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EFFECT OF TWO ESSENTIAL OILS FROM THE ASTERACEAES FAMILY AGAINST *ECTOMYELOIS CERATONIAE* ZELL. (LEPIDOPTERA, PYRALIDAE): CASE OF *ARTEMISIA HERBA-ALBA* ASSO. AND *ARTEMISIA COMPESTRIS* L.

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Abstract

The current work was done *Artemisia herba-alba* and *Artemisia compestris* essential oils harvested from the Eastern Algerian Sahara, their insecticidal characteristics against the eggs and adults of the date moth *Ectomyelois ceratoniae*. Indeed, two treatment modes were used; by contact application on eggs and by inhalation against adults. It appears from the results that the hatch rates were less than the hatching rate recorded in the control (96% ±00.00). The hatching rate reported on eggs treated by the highest dose (160 µl/ml) of *Artemisia herba-alba* and *Artemisia compestris* essential oils are 16.66 ± 08.81 and 37.77 ± 13.47 respectively. Statistical treatment results by the Chi-square test ($\chi 2$), attest that the treatment by *A. herba-alba* and *Artemisia compestris* essential oils at the same dose (160 µl/ml) affect significantly ($\chi 2 = 35.62$, $p = 0.00$ and $\chi 2 = 21.17$, $p = 0.00$ respectively) the hatching rate compared to the control. The sensitivity of adults to essential oils is expressed by 100% mortality rates obtained after 10 min of treatment by the highest doses (80 µl/ml and 160 µl/ml) of *A. herba-alba* essential oils, the same mortality rates (100%) were notified with the same doses (80 µl/ml and 160 µl/ml) after 20 min and 15 min of treatment by *Artemisia compestris* essential oils respectively. The dose-dependent mortality data revealed that there was a significant difference between the five doses of *A. herba-alba* essential oil tested except at the last treatment time (20 min) for which it was appeared $p = 0.571$, while for *A. compestris* essential oil, a significant difference was recorded with p varying between 0.00 and 0.003. The lowest LD_{50} value (0.09 µl/ml and 16.71 µl/ml) were noted during the longest treatment time (20 min), while the highest LD_{50} value (75.85 µl/ml and 263.7 µl/ml) were found during the shortest time (5 min) of *A. herba-alba* and *A. compestris* respectively.

Key words: *Artemisia compestris*, *Artemisia herba-alba*, Date moth, *Ectomyelois ceratoniae*, Essential oils, Toxicity

Introduction

Among the various parasitic attacks and diseases that affect the date, the major problem for importers is the fruit infestation by the date moth. The moth lays eggs on the surface of ripe dates. The larva penetrates inside and leaves traces which waste the fruit and reduce its commercial value (Ordines 2000). The results of

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chemical control were disappointing to the extent that there is no reduction in the infection rate, this is explained by the endophytic behavior and hanging position of the fruit on the tree that does not facilitate the contact insecticide (Khoualdia et al. 1996, Grissa et al. 2011, Peyrovil et al. 2011, Azqandi et al. 2015). Moreover, oases are a fragile ecosystem where the use of pesticides would have secondary effects for both human being and the environment (Dhouibi 2000). Continuous attack of the pest, incapacity and defects of chemical control, obliges to orient towards other means less drastic and more environmentally compatible (Escoubet 2011). Biopesticides of plant origin are called the means for a better future, because demand for safe crop protection products of low persistence and qualified as "green products" are actually on the rise which ones based on essential oils can be the tools of choice in management programs of pest resistance to pesticides (Isman 2000). The development of new control methods by the use of plants or their extracts possessing insecticidal properties constitutes a concern of the present work. The objective of this study mainly focused on *Artemisia herba-alba* Asso. and *Artemisia compestris* L. (Asteraceaes) essential oils harvested from the eastern Algerian Sahara, their insecticidal characteristics against the eggs and adults of the date moth *Ectomyelois ceratoniae.*

Material and Methods

Collection of plants

The two plants studied were harvested during October 2017 from the Ain Zaatout (Biskra, Algeria) region. The samples were dried at umber, under laboratory ambient temperature for 21 days. After drying, only the leaves were recuperated and subjected to hydro distillation to obtain the essential oils.

Extraction of essential oils

The simplest method of extracting essential oils is hydro distillation. Its principle consists of immersing the plant material in a water bath, and then the whole is brought to boiling under atmospheric pressure (Sutour 2010). Leaves were subjected to hydro distillation using a modified Clevenger type apparatus.

