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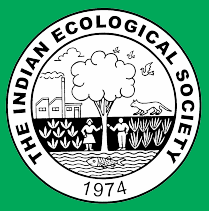

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Efficiency of Water Hyacinth Waste (*Eichhornia crassipes* (Mart) Solms) in Purifying Water Contaminated with Lead and Cadmium

Wafaa Sahib Abbood Alawsy and Shatha Majid Nafawa[1]

Al-Qadisiyah University, College of Agriculture
[1]College of Agricultural Engineering Sciences, University of Baghdad, Baghdad
**E-mail: wafaa.abbood@qu.edu.iq*

Abstract: The study included erecting columns of 75 cm long and 10.2 cm in diameter filled with up to 50 cm of water hyacinth waste (compost produced from the Ministry of Agriculture, and dried Water hyacinth powder) after placing glass cotton and fine gravel washed with dilute hydraulic acid at the bottom of the column and moistened over-saturation with tap water. Then, the contaminated water treatment (waste water W_1, industrial wastewater W_2, and drainage water W_3) was passed in addition to the comparison treatment (tap water W_4). The filtrate was collected after 24-48 hours for analysis and for a period of 30 days. The collected water was also classified before and after passing on the water hyacinth waste, and it was classified under the classes C_3-S_1, C_4-S_1, C_4-S_2, and C_4-S_3 according to the electrical conductivity and the sodium adsorption ratio SAR of the water under study. After completing the period of passing contaminated water on the water hyacinth wastes of both types, a reverse experiment was conducted, by working to wash these wastes by passing tap water for a period of no less than a month and collecting the filtrate for the purpose of analysis. The efficiency of removing heavy elements was calculated by the two types of water hyacinth waste, where the results showed that the dried water hyacinth powder and the water hyacinth compost had a high ability to reduce heavy elements (Pb, Cd) from contaminated water. Thus, reduction efficiency estimated at for lead (99.05% and 92.07%) and for cadmium (98.45% and 97.99%) to reach less than the permissible limit for irrigation and agriculture according to the Iraqi national determinants of 2012 and the Iraqi Rivers Maintenance System No. 25 of 1967, and it was the maximum removal of lead. The results of the current study show the high susceptibility of the water hyacinth plant in its non-living form to the treatment of wastewater and other polluted water containing heavy metals, and the persistence of low concentrations of heavy metals understudy in all columns after conducting the opposite experiment.

Keywords: Water hyacinth**,** *Eichhornia crassipes,* Lead, Cadmium

Water pollution include chemical or physical change in water quality, directly or indirectly, that negatively affects living organisms or makes the water unsuitable for various uses (Moselhi 2008). Heavy metals are naturally present in the aquatic environment at low levels, where these concentrations increase through the dumping of waste, industrial and agricultural waste and sewage water, which leads to a change in the water quality and harms the organisms in it (FAO 2010). Heavy elements are those elements whose specific density is five times greater than the water density (5 $g.cm^{-3}$). Besides that, their excessive use has a negative impact on the environment as well as its impact on the health of humans, animals and plants. Most aquatic plants have the ability to take heavy metals present in water or sediments through their root and due to their ability to absorb and accumulate heavy elements in their tissues. These plants can be used as vital evidence of the pollution of aquatic ecosystems with these elements (Narain et al 2011, Abdel Moneim and Al-Turki 2012). Several studies have proven the tremendous ability of the water hyacinth plant to remove elements, purify polluted water, absorb heavy elements and some mineral nutrients from the aquatic environment, and accumulate mercury, as well as other heavy elements, to a large extent in the root area of the plant. Many studies confirm the possibility of benefiting from the water hyacinth plant as a bio-accumulator of mercury in polluted lake as this plant has superior ability to desorb and extract lead. The water Hyacinth is one of the aquatic plant types that has attracted a lot of attention because of its ability to grow in highly polluted waters .Thus, water purification is one of the beneficial aspects of the water hyacinth through its ability to remove pollutants from polluted water bodies such as total suspended solids, dissolved solids, nitrogen, phosphorous, heavy metals, etc. (Barakat 2011, Guptal et al 2012). The current study aimed to evaluate the efficiency of water hyacinth waste (dried water hyacinth powder and manufactured compost) in removing and purifying polluted water from heavy metals lead and cadmium.

MATERIAL AND METHODS

Preliminary Actions

Preparation of Water hyacinth waste

Two types of water hyacinth waste were selected:

Decomposed and degraded: The compost produced by the Ministry of Agriculture.

Not decomposed: The live Water hyacinth plant was selected and collected, the plant samples were taken and washed well with tap water, then dried aerobically and using an electric oven at a temperature of 70 °C for 48 hours. Then, they were ground with a stainless electric grinder and passed through a sieve with a (0.5-1) mm holes diameter and prepared for laboratory analysis.

Collection of contaminated water samples: Three samples of contaminated water were collected in plastic containers (wastewater W_1, industrial wastewater W_2, and drainage water W_3) in addition to tap water W_4 as a comparison sample. These samples were prepared for chemical analyzes and classified in terms of salinity and alkalinity according to the classification of the American Salinity Laboratory before use.

Laboratory Procedures

Water hyacinth waste analyzes: The 0.2 g of both types of Water hyacinth waste were taken and digested using concentrated acids according to the method described by Jones et al (2001), while the heavy elements under study were estimated using the Atomic Absorption Spectrometer (AAS).

pH: It was measured directly by means of a pH-meter WTW in the extract of soil: water (5:1)

Electrical Conductivity (EC): It was measured by means of an EC-meter WTW in the extract of soil: water (7: 1).

Chemical analyzes of water understudy: The degree of reaction, electrical conductivity, dissolved ions, and sodium adsorption ratio was measured, and water quality was classified according to the American Salinity Laboratory (USDA) based on the methods mentioned in Richard (1954), where the total concentration of dissolved salts was measured according to the following equation:

$$TDS\ (ppm) = EC(^{ds}/_{m}) \times 640$$

The total concentration of each of the heavy metals (lead, cadmium) in the water understudy was estimated using the Atomic Absorption Spectrometer (AAS). Columns of 75 cm in length and 10.2 cm in diameter were erected filled with up to 50 cm of water hyacinth waste (compost produced from the Ministry of Agriculture, and dried Water hyacinth) after placing glass cotton and fine gravel washed with diluted hydraulic acid at the bottom of the column and moistened over the saturation with tap water. Then the contaminated water (wastewater, industrial wastewater, and drainage water) was passed in addition to the comparison treatment (tap water), as a funnel and a container were placed to collect the filtrate from the bottom of the column. The filtrate was collected after period of 24-48 hours for the purpose of analysis and for a period of 30 days. The collected water was also classified according to the American Salinity Laboratory (USDA) before and after its passage on the water hyacinth waste. The number of samples were 24 (4 x 2 x 3), -type of water x type of water hyacinth waste x replicates). After completing the period of passing contaminated water on the wastes of the water hyacinth of both types, a reverse experiment was conducted, by working to wash these wastes by passing tap water for a period of no less than a month and collecting the filtrate for the purpose of analysis. The efficiency of removing heavy metals from the two types of water hyacinth waste was calculated using the following equation (Argun et al 2007)

$$\text{Removal}\ \% = \frac{C_i - C_f}{C_i} \times 100$$

Where: C_i = Initial concentration of the element, C_f = Final concentration of the element

RESULTS AND DISCUSSION

Electrical conductivity: EC values of the water under study ranged between (1.110 - 15.570) ds.m^{-1} before the filtration process and (1.89 - 15.71) ds.m^{-1} after the filtration process (Table 2, Fig. 1). Thus, the highest value was recorded in the drainage water, which is due to the increase in the concentration of dissolved ions, especially sodium ions (105.575 Meq/L) as compared to tap water, (2.932 Meq/L) (Table 5). There was a significant increase in its values. Besides, the average electrical conductivity increased to

Table 1. Chemical characteristics of the two types of water hyacinth

Characteristic	Water hyacinth compost	Dried water hyacinth
pH	7.11	6.16
5:1 EC (ds.m^{-1})	9.07	13.86
Pb^{+2} (mg.kg^{-1})	414.70	685.92
Cd^{+2} (mg.kg^{-1})	70.30	14.90

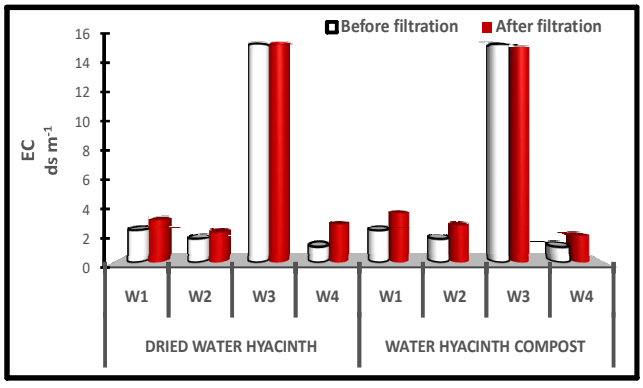

Fig. 1. Effect of water hyacinth waste on the electrical conductivity of water during the filtration process

5.87 ds.m^{-1} compared with the average electrical conductivity of the study water before the filtration process, which amounted to 5.15 ds.m^{-1}. There are significant differences in interaction between water hyacinth waste and the period (before and after filtration). The water hyacinth powder significantly increased the electrical conductivity by a small percentage than compost of the water hyacinth. This may be attributed to the high EC values of both the dried water hyacinth and the manufactured compost (13.86, 9.07 ds.m^{-1}, respectively). The field of biosorption revealed the mechanism responsible for this reaction, which is a kind of exchange interaction between positive ions such as calcium, magnesium, potassium, and sodium associated with biomass and other elements present in aqueous solutions (Naja and Volesky 2006). The biosorption process is determined by two main factors, the first is the strength of the electronegativity charge of ions, and the second factor is the diameter of the adsorbed ion (the size of the adsorbed ion). Naturally, the water hyacinth wastes contain a lot of various basic nutrients such as calcium, magnesium, potassium, and sodium, where these ions are also present in the water under study. Therefore, when the biomass of water hyacinth waste interacts with the heavy metals present in the water, the above-mentioned basic elements will be released, causing an increase in the electrical conductivity values of the water. Wang et al (2011) and Sulaymon et al (2013) observed that process of biosorption of heavy elements displaced the basic elements (calcium, magnesium, potassium, sodium) to the equilibrium solution when they adopted the technique of columns filled with dry algae.

pH: pH values that ranged between (7.070 - 8.750) before the filtration process and between (6.930 - 8.360) after the filtration process, as the lowest value was in the wastewater (Table 3). The reason for this may be attributed to the possibility of the presence and predominance of some acids such as carbonic acid, fulvic, humic, and citric acids, and others dissolved in wastewater as a result of the decomposition of rapidly decomposing organic matter (Al-Amiri 2006, Kang et al 2003). The pH values of the wastewater understudy before the filtration process with the

Table 2. Effect of water hyacinth waste on the electrical conductivity of the study water during the filtration process (ds.m^{-1})

Wastes	Water quality	Sampling period		Waste * water
		Before filtration	After filtration	
Dried Water hyacinth	Wastewater	2.28	3.04	2.66
	Industrial wastewater	1.64	2.14	1.89
	Drainage water	15.57	15.71	15.64
	Tap water	1.11	2.68	1.90
Water hyacinth compost	Wastewater	2.28	3.45	2.87
	Industrial wastewater	1.64	2.64	2.14
	Drainage water	15.57	15.41	15.49
	Tap water	1.11	1.89	1.50
LSD		0.1341		0.0948
Waste * period				
Waste		Before filtration	After filtration	Average waste
Dried Water hyacinth		5.15	5.89	5.52
Water hyacinth compost		5.15	5.85	5.50
LSD		0.0670		0.0474
Water quality* period				
Water quality		Before filtration	After filtration	Average water quality
Wastewater		2.28	3.25	2.76
Industrial wastewater		1.64	2.39	2.02
Drainage water		15.57	15.56	15.57
Tap water		1.11	2.29	1.70
LSD		0.0670		0.0670
Average sampling		5.15	5.87	
LSD		0.0474		

standards of treated wastewater used for agricultural irrigation were compared according to the Iraqi national determinants (2012). It was observed that they are within the permissible limits (6 - 8.4), as r industrial wastewater and water hyacinth powder significantly exceeded in reducing the pH so that the average pH reached 7.45 compared to the average pH of the water filtered by the water hyacinth compost of 7.85. These values are within the permissible limits when compared with the standards of wastewater and industrial waste water used for agriculture irrigation according to the Iraqi national determinants.

There were significant differences in the pH values before and after the filtration process, as the average pH of water understudy was 7.92, to record decrease after the filtration process so that its average reached 7.39. This may be attributed to the nature of the inverse relationship between pH and salinity of water hyacinth wastes. Al-Zubaidi and Hassan (1978) indicated that there is a gradual and slight decrease in pH values as the concentration of salts increases. However, the nature of the interaction between water quality had a significant effect, as can notice a decrease in pH values for all types of water except for wastewater, as the pH values increased from 7.07 to 7.80. This may be attributed to the cessation of the microorganism's activity in the wastewater, which contributes to the decomposition of organic matter and which has an active role in the increase and decrease pH of water (Fig. 2).

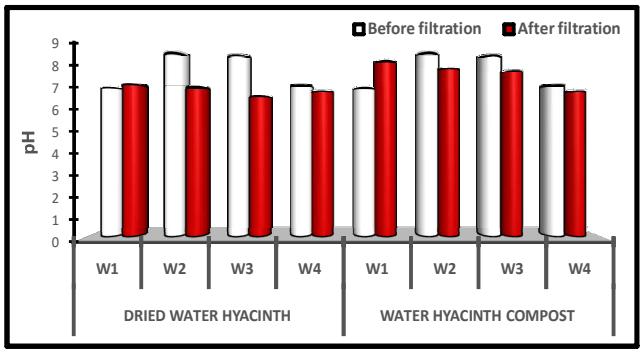

Fig. 2. Effect of water hyacinth waste on the pH of water during the filtration process

Table 3. Effect of water hyacinth waste on the pH during the filtration process

Wastes	Water quality	Sampling period		Waste * water
		Before filtration	After filtration	
Dried Water hyacinth	Wastewater	7.07	7.23	7.15
	Industrial wastewater	8.75	7.11	7.93
	Drainage water	8.65	6.68	7.67
	Tap water	7.20	6.93	7.07
Water hyacinth compost	Wastewater	7.07	8.36	7.72
	Industrial wastewater	8.75	7.98	8.37
	Drainage water	8.65	7.88	8.27
	Tap water	7.20	6.93	7.07
LSD		0.0267		0.0189
Waste * period				
Waste		Before filtration	After filtration	Average waste
Dried Water hyacinth		7.92	6.99	7.45
Water hyacinth compost		7.92	7.79	7.85
LSD		0.0133		0.0094
Water quality* period				
Water quality		Before filtration	After filtration	Average water quality
Wastewater		7.07	7.80	7.43
Industrial wastewater		8.75	7.55	8.15
Drainage water		8.65	7.28	7.97
Tap water		7.20	6.93	7.07
LSD		0.0133		0.0133
Average sampling		7.92	7.39	
LSD		0.0094		

Sodium adsorption ratio (SAR): Table 4 shows the SAR values for the water understudy before and after the filtration process ranged between 1.62-18.25 and 1.54-18.40, respectively, where the highest value was recorded in drainage water, this may be due to the high concentration of sodium compared with the concentration of calcium and magnesium (Table 5). Therefore, competition with the sodium ion decreases, thus the sodium adsorption ratio increased, as these results are agreed with earlier work (Ragab et al 2008, Mahmoud and Al-Zaidi 2011). The SAR values for wastewater and industrial waste water before and after filtration with the standards of treated wastewater and industrial wastewater were compared according to the Iraqi national determinants for the year 2012, which it was observed that they are within the permissible limits (less than 6). There were significant differences in SAR values before and after filtration. Likewise, the nature of the interaction between water hyacinth waste had a significant effect, as it powders significantly exceeded in reducing SAR values so that the average SAR values after filtration reached 6.29 compared with the average values of SAR before filtration, which was 6.65 with a decrease of 5.41% (Fig. 3). Whereas there was an increase in SAR values after passing water on the water hyacinth compost. This may be due to the ability of the water hyacinth compost, which is a humid organic substance, to adsorb calcium and magnesium ions than the water hyacinth powder that led to a higher concentration of sodium compared to the concentration of calcium and magnesium ions, thus, the SAR increased.

Water quality classification according to the American Salinity Laboratory (USDA): The water was classified according to the American classification proposed by the American Salinity Laboratory USDA (Richards 1954). The study water were classified underclasses of C_3-S_1, C_4-S_1, C_4-S_2, and C_4-S_3 according to the electrical conductivity and SAR of the water under study (Table 5). Thus, the water that was classified under the C_3 class is highly saline water and cannot be adopted for irrigation except in the presence of effective

Table 4. Effect of water hyacinth waste on the SAR values of water during the filtration process

Wastes	Water quality	Sampling period		Waste * water
		Before filtration	After filtration	
Dried Water hyacinth	Wastewater	4.12	3.49	3.80
	Industrial wastewater	2.60	2.30	2.45
	Drainage water	18.25	17.94	18.09
	Tap water	1.62	1.44	1.53
Water hyacinth compost	Wastewater	4.12	4.53	4.32
	Industrial wastewater	2.60	4.60	3.60
	Drainage water	18.25	18.40	18.32
	Tap water	1.62	1.54	1.58
LSD		0.0178		0.0126
Waste * period				
Waste		Before filtration	After filtration	Average waste
Dried Water hyacinth		6.65	6.29	6.47
Water hyacinth compost		6.65	7.27	6.96
LSD		0.0089		0.0063
Water quality* period				
Water quality		Before filtration	After filtration	Average water quality
Wastewater		4.12	4.01	4.06
Industrial wastewater		2.60	3.45	3.02
Drainage water		18.25	18.17	18.21
Tap water		1.62	1.49	1.55
LSD		0.0089		0.0089
Average sampling		6.65	6.78	
LSD		0.0063		

drainage networks and for crops with high salinity tolerance. On the other hand, the water is classified under C_4 class is the water of very high salinity and is considered unsuitable for irrigation in normal conditions and can be used only in certain cases such as soil with very high permeability, efficient drainage and crops that are very tolerant of salinity. Moreover, the study water that is classified under the S_1 class is water that can be used for most soils without any damage. Some crops that are very sensitive to sodium in plant tissues, such as fruit trees, can be affected. Similarly, the water that classified under the S_2 class could cause some risks to the characteristics of some soils, especially clay-textured soils when there is no good drainage and sufficient quantities of gypsum, and this water can be used in light-textured soils without any risks. In the role of the water classified under the S_3 class, , this type of water can cause problems because of the high percentage of sodium in the soil. This water can be used in gypsum soils without any sodic risks.

Heavy Elements

Lead: The concentrations of lead before the filtration process in the two types of water hyacinth waste, was 0.470, 0.362, 0.393, and 0.040 $mg.L^{-1}$ for wastewater, industrial wastewater, drainage water, and tap water, respectively (Table 6, 7). These concentrations are higher than the permissible limit for water drained to the water source according to the Iraqi determinants of the River and Water Conservation System from Pollution No. 25 of 1967, which amount to 0.10 $mg.L^{-1}$ and the determinants of the Hashemite Kingdom of Jordan for the year 2007, which was 0.20 $mg.L^{-1}$. Once The comparison of concentrations of lead for wastewater and industrial wastewater in the current study with the Iraqi national standards for the year 2012, exceeded the permissible limits for the use of that water for agricultural irrigation (0.1 $mg.L^{-1}$). The concentration of lead decreased after passing that water over the dried water hyacinth powder for a period of 30 days (0.000, Nil, 0.012, Nil) $mg.L^{-1}$ with a reduction efficiency estimated as 99.05%, to reach less than

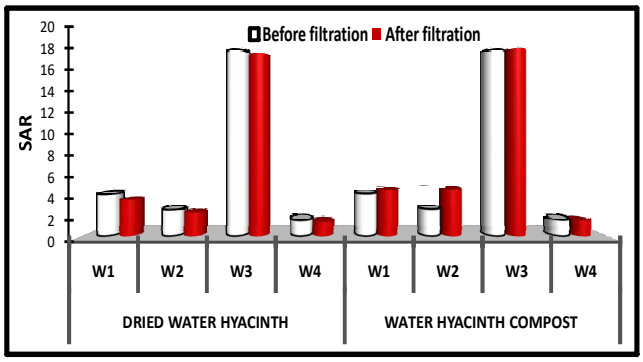

Fig. 3. Effect of water hyacinth waste on the SAR of water during the filtration process

Table 5. Water quality classification according to the USDA salinity laboratory system before and after the filtration process

Waste	Water quality	Sampling period	Characteristics with units						
			EC	TDS	Ca^{+2}	Mg^{2+}	Na^{+}	SAR	Water quality (USDA)
			µ mhos cm^{-1}	Mg L^{-1}	Meq/L				
Dried Water hyacinth	Wastewater	Before filtration	2280	1459	5.848	5.015	9.594	4.117	C_4–S_1
		After filtration	4030	1946	8.817	7.887	10.087	3.490	C_4–S_1
	Industrial wastewater	Before filtration	1640	1050	5.242	4.020	5.594	2.599	C_3 – S_1
		After filtration	2140	1370	9.800	2.332	5.663	2.2663	C_3 – S_1
	Drainage water	Before filtration	15570	9965	21.235	45.718	105.575	18.247	C_4 – S_3
		After filtration	15710	10054	33.728	47.894	114.607	17.940	C_4 – S_3
	Tap water	Before filtration	1110	710	4.207	2.344	2.932	1.620	C_3 – S_1
		After filtration	2680	1715	9.581	7.424	4.206	1.442	C_4–S_1
Water hyacinth compost	Wastewater	Before filtration	2280	1459	5848	5.015	9.594	4.117	C_4–S_1
		After filtration	3450	2208	3.752	9.781	11.788	4.532	C_4–S_2
	Industrial wastewater	Before filtration	1640	1050	5.242	4.020	5.594	2.599	C_3 – S_1
		After filtration	2640	1690	1.482	6.434	9.152	4.600	C_4–S_2
	Drainage water	Before filtration	15570	9965	21.235	45.718	105.575	18.247	C_4 – S_3
		After filtration	15410	9892	23.528	51.456	112.641	18.396	C_4 – S_3
	Tap water	Before filtration	1110	710	4.207	2.344	2.932	1.620	C_3 – S_1
		After filtration	1890	1210	5.210	6.208	3.667	1.535	C_3 – S_1

Table 6. Effect of dried Water hyacinth powder on reducing lead concentrations

Number of days	Lead concentrations ($mg.L^{-1}$)			
	Water quality			
	Wastewater	Industrial wastewater	Drainage water	Tap water
Before filtration	0.4700	0.3620	0.3930	0.040
2 days	0.0734	0.2325	0.3304	0.0332
4 days	0.3182	0.1713	0.0245	0.0244
6 days	0.1224	0.2815	Nil	0.0235
8 days	0.2692	0.1469	0.3059	0.0171
10 days	0.0612	0.0979	0.1713	0.0171
12 days	0.0503	Nil	0.2011	Nil
14 days	0.00	0.1508	0.2513	0.0276
16 days	0.1759	Nil	0.0251	0.0075
18 days	0.0503	0.1005	0.2765	0.0175
20 day	0.0979	0.0245	0.1958	0.0049
22 days	Nil	0.1958	0.1224	0.0049
24 days	0.1469	Nil	0.2692	Nil
26 days	0.000	0.0979	0.2937	0.0171
28 days	0.1469	0.0245	0.1224	0.0073
30 days	0.0000	Nil	0.012	Nil

Table 7. Effect of water hyacinth compost on reducing lead concentrations

Number of days	Lead concentrations ($mg.L^{-1}$)			
	Water quality			
	Wastewater	Industrial wastewater	Drainage water	Tap water
Before filtration	0.4700	0.3620	0.393	0.0400
2 days	0.3304	0.3427	0.3182	0.0244
4 days	0.1224	0.2203	0.1713	0.0073
6 days	0.0979	0.000	0.2448	0.0000
8 days	0.0979	0.0490	0.0979	0.0073
10 days	0.1224	0.0000	0.1469	0.0220
12 days	Nil	0.1005	0.2011	0.0050
14 days	Nil	Nil	0.0251	0.0025
16 days	0.1759	0.0251	0.2011	0.0000
18 days	0.000	Nil	0.1005	0.0050
20 day	0.1469	Nil	0.0245	Nil
22 days	0.0734	Nil	0.1224	0.0024
24 days	0.0490	Nil	0.1713	0.0049
26 days	0.0245	0.1224	0.1469	Nil
28 days	Nil	0.4161	0.1713	Nil
30 days	Nil	Nil	0.0979	0.0025

the permissible limit for irrigation and agriculture according to the Iraqi national determinants.

Hassoon and Najem (2017) also found the high efficiency of water hyacinth powder in removing lead elements, which reached 99.9%. They also confirmed high susceptibility of the water hyacinth plant in its non-living form for the treatment of wastewater and other polluted water containing heavy metals. Taha et al (2019) also reported that water hyacinth powder had the ability to remove lead from polluted water, with an efficiency of 98%. The concentrations of lead element decreased significantly after passing that water on the water hyacinth compost with a reduction efficiency of 92.07% to reach less than the permissible limit for irrigation and agriculture according to Iraqi national determinants for the year 2012. The efficiency of removal of lead element by the dried water hyacinth powder was higher than it was in the water hyacinth compost. Najem (2015) in his study on the ability of the water hyacinth roots to adsorb heavy elements that highest removal of lead was 99.66% compared to chromium.

Cadmium: The concentrations of cadmium before the filtration process was 0.2030, 0.0634, 0.0571, 0.0046 mg.L^{-1} for wastewater, industrial wastewater, drainage water, and tap water, respectively (Table 8, 9). These values exceeded the permissible concentrations in the water (0.01) mg.L^{-1} as defined by the World Health Organization (WHO/FAO 2007). It also exceeded the permissible limits (0.01) mg.L^{-1} for the use of that water for agricultural irrigation when compared with the Iraqi national determinants (2012). The cadmium concentrations decreased after passing that water on the dried water hyacinth powder for a period of 30 days (0.002, Nil, 0.0031, Nil mg.L^{-1}) with a reduction efficiency of 98.45%, to reach less than the permissible limit for irrigation and agriculture according to the Iraqi national determinants (Table 8, Fig. 5). The comparison of the concentrations of cadmium after the filtration process with the determinants of the World Health Organization and the World Food Organization indicated less than the permissible limits in the environment of water (0.01) mg.L^{-1}. Taha et al (2019) also observed that the water hyacinth powder (root and stem) can be used in removing heavy metals from polluted water with a removal efficiency of 96% cadmium. The cadmium concentrations decreased to nil, nil, 0.0061 and 0.0005 mg.L^{-1} with a reduction efficiency of 97.99% to reach suitable for irrigation and agricultural uses according to the Iraqi national determinants for the year 2012. Besides, the lowest permissible concentrations of water discharged to waterways (0.01) mg.L^{-1} according to the Iraqi Rivers Conservation System No. 25 for the year 1967 and the Hashemite Kingdom of Jordan (2007). The efficiency of removing cadmium from the dried water hyacinth powder was higher than it is in the water hyacinth compost. These results are identical to Al Bayati and Thaer (2018). The water hyacinth plant can be used to purify and remove heavy metals from polluted industrial wastewater with an efficiency of up to 100% and at a low cost. Moreover, can be used to purify industrial waste lakes in which liquid waste from factories is collected.

The concentrations of heavy metals under study decreased after passing them on water hyacinth waste (water hyacinth powder, water hyacinth compost) for a period of 30 days and with high efficiency to reach values suitable for use in agricultural irrigation (6, 7, 8, and 9). Otherwise reaches the permissible concentrations of the water discharged and drained into the waterways. Despite the fluctuation in the concentrations during that period that is attributed to the mechanisms by which ions move in the various water columns that contain the water hyacinth waste. It is represented by the mass flow, which is the movement of ions with the flow of water to the bottom of the column due to gravity, and diffusion, which includes the movement of ions

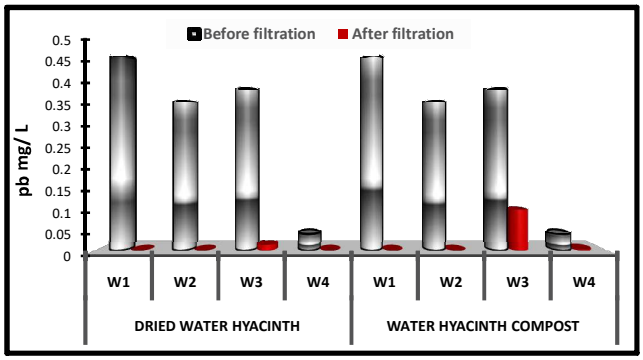

Fig. 4. Effect of water hyacinth waste on reducing lead concentrations

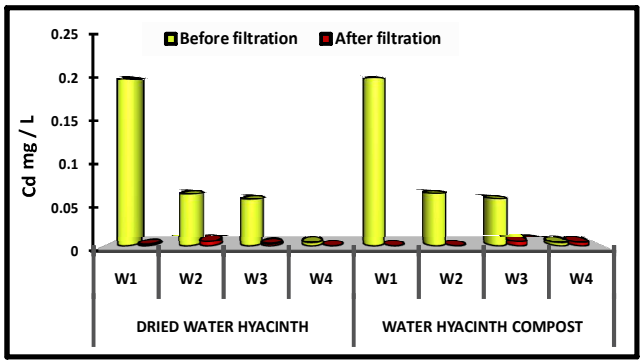

Fig. 5. Effect of water hyacinth residues on reducing cadmium concentrations

Table 8. Effect of Water hyacinth powder on reducing cadmium concentrations in polluted water ($mg.L^{-1}$)

Number of days	Lead concentrations ($mg.L^{-1}$)			
	Water quality			
	Wastewater	Industrial wastewater	Drainage water	Tap water
Before filtration	0.2030	0.0634	0.0571	0.0046
2 days	0.0061	Nil	0.0326	Nil
4 days	0.0081	0.0132	Nil	Nil
6 days	0.0132	Nil	Nil	0.0000
8 days	0.0061	Nil	0.0071	0.0012
10 days	Nil	0.0051	Nil	0.0007
12 days	0.0033	0.0000	Nil	0.0002
14 days	Nil	Nil	0.0108	0.0000
16 days	0.0008	0.0183	Nil	0.0005
18 days	0.0033	0.0033	Nil	0.0001
20 day	0.0061	Nil	0.0142	0.0011
22 days	0.0020	0.0041	Nil	0.0016
24 days	0.0112	Nil	Nil	0.0013
26 days	0.0071	0.0265	Nil	Nil
28 days	Nil	Nil	0.0102	Nil
30 days	0.0020	Nil	0.0031	Nil

Table 9. Effect of water hyacinth compost in reducing cadmium concentrations in polluted water

Number of days	Lead concentrations ($mg.L^{-1}$)			
	Water quality			
	Wastewater	Industrial wastewater	Drainage water	Tap water
Before filtration	0.2030	0.0634	0.0571	0.0046
2 days	0.1933	0.0142	0.0112	Nil
4 days	0.0092	Nil	Nil	Nil
6 days	0.0214	Nil	Nil	0.0004
8 days	0.0092	0.0204	Nil	0.0012
10 days	Nil	Nil	Nil	Nil
12 days	0.0500	0.0192	0.0208	0.0000
14 days	0.0042	Nil	Nil	Nil
16 days	0.0133	0.0200	0.0025	0.0009
18 days	Nil	0.0058	Nil	Nil
20 day	Nil	0.0092	Nil	0.0001
22 days	Nil	0.0204	Nil	Nil
24 days	Nil	Nil	0.0132	0.0003
26 days	Nil	Nil	Nil	Nil
28 days	Nil	0.0071	Nil	Nil
30 days	Nil	Nil	0.0061	0.0005

from the area of high concentration to the area of low concentration and the process of dilution resulting from adding water from time to time to the column. In addition to the occurrence of the adsorption process by the water hyacinth waste, which behaved as organic matter containing active groups of carboxylic, phenolic, and carbonyl. The dried water hyacinth powder was more efficient in removing heavy metals from the polluted water under study. This may be due to the fact that the water hyacinth compost has reached advanced stages of decomposition compared to the dried water hyacinth powder, where the basic groups of humic substances are humic acid (HA), fulvic acid (FA), and humin (HM), which contain the aromatic and aliphatic ring groups. Humic acid mainly binds with binary ions and works to restrict their movement, like the heavy elements under study are divalent, while fulvic acid is more hydrophilic than humic . However, the adsorption process is affected by several factors including the density of surface charge, the surface area, the nature of the adsorbing surface, the degree of interaction of the medium, and other factors. Furthermore, the biosorption process by the water hyacinth waste is carried out through the physicochemical reactions that take place between the heavy elements under study and the effective functional groups present on the surface of the adsorbent materials. The biomass cells are mainly composed of proteins, polysaccharides, and peptides, all of which are rich in functional groups that form bonds with heavy elements. Many studies have shown that heavy metals can be removed from polluted water by using different plant materials, as their leaves contain a lot of substances such as cellulose, pectin, carotene, which are important sites for the biosorption process. They contain active compounds or groups OH^- and CO^- and others (Votesky 2003; Ahmed et al 2016), and the biosorption method is one of the effective, inexpensive, and easily applicable methods.

Reverse experience: The concentrations of heavy metals continued to decrease in all columns after passing tap water over them for a period of 30 days (Table 10, 11). The continuous decrease in the concentration of lead and cadmium in the wastewater and industrial wastewater column containing the dried water hyacinth powder to reach unread concentrations (nil). The concentrations of lead and cadmium decreased from 0.0980, 0.0061 $mg.L^{-1}$ to 0.0061, 0.0001 $mg.L^{-1}$, respectively for the column of drainage water containing water hyacinth compost and in column containing tap water and dried water hyacinth compost, as the

Table 10. Effect of water hyacinth waste on the concentrations of Pb after passing tap water on all columns ($mg.L^{-1}$)

Waste	Column	Number of days			
		30	40	50	60
Dried Water hyacinth	Wastewater	0.0000	0.0617	0.0463	Nil
	Industrial wastewater	Nil	0.0107	0.0106	Nil
	Drainage water	0.0120	0.0123	0.0025	Nil
	Tap water	Nil	0.0093	0.0077	Nil
Water hyacinth compost	Wastewater	Nil	0.0101	0.0046	Nil
	Industrial wastewater	Nil	0.0216	0.0030	Nil
	Drainage water	0.0980	0.0215	0.0138	0.0617
	Tap water	0.0025	0.0123	0.0077	0.0010

Table 11. Effect of Water hyacinth waste on Cd concentrations after passing tap water on all columns ($mg\ L^{-1}$)

Waste	Column	Number of days			
		30	40	50	60
Dried water hyacinth	Wastewater	0.0020	0.0000	Nil	Nil
	Industrial wastewater	Nil	0.0009	0.0022	Nil
	Drainage water	0.0031	0.0129	0.0018	Nil
	Tap water	Nil	0.0002	Nil	Nil
Water hyacinth compost	Wastewater	Nil	0.0111	Nil	Nil
	Industrial wastewater	Nil	0.0022	Nil	Nil
	Drainage water	0.0061	0.0107	0.0022	0.0001
	Tap water	0.0005	0.0006	Nil	Nil

concentrations of lead and cadmium continued to decreased (0.0025, 0.0005to (0.0010, Nil mg.L^{-1} respectively). The continued decrease in the concentration of lead and cadmium elements reaches the permissible concentrations in the water environment according to the determinants of the World Health Organization (WHO/FAO 2007). The concentrations of these elements continue to decrease in the columns containing the compost of the water hyacinth after the passage of tap water on it for another 30 days. Hassoon and Najem (2015, 2017) indicated the ability of the water hyacinth plant in its non-living form to treat wastewater or industrial wastewater and other water containing heavy metals. It was found that this plant has the superior ability to remove lead and then cadmium.

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Effect of Sand, Zeolite, Ash Husk Filters, and Filtration Period in Reducing Salinity of Well Water

Baydaa A. Hassan, Hayfaa J. Hussein and Najla J. Mohammed

Department of Soil Science and Water Resources, College of Agriculture
University of Basrah, Basrah, Iraq
E-mail: baydaallawi@gmail.com

Abstract: A laboratory experiment was conducted on five groundwater wells from Al-Zubair district with primary electrical conductivity of 5.2, 8.3, 13.3, 16.9, and 22.7 $dS.m^{-1}$and was then passed on three types of filters which are rice husk ash and sand with mixing ratio of25, 50 and 75%, zeolite and sand filter with mixing ratio of 25,50 and 75% and zeolite filter and rice husk ash with a mixing ratio of1: 0.25 and 1: 0.5 and 1: 1 The water was then passed during four time periods direct, 15, 30, and 60 min The electrical conductivity of the water was measured after being passed through filters with different time periods. The type of filter had a significant effect in reducing the electrical conductivity of well water. The zeolite filter mixed with the ash of rice husk at a mixing ratio 1: 1 has resulted in lowest electrical conductivity, while the zeolite filter mixed with sand at a mixing ratio of 3: 1 recorded highest electrical conductivity compared to the original sample of well water. The direct passage of water gave the lowest values compared to other time periods 15, 30, and 60 min.

Keywords: Sand filter, Zeolite, Rice husk ash, Well water salinity

Groundwater is considered one of the most important water resources in Iraq, especially in southern Iraq, and the concentration of salts in groundwater has increased in recent times, which required using many treatments to solve the problem of salinity and reuse it for irrigation purposes (Al-Ansari 2013). The use of plant residues, mineral and organic materials have a role in treating salty water and reusing it for irrigation. Liu et al (2000) demonstrated the role of the sand filter in the formation of the biofilm on its surfaces, which has a high capacity for biodegradation of organic matter in water, which reached the removal of BOD, COD and TOC to the limits of 95%. The first attempt to treat water with natural substances containing the mineral zeolite (Mx / n [(AlO_2) x (SiO_2) y]. MH_2O was applied in the nineteenth century. This was the first practical application of using zeolite in water treatment due to the ion exchange property of zeolite. Erdem et al (2004) observed that the mineral zeolite has a positive effect on the environment through wastewater purification. Jakkula (2005) showed that zeolite has a role in treating wastewater and drinking water, removing radioactive isotopes from agricultural water, and removing ammonium from wastewater. Zeolite mineral was also used as a soil reformer, a treatment agent for wastewater, controlling heavy elements, and as a filter for water due to its low weight and low density due to a large number of cavities within its crystalline structure, which increases its efficiency in treating water and soils treated with it (Cabanilla et al 2016). Rice husks are considered agricultural wastes that are available in large quantities in rice milling factories, and when they are burned, ash forms, which are an adsorbent of many ions and an effective agent in water treatment (Feng et al 2004). Shamasdeen and Al-Kaderry (2004) observed that the ash of rice husks contains active groups on the surface of the ash, and treating soil and water with it reduces water pollution and reduces its salinity. Radi (2014) also found that the highest reduction efficiency in the electrical conductivity of the rice husk ash filter at a burning temperature of 1000°C Al-Hakim (2016) showed the efficiency of the rice husk ash filter in reducing water hardness in the wells of Al-Zubayr, Al-Barjisia and Safwan. Salman (2017) also showed the efficiency of the rice husk ash filter in reducing the turbidity and total hardness vs of drainage water at electrical conductivity. Due to the high salinity of well water in Basra province and its effect on the growth and productivity of many crops, the study demonstrated the effect of the efficiency of rice husk ash and zeolite mineral with sand filter in reducing well water salinity and improving its quality.

MATERIAL AND METHODS

The study included possibility of reclaiming saline wells water in Basra, where water samples were brought from five wells in Al-Zubair district, Al-Raha area, and Al-Mashrue with electrical conductivity of 5.2, 8.3, 13.3, 16.9, and 22.7 dS m^{-1}. The water samples were collected and kept in plastic containers in the refrigerator at a temperature of 4°C to avoid the occurrence of biological changes in them until the

required analyzes are conducted. The experiment included the use of the following factors:

1. Five different saline levels of well water (4, 8, 12, 16 and 20 $dS.m^{-1}$)
2. Mixing ratios between ash of rice husks and sand (25, 50 and 75%) for making filters
3. Mixing ratios between zeolite and sand (25 50 and 75%) for making filters.
4. Mixing ratios between zeolite and ash of rice husks (0.25: 1, 0.5: 1, and 1: 1) to make the filters.
5. Time period for passing the salt water through the filters (direct, 15, 30, 60 min).

The water was passed according to the salinity levels on the above filters according to time periods. The electrical conductivity and chemical composition were estimated according to international and Iraqi standards for irrigation purposes.

Preparation of Filters used in Well Water Treatment

Filter of rice husk ash mixed with sand: Raw rice husk (*Oriza sativa* L) was collected from agricultural fields in Al-Shamiya District, Qadisiyah province. The impurities, as well as the soil, were removed after cleaning and washing with distilled water. The samples were air dried, and were then burned in the Muffle Furnace at a temperature of 1000^{O}C for 3 hours to obtain rice husk ash (RHA). Plastic tubes with a diameter of 7.5 cm and a height of 20 cm were chosen, ending with a conical end connected to a valve to control the time of water descent from these tubes, and putting glass wool at the bottom of the tubes to prevent ash from escaping from them. The volume of ash and sand in this filter was 450 cm^3 according to the mixing ratios of ash husk and sand (25, 50and 75%). The Four different time periods were used to keep the sample on the filter, which are direct filtration and filtration after 15, 30 and 60 min, and after the specified time period had passed from placing the water sample in the filter. The valve was opened to receive the treated water. The water was collected in sealed plastic container until the necessary analyzes were conducted. The ash filter configuration was adopted according the method used by Radi (2014).

Sand-mixed zeolite filter: It was adopted the same method as for the filter of the rice husk ash and sand.

Mixing filter (rice husk ash mixed with zeolite): The same method used in the filter ash of rice husk mixed with sand was also adopt.

The electrical conductivity of the water samples was measured using a Lovibond Con200 EC meter

Estimation of ash in the rice husks: The percentage of ash in the rice husks was estimated after taking a known weight from the husks and burning them in the incineration apparatus at a temperature of 1000^{O}C, it was then left to cool down and recorded the weight after burning, the percentage ash was calculated (Table 1).

Surface area and particle size for rice husk ash and zeolite: The surface area of rice husk ash and zeolite and the size distribution of ash and zeolites particles were estimated using a Mastersizer Particle Size Analyzer (PSA) type MS2000 in the laboratories of the Geological Survey Department , Ministry of Industry (Table 1 and 2). The oxides, carbon, and other organic materials in rice husk ash and zeolite were estimated. The concentration of silica oxides and the oxides of the mineral and base elements of rice husk ash and zeolite were estimated using the X-Ray Fluorescence device (Spectro-Xepos, Germany) in the Iraqi-German laboratory at the University of Baghdad (Table 3).

RESULTS AND DISCUSSION

The type of filter had a significant effect in reducing the electrical conductivity of wells water (Table 4). The filter of zeolite mixed with the ash of rice husks at mixing ratio (1: 1) recorded the lowest electrical conductivity (0.5 $dS.m^{-1}$) and with reduction of 87.50%. The filter of zeolite mixed with the sand at a mixing ratio of 3: 1, recorded the highest electrical conductivity (2.68 $dS.m^{-1}$) and a reduction of 33.00% compared to the original sample of well water. Then ranking the filters according to their lowering of the values of electrical conductivity is in order of Rice husk ash mixed with zeolite (1: 1) <rice husk ash mixed with zeolite (1: 3) <rice husk ash mixed with zeolite (3: 1) <rice husk ash mixed with sand (1: 3) <zeolite mixed With sand (1: 3) <zeolite mixed with sand (1: 1) <zeolite mixed with sand (1: 1) <sand mixed rice husk ash (3: 1) <zeolite mixed with sand (3: 1).

The electrical conductivity increased with the advancement of the filtration time from 0.94 $dS.m^{-1}$, with a reduction of (76.50%) at direct filtration to 1.64 $dS.m^{-1}$ at a time of 60 min, with a percentage of reduction to 59.00%. This may be normal, where the mineral and organic filter (zeolite and ash of rice husks) have certain limits in absorbing dissolved ions, after which the electrical conductivity begins to increase. The interaction between the filters and the filtration time had a significant effect on the values of electrical conductivity for the water after filtration, where the interaction between direct filtration (zero time) and the filter of zeolite mixed with rice husk ash at a mixing ratio of (1: 1) recorded 0.34 $dS.m^{-1}$ while the interaction between 60 min filtration and the filter of zeolite mixed with sand at a mixing ratio of (3: 1) recorded the highest of 3.23 $dS.m^{-1}$ with a percentage of reduction of 91.5% and 19.25%, respectively. The apparent difference between the different filters is due to the difference in the mineral and organic composition of the filters, which was reflected in the nature of the interaction

between the filter materials used in the experiment and the water passing through it, where the filters operate with different mechanisms that enable them to achieve lower percentages for the concentrations of positive and negative ions from the water. The results of the current study showed an effective role for the filter of zeolite mixed with the ash of the rice husk at a mixing ratio (1: 1). This may be due combined effect for the materials used in the filter and to the different mechanisms by which these materials work in reducing the dissolved ions that cause salinity. In addition to the increase in the size of ash and its mixing with zeolite compared to the filters that contain sand within their

Table 1. Physical and chemical properties for burnt rice husk ash at 1000°C

Diameter range (mm)	Percentage for gradation of particles diameters of rice husks	Surface area ($g.cm^{-2}$)	Ash (%)
0.1-0.01	0	0.194	27.61
0.1-1.0	0.52	Bulk density ($g.cm^{-3}$)	Apparent density ($g.cm^{-3}$)
1.0-10.0	10.63	2.31	0.79
10.100	40.54	Ec ($dS.m^{-1}$)	pH
100-1000	51.69	6.14	7.92

Table 2. Physical and chemical properties of the zeolites used in the study

Diameter range (mm)	Percentage for gradation of particles diameters of rice husks	Surface area ($g.cm^{-2}$)	Cation exchange capacity ($cmol.kg^{-1}$)
0.1-0.01	0	0.136	122.31
0.1-1.0	0.96	Bulk density ($g.cm^{-3}$)	Apparent density ($g.cm^{-3}$)
1.0-10.0	8.5	2.69	1.04
10.100	39.4	Ec ($dS.m^{-1}$)	pH
100-1000	51.14	0.51	7.02

Table 3. Concentration of oxides, carbon, and other organic materials in rice husk ash and zeolite (Per cent)

Content (%)	SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO	Na_2O	K_2O	Carbon and other materials
Rice husk ash	90.13	0.41	1.03	2.61	1.14	0.26	3.07	1.35
Zeolite	38.61	13.76	11.98	11.47	3.26	2.47	1.25	17.2

Table 4. Electrical conductivity of r wells water with initial conductivity of 4 $dS.m^{-1}$ after treating with filters

Type of filters	Filtration time (min)				Average of filtered water
	Direct	15	30	60	
T_1-Zeolite + sand (3: 1)	2.08	2.58	2.82	3.23	2.68
T_2-Zeolite + sand (1: 1)	1.01	1.44	1.57	1.82	1.46
T_3-Zeolite + sand (1: 3)	0.98	1.46	1.58	1.73	1.44
T_4-Rice husk ash + sand (3: 1)	1.57	1.59	1.92	3.18	2.07
T_5-Rice husk ash + sand (1: 1)	0.78	0.89	1.05	1.23	0.99
T_6-Rice husk ash + sand (1: 3)	0.52	0.59	0.78	0.98	0.72
T_7-Zeolite + ash of rice husk (3: 1)	0.68	0.73	0.83	0.93	0.79
T_8-Zeolite + ash of rice husk (1: 1)	0.34	0.44	0.55	0.69	0.50
T_9-Zeolite + ash of rice husk (1: 3)	0.51	0.56	0.78	0.98	0.71
Time	0.94	1.14	1.32	1.64	
LSD (p=0.05)					
Type of the filter		Time			Time×type of the filtered
0.0064		0.0043			0.0129

components leads to an increase in the thickness of the ash layer that is in contact. With treated drainage water, which means an increase in the number of pores, t gives a great opportunity to trap the dissolved ions that make up the salinity, where the ash burned at high temperatures 1000^{O}C leads to the possession of the ash particles very small diameters, resulting in high surface porosity and a large number of pores, thus providing an opportunity for the treated water to penetrate into the ash body, and direct contact with the inner surface. Xiong et al (2009) and Imyim and Eakachai (2010) also showed that improving the surface area and high porosity of rice husk ash, especially when burning at a temperature higher than 700^{O}C, increases the chance of filtered water penetration within the ash structure, the crystal structure, and direct contact with the internal surfaces of the filters (Radhi (2014) and Al-Hakim (2016) also observed the same tend.

The filter of zeolite mixed with the ash of the rice husk at a mixing ratio (1: 1) had a significant effect in reducing the electrical conductivity of the filtered well water from (8 dS.m^{-1} to 0.57 dS.m^{-1}), with a reduction of 92.84 percent (Table 5). The filter of zeolite mixed with sand at a mixing ratio (3: 1) recorded the lowest percentage of reduction (39.75%), where the electrical conductivity was 4.27 dS.m^{-1} and is considered a good percentage compared to the original value of well water. The time factor, it had a significant effect on electrical conductivity as salinity increase to 8 dS.m^{-1}. The zero filtration time recorded the highest reduction of conductivity, o (1.59 dS.m^{-1}), with reduction of o 78.73%, compared to the highest value of electrical conductivity recorded by the filtration treatment after 60 min, (3.18 dS.m^{-1}), with reduction of 58.11%. Interaction between the type of filter and the filtration time had a significant effect in reducing the electrical conductivity of wells water, with electrical conductivity (8 dS.m-1decreased to 0.35 dS.m-1 at the interaction between direct filtration and a filter zeolite mixed with the ashes of rice husks at a mixing ratio (1: 1) which reduction of 95.63% The highest values of electrical conductivity was when the water passed through the filter of zeolite and sand at a mixing ratio (3: 1) (6.84 dS.m^{-1}) after 60 min of filtration, with a percentage of reduction of 14. 50%. These results agree with earlier researchers (Radhi 2014, Al-Hakim 2016, Salman 2017, Al-Aqili 2017).

The type of filter had an effect on electrical conductivity, where the filter of zeolite mixed with the ash of rice husk at a mixing ratio (1: 1) was superior (0.92 dS.m^{-1}), which approximately similar to the filter of zeolite with the ash of the rice husk at mixing ratio (3: 1) which was 0.96 dS.m^{-1}, with reduction 92.33 and 92.00% respectively (Table 6). The direct time factor excelled in reducing electrical conductivity on the rest of the experiment factors when using water wells with primary electrical conductivity (12 dS.m^{-1}). The There was an increase in electrical conductivity with the increase of the filtering time from the direct filtering (zero time) to 60 min, where an electrical conductivity gave was 2.09, 2.23, 2.84 and 3.50 dS.m^{-1} for direct, 15, 30 and 60 min, respectively with high significant differences between all treatments. The effect of the interaction between the type of filter and the filtration time significantly reduced electrical conductivity for the well water, where the electrical conductivity decreased

Table 5. Electrical conductivity for wells water with initial conductivity of (8 dS.m^{-1}) after treating with filters

Type of filters	Filtration time (min)				Average of filtered water
	Direct	15	30	60	
T_1	3.61	3.91	4.92	6.84	4.27
T_2	2.90	3.14	3.42	5.08	3.39
T_3	1.27	1.45	2.40	3.18	2.55
T_4	2.91	3.11	3.92	5.84	4.27
T_5	1.84	2.08	2.97	3.72	3.18
T_6	0.87	0.90	1.20	2.03	1.25
T_7	0.74	0.76	0.91	1.07	0.92
T_8	0.35	0.40	0.62	0.92	0.57
T_9	0.82	0.86	1.01	1.48	1.00
Time	1.92	2.07	2.33	3.18	
LSD (p=0.05)					
Type of the filter		Time			Time×type of the filtered
0.0231		0.0154			0.0462

from 12 dS.m^{-1} to 0.53 dS.m^{-1} when filtration directly with a filter of zeolite mixed with the ash of the rice husks at a mixing ratio (1: 1) and with a percentage of reduction of 95.58%). The highest electrical conductivity were at the interaction between the filter zeolite mixed with sand at a mixing ratio (3: 1) and filtration time of 60 min, which was 7.63 dS.m^{-1} with percentage of reduction of 36.42%. This may be due to the efficiency of the filter of rice husk ash when burning it at high temperatures 1000^{o}C which contributes to increasing the number of pores and the specific surface area in the ash in addition to the presence of zeolite, which has many characteristics that have an important role in reducing electrical conductivity, including the characteristic of high adsorption and selectivity, ability to absorb and lose water, and high exchange capacity, which is very suitable in the field of natural water treatment and polluted water. All these traits are due to its high porosity and fine sieves that were provided in zeolites due to the presence of mutual positive ions and the precise dimensions of internal channels and caves.

The type of filter had an effect on the values of electrical conductivity, where the filter of zeolite mixed with the ash of rice husk at a mixing ratio (1: 1) has excelled on the rest of the types of filters in reducing the electrical conductivity to 1.89 dS.m^{-1}, with a of 88.17% (Table 7), while the filter of zeolite mixed with sand (3: 1) gave the highest value of electrical conductivity which was 9.15 dS.m^{-1} reduction of 42.80The ranking the filters according to their lowering the values of electrical conductivity was in following order of Zeolite mixed with rice husk ash (1: 1) <zeolite mixed with rice husk ash (3: 1) <rice husk ash mixed with sand (1: 3) <zeolite mixed with rice husk ash (1: 3) <husk ash Sand mixed rice (1: 1) <zeolite mixed with sand (1: 3) <zeolite mixed with sand (1: 1) <sand mixed rice husk ash (3: 1) <zeolite mixed with sand (3: 1). There were significant differences between the filtration times for wells water with initial electrical conductivity of 16 dS.m^{-1}, increased with the advancement of the filtration time from (3.20 dS.m^{-1}), with a percentage of reduction of 79.98% at direct filtration to 6.56 dS.m^{-1} in 60 min, with reduction of 58.97%. There were significant differences for the interaction treatments between the filtration time and the type of filters (Table 7). The interaction treatment between zero time (direct) and the filter of zeolite mixed with rice husk ash (1: 1) achieved a significant superiority compared to all, where the maximum decrease in electrical conductivity were recorded (0.61 dS.m^{-1}). The interaction between the filtration time (60 min) and the filter of zeolite mixed with sand (3: 1) recorded the highest value of electrical conductivity (10.95 dS.m^{-1}), with a percentage of reduction of 96.19 and 31.56%, respectively.

The type of filter had a significant effect on the values of electrical conductivity, where the filter of zeolite mixed with the ash of the rice husks at a mixing ratio (1: 1) gave the lowest value of the electrical conductivity of (1.96 dS.m^{-1}, while the filter of zeolite mixed with sand (3: 1) gave the highest value of electrical conductivity (11.09 dS.m^{-1}), with reduction of 90.21 and 44.55%, respectively (Table 8). The efficiency of the rice husks ashes and zeolites may be due to increase in the size of ash and zeolites leads to an increase in the thickness of the ash layer that is in contact with the treated wastewater The increase in the number of pores in addition to

Table 6. Electrical conductivity for wells water with initial conductivity of (12 dS.m^{-1}) after treating with filters

Type of filters	Filtration time (min)				Average of filtered water
	Direct	15	30	60	
T1	4.44	4.92	5.81	7.63	5.70
T2	4.23	4.84	4.89	5.17	4.78
T3	2.75	2.50	3.50	3.92	3.17
T4	4.20	4.36	4.82	5.48	4.71
T5	0.68	0.72	1.52	2.75	1.41
T6	0.65	0.72	1. 23	1.97	1.14
T7	0.55	0.60	1.14	1.53	0.96
T8	0.53	0.57	1.12	1.47	0.92
T9	0.78	0.82	1.23	1.87	1.18
Time	2.09	2.23	2.84	3.50	
LSD (p=0.05)					
Type of the filter		Time			Time×type of the filtered
0.0733		0.0488			0.1466

the trait of the sponge composition that the ash possesses gives the ability to retain the largest number of positive and negative ions inside. The voids in the spongy composition of silica in addition to the physical structure of zeolites are porous and contain internally bound voids that hold the cations. The direct time factor in reducing the values of electrical conductivity is greater than the rest of the experiment factors when using well water with electrical conductivity of 20 $dS.m^{-1}$, where there was decrease in electrical conductivity with a decrease in the filtration time from 60 min to the direct time from 7.81 to 3.98 $dS.m^{-1}$, with percentage of reduction efficiencies amounted to 60.95 and 80.10%, respectively. The filtering time factor and the type of filters had significant differences, where the treatment of direct filtration time and the filter of zeolite mixed with rice husk ash, at a mixing ratio (1: 1), register 0.61 $dS.m^{-1}$ followed by the rest of the study factors, with a percentage of reduction of 96.95%. The interaction treatment between the filtration time (60 min) and the filter of zeolite mixed with sand (3: 1) gave a value 13.28 dS.m-1, with a percentage of reduction in

Table 7. Electrical conductivity for wells water with initial conductivity of (16 $dS.m^{-1}$) after treating with filters

Type of filters	Filtration time (min)				Average of filtered water
	Direct	15	30	60	
T_1	7.62	8.41	9.63	10.95	9.15
T_2	5.11	5.86	7.19	8.47	6.66
T_3	1.39	2.16	3.76	4.93	3.06
T_4	7.34	8.03	9.51	10.84	8.93
T_5	3.39	4.11	5.42	6.41	4.83
T_6	1.27	1.97	3.28	4.89	2.85
T_7	0.98	1.21	3.13	4.39	2.43
T_8	0.61	1.26	2.28	3.42	1.89
T_9	1.11	1.94	3.24	4.78	2.77
Time	3.20	3.88	5.27	6.56	
LSD (p=0.05)					
Type of the filter		Time			Time×type of the filtered
0.0132		0.0088			0.0265

Table 8. Values of electrical conductivity for wells water with initial conductivity of (20 $dS.m^{-1}$) after treating with filters

Type of filters	Filtration time (min)				Average of filtered water
	Direct	15	30	60	
T_1	8.96	10.17	11.95	13.28	11.09
T_2	6.88	7.83	9.64	11.18	8.88
T_3	1.48	2.27	3.83	5.73	3.33
T_4	8.52	9.63	11.42	13.19	10.69
T_5	5.83	6.31	9.01	10.27	7.86
T_6	1.27	1.83	3.03	4.51	2.66
T_7	1.06	1.21	2.72	4.01	2.25
T_8	0.61	0.87	2.34	4.01	1.96
T_9	1.21	1.67	2.87	4.11	2.47
Time	3.98	4.64	6.31	7.81	
LSD (p=0.05)					
Type of the filter		Time			Time×type of the filtered
0.712		0.475			1.424

efficiency to 44.55%. The ranking the filters according to their lowering the values of electrical conductivity was in order of Zeolite mixed with rice husk ash (1: 1) <zeolite mixed with rice husk ash (1: 3) <zeolite mixed with rice husk ash (3: 1) <rice husk ash mixed with sand (1: 3) <husk ash Sand mixed rice (1: 1) <zeolite mixed with sand (1: 3) <zeolite mixed with sand (1: 1) <sand mixed rice husk ash (3: 1) <zeolite mixed with sand (3: 1).

CONCLUSIONS

The filter of rice husk ash mixed with zeolite recorded the highest efficiency in reducing the salinity of well water, especially when passing through it directly, and the efficiency of reducing salinity decreases with the increase of the time period.

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Evaluation of Groundwater in Some Wells in Mahmoudia City during Autumn and Winter Seasons

Afrah Abdulkareem Mahdi, Saadiyah A. Dhahir, Natheer J. Imran[1] and Nahla Kadhim Salman[2]

Department of Chemistry, College of Science for Women, University of Baghdad, Iraq
[1]Environment and Water Directorate, Ministry of science and technology, Baghdad, Iraq
[2]Department of Biology, College of Science for Women, University of Baghdad, Iraq
E-mail: afrah.abd91@gmail.com

Abstract: The study was conducted on the water of five wells in the city of Mahmoudia, south of Baghdad , Iraq, to study some physical and chemical properties (pH, Turb., EC**,** TDS, TSS, Cl^-, Na^+ , K^+ , PO_4^{-3} , DO, COD, $SO_4^=$, NO_3^-, Fe^{+2}, T.H, Mg^{+2}, Ca^{+2} , HCO_3). The sampling was done for the autumn and winter seasons (November 2020 and January 2021). The pH, turbidity, NO_3, Fe^{+2} were within the permissible limits of the Iraqi standard and the WHO. The TSS values were within the permissible limits of the WHO. The EC**,** TDS, $SO_4^=$, TH shows an excess of the permissible values according to the Iraqi standard and WHO. Cl^-, Na^+, Mg^{+2} and Ca^{+2} in most of the wells were within the permissible limits. The average of the water quality index for both seasons was poor water.

Keywords: Groundwater, Wells, Winter seasons

Water is one of the most important and most precious natural resources. It is essential in the life of all living organisms from the simplest plant and microorganisms to the most complex living system. It is significant due to its unique chemical and physical properties (Reda 2016). Ground water reaches the surface of the earth through springs and springs or through drilling wells, where people benefit from this water for drinking and other uses in agricultural, animal and industrial production (Hussein 2012). Groundwater occupies a great importance as it represents 22% of the world's freshwater resources. Therefore, studying this water, exploring and developing its use has attracted the interest of researchers (Jalil 2016). Around the world, in excess of 33% of all water utilized comes from groundwater. In some regions in the world the rate is significantly higher the greater part of all drinking water overall is provided from ground water (Al-Sudani 2018). The public water assets arrive at 40% of the aggregate sum, including 8% of groundwater sources. The yearly precipitation changes from one year to another with the chance of dry spell emergency, notwithstanding quantitative variances in surface spillover The typical water assets right now accessible, including surface water entering Iraq, surface water made inside Iraq and inexhaustible groundwater is 70.86 billion m3/year utilization amount is 72.12 billion m3/year. The accessible traditional water assets in the year 2035 will diminish to 55.51 billion m3/year, which show water assets condition in Iraq. Iraq should rely upon its water assets stockpiling gathered during year (Saleh et al 2020). Climate elements, especially rain, humidity, winds and temperatures, precipitation including role of surface water resources and amount of falling water and filtration into the ground and amount of water evaporated characterized by extremes by drought, high temperatures and fluctuation in the amounts of rain with the emergence of winter and summer more than the spring and autumn seasons, which are short and transitional are key factors in determining the availability of water. Iraq is one of those affected by the water shortage problem due to the lack of rainfall as well as the increase in this population in addition to the policy pursued by neighboring countries through their control of the sources of fallen rivers as well as the construction of dams on the Tigris and Euphrates and their tributaries outside the borders of Iraq (Hussain et al 2014). The groundwater plays an important role in areas where the levels of rain are less and the real benefit desired from the groundwater compared to the surface water is that it is less polluted and not susceptible to loss. Relative temperature during different seasons due to its isolation within the layers of the earth and its remoteness from atmospheric influences, and the geological composition remains the main determinant of the quality of groundwater and the extent of concentrations of chemical elements and their suitability for different uses (Al-Sudani 2018). The aims of research study the evaluation of groundwater in some wells in the Mahmoudia city.

MATERIAL AND METHODS

Study area: The city of Mahmoudia is located in the south of

Baghdad, which is 40 km from the governorate of Baghdad between two latitude (32-50-00) and (33-15-00) north and between two longitudes (44-00-00) and (44-35-00) East (Al-Ibrahimi 2020). The study area falls within the Middle Euphrates region, as it is located administratively within the capital, Baghdad, in addition to being located at the top of the flood plain and on the eastern edge of the western plateau. Essaouira, which belongs to Wasit governorate and from the northwest, Fallujah district, in Anbar governorate. Thus, the study area is confined between the Tigris and Euphrates rivers, as well as being a link between the capital and the rest of the governorates. As the area of the district is (1410.7) km2, distributed in four areas:

1- Mahmoudia district center reaches (176.5) km^2.

2- Al-Rasheed sub-district, it is 367 km2.

3- Yusufiya sub-district, which is (423.7) km^2.

4- Latifiya (443, 5) km^2.

It constitutes an area of 30.1% of the area of Baghdad Governorate, which is 4555 km^2. The district of Mahmoudia is located at an altitude of 30 meters above sea level, while the city of Baghdad is located at an altitude of 32 meters above sea level (Alzubaidy 2011).

Water sampling and collection: Fifty (50) groundwater samples were collected from five wells have depth in range of 7m-18m (Table 1).The sampling sites were determined by using a GPS device (Fig. 1) during two seasons autumn and winter (November 2020 and January 2021). Each well was pumped for least 10 min, prior to sampling and rinsed well with sample water two or three times before being filled to the homogenate (Adimalla et al 2018). Water samples were collected by using 1.5 liter capacity polyethylene bottles for physical and chemical analysis and drops of sulfuric acid (H_2SO_4) were added to the groundwater samples to preserve their content of heavy elements for the longest storage period possible.

Water quality index (WQI): It is a single value expression that numerically summarizes multiple parameters. In this study, 11 physical and chemical variables were considered such as (pH, Turb, TDS, Cl^-, Na^+, T.H, $SO_4^=$, NO_3^-, Fe, Mg^{+2}, Ca^{+2}). And is effective tool to estimate the total groundwater quality for drinking purposes. Water quality index (Adimalla 2018 and Al-Omran et al 2018) was calculated in several stages.

Calculation relative weights (WI):

$$Wi = \frac{Wi}{\sum_{i=1}^{n} wi} \quad (1)$$

Where: Wi = the relative weight of each parameter, wi = the weight of each parameter, n = the number of parameters Quality rating scale (Qi) by using the equations:

$$Qi = \frac{Ci}{Si} \times 100 \quad (2)$$

where: Qi = the quality rating of each parameter, Ci = the concentration of each parameter in groundwater samples, Si = the Iraqi standards for drinking (IQS 2009).

Calculate water quality sub – index (SIi) of the parameter by using the equations:

$$SIi = Wi \times Qi \quad (3)$$

Finely the WQI is determined by using the equations:

$$WQI = \sum SIi \quad (4)$$

Table 1. Test methods from literature

Parameters	Method
Temperature (T)	Using a mercury thermometer
pH	Using the portable meter (model WTW Inolab 720)
Electrical conductivity (EC)	Using the portable meter (model WTW Inolab 720)
Total dissolved solids (TDS)	Using the portable meter (model WTW Inolab 720)
Turbidity (TUR.)	Using turbidity meter (Lovibond)
Total suspended solids (TSS)	Determined by filtration and afterward dried at (103–105) °C (Rahmanian et al., 2015)
Total hardness (as CaCO3)	EDTA titration method
Calcium (Ca^{2+})	EDTA titration method
Magnesium (Mg^{+2})	Measured according to the formula (Fahdawe 2020) :Mg^{+2} mg/l= [Total Hardness – Calcium Hardness] x 0.244
Na^+, k^+, Fe^{+2}, Cl^-	Measured by using ion selective electrode (inoLab pH/ION 7320)
Dissolved Oxygen (DO)	Winkler method (Singh, Jha and Jadoun, 2012) , chemical oxygen demand (COD) was determined by using colorimetric method (DR5000 HacH) (Popoola et al 2019)
Bicarbonates (HCO_3)	Sulfuric acid titration method
Sulphate ($SO4_2^=$) , Nitrate (NO_3^-) and Phosphates ($PO_4^=$)	Using UV–visible spectrophotometer(Shimadzu UV-1700) (Adimalla and Venkatayogi 2018)
Heavy metals such as Cd, Pb, Cr, Ni	Using Flame Atomic Absorption Spectrometer (AFP 100) according the standard methods (APHA 1999) (APHA 1999)

RESULTS AND DISCUSSION

Each of the samples collected from the study area was analysed (Table 7 and 8).

Temperature: The range of temperature of groundwater samples was between 19-22 °C with mean 20.6°C in autumn season. And in the winter season between 18-21°C with average of 19°C. The groundwater temperature depends on the depth of its bearing layer, the type of rock, and the source and origin of the water (Jalil 2016).

Table 2. Location of well

Well	Depth of well	Coordinates	
		Latitude	Longitude
W1	16	33.044	44.365
W2	9	33.044	44.365
W3	7	33.044	44.365
W4	16	33.044	44.365
W5	22	33.044	44.365

Table 3. Weight values (WI) adopted from the literature

Soleimani et al 2018	3	—	5	5	4	—	5	—	—	3	3
Suneetha et al 2015	2.7	2.9	4.6	4.5	2.5	2	3.3	3.1	—	2	2
Abdul Hameed M Jawad et al 2010	2.1	2.4	—	—	1	1.1	—	2.2	—	—	—
Muhammad and Islami 2020	4	—	5	—	—	—	—	—	—	—	—
Suvarna et al 2020	3	—	2	3	3	2	5	5	—	3	3
Tiwari et al 2014	4	—	5	5	—	2	5	5	—	3	3
Fathi et al 2016	3	2.4	4	—	—	1.1	—	2.2	—	—	—
Turan Kocer.(Koçer and Sevgili 2014)	1	2	2	1	—	—	2	2	—	1	1
Ramakrishnaiah (Ramakrishnaiah, Sadashivaiah and Ranganna 2009)	4	—	4	3	—	2	4	5	4	2	2
Kumar.(Kumar and Sharma 2019)	3	—	2.3	4	—	3	1	1.3	—	2.3	2.7
Howladar.(Howladar, Al Numanbakth and Faruque 2018)	4	—	5	5	—	3	5	5	—	3	3
Al-Omran.(Al-Omran et al 2018)	3	—	4	3	2	—	3	5	—	2	2
Abrahao.(Abrahão et al 2007)	1	4	2	1	1	1	2	2	—	1	1
DEEPIKA.(Deepika, Ramakrishnaiah and Naganna 2020)	4	2	4	3	—	2	4	5	4	2	2
Nabizadeh.(Nabizadeh et al 2013)	4	4	4	3	3	—	4	5	4	2	2
Yadav.(Yadav et al 2018)	4	4	5	5	—	2	5	5	4	3	3
Zaoui.(Zaoui 2020)	2	—	—	3	2	2	4	5	—	2	2
RadFard.(RadFard et al 2019)	3	—	5	3	3	3	4	5	—	2	3
Al-Paruany. (Al-Paruany 2018)	4	—	4	3	2	—	4	5	—	2	2
Khayyun. (Khayyun, Mseer and Ismaeel 2017)	4	—	3	3	3	—	4	5	—	2	2
Alhadithi. (Alhadithi 2018)	2	—	4	3	2	—	4	5	—	2	2
Ibrahim. (Ibrahim 2019)	4	3	4	5	3	3	5	5	3	—	—
∑ wi=	3.13	2.97	3.9	3.45	2.42	2.08	3.9	4.14	3.8	2.18	2.26

Table 4. Relative weight of parameters

Parameter	Water quality standard (IQS-417(2009) guideline)	Weight (wi)	Relative weights (Wi)
pH	6.5-8.5	3.13	0.09144
Turb. (NTU)	5	2.97	0.086766
TDS (mg/L)	1000	3.9	0.113935
Cl^- (mg/L)	350	3.45	0.100789
Na^+ (mg/L)	200	2.42	0.070698
T.H (mg/L)	500	2.08	0.060765
$SO_4^=$ (mg/L)	400	3.9	0.113935
NO_3^- (mg/L)	50	4.14	0.120947
Fe (mg/L)	0.3	3.8	0.111014
Mg^{+2} (mg/L)	100	2.18	0.063687
Ca^{+2} (mg/L)	150	2.26	0.066024
		$\sum w_i$= 34.23	

pH: The pH value of groundwater samples ranged between 6.4-7.5 with mean 7.16 in autumn season. And between 6.29-8.19 with mean 7.318 in the winter season .It is classified as slightly alkaline to very little acidic water (Fayydh et al 2020). The pH was within the permissible limits of the Iraqi standard and the WHO.

Turbidity: The range of turbidity of groundwater samples was between 1.6 - 6.4 NTU with mean 2.837 NTU in autumn season and between 1.82-5.2 NTU with mean 2.922 NTU in the winter season. The turbidity was within the permissible limits of the Iraqi standard.

Electrical conductivity (EC): The range of EC of groundwater samples between 1064 - 6080 µS/cm with mean 2963.9 µS/cm in autumn season and between 1714-8890 µS/cm with mean 4092.5 µS/cm in the winter season. The increase in EC is due to presence of inorganic material in groundwater and also because high dissolution of cations and anions in the study area (Fayydh et al 2020). EC was not within the permissible limits of the Iraqi and the WHO standard.

Total dissolved solids (TDS): The inorganic substances and small quantities of organic substance which are existing in water (Rahmanian et al 2015).It does not include suspended and colloidal substances and gases (Abdullah

Table 5. Status of water quality (WQI) Indian (Suneetha, Sundar and Ravindhranath 2015)

Range	Type of groundwater
< 50	Excellent water
50–99.99	Good water
100–199.99	Poor Water
200–299.99	Very poor water
≥ 300	Unsuitable for drinking/Irrigation purpose

Table 6. Water quality assessment of the five wells for autumn season

Parameter	Ci	Si	Qi=(Ci/Si)*100	Wi	SI=Wi*Qi
pH	7.16	7.5	95.466	0.091	8.729
Turb. (NTU)	2.837	5	56.752	0.086	4.924
TDS (mg/L)	1426.6	1000	142.66	0.113	16.253
Cl^- (mg/L)	366.56	350	104.731	0.100	10.555
Na^+ (mg/L)	162.8	200	81.4	0.070	5.754
T.H (mg/L)	1456.2	500	291.24	0.060	17.697
$SO_4^=$ (mg/L)	518.4	400	129.6	0.113	14.765
NO_3^- (mg/L)	2.699	50	5.398	0.120	0.652
Fe (mg/L)	0.229	0.3	76.333	0.111	8.474
Mg^{+2} (mg/L)	114.12	100	114.12	0.063	7.267
Ca^{+2} (mg/L)	397.28	150	264.853	0.066	17.486

WQI=∑SI = 112.56292 Poor Water

Table 7. Water quality assessment of the five wells for autumn season

Parameter	Ci	Si	Qi=(Ci/Si)*100	Wi	SI=Wi*Qi
pH	7.318	7.5	97.573	0.091	8.922
Turb. (NTU)	2.922	5	58.44	0.086	5.070
TDS (mg/L)	2026	1000	202.6	0.113	23.083
Cl^- (mg/L)	645	350	184.285	0.100	18.573
Na^+ (mg/L)	368.2	200	184.1	0.070	13.015
T.H (mg/L)	1438.6	500	287.72	0.060	17.483
$SO_4^=$ (mg/L)	413.16	400	103.29	0.113	11.768
NO_3^- (mg/L)	3.4196	50	6.839	0.120	0.827
Fe (mg/L)	0.0306	0.3	10.2	0.111	1.132
Mg^{+2} (mg/L)	80.64	100	80.64	0.063	5.135
Ca^{+2} (mg/L)	438.76	150	292.506	0.066	19.312

WQI=∑SI = 124.324 Poor Water

Table 8. Mean and coefficient variation of physio-chemical parameters for autumn season

Physio-chemical parameters	W1	W2	W3	W4	W5
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
PH	7.376 ± 0.755	6.67 ± 3.418	7.228 ± 2.129	7.436 ± 0.095	7.09 ± 2.018
Turb. (NTU)	2.264 ± 10.234	2.458 ± 8.413	1.714 ± 22.450	5.96 ± ٥.513	1.792 ± 9.994
Ec(µS/cm)	1594.4 ± 1.066	1817.2 ± 0.472	1068 ± 0.421	4275.2 ± 2.538	6065 ± 0.210
TDS(mg/L)	872.4 ± 0.919	972.6±0.388	636 ± 0.314	1923.8 ± 2.090	2729 ± 0.214
TSS(mg/L)	3.98 ± 3.726	11.6 ± 7.081	34.8 ± 4.721	32.6 ± 4.651	16.4 ± 10.203
Cl^-(mg/L)	252.2 ± 1.804	259.2 ± 1.261	103 ± 2.276	576 ± 1.177	642.4 ± 0.975
Na^+(mg/L)	82.4 ± 5.794	83 ± 4.507	64.8 ± 4.226	193.8 ± 1.838	390 ± 1.720
K^+(mg/L)	9.86 ± 3.092	8.72 ± 6.657	5.06 ± 5.098	21.34 ± 3.290	24.98 ±4.532
$PO_4^=$(mg/L)	0.01 ± 7	0.02 ± 5	0.0302 ± 4.911	0.0١٠٤ ± 5.267	0.0105 ± 9.524
DO(mg/L)	3.99 ± 4.884	6.34 ± 4.111	5.79 ± 4.333	4.92 ± 2.922	4.444 ± 0.002
COD(mg/L)	15.8 ± 14.432	12.8 ± 11.587	13 ± 5.439	47 ± 10.819	50.4 ± 16.483
T.H(mg/L)	904.8± 0.464	939 ± 0.361	525.2 ± 0.917	2011.2 ± 0.401	2901.2 ± 0.231
$SO_4^=$(mg/L)	460.8 ± 0.846	481± 0.906	430.2 ± 8.847	576.6 ± 0.761	643.4 ± 0.155
NO_3^-(mg/L)	1.542 ± 13.813	1.09 ± 9.146	0.411 ± 4.063	5.32 ± 5.255	5.132 ± 5.019
Fe^{+2}(mg/L)	0.094 ± 12.127	0.208 ± 10.384	0.257 ± 150.739	0.294 ± 10.340	0.292 ± 4.417
Mg^{+2}(mg/L)	67.2 ± 2.661	74 ±3.575	54.2 ± 3.999	155.8 ± 0.951	219.4 ± 0.945
Ca^{+2}(mg/L)	244 ± 1.533	269 ± 1.486	120.4 ± 1.389	551.4 ± 0.648	801.6 ± 0.463
HCO_3(mg/L)	48 ± 2.083	54 ± 1.309	35 ± 2.857	105 ± 0.673	151 ± 0.662

Table 9. Mean and coefficient variation of physio-chemical parameters for winter season

Physio-Chemical Parameters	W1	W2	W3	W4	W5
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
pH	7.396 ± 1.082	7.39 ± 2.043	6.596 ± 3.123	7.51 ± 1.678	7.698 ± 6.196
Turb. (NTU)	3.78 ± 23.015	2.518 ± 12.113	2.206 ± 16.364	1.964 ± 6.365	4.142 ± 5.577
Ec(µS/cm)	2784 ± 24.756	3091.8 ± 3.047	1738.6 ± 1.882	6763.8 ± 0.246	8840.6 ± 0.560
TDS(mg/L)	1261.8 ± 0.402	1452.4 ± 0.752	802 ± 1.132	2758.8 ± 1.478	3857.2 ± 0.702
TSS(mg/L)	20.4 ± 2.681	19.2 ± 4.354	20.2 ± 7.342	4.2 ± 19.905	4.4 ± 12.432
Cl^-(mg/L)	326.4 ± 0.349	386 ± 0.366	185.6 ± 0.817	927 ± 0.202	1400 ± 0.375
Na^+(mg/L)	187.2 ± 0.696	220.2 ± 0.380	104.2 ± 1.423	529.8 ± 0.246	799.6 ± 0.371
K^+(mg/L)	14.8 ± 5.649	16.2 ± 5.160	8 ± 8.838	39.6 ± 2.879	60.2 ± 1.389
$PO_4^=$(mg/L)	0.05 ± 6	0.061 ± 11.475	0.059 ± 11.864	0.102 ± 3.922	0.152 ± 5.263
DO(mg/L)	4.94 ± 2.308	1.698 ± 2.179	3.18 ± 2.610	3.584 ± 2.148	5.42 ± 1.531
COD(mg/L)	0.0106 ± 7.547	47.5 ± 6.981	0.0292 ± 3.425	8.8 ± 0.795	16.02 ± 0.518
T.H(mg/L)	794 ± 1.211	947 ± 1.157	550 ± 1.437	2001 ± 0.758	2901 ± 0.255
$SO_4^=$(mg/L)	361 ± 0.919	370.8 ± 0.584	306.4 ± 0.995	498.8 ± 0.519	528.8 ± 0.410
NO_3^-(mg/L)	1.378 ± 2.975	0.598 ± 2.676	1.902 ± 0.736	6.14 ± 1.857	7.08 ± 1.271
Fe^{+2}(mg/L)	0.022 ± 36.364	0.036 ± 41.667	0.017 ± 23.529	0.041 ± 12.195	0.037 ± 21.622
Mg^{+2}(mg/L)	52 ± 3.596	59.8 ± 2.480	42 ± 2.914	91.4 ± 2.268	158 ± 1.343
Ca^{+2}(mg/L)	230.8 ± 0.833	280.2 ± 1.022	130.6 ± 1.921	650.4 ± 0.279	901.8 ± 0.213
HCO_3(mg/L)	51.6 ± 2.600	30.6 ± 10.985	29.8 ± 4.375	30.4 ± 7.573	31.4 ± 4.830

and Majid 2015). The range of TDS of groundwater samples between 634 - 2736 mg/L with mean 1426.7 mg/L in autumn season and between 792-3890 mg/L with mean 2026.4 mg/L in the winter season. TDS was not within the permissible limits of the Iraqi standard and the WHO. The reason for the increase in TDS concentrations is indicates affected by soil washing operations as a result of irrigation and domestic and industrial pollutants as dissolved ions are released through the wastewater into the groundwater (AL-Fatlawy 2013).

Total suspended solids (TSS): The range of TSS of groundwater samples between 3.8-37 mg/L with mean 19.87 mg/L in autumn season and between 4-22 mg/L with mean 13.68 mg/L in the winter season. All measured of TSS was within the permissible limits of the WHO (Reda 2016).

Chloride (Cl^-): The range of Cl^- of groundwater samples between 101- 652 mg/L with mean 366.5 mg/L in autumn season and between 184-1405 mg/L with mean 645 mg/L in the winter season. Most of the wells are within the permissible limits of the Iraqi standard and WHO with the exception of well No. 4 and 5 for both seasons are out of the permissible limit due to the presence of minerals and rocks that lead to the dissolve of chloride (Abdullah and Majid 2015). also because of pollution resulting from leakage of sewage the high concentration of chloride gives water noticeable or salty test which is objectionable to numerous individuals (Channo 2012).

Sodium (Na^+): It is one of the main cation ions in the water

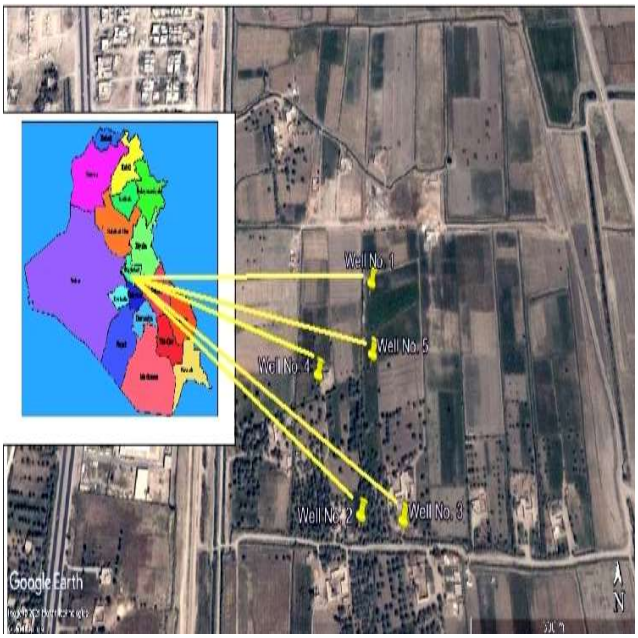

Fig. 1. Location map of the study area

Table 10. Limits of the permissible concentrations of the World Health Organization, Iraqi standard, Indian Standard and Drinking Water Contaminants US EPA

Parameter	IQS 2009 (Iraqi standard of drinking water 2009)	WHO (WHO 2011)	Indian Standard (IS, 1992, Patil, Sawant and Deshmukh 2012, Prasanth et al 2012)	EPA guidelines (EPA 2010, Patil, Sawant and Deshmukh 2012)
pH	6.5-8.5	6.5-8.5	6.5-9.5	6.5-9.5
Turb. (NTU)	5	5	—	—
Ec(µS/cm)	—	—	—	2500
TDS(mg/L)	1000	1000	500	—
TSS(mg/L)	—	≤ 30	—	—
Cl^-(mg/L)	350	250	250	250
Na^+(mg/L)	200	200	180	200
K^+(mg/L)	—	10	—	—
$PO_4^{=}$(mg/L)	—	—	—	—
DO(mg/L)	—	—	—	—
COD(mg/L)	—	—	—	40
T.H(mg/L)	500	100	300	< 200
$SO_4^{=}$(mg/L)	400	250	200	250
NO_3^-(mg/L)	50	50	45	50
Fe(mg/L)	0.3	0.3	—	—
Mg^{+2}(mg/L)	100	50	30	—
Ca^{+2}(mg/L)	150	75	75	—
HCO_3(mg/L)	—	—	—	—

Heavy Metals (Pb, Cd, Cr, Ni): The results of the samples showed that these elements are the were less than 0.001 mg/L.

(Jalil, 2016).Sodium concentration ranged of groundwater samples between 64 – 399 mg/L with mean 162.8 mg/L in autumn season and between 102-803 mg/L with mean 368.2 mg/L in the winter season. The wells are within the permissible limits of the Iraqi standard and WHO except for well No. 5 for autumn season are out of the permissible limit and also well No. 2 ,4 and 5 for winter season. The high value of Na^+ is due to many industrial wastes and domestic wastewater (Adejumo et al 2018). Sodium salts are not toxic to humans, but a high concentration affects irrigation water when the soluble sodium content in the water is higher than calcium and magnesium (Channo 2012).

Potassium (K^+): The range value of K^+ of groundwater samples between 4.7 - 26.2 mg/L with mean 13.99 mg/L in autumn season and between 7- 61 mg/L with mean 27.76 mg/L in the winter season. The results showed potassium was not within the permissible limits of the WHO, except well No.3 for autumn season and well No.1, 2 and 3 for the winter season are out of the permissible limit. Potassium enters the certain structures of clay- like minerals through the process of weathering due to its low mobility , one of its sources is leachate from fertilizers .It does not have harmful effects from the health point of view, but a high concentrations may have a laxative effect (Adejumo et al 2018). The high concentration of potassium ion is due to the human activities (Ghalib 2017).

