 60.09 mg Zn kg^{-1} dry matter). This was followed by a significant difference between the two treatments of fertilizer addition (5% and 20%) without significant differences between them, as their rates reached 59.11 and 58.66 mg Zn kg^{-1} dry matter with an increase of 8.99, 8.58, 7.79, 4.74, 3.01 and 2.24 % respectively compared to the control treatment (Table 5). The spraying with zinc and Nano and traditional boron fertilizer, the treatment of Nano scale spraying recorded the highest value without significant difference than the treatment of traditional spraying, and both recorded a significant difference with the control treatment, and their rates were (69.83%, 69.10 and 43.38 mg Zn kg^{-1} dry matter), respectively.

The interaction of the NPK addition treatments and the spraying treatments with zinc and boron Nano and traditional fertilizers indicated the highest of 72.34 mg Zn Kg^{-1} dry matter in the treatment of adding 15% - Nano NPK with the Nano zinc and boron. The lowest value of zinc (37.07 mg Zn kg^{-1} dry matter) was in the interaction of 5% Nano NPK with zero Zn + B spraying.

Concentration of boron in the vegetative part at the flowering stage: All of the treatments significantly increased percentage of boron in the vegetative part compared to the control treatment (13.67 mg B kg^{-1} dry matter), and the highest value was recorded in fertilizer addition of the NPK 20%-Nano NPK, (22.33 mg B kg^{-1} dry matter). The significance of the treatment of fertilization with NPK 15% and 100% traditional and Nano NPK, where their rates were

Table 4. Effect of fertilizers addition and spraying and their interaction on K concentration (%) in the vegetative part at the flowering stage

NPK fertilizers ground addition	Spraying			Mean of ground addition
	Without spraying Zn + B	Traditional Zn + B	Nano Zn + B	
Control	1.93	2.19	2.03	2.05
Traditional	3.22	3.55	3.57	3.45
100% Nano	3.36	3.60	3.67	3.54
5% Nano	3.05	3.30	3.34	3.23
10% Nano	2.95	3.10	3.18	3.08
15% Nano	2.89	2.86	2.91	2.89
20% Nano	2.81	2.89	2.93	2.89
Spraying mean	2.89	3.07	3.09	
LSD (p=0.05)	A: 0.12*	B: 0.08*	AB: 0.20*	

* (P≤0.05)

Table 5. Effect of fertilizers addition and spraying and their interaction on zinc concentration in the vegetative part at the flowering stage (mg Zn kg^{-1}dry matter)

NPK fertilizers ground addition	Spraying			Mean of ground addition
	Without spraying Zn + B	Traditional Zn + B	Nano Zn + B	
Control	40.68	65.24	66.19	57.37
Traditional	43.50	67.155	69.62	60.09
100% Nano	46.63	68.93	71.33	62.29
5% Nano	37.07	68.34	71.93	59.11
10% Nano	44.40	69.06	72.07	61.84
15% Nano	44.88	70.36	72.34	62.53
20% Nano	46.50	64.11	65. 37	58.66
Spraying mean	43.38	69.10	3.09	
LSD (p=0.05)	A: 1.79*	B: 1.17*	AB: 3.11*	

* (P≤0.05)

Table 6. Effect of fertilizers addition and spraying and their interaction Boron concentration in the vegetative part at the flowering stage (mg B Kg^{-1}dry matter)

NPK fertilizers ground addition	Spraying			Mean of ground addition
	Without spraying Zn + B	Traditional Zn + B	Nano Zn + B	
Control	12.0	14.00	15.00	13.67
Traditional	14.00	24.00	27.00	21.67
100% Nano	15.00	22.00	26.00	21.00
5% Nano	14.00	20.00	21.00	18.33
10% Nano	15.00	21.00	23.00	19.67
15% Nano	16.00	23.00	24.00	21.00
20% Nano	16.00	25.00	26.00	22.33
Spraying mean	14.57	21.29	23.14	
LSD (p=0.05)	A: 1.68*	B: 1.10*	AB: 2.92*	

* (P≤0.05)

21.67, 21.00 and 21.00 mg B kg^{-1} dry matter, followed by significant difference in treatment 10% and 5%, reaching 19.67 and 18.33 mg B kg^{-1} dry matter with an increase of 63.35, 58.52, 53.62, 53.62, 43.89 and 34.09 %, respectively compared with the measurement treatment.

The spraying with Nano zinc and Nano and boron fertilizer, the treatment of nanoparticle spraying recorded the highest value with a significant difference over both traditional treatment and control treatment (without spraying). The interaction of NPK addition and spraying treatments, the highest significant value of the rate of 27.00 mg B kg^{-1}dry matter was recorded in the treatment of adding the traditional 100%- NPK fertilizer with Nano-zinc and boron spraying.

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Effect of Storage and Soaking Seeds by Nanoscale Iron on Vitality and Seedling Vigor of Wheat

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Abstract: The experiment was carried at University of Baghdad for the 2018 season, with the aim of determining the effect of soaking wheat seeds with nanoparticles (FeNPs) at concentrations of 50, 100 and 150 mg L^{-1} in addition to the two comparison treatments (distilled water and dry seeds), and for a period Soaking 6, 12 and 24 hours in the germination and seedling strength and using the best concentration and the best soaking time for nano iron fertilizer. There was superiority of the nano fertilizer over the two comparison treatments (soaking in distilled water and dry seeds) in germination and seedling strength characteristics was observed. In addition, the treatment of Nan iron at a concentration of 150 mg L^{-1} outperformed the two concentrations 50 and 100 mg L^{-1}. The 24-hour soaking time outperformed the periods 6 and 12 hours in giving the highest average for all the studied traits.

Keywords: Wheat, Seed germination, FeNPs

Wheat (*Triticum aestivum L.*) is one of the most important cereal crops economically cultivated area reached about 220.4 million hectare with yield for with production of 765.0 million tons (FAO 2019). Seed germination is affected by many factors, the most important of which are internal factors such as the physiological and hormonal state of the seeds and external factors, including environmental conditions during seed development as well as harvesting, post-harvest processes and seed storage. Rajjou et al (2012). Iron is one of the important microelements necessary for plant growth, which participates in various physiological processes and photosynthesis reactions and accelerates seed germination by activating many enzymes and contributes to the synthesis of RNA and improves the performance of the photosystems in plants (Sheykhbaglou et al 2010). Many practices have been put in place to raise the viability of seeds, increase their activity and rates of emergence, which depend in their entirety on the process of activation or soaking with substances stimulating germination, especially soaking with nutrients, and the development of the situation to the use of nanoparticles and fertilizers because of this technique of many benefits as it is economical, environmentally friendly and non-toxic (Lu et al 2015). Some studies have shown that nanomaterials have the ability to penetrate the seed layer and enhance the absorption capacity and use of water, which stimulates the enzyme system and ultimately improves germination and seedling growth (Banerjee and Kole 2016.) The objectives of this study were to find the best concentration of nanoscale iron and determine the best soaking time.

MATERIAL AND METHODS

Laboratory experiment was carried out, to study the effect of soaking wheat seeds with three concentrations of all nanoparticles in addition to the two comparison treatments (dry seeds and distilled water) on standard laboratory germination characteristics and selecting the best concentration to be used in the field experiment. The experiment was according to a fully randomized design and with four iterations in the order of factor experiments. The treatments included in the experiment included, FeNPs at a concentration of 50, 100 and 150 mg liter^{-1} and the two comparison treatments (distilled water and dry seeds). The 200 seeds were soaked for each treatment for a period of 6, 12 and 24 hours. The planting was done in filter papers with a diameter of 30 cm. Alcohol was used for sterilization to avoid infection with microorganisms. Fifty seeds were placed on the germination paper and distributed mutually. These were kept at temperature of 25 ± 2°C and humidity of 70% for eight days (ISTA 2005). The following characteristics were taken:

First count: After four days, the germination percentage was calculated for the purpose of calculating the speed of germination. The natural seedlings were counted and then the results were converted into percentages:

Percentage of germination in the first count = (number of natural seedlings after 4 days / number of total seeds) x 100

Percentage of germination in the final count: The final count of the germinated seeds was taken ten days after they were placed in the plant (ISTA 2013), then the results were converted into percentages:

Germination percentage in the final count = (number of natural seedlings after 10 days / number of total seeds) x 100

Root and coleoptile length (cm): Ten seedlings were taken; the rhizome and the coleoptile were separated from their point of contact with the seed and their length was measured.

Fresh and dry weight of seedlings: After measuring the lengths of the rootstock and the coleoptile, the wet weight of the seedlings was taken and the ten seedlings were placed in perforated paper bags at a temperature of 80°C for 24 hours (Farooq et al 2006) for the purpose of drying them. The dry weight of the seedlings was recorded.

Seedling vigor index: The seedling strength index was calculated by relying on the percentage of germination in the final count and on the length of the rootstock and the coleoptile(Murti et al 2004).

RESULTS AND DISCUSSION

Germination in the first count (%): There was a significant effect of soaking treatments and soaking periods. The concentration of iron 150 mg.L exceeded the rest of the concentrations and the two comparison factors (dry seeds and distilled water), giving the highest percentage for the first count (84.42%), while the two comparison factors (dry seeds and distilled water) gave the lowest values of 71.5 and 79.08% respectively. The pre-soaking with chemical treatments is important to start the germination process in dry seeds (Panahi et al 2002). The 24-hour soaking period exceeded the 6 and 12 hours 83.75%), while the dry seeds (comparison) gave the lowest values of 71.50%. Farooq et al (2009) found that treated seeds sprouted faster compared to untreated dry seeds. The iron concentration was 150 mg. Liters -1 in the 24-hour soaking period provided the highest values of 85.25%, while the distilled water treatment in the 6-hour soaking period the lowest of 74.75%.

Germination in the final count (%): Iron treatment with a concentration of 150 mg / L^{-1} gave the highest average percentage of the final count over the rest of the iron treatments, in addition to the two comparison treatments (dry seeds and distilled water). Effect of the soaking period indicated that 24-hour period gave the highest average final count percentage of 1.69%, while the 6 and 12 hour periods gave the lowest average (87.56 and 90.19%, respectively). The treatment Fe150 mg L^{-1} at the 24-hour period the highest average (96.25%) (Table 1). The treatment of 100 ppm seeds of iron oxide nanoparticles gave the highest germination rate (41% greater than the comparison treatment-dry seeds) (Feizi et al 2013).

Root length (cm): There were significant differences between the soaking treatments and the duration of soaking and their interactions with the characteristic of root length (Table 3). The highest increase in the length of the rootstock was achieved by the treatment of iron with a higher concentration of 150 mg per L^{-1}, which gave 12.56 cm while the two comparison treatments dry seeds and distilled water the lowest average in root length (7.67 and 8.20 cm, respectively). Alidoust and Isoda (2013) found that the root length of soybean seedlings increased by 6, 8, 19 , 27, 37 and 40% when using nano-iron concentrations of 50, 100, 250, 500, 1000, and 2000 mg L^{-1}, respectively.

Yasmeen et al (2015) observed that characteristic of root length was high when iron nanoparticles were applied. The study demonstrated the catalytic effect of nanoscale iron on the germination and growth of wheat yield. Gulser et al (2019) observed when applying the highest rate of nanoscale iron 30 Mg / L^{-1} gave the highest average values of rootstock length in soybean plant. The 24-hour soaking time, gave the highest average for this characteristic (11.18 cm) while the 6-hour period gave the lowest average length of root of 9.07 cm. 150 with a 24-hour period gave the highest average length of the rootstock (13.44 cm) while the comparison treatment

Table 1. Effect of soaking the seeds with nan iron on the percentage of first and final count (%) of the wheat variety Research 10

Fe (mg.L)	First count (%)			Average	Final count (%)			Average
	6	12	24		Soaking time (hour)			
					6	12	24	
50	80.75	81.25	83.5	81.83	87.00	89.50	91.00	89.17
100	81.75	83	84.5	83.08	90.75	93.25	94.25	92.75
150	83.25	84.75	85.25	84.42	94.25	95.00	96.25	95.17
Water	74.75	80.75	81.75	79.08	78.25	83.00	85.25	82.17
Dry seeds (Comparison)		71.50				76.50		
CD (p=0.05)		1.89		1.09		1.87		1.09
Average	80.12	82.44	83.75		87.56	90.19	91.69	
CD (p=0.05)		0.94				0.94		

(distilled water) with a 6-hour soaking time gave the lowest average of 7.37 cm.

Coleoptile length (cm): Iron treatment with a concentration of 150 mg L^{-1} resulted in the highest average coleoptile length (cm) over the rest of the iron treatments, in addition to the two comparison treatments (dry seeds and distilled water. The effect of soaking time of 24 hours higher length. Average for the percentage of the final count, was 13.12 cm, while the periods of 6 and 12 hours gave the lowest (11.14 and 11.84, respectively). Maswada et al (2018) also observed the same trend. The treatment Fe150 mg L^{-1} at the 24-hour period, resulted in the highest average of 15.93 cm, while the comparison treatment (distilled water) with the 6-hour period gave the lowest average, with a non-significant difference with the 12-hour period amounting to 6.63 and 6.90 cm, respectively.

Fresh weight (mg): There were significant differences between the soaking treatments and the soaking time and their interactions for the soft weight characteristic (mg). The highest increase in the fresh weight of the seedlings was achieved by the treatment of iron with a higher concentration

of 150 mg L^{-1}, which was 19.19 mg. The two comparison treatments dry seeds and distilled water the recorded lowest average in fresh weight of the seedlings of 7.60 and 12.85 mg, respectively, and these results are consistent with Feizi et al (2013) where highest seed wet weight at 100ppm concentration of nanoscale iron was obtained. The increased concentrations of nanoparticles of iron significantly reduced the wet weight of the seedling and is highly recommended that low concentrations of the nanomaterials be applied in order to encourage seed germination and seedling growth (Table 3). The 24-hour infusion treatment gave the highest average of 19.53 mg, while the 6-hour period gave the lowest average fresh weight (mg) of 13.41 mg. The treatment of Fe150 with a 24-hour period gave the highest average fresh weight while the comparison treatment (distilled water) and the 6-hour soaking period gave the lowest average (16.12 and 8.8 mg, respectively).

Dry weight of seedlings (mg): The highest average dry weight was in iron at concentration of 150 mg L^{-1} (12.79 mg) over the rest of the iron treatments and the two comparison treatments (dry seeds and distilled water and with a non-

Table 2. Effect of soaking the seeds with nan iron on root and coleoptile length (cm) of wheat cultivar Research 10

Fe (mg.L)	Coleoptile length (cm)				Root length (cm)			
	6	12	24	Average	Soaking time (hour)			Average
					6	12	24	
50	8.625	10.375	10.675	9.892	10.88	11.64	12.43	11.65
100	9.125	10.575	11.493	10.398	13.33	14.50	15.39	14.41
150	11.185	13.070	13.44	12.565	13.72	14.33	15.93	14.66
Water	7.375	8.125	9.125	8.208	6.63	6.90	8.73	7.42
Dry seeds (Comparison)		7.675						
CD (p=0.05)		0.726		0.395		0.84		0.48
Average	11.183	10.536	9.078		13.12	11.84	11.14	
CD (p=0.05)		0.341				0.42		

Table 3. Effect of soaking seeds with nanoscale iron on fresh and dry seeding weight (mg) of wheat variety Research 10

Fe (mg.L)	Fresh seeding weight (mg)				Dry seeding weight (mg)			
	6	12	24	Average	Soaking time (hour)			Average
					6	12	24	
50	11.33	15.35	19.58	15.42	7.54	10.23	13.04	10.27
100	16.35	18.03	20.53	18.3	10.89	12.01	13.68	12.19
150	17.08	18.6	21.9	19.19	11.38	12.39	14.59	12.79
Water	8.8	12.75	16.12	12.85	5.91	8.49	10.74	8.38
Dry seeds (Comparison)		7.60				5.062		
CD (p=0.05)		1.46		1.07		0.97		0.71
Average	13.41	16.18	19.53		8.93	10.78	13.01	
CD (p=0.05)		1.15				0.76		

Table 4. Effect of soaking seeds with nano iron on the seedling vigor of wheat variety Research 10

Fe (mg.L)	Soaking time (hour)			Average
	6	12	24	
50	1697	1970	2102	1923
100	2038	2339	2534	2304
150	2347	2603	2828	2593
Water	1096	1247	1522	1288
Dry seeds (Comparison)		1070		
CD (p=0.05)		123.3		71.3
Average	1794	2040	2246	
CD (p=0.05)		61.7		

significant difference, with the treatment of iron 100 mg. Liter-1, (12.09 mg) (Table 3). Gulser et al (2019) also observed effect of the soaking period, the 24-hour period gave the highest average, reaching 13.01 g, while the 6-hour period gave the lowest average of 8.93 mg. The treatment Fe150 mg with L^{-1} at the 24-hour period recorded the highest average of 14.59 mg, while the comparison treatment (distilled water) with the 6-hour period gave the lowest average of 5.91 mg.

