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Insecticidal Activity of *Tetraclinis articulata* L. Essential Oils on *Tribolium castaneum* (Herbst 1797) (Tenebrionidae: Coleoptera) Adults

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***Abstract*:** Experimental work consists in highlighting the insecticidal properties of *Tetraclinis articulata* L. essential oils extracted from leaves and dry grains on the adults of *Tribolium castaneum*. under laboratory conditions. The fumigation treatments using four doses of *Tetraclinis articulata* leaf and dry kernels essential oils showed an insecticidal effect on *Tribolium castaneum* adults. High significant difference between grains and dry leaves *T. articulata* essential oils was observed on *T. castaneum* mortality. Mortality of 36.66 to 60% of mortality observed after 48 h of the treatment with dry grains oils. This toxicity reaches 86.66 and 96.66% respectively after the 4th and the 5th day of exposure. Whereas for dry leaves, the mortality was 36.66 to 53.33% after 48h of treatment and 83.33 and 100% mortality respectively on the 4th and 5th day of exposure. The respective LD_{50} and DL_{90} doses for dry grains oils were 10.67 and 15.99 µl; whereas, for dry leaves oils the corresponding values were 9.23 µl for the LD_{50} and 12.55 µl for the DL_{90}. Moreover, the measurement of the shorter time mortality highlights TL_{50} = 36.13 h and TL_{90} = 102.42 h for dry grain and a TL_{50}= 38.02 h and TL_{90} = 108.44 h for treatment based on dry leaves. Contact and ingestion methods can be also investigated to determine the *T. articulata* essential oils activity on other stages of this insect.

***Keywords*:** Essential oil, Inhalation, *Tetraclinis articulata*, *toxicity, Tribolium castaneum*

Damage and loss of wheat, during the storage phase, are caused by many insects, especially Tenebrionidae with *Tribolium castaneum* (Herbst) species is the most widespread one. This insect belongs to Coleoptera order and Tenebrionidae family. Its presence in wheat storage environments can cause grain deterioration and consequently qualitative and quantitative losses. Lee et al (2002) observed that red flour beetle is one of the most serious insect pests in stored grains and processed foods around the world. In Algeria, losses are estimated between 10 to 12% in storage units (Karahacane 2015). Currently, pesticides used to control these pests can have environmental and human health impacts (Ngamo and Hance 2007). The widespread use of synthetic pesticides has led several adverse effects such as food, soil and air contamination with toxic residues, which have side effects on non-target insects and other organisms (Bughio and Wilkins 2004). Faced to this problem, many studies have turned to the use of less toxic products, using non-polluting natural active substances, based on plant extracts, such as essential oils. These botanical pesticides have the advantage of proposing innovative modes of action that can reduce the risk of resistance and offer new ways to design specific molecules for the target (Isman 2006, Isman2008). Many recent studies were coducted in this direction(Mishra et al 2011, Peeyush et al 2011, Toumnou et al 2012, Delim et al 2013, Muhammad et al 2013, Theou et al 2013, Zia et al 2013, Babarinde et al 2014, Ben jemaa 2014, Mobki et al 2014, Ncibi et al 2020, Karahacane and Kaci 2021). This work aims to contribute to the enhancement of this species by evaluating the insecticidal activity of the essential oils of the leaves and seeds of *Tetraclinis articulata* (Vahl) harvested in the region of Khemis Miliana (Algeria).

MATERIAL AND METHODS

The insect mass rearing was carried out in glass container, with a capacity of 500 ml, containing 250 g of wheat. Adults of *T. castaneum* were obtained from stock cultures maintained at the laboratory. The insects were maintained in the rearing chamber at 27±2°C and 60 ±5% RH. Adults aged between 8 and 15 days were used for inhalation toxicity tests. One week after egg laying, adults are eliminated. The eggs give the first generation of adults. By repeating the same process, adults of the second generation, considered homogeneous, were tested with the extracted essential oils.