Animal rearing

Animal materials are represented by the eggs and the adults of *E. ceratoniae* collected from a rearing colony culture initiated in the laboratory of National Institute of Plant Protection (Biskra, Algeria) under following conditions: (temperature of 27±2°C, relative humidity of 65±10%) and photoperiod (16:8) (L:D) (Al-Izzi et al. 1987). The choice of stages was justified because effective control of adults limits their fertility and therefore the number of eggs and the rate of infestation would be reduced.

Toxicity tests

Two treatment modes were used to test toxicity of *Artemisia herba-alba* Asso and *Artemisia compestris* L. essential oils; one by contact application on eggs and the other by inhalation against adults. The tests were carried out according to the protocol established by McDonald et al. (1970). Several pilot tests were carried out to select the doses to be used; the doses were prepared just prior to testing, by diluting the essential oil in tween 80 at a concentration of 0.1% which is non-toxic to insects (Bokobana et al. 2014). Thus, five doses were prepared (10, 20, 40, 80.160 µl / ml) with a control (Tween 80 diluted at 0.1%).

Contact toxicity

With a total of 90 eggs (24 h old) for each concentration (30 eggs/ petri dish), the eggs were sprayed directly by the prepared doses of *Artemisia herba-alba* Asso. essential oils. The control eggs were pulverized with diluted Tween 80 (0.1%). After three days of incubation, the hatched eggs are counted by using binocular loupe during one week. The same protocol was followed for *A. compestris* L. essential oil.

Inhalation toxicity

With total of 30 adults (24 h old) for each concentration bottle of 500 ml capacity, served as fumigant chambers containing a piece of cotton soaked by *Artemisia herba-alba* Asso. essential oil, were introduced 10 adult. The control adults were introduced in the bottle containing cotton impregnated in diluted Tween 80 (0.1%), the experiment was followed until death of all the treated adults. The same protocol was followed for *Artemisia compestris* L. essential oil.

Calculation of hatching and mortality rate

For eggs, the hatching rate was calculated using the following formula: The hatching rate (%) = (number of eggs hatched / total number of eggs) × 100. Adults mortality in treated bottles (M2) were expressed according to the Schneider-Orelli (1947) formula (Xu 2004) in corrected mortalities (Mc), taking into account naturals mortalities occurring in the control bottles (M1) using the following formula: Schneider-Orelli (1947) formula: MC = [M2 - M1/ 100 - M1] × 100.

Statistical analyses

To assimilate the effect of different concentrations of the essential oil against eggs hatching rate, the results are compared pair wise by the chi-square test ($\chi 2$) using IBM SPSS statistics 22. Data of mortality rates reported by both the plants depending on the dose administered were subjected to one-way analysis of variance, then the means were compared using Tukey's test whether there were differences between the treatments at the 5% level of confidence. R^2, LD_{50} values were estimated by probit analysis using the same program.

Results

Effect of *A. herba-alba* and *A. compestris* essential oils on *E. ceratoniae* eggs

The results of the ovicidal effect of *A. herba-alba* and *A. compestris* essential oils on the hatching rate (%) of *E. ceratoniae* treated eggs depending on the doses are shown in Table 1. It appears from the results of the two studied plants that whatever the treatment doses the hatch rates were found less than the hatching rate recorded in the control in the order of 96 ± 00.00%.

Hatching rates of 88.99±02.03%; 83.33±00.00%; 75.55 ± 8.39%; 49.99 ± 8.81% and 16.66 ± 8.81% have been reported on eggs treated with *A. herba alba* essential oils at concentrations 10, 20, 40, 80 and 160 µl/ml respectively, with the same concentrations also for order of *A. compestris* essential oil, registered hatching rate were 85.55 ± 1.92%; 82.22 ± 3.84%; 73.33 ± 3.33; 65.56 ± 5.09% and 37.77 ± 13.47% respectively (Table 1).