Phosphate (PO_4^{-3}): It is considered one of the important factors for the growth of living organisms despite its importance and is less present in water due to its strong tendency to adsorb on minerals and organic compounds in the soil. The range value of PO_4^{-3} of groundwater samples was between 0.009 - 0.032 mg/L with mean 0.016 mg/L in autumn season and between 0.045- 0.16 mg/L with mean 0.057 mg/L in the winter season. The high concentration of phosphate ion is due to contamination with fertilizers (Adejumo et al 2018).

Dissolved Oxygen (DO): The range value of DO of groundwater was between 3.7 - 6.7 mg/L with mean 5.096 mg/L in autumn season and between 1.65- 5.5 mg/L with mean 3.764 mg/L in the winter season. The dissolved oxygen index is little importance in groundwater, while it is important in seas, rivers and lakes for the continued survival of aquatic organisms and fish (Fadel et al 2012).

Chemical oxygen demands (COD): Measures oxygen necessity for organic substance chemical oxidation to happen by using a strong oxidizing agent (Popoola et al 2019). The range value of COD of groundwater samples between 11 - 53 mg/L with mean 27.8 mg/L in autumn season and between 0.01- 52 mg/L with mean 14.47 mg/L in the winter season. The high concentration of COD indicates the many presence of chemically oxidizable substances of which the majority are no biodegradable (Al-Paruany 2018 and RadFard et al 2019) and also because of volume of solid waste transported into the ground by means of filtering, establishing to water contamination by increasing the organic content (Channo 2012 and Ghalib 2017)

Sulfates (SO_4^{-2}): The range value of SO_4^{-2} of groundwater samples between 457 - 645 mg/L with mean 518.4 mg/L in autumn season and between 302- 531 mg/L with mean 404.1 mg/L in the winter season. The SO_4^{-2} was not within the permissible limits of the Iraqi standard and the WHO according table-9.The high concentration of $SO4^{-2}$ due to oxidative weathering of pyrite and the dissolution of the gypsum (Tiwari et al 2016) or on the other hand of human activity and use of fertilizers in the study area (Al-Azawi 2009).

Nitrate (NO_3^-): It is the common form of nitrogen in water (Jalil 2016). The range value of NO_3^- of groundwater samples between 0.39 – 5.6 mg/L with mean 2.69 mg/L in autumn season and between 0.57- 7.2 mg/L with mean 3.41 mg/L in the winter season. All measured values were below the Iraqi standard and the WHO. It originates from several sources including pesticides, animal fertilizers, plant decomposition and wastewater. Ammonia is oxidized by ammonia-oxidizing bacteria to nitrate (Stadler et al 2008).The deficiency of nitrate in the study area due to the absence of this type of bacteria.

Iron (Fe^{+2}): Iron makes up about 5% of the earth's crust and is found as ores in soil, rocks and minerals (Popoola et al 2019). The range value of Fe^{+2} of groundwater samples between 0.08 - 0.33 mg/L with mean 0.229 mg/L in autumn season and between 0.01- 0.05 mg/L with mean 0.03 mg/L in the winter season. The values were within the permissible limits of the Iraqi standard and the WHO. The high concentration of Fe in drinking water causes some diseases such as liver-damage disease, diabetes mellitus, arteriosclerosis et al 2019.

Total Hardness (TH): The TH values in groundwater samples between 520 - 2910 mg/L with mean 1401.2 mg/L in autumn season and between 540- 2910 mg/L with mean 1438.6 mg/L in the winter season. The TH was not within the permissible limits of the Iraqi standard and the WHO. The groundwater in the study area is classified as very hardness. The high values of hardness in ground water in the study area is due to the limestone nature that characterizes Iraqi soils and also the high concentrations of total dissolved solid and sulfates increase the hardness of water (Jalil 2016).

Magnesium (Mg^{+2}): The range value of Mg^{+2} of groundwater samples between 51-222 mg/L with mean 114.12 mg/L in autumn season and between 41-160 mg/L with mean 80.64 mg/L in the winter season. The wells are within the

permissible limits of the Iraqi standard and WHO except for well No.4 and 5 for autumn season are out of the permissible limit according table 9 and well No. 5 for winter season. Most common sources of Mg^{+2} in groundwater are dolomites in the rocks (Saraswat et al 2019). The differences small in magnesium concentration is due to the limited solubility of dolomite rocks in the study area et al 2020.

Calcium (Ca^{+2}): The range value of Ca^{+2} of groundwater samples between 118-807 mg/L with mean 397.28 mg/L in autumn season and between 127-905 mg/L with mean 438.76 mg/L in the winter season and are within the permissible limits of the Iraqi standard and WHO with the exception of well No. 3 for both seasons are out of the permissible limit according table 9. The main source of calcium ion is the solubility of some minerals in sedimentary rocks and also found in igneous rocks the high concentration of calcium due to the dissolution of the rocks that contain it and the incorrect use of fertilizers and pesticides (Abdullah and Majid 2015) and also the ion exchange between sodium and calcium (AL-Fatlawy 2013).

Bicarbonates (HCO_3): The range of HCO_3 of groundwater was between 47-152 mg/L with mean 78.6 mg/L in autumn season and between 27-53 mg/L with mean 34.76 mg/L in the winter season. The sources of alkalinity in water gave an unwanted taste to water. The main source of bicarbonate in groundwater is leakage water containing dissolved carbon dioxide (Channo 2012). The pH is less than 8.2 most of the carbonates in the water convert to bicarbonate as is the case in the wells of the study area where pH value was less than 8.2.

CONCLUSION

The current study concluded some of the rates for the physical and chemical variables of groundwater samples exceeded the permissible limits of the Iraqi standard and WHO and according the water quality index (WQI) the average was poor water for both seasons.

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Development of Resistance in Susceptible *Staphylococcus aureus* and *Escherichia coli* Exposed to sub-MIC of Ampicillin

Athraa Noori Humadi and Ahmed N. Abedsalih

Department of Physiology, Biochemistry and Pharmacology
College of Veterinary Medicine, University of Baghdad, Iraq
**E-mail: azraa.nouri1206a@covm.uobaghdad.edu.iq*

Abstract: The aim of this study was to development of resistance in susceptible isolates of *Staphylococcus aureus* and *Escherichia coli* exposed to sub-minimum inhibitory concentration (MIC) of ampicillin *in vitro.* The susceptibility of these isolates was confirmed by testing its sensitivity to different antibacterial including ampicillin. MIC of ampicillin was determined, and then the tested bacteria treated with 0.5, 0.25 and 0.125 MIC continuously for 21 days. The study revealed that the *Staphylococcus aureus* was susceptible to 18 out of 20 antibacterial discs including ampicillin (90%), while *E. coli* was susceptible to 17 out of 20 antibacterial including ampicillin (85%). MIC determination showed that the values are within the range specified by the Clinical and. Laboratory Standards Institute (CLSI), which were 3±0.7 µg/ml for *S. aureus* and 4 ± 0.5 µg/ml for *E. coli*. The study s showed that the MIC of the ampicillin increased to 4 fold the breakpoint value at the end of the exposure (21 days), which became 24 µg/ml and 32 µg/ml for the *S. aureus* and *E. coli* respectively. This indicates exposing of the susceptible *S. aureus* and *E. coli* to sub-MIC led to emerging of resistance in these isolates.

Keywords: Resistance, Sub-MIC, *Staphylococcus aureus, Escherichia coli*

It is critical to quantify the active ingredient in antibiotic preparations in order to achieve pharmacological equivalence between generics and innovative medicines, because subtherapeutic concentrations lead to bacterial resistance. In addition, antibiotic treatment options for difficult-to-treat multidrug-resistant bacterial infections are limited (Holmes et al 2016). The major reasons in lack of resistance mechanisms, new drug development, and regulatory criteria that is difficult to meet (Zaman et al 2017). The results of the antibiotic sensitivity test were shown by the disc diffusion method the *E. coli* showed a high sensitivity to ciprofloxacin (Alhadrawi et al 2023). Adulteration of traditional drugs and a variety of chemical substances referred to as newer psychoactive substances are among the concerns and rising difficulties associated with drug use and trafficking (NPS), (Busardò et al 2016). Some countries have achieved significant progress in medication chemical characterization, allowing them to identify a substantial number of adulterants. Therefore, further research is needed into the impact of adulterants on drug users' health as well as their role in drug-related morbidity and mortality. A mild difference in the concentration of active ingredient in antibiotic formulations may have an effect on real efficacy. This critical quantifies the active pharmaceutical ingredient (API) in antibiotic preparations (Dafale et al 2015). The quantification of active ingredient is critical for drug regulatory agencies around the world (Marzouk et al 2023). There is need to quantify the active ingredient in antibiotic preparations in order to achieve pharmacological equivalence between generics and innovative medicines, because sub therapeutic concentrations contribute to bacterial resistance. This study aimed to investigate the effect of the sub-MIC of ampicillin on resistance development in susceptible *S. aureus* and *E. coli.*

MATERIAL AND METHODS

Confirming the susceptibility of microorganisms: *S. aureus* and *E. coli* which obtained from department of microbiology university of Baghdad were tested to confirm their sensitivity by disc diffusion method to different antibacterial including Ampicillin (AMP), Azithromycin (ATM), Cloxacillin (CX), Ceftriaxone (CRO), Amikacin (AK), Amoxicillin Clavulanic acid (AUG), Nirtofurantoin (NI), Chloramphenicol (C), Levofloxacin (LEV), Moxifloxacin (MFX), Ciprofloxacin (CIP), Enrofloxacin (ENF), Oxacillin (OX), Vancomycin (VA), Methicillin (ME), Trimethoprim and sulfamethoxazole (TS), Gentamicin (GM), Imipenem (IMP), Tetracycline (TE) and Trimethoprim(TM).

Determination of MIC: The broth macro-dilution method was used to determine the minimum inhibitory concentration (MIC) of ampicillin. The bacterium was inoculated onto 24 hour MH broth cultures, and the suspensions were adjusted to a 0.5 McFarland standard turbidity (about 1.5x108 CFU/ml). Then, in all tubes of the cultures, a two-fold serial

dilution of ampicillin ranges from (0.187–6.0 g/mL) for *S. aureus* and (0.25–8.0 g/mL) for *Escherichia coli* was applied, Incubate for 24 hours at 37 degrees Celsius. The tubes were checked visually for turbidity at the end of the incubation period. The presence of cloudiness suggested that the antimicrobial agent concentration in the medium had not been enough to stop bacterial growth. The minimum inhibitory concentration (MIC) is determined by using the lowest antibacterial concentration possible.

Development of resistance: After determination of the MIC of the ampicillin against susceptible *Staphylococcus* aureus and *Escherichia coli*, these isolates exposed to a sub-MIC (0.0.25 and 0.125 MIC) of ampicillin and incubated overnight. The next day the MIC (which may remain the same or increase) is determined and the sub-MIC concentration culture from the latest passage is used to inoculate a new series of diluted antibacterial agents. The process continues for up to 21 days (Früh et al 2022). The bacterial growth was measured by spectrophotometer at 620nm.

RESULTS AND DISCUSSION

Staphylococcus aureus: The susceptibility of isolate of the *Staphylococcus aureus* was estimated by disc diffusion method showed that the tested bacterium was susceptible to 18 out of 20 antibacterial discs including ampicillin (Table 1, Fig. 1).

Escherichia coli: Results of the confirming the susceptible isolate of the *Escherichia coli* by disc diffusion method showed that the tested bacterium was susceptible to 17 out of 20 antibacterial including ampicillin (85%) (Table 2, Fig. 2).

Determination of MIC: Results of determination of the MIC of ampicillin against the tested bacteria (*S. aureus* and *E. coli*) showed that the values are within the range specified by the CLSI, which were 3±0.7 µg/ml for *S. aureus* and 4±0.5 µg/ml for *E. coli*.

Development of resistance in *S. aureus* and *E. coli* against Ampicillin: The results showed that the MIC of the ampicillin increased to 4 fold the breakpoint value at the end of the exposure (after 21 days), which became 24 µg/ml and 32 µg/ml for the *S. aureus* and *E. coli* respectively. Measurement of the bacterial density by the spectrophotometer at different periods extended from 0-21 days showed there was significant gradual increasing in the absorbance as compared with those of the day zero, which mean increase the density, or in other words increasing in bacterial count of *Staphylococcus aureus* and *Escherichia coli*, and there was inverse relationship between concentration and absorbance, where the higher absorbance was recorded at lower concentration, while the lowest absorbance was recorded at higher concentration in both bacteria. In case of *S. aureus,* after 4 days of exposure, lowest absorbance 0.54±0.006 was observed at the higher concentration (1.5µg/ml) with significant difference as compared with the absorbance of other two concentrations 0.75 µg/ml and 0.375 µg/ml, which was 0.63±0.007 and 0.70±0.02, respectively. After 8 days of exposure the higher absorbance 1.29±0.01 observed also at the lower concentration with significant difference as compared with the other absorbance. After 14 and 21 days there was no significant difference between all observations and the values of the absorbance became very close to each other at day 21 (Table 3). In concern to *E. coli*, after 4 and 8 days of exposure, the difference was significant between absorbance of all concentrations for each period, which were 0.33±0.003, 0.46±0.001 and 0.52±0.002 after 4 days and 0.63±0.003, 0.86±0.003 and 1.11±0.001 after 8 days. After 14 days, the values of the absorbance became very close to each other and there was no any significant difference between all observations. After 21 days the higher absorbance 1.96±0.06was noticed at lowest concentration with significant difference when compared with the other concentrations (Table 4).

Increase absorbance of the tested suspensions of the both isolates *S.aureus* and *E. coli* that exposed continuously

Table 1. Zone of inhibition (mm) of the susceptible *S. aureus* by different antibacterials

Antibacterial	AM	CIP	AUG	VA	ME	ATH	IMI	OX	AK	MFX
Inhibition zone (mm)	20.8	33	23.2	20.4	23.2	21.8	37.6	23.2	29	28
Antibacterial	TM	CX	C	TE	TS	GM	CRO	NI	LEV	ENF
Inhibition zone (mm)	20.8	8.8	24.2	29	30.6	29.4	36.2	11.2	27	26.8

Table 2. Zone of inhibition (mm) of the susceptible *E. coli* by different antibacterials

Antibacterial	AM	CIP	E	VA	C	ATH	IMI	CX	AK	MFX
Inhibition zone (mm)	22.4	33.8	8	23.8	21	23	38.8	24.8	24.6	28.2
Antibacterial	TM	ME	OX	TE	TS	GM	CRO	NI	LEV	AUG
Inhibition zone (mm)	20.8	22	13.8	21.2	26.6	32.4	23.4	20	26.8	12.6

Fig. 1a. Sensitivity of susceptible *S. aureus* to different antibacterials

for 21 days to sub MIC of ampicillin is might be due to increase the numbers of these bacteria, based on optical density (OD) spectroscopy, an OD measurement characterizes the amount of light that is lost due to scattering and absorption at a single wavelength (Zhang et al 2022). The OD correlates directly with the cell concentration (Shao et al 2016). The results also showed that the MIC of the ampicillin increased to 4 fold the breakpoint values at the end of the exposure (after 21 days). So increase the bacterial count as well as reduced activity of the antibacterial agents at the MIC, means the bacteria developed certain defense mechanism made it not affected by this agents, in other words it became resistant as a result of the exposure to sub inhibitory concentrations. Bacteria are often exposed to lower concentrations of antibiotics. This occurs in the clinic due to low-dose prophylactic treatment, incorrect dosing, poor patient adherence and use of poor quality or substandard medicines, which often do not have the stated amount of active pharmaceutical ingredient (API) (Kelesidis

Fig. 1c. Sensitivity of susceptible *S. aureus* to different antibacterials

Fig. 1b. Sensitivity of susceptible *S. aureus* to different antibacterials

Table 3. Absorbance of *S. aureus* suspension after exposure to sub-MIC concentrations of ampicillin for 21 days

Drugs	Concentration (µg/mL)	Control	4 day	8 day	14 day	21 day
			A			
	1.5 (50% of MIC)	0.08±0.00Ea	0.54±0.006Dc	1.16±0.03Cb	1.46±0.01Ba	1.98±0.03Aa
	0.75 (25% of MIC)	0.08±0.00Ea	0.63±0.007Db	1.17±0.01Cb	1.52±0.003Ba	2.01±0.04Aa
	0.375 (12.5% of MIC)	0.08±0.00Ea	0.70±0.02Dab	1.29±0.01Ca	1.53±0.02Ba	2.03±0.03Aa
LSD 0.0714						

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)

Fig. 2a. Sensitivity of *E. coli* susceptible isolate to different antibiotic disc

and Falagas 2015, Fisher et al 2018). Many experiments also examined resistance development with passage of bacteria in continually increasing sub-inhibitory concentrations (Gharban et al 2022, Ibraheim et al 2023). The results of a survey of sub-inhibitory concentrations of antibiotics in environmental samples were reported by Chow et al (2021). In this study, the authors collated 40 scientific papers, covering the period from 1999 to 2018, reported measurements of antibiotic levels in diverse environments; it was found that the environmental concentrations of antibiotics in many samples fall into the MSC range and are likely to be influencing bacterial ecology and triggering the selection of antibiotic resistant bacterial cells (Chow et al 2021).

Five main aspects of bacterial virulence can be changed in *S. aureus* exposure to the sub-MIC levels of antibiotics, resulting in deformed bacterial cells to stimulate abnormal host immune responses, abnormally expressed virulence factors to alter disease development, changed bacterial

Fig. 2c. Sensitivity of *E. coli* susceptible isolate to different antibiotic disc

Fig. 2b. Sensitivity of *E. coli* susceptible isolate to different antibiotic disc

Table 4. Absorbance of *E. coli* suspension after exposure to sub MIC concentrations of ampicillin for 21 days

Drugs	Concentration (µg/mL)	Control	4 day	8 day	14 day	21 day
	2 (50% of MIC)	0.08±0.00Ea	0.33±0.003Dc	0.63±0.003Cc	1.53±0.003Ba	1.83±0.003Ab
	1 (25% of MIC)	0.08±0.00Ea	0.46±0.001Db	0.86±0.003Cb	1.55±0.004Ba	1.85±0.01Ab
	0.5 (12.5% of MIC)	0.08±0.00Ea	0.52±0.002Da	1.11±0.001Ca	1.57±0.003Ba	1.96±0.06Aa
LSD 0.0488						

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)

adhesion and invasion abilities to affect colonization and diffusion, altered biofilm formation to potentate material-related infections, and increased SCV formation to achieve persistent infection and recurrence (Chen et al 2021). Results of a study demonstrated that sub-MICs of amoxicillin can stimulate unpredictable changes in commensal bacterial strains which can be a potent source for the propagation of antibiotic resistance in *E. coli* at 1/2 MIC, thereby enhancing its colonization and survival abilities within the gut microsphere (Chadha 2021).

CONCLUSION

Exposure of the susceptible isolate of *Staphylococcus aureus* and *Escherichia coli* to sub inhibitory concentration of ampicillin for a long period led to develop resistance in these isolates against this antibacterial.

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Effect of Storage and Soaking Seeds by Nanomaterial on Vitality and Seedling Vigor of Wheat

A.L. Altayy and Saddam H. Cheyed

Agricultural Research Department, Field Crop Sciences
College of Agriculture Engineering Sciences, University of Baghdad, Iraq
E-mail: afrah3124@yahoo.com

Abstract: A field experiment was carried out in Field Crops Department, College of Agriculture, University of Baghdad for the seasons 2018 and 2019 in order to find out the effect of soaking stored wheat seeds for years 2013, 2015 and 2017 with nano fertilizers (FeNPs 150 mg L^{-1}, MnPNs and ZnPNs 100 mg L^{-1}) for a period of 24 hours, in addition to the two comparison treatments (soaking in distilled water and dry seeds). The treatments of micro-nano fertilizers were significantly superior to the two comparison treatments (soaking in distilled water and dry seeds) for all the studied traits. The treatment of nanoscale iron outperformed the two treatments of manganese and zinc for all the studied traits as well. Seed storage years, 2017 was significantly higher than the 2013 and 2015 storage years for all traits. For all the studied traits, overlap between the treatments, the results varied for the traits, as the iron treatment with the 2017 storage year was significantly superior in giving the highest average for most of the studied traits, while it was not significant in the characteristics of chlorophyll percentage and emergence rate.

Keywords: Wheat, FeNPs, MnNPs, ZnNPs

Among the most important factors for increasing the production of wheat crops is the seeds, as high-quality seeds necessary of high productivity. In successful and commercial agriculture, rapid seed germination and the emergence of healthy seedlings are important factors. In crop production seedling emergence affects the yield of crops. The 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 operations and seed storage (Rajjou et al 2012). During seed storage, the quality of the seeds may remain at the first level of viability or decrease to a level that makes the seeds unacceptable for the purpose of cultivation and this is related to several factors related to the storage conditions, including the length and age of storage, temperature and relative humidity during storage. The poor storage will lead to a loss in quantity and quality (Zahid and Saira 2019). 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 materials stimulating germination, especially soaking with nutrients, and the development condition suitable the use of nanoparticles and fertilizers because of this technology of many benefits (Lu et al 2015, Aljuthery and Saadoun 2018). Fertilizers, especially iron, manganese and zinc, play an important role in the growth and productivity of crops. Several studies have indicated that iron, when in the form of nanoparticles, leads to improved germination and although there is much research on the positive effects that nanomaterials have on germination, the underlying mechanisms The reason behind how to stimulate the seeds is still unclear, but some studies have shown that the nanomaterials have the ability to penetrate the seed layer and enhance the absorption and use capacity of water, which stimulates the enzyme system and ultimately improves germination and seedling growth (Banerjee and Kole 2016). The objectives of this study to determine the role of micro-nano fertilizers in improving the characteristics of stored wheat seeds.

MATERIAL AND METHODS

A field experiment was carried out in the field of the Field Crops Department, College of Agriculture, University of Baghdad during 2018-2019 and 2019-2020. The agricultural operations of plowing, smoothing and leveling the soil were carried out. The area of the experimental unit was 2×2 m^2. The experiment was in complete randomized block with four replications, the first factor included three types of seeds stored for different periods, in 2013, 2015 and 2017, and the second factor was concentration of fertilizer (nanoscale iron concentration was at150 mg L^{-1}, manganese and zinc nanoparticles at 100 mg L^{-1}). This is in addition to the two comparison treatments dry seeds and seeds soaked in distilled water. The seeds of wheat, class (Research 10) were obtained from the Agricultural Research Department, were

planted with a distance of 20 cm. Urea fertilizer was added at a rate of 200 kg ha^{-1} in four equal batches at planting, three whole leaves appear, second node appears on the stem and in the lining stage. The phosphate fertilizer was added at a rate of 100 kg in the form of triple superphosphate at a concentration of 45% P_2O_5 when preparing the land for cultivation.

Studied Traits

Emergence) ES): Emergence velocity represents the sum of the number of seeds germinated in each day divided by the number of days starting from the beginning of the experiment, and the emergence velocity is an important and accurate indicator of the seed viability (Yuan - Yuan et al 2010)

$Es = \Sigma Di / Ni$

Ni = number of seeds germinated per day

Di = number of days (daily germination)

Energy of Emergence (EE)%: This characteristic was calculated from the percentage of seed germination on the fourth day after sowing divided by the total number of seeds (Farooq et al 2005).

$EE = GP(4^{th}day)/TNST$

Field emergenceratio %: The number of seedlings emerging on the soil surface was calculated after 10 days of planting.

Dry weight of seedlings (mg): After taking the wet weight of the seedlings, ten seedlings were placed in a perforated paper bag and inserted into the oven at a temperature of 80^0C for a period of 24 hours and weighed again.

Chlorophyll content in seedlings: The chlorophyll content in seedlings was calculated by taking a sample of seedlings and analysis at the College of Agriculture, University of Baghdad.

RESULT AND DISCUSSION

Emergence (ES): The treatment of nanoscale iron was not significantly different from the treatment of nanoscale manganese in the first season on day^{-1}. In the second season, the treatment of nanoscale exceeded the rest significantly, gave 8.44 seedling day^{-1} followed by manganese (7.22 seedling day^{-1}). zinc treatment, for both seasons, gave 6.55 and 6.66 seedling day^{-1}, respectively. The comparison treatment (dry seeds) gave the lowest average for this trait for both seasons and these differences can be interpreted on the basis of the role played by the trace elements (iron, manganese and zinc) in promoting starch formation. Zinc, which is one of the components of important enzymes such as proteinase and peptides promotes starch formation. Laware and Raskar (2014) have shown that soaking seeds with zinc has an important physiological role in seed germination and early seedling growth (eutrophication) .The 2017 storage year gave the highest average of the emergence speed characteristic for both seasons, and the 2013 storage year gave the lowest average for the same characteristic, and for both seasons. The decrease in the speed of field emergence by increasing the storage time is due to the deterioration of the seeds, which led to a decrease in their viability and the percentage of their field emergence (Table 3). AL-Fahad (2017) also observed, that the emergence rate was higher at the shortest storage period (one year) for both seasons of cultivation, while the seeds stored for three years gave the lowest average emergence rate for both seasons of cultivation. Saeed et al (2018), also observed that the storage period (3, 6 and 9 months) greatly affected the superiority of the seeds resulting from the three-month storage period and gave highest average percentage and speed of emergence (Table 1). The interaction indicates that the parameters of the nano fertilizers and the years of storage did not differ significantly.

Energy of emergence (EE)%: The iron treatment gave the highest average of percent emergence in both seasons (31.00 and 45.25% respectively), while the comparison treatment (dry seeds) gave the lowest average for of 0.78 and 0.00 % for both seasons. The storage year 2017 gave the highest average emergence for both seasons (20.80 and 35.08% respectively) and 2013 storage year lowest average of 9.40 and 32.36% respectively. The treatment of iron with the year of storage 2017 gave the highest average of emergence energy both seasons (37.67 and 46.91% respectively). The dry seeds with the year of storage 2013, did not differ significantly with treatment of distilled water with the year of storage 2013. In the second season, the treatment of dry seeds did not differ with the storage years 2013, 2015 and 2017 significantly (Table 2).

Field emergenceratio (%): The iron treatment gave the highest average of percent emergence rate in both seasons (86.91 and 87.44 % respectively). The dry seeds gave the lowest average of 78.96 and 74.92%, for both seasons respectively. This is due to soaking wheat seeds with nanoparticles which led to an increase in the rate of field emergence. Sundaria et al (2018) confirmed that the field emergence rate of wheat seeds is greatly affected by the presence of iron nanoparticles. The field emergence rate increased in the seed treatment at 200 mg L^{-1} by 41.6%. Sharifi et al. (2016) was found that soaking wheat seeds with iron and zinc nanoparticles for 24 hours was significant with regard to the characteristic of field emergence rate, as this trait increased compared to the comparison treatment (dry seeds). The storage year 2017 gave the highest average characteristic of the field emergence rate% for both seasons

(86.12 and 86.89%) respectively, and the 2013 storage year gave the lowest average for the same characteristic, and for both seasons (79.87 and 74.12%) respectively. These results confirm the effect of the storage period on field emergence and noticed through their poor performance in the field when planted in the subsequent seasons (Hell et al 2000) Saeed et al (2018) also observed the superiority of seeds resulting from the three-month storage period in the quality of the field emergence over 6 and 9 months. Cheyed (2019) observed when seeds were stored for ten years with their spikes the ability of wheat seeds to emerge decreased with increasing storage time. The iron treatment was given with the storage year 2017 the highest average for the percentage of field emergence in both seasons (Table 3).

Dry weight of seedlings (mg): The iron treatment gave the highest average for the dry weight of seedlings in both seasons (5.97 and 7.09 mg respectively). The two comparison treatments (dry seeds and distilled water) gave

Table 1. Effect of storing and soaking seeds with nano fertilizers on the speed of emergence for wheat

Treatments	First season				Second season			
	Years in storage			Average	Years in storage			Average
	2013	2015	2017		2013	2015	2017	
Dry seeds	5.33	5.66	6.33	5.77	5.00	5.33	6.00	5.44
Water	5.66	6.33	7.00	6.33	5.00	6.00	6.66	5.88
Fe	7.66	8.00	9.00	8.22	7.00	8.33	10.00	8.44
Mn	7.66	8.00	8.66	8.11	6.33	7.33	8.00	7.22
Zn	6.00	6.66	7.00	6.55	6.00	6.33	7.66	6.66
Average	6.46	6.93	7.59		5.86	6.66	7.66	
LSD (p=0.05)	T=0.49	Y=0.38	TxY= NS		T=0.37	Y=0.28	TxY= NS	

Table 2. Effect of storing and soaking seeds with nano fertilizers on the emergence of wheat seedling (%)

Treatments	First season				Second season			
	Years in storage			Average	Years in storage			Average
	2013	2015	2017		2013	2015	2017	
Dry seeds	0.00	0.00	2.33	0.78	0.00	0.00	0.00	0.00
Water	0.00	9.00	13.00	7.33	37.58	39.75	40.75	39.36
Fe	24.33	31.00	37.67	31.00	43.53	45.25	46.91	45.25
Mn	19.67	25.33	31.33	25.44	42.41	43.83	45.31	43.86
Zn	3.00	13.00	19.67	11.89	38.25	40.75	42.41	40.47
Average	9.40	15.67	20.80		32.36	33.91	35.08	
LSD (p=0.05)	T=2.31	Y=1.79	TxY= 4.00		T=0.59	Y=0.45	TxY= 1.02	

Table 3. Effect of storing and soaking seeds with nano fertilizers on seedling emergence of wheat variety in field

Treatments	First season				Second season			
	Years in storage			Average	Years in storage			Average
	2013	2015	2017		2013	2015	2017	
Dry seeds	77.57	78.02	81.29	78.96	70.77	75.00	79.00	74.92
Water	78.40	79.01	83.08	80.16	71.50	75.67	79.66	75.61
Fe	82.52	83.65	94.55	86.91	79.33	88.67	94.33	87.44
Mn	80.88	82.20	87.72	83.60	76.00	84.50	91.43	83.98
Zn	79.97	80.21	83.97	81.38	73.00	82.00	90.00	81.67
Average	79.87	80.62	86.12		74.12	81.17	86.89	
LSD (p=0.05)	T=1.42	Y=1.10	TxY= 2.45		T=0.87	Y=0.67	TxY= 1.50	

Table 4. Effect of storing and soaking seeds with nano fertilizers on dry weight of wheat seedlings (mg)

Treatments	First season				Second season			
	Years in storage			Average	Years in storage			Average
	2013	2015	2017		2013	2015	2017	
Dry seeds	2.89	3.00	4.09	3.33	3.57	5.97	6.11	5.22
Water	3.16	3.33	4.69	3.73	3.91	6.39	6.34	5.55
Fe	4.50	4.88	8.51	5.97	6.50	7.00	7.78	7.09
Mn	3.96	4.39	6.24	4.86	5.96	6.60	7.40	6.65
Zn	3.65	3.73	4.99	4.12	5.82	6.44	7.00	6.42
Average	3.63	3.87	5.70		5.15	6.48	6.93	
LSD (p=0.05)	T=0.47	Y=0.36	TxY= 0.81		T=0.16	Y=0.12	TxY= 0.27	

Table 5. Effect of storing and soaking seeds with nano fertilizers chlorophyll content in wheat seedling

Treatments	First season				Second season			
	Years in storage			Average	Years in storage			Average
	2013	2015	2017		2013	2015	2017	
Dry seeds	8.71	11.68	14.73	11.71	8.73	13.02	15.40	12.38
Water	9.39	12.03	15.41	12.28	10.08	13.70	16.09	13.29
Fe	10.71	16.94	18.72	15.45	14.87	18.16	23.30	18.77
Mn	10.50	15.94	18.06	14.83	14.55	18.34	23.13	18.67
Zn	9.33	15.58	16.58	13.83	12.50	16.41	23.00	17.30
Average	9.72	14.34	16.07		12.14	15.92	20.18	
LSD (p=0.05)	T=1.17	Y=0.91	TxY= NS		T=1.40	Y=1.08	TxY= NS	

the lowest average (3.33, 5.22, 3.73 and 5.55 mg) for both seasons respectively, and the superiority of the nanoparticle treatments over the two comparison treatments for the dry weight characteristic. These factors resulted in the highest eruption velocity and the highest rate of emergence (Table 1, 3) and thus the highest dry weight, as the use of nano fertilizers, especially nanostructured zinc oxide in low doses, positively affects growth and physiological properties such as root elongation and dry weight (Asl et al 2019). The nanoscale iron particles positively affected the germination and growth of wheat seedlings subjected to drought and salinity, as an increase in the dry weight of seedlings was observed (Yasmeen et al 2015). The storage year 2017 gave the highest average for the dry weight of seedlings for both seasons (5.70 and 6.93 mg respectively), and the 2013 storage year gave the lowest average for the same characteristic, and for both seasons (3.63 and 5.15 mg). The iron treatment with the 2017 storage year gave the highest average dry weight for seedlings and in both seasons (8.51 and 7.78 mg respectively), and the dry seed treatment with the 2013 storage year gave the lowest average, and for both seasons of cultivation (2.89 and 3.57 mg respectively) (Table 4).

Chlorophyll content in seedlings: The iron treatment recorded maximum chlorophyll in seedling (15.45 and 18.77 in both seasons respectively). The two comparison coefficients (dry seeds and distilled water) did not differ significantly between them in giving the lowest average for the seedling content characteristic of chlorophyll (11.71, 12.38, 12.28 and 13.29 for both seasons, respectively). This is explained on the basis of influential role that iron plays in the vital processes of plants due to its participation in the enzymatic processes, as it enters in the synthesis of chloroplasts and in the synthesis of chloroplasts. Feizi et al (2013) observed the catalytic role played by nanowire oxide in increasing the proportion of chlorophyll in the wheat crop, as the proportion of chlorophyll increased by 15% over the comparison treatment as a result of using nanoscale iron. Sharifi et al (2016) also observed of soaking the seeds with nan iron for a period of 24 hours were significant for the chlorophyll content characteristic of the leaves, as it indicated an increase in the chlorophyll concentration in the leaves when the seeds were treated with nano iron.

CONCLUSION

The use of nano fertilizers, especially nano iron,

increased the viability of the seeds and increased all the characteristics of the seeds stored for years compared to untreated seeds and seeds soaked with distilled water only.

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Possibility of Cultivating Rice Crop by Dry Farming Method and Using Subsurface Drip Irrigation System

Abdrulazzak A. Jasim and Firas A. Jabbar*

Department of Agricultural Machines and Equipment
College of Agricultural Engineering Sciences, University of Baghdad, Baghdad
**E-mail: fabdullh21@gmail.com*

Abstract: The field experiment was conducted at University of Baghdad in 2020 to study the possibility of successful cultivation of rice crop by dry farming method using the drip irrigation system under the surface and to evaluate the effect of the distance between the drip irrigation tubes T-tape and the irrigation interval on some soil characteristics and the production of the rice crop *Oryza sativa* L. The distance between the tubes was at three levels 10, 15, 20 cm, and the irrigation intervals were one, two, and three days. The results showed a superiority of the distance of 20 cm and the daily watering interval by achieving the highest WUE of 1.04 kg m^{-3}, and the distance exceeds 20 cm, and the irrigation interval is every three days, resulted in lowest soil density of 1.180 Mg.m^{-3}. The distance of 10 cm and the daily irrigation interval excelled by achieving the highest PW of 23.76% and the highest grain yield reached 3.927 tons ha^{-1}. Based thesis recommend cultivating the rice crop using the dry cultivation method under the sub-surface drip irrigation system for the successful cultivation.

Keywords: Drip Irrigation, Subsurface, Rice crop, Irrigation interval, Dry farming

The world is facing an increasing demand for water as a result of the increasing population growth and more agricultural production. The water availability also declined due to scarcity of surface irrigation water and climatic changes (global warming). There is need to work seriously to reduce high water losses and improve the efficiency of water use through the use of modern systems to irrigate agricultural crops that consume large amounts of water, such as rice. In order to achieve a balance between consumption and availability for all sectors need arises to use modern farming methods and irrigation systems such as dry rice for the rice crop, which is one of the methods that consume less water during the growth stage of the crop with the use of a subsurface irrigation system with a very high water use efficiency, which may reach 95% and tested in the cultivation of dense crops such as rice, wheat and barley. The subsurface irrigation system is one of the types of localized irrigation systems, and is a relatively modern irrigation system that depends on the irrigation through the movement of water from the bottom to the top through capillary action and moisture tension. It is characterized by high efficiency in irrigating plants because the water is within an area. The roots reduce evaporation losses and spread. This is in addition to the possibility of applying fertilizers and pesticides directly to the roots by mixing them with irrigation water (Bof Bufon 2010, Jasim and Nafawah 2017). An experiment was also carried out in Japan to demonstrate the effect of sub-surface irrigation on rice plant growth, and the results showed dubsurface increases the length of the roots, their branches, and depth in the soil layers (Miyazaki et al 2020). The current research aims at the possibility of cultivating the rice crop by the dry farming method and by using the artificial subsurface irrigation system.

MATERIAL AND METHODS

A field experiment was conducted at University of Baghdad during of 2020, to study the possibility of cultivating rice (*Oryza sativa* L) by the dry farming method and using an artificial subsurface irrigation system. Two factors were studied were the distance between irrigation tubes (T-tape) at three levels, 10, 15, and 20 cm, and the irrigation interval in three levels: daily, every two days, every three days. The experiment was carried out using the nested design under the randomized complete block design with three replications. The results were statistically analyzed to compare the averages. The analysis of the soil was carried out in the central laboratory of the College of Agricultural Engineering (Table 1).

The experimental land was plowed and smoothed using a rotary plow and leveled using a plate leveling machine. Then it was divided into three sectors used for the irrigation interval with dimensions of 10 m x 10 m, then each sector was divided into three secondary sectors with dimensions of 2 m x 2 m, with three replications for each treatment, so that the number of experimental units was 27. T-tape tubes were installed in the experimental units. The rice crop (*Oryza sativa* L.) which

belongs to the grassy crops. Jasmine cultivar, was planted by dry method, and fertilized with compound fertilizer (NPK) at a rate of 140, 60, 60 kg ha^{-1}, and add urea in three batches (Ali 2012).

Parameter Studied

Water use efficiency kg. m^{-3}: The water use efficiency was measured according to the equation proposed by (Ghosh et al 2011).

$$WUE_{TWI} = \left[\frac{Y}{AW}\right] \qquad (1)$$

WUE_{TWI}: Water use efficiency relative to the total amount of water added (kg grain. m^{-3} water).

Y: grain yield (kg. ha^{-1}), AW: the total amount of irrigation water added ($m^3.ha^{-1}$).

Soil moisture content %: The soil moisture content was calculated according to the gravimetric method on the basis of dry weight, as the moist soil samples were dried by placing them in the oven and exposing them to a temperature of up to 105 ° C for 24 hours (Gardner 1965).

Pw= ((Msw-Ms)/Ms) * 100 .....(2)

Pw %: by weight moisture content.

Msw: The mass of moist soil (g).

Ms: mass of dry soil (g).

Bulk density of soil Mg. cm^{-3}: It was calculated by using the cylinder method (Core Sample) after drying the samples according to the method (Black 1965).

Pb= Ms/vt.....(3)

Pb: bulk density of the soil, $Mg.m^{-3}$, Ms: mass of dry soil, Mg, Vt: volume of the soil in its natural state, m^3.

Grain yield, tons ha^{-1}: The crop r was harvested from each experimental unitand threshing was done manually at a moisture level of 12-14%.

Table 1. Physical and chemical properties of field soil

Adjective parameter	Measuring unit	Value
True density	$Mg.m^{-3}$	2.6
Bulk density		1.6
Electrical conductivity	$ds.m^{-2}$	1.3
Ph		7.25
Sodium	$Mlg.L^{-1}$	109
potassium		98
Calcium		53
Magnesium		54
Sulfates		210
Chlorides		390
Sand	$g.kg^{-1}$	776
Green	$g.kg^{-1}$	125
Clay	$g.kg^{-1}$	99
Soil texture		Loamy sand

RESULTS AND DISCUSSION

Water Use Efficiency (WUE) $kg.m^{-3}$: Figure (1) shows the effect of the distance between the irrigation tubes T-tape and the irrigation interval on the efficiency of water use. There was significant effect of the distance between irrigation tubes T-tape on WUE as value increased from 0.44 to 0.63 to 0.79 kg. m^{-3} for distances 20,15,10 cm, respectively, The increase in the WUE was due to efficiency of water use which is directly proportional to the increase in plant production and inversely with the amount of water added. The amount of water added through tubes with a distance 20 is less from the amount of water added through the tubes for the two distances 10, 15. This leads lant exerting more effort to absorb water and causes activation and stimulation of roots to absorb more water in plants leading to an increase in the efficiency of water use.(Atiyah 2015). The irrigation interval had a significant effect on the efficiency of water use, as the daily irrigation interval recorded the highest water use efficiency of 0.8 kg m^{-3}, while the two irrigation intervals every three days recorded the (0.45 kg. m^{-3}). This may be attributed to the amount of soil moisture content and its relationship to plant productivity, as daily irrigation provided a good moisture content, which was reflected in an increase in yield (Sarkar et al 2018). The significant effect interaction between the distance between the tubes and the irrigation interval was observed, as the daily irrigation treatment and the distance 20 cm outperformed in obtaining the highest WUE of 1.04 $kg.m^{-3}$, while the lowest of WUE was in irrigation every three days, and the distance was 10 cm,(0.32 kg. m^{-3}).

Soil moisture content: Figure 2 shows the effect of the distance between the T-tape and the irrigation interval on the moisture content of the soil. The significant differences between the average distances between the T-tape irrigation tubes were observed and as the distance exceeded 10 cm

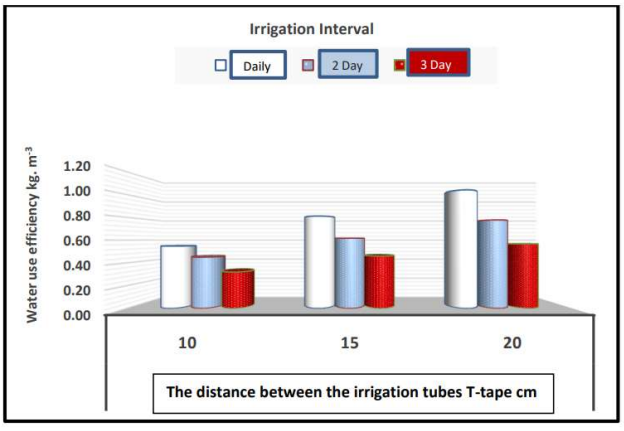

Fig. 1. Effect of the distance between subsurface drip irrigation tubes and irrigation interval on water use efficiency WUE

achieved the highest soil moisture content of 18.79 %, while the distance 20 cm achieved the lowest soil moisture content of 16.88%. This is due to the reduction of the distance between the T-tape irrigation tubes, which led to an increase in the number of drippers per unit area, and consequently, an increase in the moisture content of the soil. (Badr and Abuarab 2013). The irrigation interval had a significant effect on the soil moisture content, as the daily irrigation interval recorded the highest the soil moisture content 21.62 %, while the irrigation interval every three achieved the lowest the soil moisture content 15.67 %. The irrigation interval played an important role in depleting the soil moisture content, as daily irrigations achieved high levels of moisture compared with the soil moisture content for the two irrigation intervals every two days and every three days. (Salih and Mohammed 2011). The bilateral interaction between the distance between the T-tape and the irrigation interval did not have a significant effect on the soil moisture content. The daily irrigation interval and the distance 10 cm achieved the highest soil moisture content 23.76%, while the lowest moisture content was between irrigation interval every three days and the distance 20 cm (14.64%).

Bulk density of soil Mg. m^{-3}: The effect of the distance between the T-tape and the irrigation interval on the bulk density of the soil is given in Figure 3. The significant differences between the average distances between the T-tape irrigation tubes, as the distance exceeded 20 cm, with the lowest apparent density of 1.208 Mg m^{-3}, while the distance of 10 cm achieved the highest bulk density of 1.234 Mg m^{-3}. The reason may be attributed to the amount of irrigation water that is discharged through the irrigation tubes T-tape with a distance of 10 cm greater than the amount of water that is discharged through the tubes of T-tape with a distance of 20 cm, and this led to an increase in the moisture content of the soil (Jasim and Abdullah 2017). The irrigation interval had a significant effect on the bulk density of the soil, as the irrigation interval every three day recorded the lowest apparent density of the soil (1.193 Mg m^{-3}) while the daily irrigation interval achieved the highest bulk density of soil (1.244 Mg m^{-3}). This may be due to soil moisture content for the daily irrigation interval is high, which caused the failure of the soil particles to bond with each other in the form of aggregates compared with the soil moisture content for the irrigation interval every three days. (Omran et al 2012) and (Al Kaabi 2016). The bilateral interaction between the distance between the T-tape and the irrigation interval had a significant effect on the bulk density of the soil, as the distance of 20 cm and the irrigation interval every three achieved the least apparent density of the soil, which was 1,180 Mg m^{-3}, and the highest apparent density was between the distance of 10 cm and the daily irrigation interval amounted to 1.263 Mg m^{-3}.

Grain yield tons. ha^{-1}: Figure 4 shows the effect of the distance between the irrigation tubes T-tape and the irrigation interval on the grain yield. The significant differences between the average distances between T-tape irrigation

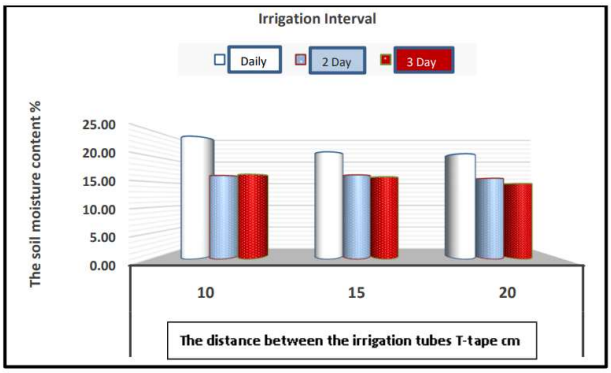

Fig. 2. Effect of the distance between the subsurface drip irrigation tubes and the irrigation interval on the moisture content of the soil (%)

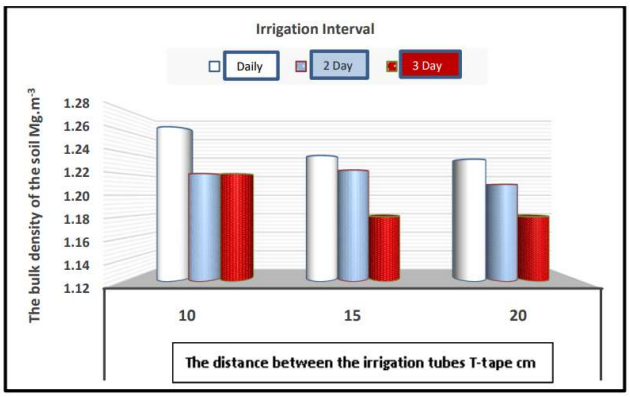

Fig. 3. Effect of the distance between the subsurface drip irrigation tubes and the irrigation interval on the bulk density of the soil

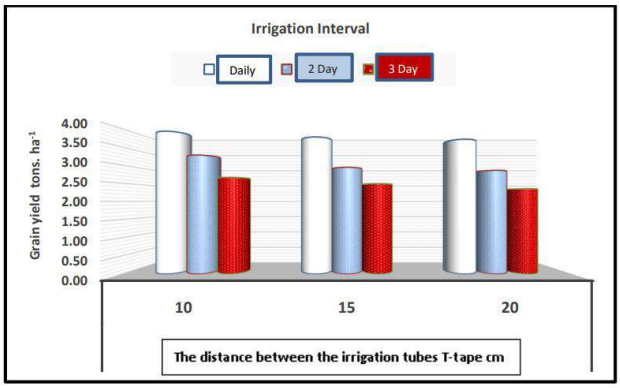

Fig. 4. Effect of the distance between the subsurface drip irrigation tubes and the irrigation interval on the grain yield of the rice crop

tubes, as the distance of 10 cm achieved the highest grain yield of 3.287 tons.ha^{-1}, while the distance of 20 cm achieved the lowest grain yield of 2.969 tons.ha^{-1}. This may be due to the fact that The number of planting lines (vegetation density) is in a distance of 10 cm more in number per unit area than irrigation tubes of 15 and 20 cm and this leads to an increase in the number of branches bearing panicle per unit area, (Al-Essawi et al 2007, Shati and Al-Ziaadee 2010) .

The irrigation interval had a significant effect between the grain yield, as the daily irrigation interval was superior with highest grain yield of 3.801 tons ha^{-1}, while the irrigation interval every three days achieved the lowest grain yield of 2.498 tons.ha^{-1}. The is due to the fact that the moisture content of the soil had clear effect on reducing the grain yield due to its effect on reducing the physiological activity of the plant, and effect on the photosynthesis process, which was reflected in reducing the absorption of nutrients (Al-Hosni et al 2016). The bilateral interaction between the distance between the T-tape tubes and the irrigation interval did not have significant effect on the grain yield, the daily irrigation interval and the distance 10 cm achieved the highest grain yield amounting to 3.927 tons.ha^{-1}, while the lowest grain yield was between the two-way overlap of the irrigation interval every three days and the distance 20 cm amounted to 2.347 tons.ha^{-1}.

CONCLUSIONS

The sub-surface drip irrigation system under the dry farming system resulted in higher production of rice crop.

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Effect of Seeds Treatment on Germination and Growth of *Acacia cyanophylla* Seedlings

Shahla A. Basher, Sumood H. Alhadedy* and Shaimaa D.H. Ali

Department of Forestry, Agriculture and Forestry College, University of Mosul, Iraq
**E-mail: sumod_husain@uomosul.edu.iq*

Abstract: This study was conducted at College of Agriculture and Forestry from February until November, 2020 to determine the effect of treatment with hot water for 24 hours and dipping with various concentration of gibberellic acid GA_3 (100-200 mg l^{-1}) on the germination of seeds and seedling growth characteristics (length and diameter of the stem, leaf surface area, number of leaves and length and diameter of the root) of *Acacia cyanophylla*. The dipping with hot water and gibberellic acid GA_3 (200 mg l^{-1}) for 24 hours, resulted in a significant increase in germination, stem length and diameter, leaf surface area and root length, but there was no significant difference in the root diameter for dipping with gibberellic acid at the concentration of 100 and 200 mg l^{-1}.

Keywords: *Acacia cyanophylla*, Gibberellic Acid, GA_3, Hot water

Acacia is considered one of the largest species that belong to the Fabaceae family, which includes about 1200-1300 species. Most important types of this species is *Acacia cyanophylla* a small tree or an evergreen and thornless and can reach eight meters in height. This species was introduced to Iraq for the purposes of afforesting in the poor lands (Dawood 1979) and also enhances fertility of soil due to the presence of the bacterial nodes that fix the nitrogen in the soil (May and Attiwill 2003). Usually, *Acacia cyanophylla* proliferatesby seeds and seeds provide most of the natural resources for the sexual propagation and preserving the genetic diversity and the spread of the plants (Taiz and Zeiger 2002). Most of the *Accacia* species suffer from dormancy due to the seed cover thickness or due to the presence of chemical compounds, which inhibit growth of plant (Ramamoorthy et al 2005). The process of dipping in hot and normal water and dipping in gibberellic acid is considered a stimulator to break the dormancy phase and accelerating the germination (Lorato et al 2014). Several studies were conducted to break the dormancy in many plants seeds in order to identify the best relevant treatment. Amongst the treatments used to break dormancy is dipping in hot water. Seeds are soaked in water either to modify the hard shield of the seed or to remove the materials that prevent germination or to shorten the period required to initiate germination or all of them (Emongor et al 2004). Mustafa (2017) indicated that treating *Delonix regia* seeds with boiling water resulted in the highest rate of germination. Male and Eun Ju (2020) mentioned that the treatment of *Dalbergia cochinchinensis* seeds with 70º degree hot water had a significant effect on increasing the germination. Edward et al (2014) observed that hot water treatment had a significant effect on the stem length, stem and root diameter and the number of leaves after the scratching treatment. Amongst other treatments to break the dormancy phase and improving the vegetative growth is plant hormones which control the growth of the plant including the gibberellins. This help the division and elongation of the stem cells and simulate certain enzymes like a-amylase, protease, peroxidase, ribonuclease, esterase and other enzymes. Mobil and Baninasab (2008) indicated that treating the seeds of *Amygdalus communs* and *A. Webbii* with gibberellic acid at of 100 mg l^{-1}, led to an increase in the vegetative growth of the seedlings and recorded better results of the growth characteristics including the stem length, leaf area, fresh and dry weight of the shoot of the seedlings compared to the control treatment. Al Hadidi (2005) found that gibberellic acid was superior at concentration of 300 mg l^{-1} in terms of seeds germination.

MATERIAL AND METHODS

Acacia cyanophylla seeds, brought from Jordan and their vitality was tested by immersing them in sterilized water and excluding the floating seeds during 2020. The experiment included two factors. The first factor is soaking in hot water for 24 hours and without soaking (control treatment), while the second factor is soaking in gibberellic acid (0, 100 and 200 mg l^{-1}) for 24 hours. Seeds were removed, dried and planted in black perforated polyethylene (30×20 cm) filled with soil. Complete randomized blocks design was followed and

results were statistically analyzed using Genstat package. At the end of the experiment, the following parameters of the characteristics studies were calculated:

Germination percentage:

Germination percentage = number of germinated seeds/total number of seeds ×100.

Height of seedlings (cm seedling^{-1}): The height was recorded in November 2020 from the bag soil surface to the top of the plant for all the seedlings.

Main stem diameter (mm seedling^{-1}): The diameter was measured one centimeter higher than the bag soil surface by vernier caliper.

Number of leaves (leaf seedling^{-1}): The number of leaves were counted for three iterations.

Leaf area: The actual area for the largest leaf was determined randomly for five seedlings in one experimental unit using the copying method which is characterized by photographing the seedling leaf on an A4 sheet of paper with real dimensions and then cutting the leaves and weighting them with a sensitive scale and then calculating the mean

Weight of the largest leaf = Weight of the largest leaf × area of the copied sheet of paper weight^{-1} of the sheet.

Length of the root (cm): The root length of *Acacia cyanophylla* seedlings was measured from the point the root attached to the stem to the end of the root growing part.

Root main diameter (mm seedling^{-1}): The diameter of the root was measured at the point at which the root is attached with the stem for all the seedlings.

RESULTS AND DISCUSSION

Germination percentage (%): Soaking in hot water was significantly superior over the treatment without soaking with 60.78% of germination (Table 1). The soaking in various concentration of gibberellic acid indicated significant effect on germination. The concentration 200 mg l^{-1} recorded the highest germination (76.00%) and was different from the concentration 100 mg l^{-1} and the control treatment (34.83%). The interaction between hot water and different concentrations of gibberellic acid had a significant effect, as the combination treatment soaking × 200 mg l^{-1} gave the highest percentage (85.67%) compared to combination treatment no soaking × 0 mg l^{-1}, which gave the lowest value (24.67%). These result is in conformity with Al-Hadidi (2007) and Mustafa (2017).

Stem length (cm): Soaking in the hot water had a significant effect as the average height of the stem (59.22 cm) compared to without soaking (Table 2). Gibberellic acid AG_3 at concentration 200 mg/l was superior (69.62 cm) from the concentration 100 mg/l and the control treatment (34.5 cm). The soaking in hot water and the gibberellic acid at the concentration 200 mg/l was superior and (75.23 cm) as compared to the control treatment (39.93 cm). This result is consistent with Mala and Eunju (2020).

Stem diameter (mm): There was a significant increase in of stem diameter and soaking with hot water treatment recorded maximum stem diameter (4.66 mm) but this increase was not significant compared to the treatment without soaking (3.96 mm) (Table 3). The treatment 200 mg l^{-1} of gibberellic acid recorded the highest average (5.16 cm), but it was not significantly different from the concentration 100 mg l^{-1} and control treatment (3.66 cm). The combination treatment soaking × 200 mg/l recorded the highest average (5.36 cm), while no soaking × 0 mg l^{-1} recorded lowest average (3.55 cm). The result was in conformity with Al-Imam (2007).

Number of leaves seedling^{-1}: There was a significant increase in the number of leaves in soaking in the hot water and was superior to without soaking in hot water achieving the highest average of 36.5 seedling^{-1} (Table 4). The treatment with gibberellic acid at concentration of 200 mg/l recorded maximum number of leaves (39.6 seedling^{-1}). The two concentrations of GA were different from the control (24.32 seedling^{-1}). The seeds treatment with water and gibberellic acid at concentration of 200 mg/l to recording the highest number of leaves (44.24 seedling^{-1}), and differed significantly from all the other treatments. The result is in conformity with Sandeep et al (2017).

Leaf area (cm^2): The soaking in hot water was superior to without soaking in terms of the leaf area (Table 5). Gibberellic acid at concentration 200 mg/l recorded largest leaf area, of 3.12 cm^2 and was superior over the concentration 100 mg/l and the control treatment. The treatment of soaking with hot water and Gibberellic acid at concentration of 200 mg/l was significantly superior (3.76 cm^2) over the other treatments and the control which achieved the lowest leaf area (24.32 cm^2). The results are in conformity with AlQaisi (2018).

Main root length (cm: There was a significant effect of the treatment of soaking in hot water (22.37 cm) compared to the treatment with no soaking (Table 6). Gibberellic acid at concentration 200 mg/l was superior with highest root length (29.05 cm) and differed significantly from the concentration 100 mg/l and the control (24.18 and 18.92 cm, respectively). The treatment without soaking and gibberellic acid at the concentration of 200 mg/l was superior with highest average root length (30.15 cm) compared with the control treatment (20.48 cm). Sandeep et al (2017) also reported similar trend.

Main root diameter (mm): The treatment of soaking in water was not significant from treatment without soaking (5.17 mm) (Table 7). Gibberellic acid, at concentration 200 mg/l recording the highest value (5.871 mm) and therefore

Table 1. Effect of soaking in hot water and gibberellic acid in the percentage of germination of *Acacia cyanophylla* seeds

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	24.67^{e}	39.33^{d}	66.33^{b}	43.44^{b}
Soaking in hot water for 24 hours	45.0^{cd}	51.67^{c}	85.67^{a}	60.78^{a}
Average	34.83^{c}	45.50^{b}	76.00^{a}	

Table. 2. Effect of soaking in hot water and gibberellic acid on the stem length (cm) of *Acacia cyanophylla*

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	28.17^{d}	39.10^{c}	64.00	43.59^{b}
Soaking in hot water for 24 hours	39.93^{c}	62.5^{b}	75.23^{a}	59.22^{a}
Average	34.50^{c}	50.80^{b}	69.62^{a}	

Table 3. Effect of soaking in hot water and Gibberellic acid in the stem diameter (mm) of *Acacia cyanophylla*

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	3.55^{b}	3.72^{b}	4.69^{ab}	3.96^{a}
Soaking in hot water for 24 hours	3.76^{b}	4.58^{ab}	5.63^{a}	4.66^{a}
Average	3.66^{b}	4.15^{b}	5.16^{a}	

Table 4. Effect of seeds soaking in hot water and Gibberellic acid in number of leaves seedling^{-1} of *Acacia cyanophylla*

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	19.26^{e}	24.07^{de}	34.97^{bc}	26.1^{b}
Soaking in hot water for 24 hours	29.39^{cd}	35.88^{bb}	44.24^{a}	36.5^{a}
Average	24.32^{c}	29.97^{b}	39.6^{a}	

Table 5. Effect of soaking in hot water and Gibberellic acid in the leaf area (cm^{2}) of *Acacia cyanophylla*

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	0.81^{e}	1.26^{de}	2.49^{bc}	1.527^{b}
Soaking in hot water for 24 hours	1.87^{cd}	2.98^{ab}	3.76^{a}	2.78^{a}
Average	1.34^{c}	2.12^{b}	3.12^{a}	

Table 6. Effect of soaking in hot water and Gibberellic acid in the main root length (cm.) of *Acacia cyanophylla*

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	20.48^{c}	26.55^{b}	30.15^{a}	25.73^{a}
Soaking in hot water for 24 hours	17.36^{d}	21.81^{c}	27.96^{ab}	22.37^{b}
Average	18.92^{c}	24.19^{b}	29.05	

Table 7. Effect of soaking in hot water and Gibberellic acid in the main root diameter (mm) of *Acacia cyanophylla*

Soaking in gibberellic acid Soaking in water	0	100	200	Average
Without soaking	4.082^{b}	5.029^{ab}	6.388^{a}	5.17^{a}
Soaking in hot water for 24 hours	4.202^{b}	4.107^{b}	5.354^{ab}	4.55^{a}
Average	4.142^{c}	4.568^{b}	5.871^{a}	

was significantly superior at the concentration 100 mg/l and the control treatment which lowest root diameter (4.142 mm). The combination treatment without soaking × 200 mg/l gave the highest average (6.388 mm), while the treatment without soaking × 0 mg/l gave the lowest of 4.0082 mm.

The increase in the percentage of germination and the rest of the characteristics studied (the stem length and diameter, number of leaves, leaf area and root length and diameter) in the treatment of soaking in the hot water is due to the breaking of the seed cover, most of the legume suffer from because of the thickness of the seed cover and the treatment with water makes the seed cover soft and permits the permeability of oxygen and water and stimulates the embryo to germinate as mentioned by Ramamoorthy et al (2005). The reason behind obtaining the highest germination percentages and the highest averages of the characteristics in question (the stem length and diameter, number of leaves, leaf area and root length and diameter) is the treatment of seeds soaking in Gibberellic acid with a concentration of 200 mg/l is due to the effect of Jiberlin in breaking the dormancy phase of the seeds and its important role in increasing the effectiveness of the decomposing enzymes (Finch-Savage et al 2006) and the increase of the cell wall flexibility and improving water absorption, which leads to activating and stimulating the embryo to early germination and also stimulating the cells to divide and elongate (Padma et al 2013).

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Effect of Glyphosate on Activity of Urease and Millet Nitrogen

Wejdan Taleb Kadhum and Karim Aubaid Hassan

Department of Soil and Water Resources, College of Agricultural Engineering Sciences
University of Baghdad, Iraq
E-mail: wejdan.Taleb1207a@coagri.uobaghdad.edu.iq

Abstract: A field experiment was conducted to find out the effect of glyphosate on the activity of urease and nitrogen concentration in millet. The herbicide glyphosate inhibited the activity of the urease enzyme when added at high levels full fertilizer recommendation at C_0 with no addition of glyphosate pesticide. This resulted in higher values than the other treatments and levels and achieved an average of 58.748 mg NH4 g kg^{-1} soil 2 hours compared to a treatment without the recommendation that achieved a mean of 41.411 mg NH4 g kg^{-1} soil 2 hours for C_o level (no addition). The concentration of N% $plant^{-1}$, the full recommendation treatment with C_0 level of the pesticide achieved a higher average of 3.797 N% $plant^{-1}$ compared with the third level of the pesticide C_3 for the same treatment, which achieved 3.298 N% $plant^{-1}$, The treatment without fertilizer addition and C_0 pesticide gave an average of 2.794 N% $plant^{-1}$ compared with the same treatment with the third level of the pesticide C_3 which achieved an average of 2.040% N $plant^{1}$. The full recommendation treatment gave higher values of urease activity than the other treatment, and the first level of the pesticide with the full recommendation treatment and without the recommendation gave the highest average enzyme activity.

***Keywords*:** Herbicide, Urease, Nitrogen, Millet

Nitrogen in nature is subject to biological processes that microorganisms participate in completing and is also exposed to the processes of loss by leaching and volatilization in the form of gases NH3, NO_2, N_2 or metabolism in the bodies of microorganisms and plants. Therefore, the nitrogen fertilizers added to the soil enter into many paths that lead to a reduction fertilizer use efficiency. In order to reduce the loss of nitrogen, expensive chemicals were used that inhibit enzymatic decomposition. The trend also included the encapsulation of nitrogen fertilizers with different materials such as sulfur and glues. The agricultural pesticides, herbicides, and pesticides are used in the agricultural system to protect crops from weeds, insect pest and diseases. The effect of pesticides on microorganisms depends on the physical, chemical and biochemical state in addition to the nature and concentration of the pesticides (Aurelio 2013, Sethi and Gupta 2013). High concentrations of pesticides in the soil may affect some processes such as plant growth, microorganism activity and enzymes, and continuing to add them will cause an accumulation of pesticides and the outcome of their decomposition in the soil system (Kumar et al 2012). Soil contains free enzymes as well as found in microbial cells. The activity of dehydrogenase enzyme in soil is an indicator of all microbial activity of soil (Mayanglambam et al 2015). The effectiveness of urease was observed in a large number of bacteria and fungi. Studies indicated decrease in the effectiveness of urease when adding different pesticides (Nawak et al 2004). In this study, the herbicide glyphosate was used to inhibit the activity of urease in order to reduce nitrogen transformations and to preserve the nitrogen fertilizer that cause nitrogen loss in the soil in millet crop.

MATERIAL AND METHODS

Field experiment was conducted at University of Baghdad in the 2019-2020 season. Millet was planted on January 7, 2020 and planted in rows (4 rows per experimental unit), and irrigation was applied after calculating the required amount of irrigation water for each experimental unit. Soil sample was taken before planting for the purpose of measuring the physical, chemical and biological characteristics. The electrical conductivity Ec, PH, available nitrogen, organic matter, calcium carbonate, soil separators, sand, silt, clay, total bacteria and total fungi were estimated, (Table 1).

The glyphosate herbicide used with three concentrations (2.548 5.096 and 7.644 ml $liter^{-1}$along with control). Urea was used at two levels, U_0 without adding nitrogen fertilizer (urea) and U1 adding nitrogen fertilizer at a complete recommendation. Glyphosate herbicide was sprayed at the specified concentrations after 15 days from germination. The addition of urea in the treatment of 100% recommended doses at three batches, the first batch is 20 kg ha^{-1}, the second batch is 40 kg ha^{-1}, and the third batch is 40 kg ha^{-1}. The activity of the urease enzyme was measured at 15, 40, 65, 90 days. The percentage of nitrogen in the plant was estimated. The vegetative index was measured at(30, 60, 90

days. The data of the experiment were subjected to statistical analysis and least significant differences (LSD) was applied to test differences between means at the 0.05 probability level with Genstat software.

RESULTS AND DISCUSSION

Effect glyphosate concentrations on urease activity: There were significant differences between urea treatments with complete recommendation with the control and achieved the highest enzymatic activity of 58.748 mg NH_4 kg^{-1} soil at 2 hours (Table 2). These results indicate that the effectiveness of the urease enzyme increases with the increase in the amount of nitrogen fertilizer added (urea). The lowest activity of the enzyme was at 90 days and may be due to the action of the inhibitor of urease, as well as to the nitrification of organisms. Subhani et al (2002) and Lin et al (2008) and stated that pesticides have negative effect on the enzymatic activity. The interaction between urea and duration showed that there were significant differences between treatments being maximum and minimum in full recommendation and without urea addition (50.803 and 37.208 mg NH_4 kg^{-1} soil at 2 hours, respectively).

The no herbicide and duration achieved an average of 48.980 mg NH_4 kg^{-1} compared to the C_3 which achieved an average of 38.533 mg NH_4 kg^{-1} soil. The enzyme activity decreased for the standard durations (40, 65, 90 days) compared to durations of 15 days, which was 53.248 mg NH_4 kg^{-1} of soil at compared to the 90-day duration that achieved 40.305 mg NH_4 kg^{-1} soil. This may be due to the effect of the herbicide on the activity of the enzyme due to the toxic effect on the enzyme and microorganisms. Ingram et al (2005) mentioned that several studies have noted a decrease in the effectiveness of urease when pesticides are added.

Effect of glyphosate on nitrogen concentration in the plant: There were significant differences between urea x

Table 1. Physical, chemical, and biological characteristics of soil

EC	Ph	N	O.M%	$CaCo_3$ %	Sand	Silt	Clay	Texture	Total bacteria	Total fungi
1.40	7.5	21	0.48	26.37	380	324	296	Clay loam	$18x10^3$	$30x10^6$

Table 2. Effect of glyphosate concentrations on urease activity (mg NH 4 kg` soil, per 2 hours)

Urea fertilizer	Glyphosate concentration	Duration (Day)				Urea * Herbicide
		15	40	65	90	
U_1	C_0	44.233	40.450	39.550	37.153	41.411
	C_1	41.550	36.260	35.470	29.220	37.760
	C_2	40.117	34.833	33.067	27.640	36.006
	C_3	38.547	31.930	30.483	23.963	33.653
U_0	C_0	64.960	60.497	50.787	45.410	58.748
	C_1	58.063	56.487	44.567	41.977	53.039
	C_2	51.760	45.823	42.127	40.357	46.570
	C_3	48.017	45.823	40.730	38.103	44.857
LSD 5%			1.302			0.231
Urea			Urea X Measurement duration			Mean
U_0		41.112	35.868	34.643	29.494	37.208
U_1		55.700	52.158	44.553	41.462	50.803
LSD 5%			0.815			0.501
Herbicide			herbicide X Measurement duration			Mean
C_0		53.248	48.467	45.227	40.305	48.980
C_1		49.138	45.040	40.329	34.960	44.836
C_2		45.595	40.134	37.101	32.749	40.943
C_3		42.755	38.093	34.752	29.773	38.533
LSD 5%			0.929			0.465
Mean		47.684	42.934	39.352	34.447	
LSD 5%			0.465			

Glyphosate pesticide application: C0 (control), C1 (2.548), C2 (5.096), C3 (7.644) ml L^{-1}

Table 3. Effect of glyphosate concentrations on N concentration in plant

Urea fertilizer	Glyphosate concentration	Duration (Day)			Urea * Herbicide
		15	60	90	
U_1	C_0	2.133	3.130	3.120	2.794
	C_1	2.103	2.927	2.950	2.660
	C_2	2.047	2.863	2.823	2.578
	C_3	1.973	2.057	2.090	2.040
U_0	C_0	3.767	4.027	3.597	3.797
	C_1	3.630	3.637	3.383	3.550
	C_2	3.403	3.637	3.150	3.397
	C_3	3.247	3.520	3.127	3.298
LSD 5%			0.223		0.125
Urea			Urea X Measurement duration		
U_0		2.064	2.744	2.746	2.518
U_1		3.512	3.705	3.314	3.590
LSD 5%			0.114		0.057
Herbicide			Measurement duration X Herbicide		Mean
C_0		3.020	3.516	3.246	3.260
C_1		2.925	3.230	3.089	3.081
C_2		2.777	2.607	2.864	2.866
C_3		2.584	2.607	2.582	2.591
LSD 5%			0.124		0.068
Mean		2.731	3.021	2.754	
LSD 5%			0.056		

measurement duration as the treatment of full recommendation recorded average of 3.590 N% plant^{-1} compared to the treatment of without urea addition (2.518 N% plant^{-1}) (Table 3). These results confirm that increasing the nitrogen fertilizer leads to an increase in the concentration of N with significant positive correlation between the soil nitrogen and the activity of urease, which is one of the enzymes that break down urea and increase nitrogen in the soil, which led to an increase in nitrogen uptake by the plant and reflected in the high concentration of N% in the plant in full recommendation treatment compared to the non-addition urea treatment. The herbicide concentrations (C_0, C_1, C_2, C_3) gave different levels of significance, and the C_0 level with highest average of 3.260 % N plants compared to the average C3 with 2.591 % N plants^{-1}. These results indicate the effect of herbicide concentrations on nitrification and the lack of inhibition of the processes of converting urea to the NH_4 ion, which is mainly absorbed by the plant. The increase in the concentration of % N in the plant at the time duration of 60 days is due to the increase in the concentration of nitrogen from the addition of fertilizer (urea) around the time of the flowering stage of 60 days from planting. There were significant differences for three-way interaction (nitrogen fertilizer, pesticide, and measurement duration) as the complete recommendation treatment at the C_0 level of the herbicide recorded average of 2.794 N% plants^{-1} compared to the C_3 level of the same treatment with average of 2.040 % N plants. These results confirm that without adding the herbicide, the activity of microorganisms, especially nitrification and urease enzymes, was high, which resulted in an increase in the nitrogen concentration in the soil and availability for absorption. Ingram et al (2005) indicated that multiple studies noted a decrease in the effectiveness of nitrification organisms and urease when pesticides were added.

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Effect of Ultraviolet Rays, Type of Sugar on Growth and Parthenolide in *Tanacetum parthenium* L. in-vitro

Z.J. Al-Mousawi and S.A. Mohammed

Medicinal and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences
University of Baghdad, Iraq
E-mail: zainab.jar@coagri.uobaghdad.edu.iq

Abstract: This research was carried out at University of Baghdad for period of February 2015, to August 2016, to evaluate the effect of ultraviolet (UV) light, type and concentration of sugar on growth characteristics and parthenolide content of leaves, The experiment was carried out by exposing the seeds to ultraviolet (UV) Rays in different periods (5, 10, 15, 20 min). The application of ultraviolet (UV) for 15 minutes gave the highest values, which was adopted in the experiment. Then the plants were planted in MS medium prepared with different concentrations of sugars (sucrose (S), fructose (F) and glucose (G) on the growth and stimulation of Parthenolide production, The food medium was prepared separately with different concentrations (25, 50, 75 g L^{-1}) and for each type of sugars mentioned in sequence. The application of different concentrations of sugars (sucrose, glucose, and fructose) to the nutrient media, gave the highest values at sucrose 25 g L^{-1} among the rest of the concentrations of plants exposed to UV with an increase in the branches number and length 13.30 branches per plant and 2.550 cm, respectively. The prepared nutrition medium with a concentration of 50 g. L^{-1} of glucose sugar was significant in the plant wet and dry weight which were exposed to UV (1.968 g, and 0.339 g). The prepared nutrition medium with concentration of 50 g L^{-1} of fructose recorded a significant hi concentration of parthenolide (13.38 mg g^{-1}) for plants exposed to UV compared to the lowest of 1.19 2 mg g^{-1} under the prepared medium with 75 g L^{-1} of sucrose for non-exposed plants.

Keywords**:** UV radiation, Fructose, Glucose, Sucrose, Parthenolide

Tanacetum parthenium L. (Feverfew), belongs to Asteraceae family, has gained pharmaceutical importance and contains parthenolide from the terpenoids group, which constitutes more than 85% of the compounds that help prevent excessive accumulation of blood platelets, and used as a treatment for migraine headache, as it works to balance the serotonin secreted in the brain (Cretnik et al 2005 , Chappell and Coates 2010). The exposure of UV rays in low doses on the plants can regulates morphological and physiological processes. Low doses stimulate the growth of UV protection genes such as genes responsible for the flavonoids formation and other phenolic compounds that accumulate in the epidermal layer and provide protection for the plant (Frohnmeyer and Steiger 2003). Sugars have many functions in plant addition to their function as a source such as a signal or antioxidant molecule (O'Hara et al 2012). In fact many researches emphasized their role in embryo size (Al-Khafaji, 2021) or as antioxidant molecule (Al-Khafaji and Al-jubouri 2022). Sucrose is the main sugar that added as a source of energy to nutrition media, which are of several types, including monosaccharides such as glucose, fructose and maltose, or disaccharides such as sucrose. This distinguishes sucrose from the rest is that fast transformation into glucose and fructose in the nutrition medium and the plant tissue absorbs glucose faster than fructose (Ramawat 2004).

MATERIAL AND METHODS

This research was carried out at University of Baghdad . The seeds were irradiated as they were 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 the seeds were exposed to ultraviolet rays with extended periods of different time (0,5, 10, 15, 20 minutes) (Shaukat et al 2013) by means of the ray-generating device. The intensity of the light emitted by it 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 that included all conditions except for exposure to radiation. The seeds that had been exposed to ultraviolet (UV-B) rays after sterilization were sown on MS food medium prepared with 30 g L^{-1} sucrose and 7 g L^{-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 them to germination (Castillon and Cornish 2000) for a period of 4 weeks. Vegetative branches with a length of 1 cm obtained from the best treatment with ultraviolet rays for a duration of 0, 15 minutes. The effect of the best type and concentration of sugars (sucrose (S), fructose (F) and glucose (G) was observed on the growth and stimulation of the production of parthenolide, which prepared

the MS food medium separately with different concentrations (25, 50, 75 g l^{-1}) and for each type of sugars mentioned sequentially.

RESULTS AND DISCUSSION

The effect of irradiation, the type, and concentration of sugar (sucrose, glucose, and fructose) and their interaction on the branches length, and number and leaves content of chlorophyll (100 mg. g^{-1} wet weight) are given in Table 1. The concentration of sugar had a significant effect on the branches length, and number and the leaves content of chlorophyll. The nutrition medium with a concentration of 25 g of sucrose (S1) gave the significant higher branches length (2,100 cm), branches number (10.65 branches), and chlorophyll content (10.31 mlg. 100 g wet weight) compared with the lowest values of branches length and chlorophyll content in leaves under the medium with a concentration of 75 g L^{-1} of sucrose (S3) (0.863 cm) and the lowest number of vegetative branches (2.00) under the medium with a concentration of 75 g L^{-1} of glucose (G3). The UV1 treatment has significantly achieved the highest values of branches length and number, and leaves content of chlorophyll (1.497 cm, 6.27, and 9.579 mg g^{-1} wet weight), respectively) in comparison with the non-irradiated (UV0) treatment, (1.141 cm, 3.87, and 6.849 mg g^{-1} wet weight, respectively).The interaction S1UV1 treatment achieved the highest branches length and number (2.550 cm and 13.30), while the interaction of S3UV0 were lowest (0.600 cm, 3.142 mg 100 g^{-1} wet weight). The lowest number of branches number (1.80) was achieved under the interaction treatment of G3UV0.

The fresh and dry weight was medium, 50 g L^{-1} of sucrose (S2), which 1.406 g and 0.180 g, respectively, while the fresh and dry weight were decreased to the lowest (0.033, 0.012 g respectively), at the prepared medium with 25 g of fructose (F1).The treatment of the prepared medium with 50 g of fructose (F2) was superior in highest concentration of parthenolide (9.911 mg. g^{-1}compared to the lowest concentration 1.891 mg. g^{-1} medium withs 75 g of sucrose). UV1 irradiation treatment achieved the highest average of fresh and dry weight and parthenolide (0.612 g, 0.071 g, and 6.143 mg g^{-1}), respectively, while the lowest rates were (0.313 g, 0.019 g, 3.563 mg g^{-1}) under the UV0 treatment. S2UV1 recorded the highest fresh and dry weight (1.968 g, and 0.339 g) respectively, compared to the lowest values of fresh and dry weight (0.012 g, 0.008 g) under the treatment of G3UV0. The interaction treatment F2UV1 was superior by giving the highest parthenolide which was 13.38 mg g^{1}, while the interaction treatment S3UV0 recorded the lowest concentration (1.192 mg g^{1}) of parthenolide.

The importance of the application of sugar to the nutrient medium comes from the fact that the photosynthesis process carried out by the plant and cultivated part doesn't meet the plant's growth requirement, as growth is limited by the applied sugar, In addition, the CO2 concentration in the cultivation tubes is increased more than the required level which causes an obstruction to the carbonation process (Fahmy 2003). Since sucrose is the source of carbohydrates, the role is essential in providing cells with the energy required

Table 1. Effect of irradiation, type and concentration of sugars (Sucrose, glucose and fructose) branch's length, number and chlorophyll of *Tanacetum parthenium* L.

Treatment	Length of vegetative branches (cm)	Number of vegetative branches	Chlorophyll (mg 100 g^{-1} fresh)
S_1	2.100	10.65	10.31
S_2	1.325	7.85	6.010
S_3	0.863	5.60	4.290
G_1	1.650	5.60	13.91
G_2	1.125	3.45	9.430
G_3	0.875	2.00	7.469
F_1	1.723	4.90	10.42
F_2	1.260	3.30	7.137
F_3	0.950	2.25	4.909
L.S.D 0.05	0.264	0.898	0.473
UV_0	1.141	3.87	6.849
UV_1	1.497	6.27	9.579
L.S.D 0.05	0.124	0.423	0.225
S_1 UV_0	1.650	8.00	10.21
S_2 UV_0	1.250	5.90	5.468
S_3 UV_0	0.600	3.40	3.142
G_1 UV_0	1.500	4.70	11.77
G_2 UV_0	0.950	2.90	7.491
G_3 UV_0	0.850	1.80	5.493
F_1 UV_0	1.595	3.90	9.078
F_2 UV_0	1.070	2.40	5.183
F_3 UV_0	0.800	1.81	3.799
S_1 UV_1	2.550	13.30	10.41
S_2 UV_1	1.400	9.80	6.552
S_3 UV_1	1.225	7.80	5.437
G_1 UV_1	1.800	6.50	16.06
G_2 UV_1	1.300	4.00	11.36
G_3 UV_1	0.900	2.20	9.500
F_1 UV_1	1.850	5.90	11.77
F_2 UV_1	1.450	4.20	9.091
F_3 UV_1	1.100	2.70	6.019
L.S.D 0.05	0.374	1.270	0.676

for growth and which is reflected on the vegetative and physiological parameters (Table 1 and Table 2). These results are in agreement with Karam et al (2003). The applied sugars also had a role in increasing and decreasing the secondary compounds in the secondary part (Table 2). The increased concentrations of sugars in the nutrition medium has decrease the production of secondary compounds. This may be due to the increment in the applied concentration that changes the water relations of the cells, which causes an increase in the pressure applied on the cells, which requires the cells to reorganize their osmotic potential to adapt to the new conditions to which the cells are exposed, because water increases the entry of solutes into the cell and then its fullness (Yao 2003). This indicates that sucrose is applied to the tissue culture medium at concentrations range of 2-3% (Feitosa et al 2007) or it may be attributed to the decrease in the production of secondary compounds at high concentrations of sugars, which is caused by a deficiency in secondary metabolism due to the decrease in the effectiveness of the enzymes responsible for building secondary metabolism (Abdul Qadir et al 1982). This can increased stress that may cause a decrease in the cells ability to absorb the nutrients they need and with high efficiency to produce secondary metabolites, and then decrease the production of secondary metabolism, which is the final product of primary metabolism (Kitamura 1998). The sugar applied to the medium is a source of energy and it must exist in appropriate concentration which is highly significant factor for plant cells, as it increases the production of secondary compounds (Yao 2003).

Table 2. Effect of irradiation, type and concentration of sugars (Sucrose, glucose and fructose) on the fresh and dry weight and parthenolide concentration of leaf *Tanacetum parthenium* L.

Treatment	Soft weight (g)	Dry weight (g)	Parthenolide concentration (mg g^{-1})
S_1	0.803	0.041	7.084
S_2	1.406	0.180	3.563
S_3	0.275	0.019	1.891
G_1	0.570	0.034	3.933
G_2	0.840	0.049	7.694
G_3	0.053	0.0166	2.731
F_1	0.033	0.012	4.427
F_2	0.135	0.043	9.911
F_3	0.049	0.014	2.440
L.S.D 0.05	0.069	0.0014	0.508
UV_0	0.313	0.019	3.563
UV_1	0.612	0.071	6.143
L.S.D 0.05	0.032	0.006	0.239
S_1 UV_0	0.391	0.018	6.604
S_2 UV_0	0.843	0.021	1.951
S_3 UV_0	0.222	0.011	1.192
G_1 UV_0	0.416	0.026	2.672
G_2 UV_0	0.783	0.034	6.252
G_3 UV_0	0.012	0.008	1.642
F_1 UV_0	0.022	0.010	3.497
F_2 UV_0	0.095	0.033	6.441
F_3 UV_0	0.033	0.011	1.814
S_1 UV_1	1.215	0.065	7.563
S_2 UV_1	1.968	0.339	5.175
S_3 UV_1	0.328	0.027	2.589
G_1 UV_1	0.723	0.043	5.194
G_2 UV_1	0.897	0.063	9.173
G_3 UV_1	0.093	0.021	3.819
F_1 UV_1	0.045	0.014	5.357
F_2 UV_1	0.176	0.053	13.38
F_3 UV_1	0.065	0.018	3.066
L.S.D 0.05	0.097	0.002	0.719

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Effect of Herbicides on Weeds and Yield in Wheat Using Different Spray Nozzles

Salma Namik Jalil Khader, Hussain T. Tahir and Abdul Mueen Shabeeb Hamad[1]

College of Agriculture, Kirkuk University, Iraq
[1] College of Agriculture, Hawija Kirkuk University, Iraq
E-mail: Salmajalil53@gmail.com

Abstract: The field experiment was carried out during 2020-2021 at the Agricultural Research Station of the College of Agriculture, University of Kirkuk, located in Sayada, in order to observed the effect of two types of weed herbicides (Topik + Granestar and Pallas OD45) by using two types of spray nozzles (conical and flat) on weeds associated with wheat. The experiment was according to the split-split-plot system, randomized complete sectors design where the main plots included two types of wheat (Aba 99 and Buhooth 22) and sub treatments included herbicides and sub- sub treatments two types of nozzles (conical and flat) . There were no significant differences between the two wheat cultivars (Aba 99 and Buhooth 22) in the characteristics of the total number of weeds and grain yield. Significant differences were observed in the dry weight of weeds, where the cultivar Abaa 99 outperformed and the average dry weight of weeds was 66.40 kg/ dunum. The conical nozzle was superior in the dry weight of weeds (64.74 g), as well as in the grain yield, with highest yield of 754.17 kg/ dunum. There were significant differences in the number of total weeds in the interaction(between varieties and herbicides), as the herbicide Pallas outperformed the two cultivars Ibaa 99 and Buhooth 22, as well as the superiority of the same interaction in the dry weight trait of Pallas herbicide in the Ibaa 99 cultivar. The grain yield in Pallas herbicide was higher in Buhooth class 22, (1125.8 kg/ dunum). The conical nozzles outperformed the flat nozzles in CV characteristics. Conical nozzles are superior in achieving a lower value of the coefficient of variation than flat nozzles (0.092 and 0.112 respectively). The conical nozzle outperformed in the characteristic of the regularity coefficient and achieved higher percentage of homogeneity than the flat nozzle, which were 91.81 and 90.012 percent respective)y. Field efficiency did not show any no significant difference between the conical and flat nozzles (62.3 and 61.6%, respectively).