Collect and plant material preparation: The leaves and grains of *T. articulata* were collected in March 2016 in the region of Khemis Miliana (Algeria) and after harvest; they were dried in the shade for ten days at the laboratory.

Essential oil extraction: The 50 g of plant material (aerial part) was placed in a one-liter flask, filled with distilled water at 1/3 of its capacity. The mixture is heated in a balloon heater. The water evaporates, bringing with it the constituents of the essential oil which are then channeled into a condenser and refrigerated at a temperature of 17°C. to 22°C. to liquefy it again. Thereafter, the oil which floats on the surface of the distillation water is recovered in a dropping funnel. The obtained essential oil was packaged in (2 ml eppendorf) tubes and stored at a temperature of 4°C until use (Karahacane 2015).

Calculation of essential oil yield: *Tetraclinis articulata* essential oil yield was calculated taking into account the mass of extracted essential oil in relation to the mass of the plant material used.

R (%) = [m1/ m2] X100

R: yield of essential oil, **m1**: mass of essential oil, **m2**: mass of the dry plant material.

Toxicity: During our experiments used the doses of 5, 10, 15, and 200 µl of pure oil. They were selected after several preliminary tests. Insecticidal tests by inhalation of essential oils were carried out in Petri dishes of 9 cm diameter and 1.5 cm depth.

Tests were performed on ten non-sexed adults placed in Petri dishes hermetically closed with parafilm. The crude essential oils (4 doses), dropped on a filter paper, were placed in a capsule of 3 cm diameter and 1 cm depth covered by aluminum and closed by a piece of tulle. There was no any contact between the insects and essential oil. The Petri dishes were placed at a temperature of 28 °C and a relative humidity of 70 %. The dead insects were counted on the Petri dishes after 24, 48, 72, 96 and 120 h of treatment. Controls receive no essential oils. To calculate the chemical concentration that kills 50% and 90% of the sample population (LD_{50} and LD_{90}) and the lethal times of 50% and 90% (TL_{50} and TL_{90}), transformed the doses and times into decimal logarithms and probit-corrected mortality percentages (Finney 1971).

These transformations make it possible to obtain regression line equations of the type:

Y = ax + b

Y: probit of corrected mortality.

x: logarithm of dose or time.

a: spawning.

b: constant value.

The corrected deaths were calculated according to the Abbott formula (1925):

MC% = (NIM-NIMT) x100 / (NTI-NIMT)

MC (%): Corrected mortality percentage.

NIM: Number of dead insects in the treated population.

NIMT: Number of dead insects in the control population

The results were interpreted by tests of the variance analysis using two classification criteria (Dagnelie 1975) to study the effect of dose and exposure time of essential oil on the percentage of *T. castaneum* adult mortality.

Factor 1: Dose with 4 levels (D1, D2, D3, and D4).

Factor 2: Exposure time with 5 levels (24h, 48h, 72h, 96h, and 120h).

Statistical analysis was done usingsoftware. In addition, the Newman-Keuls test is used to compare the maximum and minimum mortalities of the different homogeneous groups.

RESULTS AND DISCUSSION

Yield: Yields of *T. articulata* essential oils obtained are 0.37% for dry seeds and 0.2% for dry leaves (Table 1).

Mortality: The percentage mortality of *T. castaneum* has increased significantly using the essential oils extracted from dry grains a dry leaves of *T. articulata* compared to the control. Mortalities vary with dose and time of exposure (Table 2).

Influence of doses and exposure time on mortality: According to the dose and time factor in essential oils extracted from dry seeds and leaves of *T. articulata*, there is a highly significant variation in *T. castaneum* adult mortality rates (Fig. 1 and 2). Figures 1 and 2 show a variability of adult mortality according to the doses and the exposure time.