Table 1. Hatching rate (%) (Mean ± SD) of *E. ceratoniae* eggs treated with *A. herba-alba* and *A. compestris* essential oil

Dose (µl/ml)	Hatching rate (%) (mean ± SD)		*A. herba-alba*		*A. compestris*	
	A. herba-alba	*A. compestris*	$\chi 2$	P_{value}	$\chi 2$	P_{value}
10	88.99±02.03	85.55±01.92	0.21	0.64	0.74	0.389
20	83.33±00.00	82.22±03.84	1.45	0.228	1.45	0.228
40	75.55±08.39	73.33±03.33	3.26	0.071	4.32	0.038
80	49.99±08.81	65.55±05.09	13.87	0.000	6.66	0.010
160	16.66±08.81	37.77±13.47	35.62	0.000	21.17	0.000
Control (Tween 0.1%)	96±00.00		-			

It should also be noted that the hatching rate decreases with increasing concentrations of both the plants essential oils (Table 1). The unhatched eggs treated with *A. herba-alba* essential oil have a dead embryo inside the egg while the unhatched eggs treated with *A. compestris* essential oil present deformity. Statistical analysis resulted by the chi-square test ($\chi 2$) (Table 1), attest that the treatment with *A. herba-alba* essential oils at the concentrations 10, 20, and 40 µl/ml do not significantly affect the eggs hatching compared to the control ($\chi 2 = 0.21$ and $p = 0.640$, $\chi 2 = 1.45$ and $p = 0.228$, $\chi 2 = 3.26$ and $p = 0.071$ respectively), on the other hand, with the high concentrations 80 and 160 µl/ml, the hatching rate was significantly affected with $\chi 2 = 13.87$ and $p = 0.000$, $\chi 2 = 35.62$ and $p = 0.000$ respectively. Whilst *A. compestris* essential oil affect significantly the hatch rate starting at 40 µl/ml concentration with $\chi 2 = 4.32$ and $p = 0.038$.

Action of *A. herba-alba* and *A. compestris* essential oils on *E. ceratoniae* adults

The results on the effect of *A. herba-alba* and *A. compestris* essential oils on *E. ceratoniae* adults are presented in Table 2 and 3 respectively.

Table 2. Mortality rate (%) (Mean ± SD) of *E. ceratoniae* adults treated with *A. herba-alba* essential oil

Time (min.)	Dose (µl/ml)					
	10	20	40	80	160	P_{value}
5	$00.00±00.00_a$	$13.33±05.77_b$	$33.33±30.55_{ab}$	$36.66±05.77_{bc}$	$83.33±11.54_c$	0.001
10	$26.66±11.54_a$	$70.00±10.00_b$	$80.00±26.45_{bc}$	$100.00±0.00_c$	$100.00±0.00_c$	0.000
15	$83.33±05.77_a$	$96.66±05.77_b$	$100.00±0.00_b$	$100.00±0.00_b$	$100.00±0.00_b$	0.001
20	$96.66±05.77_a$	$100.00±0.00_a$	$100.00±0.00_a$	$100.00±0.00_a$	$100.00±0.00_a$	0.571

Means in the same line with the same letter are not significantly different at $p \leq 0.05$.

E. ceratoniae adult's treated with different doses of *A. herba-alba* essential oil, it occur that with the lowest dose (10 µl/ml), the highest mortality rate (96.66 ± 5.77) is obtained during the longest period of treatment and which is 20 min (Table 2). At half of this period (10 min), it was reported a mortality rate of 100% with the highest doses (80 and 160 µl/ml). During the same treatment period, the mortality rates are 70±10% recorded with the 20 µl/ml dose and 80±26.45% with the 40 µl/ml dose. No mortality is achieved for the control, whatever the duration of treatment. According to the results (Table 3) of adult's treatment with the *A. compestris* essential oils, it appears that the most important mortality recorded on adults treated with the lowest dose (10 µl/ml) was 10±10% after 20 min of treatment. During the same treatment period, maximum mortalities rates of 76.67 ± 5.77 and 90±10% were notified with the 20 and 40 µl/ml doses respectively. With high doses, 80 and 160 µl/ml, the mortality rates of 100% are recorded for 20 and 15 min respectively. No mortality is achieved for the control, whatever the duration of treatment (Table 2).