Seedling vigor index: The superiority of the treatment of iron with a concentration of 150 mg L^{-1} observed with highest average for the seed strength characteristic over the rest of the iron treatments (2593) in addition to the two comparison treatments (dry seeds and distilled water) with average of 1070. The soaking period, the 24-hour period gave the highest average for the seed strength characteristic, reaching 2246, while the 6-hour period gave the lowest average for the same characteristic (1794). Fe150 mg L^{-1} at the 24-hour period recorded the highest average of 2828, while the comparison treatment (distilled water) with the 6-hour period gave the lowest average of 1096. The seeding strength characteristic can be interpreted on the basis of root length, coleoptile and germination percentage.

CONCLUSION

The Nano fertilizers have a large surface area and the particle size of them is less than the pore size of the seeds, thus the penetration of the iron nano fertilizer to the surface is applied increased. The efficiency of absorption and the use of nano-nutrients increases germination, vigor and initiation characteristics significantly.

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Response of Orange Transplants to Spraying with Moringa Leaves and Ginger Extracts

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Abstract: The study was carried out in canopy of Kirkuk Agriculture Directorate during 2020-2021 growing season to study effect of Moringa and ginger extracts spray on growth and some leaf mineral content of local orange transplants *Citrus sinensis* L. This experiment was carried out on 81 transplants of orange transplants of homogeneous vegetative growth as much as possibility and two factors were used in this experiment. The two factors were used in this experiment first Moringa leaves extract spray (M) at concentration of 10 and 20 g.L^{-1} (M_{10}, M_{20}) along with without spray (M_0). The second ginger extract spray (G) at 10 and 20 g.L^{-1} (G_{10}, G_{20}) along with without spray (G_0). The moringa leaves extract at 20 gm.L^{-1} significantly increased in transplant height of 5.92 cm and highest leaf area of 20.43 cm^2 and highest leaf chlorophyll content of 29.03 mg.g^{-1}, highest leaf nitrogen content of 1.309 %, highest leaf phosphorus content of 0.373%, highest leaf potassium content of 1.559 % and highest leaf iron content of 180.3 %. The ginger extract spray at 20 gm.L^{-1} gave the highest increased in transplant height of 5.63 cm and highest leaf area of 22.79 cm^2 and highest leaf chlorophyll content of 30.92 mg.g^{-1}, highest leaf nitrogen content of 1.322 % , highest leaf phosphor content of 0.381 % , highest leaf potassium content of 1.544 5 and highest leaf iron content of 184.1 %. The interaction between treatments $M_{20}G_{20}$ gave a significant increased in all traits studied except leaf dry weight.

Keywords: Orange transplants, Moringa leaves, Ginger extracts

Citrus belongs to Rutaceae family, which is characterized by the presence of oil glands with an aromatic odor in most parts of the plant that distinguishes it from other types of fruits.This family includes many genera that spread in tropical and subtropical regions between latitudes 40° north and south of the equator .The genus *Citrus* is one of the most important of these genera, as it includes most species and varieties of economic importance for citrus because they are adapted to a wide range of environmental conditions ranging from hot, humid tropical climates and warm subtropical regions to cold regions. Citrus trees have a distinguished position among fruit trees due to their nutritional, economic, medical and aesthetic importance, as they are rich in vitamins, especially vitamin C, as well as vitamins A, B1, B2 and are also rich in mineral elements, especially calcium, phosphorous, potassium, iron, manganese, chlorine, sodium, sulfur, copper and other elements that lead. It plays an important role in activating the work of enzymes within the human body and providing energy that enhances its health, as well as the fact that fruits are a good source of dietary fiber with a low percentage of proteins and a very low content of fats (Liu et al 2010).

The number of fruitful orange trees in Iraq is estimated at approximately 6, 383, 881 trees and produces up to 142,717 tons, and average production of one tree is about 22.4 kg. Salah al-Din governorate is the first in orange production,

while Baghdad governorate was second in production, followed by Diyala governorate. As for the global production in 2019 was 78699,604 tons on area of 4060129 hectares. Brazil is first in orange production with a production of 17,073,593 tons, followed by China, India, the United States of America and Spain, and Egypt was the first country in production in the Arab world, followed by Algeria. The trees productivity is low in Iraq compared to the production of other countries, perhaps due to the lack of information on fertilization, irrigation, disease and insect control, as well as the lack of interest in post-harvest operations such as harvesting, transportation, storage and marketing. Recently, plant extracts have begun to be used as alternatives or supplements to fertilizers and pesticides, and may be added to soil or sprayed on plant. Some plant extracts contain some nutrients and some alternative materials for growth regulators, as well as some compounds that are used for control of diseases and insects. Among these extracts are turmeric, ginger, licorice root, garlic, onion, and moringa extract, in addition to algae extract (Bulgari et al 2019).

Moringa oleifera L. is one of the 13 species belonging to the genus Moringa and is the only genus in the family Moringaceae. This tree is called al-Yusr tree and in English it is called the Drumstick tree, or it is called the Horseradish tree because the taste of roots of this tree is similar to that of radish roots (Sanjay and Dwivedi 2015, Rani et al 2018).

Moringa is in Africa, especially in Sudan, Kenya and Ethiopia, and some sources indicate that the original home of this tree is India and from it moved to Africa, while other sources indicate that its original home is South Africa (Padayachee and Baijnath 2012, Rani et al 2018). This tree is rich in nutrients, especially in its leaves, as it contains high levels of carbohydrates, protein and minerals such as phosphorous, potassium, magnesium, zinc and iron, as well as flavonoids, phenols, carotenoids and amino acids. It also contains a high percentage of zeatin, which is one of the most The forms of cytokinins found in plants and auxins, and therefore the use of Moringa leaf extract rich in these components in spraying or in addition to other plants will contribute directly to encouraging the growth and development of the plant and increasing the enzymatic and hormonal activities within it (Meireles et al 2020). Considering the elements and hormones contained in Moringa leaves, many studies have been conducted and indicated that spraying or adding Moringa leaf extract to the plant in general and to fruit trees in particular plays a positive role in fruit trees growth. Abd El-Hamied and El-Amary (2015) included spraying pear trees of Le-Conte cultivar for two seasons, with three plant extracts (garlic extract, moringa extract and licorice root extract) and that spraying three concentrations of moringa leaf extract (2 and 4%) in addition to control treatment, spraying with this extract had a significant effect on leaf area and length and diameter of branches compared to the control treatment, especially when spraying with a concentration of 4%. Nasir et al (2020) conducted that spraying with Moringa leaf extract significantly affected the leaves potassium, calcium and zinc content, while spraying with this extract did not affect on leaves manganese and chlorophyll content.

Ginger is a perennial herbaceous plant belonging to Zingiberaceae family, which includes cardamom and turmeric. It is characterized by its fragrant smell, taste and color, white or yellow. It is considered a medicinal herb often used for medicinal purposes. Ginger rhizomes grow below soil surface and contain 9% protein and 50% starch, when delving into nutritional and chemical composition of ginger rhizomes, in addition to the minerals and vitamins contain amino acids, tannins, carbohydrates, terpenes, flavonoids, saponins, glycosides and sterols. Ginger extract plays a major role in promoting aspects of vegetative growth of fruit trees. AL-Shujairy and Al-Hadethi (2021) found that ginger extract spray at 10 and 20 g.L^{-1} caused significant increases in stem diameter, leaves dry weight and leaves mineral content for "Hollywood" plum trees. This study aims to determine effect of moringa leaves and gingers extracts spray on growth and leaves mineral content of "Local" orange transplants.

MATERIAL AND METHODS

The study was carried out in canopy of Kirkuk Agriculture Directorate during 2020-2021 growing season to study effect of Moringa and ginger extracts spray on growth and some leaf mineral content of local orange transplants *Citrus sinensis* L. This experiment was carried out on 81 transplants of orange transplants of homogeneous vegetative growth. The two factors were used in this experiment; first Moringa leaves extract spray (M) at concentration of 10 g.L^{-1} and 20 g.L^{-1} (M_{10}, M_{20}) along with without spray (M_0. The second ginger extract spray (G) at 10 and 20 g.L^{-1} (G_{10}, G_{20}) along with without spray(G_0) .Each treatment replicated three times with three transplants in experimental unit with a factorial experiment using RCBD. The following parameters were determined.

Transplant height (cm): Transplant heights were measured at the beginning and end of the experiment.

Leaf area (cm^2): Five leaves were taken from the middle position of shoot randomly and measuring leaf area (cm^2) using a Digimizer program Windows 7 operating system.

Leaf chlorophyll contents (mg.g^{-1} fresh weight): Representative fresh leaf sample at middle part of shoots were taken in the first week of June and used for analysis of chlorophyll by calorimetrically method according to Mackinny (1941).

Leaf dry weight (%): Various leaves were taken from the sampling were weighed and then dried. After stability of weight, the percentage of dry matter was calculated by dividing the weight after drying on the weight before drying× 100.

Leaf mineral content: Samples of ten leaves from middle shoots were selected at random from each replicate (1st week of April) to measure their content of N, P, K according to Wilde et al (1985) on dry weight basis. Iron was determined using atomic absorption as (Black 1965). The obtained results were subjected to analysis of variance by comparing differences between various treatment means.

RESULTS AND DISCUSSION

Effects on increase in transplant height, leaf area, leaves chlorophyll contents and leaves dry weight: The moringa leaves extract at 20 gm.L^{-1} (M_{20}) significantly increased in transplant height to 5.92 cm and highest leaf area of 20.43 cm^2 and highest leaf chlorophyll content of 29.03 mg.g^{-1} (Table 1). Moringa leaves extract did not significant effect on leaf dry weight. The lower values of these traits was in control treatment (M_0). The ginger extract spray at 20 gm.L^{-1} (G_{20}) gave the highest increased in height of 5.63 cm and t leaf area of 22.79 cm^2 and highest leaf chlorophyll content of 30.92 mg.g^{-1} while the spray treatments with ginger extract

did not affect in leaf dry weight .The lower values of these traits were in control treatment (G_0). The interactions between moringa leaves and ginger extracts significantly affected in all studied traits except leaf dry weight especially the interaction treatment ($M_{20}G_{20}$).

These results may be due to increase carbohydrates as a result of spraying moringa leaves extract, which can be used to provide the energy needed for vegetative growth processes, including increase in growths and leaf area as well as leaves number (Chen and Chen 2004). In addition to the fact that the extract of Moringa leaves contains IAA hormone and contains a number of amino acids, including the amino acid Tryptophan, which is the initiator in construction of natural auxin IAA, as the latter works to increase vegetative growth due to its role in increasing divisions and thus its reflection on vegetative growth. These results are in harmony with Nasir (2018). The ginger extract contain good amounts of proteins, fats and some necessary nutrients, as proteins and fats are used to provide energy needed for growth, in addition to the many nutrients inside plant, which may contribute to increasing vegetative growth, as well as increasing leaves chlorophyll content, because ginger extract contains good amounts of some micro-nutrients, especially iron, which may increase construction of chlorophyll and reduce its oxidation. These results are in agreement with AL-Shujairy and Al-Hadethi (2021) and Al-Dulaimi (2021) on plum trees and observed significant increase in vegetative growth when spraying with ginger extract.

Effects on mineral content in leaves: The moringa leaves extract at 20 gm.L^{-1} (M_{20}) significantly increased and gave the highest leaf nitrogen content of 1.309%, leaf phosphorus content of 0.373%, highest leaf potassium content of 1.559 % and highest leaf iron content of 180.3 % (Table 2). The ginger extract spray at 20 gm.L^{-1} (G_{20}) showed significantly superiority with highest leaf nitrogen content of 1.322 %, highest leaf phosphorus content of 0.381 % , highest leaf potassium content of 1.544 5 and highest leaf iron content of 184.1%. The interaction between treatments especially interaction treatment $M_{20}G_{20}$ gave the highest leaf nitrogen content of 1.340 %, highest leaf phosphorus content of 0.410 %, highest leaf potassium content of 1.612 % and highest leaf iron content of 194.2 %.

This increase may be due to contain these extracts on macro nutrients, especially nitrogen, phosphorus and potassium, as well as micro elements, which are directly absorbed when sprayed on leaves, thus increasing their percentage in plant. This may be attributed to increase in vegetative growth (Table 1) and photosynthesis process efficiency, which leads to absorption of these elements to vegetative growth and strength of tree growth. These results agree with those obtained by Abd Al Rhman et al (2018) on orange trees and observed significant increase in leaves mineral content when spraying with moringa leaves extract. AL-Shujairy and Al-Hadethi (2021) on plum trees found significant increase in leaves mineral content when spraying with ginger extract.

Table 1. Effects of spraying with moringa leaves and ginger extracts on increase in transplant height, leaf area, leaf chlorophyll contents and leaves dry weight of "Local" orange transplants

M	Increase in transplant height (cm)				Leaf area (cm^2)			
	G				G			
	0	10	20	Mean	0	10	20	Mean
M_0	4.15	4.36	4.82	4.44	16.25	17.30	19.22	17.59
M_{10}	4.56	5.13	5.59	5.09	16.60	17.79	23.26	19.22
M_{20}	5.41	5.88	6.47	5.92	17.12	18.28	25.90	20.43
Mean	4.71	5.12	5.63		16.66	17.79	22.79	
L.S.D 5%	G	M	Int.		G	M	Int.	
	0.42	0.42	0.73		0.91	0.91	1.58	
	Leaf chlorophyll contents (mg.g-1 fresh weight)				Leaf dry weight (%)			
M_0	22.19	24.73	29.22	25.38	29.14	32.53	29.90	30.52
M_{10}	24.12	26.56	30.36	27.01	30.72	29.56	28.31	29.53
M_{20}	24.84	29.07	33.17	29.03	28.04	31.64	30.18	29.95
Mean	23.72	26.79	30.92		29.30	31.24	29.46	
L.S.D 5%	G	M	Int.		G	M	Int.	
	1.17	1.17	2.03		N.S	N.S	N.S	

Table 2. Effects of spraying with moringa leaves and ginger extracts on leaves mineral content of "Local" orange transplants

M	Increase in transplant height (cm)				Leaf area (cm^2)			
	G				G			
	0	10	20	Mean	0	10	20	Mean
M$_0$	1.251	1.270	1.309	1.277	0.311	0.320	0.354	0.328
M$_{10}$	1.260	1.278	1.318	1.285	0.325	0.338	0.379	0.347
M$_{20}$	1.292	1.294	1.340	1.309	0.346	0.363	0.410	0.373
Mean	1.268	1.281	1.322		0.327	0.340	0.381	
L.S.D 5%	G	M	Int.		G	M	Int.	
	0.015	0.015	0.026		0.011	0.011	0.019	
	Leaf chlorophyll contents (mg.g-1 fresh weight)				Leaf dry weight (%)			
M$_0$	1.418	1.437	1.452	1.436	157.4	164.0	171.6	164.3
M$_{10}$	1.505	1.531	1.569	1.535	162.2	170.8	186.4	173.1
M$_{20}$	1.522	1.542	1.612	1.559	164.6	182.8	194.2	180.3
mean	1.482	1.503	1.544		161.4	172.3	184.1	
L.S.D 5%	G	M	Int.		G	M	Int.	
	0.052	0.052	0.090		5.16	5.16	8.93	

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Response of Varieties of Sweet Corn to different Glutamic Acid Concentrations and Planting Distances

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Abstract: Glutamic acid plays an important role in plant growth and development by its impact on the biosynthesis of proteins. An experiment was conducted in clay soil in fall 2020 at Baji, Salah Aldeen, Iraq to investigate the effect of three concentrations of glutamic acid (0, 150, 200 ml / L) and three planting distances (10, 20, and 30 cm) on two sweet corn cultivars (Arma, and Soleil.) The number of corn ears per plant, weight of 300 kernels, and plant yield were higher at 30 cm between plants than 10 and 20 cm. The glutamic acid, 200 ml / L, increased the number of kernels per ear, number of ears per plant, weight of 300 kernels, and plant yield. The yield of Soleil cultivar was higher than Arma cultivar. The interactions 20 cm with Arma cultivar and 200 ml / L glutamic acid gave higher plant yield.

Keywords: Sweet corn, Planting distances, Amino acid, Yield and components

Sweet corn (*Zea mays* L.) is one of the most popular crops in Australia, the United State of America, Canada, Brazil, and some other South American countries. In Iraq, this crop is not getting commercial importance due to low yields compared with the global producers. Therefore, to deal with this low yield scientific method to increase yield are required. The plants geometry plays an important role to increase yield. Knowing the best distances between plants is a useful tool to maximize yield quantity and quality (Rafiqet al2010). The high plant densities per unit of the cultivated area have a negative effect on producing high yield. The nutrition that available to a single plant will be less as well as the amount of radiation decreasing because of shading plants, which is reducing the photosynthesis process. Thus by adding nutrition through leaves such as amino acids can be used to improving plant growth and improving physiological processes (Jumaili 2016). This study aims study the effect of planting distances on the growth and yield of sweet corn.

MATERIAL AND METHODS

The experiment was conducted in fall 2020 in Baiji, Salah Al-Deen Iraq. The aims were understanding the effect of planting distances and glutamic amino acid spraying on the growth and yield of sweet corn for the two cultivars (Arma and Soleil). The crop was sown on July 1, 2020 and harvesting on September 28, 2020. The experiment was in RCBD with three replications. Each experimental unit had nine rows, three meters long. The distance between rows was 0.75 meters. The distance between plants was10,20 and 30 cm.The physical and chemical properties of the experiment soil are given in Table 1. The crop was raised as per standard recommendation Glutamic acid with three levels, 0, 150, and 200 m.L^{-1} was sprayed before the flowering period and when the sweet corn plants reached the flowering stage.