Keywords: Wheat, Nozzles, Herbicides, Pallas, Topik + Granestar, Conical nozzle, Flat nozzles

The wheat crop (*Triticum aestivum* L.) is the strategic crop in the world and Iraq due to its importance as a major source of food and its role in economic and social development. Despite the strategic importance of this crop in Iraq, the yield is much lower than global production (Ahmad et al 2005). The different types of weeds in wheat fields in Iraq, especially in irrigated areas, and their intense competition for this crop is one of the most important reasons for the decline in production. On washing from the surface of leaf due to photodegradation and chemical degradation may remain in the form of viscous or crystalline liquids which is not absorbed (Alan 2008). The bush weed is one of the most important problems facing wheat cultivation and production in Iraq, as crop plants compete with high efficiency for the most important growth requirements such as water, light, location and nutrients, especially in the early stages of their growth, and as a result of this competition. The growth of the wheat crop is weak and causes a loss in yield ranging from 30-50% and sometimes 70, depending on the density of weeds (Shati and Al-Ami 2010)). There are more than 12 species of thin-leaved bush and 16 species of broad-leaved bush spread within the wheat crop in Iraq. These plants compete with crops for nutrients, water, light and other determinants of growth, and these factors all lead to lower yields and lower quality (Cheem and AKhtar 2005). Various weed herbicides have been used to limit the growth of the weed and its spread in the wheat fields, and they gave positive results and caused an increase in the yield by a large percentage. However, the excessive use of herbicides in a large way led to the presence of some problems in the environment, in addition to the fact that following one method in managing the weed is not efficient in most of the cases. It is preferable to apply integrated management in bush control, including employing one or more control methods in order to achieve economic and control of the weed (Kong et al 2009). The study also showed the presence of variation in the competitiveness of wheat varieties for weeds (Al-Akidi 2010). Many weed developed resistance to many herbicide or may not control different weed at same time. Therefore, it became necessary to search for mixtures of herbicides to combat the weed in one spray in order to increase the efficiency and effectiveness of control operations on the one hand, and reduce their environmental impacts on the other hand (Abadi 2010). This research aims to know the response of the

accompanying weeds to some cultivars of wheat (Ibaa 99, Buhooth 22) of mixtures of the herbicides Pallas and Topik + Granestar on growth, yield and quality by using two types of nozzles.

The most widely used type of equipment for spraying herbicides is the spray arm and that about (90%) of the herbicides are manufactured for use in spraying, and hydraulic sprinklers use water or any other material to carry the herbicide to the application site to be sprayed (Nasada and Justin 2014). The choosing the nozzle and the appropriate action is one of the keys to a successful weed control, as there are many sprays available for the application of weed killers, and each type is designed for a specific use and is also designed to be used in a specific way. The jets work to control the flow, the formation of the droplets and the diffusion of the droplets in a certain way, and these functions are affected by several factors that affect the flow rate of the jets, the size of the jet hole, pressure and specific weight of spray solution and viscosity of the spray solution (YorK 2008).

MATERIAL AND METHODS

Field experiment was carried conducted at the Agricultural Research and Experiment Station of the College of Agriculture , University of Kirkuk in Al-Sayada complex during the winter season 2020-2021 in order determine response of weeds and some cultivars of wheat (Ibaa 99 and Buhooth 22) to the mixtures of chemical herbicide (Topik+ Granestar and Pallas 45 OD herbicide). The experiment was according to the split-split-plot system, randomized complete sectors design and with three replications, where the main plots included two types of wheat (Aba 99 and Buhooth 22) and sub treatments included herbicides and sub- sub treatments two types of nozzles (conical and flat). There were 12 treatments each replicated thrice units. Then, the physical and chemical properties were examined (Table 1). The seed rate was 35 kg/ dunum and using two types of wheat, namely Ibaa 99 and Buhooth 22 approved by the Iraqi Ministry of Agriculture. The 80 kg/dunum containing NPK was added (N=18%, P=46%, K= 0%) in one batch. The crop was sown on November 11, 2020. All agricultural operations were carried out as needed according to the recommendations and crop was harvested on May 11, 2021.

Observations

Total number of weeds: The number of weeds was calculated after 30 days (after the spraying of weed herbicides (Pallas 45 OD, Topik+ Granestar).

Dry weed weight/m^2: The weeds were cut at harvest at the level of the soil surface and dried in an electric oven at a temperature of 70°C until stability weed dry weight. The percentage of inhibition was estimated (Al-Chalabi 2003)

Grain yield/donm: Samples were harvested from a square meter area, and then separated the straw and weighing of grains was done.

Coefficient of variation cv%: This was calculated using the following equation (FAO 2001):

CV = SD/qm

Whereas: CV: coefficient of variation %, SD: standard deviation, qm: average sample discharges.

Field efficiency: It is the ratio between the field capacity over the theoretical field capacity (Sahay 2008).

Field efficiency = (Actual field capacity)/(Theoretical field capacity) x 100

Table 1. Effect of cultivars, herbicides, spray nozzles on the total number of weeds

Varieties	Herbicides	Nozzles		Varieties × Herbicides	Herbicides	Varieties
		Conical	Flat			
Ibaa 99	Control	95.00a	94.00a	94.50a		
	Topik+ Granestar	11.66b	8.66bc	10.16b		
	Pallas 45 OD	7.33bc	5.00c	6.16c		
Buhooth 22	Control	93.66a	94.00a	93.83a		
	Topik+ Granestar	7.33bc	4.66c	6.00c		
	Pallas 45 OD	7.33bc	6.33bc	6.83bc		
Herbicides × Nozzles	Control	94.33a	94.00a		94.16a	
	Topik+ Granestar	9.50b	6.66bc		8.08b	
	Pallas 45 OD	7.33bc	5.66c		6.50b	
Varieties × Nozzles	Ibaa 99	38.00a	35.88ab			36.94 a
	Buhooth 22	36.11ab	35.00b			35.55 a
Nozzles		37.05a	35.44a			

Similar letters indicate no differences at the 5% probability level

Uniformity coefficient of water distribution UC (%): The uniformity parameter was measured by collecting a given volume of water falling from the nozzle at fixed points with fixed distances (Christiansen 1942).

$$UC = \left(1 - \frac{\Sigma_1^n (xi - \bar{x})}{nx}\right) \times 100$$

RESULTS AND DISCUSSION

Effect of cultivars, herbicides and spray nozzles on number of weeds: There were no significant differences between the two wheat cultivars (Ibaa 99 or Buhooth 22) and herbicides and nozzles in this characteristic. The interaction between the cultivars indicated differences between the interaction of the weedicide with the cultivars, where the Pallas 45 OD outperformed the mixture Topik + Granestar in Buhooth 22. The interaction of cultivars with the nozzles used in spraying, indicate that the conical nozzle was superior in Buhooth 22, as the percentage of control in the second flat nozzle reached 94,66%. The triple interaction between cultivars, herbicides, and spray nozzles on the number of weeds showed that there are significant differences where the herbicide Pallas was superior to the flat nozzle in the cultivar Buhooth 22. These results are in agreement with Benegas (1998) and Mishra and Singh(2012).

Effect of cultivars, herbicides and spray nozzles on the dry weight of weeds: Effect of cultivars and herbicides on the weight of the weeds, showed the superiority of Ibaa 99 over Buhooth 22 Effect of spray nozzles indicate a significant difference between the two types of nozzles, where the flattening nozzle with average dry weight of 54.56 g, was superior. The interaction between the cultivars and herbicides was significant differences, where Pallas in Buhooth 22 was superior to the Topik + Granestar in the cultivar Ibaa 99 where the average dry weight of the weed was 25.96 and 21.32 g, respectively. The interaction between herbicides and spray nozzles, indicate that flat nozzle with Pallas was superior to Topik + Granestar.

Effect of cultivars, herbicides, spray nozzles on grain yield (kg/donum): There was no significant differences between the cultivars in the yield trait, as well as the pesticides did not differ significantly in the grain yield but differed from the comparison treatment. There was no significant difference in two types of spray nozzles. The interaction of cultivars and nozzles, showed that significant differences between the treatments, where the Iba 99 variety by using flat nozzles excelled over control, which did not differ significantly from Bohouth 22 in the use of both types of spray nozzles, with the highest mean of 763.56 kg/ dunum. Al-Hayani (2009) and Al-Jalabi and Al-Akidi (2010) indicated that the use weedicides in controlling the wheat bush leads to an increase in the grain yield. The interaction of varieties and pesticides, showed no significant differences between the pesticides and for both types but were superior to comparison. The interaction of pesticides and spray nozzles indicated significant differences between the treatments where the mixture of Topik + Granestar excelled by using the flat nozzle. This can be attributed to rapid flowing from the nozzle. The triple interaction between the three factors (varieties, pesticides and spray nozzles showed the

Table 2. Effect of cultivars, herbicides and spray nozzles on the dry weight of weeds (gm)

Varieties	Herbicides	Nozzles		Varieties × Herbicides	Herbicides	Varieties
		Conical	Flat			
Ibaa 99	Control	134.9a	129.3ab	132.10a		
	Topik+ Granestar	41.6d	22.3ef	32.01cd		
	Pallas 45 OD	42.7d	27.5ef	35.10c		
Buhooth 22	Control	123.4b	99.4c	111.41b		
	Topik+ Granestar	21.12.ef	30.8e	25.96ed		
	Pallas 45 OD	24.66ef	17.9f	21.32e		
Herbicides × Nozzles	Control	129.15a	114.36b		121.75a	
	Topik+ Granestar	31.40c	26.58cd		28.99b	
	Pallas 45 OD	33.68c	22.74d		28.21b	66.40a
Varieties × Nozzles	Ibaa 99	73.09a	59.71b			52.90b
	Buhooth 22	56.39b	49.40c			
Nozzles		64.74a	54.56b			

Similar letters indicate no differences at the 5% probability level

superiority of the Aba 99 class by using Topik + Granestar with flat nozzle, which did not differ significantly from Bohouth class 22 and Pallas with flat nozzle.

Sprayer related characteristics: Figures 1 and 2 show the pattern of spray distribution for conical and flat nozzles, respectively. The conical nozzle is closer to homogeneity than the flat nozzle (0.0921 and 0.112%, respectively) which was reflected on the spraying pattern (Fig. 4). The two patterns reflect the deterioration of the spray pattern, which causes erosion and significant unevenness in the distribution of the pesticide and is consistent with observations of Bolat and Ozluovmak (2020).

The effect of a winged spray machine and two types of conical CO and flat FL nozzles on some characteristics

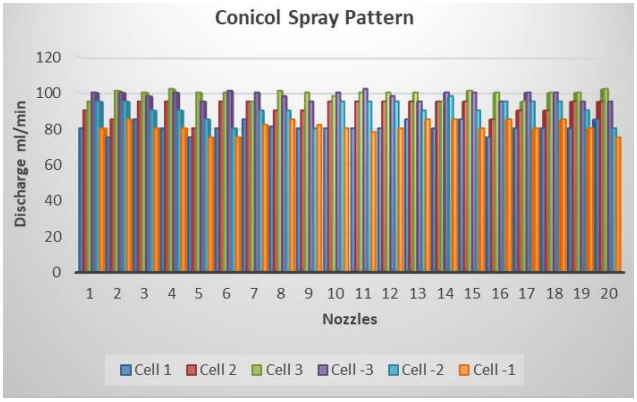

Fig. 1. Spray pattern of conical type nozzles

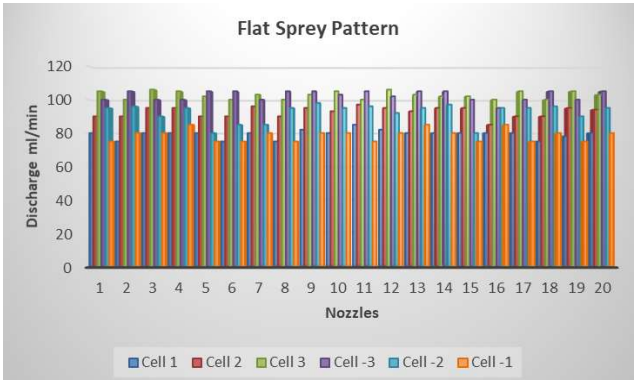

Fig. 2. Spray pattern of flat type nozzles

Table 3. Effect of cultivars, pesticides, spray nozzles on grain yield (kg/dunum)

Varieties	Herbicides	Nozzles		Varieties × Herbicides	Herbicides	Varieties
		Conical	Flat			
Ibaa 99	Control	137.67c	152.33c	145.00b		
	Topik+ Granestar	913.67b	1125.00a	1019.33a		
	Pallas 45 OD	1013.33ab	1013.33ab	1013.33a		
Buhooth 22	control	127.33c	135.33c	131.33b		
	Topik+ Granestar	1028.33ab	1022.33ab	1025.33a		
	Pallas 45 OD	1018.33ab	1035.33ab	1026.83a		
Herbicides × Nozzles	Control	132.50c	143.83c		138.17b	
	Topik+ Granestar	971.00b	1073.67a		1022.33a	
	Pallas 45 OD	1015.83ab	1024.33ab		1020.08a	
Varieties × Nozzles	Ibaa 99	688.22b	763.56a			725.89a
	Buhooth 22	724.67ab	731.00ab			727.83a
Nozzles		706.44a	747.28a			

Similar letters indicate no differences at the 5% probability level

Table 4. Effect of a winged spray machine and nozzles on some characteristics related to spraying

Characteristics	Nozzle type		Calculated T value	P. value
	Conical	Flat		
Coefficient of variation CV (%)	0.09212064	0.11248729	-4.96	0.0025
Uniformity coefficient UC (%)	91.8171328*	90.0182398	3.12	0.0207
Field efficiency FE (%)	62.3	61.6	1.21	0.2709

* Denotes that there are significant differences between the two averages
The tabular value of the T-test corresponding to the degree of freedom 4 and the probability level of 0.05 = 2.77
P: Significant differences in the T-test analysis at the 0.05 probability level

related to spraying are given in Table 4. There was no statistically significant difference but the conical nozzle is considered superior in achieving a lower value for the coefficient of difference than the flat nozzle (0.09212064 and 0.11248729, respectively) and this is consistent with recommendation of FAO (2001) as it should not increase the coefficient of variation about 10%. There was significant difference in the characteristic of the uniformity coefficient, as the conical nozzle achieved a higher percentage of homogeneity than the flat nozzle (91.817% and 90.018%, respectively). There was no significant difference between field efficiency of conical and flat nozzles, which were 62.3 and 61.6%, respectively, which were equivalent to the range 55-65% within the limits recommended by Hunt (1983). Sanchavat et al (2020) emphasized need to determine the ideal operating pressure and discharge rate to reduce losses in the spray and thus increase field efficiency.

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Effect of Nitrogen Fertilization and Cutting Dates on Growth Characters and Green Forage Yield of Maize

Saadoon Jaber Haydar and Hameed K.H. Khrbeet[1]

Diyala Agriculture Directorate, Baghdad, Iraq
[1]Field Crop Sciences College of Agriculture Engineering Sciences, University of Baghdad, Iraq
**E-mail: saadoonj0@gmail.com*

Abstract: Field experiment was carried out during the spring season of 2021 at the research station of the College of Agricultural Engineering Sciences, University of Baghdad, to observe the effect of nitrogen fertilization and cutting dates on vegetative growth characters and green maize forage yield. The experiment was in randomized complete blocks design according to split plots the arrangement. The main plots included four levels of nitrogen fertilizer (0, 90, 180 and 270 kg N ha^{-1}), while the sub-plots included the three dates of cutting (60, 75 and 85 days after sowing). The adding of nitrogen fertilizer at a 270 Kg N ha^{-1} was significantly superior in the plant height (207.7 cm), stem diameter (1.61 cm), leaves number (13.4 leaves $plant^{-1}$) and green forage yield (61.40 ton ha^{-1}), while the nitrogen fertilizer at a 180 Kg N ha^{-1} was significantly superior in the leaves to stems ratio (0.62). The cutting after 85 days of sowing was significantly superior in the plant height (200.3 cm), stem diameter (1.64 cm), leaves number (13.7 leaves $plant^{-1}$) and green forage yield (65.39 ton ha^{-1}), whereas the cutting after 60 days of sowing was significantly superior in the leaves to stems ratio (0.67).

***Keywords*:** Maize fodder, Nitrogen fertilization, Cutting dates, Growth characters, Green forage yield

Maize (*Zea mays* L.) is a summer grass crop that is considered among the most important forage crops in Iraq and globally ranks third in terms of production after wheat and rice. Maize grain's is used for human and animal nutrition, especially in poultry and ruminant diets, and has an important place in the global economy and trade as a grain and feed crop (Iderawumi and Charles 2018). Maize is also used either as green fodder or made into hay or silage or its grain is used to feed animals due to its high carbohydrate and protein content. The provision of green forage is an important element in the animal nutrition especially as maize fodder , which is characterized by rapid growth, high productivity, rich in protein and carbohydrates and easy to digest by the animal. The availability of green forage contributes to reducing the costs of producing meat, milk and its derivatives (Salam 2019) but cannot meet the demand of animal nutrition. Therefore, maize can contribute to reducing the problem of the shortage of green forage, especially in the summer period, if this is accompanied by the use of scientific methods in production Al-Jubouri and Anwar (2009) mentioned an increase in the leaves / stems ratio at a high level of nitrogen fertilizer. Shehzad et al (2012) indicated that there were significant differences in stem diameter and green forage yield when the levels of nitrogen fertilization increased, as well as an increase in stem diameter and green forage yield whenever cutting time was delayed. Shaalan et al (2015) found that there was a significant increase in plant height when the nitrogen fertilizer was added, as well as at the cutting period after 75 days of sowing. Woldesenbet and Haileyesus (2016) observed increased the leaf number of maize at higher nitrogen level. Karki (2020) showed a significant increase in the number of leaves after 90 days of emergence. Therefore, study aims to observe the effect of nitrogen fertilization and cutting dates on vegetative growth characters and green forage yield of forage maize.

MATERIAL AND METHODS

Field experiment was carried out during the spring season of 2021 at the research station of the College of Agricultural Engineering Sciences, University of Baghdad which is located at 44° east longitude and 33° north latitude. Soil samples were randomly collected from the experimental land before sowing at a depth of 30 cm to analyze some physical and chemical characters of soil (Table 1). A randomized complete blocks design according to split plots the arrangement at three replications was used. The main plots included four levels of nitrogen fertilizer (0, 90, 180 and 270 kg Nha^{-1}) symbol as N0, N1, N2 and N3 respectively, while the sub-plots included the three dates of cutting (60, 75 and 85 days after sowing), symbol as (D1, D2 and D3) respectively. The experimental field was divided into 36 experimental unit sand the area of each experimental unit was 5 m^2 (2.5 m long x 2 m width) which contained 8 lines, 25 cm apart. The seeds of maize Baghdad-3 cultivar were sown

in mid-March 2020 at rate of 80 kgha^{-1}. Nitrogen fertilizer was added according to the treatments as a urea (46% N) at two doses, the first dose after two weeks of emergence and second dose a after 30 days of the first dose, whereas phosphate fertilizerwas added as a triple superphosphate (45% P_2O_5) at an average of 100 kg P ha^{-1} before sowing. Crop service operations were conducted as needed.

Observations

Plant height (cm): Plant height was measured from the soil surface until the end of the vegetative growth of the first cutting stage, while it was measured from the soil surface to tassel of the second and third cutting stagesby randomly taking of five plants at each stage of cutting.

Stem diameter (cm): Stem diameter was measured from the middle of the first internode above the soil surface by randomly taking of five plants at each stage of cutting.

Leaves number plant^{-1}: The total leaves number was calculated from the first leaf at the soil surface to the last leaf by randomly taking offive plants at each stage.

Leaves / stems ratio: It was calculated based on dry weight of five plants that were randomly taken. Leaves were separated from the stems and then the leaves weight divided into the stems weight.

Table 1. Physical and chemical soil properties in spring season (2021)

Trait	Value	Unit
Sand	240	g kg soil^{-1}
Loam	260	
Clay	400	
Texture	Clay loam	
Ec 1:1	1.2	ds m^{-1}
pH 1:1	7.31	-----
Available N	26.0	mg kg Soil^{-1}
Available P	3.24	
Available K	145.3	

Green forage yield (ton ha^{-1}): The plants of the two middle lines were cut of each experimental unit, directly weighed and the green forage yield was calculated.

Statistically analysis: The data were statistically analyzed for all the studied traits by using Gnestat program.

RESULTS AND DISCUSSION

Plant height (cm): The nitrogen fertilizer levels significantly differed in the plant height, N3 treatment (270 Kg N ha^{-1}) gave a highest mean 207.7 cm with non-significant difference with N2 treatment (180 Kg N ha^{-1}) while N0 treatment (0 Kg N ha^{-1}) gave a lowest ١٥٩.1 cm (Table 2). The increase may be due to the role of nitrogen in increasing chlorophyll content of leaves of thus increasing the rates of photosynthesis, which positively reflected on the division and expansion of cells and then increased the length of the internodes. In addition to, an increase the root hairs, thus increasing their ability to absorb mineral elements and water from the soil resulting in increase of plant height. These results are in agreement with Gaire et al (2020) and Adhikari et al (2021). There were significant differences among cutting dates in the plant height. D3 treatment (cutting after 85 days of sowing) achieved maximum height of 200.3 cm compared with D1 (cutting after 60 days of sowing) (174.1 cm).The difference among cutting date treatment may be due to the fact that the plant increases in height as progresses in the growth stage, i.e. there is an opportunity of the plant to continue growing and elongate the internodes as a result of the availability of suitable conditions for growth. These results are in agreement with Shaalan et al (2015) and Paul et al (2019). The interaction between two factors had a significant effect on the plant height and this could be attributed to the difference in the relative response to the effect of nitrogen fertilizer and the cutting dates on plant height. The response at the D1 treatment (cutting after 60 days of sowing) was low and was increased when the levels of fertilizer increased and the cutting dates were delayed.

Table 2. Effect of nitrogen fertilization and cutting dates on plant height (cm)

Nitrogen fertilizer (Kg ha^{-1}) (N)	Cutting dates (D)			Mean (N)
	60 (D1)	75 (D2)	85 (D3)	
0 (N0)	134.5	169.8	172.7	159.0
90 (N1)	173.1	198.0	201.3	190.8
180 (N2)	191.8	201.5	210.7	201.3
270 (N3)	197.0	209.5	216.8	207.7
LSD (p=0.05)		11.03		8.12
Mean (D)	174.1	194.7	200.3	
LSD (p=0.05)		5.36		

Stem diameter (cm): The nitrogen fertilizer levels significantly differed in the stem diameter, N3 treatment (270 Kg N ha^{-1}) gave a highest mean of ١.61 cm with non-significant difference with N2 treatment (180 Kg N ha^{-1}) while N0 treatment (0 Kg N ha^{-1}) gave a lowest 1.48 cm with non-significant difference with N1 treatment (90 Kg N ha^{-1}) with height of 1.52 cm (Table 3). The increase may be due to the role of nitrogen in the division and expansion of cells, which leads to an increase the stem diameter, which is an important for increasing the yield of dry matter and the resistance of the plant to lodging. These results are in agreement with Awadalla and Morsy (2016). There were significant differences among cutting dates in the stem diameter. D3 treatment (cutting after 85 days of sowing) recorded highest mean 1.64 cm compared with D1 treatment (cutting after 60 days of sowing) which recorded lowest of ١.44 cm. The increase in the stem diameter when the cutting date is delayed may be due to the availability of sufficient opportunity for plant growth, thus increasing the division and expansion of cells, which leads to an increase the stem diameter. These results are in agreement with Sangma et al (2017).

The interaction between two factors had a significant effect on the stem diameter. The reason could be attributed to the difference in the relative response to the effect of the nitrogen fertilizer levels and the cutting dates on stem diameter. D1 treatment (cutting after 60 days of sowing) recorded minimum stem diameter but the response was increased when the levels of fertilizer increased and the cutting dates were delayed.

Leaves number $plant^{-1}$: The nitrogen fertilizer levels significantly differed in the leaves number per plant, N3 treatment (270 Kg N ha^{-1}) achieved highest mean of 13.4 leaf $plant^{-1}$ compared with N0 treatment (0 Kg N ha^{-1}) which achieved lowest of 10.7 leaf $plant^{-1}$(Table 4). The increase the number of leaves when increasing the levels of nitrogen fertilizer may be due to the role of nitrogen in encouraging vegetative growth by increasing the activity of cell division and expansion, which leads to the elongation of the internodes of the developing nodes under the surface of the soil and their appearance above the surface of the soil and an increase the nodes number increase the leaves number per plant. These results are in agreement with Afolabi et al (2020). There were significant differences among cutting dates in the leaves number per plant. D3 treatment (cutting after 85 days of sowing) recorded highest mean of 13.7 leaf $plant^{-1}$compared with D1 treatment (cutting after 60 days of sowing) which achieved a lowest of ١٠.1 leaf $plant^{-1}$. These results are in agreement with Paul et al (2019). The interaction between nitrogen fertilizer levels and cutting dates wasn't significant on the number of leaves per plant.

Table 3. Effect of nitrogen fertilization and cutting dates on stem diameter

Nitrogen fertilizer (Kg ha^{-1}) (N)	Cutting dates (D)			Mean (N)
	60 (D1)	75 (D2)	85 (D3)	
0 (N0)	1.41	1.52	1.51	1.48
90 (N1)	1.43	1.53	1.61	1.52
180 (N2)	1.46	1.59	1.70	1.58
270 (N3)	1.46	1.65	1.73	1.61
LSD (p=0.05)		0.08		0.07
Mean (D)	1.44	1.57	1.64	
LSD (p=0.05)		0.02		

Table 4. Effect of nitrogen fertilization and cutting dates on leaves number $plant^{-1}$

Nitrogen fertilizer (Kg ha^{-1}) (N)	Cutting dates (D)			Mean (N)
	60 (D1)	75 (D2)	85 (D3)	
0 (N0)	8.9	11.4	11.8	10.7
90 (N1)	9.8	12.3	14.0	12.0
180 (N2)	10.5	13.1	14.3	12.6
270 (N3)	11.1	14.2	14.9	13.4
LSD (p=0.05)		NS		0.39
Mean (D)	10.1		13.7	
LSD (p=0.05)		0.50		

Leaves / stems ratio: The nitrogen fertilizer levels significantly differed in the leaves / stems ratio. N2 treatment (180 Kg N ha^{-1}) recorded highest mean of 0.62 compared with N0 treatment (0 Kg N ha^{-1}) which hada lowest 0.53 leaf $plant^{-1}$(Table 5). The increase may be due to the role of nitrogen in increasing the leaf area and raising the efficiency of photosynthesis, which increased the plant's ability to grow and thus increase the plant height (Table 2), stem diameter (Table 3) and leaves number per plant (Table 4) which positively reflected on the increase the leaves to stem ratio. These results are in agreement with Nabooji et al (2018). There were significant differences among cutting dates in the leaves / stems ratio. D1 treatment (cutting after 60 days of sowing) gave highest mean of 0.67compared with D3 treatment (cutting after 85 days of sowing) ٠,٤٩(). The decrease in the leaves to stems ratio when cutting after 85 of sowing may be due to the slow vegetative growth when the plant grows old, as well as the plant's tendency towards reproductive growth, whereas at the early stages, the plant is at the top of its vegetative growth and then an increase the leaves to stems ratio. These results are in agreement with Saleh (2020). The effect of the interaction between nitrogen fertilizer levels and cutting dates was not significant on the leaves / stems ratio.

Green forage yield (ton ha^{-1}): The nitrogen fertilizer levels significantly differed in the in the green forage yield, N3 treatment (270 Kg N ha^{-1}) achieved a highest mean 61.40 ton ha^{-1} compared with N0 treatment (0 Kg N ha^{-1}) with lowest of 46.07 ton ha^{-1} (Table 6). This may be attributed to the positive effect of nitrogen fertilizer levels on increasing plant height, stem diameter and number of leaves (Tables 2, 3 and 4) which have a role in increasing the yield of green forage. Nitrogen has a high mobility within the plant, so the nitrogen feeding greatly controls the rate of plant growth during the vegetative stage, which leads to an increase in the yield of green forage. These results are in agreement with Hafez et al (2015).

There were significant differences among cutting dates in the green forage yield. D3 treatment (cutting after 85 days of sowing) recorded highest mean of 65.3 9ton ha^{-1}compared with D1 treatment (cutting after 60 days of sowing) which recorded lowest of ٣٦.78 ton ha^{-1}. The increase the green forage yield with the progression of cutting dates may be due to the provision of sufficient opportunity for the plant to grow and increasing the leaf area, which increases the efficiency of photosynthesis and thus increases the plant height, leaves number and the stem diameter (Tables 2, 3 and 4), which leads to an increase the vegetative size of the plant and then an increase the green forage yield. These results are in agreement with Shaalan et al (2015).

Table 5. Effect of nitrogen fertilization and cutting dates on leaves/stems

Nitrogen fertilizer (Kg ha^{-1}) (N)	Cutting dates (D)			Mean (N)
	60 (D1)	75 (D2)	85 (D3)	
0 (N0)	0.60	0.54	0.46	0.53
90 (N1)	0.65	0.56	0.48	0.56
180 (N2)	0.70	0.62	0.54	0.62
270 (N3)	0.72	0.59	0.49	0.60
LSD (p=0.05)		NS		0.023
Mean (D)	0.67		0.49	
LSD (p=0.05)		0.025		

Table 6. Effect of nitrogen fertilization and cutting dates on green forage yield

Nitrogen fertilizer (Kg ha^{-1}) (N)	Cutting dates (D)			Mean (N)
	60 (D1)	75 (D2)	85 (D3)	
0 (N0)	33.10	51.37	53.73	46.07
90 (N1)	35.27	60.27	61.57	52.37
180 (N2)	37.53	62.03	70.70	56.76
270 (N3)	41.23	67.40	75.57	61.40
LSD (p=0.05)		5.02		3.08
Mean (D)	36.78		65.39	
LSD (p=0.05)		2.68		

The interaction between two factors had significant effect on the green forage yield and could be attributed to the difference in the relative response to the effect of the nitrogen fertilizer levels and the cutting dates of green forage yield.

CONCLUSION

There was a high response of the maize crop to the high levels of added nitrogen, as the growth indicators and the yield of green fodder increased as a result of the added nitrogen levels and delaying the date of cutting plants to 85 days from planting was the best in obtaining the highest yield of green fodder. This study may give an indication of the possibility of maize responding to nitrogen fertilization levels higher than 270 kg ha^{-1}, or delaying the cutting date to more than 85 days.

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Determine Genetic Distance among Some Okra (*Abelmoschus esculentus*) types using SCot Markers in Iraq

Melath Kadhum Farhood AL- Ghufaili and Attyaf, Jameel Thamir Al-Tamimi

Faculty of Science, University of Kufa, Iraq
E-mail: melathkadhum1@gmail.com

Abstract: Seven molecular markers SCoT (Start Codon Targeted) were used to find genetic diversity, genetic relationship, and DNA fingerprint for nine Okra genotypes. Primers varied among them in giving unique DNA fingerprints. The low genetic distance was 0 between Hasnawia and Soutl but the high genetic distance was 0.28862 between (Lahluba and Zasco seed and Lahluba and Egypt. Cluster analysis (Phylogenetic tree) grouped studied genotypes into two main clusters. SCoT marker could reveal a genetic relationship in studied genotypes according to their origin, thus it gave an excellent tool to breeding programs help breeders.

Keywords: Okra, SCoT markers, Genetic diversity, Dendrogram, Cluster analysis

Okra (*Abelmoschus esculentus*) is a dicotyledonous herb of Malvaceae family. It is a diploid with somatic chromosome numbers 2n. Okra is a healthy vegetable with lots of fibers and folate (Bello et al 2015 and Fufa, 2019). A fragment of DNA knows as DNA markers are used to indicate mutations/variations, help to find out polymorphism between alleles of a gene for a private series of DNA or various genotypes. Such fragments are detected by using particular molecular technology when it linked with a critical site within the genome (Lateef 2015). The markers in recent years are used to determined genetic* diversity*, such as Amplified Fragment*Length*Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), SCoT (start codon targeted marker) and DNA amplification finger (DAF), (Yuan et al 2015). SCoT is the dominant markers developed by Collard and Mackill (2009) from transcribed and conserved regions flanking the initiation codon (the ATG start codon) and sequences of genes (Penedo et al 1999, Luo et al 2014, Nouairia et al 2015). The SCoT Polymorphism technique is similar to RAPD (Randomly Amplified Polymorphic) DNA and ISSR (Inter Simple Sequence Repeat) because it uses a single forward and reverses primer. SCoT markers are expected to be linked to functional genes and corresponding traits, (Bhattacharyya et al 2013). This marker technique uses 18-mer single primer in PCR and an annealing temperature of at 50C, and PCR products are resolved using standard agarose gel electrophoresis. The primers are very easy to design based on the preserved region surrounding the translation initiation codon without the requisites of genomic sequence information (Joshi et al 1997, Milbourne et al 1997, Sawant et al 1999). SCoT markers are generally highly reproducible, targeted marker and could be used in studying cultivars genetic relationship, the SCoT markers were employed to target the polymorphism in sequences near the genes (Gorji et al 2011, Nouairia et al 2015, Vivodik et al 2016, Vivodik et al 2017, Sadek and Ibrahim, 2018). This is possible to use directly in marker-assisted breeding programs (Mulpuri et al 2013) and application study of genetic diversity in crop plants i.e., date palm (AL-Qurainy et al 2015), summer squash (Xanthopoulou et al 2015), durum wheat (Etminan et al 2016), grape variety discrimination (Miro et al 2017), pistachio (Baghizadeha and Dehghan, 2018) and maize (*Zea mays* L.) (Al-Tamimi (2020).The exploitation of genetic variability in the germplasm of any crop species is considered the key point for making further genetic improvement in economically important traits. Production and evolution of high-yielding and well-adapted cultivars with desirable characters generally remain the prime objective of plant breeding programs (Al-Tamimi 2020). The information based on DNA marker and their consequence variation would be of huge pleasure in okra cultivation programs facts to limit the genetically identical and morphology analogous plant variety or not (Duzyaman 2005).

MATERIAL AND METHODS

Plant genotypes: Okra genotypes seeds were procured from the local market and were soaked overnight and then planted on a 25m x 10m plot of land using the randomized complete block design with three replications at Department of Horticulture and Forests of the Ministry of Agriculture in Najaf Governorate. Their pedigree is shown in Table (1).

Okra germplasm and procedure: DNA extraction and PCR amplification: nine Okra genotypes with diverse characterization and pedigree were used for DNA extraction **(table.1)**. Powder dry leaves were used for DNA extraction using I-genomics plants (DNA extractions Mini Kit). Intron Biotechnology/ Korea.

Polymerase chain reaction (PCR) and amplification: PCR reaction mixture was prepared as follows: 5µl template DNA and5 µl of primer (10 pmole/µl), 5 µl Sterilized deionized distilled water was added to AccuPower® TLA PCR PreMix tubes to the final volume of (20 µl)., seven SCoT markers were examined for fingerprinting genotypes as listed in Table 2. Amplification was performed in thermos cycler programmed according to annealing temperatures produced in Table 3. The amplified DNA product was separated by electrophoresis on 1.2 % agarose gels stained with ethidium bromide (3 µl of ethidium bromide solution) electrophoresis runs for 3-4 hr at 70V and then visualized under UV light and photographs.

Statistical analysis: The presence of a product was identified as (1) and absence was identified as (0). Data were scored for all genotypes, their amplification reproduction, and primers. The result then scrod into NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), Version 1.8 (Applied Biostatistics) program [19].

A Dendrogram design was based on genetic distance: genetic distance (GD) =1- genetic similarity (GS) using the Unweighted Pair-Group Method with Arithmetical Average (UPGMA).

RESULTS AND DISCUSSION

Using Bio drop apparatus the concentration of isolated DNA was 75.92µg/ml with purity 1.9 (Fig. 2). SCoT profile results showed variation among studied genotypes through the presence of monomorphic, polymorphic and unique bands. Primer SCoT23 gives a unique fingerprint for seven genotypes (Batra, Lahluba, Hasnauia, Musiliaa, Khnisiraa, Houseagrl seed, Okra hybrid F1and Soutl), and primers SCot39, SCoT61 give unique fingerprint for one genotype (Khnisiraa and Lahluba) respectively. Primers SCoT9 and SCoT59 failed to give a unique fingerprint. The primer (SCoT23) gave the higher value for polymorphism was (76.923%). This showed the ability of this primer to reveal genetic variation among individual germplasm. Primers differ in their ability to recognize different regions (annealing sites) in the genome. Generally, the primers which recognize more annealing sites are considered more useful for genetic polymorphism studies than the primers recognizing a low number of annealing sites. The recognition of more annealing sites will result in the production of a high number of amplified fragments, and thus high chances of polymorphism among individuals of a population (Al-Tamimi 2020), and primer SCoT61 give the lower value of polymorphism (50%). This low polymorphism might be due to the close pedigree and geographical origin of the studied genotypes (Fig. 3-6). The higher molecular size was1709 bp in primer SCoT61 and the lower molecular size was 140 in primer SCoT9 (Table 5). Variation in molecular size of amplified products was found related to an insertion or deletion between primer annealing sites (Hurtado and Rodriguwz 1999). The reason is that the SCoT primers amplify only functional gene sequences, therefore, a low number of bands per primer are expected. On the contrary, RAPD markers are independent of coding regions, therefore more bands are expected (Vivodik et al 2016). Higher calculate of main bands was13 in primer SCoT23 while the lower was 1 band in primer SCoT59. The higher number of amplified bans was43 bands in primer SCoT26 while the lower number of amplified bans was 1 band

Table 1. Genotypes

Place	Genotypes
Iraq	Batra, Lahluba, Hasnauia, Musiliaa, Khnisiraa Houseagrl seed, Okra hybrid F1
Turkish l	Sout
Holland	Zasco seeds
Egypt	Egypt

Table 2. Primers name and their sequences which have been used as SCoT markers

SCoT primer names	Sequence (5´ – 3´)
SCoT 9	CAACAATGGCTACCAGCA
SCoT 23	CACCATGGCTACCACCAG
SCoT 26	ACCATGGCTACCACCGTC
SCoT 29	CCATGGCTACCACCGGCC
SCoT 36	GCAACAATGGCTACCACC
SCoT 59	ACAATGGCTACCACCATC
SCoT 61	CAACAATGGCTACCACCG

Collard and Mackill (2009)

Table 3. SCoT primers with their PCR amplification programs

SCoT primers	Step	Temperature	Time
SCoT 9	Initial denaturation	94 C˚	3min
SCoT 23	No. of Cycles = 35 cycles		
SCoT 26	Denaturation	94C˚	1min
SCoT 29	Annealing	50C˚	1min
SCoT 36	Extension	72C˚	1min
SCoT 59	Final extension	72C˚	10 min
SCoT 61			

Vivodik et al 2017

in primer SCoT59.Variation in several main and amplified bands are mainly due to primer structure and a large numeral of annealing site for primers determined which is farther benefit rather primers have the lowest numeral of annealing sites. In this status the enumerate of amplified bands will be larger thus awarding the best opportunity for determining DNA polymorphisms through a population (Tahir 2014). Monomorphic bands were three in primers (SCoT9 and SCoT61) while in primers (SCoT36, SCoT59) were only one band, but primers (SCoT23, SCoT29) failed to give a monomorphic band. Presence of monomorphic bands related to that all studied genotypes belong to one particular species, thus they share several genome sequences called constant or conserved sequence appear as monomorphic bands. The stability of these conserved sequences is important to study plant response to different stress factors. The presence of these common sequences among some genotypes only without other genotypes may refer to certain characters between these genotypes like resistance to unsuitable environmental factors and disease. The higher polymorphic bands were ten in (SCoT23) while primers (SCoT61) gave three polymorphic bands, and primers (SCoT9, SCoT59) failed to give a polymorphic band. Studies reported that primers with di-nucleotide GA sequence give low polymorphism (Singh and Jaiswal 2016), this may result from nucleotide sequence changes by deletion or insertions that may change the level of polymorphism by changing primer annealing sites (Powell et al 1996 and Fadoul et al 2013). The greater measure of unique bands was three at primer (SCoT23) but primers (SCoT26, SCoT29, SCoT36) gave just alone band. The rest (SCoT9, SCoT59) failed to give an alone band. The existence of like bands refer to that primer familiar in the genome a unique annealing site, so excess possibility of produce a unique plant variety fingerprint (Grewal et al 2007, Vishwanath et al 2010, Fadoul et al 2013). Primer (SCoT61) gave the lowest value for efficiency (0.08108) in contrast to primerSCoT23 which gave a higher value (0.2857). Discriminatory value in primers SCoT23 was the highest (37.037) while primer (SCoT61) gave the lowest value (11.11), and primers (SCoT9, SCoT59) failed to give discriminatory value. Both efficiency and discriminatory value of primer concerned with its ability to give a unique fingerprint. (Arif et al 2010). The low genetic distance was 0 between Hasnawia and Soutl but the high genetic distance was

Table 4. Okra genotypes fingerprinting (DNA profile) using seven SCOT primers and their sequences

Primers	Sequence (5'-3')	Varieties fingerprinting	No. of varieties fingerprint
SCoT 9	CAACAATGGCTACCAGCA	-	-
SCoT 23	CACCATGGCTACCACCAG	1-2-3-4-5-6-7	7
SCoT 26	ACCATGGCTACCACCGTC	3-5	2
SCoT 29	CCATGGCTACCACCGGCC	4-6-8	3
SCoT 36	GCAACAATGGCTACCACC	5	1
SCoT 59	ACAATGGCTACCACCATC	-	-
SCoT 61	CAACAATGGCTACCACCG	2	1

Table 5. Summarized results of SCOT amplification product

Primers	Amplified bands molecular size in bp	Number of Main bands	Number of amplified bands	Number of monomorphic bands	Number of polymorphic band	Number of Uniqueubands	Primer polymorphism (%)	Primer efficiency	Primer discriminatory value (%)
SCoT9	270-140	3	27	3	0	-	0	0	0
SCoT23	990-179	13	35	0	10	3	76.923	0.2857	37.037
SCoT26	1338-187	8	43	2	5	1	62.5	0.1162	18.5185
SCoT29	707-191	6	20	0	4	1	66.666	0.2	14.814
SCoT36	1062-320	7	32	1	5	1	71.428	0.156	18.518
SCoT61	1709-202	6	37	3	3	0	50	0.08108	11.11
SCoT59	187	1	0	1	0	0	0	0	0
Total no. of bands		44	194	10	27	6	-	-	
Average bands per primer		6.285	27.714	1.428	3.857	0.857	-	-	

0.28862 between Lahluba and Zasco seed and Lahluba and Egypt (Table 6). The genealogy of the cultivars may be causing the variation in genetic similarity because some of them have a general ancestry or not, scholars assurance that there is the ability to form collection could be joint to the participation of hereditary materials from one separate universal predecessors, it would elucidate the genetic resemblance, the presence of universal predecessors might

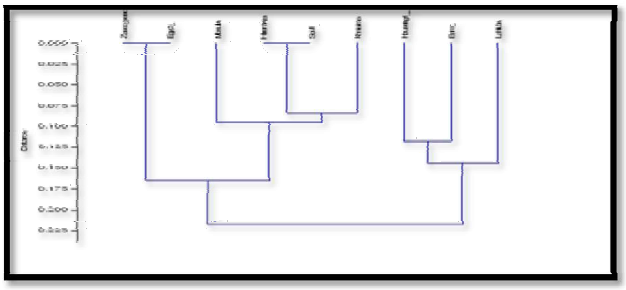

Fig. 1. UPGMA dendrogram of phylogenetic relationship among nine Okra genotypes by seven SCoT primers

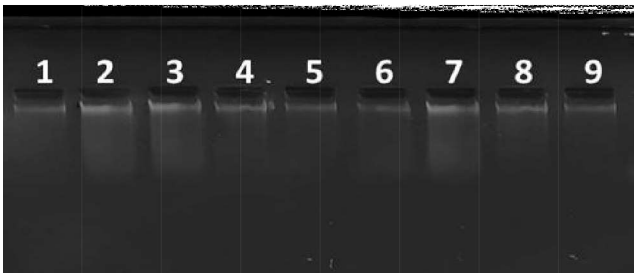

Fig. 2. Agarose gel electrophoresis of total genomic DNA of okra genotypes: 1.Batra 2. Lahluba 3.Hasnauia 4. Musiliaa 5. Khnisiraa 6. Houseagrl seed 7. Soutl 8. Zasco seeds 9. Egypt and lane M for 1kb DNA ladder. Using 0.9% agarose for half an hour at 90 volts

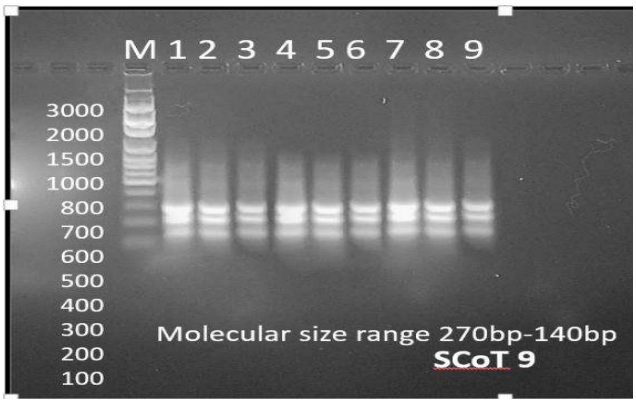

Fig. 3. PCR amplification products of nine okra genotypes produced with SCoT marker (primer SCoT9). Lanes 1-9 Okra genotypes: 1. Batra 2. Lahluba 3. Hasnauia 4. Musiliaa 5. Khnisiraa 6.Houseagrl seed 7. Soutl 8. Zasco seeds 9. Egypt and M 100 bp ladder

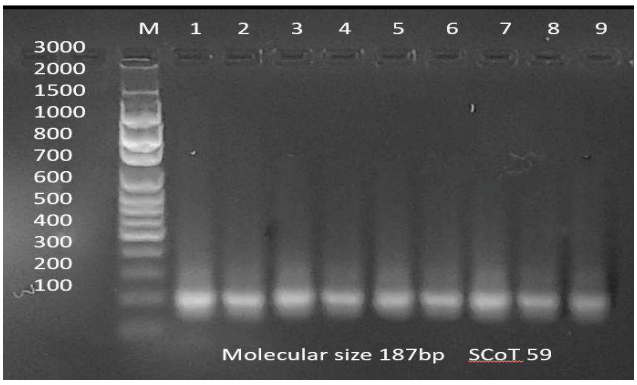

Fig. 6. PCR amplification products of nine okra genotypes produced with SCoT marker (primer SCoT59). Lanes 1-9 Okra genotypes: 1. Batra 2. Lahluba 3. Hasnauia 4. Musiliaa 5. Khnisiraa 6. Houseagrl seed 7. Soutl 8. Zasco seeds 9. Egypt and M 100 bp ladder

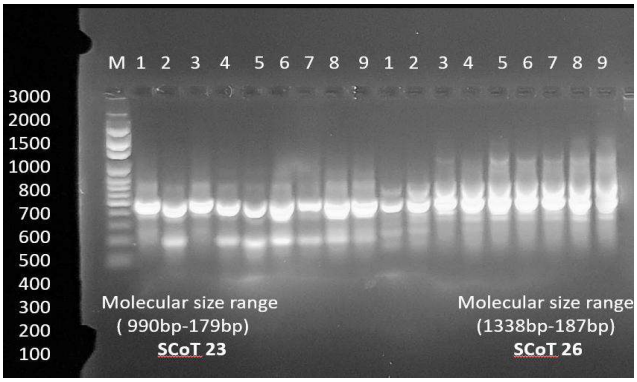

Fig. 4. PCR amplification products of nine okra genotypes produced with SCoT markers, (primer SCoT23, SCoT26). Lanes 1-9 Okra genotypes: 1. Batra 2. Lahluba 3. Hasnauia 4. Musiliaa 5. Khnisiraa 6. Houseagr seed 7. Soutl 8. Zasco seeds 9. Egypt and M 100 bp ladder

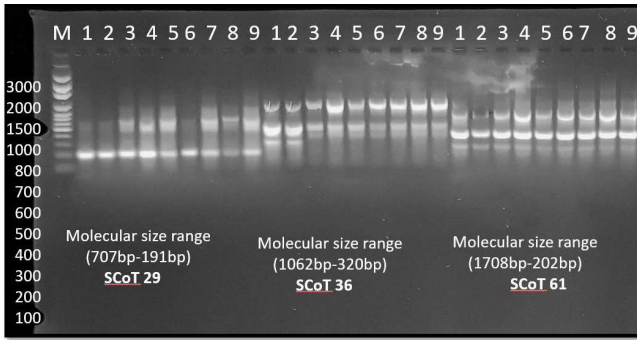

Fig. 5. PCR amplification products of nine okra genotypes produced with SCoTmarkers, (primer SCoT29, SCoT36, SCoT61). Lanes 1-9 Okra genotypes: 1. Batra 2. Lahluba 3.Hasnauia 4. Musiliaa 5.Khnisiraa 6.Houseagrl seed 7. Soutl 8. Zasco seeds 9. Egypt and M 100 bp ladder

Table 6. Genetic relationship among Okra genotypes

0	Batra	Lahluba	Hasnawia	Mosulia	Khnisiraa	Houseagrl	Soutl	Zasco	Egypt
Batra	0								
Lahluba	0.14428	0							
Hasnawia	0.20412	0.22047	0						
Mosulia	0.14431	0.16663	0.08334	0					
Khnisiraa	0.22047	0.20412	0.0833	0.11783	0				
Houseagrl	0.1178	0.14428	0.20412	0.14431	0.18635	0			
Soutl	0.20412	0.22047	0	0.08334	0.0833	0.20412	0		
Zasco	0.22047	0.28862	0.14428	0.16662	0.20404	0.27634	0.14428	0	0
Egypt	0.22047	0.28862	0.14428	0.16662	0.20404	0.27634	0.14428	0	0

affect the resemblance through the crops in the present study. (Morale et al 2011). According to the dendrogram (Fig. 1) cluster analysis (Phylogenetic tree) grouped studied genotypes into two main clusters, the first large one included six genotypes (Mosulia, Soutl, Khnisiraa, Hasnawia, Zasco seed, and Egypt) and other small one includes three genotypes (Houseagrl, Lahluba, and Batra), varieties in clusters group according to their origin. Plant verities originating from similar or nearby geographic origins would give a higher level of homogeneously between them so agrees with the thesis of autochthonal origin (Belaj et al 2001) and agrees with this study that the presence of genetic similarity between certain varieties present in the same location.

CONCLUSION

As SCoT markers proceeds all markers in fingerprinting ability, it can be success in targeted fingerprinting or QTL mapping purposes since it targeted coding sequence, those characteristics also consent their use for genetic diversity assessment in okra germplasm.

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Effect of Variety and Plant Density on Vegetative Growth and Yield of Lettuce (*Lactuca sativa* L)

Aziz Mahdi Abd Al-Shammari and Jamal Nather Naji Al-Sumaidaie*

College of Agriculture, University of Diyala, Iraq
**E-mail: jmalndhyr52@gmail.com*

Abstract: Field experiment was conducted in Diyala Iraq during the autumn season (2020-2021) to study the effect of plant density on growth and yield of five varieties of lettuce. The study included two factors, the first was lettuce varieties (Romana, the local variety, Teresa, Fajer and Nader), and second factor included three levels of plant density (8, 12 and 16 plants.m^{-2}). There were no significant differences between the varieties in the percentage of total soluble solids, and the local variety gave the highest plant height of 39.66 cm and the early maturity (41.88 days), while Teresa variety gave maximum total chlorophyll content in the outer leaves, plant circumference, number of outer leaves, total leaf area of outer leaves, total plant weight, head weight, total yield and protein percentage (20.27 mg / 100 g fresh weight, 103.3 cm, 19.08 leaves.plant^{-1}, 101.7 dm^{-2}, 878.1 g, 468.7 g, 53. 38 tons. h^{-1} and 13.51%, respectively). Nader variety recorded the highest carbohydrate (1.083%) and an oil content (0.481%) whereas density of 8 plants. m^{-2}recoded maximum growth and yield (51.84 ton. h^{-1}).

Keywords: Lettuce, Varieties, Plant density

Lettuce (*Lactuca sativa* L.) belonging to Asteraceae family and it is one of the important winter vegetable crops in Iraq due to its high nutritional value. Its native is the Mediterranean region and Europe (Hassan 2003). The local varieties and majority of foreign varieties grown in Iraq belong to the group of lettuce with elongated heads, with high nutritional value .Lettuce leaves are a natural and rich source of antioxidants, vitamins A and C, and anti-cancer phytochemicals (Masarirambi et al 2012). The environmental diversity of different agricultural areas impacts on the performance of agricultural varieties, especially most of the economic characteristics of crops are quantitative characteristics and are greatly influenced by environmental factors, therefore choosing the appropriate variety plays an important role in increasing the yield (Al-Shammari and Saud 2014). The selection of the appropriate plant density (planting distance between plants) is one of the most important methods that increase the production of plants and also it determines the extent of plants benefit from various environmental factors such as lighting, temperature, nutrition, humidity, ventilation and others, thus ensuring that plants obtain their needs from these factors, which is reflected in the plant growth, increasing (Al-Hamdani and Hadi 2017). The present study was undertaken to evaluate the effect of plant density on the growth and yield of five varieties of lettuce.

MATERIAL AND METHODS

Field experiment was conducted in factorial layout based on a randomized complete block design with three repetitions, first factor was lettuce varieties (Romana, the local variety, Teresa, Fajer and Nader), and the second factor was the plant density (8, 12 and 16 plants. m^{-2}). The experiment was laid out in the autumn season 2020-2021 in the Hamrin dam region, Khanaqin, Diyala Governorate, Iraq. Field land was prepared through plowing at a depth of 30 cm, followed by leveling. The organic manure (poultry) was added to the soil before planting as recommended. Soil samples representing depth of 0 - 30 cm were taken to record the physical and chemical properties (Table 1).

The compound fertilizer NPK 18-18-18 was added at a rate of 50 kg dunum^{-1} before planting, and nitrogen fertilizer (urea, 46% nitrogen) was added in two equal batches, the first quantity after 14 days of sowing and the second after 10 days of the first. The land was divided into three sectors and each sector into fifteen experimental units with dimensions 3 × 1 m with space between units 25 cm and space between sectors 100 cm. The crop was sown in cork dishes on August 20, 2020 and peat moss was used as medium for the growth of seedlings, then the seedlings (5 real leaves) were transferred to the designated field for the experiment on September 30, 2020. The irrigation was done with drip irrigation system.

Studied traits: The harvesting started on November 11, 2020 and five plants were randomly selected from each experimental unit for each treatment. The percentage of chlorophyll in the outer leaves (mg per 100 grams of fresh weight), the plant length with leaves (cm), the plant

Table 1. The physical and chemical properties of soil

Measurements	Value	Unit of measurement
N	56	mg. kg^{-1}
P	10	mg. kg^{-1}
K	129	mg. kg^{-1}
Ca	548	mg. l^{-1}
Mg	237	mg. l^{-1}
SO_4	1078	mg. l^{-1}
CaCo3	28.8	%
Ec	6.86	m \ ds
Ph	7.7	-
Clay	36.0	%
Silt	42.5	%
Sand	21.5	%
Texture of soil	Clay mixture	-
Organic matter	1.19	%

circumference (cm), the number of outer leaves (leaf.$plant^{-1}$), the total leaf area of the outer leaves (dm^{-2}), early maturity (day), total plant weight (g. $plant^{-1}$), head weight (g. $head^{-1}$), total yield (ton. h^{-1}), percentage of total soluble solids in head leaves (%), oil percentage in head leaves (%), carbohydrate percentage in head leaves (%) and protein percentage in head leaves (%) were estimated.

Statistical analysis: The results were analyzed using the statistical program, SAS (2001).

RESULTS AND DISCUSSION

The local variety was significantly superior in recording highest mean plant length 3 (9.66 cm) whereas Teresa gave the lowest length (35.46 cm). The density D3 was significantly superior with highest mean plant length of 38.67 cm as compared with the first density (D1) which recorded the lowest mean in plant length of 36.27 cm. The interaction

Table 2. Effect of varieties and plant density on plant length, plant circumference, leaves number and total leaf area of lettuce

Variety	Plant density (cm)			Mean
	D1 (8)	D2 (12)	D3 (16)	
Romana	36.00 ^{cde}	37.60 ^{abcde}	38.60^{abcd}	37.40 ^{B}
Local variety	38.60 ^{abcd}	39.60 ^{ab}	40.80 ^{a}	39.66 ^{A}
Teresa	34.40 ^{e}	35.13 ^{de}	36.86 ^{bcde}	35.46 ^{C}
Fajer	36.03 ^{bcde}	37.33 ^{abcde}	38.33 ^{abcd}	37.23 ^{B}
Nader	36.33 ^{bcde}	37.30 ^{abcde}	38.80 ^{abc}	37.47 ^{B}
Mean	36.27 ^{B}	37.39 ^{AB}	38.67 ^{A}	
Plant circumference (cm)				
Romana	104.2 ^{b}	92.26 ^{def}	84.33 ^{fg}	93.59 ^{BC}
Local variety	104.4 ^{b}	103.6 ^{bc}	87.13 ^{efg}	98.37 ^{B}
Teresa	116.4 ^{a}	101.0 ^{bcd}	92.73 ^{cdef}	103.3 ^{A}
Fajer	99.20 ^{bcd}	97.20 ^{bcde}	80.73 ^{g}	92.37 ^{C}
Nader	95.40 ^{bcde}	93.60^{bcdef}	79.60 ^{g}	89.53 ^{C}
Mean	103.9 ^{A}	97.53 ^{B}	84.90 ^{C}	
Number of outer leaves (leaf.$plant^{-1}$)				
Romana	20.06 ^{ab}	17.66 ^{bc}	12.80 ^{de}	16.84 ^{B}
Local variety	16.33 ^{bcde}	15.40 ^{bcde}	13.26 ^{cde}	14.99 ^{B}
Teresa	23.00 ^{a}	18.33 ^{ab}	15.93 ^{bcde}	19.08 ^{A}
Fajer	18.30 ^{ab}	16.86 ^{bcd}	12.06 ^{e}	15.74 ^{B}
Nader	16.60 ^{bcde}	15.93 ^{bcde}	13.16 ^{de}	15.23 ^{B}
Mean	18.85 ^{A}	16.83 ^{A}	13.44 ^{B}	
Total leaf area of the outer leaves (dm^{-2})				
Romana	87.02 ^{bc}	69.36 ^{cde}	45.91 ^{ef}	67.43 ^{BC}
Local variety	83.16 ^{bcd}	80.49 ^{bcd}	58.27 ^{def}	73.97 ^{B}
Teresa	129.7 ^{a}	99.51 ^{b}	75.97 ^{bcd}	101.7 ^{A}
Fajer	73.13 ^{cd}	67.90 ^{cdef}	44.37 ^{f}	61.80 ^{C}
Nader	68.53 ^{cdef}	60.05 ^{def}	44.75 ^{ef}	57.77 ^{C}
Mean	88.30 ^{A}	75.46 ^{A}	53.85 ^{B}	

between the variety and the plant density was significant, where the local variety with the third density (D3) achieved the highest plant length (40.80 cm). The Teresa variety was significantly superior in recording the highest mean in plant circumference, leaves number and total leaf area (103.3 cm, 19.08 and 101.7 dm^{-2} respectively) as compared with other varieties. The D1 was significantly superior with highest mean in the previous traits (103.9 cm, 18.85 and 88.30 dm^{-2} respectively). Teresa variety with D1 gave the highest plant circumference, leaves number and total leaf area (116.4 cm, 23.00 and 129.7 dm^{-2} respectively).

The Teresa variety significantly increased maturity, total plant weight, head weight and total yield (51.21 day, 878.1 g, 468.7 g and 53.38 ton respectively) (Table 3). There was no significant differences in early maturity between the plant density D1, D2 and D3, but first density D1 recorded the highest mean in total plant weight (907.1 g) and head weight (513.7 g) as compared with other plant density (D2 and D3). The D3 recorded the highest yield of 51.84 ton followed by D2 (50.01 ton). Teresa variety with D1 gave the highest increase in maturity, total plant weight and head weight (51.66 day, 1.077 g and 573.2 g, respectively). Teresa variety with the D3 recorded the highest yield 57.34 of ton.

There was significant effect of the variety on the total chlorophyll content of the outer leaves. Teresa variety was superior with the highest mean of 20.27 mg, while the Nader variety recorded the lowest mean of 15.58 mg, with non-significant differences between the other three varieties.

Table 3. Effect of varieties and plant density on maturity, total plant weight, head weight and yield

Variety	Plant density (cm)			Mean
	D1 (8)	D2 (12)	D3 (16)	
Maturity (Days)				
Romana	48.00 ^{abc}	47.33 ^{bcd}	46.00 ^{cdef}	47.11 ^{B}
Local variety	42.33 ^{efg}	42.00 ^{fg}	41.33 ^{g}	41.88 ^{D}
Teresa	51.66 ^{a}	51.33 ^{ab}	50.66 ^{ab}	51.21 ^{A}
Fajer	46.00 ^{cdef}	46.33 ^{cde}	43.66 ^{defg}	45.33 ^{BC}
Nader	46.00 ^{cdef}	45.00 ^{cdefg}	44.33 ^{cdefg}	45.11 ^{C}
Mean	46.79 ^{A}	46.39 ^{A}	45.19 ^{A}	
Total plant weight (g. $plant^{-1}$)				
Romana	982.8 ^{ab}	800.9 ^{cd}	598.0 ^{ef}	793.9 ^{B}
Local variety	801.0 ^{cd}	725.6 ^{cde}	589.2 ^{ef}	705.2 ^{C}
Teresa	1.077 ^{a}	887.6 ^{bc}	669.8 ^{def}	878.1 ^{A}
Fajer	884.5 ^{bc}	724.6 ^{cde}	565.0 ^{ef}	724.7 ^{BC}
Nader	790.5 ^{cd}	686.0 ^{def}	532.5 ^{f}	669.6 ^{C}
Mean	907.1 ^{A}	764.9 ^{B}	590.9 ^{C}	
Head weight (g. $head^{-1}$)				
Romana	557.9 ^{a}	438.4 ^{abcde}	328.6 ^{cde}	441.6 ^{AB}
Local variety	462.8 ^{abcd}	393.8 ^{bcde}	306.3 ^{de}	387.6 ^{B}
Teresa	573.2 ^{a}	474.7 ^{abc}	358.4 ^{bcde}	468.7 ^{A}
Fajer	519.4 ^{ab}	435.4 ^{abcde}	334.2 ^{cde}	429.6 ^{AB}
Nader	455.6 ^{abcd}	374.8 ^{bcde}	292.7 ^{e}	374.3 ^{B}
Mean	513.7 ^{A}	423.4 ^{B}	324.0 ^{C}	
Total yield (ton. h^{-1})				
Romana	44.63 ^{ab}	48.61 ^{ab}	52.58 ^{ab}	48.60 ^{AB}
Local variety	37.02 ^{b}	47.26 ^{ab}	49.01 ^{ab}	44.43 ^{B}
Teresa	45.85 ^{ab}	56.96 ^{a}	57.34 ^{a}	53.38 ^{A}
Fajer	41.55 ^{ab}	52.25 ^{ab}	53.47 ^{ab}	49.09 ^{AB}
Nader	36.45 ^{b}	44.97 ^{ab}	46.83 ^{ab}	42.75 ^{B}
Mean	41.10 ^{B}	50.01 ^{A}	51.84 ^{A}	

There were no significant differences between plant varieties in the percentage of total soluble solids. Nader variety gave the highest mean in oil content in the head leaves (0.481 %) which did not differ significantly from the Romana variety (0.453 %) while the oil content decreased to 0.305 % in the Teresa variety. The Nader variety was significantly superior with the highest mean of carbohydrate (1.083%) in head leaves as compared with other varieties. Teresa variety was distinguished with the highest mean in protein percentage in head leaves (13.51%) which was not significantly different from Fajer variety (12.98%) and Romana variety (12.45%). Nader variety recorded the lowest mean of 9.974%. The D1

Table 4. Effect of varieties and plant density on total chlorophyll, total dissolved solids, oil percentage, carbohydrate percentage and protein percentage of lettuce

Variety	Plant density (cm)			Mean
	D1 (8)	D2 (12)	D3 (16)	
Total chlorophyll (mg per 100 grams fresh weight)				
Romana	19.96 ^{bc}	17.46 ^{def}	14.65 ^{gh}	17.35 ^{B}
Local variety	20.35 ^{b}	17.92 ^{cde}	15.28 ^{fgh}	17.85 ^{B}
Teresa	24.12 ^{a}	20.69 ^{b}	16.01 ^{efgh}	20.27 ^{A}
Fajer	19.66 ^{bcd}	16.90 ^{efg}	15.23 ^{fgh}	17.26 ^{B}
Nader	17.25 ^{ef}	15.61 ^{fgh}	13.88 ^{h}	15.58 ^{C}
Mean	20.26 ^{A}	17.71 ^{B}	15.01 ^{C}	
Total soluble solids in head leaves (%)				
Romana	3.36 ^{ab}	3.03 ^{ab}	2.63 ^{b}	3.01 ^{A}
Local variety	4.17 ^{a}	3.76 ^{ab}	2.93 ^{ab}	3.62 ^{A}
Teresa	4.16 ^{a}	3.66 ^{ab}	2.80 ^{ab}	3.54 ^{A}
Fajer	3.70 ^{ab}	3.26 ^{ab}	2.83 ^{ab}	3.26 ^{A}
Nader	4.13 ^{a}	3.83 ^{ab}	2.76 ^{ab}	3.57 ^{A}
Mean	3.90 ^{A}	3.51 ^{B}	2.79 ^{C}	
Oil percentage in head leaves (%)				
Romana	0.51 ^{abc}	0.49 ^{abcd}	0.35 ^{gh}	0.45 ^{AB}
Local variety	0.47 ^{bcd}	0.45 ^{cde}	0.36 ^{fgh}	0.42 ^{B}
Teresa	0.33 ^{ghi}	0.30 ^{hi}	0.27 ^{i}	0.30 ^{D}
Fajer	0.43 ^{edf}	0.40 ^{efg}	0.35 ^{gh}	0.39 ^{C}
Nader	0.54 ^{a}	0.52 ^{ab}	0.37 ^{fg}	0.48 ^{A}
Mean	0.45 ^{A}	0.43 ^{A}	0.34 ^{B}	
Carbohydrate percentage in head leaves (%)				
Romana	1.19 ^{a}	1.03 ^{d}	0.96 ^{efg}	1.06 ^{B}
Local variety	1.18 ^{a}	0.99 ^{de}	0.94 ^{fgh}	1.04 ^{C}
Teresa	1.11 ^{bc}	0.97 ^{efg}	0.91 ^{h}	1.00 ^{D}
Fajer	1.13 ^{b}	0.98 ^{ef}	0.93 ^{gh}	1.01 ^{D}
Nader	1.20 ^{a}	1.07 ^{c}	0.97 ^{efg}	1.08 ^{A}
Mean	1.16 ^{A}	1.01 ^{B}	0.94 ^{C}	
Protein percentage in head leaves (%)				
Romana	14.27 ^{abc}	13.49 ^{abcde}	9.64 ^{ef}	12.45 ^{AB}
Local variety	12.95 ^{abcdef}	11.99 ^{bcdef}	9.76 ^{def}	11.57 ^{BC}
Teresa	16.31 ^{a}	14.30 ^{abc}	9.91 ^{def}	13.51 ^{A}
Fajer	14.83 ^{ab}	13.97 ^{abcd}	10.14 ^{cdef}	12.98 ^{AB}
Nader	11.45 ^{bcdef}	9.70 ^{def}	8.76 ^{f}	9.97 ^{C}
Mean	13.95 ^{A}	12.69 ^{B}	9.64 ^{C}	

was significantly superior in total chlorophyll content (20.26 mg) percentage of total soluble solids (3.90%) oil percentage (0.45%) carbohydrate percentage (1.16%) and protein percentage (13.95%) as compared with the other plant density. The interaction between the variety and the plant density was significant, where Teresa variety with D1 gave the highest total chlorophyll content (24.12 mg) and protein percentage (16.31 %) while Nader variety with D1 recorded the highest increase in oil percentage (0.54 %) and carbohydrate percentage (1.20 %) whereas the Local variety with D1 achieved the highest total soluble solids (4.17 %)

The results indicate that there is a significant difference between the varieties in most of the vegetative growth characteristics and the yield, due to the difference in the nature of growth between the varieties due to genetic factors (Al-Dabbagh and Dawood 2013). Farag et al (2013) showed that the difference in a number of chemical characteristics due to the difference of lettuce varieties. Boroujerdnia and Ansari (2007) and Shahein et al (2015) found that significant differences between the studied lettuce varieties in head length. Similar trend was observed by earlier researchers (Jerry et al 2011, Al-Habbar et al 2019, Al-Khazraji and Al-Hamdani 2019 Al-Shammari et al 2020). The plant density D1 recoded significant increase in the number of outer leaves, plant circumference, total leaf area, total plant weight, head weight, total chlorophyll content, percentage of total soluble solids, oil percentage, protein percentage and carbohydrate percentage in the head, due to the reduction of competition between plants for nutrients and light as a result of increasing the plant growth area, which impacts on the process of photosynthesis and thus increase the vegetative growth of the plant and accumulation of dry matter in the leaves. Moniruzzaman (2006) and Makhadmeh et al (2017) also observed that low density led to a significant increase in stem, number of inner and outer leaves, head weight and plant height. The plant density D3 was superior in plant height with leaves and total yield compared to the first and second densities, due to the high plant density led to plants competition for light and resulted in decreasing the intensity of the received light between the competing plants and decrease the photo-oxidation of auxin, thus an increase in plant height, also the increase in yield is due to the increase in the number of plants per unit area, as well as leaf area, number of leaves and other characteristics of vegetative growth Hassan et al (2017) found that the planting distances 30 and 25 cm between lettuce plants was a superior in total yield and market head weight. Earlier studies also indicated the same trend (Sharma et al 2001, Changchien and Hsu 2003, McDougall 2006).

CONCLUSION

Teresa variety was the best in productivity and growth compared with other varieties, also planting of plants with a density of 16 plants m^{-2} led to increase the productivity of the crop without affecting its quality. Teresa variety and 16 plants m^{-2}performed well.

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Effect of Foliar Spraying with Nano-Fertilizer (Loenergy Plus) on the Growth and Yield of Cabbage

Aziz Mahdi Abd Al-Shammari and Wasam Habeib Abdala Al-Tamimi*

College of Agriculture, University of Diyala, Iraq
**E-mail: agrihorth24@uodiyala.edu.iq*

Abstract: A field experiment was conducted at the research station, Department of Horticulture at the College of Agriculture, Diyala University, during 2020-2021. To study the effect of foliar spraying with Nano-fertilizer (Loenergy Plus) on the growth and yield of cabbage. The study included two factors, first factor was hybrid varieties of cabbage (Bordeaux, Globe Master, Mighty, Zuha and Samanta) and second factor was three levels of foliar fertilizer Loenergy Plus (0.0. 0.5 and 1.0 ml. l^{-1}). Globe Master variety was significantly superior with maximum number of leaves (14.87) and leaf area (158.18 dm^2). Samanta variety recording the highest yield of 91.12 tons, while Bordeaux variety was significantly superior with respect to percentage of nitrogen (1.49 %), phosphorus (0.50 %,) protein(9.31%) and carbohydrate (5.21%) in the cabbage head leaves. The spraying of Nano fertilizer (Loenergy Plus) at 1.0 ml was significantly superior in recording the highest mean of all the previous mentioned traits 14.81, 172.15 dm^2, 89.14 tons, 1.62 %, 0.56%, 10.14 % and 5.37%, respectively.

Keywords: Cabbage, Nano fertilizer, Loenergy Plus, Nitrogen, Phosphorus, Protein, Carbohydrate

Cabbage (*Brassica oleracea* var. *capitata* L.) is considered one of the important winter vegetable crops in Iraq and grown in most of its regions (Al-Quzat et al 2007, Decoteau 2013). Each 100 g of fresh leaves contains 6.1-11.2 % dry matter, 3-4% carbohydrates, 1-2% proteins, 0.2% fats, 30-50% vitamins, 0.05 mg thiamine, 238 mg potassium, 49 mg phosphorus, 9 mg magnesium, 102 mg iron, 24 calories and contains B vitamins in the inner leaves (Badii et al 2013). The production average in Iraq less than the production rate in the world, and this is related to many factors that are affected by the plant growth, including genetic factors for the variety, environmental conditions and agronomic practices. In Iraq cultivated area of the cabbage crop is 3270 dunams, with a productivity rate 5769 tons during 2019 (Central System for Statistics 2019). The environmental diversity of agricultural areas greatly effects on the performance therefore, selection of the appropriate variety plays an important role in increasing the yield, and comes first among the factors affecting in increase of production (Al-Shammari and Saud 2014). The superiority of the cultivar in the yield indicates its high efficiency in exploiting the surrounding environmental factors to serve the process of carbon metabolism and then converting the metabolism products into an economic yield. In order to increase production, must adopt the use of varieties which are efficient in absorbing nutrients, highly productive and tolerance to biotic and abiotic stresses (Al-Shammari et al 2019). The scientific experiments showed the foliar spray is highly efficient and effective for increasing nutrient access to leaf tissue compared to the soil fertilization (Kuepper 2003). The Nano-fertilizers are oxides or salts with a diameter of less than 100 nanometers, which are added as a spray to the vegetative system and provide the plant with the most nutrients, especially macro and micro nutrients and amino acids, thus contributing significantly to higher production (Singh et al 2016). The study aimed to evaluate the effect of foliar spraying with Nano-fertilizer (Loenergy plus) on the growth and yield of five varieties of cabbage.

MATERIAL AND METHODS

The field experiment was conducted at Department of Horticulture at the College of Agriculture, Diyala University during 2020-2021. The experiment was in a factorial layout randomized complete block design by using the split-plot with three replications. The first factor was hybrid varieties of cabbage (Bordeaux, Globe Master, Mighty, Zuha and Samanta) and second factor was three levels of foliar feeding with Nano fertilizer Loenergy Plus (0.0, 0.5 and 1.0 ml. l^{-1}).

The field was prepared using recommended agronomic practices with the drip irrigation system. Random samples were collected at depth of 0-30 cm before fertilizer application and were mixed together and a representative sample was taken, then passed through a sieve with holes 2 mm in diameter. The chemical and physical analyzes were carried out in the central laboratory of College of Agriculture, University of Baghdad in (Table 1).

The seeds of the hybrid varieties of cabbage were planted on August 17, 2020 using peat moss medium for the growth

of seedlings. The seedlings were transferred with 4-5 leaves to in field on September 29, 2020. The field was divided into forty-five experimental units, with an area 2.4 m^2 and dimensions of 4 m x 0.6 m per unit. The distance between plants was 0.4 m and replication contained 10 plants. The spraying of plants was carried out three times until full wetness during the growing season, starting from October 18, 2020 at 10 days interval.

Studied traits: Five plants were randomly selected from each experimental unit for each replication. The estimation of nitrogen, phosphorus and content in the outer leaves (%):

Nitrogen content (%): The wet digestion process was carried out for estimation of nitrogen (Cresser and Parsons 1979). The total nitrogen percentage was estimated according to the Kjeldahl method using the Micro Kjeldahl device (Al-Sahaf 1989) and percentage of total nitrogen was calculated from the following equation:

$$\text{Nitrogen percentage (\%)} = \frac{\text{Volume of the consumed acid x acidity titration x 14 x volume of dilution}}{\text{Volume of sample taken at distillation x Weight of the digested sample x 1000}} \times 100$$

Phosphorus (%): The percentage of the total phosphorous element was estimated by digestion, as in the estimation of nitrogen by using ammonium molybdate, and then measuring it with a spectrophotometer at a wavelength of 882 nm (Al-Zoghbi et al 2013).

3- Leaves number (Leaf.plant^{-1})

4- Leaf area (dm^2)

5- Total yield (ton. h^{-1})

Protein percentage (%): The protein percentage was calculated in head as:

Protein percentage (%) = Nitrogen content x 6.25 (Dalali and Al-Hakim 1987).

Carbohydrate content in the head)%(;It was estimated according to Ibrahim's method (2010) .

Statistical analysis: The results were analyzed using the (SAS) program 2001 and the averages were compared with Duncan's polynomial test at a probability level of 0.05 (Al-Rawi and Khalaf Allah 2000).

RESULTS AND DISCUSSION

The Globe Master variety was significantly superior recording the highest mean in leaves number and leaf area, which was 14.87 and 158.18 dm^2 respectively as compared with other varieties, while spraying of Nano fertilizer at 1.0 ml led to increase in leaves number of previous traits 14.81 and 172.15 dm^2 respectively as compared with other concentrations (Table 1). The interaction between the cabbage variety and the spraying of Nano fertilizer was significant, where Globe Master variety with the spraying of Nano fertilizer at 1.0 ml gave the highest increase in leaves number 16.90 and leaf area 194.93 dm^2. Samanta variety was significantly superior in recording the highest mean of total yield 91.12 tons, followed by a Globe Master variety 86.82 tons . The spraying of Nano fertilizer at 1.0 ml led to increase in total yield 89.14 tons followed by spraying of Nano fertilizer at 0.5 ml (81.10 tons (as compared to spraying with distilled water, which recorded lowest mean 58.83 tons, whereas through the interaction between the cabbage variety and the spraying of Nano fertilizer, the Globe Master variety with the spraying of Nano fertilizer at 1.0 ml gave the highest increase in total yield 101.87 tons.

Bordeaux variety was significantly superior in recording the highest mean in nitrogen, phosphorous, protein and carbohydrate which was 1.49, 0.50, 9.31 and 5.21 per cent respectively as compared with other varieties. The spraying of Nano fertilizer at 1.0 ml was significantly superior in recording the highest mean in the previous traits as 1.62, 0.56, 10.14 and 5.37%, respectively as compared with other concentrations. The interaction between the cabbage variety

Table 1. Physical and chemical properties of soil

Measurements	Value	Unit of measurement
Texture of soil	Clay mixture	-
Clay	37.6	%
Silt	45.0	%
Sand	17.4	%
True density	1.47	$g.cm^3$
Porosity	42.4	-
Ph	7.59	-
Ec	12.28	m / ds
N	73	mg. kg^{-1} or ppm
P	10.5	mg. kg^{-1} or ppm
K	112	mg. kg^{-1} or ppm
Organic matter	1.22	%
CaCo3	29.4	%
Ca^{+2}	941	mg. l^{-1} or ppm
Mg^{+2}	410	mg. l^{-1} or ppm
Na^{+}	1025	mg. l^{-1} or ppm
K^{+}	81	mg. l^{-1} or ppm
Cl^{-}	2145	mg. l^{-1} or ppm
$SO4^{-2}$	2075	mg. l^{-1} or ppm
HCO3	582	mg. l^{-1} or ppm
CO3	55	mg. l^{-1} or ppm
N03	64	mg. l^{-1} or ppm
PO4	0.63	mg. l^{-1} or ppm

and the spraying of Nano fertilizer, the Bordeaux variety with the spraying of Nano fertilizer at 1.0 ml gave the highest increase in percent nitrogen, protein and carbohydrate, which was 1.76, 11.02 % and 5.48 %, respectively, while the Zuha variety with the spraying of Nano fertilizer at 1.0 ml gave the highest increase in phosphorous (0.59 %).

The difference between cabbage varieties in increasing the vegetative and chemical characteristics of cabbage may be due to the variation in the genetic composition of these varieties, as their genes were expressed differently from the other variety, which effects physiological capability and efficiency in converting the products of the carbon metabolism process to the process of growth and elongation of stem cells, thus increasing the height of the plant, which was positively reflected in other vegetative growth indicators, and the control of the genetic factors of the varieties in showing the superiority of any of the aforementioned characteristics. The and the difference in response of the varieties to the prevailing environmental conditions may be the reason for the differences in the vegetative characteristics, this is consistent with the findings of earlier scientist (Olaniyi and Objetayo 2011, Uddain et al 2012, El-Bassiony et al 2014, Nagar 2016) .

The significant effect of foliar feeding treatment with Nano-fertilizer Loenergy Plus at 1 ml per liter^{-1} on the leaves number and leaf area, may be attributed to the role of the elements inside this Nano-fertilizer, especially nitrogen, which works in the elongation and division of cells, and the role of phosphorus comes in increasing the activity and growth of the roots and thus contribute to increasing the absorption of nutrients, and accordingly, these elements play a major role in the increasing vegetative growth and activating the photosynthesis process and the manufacture of nutrients necessary for plant growth, the leaf area increased as a result of the increase in the number of leaves because there is a significant correlation between them. These agree with Al-Sahaf (1989), Al-Hamdani (2008), Al-Hasany (2019). The superiority of the Bordeaux variety in most of the above chemical characteristics was due to the most efficient in the process of food conversion from the outer leaves to the head, which led to an increase in the nutritional components from nutrients, protein and carbohydrates. This

Table 2. Effect of foliar spraying with Nano-fertilizer (Loenergy plus) and variety leaves number, leaf area and total yield of cabbage

Cabbage variety	Spraying of Nano fertilizer (Loenergy Plus)			
	Distilled water	0.5 ml. l^{-1}	1.0 ml. l^{-1}	Mean
Leaves number (Leaf plant^{-1})				
Bordeaux	11.93^{efg}	12.33^{def}	13.53^{cde}	12.60^{B}
Globe master	11.16^{fg}	16.56^{a}	16.90^{a}	14.87^{A}
Mighty	11.80^{efg}	12.96^{cdef}	14.10^{bcd}	12.95^{B}
Zuha	10.26^{g}	14.60^{bc}	15.83^{ab}	13.56^{B}
Samanta	11.86^{efg}	13.46^{cde}	13.70^{cde}	13.01^{B}
Mean	11.40^{C}	13.98^{B}	14.81^{A}	
Leaf area (dm^{2})				
Bordeaux	116.50^{e}	148.64^{cd}	169.53^{b}	144.83^{BC}
Globe master	112.36^{e}	167.26^{b}	194.93^{a}	158.18^{A}
Mighty	88.98^{f}	149.40^{cd}	153.20^{c}	130.52^{D}
Zuha	108.33^{e}	173.00^{b}	170.36^{b}	150.56^{B}
Samanta	103.20^{e}	137.40^{d}	172.73^{b}	137.77^{CD}
Mean	105.87^{C}	155.10^{B}	172.15^{A}	
Total yield (ton. h^{-1})				
Bordeaux	54.08^{g}	73.66^{de}	76.91^{cde}	68.21^{C}
Globe master	70.77^{def}	87.83^{bc}	101.87^{a}	86.82^{AB}
Mighty	33.73^{h}	63.87^{efg}	69.28^{def}	55.63^{D}
Zuha	59.16^{fg}	82.53^{cd}	98.30^{ab}	80.00^{B}
Samanta	76.14^{cde}	97.63^{ab}	99.33^{ab}	91.12^{A}
Mean	58.83^{B}	81.10^{A}	89.14^{A}	

Table 3. Effect of foliar spraying with Nano-fertilizer (Loenergy Plus) and variety on percentage of nitrogen, phosphorus , protein and carbohydrate in the cabbage head leaves

Cabbage variety	Spraying of Nano fertilizer (Loenergy Plus)			
	Distilled water	0.5 ml. l^{-1}	1.0 ml. l^{-1}	Mean
Nitrogen percentage				
Bordeaux	1.17^{i}	1.53^{de}	1.76^{a}	1.49^{A}
Globe master	1.15^{ij}	1.47^{f}	1.67^{b}	1.43^{B}
Mighty	1.06^{k}	1.37^{h}	1.51^{e}	1.31^{E}
Zuha	1.16^{ij}	1.45^{f}	1.62^{c}	1.41^{C}
Samanta	1.13^{j}	1.41^{g}	1.54^{d}	1.36^{D}
Mean	1.13^{C}	1.45^{B}	1.62^{A}	
Phosphorus percentage				
Bordeaux	0.41^{fgh}	0.50^{cde}	0.58^{ab}	0.50^{A}
Globe master	0.41^{fgh}	0.47^{cdef}	0.57^{ab}	0.48^{AB}
Mighty	0.35^{h}	0.46^{def}	0.54^{abc}	0.45^{B}
Zuha	0.38^{gh}	0.45^{ef}	0.59^{a}	0.47^{AB}
Samanta	0.43^{efg}	0.49^{cde}	0.52^{bcd}	0.48^{AB}
Mean	0.39^{C}	0.47^{B}	0.56^{A}	
Protein percentage				
Bordeaux	7.31^{f}	9.60^{cd}	11.02^{a}	9.31^{A}
Globe master	7.21^{f}	9.23^{de}	10.46^{ab}	8.96^{AB}
Mighty	7.64^{f}	8.60^{e}	9.44^{cde}	8.56^{B}
Zuha	7.27^{f}	9.08^{de}	10.12^{bc}	8.82^{B}
Samanta	7.06^{f}	8.81^{de}	9.67^{bcd}	8.51^{B}
Mean	7.30^{C}	9.06^{B}	10.14^{A}	
Carbohydrate percentage				
Bordeaux	4.88^{f}	5.28^{b}	5.48^{a}	5.21^{A}
Globe master	4.81^{g}	5.20^{c}	5.41^{a}	5.14^{B}
Mighty	4.52^{i}	4.94^{f}	5.25^{bc}	4.90^{E}
Zuha	4.78^{g}	5.10^{d}	5.41^{a}	5.10^{C}
Samanta	4.71^{h}	5.02^{e}	5.31^{b}	5.01^{D}
Mean	4.74^{C}	5.11^{B}	5.37^{A}	

agree with Boroujerdnia et al (2007), Arin et al (2003) and Abdel-Hadi et al (2009) on Kohlrabi and Zaki et al (2015) on broccoli.

The superiority of spraying with Nano-fertilizer 1.0 ml L^{1} in all chemical characteristics of the head leaves is due to the Nano-fertilizer containing the major nutrients for plant growth, which has great importance in the biological construction of the plant which leads to an increase in its concentration in the plant, which increases the efficiency of the photosynthesis process due to the increase in the leaf area and the increase in carbohydrates and protein in the leaves and their transported and stored in the leaves of the heads, which is reflected positively on improving quality characteristics also absorbed elements, which increases the amount of nutrients transmitted to the heads. These results are consistent with Eris et al (2004), Hasan et al (2017), Roosta (2017), Rostami (2017), Mohammed et al (2018), Merghany et al (2019) , Salman and Mahmoud (2020).