Table 3. Mortality rate (%) (Mean ± SD) of *E. ceratoniae* adults treated with *A. compestris* essential oil

Time (min.)	Dose (µl/ml)					
	10	20	40	80	160	P value
5	$00.00\pm00.00_{a}$	$00.00\pm00.00_{a}$	$00.00\pm00.00_{a}$	$06.67\pm11.54_{a}$	$30.00\pm17.32_{b}$	0.003
10	$00.00\pm00.00_{a}$	$00.00\pm00.00_{a}$	$13.33\pm23.09_{ab}$	$60.00\pm26.45_{bc}$	$80.00\pm20.00_{c}$	0.001
15	$00.00\pm00.00_{a}$	$36.67\pm05.77_{b}$	$80.00\pm20.00_{bc}$	$90.00\pm10.00_{c}$	$100.0\pm00.00_{c}$	0.000
20	$10.00\pm10.00_{a}$	$76.67\pm05.77_{b}$	$90.00\pm10.00_{bc}$	$100.0\pm00.00_{c}$	$100.0\pm00.00_{c}$	0.000

Means in the same line with the same letter are not significantly different at $p \leq 0.05$.

The results of statistical analyses of the dose-dependent mortality data revealed that there is a significant difference between the five doses of *A. herba-alba* essential oils tested except at the last treatment time (20 min) for which we was reported a p = 0.571 (Table 2), while for *A. compestris* essential oil, a significant difference was recorded between the five doses tested for all the treatment times with p varying between 0.00 and 0.003 (Table 3). The discrimination of the means by the Tukey test at the significance level of 5% proves that *A. herba-alba* essential oils have an identical activity at the different doses after 20 min of exposure to the treatment (Table 2). On the other hand, the treatment with *A. compestris* essential oils for 20 min permits distinguished four homogeneous groups; group "a" contains the first dose (10 µl/ml), group "b" contains the second dose 20 µl/ml), group "bc" includes the third dose (40 µl/ml) and group "c" which regroups the fourth (80 µl/ml) and fifth dose (160 µl/ml) (Table 3).

LD_{50} of essential oils tested: The results of calculated lethal doses 50 (LD_{50}) are presented in Table 4, the essential oils of the plants studied become more effective when the exposure duration to the treatment increases.

Table 4. Toxicological parameter of *A. herba-alba* and *A. compestris* effect on *E. ceatoniae* adults (y: probits of mortality rates, x: decimal logarithm of concentrations)

A. herba-alba				
Time (min.)	Regression equation	R^2	LD_{50} (µl/ml)	P_{value}
5	Y = 4.23X – 2.95	0.78	75.85	0.044
10	Y = 4.29X – 0.43	0.88	18.00	0.016
15	Y = 2.46X + 3.84	0.80	2.95	0.041
20	Y = 1.26X + 6.32	0.50	0.09	0.182
A. compestris				
Time (min.)	Regression equation	R^2	LD_{50} (µl/ml)	P_{value}
5	Y = 4.14X – 5.04	0.79	263.70	0.043
10	Y = 5.64X – 6.03	0.89	90.13	0.014
15	Y = 6.35X – 5.07	0.88	38.40	0.018
20	Y = 4.33X – 0.92	0.93	16.71	0.008

The LD_{50} values are inversely proportional to the treatment times. The lowest LD_{50} (0.09 and 16.71 µl/ml) were noted during the longest treatment time (20 min.), while the highest LD_{50} (75.85 and 263.7 µl/ml) are found during the shortest treatment time (5 min.) by the essential oils of *A. herba-alba* and *A. compestris* respectively. Probit analysis proves that there is a significant correlation between mortality rate and the dose of treatment with *A. herba-alba* and *A. compestris* oils (Table 4).

Discussion

The results prove that essential oils extracted from *A. herba-alba* and *A. compestris* have insecticidal properties which on one hand lead a reduction in hatchability and also exert a lethal effect on adults. Previous studies demonstrate that *A. herba-alba* and *A. compestris* have very important insecticidal activity such as study of Ben Chaaban et al. (2019), showed that fumigant toxicity of *A. herba-alba* essential oil depending on concentration and exposure time. The exposure to vapors of *A. herba-alba* essential oil caused 0% of hatching rate of *E. ceratoniae* eggs at the concentration of 150 µl/l air during 48 h, the same concentration lead 94% mortality of adults after 24 h with LC_{50} of 0.3 µl/l air. Delmi et al. (2013), after submission of *Ephestia kuehnilla* (Lepidoptera) adults at various doses of *A. herba-alba* essential oil reduced a significant mortality compared with control according to the dose and duration of exposure. In addition, disruption of adults' reproduction by extending the preoviposition period and reducing the period for depositing eggs as fertilized females couldn't who can't live more than one or two days, reduces the number of eggs deposited. *A. herba-alba* essential oil inhibits completely the fertility of *Tineola bisselleilla* (Lepidoptera) from 3 µl dose. This oil, causes mortality rate of 100% with the dose of 2 µl after four days of treatment with the LD_{50} was 1.25 µl (Bouchikhi et al. 2010). Hannour et al. (2016) demonstrate that *A. herba-alba* essential oil passed ovicidal and adulticidal effect against *Phthorimaea operculella* (Lepidoptera). The LD_{50} for eggs was 39.54 µl/l airs, lethal concentrations ranged from 39.33 to 6.80 µl/l air among adult males