Study traits: The traits were measured from ten plants that were selected randomly. The number of days from planting to 75% tasseling, and the number of days from planting to 75% silking were calculated when the plants reached that stages. At the harvest time the number of ears plant^{-1} was measured, and the number of kernels.ear^{-1} was counting the average of the ten plant kernels sample. The weight of 300 kernels was taken a sample from the yield from ten plants after moisture reached 15%, and the average yield of kernels. plant^{-1} was calculated from the weight of the yield of the ten plants. Finally, protein percentage was estimated using the Kjeldahl device in the laboratory of the grain manufacturing company in Tikrit and the nitrogen was estimated by Kjeldahl for its percentage, and then the protein percentage was calculated as follows.

Proteinratio (%) = Nitrogenratio×6.25) (Hart & Fisher 1971).

Carbohydrates percentage were estimated according to Joslyn (1970). Two grams of fresh corn kernels were taken and put in a test tube, 80 ml of ethyl alcohol was added to the test tube and the mixture was placed in a water bath for 30 minutes at 60 ° C. The mixture was placed for 15 minutes in a centrifuge, and then the clear solution was extracted and the volume was completed. The solution was to 25 ml using

perchloric acid. 1 ml of the solution was taken and 1 ml of 5% phenol was added to it with 5 ml of sulfuric acid and the brown color was seen.

Statistical analysis: The data were analysed according to the Duncan test and at the level of 5% significance.

RESULTS AND DISCUSSION

There were significant differences between the cultivars in the number of days from planting until 75% of plants flowering (Table 2). The cultivar Soleil gave shorter period, 55.70 days, compared to cultivar Arma. The average of duration of the trait was 56.74 days. The 20 cm distance between plants gave lower average for the trait (55.55 days) and did not differ significantly from the average distance 30 cm (55.66days). The distance 10 between plants reached duration was 57.66 days as duration, and was the longest compared with other traits. The control trait, 0 ml.L^{-1} glutamic acid, gave an average of 55.22 days compared to the concentration 200 ml.L^{-1} with longest duration of 57.38 days. The interaction between cultivars and plants distances were significant. The cultivar Soleil with 20 cm distance recorded lowest flowering period (52.66 days) compared with the cultivar Arma and 30 cm distance, (58.44 days). The delay in tasseling may be due to its impacted by soil temperature

more than the air temperature. The increasing in plant density resulted in a decrease in soil temperature. Farther more, there was a competition between plants in obtaining the necessary light to complete physiological processes (Sangoi2000).

Number of days from planting to 75% silking: There was a significant difference between cultivars in the time to 75% of silking (Table 3). Cultivar Soleil gave the shortest period for the trait of 63.25 days compared with the cultivar Arma (64.33 days). The 30 cm distance gave a shorter duration of 63.05days, while the 10 cm distance gave the longest duration of 64.50 days. The control treatment, 0 ml.$^{-1}$ gave the lowest duration of the trait (63.22 days) and did not differ significantly from theconcentration of 150 ml. $^{-1}$(63.55 days). The concentration of 200 ml $^{-1}$ gave the longest period of 64.611 days. Cultivars and distance interaction was significantly different. The combination Soleil cultivar and 20 cm distance gave the lowest duration of 60.77 days while cultivar Arma with 20 cm distance show the longest period, 66.88 days and may depend on the genetic variation between cultivars (Zaborsky 2004).

Number of ears per plant: There were no significant differences between cultivars in the number of ears per plant (Table 4). The 10 and 20 cm distances gave the lowest average of number of ears per plant (1.06 and 1.08 respectively), however, the highest mean of the trait at 1.14 appear at 30 cm distance. The concentration of 200 ml.L^{-1} gave the highest number of ears per plant (1.17 ears); however, concentration 0 ml. L^{-1} gave the lowest mean for the trait (1.03 ear per plant) while the combination between the cultivar Soleil and the 30 cm distance gave a higher average of 1.15 ears per plant. The combination cultivar Arma and 10 cm distance gave the lowest average of 1.03 ears plant. Decreasing the plants number in certain area increasing the light density that plant received and that might be the reason

Table 1. Some physical and chemical properties of soil

Properties	Value
Soil texture	Silty clay
Electrical conduction (EC)	1.96 (dc/m)
Nitrogen	1.764%
Available phosphorous	0.02mg Kg^{-1}
Available potassium	0.04 mg Kg^{-1}
pH	6.8
Organic matter	0.8%

Table 2. Effect of amino acid spraying, planting distances and varieties onnumber of days from planting to 75% tasseling

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	56.33cd	57.00bc	57.00bc	52.00f	54.00e	55.00de	55.22c
150	56.00cd	57.33bc	58.00b	52.00f	56.00cd	57.00bc	56.05b
200	58.00b	60.00a	60.33a	54.00e	55.00de	57.00bc	57.38a
Average	56.77b	58.11a	58.44a	52.66d	55.00c	56.33b	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	56.66bc	54.50d	54.50d	0		55.77c	54.66d
150	56.66bc	55.00d	56.50bc	150		66 56b	55.44cd
200	59.00a	57.16b	56.00cd	200		57.77a	57.00ab
Average	57.44a	55.55b	55.66b	Average		56.74a	55.70b

* Similar letters mean indicate no significant differences

for increasing the photosynthesis and contributes to increase in the number of ears per plant and the high plant density caused lowering pollination, which might be reduced the number of ears per plant (Arif et al 2010).

Number of kernels per ear: There were no significant differences between the two cultivars in the number of kernels. Ear[-1] while 20 cm distance gave the highest average for the characteristic, 331,16 kernels. Ear[-1], and it did not differ significantly from the 30 cm distance. The average of the kernels. Ear[-1] was 328,38. The concentration 200 ml. L[-1] gave the highest average for the trait, and it was 332,33 kernels. Ear[-1]. The control treatment, 0 ml. L[-1] the lowest average, 309,88 kernels. Ear[-1]. The combination cultivar Arma and 20 cm distance was the highest average for the trait, and it was 372.11 kernels. Ear[-1] while the combination cultivar Arma and the 10 cm distance gave the lowest average for the trait, and it was 284.56 kernels. Ear[-1]. The number of kernels. Ear[-1] is impacting by environmental factors such as light density, temperature, and soil moisture. Increasing plants number per area will increase the competition between plants for water sources, light, which might be reduce the number of kernels per plant. Also, that could be reduce the leaf area, which play an importance role for plant growth and development (Monnevuex et al 2005).

Weight of 300 kernels: There were no significant differences between the two cultivars in the weight of 300 kernels (Table 6). The 30 cm distance gave the highest average for the trait (60.33 g) and did not differ significantly from the 20 cm distance (57.44 g.) The 10 cm distance gave the lowest average for the trait (50.889 g) and 200 ml.L^{-1} concentration showed the highest mean of 63.11 g. The control treatment, 0 ml.L^{-1} showed the lowest mean for the trait (48.72 g). The combination Soleil cultivar and the 30 cm distance reached the highest average of 61,55 g, while the combination soleil cultivar and the 10 cm distance of 10 cm reduced average to 50.22 g. The reason might be increasing the photosynthesis, which lead to increase the weight of 300 kernels (Al-Nouri and Al-Abadi 2013).

Plant yield (gm per plant): There was no significant to increase in the yield plant yield between the two cultivars.

Table 3. Effect of amino acid spraying, planting distances and varieties on flowering characteristics of 75% silking

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	63.33c-f	66.33ab	61.33hi	64.66cd	61.00ij	62.66e-h	63.22b
150	63.63c-f	66.66a	63.00dg	64.33cd	59.66j	64.00cde	63.55b
200	64.66cd	67.66a	63.33f-I	66.33ab	61.66ghi	65.00bc	64.61a
Average	63.88c	66.88a	62.22d	65.11b	60.77e	63.88c	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	64.00bc	63.66bc	62.00d	0		63.66b	62.77c
150	64.00bc	63.16c	63.50bc	150		64.44ab	62.66c
200	65.50a	64.66ab	63.66bc	200		64.88a	64.66ab
Average	64.50a	63.83b	63.05c	Average		64.33a	63.25b

* Similar letters mean indicate no significant differences

Table 4. Effect of amino acid spraying, planting distances and varieties on number of earper plant

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	1.00d	1.06bcd	1.06bcd	1.00d	1.03cd	1.06bcd	1.03c
150	1.00d	1.13abc	1.10bcd	1.03cd	1.03cd	1.16ab	1.07b
200	1.10bcd	1.20a	1.23a	1.23a	1.06bcd	1.23a	1.17a
Average	1.13d	1.13ab	1.13ab	1.08bc	1.04cd	1.15a	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	1.00e	1.05de	1.06cde	0		1.14b	1.04b
150	1.01de	1.08cd	1.13bc	150		1.07b	1.07b
200	1.16b	1.13bc	1.23a	200		1.17a	1.17a
Average	1.06b	1.08b	1.14a	Average		1.10a	1.09a

* Similar letters mean indicate no significant differences

The 30 cm distance increased yield per plant to 104.72 gm. The 10 cm distance decreased the average yield of kernelsto 88.88 gm per plant. The concentration of 200 ml.L^{-1} provided the highest average of 102.11gm per plant while the combination Arma cultivar and the 20 cm distance showed the highest average of 120.77 gm per Plant^{-1}, but it did not show a significant differ from the combination soleil and the 30 cm distance (119.33 gm perplant). The combination Arma and the 10 cm distance and the combination Soleil cultivar and the 20 cm distance gave the lowest average of 84.33 and

Table 5. Effect of amino acid spraying, planting distances and varieties number of kernels

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	de275.67	a366.67	cde291.33	cde306.00	e268.67	ab351.00	b309.88
150	cde289.33	a363.00	cde298.33	cde300.33	de278.67	ab249.00	b313.11
200	cde288.56	a386.67	cde308.33	bcd314.67	bc323.33	a372.33	a332.33
Average	b284.56	a372.11	b299.33	b307.00	b290.22	a357.44	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	d290.83	bcd317.67	bc321.17	0		b311.22	b308.56
150	cd294.83	bc320.8	bc323.67	150		ab316.89	b309.33
200	cd301.6	a355.00	ab340.33	200		ab327.89	a336.78
Average	b295.77	a331.16	a328.38	Average		a318.6	a318.22

* Similar letters mean indicate no significant differences

Table 6. Effect of amino acid spraying, planting distances and varieties on weight of 300gm grains

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	h44.00	fgh50.00	e-h52.00	h46.66	h46.66	d-h 53.00	c48.72
150	gh48.66	abc63.00	a-d60.66	gh48.66	c-g58.00	a-d62.00	b56.83
200	c-g58.00	ab67.66	abc64.66	b-f59.33	b-f59.33	a69.66	a63.11
Average	c50.22	a60.22	ab59.11	c51.55	bc54.66	a61.55	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	d45.33	cd48.33	c52.50	0		c48.66	c48.77
150	cd48.66	b60.50	ab61.33	150		b57.44	b56.22
200	b58.66	ab63.63	a67.16	200		a63.44	a62.77
Average	b50.88	a57.44	a60.33	Average		a56.51	a55.92

* Similar letters mean indicate no significant differences

Table 7. Effect of amino acid spraying, planting distancesandvarieties on plant yield

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	de82.00	a119.66	cde 85.66	bcd92.66	e80.33	a119.00	b96.55
150	cde84.66	a118.00	b-e90.00	b-e89.66	cde86.00	a118.33	b97.77
200	cde86.33	a124.66	bc94.66	b98.00	b-e88.33	a120.66	a102.11
Average	c84.33	a120.77	bc90.11	b93.44	c84.88	a119.66	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	b87.33	a 100.00	a102.33	0		b95.78	ab97.33
150	b87.16	a102.00	a104.16	150		ab97.55	ab98.00
200	b92.16	a106.50	a107.66	200		ab101.88	a102.33
Average	b88.89	a 102.83	a104.72	Average		a98.40	a99.22

* Similar letters mean indicate no significant differences

Table 8. Effect of spraying amino acid concentrations, planting distances on protein (%)

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	g4.93	efg5.10	d5.50	fg5.06	efg5.16	c5.80	c5.26
150	fg5.00	de5.53	d5.46	efg5.16	de5.33	b6.10	b5.40
200	ef5.20	de5.33	c5.76	de5.33	c5.80	a6.53	a5.69
Average	d5.04	c5.32	b5.57	d5.18	c5.43	a6.14	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	e5.00	de5.13	b5.65	0		c5.20	c5.32
150	e5.08	c5.33	b5.78	150		c5.26	b5.53
200	cd5.26	b 5.66	a6.15	200		b5.58	a5.80
Average	c5.11	b5.36	a5.86	Average		b5.35	a5.55

* Similar letters mean indicate no significant differences

Table 9. Effect of spraying amino acid concentrations, planting distances and varieties on carbohydrates (%)

Varieties	Arma			Soleil			Average
Concentrations	10	20	30	10	20	30	
0	k7.30	h-j7.93	efg8.83	ijk7.43	f-j8.23	def9.03	c8.12
150	jk7.40	g-k7.96	cde9.16	ijk7.83	e-h8.80	ab10.56	b8.62
20	h-k7.93	f-j8.20	cd9.73	f-h8.30	bc9.90	a11.03	a9.18
Average	d7.54	c8.03	b9.24	cd7.85	b8.67	a10.21	
Concentrations	10	20	30	Concentrations		Arma	Soleil
0	e7.36	cd8.08	b8.93	0		c8.12	c8.13
150	de7.61	c8.38	a9.86	150		bc8.45	ab8.78
200	cd8.11	b9.05	a10.38	20		a9.18	a9.17
Average	c7.70	b8.50	a9.72	Average		a8.58	a8.70

* Similar letters mean indicate no significant differences

84.88 gm plant , respectively(Tables 5 and 6).

Protein (%): There were no significant differences between the two varieties of Sweet corn. This result agreed with finding of Bylkam et al (2010) and Llyas et al (2014). The distance 30 cm gave the highest mean of the trait, which was 5.86 % compared with 10 cm. The higher concentration of glutamic acid gave recorded 5.69% compared to the comparison treatment 0m.L^{-1} (5.26%). This result was consistent with Fadroset al(2019) .The interaction treatment cultivar Soleil with distance 30cm gave the highest mean of 6.14% compared with the interaction treatment cultivar Arma with distance 10cm was gave the lowest average of 5.04%.

Carbohydrates (%): There was non-significant difference between the two varieties of sweet corn. The distance 30 cm gave the highest mean (9. 72 %) compared with the distance 10 cm which gave the lowest for the trait (7.70%). The higher concentration of glutamic acid (200 m.L^{-1}) gave the highest average for the trait (9.18%) compared with the control treatment which gave the lowest average (8.13%). The

interaction combination cultivar Soleil and distance 30cm gave the highest mean of the trait which reached 9.18 % and interaction Arma with distance 10cm gave the lowest average for the trait of (7.54%).

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Effect of Bacterial Inoculum and Spraying with Salicylic Acid on *Trigonella foenum-graecum* L.

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Abstract: Field experiment was carried out during the season 2020-2019 at Tikrit University which included 16 treatments resulting from the interaction of the inoculation with bacteria (*Sinorhizobium meliloti*). The treatments were inoculated and non-inoculated plants, spraying with plant growth stimulants at four concentrations (100 mg.l^{-1} salicylic, 100 mg.l^{-1} proline, salicylic 50 mg.l^{-1}+ proline 50 mg.l^{-1} and control) and the use of two types of irrigation water (well water and river water) to study the effect of factors and their interaction on the physiological characteristics of fenugreek plant (*Trigonella foenum-graecum* L). The results of the statistical analysis showed the superiority of bacterial pollination over unpollinated plants in each of the physiological growth traits represented in leaf area (8024 cm^2), dry matter production (147.095 g.) and the relative growth rate (15.0459 mg. g.$^{-1}$ day^{-1}) and the crop growth rate (0.86577 mg. cm^{-2}.day^{-1}) and absolute average growth rate (162.437 g. day^{-1}). The combination (salicylic 50 mg.l^{-1}+ proline 50 mg.l^{-1}) was superior to the rest of the concentrations used in the experiment for all traits with significant higher leaf area (8991.2 cm^2), dry matter production (165.673 g.) and relative growth rate (15.4831 mg. g^{-1}.day^{-1}), crop growth rate (0.98029 mg.cm^{-2}.day^{-1}) and the absolute growth rate (183,922 g. Day^{-1}). Irrigation with well water was superior to river water used in the experiment for all traits, with maximum leaf area were (8797.7 cm^2), the dry matter production (14 6.731 g), relative growth rate (14.879 mg. g.$^{-1}$. day^{-1}), crop growth rate (0.855 mg. cm^{-2}. day^{-1}) and absolute growth rate (160.522 g. day^{-1}).