LD_{50} and LD_{90} : The transformation of the percentages of mortality after 5 days of exposure to probits and the regression of these data as a function of the logarithm of the dose of the essential oils made it possible to obtain the following equations successively Y1, Y2, Y3, Y4 and Y5 for Seeds and leaves (Table 5). The LT_{50} and LT_{90} are calculated directly from the right regression (Table 6) at different doses applied essential oils of seeds and leaves of the studied plant. The best lethal times found for the grains are 36.13 hours for the TL_{50} and 102.42 hours. For dry leaves, they are 38.02 h for the TL_{50} and 108.44 h for the TL_{90} (Table 6) the 4 doses.

Essential oil yield extracted from seeds and leaves of *T. articulate* are respectively 0.37% and 0.2%. Previous work reported that yield of *T. articulata* essential oil in vary from 0.11 to 0.70% for oils extracted from leaves and from 0.32 to 0.41% for oils extracted from twigs. Larabi (2015) observed the yield of the essential oils extracted from leaves of *T. articulata* is 0.11%. Additionally, Bourkhiss et al (2007a) found a yield of 0.22% in the Khmisset region in Morroco. In the same region, a yield of 0.41% was obtained from twigs (Bourkhiss et al 2007b). The higher yield of 0.70% extracted from the leaves, collected at Tetouane in Morocco, (Barrero et al 2005). In Algeria, yields of essential oils extracted found leaves achieved respectively 0.35% in Frenda (Wilaya of Tiaret), 0.75% in EL-Hacaiba (Wilaya of SidiBel Abbes) and 0.78% in Ouled Mimoun (Wilaya of Tlemcen) (Toumi et al 2011).

Several works have revealed that alpha pinène is the main compound of the essential oils of the majority of plant species of the Cupressaceae and in particular the genus *Tetraclinis* and *Juniperus* (Jemli et al 2018, Fierascu et al 2020, Boukraa et al 2022). Inhalation treatment of *T. castaneum* adults with essential oils extracted from seeds and leaves of *T. articulate* showed mortalities exceeding 50%. Mortalities began to appear from the first day with a maximum of 63.66% in dose 4. Then they evolved to 60% on the second day, to arrive at 96.66% at the 5TH day with the dose 4 from seeds oils. With regard to leaves oils, mortalities are also observed from the first day with 36.66%. Then they started to evolve to 53.33% on the second day, 73.33% on the third day, to reach 83.33% and 100% respectively during the 4th day and the 5th day with dose 4. There was no mortality was obtained in the controls. The lethal doses (LD_{50}) are 6.57 and 10.67 µl of the essential oil extracted from the seeds and 5.05 and 6.06 µl of the oil extracted from the leaves. For LD_{90}, only 15.99 µl for seeds and 12.55 µl are retained. The

Table 1. Essential oils yields (%) for grains and leaves of *Tetraclinis articulata* collected from Khemis Miliana, Algeria

Part of plant	Dry matter (g)	Essential oil (g)	Yield (%)
Dry seeds	800	3	0.37
Dry leaves	1500	3	0.2

Table 2. Mortality of *Tribolium castaneum* adults exposed for various periods to essential oils from dry seeds and leaves of *Tetraclinis articulata* (%)

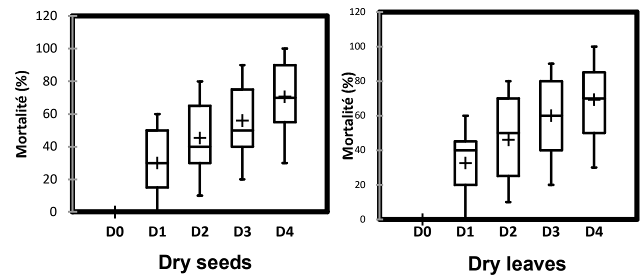
Hours	Dry seeds					Dry leaves				
	T	D1	D2	D3	D4	T	D1	D2	D3	D4
24 h	0	0	13.33	26.66	36.66	0	3.33	13.33	30	36.66
48 h	0	20	33.33	40	60	0	23.33	26.66	43.33	53.33
72 h	0	30	46.66	53.33	73.33	0	36.66	46.66	63.33	73.33
96 h	0	43.33	60	73.33	86.66	0	43.33	66.66	76.66	83.33
120 h	0	56.66	73.33	86.66	96.66	0	56.66	76.66	86.66	100