CONCLUSION

Globe Master variety was the best in increasing leaves number, leaf area and total yield of cabbage, while Bordeaux variety in percentage of nitrogen, phosphorus, protein and carbohydrate in the cabbage leaves. The spraying of Nano fertilizer (Loenergy Plus) at 1.0 ml led to increase in all traits.

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Evaluating the Extension Program for Developing the Capacities of Beekeepers for Rural Youth in the Governorates of Baghdad and Diyala

Salam Attia Abbas and Ashwaq A.R. Naji[1]

Diyala Agriculture Directorate, Baghdad, Iraq
[1]Agricultural Extension and Technology Transfer, College of Agricultural Engineering Sciences, University of Baghdad, Iraq
[*]E-mail: sallam.atya1209a@coagri.uobaghdad.edu.iq

Abstract: The research aimed at evaluating the extension program for developing the capacities of beekeepers for rural youth in the governorates of Baghdad and Diyala through identifying the reality of the program (organization, planning, implementation, monitoring, evaluation). Besides, categorizing the degree of program application of total quality standards in its fields, identifying satisfaction of rural youth who are involved in the extension program for developing the capacities of beekeepers on extension service, processing service, program managers and results achieved from the program. In order to achieve the research objectives, the reality of the program was identified by referring to the annual reports, records and documents of the extension program, as well as personal interviews with some officials and employees working in the program and the targeted rural youth in the governorates of Baghdad and Diyala. Two questionnaires were designed, the first was to collect data from the employees working in the program, which included a five-point scale with degrees ranges between (0-4) to measure the degree of the program's application of total quality standards in its fields. The scale included paragraphs (68) distributed on axes(30), which distributed on fields(5). Along with, another questionnaire for collecting data from the rural youth involved in the program, which included a three-point scale to measure the satisfaction of rural youth about extension service, processing service, program managers, results achieved from the program. The scale included paragraphs (28) distributed on axes (4) and also distributed on the satisfaction field. The governorates of Baghdad and Diyala were selected as an area to conduct the research. The research results concluded that the weighted average for the program fields in general reached 1.744 degrees, which is much less than the hypothetical mean for the scale of 2 degrees, with a percentage weight of 43.60 degrees. This confirms the low degree of the extension program application for developing the capacities of beekeepers for rural youth in the governorates of Baghdad and Diyala for the total quality standards in its fields. The weighted averages of satisfaction of extension service= and program managers were 2,478 and 2,508 degrees, respectively. This result is higher than the hypothetical mean of the scale of 1.5 degree, with percentage weights of 82.6 and 83.6 degree, respectively, which confirms that the involved rural youth are satisfied with the axes (extension service, program managers). The weighted averages of satisfaction of the axes processing service, and the results achieved from the program were 1.254, 1.632 degrees, respectively, and the first value is much less than the hypothetical mean of the scale (1.5 degree)s, and the second value is much close to the hypothetical mean, with percentage weights of 41.8, 54.4 degree respectively. This confirms the low satisfaction of those included in the aforementioned axes, as satisfaction is one of the total quality standards and an important indicator of the efficiency and effectiveness of the extension program.

Keywords: Rural youth, An extension program, Evaluation, Total quality

Bees of long ages benefit humans, plants, and the environment, as they are among the most dedicated organisms to their work on earth. Bees and other pollinators help to obtain an abundance of grains, nuts, and fruits, as well as greater diversity, quality, and better productivity, which contributes to providing food security. Pollinators such as bees and birds affect 35% of global production, increasing the yields of 87 major food crops around the world (FAO 2020). Apiculture have great potential to develop them as a specialized profession for smallholders with low input costs to expand rapidly, which in many cases improve the productivity of cropping systems through improved pollination (Australian Center for International Agriculture Research 2019). Recently, the agricultural extension agency in Iraq has increased interest in rural youth by encouraging and assisting them in practicing and adopting agricultural projects (Hassoun 2015). Beekeeping needs skills, information and capabilities that can be developed among the rural youth, because their characteristics and levels of knowledge and education qualify them to adopt the profession of management and beekeeping better than others. (Al-Tohamy and Ahmed 2019). The Agricultural Extension and Training Authority performs its educational role for rural youth through the extension programs that it prepares and implements for them, and the success of the extension agency in its work and the achievement of its goals. Likewise, its contribution to agricultural development depends on the success of its extension programs and

activities and the achievement of its objectives, which in turn depends on the proper organization, planning, implementation, monitoring and evaluation of these programs. In addition to determining what goals they have achieved in order to reach correct decisions regarding the continuation of their implementation, modification or discontinuation (Al-Taie Hussein Khudair 1998). Among these extension programs that are provided to rural youth, the extension program for developing the capacities of beekeepers for rural youth provided by the Agricultural Extension and Training Department since 2007 until now in all governorates of Iraq except the Kurdistan region. This program aims to support the rural youth in rural societies by developing their knowledge and skills in the field of beekeeping and management, employing their energies and employing them in beneficial projects, thus improving their economic situation (Agricultural Extension and Training Department 2020). The evaluation is a fundamental process in any project or extension program to determine its feasibility and suitability for the targets, as well as revealing the strengths and weaknesses (Al-Taie, Hussain Khudair Abdul-Hussein 2020). The main objectives include understanding the reality of the extension program for developing the capacities of beekeepers for rural youth and what degree does the extension program for developing the capacities of beekeepers for rural youth apply total quality standards including the satisfaction of the rural youth involved in the extension program for developing the capacities of beekeepers .

MATERIAL AND METHODS

The current research is within the framework of survey research that falls within the descriptive approach (Elyan and Rabhi 2001)

Research area: The governorates of Baghdad and Diyala were chosen as the research area, as they are among the governorates in which beekeeping is widespread and in which the activities of the extension program were carried out to develop the capacities of beekeepers for rural youth. Furthermore, the presence of government apiaries are good places to implement program activities and educate and train rural youth in the field of management and breeding bees.

Research community: The research community includes the following:

1. The agricultural divisions distributed over the research area, which are 18 agricultural divisions in the Baghdad governorate and 19 agricultural divisions in the Diyala governorate.
2. Agricultural employees working in the extension program to develop the capacities of beekeepers for rural youth in the governorates of Baghdad and Diyala, which amounted to 182 respondents.
3. The rural youth involved in the extension program for developing the capacities of beekeepers in the research area were 180 from Baghdad governorate, 235 from Diyala governorate.

Research sample: The governorates of Baghdad and Diyala included 36 divisions and the employees working in the program were 182.

Data collection tool: The questionnaire form was adopted and initial questionnaire (were developed for rural youth to identify the satisfaction of the rural youth

Validity of the questionnaire: The two questionnaires (the employees' questionnaire and the rural youth questionnaire) were presented, in their initial form, to a group of experts in the fields of agricultural extension, management, and beekeeping, to survey their views on (fields, axes, and paragraphs). This procedure was carried out to measuring the face validity and content validity through the questionnaire of experts, and in light of a three-point scale (Agree, agree with the amendment, disagree). Combined with the ideas and amendments issued by them to come out with the final form of the extension program evaluation for developing the capacities of beekeepers for rural youth in light of total quality standards and before starting to present it to a group of respondents for a pre-test. In order to verify the face validity, the two questionnaires were presented to a number of professors specialized in the field of agricultural extension, totaling 4 experts.

Questionnaires in their final form: The final form (Employees questionnaire) was prepared to evaluate the extension program for developing the capacities of beekeepers for rural youth in light of total quality standards, which consists of 68 paragraphs distributed on 30 axes and in 5 fields (Table 1).

The final form (questionnaire of rural youth) was prepared to identify the satisfaction of the rural youth involved in the extension program about (extension service, processing service, program managers, the program results), consisting of one field and 4 axes that included 28 paragraphs (Table 2).

Reliability analysis: To measure the reliability of the (employees questionnaire), the researcher conducted a pre-test in September of 2020 in Babylon Governorate on a sample of 5 employees in the Babylon Agriculture Directorate. It included Director of Agriculture, Director of the Extension Department, Head Training Division, Head of the Rural Youth Division, Head of the Agricultural Division. Besides, 6 employees in the Agricultural Training Extension Center affiliated with the Agricultural Extension and Training Department, which included head of the center, the head of

Table 1. Details of fields, axes, and paragraphs for the employees questionnaire

A. Organization

Axes	Sequence	Paragraphs
Organizing the program's work	1	Existence of a specific organization concerned with the responsibility of working in the program
Organizational structure	2	Existence of a modern organizational structure that organizes relationships and defines responsibilities in the extension program
	3	Implemental decision-making rapidly and responding to the needs of those involved in the program
	4	Flexibility of work (decentralization) in the program and the ability to make decisions without reference to the program's senior management
	5	Using modern technology and reducing red tape in dealing with the program
	6	An integrated database exists to organize the extension program
Objectives of the organization	7	Existence of educational goals for the program
	8	Existence of processing targets for the program
	9	Existence of research objectives for the program
	10	Objectives of the organization are consistent with the directives of the Ministry of Agriculture to develop the performance of rural youth
	11	Employees of the extension program have knowledge of the organization's objectives
	12	Objectives of the organization are consistent with the systems of the target area
Coordination of the organization	13	Communication mechanism with the participation of all stakeholders involved in the extension program
	14	The existence of continuous meetings between the organizing authorities concerned with the extension program
Tasks and duties of the program employees	15	Existence of specific tasks and duties for the extension program employees
	16	Tasks and duties of the program employees are consistent with their academic qualifications
	17	Existence of continuous training to develop the knowledge and skills of the extension program employees
Tasks and duties of programme participants	18	Existence of specific tasks and duties for the participants in the extension program
	19	
	20	Program participants' awareness of the importance of working in a single multidisciplinary team style

B. Planning

Axes	Sequence	Paragraphs
Existence of a plan	1	Existence of a written and clear plan for all authorities related to the program
	2	Having a time-bound plan to achieve program-related goals
Objectives of the plan	3	Existence of clear written objectives for the extension program work plan
	4	Existence of specific goals for each entity participating in the extension program
	5	Adapt the objectives of the extension program in meeting the needs and solving the basic problems of those involved by it
	6	Objectives of the extension program are consistent with the goals of sustainable agricultural development to preserve natural resources
Participate in preparing the plan	7	Participation of those involved in the extension program in preparing the plan
	8	Participation of the relevant authorities in preparing an action plan for the extension program
Plan activities	9	Several extension activities included in the extension program
	10	Existence of multiple processing activities included in the extension program
	11	Existence of multiple research activities included in the extension program
Finance	12	Existence of sufficient financial allocations to cover the requirements of the plan set for the program

C. Monitoring

Axes	Sequence	Paragraphs
Organizing monitoring	1	An organization to monitoring the extension program
Who carry out the monitoring process	2	Adequacy of monitoring employees
	3	Adequacy of the physical supplies (cars, gasoline, stationery) for the monitoring process
Monitoring period	4	Period to monitoring the program (bi-monthly, monthly, quarterly, annually)
Monitoring mechanism	5	Existence of a monitoring mechanism (field visits, reports)
Feasibility monitoring	6	Existence of a diagnosis of deviations and problems in implementing the extension program
	7	Existence of treatment for deviations of the extension program in light of the available capabilities
	8	Existence of an integrated database of those involved by the extension program, including rural youth

D. Implementation

Axes	Sequence	Paragraphs
Implementation of program activities	1	Activities were carried out according to plan
	2	Activities were carried out in light of the available capabilities
Number and type of activities	3	Adequacy of the activities included in the program for those involved in it
	4	Diversity of activities (seminar, course, field viewing, field day, exhibition, publications, conferences, lectures) included in the program for those involved
	5	Suitability of activities to the characteristics of the involved (educational level, socio-economic)
	6	Keeping up with the latest activities in the field of beekeeping and management
Site of implementation	7	Implementation of activities according to their specific location
	8	Presence of all requirements for implementation at the specified time and place
Timings of implementation	9	Implemented according to their timing in the plan
	10	Appropriateness of implementation timings for those involved in the program
Implementers	11	Presence of specialized cadres to implement the activities
	12	Presence of trained cadres to implement the activities
	13	Presence of cadres that are considered a work environment based on discussion, partnership, and mutual trust between them and those involved in the program and stimulate creativity and innovation in the field of beekeeping and management
	14	Adequacy of the extension program employees for implementation
Participation of the targeted	15	Rural youth participation in the extension program activities
Coordination with the participating authorities	16	Coordination with the authorities participating in the extension program
Implementation requirements	17	Having the requirements and material supplies (beehives, wax, boxes, clothes)

E. Evaluation

Axes	Sequence.	Paragraphs
Organizing the evaluation	1	Existence of an organization to evaluate the extension program
The program managers of evaluation process	2	Existence of people who evaluate the extension program
	3	Existence of evaluators from within or outside the organization qualified and trained to carry out the evaluation tasks
	4	Existence of criteria for evaluators to evaluate the program
	5	Adequacy of the extension program evaluation employees
	6	Sufficiency of physical supplies (cars, petrol, stationery, cameras)
Evaluation type	7	Existence of a constructive evaluation for the program (inputs and processes)
	8	Existence of a synthesis evaluation of the program (outputs)
Evaluation of results	9	The results of the program work evaluation
Writing evaluation results	10	Existence of documented and written evaluation results of the extension program
Making use of the evaluation results	11	Benefit from the results of the evaluation in making decisions related to the work and development of the program in terms of its organization, planning, implementation and monitoring

the training division, and four directors of extension farms. To measure the reliability researcher conducted a pre-test in September of 2020 in Diyala Governorate on a sample consisting of 11 respondents of rural youth who were involved in the extension program for developing the capacities of beekeepers in the Baladruz Agriculture Division of the Diyala Agriculture Directorate, which was excluded from the research sample. The researcher has used the Cronbach Alpha Test to measure reliability, which is one of the most accurate reliability tests to ensure the validity of the scale. The value of the reliability coefficient for all fields, axes and paragraphs for the employee's questionnaire was90%, and the value of the reliability coefficient for all paragraphs of the rural youth was 82%.

Table 2. Distribution of the fields, axes, and paragraphs of the questionnaire of rural youth to identify the satisfaction of the rural youth involved

Field	Axes	Paragraphs
Satisfaction of the rural youth involved by the extension program to develop the capacities of beekeepers	Satisfaction with the extension service of the extension program for developing the capacities of beekeepers	Covering all topics of the service provided by the program, including beekeeping and management
		Relevance of the service topics provided by the program to your basic needs in beekeeping and management
		Service provided by the program is relevant to your educational level and experience
		Service provided by the program is appropriate to your economic level
		Realism and simplicity of the service provided by the program and the possibility of applying it in your activity
		Modernity of messages, information, and recommendations provided by the program
		Presenting the messages, information, and recommendations provided by the program in a clear and understandable manner, with the use of illustrative means
		Get messages, information, and recommendations from the program about your need
		Creating a suitable atmosphere for discussion and providing opportunities to present your problem, find a solution, and apply it
	Satisfaction with the processing service for the extension program for developing the capacities of beekeepers	Adequate quantity of supplies for your needs in beekeeping and management
		Appropriate timing for preparing the requirements provided by the extension program for developing the capacities of beekeepers
		Modernity and sobriety of the requirements provided by the extension program
		Continuous supply of supplies when you need them
		Adapting the supplies to your needs in bee management and breeding
Satisfaction of the rural youth involved in the extension program for developing the capacities of beekeepers	Satisfaction with the managers of the extension program to develop the capacities of beekeepers	Good treatment by the managers of the extension program
		Paying attention to your ideas and motivating you to creativity and innovation
		Keenness of the program managers to meet your needs and desires in the management and breeding of bees
		Keenness of the program managers in diagnosing the problems you face in the management and breeding of bees
		Program managers possess the knowledge and skills necessary to develop and improve your knowledge and skills
		Keenness of the program managers to keep up with modernity, development, and continuous improvement in the management and breeding of bees
	Satisfaction with the results achieved from the extension program for developing the capacities of beekeepers	Improve and develop your knowledge, skills and experiences in bee management and breeding
		Improvement and development of the knowledge, skills and experiences of some of your family members who are involved with you in the management and breeding of bees
		Extension program to develop the number of your beehives
		Increasing the number of bee apiaries in the area covered by the extension program
		Improving the quality and productivity of beehives
		Improving the economic returns from your activity in beekeeping and management
		Attending continuity and participate in the extension program activities
		Implementation continuity and practice of the information and recommendations that were presented to you through the extension program to develop the capacities of beekeepers

Quantization of scale: The researcher used in the employees questionnaire a graded five-point scale consisting of degrees (too many, many, to some extent, few, does not apply never) and were categorized as 0,1,2,3,4, respectively, for all five fields and 68 paragraphs to evaluate the extension program for developing the capabilities of beekeepers by identifying the degree of program implementation of total quality standards. Thus, the weighted mean and percentage weight for each paragraph of the employee's questionnaire were calculated, then the weighted average and percentage weight were calculated for each area of the extension program for developing the capacities of beekeepers for rural youth.

The researcher used questionnaire of rural youth a graded three-point scale consisting of (satisfied, somewhat satisfied, dissatisfied). The weights were given as 1,2,3, respectively, for all of the 28 paragraphs of the rural youth satisfaction scale those who are involved in the extension program for developing the capacities of beekeepers about (extension service, processing service, program managers, the program results). Therefore, the weighted mean and percentage weight for each paragraph of the questionnaire of rural youth were calculated, then the weighted mean and percentage weight for each axis of the respondent's satisfaction from rural youth were calculated for extension service, processing service, program managers, and program results.

Data collection: Data on the extension program for developing the capacity of beekeepers were collected using tables and data, as well as access to records, official documents, and reports of the program. Meetings with a number of officials and employees working in the program, including rural youth, through a personal interview as a tool to obtain the necessary data to achieve the research objectives. The questionnaire form was used (For employees) and another (Rural youth), and the data collection was between September 20 to December 20, 2020, after clarifying the subject under discussion to the respondents and focusing on the data being used for scientific research purposes only.

Data analysis: The data were analyzed manually and electronically using the Statistical Package for the Social Sciences (SPSS) program, after being classified, unpacked, and analyzed in tables in order to present the results and interpret.

RESULTS AND DISCUSSION

The weighted average for the program's fields in general was 1.744 degrees, which is much less than the hypothetical mean 43.60 degrees. This result confirms the low degree of applying the extension program for developing the capacities of beekeepers for rural youth in the governorates of Baghdad and Diyala for total quality standards in its fields (Table 3).

The implementation field ranked first among the fields in order of importance in terms of the application of total quality standards with a weighted average of 2,361 degrees and percentage weight of 59.02 degrees. This may be attributed to the endeavor and interest of the program management and its employees to apply and implement the extension plan decision-making on the ground. Thus, the extension plan is considered a statement or a written document (for whom the activities are carried out, who carries them out, how, when, and where they are carried out), as one of the priorities of the program employees is to implement the plan activities and proceed according to its vocabulary. However, the evaluation field was ranked last according to importance in terms of the application of total quality standards with a weighted mean of 0.291 degrees and percentage weight of 7.28 degrees. This may be attributed to the absence of an organization concerned with evaluating the program, in addition to the fact that no kind of evaluation was conducted on the program and consequently the absence of evaluation results. The weighted mean for the fields in general reached 1.744 degrees, which is less than the hypothetical mean of 2 degrees, which confirms the low application of total quality standards in the fields of extension program for developing the capacities of beekeepers in the governorates of Baghdad and Diyala.

The weighted means for the satisfaction of the axes extension service and program managers were 2.47 and 2.50

Table 3. Distribution of respondents according to their answers to applying total quality standards

Field arrangement according to the questionnaire	Field arrangement according to the importance	Fields	Weighted average	Percentage weight	Weighted average of fields	Percentage weight of fields
3	1	Implementation	2.361	59.02	1.744	43.60
1	2	Organization	2.240	56		
2	3	Planning	2.231	55.78		
4	4	Monitoring	1.598	39.95		
5	5	Evaluation	0.291	7.28		

Table 4. Distribution of the respondents according to their satisfaction

Arrangement according to the questionnaire	Arrangement according to the importance	Axes of the satisfaction field	Weighted mean	Percentage weight
2	1	Satisfaction with the program managers	2.50	83.6
1	2	Satisfaction with the extension service of the program	2.47	82.6
4	3	Satisfaction with the results achieved from the program	1.63	54.4
3	4	Satisfaction with the processing service of the program	1.25	41.8

degrees, respectively, which is higher than the hypothetical mean of the scale of 1.5 degrees, with percentage weights of 82.6, 83.6 degrees, respectively. This confirms that those rural youth are satisfied with the axes (extension service, program managers), whereas the weighted means of satisfaction with the axes (processing service and program results were 1.25, and 1.63 degrees, respectively.

The much less than the hypothetical mean 1.5 degrees, and the second value is much close to the hypothetical mean of 41.8, 54.4 degrees, respectively, which confirms the low satisfaction of those involved of the aforementioned axes, as satisfaction is one of the criteria of total quality and an important indicator of the efficiency and effectiveness of the extension program (Table 4). Satisfaction with the program managers ranked first among the axes in order of importance, with a weighted average of 2.50 degrees and with percentage weight of 83.6 degrees. This may be attributed to the keenness of the program managers to prepare the appropriate atmosphere and climate of effective and influential communication and discussion between them and those involved in the program from the rural youth. Besides that, most of them resided in the areas in which the activities of the program are carried out and therefore there are mutual relations between them and the involved in respect and goodness treatment. Satisfaction with the processing service of the extension program ranked the last with a weighted average of 1.25 degrees and percentage weight of 41.8 degrees. This may be attributed to the suspension of the processing service for the extension program to develop the capacities of beekeepers for rural youth since 2013 and the program's restriction to extension service only.

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Evaluation of Water Stress and Growth Function for Proso Millet (*Panicum miliaceume* L.) Based on Evaporation Pan in a Silt Loam Soil

Sama Mohammed Noori and Salloom Barghooth Salim

Department of Soil and Water Resources Sciences
College of Agricultural Engineering Sciences, University of Baghdad, Iraq
E-mail: salloom.salim@coagri.uobaghdad.edu.iq

Abstract: A field experiment was conducted during the 2020 summer season at the Agricultural Research and Experiments Station of the College of Agricultural Engineering Sciences, University of Baghdad to evaluate the effects of continuous and stage-water stress of proso millet. The study site was situated at a latitude of 81°29'26.33" North, a longitude of 44°37'91.56" East, and an `altitude of 31.8 meters above sea level. The study area exhibited a predominantly flat to semi-planar topography, with a slope of less than 2%. The soil was classified as sedimentary, with a silt loam texture. The experiment involved two factors' depths of applied irrigation water (DAIW) based on three levels of the amount of water evaporated from the evaporation pan (PEL) 40% (P1), 80% (P2), and 120% (P3) and the stage of water stress no water stress (a control treatment) (S1), vegetative stage (S2), appearance of the flag leaf stage (S3), and flowering and grain filling stage (S4). The DAIW for the full irrigation treatment were 289.0 mm, 476.6 mm, and 753.8 mm, with deep percolation losses of 0.0 mm, 82.4 mm, and 318.0 mm for the 40, 80 and 120% PEL, respectively. The average grain yield exhibited significant differences among the various PEL treatments, with values of 4098, 3802 and 1147 kg ha^{-1} for the 80, 120 and 40% PEL treatments, respectively. The 40% PEL resulted in the lowest average values for plant height, number of leaves. The actual evapotranspiration (ETa) values were highest for the 120% PEL, compared to the 80% and 40% PEL treatments with the 80% PEL treatment demonstrated the highest water use efficiency. The growth function was accurately represented by a second-degree polynomial function, demonstrating a highly significant correlation coefficient. Analysis of the growth functions further reveals that the rate of plant height increase was the highest for the 120% PEL treatment consistently throughout the entire growing season. These findings emphasize the substantial impact of different PEL levels on growth and grain yield of millet.

Keywords**:** Water stress, Proso millet, Pan evaporation, Growth function

Water stress, also known as drought stress, is one of the main causes of severe food shortages worldwide as it causes severe losses in agricultural crop production. Water stress negatively affects the productivity of agricultural crops by influencing the chemical, biological and physiological processes that occur in the soil (Hadi and Salim 2016, Gracia et al 2020). Plants experience water stress when there is a limited water supply to the roots or a high rate of transpiration (Shrestha et al 2023). This condition can persist throughout the entire growth period or during specific stages of growth. Considering that 40% of agricultural production relies on irrigated lands, which constitute 70% of the world's water reservoirs, insufficient production remains a major hurdle, particularly with the increasing global population and the escalating impact of global warming. Water stress is projected to worsen in the future due to the limited availability of water resources (Zhao et al 2006, Al-Ansari et al 2015). To ensure survival, understanding the mechanisms underlying drought resistance of drought-tolerant field crops requires understanding. It is critical to identify critical periods and responses to water scarcity (Sarwata et al 2017). Millet, specifically the local variety Prosso (*Panicum miliaceum* L.), is one of the summer crops known for its resistance to drought. It has been cultivated for centuries in hot, dry and tropical regions, which makes it successfully suitable for cultivation in Iraq. As the water requirements of millet increase with the progression of the growth period, to reach peak during the flowering stage and gradually decrease until the final irrigation date. Given the economic and nutritional importance of millet, as well as being one of the most productive cereal crops in the world after rice, wheat and maize (FAO 2021), especially in arid and semi-arid regions. This is necessary to study the performance of the crop and note its response to stress under conditions of water stress, specifically at the critical growth stages. Plants may be exposed to water stress due to climatic factors, a sharp decrease in renewable water sources, or the high cost of water, which leads to the plant being exposed to water stress continuously throughout the plant growing season or during a specific growth stage. The method used to manage water stress is of great importance in understanding the plant's response to water stress and evaluating its adaptive

capacity. In most experiments, water is temporarily withheld for a short period. However, to accurately simulate the plant's response to drought, it is necessary to subject the plant to a prolonged period of stress, which may require implementing staged stress (cyclic water stress).\

MATERIAL AND METHODS

The field experiment was conducted during the summer season of 2020 in an agricultural field designated at the College of Agricultural Engineering Sciences, University of Baghdad, located in Al-Jadriya. The site is situated at latitude 81.29°26'33" north and longitude 44.37°91'56" east, with an elevation of 31.8 meters above sea level. In order to evaluate the physical and chemical properties of the soil, soil samples were collected from five distinct locations at depths ranging from 0.0 to 0.40 m. These soil samples were thoroughly mixed, air dried in the laboratory then grinded and passed through a 2 mm sieve to determine the physical and chemical properties of the soil before planting. The study area is characterized by mostly flat to semi-flat topography, with a slope of less than 2%. Soils in the field were classified as sedimentary, with a soil texture as loamy loam, and fell under the typical Torrifluvent group according to the soil survey manual (2014).

To estimate the relationship between volumetric moisture content and water potential of the field soil, the moisture retention curve was determined by measuring the moisture content at different potential values ranging from 33 to 1500 kPa. To estimate this relationship, a pressure plate apparatus was used following the method defined by Kulte (1986). The experimental data were fitted to van Genuchten equation (Van Genuchten, 1980) to determine the functional relationship between laboratory-measured moisture content and water potential data using nonlinear regression analysis according to the following equation

$$\theta = \theta_r + (\theta_s - \theta_r)\,[1 + (\alpha\varphi)^n]^{-m} \quad (1)$$

Field experiment parameters: The experiment consisted of the following treatments:

Irrigation levels: Three levels of irrigation were applied based on the percentage of evaporation pan data; 40% of evaporation pan data (P1), 80% of evaporation pan data (P2) and 120% of evaporation pan data (P3).

Stage water stress treatments: Four levels of water stress treatments were implemented; well irrigated (control treatment) without exposing the plants to any water stress (S1), 10 days of water stress treatment during the vegetative stage (S2), 10 days of water stress treatment during the appearance of the flag stage (S3) and 10 days of water stress during flowering and grain filling stages (S4).

The experimental design was in split-plot arrangement within a randomized complete block design with three replicates.

The water content at field capacity (θ_{fc} =0.391 cm^3cm^{-3}) and the permanent wilting point (θ_{pwp} = 0.178 cm^3cm^{-3}) were estimated to determine the available soil water content (θ_{avil} = cm^3cm-3). The DAIW for the initial germination irrigation (d_i) was determined as the difference between the water content at field capacity and the initial water content before irrigation (θ_i) for the 0-30 cm layer, according to the following equation:

$$d_i = (\Theta_{fc} - \Theta_i) \times 30 \quad (2)$$

The second irrigation was applied after depleting 40% of the available water from the first irrigation. The third irrigation was applied to all treatments at the same time after depleting 40% of the available water from the second irrigation. For the subsequent irrigation water was applied based on PEL at 50% depletion of the moisture storage of the available water in the root zone. Stage-water stress treatments were implemented for 10 days at mid stage period. Irrigation period for each treatment varied depending on soil moisture content at 50% PEL depletion. Soil moisture content measurements were conducted during the growing season using the gravimetric method. Hand-held soil sampler (2 cm diameter and one meter length) was used to collected soil samples then the samples were dried in a microwave oven to determine gravimetric water content. Iirrigation water was delivered using a PVC piping net consisted of main (0.05 m) , submain (0.03 m) for each block and laterals (0.016 m) for each plot with control valves. Water meter measuring +0.001 m^3 volume differences was fixed directly after the electric water pump.

RESULTS AND DISCUSSION

The number of irrigations during the growing season were 7, 9 and 13 for 40, 80 and 120% PEL, respectively (Table 1). Evaporation rate and transpiration decrease at low water potential values (negative value) since soil water is adhered more tightly to soil particles, resulting in low rate of change in water content over time ($d\Theta/dt$) and extended periods between irrigations for the 40% PEL. While no deep percolation losses occurred in the 40% PEL, 82.4 mm and 318.0 mm occurred as deep percolation in the 80% PEL and 120% PEL, respectively during the growing season as a large amount of water was added so that it exceeded the ability of the soil to store water (Table 1). In soil profile water moves from high water potential to low water potential (Salim and Khudhair 2015). The water content at 50% depletion from the 80% and 120% PEL was higher than the moisture content at which 50% was depleted based on the moisture storage of the available water of the root zone. The water content deficit

of the root zone at 50% depletion equals 0.109 cm^3cm^{-3} which stands to 4.36 cm of water for the 40 cm effective root zone, so that deep percolation occurred when DAIW based on PEL is greater than 4.36 cm. DAIW for most 120% PEL exceeded 4.3 cm resulting in deep percolation losses. As a result, water loss from the root zone increased and moved below the root zone with the increase in the number of irrigations. On the other hand, the water deficit increased with increasing the number of irrigations for the 40% PEL when 50% of the moisture storage of the available water of the root zone was depleted. Based on PEL, the ETa value of the 120% PEL treatment was the highest at 435.8 mm $season^{-1}$, while the ETa value of the 40% PEL treatment was the lowest at 280.0 mm $season^{-1}$ (Table 1). The difference in ETa values for different treatments is due to the difference in the amount of Irrigation water added based on pan evaporation levels (Fabre 2008).

The actual evapotranspiration rate for the control treatment (full irrigation) was the highest compared to the ETa for the different water stress treatments. The reason for this may be attributed to the fact that the soil water content of the control treatment is higher than the rest of the treatments with higher rates of evaporation from the soil surface and higher plant transpiration. These results are consistent with the findings of Ahmed et al (2013) also observed that ETa and DAIW in full irrigation treatment was larger compared to water stress treatments. In field studies, effective root zone depth and DAIW should be considered to prevent over wetting of the root system and deep percolation losses due to the addition of irrigation water greater than the actual plant need.

Growth functions and plant heights: Figure 1 illustrates the presence of inflection-deflection points in the crop growth function: the inflection point indicates an increase in growth stage and the deflection point indicates a decline in growth stage. The second stage is characterized by an immediate decrease in growth rate after flowering and grain filling stage. Initially, the three levels of irrigation water application exhibit similar growth patterns, but over time, the rate of plant height changes for each level varies until it reaches its maximum potential level, after which it gradually decreases. The growth function was described by a second order polynomial function, with very significant correlation coefficient (Fig. 1). The change in plant height (dh) per unit time (dt), signifies the rate at which plant height increases during different time intervals throughout the growing season. For 120 and 80% PEL, after 10 days of growth, the rate of change in plant height (dh/dt) was 1.8 cm/day. It can be observed from that the growth functions for the 120 and 80% irrigation levels align across all treatments, including full irrigation level, indicating that water stress has no significant impact on millet growth (Fig. 1). Conversely, the growth function for the 40% irrigation level demonstrates a continuous decrease in the rate of plant height throughout the growing season due to a sharp decline in water content in the root zone. This decline consequently affects plant nutrient absorption, leading to reduced water content in plant tissues, limited leaf elongation, diminished leaf photosynthesis, protein synthesis, and ultimately resulting in a significant decrease in plant height.

The highest average of plant height, 95.13 cm, which was not significantly different from the 120% PEL, where the average height was 94.78 cm (Table 2). Conversely, the treatment with 40% PEL resulted in the lowest height average of 56.95 cm. During the flowering and grain filling stages, the water stress treatments exhibited good performance, as they produced the highest average height of 83.55 cm. Subsequently, the remaining water stress treatments did not significantly differ from flag leaf and the control treatments, yielding average plant heights of 82.72 cm and 82.29 cm, respectively (Fig. 2). The treatments

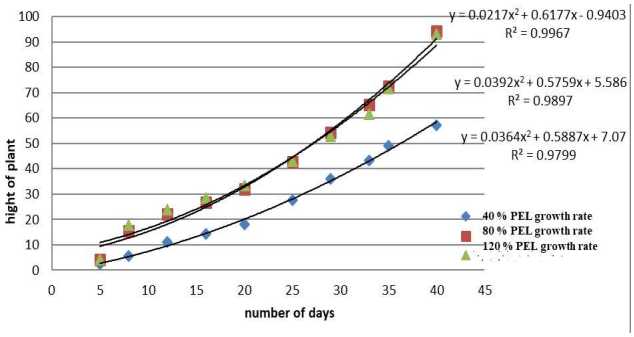

Fig. 1. Growth functions for the 40, 80 and 120% pan evaporation levels

Table 1. Average values of the parameters of the soil water balance equation for the 40, 80 and 120% pan evaporation levels

Eta mm $season^{-1}$	Water deficit Mm	Deep percolation Mm	Rainfall Mm	DAIW mm $season^{-1}$	Number of irrigations	PEL (%)
280.0	138.8	0.0	0.0	0.028	7	40
394.2	12.0	82.4	0.0	476.6	9	80
435.8	0.0	318.0	0.0	753.8	13	120

involving stress during the vegetative growth stage resulted in lowest plant height of 80.59 cm. Treatment P2S4 produced the greatest height of 96.81 cm (representing the 80% PEL irrigation treatment during flowering and grain filling stages). Conversely, the interaction between 40% PEL and water stress during the vegetative growth stage (P3S2) produced the lowest height of 55.57 cm. Water availability significantly impacted vegetative growth, as an adequate supply of soil water prompted an increase in plant height through enhancing cell size and division. Water stress typically arises in plants when transpiration rates surpass root water uptake rates, leading to growth cessation in developing plant tissue structures particularly in hot weather. This finding is consistent with the outcomes reported by Enneb et al 2020).

The presence of water stress triggers a sharp and rapid decline of 75% in photosynthesis within leaves compared to those subjected to low water stress. Water stress also reduces the concentration of auxins in the plant, which play a crucial role in cell elongation and division, ultimately hindering stem growth and carbon assimilation. Additionally, increased exposure to water stress results in significant inhibition of Indole-3-Acetic Acid (IAA) function. These findings align with the studies conducted by Blossey and Hunt (2003) and Harikrishnan et al (2014).

Grain yield (kg ha^{-1}): Water stress had a substantial impact on grain yield, as indicated by the results of the statistical

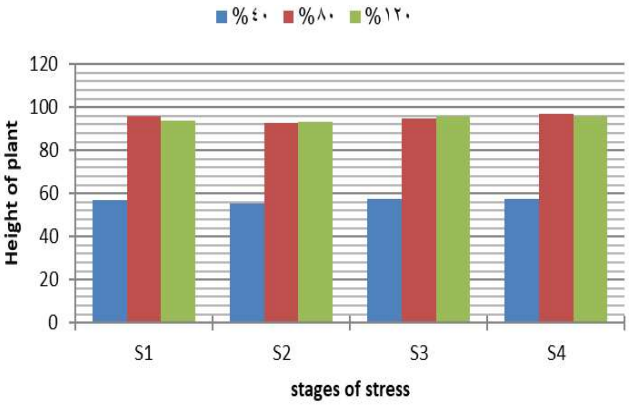

Fig. 2. Effect of pan evaporation level PEL and stage-water stress on plant height (cm)

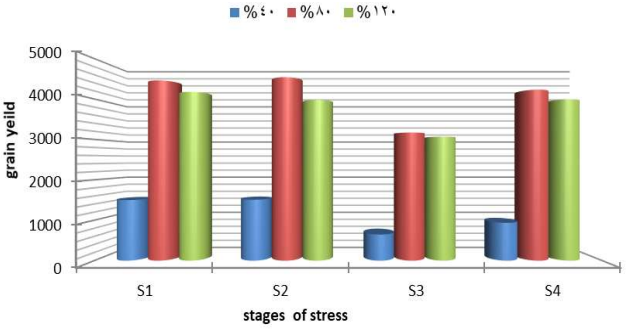

Fig. 3. Effect of pan evaporation level PEL and stage-water stress on average grain yield (kg ha^{-1})

Table 2. Effect of pan evaporation level PEL and stage-water stress and on plant height (cm)

Irrigation level based on percent pan evaporation	Stress stage				Average
	S1	S2	S3	S4	
40%	57.07	55.57	57.48	57.67	56.95
80%	95.97	93.03	94.70	96.81	95.13
120%	93.83	93.17	95.97	96.16	94.78
LSD			4.2		2.55
Average of stress stage	82.29	80.59	82.72	83.55	
LSD			1.19		

Table 3. Effect of pan evaporation level PEL and stage-water stress on average grain yield (kg ha^{-1})

Irrigation level based on percent pan evaporation	Stress stage				Average
	S1	S2	S3	S4	
40%	1508	1498	647	937	1147
80%	4537	4458	3169	4229	4098
120%	4175	3977	3006	3991	3802
LSD			204		2.55
Average of stress stage	3406	3311	2294	3053	
LSD			119		

analysis, which revealed significant differences in average grain yield among treatments which agree with the findings of Armand (2016). Present the average grain yield was 4098, 3802, and 1147 kg ha^{-1} for the 80%, 120%, and 40% PEL treatments, respectively. Furthermore, the stage of water stress also exerted a significant effect on the average grain yield (Table 3, Fig. 3). The treatment with full irrigation outperformed the other stage-water stress treatments, yielding the highest grain yield of 3406 kg ha^{-1}, which did not significantly differ from the treatment with stress during vegetative growth, having an average yield of 3311 kg ha^{-1}. Conversely, the stress treatments during the growth of the flag leaf and flowering and grain filling exhibited significantly lower grain yields of 2294 kg ha^{-1} and 3053 kg ha^{-1}, respectively. In terms of interaction effects, the P2S1 treatment demonstrated the highest yield, with a grain yield of 4537 kg ha^{-1}, while the P1S3 treatment demonstrated the lowest yield, 647 kg ha^{-1}, in the treatment involving water stress during flag leaf stage. The growth stage of the flag leaf is crucial for flower pollination and seed formation. Insufficient water availability during this stage hinders proper flower pollination, leading to seed abortion. Moreover, the flag leaf plays a pivotal role in synthesizing 80% of the accumulated dry matter in the seeds.

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Depth Function of Boron Distribution in Some Iraqi Salt Affected Soils

Sabreen A. Ali and Kusay A. Wheib

Department of Soil sciences and Water Resources, College of Agricultural Engineering Sciences
University of Baghdad, Iraq
E-mail: sabreen.abdalhusain1207a@coagri.uobaghdad.edu.iq

Abstract: The study area was chosen in the salt affected soils spread between the two agricultural projects of Al-Wahda and Al-Suwaira, which includes the Al-Suwaira Model Farm as part of its lands, in order to provide soils with different stages of salinization. Eight pedons were selected for the purpose of studying the distribution and genesis of the available and total boron in salt-affected soils of the type of shurah, sabkha, shura-sabkha, and other unaffected soils for comparison. As well as studying soil properties to reveal the relationship between them and the boron content in the soil. The highest total boron content was 31.52 mg kg-1 in the C3 horizon of the DM95 series, followed by the A1 horizon for the same series with a content of 25.36 mg kg-1, as this series represents salt affected soils of the type of sabkha with a salinity value of 82.6 dS m^{-1} and 27.00 dS m^{-1} for horizons A1 and C1 respectively. Whereas, the lowest boron content in the horizon was A1 for soil series DF97, which represents soil unaffected by salts. The total boron content showed a positive and significant correlation (R^2 = 0.26, $p<0.05$) with the total sand content in the soil. As the total boron content increased with the increase in the sand content, the reason is due to the presence of the tourmaline minerals in the coarse separate and containing boron in its chemical composition. The total boron content showed a highly significant relationship, R^2 = 0.64 and R^2 = 0.51 for the A1 and C1 horizons, respectively, with the presence of tourmaline mineral in the soil. The total boron content increased with the increase in the boron content in the fine sand separate. The available boron content was significantly related to the total clay content in the soil (R^2 = 0.3833, $p<0.05$), as the increase in the total clay content in the soil leads to an increase in the available boron content. The available boron content showed a simple positive exponential relationship with soil salinity. The available boron content extracted with hot water increased with the increase in soil salinity of the saturated paste extract. The upper horizons showed a clear variation in the content of the organic matter, as it was found that the standard deviation of the organic matter content in the upper horizons was 4.5, while it was 1.6 for the rest of the horizons of the selected soil series, so the total horizons of the series combined is a general trend of the relationship between the content of organic matter and the content of available boron (R^2 = 0.1963), but when the upper horizons were isolated from the subsurface ones, the values of the organic matter content showed a highly significant positive relationship indicating the effect of organic matter content on the available boron content in the soil (R^2 = 0.6173).

Keywords: Boron, Soil, Salt, Iraq

The salt affected soils are defined as the soils that contain a concentration of dissolved salts in a quantity that negatively affects the growth of cropsor may be defined as the soils in which the electrical conductivity of the saturated paste extract (ECe) exceeds (4 dS m^{-1}), the exchangeable sodium percentage ESP is less than 15%, and soil reaction degree for these soils is usually less than 8.5, since most of the salts accumulated in them are neutral, in addition to the presence of carbonate minerals in abundant quantities in them. Such soils arise in dry and semi-arid areas where the annual rainfall rate is lowof 100 -200 mm per year. In such conditions, the processes of washing and transferring salts from the soil sector to the ground water and then to the main waterways are very weak, and the increase in evaporation and transpiration under drought conditions helps to collect salts in the soil column and thus the so-called salt-affected soils arise. The concentration of salts, especially the neutral ones, may increase in the soil extract (Saline soil). The increase in the concentration of salts in the soil solution is usually varied, as it consists of sodium, calcium, magnesium, chlorides and sulfates mainly and potassium, bicarbonate, nitrates, and boron in secondly. The salt compounds dissolve in the soil solution to their roots, which consist of a number of ions, and these ions move in the soil column to the bottom or top according to the abundance or scarcity of water, and the factor affecting its movement is the degree of solubility of these salts. Boron salts are found in the soil in the form of borate ions, which is the soft form of the plant $B(OH)^{-4}$ or boric acid H_3BO_3. Therefore, it is subjected to loss from the soil through washing, and this makes dry lands on the one hand and salt-affected on the other hand with a higher content of this element than the lands Boron is easily lost from soil compared to other microelements. Despite the common prevalence of high boron content associated with high salinity in many parts of the world, there is a dearth of research on the study of the interaction between these two

terms (Ferreyra et al 1997). Boron analysis in rocks and sedimentary soils is related to the estimation of boron content in soil as an indication of ancient salinity in it. Studies to detect the presence of salinity in soils of ancient environments are an important factor in prospecting for the presence of petroleum in an area (Wei and Algeo 2020). When Boron is abundantly present, poses a threat to the ecosystem. The problem of boron arises by increasing the content in saline sewage and sewage waste as part of the hydrological cycle, to collect later in the food chain, causing problems in the ecosystem. Boron can be present in significant quantities in groundwater or wash water, but it is rarely high in surface water sources (Ravenscroft and McArthur 2004). Manchanda and Sharma (1991) concluded that the occurrence of the salinization process in the soil is accompanied by an excess of boron in the soil solution, and that there is a joint effect between soil salinity and boron content in affecting plant growth in salt-affected soils.

The objective of study includes studying the distribution of boron in the pedunas of some salt affected sedimentary soils and effect of the type of salinity on the distribution of boron in some sedimentary soils affected by salts.

MATERIAL AND METHODS

The study area is located in the salt-affected soils spread between the two agricultural projects of Al-Wahda and Al-Suwaira, which occupies the Suwaira model farm as part of its lands, due to the availability of soils with different stages of salinization. The Al-Suwaira agricultural project was chosen, which belongs to the administrative borders of Wasit Governorate, and this project is characterized by the presence of a variety of salt-affected soils between white salt soils (Shura) and dark-colored salts (Sabkha). The project is located between longitudes 44º 39′ and 44º 57′ east and latitudes 32º 54′ and 33º 14′ north (Fig. 1).

Soil samples: For the purpose of conducting a preliminary survey of the levels of boron in Iraqi soils, 32 representative soil samples were collected from eight pedons of different soil series from the middle of the Iraqi alluvial plain. Two Shura soil, two Sabkha, and two shura-sabkha soils, besides, two unaffected soils with salt. The pedons were morphologically described according to the soil survey manual and its appendices. Samples were taken from each horizon basically, dried, crushed and sieved through a sieve with a diameter of 2 mm and kept in plastic bags for the purpose of laboratory analysis. Soil samples were taken from each horizon and transferred to the laboratory for the purpose of estimating some physical and chemical properties, as well as ground water samples, if any. Table 1 shows the number and coordinates of soil series with type of salinity depending on the morphological characteristics of the soil.

Chemical and physical properties: After obtaining the soil samples, they were transferred to the laboratory for the purposes of some chemical and physical properties on them:

Volume distribution of soil particles: Estimate the volume distribution of soil particles by hydrometer method, according to Day's method (Black 1965).

Bulk density: It was measured by paraffin wax wrapping method according to the method mentioned in Black (1965).

Soil reaction (pH): The process of measuring soil reaction (pH) was carried out with a pH meter according to the method (Jackson 1958).

Electrical conductivity (ECe): The electrical conductivity (ECe) was measured in a 1:1 extract and converted to saturated soil paste extract using electrical conductivity bridge (Wimmer et al 2003).

Cations and anions: Cations (K^+, Na^+, Mg^{+2}, Ca^{+2}) and negative ions ($CO3^{-2}$, HCO^{3-}, SO_4^{-2}, Cl^-) were determined in a 1:1 extract and converted to the saturated paste extract for soil (Wimmer et al 2003).

Cation exchange capacity (CEC): 1N NH4OAc ammonium acetate was used at (pH = 7.0) (Black 1965).

Carbonate minerals: Calcium carbonate was determined by calcimeter method using HCL acid.

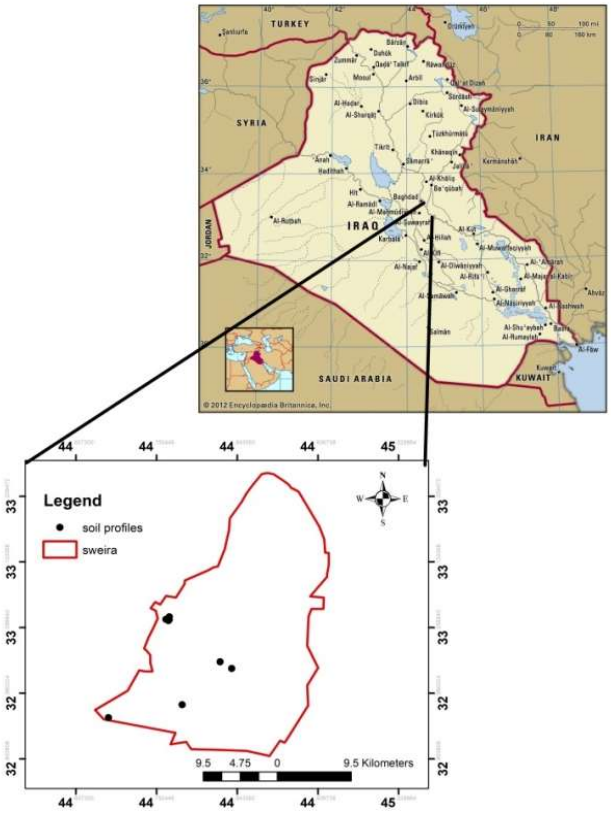

Fig. 1. Location of the Al-Sweira projects from the map of Iraq and the sites of the Pedons

Soil organic matter content (OM): OM was estimated by wet digestion method according to Walkly and Black method (Jackson 1958).

Available boron: the available boron was extracted with hot water and it was determined by the colorimetric method described in (Ferreyra et al 1997). After extracting the prepared boron with hot water, the sample size was completed to 25 ml, and then it was estimated by the method of Isomethan H and chromatic development.

Total boron content: Soil was digested and the boron content was estimated by the colorimetric (Ferreyra et al 1997).

Sodium adsorption ratio of SAR and the percentage of exchanged sodium (ESP): It was estimated through the following equations and according to the methods of Wimmer et al (2003):

$$SAR = \frac{Na^{+}}{\sqrt{(Ca^{+2} + Mg^{+2}/2}}$$

$$ESP = \frac{100(-0.1226 + 0.1475\,SAR)}{1 + (-0.1226 + 0.1475\,SAR)}$$

The salt affected soils were also classified based on the aforementioned criteria and according to the classification of the American Salinity Laboratory (Wimmer et al 2003):

Heavy sand minerals: Sand minerals were estimated by counting under a polarized microscope according to the following steps:

1. Obtaining fine sand separated 50-100 µm for the soil sample.
2. Separation of heavy and light metals for fine sand fractions using bromoform liquid with a specific weight of 2.89 g/cm3.

- Prepare glass slides using Canada balsam as a display medium with a refractive index of 1.54.
- Identification and quantitative determination of minerals using the polarized microscope technique and by counting method according to (Ravenscroft and McArthur 2004).

RESULTS AND DISCUSSION

Some morphological characteristics of the study soils for the salt-affected sites such as shura, sabkha, shura sabkha and unaffected soils are given in Table 3.

Boron content

Total boron content: The highest total boron content was 31.52 gm kg^{-1} in the C3 horizon for the DM95 series, followed by the A1 horizon for the same soil basket with a content of 25.36 mg kg^{-1}(Table 4). This series represents soil affected by salts of the type of sabkha. The salinity was 82.6 and 27.00 dS m^{-1} for the A1 and C1 horizons, respectively, while the lowest boron content was at the A1 horizon for the DF97 soil series, which represents unaffected soils of salt.

The total boron content showed a positive and significant correlation (R^2 = 0.26 with the total sand content in the soil (Fig. 2). The total boron content increased with the increase in the sand content and is due to the presence of the mineral tourmaline in the coarse separation and containing boron in its chemical composition. Boyd (2002) observed that the source of boron in sedimentary crumbs is mostly tourmaline. The total boron content also showed a highly significant relationship of for the A1 and C1 horizons (R^2 = 0.64 and R^2 = 0.51, respectively), with the presence of the mineral tourmaline in the soil, as the total boron content increased

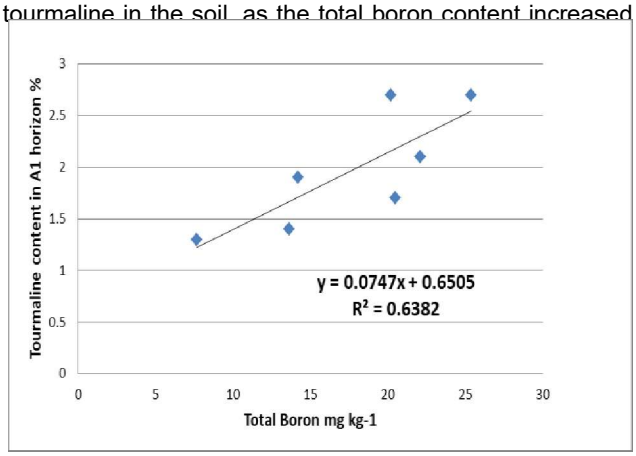

Fig. 2. Relationship between the content of sand separate and total boron

Table 1. Coordinates of study pedons and type of salinity

Pedon No.	Soil series	Longitude	Latitude	Soil type
1	DF126	44.76135	33.06629	Unaffected
2	DM97	44.82391	33.01677	Unaffected
3	TM1175	44.83734	33.00899	Shura Sabkha
4	DF46	44.78011	32.96671	Shura
5	DM47	44.76513	33.06582	Sabkha
6	DM95	44.7643	33.06474	Sabkha
7	DF86	44.76529	33.06908	Shura Sabkha
8	DW55	44.69498	32.95176	Shura

with the increase in the boron content in the fine sand separated (Fig. 3).

Available boron content: The highest value of the prepared boron was 12.96 mg kg^{-1} in the C2 horizon of the TM1175 soil series, the highest salinity on the one hand and the highest in the clay content on the other hand (Table 4). The salinity of the highest horizon of the same series was 154.2 dSi-Siemens m^{-1}. The available boron content was significantly related to the total clay content in the soil (R^2=0.3833) the increase in the total clay content in the soil leads to an increase in the available boron content (Fig. 4). The available boron adsorbs on the surfaces of colloids (clay and organic matter), so the increase in the content of clay leads to an increase in the content of available boron, and this was confirmed by Arora and Chahal (2007, 2014).

In soil sample highest available boron content, was in C2 horizon, and total boron content of 15.82 mg kg^{-1} and reached 40 g kg^{-1}, which is reflected on the content of boron-bearing

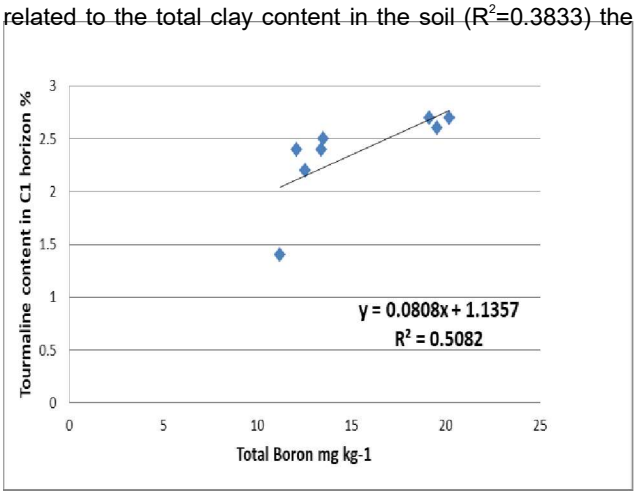

Fig. 3. Relationship between the total boron content with tourmaline in the A1 and C1 horizon samples

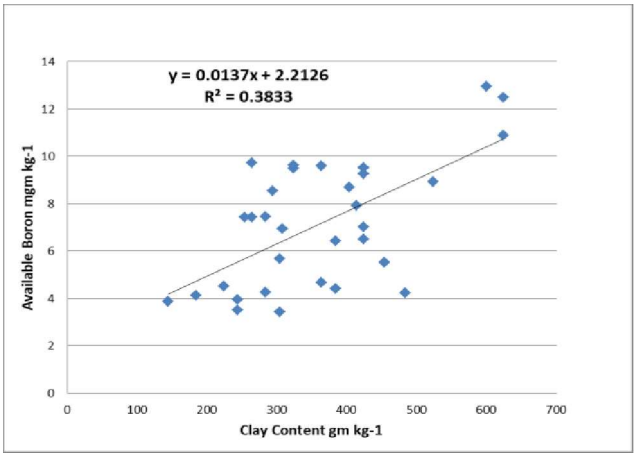

Fig. 4. Relationship between the content of clay and available boron

Table 2. Saline soils classification according to US soil salinity lab

Classification of salt affected soil	Electrical conductivity ECe dS m^{-1}	Reaction pH	Exchangeable sodium Percentage ESP
Non-saline soils	Less than 4	Less than 8.5	Less than 15%
saline soils	More than 4	Less than 8.5	Less than 15%
Alkaline saline soils	More than 4	More than 8.5	More than 15%
Soils alkalinity	less than 4	more than 8.5	more than 15%

Table 3. Some morphological properties of the study soils

Series	Color	Texture	Structure	Remarks
DF126	Very pale brown when dry to dark yellowish brown when moist	Silty clay over clay and silty clay loam	Medium, strong, angular to sub angular blocky	Extremely hard, firm, very sticky, very plastic
DM97	Very pale brown when dry to dark brown when most	Silty clay over silty clay loam and silty clay	Strong granular and sub angular blocky	Firm, sticky and plastic
TM1175	Very pale brown when dry to dark and yellowish brown when moist	Clay over silty clay, clay and loam	Medium strong and moderate angular and sub angular blocky	Extremely hard and firm and friable, sticky and plastic
DF46	Light yellowish brown when dry to yellowish brown when moist	Clay loam over loam and silty clay loam, and lens of clay	Strong and moderate angular and sub angular blocky	Hard, firm and friable, sticky and plastic
DM47	Light yellowish brown when dry to brown and dark brown when moist	Loam over clay and silty clay	Weak and strong, medium, sub angular blocky	Slightly hard, friable and firm, sticky and plastic
DM95	Yellowish brown when dry to brown and pale brown when moist	Loam over silty clay loam and loam	Strong, moderate and weak, medium, angular and sub angular blocky	Slightly hard, friable and firm, sticky and plastic
DF86	Pale yellowish gray when dry to Dark yellowish brown and very dark brown when moist	Clay loam over sandy clay	Weak and moderate, medium, sub angular blocky	Friable and sticky and very sticky, plastic and very plastic
DW55	Yellowish brown when dry to brown to dark brown when moist	Clay loam over slit loam and loam	Weak and moderate, medium, sub angular blocky	Friable, sticky and plastic

minerals in their chemical composition, such as tourmaline, and therefore the total content of boron will be low.

The content of the available boron showed a simple positive exponential relationship with the soil salinity, as the content of the prepared boron extracted with hot water increased with the increase of soil salinity in the saturated paste extract (Fig. 5). The increase in the salt concentration is often accompanied by an increase in the content of available boron due to presence of dissolved boron with an increase in the salt concentration in the soil solution and ground water in the soil, as the boron content increases in the salt-affected soils, and this was confirmed by a number of researchers (Wimmer et al 2003, Naz et al 2006, Zhao et al 2019).

The content of carbonate minerals did not show a significant relationship with the content of available boron, although a number of researchers confirmed the existence of an inverse relationship between the soil content of carbonate minerals and the prepared ones of boron. The reason is that all of the study soils are characterized as lime, and that the lime content in them is high in the horizons of the study soil series, which led to the disappearance of the effect of these compounds in the soil on the content of available boron in it.

As for the organic matter, the distribution of organic matter in the upper horizons of the saline soil series was somewhat high compared to the rest of the horizons of the study soils. Salt, reduces the decomposition of the accumulated substances in the soil body with the presence of salts. Therefore, the upper horizons showed a clear variation in the content of organic matter. The organic matter content in the upper horizons was 4.5, while it was 1.6 for the rest of the horizons of the selected soil series. Therefore, the total horizons of the series combined showed a general trend of the relationship between the content of organic matter and the content of available boron (R^2 = 0.1963), but when the upper horizons were isolated from the subsurface, the values of the organic matter content showed a highly significant positive relationship indicating the effect of the content of organic matter on the content of available boron In soil (R^2 = 0.6173) (Fig. 6 and 7).

Depth function of the of available boron distribution in soil: The distribution of available boron in the soil as a function of depth for soils of shura, sabkha, shura sabkha and soils unaffected by salt given in Table 8. All series of soils were characterized by having a relatively high content of available boron in the upper horizons, after which the boron

Table 4. Total and available boron content in mg kg^{-1} in soil series

Soil series	Horizon	Total boron	Available boron
DF126	Ap	14.23	6.49
	C1	19.52	4.23
	C2	22.11	4.42
	C3	8.39	4.68
DM97	A1	7.68	8.94
	C1	12.08	6.42
	C2	12.54	5.51
	C3	16.54	9.51
TM1175	A1	20.48	12.5
	C1	13.49	8.69
	C2	15.82	12.96
	C3	19.71	3.94
	C4	19.11	7.42
DF46	A1	20.22	9.49
	C1	11.17	10.9
	C2	22.15	7.03
	C3	19.29	4.26
DM47	A1	13.65	9.73
	C1	19.11	7.42
	C2	19.37	9.27
	C3	13.54	7.91
DM95	A1	25.36	3.87
	C1	20.18	9.63
	C2	17.23	7.45
	C3	31.52	4.13
DF86	A1	22.11	9.61
	C1	13.39	5.68
	C2	11.68	6.94
	C3	18.08	3.42
DW55	A1	12.54	8.54
	C1	12.54	4.51
	C2	17.48	3.5

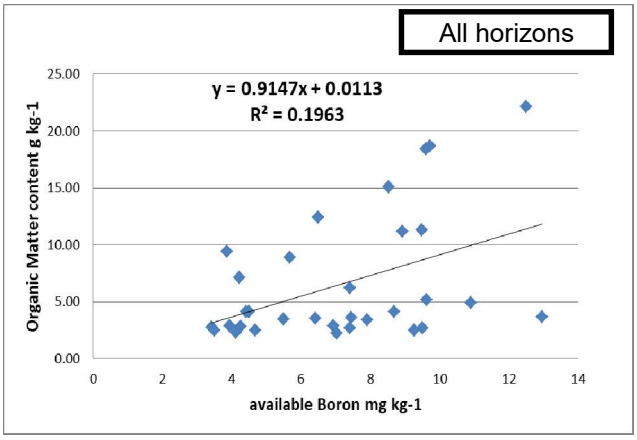

Fig. 6. Effect of organic matter in all horizons of the study soils on boron

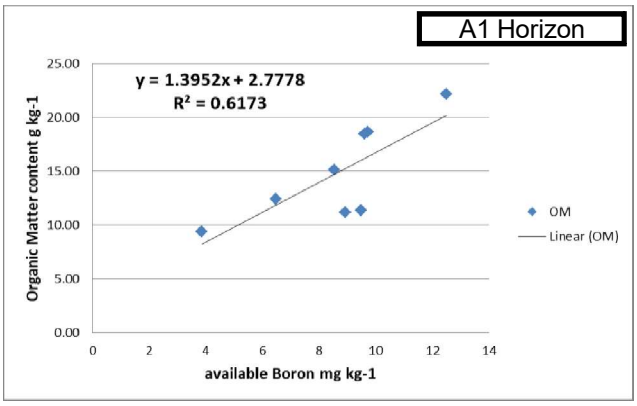

Fig. 7. Effect of organic matter in horizon A on boron

begins to decrease with depth, but except for the DM95 series of soils, in which the content of available boron decreased in the upper horizons compared to the horizons that follow, and is due to the fact that the texture of the soils of the horizon A1 in this series was mixed and with a clay content less than its content in the next horizons, which was reflected on the prepared boron element in the soil. The content of available boron was the highest in the series of soils with salinization of the type of Shura sabkha in the surface and subsurface horizons, followed by the chains of soils with salinization of the type of sabkha and then the shura and finally the soils unaffected by salts. The content of available boron decreased in the horizons. Deep subsurface may be evidence of the contribution of ground water to soil

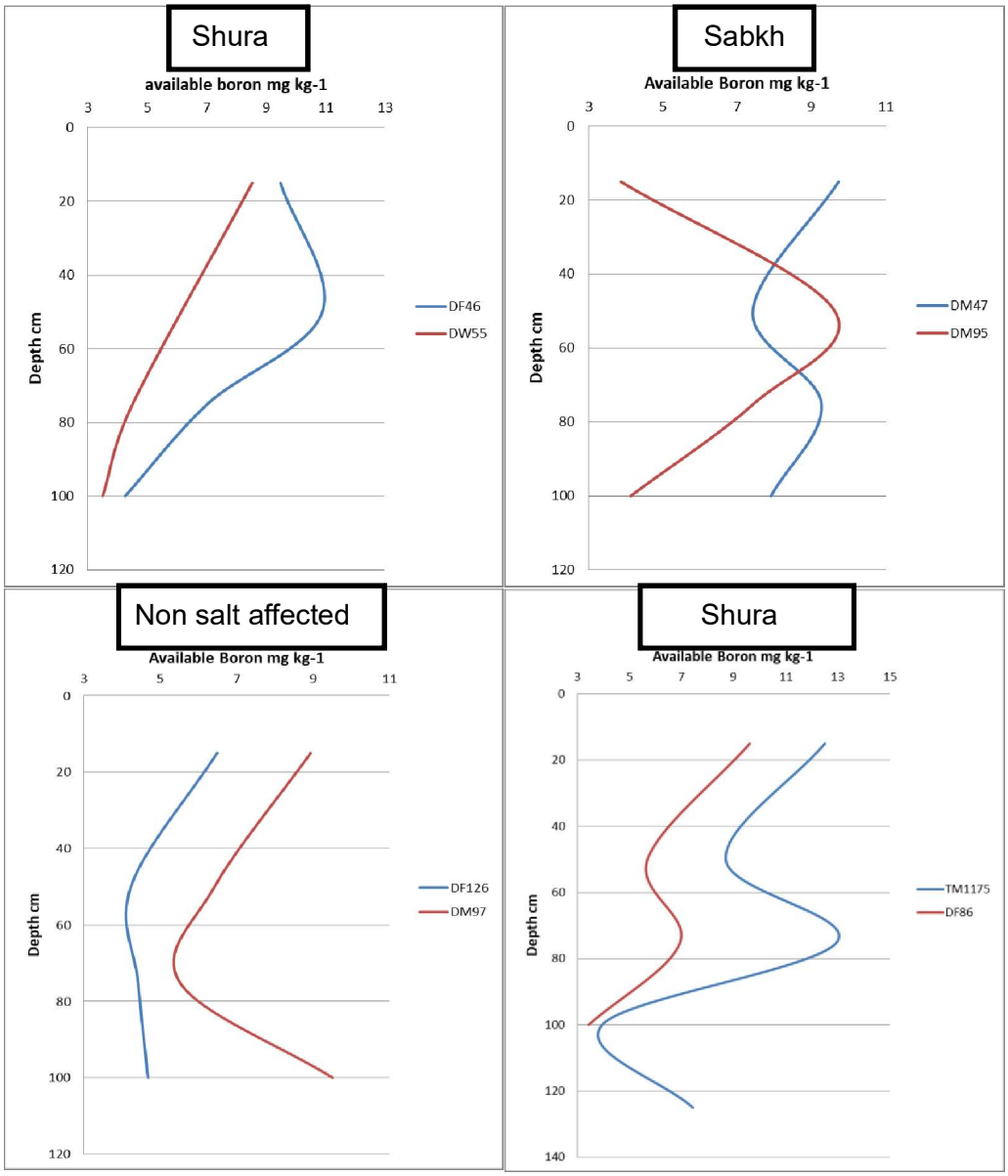

Fig. 8. Depth function of the available boron content in soil series

salinization through the rise of capillary salts to the upper horizons and at the same time leading to an increase in boron carried from ground water to those higher horizons by means of the contribution of ground water to soil salinization, especially since most of the tissues of horizons are soft and that the clay separated in them is relatively high. This was confirmed by the results of the analysis of the boron content in the ground water of the pedons of soils of shore, sabkha, shura sabkha and not affected by salt.

CONCLUSIONS

Total boron content was significantly correlated with the total sand content in the study soil series. The total boron content increased in soils with a higher content of tourmaline minerals, meaning that the increase in the tourmaline content in the heavy metal group led to an increase in the total boron content. The increase in the total clay content also led to a significant increase in the prepared boron content. Soil salinity did not show a highly significant relationship with the available boron content, but the general trend was in a positive relationship whereby the increase in soil salinity increased the available boron content. The increase in the organic matter in the upper horizons of the soil series gave an increase in the available boron content with a highly significant relationship. The relationship was positive in the rest of the horizons of the study soils. The boron is distributed vertically with depth, with a clear increase in the surface horizons and decrease in the subsurface horizons.

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Effect of Land Perspective on Soil Drifted by Wind Erosion in Al-Heliwat Region-Anbar Governorate, Iraq

Lateif Naseif Jassim and Farhan Mohammad Jasim Al-Dulamiy

Department of Soil and Water Resources, Agriculture College, University of Anbar, Iraq
E-mail: lateefnas671@gmail.com

Abstract: The effect of the nature of the land perspective on the amount of soil erosion by wind erosion was carried out from 10 May to 1 September 2021, in a part of the desert lands located within Al-Haliwat region, district of Ramadi, Anbar Governorate, which is located between latitudes 24 37 33 and 0838 33N, and longitudes 231043 and 481143 E. Five variable locations were identified in the land perspective for the study which are: non-plowed land and uncultivated, surface plowed land by spike tooth harrow and uncultivated, surface plowed land by spike tooth harrow and cultivated, plowed land by moldboard plow and uncultivated and land plowed by moldboard plow and uncultivated. The highest mean of the amount of drifted soil at uncultivated location, while the lowest at surface plowed land by spike tooth harrow and cultivated and the rate of drifted soil at the surface plowing by spike tooth harrow was 36.25 Kg compared with 55.3 Kg when plowed land by moldboard plow, which means a decrease by 58.8% and 37.1% for both of the two land perspectives respectively, with a 21.7% superiority of the surface plowed compared with common plowed.

***Keywords*:** Soil erosion, Wind erosion, Ramadi district

Wind erosion is a common phenomenon in arid and semi-arid regions, through which soil particles are transferred from one place to another by the wind. This phenomenon naturally occurs if the appropriate conditions are available for its occurrence, such as dry brittle soil with soft texture, scarcity of vegetation cover, high temperatures, strong winds and low rainfall rates. Wind erosion harms the soil, crops and the environment by reducing soil productivity, plant growth and crop quality and increasing suspended particles in the atmosphere, as it affects the properties of the soil through the transfer of small-sized soil particles (silt and clay) and nutrients necessary for plant growth as well as organic matter, causing a decrease in the fertility level of the soil and thus reducing its productivity The type of soil management, method of plowed, type of plow and method of management are among the important factor in wind erosion, as well as the type of agricultural system has an impact on soil characteristics according to the difference of the plant type and its residues, the quick of decomposition in the soil and chemical properties of the residues plants. Agriculture leads to many changes in the physical and chemical properties of the surface layer of the cultivated soil, which reduces the amount of soil losses compared with agriculturally unexploited areas. Zaho (2006) mentioned that the process of wind erosion is greatly affected by the degree of land gradient, gradient direction, the type of land use and the characteristics of the soil affected by erosion, and the variation in the intensity of wind erosion depends on the variation of the soil particles capable of wind erosion, as well as the difference in the rate of wind speed, as the wind strength pressure is applied to the surface layer of the soil directly proportional to the square of its speed. Soil particles begin to separate from the soil surface when the pressure force applied to them is greater than the earth's gravitational force, which leads to the transfer of soil particles and their deposition away from their locations. Luo et al (2016) reported that when the land is covered with vegetation, it provides a barrier to prevent the uplift of soil separators, reduce wind erosion and reduce the amount of dust emitted, and that arid and semi-arid regions are characterized by scarce vegetation which affects the type and amount of atmospheric particles. Fallahzade et al (2020) observed that turning a virgin desert into agricultural land can change some soil properties, but little information is known about how this transformation affected the mechanical properties of soil and wind erosion. Li et al (2020) showed that increasing the efficiency of ecosystems is an important barrier to slowing the expansion of desertification and limiting the wind erosion. Therefore, this study aims to know the effect of the nature of the land perspective on the amount of soil erosion by wind erosion in Al-Haliwat region, the district of Ramadi of Anbar Governorate as a model for the arid areas exposed to wind erosion in western Iraq.

MATERIAL AND METHODS

This study was carried out from May 10 to

September,2021in a part of the desert lands located within Al-Haliwat region, district of Ramadi, Anbar Governorate, which is located between latitudes 24 37 33 and 0838 33N, and longitudes 231043 and 481143 E. It is bordered by Tharthar Lake on the north, Fallujah district on the east, The Euphrates River on the south and Hit distraction the west (Fig. 1).

Depending on the variations in the study area, which are related with the nature of the land perspective and the nature of agricultural exploitation, five different locations were identified for the study, non-plowed land and uncultivated (V), surface plowed land by spike tooth harrow and uncultivated (T1), surface plowed land by spike tooth harrow and cultivated (T2), plowed land by moldboard plow and uncultivated (T3) and land plowed by moldboard plow and uncultivated (T4). Two districts, representing the soil of the study area, was excavated after determining its locations by digging four perforated holes and the soil texture was examined in the field to a depth of 100 cm (Fig. 2), as well as study locations of the indicators of wind erosion within the study area.

After determining the locations based on the nature of the exploitation in the area, horizontal devices of boxes with dimensions of 10 x 20 x 70 cm were placed (Wasif and Al-Askar 1996) in a vertically direction to the prevailing winds direction in the study area at three devices and three replicates, a distance among them 500 m to estimate the weight of the drifted material at each location every ten days. The drifted materials were collected from each replicate and placed in polyethylene bags thus transported to the laboratory and weighed to determine the amount of soil drifted at each measurement period. At the end of the experiment, the drifted materials were mixed from each examination location and a composite model was obtained to identify the nature of the drifted materials, in which the size distribution of the particles was determined >1 mm, 0.5-1 mm, 0.25-0.5 mm, 0.1-0.25 mm, 0.05-0.1 mm and < 0.05 mm..

RESULTS AND DISCUSSION

Natural Components of the Study Area

Climate: The annual average temperature in the study area was 22.86°C during the period of analysis of climatic data, and that the highest average temperature was during July (35.89°C), while the lowest average temperature was in January (8.34°C).

The variation in the temperature of study area increases the amount of drifted materials through the activity of the physical weathering process, and the location of the area within the dry desert range, as it has been characterized by the characteristics of arid areas. There was difference between the temperature of the summer and winter months of 25.23ºC, so the soil temperature regime is classified as hyperthermic, because the average annual temperature in the soils of the study area is more than 22ºC, and the difference between the temperature of the soil in summer and winter more than 5°C (Soil Survey Staff 1999).

The highest amount of rainfall was in January (20.89 mm), while there was no rainfall during the period from May to October, and the annually total amount of rainfall in the study area was 114.86 mm. Therefore, the study area is characterized by a semi-arid continental climate, which is characterized by a long hot dry summer with a high amount of evaporation and a short cold winter with little rainfall. The lack of rainfall increases the process of soil loss by wind erosion, especially during the dry season and the lack of rain precipitation. The highest relative humidity was in January (59.65%), while it decreased in the summer during the months

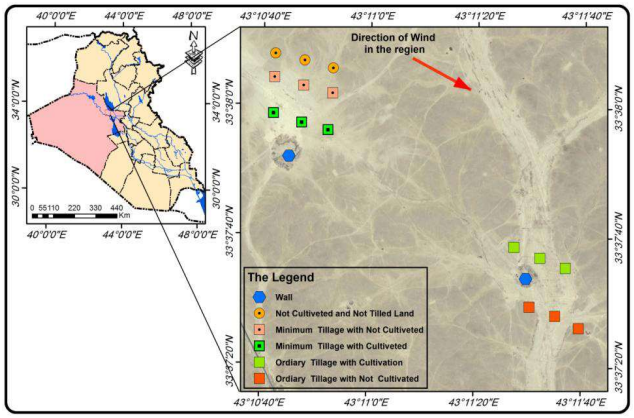

Fig. 1. Location of the administratively study area of Anbar Governorate, Iraq

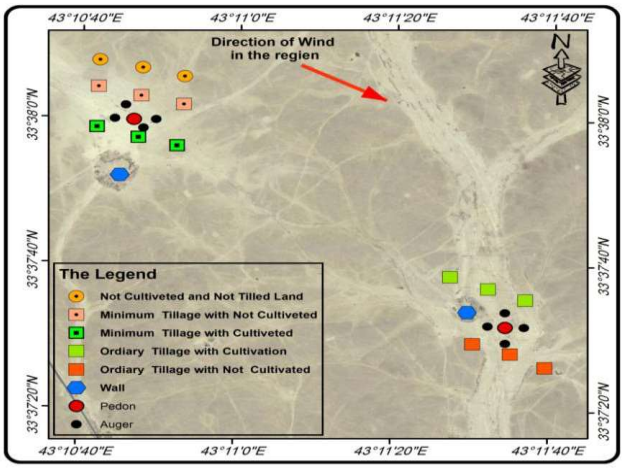

Fig. 2. Perforated examination locations, the two morphological examination location sand study locations of the indicators of wind erosion within the study area

of June, July and August (19.48, 18.68 and 19.78%, respectively), and the annual average relative humidity was 35.81%. Moisture plays an effective role in keeping the surface layer of the soil moist, which contributes to reducing the loss of soil particles by wind erosion the soil moisture regime of the study area falls within the dry variety Toric (Aridic) as a result of the soil remaining in dry conditions for more than 90 days continuously, and the difference between the temperature of the soil in summer and winter more than 5 °C. The annual average of wind speed in the study area was 2.84 m sec^{-1}, the highest monthly average of wind speed was recorded in July (4.13 m sec^{-1}), while the lowest was in December (2.21 m sec^{-1}). Wind is one of the most important factors that contribute to the wind erosion process, and the active factor in the transferring and re-deposition of soil separations in arid areas. The prevailing wind direction and frequency in the study area that the prevailing direction is the northwest wind with its frequency reaching 23.39%, and the lowest frequency of the wind direction was for the north east which amounted to 6.58% (Table 2), and the total frequency of the northwest and western winds amounted to 40.83% compared with the occurrence frequency in other directions (Figure 3). Therefore, the direction of winds from northwest to southeast was adopted in the field, as the northwest winds are dry and not rainy because they blow from isolated lands from marine influences.

Natural plants and agricultural use of the land: Some types of shrubby plants that grow within the study area are *Haloxylonsali cornicum*, *Haloxylonarti culatum*, *Artemisia seberi*, *Iris sisyrinchium* L., *Drimiam aritime*, *Atriplex* spp., *Erncadeserti* and *Achillea fragrantissima*. The seasonal plants that grow during the spring season of the study area, including: *Platycerium bifurcatum, Urticadiocia*, *Ranunculus*

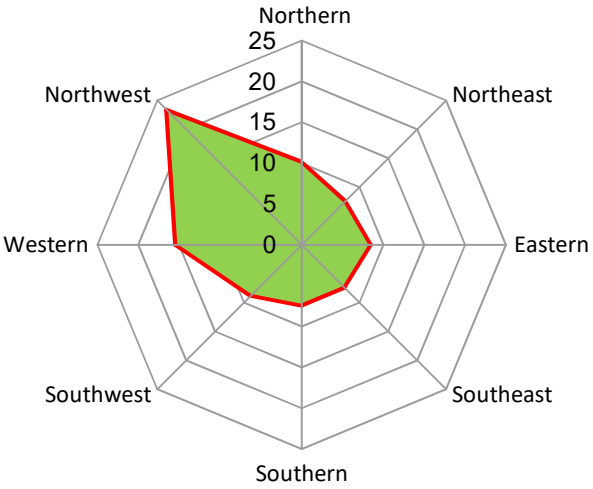

Fig. 3. Wind rose of the study area

Table 1. Climatic parameter of the study area from 1990-2020*

Month	Temperature (°C)			Rainfall (mm)	Humidity (%)	Wind speed (m sec^{-1})
	Min	Max	Average			
January	3.34	14.82	8.34	20.89	59.65	2.30
February	5.04	17.64	10.80	19.71	52.87	2.42
March	9.50	23.14	15.94	17.52	42.26	2.70
April	15.36	29.69	22.39	11.15	34.02	2.75
May	21.46	36.02	28.81	4.13	25.98	2.99
June	26.00	40.85	33.53	0.11	19.48	3.80
July	28.51	43.27	35.89	0.00	18.68	4.13
August	28.13	43.20	35.60	0.25	19.78	3.41
September	24.24	39.09	31.37	0.48	22.20	2.75
October	19.14	32.56	25.32	7.83	30.86	2.38
November	10.98	22.65	16.13	14.96	45.96	2.23
December	5.57	16.37	10.19	17.82	57.97	2.21
Annual rate	16.44	29.94	22.86		35.81	2.84
Annual total				114.86		

Data were obtained from the Ramadi meteorological station (33.27333°N – 43.01667° E)48 m above sea level

Table 2. Direction and frequency of the prevailing winds of the Ramadi station

Wind direction	Northern	Northwest	Western	Southwest	Southern	Southeast	Eastern	Northeast	Clam
Wind frequency (%)	9.17	23.39	17.44	7.84	7.46	7.43	7.5	6.58	13.19

asiaticus L., *Anemone coronaria*, *Gundelia tourneforti*, *Silbumm arianum* and *Carthamus oxybcanthus*. The desert area is far from water sources, which is limited, except for the areas surrounding the wells spread, which are separated from each other by great distances. Therefore, most of the land uses have been confined to the rainfall cultivation of cereal crops (wheat), while the lands that are irrigated with well water they are also planted with cereal crops and some trees such as palms and eucalyptus, and the most of the lands of the region are exploited as natural pastures, due to the growth of some types of natural plants during winter and spring seasons.

Characterization and classification of soils: Soils of the region are desert soils of moderate coarse texture, characterized by the absence of a diagnostic horizon under the surface of the argillic clay as a result of the weak processes of genetic clay transfers from the upper to the lower horizons due to the lack of activity. The soil texture in all horizons is sandy loam, and the lands of this MW3 series are located within the lands with slope less than 2%. The electrical conductivity of the soils ranged between 2.7-4.7 ds m^{-1}, pH between 7.7-8.1 and the soil content of organic matter between 2.0-2.4 g Kg^{-1}, while the soils content of calcium carbonate was between 367-391 g Kg^{-1}. Soils had ow content of calcium sulfate (25 g Kg^{-1}). The soils were classified as Typic Torrifluvents.

Amount of drifted soil material: The amount of soil accumulated in the horizontal devices ranged between 29.7 and 91.2 Kg (2.12 and 6.51 tons ha^{-1}) respectively (Table 3). This difference was as result of the land use variation. The V location (non-plowed land and uncultivated) recorded the highest rate of drifted soil amounting to 89.7 Kg (6.28 ton ha^{-1}), while the T2 location (surface plowed land by spike tooth harrow and cultivated) recorded the lowest amount of drifted soil amounted to 31.3 Kg (2.23 ton ha^{-1}), which indicates the role of administrative processes in determining the amount of drifted soil materials.

The plowing method has a clear effect on the amount of drifted soil materials, the plowed surface by spike tooth harrow achieved the lowest amount of drifted soil materials was 36.25 Kg (2.59 ton ha^{-1}) as compared with the plowed by moldboard plow which achieved 55.3 Kg (3.95 ton ha^{-1}), while when comparing the drifted amounts with uncultivated land a decrease of 58.8% and 37.1% for both perspectives of the land was shown respectively, with a 21.7% superiority of surface plowed compared with common plowed (Fig. 4). This may be attributed to the fact that the use of the moldboard plow reduces the soil's ability to resist wind compared with surface plowing. Al-Mousawi (1997) also indicated that there is a negative effect of plowing with the moldboard plow on the soil's susceptibility to wind erosion as a result of increasing the amount of moisture lost from the soil thus increasing the amount of soil lost.

The agriculture affected the amount of drifted soil materials, the amount of drifted materials decreased by 27.4% when the land was agriculturally used compared with the unexploited land (Fig. 4). This may be due to the positive role of vegetative cover and crop residues in reducing the susceptibility of land to erosion, and reducing the effect of wind strength on the soil surface. Franco et al (2003) attributed to the fact that agriculture or afforestation has an important role in improving the soil structure and enhancing the process of water tipping and storage in the soil, which makes it more capable of retaining moisture thus increasing its resistance to transferring and erosion factors. The amount of drifted material increased with the progression of time (Fig. 5). This is attributed to an increase in temperatures between May and August, which caused moisture loss from the soil and reduced the ability of the soil to resist the forces of separation and transferring (Cyranoski 2009).