Keywords: Salicylic acid, Proline acid, Bacterial inoculation, Fenugreek

Trigonella foenum-graecum L. belongs to Fabaceae family. Fenugreek plants are widely cultivated around the world for food and medicinal uses, where fenugreek is known for its pharmacological effect as an anti-inflammatory and is a large stockpile for many raw materials used in the pharmaceutical industry (Snehlata and Payal 2012). Among those substances are resins, tannins, flavonoids, coumarins, soaps, vitamins and alkaloids in varying proportions in fenugreek seeds. It also contains moisture, protein, saponins, ash and carbohydrates, fixed oil, and volatile oil (Taqi et al 2010). Fenugreek plant increases soil fertility by about 95% and ability to retain water and nutrients for plant growth, whether accompanying or following the crop (Rajib 2011). Higher plants do not benefit from atmospheric nitrogen until after it is fixed by prokaryotic microorganisms (Procaryotes) due to their possession of the nitrogenase enzyme, which works to reduce atmospheric nitrogen and convert it to ammonia (Tisdal et al 1993). The process of biological fixation by symbionts such as rhizobium bacteria (*Rhizobium* spp.), coexist symbiotically with leguminous plants, as it turns nearly 20 million tons of atmospheric nitrogen into ammonia (Simon et al 2014), Khalaf (2021) mentioned that biofertilization with bacteria (*Sinorhizobium meliloti*) is estimated to improve Physiological traits, growth and yield of fenugreek plants. As for the use of the

underground water source stored in the subsoil of those soils and is useful for irrigating these plants due to the ions and nutrients they contain important for plant growth, as these ions and elements lead to the dissolution of the ions and elements caught with the soil granules, liberating them and making them viable. For absorption by the plant, which affects the physiological processes and the yield, both quantitatively and qualitatively, the tendency to use modern methods in agriculture to reduce the environmental stress to which the plant is exposed and to increase the concentration of effective medicinal substances by adding growth regulators that are characterized by their ability to increase the formation of these substances in the plant, including the use of both salicylic acid. Proline, positive role in raising the efficiency of plants to reduce the negative effects of environmental stresses, which negatively affect the quantity and quality of yield.

MATERIAL AND METHODS

A field experiment was carried out in the winter season (2019-2020) at Research Station of Field Crops to study the effect of irrigation water quality (river and well) and spraying with salicylic acids and proline and the interaction between them as well as adding Bacterial inoculation to know the effect of treatments on growth characteristics, yield and

active substance of fenugreek plant. The experiment land was plowed by two orthogonal plows using disc harrows, then leveling and smoothing operations were carried out in order to prepare a suitable bed 10 days before planting. Then, phosphate and potassium fertilizers were added scattered before planting to the soil in one batch according to the treatments, where 120 kg ha^{-1} was added in one batch before planting (Al-Mosali and Abdel-Wahab 2010). The nitrogen fertilizer was added in two batches, the first with half the recommended amount when planting and the other half after a month of planting, at a rate of 90 kg. H^{-1} nitrogen in the form of urea (34% N) (Abdul-Hussein et al 2013). Then the land was divided according to the field plan of the experiment, 48 experimental units distributed in three replicates, with 16 experimental units and the area of the experimental unit (4 square meters).

Studied Traits

Leaf area duration (cm^2. measurement time $^{-1}$):

LAD = (LA1-LA2)*(T2-T1)/2

LA= Leaf Area, T= Time

Dry matter production duration (g. measurement duration^{-1}): This characteristic refers to the function of biological weight and temperature, and is useful in obtaining the value of the dry matter in time, and it is estimated Hunt 1982.

B.M.D = (T2-T1) (W2+W1) / 2

T= Time, W= Weight.

Relative growth rate of plant (mg day^{-1}): It is estimated from the equation according to Hunt (1982).

R.G.R=(Lin W2 – Lin W1) /(T2-T1)

Lin= logarithm W= Weight, T= Time

Crop growth rate (mg. cm^{-2}. day^{-1}): It increase in the dry weight of the plant per unit area of land per unit time or increase in the dry duration production rate per unit area of land per unit time (mg. cm^{-2}.day^{-1}) (Ashwini 2005). The growth rate of the crop is calculated according to the equation (Hunt 1982).

C.G.R = (1/AG) (W2-W1) (T2-T1)

AG= Ground Area occupied by plant

Absolute growth rate (AGR): This criterion indicates that at what rate is the growth of the crop whether the growth of the crop is at a faster or slower rate than normal and is expressed in grams of dry matter produced during the day (Hunt 1982).

AGR= (W2-W1) (T2-T1)

W= Weight , T= Time.

RESULTS AND DISCUSSION

Leaf area: The bacterial inoculum (*Sinorhizobium meliloti*) had a significant effect on the survival time of the leaf area of fenugreek plant compared to the non-inoculated plants (the

comparison 8024.2 cm^2). The leaf are in plants not inoculated with bacteria has significant higher area (12.36%) compared to the control treatment (7032.2 cm^2) to due to pollination with bacteria (*Sinorhizobium meliloti*), which fixes atmospheric nitrogen through the symbiotic relationship between the plant and the bacteria and thus leads to an increase in the efficiency of the metabolic processes of the plant. This results in an increase in dry matter production of the plant. The increase in the dry matter production using the inoculum is due to the ability of *Sinorhizobium meliloti* bacteria. On fixing atmospheric nitrogen bacteria inimproving plant growth through the production of growth-regulating substances that lead toincreased nitrogen uptake from the soil in addition to other nutrients such as phosphorous andpotassium (Vande Broke et al 1997). This effect is reflected in an increase in plant growth and dry weight with significant differences between the treatments. The combinationsalicylic 50 mg.l^{-1} + proline 50 mg.l^{-1} performed significantly better than other treatments. The reason for this increase is attributed to the important role of salicylic acid in revitalizing meristematic tissues, increasing cell division, increasing the production and effectiveness of growth regulators, especially auxins and cytokinin (Kaydan et al 2007) and increasing vegetative growth characteristics, including the number of vegetative branches and plant height. Salicylic acid has an important role in raising the efficiency of photosynthesis and increasing the concentration of hormones such as cytokines, gibberellins and auxins (Yanova 2010). The amino acid proline shows its effect in building proteins, which consist of the union of different amino acids, including proline acid, and thus building plant tissues, and that its addition leads to an increase in the duration and number of cell divisions and their expansion, as well as the role of proline acid in increasing the plants ability to photosynthesis through controlling the process of opening and closing stomata and increasing the plant's ability to build chlorophyll pigments (Raven 2002). The quality of the irrigation water (well water and river water) affected leaf area of fenugreek plants and there were significant differences between the use of well water, as the well water recorded an average of 8797.7 cm^2 with a significant difference for the river water, recorded the lowest average (6258.8 cm^2). The difference in irrigation water has a moral effect on the studied characteristic, and the reason for this is due to the presence of nutrients in the well water, which clearly affect the processes of photosynthesis and metabolic building processes of cells and tissues through the participation of these elements in building proteins and increase the effectiveness of enzymes, hormones and other vital compounds that increase the vegetative growth of the plant. The bilateral interaction between the treatment of the

inoculum and the growth stimulants showed significant difference in favor of the treatment of bacterial inoculation and the combination (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}), which recorded the highest significant value compared to the rest of the treatments . The lowest mean was in plants not inoculated with bacteria and the comparison treatment for growth stimulants, with a difference of (45.71%). The binary interaction of the treatment of the inoculum and the quality of the irrigation water varied among different combinations. . The treatment of the inoculum and the well water used in irrigation gave the highest significant value for the studied trait with an average of 9353.6 cm^2. with a difference from the lowest value of the treatment of unpollinated plants and water the river. The dual interaction of growth stimulants and the quality of irrigation water recorded a significant superiority with the treatment salicylic 50 mg.l^{-1} + Proline 50 mg.l^{-1} for growth stimulants with the use of well water, a significant superiority (10740.5 cm^2.) over the comparison treatment of growth stimulants and river water. The results of the triple interaction of the bacterial inoculum factor and the growth stimulator for the combination (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) and well water gave a significant difference over the rest of the treatments applied (11904.8 cm^2.) compared to the lowest average of the triple interaction of non-inoculated plants and the comparison treatment of growth stimulator and river water.

Dry matter production duration (gm): The bacterial

inoculum had a significant effect on the duration of dry matter production of fenugreek plants compared to the non-inoculated (control) plants with average of 147,095 g. compared to the non-inoculated plants with bacteria (Table 2). There were significant differences between the treatments of adding growth stimulants (salicylic and proline), where the combination gave (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) highest value for the mean of the studied characteristic compared to the rest of the blends (100 Salicylic, 100, Proline, 0) mg. liter^{-1} which averaged (165.673 g) with an increase of 55.93% over the comparison treatment. The quality of irrigation water affected the quality of the dry matter survival of fenugreek plants, The statistical analysis indicated that there were significant differences between the use of well water and river water, as the well water recorded an average of (146.731 g.^{1}) with a significant difference of 18.67%) for river water, which recorded the lowest average (119.332 g). The difference in irrigation water has significant effect on the studied trait. As for the bilateral interaction between the treatment of bacterial inoculum and growth stimulants, the treatment of bacterial inoculation and the combination (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) gave the highest significant value compared with the rest of the treatments with an average amount of 185,302 g compared to the lowest mean recorded by the treatment of plants not inoculated with bacteria and the comparison for growth stimulants, (69.512gm with a difference of 62.48%. The The

Table 1. Effect of bacterial inoculum, growth stimulator and irrigation water quality onleaf area duration (cm^2)

Stimulants	Well		River		Mean	Inoculation x stimulants		Irrigation water x stimulants	
	Non inoculated	Bacterial inoculum	Non inoculated	Bacterial inoculation		Non inoculated	Bacterial inoculation	Well	River
Control	5785.5 ghi	6073.7 fgh	5027.8 j	5359.7 ij	5561.7 d	5406.7 e	5716.7 e	5929.6 f	5193.8 g
Salicylic	9124.4 c	10279.6 b	6132.7 fgh	7090.5 e	8156.8 b	7628.6 c	8685.1 b	9702.0 b	6611.6 e
Proline	84840.7 d	9156.2 c	5661.8 hi	6313.9 fg	7403.1 c	7071.3 d	7735.0 c	8818.5 c	5987.8 f
SA-Pro	9576.2 c	11904.8 a	6468.6 f	8015.4 d	8991.2 a	8022.4 c	9960.1 a	10740.5 a	7242.0 d
Mean	8241.7 b	9353.6 a	5822.7 d	6694.9 c		7032.2 b	8024.2 a	8797.7 a	6258.8b

*Similar letters indicate that there are no significant differences at the 5% probability level

Table 2. Effect of bacterial inoculum, growth stimulator and irrigation water quality on the dry matter production (gm)

Stimulants	Well		River		Mean	Inoculation x stimulants		Irrigation water x stimulants	
	Non inoculated	Bacterial inoculum	Non inoculated	Bacterial inoculation		Non inoculated	Bacterial inoculation	Well	River
Control	74.886 jk	80.081 j	64.138 k	72.989 jk	73.008 d	69.512 e	76.505 e	77.452 e	68.564 f
Salicylic	140.65 fg	196.009 b	125.243 h	149.857 ef	152.94 b	132.947d	172.933b	168.329 b	137.550 d
Proline	122.347 hi	168.421 d	132.393 gh	138.86 fg	140.51 c	127.37 d	153.641	145.384 c	135.627 d
SA-Pro	180.620 c	210.898 a	111.471 i	159.71 de	165.673a	146.045c	185.302a	195.759 a	135.588 d
Mean	129.626 b	163.837 a	108.311 c	130.35 b		118.968	147.095	146.731 a	119.332b

*Similar letters indicate that there are no significant differences at the 5% probability level

treatment of bacterial pollen and well water used in irrigation gave the highest significant value for the studied trait with an average of (63.837 g with a difference from the lowest value of the treatment of unpollinated plants and rive water (33.89%).The dual interaction of growth stimulants and irrigation water quality recorded, the treatment salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1} with the use of well water was significantly superior to the mean for the characteristic (195.759 g) over comparative treatment of growth stimulants and river water.The triple interaction of bacterial inoculum factor and growth stimulator for the combination (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) and well water gave a significant difference over the rest of the treatments applied in the experiment with an average of 210,898 gm. compared to the lowest average of the triple interaction of non-inoculated plants and the comparison treatment of growth stimulator and river water (64.138 g) .

Relative growth rate (mg.gm^{-1}.day^{-1}): The inoculum with (*Sinorhizobium meliloti*) had a significant effect on the relative growth rate of fenugreek plant compared to the non-inoculated plants (the comparison), as it recorded an average of 15.0459 mg.g^{-1}.day^{-1}) for plants not inoculated with bacteria with a significant difference 13.31%) compared to the control treatment (non-inoculated), which recorded the lowest mean of 13.0424 mg.gm^{-1}.day^{-1} (Table 3). There were significant differences between the treatments of adding growth stimulants (salicylic and proline). The concentrations 50 salicylic + 50 proline, 100 salicylic and 100 proline mg.l^{-1} was significantly superior in studied trait compared to concentration. The average was 15.4831 mg.gm^{-1}day^{-1} with anincrease of 0.81% compared to the comparison treatment. The quality of irrigation water affected the relative growth rate of the fenugreek plants and there were significant differences between the use of well water and river water. The well water recorded an average of 14.8792 mg.g.g^{-1}.day^{-1} with a significant difference of 18.67% for river water. The bilateral interaction between the treatment of bacterial inoculum and growth stimulants, indicated bacterial

inoculation and combination (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) and the concentration (salicylic 100 mg.l-1) gave the highest significant value compared with the rest of the treatments (17.2197 and 16.6303 mg.g.g^{-1}.day^{-1}, respectively), compared to the lowest mean recorded in plants not inoculated with bacteria and compared to growth stimulants (37.19 and 34.96%). The binary interaction of the inoculum treatment and irrigation water quality recorded significant differences in the relative growth rate. The treatment of pollen with bacteria and well water used in irrigation gave the highest significant value for the studied trait (16.0033 mg.gm^{-1}.day^{-1}) with a difference of 16.0033 mg.gm^{-1}.day^{-1}. The lowest value for the treatment of unpollinated plants and river water was 22.95%, (12.3296 mg.gm^{-1}.day^{-1}). The dual interaction of growth stimulants and irrigation water quality recorded a significant superiority of salicylic 50 mg.l^{-1} + Proline 50 mg.l^{-1} with the use of well water (17,243 mg.g.$^{-1}$.day^{-1}). The   comparison treatment with growth stimulants and river water, which recorded the lowest average (10,312 mg.gm^{-1}.day^{-1}). The results of the triple interaction of the bacterial inoculum factor and the concentrations of growth stimulator (50 salicylic + 50 proline, 100 salicylic and 100 proline) and well water showed a significant difference over the rest of the treatments (18,043 mg.gm^{-1}.day^{-1}) compared to the lowest average of the triple interaction of unpollinated plants and the comparison treatment of growth stimulator and river water (10.202 mg.gm^{-1}.day^{-1}) with a difference of 43.45%.

Crop growth rate (mg. cm^{-2}. day^{-1}): The bio-inoculation with *Sinorhizobium meliloti* had a significant effect on the crop growth rate of fenugreek plants compared to the non-inoculated plants (the comparison), with average of 0.86577 mg. cm^{-2}. day^{-1} (Table 4). As compared to plants not inoculated with bacteria (0.61133 mg.cm^{-2}.day^{-1}). There were significant differences between the treatments of adding growth stimulants (salicylic and proline) and combination salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1} (0.98029 mg.cm^{-2}.day^{-1}) compared to the rest of the concentrations with an increase of

Table 3. Effect of bacterial inoculum, growth stimulator and irrigation water quality on relative growth (mg.gm^{-1}.day^{-1})

Stimulants	Well		River		Mean	Inoculation x stimulants		Irrigation water x stimulants	
	Non inoculated	Bacterial inoculum	Non inoculated	Bacterial inoculation		Non inoculated	Bacterial inoculation	Well	River
Control	11.014 d	11.207 cd	10.202 d	10.424 d	10.7116 b	10.6081 c	10.8151 c	11.1103 d	10.3128 d
Salicylic	13.737 bc	17.837 a	14.045 b	15.424 ab	15.2604 a	13.9243 b	16.6303 a	15.7865ab	14.734 bc
Proline	13.827 bc	16.927 a	14.022 b	14.110 b	14.7214 a	13.9243 b	15.518 ab	15.3768bc	14.066 bc
SA-Pro	16.443 ab	18.043 a	11.050 d	16.396 ab	15.4831 a	13.7466 b	17.2197 a	17.2431 a	13.7232 c
Mean	13.7551bc	16.0033 a	12.3296c	14.0885 b		13.0424 b	15.0459 a	14.8792 a	13.2091b

*Similar letters indicate that there are no significant differences at the 5% probability level

69.04%. The quality of irrigation water affected the crop growth rate of the fenugreek plant, with significant differences between the use of well water and river water, as the well water recorded an average of (85556 mg.cm^{-2}.day^{-1} with a significant difference of percentage 18.67% for river water. The bilateral interaction between the treatment of the bacterial inoculum and the growth stimulants, the treatment of bacterial inoculation and the combination salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1} gave the highest significant value compared to the rest of the treatments with an average of 1.17325. mg.cm^{-2}.day^{-1}. The lowest mean was recorded by the treatment of plants not inoculated with bacteria and the comparison treatment for growth stimulants, which recorded an average of (0.28400 mg.cm^{-2}.day^{-1}). The binary interaction of the inoculum treatment and the quality of the irrigation water showed significant differences in the growth rate of the crop. The treatment of the bacterial pollen and the well water used for irrigation gave the highest significant value for the studied trait with an average of 1.01279 mg. cm^{-2}.day^{-1} with a difference from the lowest value for the treatment of unpollinated plants and river water which amounted to 48.22%. The dual interaction of growth stimulants and irrigation water quality, the treatment salicylic 50 mg.l^{-1} + Proline 50 mg.l^{-1} for growth stimulants with the use of well water with significant superiority (1.23867 mg. cm^{-2}.day^{-1}) in the comparison treatment of growth stimulants and river water. The results of the triple interaction of the bacterial

inoculum factor and the concentrations of the growth stimulator (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) and (salicylic 100 mg.l^{-1}) and well water showed a significant difference over the rest of the treatments. The experiment had an average of the highest value of (1.38067 and 1.27583 mg. cm^{-2}.day^{-1}) as compared to the lowest value of the triple interaction of unpollinated plants and the comparison treatment of growth stimulator and river water with a difference of 81.63% from the highest value.