Table 3. Impact of *Tetraclinis articulate* essential oil doses from dry seeds and leaves on *Tribolium castaneum* mortalities

Parameter	D0	D1	D2	D3	D4
Dry seeds					
Number of observations	15	15	15	15	15
Minimum	0.000	0.000	10.000	20.000	30.000
Maximum	0.000	60.000	80.000	90.000	100.000
Amplitude	0.000	60.000	70.000	70.000	70.000
Aérage	0.000	30.000	45.333	56.000	70.667
Standard déviation	0.000	21.381	22.636	22.928	22.509
Coefficient of variation		0.689	0.482	0.396	0.308
Dry laves					
Numbers of observations	15	15	15	15	15
Minimum	0.000	0.000	10.000	20.000	30.000
Maximum	0.000	60.000	80.000	90.000	100.000
Amplitude	0.000	60.000	70.000	70.000	70.000
Aérage	0.000	32.667	46.000	60.000	69.333
Standard déviation	0.000	19.445	25.014	22.361	23.442
Coefficient of variation		0.575	0.525	0.360	0.327

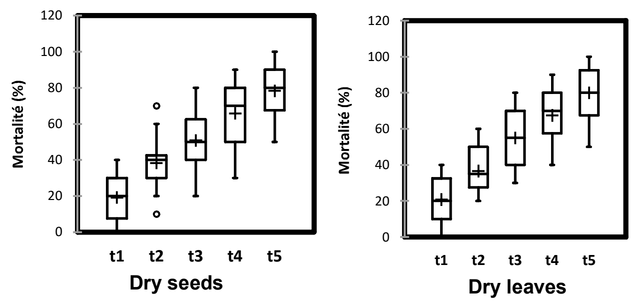
shortest lethal time for killing 50% of the *T. castaneum* population is 52.24 h for the dose 3 and 36.13 h for the dose 4 for the seeds.

Fumigation tests have shown that essential oils obtained from the cones of *Tetraclinis articulata* show high toxicity against *Tribolium castaneum* and *Sitophilus granarius* (Sadik et al 2022). For leaves oils, the TL_{50}s values are 46.66 h for dose 3 and 38.02 h for dose 4. In addition, LT_{90} As for the LT_{90} values were 102.42 and 108.44 hr espectively for grains and leaves oils. Guo et al (2016) found that the essential oil leaves of *Juniperus formosana (*Cupressaceae) same botanical family, as *Tetraclinis* had a strong contact and repellent activity against adults of *T. castaneum* and *Liposcelis bostrychophila* (Liposcelididae- Psocodea) with respective LD_{50}s values of 7.65 g/adult and 81.50g/cm^2., Athanassiou et al (2013) reported a strong insecticidal activity of *Juniperus oxycedrus* (Cupressaceae) leaves essential oils associated with silica gel on adults of *T. confusum* at doses 0.125, 0.250 and 0.5g/kg. Hedjal-chehheb et al (2013) showed that the fumigant action, of *Cupressus sempervirens* essential oil collected from Algeria and Tunisia on *Callosobruchus maculatus* adults after 24 hours of exposure to the dose 37.5 µl. Giatropoulos et al (2013) demonstrated that the leaf essential oils from *Tetraclinis* articulata and other species of the genus *Cupressus* and *Juniperus* gave a strong repulsive activity on *Aedes albopictus* larvae (Culicidae) withs LC_{50}s values ranging from 47.9 to 70.6 mg/l. Harmouzi et al (2016), noted that essential oils, extracted from the leaves and twigs of *T. articulata*, show a toxicity towards *Aphis citricola* which increases with concentration and the exposure time. Kellouche and Chehbab (2014) reported the impact of *T. articulata* essential oils on embryonic, pos-embryonic and