Particle size distribution of soil: The particle size distribution of the soil separations which collected in the horizontal devices placed in the modeling area is presented in Table 4. The separation ratios were calculated at the end of

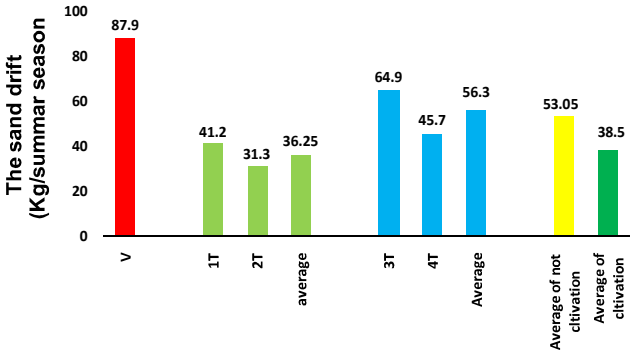

Fig. 4. Effect of plowing method and agricultural exploitation on the amount of drifted soil (Kg/experimental unit)

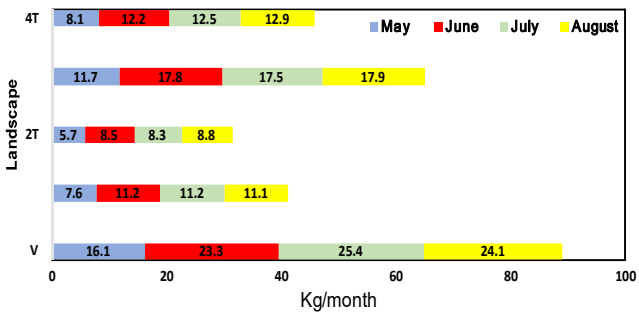

Fig. 5. Total amount of drifted materials (kg/experimental unit) and accumulation in the horizontal devices at each the land perspective

Table 3. Amount of soil accumulated in the horizontal devices placed in the modeling area

Land scape		Sample No.	Date of sampling											Location	Total accumulation soil (Kg/sampling unit)
			20/5	30/5	10/6	20/6	30/6	10/7	20/7	30/7	10/8	20/8	30/8		
V	1	1	7.8	8.0	6.9	6.5	7.9	8.5	8.7	7.5	7.8	7.9	8.5	86.0	86.0
		2	8.0	8.3	8.0	6.8	8.2	7.9	8.5	6.7	8.1	7.5	8.7		86.7
		3	7.6	8.1	7.5	7.0	8.1	8.3	8.5	7.4	8.0	7.7	7.2		85.4
	2	1	7.6	8.0	7.8	7.5	7.7	8.0	8.6	7.8	8.1	7.8	7.8	87.0	86.7
		2	7.8	8.4	7.9	7.6	7.2	8.1	8.7	7.8	8.0	7.9	7.6		87.0
		3	8.0	8.2	8.0	7.7	7.2	8.0	8.5	8.0	8.1	8.0	7.6		87.3
	3	1	8.2	8.3	8.2	8.2	8.4	8.3	8.0	8.1	8.2	8.3	7.9	90.6	90.1
		2	8.0	8.4	8.2	8.0	8.9	8.5	8.1	8.3	8.0	8.6	8.2		91.2
		3	8.1	8.1	8.0	8.1	8.3	8.4	8.3	8.5	8.0	8.3	8.3		90.4
Mean of landscape			7.9	8.2	7.8	7.5	8.0	8.2	8.4	7.8	8.0	8.0	8.1		87.9d
T1	1	1	3.7	3.6	3.5	3.6	3.7	3.6	3.7	3.6	3.5	4.0	4.1	40.5	40.6
		2	3.8	3.6	3.6	3.5	3.8	3.7	3.7	3.5	3.6	4.0	4.0		40.8
		3	3.5	3.7	3.7	3.8	3.7	3.5	3.3	3.8	3.3	3.5	3.2		40.2
	2	1	3.8	3.7	3.8	3.6	3.6	3.8	3.9	3.8	3.5	3.9	3.9	41.3	41.3
		2	3.7	3.8	3.7	3.6	3.5	3.7	3.7	3.5	3.6	4.0	4.1		40.9
		3	3.9	3.8	3.6	3.8	3.6	3.9	3.5	3.7	3.5	4.1	4.3		41.7
	3	1	3.8	3.7	3.6	3.8	3.7	4.0	3.7	3.9	3.6	3.9	4.0	41.9	41.7
		2	4.0	4.0	3.8	3.9	4.0	4.1	3.8	3.9	3.6	3.3	3.5		41.9
		3	4.0	3.9	3.9	4.1	4.0	3.9	3.9	3.8	3.5	3.5	3.5		42.0
Mean of landscape			3.8	3.8	3.7	3.8	3.7	3.8	3.7	3.7	3.5	3.9	3.8		41.2ab
T2	1	1	2.7	2.6	2.6	2.7	2.5	2.6	2.6	2.7	2.6	3.0	3.4	30.2	30.0
		2	2.7	2.7	2.8	2.8	2.7	2.6	2.7	2.5	2.6	2.6	3.0		29.7
		3	2.8	2.9	2.8	2.7	2.6	2.7	2.8	2.8	2.8	3.0	3.1		31.0
	2	1	2.9	2.8	2.7	2.9	2.8	2.9	2.8	2.7	2.8	2.9	3.4	31.4	31.6
		2	2.9	3.0	2.8	2.9	2.8	2.9	2.8	3.0	3.0	3.0	2.9		32.0
		3	2.7	2.8	2.9	2.8	2.6	2.7	2.6	2.7	3.0	2.6	3.3		30.7
	3	1	2.9	3.0	3.1	2.9	2.8	2.7	2.6	3.0	3.0	3.0	3.0	32.2	32.0
		2	2.9	3.0	3.0	2.9	2.8	2.9	2.6	3.0	2.9	3.0	2.8		31.8
		3	3.0	3.2	3.1	3.1	2.9	3.1	2.9	2.8	2.7	2.8	3.3		32.9
Mean of landscape			2.8	2.9	2.9	2.9	2.7	2.8	2.7	2.8	2.8	2.9	3.1		31.3a
T3	1	1	5.8	5.7	5.8	5.9	5.7	5.8	5.7	5.9	6.2	5.8	5.8	64.1	64.1
		2	5.9	5.8	5.8	6.0	5.8	5.8	5.7	5.9	6.1	5.9	5.8	64.5	
		3	5.9	5.7	5.8	5.9	5.7	5.7	5.7	5.8	6.0	5.7	5.7	63.6	
	2	1	6.0	5.9	6.0	6.0	5.9	5.8	5.8	6.0	5.8	6.0	6.0	65.2	64.9
		2	5.6	5.8	5.8	6.0	5.8	6.0	5.8	6.0	5.9	5.9	6.0	64.6	
		3	6.0	5.9	5.8	6.0	5.8	5.8	5.9	6.0	6.0	6.0	5.8	65.0	
	3	1	6.1	6.0	5.9	6.2	6.0	5.9	5.8	5.8	6.1	6.1	5.9	65.8	65.6
		2	5.8	5.8	6.0	5.9	6.0	6.0	6.1	6.0	6.1	6.3	6.0	66.0	
		3	5.8	5.8	5.8	5.8	6.0	5.7	6.1	6.1	5.9	6.0	5.9	64.9	
Mean of landscape			5.9	5.8	5.9	6.0	5.9	5.8	5.8	5.9	6.0	6.0	5.9	64.9c	
T4	1	1	4.0	3.9	4.0	3.8	4.0	4.1	4.0	4.1	4.1	4.6	4.4	45.0	45.0
		2	4.0	3.9	4.0	3.8	4.0	4.1	4.1	4.0	4.1	4.4	4.2	44.7	
		3	4.1	4.0	4.1	4.0	4.0	4.2	4.1	4.1	4.1	4.2	4.4	45.3	
	2	1	4.1	4.0	4.1	4.0	4.1	4.2	4.1	4.0	4.2	4.4	4.2	45.4	45.6
		2	4.2	4.1	4.2	4.0	4.1	4.1	4.1	4.1	4.2	4.2	4.2	45.5	
		3	4.2	4.1	4.2	4.0	4.2	4.0	4.2	4.3	4.3	4.2	4.3	46.0	
	3	1	4.0	4.0	4.3	4.0	4.3	4.4	4.2	4.2	4.2	4.2	4.2	46.0	46.3
		2	4.1	4.2	4.3	4.0	4.2	4.4	4.2	4.0	4.3	4.3	4.3	46.3	
		3	4.0	4.0	4.1	4.1	4.3	4.4	4.3	4.3	4.4	4.3	4.4	46.7	
Mean of landscape			4.1	4.0	4.1	4.0	4.1	4.2	4.2	4.1	4.2	4.4	4.3	45.7b	
L.S.D (p=0.05) between sampling replications														2.1	
L.S.D(p.0.05) between landscape locations															14.2

Table 4. Particle size distribution of the soil separations collected in the traps

Land scape	Sand						Silt				Clay
	VF	F	M	C	VC	Total sand	F	M	C	Total silt	Total
	gm Kg^{-1}										
V	261	183	115	85	65	709	135	90	15	240	51
T1	224	175	122	82	67	670	128	87	40	255	75
T2	191	163	137	87	72	650	120	79	31	230	120
T3	239	167	128	84	65	683	128	83	14	225	92
T4	216	170	130	87	68	671	120	91	19	230	99
L.S.D.(p=0.05)	12.10	4.93	6.76	4.41	4.22	25.42	10.02	4.25	4.04	12.78	8.53

the experiment as a composite sample of the total amount of the drifted soil materials, and it was of a texture similar to the field soil at each modeling area (sandy loam). There is an increase in the proportion of separated sand, especially fine and very fine sand, as well as fine and medium silt within the drifted materials (Fig. 6), which indicates that the proportion of sand in the soils accumulated in the horizontal devices has exceeded its proportion in the soil. This may be attributed to the fact that sand is easy to separate from the soil because it doesn't form bonds with other soil particles, therefore is the first material separated by the strength of the wind (Al-Dhiabi 2005). There are significant differences between the locations in the amount of sand separation, T2 location (surface plowed land by spike tooth harrow and cultivated) recorded the lowest amount of sand separation compared with V location (non-plowed land and uncultivated) which recorded the highest value of the amount of sand drifted. This indicate that agriculturally exploited lands reduced the proportion of drifted sand in the soil collected in the horizontal devices. This is could be attributed to the positive effect of vegetation cover in reducing soil erosion, thus reducing the proportion of sand transported by crawling method in the drifted soil. The silt content of soil significantly decreased in all locations (Fig. 7). The percentage of decrease ranged between 11.1-17.9. The decrease in the content of the soil accumulated by the traps is due to silt separated is characterized by the small size of its grains, so it is more likely to transfer in a suspended form than in the form of crawling.

The decrease ranged between 25.1-57.1% in the content of the soil accumulated in the horizontal units of separated clay compared with the soil of the modeling areas, and the largest decrease was recorded in the agriculturally

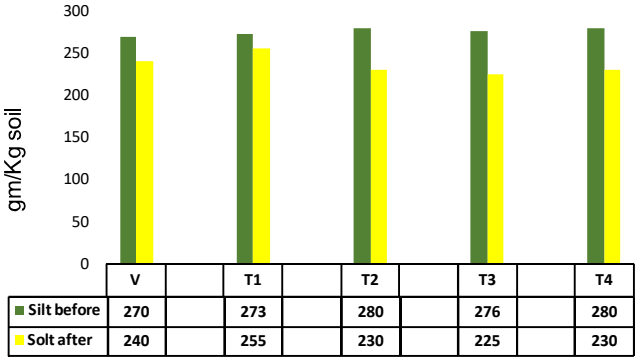

Fig. 7. Variation in the soil content of silt separation compared with the content of soil accumulated in sand traps at each land perspective

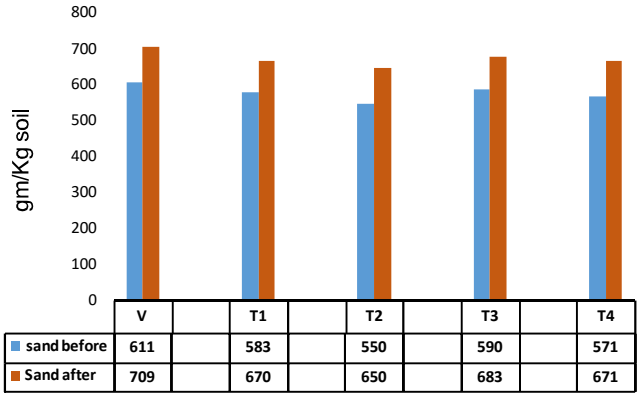

Fig. 6. Variation in the soil content of sand separation compared with the content of soil accumulated in sand traps at each land perspective

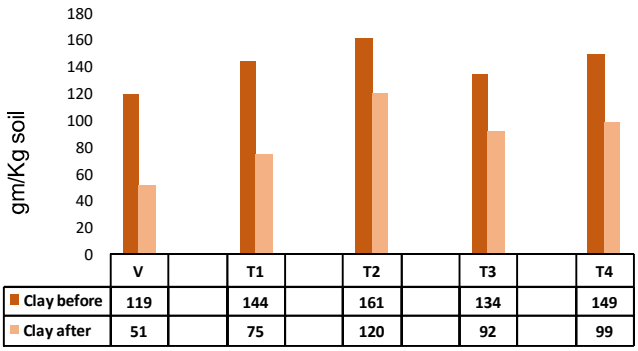

Fig. 8. Variation in the soil content of clay separation compared with the content of soil accumulated in sand traps at each land perspective

unexploited soils (Fig. 7). This may be due to the fact that the separated clay is more susceptible to suspended transport than sand and the transferring takes place at longer distances compared to transferring by crawling method. This result indicates the importance of management methods in limiting the loss of this important component in the soil, which is responsible for maintaining moisture and important nutrients.

CONCLUSION

Land perspective has a clear effect on the amount of soil drifted by wind erosion, and effect decreased in the presence of vegetation cover. The plowing affected the amount of drifted soil by wind erosion, and surface plowing reduced the amount of drifted soil more than plowing by using a moldboard plow. There was an increase in the proportion of separated sand, especially fine and very fine sand, as well as fine and medium silt within the drifted materials.

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Effect of adding Organic Fertilizer and Spraying IQ Combi on Physical and Chemical Properties of Soil in Rice Cultivated Under Intensification (SRI) System

Mohammed Muayid Sahib Al-Lami and Waleed Flieh Hassan

Department of Soil and Water Sciences, Faculty of Agriculture, University of Kufa, Najaf, Iraq
E-mail: mohamedmo9017@gmail.com

Abstract: A study was conducted in Jazra 4 and Nahar Saad region in Amarah, Maysaan province in a sandy-loamy soil during 2020-2021 season to determine the effect of adding organic fertilizer and spraying IQ combi on the physical and chemical properties of the soil cultivated with rice crop under intensification system (SRI). The factorial experiment included three main factors, 18 treatments. The first factor was adding three levels of organic fertilizers (0, 8 and 16 ton.hectare^{-1}) represented as MO1, MO2 and MO3, the second factor was spraying IQ combi in three levels (0, 75 and 150g.100 L^{-1}) represented as N1, N2 and N3 respectively, while the third factor was two agricultural methods including traditional method (G1) and intensification system (G2). Results indicated that adding MO3 level of organic fertilizer was significantly exceeded MO1 level in providing nitrogen, phosphorous, potassium, iron, and zinc in the soil with an increasing amounted 71.45, 104.52, 173.87, 39.57 and 51.51%, respectively. Spraying of IQ combi also had significant effect as N3 level was exceeded N1 in an increasing of nitrogen, phosphorous, potassium, iron, and zinc in the soil (19.88, 22.99, 45.76, 53.66 and 108.04% respectively). The outcome indicated that the method G2 was exceeded G1 significantly by providing better nitrogen and potassium in soil and increased by 5.06 and 15.96% respectively. The results showed that no improvement of G2 in providing phosphorous, iron and zinc in soil.

Keywords: Rice, IQ combi, Organic fertilizer, Intensification system, Soil properties

Rice (*Oryza sativa* L.) is a most important crop worldwide, and is ranked in the second place after wheat in terms of economic important and cultivated area and is cultivated in more than 114 countries out of 193 worldwide (Al-Hasanie and Al-Maadhedi 2017). Recent studies are focused on the use of organic fertilizers in order to improve soil properties, increase organic compounds and enhance microbial activities in decomposing organic matter and providing nutrients that necessary for plant to growth (Nyalemegbe et al 2010). These fertilizers are environmentally friendly and provide a slow release of nutrients due to its direct impact on microbial activities and this is reflected positively on the stages of plant growth (Alwan et al 2017). Nano-fertilizers are aimed to release nutrients into the soil gradually and in a controlled manner, thus avoiding environmental damage and improving crop growth and productivity (Qureshi et al 2018). In recent years, Iraq has suffered from the problem of water scarcity when planting rice and one of the most innovative ways to solve this issue is rice intensification system method, which aims to increase rice productivity per unit area and reduce the amount of seeds to a third of the amount used in traditional agriculture (Anchal 2015). The controlling of distances between plants is best in terms of performance and production (Thakur et al 2014).

MATERIAL AND METHODS

A field experiment was carried out within Jazra 4 and Nahar Saad region in Amarah, Maysaan province during 2020- 2021 season to examine the effect of adding organic fertilizer and spraying IQ combi on the physical and chemical properties of soil that cultivated by rice crop under SIR system in sandy-loamy soil. The soil of field was prepared by tilling and levelling then soil samples from different site of field were taken randomly from 0-30cm depth, samples were mixed very well and dried after that grinded and sifted with a 2mm hole sieve. Samples of soil were analysed in Faculty of Agriculture, University of Kufa laboratories to measure some of their chemical and physical properties before planting (Table 1).

Chemical and physical analysis of soil: Samples of soil were randomly taken from different site of studied field then mixed very well and dried after that grinded and sifted with a 2mm hole sieve then some physical and chemical properties was estimated.

pH: The level of pH was estimated in 1:1 ratio of water : soil using pH meter Jackson 1958.

Electrical conductivity (EC): It was estimated in in 1:1 ratio of water: soil using conductivity bridge Jackson 1958.

Nitrogen: Nitrogen was extracted by Microkjeldahl (Page et al 1982).

Phosphorous: It was extracted by sodium bicarbonate ($NaHCO_3$) at 0.5 mol and 8.5 level of pH then estimated using Spectrophotometer at 882nm according to Olsen method (Page et al 1982).

Potassium: Potassium was extracted by ammonium acetate at 1mol then estimated using flame photometer (Page et al 1982).

Zinc and iron: A suspension of 1:2 soil: water was prepared then 10g of it was dissolved in 20cm3 of DTPA solution at 7.3 level of pH, after that, the mixture was shaken for 2 hours by centrifuge then filtrate and the studied elements were estimated using Atomic Absorption Spectroscopy (Page et al 1982).

Table 1. Some chemical and physical properties of soil field before planting

Properties	Unit	Value
EC 1:1	$ds.m^{-1}$	4.4
pH 1:1	$mg.kg\ soil^{-1}$	7.94
Nitrogen	$mg.kg\ soil^{-1}$	60.32
Phosphorous	$mg.kg\ soil^{-1}$	10.2
Potassium	$mg.kg\ soil^{-1}$	90.11
Iron	$mg.kg\ soil^{-1}$	5.44
Zinc	$mg.kg\ soil^{-1}$	0.97

RESULTS AND DISCUSSION

There were significant effect of adding organic fertilizer, the combination MO3N3G2 was superior to MO1NIG1 and achieved the highest average of nitrogen, phosphorous, potassium, iron and zinc (118.34, 179.48, 276.73, 251.73 and 354.54% respectively). The increasing of these elements may attribute to the adding of organic fertilizer and its high content of nutrients that contributed in increase its concentrations in soil (Hassan and Issa 2021). Spraying micro Nano fertilizer had significant effect on green parts of plant and stimulating roots to increase the secretions that microorganisms feed on which led to the decomposition of carbonic organic matter and release more nutrients to the soil (Al-Maamouri and Al-juthery 2020). G2 method showed no significant effect of increasing phosphorous, iron and zinc in soil.

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Table 2. Effect of adding organic fertilizer, spraying IQ Combi and the method on the concentration of nitrogen, phosphorous, potassium, iron and zinc in soil

Combinations	Nitrogen	Phosphorous	Potassium	Iron	Zinc
MO1N1G1	14.45	8.48	70.87	4.33	0.86
MO1N1G2	14.65	8.73	82.35	5.35	1.15
MO1N2G1	15.22	8.71	73.98	8.24	0.95
MO1N2G2	15.55	9.05	79.70	8.18	0.90
MO1N3G1	15.80	11.24	74.90	8.38	1.09
MO1N3G2	16.40	10.90	82.75	10.37	0.97
MO2N1G1	16.83	10.33	90.07	10.09	0.61
MO2N1G2	17.50	14.89	90.29	9.10	0.55
MO2N2G1	17.80	14.12	133.14	8.06	0.90
MO2N2G2	18.76	13.94	199.61	10.04	0.97
MO2N3G1	19.48	12.21	152.86	8.69	0.97
MO2N3G2	20.43	15.38	166.18	10.91	2.00
MO3N1G1	23.02	18.23	168.42	8.18	0.87
MO3N1G2	24.00	16.53	187.90	7.21	1.17
MO3N2G1	24.70	17.41	163.40	8.57	0.95
MO3N2G2	26.13	19.41	223.71	8.95	1.23
MO3N3G1	28.77	21.56	261.94	15.23	2.50
MO3N3G2	31.55	23.70	266.99	14.48	2.29
LSD (p=0.05)	0.6162	394. 3	01.20	605.2	6312. 0

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Effect of Leveling and Landing Implement and Some Tillage Equipment on Some Physical Soil Properties

Ali J. Yousif and Abdul Razzak A. Jasim

Department of Agricultural Machines and Equipment
Collage of Agriculture Engineering Sciences University of Baghdad, Iraq
E-mail: alij12241@gmail.com

Abstract: A field experiment was carried out on silty loam soil in the autumn season of 2020, in the Region of Saad River which is located in the Southern part of Iraq almost 340 km from the North of the Capital Baghdad and approximately 27 km from the Center of Maysan Province. In this experiment two factors were used, the first is the laser scraper leveling equipment, graders and leveling implement and the second factor is the tillage system using moldboard plow, chisel and no-till system. The indicators studied were (bulk density, slope, total porosity and soil penetration resistance). The highest bulk density of soil was recorded for the grader, which was significantly superior to the rest of the used systems. The leveling implements recorded the highest significant of total porosity and moisture content compared to the other equipment. The moldboard plow recorded the highest significant bulk density compared to the other equipment. Regarding the slope, the results showed that there were no significant differences between the equipment of the leveling systems and the used plowing systems. The moldboard plow recorded its superiority in the bulk density (1.40 megagrams m^{-3}), while the no-till system was superior in terms of total porosity and moisture content, which amounted to 48.68 and 17.38%, respectively over the rest of the treatments.

Keywords: Chisel plow, Laser scraper, Leveling equipment, Mold board plow

Agricultural mechanization has become one of the basic components of agriculture because it provides the possibility to control the various factors affecting productivity and thus increase production in quantity and quality. In addition, agricultural mechanization has a clear role in speeding up the completion of agricultural operations and increases the areas planted with different crops, also with reducing costs and reducing working hours. The use of primary agricultural equipment causes uneven land surface, which necessitates the use of machines or equipment to level the land for the purpose of regular irrigation without water erosion. Heavy equipment such as a bulldozer or grader and scraper can be used, and a laser scraper with GPS can be used in case the slope is severe and the field area is large (Kaur et al 2014). The benefit of laser leveling proper preservation slope rate and increase the efficiency of irrigation and thus the best productivity of the crop, along with saving the effort and time (Adamala et al 2014). The selection of inappropriate plowing systems works to compact the soil surface as a result of the passage of tractors, which has a detrimental effect on the physical condition of the soil by increasing the bulk density and poor soil productivity (Jassim 2018). Therefore, the research aims to be noticed effect of leveling machines and plowing systems on some physical properties of soil.

MATERIAL AND METHODS

The field experiment was carried out in the Nahr Saad area, north of Maysan governorate center, about 27 km and south of the capital, Baghdad during July 7, 2020 to August 10, 2020. The field of experiment is characterized by a semi-flat topography, and the soil of the field was classified as a silty loam soil.

The results of the soil analysis are presented in Table 1 (Black and Hartge 1986).

The factors included were:

First parameter includes the following levels:

1. Leveling using the laser scraper
2. Leveling using the grader (leveling equipment or graded).
3. Leveling using the flat leveling machine (leveling implement).

Second parameter, which is the tillage systems and includes:

1. Agricultural system using the mold board plow + disc harrows.
2. Plowing system using the chisel plow + disc harrows.
3. No-till agricultural system + disc harrows.

The experiment was carried out on a land area of 5000 m^2 with a width of 50 m and length of 100m. The field's land was divided into three main sectors to carry out the leveling process by means of the laser scraper, leveling equipment and grader. Each sector contains 9 replicates, so the total experimental units are 27 units.

The area of each unit was 20 m in length and 3 m in width and a distance of 10 m was left before and after each experimental unit in order to achieve stability in the mechanical

unit.

The distance of 2 m was left horizontally between sectors and treatments. Each sector was separated from the other by placing iron marks to avoid overlap between the sectors.

The following characteristics were studied:

Bulk density, g cm^{-3}: The bulk density was calculated before and after leveling, as well as after tillage by the core sample method from the following relationship (Black et al 1986)

fb = Ms/Vt, fb: Soil bulk density, Ms: Mass of kiln-dried soil sample gm, Vt: The total volume of the soil with its natural structure cm^3

Slope %: The slope of the land is calculated in two ways (Chandra 2005):

A. If you know the slope ratio:

Slope of the unknown point = Slope of the known point ± slope X distance (+) if the slope is up, (-) if the slope is down

B. If you know the degree of the slope and the distance between any two points.

Slope = difference between the slope of two points / difference between the distance between them

Soil total porosity (%): The percentage of porosity was estimated mathematically based on the values of the true density and the bulk density of the soil (Odeh, 1990).

$TP = (1 - (PB / PS)) \times 100$

TP: Soil total porosity,

PS: The true density of soil (mcg/m^3) is 2.65 (mcg/m^3).

PB: The bulk density of the soil (mcg/m^3).

Soil resistance to penetration (%): Used a pocket or manual penetration resistance CL-700 device made in America (Chicago) and the force is measured in tons (feet-^{2} or in kg. cm^{-2}) (Fig. 1).

RESULTS AND DISCUSSION

Bulk density (mcg/m^3): The effect of leveling equipment, machines and tillage systems on the bulk density of soil contained given in Table 2. The laser scraper and leveling equipment recorded to 1.34 and 1.29 Mg.m^{-3}, respectively and are significantly lower than the grader leveling machine, which recorded the highest bulk density (1.5 megagrams M^{-3}.) The reason for the increase in bulk density when using the grader may be due to an increase in its weight and pressure of the knife. These results agree with the results obtained by Jassim et al (2000) and Humphreys et al (2010).

Slope (%): There were no significant differences in slop between the flatting systems and the tillage systems (Table 3). However, highest percentage of slope was in the leveling

Fig. 1. Soil penetration resistance meter

Table 1. Some physical and chemical properties of field soil before conducting the experiment

Physical property	Soil penetration resistance (kg cm^{-2})	Moisture content (%)	Porosity (%)	Bulk density (Mega grams m^{-3})	Soil texture	Soil separators (gm km^{-1})		
						Clay	Silt	Sand
Value	1.25	18 – 20	54	1.21	Silty Loam	315	478	90

Chemical property	Mineral element (Millimole)						Bicarbonate (Millimole liter^{-1})	$CaCO_3$ (gm km^{-1})	Organic matter	EC1:1 (Dcm m^{-1})	H
	Na	Ma	Ca	K	P	N					
Value	9.45	0.22	1.85	1.18	1.21	28.12	10.74	2.5	8.3	2.5	7.5

Table 2. Effect of leveling equipment and tillage systems on the bulk density (Mg m^{-3})

Leveling system	Average leveling machines	Tillage systems		
		Interaction between leveling systems and tillage system		
		No tillage	Chisel plow	Mold board plow
Laser scraper	1.34	1.33	1.33	1.37
Grader	1.50	1.48	1.49	1.53
Leveling equipment	1.29	1.27	1.28	1.31
L.S.D (p=0.05)	0.029		0.0293	
		1.36	1.37	1.40
L.S.D (p=0.05)			0.0059	

implement (6.64%) and the mold board plow (6.67%). Rajput et al (2004) also indicated that the highest percentage of leveling was achieved with the use of the laser scraper and therefore the best values for productive efficiency and uniformity of irrigation water distribution was observed.

Total soil porosity (%): The leveling equipment outperformed scraper and grader, with a very high significance (Table 4). Likewise, the scraper treatment outperformed the grader significantly. Likewise the agricultural systems also recorded significant differences between these systems. The no-till treatment was outperformed each of the other two systems. Jasim and Al-saadi (2010) also observed the superiority of the deep-tillage or no-till treatment and chisel plow. The Chisel plow outperformed the mold board plow and this case agrees with the results of Jassim and Saadoun (2016). The interaction between the two rates and the no-till treatment recorded highest value, while the lowest value was for the interaction between the grader and the plow.

For the interaction between the grading and tillage systems, the highest value was recorded for the interaction between leveling equipment and the no-till treatment, while the lowest was for the interaction between the grader and the mold board plow. There was significant difference in the bilateral overlap between the leveling systems and the tillage systems. The highest percentage of slope was recorded as a

Table 3. Effect of leveling equipment and tillage systems on the slope after flatting (%)

Leveling system	Average leveling machines	Agricultural systems		
		Interaction between leveling systems and tillage system		
		No tillage	Chisel plow	Mold board plow
Laser scraper	6.54	6.5	6.51	6.60
Grader	6.58	6.54	6.56	6.65
Leveling equipment	6.64	6.57	6.69	6.68
L.S.D (p=0.05)	$0.248^{N.S}$	0.354		
Average of tillage system		6.54	6.59	6.67
L.S.D (p=0.05)		$0.207^{N.S}$		

Table 4. Effect of leveling equipment and tillage systems on the total porosity of the soil (%)

Leveling system	Average leveling machines	Agricultural systems		
		Interaction between leveling systems and tillage system		
		No tillage	Chisel plow	Mold board plow
Laser scraper	49.31	49.81	49.81	48.30
Grader	43.39	44.15	43.77	42.25
Leveling equipment	51.45	52.08	51.70	50.57
L.S.D (p=0.05)	0.04		0.09	
Average of tillage systems		48.68	48.43	47.07
L.S.D (p=0.05)			0.06	

Table 5. Effect of grading equipment and plowing systems on the coefficient of penetration (kg cm^{-2})

Leveling system	Average leveling machines	Agricultural systems		
		Interaction between leveling systems and tillage system		
		No tillage	Chisel plow	Mold board plow
Laser scraper	2.09	1.75	2	2.50
Grader	3.87	3.34	3.75	4.53
The leveling equipment	1.88	1.65	1.75	2.25
L.S.D (p=0.05)	0.01		0.17	
Average of tillage systems		2.25	2.5	3.10
L.S.D (p=0.05)			0.11	

result of the interaction between the leveling implement and the chisel plow (6.69%), while the lowest percentage was as a result of the interaction between the no-till leveling treatment and the laser scraper. Ratan et al (2011) indicated that flattening in laser scraper achieved the best gradient ratio (the lowest ratio) when applied to different gradient levels (0.5 - 2.5 - 4.5 and 9.5%). Likewise, Winkler et al (2018) indicated the necessity of applying the laser skimmer with or without plowing systems in order to obtain a settlement rate of only 1%.

Soil resistance to penetration (%): The effect of the leveling equipment, tillage systems and the interactions between them on the soil penetration coefficient presented in Table 5. The grader leveling machine recorded the highest value (3.87 kg.cm-2), followed by the laser scraper (2.09 kg.cm-2), while the lowest value of the penetration coefficient when using the plate leveling implements (1.88 kg.cm^{-2}). The grader achieved the highest value and based on the above shows the effect of the laser scraper in recording the lowest specific percentage of the penetration coefficient when using different tillage systems after leveling compared to the grader (Abdullayev et al 2007). The tillage systems lead to increase soil penetration resistance (Horn et al 2003). The chisel plow and no-till plow systems recorded 2.5 and 2.25 kPa, respectively to show difference between these two treatments. The mold board plow system achieved the highest of 3.10 kPa was significantly superior to the rest two. Al-Janabi (2000) also indicated the high resistance of the soil to penetration by 38% when using the mold board plow compared to the no-till agricultural system. This is because of the increased the soil deep volume and thus the high bulk density ratio with increasing speed. Al-Mousawi and Al-Tamimi (2011) indicated that there is a positive correlation between the resistance of the soil to penetration and the bulk density of the soil, which was the highest value for the mold board plow. There was a clear effect of the interaction between the leveling machines and the tillage systems on penetration coefficient. The interaction between the landing machine and the mold board plow had the highest value (4.53 kPa), while the lowest value (1.65 kPa) was recorded for the interaction between the leveling implement and the no-till farming system. This may be due to increase in the rate of the soil masses formed when using the mold board plow system compared to the no-till system, and consequently increasing the rate of soil deep volume and then causing to an increase in the bulk density (Al-Janabi 2000).

CONCLUSION

The use of the laser scraper and the plate leveling equipment in order to perform the landing and leveling process is recommended. The use of tillage system in chisel plow and laser leveling implements, chisel plow and no-till farming system give the best physical properties of the soil.

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

Israa Obaid Al-karaquly and Aswan H. Bayar[1*]

Departmentof Plant Protection, Agricultural Research Office, Baghdad, Iraq
[1]Department of Food Science, College of Agricultural and Engineering Sciences, University of Baghdad, Iraq
**E-mail: aswan@coagri.uobaghdad.edu.iq*

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

Among the most important factors for increasing the production of wheat crops is the seeds used as high-quality seeds are the key to high economic return. In successful and commercial agriculture, rapid seed germination and the emergence of seedlings are among the specific and important factors for establishing a successful crop. In crop production and standardization of seedling emergence, which in turn affects the yield of crops, 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 operations and seed storage (Rajjou et al 2012). During seed storage, the quality of the seeds may remain with higher viability or decrease to a level that makes the seeds unacceptable for the purpose of cultivation. This is related to several factors related to the storage conditions, including the length and age of storage, temperature and relative humidity during storage and poor storage will lead to a loss in quantity and quality. The stored seeds, which affects the yield of and quality of crops for human and animal feeding (Zahid and Saira 2019). Many practices have been put in place to raise the viability of seeds, increase their activity and rates of emergence, which depend entirely on the process of activation or soaking with materials stimulating germination, especially soaking with nutrients, and use of nanoparticles and fertilizers because many benefits. The nano fertilizers, are economical, environmentally friendly and non-toxic (Lu et al 2015). Fertilizers, especially iron, manganese and zinc, play an important role in the growth and productivity of crops. Several studies have indicated that iron, when in the form of nanoparticles, leads to improved germination. The reason behind how to stimulate the seeds is still unclear, but some studies have shown that the nanomaterials have the ability to penetrate the seed layer and enhance the absorption and use capacity of water, which stimulates the enzyme system and ultimately improves germination and seedling growth (Banerjee and Kole 2016). The objectives was to study the role of micro-nanofertilizers in improving the characteristics of stored wheat seeds.

MATERIAL AND METHODS

Field experiments for the seasons was carried out in the College of Agriculture, University of Baghdad during 2018-2019 and 2019-2020. The agricultural operations of plowing, smoothing and leveling the soil were carried out, and then the experiment land was divided into four replications, and the area of the experimental unit was 2 x 2 m^2, in randomized complete sectors (RCBD) with four replications, where the first factor included three types of seeds stored for different periods, which are 2013, 2015 and 2017, and the second factor was the was nano fertilizer (iron concentration 150 mg/liter, manganese and zinc nanoparticles at concentration of 100 mg/liter). This is in addition to the two comparison treatments of dry seeds and seeds soaked in distilled water. The seeds of wheat, class (Research 10), which were obtained from the Agricultural Research Department, were planted on lines with a distance of 20 cm, and 400 seeds were placed in each line. Urea fertilizer was added at a rate of 200 kg in four equal batches (when planting, three whole leaves appear, the second node appears on the stem and in lining stage) and phosphate fertilizer was added at a 100 kg in the form of triple superphosphate at a concentration of 45%. P_2O_5 was added at once when preparing the land for cultivation.

Emergence(Es): Emergence velocity represents the sum of the number of seeds germinated in each day divided by the number of days starting from the beginning of the experiment, and the emergence velocity is an important and accurate indicator of the seed viability (Yuan - Yuan et al 2010)

Es= Σ Di / Ni

Ni = number of seeds germinated per day, Di = number of days (daily germination)

Energy of emergence (EE %): This characteristic was calculated from the percentage of seed germination on the fourth day after sowing divided by the total number of seeds (Farooq et al 2005): EE=GP(4^{th}day)/TNST

Field emergence ratio (%): The number of seedlings emerging on the soil surface was calculated after 10 days of planting, then the results were converted into a percentage.

Dry weight of seedlings (mg): After taking the wet weight of the seedlings, the ten seedlings were placed in a perforated paper bag and inserted into the oven at a temperature of 80 m for a period of 24 hours for the purpose of drying, after that the weighing was carried out and the resulting number was divided by ten to obtain the dry weight of one seed.

Chlorophyll content in seedlings: The chlorophyll content in seedlings was calculated by taking a sample of seedlings and performing analysis in the Graduate Studies Laboratory at the College of Agriculture, University of Baghdad.

RESULTS AND DISCUSSION

Emergence (ES): The speed of emergence of seeds and their growth play important roles in the proper field establishment of the wheat crop. The treatment of nanoscale iron was not significantly different from the treatment of nanoscale manganese in the first season with highest average of the emergence rate of 8.22 and 8.11 percent. In the second season, the treatment of nanoscale exceeded the rest of treatments significantly (8.44 %) followed by manganese treatment, which amounted to 7.22 %. Zinc treatment for two seasons, gave 6.55 and 6.66 percent, respectively. The comparison treatment (dry seeds) gave the lowest average for this trait, reaching 5.77 and 5.44 gestures for both seasons, respectively, and these results can be interpreted on the basis of the role played by the trace elements (iron, manganese and zinc) in promoting starch formation, especially Zinc, which is one of the components of important enzymes such as proteinase and peptides, which promotes starch formation and seed maturation and production. Many studies have shown that soaking seeds with zinc has an important physiological role in seed germination and early seedling growth (eutrophication) (Laware and Raskar 2014). As for the storage years, the 2017 storage year gave the highest average of the emergence speed characteristic for both seasons (7.59 and 7.66 % respectively) and 2013 storage year gave the lowest average for the same characteristic. Consequently, the reason for the decrease in the speed of field emergence by increasing the storage time is due to the deterioration of the seeds, which led to a decrease in their viability and the percentage of their field emergence (Table 3). AL-Fahad (2017) observed that the emergence rate was higher than at the shortest storage period (one year) for both seasons of cultivation, while the seeds stored for three years gave the lowest average emergence rate for both seasons of cultivation. Saeed and others (2018) observed that the storage period (3, 6 and 9 months) greatly affected the superiority of the seeds resulting from the three-month storage period and gave highest average for the percentage of field emergence. The nanofertilizers and the years of storage did not differ significantly between them and in both seasons of cultivation.

Energy of emergence (EE)%: The iron treatment gave the highest average percent emergence capacity and in both seasons (31.00 and 45.25%, respectively) (Table 2) followed by manganese treatment and then zinc treatment for both seasons, where the average for these treatments was 25.44, 43.86%, 11.89 and 40.47% respectively, while the comparison treatment (dry seeds) gave the lowest average for this characteristic of 0.78 and 0.00% for both seasons. The 2017 storage year gave the highest average of emergence for both seasons, as it gave 20.80 and 35.08% respectively, and the 2013 storage year gave the lowest average for both seasons (9.40 and 32.36%, respectively). The interaction of iron with the year of storage 2017 gave the highest average of emergence energy in both seasons (37.67 and 46.91% respectively). The treatment of dry seeds with the year of storage 2013 did not differ significantly with treatment of distilled water. The emergence energy % in the first season was 0.00 and 0.00%, respectively. In the second season, the treatment of dry seeds did not differ with the storage years 2013, 2015 and 2017 significantly in giving the lowest average (0.00 and 0.00 0.00%, respectively).

Field emergence ratio (%): The seeds are subject to a combination of biological and environmental stresses, which ultimately reduces the field emergence of these seeds and thus reduces the final yield of the crops. The the iron treatment gave the highest average emergence rate in both seasons (86.91 and 87.44% respectively), followed by manganese treatment, and then zinc treatment, for both seasons. The comparison treatment (dry seeds) gave the lowest average for this trait, which was 78.96 and 74.92%, for both seasons respectively. This is explained by the fact that

soaking wheat seeds with nanoparticles has led to an increase in the rate of field emergence by stimulating the components of the seed to switch from the related state to the simple state and the seed benefiting from these materials necessary for germination faster compared to untreated seeds (dry seeds). Sundaria et al (2018) confirmed that the field emergence rate of wheat seeds is greatly affected by the presence of iron nanoparticles and field emergence increased in the seed treatment at 200 ppm by 41.6% and then decreased in treatments with iron at concentrations 300 and 400 ppm. Sharifi et al (2016) found that soaking wheat seeds with iron and zinc nanoparticles for 24 hours was

Table 1. Effect of storing and soaking seeds with nano fertilizers on speed of emergence for wheat (seedling/ day) (2018-2019)

First season					Second season				
Treatments	Years of storage			Average	Treatments	Years of storage			Average
	2013	2015	2017			2013	2015	2017	
Dry seeds	5.33	5.66	6.33	5.77	Dry seeds	5.00	5.33	6.00	5.44
Water	5.66	6.33	7.00	6.33	water	5.00	6.00	6.66	5.88
Fe	7.66	8.00	9.00	8.22	Fe	7.00	8.33	10.00	8.44
Mn	7.66	8.00	8.66	8.11	Mn	6.33	7.33	8.00	7.22
Zn	6.00	6.66	7.00	6.55	Zn	6.00	6.33	7.66	6.66
Average	6.46	6.93	7.59		Average	5.86	6.66	7.66	
L.S.D 5%	T	Y	T×Y		L.S.D 5%	T	Y	T×Y	
	0.49	0.38	N.S			0.37	0.28	N.S	

Table 2. Effect of storing and soaking seeds with nano fertilizers on the emergence capacity of wheat (%) (2018-2019)

First season					Second season				
Treatments	Years of storage			Average	Treatments	Years of storage			Average
	2013	2015	2017			2013	2015	2017	
Dry seeds	0.00	0.00	2.33	0.78	Dry seeds	0.00	0.00	0.00	0.00
Water	0.00	9.00	13.00	7.33	Water	37.58	39.75	40.75	39.36
Fe	24.33	31.00	37.67	31.00	Fe	43.53	45.25	46.91	45.25
Mn	19.67	25.33	31.33	25.44	Mn	42.41	43.83	45.31	43.86
Zn	3.00	13.00	19.67	11.89	Zn	38.25	40.75	42.41	40.47
Average	9.40	15.67	20.80		Average	32.36	33.91	35.08	
L.S.D 5%	T	Y	T×Y		L.S.D 5%	T	Y	T×Y	
	2.31	1.79	N.S			0.59	0.45	1.02	

Table 3. Effect of storing and soaking seeds with nano fertilizers on the proportion of field emergence of the wheat (%) (2018-2019)

First season					Second season				
Treatments	Years of storage			Average	Treatments	Years of storage			Average
	2013	2015	2017			2013	2015	2017	
Dry seeds	77.57	78.02	81.29	78.96	Dry seeds	70.77	75.00	79.00	74.92
Water	78.40	79.01	83.08	80.16	Water	71.50	75.67	79.66	75.61
Fe	82.52	83.65	94.55	86.91	Fe	79.33	88.67	94.33	87.44
Mn	80.88	82.20	87.72	83.60	Mn	76.00	84.50	91.43	83.98
Zn	79.97	80.21	83.97	81.38	Zn	73.00	82.00	90.00	81.67
Average	79.87	80.62	86.12		Average	74.12	81.17	86.89	
L.S.D 5%	T	Y	T×Y		L.S.D 5%	T	Y	T×Y	
	1.42	1.10	2.45			0.87	0.67	1.50	

significant with regard to emergence rate as this trait increased as compared to the comparison treatment (dry seeds). The storage years indicated that the storage year 2017 gave the highest average emergence rate for both seasons (86.12 and 86.89% respectively), and the 2013 storage year gave the lowest average of 79.87 and 74.12% respectively. These results confirm the effect of the storage period on emergence as the loss of viability and activity of the seeds as a result of inappropriate storage conditions can only be noticed through their poor performance in the field when planted in the subsequent seasons (Hell et al 2000). Saeed et al (2018) observed the superiority of seeds resulting from the three-month storage period on emergence rate over the 6 and 9 months. Giad (2019) also observed that seeds were stored for ten indicated that the ability of wheat seeds to emerge decreased with increasing storage time. The iron treatment in storage year 2017 gave highest average for the percentage of field emergence in both seasons (94.55 and 94.33% respectively).

Dry weight of seedlings (mg): The iron treatment gave the highest average for the dry weight of seedlings and in both seasons (5.97 and 7.09 mg respectively), followed by manganese treatment and zinc treatment (4.86, 6.65 mg and 4 12, 6.42 mg, respectively) .The two comparison treatments (dry seeds and distilled water) gave the lowest average of 3.33, 5.22, 3.73 and 5.55 mg for both seasons respectively. There was superiority of the nanoparticle treatments over the two comparison treatments for the dry weight characteristic and at this gave the highest eruption velocity and highest rate of emergence. The highest dry weight, with the use of nano fertilizers, especially nanostructured zinc oxide in low doses, positively affects growth and physiological properties such as root elongation and dry weight (Asl et al 2019). The using nanoscale iron particles, positively affected the germination and growth of wheat seedlings subjected to drought and salinity, as an increase in the dry weight of seedlings was observed (Yasmeen et al 2015). The storage years indicated that the storage year 2017 gave the highest average for the dry weight of seedlings (mg) for both seasons (5.70 and 6.93 mg respectively), and the 2013 storage year gave the lowest average for the same characteristic, and for both seasons it was 3.63 and 5.15 Amalgam sequentially.

Table 4. Effect of storing and soaking seeds with nano fertilizers on dry weight of seedlings of wheat variety (mg) (2018-2019)

First season					Second season				
Treatments	Years of storage			Average	Treatments	Years of storage			Average
	2013	2015	2017			2013	2015	2017	
Dry seeds	2.89	3.00	4.09	3.33	Dry seeds	3.57	5.97	6.11	5.22
Water	3.16	3.33	4.69	3.73	Water	3.91	6.39	6.34	5.55
Fe	4.50	4.88	8.51	5.97	Fe	6.50	7.00	7.78	7.09
Mn	3.96	4.39	6.24	4.86	Mn	5.96	6.60	7.40	6.65
Zn	3.65	3.73	4.99	4.12	Zn	5.82	6.44	7.00	6.42
Average	3.63	3.87	5.70		Average	5.15	6.48	6.93	
L.S.D 5%	T	Y	T×Y		L.S.D 5%	T	Y	T×Y	
	0.47	0.36	0.81			0.16	0.12	0.27	

Table 5. Effect of storing and soaking seeds with nano fertilizers on chlorophyll content for wheat variety (2018-2019)

First season					Second season				
Treatments	Years of storage			Average	Treatments	Years of storage			Average
	2013	2015	2017			2013	2015	2017	
Dry seeds	8.71	11.68	14.73	11.71	Dry seeds	8.73	13.02	15.40	12.38
Water	9.39	12.03	15.41	12.28	Water	10.08	13.70	16.09	13.29
Fe	10.71	16.94	18.72	15.45	Fe	14.87	18.16	23.30	18.77
Mn	10.50	15.94	18.06	14.83	Mn	14.55	18.34	23.13	18.67
Zn	9.33	15.58	16.58	13.83	Zn	12.50	16.41	23.00	17.30
Average	9.72	14.34	16.07		Average	12.14	15.92	20.18	
L.S.D 5%	T	Y	T×Y		L.S.D 5%	T	Y	T×Y	
	1.17	0.91	N.S.			1.40	1.08	N.S.	

During 2017 storage year the highest average dry weight for seedlings in both seasons was 8.51 and 7.78 mg respectively, and the dry seed treatment with the 2013 storage year gave the lowest average and for both seasons of cultivation (2.89 and 3.57 mg, respectively).

Chlorophyll content in seedlings: The results showed that compared with the comparison treatment, percentage increased significantly in plants grown using nanoscale iron and with non-nano iron (Table 5). This indicates the superiority of the iron treatment over the two comparison treatments (dry seeds and distilled water) in chlorophyll content of seedlings, which amounted to 15.45 and 18.77 in both seasons respectively. In magnesium was 14.83 and 18.67 respectively, noting that there was no significant difference between nanoscale manganese and nano-zinc in both seasons. The latter gave 13.83 and 17.30 respectively. In two comparison coefficients (dry seeds and distilled water), they did not differ significantly between them in giving the lowest average for the seedling content characteristic of chlorophyll, which amounted to 11.71, 12.38, 12.28 and 13.29 for both seasons, respectively. This is explained on the basis of the great and influential role that iron plays in the vital processes of plants due to its participation in the enzymatic processes, as it enters in the synthesis of chloroplasts and in the synthesis of chloroplasts, and that well-prepared plants from Alkhadid have high chlorophyll content in them. This is confirmed by Feizi et al (2013) in a study to find out the catalytic role played by nanowire oxide in increasing the proportion of chlorophyll in the wheat crop, as the proportion of chlorophyll increased by 15% over the comparison treatment as a result of using nanoscale iron. This is consistent with Sharifi et al (2016). The seeds with nan iron for a period of 24 hours were significant for the chlorophyll content characteristic of the leaves, as it indicated an increase in the chlorophyll concentration in the leaves when the seeds were treated with nano iron compared to other fertilizers and the comparison treatment and the superiority of the nanostructured zinc treatment over the comparison treatment. Nano manganese affects the chlorophyll ratio. Al-juthery and Saadoun (2018) observed that use of nanoscale manganese concentrations increased the chlorophyll content. Soaking the seeds may be an appropriate way to enhance plant growth and increase the percentage of chlorophyll, as when soaking wheat seeds with different concentrations of nanocrystalline zinc, an increase in the percentage of chlorophyll in the leaves was observed with an increase in the concentrations of nanocrystalline zinc, where the percentage of increase in concentration was 100 mg / liter was 58% compared to the comparison treatment (distilled water) (Munir et al 2018).

CONCLUSION

The use of nano fertilizers, especially nano iron increased the viability of the seeds and increased all the characteristics of the seeds stored for years compared to untreated seeds and seeds soaked with distilled water only.

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Use of Star Anise (*Illicium Verum*) Essential Oil to Enhance The Specific Properties of Cold-Stored Beef Burger

Enas Najm Abdullah and Batool A.A. Abdulrahem

Department of Food Science
College of Agricultural Engineering Science, University of Baghdad, Iraq
E-mail: batool.a@coagri.uobaghdad.edu.iq

Abstract: The essential oil of star anise was extracted using the Clevenger apparatus and the active compounds in were identified using gas chromatography-mass spectrometry (GC-MS) technology. Besides, the physicochemical properties of the oil were studied by using it in the mixtures of manufactured meat tablets with different concentrations to examine its preservative and anti-oxidation effect in the manufactured burger throughout cold-stored at a temperature of 4°C and for periods of 1, 5, and 10 days. The percentage of the extracted essential oil was 6.09%, where the essential oil contained several active compounds estimated with GC-MS .The highest percentage of the compound was Anethol by 65.2%. and other compounds 1-{(3-Methyl-2-butenyl) Oxy}-Estragole, D-limonene and Linalool of 13.00, 9.76, 6.53, and 2.75%, respectively). The refractive index was 1.5580 and the specific gravity was 0.842 recorded high solubility in all organic solvents (ethanol, methanol, hexane) as well having good and desirable color and taste like the smell and taste of anise to which the essential oil is added at the rate of(0, 0.5, 1%. Moreover, chemical tests for the treatments indicated that the percentage of fat, protein, and ash in the samples increased insignificantly throughout the storage period corresponding to an insignificant decrease in the percentage of moisture compared to the control treatment. The thiobarbituric acid (TBA), peroxide value (PV), free fatty acid (FFA), and total volatile nitrogen (TVN), decreased significantly with an increase in the added oil concentrations compared to the control treatment throughout the storage period. The physical tests also showed that the treatments to which the essential oil was added recorded the highest values in the meat's water-holding capacity (WHC), which increased with an increase in the concentrations of the added essential oil compared to the control treatment. However, the pH was slightly increase with the increase of the added concentrations, while the cooking loss was recorded slight decrease by increasing the oil concentration throughout the storage period.

Keywords: Star Anise, *Illicium Verum*, Essential oil, Cold – Stored beef burger

Some aromatic plants are used in food preservation, including meat products because they contain active compounds that act as antioxidants, maintain meat quality and inhibit enzyme activity. and also contains esters, phenols, and flavonoids Star anise is one of the common medicinal plants, which used since ancient times, as it contains several active compounds (estragole, limonene, anethol). In addition to flavonoids, terpenes, sterols, and many elements such as iron, calcium, copper, potassium, and vitamins such as vitamin C and vitamin B indicated that red meat contains proteins by 20-25 g/100 g, where this percentage increases at cooking meat and varies between 28-36 g/100 g because the moisture content is reduced, and thus the nutrients become more concentrated. Meat also contains fats, which mostly consist of saturated fatty acids such as stearic acid as well as containing unsaturated fatty acids that contain a single double bond such as oleic acid. Avoiding the occurrence of oxidation has become an important matter for food manufacturers in order to protect the consumer from the dangers of oxidation by using compounds that have the ability to prevent and delay the oxidation process. It has been known as an antioxidant, as it works to protect oils, fats, and foods containing a high percentage of fat from damage caused by oxidation, and then prevents the change in color, taste, smell, and loss of nutritional value. These compounds also prevent the formation of breakdown products that include aldehydes, ketones, alcohols, and acids. Although industrial additives have been widely used in the meat industry to prevent fat oxidation, the modern trend is to reduce their use because of their negative impact on consumer health (Holtekjolen et al 2008). Shah et al (2014) indicated that the use of some plant extracts had a significant effect on the quality characteristics of meat, as are effective as an antioxidant in minced meat products compared to the industrial antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).They had an inhibitory effect on the formation of peroxides and prevented the oxidation of meat dyes when added with concentrations ranging from 0.02-0.10% to fresh minced beef, cooked and refrigerated for 4 weeks. Water Holding Capacity (WHC) is the meat's ability to retain its own water when any external force is applied to it, such as mincing, chopping, and heating (Sarica et al 2011). Al-Doukhi (2014) found during studying the effect of some plant extracts

on the water holding capacity of minced meat tablets stored in cold storage that the water holding capacity and pH increased in the meat tablets to which the extract was added compared to the control sample. Thus, the WHC value was 24.32% and increased to 34.30% when adding the rosemary extract at 0.05%, but adding onion extract at a concentration of 0.1% increased to 43.92%. The research aims to extract the essential oil from the fruits of star anise to use it as a natural source of antioxidants. In addition to identifying the chemical compounds present in the essential oil by GC-MS technology, as well as to estimate some of the physicochemical properties of the essential oil. Effect on prolonging the shelf life of cold-stored burgers for a period of 10 days was also estimated.

MATERIAL AND METHODS

The essential oil of star anise was extracted by the method of water distillation (Clevenger) while the identification of the active compounds in it by GC-MS technology (Stein 2011). The specific physicochemical properties of the essential oil were observed as refractive index at a temperature of 20°C with an Abbe-Refractometer (AOAC 2010). The specific gravity was also estimated by using the density vial at a temperature of 25°C. Conversely, the color was estimated and a sensory evaluation was conducted to determine the taste and color of the oil by specialists in the quality control department. The essential oil was added with different concentrations (0, 0.5, and 1%) in the beef burger mixture and the burger tablets are then cold-stored at a temperature of 4°C for 1, 5, and 10 days to investigate their chemical and physical properties, using 80% beef (1600 g) by 400 g for each sample, and minced animal fat (abdominal fat) was added at a rate of 10% (200 g). Furthermore, 5% of the filler material (crushed cookies) (100 g), pure table salt by 0.6% (12 g), black pepper by 0.4% (8 g), garlic powder by 0.3% (6 g), and kneading water by 3% (50 ml) were added, and the mixture was divided into four equal parts, weighing 500 g per treatment. The essential oil was added to the components of the mixture, as follows:

T_1: (Control treatment) without adding essential oil.

T_2. T_3: addition of 0.5% essential oil is equivalent to 2.5 and 5 ml of oil.

This was mixed well and formed in the form of tablets weighing 50 g per tablet. The tablets were kept in polyethylene bags separately and closed in the refrigerator at a temperature of 4° for chemical and physical tests after 1,5,10 days.

Chemical tests: The percentage of moisture, protein, fat, and ash in beef burger samples was estimated (AOAC 2010), where the value of thiobarbituric acid (TBA), peroxide value (PV) and total volatile nitrogen (TVN) and free fatty acids were estimated (AOAC 2010).

Physical tests: Method was followed in measuring the water holding capacity of meat (WHC) as well as the cooking loss percent. The pH was estimated according to Capita et al (2006).

Statistical analysis: The statistical program Statistical Analysis System (SAS), 2012 was used in data analysis.

RESULTS AND DISCUSSION

The star anise oil extracted by the water distillation method in a Clevenger apparatus was 6.09%, which was higher than the percentage obtained by Al-Younes (2019) when extracting the essential oil from star anise in the same way, (4.5 %) .However, it is less than what was mentioned by Qin et al (2007) when they extracted the oil from the same source and in the same way, (8.2%). The reason for the difference in the yield of the essential oil may be due to the difference in the temperatures used in the extraction and the extraction conditions in general. This was confirmed when extracting essential oils from some plant sources, as they showed that the dry plant parts such as seeds, stems, and fruits need steam with higher temperatures at the extracting for the purpose of obtaining a higher percentage of the extracted essential oil. The refractive index of the extracted star anise oil at a temperature of 20 °C was 1.5580 which is very close to Tuan and Ilangantileke (1997), which was 1.5553. Yadav et al (2015) observed 1.5500, and agrees with Al-Younes (2019) at 20°C, which was 1.5584 for the same essential oil. The specific gravity of the star anise oil extracted with Clevenger, was 0.842 and this ratio is close to what observed by Wong and Wannurdiyana (2014), indicating that the specific gravity of all types of extracted essential oils is less than the density of water as it floats on the surface of the water, while there are other types of oils such as the oil of cinnamon and cloves that have a specific gravity of more than 1 and it is heavier than water. This difference is due to the specific gravity of the extracted essential oils depends on the difference in the environmental conditions surrounding the plants and the extraction method used. The density varies slightly according to the extraction method, and the specific gravity when it is less than (0.9) means that the oil carries high terpene and aliphatic compounds, and if more than one oil contains compounds with many chemically different aromatic rings. The color of star anise oil was pale yellow color and taste was sweet which is identical to observation of Yadav et al (2015) and Al-Younes (2019).

The chemical compounds present in star anise oil extracted by the water distillation method using Clevenger

and separated using GC-MS technology are given in Table 2 and Figure 1. The oil contained compounds Anethol, 1-{(3-methyl-2). -butenyl) oxy}-, Estragole, D-Limonene, Linalool at were 65.28, 13.00, 9.76, 6.53 and 2.75%, respectively. The Anethol was in highest percentage in the essential oil of star anise. Zhang et al (2015) also reported that Anethol was 75.76%, and the percentage of D-Limonene and Linalool was 1.03% and 0.81%, respectively. Al-Younes (2019) also confirmed that star anise oil contains Anethol, with the highest percentage in the essential oil, reaching 86.88%, in addition to D-Limonene and Linalool, (1.03 and 0.81%, respectively). Mohamad et al (2020) confirmed that star anise oil contains Anethol, Limonen D- and Linalool, with a percentage of 75.70, 1.01 and 1.44, respectively.

Table 1. Physical properties and yield of star anise oil

Yield and physical properties	Star anise oil
Yield	6.09%
Refractive index	1.5580
Specific gravity	0.842
Color	Pale yellow
Taste	Sweet

The effect of adding star anise oil on the chemical content of cold-stored beef burgers resulted in the percentage decreased of moisture with the increase in the

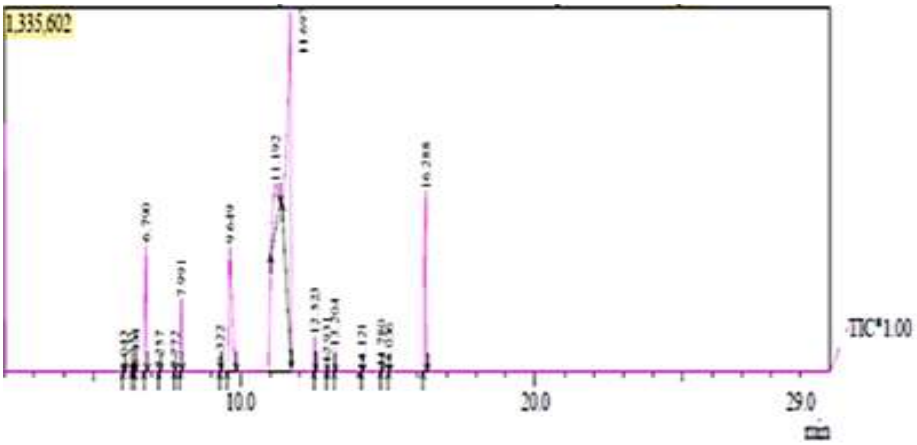

Fig. 1. Separation of chemical compounds by GC-MS technology of star anise oil

Table 2. Chemical compounds present in star anise oil

Peak number	Compound na	Retention time	Percent compound
1	1,6 – Heptadiene , 2- methyl	6.043	0.03
2	1, 4- Methano – 1H-Cyclopropa(d) pyridazine 4, 4a,5,5a- tetrahydro -	6.322	0.03
3	6,6 dimethyl- (1.alpha . ,4 alpha . ,4a.alpha., 5a. alpha.) -	0.03	0.11
4	Bicyclo(3.1.0) hex – 2- ene , 2 – methyl-5-(1-methylethyl)	6.790	6.53
5	D- Limonene	7.257	0.05
6	Bicyclo(3.1.0) hex- 2- ene,4-methyl-1(1- methylethyl)	7.772	0.04
7	(+) -4- Carene	7.991	2.75
8	Linalool3- Cyclohexen-1-0l,4- methyl	9.649	0.19
9	Anethol	11.192	16.25
10	Estragole	11.697	9.76
11	Anethol	12.523	49.03
12	2- propanone, 1-(4-methoxyphenyl)-	12.931	1.23
13	1,3,6,10-Dodecatetraene,3,7,11-trimethyl-,(z,e) -	13.204	0.19
14	Bicyclo(3.1.1) hept-2-ene , 2,6- dimethyl-6-(4-methyl-3-pentenyl)-	14.121	0.66
15	1,5-Heptadiene,3,4-dimethyl-	14.780	0.04
16	1,6,10-Dodecatrien-3-01,3,7,11-trimethyl-	15.036	0.08
17	Perfluoro-2-azapropene	16.288	0.03
18	1-{(3-methyl-2-butenyl) oxy}-		13.00

concentrations of the added oil. The highest moisture content was in treatment T_1 (65.24%) as compared to T_2 and T_3, (65.02, 64.74, respectively), in 1 day. The percentage of moisture decreased slightly with an increase in the concentration of the added essential oil with the continuation of the storage period up to 10 days. This decrease may be attributed to the occurrence of moisture evaporation from the surface of the meat tablets stored in the refrigeration during the storage period. These results agreed with what was mentioned by Naif (2019), where percentage of moisture decreases as the storage period progresses and the percentage of added dry matter such as protein, fat, and ash increase. Moreover, the study also indicated that decrease was significantly reduced with the increase in the percentage of adding essential oil compared to the control treatment. The percentage of protein increased insignificantly with the increase in the concentrations of the essential oil as the storage period progresses. The high percentage of protein is affected by adding in some natural additives to meat and its products when cold-stored. The percentage of fat increases slightly with an increase in the concentrations of the added essential oil compared to the control treatment T_1 (Table 3). However, storage period affected the percentage of fat, which increased from its value in the storage period of 1 day and for all concentrations under study. The slight increase in the percentage of fat may be due to a decrease in moisture, which led to an increase in the percentage of dry matter, which includes both fat, protein, and ash, and this is consistent with Siraj (2011), Al-Alwani (2017) and Naif (2019). Similarly, was observed that the percentage of ash increased with the increase in the concentration of added essential oil and the increase in the storage period up to 10 days. This indicates a positive effect of the essential oils added to the beef burger in maintaining the moisture content and raising the ash and protein content throughout the storage period. These results present a good agreement Naif (2019) that ash percentage is at its lowest level in a period of 1 day and then begins to increase as the storage period progresses until it reaches highest level in a period of 21 days.

The effect of adding star anise oil on thiobarbituric acid (TBA) for a cold-stored beef burger indicated that in T_1 decreased significantly with increasing concentrations of the added essential oil, which differs significantly from T_2 and T_3, (0.95, 0.92 mg MDA/ kg meat). The acid slightly increased in the treatment T_2 and T_3, which differed with significant differences in the period 10 days (1.25 and 1.19 mg MDA / kg meat) compared to the control treatment (1.50 mg MDA / kg meat). The reason for the decrease in thiobarbituric acid with an increase in the added oil concentrations as essential oils contain flavonoids, which contribute to the protection of fats from oxidation and react quickly with the peroxide radical by giving them a hydrogen atom from the phenolic hydroxyl group. The results agreed with Arora et al (2000). The

Table 3. Effect of adding star anise oil on the chemical content of the cold-stored beef burger

Chemical content	Treatments	Storage period (days)			LSD (p=0.05)
		1	5	10	
Moisture (%)	T1	65.24 ± 2.73	64.38 ± 2.28	63.57 ± 1.94	3.39*
	T2	65.02 ±2.94	63.14 ±3.08	62.45 ±2.35	3.57*
	T3	64.74 ± 2.78	62.72 ± 1.67	61.26 ± 2.37	NS3.07
LSD (p=0.05)	...	NS 3.93	NS 3.72	NS 3.68	...
Protein (%)	T1	17.50± 0.68	17.77 ± 0.82	16.58 ± 0.71	NS 1.17
	T2	18.04 ± 0.93	18.35 ± 0.66	17.93 ± 0.61	NS 1.55
	T3	18.44 ± 0.74	18.75 ± 0.92	18.60 ± 0.71	1.49
LSD (p=0.05)	...	NS 1.46	NS 1.27	NS 1.52	...
Fat (%)	T1	13.28±0.54	13.86 ± 0.62	15.33 ± 0.59	1.19 *
	T2	13.94 ± 0.38	14.43 ±58	14.92 ± 0.68	1.07
	T3	14.55 ± 0.51	14.91 ± 0.62	14.34 ± 0.51	1.18
LSD (p=0.05)	...	NS 1.26	NS 1.18	NS 1.16	...
Ash (%)	T1	1.50 ± 0.08	1.76 ± 0.10	1.90 ± 0.11	0.328*
	T2	1.60 ± 0.09	1.80 ± 0.08	1.94 ±0.09	0.371*
	T3	1.70 ± 0.08	1.83 ± 0.08	1.99± 0.12	0.288*
LSD (p=0.05)	...	NS 0.419*	NS 0.361*	NS 0.383*	...

presence of phenolic and flavonoids compounds in essential oils acted as natural antioxidants that did not differ from the effectiveness of industrial antioxidants (Mustafa et al 2010). These results agreed with the Iraqi standard issued by the Central Organization for Standardization and Quality Control No. 2688 for the year 1987 concerning chilled and frozen red meat and poultry products, which stipulates that the TBA value should not exceed 2.0 mg MDA / kg meat because it is considered unacceptable. The effect of adding star anise oil on the PV values for a beef burger showed significant decrease in the T_3 in the period (0 days compared to the control treatment). The effect of storage periods on PV values increased significantly in T_1 during the storage period, reaching 3.91 Meq/kg fat in 5 days, and in 10 days was 4.35 Meq/kg fat. In T_2, PV value increased slightly with non-significant increase in the storage period 1, 5, and 10 days reaching 2.13, 2.80, 3.21 Meq/kg fat. However, the storage period did not have a clear effect on increasing the PV value throughout the storage period of the treatment T_3, as recorded a slight insignificant increase throughout the storage period up to 10 days. The addition of the essential oil recorded a significant decrease in the PV values compared to the control treatment T_1 throughout the storage period under study. The reason for the decrease in the PV values may be due to the effect of the natural additives of the treatments in reducing the oxidation of fats and decrease in the PV values. It is consistent with the Iraqi standard issued by the Central Organization for Standardization and Quality Control No. 2688 for the year (1987) regarding red meat products, chilled and frozen poultry that the permissible PV values should not exceed 10 Meq/kg fat. This was confirmed by Al-Yunus (2019) that adding essential oils (star anise and thyme) to beef tablets at a ratio of 0.1, 0.5 and 1% at a temperature of 4°C, led to a decrease in the PV values by increasing the concentrations of the added essential oil. Al-Zaidi (2020) confirmed that the addition of rosemary leaf oil, lemon peel oil, and orange-peel oil recorded a significant decrease in the peroxide compared to the control treatment. The effect of adding star anise oil on the percentage of free fatty acids FFA percentage of FFA decreases insignificantly with an increase in the concentrations of the added essential oil compared to the control treatment. The storage period had a clear effect in increasing the percentage of FFA, as the treatment T_1 recorded the highest rates in the storage period of 10 days, amounting to 0.68%, which differs with significant differences from its value in the period 1 and 5 days. The treatments T_2 and T_3 recorded a significant decrease in the percentage of FFA compared to the treatment T_1 during the storage periods. The reason is that medicinal plant extracts contain multiple phenolic compounds that have an antioxidant action by breaking the chain reaction by donating a hydrogen atom to fatty acid and free radicals Jordan et al (2014). AL-Rubeii et al (2009) also observed that some natural antioxidants have an effect on beef during cold storage, as there was a decrease in

Table 4. Effect of adding star anise oil on the values of TBA, PV, percent FFA, and TVN of stored burger

Chemical content	Treatments	Storage period (days)			LSD (p=0.05)
		1	5	10	
PV	T1	0.06 ± 2.17	0.11 ±.913	4.35 ± 0.15	1.06*
	T2	2.13 ± 0.08	2.80 ± 0.09	3.21 ±0.10	1.18*
	T3	2.11 ± 0.09	2.20± 0.09	2.61± 0.08	0.866
LSD (p=0.05)	...	0.428	0.398	1.19*	...
TBA	T1	1.18 ± 0.05	1.20 ± 0.08	1.50 ± 0.05	0.286*
	T2	0.95 ± 0.03	1.15 ±0.06	1.25 ±0.03	0.271*
	T3	0.92 ±0.08	1.12 ± 0.068	1.19 ± 0.06	0.307*
LSD (p=0.05)	...	0.272	0.293*	0.307 *	...
FF (%)	T1	0.28 ± 0.02	0.56 ± 0.06	0.68 ± 0.05	0.291*
	T2	0.14 ±0.02	0.42±0. 04	0.59 ±0.03	0.315*
	T3	0.12±0.03	0.14 ±0.02	0.28±0.03	0.266*
LSD (p=0.05)	...	0.225	0.288*	0.307 *	...
TVN (%)	T1	10.92 ± 0.56	10.95 ± 0.47	10.98 ± 0.75	0.773
	T2	8.34 ± 0.37	8.45 ± 0.52	8.51 ± 0.57	0.604
	T3	8.26 ± 0.53	8.28 ± 0.66	8.47± 0.48	0.637
LSD (p=0.05)	...	1.274*	1.086*	1.237*	...

the percentage of FFA for all the addition treatments, especially when the rosemary was added to it, which led to a prolongation of the storage period compared to the control treatment that recorded the highest percentage of FFA. These results agree with the Iraqi standard issued by the Central Organization for Standardization and Quality Control No. 2688 for the year (1987), which stipulates that the percentage of FFA does not exceed 1.5% because it is considered unacceptable. Furthermore, can be observed the effect of adding essential oil on the concentration of TVN for a beef burger, as TVN decreased by increasing the concentrations of the added essential oil, which was reduced by significant differences in treatment T_2 and T_3 compared to the control treatment. Similarly, storage periods had a clear effect on the TVN values, as treatment T_1 recorded its highest value in the 10 days storage period, which was 10.98 mg N/100 g meat. In T_2 TVN valuesincreased slightly, insignificantly up to 10 days, which does not differ with significant differences in treatment T_3 during the storage periods. The addition of the essential oil with different concentrations led to a decrease in the TVN values throughout the storage period compared to the treatment T_1. The reason for the decrease in TVN is due to essential oils have an active compound that has a natural antioxidant effect in beef when cold-stored that adding essential oils to cold-stored beef leads to a decrease in TVN, because of its natural antioxidant effect compared to the control treatment. This was confirmed by Andres et al (2014) that the storage period affected TVN values, was lowest level in 1 day and then begins to increase with the storage period progresses until it reaches highest level in 15 days within the permissible limits. These results agreed with the Iraqi standard specification issued by the Central Organization for Standardization and Quality Control No. 2688 for the year 1987 which stipulated that TVN values should not exceed 14 mg N/100 g of meat and thus it is considered unacceptable.

Effect on meat's WHC on a cold-stored beef burger: WHC values increased significantly with the increase in the concentrations of the added essential oil compared to the control throughout the storage period. The high value of this trait may be due to the increase in pH and protein content in the meat to which the essential oil is added, which contributes to the meat's ability to retain and hold water in a large amount, that is reflected in raising the value of WHC Taher (1990). Viuda- Martos et al (2015) also observed that adding essential oils to meat increases the meat's WHC. This is because it contains active compounds that can protect cell membranes from breaking down and then protect proteins from decomposition and prevent water from leaving outside and bound with the protein by a protein-water bond. Al-Zaidi (2020) indicated that addition of rosemary leaf oil T_4 recorded a significant increase in WHC values compared with the addition treatments T_3 orange peel oil and T_2 lemon peel oil and compared to the control treatment T_1 which recorded the lowest values of WHC for the periods cooling storage. The effect of adding star anise oil on the pH of cold-stored beef burger showed that pH increased insignificantly with the increase in the concentrations of the added essential oil, as in the treatment T_1 reached 5.04 and in t T_2 5.17, which significantly increased in T_3 to 5.87 in 1 day. The pH values increase the as storage period progresses and increasing the concentration of the added essential oil and increase of pH is

Table 5. Effect of adding star anise oil on the physical properties of cold-stored beef burger

Chemical content	Treatments	Storage period (days)			LSD (p=0.05)
		1	5	10	
pH	T1	5.04±0.09	6.19±0.12	6.34±0.18	O.572
	T2	5.17±0.07	6.26±0.25	6.45±0.21	O.482
	T3	5.87±0.11	6.35±0.17	6.46±0.24	* 0.669
LSD (p=0.05)	...	0.805*	0.473	0.491*	...
WHC (%)	T1	40.21±1.07	41.48±1.69	43.89±1.53	5.28
	T2	49.36±1.35	50.41±1.83	51.19±1.79	4.33
	T3	54.60±2.12	55.21±2.06	55.40±2.19	4.16
LSD (p=0.05)	...	6.73*	7.02*	6.89*	...
Cooking loss percent (%)	T1	32.57±1.09	31.49±1.22	30.09±1.46	*2.85
	T2	32.29±1.26	31.43±1.07	29.87±1.14	*2.91
	T3	32.21±2.05	31.26±1.37	29.42±1.06	*2.88
LSD (p=0.05)	...	1.96	1.67	1.33	...

due to the decomposition of meat proteins with the progression of the storage period, which causes an increase in nitrogen compounds which leads to an increase in the pH value Al-Alwani (2017). Zangana (2015) observed that adding the essential oil of rosemary and black seed to minced chicken meat cold-stored for 7 days led to a significant increase in pH values. The addition of watercress, rosemary and radish extracts to minced tablets meat led to increase pH. The addition star anise oil on the cooking loss percent for a cold-stored beef burger indicated no significant differences in the cooking loss percent for the added essential oil concentrations. However, the cooking loss percent decreased slightly, insignificantly for the treatmentsT_1, T_2, and $T_3$by 2.57, 32.30, and 32.21%, respectively in 1 day and they did not differ significantly in 5 days, in T_1, T_2, and T_3 (31.49, 31.43, 31.43%, respectively). It decreased slightly insignificantly in 10 days, which was 30.09 29.87, and 29.42 for T_1, T_2, and T_3. There was significant decrease in the cooking loss percent for added treatments and different storage periods compared to the control treatment. This is due the low percentage of moisture due to the water evaporation on the meat surface, and on the other hand, the decomposition of meat proteins by the decomposing enzymes that lead to the breaking of the bonds that link the protein with water, and then the meat's WHC decreases, so subject to evaporation Juarez et al (2012). These results agreed with indicated that there was a significant decrease in the cooking loss percent and the percentage of shrinking in the meat tablets treated with plant extracts compared with the control treatment. Moreover, the results agree with the findings of Al-Zaidi (2020) that adding rosemary leaf oil to frozen and chilled beef sausages, led to a decrease in the cooking loss percent compared to all addition treatments and control that recorded the highest percentage of cooking loss and for different storage periods.

CONCLUSIONS

The results of identifying he active compounds using GC-MS technology showed the appearanceof active compounds with a high percentage, especially Anethol, {(3-Methyl-2-butenyl)oxy}-1, Linalool, D-Limonene and Estragole in the essential oil of star anise. The addition of star anise essential oil to the beef burger showed high efficacy as antioxidants by decreasing the oxidation indicators thiobarbituric acid value (TBA), peroxide value PV, free fatty acids FFA, and total volatile nitrogen TVN). Moreover, the addition of the essential oil to the burger tablets led to an improvement in the nutritional value by decreasing the moisture content and increasing the chemical content such as protein, ash, and the rest of the dry ingredients.

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Efficacy of Chlorine and Ammonia Gases on Corrosion of Pure Copper Metals

Iman N. Mahdi and Jhad D. Mahl

Department of Biology, College of Education for Pure science, University of Tikrit, Iraq
E-mail: em.an2022@gmail.com

Abstract: The study included the effects of two gases (chlorine, ammonia) on pure copper metal and coated with aluminum. Each plate was cut into 16 pieces with a length 2.5 * 2.5 cm, the thickness of one piece of copper is 3 mm. The metal pieces were placed inside the flasks, then the flasks were closed with tightly closed rubber, and the gases were injected at 1 and 3 volumes into the flasks, some of which were exposed to dry media and others to wet media. The flasks were divided into two groups. The first group was provided with protection through the use of aluminum coating for the metals used, and the other group did not provide protection, and was then left for 6 months. The effect of chlorine gas on copper metal was clear, and the loss weight and corrosion percentage were increased at 3 volume. Ammonia gas also has a negative effect on copper metal, and the negative effect was increased at 3 volume. The synergistic efficacy of chlorine and ammonia gases was recorded at a high loss weight and percentage of corrosion compared with the single effect of gases on copper metal. The moisture in wet media accelerated the corrosion of copper compared with dry media. The aluminum coating that was used in this study decreased the loss weight and corrosion percentage in dry and wet media, and also approved its activity against the effect of single and synergistic chlorine and ammonia gases on copper metal corrosion.

Keywords: Chloarine, Ammonia, Copper metal, Corrosion

Corrosion may occur on non-metal materials such as concrete and plastic. The factors on which corrosion depends are temperature and environmental factors, in addition to pressure and erosion, which increase the rate of corrosion. Corrosion also caused material losses (Kruger and Begum 2019). Copper is an important metal in many industries. Copper is a chemical element that is used in the composition of many alloys. The alloy that is made from zinc and copper is called brass, and it is used in some musical devices and equipment (Bell 2020). Because it is a good conductor of electricity, copper is used today in the manufacture of electrical wires, cables, and electronic devices, and it is also used in building materials because of its resistance to corrosion (Li et al 2017). Gas corrosion is the direct reaction between a gas and the surface of a metal, which usually occurs at high temperatures. Scientists have differed on this type of corrosion and whether it is chemical or electrochemical corrosion. The mechanism of gas corrosion is at begging chemical and when a complete layer is formed on the surface, in the absence of direct contact of the metal with the corrosive gas, then electrochemical corrosion occurs. Wet gas corrosion occurs when the surrounding medium is aqueous solutions or electrolytes, and the corrosion product in the electrochemical reaction is more dangerous than the corrosion product in the dry reaction (Mansoori 2017). Due to the large losses resulting from metal corrosion, coatings were used on many metals to protect against corrosion (Al-Hamdani 2018). The aims of the current study were to study the effect of corrosion media on copper metal, the effects of dry and wet atmospherics on copper metals, and finally, the protection of copper from corrosion using aluminum coating.

MATERIAL AND METHODS

Collection of copper metal plates: Metal plates were purchased from the specialized scientific offices. Four copper plates were purchased, each plate has 3 mm thick and with dimensions of 10 * 10 cm.

Preparing copper metal plates for the experiment: Each plate of metal was cut into 16 pieces. The dimensions of each piece were 2.5 * 2.5 cm, and then the metal pieces were polished using polishing with sandpaper sequentially from size 250, then 400, and 1000, in order to get the perfect shape and more smoothness for each piece. The methods for preparing metal pieces were used as described in Mahdi and Mahl (2021), with some modifications, such as using chlorine and ammonia gases on the copper metal instead of sulfur trioxide and chlorine gas on the lead metal.

Preparing gas storage bottles: Gas bottles were prepared to save gas (ammonia and chlorine gas). The bottles were obtained from specialized offices. Each bottle has a tight

outlet hole with a specific pressure-bearing device and a manual control tap to make it easier to fill the bottles with the required amount of gas.

Preparation of gas: Ammonia gas of 99.9% purity was purchased from the offices supplying chemicals in Baghdad governorate at a rate of 3 L for each gas storage bottle. A chlorine gas of 99.9% purity was bought from scientific offices, where 3 liters were filled in each bottle, three bottles for each gas. All gas bottles were stored at a moderate temperature of 25–30 °C with ventilation. After filling the bottles with the required gases, the high-pressure tap was separated from each bottle and replaced with a sterile 2-way latex Foley Catheter with a thickness of 0.8 mm to pull the gas using medical syringes.

Preparation of corrosion media: Thirty-six conical flasks with a volume of 500 ml were prepared. The flasks were cleaned carefully, then dried. After that, it was kept in an oven for two hours at a temperature of 160°C for sterilization (Casolari 2018). The metal copper pieces were dried in the oven at 60°C for 48 hours and weighed using a sensitive balance after drying. The flasks were closed with a tight rubber seal and coated with silicone to prevent the possibility of gas leakage. Sterile medical syringes with a volume of 3 ml were used to pull the air inside the flasks to create anaerobic conditions to ensure that the air does not interact with copper metals as well as gases.

Design of a corrosion media experiment: After preparing the metal for the required shape and size, it was cleaned and dried. After the weighing was completed, it was distributed among the corrosion media. Dry and wet corrosion media were prepared. The dry corrosion media included only the presence of gases with metals without adding hot water vapor, while in the wet corrosion media, hot water vapor was used to provide moisture with the gases used. Group one consisted of injecting 1 volume of chlorine gas into the dry medium and 1 volume into the wet medium containing copper metal. The second group consisted of injecting ammonia gas with 1 volume into the dry medium and 1 volume into the wet medium containing copper metal. The third group included injecting chlorine gas with ammonia gas for a total of one volume in the same media as the first group. Group four included the use of control media, a dry medium, and wet media containing only copper metal. The same groups were repeated, but with a 3 volume. The metals were used without a protective coating.

Coating copper metals with aluminum: The pieces of copper metal were put inside the vacuum thermal device. The Dunkston wire was used to place the aluminum wire around it. The Dunkston wire was heated by the electrons used by the device, which led to the heating of the aluminum wire and its evaporation towards the pieces of copper metal that needed to be coated. After the temperature was reduced, the coated metal pieces were taken out and put in the oven for 48 hours at a temperature of 60°C. Immediately after that, they were weighed. The coated metals were put in the corrosion media, and then the steps were repeated as mentioned in 2-6, but with some difference, through the use of aluminum-coated metal. The thickness of the aluminum coating ranges from 800 to 1000 micrometers.

Preparing the copper metal pieces to measure weight loss: The metal pieces were cleaned with a hard plastic brush under running tap water to remove suspended matter. The samples were dipped and rinsed in concentrated hydrochloric acid to remove the substances that could not be removed by the first step. The method was used as in (Hasan 2015), with some modifications, including the use of concentrated hydrochloric acid only. The samples were cleaned using ethanol to remove the hydrochloric acid and the remaining deposits on the copper metal. After that, the samples were kept in the oven at 60 °C for 48 hours to get rid of moisture. Samples were weighed, and their weight was recorded.

Data analysis: This was carried out using the Excel 2016 program by using the equations required to obtain the difference in weight and percentage of corrosion (Al-Hamdani 2018) .

RESULTS AND DISCUSSION

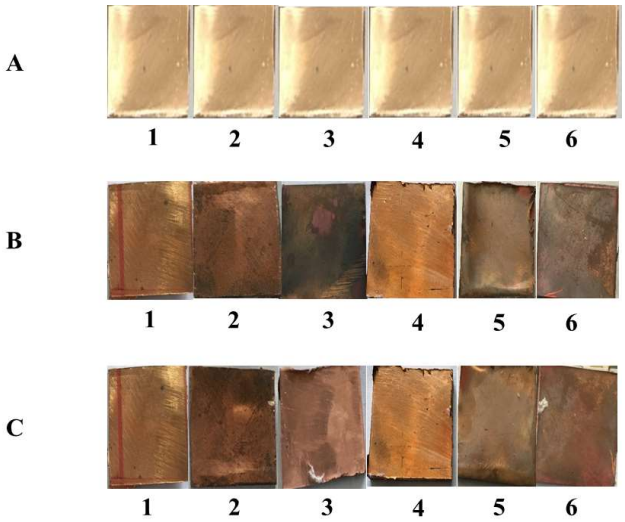
Effect of chlorine gas on copper metal: The the weight lost was 0.0155 mg, and the percentage of corrosion was 0.08% by adding of 1 volume chlorine gas to the corrosion medium containing copper metal for 6 months in dry media (Table 1). The loss in weight for the same metal was increased by 0.025 mg and a percentage of 0.16% at 3 volumes, the control sample in dry media was not affected (Fig. 1). When adding water vapor to the corrosion medium containing copper metal and chlorine gas, the weight loss was 0.0358 and 0.0367 mg, and the percentage of corrosion was 0.201% and 0.196%, respectively. The increase in the corrosion percentage in dry media may be attributed to the formation of a protective layer on the surface of the copper metal used, which is a copper oxide layer (I) (Cu_2O, cuprite), which is green in color, and another layer of copper dioxide (CuO, tenorite) (Suh et al 2016). Although the corrosion continues, possibly due to the protective layer formed not fully adhering to the metal surface, allowing the passage of electrons, while the corrosion percentage in wet media is higher than that in dry media. García-Ávila et al (2019) also found that chlorine gas used in copper pipes used to transport water for long periods leads to an increase in the

corrosion rate. The interaction of molecular water with chlorine gas results in the formation of hydrochloride acid, as is clear from equation 1. Therefore, the corrosion percentage in wet media is higher than the percentage of corrosion in dry media.

$H_2O + Cl_2 = O_2 + HCl$ -------1

Effect of ammonia gas on copper metal: When copper was exposed to ammonia gas at 1 volume, the weight loss was 0.0209 mg, and the percentage of corrosion was 0.136%. At 3 volumes, the weight loss increased to 0.0499 mg, and the corrosion percentage also increased to 0.34% (Table 2). This result is almost similar to the results of Davalos-Monteiro (2018). In study copper pieces were placed in a medium containing ammonia vapor in semi-dry conditions, and the formation of corrosion products of a dark gray color uniformly covering the entire copper sample was observed (Fig. 2). This corrosion product is copper oxide (Cu_2O) and the process of its formation is called coloring. The presence of copper samples in wet media with 1 volume of ammonia gas. The loss in weight was observed at 0.0244 mg and the corrosion percentage was 0.134%, while when the volume of ammonia gas was increased to 3 volume, the loss in weight was observed at 0.0992 mg and the percentage was 0.611%. The presence of moisture accelerated the corrosion process by increasing the concentration of ammonia gas (Davalos-Monteiro 2018).