Absolute growth rate (g. day^{-1}): The inoculum with (*Sinorhizobium meliloti*) had a significant effect on the absolute growth rate of fenugreek plant compared to the non-inoculated plants (the comparison), average of 162.437 g. day^{-1} for plants not inoculated with bacteria with a significant difference (29.38%) as compared to the control treatment (non inoculated) (114,697 g. day^{-1}) (Table (5). There were significant differences between the treatments of adding growth stimulants (salicylic and proline). The combination salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1} (183.922 g. day^{-1}) significant superior compared to the rest of the concentrations. The comparison treatment recorded the lowest mean (56.927 g. day^{-1}). The quality of irrigation water affected the absolute growth rate of the fenugreek significant differences between the use of well water (160.522 g. day^{-1}) over river water. The bilateral interaction between the treatment of bacterial inoculum and growth stimulants, combination (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) was

Table 4. Effect of bacterial inoculum, growth stimulator and irrigation water quality on crop growth rate (mg. cm^{-2}. day^{-1})

Stimulants	Well		River		Mean	Inoculation x stimulants		Irrigation water x stimulants	
	Non inoculated	Bacterial inoculum	Non inoculated	Bacterial inoculation		Non inoculated	Bacterial inoculation	Well	River
Control	0.314 g	0.347 g	0.253 g	0.297 g	0.303 d	0.284 f	0.322 f	0.331 e	0.275 e
Salicylic	0.737 e	1.275 a	0.668 e	0.865 d	0.886 b	0.702 e	1.070 b	1.006 b	0.766 d
Proline	0.645 e	1.046 bc	0.697 e	0.746 e	0.783 c	0.671 e	0.896 c	0.846 c	0.721 d
SA-Pro	1.096 b	1.380 a	0.478 f	0.965 cd	0.980 a	0.787 d	1.173 a	1.238 a	0.721 d
Mean	0.698 b	1.012 a	0.524 c	0.718 b		0.611 b	0.865 a	0.855 a	0.621b

*Similar letters indicate that there are no significant differences at the 5% probability level

Table 5. Effect of bacterial inoculum, growth stimulator and irrigation water quality on absolute growth rate (g. day^{-1})

Stimulants	Well		River		Mean	Inoculation x stimulants		Irrigation water x stimulants	
	Non inoculated	Bacterial inoculum	Non inoculated	Bacterial inoculation		Non inoculated	Bacterial inoculation	Well	River
Control	59.02 g	65.26 g	47.53 g	55.90 g	56.927 d	53.275 f	60.580 f	62.141 e	51.713e
Salicylic	138.27 e	239.36 a	125.32 e	162.34 d	166.32 b	131.792e	200.851b	188.815 b	143.828 d
Proline	121.08 e	196.40 bc	130.93 e	139.98 e	147.097c	126.005e	168.190c	158.742 c	135.453d
SA-Pro	205.73 b	259.05 a	89.70 f	181.20 cd	183.922a	147.716d	220.127a	232.391 a	135.452 d
Mean	131.025 b	190.020 a	98.369 c	134.854 b		114.697b	162.437a	160.522 a	116.611b

*Similar letters indicate that there are no significant differences at the 5% probability level

significantly superior over rest of the treatments with an average of 220.127 g day^{-1}. The binary interaction of the inoculum treatment and the quality of the irrigation water recorded significant differences in the absolute growth rate, and the treatment of the bacterial inoculum and the well water used in irrigation gave the highest significant value for the studied trait with (190.020 g. day^{-1}) as compared with lowest value in unpollinated plants and river water. The treatment salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1} with well water recorded significant superiority (232.319 g. day^{-1}) over comparison treatment of growth stimulants and river water. The results of the triple interaction of the bacterial inoculum factor and the concentrations of growth stimulator (salicylic 50 mg.l^{-1} + proline 50 mg.l^{-1}) and (salicylic 100 mg.l^{-1}) and well water showed a significant difference over the rest of the treatments with maximum of (259.05 and 239.36 g. day^{-1}) compared to the lowest value of the triple interaction of unpollinated plants and the comparison treatment of growth stimulator and river water.

CONCLUSIONS

The response of the fenugreek plant to the bacterial inoculum, well water and spraying with salicylic acid and proline in combination (salicylic 100 mg. l^{-1} + proline 100 mg. l^{-1}) was positively reflected in all studied traits by giving it a significant increase in all physiological characteristics of the fenugreek plant compared to non-inoculated plants, no-spray treatment and river water.

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Effect of Clove and Lemon Essential Oil Mixture on Gene Expression SIX3 and SIX5 of *Fusarium oxysporum f. sp. lycopersici* Wilt in Tomato

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Abstract: This study was carried out with the aim of evaluation the efficiency of essential oil mixture (EOM) in normal and nano state of clove and lemon peel essential oil in gene expression of SIX3 and SIX5 of *Fusarium oxysporum f. sp. lycopersici* that causes tomato seedling wilt. Three concentrations 600, 800 and 1000 ppm of normal and nano mixture and two concentrations of fungicide (Topsin) 25 and 50 ppm were evaluated. Normal and nano mixture had more effect on inhibiting the gene expression of the virulence genes SIX3 and SIX5 than the fungicide. SIX3 gene expression was more affected than SIX5 expression. Where the concentration of 1000 ppm of EOM, whether in nano or normal state, recorded the highest inhibition in gene fold expression of the SIX3 gene (0.42337 and 0.45376, respectively) comparing to other treatment.

Keywords: *Fusarium oxysporum f. sp. lycopersici* , Tomato, SIX3 and SIX5 gene, Clove and lemon essential oil

Fusarium oxysporum is the main cause of wilt, rotting, and seedling damping-off diseases for more than 100 species of economically important plants (McGovern 2015 and Sidharthan et al 2018). The genus *Fusarium* is a soil fungus facultatively parasitizing and prefers to parasitize on living tissues more than saprotrophic (Francia et al 2007), which is found in all types of soils, remain in soils for a period ranging from 10-15 years without the presence of a host (McGovern 2015 and Bawa 2016). The genus *Fusarium spp*. is widespread in almost every country in the world and has a wide family range, infecting vegetables, cereal crops, ornamental plants, orchards, and forest trees identified as the fifth most important plant pathogen (Dean et al 2012). Furthermore, this genus produces a group of mycotoxins such as Tirchothecenes and Fumonisins, which can contaminate agricultural products and make them unhealthy for human consumption. These toxins can act as virulence factor of the pathogen (Desmond et al 2008 and Ilgen et al 2008). The genus *F. oxysporum* includes a group of species, some of them are pathogenic or non-pathogenic species. Non-pathogenic species or strains are commonly found in the soil and live saprotrophic on dead plants and decomposing materials. On the other hand, specific species have been identified as biocontrol agents (Alabovette et al 2009). Though, pathogenic strains are classified into their formae specials based on their host specificity such as lycopersici that causes infection to tomato plants (Leslie and Summerell 2006). It is one of the pathogens that are difficult

to control and therefore avoiding the occurrence of infection by pathogenic fungi using traditional methods is almost impossible. The pathogen attacks plants through root system, then the xylem, which prevents the optimal growth and development of host plant (Berges et al 2013, Giurgiu et al 2018). In general, more than 120 forms of *F. oxysporum* have been recorded (Michielse and Rep 2009). The identification of the genome sequences of FOL fungus that causes fusarium wilt on tomato helped greatly to discover the chromosomes that contain pathogenic genes, which include Xylem (SIX) genes in many cases that called virulence genes, through which pathogenic types are distinguished from non-pathogenic ones. The products of pathogenic genes are small proteins called effector proteins that are secreted when they attack the plant cells to prevent plant resistance to the pathogen (Ma et al 2010). Fourteen six pathogenicity genes have been identified in FOL fungi (Houterman et al 2007, Schmidt et al 2013), most of which are on chromosome 14 (Schmidt et al 2013). Both SIX3 gene known as Avr2 and SIX5 gene are the genes that actively contribute to virulence of the pathogen (Houterman et al 2009, Rep Takken 2010, Gawehns et al 2014 and Ma et al 2015). The aim of this study was to evaluate the mixture of Clove and Lemon peel essential oil in gene expression of SIX3 and SIX5 compared to fungicide Topsin

MATERIAL AND METHODS

Fungal strain: Isolate of *F. oxysporum f. sp .lycopersici* were

obtained from the Mycotoxins Laboratory, Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. These isolates were identified and deposited in the gene bank, Accession Number MH458918 (Al-Dulaimi 2019). The morphological and microscopic characteristics of the fungus were re-identified and studied for the level of genus and species, depending on the characteristics of the fungal colony, and the nature and color of the mycelium. Besides, the conidial compositions that it forms, using the approved taxonomic keys (Booth 1977, Leslie and Summerell 2006).

Fungus pathogenicity: The pathogenicity of the fungus (FOL) was tested in vitro using radish and tomato seeds. Nutrient medium (PDA) was prepared and sterilized by placing in an autoclave at a temperature of 121°C and a pressure of 15 lb/in^2 for 15 minutes. Then was left to cool and before it hardens, an antibiotic Tetracycline at a concentration of 2 g/L was added to prevent the bacteria growth, the medium was poured into sterile Petri dishes with a diameter of 9 cm**.** The dishes contaminated with pathogenic fungi (FOL). Twenty sterilized radish and tomato seeds were placed separately, for each dish in three replicates with control treatments. The dishes were placed in the incubator at a temperature of 25 ± 2°C for seven days, after which germination percentage was calculated.

Essential oils: The essential oils of limon (*Citrus limon L. Burm*) and cloves bud (*Dianthus chinensis L.*) extracted using clevenger apparatus. The essential oils mixture 1:1 were transferred from normal state to nano state (green synthesized) by exposure to a 20 kHz and 400 watts. Ultrasonic prpbe probe Ultrasonicator (U.K Soniprep) for four minutes. The nano state was examined using: Atomic Force Microscopy-(AFM), UV-Vis Spectroscopy and Particle Size Analyzer (PSA) (AL-Muttairi et al 2021).

 Sample preparation: Sabouraud Dextrose Broth (SDB) medium was prepared according to the instructions of the manufacturer Hi-media and 30 g/l and placed in an autoclave for sterilization. After the sterilization process was completed, the antibiotic Tetracycline at a concentration of 2 g / l was added. The sterile medium was distributed on a set of 10 ml test tubes and before contaminating the tubes with the pathogenic fungus, three selected concentrations of the nano and normal essential oil mixture (600, 800, 1000 ppm) and two concentrations of the fungicide Topsin (25, 50 ppm) were added separately. The tubes without contamination were kept as control**.** Test tubes were contaminated with the pathogen by taking mycelium from a colony of FOL at the age of 7 days with a sterile needle tip, the colony was prepared in advance on a medium of Potato Dextrose Agar (PDA). Then, the tubes were tightly closed and placed in an incubator at

25°C. After six days, 1.5 ml of each sample was withdrawn and placed in new tubes and centrifuged at a speed of 10,000 rpm for five minutes, the precipitate was taken and the raw material was discarded. Then 1 ml of TransZol reagent was added and the tubes were kept in a deep freezer until use.

Total RNA extraction: The TransZol reagent was used to isolate total RNA from fungal cells according to the extraction program described by (Gautam et al 2016). The extraction process includes leaving the preserved samples five minutes at room temperature to melt and disintegrate. Then, the samples were mixed to homogenized, where 0.2 ml of chloroform was added for every 1 ml of TransZol, the tube was manually shaken vigorously for 30 seconds. The tubes were incubated for 3 minutes at room temperature, as the samples were centrifuged at a speed of 10,000 rpm for 15 minutes at a temperature of 2-8°C. The mixture was separated into an organic pink lower phase and colorless aqueous interphase and upper phase, which was the phase containing RNA. The volume of the aqueous phase was about 60% of the TransZol volume. The aqueous phase of each sample was transferred using a micropipette to clean, sterile, new tubes. 0.5 ml of isopropanol was added for every 1 ml of TransZol. The mixture was mixed by turning the tube up and down by hand. Then the mixture was incubated at room temperature for 10 minutes. Moreover, 1 ml of ethanol at a concentration of 75% was added. samples were placed in a centrifuge at a speed of 7500 rpm for 5 minutes at a temperature of 2-8°C. Then, the supernatant was removed and the precipitate RNA was retained. The RNA was left for 5 minutes exposed to the room air to dry then was dissolved by adding 50-100 µL of RNase-free water to each sample. Finally, pure RNA was incubated for 10 minutes at 55-60°C and that can be stored for a longer time at -70°C.

Primer preparation: The primers were prepared by company Alpha and, S.E.N.C, and they were lyophilized (Table 1). Stock solution was prepared by adding different concentrations of Nuclease-free water to reach the concentration of 100 pmol/ µL. After that, the solution centrifuged and left for 2-24 hours to be ready for use and can be kept at -20°C until uses according to the manufacturer's instruction.

Working solution: A small divisions known as the working solution was prepared, which included a solution with a concentration of 10 pmol/ µL, 10 µL of the storage solution was added to 90 µL of Nuclease free water according to the company instruction.

cDNA synthesis: The RNA of the studied samples was converted to cDNA according to the method included with the EasyScript® One-Step gDNA and cDNA Synthesis Removal SuperMix kit supplied by TransGen Biotech Co., The tubes

were placed in a Thermal cycler Real-Time PCR (RT-PCR)) device (Table 2).

Quantitative Real-Time PCR (qRT–PCR): According to the work program attached to the Kit supplied by TransGen Biotech Co., Ltd, the final volume of the reaction mixture should be 20 μl. Thus, 1 μl of Forward primer was added for TUB2, SIX3, and SIX5 genes separately. Then, 1 μl of Reverse primer was added for TUB2, SIX3, and SIX5 genes separately. Besides, 10 μl of TransSrartY Top Green qPCR SuperMix, and 5 μl of Nuclease free water were added. The above mixture was added to 3 μl of cDNA for each of the samples of study, where all tubes were placed in a centrifuge at a speed of 3000 rpm for two minutes. Finally, the samples were placed in a Real Time qPCR machine. The reaction conditions were enzyme activation at 94°C for 30 sec, 40 cycles of 94°C for 5 sec for denaturation, 62°C for 15 sec for annealing, 72°C for 10 sec for extension and melting curve range from 76°C-83°C.