D0, D1, D2, D3 and D4 = Doses of Treatment

Fig. 1. Variability of mortality induced by different doses of dry seeds and leaves *T. articulata* essential oil



t1, t2, t3, t4, and t5 = Time exposures)

Fig. 2. Variability of mortality induced by different times of dry seeds and leaves *T. articulata* essential oil

Table 4. Impact of *Tetraclinis articulate* essential oil doses from dry seeds and leaves of *T. castaneum* mortalities

Parameters	t1	t2	t3	t4	t5
Numbers of observations	12	12	12	12	12
Dry seeds					
Minimum	0.000	10.000	20.000	30.000	50.000
Maximum	40.000	70.000	80.000	90.000	100.000
Amplitude	40.000	60.000	60.000	60.000	50.000
Aérage	19.167	38.333	50.833	65.833	78.333
Standard déviation	15.050	16.422	17.816	18.320	16.422
Coefficient of variation	0.752	0.410	0.336	0.266	0.201
Dry laves					
Numbers of observations	12	12	12	12	12
Minimum	0.000	20.000	30.000	40.000	50.000
Maximum	40.000	60.000	80.000	90.000	100.000
Amplitude	40.000	40.000	50.000	50.000	50.000
Aérage	20.833	36.667	55.000	67.500	80.000
Standard déviation	15.050	13.707	15.667	16.583	17.056
Coefficient of variation	0.692	0.358	0.273	0.235	0.204

Table 5. Regression lines equations obtained for *T. articulata* seeds and leaves

Part of the plant	Right of regression	LD_{50} (µl)	LD_{90} (µl)
Seeds	Y1=7.7502x – 4.8402 (R^2 = 88.7%)	18.60	27.21
	Y2=1.6496x +2.9644 (R^2 = 92.3%)	17.13	102.31
	Y3=2.4401x + 3.3423 (R^2 = 92.5%)	10.67	60.42
	Y4=2.0177x + 3.3491 (R^2 = 94.5%)	6.57	28.35
	Y5=2,4401x + 3.3423 (R^2 = 92.5%)	4,77	15,99
Leaves	Y1=2.5043x + 1.4305 (R^2 = 98.3%)	26.62	86.39
	Y2=1.3500x + 3.2285 (R^2 = 85.8%)	20.52	182.12
	Y3=1.604x + 3.4518 (R^2 = 94.4%)	9.23	57.97
	4=1.8667x + 3.5386 (R^2 = 99.9%)	6.06	29.41
	Y5=3.2392x + 2.7212 (R^2 = 82.8%)	5.05	12.55

LT_{50} and LT_{90}

Table 6. Different equations obtained from the right regression relating to the calculation of TL_{50} and TL_{90} of dry grains and dry leaves of *T. articulata*

Part of the plant	Right regression	LT_{50} (h)	LT_{90} (h)
Seeds	Y1=7.1376x – 9.0706 (R2 = 85.20%)	93.61	141.47
	Y2=2.4208x + 0.5039 (R2 = 98.99%)	71.99	243.23
	Y3=2.3520x + 0.9592 (R2 = 91.16%)	52.24	182.91
	Y4=2.8292x + 0.5922 (R2 = 94.13%)	36.13	102.42
Leaves	Y1= 2.7644x – 0.5519 (R2 = 97.66%)	101.94	296.06
	Y2=2.5993x + 0.18 00 (R2 = 97.04%)	71.50	222.22
	Y3=2.2917x + 1.1752 (R2 = 94.60%)	46.66	168.85
	Y4=2.8123x + 0.5564 (R2 = 90.99%)	38.02	108.44

larval survival on eggs and larvae of *Callosobruchus maculates* when the dose and the time of exposure increase from 0 to 75µl/l of air and from 24 to 96h.

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