Synergistic effect of ammonia and chlorine gases on the corrosion of copper metal: The effect of combining two gases at 1 and 3 volumes on the corrosion of copper metal for 6 months in dry and wet media showed that when copper metal was put in a space containing chlorine gas and



1. A dry-medium control sample of copper metal without air and chlorine gas. 2. The copper metal was exposed to chlorine gas at a volume of 1 in dry medium. 3. Copper metal was also exposed to chlorine gas, but at a volume of 3 in dry medium. 4. A control sample of copper metal without air and chlorine gas in a wet medium. 5. Exposed copper metal to 1 volume of chlorine gas in a wet medium. 6. The copper metal was exposed to chlorine gas at a volume of 3 volume in a wet medium

Fig. 1. Exposed copper metal to chlorine gas for 6 months in dry and wet media. A before test, B after test and before cleaning and C after cleaning

Table 1. Effect of corrosion of copper metal by chlorine gas in dry and wet media on weight loss and corrosion percentage

Metal No.	Metals	Gas type	Media	Concentration (ml)	Before (mg)	After (mg)	Loses (mg)	Percentage of corrosion
1	Control	---	dry	---	17.9352	17.9352		---
2	Copper	Cl_2	dry	1	19.4256	19.4101	0.0155	0.08
3	Copper	Cl_2	dry	3	15.6953	15.6703	0.025	0.16
4	Control	---	wet	---	14.4212	14.4212		---
5	Copper	Cl_2	wet	1	17.8728	17.8370	0.0358	0.201
6	Copper	Cl_2	wet	3	18.7741	18.7374	0.0367	0.196

Table 2. Effect of corrosion of copper metal by ammonia gas in dry and wet media on weight loss and corrosion percentage

Metal No.	Metals	Gas type	Media	Concentration (ml)	Before (mg)	After (mg)	Loses (mg)	Percentage of corrosion
1	Control	---	Dry	---	17.9352	17.9352	---	---
2	Copper	NH_3	Dry	1	15.4293	15.4084	0.0209	0.136
3	Copper	NH_3	Dry	3	14.6786	14.6287	0.0499	0.34
4	Control	---	Wet	---	14.4212	14.4212	---	---
5	Copper	NH_3	Wet	1	18.2891	18.2647	0.0244	0.134
6	Copper	NH_3	Wet	3	16.2452	16.1460	0.0992	0.611

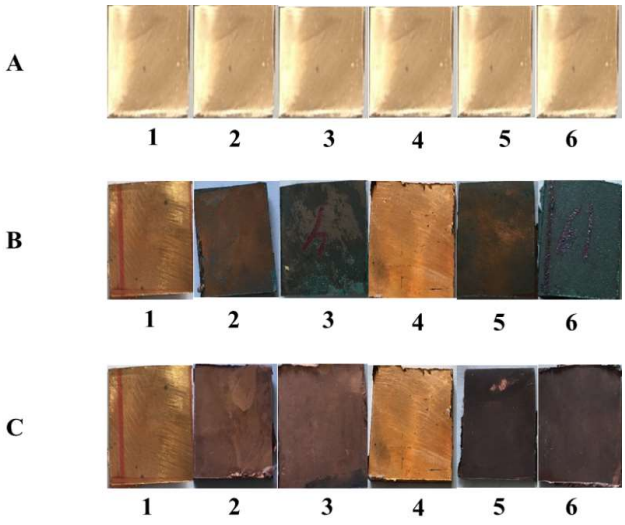
ammonia gas at a total 1 volume for both gases for 6 months, a weight loss of 0.0299 mg and the percentage of corrosion was 0.189% (Table 3). When the total volume of chlorine and ammonia gas was increased to 3, the weight loss increased to 0.0501 mg and the percentage also increased to 0.316% in dry conditions. Ammonia gas reacts with chlorine gas to produce nitrogen gas and hydrogen chloride vapor, equation No. 2, hydrogen chloride vapor reacts with the remaining ammonia gas molecules from the first reaction to produce ammonia chloride, which is in the form of a white solid fog, equation No. 3. Therefore, the compound NH_4Cl is responsible for the corrosion product in copper metal.

$2NH_3 + 3Cl_2 = N_2 + 6HCl$ -------- 2

$NH3 + HCl = NH_4Cl$ ---------- 3

The wet media weight loss was observed at 1 volume(0.0379 mg) and a percentage (0.239%), while at 3 volume, the weight loss of copper metal was 0.0372 mg and percentage of corrosion was 0.2088%. When comparing the weight loss and percentage at 1 volume in both dry and wet media weight loss and percentage of corrosion were increased in the wet medium. This is due to the moisture factor and synergistic effect of the gases used, which speed up the corrosion process (Table 3). These results are in agreement with those of Brimblecombe (2003), where adsorbed water layer present on the surface of the metal acts as an electrolyte medium and thus accelerates the corrosion process in addition to the effect of the NH_4Cl compound. In the dry medium, there is no moisture, so the weight loss and percentage of corrosion are not high. In the wet medium, when comparing the 1 volume and the 3 volume, the weight loss and the percentage of corrosion of copper metal do not differ between them. This is due to the fact that the corrosion layer formed on the surface of the metal at 1 volume may act as a protection for the metal. Therefore, the percentage of corrosion does not increase at 3 volumes compared to 1 volume (Fig. 3).

Influence of chlorine gas on coated copper with aluminum: When the metal was exposed to chlorine gas, the weight loss and corrosion percentage were measured at 1 volume and 3 volume, the weight loss was 0.017 and 0.0178 mg with percentages loss of 0.1% and 0.092% in the dry medium (Table 4). When compared with the results of non-coating copper with coating copper in dry media, no clear effect was observed on copper metal, as the color of the metal returned to the original color of the metal after cleaning, which was a reddish-yellow color. The loss in weight and percentage with this volume is due to the loss of the aluminum coating layer, and no obvious corrosion was seen. In wet medium, the weight loss was recorded at 0.01 and the percentage was 0.064% at 1 volume of chlorine gas. At 3 volume the weight loss was recorded at 0.0179 and the percentage of corrosion was 0.123%. The copper samples that were coated with aluminum in a wet medium and



1. A dry-medium control sample of copper metal in space without air and ammonia gas. 2. The copper metal was exposed to ammonia gas at a volume of 1 in dry medium. 3. Copper metal was also exposed to ammonia gas, but at a volume of 3 in dry medium. 4. A control sample of copper metal in space without air and ammonia gas in a wet medium. 5. exposed copper metal to 1 volume of ammonia gas in a wet medium. 6. The copper metal was exposed to ammonia gas at a volume of 3 volume in a wet medium

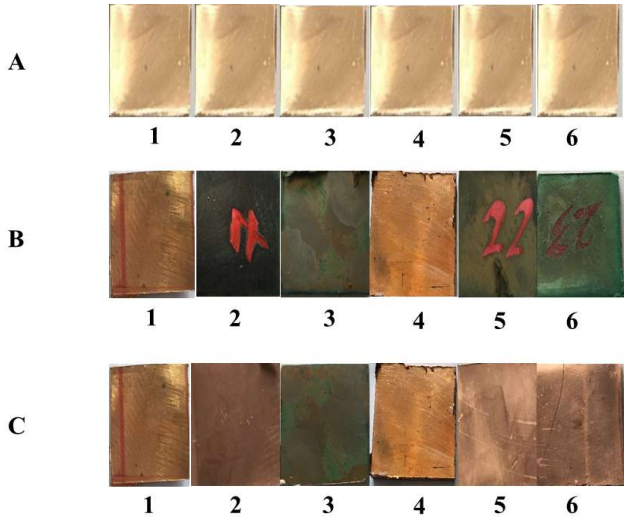
Fig. 2. Exposed copper metal to ammonia gas for 6 months in dry and wet media. A before test, B after test and before cleaning and C after cleaning

Table 3. Corrosion of copper metal by ammonia and chlorine gas in dry and wet media on weight loss and corrosion percentage

Metal No.	Metals	Gas type	Media	Concentration (ml)	Before (mg)	After (mg)	Loses (mg)	Percentage of corrosion
1	Control	---	Dry	---	17.9352	17.9352	---	---
2	Copper	$NH_3 + Cl_2$	Dry	1	15.8419	15.8120	0.0299	0.189
3	Copper	$NH_3 + Cl_2$	Dry	3	15.8601	15.8100	0.0501	0.316
4	Control	---	Wet	---	14.4212	14.4212	---	---
5	Copper	$NH_3 + Cl_2$	Wet	1	15.8622	15.8243	0.0379	0.239
6	Copper	$NH_3 + Cl_2$	Wet	3	17.9563	17.9191	0.0372	0.208

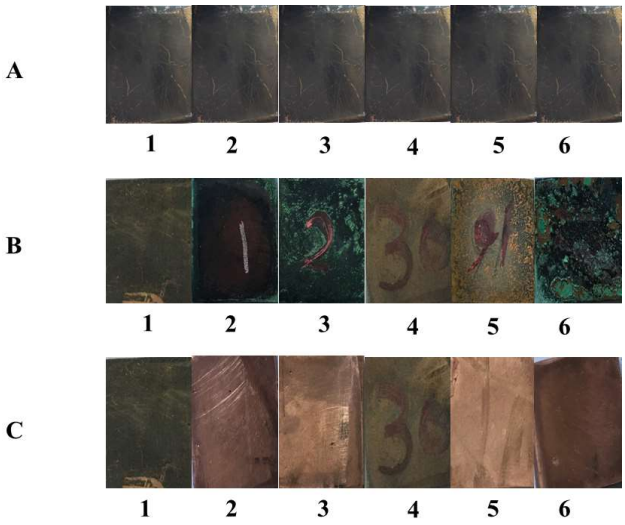
exposed to chlorine gas at different volumes were not affected by the gas. It was also seen that the copper recovered its reddish-yellow color after cleaning. There was also no difference between the copper coated samples after cleaning and the control sample (Figure.4). The reason for this may be due to the formation of the aluminum oxide layer as a result of the interaction of the aluminum coating surface with gases and moisture. This layer protected the copper metal from corrosion (López-Ortega et al 2018).

Influence of ammonia gas on coating copper with aluminum: The weight loss and percentage of corrosion for coating copper that has been exposed to ammonia gas is given in Table 5. At 1 volume, weight loss was recorded at 0.0022 mg and a percentage of 0.012% in the dry medium. At this volume, the effect of corrosion was not observed on the coated metal while at 3 volumes, weight loss was increased to 0.0053 mg and the percentage of corrosion to 0.035% (Fig. 5). In a wet medium, the loss weight at 1 volume was 0.0124 mg and the corrosion percentage was 0.103%. When the volume was increased to 3 volume, the loss weight increased to 0.0243 mg and the corrosion percentage also increased to 0.123%. When the results of table 2 and table 5 are compared, the corrosion percentage in dry and wet corrosion media has decreased. This means the aluminum coating on copper metal provides protection for copper metal against the negative effects of ammonia gas. After cleaning the



1. A dry-medium control sample of copper metal in space without air and gases. 2. The copper metal was exposed to ammonia gas and chlorine gas at a volume of 1 in dry medium. 3. Copper metal was also exposed to ammonia gas and chlorine gas, but at a volume of 3 in dry medium. 4. A control sample of copper metal in space without air and ammonia gas and chlorine gas in a wet medium. 5. Exposed copper metal to 1 volume of ammonia gas and chlorine gas in a wet medium. 6. The copper metal was exposed to ammonia gas and chlorine gas at a volume of 3 volume in a wet medium

Fig. 3. Exposed copper metal to synergism effect of ammonia gas and chlorine gas for 6 months in dry and wet media. A before test, B after test and before cleaning and C after cleaning



1. A dry-medium control sample of coated copper metal in space without air and chlorine gas. 2. The coated copper metal was exposed to chlorine gas at a volume of 1 in dry medium. 3. Coated copper metal was also exposed to chlorine gas, but at a volume of 3 in dry medium. 4. A control sample of coated copper metal in space without air and chlorine gas in a wet medium. 5. Exposed coated copper metal to 1 volume of chlorine gas in a wet medium. 6. The coated copper metal was exposed to chlorine gas at a volume of 3 volume in a wet medium

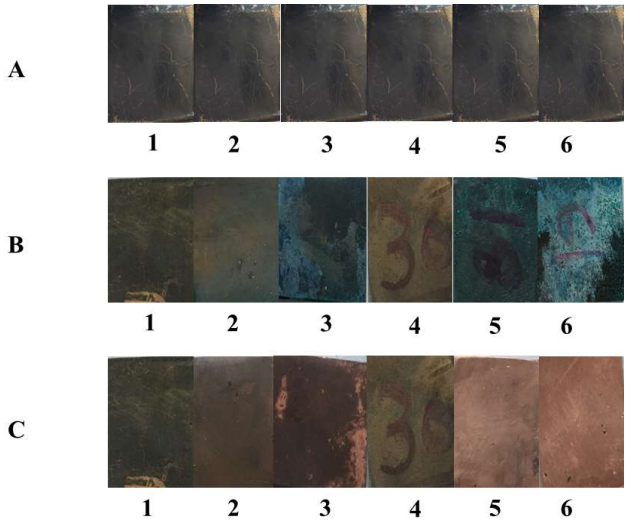
Fig. 4. Exposed coated copper metal to chlorine gas for 6 months in dry and wet media. A before test, B after test and before cleaning and C after cleaning

Table 4. Effect of corrosion of coated copper metal by chlorine gas in dry and wet conditions on weight loss and corrosion percentage

Metal No.	Metals	Gas type	Media	Concentration (ml)	Before (mg)	After (mg)	Loses (mg)	Percentage of corrosion
1	Control	---	Dry	---	17.1981	17.1981	---	---
2	Copper	Cl_2	Dry	1	17.1164	17.0994	0.017	0.1
3	Copper	Cl_2	Dry	3	19.5588	19.5410	0.0178	0.092
4	Control	---	Wet	---	15.6528	15.6528	---	---
5	Copper	Cl_2	Wet	1	15.7340	15.7240	0.01	0.064
6	Copper	Cl_2	Wet	3	14.5930	14.5751	0.0179	0.123

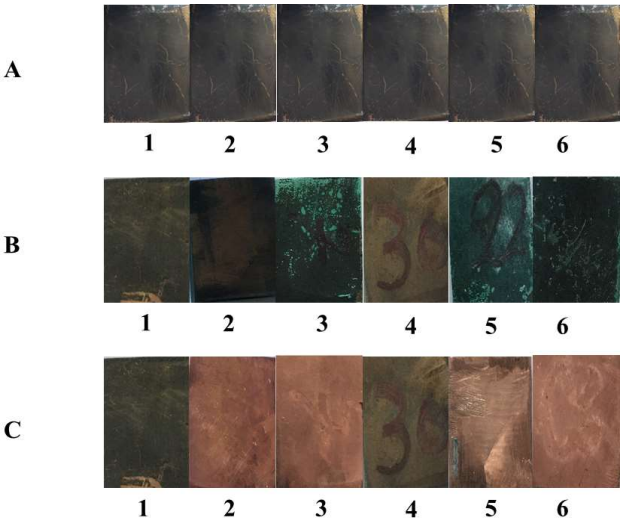
copper metal pieces from deposits with the cleaning solution, the copper metal keeps its reddish yellow color.

Synergistic effect of ammonia and chlorine gases on the corrosion of coated copper metal: The loss weight and corrosion percentage were 0.0246 mg and 0.139% at 1 volume, while at 3 volume the loss weight and corrosion percentage decreased to 0.0075 mg and 0.053%



1. A dry-medium control sample of coated copper metal in space without air and ammonia gas. 2. The coated copper metal was exposed to ammonia gas at a volume of 1 in dry medium. 3. Coated Copper metal was also exposed to ammonia gas, but at a volume of 3 in dry medium. 4. A control sample of coated copper metal in space without air and ammonia gas in a wet medium. 5. exposed coated copper metal to 1 volume of ammonia gas in a wet medium. 6. The coated copper metal was exposed to ammonia gas at a volume of 3 volume in a wet medium

Fig. 5. Exposed coated copper metal to ammonia gas for 6 months in dry and wet media. A before test, B after test and before cleaning and C after cleaning



1. A dry-medium control sample of coated copper metal in space without air and gases. 2. The coated copper metal was exposed to ammonia gas and chlorine gas at a volume of 1 in dry medium. 3. Coated copper metal was also exposed to ammonia gas and chlorine gas, but at a volume of 3 in dry medium. 4. A control sample of coated copper metal in space without air and ammonia gas and chlorine gas in a wet medium. 5. Exposed coated copper metal to 1 volume of ammonia gas and chlorine gas in a wet medium. 6. The coated copper metal was exposed to ammonia gas and chlorine gas at a volume of 3 volume in a wet medium

Fig. 6. Exposed coated copper metal to synergism effect of ammonia gas and chlorine gas for 6 months in dry and wet media. A before test, B after test and before cleaning and C after cleaning

Table 5. Effect of **c**orrosion of coated copper metal by ammonia gas in dry and wet media on the weight and percentage of corrosion

Metal No.	Metals	Gas type	Media	Concentration (ml)	Before (mg)	After (mg)	Loses (mg)	Percentage of corrosion
1	Control	---	Dry	---	17.1981	17.1981	---	---
2	Copper	NH_3	Dry	1	18.0653	18.0631	0.0022	0.012
3	Copper	NH_3	Dry	3	15.2201	15.2148	0.0053	0.035
4	Control	---	Wet	---	15.6528	15.6528	---	---
5	Copper	NH_3	Wet	1	12.1539	12.1415	0.0124	0.103
6	Copper	NH_3	Wet	3	19.9024	19.8781	0.0243	0.123

Table 6. Corrosion of coated copper metal by synergism effect of ammonia and chlorine gas in dry and wet media

Metal No.	Metals	Gas type	Media	Concentration (ml)	Before (mg)	After (mg)	Loses (mg)	Percentage of corrosion
1	Control	---	Dry	---	17.1981	17.1981	---	---
2	Copper	$NH_3 + Cl_2$	Dry	1	17.7268	17.7022	0.0246	0.139
3	Copper	$NH_3 + Cl_2$	Dry	3	14.4032	14.3957	0.0075	0.053
4	Control	---	Wet	---	15.6528	15.6528	---	---
5	Copper	$NH_3 + Cl_2$	Wet	1	18.0492	18.0244	0.0248	0.138
6	Copper	$NH_3 + Cl_2$	Wet	3	15.3315	15.3046	0.0269	0.176

respectively (Table 6). Whereas when coated copper metal was exposed to the synergistic effect of the gases using wet media, at 1 volume the loss weight was 0.0248 mg and the percentage of corrosion was 0.138 mg, while at 3 volume the loss weight and corrosion percentage were 0.0269 mg and 0.176%, respectively. The aluminum coating helps to decrease the loss weight and corrosion percentage. The aluminum coating also provides protection against corrosion for coated copper metal, and the higher corrosion percentage may be due to the aluminum coating that is used for protection against corrosion after removing it from the copper metal (Mirhashemihaghighi et al 2016; López-Ortega et al 2018).

CONCLUSION

The copper metal not protected by coating in dry and wet media suffered from corrosion when exposed to the single and synergistic effects of chlorine gas and ammonia gas at a volume of 1 and 3. The aluminum coating used in coating pure copper metal protected coated copper metal from corrosion in dry and wet media when exposed to the single and synergistic impact of chlorine gas, ammonia gas at a volume of 1 and 3.

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Biodegradation of Chrysene by *Aspergillus niger* and *Penicillium funiculosum*

Ahamed Farhan Suker, Ihsan Flayyih Hasan Al-Jawhari and Yas Khuthaair Abas

College of Education for Pure Sciences, University of Thiqar, Iraq
E-mail: ahmed2020.bio@utq.edu.iq

Abstract: Filamentous fungi were isolated from the upper surface of the sediments of the central marshes (Abu Zarak marsh) and (Abu--Subat marsh) at six stations, three of which were in Abu zarak marsh and three stations in Abu--Subat marsh in AL-Nasiriyah governorate using the dilution method. Environmental factors such as temperature, dissolved oxygen, pH and organic carbon were measured in the study stations to determine the distribution of these fungi. The results showed that *Aspergillus niger* and *Penicillium funiculosum* were the most common fungi among than other fungi isolated with this high-frequency study. The incidence of *Aspergillus niger* was 100% and in *Penicllium funiculosum*, was 92%. The colony diameters were calculated in the solid medium, and both fungi showed their ability to adapt and grow in the chrysene-containing medium. The results showed that *Aspergillus niger*, *Penicllium funiculosum* has ability to degrade chrysene at a concentration of 60 ppm. The metabolites of the decomposition and the remaining concentration were identified by Fourier-transform infrared spectroscopy and gas chromatography-mass spectroscopy. The statistical methods showed significant differences between the fungi.

Keywords: Biodegradation, Chrysene, *Aspergillus niger*, *Penicillium funiculosum*

Polycyclic aromatic hydrocarbons (PAHs) are worldwide environmental contaminants of anthropogenic origin released into aquatic systems mainly due to accidental fuel spills or leakages from corroded tanks at petrol stations or refineries. PAHs are ubiquitous contaminants in the environment and their fates in nature are of great environmental concern due to their potential toxicity, mutagenicity, and carcinogenicity. Chrysene is a polycyclic aromatic hydrocarbon (PAHs) with the molecular formula $C_{18}H_{12}$ that consists of four fused benzene rings. It is a natural constituent of coal tar, from which it was first isolated and characterized. It is also found in creosote, a chemical used to preserve wood. Chrysene is formed in small amounts during the burning or distillation of coal, crude oil, and plant material (Harvey 1991). Larger PAHs have also been used as models to determine factors that affect the bioavailability, biodegradation potential, and rate of microbial degradation of PAHs in the environment (Kanaly and Harayama 2000). The metabolism of more complex PAHs with four or more rings has been less extensively studied when they are used as a sole carbon source. However, the very low solubility of complex PAHs, in fact, strongly reduces their bioavailability and makes microbial growth and biodegradation difficult (Boldrin et al 1993). The use of fungi as a method of biodegradation provides an option to clean up environmental pollutants' biodegradation using fungi have drawn little attention in the past two decades since most of the bioremediation researches focused mainly on the use of bacteria. Nevertheless, recently fungi have received considerable attention for their bioremediation potential that is attributed to the enzymes they produced that are involved in lignin breakdown which degrade a wide range of recalcitrant pollutants such as polyaromatic hydrocarbons, chlorophenols, and pesticides. Batelle (2000) showed that fungi were better degraders than traditional biodegradation techniques including bacteria. The purpose in present investigation was to study filamentous fungi (*A.niger* and *P. funiculosum*) to degrade chrysene. The selected fungi in study because these fungi were highly frequency among other fungi isolated in this investigation, are due their ability to remove chrysene. This study was carried out on fungi isolated from the surface of sediments in Abu- Subat and Abu Zarak marshes, in the central marshes south of Iraq.

MATERIAL AND METHODS

Collection of sediments samples: Forty-eight sediments sample were collected from six stations during August 2020 to March 2021 from the center marshes. Three stations S_1, S_2, S_3 were in Abu Subat marsh, Abu Zarak marsh in Thiqar governorate. The methods used for collection of the sediment samples were the same as described by Hohnk (1972).

Isolation of fungi: The technique is applied to isolated fungi from the surface (15-30 cm depth of sediments samples) by using dilution plate method (Al-Nasrawi 2012). Two media were used in this study, Potato dextrose agar (PDA), and

Mineral salts medium (MSM). Each media was supplemented with 250 mg chloramphenicol to kill bacteria. One lapful was transferred from the plates and examined under a dissecting microscope. Mineral salts medium containing (g/L): K2HPO4, 1.71; KH2PO4, 1.32; NaNO3, 0.42; MgSO4.7H2O, 0.42; CaCl2, 0.02 was used for fungi growth. General and specific taxonomic references were used for the identification for fungal species (Klich and Pitt 1992). Chrysene at 60 ppm was used as a sole of carbon and energy.

Ability of isolated fungi to grow on solid medium supplemented with chrysene: The growth examined to find the resistant isolated fungi to chrysene in solid medium, and the growth were comparing with control. The chrysene at a concentration of 60 ppm was added to warm PDA before solidify in all plates. Dishes without added chrysene were used in control plates. This experiment was done duplicate. All dishes were inoculated with 5mm from 7-day old colonies. The dishes were transferred to incubator with 25°C. The colony diameter of all fungi under study were calculated after 7 days and compared with control.

Ability of isolated fungi to grow in mineral salts medium supplemented with chrysene: The growth examined to find the resistant isolated fungi to chrysene in liquid medium. The growth was comparing with control, as a weight of mycelium the mineral salts medium 250 ml containing chrysene at a concentration of 60 ppm added as a source of carbon. The liquid mineral salts medium was inoculated with 5mm disk from the mycelial of the old 7 days. The control flasks were not inoculated with mycelial of fungi colony isolated. All flasks were covered with non- absorbent cotton wool and incubated 7 days in 25°C. The flasks were shaken with orbital shaker (150 rpm) to mixed content. The mycelial dry weight of isolated fungi was calculated by using sensitive balance after filtration through Whattman No.1 filter paper. Hydrogen ion concentration was calculated by using pH meter.

Biodegradation of chrysene: Calculation of residual chrysene was done by using by gas chromatography-mass spectroscopy after 7 days incubation. Residual chrysene were extracted with hexane (1:2), and centrifuged for 10 min at 10000g, after separation 1 ml of hexane was filtered with Millipore filters paper (0.45 µm) and transported into a sterile vial for evaporation of hexane. After complete evaporation of hexane, 1ml of acetonitrile was added to the residue and the remaining chrysene was analyzed by Fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectroscopy (GC-MS). (The injector volume was 1µl and the oven temperature started at 60°C (1min), and increased by 25°C min-1 to 150°C, and 10min-1 to 260°C field for to 20 min), and increased to 270°C (held for 20min). The carrier gas was 1 ml min^{1} helium, in the same time the remaining chrysene was determined by Fourier-transform infrared spectroscopy.

Statistical analysis: The analysis was done by using SPSS (version 23.0).

RESULTS AND DISCUSSION

Isolation fungi: Filamentous fungi were isolated from the upper surface of the sediments of the central marshes (Abu Zarak marsh) and (Abu- Subat marsh) at six stations. The total numbers of fungal genus in all samples with frequency of genus in a sediment are given in Table 1 showed that *Aspergillus niger* and *PenicIllium funiculosum* were more common fungi among other fungi isolated with high frequency (Table 2). The appearance of *A. niger* was 100% and of *PenicIllium funiculosum* 92%. However, Al-Jawhari (2015) showed that the appearance percent of *P. funiculosum* was 83% in Suq-Al Shuyukh marshes. The results in present study was similar to the findings of Al-Jawhari (2016) where appearance of *A. niger, P. funiculosum* was 100% in Abu- Subat marsh . The differences of stations in present study did not affect fungi diversity in sediments because the similarity of environmental such as temperature, dissolved oxygen, pH and organic carbon. Environmental factors such as temperature, dissolved oxygen, pH and organic carbon were measured in the study stations to determine the distribution of these fungi (Table 3).

Ability of isolated fungi to grow on solid medium supplemented with chrysene: The results indicated in Table (4) that the fungi grow well in the medium of PDA supplemented with Chrysene, as it was found that there is a daily increase in the colony diameters of the isolated fungi, and this indicates that these fungi can grow in the presence of the aromatic compound. The results of the study showed that measuring the average colony diameter of the fungus *A. niger*

Table 1. Total numbers of fungal genus in all samples with frequency to all genus in sediments

Genus of fungi	Total numbers of genus in all samples	Frequency (%)
Aspergillus	10^4×132	70
Penicillium	10^4×44	24

Total numbers of all fungi genus 176×10^4

Table 2. Frequency of species isolated from the upper surface of sediments

Fungi species	Numbers of fungal species appear	Frequency (%)
A. niger	48	100
P. funiculosum	44	92

on the fifth day of the experiment in the culture medium containing chrysene at a concentration of (60) ppm was (7.0) cm. While the diameter of the *A. niger* in the control on the fifth day was 8.5 cm. The results of the study also showed that measuring the average colony diameter of the fungus *P. funiculosum* on the fifth day of the experiment in the culture medium containing chrysene at concentrations of (60) ppm was (6.5) cm. While the diameter of the colony in the control on the fifth day was 8.5 cm. The results showed that both fungi were not affected by the presence chrysene. The results in present study was similar to the findings of (Al-Jawhari 2016) which showed that the appearance percent of *A. niger, P. funiculosum* reached to 100% in (Abu- Subat marsh).

Ability of isolated fungi to grow in Mineral salts medium supplemented with chrysene: Table 5 showed that the meycelial dry weight of the P*. funiculosum* reached to 4.2 when compared with control 4.5 and the meycelial dry weight of *A. niger* reached to 3.2 when compared with control 3.5 . The results showed that the pH in liquid medium inoculated with *A.niger* reached to 6.3 also showed that the pH to *P.funiculosum* reached to 6.3 (Table 6). This result was similar the findings of Al-Jawhari (2015). Many fungi can

Table 4. Ability the growth of *A. niger and P. funiculosum* in solid medium

Fungi	Colony diameter	Control
A. niger	7.0	8.5
P. funiculosum	6.5	8.5
L.S.D. (p=0.05)	1.54	

Table 5. Ability the growth of *A. niger and P. funiculosum* in mineral salt medium

Fungi	Dry weight	Control
A. niger	3.2	3.5
P. funiculosum	4.2	4.5
L.S.D. (p=0.05)	0.06	

Table 3. Environmental factors in Abu Zarak marsh and Abu Subat marsh

		Abu Zarak marsh				Abu Subat marsh			
Stations	Date	Temperature (C°)	O_2	pH	T°C	Temperature (C°)	O_2	pH	TOC
St1	August 2020	28*	6.8	8.1	1.24	31	5.7	7.80	1.88
	September 2020	28	7.9	7.95	1.16	28	6.3	8.00	1.59
	October 2020	25	7.2	8.45	1.3	23	7.5	8.33	1.22
	November 2020	23	8.7	8.3	1.3	21	7.2	8.33	1.96
	December 2020	17	8	8.4	1.12	17	8.8	8.42	1.92
	January 2021	16	9.5	8.45	1.28	16	9.2	8.45	2.21
	February 2021	17	10.2	8.55	1.27	17	8.1	8.38	2.35
	March 2021	21	9.8	8.4	1.4	22	7.9	8.20	2.49
St2	August 2020	29	6.7	8.2	1.78	32	5.9	7.95	1.20
	September 2020	27	8	8.25	1.59	28	5.5	8.10	2.55
	October 2020	25	8.5	8.46	1.09	23	6.9	8.29	2.21
	November 2020	22	8.8	8.48	1.3	21	7.5	8.33	2.92
	December 2020	17	9.2	8.5	1.2	16	9	8.41	2.96
	January 2021	16	9.6	8.55	1.05	16	9.2	8.55	2.34
	February 2021	16	10.5	8.4	1.15	17	8.4	8.48	2.86
	March 2021	21	10	8.38	1.65	22	7.9	8.41	2.89
St3	August 2020	28	6.6	7.7	1.34	31	5.9	7.95	2.17
	September 2020	28	6.4	7.92	1.31	28	6.6	8.20	2.38
	October 2020	24	7	8.1	2.03	23	6.8	8.25	2.63
	November 2020	21	7.5	8.39	2.13	21	7.3	8.33	2.75
	December 2020	18	8.5	8.4	1.8	16	9.4	8.42	2.79
	January 2021	16	9	8.46	1.08	15	9.4	8.50	2.33
	February 2021	17	9	8.39	1.2	16	8.8	8.43	2.54
	March 2021	22	9.2	8.33	1.45	22	7.7	8.39	1.65

(*) Non-significant

metabolize a wide range of pH. Microbial degradation produces organic acids and different compounds (Nwachukwu and Ugoji 1995).

Biodegradation of chrysene: The results of the current study showed the susceptibility of fungi isolated from the sediments of the marshes in the study area from the analysis of the polycyclic aromatic compound (chrysene) at a concentration of (60) ppm in the mineral salts medium. The Figure 1 and 2 show biodegradation of chrysene after 7 days incubation with A. *niger* and *P. funiculosum*. Many peaks were appearing when were compared with standard chrysene (Fig. 3). The disappearance of many peaks in the region 500-1500 and 2500-3000 where number of peaks disappeared and two new peaks appeared and the

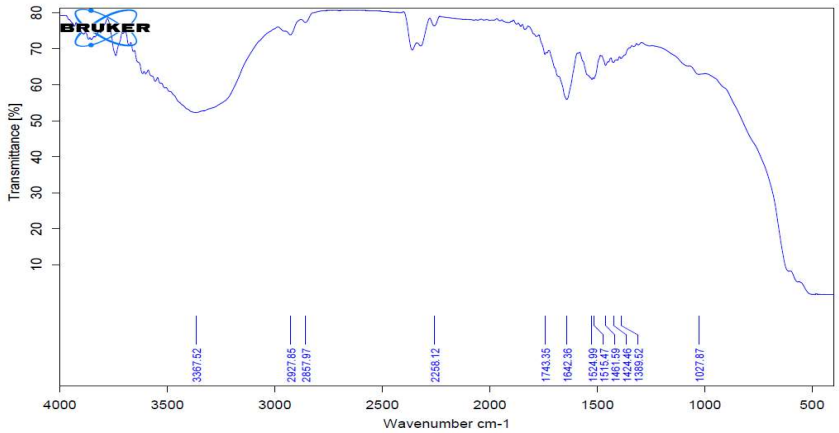

Fig. 1. Biodegradation of chrysene by *A. niger* after 7 days incubation

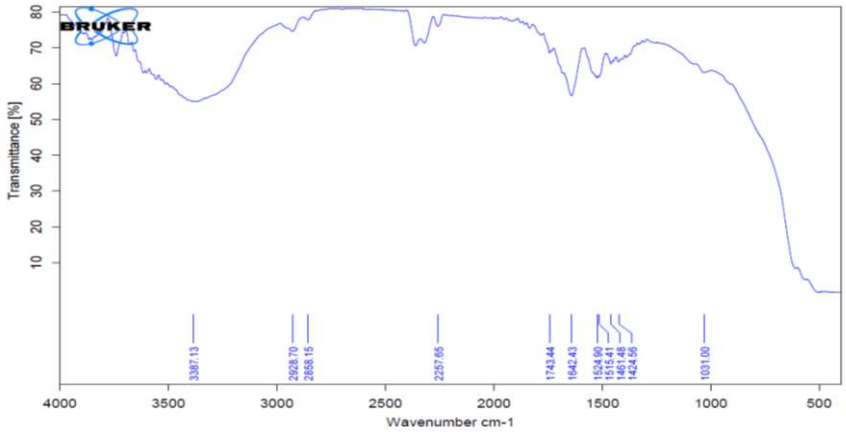

Fig. 2. Biodegradation of chrysene by *P . funiculosum* after 7 days incubation**.**

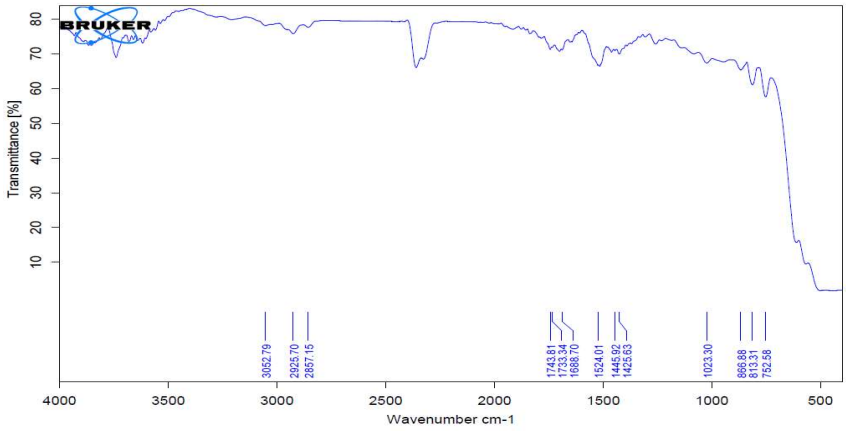

Fig. 3. Chrysene (Standard)

emergence of a wide band in the region 3000-3500 indicates the presence of an OH group. This means the presence of acid, and may be due to the fermentation of glucose sugar in the culture medium These results are in agreement with the findings Al-Jawhari (2016).

The results of the analysis with a gas chromatographic technique (GC-MS) showed the susceptibility of fungi isolated from the sediments of the marshes based on analysis of the aromatic compound (chrysene) at a concentration of 60 ppm in the mineral salts medium during 7 days of incubation and at temperature of 25°C. Figure 4, 5 showing that chrysene degradation when compared with standard chrysene. The concentration of chrysene reached to 0.03 ppm with culture of *A. niger*, and the removal percentage reached to 99.95%. In the same time the concentration of chrysene reached to 0.01 ppm with culture of *P. funiculosum* and the removal percentage reached to 99.98% (Fig. 6). All figures showing the chrysene degradation by *A. niger* and *P. funiculosum*, and chromatographic peaks were also observed. These new peaks suggest formation of degradation products and that chrysene transformed to anthracene, and phthalic acid and finally produced phenol (Fig. 7). In this study, the tested fungi showed their ability to remove the aromatic compound in different proportions within only 7 days. This study agrees with various studies that showed that many Ascomycota

Table 6. Variation in pH in mineral salts medium supplemented with chrysene

A. niger	*P. funiculosum*	Control
6.3*	6.3	7.95

(*) Non-significant

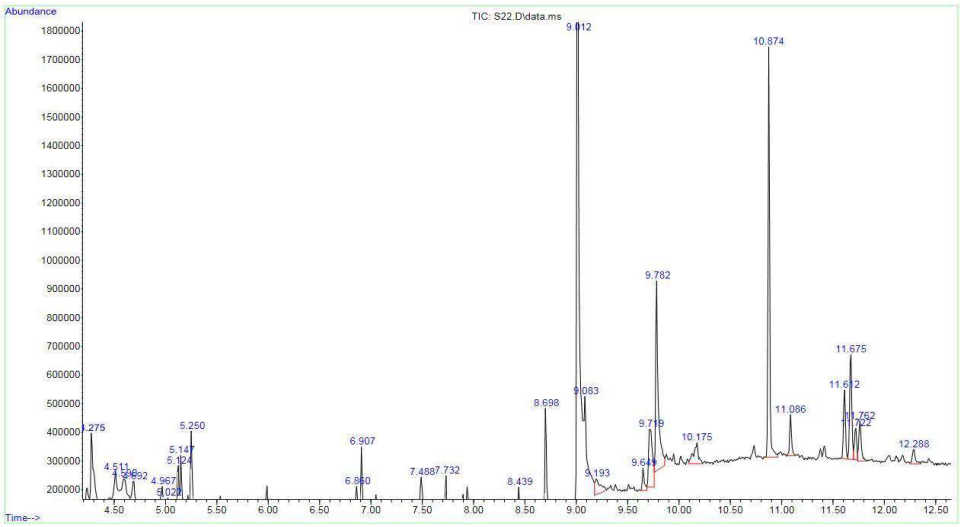

Fig. 4. GC- MS chromatogram of *A. niger* with chrysene treatment after 7 days incubation

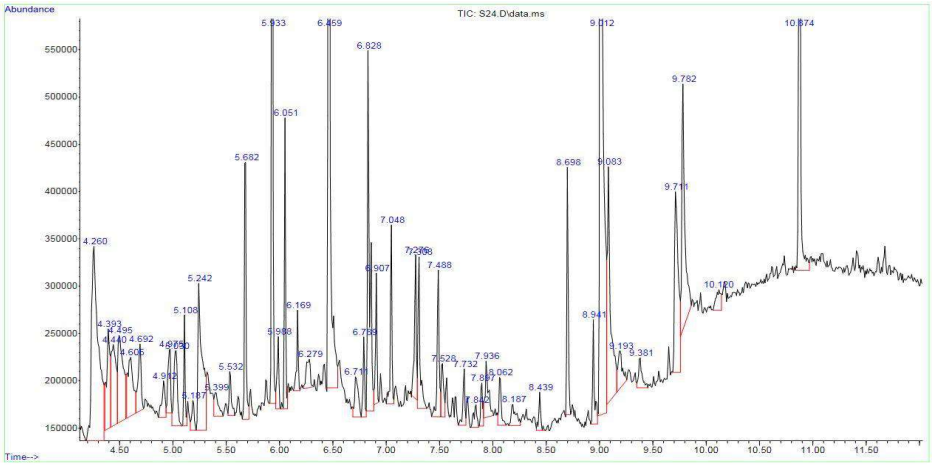

Fig. 5. GC-MS chromatogram of *P. funiculosum* with chrysene treatment after 7 days incubation

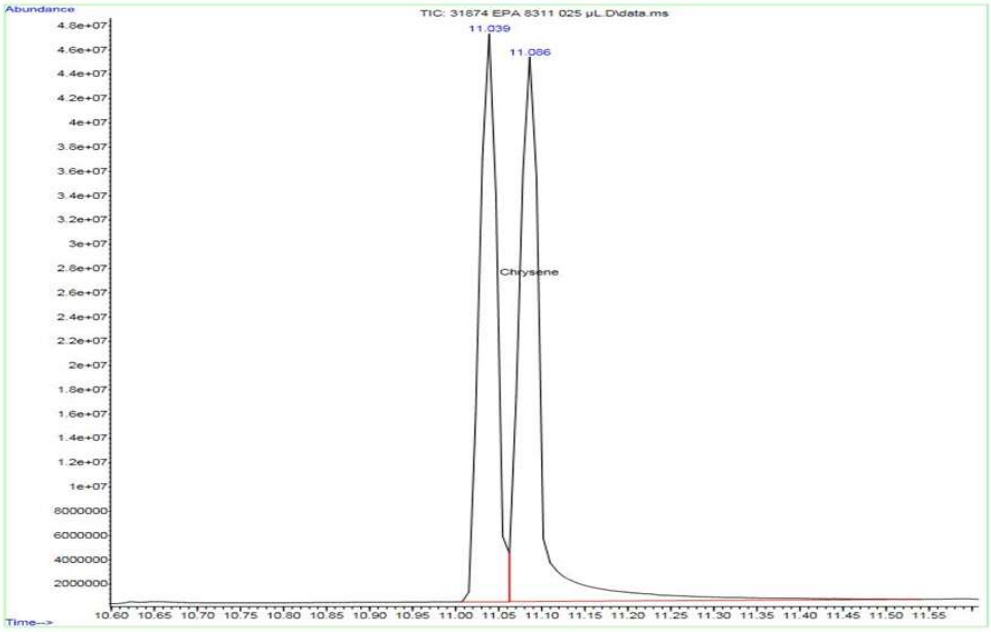

Fig. 6. Gc-MS chromatography showed standard chrysene (control)

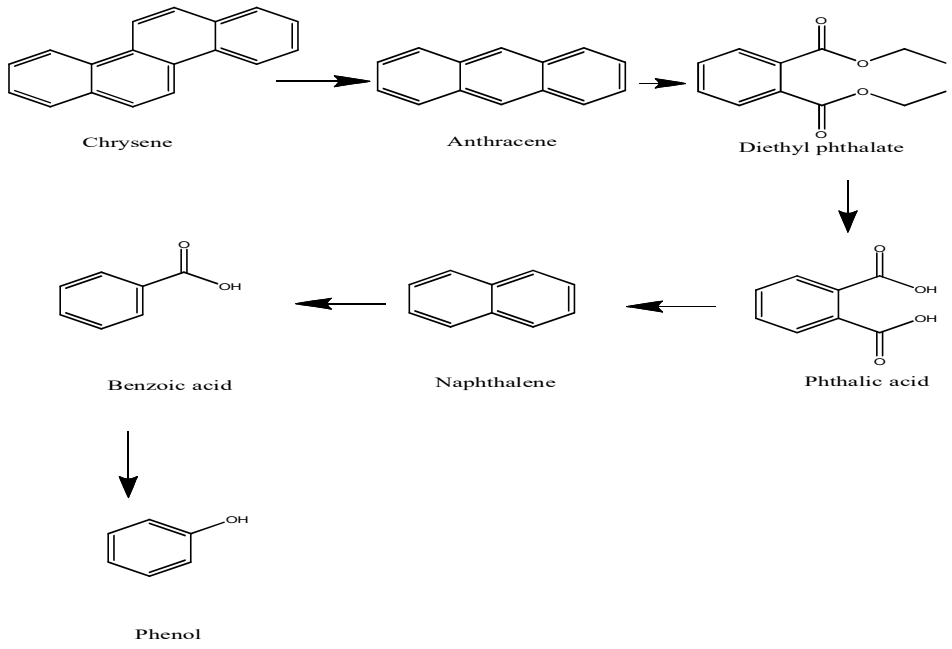

Fig. 7. Proposed pathway of chrysene degradation after 7 days incubation with *A. niger* and *P. funiculosum*

fungi, including *Penicillium*, *Aspergillus*, *Pseudallascheria* and *Fusarium* not only spread widely in contaminated areas, but it is also even capable of removing contaminants in soil contaminated by industrial spills or gas station tanks (Zafra et al 2014). This study also agrees with the study of Wu et al (2010) and Al-Jwhari (2016).

CONCLUSIONS

Biodegradation of chrysene by *A. niger* and *P. funiculosum* after 7 days incubation in mineral salts medium and the concentration of chrysene reached to 0.03 ppm with culture of *A. niger*, and the removal percentage reached to 99.95%, in the same time the concentration of chrysene reached to 0.01 ppm with culture of *P. funiculosum* and the removal percentage reached to 99.98%. The differences of

stations in present study did not affect in fungi diversity in sediments because the of similar environmental factors .These data in the present study was advanced our knowledge of polyaromatic hydrocarbons and behavior of fungi in polluted marshes in different location, and how these fungi breakdown or biodegradation of pollutants in environment and can use these organisms to removal pollution now and in future.

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Molecular Investigation of Infectious Bursal Disease Virus in Iraqi Poultry Farms

Azhar Hamza Hussein and Aida Bara Allawe

Veterinary Microbiology Department, Veterinary College, Baghdad University, Iraq
E-mail: azharhamza171@gmail.com

Abstract: In this study 250 bursa of fabricius samples were collected from 50 infected flocks from different areas in Iraq .The age of infected chicken was 20-35 days . Real time Rt-PCR was used to detected IBDV in infected collected samples by amplification of viral protein2 (VP2). The results of real time rt-PCR revealed that 36 samples out of 50 samples from infected farms were positive with classical IBDV. However 14 farms were positive with vvIBDV. Real time RT-PCR revealed that 7 farms had mixed infected with vvIBDV and clIBDV. Collected samples were sent to Korea for sequencing of VP1 gene and VP2 gene. The phylogenetic tree analysis showed that the VP2 analysis of Iraqi IBDV was similar to that of Jordan strain with 98.3% identity and VP1 gene analysis was similar to Jordan strain with 99.1 identity . Five positive samples by real time RT-PCR were used for isolation of the virus in chicken embryos after preparation of samples were inoculated chorioallantoic membrane (CAM) of chicken embryos. After 96hr the chorioallantoic membrane and allantoic fluid were harvested. Real time RT-PCR was used to detect IBDV in harvested chorioallantoic membrane and allantoic fluid.

Keywords**:** Infectious bursal disease, vvIBD, clIBD, RT-PCR

Infectious bursal disease (IBD) is an acute and highly contagious viral disease infected poultry consider immunosuppressive disease of young chickens and cause severe economic losses for the poultry industry in world (Caston et al 2008, Dey et al 2019). Infectious bursal disease virus (IBDV), member of the genus Avibirnavirus in family Birnaviridae, is a non-enveloped, icosahedral virus with diameter 60-65 nm. There are two serotypes of IBDV, namely serotypes 1 and 2; the strains of serotype 1 is consider as classical virulent IBDV (cvIBDV), antigenic variant IBDV (avIBDV),(atIBDV), and very virulent IBDV (Le et al 2019). The disease was first discovered in Gumboro, Delaware in 1962, is usually sub-clinical in birds less than two weeks of age and clinical disease is generally observed in birds over two weeks of age. It is economically important to the poultry industry worldwide due to increased susceptibility to other disease and negative interference with effective vaccination (Adamu et al 2013). There are two forms of disease the first form didn't produce clinical signs with severe immune-suppression and infected chicks less than three weeks of age (Lukert and Saif 2015). The second form of the disease characterized by sudden onset with short incubation period and mainly affected (3-6) week old chicken and in the same time this form of disease produce high morbidity and mortality may be arrived to 100% depending on the pathogenicity of the virus and the susceptibility of the flock depended to the pathogenicity of the infected virus along with susceptibility and immune status of the flock (Lukert and Saif 2015, Jackwood et al 2018). The aim of this study is conventional and molecular detection of IBDV isolates in Iraqi farms.

MATERIAL AND METHODS

The 250 samples were collected from different areas of Iraqi and (9-11) days old chicken embryos were used for virus isolation from Barakat-Alhusian hatcher. High pure viral nucleic acid and purification kit (Roche /USA) commercial kit was used for obtaining complementary DNA(cDNA) from total RNA. The oligonucleotides sequence primers (AlphaDNA /Canada) are listed in Table 1. The sequencing service was provided from macrogene Laboratories/ Korea, the microcentrifuge tubes containing the PCR products and corresponding primers (17 Picomoles) were transferred in cold chain.

Samples collection: Between December 2020 to July 2021, a total of 250 samples (*Bursal fabricius*) were collected from 50 poultry farms (broilers and layers) suffer from high morbidity with mortality of 20-70% from Iraq which include 5 cities (Karbala, Al-Najaf, Babylon, Al-Qadyssia and Baghdad), Fifty pooled tissue samples were collected from fifty vaccinated broilers and layers flocks. These flocks suffered from sudden death and sudden increase mortality with dehydration, cloacae pecking, ruffled feathers and white watery diarrhea with high morbidity. Disease birds were subjected to necropsy and tissue samples were collected including *Bursa fabricius*. These samples were stored in labeled falcons and transported immediately with ice to the

laboratory and stored at -80°C until processing and virus isolation.

RNA extraction: The RNA was extracted and purified according to the instructions of High Pure Viral RNA extraction kit, ROCHE

Reverse transcription: Reverse the transcription process was performed according to the method reported by the manufacturer company. For this purpose the following procedure were performed.

- Before use, all frozen materials were thawed and briefly centrifuged, 9.4 µl of total RNA and 2 µl of random hexamer for target RNA primer mix for reaction primer were used in single tube.
- The target-primer was heated in a thermal block at 65°C for 10 minutes.
- Denaturation of the mixture was achieved. The tube was quickly cooled on ice. Then, 4 µl of THF reverse transcriptase was added per reaction to the tubes containing the target primer mix.
- The reaction buffer, 0.5 µl protector RNase inhibitor, 2 µl deoxyribonucleotide mix, RT mixture containing 1µl of DTT and 1.1 µl THF reverse transcriptase were added.
- The tubes thermal was placed in the block. Then it was incubated at 55 0C for 30 minutes. THF reverse transcriptase was inactivated by heating at 850C for 5 minutes. The reaction was stopped by placing the tubes on ice. In hand until the extracted cDNAs are used in subsequent real time PCR steps.

Amplification of nucleic acid by RT -PCR: Amplification of cDNAs by real time PCR was used and the amplification procedure was done according to the manufacture company.

- 10 µl TaqMan® Fast Advanced Master Mix (2X)
- For each sample,1.0 µl of TaqMan® Assay primer/probe (20X)
- 2.0 µl of cDNA template.
- 7.0 µl of nuclease free water
- Pre-incubation at 95°C for 10 min.

This was followed by amplification of cDNA in real-time - thermocycler and start the program as in Table 2.

Sequencing: Sequencing was performed by Sanger methods with a fully automatic DNA sequencing device and the results were obtained through the virtual laboratory on the website.

Phylogenetic analysis: All nucleotides and amino acid sequences were compared between strain isolated and selected strains representing established IBDV genotypes were performed using the software ApE- Aplasmid Editor Program version 2 .0.51. (Thompson et al 1994). Phylogenetic analysis was accomplish using the MEGEX software (Version, 6.0.6) (Tamura et al 2013). The sequences was differentiated against IBDV around the world, complete and near complete reference genomes sequences of virus strains obtainable and analyzed via BLAST search(http://blast.ncbi.nlm.nih.gov/) on the GenBank database.

Virus isolation: Virus isolation was performed according to the protocol adopted by OIE (2012). One gram of positive tissue samples (*Bursa fabricius*) from each flock were grinding in an autoclaved mortar and pestle with sterile phosphate buffer saline pH (7.0–7.4). Suspensions of organs were first centrifuged in a cooling centrifuge at 3000 rpm for 15 min at 4°C, The 10-11 days old embryonated eggs was marked with suitable site of chorioallantoic inoculation on the egg shell and 0.2 ml (200µl) of the supernatant was inoculated into the chorioallantoic membrane of (10-11) days-old embryonated chicken eggs . The negative control was inoculated with 0.2µl PBS only. The puncture hole in the egg was sealed by melted paraffin and incubated at 37°C for 4 days with daily checking. Deaths on the first 24hr post inoculation (PI) were considered nonspecific death and neglected. After 96hr the chorioallantoic and allantoic fluid was collected and keep in refrigerator at 4°C with a sterile syringe and centrifuged at 3000rpm for 3 minutes to remove blood and tissues debris and stored in sterile screw-capped vials at (-20 or - 80°C) till further detection by RT-PCR again.

RESULT AND DISCUSSION

Prevalence of IBD virus among Iraqi cities: A total of 250 samples from 50 poultry farm in different Iraqi cities were observed for bursal disease virus. The 36 of 50 were positive for IBDV (72%). Overall, only 22 (44 were positive for Classical IBDV, However, 14 (28 %) had at least one flock positive for vvIBDV, on the other hand , (14%) farms have

Table 1. Oligonucleotide sequences primers with probes for detection of IBD virus (Tomás et al 2012)

Primer	Sequence (5´→ 3´)
Forward primer	5'-GAGCCTTCTGATGCCAACAAC-3'
Reverse primer	5'-TCAAATTGTAGGTCGAGGTCTC TGA-3'
Forward primer	5'-GGGACAGGCCGTCAAGGC-3'
Reverse primer	5'-ATTTTGTCGTTGATGTTGGCTGTTG-3'
Probe for vvIBDV	FAM-5'-ACACCCTAGAGAAGC-3'-MGB
Probe for NovvIBD	VIC-5'-ACACCCTGGAGAAGC-3'-MGB

Table 2. PCR amplification program for Vp2 gene detection

Step	Temperature (C˚)	Time	No. of cycle
Initial denaturation	95	10 min.	1
Denaturation	95	20 sec.	45
Annealing and extension	60	30 sec.	

been recorded mixed virus infection vvIBDV and cIBDV There are no viral infections IBD in the rest of the fields (28 %) (Fig. 1). The current study also aimed to diagnosis the virus at the level of the governorates of Iraq. It was more prevalence in Babylon and Karbala city (25 and 22.22% respectively). The lowest infection of IBDV in Baghdad and Al-Qadissyia (16.66%) (Table 3).

Clinical and pathological observation: All samples were collected from different poultry flocks from infected farm were observed. Clinically, characterized by short incubation period of disease arrived to 5-7 days, vent picking, white to cream watery diarrhea, anorexia, prostration, ruffled feather, dehydration and finally, subnormal temperature and death. These infected flocks grossly characterized by severe obvious hemorrhagic lesion in the *bursa fabricius* , poetical hemorrhage in thigh and in some cases enlargement with consolidation with double size than normal in the bursa of fabricius , sever congestion with hemorrhagic bursa of fabricius, contain series to caseous materials due to inflammation of bursa (Fig. 2). This clinical signs and gross lesion also were also observed by earlier researchers (Mutinda et al 2015, Al-Gubori et al 2020).

Real time - PCR for detection of IBDV: Real time PCR was used to detected IBDV in 36 samples out of 50 samples from different areas in Iraq. Al-Gburi et al (2020) observed

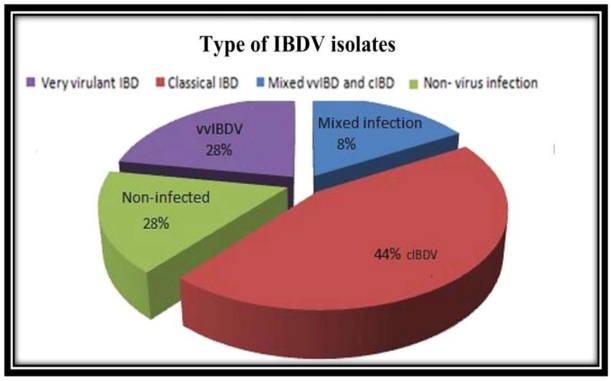

Fig. 1. Types of IBDV isolates according to the study areas

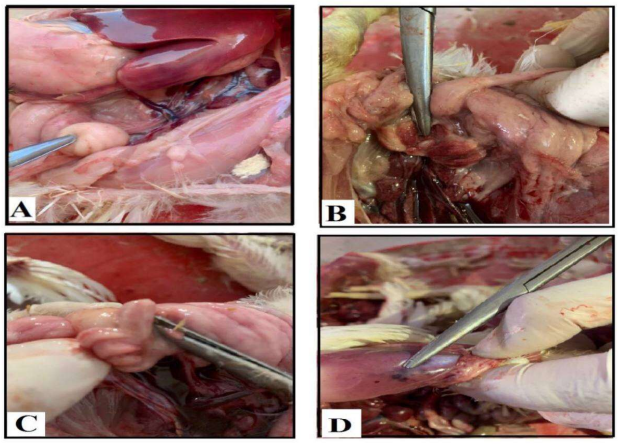

Fig. 2. A-enlargement with consolidation with double size than normal in the bursa of fabricius due to IBDV infection B- sever congestion with hemorrhagic bursa of fabricius due to IBDV infection, C- bursa of fabricius contain series to caseous materials due to inflammation of bursa D- petechial hemorrhage in thigh due to IBDV infection

Table 3. Distribution of IBDV among Iraqi cities

Province	Number farm	Positive infected farm	Type of strain	Number
Baghdad	10	6 (16.66%)	cIBDV	3
			Mixed	2
			vvIBDV	3
Babylon	10	9 (25%)	cIBDV	5
			Mixed	3
			vvIBDV	4
Al-Qadissyia	10	6 (16.66%)	cIBDV	4
			Mixed	2
			vvIBDV	2
AL-Najaf	10	7 (19.44%)	cIBDV	5
			Mixed	0
			vvIBDV	2
Karbala	10	8 (22.22%)	cIBDV	5
			Mixed	1
			vvIBDV	3
Total	50 farm	36 farm	-	44

prevalence of vvIBDV in 30% from chicken in Iraq. The study also proved this technique is important for diagnosis vvIBDV and cIBDV strains.

There are various diagnostic tests for IBDV enzyme-linked immunosorbent assay, monoclonal antibody test, virus neutralization test, virus isolation, electron microscopy, immunodiffusion, Agar gel deposition and immunofluorescence assay. Some indicated that all these tests have disadvantages, such as labor-intensive, expensive, time consuming, indefinite, or insensitive. More importantly, these methods lack the ability to detect low-level IBDV antigens organize. Therefore, in order to control the disease, fast, specific and sensitive methods, such as RRT-PCR for determination of IBDV are absolutely essential (Thenamutha et al 2017).

Sequencing of IBD: Real time PCR products for four samples were sent to the NICEM company in South Korea to perform partial VP1 and VP2 -gene nucleotide sequencing and these sequences reacted twice with either forward or revers primers within selected of major capsid protein VP1 and VP2 region. All isolated strains showed positive results in sequencing. The result of sequencing of four positive samples by real time PCR revealed that the detected IBDV in Iraqi farms was positive and similar to Jordan strain by sequencing of VP1 and VP2 protein (Table 4).

Phylogenic tree: The results of partial nucleotides sequences of the selected strain VP1 and VP2 gene were edited and verified using ApE-Aplasmid Editor Program version 2 .0.51. The selected sequences were compiled and compared with the different IBD virus nucleotides sequences data base around the globe in the GenBank NCBI. All reported sequences were aligned with present sequences in this study. The tree was constructed using the Maximum Likelihood method in MEGA X. The phylogenetic analysis of partial sequences of the isolated strains Vp2 gene and VP1

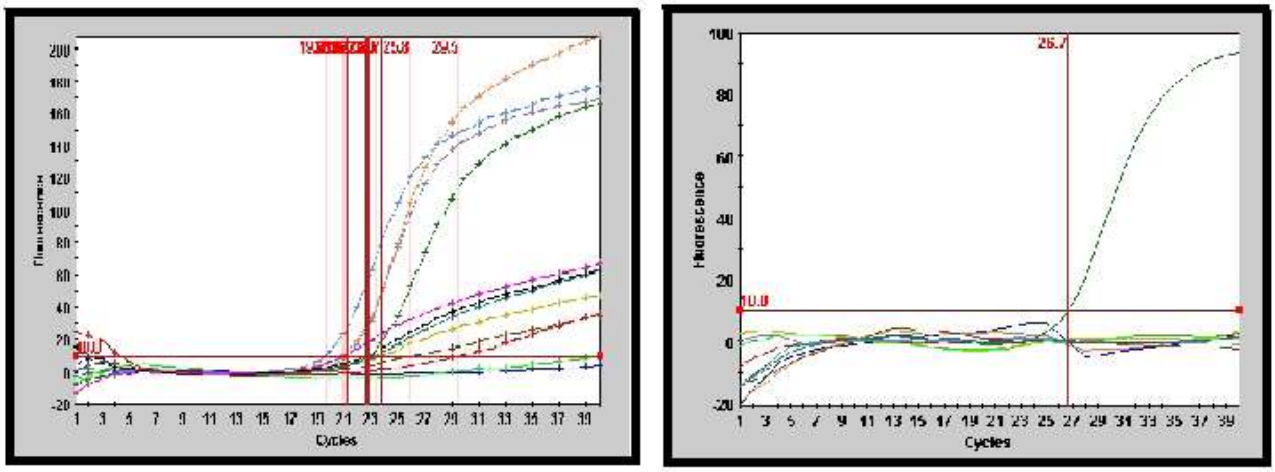

Fig. 3. Amplification plots of real time PCR showed positive samples of RT-PCR with amplification of VP2 gene with samples of IBDV for collected samples

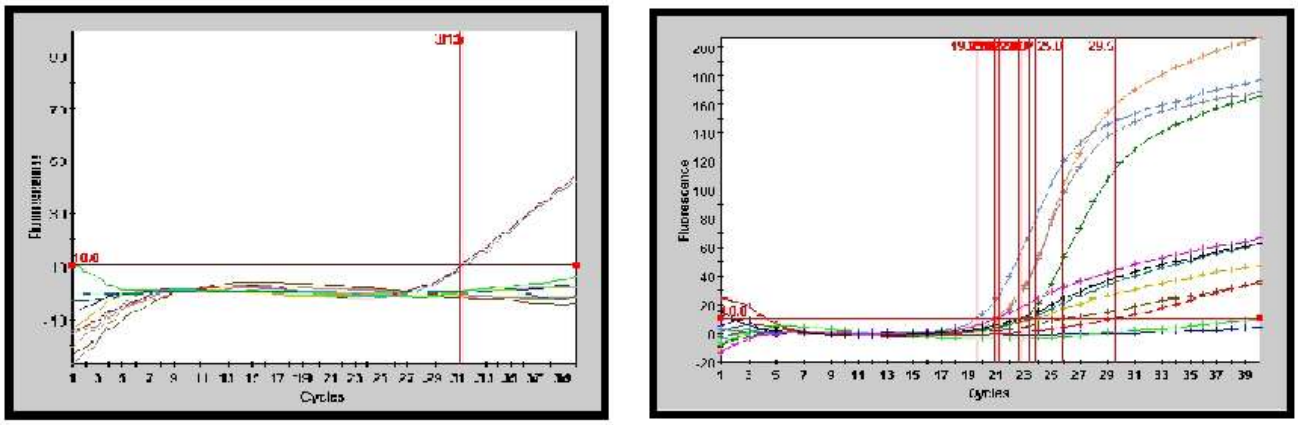

Fig. 4. Amplification plots of real time PCR showed positive samples of RT-PCR with amplification of VP2 gene with positive samples of IBDV for collected samples

gene showed isolated viruses after comparison with known vaccine and field strains the isolated RNA from sample A2110024.001 and most related to isolate 710_Jordan (MF142560.1) and to isolate 276_Jordan (MF142517.1) (each 98,3%). The clusters were with genogroup A3 ("very virulent") according to Islam et al (2021) (Fig. 6). The sequencing analysis of IBDV isolates are closely related / similar to Jordanian strains. The reason behind that is Iraq and Jordan is neighbors countries and birds can easily cross between them as well as vehicles that act as vector to the virus.

Virus isolation from chicken embryos inoculation: Five positive samples by real time RT PCR inoculated with two passage into the chorioallantoic membrane of three embryonated eggs in 10-11 days old. The result revealed that the first passage did not show any pathological lesion effect in the embryos compared with control embryo, but the second passage showed death of embryos with congestion of embryonic chorioallantoic membrane. This indicated that

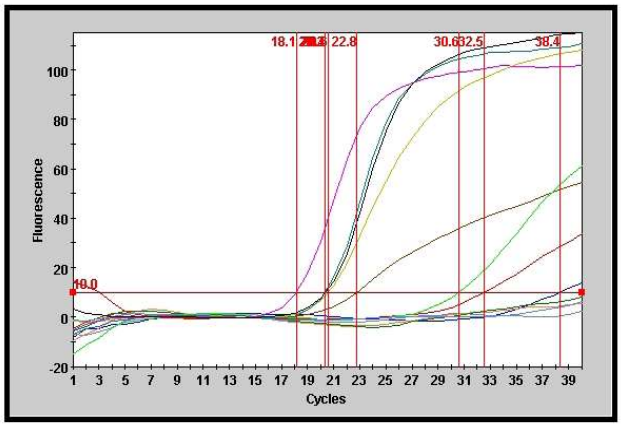

Fig. 5. Amplification plots of Real Time PCR showed positive samples of Real Time PCR with amplification of VP2 gene with positive samples of IBDV for collected samples

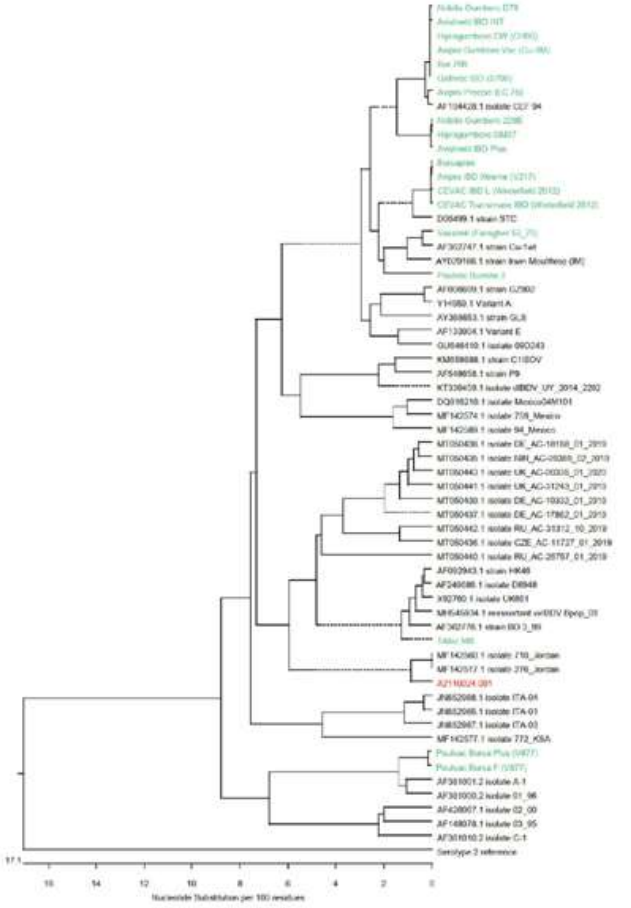

Fig. 6. Phylogenetic tree of nucleotides sequences of the major capsid protein VP1 and VP2 gene of IBDV isolates

Table 4. Nucleotide sequencing for IBDV isolates

Major capsid protein	Sequencing
VP1	GTCTGGGTGCCACCTGAAGATCCACTGGCCAGTCCTAGTCGACTAGCAAAGTTCCTCAGAGAGAACGGCTA CAAAGTCCTGCAGCCACGGTCTCTGCCCGAGAATGAGGAGTATGAGACCGACCAGATACTTCCAGACCTAG CATGGATGCGGCAGATAGAGGGGGCTGTTTTAAAGCCTACTCTATCTCTCCCCATTGGAGACCAGGAATACT TCCCAAAGTATTACCCAACTCACCGCCCTAGCAAGGAGAAGCCCAATGCGTACCCGCCAGACATTGCATTAC TCAAGCAGATGATCTACCTGTTTCTCCAGGTTCCAGAGGCCGACGAAGGCCTAAAGGACGAGGTTACCCTC TTGACTCAAAATATAAGAGACAAGGCCTATGGAAGTGGGACCTACATGGGACAAGCAACTCGGCTTGTTGCC ATGAAAGAGGTTGCCACCGGAAGAAACCCAAACAAGGATCCTCTAAAACTTGGGTACACTTTTGAGAGTATT GCACAGCTGCTTGACATCACTCTACCGGTAGGCCCACCCGGTGAGGATGAAAAGCCCTGGGTACCACTCAC AAGAGTGCCGTCGAGGATGCTAGTGTTGACGGGAGACGTAGATGGGGACTTTGAGGTTGAAGACTACCTTC CCAAAATCAACCTCAAGT
VP2	GCAACAGCCAACATCAACGACAAAATCGGGAACGTCCTAGTAGGGGAAGGAGTGACCGTCCTCAGCTTACC CACATCATATGATCTTGGGTATGTGAGACTCGGTGACCCCATTCCTGCTATAGGGCTCGACCCAAAAATGGTA GCAACATGTGACAGTAGTGACAGGCCCAGAGTTTACACCATAACTGCAGCCAATGATTACCAATTCTCATCAC AGTACCAAGCAGGCGGAGTAACAATAACACTGTTCTCAGCCAATATTGATGCCATCACAAGTCTCAGCATTGG GGGAGAGCTCGTGTTTCAAACAAGTGTCCAAGACCTTATACTGGGCGCTACCATCTACCTTATAGGCTTTGAT GGGGCTGCGGTAATCACCAGAGCTGTAGCCGCAAACAATGGGCTAACGGCCGGCACTGACAACCTTATGCC ATTCAATATTGTGATTCCGACCAGTGAGATAACCCAGCCAATTACATCCATCAAACTGGAGATAGTAACCTCCA AAAGTGGTGGTCAGGCGGGGGATCAGATGTCATGGTCAGCTAGTGGGAGCCTAGCAGTGACGATCCACGG TGGCAACTATCCAGGGGCCCTCCGTCCCGTCACACTAGTAGCCTACGAACGAGTGGCAACAGGATCTGTCG TTACGGTCGCT

Fig. 7. Choiroallantoic membrane of inoculated eggs with IBDV with congested chorioallantoic membrane

Fig. 8. Infected embryo with IBDV, congestion with dwarfing embryos

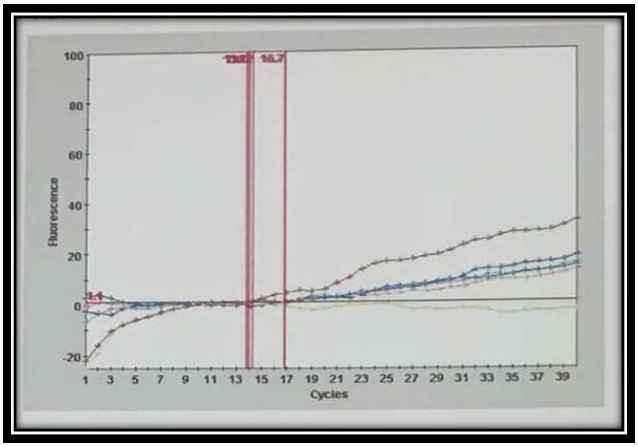

Fig. 9. Amplification plots of real time PCR revealed positive IBDV in chorioallantoic membrane and allantoic membrane against cycle number, This showed positive samples level of signal that reflects a statistically significant increase over the calculated baseline signal with different color, and some of them not above ct (Threshold) probes as negative result

IBDV was replicated in the chorioallantoic membrane of chicken embryo. (Mutinda et al (2015), Al-Zuhair et al (2016) and (Al-Gburi et al (2020), also observed the dwarfing and death of embryos, congestion and hemorrhage of chorioallantoic membrane after inoculation of chicken embryos with IBDV and embryonated chickens eggs supported the growth of IBDV and the virus can produce the pathological lesion (Fig. 7, 8). The PCR technique was performed on six samples and gave positive result for IBDV in allenoic fluid and chorioallantoic membrane (Fig. 9). Wang et al (2009) also observed that RT-PCR approach provided a powerful diagnostic tool with highly specificity and sensitivity for identification and quantification of IBDV.

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Protective Role of Aqueous Extract of *Malva parviflora* Seeds against Cholesterol-induced Hyperlipidemia in Male Albino Rats

Rania Nazim Sobhi and Qasim Azeez Razooqi

College of Education, Department of Life Sciences, Tikrit University, Iraq
E-mail: Qasimrazooqe80@yahoo.com

Abstract: The current study aimed detemine protective effect of the aqueous extract seeds of *Malva parviflora* in reducing cholesterol-induced oxidative stress and comparing its effect with rosuvastatin on some physiological and biochemical parameters in the serum of white male rats. It included estimation of lipid profile levels (cholesterol, triglycerides, VLDL-C, LDL-C, HDL-C) and leptin level. Cholesterol was given with a natural diet for 6 weeks. In the group treated with a diet rich in cholesterol (1 g/kg) showed a significant increase of in the concentration of CHO, LDL-c, VLDL-c and a significant decrease in HD and significantly high leptin hormone, specifically in muscle layer. In the group dosed with a diet rich in cholesterol (1 g/kg) and aqueous extract of *M. parviflora* seeds together, and the group dosed with a diet rich in cholesterol (1 g/kg) and rosuvastatin together showed a significant decrease in CHO,LDL, VLD and significant increase in concentration of HDL and a significant decrease in leptin hormone. The histological study indicated rupture in the layers of the aorta wall in the infective control group and the layers of the aorta appeared naturally in aqueous extract of *M. parviflora* seeds.

Keywords**:** Aqueous extract, *Malva parviflora*, Cholesterol-induced hyperlipidemia, Rat

Hyperlipidemia is a variety of disorders characterized by elevated levels of plasma lipids and lipoprotein molecules or lipoproteins, and an abnormal increase in the level of lipoproteins that transport lipids in the blood (Machaba 2014) and is important risk factor for predicting atherosclerosis, coronary artery diseases and cerebral vascular diseases (Chu et al 2018). These diseases are important cause of death in the world (WHO 2017). Medicinal plants play a very active role in traditional medicines for the treatment of various diseases (Mohammad Saleem et al 2008). *Malva parviflora* is a common plant in Asia and is an annual herbaceous plant belonging to the family Malvaceae. Plants of this family make a significant contribution in the treatment of cough, sore throat and other bronchial problems, as well as stomach and intestinal irritation. Flowers and leaves are emollient for the digestive system. It is also used to reduce swelling and extract toxins and when combined with eucalyptus and is also good remedy for cough and other chest ailments (Bhat et al 2020).

MATERIAL AND METHODS

In this study, 20 white male Sprague Dawley rats, aged (18-16 weeks), obtained from the College of Veterinary Medicine, Tikrit University and weight ranged between 150-200 g. They were bred in animal house of the College of Veterinary Medicine, Tikrit University. The experiment lasted for six weeks, starting on September 28, 2020 to November 12, 2020**.** The groups were divided into four groups, each group consisting of 5 rats, the control group, the hyperlipidemic group fed on diet rich in fat (1% g/kg), hyperlipidemic group with aqueous extract of *M.parviflora* seeds and rosuvastatin.

Plant extraction: 25 gm of dried and finely ground *M. parviflora* seeds were soaked in 250 ml of distilled water, then the mixture was placed on magnetic stirrer at a speed of 70 for 24 hour sand beaker was covered with a black (opaque) lid. The nozzle was closed to prevent contamination. The next day the process of filtering the solution was carried out by several layers of medical gauze and filtrate was placed in Petri dishes. It was placed in the electric oven at a temperature of 40 degrees Celsius. After 24 hours extract was obtained by scraping, collecting from the dishes and placing in opaque black glass bottles and kept in the refrigerator.

Determination of cholesterol concentration in blood serum: The estimation of cholesterol concentration in blood serum was done by using the enzymatic method, using ready-made analysis kit from Biolabo company, manufactured in France (Tietz 1999). The concentration of HDL-C in serum was determined by using the ready-made HDL-C assay kit supplied by Biolabo company, manufactured in France, (Badimon et al 1990)

Determination of VLDL–C concentration in blood serum: The very low-density lipoprotein cholesterol concentration was calculated according to the relationship:VLDL-C conc.(mg/dL)= TGs (mg/dL)/5**(**Burtis and Ashwood 1999).

Determination of LDL-C concentration in blood serum: The concentration of low-density lipoproteins for cholesterol was calculated (Andreoli et al 2001).

LDL-C Conc. (mg/dL) = Total cholesterol – (HDL + VLDL.)

Determination of leptin concentration in serum:The leptin concentration was estimated by assay kit supplied by Shanghi Company of China by ELISA technique(Finck 2000).

RESULTS AND DISCUSSION

There significant increase in the concentration of CHO, LDL, VLDL was in the group that was fed a diet rich in cholesterol (Table 1) as compared with the control group. The group that was fed on diet rich in cholesterol and *M. parviflora* seed extract, and the group that was fed a diet rich in cholesterol and rosuvastatin significantly decreased (CHO, LDL, VLDL). There was significant decrease in the level of high-density lipoprotein HDL-c in the group that was fed a diet rich in cholesterol when compared with the control group. In the group that was fed a diet rich in cholesterol and aqueous extract of the seeds of *M. parviflora*, and the group that was fed a diet rich in cholesterol and rosuvastatin significant increase in of high-density lipoprotein HDL-c was observed when compared with the infected control group. There was significant increase in the level of leptin in the group that was fed a diet rich in cholesterol (1 % g/kg) when compared with the control group (Table 2). In group fed a diet rich in cholesterol, aqueous extract of *M. parviflora,* and the group that was fed a diet rich in cholesterol and rosuvastatin significantly decreased was observed when compared to the infected control group.

There was significant increase in the level of cholesterol, triglycerides, LDL and VLDL and a significant decrease in HDL in the group treated with a diet rich in cholesterol. This is consistent with the study of Khan and Makki (2017). The cause of high cholesterol concentration is due to a defect in the lipid metabolism process, or a defect in the absorption and excretion of steroids, or perhaps due to a decrease in the concentration of bile salts (Dudzik et al 2018). Fat oxidation increases the possibility of atherosclerotic disease formation by 100% by increasing the oxidation of lipids in c-LDL particles and chylomicron remnants and decreasing the level of antioxidants (Delgado et al 2021). The aqueous extract of baker's seed curb free radicals caused by oxidation of fats and play effective role in reducing lipid levels and other metabolic processes because it contains flavonoids that have strong antioxidant properties. This has the ability to neutralize free radicals and prevent cell damage due to them (Amawi et al 2017). The *M. parviflora* plant contains polyunsaturated fatty acids (PUFAs), such as linoleic acid (di-unsaturated) and linoleic acid (tri-unsaturated (Zoufan et al 2020). These acids play a major role in reducing triglyceride levels. In the blood plasma the concentration of high-density lipoprotein HDL was increased, the concentration of low-density lipoprotein LDL (LDL) and very low-density lipoprotein (VLDL) was decreased (Garelnabi et al., 2017) as well as a decrease in the level of peroxides in the liver. This agree with Gutiérrez (2017). The *M. parviflora* plant contains rosmarinic acid and is one of the highly effective phenolic compounds in treating atherosclerotic diseases and heart

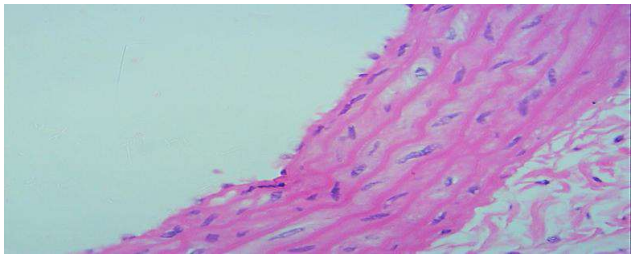

Plate 1. Cross-section of the aorta of the cholesterol-treated group showing a bulge (Vg) in the tunica mediastinum region. H & E 400X

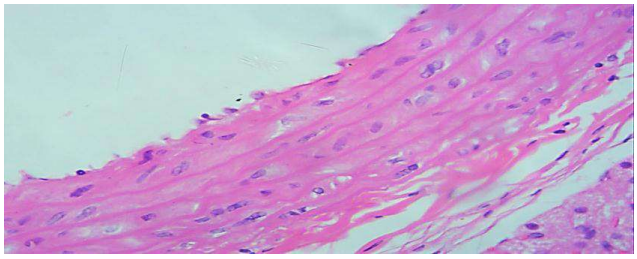

Plate 2. Cross - section of aorta of group treated with cholesterol and baker's seed extract showing the endothelial tunica (TE) and muscular tunica media (TM) containing smooth muscle fibers and adventitial tunica (TA) normally. H & E 400X

Table 1. Level of cholesterol, LDL-C, VLDL-C, HDL-C and leptin

Groups	CHO	LDL-C	VLDL-C	HDL-C	LEPTIN
Control	63.2	13.0	12.60	40.0	415.6
Hyperlipidemia	131.6	38.60	15.8	34.20	829.5
Hyperlipidemia +*M. parviflora*	88.20	32.50	9.20	41.50	478.6
Hyperlipidemia + rosuvastatin	83.25	34.7	13.25	37.50	603.0

diseases through ability to scavenge free radicals, prevent oxidative stress and reduce fat oxidation (Cani 2018). There is evidence to support that lowering LDL-C reduces cardiovascular disease and atherosclerosis, and are the most widely prescribed drugs for lowering LDL-C and reducing cardiovascular disease and mortality through their anti-inflammatory effects (Ference et al 2017). The mechanism of action is by blocking the active site of the first and major enzyme (HMG-CoA reductase) in the mevalonate pathway (Purohit et al 2012). Inhibition of this site prevents substrate access and thus prevents the conversion of HMG-CoA to mevalonic acid in the liver from the bloodstream and the subsequent reduction of LDL levels in the blood plasma by 20 to 50% (Ward et al 2019).

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Antibacterial Activity of Ciprofloxacin against Susceptible and Resistant Isolates of *Klebsiella pneumoniae* and *Enterococcus faecalis*

Mahmood Shaker and Ahmed N. Abedsalih

Department of Physiology, Biochemistry and Pharmacology
College of Veterinary Medicine,University of Baghdad, Iraq
E-mail: mahmoodshakir507@gmail.com

Abstract: This study was conducted to investigate the antibacterial activity of ciprofloxacin against isolates of *Enterococcus faecalis* and *Klebsiella pneumoniae* and resistance emerging in those susceptible after exposure to sub-MIC *in vitro*. e MIC of ciprofloxacin and inhibition zone was determined, then the susceptible isolates were exposed to sub-MIC continuously for 21 days. The results showed that the MIC were 0.25, 0.45, 28.80 and 11.20 µg/ml for susceptible *Enterococcus faecalis* and *Klebsiella pneumoniae*; resistant *Enterococcus faecalis* and *Klebsiella pneumoniae,* respectively. The inhibition zones were (7.20, 23.20 and 26.40 mm for 0.5 MIC, 1 MIC and 2 MIC of ciprofloxacin against susceptible *Klebsiella pneumoniae* with significant difference (between all concentrations. In the resistant *Klebsiella pneumoniae* the zones of inhibition were 12.00, 14.80 and 16.2058 mm with significant difference between 2 MIC and other two concentrations. In susceptible *E. faecalis,* the inhibition zones were 16.20, 22.00, 1.04 and 24.009 mm) with significant difference (except between 0.5 and 1MIC, while those of the resistant *E. faecalis* were 10.90, 15.4024 and 21.0083 mm. with significant difference between all concentrations. Measurement of the bacterial density by the spectrophotometer at different periods extended from 0-21 days showed there was significant gradual increase in the absorbance as compared with those of the day zero. In conclusion the exposure of the susceptible *Enterococcus faecalis* and *Klebsiella pneumoniae* to sub-MIC for 21 days led to emerging of resistance against ciprofloxacin.

Keywords: Resistance, Ciprofloxacin, *Enterococcus faecalis* and *Klebsiella pneumoniae*

Antimicrobials are particularly very important drugs, but the increasing evidence of resistant strains to old and modern antibiotic molecules has become a global challenge (Davies 2014). Antimicrobial resistance (AMR) is a growing global concern, with several reports indicating that it is a massive problem that affects both developed and developing countries. From 2017 to 2050, according to a World Bank simulation, antimicrobial resistance (AMR) may have a catastrophic effect on millions of people as well as a negative impact on the global economy (Bank 2017). AMR has emerged as a major risk to public health estimated to cause 10milions deaths annually by 2050 (Dixit et al 2019). Annually, more than 50,000 newborns die from sepsis due to resistance against first-line antibiotics (Dixit et al 2019). O'Neil (2019) estimated antimicrobial resistance to escalate tenfold globally by 2050, with the predicted number of deaths ranging across continents (O'Neil 2019) and antibiotic resistance genes (ARGs) are regarded as a new environmental pollutant (Sabri et al 2018). Antibiotic drug effectiveness can be improved and the lifespan of antibiotics used in medicine can be extended by slowing the rise of antibiotic resistance (Laureti et al 2013). Sub-therapeutic dose is one of the recognized reasons behind failed therapies or reduced its effect, which leads to the survival of bacterial population or induces antibiotic resistance. Therefore, it is very important to use antibiotics in true bacterial infections and in such doses so as to increase the likelihood of therapeutic effectiveness (Cantón and Morosini 2011). It is estimated that the MSC for a diverse range of microorganisms ranges between 1/4 and 1/230 of MIC values (Bengtsson-Palme and Larsson 2016). The number of studies have examined the results of the long-term exposure of environmental bacterial strains to low concentrations of antibiotics. Such exposure appears to have a significant influence on bacterial genomes, for example, this exposure has been found to modulate the transcription levels of about 5-10% of bacterial genes. These genes are associated with a variety of cellular processes, such as protein synthesis and carbohydrate metabolism, not just processes associated with the target of antibiotic action (Laureti et al 2013). Sub-inhibitory concentrations of antimicrobials can induce the bacterial repair system, which, in turn, increases the frequency of genome mutation and horizontal gene transfer and mobile genetic elements, including those responsible for antimicrobial resistance (Blázquez et al 2012). This study aimed to investigate the activity of the ciprofloxacin against susceptible and resistant isolates of *Klebsiella pneumoniae* and *Enterococcus faecalis*

and developing resistance in the susceptible by exposing to a sub inhibitory concentration of ciprofloxacin.