RESULTS AND DISCUSSION

Pathogenicity of *Fusarium oxysporum f. sp. lycopersici* on Radish and tomato seeds *in vitro*: The isolate of fungus (FOL) caused a significant decrease in the germination rate of both radish and tomato seeds *in vitro* (Table 3) compared with the pathogen-free control treatment. This indicate fungus ability to influence germination   percentage, which was 3.33 and 1.66%. in radish and tomato. The germination percentage in control was 100 and 98.33%, respectively (Fig. 1, 2). The FOL significantly reduce the percentage of germination is due to the ability of secreting enzymes such as chitinase and cellulase, which are characterized by their ability to degrade cell walls. These enzymes work to break down chitin and cellulosic substances in addition to their

important role in the virulence of the causative agent or may be due to other secretions secreted by the fungus such as fusaric acid, dehydro fusaric acid, and lycomarsmin, which are toxic metabolic substances that can lead to the failure of

A   **a**

Fig. 1A. Radish seeds germination (control without FOL)
a: Failure of radish seed germination (with FOL)

B   **b**
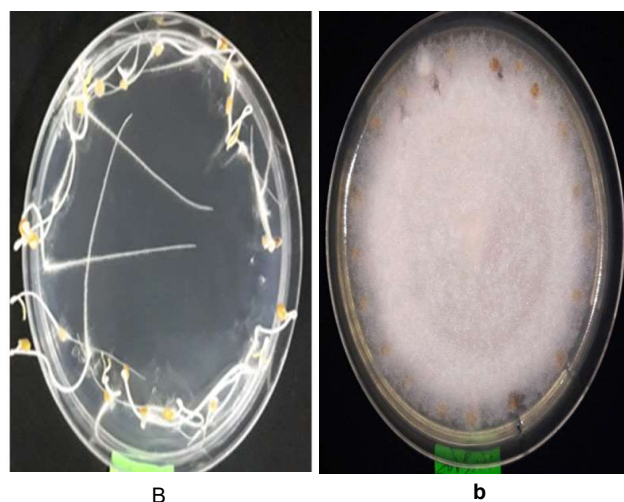
* **FOL:** *Fusarium oxysporum f. sp. lycopersic*

Fig. 2A. Tomato seeds germination (control without FOL)
a: Failure of tomato seed germination (with FOL)

Table 1. Primers used in the study

Gene	Primers	Primer sequences 5'–3'	Product size (pb)	Annealing temperature	Source
TUB2	Forward primer	TTCTGCTGTCATGTCCGGTGT	134	63	(Taylor et al 2016)
	Reverse primer	TCAGAGGAGCAAAGCCAACCA			
SIX3	Forward primer	GGCCGTCTTCTACTTCATTTAC	69	63	
	Reverse primer	GGGAGAATGTTCTAGCATAACC			
SIX5	Forward primer	TGGGCTCGAAAAGTCCAGCAT	114	63	
	Reverse primer	TGTTTCGCCGTCAATGTCGCC			

Table 2. RT-PCR reaction conditions for reverse transcription of cDNA synthesis

Parameter	Annealing	Reverse transcription	Reverse transcriptase enzyme inactivation	Hold
Temperature	25°C	42°C	85°C	4°C
Time	10 Min.	30 Min	5 Min	∞

The cDNA produced used directly in the RT-PCR reaction and can be stored for a longer period at -20°C

germination (Langlcaces Drysdale 2005, Calero-Nieto et al 2007, Ramanathan et al 2010).

SIX3 and SIX5 Gene expression of *Fusarium oxysporum f. sp. Lycopersici*: RNA was successfully isolated from the mycelium of all the samples. The concentration of the isolated RNA ranged between 42-88 ng/μl, and the purity ranged between 2.01 - 2.12 according of the absorbance reading of the Nanodrop spectrophotometer. This is evidence of the RNA isolation efficiency, as the ratio 260/28) of pure RNA is greater or equal to 2.

Gene expression study using quantitative real time PCR technology: The main objective of this step is to determine the gene expression of the virulence genes SIX3 and SIX5 of the pathogen *Fusarium oxysporum f. sp. lycopersici* with the use of the essential oil mixture (nano and normal) or a fungicide with specific concentrations to compare them with the (control). The dye SYBR Green was used in the reaction of Quantitative real time PCR, which is a fluorescent dye that can identify the DNA, including cDNA, as the amplification process is adopted as the value of the Ct (cycle threshold). The low (Ct) value indicates the presence of more copies of the target gene and vice versa. In the case of gene expression, the relationship is inverse between gene expression and the value of (Ct), where a high value indicates low gene expression and a low value indicates high gene expression (Livak and Schmittgen 2008, Al-Saedi 2016). Due to the very accurate and fast results that the quantitative real time PCR technique characterized in the study of gene expression, this technique has proven itself as a distinctive method in the analysis of gene expression. It is very important to realize that in relative quantification studies, most experiments are concerned with comparing the expression level of a specific gene among different samples (Derveaux et al 2010).

Gene expression of TUB2)by quantitative real time (PCR) technique: The purpose of using the TUB2 gene and its amplification is to stabilize the optimal experimental conditions and evaluate the reaction efficiency, as this gene is considered an endogenous control or Housekeeping element that maintains the integrity of cells and prevents the protein denaturation of the studied samples. In addition to that, in molecular studies, the gene expression of Housekeeping genes remains stable in the investigation cells or tissues (Freire et al 2015, Yu et al 2016 a, Taylor et al 2016). Quantitative Real Time PCR (RT-qPCR) technology was used to amplify TUB2 gene, study the melting point and gene expression of TUB2 and determine the value of the cycle threshold Ct. The Figure (3) reveals the amplification of the reaction product of TUB2 gene, which appeared in the form of a line or high lines and from almost one point for all the study samples. This confirms that the gene was expressed by approximately equal values in all samples in different concentrations, whether for the essential oil mixture in nano and normal states or for the two concentrations of (Topsin) that were used as comparison to the control, the (Ct) value ranged between 14.14-14.21 for all samples (Table 4). It also

Table 3. Effect of *Fusarium oxysporum f. sp. lycopersic* (FOL) pathogenicity in germination of radish and tomato

Seq.	Treatments	Germination rate	Germination percentage
1	Radish seeds with FOL	*0.67 b	3.33
2	Tomato seeds with FOL	0.33 b	1.66
3	Radish control without FOL	20.00 a	100.00
4	Tomato control without FOL	19.67 a	98.33
	L.S.D	0.66	————

*Different letters indicate a significant difference at (0.05) probability

Table 4. Ct and the comparison of TUB2 gene expression

Group	Ct of *TUB2*	2^{-Ct}	Experimental group/ Control group	Fold of gene expression
A1	14.18	5.3876E-05	5.387E-05/5.425E-05	0.97
A2	14.15	5.5008E-05	5.500E-05/5.425E-05	0.99
B1	14.19	5.3504E-05	5.350E-05/5.425E-05	0.96
B2	14.19	5.3504E-05	5.350E-05/5.425E-05	0.96
D1	14.14	5.5391E-05	5.539E-05/5.425E-05	1.02
D2	14.14	5.5391E-05	5.539E-05/5.425E-05	1.02
T1	14.16	5.4628E-05	5.462E-05/5.425E-05	1.00
T2	14.21	5.2767E-05	5.276E-05/5.425E-05	0.97
C1	14.18	5.3876E-05	5.387E-05/5.425E-05	0.99
C	14.17	5.4251E-05	5.425E-05/5.425 E-05	1

Where: - A1 = Treatment with 1000 ppm nano-essential oil mixture. A2 = Treatment with 1000 ppm normal essential oil mixture. B1 = Treatment with 800 ppm nano-essential oil mixture. B2 = Treatment with 800 ppm normal essential oil mixture. D1 = Treatment with 600 ppm nano-essential oil mixture. D2 = Treatment with 600 ppm normal essential oil mixture. T1 = Treatment with fungicide Topsin 50 ppm. T2 = treatment with the fungicide Topsin 25 ppm. C = Control, the fungus growth on the SDB medium. C1 = Control, fungi growth on PDA medium.

indicates that the reaction efficiency was high compared to negative control, which was observed in a straight line because there was no amplification of the TUB2 gene due to its lack of a cDNA template. Thus it confirms the safety of used primers and their purity (Svey 2001). Figures 3-A and 3-B show the melting curves, where the melting temperature of TUB2 gene for all samples was 79.5°C. The reason for analyzing the melting curve to ascertain the efficiency of (RT-qPCR) reaction and the absence of a primer-dimer. The melting curve can be seen on the computer screen of the (RT-qPCR) device and it shines by the present dye that gives direct results in each step. Moreover, e values of 2^{-Ct} ranged between (5.2767 - 5.5391), as the differences were not significant between the samples and the control, which was reflected on the fold of gene expression of TUB2 gene. According to the value of 2-Ct, the TUB2 gene expression values for all ten samples specified in the study ranged between 0.96 - 1.02. These simple differences make TUB2 gene a good and useful endogenous control gene.

Gene expression of SIX3 gene using quantitative real time (PCR) technology: The SIX3 gene was amplified by RT-qPCR technique in the same way as the TUB2 gene to study the effect of essential oil mixture in both nano and normal forms. Combined with, the effect of Topsin at specific concentrations on the gene expression of the pathogen *F. oxysporum f. sp. lycopersici*. Identifying the Ct values for each concentration of the essential oil or fungicide and calculating the difference between the target gene and the housekeeping gene (ΔCt) and by applying the equation ($2^{-ΔCt}$). Thus, the fold of gene expression of SIX3 gene can be obtained for each treatment (Table 5). The results of data analysis of the studied samples indicated that the essential
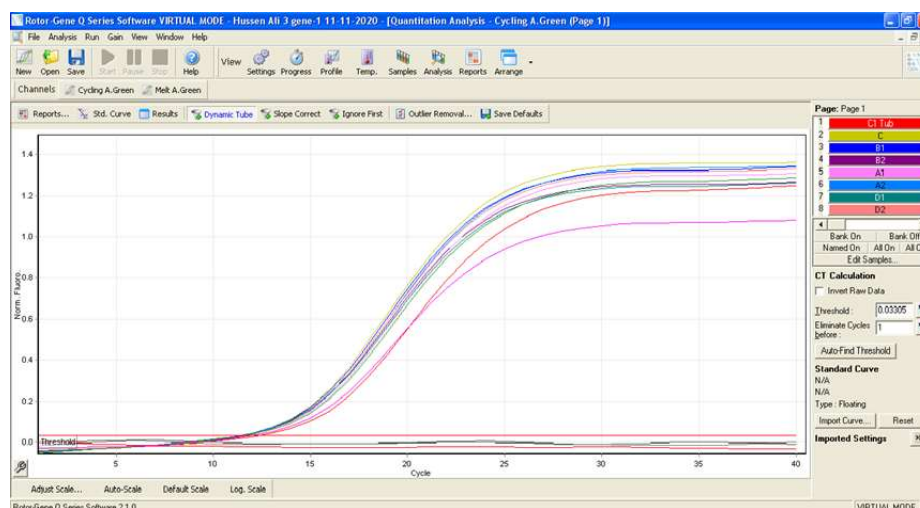
Fig. 3A. TUB2 gene Amplification by qPCR
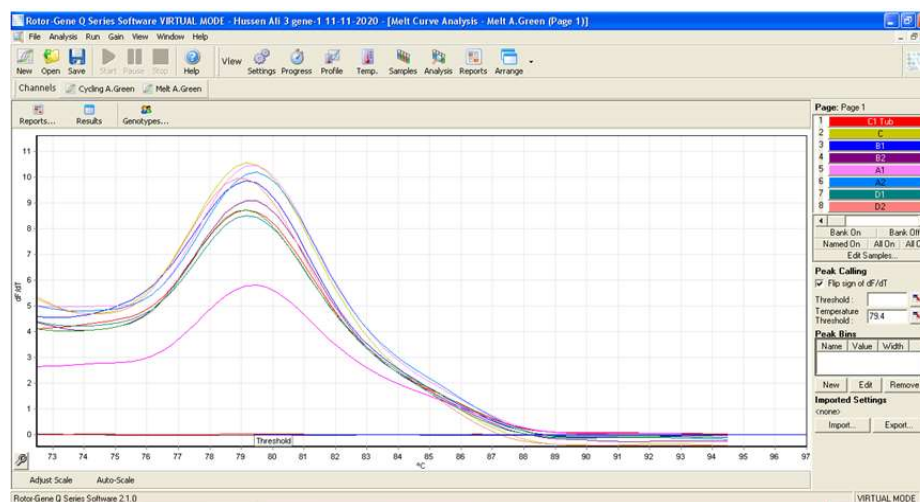
See Table 4 for details. Image was taken directly by Rotor gene for qPCR device

Fig. 3B. TUB2 gene dissociation curves by qPCR Melting temperature 79.5°C
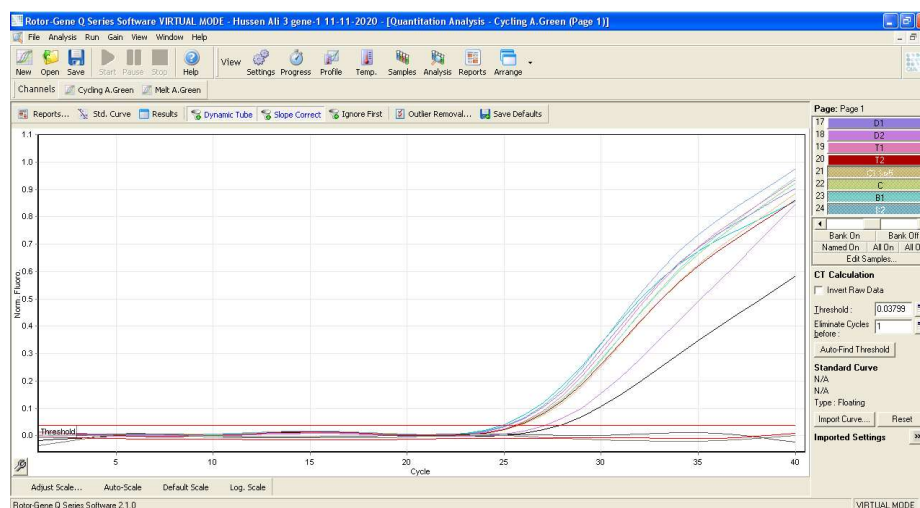
Fig. 4A. SIX3 gene Amplification by qPCR where
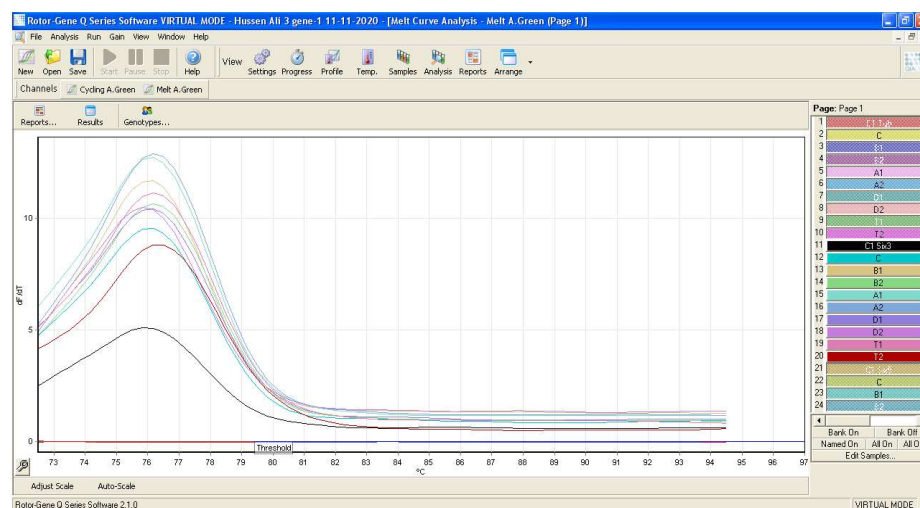
See Table 4 for details . Image was taken directly by Rotor gene for qPCR device

Fig. 4B. SIX3 gene dissociation curves by qPCR, melting temperature was 76°C

Table 5. Ct values and gene expression of SIX3 gene based on ($2^{-\Delta Ct}$) method

Groups	Ct of *SIX3*	Ct of *TUB2*	ΔCt (Ct of *SIX3* - Ct of *TUB2*)	$2^{-\Delta Ct}$	Experimental group/ Control group	Fold of gene expression
A1	26.9	14.18	12.72	0.0001482	0.000148/0.0003501	0.42337
A2	26.77	14.15	12.62	0.0001589	0.0001589/0.0003501	0.45376
B1	26.62	14.19	12.43	0.0001812	0.0001812/0.0003501	0.51763
B2	26.39	14.19	12.2	0.0002125	0.0002125/0.0003501	0.6071
D1	27.01	14.14	12.87	0.0001336	0.0001336/0.0003501	0.38156
D2	26.92	14.14	12.78	0.0001422	0.0001422/0.0003501	0.40613
T1	26.56	14.16	12.4	0.000185	0.000185/0.0003501	0.52851
T2	26.21	14.21	12	0.0002441	0.0002441/0.0003501	0.69737
C1	25.75	14.18	11.57	0.0003289	0.000328/0.000351	0.93952
C	25.65	14.17	11.48	0.0003501	0.0003501/0.0003501	1

See Table 4 for details

oil mixture (nano and normal) had a clear effect on inhibiting the expression of the SIX3 gene. The 1000 ppm of the essential oil mixture, whether in the nano or normal state, recorded the highest inhibition of fold of the SIX3 gene expression compared to other samples 0.42337, 0.45376 respectively, 50 and 25 ppm of Topsin had less inhibition fold of gene expression of SIX3, 0.52851 and 0.69737 respectively, when compared to the essential oil mixture with most of nano and normal concentrations.

Similarly, Figure 4-A shows the amplification of the reaction product of SIX3 gene, which shown in the form of elevated lines from different points of the studied samples. This indicates that the gene was expressed with different values affected by concentration of essential oil mixture (600, 800 and 1000 ppm) and the concentration of the fungicide

(25, 50 ppm) used in the model, while Figure (4-B) shows that the melting curves of SIX3 gen was 76°C.