and from 39.72 to 6.39 µl/l air among adult females. *A. herba-alba* essential oils showed a nematicidal activity toward *Meloidogyne incognita* and this activity increases with the concentration and the period of exposure. Thus, the high dose (800 µl/l) inhibits the hatching of eggs by 62.8% after 12 days of treatment (Sellami et al. 2010). The acridicide test revealed that *A. herba-alba* essential oil proved effective against *Schistocerca gregaria* adults for which a mortality rate of 100% was reached on the 16th day with the highest dose (0.1 µg/g) (Figuigui et al. 2014). There are few studies that have investigated the insecticidal effect of *A. compestris* essential oil compared to *A. herba-alba* essential oil. According to Al-Harbi et al. (2021) the high doses (8 and 16 µl/ml) of *A. compestris* essential oils applied by inhalation on the rice weevil adults (*Sitophilus oryzae*) provokes mortality rates of 100% after 48 h. The LD_{50} was 10.59 h for the highest dose (16 µl/ml). The essential oil from *A. campestris*, containing β-pinene (15.2%), α-pinene (11.2%), myrcene (10.3%), germacrene D (9.0%) (*Z*)-β-ocimene (8.1%) and γ-curcumene (6.4%), showed remarkable toxicity against *Culex quinquefasciatus* (LC_{50} of 45.8 mg/l) and moderate effects (LD_{50} of 99.8 µg/adult) against *Musca domestica* (Sassoui et al. 2020). The ovicidal and adulticidal effect of essential oils against *E. ceratoniae* has been proven by several studies including the study of Ben Abada et al. (2020), showing that *Rosmarinus officinalis* essential oils inhibits adult emergence with inhibition rates ranged between 22% and 100%. Lebbouz et al. (2016), report that *Peganum harmala* essential oils have an ovicidal and adulticidal effects against *E. ceratoniae*, which reflected by decreasing of hatching rate to 5.65% compared to the control which is 87.35% and causes 100% mortality after 5 days of treatment of *E. ceratoniae* adults with a LT_{50} of 1.45 days. Amri et al. (2014), indicate that the topic aplication of *Thymus capitalus* essential oils induced at the dose of 8 µl/ml causes 100% mortality of *E. ceratoniae* adults after 24 h exposure. As stated by Bachrouch et al. (2010), the highest concentration (136 µl/l air) of *Pistacia lentiscus* essential oils induces 100% mortality after 48 h exposure, the corresponding LC_{50} was 40.2 µl/l air. The fecundity and hatching rates decreased with increases in concentration or exposure time to the oil, at the concentration of 136 µl/l air, fecundity and hatching rates were respectively 35 eggs/ female and 42.86% adds Bachrouch et al. (2010). Haouel et al. (2010), showed that *Eucalyptus rudis* essential oils achieved 100% mortality of *E. ceratoniae* adults treated by fumigation after 12 h of treatment. The LT_{50} was 36.10 h.

The toxicity of essential oil is related to their compositions in oxygenated mono terpenes which are major compounds and which prove an insecticidal activity against different species of insects (Papachristos and Stamopoulos 2002). However, Deletre et al. (2014), have shown that the biological effect of an essential oil is not always due only to the activity of the major compound. Indeed, synergistic effects can occur between major and/or minor compounds, suggesting that the mechanisms of action are different. In general, essential oils reduce the phytophagous insect populations by a double action: inhalation toxicity exerted on the adults as well as an inhibition of the reproduction (Regnault- Roger and Hamraoui 1997).

Conclusion

The result shows that *A. herba-alba* and *A. compestris* essential oils have an ovicidal and adulticidal effects aginst *E. ceratoniae*. They could be good bio-insecticide alternatives to chemical control, while preserving human health and the environment. These new molecules are biodegradable and less likely to cause the resistance of the target species.

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