MATERIAL AND METHODS

Experimental microorganisms: Antibacterial agent ciprofloxacin was procured from veterinary clinic. The susceptible microorganism *Klebsiella pneumoniae* and *Enterococcus faecalis* were obtained from Diwaniyah General Hospital, while the resistant isolates obtained from College of Veterinary Medicine, University of Baghdad.

Minimum inhibitory concentration: The minimum inhibitory concentration (MIC) of the ciprofloxacin was determined by using the broth macrodilution method (CLSI 2000). Briefly, the inoculation of the microorganisms was carried out on 24 h MH broth cultures, and the suspension was adjusted to a 0.5 McFarland standard turbidity (approximately $1.5x10^8$ CFU/ml). Then two fold serial dilution of ciprofloxacin that ranged from (0.03–1 µg/mL) for susceptible *Enterococcus faecalis* and *Klebsiella pneumoniae* and (2–32 µg/mL) for the resistant isolates were made and added to all tubes of the cultures, then incubate at 37˚C for 24 h. At the end of the incubation period, the tubes were visually examined for turbidity. Cloudiness indicated that bacterial growth had not been inhibited by the concentration of the antimicrobial agent contained in the medium. MIC defined at the lowest concentration of antibacterial.

Determination of the inhibition zone: The agar well diffusion method was adopted (Kavanagh 1972, Perez et al 1990), for assessing the antibacterial activity of the ciprofloxacin against *Enterococcus faecalis and Klebsiella pneumoniae*. Five ml of standardized bacterial stock suspension (1.5 × 108 cfu/ml) of tested bacteria was thoroughly mixed to 500 ml of sterile Mueller Hinton agar. The 25 ml of the inoculated Mueller Hinton agar was distributed into sterile Petri dishes of each. The agar were left to set for 10 minutes to allow solidifying the agar, and in each of these plates, 3 well 6 mm in diameter were made, after that, wells were filled with 100 microliter of three concentrations of ciprofloxacin. Then allowed to diffuse at room temperature for two hours. The plates were incubated at 37°C for 24 hours. Three replicates were carried out for each concentration of antibacterial and the activity was determined by measuring the diameter of inhibition zone around each well by millimeter against the tested organism.

Development of resistance: After determination of the MIC of the ciprofloxacin against susceptible *Enterococcus faecalis* and *Klebsiella pneumoniae*, these isolates exposed to a sub-MIC of ciprofloxacin and incubated overnight. The next day the MIC (which may remain the same or increase) is determined and the sub-MIC concentration culture from the latest passage is used to inoculate a new series of diluted antibacterial agents. The process continues for up to 21 days (Mani et al 2006).

RESULTS AND DISCUSSION

Minimum inhibitory concentration: MIC of the ciprofloxacin against the tested bacteria (*Enterococcus faecalis* and *Klebsiella pneumoniae*), showed that the values are within the range specified by the CLSI, where the MIC against susceptible and resistant *Enterococcus faecalis* were 0.25 and 28.80/ml respectively, while were 0.45 and 11.20 µg/ml against susceptible and resistant *Klebsiella pneumoniae* ,respectively. In comparison between all the tested bacteria, the resistant *Enterococcus faecalis* was the less affected by ciprofloxacin, while the more affected bacterium was the susceptible *Enterococcus faecalis* with significant difference except between the susceptible *Enterococcus faecalis* and *Klebsiella pneumonia* (Table 1).

Determination of the inhibition zone by ciprofloxacin against *Enterococcus faecalis* and *Klebsiella pneumoniae*: MIC, 0.5 MIC and 2 MIC of the ciprofloxacin were used to determine the inhibition zone against the susceptible isolate of *Enterococcus faecalis* and *Klebsiella pneumoniae*. The inhibition zones were 17.20±, 23.20 and 26.4021 mm for 0.5, 1 and 2 MIC of ciprofloxacin against susceptible *Klebsiella pneumoniae* with significant difference (between all concentrations). In resistant *Klebsiella pneumoniae* the zones of inhibition were 12.00, 14.80 and 16.20 mm with significant difference between 2 MIC and other two concentrations. In susceptible *E. faecalis,* the inhibition zones were 16.20, 22.00 and 24.009 mm with significant difference except between 0.5 and 1MIC, while those of the resistant *E. faecalis* were 10.90, 15.40 and 21.00

Table 1. Minimum inhibitory concentration (µg/ml) of the ciprofloxacin against *Enterococcus faecalis* and *Klebsiella pneumoniae* (Means± SE)

Bacteria drugs	*Entero faecalis* susceptible	*Entero faecalis* resistant	*Kleb pneumoniae* susceptible	*Kleb pneumoniae* resistant
		MIC µg/ml		
Ciprofloxacin	C 0.25±0.06	A 28.80±9.32	C 0.45±0.05	B 11.20±1.95
		LSD 13.339		

Means with a different capital letters are significantly different (P<0.05)

mm. with significant difference between all concentrations (Table 2, Fig. 1).

Development of resistance in *Enterococcus faecalis* and *Klebsiella pneumoniae* against ciprofloxacin: The, susceptible isolates of *Enterococcus faecalis* and *Klebsiella pneumoniae* were exposed continuously for 21 days to 50, 25 and 12.5 % of MIC of ciprofloxacin. The MIC of the ciprofloxacin increased to 4 fold the breakpoint value at the end of the exposure (after 21 days), which became 1 and 1.8µ g/ml against *Enterococcus faecalis* and *Klebsiella pneumoniae*, respectively. Measurement of the bacterial density by the spectrophotometer at different periods extended from 0-21 days showed there was significant gradual increase in the absorbance as compared with those of the day zero, which mean increase the density, or increasing bacterial count of these bacteria. There was inverse relationship between concentration and absorbance. In both bacteria, *Enterococcus faecalis,* and *Klebsiella pneumoniae* after all periods of exposure (7, 14 and 21 days), higher absorbance was observed at the lower concentration (0.125 MIC), while the lowest absorbance was at the higher concentration (0.5 MIC) with significant difference between absorbance of all concentrations (Table 3 , 4).

MIC is defined as the minimum concentration of the antibiotic which prevents visible growth of a microorganism in an agar or broth dilution susceptibility test. These MIC values in combination with bacterial identification are required to obtain antibiotic susceptibility interpretations and breakpoints. A breakpoint is defined as the selected concentration of the antibiotic which provides interpretation of whether the species of the bacteria is susceptible or resistant to the antibiotic (MacGowan and Wise, 2001).

Table 2. Inhibition zone resulted from different concentrations of ciprofloxacin against susceptible and resistant isolate of *Enterococcus faecalis* and *Klebsiella pneumonia* (Means± SE)

Group		*K. pneumoniae* susceptible	*K. pneumoniae* resistant	*E. faecalis* susceptible	*E. faecalis* resistant
			Zone of inhibition (mm)		
Cipro	1/2mic	17.20±0.58c	12.00±0.31c	16.20±0.34c	10.90±0.40c
	1mic	23.20±0.58b	14.80±0.58bc	22.00±1.04ab	15.40±0.24b
	2mic	26.40±0.21a	16.20±0.58a	24.00±1.09a	21.00±0.83a
LSD		1.3711	1.2034	2.1271	1.3866

Means with a different letter in the same column are significantly different (P< 0.05)

Table 3. Absorbance of *Enterococcus faecalis* suspension after exposure to sub MIC concentrations of ciprofloxacin for 21 days

Drugs	Concentration (µg/ml)	Day			
		0 (control)	7	14	21
			Absorbance		
Cipro	0.5 MIC	D0.08±0.00a	C0.08±0.001c	B0.39±0.001c	A1.20±0.001c
	0.25 MIC	D0.08±0.00a	C0.15±0.001b	B0.80±0.002b	A1.85±0.001b
	0.125 MIC	D0.08±0.00a	C0.56±0.001a	B1.48±0.001a	A3.11±0.002a
LSD			0.0031		

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)

Table 4. Measurement of absorbance of *Klebsiella pneumoniae* suspension after exposure to sub MIC concentrations of ciprofloxacin for 21 days

Drugs	Concentration (µg/ml)	Day			
		0 (control)	7	14	21
			Absorbance		
Cipro	0.5 MIC	D0.08±0.00a	C0.10±0.001c	B0.64±0.001c	A1.77±0.001c
	0.25 MIC	D0.08±0.00a	C0.26±0.001b	B1.01±0.001b	A3.16±0.001b
	0.125 MIC	D0.08±0.00a	C0.71±0.001a	B1.96±0.001a	A4.88±0.002a
LSD			0.0031		

Means with a different small letter in the same column are significantly different (P<0.05)

Bacteria are considered susceptible if the MIC value for the antibiotic bacterial pair is lower than the breakpoint and are considered resistant if the MIC value is above the breakpoint, while for the MIC values in between is declared as intermediate susceptible. These breakpoints for each bacteria-antibiotic pair are predetermined in accordance with the Clinical Laboratory Standards Institute (CLSI) in USA and European Committee on Antibiotic Susceptibility Testing (EUCAST) in Europe. These numbers provide valuable information to physicians to determine the appropriate targeted antibiotic to be administered to the patient.

Increase absorbance of the tested suspensions of the both isolates *Staphylococcus aureus* and *Escherichia coli* that exposed continuously for 21 days to sub MIC of ciprofloxacin might be due to increase the numbers of these bacteria, based on optical density (OD) spectroscopy, an OD measurement characterizes the amount of light that is lost due to scattering and absorption at a single wavelength (Myers et al 2013). The OD correlates directly with the cell concentration, as the number of cells in a sample increase, the transmission of light through the sample will decrease (Shao et al 2016). The results also showed that the MIC of the ciprofloxacin increased to 4 fold the breakpoint values at the end of the exposure (after 21 days). The increase the bacterial count as well as reduced activity of the antibacterial agents at the MIC, means the bacteria developed certain defense mechanism which made it not affected by this agents as this became resistant as a result of the exposure to sub inhibitory concentrations which disagreed with study conducted by Al-Samarraae (2019) that the lowest resistance rate was to the ciprofloxacin .

During antibiotic therapy pathogens may encounter the sub-inhibitory concentrations (sub-MICs) of antibiotics due to several situations (Yang et al 2020). First, antibiotic

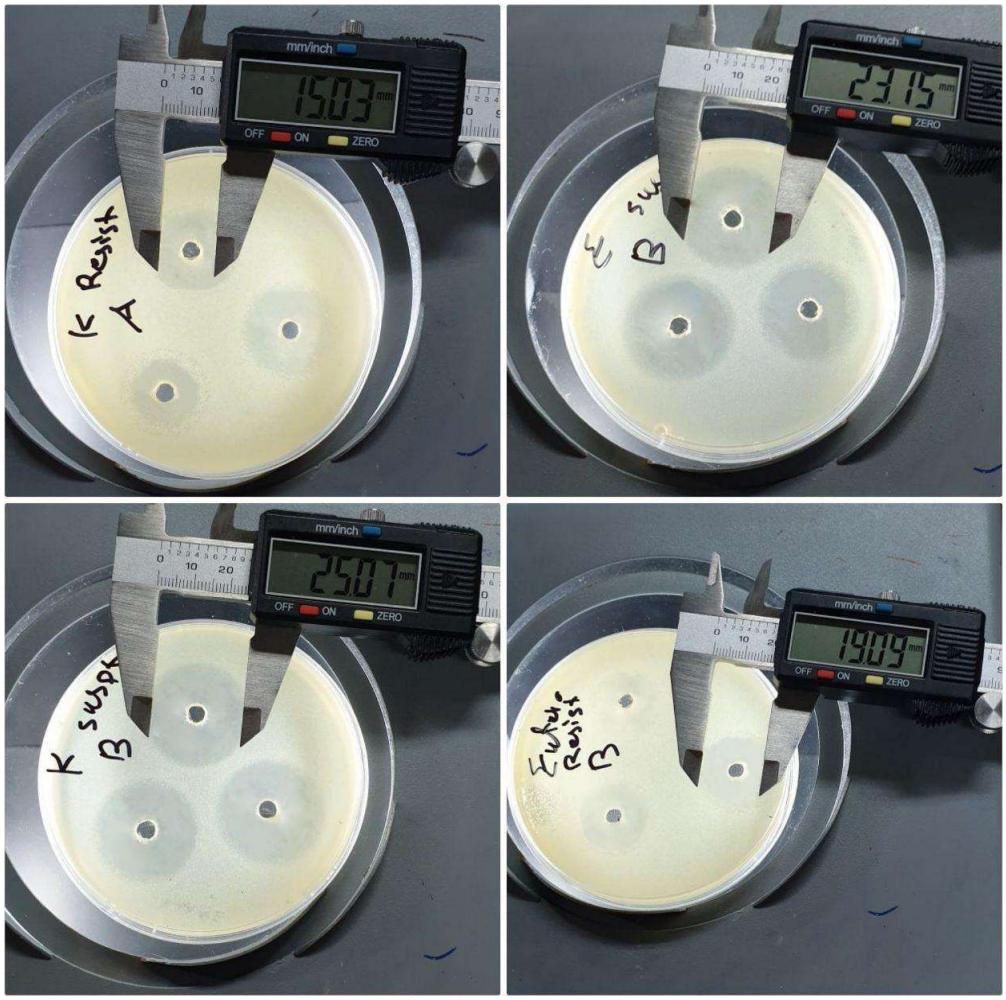

Fig. 1. Inhibition zone resulted from different concentrations of ciprofloxacin against susceptible and resistant isolate of *Enterococcus faecalis* and *Klebsiella pneumoniae*

concentrations decrease to sub-MICs over time after administration as a result of the pharmacokinetics of antibiotics (Sasso et al 2003). Second, the occurrence of antibiotic sub-MICs in tissues or the internal milieu of bacterial biofilms is attributed to physical and chemical barriers to antibiotic penetration, as well as to drug–drug interactions in sites other than in the blood where pathogens are in direct contact with antibiotics (Kümmerer, 2009). Third, antibiotic sub-MICs may be generated after waste emissions from hospitals and other treatment facilities or pharmaceutical manufacturers. Fourth, antibiotic sub-MICs can occur owing to bacterial evolution and drug-resistant organism formation (Yang et al 2020). Fifth, in the clinic due to incorrect therapy, poor adherence and poor-quality medicines (Chatterjee et al 2018; Fisher et al 2018). Lastly, in stock farming, antimicrobial drugs used at sub-MICs are still allowed in some countries to promote animal production or prevent bacterial infections (Hao et al 2014).

The important effects of sub-MICs of antibiotics on the morphology, biofilm formation, and virulence expression of Gram-positive and Gram negative bacteria are of interest and have been intensively investigated in the last decade (Hodille et al 2017). Sub-MICs of antibiotics from diverse classes and thus different modes of action can induce similar alterations in a variety of bacterial phenotypes such as virulence, biofilm formation, quorum sensing, gene expression and gene transfer (Andersson and Hughes 2014). In sub-inhibitory concentrations (concentrations below the MIC), the susceptible bacterial cells continue growing at a reduced growth rate, and the lowest antibiotic level that drives selection of a resistant mutant over the wild type cells is determined as the Minimal Selective Concentration (MSC).

CONCLUSION

The exposure of the susceptible *Enterococcus faecalis* and *Klebsiella pneumoniae* to sub-MIC for 21 days led to emerging of resistance against ciprofloxacin.

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Effect of Adding Zeolite to Diets Containing Yellow Corn Contaminated with Aflatoxin on Production Performance of Broilers

Ahmed A. Allaw and Basim M.K.H. Jasim[1]

University of Tikrit College of Agriculture, Tikrit, raq
[1]Ministry of Agriculture, Baghdad, Iraq
**E-mail: Drallaw@tu.edu.iq*

Abstract: This experiment was conducted at University of Tikrit for 35 days in order to study the ability of Zeolite to inhibit effects of aflatoxicosis on broiler performance. Two hundred and seventy one day old broiler chicks (Ross 308) were randomly distributed into six treatments. The treatments were: T1- control treatment, T2-Contaminated yellow corn included 0.45 ppm toxin. T3 and T4 addition of Zeolite only 0.3 and 0.4%, T5-Zeolite addition at 0.3% + contaminated corn 0,45 ppm. T6-Zeolite addition of 0.4% + contaminated corn 0.45 ppm. There was a no significant difference between T1 and T6. There was significant decrease in T2 compared to other experimental treatments in live body weight and weight gain.

Keywords: Zeolite, Broiler, Aflatoxin, Performance

Aflatoxins cause a variety of effects in poultry, including poor performance, liver pathology, immunosuppression, and changes in relative organ weights. The severity of aflatoxicosis is related to dose and duration of the intake of aflatoxins by a flock. Low levels of aflatoxin cause listlessness, ruffled feathers and morbidity in chicks. In layers the adverse effects of aflatoxins are characterized by lack of appetite, stunted growth, decreased egg production and reproductive disorders. Several strategies for the reduction or inactivation of aflatoxins have been previously reviewed and include diverse physical, chemical, and biological methods (Phillips et al 2002, Shahad and Allaw 2020). One of the strategies of the inclusion of non-nutritive sorbents in contaminated feeds for remove of aflatoxins. This approach is considered to be practical and cost effective for the detoxification of contaminated feedstuffs on a large scale.

Natural Zeolites have considerable freedom of movement as such, can undergo the processes of exchange, absorption, diffusion, dehydration, reversible dehydration, and catalysis. The absorbent, Zeolite eliminates a number of toxic substances and acts as detoxicant, maintaining ion balance in the gastrointestinal tract, increasing nutrient conversion and reducing oxidative stress (Wu et al 2013). Zeolite incorporated into the diet has the ability to reduce the deleterious effects of aflatoxin on broiler chickens. Studies have revealed that Zeolite is able to absorb damaging toxins that can potentially reduce the growth of animals (Oguz and Kurtoglu 2000). The purpose of this experiment was to evaluate the ability of dietary of Zeolite to suppress the deteriorates effects of aflatoxicosis

MATERIAL AND METHODS

This study was conducted at Tikrit University for a period of 35 days. 270 one day old chicks with average weight of 34 g were used. The experiment included six treatments and each treatment included three replications of 15 chicks. Chicks were distributed in randomly and there were six treatments.

T1- control treatment, without adding Zeolite or contaminated corn

T2 - Contaminated yellow corn included 0.45 ppm toxin.

T3- Zeolite addition of 0.3%.

T4 - Zeolite addition of 0.4%

T5 - Zeolite addition of 0.3% + contaminated corn 0.45 ppm.

T6 - Zeolite addition of 0.4% + contaminated corn 0.45 ppm.

The addition of Zeolite began in the second week. The amount of toxins in corn was 0.45 ppm, and the aflatoxin B1 type was determined. (AOAC 2005). The Zeolite grains were supplied by Zeo Green Company/ Jordan. The birds were housed in a semi-closed hall with dimensions of 45 x 10 x 3 M, containing cages (pens), the lighting was continuous at a rate of 23 hours per day . Birds allowed to consume feed and water *ad libitum*, the three diet were starter, grower and finisher (Table 1).

RESULTS AND DISCUSSION

The effect of dietary treatments on live body weight , body weight gain, feed consumption and feed conversion rate, are presented in Tables 2, 3, 4 and 5 respectively. There was no significant effect on body weight up to 2 week. In third week T3 was significantly superior than in other treatments. In

Table 1. Formulation and calculated composition of broiler diets (Basal diet)

Ingredients (%)	Starter (1-10) days	Grower (11-24) days	Finisher (25-35) days
Yellow corn	46.18	50.08	53.88
Soybean meal (48% C.P.)	36.9	33	29.5
Wheat	9.92	10.22	9
Premix*	2.5	2.5	2.5
Sunflower oil	2.8	3.1	4.2
Limestone	0.3	0.3	0.5
D-methionine	0.1	0.1	0.1
Dicalcium phosphate	1	0.5	0.2
L-Lysine	0.3	0.2	0.12
Total	100	100	100
Calculated chemical compassion**			
Crude protein. C.P%	23.02	21.52	20.02
AME (kcal/kg)	3027.74	3099.59	3202.42
Calcium (%)	0.97	0.84	0.83
Av. phosphorous (%)	0.61	0.52	0.45
Lysine(%)	1.48	1.28	1.12
Methionine (%)	0.57	0.56	0.54
Meth +Cyst (%)	0.92	0.89	0.85

* Premix provided per kilogram of the diet: Vit. A, 1000 IU; D3 2000 ICU; Vit. E, 10 mg; Vit. K, 1mg; B1, 10 mg; B2, 5 mg; B6, 1500 mg; B12, 10mg Pantothenic acid, 10 mg; Nicotinic acid, 30 mg; Folic acid, 1mg; Biotin, 50 mcg; Chloride, 500 mg; copper, 10 mg; iron, 50 mg; Manganese, 60 mg; Zinc, 50mg, and selenium, 0.1 mg; **The chemical composition was calculated according NRC (1994).

fourth week T1, T3, T4 and T6 an increase was observed over T2 and T5. In fifth week body weight in T1, T3, T4 and T6 was more than in other treatments (Table 2 and 3). There were no significant differences between treatments T6, T4, and T3 in the rate of total body weight gain and in T2 was significantly low as compared to the other treatments. The total feed consumption of the broilers of T2 was the lowest compared to the broilers of the other treatments. There were no significant differences between the treatments T1, control, T3, T4, and T6 (Table 4). There were no significant differences between the control treatment T1 and treatment that contains yellow corn contaminated and with Zeolite (T5, T6).

The decrease that occurred to the birds of the second treatment, whose feed included yellow corn contaminated with aflatoxin B1 in body weight and weight gain was due to the decrease in feed consumption due to a problem on metabolism as a result of its negative impact on the liver and digestive system because B1 toxins affect the production of enzymes of both the liver and pancreas. This is reflected in decreased appetite generally (Tedesco et al 2004, Bailey et al 2006, Shi et al 2006). The decrease in the live body weight and the increased weight may be due to the decrease in absorption of mineral elements, which caused the lack of utilization of protein due to the inhibition of protein and energy building. These toxins cause anemia as a result of inhibiting

Table 2. Effect of Zeolite on live body weight for broiler chicks fed with aflatoxin contaminated diet

Treatment	Week				
	1	2	3	4	5
T1	114.38	317.22	688.82b	1197.20a	1711.71a
T2	112.95	311.35	656.15c	1098.13b	1544.56c
T3	112.82	322.02	772.04a	1194.34a	1779.25a
T4	112.84	320.04	698.78b	1215. 73a	1807.71a
T5	111.71	310.73	678.98bc	1123.62b	1672.89b
T6	113.02	319.49	684.16bc	1199.42a	1799.00a

T1 - control treatment . T2- contaminated corn 0.45 ppm. T3 - Zeolite 0.3%. T4- Zeolite 0.4% T5- Zeolite 0.3% + contaminated corn 0.45 ppm. T6 - Zeolite 0.4% + contaminated corn 0.45 ppm

Table 3. Effect of Zeolite on body weight gain for broiler chicks fed aflatoxin

Treatment	Week				
	2	3	4	5	Total
T1	202.84	371.60a	508.38a	514.44b	1597.27b
T2	198.400	344.80c	441.98b	446.42c	1431.60c
T3	209.200	450.02a	422.30b	584.90a	1666.43a
T4	207.200	378.73a	516.95a	591.98a	1694.87a
T5	199.02	368.24b	444.64b	549.27ab	1561.18b
T6	206.46	364.68ab	515.25a	599.57a	1685.97a

See Table 3 for treatment details

Table 4. Effect of Zeolite on feed consummation for broiler chicks fed with aflatoxin contaminated diet

Treatment	Week				
	2	3	4	5	Total
T1	244.89a	507.90a	800.00Ab	947.33a	2500.12a
T2	219.73b	496.19b	646.20c	753.42c	2115.94c
T3	240.44a	510.42a	760.67ab	936.78a	2448.31ab
T4	249.44a	504.91ab	750.60ab	961.59a	2466.54ab
T5	257.67a	502.79ab	746.22b	884.11b	2390.79b
T6	260.00a	505.65ab	809.55a	958.04a	2533.25a

See Table 3 for treatment details

Table 5. Effect of Zeolite on feed conversion rate for broiler chicks fed with aflatoxin contaminated diet

Treatment	Week				
	2	3	4	5	Total
T1	1.21ab	1.37b	1.57bc	1.84a	1.56a
T2	1.10b	1.43a	1.46cd	1.69ab	1.47b
T3	1.15ab	1.13c	1.80a	1.60b	1.46b
T4	1.20ab	1.33b	1.45d	1.63b	1.45b
T5	1.30a	1.36b	1.67b	1.61b	1.53ab
T6	1.25ab	1.39ab	1.57bc	1.60b	1.50ab

See Table 3 for treatment details

the process of blood formation. Since the digestive system is the first system to come into contact with mycotoxins and affected by the effectiveness of toxins more than other systems and may be due to the fact that the absorption surface of the small intestine deteriorates during chronic exposure to low levels of B1 as the nutrients are absorbed primarily by the epithelial cells in the small intestine so that the toxin B1 is easily absorbed through these. Most of the B1 toxins are transported directly into the blood. Diets containing Zeolites are more effectively absorbed by the intestinal villi in the duodenum than in other parts of the small intestine, and the elevation of large villi and many mitotic cells in the intestine are indications of activation of the function of the intestinal villi (Incharoen et al 2010). Zeolite functions may stimulate these enlarged intestinal villi and epithelial cells (Khambualai et al 2009).

CONCLUSION

This study indicate that adding Zeolite (0.3, 0.4%) to the diet of broilers did not negatively affect the performance of chicks. Growth performance of broiler chicks was significantly decreased by administration of aflatoxin (0.45 ppm) in feed for 3 weeks. Addition of Zeolite (0.4%) to a diet containing aflatoxin A (0.45 ppm) reduces the adverse effects of aflatoxin on production performance. These results may be helpful in finding a solution to the aflatoxin problem in broilers diets.

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Prevalence of Head Lice (*Pediculus humanus capitis*) Among School Children in Baghdad City

Russl Sameer Mhana and May Ibrahim Younis[1]

College of Science, Al_Mustansiriyah University
[1]College of Science, University of Baghdad
E-mail: Russl.s.1996.s@gmail.com

Abstract: Head lice infestation is a common public health problem is a contagious caused by the human louse *Pediculus humanus capitis*, an obligate ectoparasite. The study was aimed to determine the prevalence of head lice in public school in eastern region of Baghdad city and to study the risk factors that increase the lice infestation. Survey was carried out during the period from December 2019 to February 2020 in 35 primary public school in Baghdad , a total of 4000 school student (Boys and girls) an aged 6-11 years old examined carefully using head lice remover device and wet fine comb method. Totally 856 child (Boys and girls) were found to be infected, prevalence rate was higher in female than male, in age group 6-8 than 9-11. The highest infestation rate was in December, and moderate in January while in February there was not any cases of pediculosis . The head lice infestation was higher in long dark and straight hair. Some behaviors as combing, washing of hair and sharing room was strongly correlated to the infection. The family size was considered as an effective agent for infestation. The results obtained in the current study showed the spread of lice in the elementary schools in Baghdad due to personal hygiene and lack of awareness to head lice among teachers and parent.

Keywords: *Pediculus capitis*, School children, Baghdad, Head lice

Head lice infestation (*Pediculosis capitis*) is a common public health problem worldwide and is an obligate ectoparasite. These blood-sucking insects can cause many physiological, psychological, and social complications (Değerli et al 2013). They can spread rapidly in closed environments. The hallmark of *P. capitis* is pruritus, itching which happen when the infested person becomes sensitized to the antigenic components of the louse saliva that is injected as the louse feeds (Okoh et al 2013) .The main route of transmission is direct head-to-head contact, however, the indirect spread can occur through shared items, such as combs, scarfs, and hats (Elserite 2016). Various factors contribute to the prevalence rate of head lice including age, gender, race, socioeconomical state, and hair characteristics. Moreover, living in crowded populations and lice resistance to common pesticides may lead to increase infestation rate nowadays (Nazari et al 2006). In Iran the prevalence of head lice infestation among primary school was 56.15%, and was higher in girls and women (Sanei-Dehkordi et al 2017). In Makkah, Saudi Arabia, infestation rate was 31.2%. Girls were significantly more infested than boys (Mohamed et al 2018) In Baghdad, Iraq showed the endemicity of the disease among elementary school children from eight elementary schools, including 540 boys and girls. The total infestation rate was 13.5%, and among girls was higher compared to boys (Mahmood 2010). The current study aims to estimate the prevalence of head lice infestation and assess the factors affecting it among elementary school children in eastern region of Baghdad.

MATERIAL AND METHODS

This study done to find out the prevalence of head lice from December 2019 to February 2020 with daily visit randomly to 35 primary school (public school) located in the eastern region of Baghdad. Four thousands student (boys and girls) an aged between 6-11 years old were examined. Two methods were used to collect the insect, fine comb method and head lice remover device. The detailed questionnaire contained data on age, gender, sharing same room, family member , showering, brushing habit and individual's hair characteristics (color, length, and type). Data were analyzed by statistical analysis system-SAS (2012) program.

RESULTS AND DISCUSSION

The 1500 student were investigated during December between 6-8 years old , and 41.12% were infested. There were significant differences between male and female (12.14 and 28.9% respectively) . The number of infested student during the same month among 9-11years age group was 23.36% with no significant difference between male and female. In January, out of the 1401 student in age 6-8 years

old infestation was 26.98%. being significantly low in male (3.5%) than in females (23.4%). The number of infested student for age group 9-11 age group was 8.52%. The non-significant difference was observed between male and female among this age group. During February in 87 student with age 6-8 years old and in 13 student with age 9-11 years old infestation was nearly zero which mean there was not any cases of pediculosis.

The prevalence was higher in long hair than short and medium hair length being 60.0, 28.2 and 11.6 respectively. Prevalence rate for dark hair was 72.8% and for light hair was 27.1%. The relation of pediculosis and hair style were investigated and there was a significant relationship between the infection and hair style, infection rate was 59.6% and 40.3% of case had straight and curly hair. The head lice infestation was significantly related to showering and brushing habit, moreover, there was a strong correlation between incidence of infestation with family size and sharing of rooms.

Almost in all this studies in Iraq and other parts of the world, the prevalence of head lice infestation in female pupils was more than the male pupils. There was highly significant associated between gender and head lice infestation ,which is similar with the finding of previous studies in various country such as Makkah, Saudi Arabia (Mahmood et al 2018), turkey (Gulgun et al 2013) and Iran (Moosazadeh et al 2015). In this study the high prevalence of head lice in female population can be attributed to the length and quality of their hair, in addition to the fact that the males tend to shave their hair so that ,the head lice cannot find the appropriate place of warmth, moisture and darkness that are available in long hair. Thus, the spread of lice increase among females was more. In contrast, Olaitan (2006) found that the prevalence of head lice was higher in boys in both urban and rural primary schools in Nigeria. Furthermore, in present study the highest rate of infestation was seen in (6-8) years old students. The low infestation rate in older age groups observed in the present study may be indicate that better personal hygiene practices, including the regular combing and washing of the hair. Mohammed (2012) in Jordan reported a higher infestation rate in younger children (<9 years). This is in disagreement with the results of a number of previous studies as Bibi (2011) in Pakistan showed the prevalence of *P. capitis* was highest (80.0%) in age group of (61-75) years and lowest (46.2%) in age group of (31-45) years. High population rate, socioeconomic status , and some of environmental factor among different countries like temperature and humidity, also play role for this variation. The results of the study showed the highest incidence of head lice in December , moderate in January, and the lowest in February Head lice infestation occurred more in the winter. Frequent and high rainfall in winter provides suitable conditions for the growth of insects this increases the extent and severity of head lice dissemination in communities in winter. Counahan et al (2004) conclude that in humid and relatively cold weather, many children wear woolen hats and earmuffs when they are outside during winter time. This finding was similar with Kassiri and Mardani (2018). But mismatched with Ibrahem and Tawfeeq (2008) study .The current showed long hair was much more infested with *P. capitis* than those with medium or short hair Similar results were observed by Bosely and El-Alfy (2011) and Tappeh et al (2012). However Counahan et al (2004) observed that hair length did not appear to be an independent risk factor. The lower head lice prevalence in light hair group student than dark one this is related to the common hair color for most of student in Iraq is dark and little percentage belong to light hair color. Borges and Mendes (2002) observed highest rates with long and dark hairs. In Iran, Doroodgar et al (2014) recoded that in 88.2% of the cases were with straight hair and

Table 1. Prevalence of *Pediculus humanus capitis* infestation in male and female school student in eastern of Baghdad city

Month	Age (Year)	No. of examined	Infested		Male		Female		Chi-Square (χ^2)
			No	Percent	No	Percent	No	Percent	
Dec.	6-8	1500	352	41.12	104	12.14	248	28.9	5.488 *
	9-11	567	200	23.36	100	11.6	100	11.6	0.772 NS
Jan.	6-8	1401	231	26.98	30	3.5	201	23.4	7.694 **
	9-11	432	73	8.52	8	0.93	65	7.5	1.794 NS
Feb.	6-8	87	Zero	0	Zero	0	Zero	0	0.00 NS
	9-11	13	Zero	0	Zero	0	Zero	0	0.00 NS
Total		4000	856						
Chi-Square (χ^2)	---	---	---	9.548 **	---	5.027 *	---	8.946 **	---

* (P≤0.05), ** (P≤0.01).

Table 2. Relation between *Pediculus humanus capitis* infestation and the personal characteristics of students in eastern Baghdad

Variables	No. of examined	Total	Percent	No. of infested	Total	Percent
Hair length: short	1068	4000	26.7	242	856	28.2
Medium	832	4000	20.8	100	856	11.6
Long	2100	4000	52.5	514	856	60.0
Chi-Square (χ^2)	---	---	8.95 **	---	---	10.74 **
Hair color:						
Dark	3126	4000	78.15	624	856	72.8
Light	874	4000	21.85	232	856	27.1
Chi-Square (χ^2)	---	---	11.07 **	---	---	10.63 **
Hair type:						
Straight	3094	4000	77.3	511	856	59.6
Curley	906	4000	22.6	345	856	40.3
Chi-Square (χ^2)	---	---	11.17 **	---	---	7.29 **
Showering:						
Daily	2002	4000	50.0	50	856	5.8
Weekly	1273	4000	31.8	216	856	25.2
Never	726	4000	18.15	590	856	68.9
Chi-Square (χ^2)	---	---	10.68 **	---	---	12.04 **
Brushing:						
Daily	3032	4000	75.8	245	856	28.6
Weekly	220	4000	5.5	100	856	11.6
Never	748	4000	18.7	511	856	59.6
Chi-Square (χ^2)	---	---	12.56 **	---	---	11.73 **
Family size:						
≤3	1274	4000	31.85	141	856	16.47
>3	2725	4000	68.12	715	856	83.53
Chi-Square (χ^2)	---	---	10.027 **	---	---	13.459 **
Sharing same room :						
Yes	3226	4000	80.65	554	856	64.72
No	774	4000	19.35	307	856	35.28
Chi-Square (χ^2)	---	---	13.171 **	---	---	9.036 **

** (P≤0.01)

11.8% had curly hair. Mahmud et al.(2011) have not emphasized the importance of personal cleanliness in *P. capitis*. This study also showed a positive relationship between head lice infestation and family size and sharing same room, in overcrowded families the incidence of infestation increase. Sim et al (2011) in studies conducted in Korea, concluded that factors such as hygiene and the number of family who share same room, are related with head lice infestation. In Yemen a relationship was found between the rate of infestation and family member (Bassam 2012).

CONCLUSION

Pediculus humanus capitis are most likely to flourish in winter, especially in December, due to low temperatures and high humidity and was considered an important pest in primary school student in eastern Baghdad. Providing more medical services and periodic inspection for student is necessary in the future.

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Soil Evaluation Extraction Methods of Available Boron in Soil

Khalida A.K. AL-Jumaily and Kahtan J. Abdul Rasool

Department of Soil sciences and Water Resources
College of Agricultural Engineering Sciences, University of Baghdad, Iraq
Email: khalida.abdalmoeen1207a@coagri.uobaghdad.edu.iq

Abstract: The field experiment was conducted to evaluate the methods of extracting available boron in soil (hot water, Mannitol-$CaCl_2$, 0.005*M* DTPA, NH_4HCO_3 + 0.005*M* DTPA) in correlation with absorbed boron from green bean plant (*Phaseolus vulgaris* L*)* at College of Agricultural Engineering Sciences, University of Baghdad. The experiment was carried out by adding five levels of boron 0, 0.5, 1, 2, 3 kg B ha^{-1} in form of boric acid . The results showed correlation between the extracted and absorbed boron . The extractants was in order of DTPA > Mannitol - $CaCl_2$ > Hot Water > DTPA + NH_4HCO_3 according to r values which were 0.89,0.8,0.82 and 0.72, respectively, with significant differences for all extractants except DTPA+NH_4HCO_3 extractant. The concentration of available boron extracted from soil with hot water ranged between 0.99 - 12.0 mg B kg^{-1} soil and the extracted by mannitol-calcium chloride was 2.76 -6.24 mg B kg^{-1}soil, and ammonium bicarbonate + DTPA was 0.04 - 2.0 mg B kg^{-1}soil. The concentration of boron in the pods varied from 15.to 55.1 mg B kg^{-1}.

Keywords: DTPA , Mannitol.$CaCl_2$, Hot Water , DTPA + NH_4HCO_3 ,Soil, Boron , *Phaseolus vulgaris*

Estimation and extraction methods and chemical extractants that extract available forms of plant nutrients in soil are the right first steps in assessing soil fertility. The proper assessment of soil fertility results in accurate fertilizer recommendations based on the actual plant nutrients in the soil, as well as the actual plant need of these nutrients. There are many chemical extractants that may extract quantities of available plant nutrients that may exceed or less than the actual content in the soil due to the quality of these chemical extracts (mineral acids and bases, organic acids, chelating materials, mineral and organic salts) as well as the concentration of these extracts (Abdul Rasool 1997). Boron has an important role in the absorption of water from the soil and movement of sugars inside the plant to its storage places, as well as its effect on the absorption of nitrogen, potassium and calcium and formation of the cell wall and importance in the formation of plant hormones .Boron helps to form the pollen tubes that's necessary in development and formation of grains, and the plant needs more during the flowering and fruiting stage (Havlin et al 2014).

The green bean (*Phaseolus vulgaris* L.) is one of the plants belonging to the family (Fabaceae). The genus *Phaseolus* includes about 150 species of annual and perennial plants, and it is one of the main legumes throughout the world. The percentage of protein in the pods ranges between 22-37% of the dry weight and the percentage of carbohydrates between 13-18% (Al-Akidi 2015). The importance of boron in plant nutrition, and estimation and extraction methods due to narrow range between deficiency and toxicity is well documented. Due to the lack of studies on estimation and extraction methods of available boron in Iraq, current study aims to evaluate extraction methods of available soil boron in correlation with absorbed boron.

MATERIAL AND METHODS

A field experiment was carried out at University of Baghdad on green bean crop, variety Baritone. Soil samples were taken from the study site during the preparation of the field soil for cultivation. After preparing soil for the analysis some chemical and physical properties of the soil were estimated according to Page et al (1982) (Table 1). The experiment included adding boron to the soil at five levels (0 , 0.5, 1, 2, 3 kg B ha^{-1}) in form of boric acid after dissolving the required quantities with water with the fertilizer recommendation of 28 kg K, 28 kg P and 84 kg N ha^{-1} (Havlin et al 2014). Triple super phosphate was added as a source of phosphate, potassium sulfate as a source of potassium, urea as a source of nitrogen and boric acid as a source of boron. The fertilizers were added all at once at the bottom of the plants at a depth of 5 cm from the plants after 10 days of germination. The randomized complete block design with three replicates was used to carry out the experiment. Available boron was extracted by the hot water (Page et al 1982), by 0.05M Mannitol + 0.01M $CaCl_2.2H_2O$ (Cartweight et al 1983), by 0.005M DTPA +1M NH_4HCO_3 (Soltan pour and Schwabb 1977) and by 0.005M DTPA (Lindsay and Norvell 1978). The available boron was determined by the Azomethine-H method and by using a spectrophotometer at

a wavelength 420 nm (Shaine et al 1967). Boron was determined in beans pods by curcumin method (Page et al 1982, Al-Sahaf 1989). Green pods were collected five times on November 15, November 24, December 6, December 14 and December 22 for estimation

Available Boron Extraction

Hot water method: Available boron extracted from the soil increased with time from 2.79, 4.29, 7.89, 7.93, 7.62 mg B kg^{-1} soil during November 15, November 24, December 6 and December 14 and December 22 decreased slightly. The November 15 recorded the lowest while December 14 highest. The differences were significant between the first and second observation and third, fourth and fifth .The available boron extracted from the soil increased with the fertilizer levels. The differences were significant between the fourth level and the rest levels except the third level. There were no significant differences between the control treatment and T_1.

Method mannitol - calcium chloride: The effect of adding different levels of boron fertilizer shows that available boron extracted with mannitol - calcium chloride over times was 5.67, 4.91, 4.06, 3.63, 3.32 mg B kg^{-1} soil during November 15, November 24, December 6 and December 14 and December 22. The November 15 gave the highest values and December 22 the lowest values. The differences were significant among dates**.** The effect of boron fertilizer levels on available boron extracted by mannitol-calcium chloride extractant indicate that an increase with increasing the levels of the added fertilizer and was 3.97, 4.16, 4.32, 4.53, 4.63 mg B kg^{-1} soil according to the levels of fertilizer sequentially, The control treatment gave the lowest values 3.97 mg B kg^{-1}-soil, while the treatment T_4 gave the highest value 4.63 mg B kg^{-1} soil. There were no significant differences between the control treatment and the levels T_1 and T_2, while there were significant differences between T_4 and the control treatment and also between T_1 and T_2.

Method 1M NH_4HCO_3 + 0.005M DTPA: The values of available boron extracted with ammonium bicarbonate + 0.005*M* DTPA tended to decrease with time (fairies) (Table 4). The values were 1.91, 1.91, 0.55, 0.11, 0.074) mg B kg^{-1} soil on during November 15, November 24, December 6 and December 14 and on December 22. The first and second fairies gave the highest 1.91 mg B kg^{-1} soil, while the fifth fairy gave the lowest values 0.074 mg B kg^{-1}soil. The differences were significant between the first and second fairies on the one hand and rest fairies on the other hand. The results also showed an increase in the values of the Available boron extracted from the soil, according to the increasing in the levels of the added boron fertilizer, The values were 0.83, 0.86, 0.89, 0.95, 1.02 mg B kg^{-1} soil, according to the levels of boron added sequentially. The control treatment gave the lowest values 0.83 mg Bkg^{-1} soil and T_4 treatment the highest values 1.02 mg B kg^{-1} soil. The differences were not significant between the control treatment and the levels T_1 and T_2 while they were significant with the T_4 level.

Methos 0.005M DTPA: Available boron extracted from soil by DTPA recorded a decrease with time (Fairies) in general especially in the third fairy with an increase in the fourth pound and the values were 3.26, 2.82, 1.95, 3.11, 2.43 mg B kg^{-1} soil on during November 15, November 24, December 6 and December 14 and on December 22 fairies respectively (Table 5). The highest value was in the first fairy 3.26 mg B kg^{-1} soil, and the lowest in the third fairy 1.95 mg Bkg^{-1} soil, The differences were significant between the first , second fairies and fourth, fifth fairies. There were no significant differences between the third, second and fifth fairies..

There was an increase in the values of the extracted available boron with the fertilizer levels and were as follows 2.22, 2.64, 2.74, 2.85, 3.13 mg B kg^{-1} soil with the fertilizer levels sequentially. The control treatment recorded the lowest value 2.22 mg B kg^{-1} soil, while the T_4 treatment recorded the highest value 3.13 mg B kg^{-1} soil. The results showed a significant difference between the two treatments T_3 and T_4 on the one hand and the control treatment on the other.

The available boron extracted from the soil with time (the fairies) tended to decline in general with all extractants except for the hot water extractant which recorded the

Table 1. Some chemical and physical properties of field soil

Properties	Value			Unit
pH $_{1:1}$	7.4			
EC 1:1	1.23			dS m^{-1}
Soil organic matter OM	30.1			G kg^{-1}
Carbonate equivalent	21.25			
bulk density	1.35			M gm^{-3}
Anions		Cation		mmol L^{-1}
$^{-}SO_4^{2}$	3.3	$^{+}Ca^{2}$	6	
CO_3^{2-}	Nil	$^{+}Mg^{2}$	3.9	
$^{-}HCO_3$	6.2	^{+}Na	2	
Cl^{-}	3	^{+}K	0.6	
Available boron	5.5			mg kg^{-1}
Available nitrogen	53			
Available phosphorous	10			
Available potassium	190			
Sand		150		g kg^{-1}
Silt		650		
Clay		200		
Texture				Silty loam

opposite. The decrease may be due to the absorption of boron from the plant as well as the adsorption and sedimentation processes (Al-Falahi 2000, Majidi et al 2010). The available boron extracted from the soil with the fertilizer levels have increased with all extractants without exception and this is normal due to the increasing in the content of the soil solution from dissolved boron in addition to the adsorbed boron as a result of fertilizer additions (Alwan 2012)

The available boron extracted cumulatively with the four extractants were (22.74, 67.85, 108.08, 152.58 mg B kg^{-1} soil for the extractants of ammonium bicarbonate + DTPA, DTPA, mannitol-calcium chloride and hot water sequentially. This is

Table 2. Available boron extracted from the soil by hot water (mg kg^{-1})

Treatments	Available boron extracted from the soil by hot water					Average
	November 15	November 24	December 6	December 14	December 22	
T_0	0.99	1.5	3.64	5.33	5.07	3.31a
T_1	1.38	3.27	8.04	5.89	5.81	4.88ab
T_2	2.16	3.95	8.89	6.7	8.53	6.05bc
T_3	4.23	4.67	9.24	9.66	8.9	7.34cd
T_4	5.2	8.04	9.65	12.05	9.77	8.94d
Average	2.79a	4.29a	7.89b	7.93b	7.62b	

Table 3. Available boron extracted from the soil by mannitol-calcium chloride (mg kg^{-1})

Treatments	Available boron extracted from the soil by mannitol - calcium chloride					Average
	November 15	November 24	December 6	December 14	December 22	
T_0	5.23	4.53	3.89	3.46	2.76	3.97a
T_1	5.45	4.58	3.99	3.56	3.22	4.16ab
T_2	5.5	4.97	3.99	3.69	3.48	4.32abc
T_3	6.01	5.17	4.19	3.7	3.57	4.53c
T_4	6.24	5.29	4.25	3.76	3.59	4.63cd
Average	5.67e	4.91d	4.06c	3.63b	3.32a	

Table 4. Available boron extracted from soil by ammonium bicarbonate + 0.005M DTPA mg kg^{-1}

Treatments	Available boron extracted from soil by ammonium bicarbonate + 0.005M DTPA					Average
	November 15	November 24	December 6	December 14	December 22	
T_0	1.8	1.8	0.44	0.06	0.04	0.83a
T_1	1.81	1.85	0.52	0.07	0.06	0.86ab
T_2	1.87	1.86	0.52	0.13	0.07	0.89ab
T_3	2.0	1.95	0.59	0.13	0.06	0.95bc
T_4	2.05	2.07	0.7	0.15	0.14	1.02bc
Average	1.91c	1.91c	0.55b	0.11a	0.074a	

Table 5. Available boron extracted from soil by 0.005*M* DTPA mg kg^{-1}

Treatments	Available boron extracted from soil by 0.005M DTPA					Average
	November 15	November 24	December 6	December 14	December 22	
T_0	2.99	2.53	1.74	1.67	2.15	2.22a
T_1	3.17	2.6	1.86	3.27	2.28	2.64a
T_2	3.24	2.95	1.9	3.29	2.3	2.74ab
T_3	3.31	2.97	2.2	3.43	2.35	2.85b
T_4	3.6	3.04	2.06	3.9	3.05	3.13b
Average	3.26cd	2.82ab	1.95a	3.11cb	2.43a	

due to the nature of the chemical solutions used in the extraction in the first class, bearing in mind that the dilution ratio is constant for all extractants. The concentrations of boron extracted by hot water and estimated by the azomethine –H ranged between 3.74 -15, 23 mg B kg^{-1} for soils planted in Diyala Governorate. Available boron extracted with mannitol-calcium chloride extractant were within the values obtained by Goldberg and Suarez (2013), and ranged between 0.059 -30.9 mg B kg^{-1}. Available boron extracted with ammonium bicarbonate + DTPA were among the values obtained by Samiulah et al (2013), as the values ranged between 0.44-6.89 µg g^{-1} soil. .

Boron concentration in pods: The effect of the levels of added boron and fairies on the boron concentration in the pods as were 39.1, 47.0, 38.0, 22.5, 20.1 mg B kg^{-1} dry weight on during November 15, November 24, December 6 and December 14 and on December 22, respectively (Table 6). The second fairy gave the highest value 47.0 mg B kg^{-1} dry weight while the fifth fairy gave the lowest value 20.1 mg B kg^{-1} dry weight. The differences were significant between the second fairy and rest fairies and between the first and third fairies on the one hand and the fourth and fifth fairies on the other hand. The values of boron concentration in the pods increased with the increasing the levels of the added fertilizer and was 27.6, 29.5, 32.1, 37.4, and 40.0 mg B kg^{-1} dry weight with the fertilizer levels, respectively. The control treatment gave the lowest concentration 27.6 mg B kg^{-1} dry weight, while the T_4 treatment gave the highest of 40.0 mg B kg^{-1} dry weight. The differences were significant between the two treatments T_3, T4 on the one hand and the rest treatments on the other hand. The differences were not significant between T_1 and T_0. The reason of decrease in the values of boron concentration in the pods after the third fairy may be due to the decrease in the concentration of the available boron in the soil with the fairies which negatively affected the concentration of boron in the pods as it appeared from the decrease in available boron extracted from the soil with time in all extractants except for hot water. The increasing boron concentrations in the pods with the fertilizer levels is due to increasing in the available boron in the soil with the increasing in the added fertilizer levels (Alwan 2012).

Absorbed boron content of pods: The effect of adding different levels of boron on the amount of boron absorbed from the plant in the pods with time (the fairies), was 8.2, 15.0, 14.1, 6.1, 3.5 mg B experimental $unit^{-1}$ on during November 15, November 24, December 6 and December 14 and on December 22, respectively. The highest absorption value was in the second fairy 15.0 mg B experimental $unit^{-1}$ and the lowest absorption value was in the fifth fairy 3.5 mg B experimental $unit^{-1}$. There were significant differences between the second and third fairies on the one hand and the rest of the fairies on the other.

The results also showed the effect of adding boron fertilizer levels in the absorbed boron with the fertilizer levels

Table 6. Concentration of boron in pods in green bean plant (mg kg^{-1})

Treatments	Fairies					Average
	November 15	November 24	December 6	December 14	December 22	
T_0	31.8	41.7	32.3	16.7	15.6	27.6a
T_1	33.4	43.4	36.2	18.3	16.3	29.5ab
T_2	41.2	43.4	39.0	19.6	17.4	32.1b
T_3	42.3	51.2	40.7	29.0	38.8	37.4c
T_4	46.8	55.1	41.7	29.0	27.4	40.0c
Average	39.1b	47.0c	38.0b	22.5a	20.1a	

Table 7. Boron absorbed from the plant (Pods) in green beans (mg B)

Treatments	Fairies					Average	Absorbent kg ha^{-1}
	November 15	November 24	December 6	December 14	December 22		
T_0	4.5	5.0	3.9	1.7	1.4	3.3a	0.008
T_1	8.0	13.9	14.1	5.5	2.4	8.8b	0.002
T_2	11.1	17.8	19.5	5.1	3.3	11.4b	0.029
T_3	9.7	19.5	18.7	7.8	5.5	12.2b	0.031
T_4	7.5	18.7	14.2	10.4	4.9	11.1b	0.028
Average	8.2a	15.0b	14.1b	6.1a	3.5a		

Table 8. Correlation coefficient (r) between the available boron extracted from the soil and absorbed from the plant (pods)

Extractants	Available boron extracted from soil mg (kg^{-1} soil)					Absorbed boron mg experimental $unit^{-1}$ ($4m^2$)					Correlation coefficient
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	
Hot water	3.3	4.9	6.1	7.3	8.9	3.3	8.8	11.4	12.2	11.1	0.82*
Mannitol+$CaCl_2$	4.0	4.2	4.3	4.5	4.6						0.84*
DTPA	2.2	2.6	2.7	2.9	3.1						0.89*
DTPA+NH_4HCO_3	0.83	0.86	0.89	0.95	1.0						0.72

indicate 3.3, 8.8, 11.4, 12.2, 11.1 mg B experimental $unit^{-1}$ with the fertilizer levels sequentially, as the highest value of absorbed B appeared in treatment T_3 and the lowest absorption in control. The differences were significant between all fertilizer levels on the one hand and the control treatment on the other while the differences were not significant between the levels of fertilizer itself**.** The uptake of any nutrient ion is a reflection of two parameters the dry weight of the plant part and the concentration of the nutrient ion in that plant part (Havlin et al 2014).

Correlation relationship between available boron extracted from the soil and absorbed from the plant: The simple correlation coefficient (r) between the rate of available boron extracted from the soil with the four extractants of five fairies and the rate of boron absorbed from the plant (pods) for five fairies (Table 8). The values of the correlation coefficient were 0.72, 0.89, 0.84, 0.82 for extractants DTPA + NH_4HCO_3, DTPA, Mannitol + $CaCl_2$, and hot water, sequentially**.** The results showed that DTPA extract gave the highest significant correlation coefficient r 0.89* while DTPA+NH_4HCO_3 extractant gave the lowest correlation coefficient 0.72. The extractants of mannitol and hot water also recorded a significant correlation 0.84*, 0.82* in sequence. The extractants can be arranged according to their preference according to the correlation coefficient values was PA > Mannitol + $CaCl_2$ > Hot Water > DTPA + NH_4HCO_3.

The results can be interpreted according to characteristics of the boron ion and the characteristics of these extractants. Boron is one of the anions as it carries a negative charge which means that it is not held on the exchange surfaces and as a result it is found in the soil solution in the form of boric acid or it is adsorbed in the basic conditions, The main adsorption sites for boron are Si-O and Al-O bonds at the edges of clay minerals and the surfaces of iron and aluminum oxides (Havlin et al 2014). As for the role of extractants is through the composition of the chemical or organic extractants and since boron does not adhere to colloidal surfaces. So it does not need a negative ion to replace it and here comes the role of organic and chelating substances in chelating the boron ion and preventing it or reducing its adsorption on the above-mentioned surfaces. This is why both the extract of DTPA and Mannitol showed a high efficiency in extracting available boron because the two are organic materials, especially DTPA, which is a chelating substance. Mannitol is an organic substance that has a sucrose-like structure that chelates boron and increases its availability. According to these results DTPA can be adopted as an extractant of boron in addition to the rest of the micro-nutrients as well as mannitol. As for the hot water extractant which is one of the main boron extractants its efficiency can be attributed to the role of heating and heat in releasing the adsorbed boron on the aforementioned surfaces, as it is known in chemistry that chemical reactions accelerate by heating and this is what actually happens during the extraction process. It can be said that the effectiveness of these three extracts is due to their simulating the action of plant roots in extracting plant nutrients from the soil.

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Effect of Chemical Fertilization and Foliar Spray with Auxine (IAA) and Benzyl Adenine on Growth and Flowering of Gerbera Jamesonii H. Bolus

Maad Yahya Dari and Abdulrahman A. Raheem

Collage of Agriculture, University of Diayla, Diayla, Iraq
E-mail: mdalhmyl@gmail.com

Abstract: The experiment was conducted at Diyala University during 2019-2020 season .The objective of the experiment were to investigate the effect of chemical fertilizing at four levels of NPK (0-50-100-150 mg L^{-1}) and foliar spray of Auxin (IAA) with two concentrations (50, 100 mg L^{-1}) and Benzyl adenine (BA) sprayed with two concentrations (100, 200 mg L^{-1}) and control treatment which sprayed with distilled water. Crop was sprayed two times on December 25, 2019 and January 25, 2020 on during growth and flowering stage of *Gerbera jamesonii* H. Bolus cv Great smokey mountain. Chemical fertilization at 150 mg L^{-1} provided the best results regarding plant height (19.41 cm) , number of inflorescences $plant^{-1}$(4.93 inflorescences) and nitrogen, phosphorus, potassium percentage in the leaves which are (2.7 , 0.36 and 2.41%), while treatment with 100 mgL^{-1} NPK registered the highest inflorescences diameter (8.96 cm) and stalk length (35.94 cm). The result of the foliar spray with Benzyl adenine at 200 mg L^{-1} recorded the maximum plant height (17.35 cm) and nitrogen, phosphorus, potassium in the leaves (0.37, 2.33 and 2.29%), while treatment with 50 mg L^{-1} IAA 50 and 100 registered the highest inflorescences diameter (8.72cm) and stalk length (35.64 cm) and number of inflorescences $plant^{-1}$ (4.19 inflorescences).

Keywords: Benzyl adenine, IAA, Gerbera, Fertilization

The cultivation of economic ornamental plants with suitable flowers for picking has become a wide and large trade in the world after the development of its production and marketing. *Gerbera jamesonii* H. Bolus, also known as Transvaal Daisy, belongs to the Asteraceae family and is one of the most important cut flowers cultivated around the world (Pattanashetti 2012). There are hundreds of cultivated genotypes which are hybrids resulting from the hybridization between the two species *jamesonoo X G* and *viridifolia*. The production of ornamental plants and cut flowers has now become a large and wide industry and trade in developed countries also and companies compete to a large degree for this resource, as gerbera is one of the most important cut flowers. Gerbera is produced in many countries, including America, Canada and Philippines and India (Pattanashetti 2012) and its economic importance comes in fifth place after rose, davidii, tulip and lily in global production (Lim et al 2012). Important to assess the quality of flowering after harvesting, and the phenomenon of curvature of the flowering stalks. These may be due to hereditary factors, water relations, storage temperature, post-harvest treatments, plant nutrition, or some hormones (Nazarideljou and Azizi 2015). Chemical fertilization is an important factor affecting plant growth by providing plants with the nutrients necessary for their growth. The present study was conducted with the aim of finding out the effect of spraying the gerbera plant at different concentrations of growth regulators, (benzyl adenine BA and IAA) and fertilizing with chemical fertilizers on the vegetative and flowering growth of gerbera and the possibility of improving the quality of flowers.

MATERIAL AND METHODS

The experiment was carried out during the fall season 2019-2020 at Diyala University during 2019 - 2020. The planting took place in Sanadsen with a diameter of 22 cm. The cultivation medium used in the experiment was prepared from a mixture of sandy soil and Albeetmos in a ratio of 3^{-1}.

The experiment included a study of two factors. The first factor represented chemical fertilization with NPK at four levels were added with irrigation water, where the comparison treatment that uses irrigation with water only, F0 and 50,100 and 150 mgL^{-1} as F1, F2 and F3. The fertilizer solutions are prepared for watering into the soil throughout the experiment period and using fertilizer NPK (20: 20.20). The second factor included is the foliar spraying with growth regulators auxin (IAA) at 50 and 100 mgL^{-1} and benzyl adenine (BA) at 100 and 200 mgL^{-1} in addition to the comparison treatment. The growth regulators were prepared by dissolving the materials according to concentrations of auxin benzyl adenine and was dissolved using (several

drops) of (NAOH) (1 standard) and then the volume was completed to 1 liter distilled water and 1 ml. Liter^{-1} from the diffuser Tween-20 to spray solutions. The plants were sprayed with twice the first spray was in December 25, 2019 and January 25, 2020 and the plants were sprayed until complete wetness in the early morning. The experiment was carried out as a factorial experiment (4 x 5) according to the randomized complete block design. The experiment included 20 treatments with three replications, thus the number of experimental units would be 60 experimental units. The data were analyzed according to the SPSS statistical and compared using the Duncan's multiple Range test at a 5% probability level.

RESULTS AND DISCUSSION

Plant height: The chemical fertilization and 150 mgL^{-1} was significantly superior (19.41 cm) to 50 ,100 mg liter^{-1} (15.79 and 17.46 cm, respectively). The control treatment gave the lowest height (15.06 cm). Foliar spraying with growth regulators had a significant effect on plant height and auxin at a 100 mgL^{-1} resulted maximum height (17.79 cm), and but did not significantly differ from the rest of the treatments except for the spraying with benzyl adenine of 100 mg^{-1} liter (16.05 cm). The interaction treatment BA 200 × F3 was significantly superior with plant height of 21.66 cm, while the treatment BA200 × F0 gave the lowest height (14.11 cm) (Table 2).

Inflorescence numbers: There significant effect of chemical fertilization on inflorescence number (Table 2). The treatment with concentration 150mg liter^{-1} was significantly superior with highest number of inflorescences (4.93 flower-plant^{-1}), which did not differ significantly from 50 to 100 mg liter $^{-1}$ (3.61 and 4.45 inflorescence. plant^{-1} respectively), while the comparison treatment gave the lowest number of inflorescences, 2.51 inflorescence plant^{-1}.Foliar spraying with growth regulators had a significant effect on the number of inflorescences. The IAA treatment with a concentration of 100 mg^{-1} resulted in the highest number of inflorescences (4.19 plant^{-1}) and did not differ significantly from the rest of the treatments except for the BA treatment with a concentration of 100 mgL^{-1} which gave the least number of inflorescences (3.55 inflorescence plant^{-1}). The interaction showed significant effect, the treatment IAA 100x F3 significantly outperformed with the highest number of inflorescences (5.88 plant^{-1}) while the treatment C0x F0 gave the lowest number of inflorescences (2.16).

Stalk length: There was significant effect of chemical fertilization, as the treatment with concentration 100 mgL^{-1} was significantly superior to giving the highest inflorescence stalk length of 35.94 cm, which was not significantly different from the two fertilization treatments with two concentrations, 50 and 150 mg per L^{-1} (Table 3). The comparison treatment gave the lowest stalk length, which was 22.56 cm. Foliar spraying with growth regulators had a significant effect on the stalk length, as the treatment IAA with a concentration of 100 mgL^{-1} resulted in the stalk length of 35.64 cm and did not significantly differ from the rest of the treatments except for the BA treatment with a concentration of 200 mgL^{-1} which gave the lowest stalk length of 33.51 cm. The interaction

Table 1. Chemical and physical properties

Parameter		Value
Electrical conductivity EC 1: 1		2.4
PH 1: 1		7.11
Ready nitrogen N		35 mg k^{1}
Ready phosphorous P		1.94 mg kg^{-1}
Ready potassium K		126.29 mg kg^{-1}
Organic matter OM		7.5g kg^{-1}
Carbonate calcium CaCo3		292.1g kg^{-1}
Dissolved calcium Ca^{+2}		8.8 Milli equivalent liter^{1-}
Dissolved magnesium Mg^{+2}		6.8 Milli equivalent liter^{1-}
Dissolved sodium Na^{+2}		4.65 Milli equivalent liter^{1-}
Dissolved bicarbonate HCO_3		1.3 Milli equivalent liter^{1-}
Dissolved chlorine Cl		18.4
Dissolved potassium K		0.18
Soil separators	Sand	77.6 Grams kg^{-1}soil
	Silt	12 Grams kg^{-1}soil
	Clay	10.4 Grams kg^{-1}soil
Class		Sandy loam

Table 2. Effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine on the plant height (cm)

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
NPK control	16.66Cdef	15.11Def	14.66Ef	14.77Ef	14.11f	15.06b
NPK50	16.33Cdef	15.66Cdef	16.77cdef	15.00Ef	15.22def	15.79ab
NPK100	15.66Cdef	17.77Bcdef	18.88abcd	16.55cdef	18.44abcde	17.46ab
NPK150	19.11Abc	17.55Bcdef	20.88Ab	17.88bcdef	21.66a	19.41a
Growth regulators	16.94Ab	16.52Ab	17.79A	16.05b	17.35a	

between the two studied factors showed a significant effect, as the treatment IAA 100 × F2 was significantly superior, as it gave the highest stalk length of 37.38 cm, while the treatment BA200 × F0 gave the lowest Stalk Length of 29.16 cm.

The results showed that there were significant differences in the diameter of the flowerpot, and it may be due to the fact that both nitrogen and phosphorous lead to nutrient absorption and stimulate blooming and increase the diameter of the flowering flower in Gerbera. This may be due to the presence of potassium, which is a major active component in plant cells that contributes to cell division and enhances the ability of the plant cell to retain water and nutrients. Potassium also results in superior inflorescence diameter due to improved photosynthesis efficiency. It was agreed by Mohariya (2004) for the same results on Gerbera.

Inflorescence diameter: There was significant effect of chemical fertilization, treatment with concentration 100 mgL^{-1} was significantly superior by with highest diameter of inflorescence of 8.96 cm, which was not significantly different from the two fertilization treatments with two concentrations, 50 150 mgL^{-1} (Table 4). The comparison treatment gave the lowest inflorescence diameter which was 7.89 cm. Foliar spraying with growth regulators had a significant effect in the diameter of the flower, where the IAA treatment with a concentration of 50 mgL^{-1} resulted in the highest inflorescence diameter of 8.72 cm, and did not significantly differ from the rest of the treatments except for the BA treatment with a concentration of 200 mg L^{-1}, which gave the lowest inflorescence diameter of 8.50 cm. The interaction between the two studied factors showed a significant effect, treatment IAA 50 × F3 significantly outperformed with highest inflorescence diameter of 9.24 cm, while the treatment BA200 × F0 gave the lowest inflorescence diameter of 7.73 cm.

Nitrogen: There was significant effect of chemical fertilization, where the treatment with concentration 150 mgL^{-1} was significantly superior with the highest percentage of nitrogen in the leaves of the plant (2.7%), which did not differ significantly from concentrations 50 and 100 mgL^{-1} while the comparison treatment gave the lowest nitrogen percentage (1.47%). Foliar spraying with growth regulators had a significant effect on nitrogen ratio, as the BA treatment with a concentration of 200 mgL^{-1} resulted in the highest nitrogen percentage reaching 2.29% and did not significantly differ from the rest of the treatments except for the comparison treatment (1.50%). The interaction showed a significant effect, as the treatment BA 200 × F3 significantly outperformed with highest nitrogen percentage for the plant (3.38%), while the treatment C0 × Fo gave the lowest nitrogen ratio of (1.28%).

Phosphorous: There was significant effect of chemical fertilization, where the treatment with concentration 150 mgL^{-1} was significantly superior by giving the highest percentage of phosphorus in the leaves of the plant, (0.36%), which did

Table 3. Effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine and their interactions on the inflorescence numbers

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
NPK Control	2.16f	2.27f	2.44ab	2.83abc	2.88abc	2.51b
NPK50	3.71def	4.27a	3.56abcd	3.55abcd	2.99abc	3.61ab
NPK100	4.83ef	4.49ef	4.88ef	3.72def	4.33ef	4.45ab
NPK150	5.22ef	4.72ab	5.88a	4.10def	4.77ef	4.93a
Growth regulators	3.98ab	3.93ab	4.19a	3.55b	3.74ab	

Table 4. The effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine and their interactions on the Stalk Length (cm) of Gerbera plant

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
NPK control	34.38cd	32.50d	34.11bc	32.66d	29.16d	32.56B
NPK50	34.66abc	35.83abc	34.27abc	35.44abc	33.11cd	34.66Ab
NPK100	35.61cd	36.05ab	37.38a	35.33cd	35.33cd	35.94A
NPK150	34.33abc	36.33ab	36.83ab	35.33ab	36.44ab	35.85Ab
Growth regulators	34.74ab	35.177ab	35.64a	34.69ab	33.51b	

not differ significantly from the two fertilization treatments with two concentrations 50 and 100 mgL^{-1} (Table 7). The comparison treatment gave the lowest percentage of phosphorus (0.15%). Foliar spray with growth regulators had a significant effect on the percentage of phosphorous, as the BA treatment with a concentration of 200mgL^{-1} resulted in the highest phosphorous percentage reaching 0.37% but did not differ significantly from the rest of the treatments except for the comparison treatment(0.17%). the interaction showed a significant effect, as the treatment BA 200 x F3 significantly outperformed it, as it gave the highest percentage of phosphorus (0.54%,) while the treatment C0x Fo gave the lowest percentage of phosphorus (0.13%).

Potassium: There was significant effect of chemical fertilization, where the treatment with concentration 150 mgL^{-1} was significantly superior with highest percentage of potassium in the leaves of the plant (2.41%), which did not differ significantly from two fertilization treatments (Table 7). The comparison treatment gave the lowest percentage of potassium (1.42%). Foliar spraying with growth regulators had a significant effect on the percentage of potassium, treatment BA with a concentration of 200 mgL^{-1} resulted in the highest potassium content reaching 2.33% and did not differ significantly from the rest of the treatments except for the comparison treatment (1.45%). The interaction showed a significant effect, as the treatment BA 200 x F3 significantly outperformed (3.03%) while the treatment F0 x C0 gave the lowest percentage of potassium (1.24%)

The significant increase in, nitrogen, phosphorus and potassium levels is due to the levels of NPK present in the soil, because NPK is effective in providing balanced and favorable nutritional requirements in both soil roots and in the plant system (Singh and Singh 2004). Where the vital components such as nucleic acids and components increase to produce catalytic and that increase both the carbohydrate contents and NPK Also, phosphorus is an essential nutrient

Table 5. Effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine on the inflorescence diameter (cm)

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
NPK Control	7.88e	7.78e	8.12de	7.94e	7.73e	7.89B
NPK50	8.76abc	8.75abc	8.45de	8.78abc	8.50de	8.64Ab
NPK100	8.97abc	9.14abc	8.94Abc	8.90abc	8.86abc	8.96A
NPK150	8.64Cde	9.24a	9.03Abc	8.63cde	8.94abc	8.89Ab
Growth regulators	8.56Ab	8.72a	8.63Ab	8.56ab	8.50b	

Table 6. Effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine on the percentage of nitrogen in leaves

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
Control NPK	1.28G	1.48Fg	1.58Efg	1.44fg	1.57efg	1.47B
NPK50	1.53Fg	1.87Def	2.06Cde	2.23cd	1.85def	1.90Ab
NPK100	1.59Efg	1.78Defg	1.82Def	2.12cd	2.39c	1.94Ab
NPK150	1.61Efg	2.48Bc	2.92Ab	3.11a	3.38a	2.7A
Mean growth regulators	1.50b	1.90ab	2.09Ab	2.22ab	2.29a	

Table 7. Effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine on the percentage of phosphorus

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
Control NPK	0.13I	0.15I	0.17Ghi	0.16hi	0.18ghi	0.16B
NPK50	0.18Ghi	0.23Efg	0.22Efgh	0.26de	0.31cd	0.24Ab
NPK100	0.19fghi	0.25De	0.30Cd	0.35c	0.46b	0.31Ab
NPK150	0.19fghi	0.28De	0.35C	0.45b	0.54a	0.36A
Growth regulators	0.17b	0.22ab	0.26Ab	0.30ab	0.37a	

Table 8. Effect of chemical fertilization and foliar spraying with auxin (IAA) and benzyl adenine on the percentage of potassium in of leaves

NPK	Growth regulators					NPK mean
	Control	IAA50	IAA100	BA100	BA200	
Control	1.24I	1.42Hi	1.50Ghi	1.47ghi	1.50ghi	1.42b
NPK50	1.50Ghi	1.69Fgh	1.76Fgh	2.04def	2.30cde	1.85ab
NPK100	1.54Ghi	1.80Fg	2.03Ef	2.38cd	2.51bc	2.05ab
NPK150	1.55Ghi	2.20Cde	2.52Bc	2.78ba	3.03a	2.41a
Growth regulators	1.46B	1.77ab	1.95Ab	2.16ab	2.33a	

in the various metabolic processes and a major component of energy compounds, nucleic acids, phospholipids and coenzymes (Nair 2002). Moreover, phosphorous plays a central and regulatory role in metabolism and the relationship between many physiological and biochemical processes in plants, in addition to photosynthesis, energy conservation, and intercellular coordination of carbohydrate metabolism and energy transfer processes .K plays an essential role in activating enzyme and protein synthesis, photosynthesis, regulation of osmosis, energy transport, cation balance, and stress resistance, thus increasing the NPK level leads to an increase in the level of carbohydrates, nitrogen, potassium and phosphorous

CONCLUSIONS

Gerbera plant responded to fertilization with a concentration of 150 mg L^{-1} in all studied traits. This response was directly proportional to the increase in concentration. Treatment of plants with benzyl adenine at a concentration of 200 mg L^{-1} gave the best results and for most of the studied traits. Treatment of plants with bandol acetic acid at two concentrations of 100 and 50mg^{-1} liter gave significant results. The data of most traits indicate that the benzyl adenine is 200 mg L^{-1} and the chemical fertilization concentration of 150 mg. L^{-1} was significantly superior for most of the traits.

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Influence of Qalat Sukkar on Al-Gharraf River Water Quality

Hussein Khalefa Chlaib, Israa Najim Abdulla, Ahmed A. Saheeb and Jalal Hameed Ali

Department of Soil Sciences and Water Resources, College of Agriculture,
University of Sumer, Al Refaee, Thi-Qar, 64005, Iraq
E-mail: hkaldobayany@yahoo.com

Abstract: Al-Gharraf River is the main source of water in Qalat Sukkar city. The city influence on river water quality is the main aim of this study. Three water samples were taken from the entry point of Al-Gharraf River to the city and three samples were taken from the exit point on the length of the river passing the city. pH, turbidity, electrical conductivity ,dissolved solids, calcium, carbonate, bicarbonate, dissolved oxygen and chlorine concentrations. The city has added an amount of salts to the river, as well as the electrical conductivity increased. The study also revealed a decrease in calcium concentration, dissolved oxygen, turbidity and bicarbonate after the river exits from the city. There was no increase or decrease was recorded for chlorine ions concentration. Comparing results of the positive and the negative ions and TDS with the World Health Organization standards shows that Al-Gharraf water is polluted and alkaline in the city. According to WHO (2004) and Iraqi drinking water specification, the studied elements were beyond the permissible limits except the TDS and Cl, which were within the limits. The river water suitable for irrigation and animal consumption.

Keywords: Al-Garraf River, Qalat Sukkar, Water quality, pH, TDS, Dissolved Oxygen

Water is one of the most important elements necessary for life on the earth planet, so no internal biological process takes place in the body of any living organism without water. Water is considered one of the basic elements in the formation of the human, animal and plant. The economies many of countries' depend on water. Water covers about 70% of the earth surface, most of this water is salt water that full seas and oceans. The fresh water, that humans and animals used is about 3% of the water covers the surface of the earth (Naseem 2007). Rivers and lakes are the most important sources of fresh water. Rivers water is still the main source for meeting human needs and reduces water pollutants resulting from human action. Pollution is a quantitative or qualitative change in biological and biological components outside the natural ranges that leads to an ecological imbalance (Al-Saadi et al 1986, Mawloud et al 1991). The problem of pollution has been increasing continuously in recent years (Ramprasad et al 2021) in many countries, including Iraq. The natural environment, including water resources, is suffering from pollution as a result of neglect and lack of maintenance of water resources. The increase in the population and urban expansion in Iraq in recent years has led to an increasing in the demand for water resources.

The percentage of pollutants increases with the increase in population, urban and industrial development and agricultural activity, because most of the water uses are non-consumptive uses for it, and large amount of water may return to the water source in the form of heavy water loaded with various types of pollutants. Household wastewater is a major source of pollution, as there is a difference in the nature and contents of heavy water (Al-Khair 2001).The pollution in rivers and lakes makes them unfit for human use. As the sewage that is thrown into the river from houses, factories, hospitals, slaughter sites, etc., contains many microorganisms that pollute the water, which was the cause of many of the epidemic diseases that hit and sweep the world (Al-Rubaie 2002).

MATERIAL AND METHODS

Study area: The study area is located in Qalat Sukkar on of Dhi Qar governorate cities, in southern Iraq, the distance between the samples taken from the beginning of Al-Gharraf River (the location where the river entered the city) and the samples taken from the end of the river (the location of its exit from the city) is about 8 km (Fig. 1). The Tigris River divided after Al-Kut Dam into two branches, the first one goes towards Maysan governorate and the second goes towards the city of Al-Shatrah in Dhi Qar governorate, which is called Al-Gharraf River and is located in the southern part of Iraq within the alluvial plain of Mesopotamia. The slope and speed of Tigris River decreases as moves towards the south. It loaded with large amounts of sediments. Al-Gharraf River is located between the Tigris and Euphrates rivers between latitudes 31.2-32.27 and longitudes 45.45-46.43. Its length about 230 km and continues to run to the southwest from Al-Kut dam passing through the cities of Al-Hayy, Al-Fajr, Qalat Sukkar, Al-Rifai, then Al-Gharraf city, down to its estuary in the marshes of Dhi Qar Governorate (Hassan 2004). This

research aims to assess the quality of the surface water represented by the water of Al-Gharraf River and whether Qalat Sukkar has a role or influence in the pollution of the river's water as well as determine the water use for all purposes according to local and international specifications.

Modeling method: Three samples were taken for the river's east bank, the middle of the river, and the west bank, with three replications for each sample and at November 14, 2020 in the morning from the entry point of the river into Qalat Sukkar city (Fig. 1). In the next day, three samples were taken from the river's east bank, the middle of the river, and the west bank, with three replications for each sample from the exit point of the river (Fig. 1). The water samples were placed in tightly closed (1 liter) plastic bottles and stored inside the refrigerator at an appropriate temperature. Through the exploratory and field visits to Al-Gharraf River in Qalat Sukkar city, a small number of sewage channels were observed, which dump their water into the river (Fig. 2 a, b). In addition to there was many piles of waste inside the river (Fig. 2.c). A pipe was also observed, it drains out the water of Qalat Sukkar water purification station (represented by the sample C (Fig. 2.d).

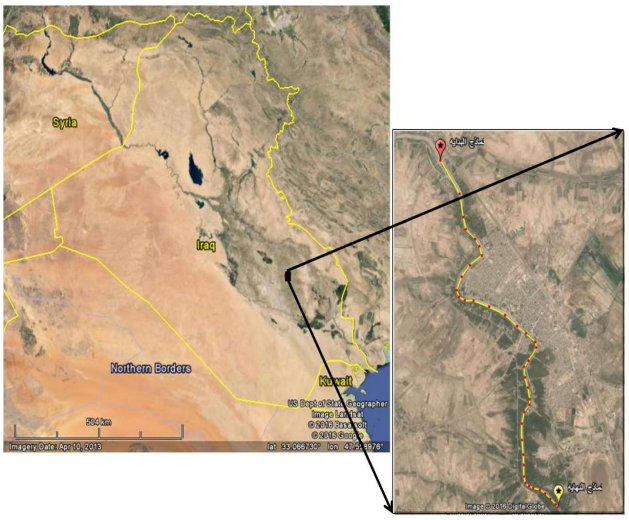

Fig. 1. Google earth image represents the location of the study area on the map of Iraq

Estimation of chloride: Chloride enters the human body through water and food, and its amount excreted from human body is about 6 g / person / day. Chloride was estimated in water using the Mohrs method. This method depends on the formation of insoluble silver chromates when the chloride is titrated with silver nitrate using the potassium chromate indicator (K_2CrO_4).

Silver Nitrate 0.01N $AgNO_3$ was prepared by dissolving 0.42 g of dry material in water, then the volume was supplemented with 250 ml of distilled water. Potassium chromate indicator is prepared by dissolving 20 g of the substance in water and the volume is supplemented with 200 ml of distilled water. The blank was prepared by taking 5 ml of distilled water and adding 4 drops of potassium chromate indicator to it and titrate with silver nitrate (0.01 N) until the color changed from yellow to orange according to the volume of silver nitrate coming from the burette. 5ml were taken from each sample and added 4 drops from the potassium chromate indicator to each sample, then titrate with silver nitrate until the color changed from yellow to orange to obtain the volume of silver nitrate and calculate the amount of chloride present in each sample as according to the equation (Table 1).

Cl Meq / l = (($AgNO_3$ volume of the sample- $AgNO_3$ volume of the blank * $AgNO_3$ Normality) / (water sample volume)) x 1000

pH: The pH meter device was used to measure the pH of the studied samples.

EC measurement: EC depends on the water temperature, increasing the water temperature by one degree celsius causes an increase in EC by 2%. EC increases by increasing

Table 1. Chemical and physical properties of samples of Al-Gharraf River water

Sample/Property	Beginning of the city samples			Ending of the city samples			Sample C
	1	2	3	1	2	3	C
pH	7.4	7.4	7.5	7.5	7.4	7.3	7.6
EC (mmho/cm)	1.02	1.02	1.02	1.23	1.24	1.23	1.05
TDS (ppm)	510.3	511.3	512.3	616.6	624.3	618.6	529
Cl (meq/l)	3.4	3.5	3.06	3.4	3.06	3.3	3.06
HCO_3 (meq/l)	53	43	46	43	46	36	30
CO_3 (meq/l)	0	0	0	0	0	0	0
Ca (meq/l)	32.2	34.32	22	23.92	17.86	15	16.8
DO (ppm)	6.53	7.25	6.92	6	4.72	4.73	6.96
TUR (NTU)	14.33	47.3	45.04	29.3	30.02	14.3	62

the dissolved salts (Detay 1997). EC meter device was used to measure the EC of the samples.

Total dissolved salts (TDS): TDS includes the total dissolved salts (both ionized and non-ionized) in solution and does not include suspended and colloidal substances and dissolved gases. TDS consists of the total of negative and positive ions. The concentration of dissolved ions in natural waters depends on the type of rocks and soils that are in contact with them and on the time period that the contact process takes (Hem 1970). TDS device was used to measure the total dissolved salts of the samples.

Turbidity: Turbidity is formed as a result of the presence of a proportion of suspended matter and water float organisms. The degree of turbidity depends on the amount, type, color and size of suspended substances. It is important to estimate the turbidity in the water because of its importance in the growth of fish and has been observed that the turbidity of the water causes some problems for shallow fish ponds when blocked sunlight from reaching plant organisms, therefore these plants will not able to producing oxygen (Naseem 2007). 10 ml of the sample was withdrawn by pipette and put into the test tube of the turbidity device, after cleaning the tube well with a clean piece of cloth and then the turbidity device was calibrated. The test tube was placed inside the device chamber and the device is turned on and wait until the readings are taken. The tube is washed with distilled water before and after each measurement.

Carbonate and bicarbonate: The natural source of alkalinity is limestone and dolomite sedimentary rocks from which carbon and sodium, calcium, and magnesium bicarbonate, are generated. Bicarbonate represents the general or predominant form of the base compounds. The interaction of water with limestone results in bicarbonate. 10 ml was taken from each sample and 3 drops of phenolphthalein were added to each sample, and no pink color was noticed, indicating the absence of carbonates, meaning that the value of (Y), which is the volume of acid required for titrations, is equal to zero (Table 1). Then, 5 drops of orange methyl were added to the same solution and titrated with 1N of sulfuric acid until the color changed from yellow to pink to obtain the volume of acid coming down from the burette (Z), the amount of bicarbonate in the sample is calculated.

HCO_3^- meq/L = ((Z-2Y)) x H_2SO_4 Normality) / (ml in a liquot) x 1000

Dissolved oxygen (DO): DO represents the dissolved oxygen in water and the oxygen factor plays an important role in the vertical distribution of animals. The 95% of all animals are located in the upper 2cm and 5cm of the upper layer, respectively. The decrease in oxygen concentration has a great effect on the degree of tolerance to other environmental factors such as temperature and salinity (Al-Saadi et al 2000). The DO device was used to measure the DO of the samples.

Calcium: Calcium is one of the most abundant alkaline earth elements in the earth's crust, and it is an essential element for plants and animals. Calcium ion is produced from dissolving processes of carbonate and gypsum sedimentary rocks, as well as from erosion of pyroxene and amphibole minerals group and feldspar (Hem 1989). EDTA (0.01 N) was prepared by dissolving 0.73 g of the substance in a volume of distilled water and then the volume was supplemented to liter. 5 ml were taken from each sample, 20 ml of distilled water were added to each sample, then 5 drops of sodium hydroxide NaOH (4N) were added and a little phenolphthalein indicator was added and then titrated with EDTA until the color changed from pink to purple to know the volume of EDTA goes down from the burette, calculation the amount of calcium in each sample.

Meq Ca / L = ((EDTA normality x EDTA volume goes down from burette) / sample volume)) x 1000

RESULTS AND DISCUSSION

The results of the current study, which included measuring some chemical and physical variables for the water of some wells in Al-Rifai district are shown in Table 1. pH values recorded a small change and the values were close to each other, the highest value of the pH was 7.5, while its lowest value was 7.3. This is due to the small change in the amount of dissolved salts in the water, the acidity increases with the increase of water salts. Iraqi soils are rich in calcium salts and hence they are the predominant salts in water that lead to giving Iraqi water the basic characteristic (Hassan 2004).

The EC values were ranging between 1.02- 1.24 mmhos / cm, the increasing in the EC was so slight which was due to the slightly increasing in the ratio of salts, as well as the geological nature and salt content of the lands were the river located. The highest value of the TDS was 624.3 ppm and lowest was 510.3 ppm, the reason for this is due to the slight increase in the concentration of ions (Al-Asadi 1983). The human activities such as drainage of agricultural lands, sewage and industrial wastewater (WSC 2007), as well as the dissolution of limestone leads to an increase in salinity (Al-Saadi et al 2000, Al-Lami et al 2002). The river water was classified as of good quality according to Bouwer (1978) (Table 2).