Expression of the SIX5 gene using quantitative real time (PCR) technique: SIX5 gene expression was quantified by RT-qPCR, (Fig. 5-A, B). The presence of amplification of the gene at Ct values ranged from 27.22-28.75. Table 6 shows the calculating of the fold value of the SIX5 gene expression by following the same method used in calculation the gene expression of SIX3. The highest inhibition of gene expression was recorded at two concentrations of the normal essential oils mixture 1000 and 600 ppm (0.47632 and 0.47963, respectively). However, the concentrations of fungicide did not affect the gene expression of SIX5 compared to the control, where both concentrations 50 and 25 ppm recorded gene expression fold 0.82932 and 1.43396, respectively. It
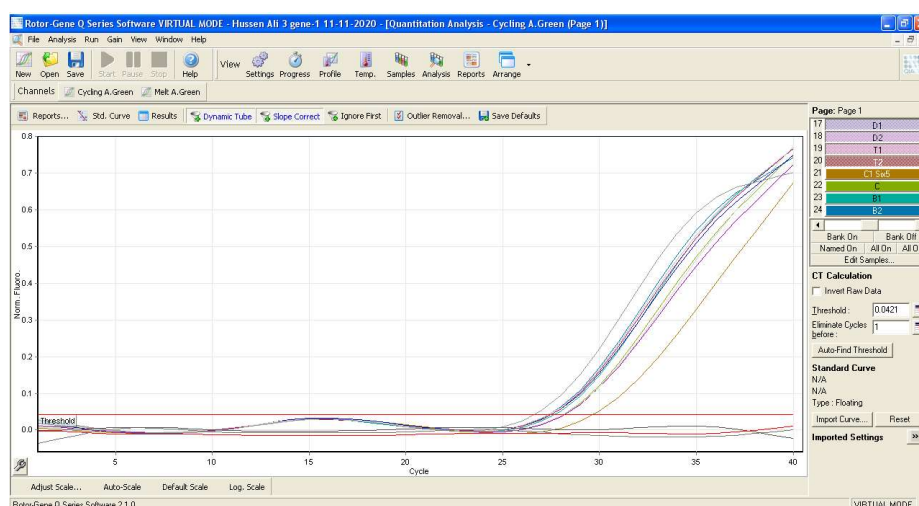
Fig. 5A. Amplification of SIX5 gene by qPCR
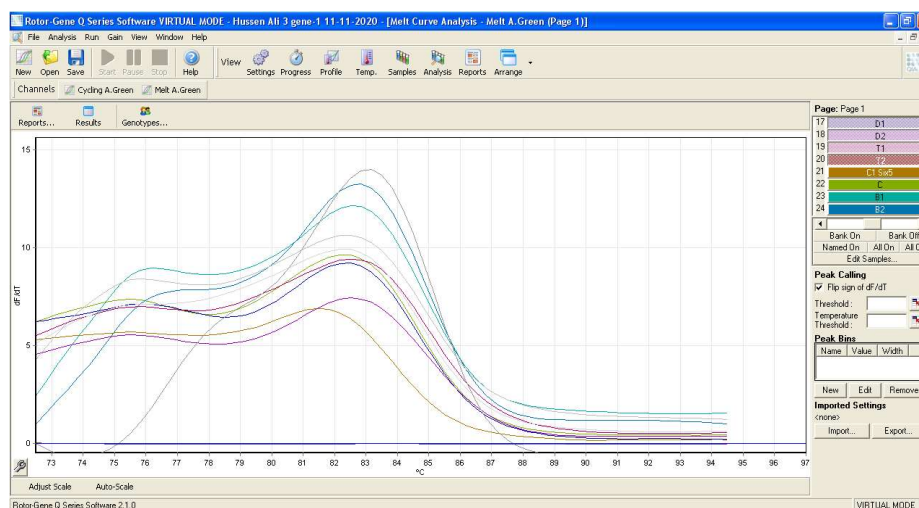
See Table 4 for details. Image is taken directly by Rotor gene for qPCR apparatus

Fig. 5B. SIX5 gene dissociation curves by qPCR, melting temperature is 83°C

Table 6. Ct values and gene expression of SIX5 gene based on ($2^{-\Delta Ct}$) method

Groups	Ct of *SIX5*	Ct of *TUB2*	ΔCt (Ct of *SIX5* - Ct of *TUB2*)	$2^{-\Delta Ct}$	Experimental group/ Control group	Fold of gene expression
A1	28.09	14.18	13.91	6.496E-05	8.454E-05/ 8.454E-05	0.76844
A2	28.75	14.15	14.6	4.027E-05	4.027E-05/ 8.454E-05	0.47632
B1	28.26	14.19	14.07	5.814E-05	5.814E-05/ 8.454E-05	0.68777
B2	27.95	14.19	13.76	7.208E-05	7.208E-05/ 8.454E-05	0.85263
D1	28.06	14.14	13.92	6.452E-05	6.452E-05/ 8.454E-05	0.76313
D2	28.73	14.14	14.59	4.055E-05	4.055E-05/ 8.454E-05	0.47963
T1	27.96	14.16	13.8	7.011E-05	7.011E-05/ 8.454E-05	0.82932
T2	27.22	14.21	13.01	0.0001212	0.0001212/ 8.454E-05	1.43396
C1	27.62	14.18	13.44	8.998E-05	8.99E-05/ 8.454E-05	1.06437
C	27.7	14.17	13.53	8.454E-05	8.454E-05/ 8.454E-05	1

See Table 4 for details

seems that this gene requires the use of a higher concentration to influence gene expression. The melting point of the gene was 83°C.

CONCLUSIONS

There was an effect of EOM in nano and normal state on gene expression of virulence genes SIX3 and SIX5 compared to fungicide (Topsin) and the expression of SIX3 gene was more affected that of SIX5 gene. The Clove and Lemon peel (EOM) in both nano and normal state could be good bio fungicide to replace the chemical fungicide (Topsin) that pollute soil and environment.

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Capacity Factor of Wind Turbine by Using Least Squares and Bayesian Estimation in Water Bodies (Marshes)

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Abstract: The marshes are considered attractive areas due to their beautiful nature, so it was important to exploit the natural resources in them to benefit from them in the field of energy and water. The aim of this paper is to evaluate the capacity factor of wind power turbines using three important Weibull distribution parameters in analyzing wind characteristics in addition to estimating the frequency distribution of wind speed for the Hawizeh site in southern Iraq at altitudes 10m and 50m within the period 2017 to 2019. The probability was indicate average wind speed 3.5 m/s at 10m and 4.8 m/s at 50 m is prevalent in the site. The Bayesian method was used to estimate the scale parameter for Weibull distribution. It was observed that when shape parameter was greater than one, the total time of test decreases with increasing duration. The test also found the highest rated capacity factor of the Enercon 330kw wind turbine was 43.1% in June.

Keywords: Wind energy, Capacity Factor, Weibull distribution, Bayesian, Location parameter

Wind energy is a naturally sustainable source of energy and is becoming more and more popular as a future clean energy source in the world. Knowing the probability of the mean wind speed is important at a given site. The Mesopotamian marshes in southern Iraq are considered the largest complex of wetlands in Iraq and one of the most important water systems in the Middle East. During the fortieth session of the world heritage committee according to cultural criteria. maybe a candidate for building a sustainable city due to the availability of water, solar radiation and wind almost all year round (Al-Thahaibawi et al 2019). Wind speed is known to be variable and unstable with time and location, and is affected by weather phenomena and site topography. Due to this random nature, their properties and behaviors at a given location can be determined by matching specific probability distributions to a given sample of wind velocities collected over time. The two-parameter of Weibull distribution has become the most widely used probability distribution in wind speed analysis and statistics. The function of Weibull distribution uses them comprehensively for demarcation of the wind energy potential at the concerned place. Three-parameter Weibull the use also function in some studies and found that they provide improved flexibility and fitness of classic two function Weibull parameter. Thus there is an opportunity to improve the Weibull distribution which includes the three variables in the wind resource assessment. A few researchers specialized in assessing wind resources used the three-parameter Weibull distribution, and most of them assumed that the site

parameter value ε ≠ 0, while many researchers relied on the two-parameter Weibull distribution to determine and evaluate wind resources and who took into consideration the value of = 0 (Sukkiramathi K et al 2020). The Weibull estimators obtained using statistical methods are generally more accurate but complex to deal with than their graphical counterpart. Some commonly used statistical methods include: method of moment and method of, maximum likelihood estimation and method of energy, pattern factor, Least Squares and Bayesian method (Osatohanmwen et al 2017, Altmimi et al 2017). Because of the wind blowing inconsistently, the wind turbines barely operate the estimated strength. So it is usually used the concept of capacity factor to evaluate the delivery of wind power turbines expected. In order to calculate capacity factor, you need a function of wind probability distribution function and the curve of the turbine power performance (Ditkovich et al 2014). In this paper, three-parameter of Weibull distribution it used, to estimate the capacity factor for Huwaizah Marsh south of Iraq at different heights, so introduce statistical comparison between the Least Squares and Bayesian estimation procedures for estimating Weibull shape parameter.

MATERIAL AND METHODS

Wind profile law: The logarithmic wind power law give a profile wind which is important to quantify and express it officially, when integrated, with respect to height (z) the law of logarithmic wind profile is (Sointu 2014):

$$u(z) = \frac{u_*}{K} \ln \ln \frac{z_o}{z}$$

Where µ is friction, velocity, K is von Karman's, constant so which has an experimentally determined equal 0.4 and Z_0 the, roughness length.

Weibull distribution probability function: Weibull distribution function is described by three-parameters can be expressed as (Sukkiramathi K et al 2020).

$$f(u) = \frac{k}{c-\varepsilon}\left(\frac{u-\varepsilon}{c-\varepsilon}\right)^{k-1} exp\left[-\left(\frac{u-\varepsilon}{c-\varepsilon}\right)^k\right]$$

Where u wind speed and k no dimensional shape parameter, ε location parameter, c in (m/s) is the scale parameter. Parameter of location ε is the minimum, of wind speed $u \geq \varepsilon$. The dimensions, of ε and c are the same as u in (m/s). Weibull distribution, with parameters c, k, $\varepsilon > 0$ has cumulative, density function it given by (Sukkiramathi et al 2020).

$$F(u) = 1 - exp\left[-\left(\frac{u-\varepsilon}{c-\varepsilon}\right)^k\right], \quad u \geq 0$$

Location parameter effects (ε): Location parameter, as the, name implies and also called the waiting time parameter or sometimes the shift parameter, locates distribution along of the abscissa. Change the value of location parameter has effect of sliding distribution, and associated, function either to right (if $\varepsilon > 0$) or to left (if $\varepsilon < 0$).

If $\varepsilon > 0$, the distribution starts, at the location parameter ε to right of the origin.

If $\varepsilon < 0$, the distribution starts, at the location parameter ε to left of the origin.

Estimation of Weibull parameters: Many different researchers have made several attempts to define Weibull parameters and wind frequencies that are used in evaluating wind resources for use in generating energy by wind turbines. It has been found that there is a method used to define Weibull parameters called the Least Squares Method, (LSM).

Least, squares method (LSM): The least, squares method second place in the line have found a large number of applications, in engineering, and mathematics. The wind speed, data represented by the cumulative, frequency distribution, satisfies the requirements of this method. In this distribution method, the data of wind speed, are interpolated in straight, line using the least squares regression concept (Soman et al 1992):

$$F(u_i) = 1 - exp\left[-\left(\frac{u_i-\varepsilon^k}{c-\varepsilon}\right)\right]$$

$$ln \; ln \quad ln\left\{\frac{1}{1-F(u_i)}\right\} = kln\,(u_i - \varepsilon) - kln(c - \varepsilon)$$

Bayesian estimation: The Bayesian estimation has received great attention because it is important in analyzing failure time data, which is a newly proposed method in place of the traditional methods. Albaaza assessment approach uses prior knowledge about data available and parameters. When the prior knowledge of parameter is not available. It is possible to take advantage of non-informative priors in Bayesian analysis. Bayesian analysis can make less strict assumptions about sampling distributions, and, thus, it has potential advantages in circumstances. The Bayesian estimation method is theoretically, fundamentally different from maximum likelihood or another classical significance testing "frequents" approaches. The Bayesian estimation used in prior information about distribution to seek a posterior probability of parameters given the data, when p (θ\y) (Leren et al 2018).

$$p(\theta \setminus y) = \frac{p(\theta)p(y\setminus\theta)}{p(y)}$$

Where θ is a random parameter (or set of parameters) and y represents a random variable (or set of variables). If parameter shape is greater than one, the total test time decreases as the test duration increases, if parameter shape is less than one, the total test time decreases as the duration decreases.

Capacity, **factor:** The capacity, factor on a given site is defined, as the ratio of the output power, to the rated power, of a wind, turbine, over a given period, of time. Thus, it is considered the main factor for estimating energy production and it has a major role in evaluating economic projects for wind energy. The capacity factor ratio of 30 to 40% is considered high for coastal areas. The capacity factor can be expressed in the following equation (Albani et al 2017).

$$CF = \frac{1}{P_R} \int P(u)f(u)du$$

Where P_R, is rated, power and power curve of turbine, f (u) is wind probability distribution function. Power curve, of the turbine is, discrete series, rather than a continuous, function, and, the wind probability distribution function is the result, of fitting, the discrete raw, wind data, to an a priori, assumed probability distribution function.

Study area: The Huwaizah Marsh is located east of, the Tigris River adjacent to the Iraq-Iran border, and about 70 km away from Amara. The area is distributed by 79% for the Iraqi part and by 21% for the Iranian part (Mohamed ARM et al 2008). Its length is about 80 km, from the Iraqi-Iranian borders to the east of the Tigris, River from its western part, with wide of 30km (Fig. 1).

RESULTS AND DISCUSSION

In the statistical analysis data from the wind data tower station was used to analyze the least squares method in addition to the Weibull, distribution parameters. The most important, results of this analysis are presented based on hourly, monthly and yearly data borrowed from the MERRA-2 Meteorological website. Tables 2, 4 show the most important statistics of wind data, which includes both the monthly and

annual average of wind speed, median, standard deviation, minimum, and maximum, wind speed, at a height of 10 m and 50 m within the period 2017-2019.

The highest mean value about 6 m/s in June with the maximum 13.66 m/s which is, the mean wind, speed within the period 2017-2019. Table 3 shows the cumulative frequency distribution and sample data frequency. As shown from the table that the highest frequency of wind speed (2-4) with 3671 times at 10 m height.

The highest mean value about 8 m/s in June months with the maximum 17 m/s which is mean wind speed within the period 2017-2019 at height 50m. By using the Least Squares Method to give better results, where the value of *k, c* and ε value becomes approximately the same in this way and is, therefore, larger than the measured value (Table 5).

The highest value of scale and shape parameters were 6.942 m/s, 2.756 at height 10m and 8.677 m/s, 2.756 at height 50m in June. Three-parameter Weibull, probability density, function curve by Least Squares Method histogram, of observed, wind speed at 10, 50 m height (Fig. 2 and 3).

Thus it is possible to estimate the scale parameter if the shape parameter is known. The shape parameter is also used to represent failure data exponentially. The scale parameter is estimated with the Lower Confidence limit and Upper Confidence Limit 90% (Tables 6 and 7) and is useful for designing reliability verification tests that anticipate no failures.