The highest chloride was 3.5 meq / l and the lowest was 3.06 meq / l. The reason for the presence of chlorine in the water is due to sewage water and pollution from plant

seepages, chemical industry waste and oil well operations (FDSDW 1996). The differences in chloride values are small, and the reason is the decreasing of sewers thrown in the river in Qalat Sukkar and the decreasing of industrial movement in the city. The atmosphere is considered a major source of bicarbonate in addition to its primary sources of minerals and sedimentary rocks. The highest value of bicarbonate in the studied samples was 53 meq / l and the lowest was 36 meq / l and accompanied that in the first case the pH was 7.4, while in the second case was 7.3. This bicarbonate concentration led to an increase in the pH and direction of the water to be basic. The highest value of calcium was 34.32 meq / l and the lowest was 15 meq / l. The reason is the solubility of limestone in water (Al-Adly 1992), also, the type of the salts that make up the soil surrounding the well, Iraqi soils are of a limestone nature (Buringh 1960). The rates of turbidity concentration (TUR) at the beginning of the Qalat Sukkar district was 35.5 FTU, and at the end of the Qalat Sukkar was 24.5 FTU. This decrease is due to presence of the colloidal substances, silt, clay, humic substances, organic matter, and the various plants and animals found in the water. DO values were ranging between 7.25 ppm, at the beginning of the river entering the city, and 4.72 ppm, when the river leaves the city. The reason for this decreasing in the DO values is due to the oxidation of materials and the activities of microorganisms which lead to the decomposition of organic materials reduce the level of dissolved oxygen in the water (Maitland 1990). The decrease may be attributed to an increase in the water current velocity due to the rise in the water level, the speed of wind movement and precipitation (Al-Zubaidi 1985, Al-Araji 1988). The results of the chemical analyzes of the water samples (Ca, Cl, HCO_3, TDS) were compared with the limits of positive and negative ions and salinity limits according to the specifications of the World Health Organization (WHO 2004). The concentrations of TDS, Cl confirm to the specifications of the World Health Organization, while the Ca, HCO_3 in all samples were exceeding the limits for WHO specifications as shown in Table 3. Since the pollution is a quantitative change in the biological and biological components outside the natural ranges, this means that the water of the river in Qalat Sukkar is contaminated.

The study also included a study of the water coming out of "Qalat Sukkar water purification station" (sample C), pH value was 7.6 which was larger than the pH values of the river water samples, The reason for this is due to the increase in the salts that increase its pH resulting from the purification processes. EC value of C sample was 1.05 mmhos / cm. TDS was also estimated, and it was close to the TDS value of the water of the Al-Gharraf River when it entered the city. Cl concentration of the C sample was equal to what is found in the river water samples. The bicarbonate of the C sample was about 30 meq / l, which was lower than the river samples values. DO for the C sample was close to that in the river samples. The C sample's turbidity was higher than what is found in river samples, the reason for this is due to the purification processes that throw impurities into this pipe. When comparing the results of the chemical analyzes of sample (C) with the specifications of WHO (2004). The values of Cl, TDS confirm to the specifications shown in Table (3). The values of Ca, HCO_3 do not comply with these specifications.

Assessment of water quality for drinking purposes: The basic determinants of drinking water depend on the basic elements (cations, anions), inorganic chemical properties, organic compounds, biological and radiological properties. The analysis process included only the basic elements, so it relied upon to assess the validity of the water of Al-Gharraf River for human drinking purposes after comparing it with the specifications proposed by the World Health Organization (WHO 2004) and the Iraqi specifications IEPID (1998) issued by the Iraqi Environment Protection and Improvement Department. All water samples do not meet the specifications, and the water is unfit and unsuitable for human drinking (Table 4).

Table 2. Bouwer classification of water according to its TDS content

TDS (ppm)	Water quality
1000>	Fresh water
10000-1000	Brackish water
35000-10000	Salty water
35000<	Brine

Table 3. Specifications of the World Health Organization for water quality according to the limits of negative and positive ions and salinity (WHO 2004)

Unit	Ca	Cl	HCO_3	TDS
ppm	75	250	125-350	500-1000

Table 4. The specifications of the World Health Organization (WHO 2004) and the Iraqi specifications of Iraqi Environment Protection and Improvement Department (IEPID 1998) of the drinking water

Property	Specification WHO 2004	Iraqi specification 1998
TDS	1000	1000
Ca	75	75
Cl	200	250
pH	7.5-8.5	6.5-8.5

Table 5. International physical standards for drinking water (Drinking water standards and science 2006)

Property	Unit	WHO specification	Iraqi specification	European specification	USA specification
pH		6.5 - 9.2	6.5 – 8.5	6.5 – 8.5	6.5 -9.5
Turbidity	NTU	2.5 - 5	5>	1	< 5

Fig. 2 a, b. Pictures of some sewage streams that drain their water into the river, c shows the piles of waste in the river, while d shows the pipe coming out of Qalat Sukkar water purification station

Table 6. Standard specifications of the general veterinary services in the United States of America (Crist and lower 1972)

Animals	TDS (ppm)
Domestic birds	2860>
The horses	6435>
Cattle (for milk purpose)	7150>
Cattle (for meat purpose)	10000>
Sheep	12900>

Table 7. Specifications of water for animal consumption (ppm) (Altovisiki 1962)

Ions	Very good water	Good water	Permitted water to be used	Water can be used	Upper limit
Ca	350	700	800	900	1000
Cl	900	2000	3000	4000	6000
TDS	3000	5000	7000	10000	15000

Table 8. Classification of irrigation water based on EC and TDS

	Class of the irrigation water		
	Good	Medium to good	Bad
EC (mmhos/cm)	> 0.7	3.0-0.7	< 3.0
TDS (ppm)	> 450	2000-450	< 2000

Table 9. Classification of water according to the concentration of chlorides

Chloride limits (ppm)	Water type
> 142	Excellent
142-250	Good
250-425	Allowed
425-710	Doubtful
< 710	Inappropriate

The results of the physical characteristics obtained from the study were compared with the international physical specifications for drinking water (Drinking water standards and science 2006), which are shown in (Table 5). It is clear from the comparison that the pH meets with all specifications. As for turbidity, in the other hand, it does not meet with all of these specifications, which confirms that water is not suitable for human drinking.

Water quality for irrigation and animal drinking: To demonstrate the validity of the water of Al-Gharraf River for the purposes of animal husbandry, the results of the chemical analysis were compared with the standard specifications of the general veterinary services in the United States of America (Crist and lower 1972) depending on TDS (Table 6) and was found that the water of Al Gharraf River is suitable for all animals drinking.

The result of chemical analyzes of water samples were also compared with specifications of Altovisiki (1962) for animal consumption (Table 7) and was observed that the water quality type was good - very good.

The classification of Ayers and Westocot (1994) was used for irrigation water, which depends on the values of EC and TDS. Al Gharraf water in Qalat Sukkar is good for medium use for irrigation (Table 8).

When comparing the results of chlorides for water samples with the classification of Scofield (1936) for irrigation based on chlorides in the water (Al-Muzaffar 2007) (Table 9). All water samples indicate that the water of river in the city is of excellent quality for irrigation.

CONCLUSIONS

The water of Al-Gharraf River in Qalat Sukkar is a polluted water, where pH, TDS, Cl were in compliance with

the specifications of the World Health Organization, while the concentrations of (TUR, Ca, HCO_3) exceeded the permissible limits. The river water when entering the city and after leaving it was polluted, city added an amount of salts, while the calcium ion and bicarbonate concentrations decreased, as well as the turbidity and DO, while the chlorine concentrations were almost equal. Qalat Sukkar city adds pollutants to Al-Gharraf River water, but to a limited degree. Water quality is suitable for irrigation and drinking of animals. The periodic measurements of Mg, SO_4, Na, TH, NO_3, PO of Al Gharraf River water should be recorded. The diversion of drainage water or agricultural lands water in the river should be avoided.

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Green Synthesis and Characterization of Zinc Oxide Nanoparticles by Iraqi Propolis Extract

Aseel A. Obaid, Suad S. AL-ghbban[1] and Ryaidh S.H. AL-Hussain[1]

Department of Chemistry, College of Science for Women,
University of Baghdad, AL-Jadriya, Baghdad, Iraq
[1]Minstry of Science and Technology, Director of Material Research, Baghdad
E-mail: aseel.abd1205a@csw.uobaghdad.edu.iq

Abstract: The present study was aimed at the biosynthesis of zinc oxide nanoparticles (ZnONPs) using an aqueous extract of Iraqi propolis, a straightforward, low-cost, and fast method. Zinc oxide nanoparticles were formed by adding an aqueous extract of Iraqi propolis to zinc acetate dihydrate solution with adjusted pH to 10 and temperature 70^0 C, changing color from yellow to light brown after drying. ZnONPs were confirmed using various techniques. UV-Vis spectrum absorption peak was observed at (271nm). Fourier transform infrared (FT-IR) Result showed functional group related to ZN-O at 619 cm^{-1}. Whereas the X-ray diffraction (XRD) pattern revealed well-defined peaks appearing for ZnO. The average size of the crystalline plane of the nanoparticles at 2 thetas was 17.79 nm. The particles size of atomic force microscopy (AFM) showed the ZnONPs nanoparticles was 7of 7.3 nm. The scanning electron microscope (SEM) analysis indicated the average particle size was 44,79 nm and the shape of the particles is spherical. These study indicate the production of zinc oxide nanoparticles in green synthesis.

Keywords: Green synthesis, ZnONPs, Iraqi propolis, Aqueous extract

Nanoparticles are used for many biomedical applications, including biological imaging. Biosensors deliver targeted drugs and photothermal therapy (Vickers et al 2017). There are many methods for producing nanoparticles, such as chemical, physical, and biological routes to reach the favorite product as metal or oxide nanoparticles. Physical routs require much excess energy, in chemical procedures, and unwanted by-products; for these reasons, biosynthesis methods are developed (Agarwal et al 2017). Nanoparticles (NPs) are formed by the reaction of chemicals present in bioactive natural and plant products where the minerals are reduced to their oxide to demonstrate good stability in the formation of NPs (Mishra et al 2015). Biological methods are included in the use of plants (Salem et al 2016), natural products (Hoseini et al 2015), microorganisms (Mortazavi et al 2017) by the technique of self-assembly from new nuclei of atoms that grow into a nanoparticle. Zinc oxide (ZnO) obtained a distinguished position from these minerals due to its wide applications in various fields (Dhanemozhi et al 2017). Zinc oxide (ZnO) is an inorganic metal oxide available for a wide range of nanostructures (Parthasarathy et al 2016), drug delivery, cosmetics and electronics (Andrade et al 2017). It is also more preferred due to its ability to stimulate, adsorb, and magnetic properties in addition to its filtering of ultraviolet rays, anti-cancer, drug delivery, antibacterial and antifungal applications (Vickers et al 2017). Propolis (bee glue) is one of the most intriguing honey bee products. It is a sticky dark-colored substance collected by honey bees from plants, which they mix with wax and use in the construction and modification of their nests. It is also a key component of honey bee social immunity, and it is frequently utilized in nutraceuticals, over the counter medicines, and cosmetics by humans (Al-hussain et al 2018).

Propolis is a lipophilic substance that is stiff and fragile at room temperature but stretchy, mushy, and highly sticky when heated (Hassan et al 2019). It has a pleasant aromatic odor and colors, including brown, green, and red (Zaccaria et al 2017). Propolis differs in chemical composition, varies with season, and region (Martinotti et al 2015) and for this reason, more than 300 different combinations have been identified so far (Huang et al 2014). Many compounds have been isolated from propolis, including flavonoid a glycans, volatile organic compounds, phenolic acids, and their esters, alcohols, and ketones, phenolic aldehydes, sesquiterpenes, quinones, coumarins, steroids, and amino acids (Šturm et al 2018). Propolis is a good source of natural antioxidants play an important role in reducing the risk of developing a wide range of chronic diseases (Yang et al 2011). It acts as an anticancer because it contains chemicals such as caffeic acid phenethyl ester, which have a role in the process of programmed cell death and its effect on cell proliferation (Forma et al 2021). The aim of the current study is to produce zinc oxide nanoparticles in an environmentally friendly manner from a natural product.

MATERIAL AND METHODS

Zinc acetate dehydrate (Zn (CH3COO) 2 2H2O) with purity 99.5% was obtained from BDH, England and Iraqi propolis from local markets in Baghdad. The distilled water was used throughout the synthesis of ZnO NPs, sodium hydroxide (NaOH).

Preparation of Iraqi propolis extracts: Iraqi Propolis was obtained from local markets in Baghdad city. It was kept in the freezer for 24 hours, then quickly cut. The impurities were removed, returned to the freezer for one hour, then crushed into small pieces. Aqueous extracts were obtained after mixing 50 gm from Iraqi Propolis with 500 ml distilled water in a conical flask (1000ml), and put on a heating magnetic stirrer, with continuous stirring at a temperature of 70^{0}C for 48hour, then this suspension solution separated by Whatman No. 1 filter paper, cooled to room temperature and stored in airtight volumetric bottles until use (Al-hussain et al 2018).

Synthesis of ZnO nanoparticles: ZnO NPs were synthesized by use of 0.1M of zinc acetate dihydrate (Zn(CH3COO)^2 2H2O) (2.195 gm) with 99.5% purity was dissolved in 100ml distilled water and stirred for two minutes (Dhanemozhi et al 2017). The 50ml aqueous extract of Iraqi propolis was mixed with 100ml of zinc acetate dihydrate in volume ratio 2: 1 and stimulated on a heating magnetic stirrer at 70^{0}C. The pH of the mixture was adjusted to10 by NaOH (1M). The color was changed, the generated nanoparticles were separated for 20 min in the centrifuge (4000 rpm). The pellets were collected and washed three times, twice with distilled water and once with ethyl alcohol. The separated pellets were dried in the oven at 70C$^\circ$ for 12 h and store in airtight bottles for further studies.

Characterization of ZnO nanoparticles: The characterization of ZnO nanoparticles using UV-Vis spectra of ZnO Nps was done by using a UV visible spectroscopy, Shimadzu UV - 1650, Japan with wavelength range 190.00 - 800.00 nm. FTIR spectra of the as-synthesized ZnO Nps were recorded using Fourier transform infrared FTIR spectroscopy (ABB / Spectro, lab / MB3000 / UK). The crystal structure of ZnO was analyzed by X-ray diffraction (XRD) to analyze diffractometer by XRD (6000/Shimadzu, Japan). AFM images of the ZnO NPs were recorded in AFM (NT-MDT/ Russia). All previous analyses were performed in the Ministry of Science and Technology. The surface morphology was examined using scanning electron microscopy (SEM) (Zeiss supra 55vp /Germany electron microscope unit) and was analyzed at Basrah University/ pharmacy college.

RESULTS AND DISCUSSION

Color changes: The results showed an alteration in color 15 minutes after mixing propolis extract with zinc nanoparticles from yellow to pale white after an hour and a half, in addition to increasing the intensity and density of the precipitate which steadily grows to achieve the highest degree of pale whiteness and density after 24 hours of mixing propolis extracts with zinc acetate combination, then into light brown granules after drying for Pr ZnONP (Fig. 1). The results agreed with studies that indicated that ZnONPs had been completely synthesized, metal ions are reduced to metal nanoparticles with the help of phytochemicals such as polyphenols, polysaccharides, alkaloids, vitamins, and amino acids (Ishak et al 2019, Agarwal et al 2017) and the stability of ZnONP was in an aqueous solution wave range of 190.00-800.00 nm.

UV-Vis spectrum studies: Figure 2 shows the UV-Vis spectrum of the as-synthesized ZnONPs, and maximum absorption occurred at 279nm indicates. Similar result were previously by Ghidan et al (2017) and Ekennia et al (2021).The asymptotic value was also observed by Ifeanyichukwu et al (2020).

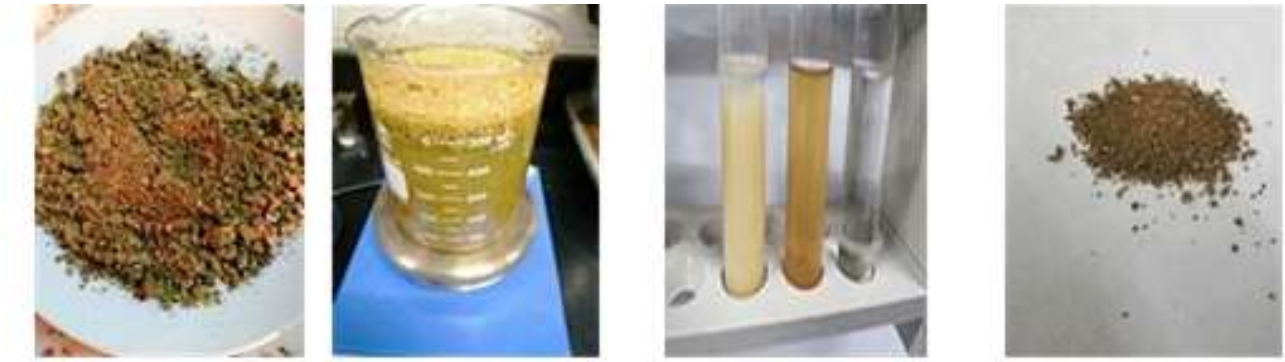

Fig. 1. Steps of preparing nano-zinc oxide as shown in the picture, where (1) Iraqi propolis, (2) Propolis with water, (3) Zinc acetate dehydrate dissolved in distilled water, B propolis extract, and C zinc acetate dihydrate mixture with propolis extract. (4) Zinc oxide nanoparticles

Fourier transform infrared (FTIR) spectroscopy: The absorption bands occur at 3474 cm^{-1} because of O–H stretching vibrations in water, phenols, and alcohol (Fig. 3) (Irshad et al 2018, Nezamabadi et al 2020). This absorption range confirms the propolis contains flavonoids, polyphenols, and alcohol functional groups that have different absorbances (Ifeanyichukwu et al 2020). The strong band at 1640cm^{-1} is due to the C=C stretch in the aromatic ring and C=O period in polyphenols (Abdullah et al 2020). The C-N stretch of amide-I in protein gives the band at 1414 cm^{-1} (Dhanemozhi et al 2017). The peak at 1048 cm^{-1} may be assigned to C-O and C-O-C stretching vibrations (Mohammadian et al 2018). The peak at 797 can be assigned to aromatic compounds (Ghidan et al 2017). The additional peaks appearing at 616,479 cm^{-1} in the IR spectrum of the ZnO NPs are the characteristic peaks of ZnO molecules. The presence of a higher percentage of phenolic group molecules is responsible for the reduction process. At the same time, amino acids and amide linkages in protein are shown to be necessary for the stabilization of ZnO nanoparticles. These results are in agreement with Dhanemozhi et al (2017).

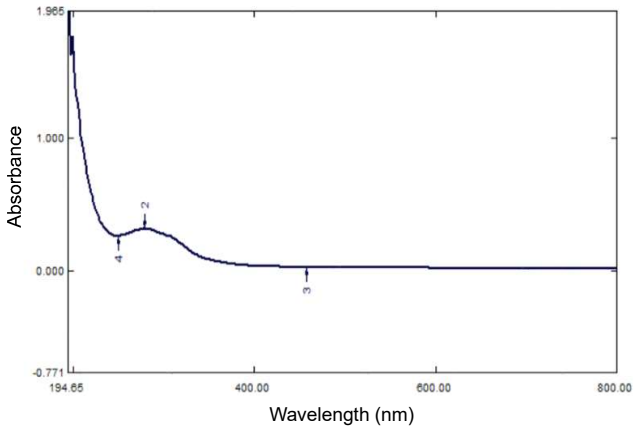

Fig. 2. UV-visible spectra of zinc oxide nanoparticles synthesized from Iraqi propolis extracts

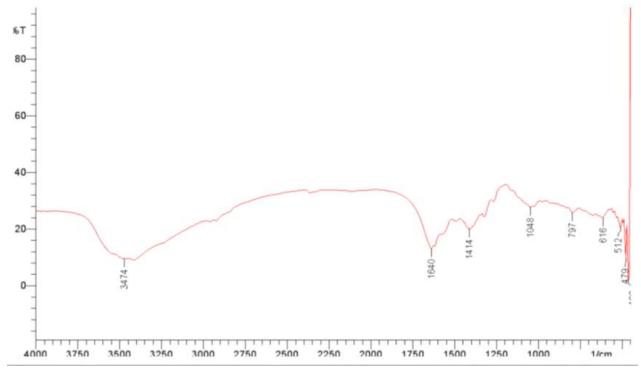

Fig. 3. FTIR spectrum of zinc oxide nanoparticles synthesized from Iraqi propolis extracts

X-ray diffraction analysis: The patterns of -ray diffraction (XRD) has been widely employed to characterize crucial materials properties of the compound, such as the types and nature of crystalline phases present (Umamaheswari et al 2018, Khatami et al 2018). Figure 4 represents the XRD pattern of biosynthesized ZnO NPs. The peak position with 2θ values of 31.39°, 34.61°, 36.41°, 47.72, 56.27, 62.85, 66.35, 67.98, 69.95 are indexed as 100, 002,101,102,110, 103,200,112 and 201 planes which are in agreement with those of ZnO powder obtained from data label (JCPDS-36-1451). This confirms the formation of a monocrystalline structure wurtzite structure of the ZnO NPs (Soliman et al. 2020). Zinc oxide nanoparticles were calculated in the crystal plane using the Debye-Scherrer equation (Ghidan et al 2017, Taherian et al 2019). The XRD pattern revealed the orientation and crystalline nature of zinc oxide nanoparticles average size of the synthesized nanoparticle was 17.79 nm and was similar to Sultana et al (2017) and Khanal et al (2020).

Atomic force microscopy (AFM): AFM is a technique that

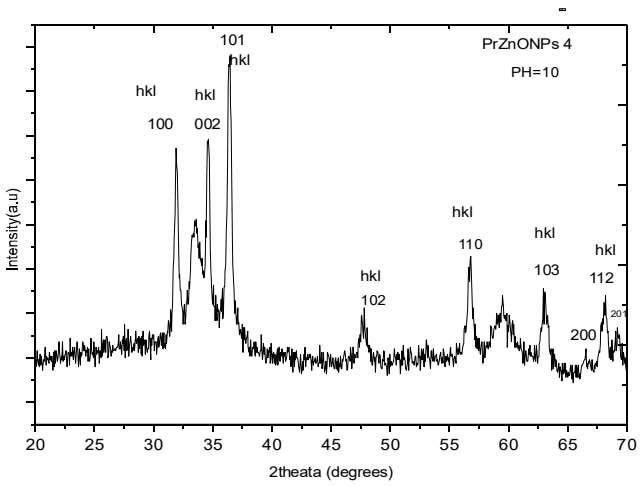

Fig. 4. X-ray diffractogram of zinc oxide nanoparticles synthesized from Iraqi propolis extracts

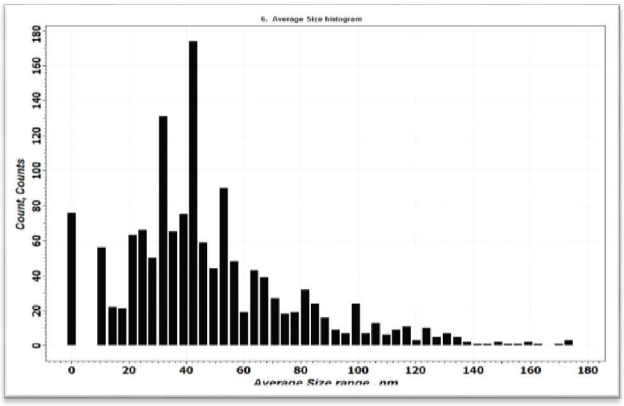

Fig. 5. Size distribution of zinc oxide nanoparticles synthesized from Iraqi propolis extracts

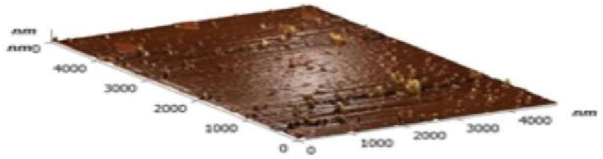

Fig. 6. AFM image showing the topographical 3D image of Zinc oxide nanoparticles synthesized from Iraqi propolis extracts

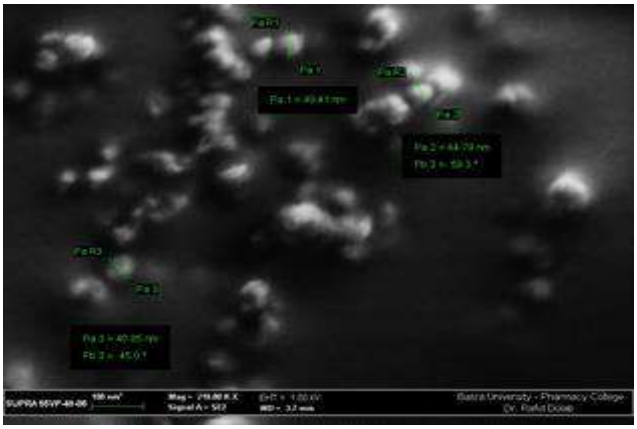

Fig. 7. SEM image of ZnO nanoNPs synthesized from Iraqi propolis extracts

uses extreme temporal and spatial resolution to investigate the structures and properties of biological systems and native biomaterials and study the shape, size, and surface area of produced nanoparticles (Li et al 2017). The size of ZnONPs was in the range 5 to 100 nm (Fig. 5). The average particle size was 77.3 nm. This indicates that the material is within the nanoscale. Through the slide assays, small nanoparticles were observed, and this was evident in the 3 dimensional slide and show the arrangement of ZnONPs (Fig. 6).

Scanning electron microscope analysis (SEM): The scanning image of ZnO NPs prepared from Iraqi propolis extract was explored using SEM (Fig. 7). The particles are spherical correspond in terms of shape with as reported by Umar et al (2019) and Muhammad et al (2019). Furthermore, have a wide surface area and the average particle size of 44,79nm. Similar results were reported by Husain et al (2019).

CONCLUSION

Biosynthesis of ZnO nanoparticles is easy, very low cost, fast, environmentally friendly, and non-toxic, synthesized by the aqueous extract of Iraqi propolis. ZnONPs particle size was 44,79 nm and the shape of the particles is spherical.

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Effect of Antibiotics on Biofilm Formation for *Pseudomonas aeruginosa* Isolates

Neyaf Majid Alageedi and Kareem Ibrahim Mubarak

Department of Biology, College of Science, Diyala University, Iraq
E-mail: Kareemmubarak9@gmail.com

Abstract: *Pseudomonas aeruginosa* is a gram-negative bacteria, belongs to the family of Pseudomonadaceae, non-fermenting, rod-shaped, and aerobic. That grows well at 37-42°C, positive for oxidase, measuring around 1-5µm long and 0.5-1µm wide. The study aims to explore the formation of biofilms and the effect of sub-minimum inhibitory concentrations (sub-MIC) antibiotics on their formation and investigates which genes are associated with this biofilm formation. Total of 200 specimens collected from different sources (urine, wounds, ear, burns and sputum) from governmental hospitals in Baqubah Diyala. The formation of biofilm was detected by the microtiter plate quantitative method. The serial dilution method was used to determine the minimum inhibitory concentration (MIC), sub minimum inhibitory concentration (Sub MIC) to study the change in the ability of *P. aeruginosa* isolates in the biofilm formation. Polymerase Chain Reaction (PCR) was carried out for detection of *pelA*,*pslA* and *pslD* genes. The 26 isolates 13% were identified as *P. aeruginosa* and 92.3% of isolates produced biofilm. Sub _MIC of imipenem and ceftazidime affected biofilm by decreasing the density of biofilm formation in most isolation after incubating for 24 hours. The prevalence rate of genes was 96.1% for *PslA* and *PslD* genes while 88.4% for *PelA* gene.

Keywords*: Pseudomonas aeruginosa,* Biofilm formation, Imipenem, Ceftazidime*, PelA, PslA, PslD*

Pseudomonas aeruginosa is an omnipresent gram negative aerobic bacterium, an opportunistic pathogen, widely spread and causes nosocomial infections (Al-Mayali and Salman 2020). This is in addition to fatal infections in immunocompromised individuals despite the emergence of newer and stronger antibiotics (Amoon *et al* 2018), such as patients with cancer, severe burns, or post-surgery or those with human immunodeficiency virus (HIV) (Gomila et al 2018). The wide spectrum of infection caused by bacteria depends on the presence of many virulence factors including biofilms. Biofilms are a virulence factor that has an advantage in several infections and significantly boosts the ability to be resistant to antibiotics and harsh environmental conditions (Samee et al 2020). They are sessile and organized communities of mono or multi species bacteria that adhere to biotics or abiotic surfaces (Costa et al 2021) and has ability to form biofilms on both biotics and abiotic surfaces which is an important factor contributing to the pathogenesis of *P. aeruginosa* (Saffari et al 2017). The matrix of bacterial biofilm consists of various polymers such polysaccharide, proteins, and extracellular DNA (eDNA). The core component of biofilm production in *P. aeruginosa* is the biosynthesis of exopolysaccharides (EPS) known as polysaccharide encoding locus (Pel) and polysaccharide synthesis locus (Psl), which are involved in the development and maintenance of structural biofilm scaffolding and protection against antimicrobials and host defenses and are the most important exogenous polysaccharides exploited in the formation of biofilms (Moradali and Rham 2019). Understanding the effects of antibiotics on biofilms is of paramount importance in clinical practice due to the increased resistance of antibiotics and dissemination of resistance in biofilms. Accordingly, the current study was proposed to explore the formation of biofilms and the effect of sub-MIC of antibiotics on their formation and investigate which genes are associated with this biofilm formation in this species of pathogenic bacteria.

MATERIAL AND METHODS

Collection of specimens and bacteriological identification: This study conducted from September 2020 to January 2021. The pathological specimens taken from different sources included urine, wound, ear, burn, sputum from Baqubah General Teaching Hospital include males and females of different ages. The specimens were cultured on Maconkey's agar and Blood agar and isolates were confirmed on *Pseudomonas* agar and incubated at 37C for 24 hours. The colony characteristics including colony texture, shape and colour, and edges were observed in addition to its ability to lysis of red blood cells on the medium of blood agar and lactose non-fermentation on the medium of the Mackonkey agar (Tille 2017). Biochemical tests, including gram-stain, IMVIC tests, catalase, oxidase, growth on pseudomonas agar, growth at 42°C and 4°C and then isolates were confirmed with the VITEK-2 Compact system. The biochemical tests were carried out to confirm the

identification of isolates according to (Cappuccino and Welsh 2020).

Biofilm formation: The formation of biofilm was detection by Micro Titer Plate assay according to Almeida et al (2013). Bacteria were inoculated on nutrient broth medium at 37°C for 24 hours. Thereafter, the broth cultures were compared with a McFarland Standard using the same medium as the diluent. 200 µl of an isolate suspension were transferred into each of three wells of a 96-well flat-bottomed polystyrene plate and incubated for 24 hours at 37°C. Then washed each well three times with distilled water with rough shaking and later dried thoroughly. The adhering bacterial cells were fixed with 200 µl of absolute methanol. Each well was stained with 200µl of 0.5% Crystal Violet for 15 minutes. Repetitive washing was performed to remove the excess stain. Later, the crystal violet associated with adherent cells was retained with 200µl of ethanol per well. The test was made in triplicates, and the absorbance of wells filled with bacteria-free nutrient broth served as a negative control. The amount of crystal violet removed by 95% ethanol in each well was quantified by measuring the OD 630 using ELISA reader (Tang et al 2011). The absorbance values represented the intensity of the biofilm formed by well-studied isolates on the surface of the microtiter. The results obtained were categorized into three groups (i.e., non-biofilm producer, moderately and strongly). The absorption of the cultivated pit was compared with the control pits. If OD≤ ODc , ODc ≤ OD ≤2*ODc, and 2*ODc ≤ OD considered non-biofilm, moderately biofilm and strongly biofilm producer. Where OD represent the tested isolates and ODc represent control pits.

Minimum inhibitory concentration (MIC) and sub-minimum inhibitory concentrations (sub-MICs): Minimum inhibitory concentration was determined for *P. aeruginosa* strains sensitive to imipenem(IMI) and ceftazidime(CAZ) by the serial dilution method on Mueller-Hinton broth. Serial dilution of antibiotics were between 2-1024µg/ml of antibiotics. Used ten isolates selected according to biofilm formation (strong biofilm). Bacterial suspension with turbidity equivalent to 0.5 MacFarland was added to the tubes contained a different concentration of antibiotics. After incubation at 37°C for 24hours, the MIC was determined as the lowest concentration of the antibiotic that inhibits bacterial growth and generally determined by turbidity. Sub-minimum inhibitory concentrations (Sub-MICs) represents the lowest inhibitory concentration at which bacteria can grow (Andersson and Hughes 2014).

Effects of Sub-MICs Imipenem and Ceftazidime on the formation of biofilm: The antibiotic (imipenem and ceftazidime) was tested at sub-MICs to study the change in the ability of *P. aeruginosa* isolates in the biofilm formation. Different concentrations of each antibiotic were applied to measure biofilm formation at sub-MIC by microtiter plates as previously mentioned and the microtiter plates were prepared and incubated at a temperature of 37°C for 24 hours. Control plates were prepared of a free antibiotic-microtiter plate which was dispensed to the wells with 200µL of nutrient broth without antibiotics (Hemati et al 2016).

Detection of pelA and pslA,pslD

Extraction of DNA and polymerase chain reaction amplification: Genomic DNA was isolated from bacterial growth according to the protocol of Geneaid Extraction and the quantitation of DNA was estimated by a quantum fluorometer.

Primer selection: These primers were mentioned in Table 1 supplied by Macrogen company in a lyophilized form ere used. Lyophilized primers were dissolved in Nuclease-free water to give the final concentration of 100pmol/µl as a stock solution.

Reaction setup and thermal cycling protocol: PCR mixture from GO Taq Green Master Mix prepared by Promega USA, PCR mixture was thawed by exposing it to a laboratory temperature, and then placed in a centrifuge so that the components collided at the bottom of the tube. The final volume of the reaction mixture became 20 µl. The PCR tubes containing the mixture were transferred to thermo-cycler and DNA was amplified using the protocol 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 sec, 52°C for 30 second, 72°C for 30 second, and final extension at 72°C for 7 minutes. Amplicons were resolved in1.5% agarose gel.

Statistical analysis: The data were analyzed in SPSS (statistical package for social science) version 26 software. The chi-square was used to find the significant difference between the number of isolates and according to the source of isolation. T-test was used in evaluating the effect of sub-MIC of antibiotics on biofilm formation.

RESULTS AND DISCUSSION

Distribution of *P. aeruginosa* according to the source: Two hundred specimens were collected from different infections included urine 67, wounds 55, ear 41, burns 25 and sputum 12. That the 26 isolates (13%) were primarily identified as *P. aeruginosa*. The highest percentage of *P. aeruginosa* was in urine and wound infections 26.92%, followed by ear specimens 23.07%. There were highly significant difference between the number of isolates and according to the source of isolation in the current study (Table 2).

Isolation and bacteriological Identification: All the specimens were cultured on MacConkey agar and blood agar and the isolates were then confirmed on *Pseudomonas*

agar at 37°C for 24 hours. The colonies were appeared as pale greenish and lactose non-fermenter on MacConkey agar and colonies were round, convex, and surrounded by transparent halo on blood agar indicate complete hemolysis (β-hemolysis) and ability of all isolates to grow at a temperature of 42°C but all isolates did not grow at 4°C. All the isolates showed positive results for the oxidase test, catalase test, and citrate utilization tests, while negative results for the indole production test, methyl red test, Voges-Proskauer, and negative for gram's stain. VITEK-2 system using the GN-ID cards which contains 64 biochemical tests confirm that all isolates were of *P. aeruginosa* (Table 3).

Biofilm formation: The majority of isolates produced biofilm (92.3%). Among them, 38.4% of isolates were strongly biofilm producer and 53.8% moderately biofilm producer and only two isolates 7.6% represented non-biofilm producer (Table 4).

The differences in biofilm density between isolates of the current study may be due to several reasons. Differences in the capability of isolates to the formation of biofilms were perhaps differences in the initial number of cells that successfully adhere to and differences in the quality and quantity of quorum sensing signaling molecules produced from each isolate (Abdulammer 2018). Heydari and Eftekhar (2014) indicated that the variation in the capability of isolates to the formation of biofilms is due to the correlation of production with ability to produce different types of beta-lactamase, which leads to the formation of a strong biofilm compared to the isolates that produced one type of enzyme. Conversely, the isolates did not produce this enzyme and were not able to form a biofilm. Biofilm is representing aggregates encased in a self-produced extracellular matrix that is impossible or difficult to eradicate with antibiotics. Its matrix provides a protective border that allows it to adhere to an environmental substrate. This coating plays a role in

Table 3. The results of diagnostic tests for *Pseudomonas aeruginosa*

Medium and tests	Results
Growth on MacConkey agar	+
Lactose fermentation	-
Catalase	+
Oxidase	+
Gram stain	-
Growth on Pseudomonas agar	+
Pigment	+ (bluish green pigmentation)
Growth at 42°C and 4°C	+/-
Indol test	-
Methyl red test	-
Voges-Proskauer	-
Citrate	+

Table 4. The percentage of Biofilm formation of for *Pseudomonas aeruginosa* isolates

Biofilm level compared to (ODc=0.038)	NO (%)	Absorbency at 630 nm
Biofilm-produced	24 (92.3%)	0.041-0.0153
Non-biofilm-produced	2 (7.6%)	0.034-0.036

Table 1. The primers used in the current study for genes detection

Primer name	Sequences	Ref.	Annealing Temp. (oC)	Size (bp)
pelA-F	5'- CCTTCAGCCATCCGTTCTTCT-3'	Colvin et al (2011)	52	118
pelA-R	5'- TCGCGTACGAAGTCGACCTT-3'			
pslA-F	5'- TGGGTCTTCAAGTTCCGCTC -3'	Maita and Boonbumrung (2014)		119
pslA-R	5'- ATGCTGGTCTTGCGGATGAA -3'			
pslD-F	5'- CTCATGAAACGCACCCTCCT -3'	Maita and Boonbumrung (2014)		295
pslD-R	5'- TGCGACCGATGAACGGATAG -3'			

Table 2. Numbers and percentages of *P. aeruginosa* among different clinical specimens

Type of specimens	No. of specimens (%)	No. of *P. aeruginosa* (%)	Percentage of isolates to specimens
Urine	67 (33.5%)	7 (26.92%)	7*** (10.44%)
Wounds swabs	55 (27.5%)	7 (26.92%)	7 (12.72%)
Ear swab	41 (20.5%)	6 (23.07%)	6 (14.63%)
Burn swab	25 (12.5%)	4 (15.38%)	4 (16%)
Sputum	12(6%)	2 (7.69%)	2 (16.66%)
Total	200 (100%)	26 (100%)	26 (13%)

***P=0.008

bacteria's resistance to antibiotics. Biofilms can provide 10 to 1,000 fold more protection against antibiotic treatment. The risks of biofilm arose from the fact that it is a major driver of the persistence of chronic infection (Ciofu and Tolker-Nielsen 2019).

Minimum and subminimum inhibitory concentration (MIC, Sub MIC): MIC values of imipenem were 16-512 µg /ml while MIC for ceftazidime was 16-1024 µg /ml (Table 5).

Effects of antibiotics on the formation of biofilm: Ceftazidime and imipenem after incubating for 24 hours decreased the density of biofilm formation in eight isolates for both antibiotics (Table 6). But, no change in biofilm density was detected in two isolates (PA3, PA20) for ceftazidime antibiotics and (PA20, PA25) for imipenem antibiotics with statistical difference in the capacity of isolates to formation of biofilms between the non-use of the antibiotic (free from antibiotic) and the treatment of isolates with Mic for each antibiotic (after antibiotic treatment). The high MIC may be due to the higher production of the enzyme b-lactamase that breaks the b-lactam ring and the presence of resistance genes that play a role in inhibiting antibiotic activity (Gonçalves et al 2017). The overproduction of AmpC and the mutagenic inactivation of oprD are the main mechanisms of carbapenem resistance especially to imipenem in the absence of acquired carbapenemase. The overproducer of AmpC, usually when combined with efflux systems overexpression and/or down-regulation systems, leads to carbapenem resistance. Overproduction of AmpC caused an increase in MICs of imipenem (Mirsalehian et al 2017) and associated with resistance to ceftazidime **(**Emaneini et al 2019). Ceftazidime resistance was more common with overexpression of AmpC alone (Hawkey et al 2018).

Table 5. Sub MIC and MIC values of ceftazidime and imipenem

Isolates No.	Antibiotic / breakpoint µg/ml			
	Ceftazidime		Imipenem	
	Sub_ MIC ≤8(S)	MIC ≥32(R)	Sub_MIC ≤2(S)	MIC ≥8(R)
PA3	8	16	128	256
PA4	16	32	8	16
PA10	512	1024	256	512
PA12	32	64	32	64
PA13	512	1024	32	64
PA15	16	32	256	512
PA18	256	512	32	64
PA19	256	512	64	128
PA20	16	32	8	16
PA25	16	32	8	16

Table 6. Formation of biofilm by *P. aeruginosa* treated with ceftazidime (CAZ) and imipenem (IMI) at sub-MIC

Isolates No.	Free from antibiotic		After antibiotic treatment (CAZ)		After antibiotic treatment (IMI)	
	Absorbency at 630 nm	Biofilm level compared to (ODc=0.038	Absorbency at 630 nm	Biofilm level compared to (ODc=0.038 (24h)	Absorbency at 630 nm	Biofilm level compared to (ODc=0.038 (24h)
PA3	***0.096	Strongly	***0.084	Strongly	***0.046	Moderately
PA4	0.105	Strongly	0.044	Moderately	0.041	Moderately
PA10	0.077	Strongly	0.042	Moderately	0.058	Moderately
PA12	0.131	Strongly	0.051	Moderately	0.052	Moderately
PA13	0.081	Strongly	0.032	non-biofilm producer	0.035	Non-biofilm producer
PA15	0.153	Strongly	0.046	Moderately	0.061	Moderately
PA18	0.098	Strongly	0.051	Moderately	0.032	Non-biofilm producer
PA19	0.153	Strongly	0.067	Moderately	0.033	Non-biofilm producer
PA20	0.092	Strongly	0.083	Strongly	0.082	Strongly
PA25	0.082	Strongly	0.056	Moderately	0.080	Strongly

***P=0.001

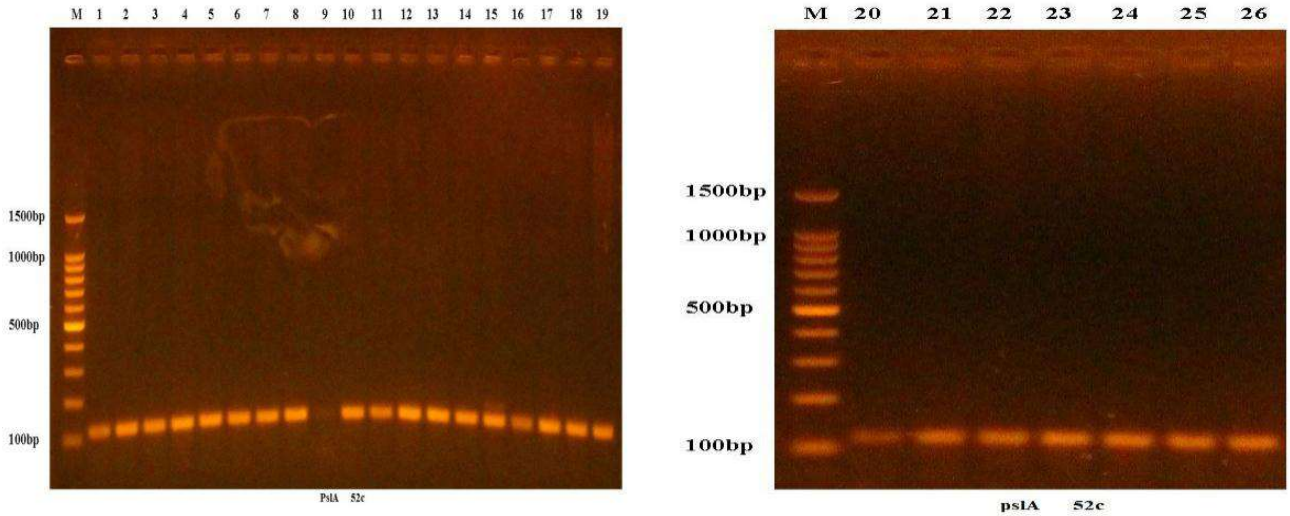

Fig. 1. Amplification of *PslA* region in *P.aeruginosa* species fractionated on 1.5% agarose stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 119bp PCR products

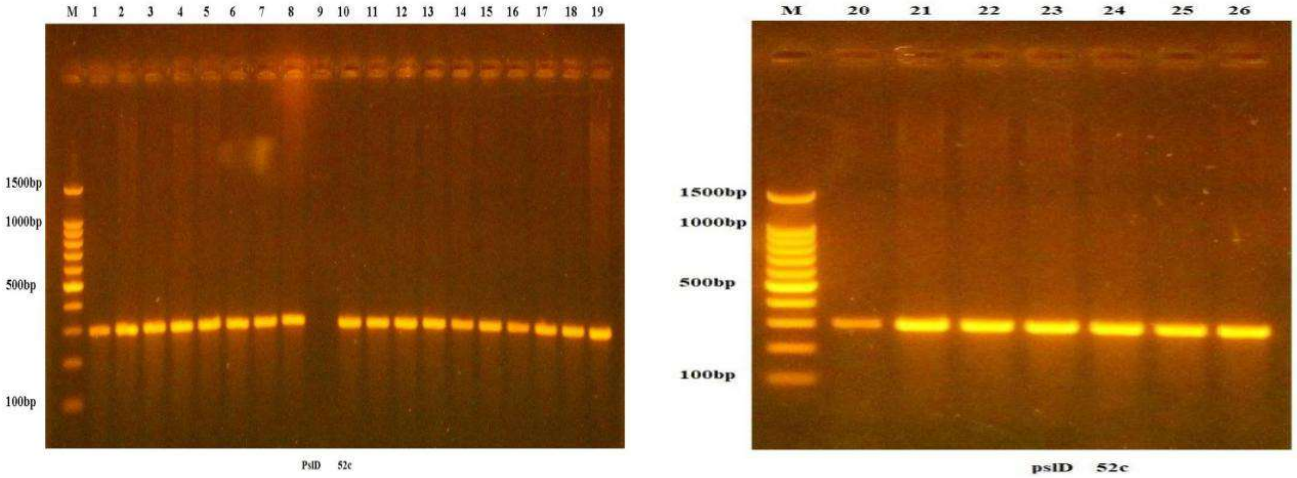

Fig. 2. Amplification of *PslD* region in *P. aeruginosa* species fractionated on 1.5% agarose stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 295bp PCR products

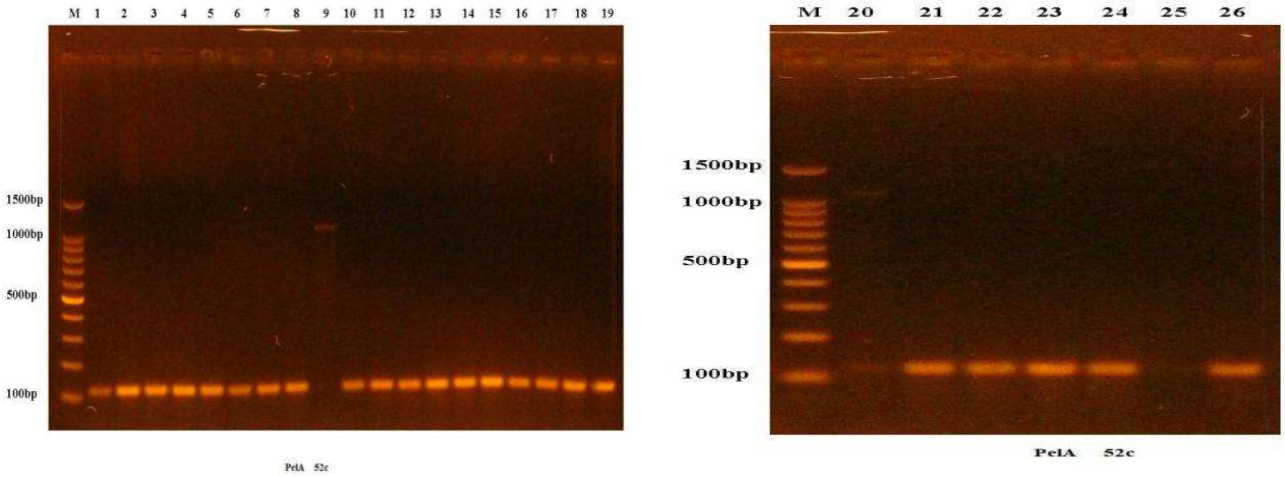

Fig. 3. Amplification of PelA region in *P. aeruginosa* species fractionated on 1.5% agarose stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 118bp PCR products

Despite the extensive biofilm tolerance to antimicrobials, some conventional antibiotics still show activity against bacterial cells that grow in the state of biofilms. Otani et al (2018) showed that sub-MICs of ceftazidime reduce biofilm formation after 24 h incubation. A sub-MIC ceftazidime may inhibit biofilm formation by altering the effect exerted on the cellular membrane of *P.aeruginosa* (Otani et al 2018). Such differences in the present study may be considered normal due to the types of isolates studied and their source in addition to the genetic makeup of the isolates or the laboratory conditions that accompanied the detection of sub-MIC (AL-Sheikhly et al 2019). Moreover, biofilm inhibition by *P. aeruginosa* in response to antibiotics is consistent with the hypothesis that metabolic stress is the main signal mediating the response. The disruption of the biofilm structure may result in the separation of cells that can act as a vaccine for new infection points and thus cause the spread of the infectious agent (Penesyan et al 2020). The study concluded that incubating the *P. aeruginosa* isolates in sub-MIC of antibiotics incubating for 24 hours exhibited reduced the ability of bacteria to formation of biofilm in most isolation.

DNA extraction: Using the Presto™ Mini gDNA Bacteria Kit, the genomic DNA was extracted from *P.aeruginosa* isolates. DNA concentration was determined using the quantum fluorometer. All isolates have DNA concentration between 19.8-35.6 ng /µl.

Detection of *PslA, PslD and PelA:* PCR was carried out for the detection of *PslA, PslD* and *PelA*genes for isolates. The detection showed the percentage of *PslA* and *PslD* genes in isolates was 96.1%. In *PelA* gene was 88.4%. Maita and Boonbumrung, (2014) also stated that the percentage of *pslA*, *PslD* and *PelA* was 94, 95.9 and 97 respectively. These were found in nearly all clinical isolates of *P. aeruginosa*. The genes mentioned above were found in nearly all clinical strains of *P. aeruginosa*, but not all of these genes can contribute to biofilm production, and their presence cannot predict which strains will produce biofilm because many factors influence biofilm formation. In the current study that two isolates were not able to form biofilm despite them carrying each of the three genes. This indicates that there are other genes responsible for the formation of biofilms. One of the isolates did not carry the three genes despite being a producer of the biofilm. The ability to produce biofilms despite the absence of the studied biofilm genes indicates that other genetic determinants of biofilms are involved in matrix formation in *P. aeruginosa* (Moradali et al 2017). In contrast, the presence of genes without the production of biofilms perhaps the result of chromosomal mutations in different regulatory systems, affecting the production of functional proteins correlating with the biofilm (Kamali et al 2020).

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Morphological and Molecular Identification of *Hyalomma rufipes* in Al-Anbar Province, Iraq

Ibrahim Abduladheem Flayyih and Ali Issa Fadhil

Department of Parasitology, College of Veterinary Medicine
University of Baghdad, Baghdad, Iraq
E-mail: ialheety92@gmail.com

Abstract: Samples of ticks were collected from 180 cattle during October, 2020 to March, 2021 to identify their morphological and molecular characteristics. The total infection rate for all *Hyalomma* spp. was 33.3% and for *Hyalomma rufipes* (*H. rufipes*) was 20% . Targeting the Cytochrome c oxidase subunit 1 (COX 1) gene, the molecular and DNA sequencing were performed, and the local isolate was documented in the National Centre For Biotechnology Information (NCBI) under the accession number MK551197.1. Homology sequence identity of local isolate showed significant high similarity with the South Africa (MK551197.1) and Australia (AF132823.1). In conclusion, the local isolate considered the first Iraqi isolate registered in the NCBI.

Keywords: Tick, Cattle infestation, PCR, Sequence, *Hyalomma*

Tick is an obligate blood-sucking arthropod classified under Acari subclass as a nonpermanent ectoparasite infested several animal species (Keirans and Durden 2005). It distributed in particular throughout the tropical and subtropical areas with great species diversity (Adham et al 2009). *Hyalomma* spp. conceder is of economic veterinary importance especially hard ticks that infested the livestock in tropical regions because act as a vector for transmitting a wide variety of pathogens such as hemoparasite (*Theileria* spp., *Babesia* spp. and *Anaplasma* spp.) (Gharban et al 2023). In addition, *Hyalomma* spp. have a severe hazard effects on animal health resulting in economic impact through morbidity or mortality (Magnarelli et al 2000, Stiller et al 2002, Rezaei and Mirzaei 2011), as well as, their direct effect through blood loss, skin wounds, tick-induced paralysis, allergy (Jongejan and Uilenberg 2004, Nicholson et al 2009, Pfäffle et al 2009). Champour et al (2016) mentioned that about 80% of the world's cattle are infested with different types of ticks and classified as economically important ectoparasites of livestock. *Hyalomma rufipes* can parasitize on different types of organisms, and adults parasitize cattle, horses, sheep, goats, wild ruminants and humans while, the nymph and larval stages were recorded to feed on birds and hares (Tomassone et al 2004, Ruiz-Fons et al 2006). This tick is the primary vector of many diseases like the Crimean-Congo hemorrhagic fever in South Africa (Horak et al 2001). It also transmits bovine anaplasmosis, tick typhus in humans, and bovine babesiosis (Jongejan and Uilenberg 2004, Apanaskevich and Horak 2008).

MATERIAL AND METHODS

Samples: Ticks samples collection started from the beginning of October 2020 until the end of March 2021. Samples were gained from 180 cattle from both sexes from different regions from Al-Anbar province and transported directly to the Lab of Parasitology at the College of Veterinary Medicine, University of Baghdad and all required data were collected (Barmon et al 2010).

Slide preparation: Sample of ticks were selected after the tick was cleaned well to remove suspensions in the mouth parts of the host's tissues, and placed in glass tubes 10% (KOH) for 3-5 days, and slide were prepared. Light microscope was used for primary identification under X4 and X10 (Soulsby 1982) and samples were sent to the Museum of Natural History to confirm the diagnosis.

Molecular examination: Extraction of whole genome form tick DNA was done followed the manufactural procedure by using special Kit G- spin DNA extraction kit , intron biotechnology /Korea, cat.no. 17045. The purity and quality of tick DNA samples was evaluated by using a nanodrop spectrophotometer and by running of samples on gel electrophoresis, these was done by preparing 1% of agarose gel (Crowder et al 2010).

Cytochrome oxidase subunit 1 gene: The gene fragment of size 415 bp), was used to catch different species of hard tick [foreword (5'-AGGGTCCCCAGATATAGCATT-3') and reverse (5'-ACCGCCTGAAGGGTCAAAAA-3')] (AL-Fatlawi et al 2018). The PCR mastermix for extracted DNAs was prepared as mentioned in (Table 1), whereas, the Thermal

cycler conditions were applied as mentioned in (Table 2).

RESULTS AND DISCUSSION

Morphological study: Out of 180 cattle examined, tick infestation was 33.3% with different species of *Hyalomma* spp. *H. rufipes* recorded 20% depending on the morphological characters (Fig. 1).

Molecular study: DNA amplification of the *COX 1* gene yielded a single fragment of approximately 415bp in 15 samples (Fig. 2). The DNAs of some positive samples were sequenced and the received data were recorded in the GenBank-NCBI under the accession number MK551197.1. Comparative analysis of nucleotides sequence of study samples with the strains existed in GenBank database was constructed using the Clustal W Alignment of MEGA-6 software, and the results were showed a high identity (99%) to the *H. rufipes* isolate of South Africa (MK551197.1), and Australia (AF132823.1) with 94% similarity to *H. rufipes* isolate of China (JQ737074.1) (Fig. 3).

Hyalomma rufipes was first recorded by Koch in 1844 and named as the hairy *Hyalomma* and known also as the coarse-legged *Hyalomma*, *H. rufipes* is one of the widely distributed ticks in Africa, Middle East, Russia, and central parts of Asia. The recent study focused on this species due to the lack of information about it and conceder the first molecular identification for this spices in Al-Anbar province. The incidence was relatively higher than obtained by Barkirci et al (2011) in turkey during (0.07%). Furthermore, results disagreed with the findings reported by Tuma et al (2007) in southern part of Iraq, which might be attributed to differences in the environmental conditions and type of management. Hosseini-chegeni et al (2013) mentioned that the morphological identification for *H. rufipes* need expert

Table 1. Mixture of the specific interaction for diagnosis gene

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table 2. Optimum condition of detection gene

Phase	Tm (ºC)	Time	No. of cycle
Initial denaturation	95	5 min.	1 cycle
Denaturation	95	30 sec	35 cycle
Annealing	58	30 sec	
Extension	72	1 min	
Extension	72	5 min.	1 cycle

Fig. 1. *Hyalomma rufipes* collected from cattle in the udder

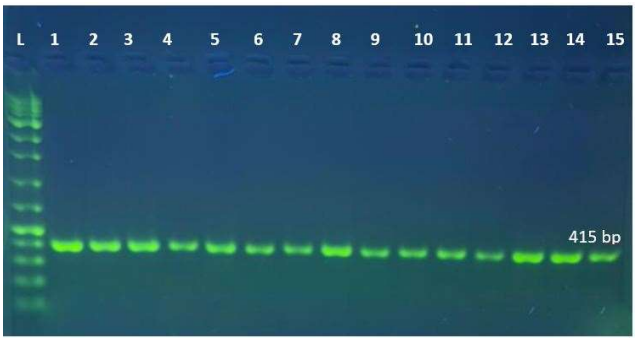

Fig. 2. Agarose-gel electrophoresis (2%) for PCR products; Lane L: DNA ladder (100-1500bp); Lanes 1-15: PCR products for positive samples at 415bp product size

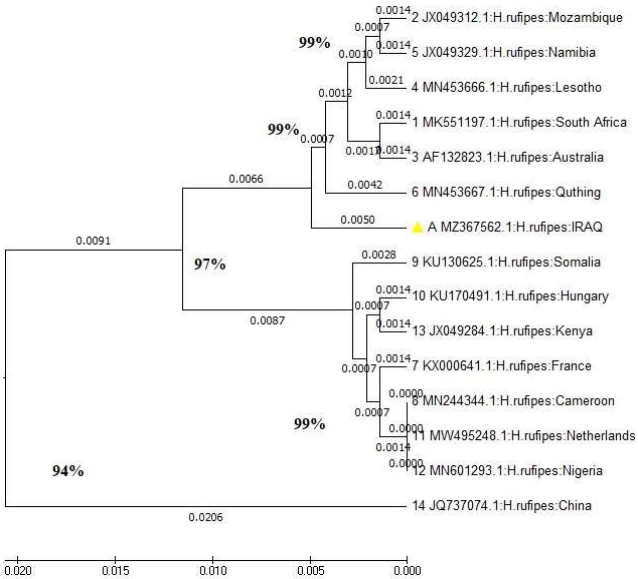

Fig. 3. Phylogenetic tree similarities based on the COX-1 gene sequences *H. rufipes* identified in cattle in this study are indicated by yellow triangle

taxonomic for morphological identification for this spices. In our study two methods were used to confirm our results, the Museum of Natural History and molecular diagnosis. Phylogenetic tree analysis for partial sequenced amplicon for the *Cox 1* gene for r isolates by using BLAST program and GenBank data base showed high similarities with other isolates with 99% identity and this also confirm our diagnosis.

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Purification and Characterization of Collagenase of *Staphylococcus aureus* Isolated from Different Clinical Source

Mohammed. A. Mahdi and Sawsan H. Authman[1]

College of Dentistry, Al iraqia University, Baghdad, Iraq
[1]Department of Biology, College of Science, Al–Mustansiriya University, Baghdad, Iraq
E-mail: mohammed94630@gmail.com

Abstract: The main objective of this study is to comprehensively investigate the extraction and purification processes of collagenase from *S. aureus*. The ability of *S. aureus* to produce collagenase was examined phenotypically on medium containing (g/L): K_2PHO_4 7g, KH_2PO_4 2g, $MgSO_4.7H_2O$ 0.1g, yeast extract 1g, $CaCl_2$ 2 H_2O 0.1g, peptone 1g, gelatin 15g and 15 g agar. Quantitative assay of collagenase that revealed 34(70.8%) of *S. aureus* were able to produce collagenase, The optimum pH for collagenase production was 6.5. while the optimum temperature was 37ºC. The collagenase produced by *S. aureus* was purified at 13.54-folds through application of chromatography with DEAE-Cellulose and Sephadex G150 columns. The molecular weight of collagenase was estimated to be 69 KDa by SDS-PAGE. The optimum pH for collagenase was 8 while the enzyme was stable at wide range of pH 6, 7 and 8 with activity 91, 97 and 100% , respectively. The optimum temperature for collagenase activity was 40ºC while the stability was below 37ºC. These findings not only contribute valuable insights into the characteristics of S. *aureus* collagenase but also hold implications for its potential applications in various fields, such as medicine or biotechnology.

Keywords: *Staphylococcus aureus,* Purification, Characterization, Extraction, Collagenase

Staphylococcus aureus is a gram-positive bacterium that live in mouth, upper respiratory system and skin, as a commensal, making it a risk factor for opportunistic and nosocomial infections (Gitau et al 2018). The ability of *S. aureus* to produce a wide range of exoenzymes is one of its distinguishing characteristics (Bronner et al 2004). *S. aureus* has a variety of virulence factors and exoenzymes such as exotoxins and enzymes such as nucleases, lipases, hyaluronidase, proteases, and collagenase (Bien et al 2011). Collagenase is enzyme that hydrolysis of collagen are fibrous protein found in the connective tissues and consists of three peptide chains in a triple helix structure. Many microorganisms that produce collagenase include pathogenic and non-pathogenic microorganisms which secreted collagen as a source of nutrient (Chauhan et al 2017). Collagenase contains a saddle-shaped tertiary structure, and the active site contains zinc moiety. Bacterial collagenases are metalloproteases can breakdown extracellular matrices, making them important virulence agents (Vachher et al 2021). The first attempt to isolate the collagenase enzyme was demonstrated by Mandel and his group in 1953 from the bacterium *Clostridum histolyticum* (Gabrielson et al 2017). Due to the many therapeutic applications of collagenase enzyme such as Dupuytren's disease, wound burns treatment, chronic total occlusions (CTO), debridement, gene delivery, cancer gene therapy and production of pharmaceutical compounds make the collagenase characterization and determined the best production collagenase bacteria important (Chauhan et al 2017).

MATERIAL AND METHODS

Isolation of bacteria: From 302 samples of different clinical sources 48 isolate primary diagnosed as *S. aureus* depending on cultural morphological and biochemical test. These characteristics include colonial morphology, size of colony, ability to ferment mannitol. Bacterial isolates were examined and identified by microscopic, biochemical test and API staph strip system characteristics (Brook and Foote 2006).

Phenotypic detection of collagenase production: Screening for collagenase producing isolates was performed by inoculating *S. aureus* isolates by stabbing on the collagenase screening agar medium. This medium was prepared according to Lee et al (2006) with some modification by added (g/L) K_2PHO_4 7g, KH_2PO_4 2g, $MgSO_4.7H_2O$ 0.1g, yeast extract 1g, $CaCl_2$ 2 H_2O 0.1g, peptone 1g, gelatin 15g and 15 g agar in 1000 ml of DW and pH was adjusted at 7±0.2 and sterilized by autoclaving at 121 ºC for 15 minutes After incubation for 18-24 hrs at 37C °, collagenase production was evident in the clear halo zone around the colonies. The clarity of the hydrolyzed zone around the microbial colony can be improved by precipitating the proteins with trichloroacetic acid (TCA). After incubation

flooded Petri dish with 30% of TCA produced a very sharp and clear zone gelatin hydrolysis (Medina and Baresi 2007).

Quantitative assay of collagenase: The bacteria with larger clear zone were obtained and then inoculated concentration comparable to McFarland standard no. 0.5 in the broth medium contain (g/L): K_2PHO_4 (7), KH_2PO_4 (2), $MgSO_4.7H_2O$ (0.1), yeast extract (1), $CaCl_2$ 2 H_2O (0.1), peptone (1) and gelatin (15), pH=7.0-7.2 at 37°C (Lee et al 2006). After 24 h of incubation, the culture broth was centrifuged at 4°C and 12000 g for 10 min and the superannuate was taken for enzyme activity measurement (Nagano and To 2000).

Determination of Optimum Conditions for Collagenase Production

Determination of optimum temperature for collagenase production: The selected isolates were cultured on broth medium and incubated in different temperatures (25, 30, 35, 37, 40 and 45ºC) for 24 hrs, then centrifuged at 4ºC and supernatant was taken to determined collagenase activity.

Determination of optimum pH for collagenase production: The selected isolates were cultured on broth medium with pH to 5, 6 ,6.5,7,7.5, 8 and 9 at 37 °C for 24 hrs, then centrifuged at 4ºC and supernatant was taken to determined collagenase activity.

Extraction of collagenase: The method described by was followed with some modifications. The selected isolates *S. aureus* was cultivated in 500 ml of medium contain (g/L): K_2PHO_4(7), KH_2PO_4 (2), $MgSO_4.7H_2O$ (0.2), yeast extract (2), $CaCl_2$ 2 H_2O (0.1), peptone (1) and gelatin (10). pH 6.5 at 37 C° in shaking incubator at 200 rpm for 24 hrs. followed by 10 min at 4°C and 12,000 rpm the supernatant was obtained. Then took to assay collagenase activity and protein concentration and used for purification of enzyme.

Purification of collagenase: The crude DNase was subjected to different steps of purification including ammonium sulphate (NH4)2SO4 precipitation, dialysis, DEAE-Cellulose ion-exchange chromatography and gel filtration by using gradient elution buffer.

Determination of optimum pH for collagenase activity and stability: The optimum pH was examined using 50 mM of Citrate buffer and 50 mM Tris-HCl buffer over the pH ranges 3.0-6.0 and 7.0-9.0, respectively. The collagens are dissolved with buffer at different pH from (3.0-9.0) and react with collagenase for 30 min at 37°C, then measure the collagenase activity to examine the effect of pH on the enzyme activity (Rochima et al 2016). pH effect on collagenase stability was done by using equal volumes of purified enzyme solution was reacted with different pH buffers ranges (3 to 9) were incubated at a room temperature for 30 min. The enzymatic activity was measured for each one. The remaining activity for collagenase was plotted against the pH value of solutions to determine the optimal pH for collagenase stability (Abood et al 2018).

Determination of optimum temperature for collagenase activity and stability: To Determining the optimum temperature collagenase carried out by reacting the collagenase with collagen at different temperature (20, 30, 40 and 50°C). Then measured the collagenase activity to examine the effect of temperature on the enzyme activity, while for thermal stability, equal volumes of collagenase were incubated in water baths at (20, 30, 40 and 50°C) for 30min and immediately transferred into an ice bath. Enzymatic activity was measured and the remaining activity was plotted against the temperature (Abood et al 2018).

Determination of molecular weight of collagenase: Collagenase enzymes molecular weight was determined by used SDS-PAGE 12% (Brunelle and Green 2014).

RESULTS AND DISCUSSION

Isolation and identification of *Staphylococcus aureus*: Three hundred and two samples were collected from different clinical sources include: wound, urine, burn, wound, blood and stool (Table 1). Forty-eight (15.89%) isolates of were identified as *S. aureus* according to culture, microscopic examination, biochemical tests, Api 20 staph and VITEK® 2 Compact system.

Phenotypic detection of collagenase production: Forty-eight isolate of *S. aureus* culture by stabbing method on of collagenase screening agar based on gelatin hydrolysis method. Results showed that 34 (70.8%) of *S. aureus*

Table 1. Percentage of S. *aureus* according to the site of infection

Type of sample	Total number	No. of isolate	S. *aureus* percentages according to source	S.aureus percentages according to isolate
Urine	70	14	30	29.1
Wounds	67	13	19.4	27.1
Burns	65	12	18.5	25
Stool	56	6	10.7	12.5
Blood	44	3	6.8	6.2
Total	302	48		

isolates give positive results with halo zone around colony which produce collagenolytic enzyme after added TCA while only 14 (29.2 %) of *S. aureus* were non-producer (Fig. 1).

Quantitative assay of collagenase: The quantitative assay was done for thirty-four isolates of *S. aureus* . The 34 (100%) of *S. aureus* were able to produce collagenolytic activity and 10 was chosen for collagenase extraction since that it accomplished the high enzyme activity.

Optimal Conditions for Collagenase Production

Optimal incubation temperature: Different incubation temperatures (25-45ºC) were tested to determine the optimum temperature for collagenase production by *S. aureus* (10). The best production of collagenase was reached at range between 35-40ºC while the optimal temperature was 37ºC. The collagenase activity was 5.93 U/ml for *S. aureus* (10) at 37ºC. However, other temperatures led to decreasing the enzyme activity (Fig. 2). Suphatharaprateep et al (2011) mentioned that temperature is one of the most important factors affecting bacterial growth and most types of bacteria have a special temperature range that they can grow. Furthermore, an increase or decrease in incubation temperature leads to a decrease microbial growth and enzyme production was observed by for *Bacillus cereus* CNA1 and *Klebsiella pneumoniae* CNL3 which was 37ºC for both strains. Hamdy (2008) observed the optimum collagenase production in *Rhizoctonia solani* was 30ºC while Gautam and Azmi (2017) was observed the optimum incubation temperature was 37ºC in *P. aeruginosa*.

Optimal pH for collagenase production: The productivity of collagenase was variable at different pH from 5 to 9. The optimal pH for collagenase production by *S. aureus* (10) was 6.5 with collagenase activity 5.65 U/ ml in comparison with other pH values which led to reduce the enzyme activity to 3.76 and 3.01 U/ml when the pH of the medium was 5 and 8, respectively and no collagenase activity in pH 9 (Fig. 3). Chauhan and Azmi (2017) also showed that the optimal pH for collagenase produced by *P. aeruginosa* was 6.5. While Pequeno et al (2019) observed the optimum pH for collagenase production was 7.5 in *Bacillus cereus*. The intake of nutrients from the medium is strongly affected by changes in medium pH, which helps in growth and metabolite production (Olson 1993). *S. aureus* can grow in a wide range of pH 4.2 to 9.3, the optimum pH was 7-7.5 (Kadariya and Thapaliya 2014).

Extraction of the enzyme: The selected isolates S. *aureus* (10) were grown in shaking incubator in 500 ml of medium and incubation at 37°C for 24 hrs. Bacterial Cells were removed by centrifugation at 12,000 rpm for 10 min at 4°C. Present study revealed that the supernatant (crude enzyme) for *S. aureus* collagenase was 5.9 U/ml.

Purification of collagenase enzyme: After extraction, the supernatant was processed for 40-80% ammonium sulphate precipitation. The collagenase activity for S. *aureus* was 15.8U\ml, while the specific activity was 49.1 U/mg. The sample was subjected to DEAE-Cellulose column by linear gradient of NaCl (0.1-0.7 M). Ion exchange chromatography pattern for *S. aureus* showed two peaks in wash step but had no collagenase activity thus it was neglected. Third protein peak with 0.3M of NaCl at fraction numbered 41 to 48 showed the highest collagenase activity reached to 13.45 U/ml. The

1- *S. aureus* NO (5) & 2- *S. aureus* NO (10)

Fig. 1. Collagenase activity based on gelatin hydrolysis

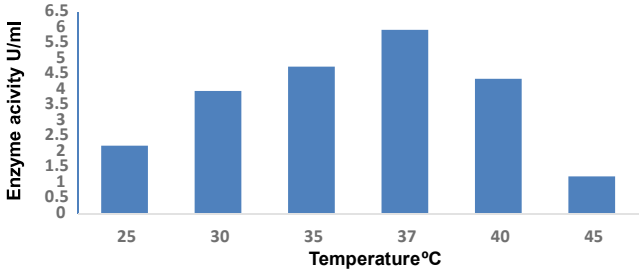

Fig. 2. Effect of temperature on collagenase production

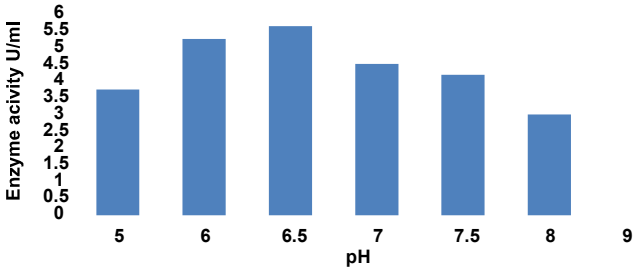

Fig. 3. Effect of variable pH on collagenase production

fourth protein peak with 0.7 M of NaCl at fraction numbered 82 to 90 also don't have collagenase activity thus it was neglected (Fig. 4).

DEAE-Cellulose ion exchange column has been used to purify the collagenase enzyme from different sources, It was used to purify the enzyme from *Rhizoctonia solani* yielded 62.8 % with purification fold 9.1. Hamdy(2008), also used to purify enzyme from *Lysinibacillus sphaericus* yield 38.9 % with purification fold reach 12 (Hoa Bach et al 2020). Mahmoud et al (2007) also used of DEAE-Cellulose to purification of collagenase from *Aspergillus flavus* with purification fold reach 253.9. Further purification carried out by a gel filtration using Sephadex G150. Enzymic fractions from DEAE-Cellulose were pooled and passed through gel filtration column. The fractionation yielded one protein peaks as absorbance reading at 280nm. The collagenase activity pooled and concentrated in third peak with activity reach to 14.5 U\ml (Fig. 5).

Enzyme activity, specific activity and protein concentration of collagenase were measured, and there was an increase in the specific activity of the purified enzyme (175.8 U/mg) with 13.54 purification fold and 13.44 % overall yield (Table 2).

Characterization of Purified Collagenase

Determination of molecular weight for collagenase: The molecular weight was estimated by SDS-PAGE according to the logarithm molecular weight and Rf curve figure. The molecular weight for collagenase 10 was 69 KDa (Fig. 6). Most of the collagenases have a high molecular weight within the range of 50-120 kDa (Pal and Suresh 2016). SDS-PAGE indicated the molecular weight of purified collagenase from *S. aureus* is 69 KDa. Lee et al (2006) stated that the molecular weight of purified collagenase extracted from *S. aureus* was 62 KDa by using SDS-PAGE. According to Nagano and To (2000) the molecular weights of collagenases enzyme are variable according to the type of microorganisms

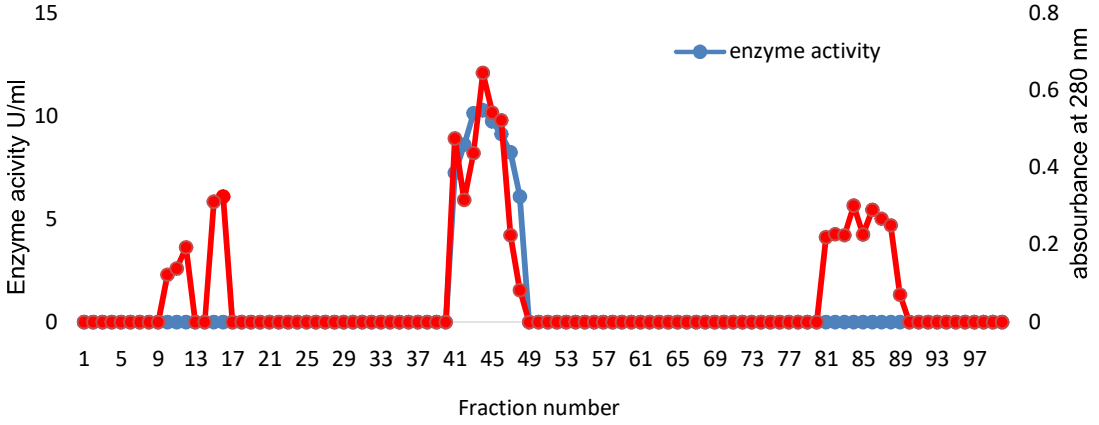

Fig. 4. Ion exchange chromatography of collagenase produced by *S. aureus 10* using DEAE Cellulose column (3 × 15). The flow rate was a 30 mL / hrs, 5 mL per tube; at temperature 25°C.

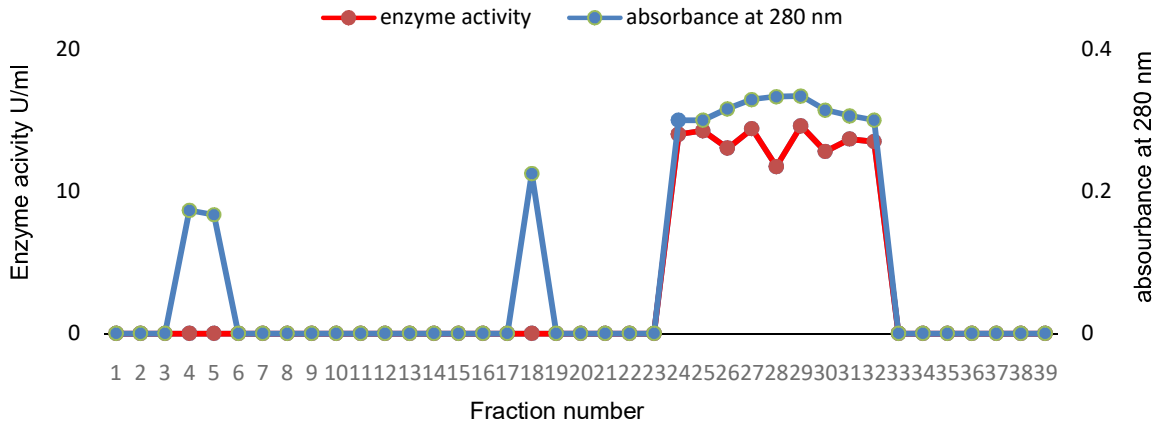

Fig. 5. Gel filtration chromatography of collagenase purified from; *S. aureus* by using Sephadex G-150 column (2x40) cm

Table 2. Purification steps for collagenase produced by *S. aureus*

Purification steps	Volume (ml)	Enzyme activity (U\ml)	Protein concentration (mg\ml)	Total activity (U)	Specific activity (U\mg)	Purification (fold)	Yield (%)
Crude enzyme	490	5.98	0.46	2,930.2	12.98	1.00	100
Ammonium sulphate	50	15.836	0.322	791.8	49.18	3.78	27
Ion exchange chromatography on DEAE Cellulose	40	13.45	0.120	538	112.08	8.63	18.36
Gel filtration chromatography Sephadex G-150	27	14.592	0.083	393.98	175.8	13.54	13.44

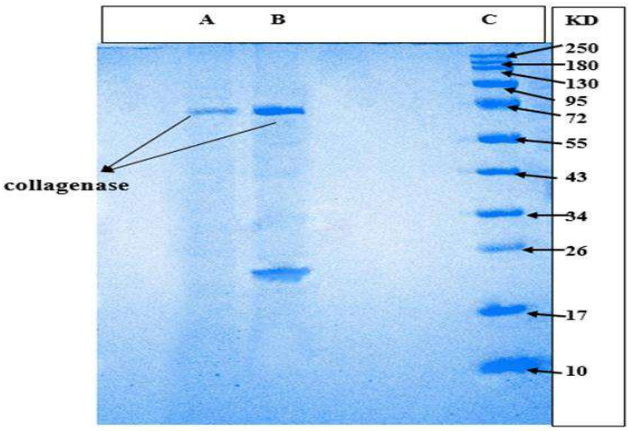

Fig. 6. SDS-PAGE of the purified collagenase from *S. aureus,* (A) collagenase after gel filtration (B) collagenase after ion exchange chromatography (C) protein marker.

as follows: 125 KDa of *B. subtilis* FS-2. The molecular weights of collagenases extract from *Streptomyces* sp. 3B was 97 and 116 KDa (Petrova Derekova and Vlahov 2006), 50 KDa of *Thermoactinomyces* sp 21E (Petrova, Shishkov, and Vlahov 2006) from *Pseudomonas aeruginosa* 34 KDa (Gautam and Azmi 2017) and 110 to 100 of KDa *C. histolyticum*, 82 KDa of *Vibrio alginolyticus* (Di Pasquale et al 2019).

Effect of pH on collagenase activity: The optimum pH of collagenase purified from *S. aureus* was determined at variable pH (3, 4, 5, 6, 7, 8, 9). The optimal pH for purified collagenase activity was at pH= 8. However, collagenase of *S. aureus* was still active over a wide range (7 to 9) of pH values. On the other hand, collagenase activity was decreased at pH 6 and below (Fig. 7).

Lee et al (2006) stated that the optimum pH of collagenase extract from *S. aureus* was 7 while Chauhan and Azmi (2017) s found optimum pH for the activity of purified collagenase from *Pseudomonas* spp with collagen substrate was 8.5. Pequeno et al (2019) revealed that maximum activity of collagenase extract from *Bacillus cereus* under alkaline conditions (pH from 7.2 to 9.0).

Effect of pH on collagenase stability: The pH profile for collagenase stability was shown in Figure 8, where the purified enzyme was incubated at different pH values for 30 min at 40°C. Collagenase of S. *aureus* was stable in a wide range of pH 6, 7 and 8 with activity 91, 97 and 100% respectively.

Effect of temperature on collagenase activity: To determine the optimum temperature of collagenase activity, enzymatic reaction at different temperature (20, 30, 40, 50ºC) (Fig. 9) illustrated the optimum temperature, Purified collagenase from *S. aureus* showed the highest activity at 40ºC with enzyme activity 17.2 U\ml. De Albuquerque Wanderley et al (2020) reported that the optimum temperature for purified collagenase at 37 ºC from *Chlorella vulgaris* , while Danial et al (2019) indicate the optimum temperature of collagenase from *Bacillus* sp. is 40ºC. The temperature is an energy key for enzymes was discussed by Segel (1976). As the temperature increases, the reactant molecules acquired more kinetic energy, resulting in more

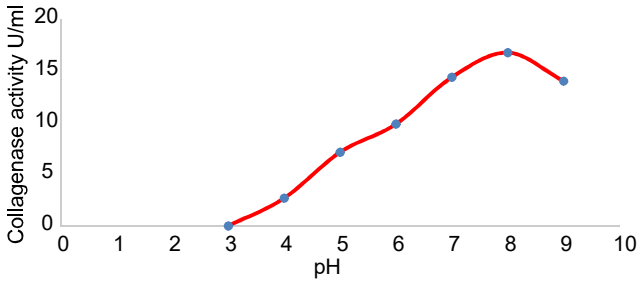

Fig. 7. Effect of pH on collagenase enzyme activity

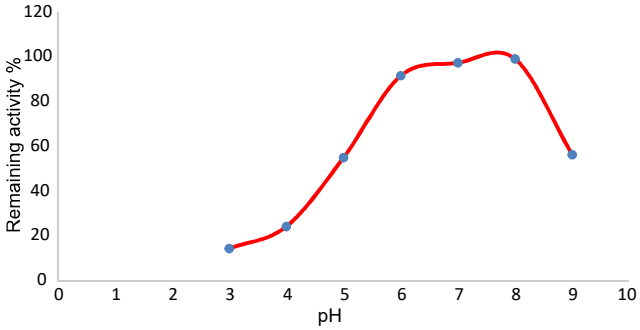

Fig. 8. Effect of pH on collagenase enzyme stability

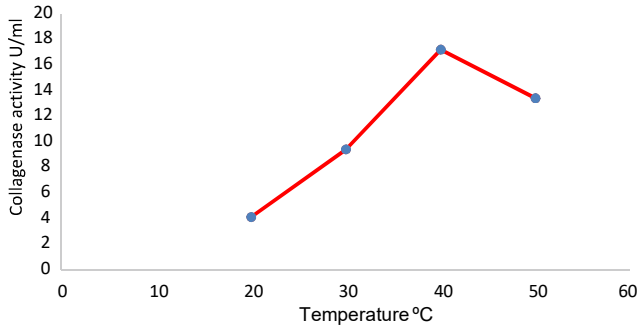

Fig. 9. Effect of temperature on collagenase enzyme activity

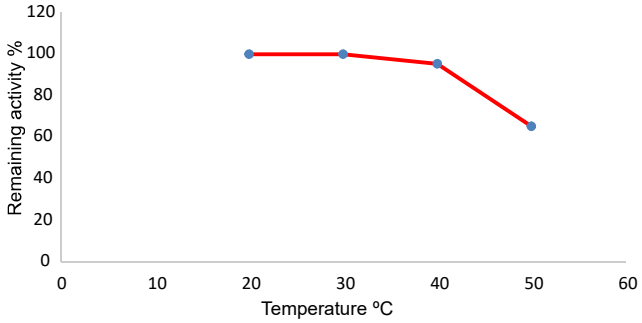

Fig. 10. Effect of temperature on collagenase enzyme stability

productive collisions per unit time. However, if the molecule absorbs too much energy, the tertiary structure will be disrupted, and the enzyme's catalytic activity will be lost when it is denatured.

Effect of temperature on collagenase stability: Effect of temperature on collagenase stability was assayed by incubation purified collagenase in temperatures ranging from 20 to 50ºC. The enzyme retained all its activity (100%) at temperature from 20-30ºC, but began to lose its activity beyond this temperature and activity reached 65 % at 50ºC (Fig. 10). Lee et al (2006) indicate that collagenase of *S. aureus* shows stable activity at 40ºC. while Suphatharaprateep et al (2011) observed when the temperature was increased up to 37ºC, beyond that temperature began to lose activity and at 50ºC lost about 50% of maximum activity of collagenase extract from *B. cereus.* Large number of bonds, such as van der Waal interactions, hydrogen bonds and others, were required for protein's three-dimensional structure. Increase temperature effect on these bonds, causing protein denaturation and loss of all or part of its activity (Roy et al 1996).

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