Since shape parameter is greater than one, total test time

Table 3. Frequency of wind speed at 10 m

Wind speed (m/s)	Count	Cumulative count
0≤x≤2	1066	02
2≤x≤4	3671	4737
4≤x≤6	2625	7362
6≤x≤8	954	8316
8≤x≤10	322	8638
10≤x≤12	91	8729
12≤x≤14	31	8760
14≤x≤16	0	8760
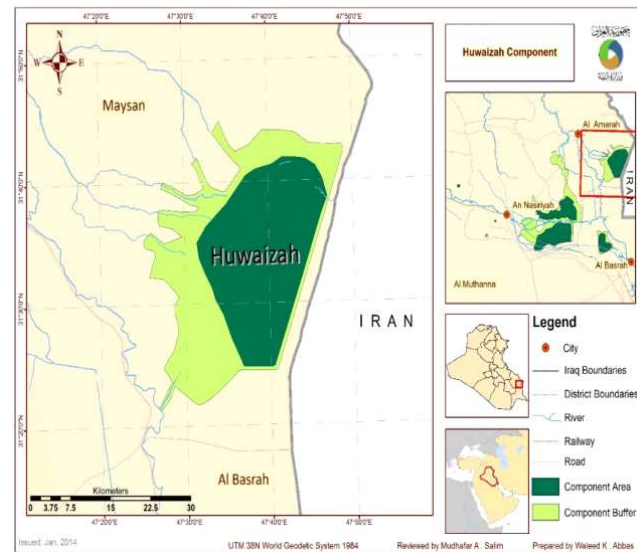
Fig. 1. Location of Huwaizah Marsh

Table 1. Statistical characteristics of Huwaizah Marsh

Sites coordinates			Standard deviation of wind speed	Ave. Wind Speed at height 10m (m/s)	Relative humidity (%)	Ave. temperature (°C)
Elev (m)	Lon. (°)	Lat. (°)				
5	47.34	31.38	2.27	4.31	32.55	24.98

Table 2. Monthly of the mean wind speed in m/s for selected wind station at height 10m

Month	Mean	Median	S.D	Minimum	Maximum
January	3.754	3.805	1.505	0.25	8.32
February	3.620	3.415	1.482	0.26	9.02
March	3.832	3.395	2.051	0.20	10.89
April	4.204	4.100	2.024	0.08	13.35
May	4.810	4.505	2.298	0.14	13.51
June	6.273	6.005	2.345	0.07	13.66
July	4.682	4.140	2.480	0.09	12.05
August	3.618	3.510	1.808	0.19	10.77
September	3.923	3.715	1.802	0.18	9.13
October	4.978	4.712	2.081	0.15	11.43
November	4.074	3.918	1.724	0.30	11.13
December	4.492	4.506	1.777	0.22	10.25
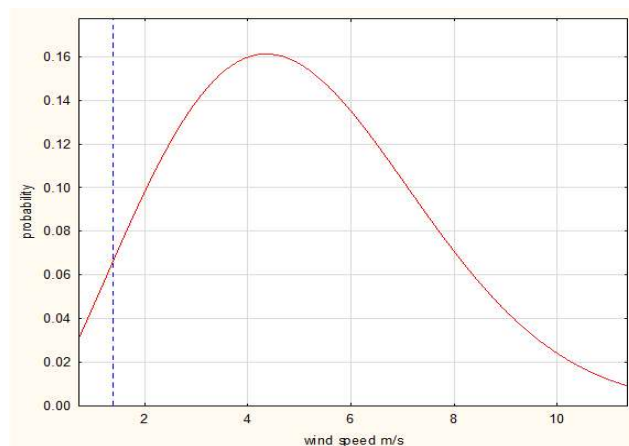
Fig. 3. Three-parameter Weibull probability density function curve by Least Squares Method for wind speed at height 50m at Huwaizah Marsh
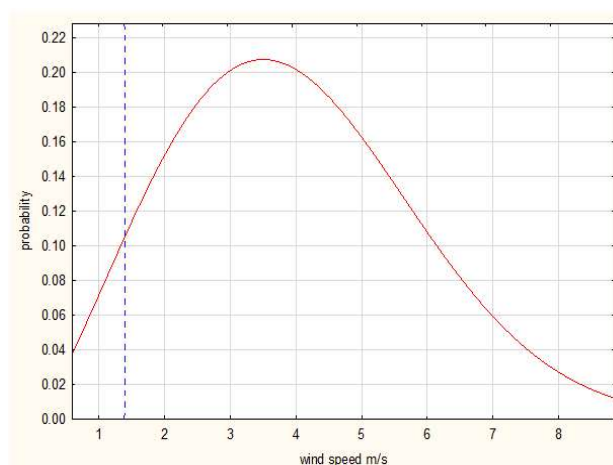
Fig. 2. Three-parameter Weibull probability density function curve by Least Squares Method for wind speed at height 10m at Huwaizah Marsh

Table 4. Monthly of the mean wind speed in m/s for selected wind station at height 50m

Month	Mean	Median	SD.	Minimum	Maximum
January	4.692	4.756	1.881	0.312	10.400
February	4.525	4.268	1.853	0.325	11.275
March	4.790	4.243	2.562	0.250	13.612
April	5.255	5.125	2.530	0.100	16.687
May	6.013	5.631	2.872	0.175	16.075
June	7.841	7.506	2.932	0.087	16.887
July	5.853	5.175	3.100	0.112	15.062
August	4.523	4.387	2.261	0.237	13.462
September	4.903	4.643	2.253	0.225	11.412
October	4.978	4.712	2.081	0.150	11.437
November	4.074	3.918	1.724	0.300	11.137
December	4.492	4.506	1.777	0.225	10.250

Table 5. Scale, shape and location parameters for Weibull distribution at height (10, 50 m).

Month	10 m			50 m		
	c m/s	k	ε	c m/s	k	ε
January	3.925	2.417	0.248	4.9060	2.417	0.312
February	3.848	2.412	0.209	4.806	2.408	0.261
March	4.131	1.88	0.173	5.166	1.880	0.216
April	4.751	2.19	0.079	5.804	2.112	0.099
May	5.437	2.204	0.063	6.698	2.168	0.085
June	6.942	2.756	0.069	8.677	2.756	0.087
July	5.274	1.982	0.017	6.648	1.997	0.237
August	4.079	2.088	0.189	6.592	1.982	0.022
September	4.191	2.096	0.179	4.785	1.885	0.237
October	4.331	2.399	0.119	5.239	2.096	0.224
November	3.547	2.392	0.110	4.434	2.392	0.138
December	3.824	2.514	0.179	4.780	2.512	0.224
Mean	4.523	2.272	0.136	5.711	2.217	0.178

Table 6. Bayesian estimation with the Weibull distribution at 10m

Months	Scale parameter	Lower confidence limit 90%	Upper confidence limit 90%
January	4.956	4.86	5.055
February	4.955	4.858	5.054
March	4.794	4.675	4.918
April	4.889	4.785	4.997
May	4.894	4.789	5.001
June	5.052	4.966	5.141
July	4.826	4.712	4.944
August	4.859	4.749	4.971
September	4.861	4.742	4.973
October	4.951	4.854	5.051
November	4.949	4.852	5.049
December	4.984	4.891	5.08

Table 7. Bayesian estimation with the Weibull distribution at 50m

Months	Scale parameter	Lower confidence limit 90%	Upper confidence limit 90%
January	4.956	4.86	5.055
February	4.954	4.857	5.053
March	4.794	4.675	4.918
April	4.866	4.758	4.977
May	4.883	4.777	4.992
June	5.052	4.966	5.141
July	2.646	1.621	5.136
August	2.647	1.616	5.165
September	2.658	1.581	5.367
October	2.638	1.654	4.963
November	2.624	1.743	4.565
December	2.621	1.775	4.441

Table 8. Capacity factor at height 50m

Month	Capacity factor (%)
January	10.1
February	9.5
March	14.3
April	25.0
May	19.5
June	43.1
July	25.7
August	25.2
September	11.5
October	12.5
November	7.4
December	9.1

decreases as the test duration increases. Estimated values of capacity factor by using equation (7) for wind turbine (Enercon 330kw with rotor diameter 33.4 m and tower maximum 50m which presents, the capacity factors, in percentages at, hub height 50 m). It is observed, that the highest, capacity factor was obtained 43.1% in June at 50 m hub height (Table 8). The highest value of capacity factor is 43.1% and it's considered very high for coastal areas.

CONCLUSION

The capacity, factor of wind energy output at height 50m for the wind turbine type (Enercon 330 kw with rotor diameter 33.4 m and tower maximum 50 m) at Huwaizah Marsh have been calculated in this research using least squares method to estimate three Weibull distribution functions parameters (c, k, ε). From the cumulative frequency distribution probability of wind speed 3.5 m/s at 10 m and 4.8 at 50 m was predominant in the selected region. The Bayesian method use to estimate the scale parameter, for Weibull distribution, when the shape parameter was known. Since it is the shape parameter is greater than one, total test, time decreases as, the test duration increases.

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Molecular Detection of *Trypanosoma evansi* Isolated from Iraqi Camels Inoculated in Rabbits

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Abstract: In developing countries, trypanosomiasis of animals is one of the most serious problems facing the livestock industries. This is the first study in IRAQ by which COX 1 gene was used for *Trypanosoma evansi* diagnosis and registered in GenBank at NCBI by the following code: QQZ19798.1. This study was aimed for molecular diagnosis of *T. evansi* in Iraqi camel by polymerase chain reaction (PCR) depending on specific *T. evansi* gene which is cytochrome c oxidase subunit I (COX1). Study was conducted on 85 blood samples collected from Dromedary camel of different ages and sexes in Iraq. *T. evansi* was isolated and identified by wet blood film examination and thin blood film stained with Giemsa. The overall 17.6% of study camels were infested with this parasite. Blood of infested camel were injected intraperitoneally (i/p) with 0.5 ml containing 1×10^5 trypanosome in 12 male rabbit. At 7 and 10 days post infection blood sample were collected from these rabbits and the gene cytochrome c oxidase subunit I (COX1) related to *T. evansi* was identified by polymerase chain reaction (PCR). In conclusion, the prevalence of *T. evansi* infestation in Iraqi camel is 17.6% and PCR is an effective technique for *T. evansi* diagnosis.

Keywords: *Trepanosoma evansi*, Camel, PCR, COX1, Rabbit

The sickness is caused by *Trypanosoma evansi*, a protozoan parasite that lives in both intra- and extra-vascular fluids of mammals. Surra can be found all over the world in tropical and subtropical climates. *T. evansi* infects a wide variety of species, however, it is notably frequent in horses and camels. The hematophagous flies *Chrysops, Tabanus, Atylotus, Haematopota, Lyperosia,* and *Stomoxys* transfer it mechanically. Reduced milk supply, anemia, weight loss, and abortion are all symptoms of the disease, which can be fatal. It found all throughout the world, including Asia, Africa, the Canary Islands, Central and South America, and, more recently, France and Spain (Luckins and Dwinger 2004, Desquesnes et al 2013). Surra is a severe, difficult-to-treat disease for which there is no vaccination. It is the deadliest protozoan illness that affects camels, and it has the potential to cause significant economic losses. Thousands of live camels are imported each year from countries in the Arabian Gulf, Somalia, Sudan, and Djibouti (Alghamdi 2016). Camels, which are primarily employed as pack animals and sources of milk, meat, leather and skin, play a significant part in desert and rural transportation. The prevalence of the disease was determined using ELISA and haematocrit centrifugation technique (Elamin et al 1998). *T. evansi* infections were detected using the card agglutination test (CAT) (Chappuis et al 2002). Molecular markers are required to detect accurately *T. evansi* infections in camel. As a result, the study's goal is to evaluate the diagnosis of *Trypanosoma evansi* in Iraqi camel by blood picture and confirmed conventional by polymerase chain reaction (PCR) depending on specific *T. evansi* gene which is cytochrome c oxidase subunit I (COX1).

MATERIAL AND METHODS

From January to October 2020, the study was conducted in various Iraqi provinces. A total 85 blood samples were obtained from camels of various ages, genders and sexes. Five milliliters of blood were drawn from jugular vein into plastic tubes containing the anticoagulant ethylene diamine-tetra acetic acid (EDTA). All samples were transported to the laboratory in chilled circumstances for the detection of trypanosomes based on their movement among red blood cells (RBCs) in a wet blood film analysis. Thin blood film was stained with Giemsa prepared according to method of Chaudhri and Gupta (2003). Blood of suspected infested camel were injected intraperitoneally (i/p) with 0.5 ml containing 1×10^5 trypanosome in 12 male rabbit. At 7th and 10th days post infection, blood sample were collected from these rabbits and the gene cytochrome c oxidase subunit I (COX1) related to *T. evansi* was identified by polymerase chain reaction (PCR) for confirmation of diagnosis. The protocol of gene detection was used according to manufacture and Scherczinger (1997).

Sequencing and sequence alignment: After staining with ethidium bromide or red stain, the PCR products were separated on a 2 percent agarose gel electrophoresis and examined using ultraviolet light (302 nm). The National

Center for Biotechnology Information (NCBI) and the BioEdit program released this article online at (http://www.ncbi.nlm.nih.gov). The Basic Local Alignment Search Tool was used to do the homology search (BLAST).

RESULTS AND DISCUSSION

This is the first study in IRAQ by which COX 1 gene was used for *T. evansi* diagnosis and registered in GenBank at NCBI under QQZ19798.1accession number. Our results were revealed 17.6% prevalence of trypanosomiasis in Iraqi camel depending on wet blood film examination and thin blood smear stained with Giemsa stain in camel (Fig. 2) and rabbits (Fig. 3). Most positive cases were concentrated at Al-

Table 1. Optimum condition of detection of COX 1 gene

Phase	Tm (°C)	Time	No. of cycle
Initial Denaturation	95°C	5 min.	1 cycle
Denaturation -2	95°C	45sec	35 cycles
Annealing	58°C	45sec	
Extension-1	72°C	45sec	
Extension -2	72°C	7 min.	1 cycle

Samawah Desert / Al-Muthanna Province followed by Rabbit inoculation test and confirmed by conventional PCR using COX1 gene (Fig. 4).

Numerous researches on the characterization of trypanosomes in general and *T. evansi* in particular (Li et al 2009). The COX1 gene of *T. evansi* was one of the molecular targets used in this study. For the diagnosis of trypanosomiasis, PCR analysis was indicated since it has a higher sensitivity than microscopic analysis of blood smears

Fig. 1. DNA sequencing of *T. evansi* by PCR using COX 1 gene

Table 2. Percent of identification of *Trypanosoma evansi* COX1 gene

Score	Expect	Identities	Gaps	Strand
563 bits(624)	3e-162	312/312(100%)	0/312(0%)	Plus/Plus

```
Sbjct  1    ............................................................
60
Query  61   CTTGCAGGAGCCTTGTTTGATCTAACTATTTTCTCTCTTCACTTGTCTGGGGTGTCCTCT
120
Sbjct  61   ............................................................
120
Query  121  ATTCTAGGGGCCATTAATTTTATCACAACTGTCATCAATATAAAACCCCCTGCTATATCT
180
Sbjct  121  ............................................................
180
Query  181  CAGTGTCAAACCCCCTTATTCGTATGGTCTGTTCTCATAACAGCCGTTCTTCTTCTTTCC
240
Sbjct  181  ............................................................
240
Query  241  CTGCCATTTCTAGCCGCTGGCATTACAATACTCTTAATGTACCAAAATCTAAACACAACG
300
Sbjct  241  ............................................................
300
Query  301  TTTTTTGACCCT  312
Sbjct  301  ............  312
```

Trypanosoma evansi isolate Raln-1 COX1 gene, partial cds; mitochondrial
Sequence ID: MW513372.1Length: 312Number of Matches: 1
Range 1: 1 to 312 GeneBank Graphics Next Match Previous Match

(Barghash et al 2016). A total 15 camels out of 85 were positive for trypanosome infection, according to a rabbit inoculation test (17.6% prevalence rate). Amer et al (2011)
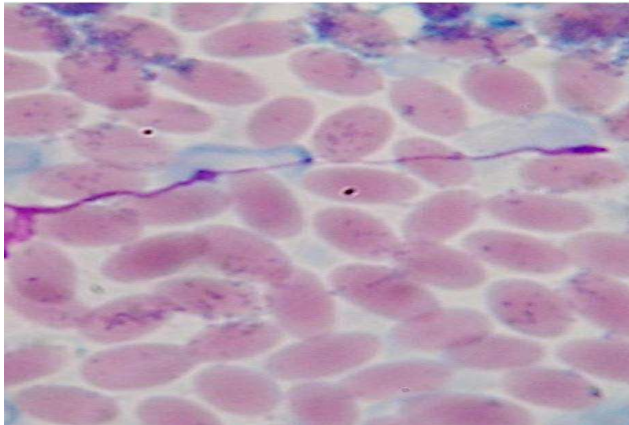
Fig. 2. Presence of *Trypanosome evansi* in blood smear of camel stained by Giemsa stain (100X)
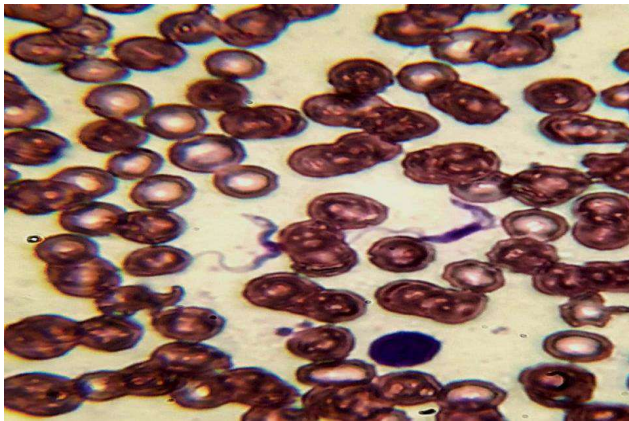
Fig. 3. Presence of *Trypanosome evansi* in blood smear of rabbit stained by Giemsa stain (100×)

M: Ladder marker (100-1500)
Lanes 1-4: Positive PCR products to *T. evansi* COX1 gene at a product size of 720 bp

Fig. 4. Agarose gel electrophoresis at concentration of 1.5% with using 1X at 5 volt/cm^2, 1:30-hour TBE buffer

recorded 4.7% prevalence of trypanosomiasis while, less than the prevalence recorded by Metwally et al (2021) who suggested 39.5% and 49% prevalence rate respectively in two group of camel. In Iraq, blood smears indicated the incidence of *Trypanosoma* spp. as 31.87% (Al-Amery et al 2017). The prevalence was revealed 4.5 – 74.4 % in studies in *Trypanosoma* spp. in Egypt (Abdel-Rady 2006, Elhaig et al 2013). *Trypanosoma evansi* infection in camels has been investigated using several microscopy and serological assays. Camels were found to have a 5.5 percent infection rate in another investigation (Omer et al 1998). *Trypanosoma evansi* infection rates in camels have been found to range from 0 to 40% in different parts of Saudi Arabia (Al-Khalifa et al 2009). The results of epidemiological molecular analysis range from 33.9 t to 42.1 percent (Salim et al 2011). These variations could be related to changes in management, the climate in which the specimens were gathered, or the sample sizes of the animals studied. The study's limitation is that it only used the COX1-PCR to analyze the samples for *T. evansi*. As a result, further genes for trypanosomiasis detection are recommended. In addition, future studies should investigate more samples.

CONCLUSION

Infestation with *T. evansi* is found in 17.6% of Iraqi camels and PCR is a special technology to detect *T. evansi* in blood samples of camel that is sensitive and dependable.

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CONTENTS

CONTENTS

CONTENTS

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