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Ovicidal, larvicidal and adulticidal activities of essential oils from *Peganum harmala* L. (Zygophyllacae) against date moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)

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Abstract

The date moth *Ectomyelois ceratoniae* is the major insect pest of Algerian palm gardens. All phoenicicol regions are infested. The study of toxic activity of essential oils from *Peganum harmala* by topical application on eggs forth instar larvae and by fumigation on adults, showed perceptible effects against this insect. The hatching rate recorded for eggs treated by *Peganum harmala* essential oils is a low (5.56%) against the control (87.35%). The topical application of essentials oils on forth instar larvae (L_4) achieved 56.66% mortality after five days of treatment with lethal Time 50(TL_{50}) of 2.57 days. For adults, 100% mortality was recorded after 5 days of treatment with a lethal Time 50 (TL_{50}) of 1.45 days. These results indicate that *Peganum harmala* essential oils have an ovicidal, larvicidal and adulticidal effects against *Ectomyelois ceratoniae* and it can be used as an alternative of chemical pesticides.

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Introduction

The date moth *Ectomyelois ceratoniae* infest date both in the field, on the date palms and the proliferation continuing during storage (Munier, 1973). In the fact, the presence of larvae and their excrements in date making theme unfit for human consumption, conditioning so, sever measurement in the marketing including exportation (Mokhtar *et al.,* 2010). In Algeria, chemical control was first tool used by using DDT (Wertheimer, 1958) and various products are also applied in the field, in particular, Malathion 2%, Parathion 1,25%, Phosalon 4%, and Bactospeine 1% (Bounaga and Djerbi, 1990). However, the observation of adverse effects of chemical control on the environmental and human health requires us to consider some rules when using them and oriented researches towards other less drastic and more compatible solutions with environment (Escoubet, 2011). That's why the resorts to natural compounds from plant, resulting from secondary metabolism is revealed promoter. The structural diversity and bioactivity of plant alkaloids such as in *Peganum harmala* make them, one of the most important natural substance origins (Salari *et al.*, 2012). Numerous studies have proven insecticidal activity of *Peganum harmala* alkaloids against different species such as *Schistocerca gregaria* (Orthoptera. Acrididae) (Idrissi Hassani *et al.*, 2002 ; Abbassi *et al.,* 2003 ; Abbassi *et al.*, 2005 ; Idrissi Hassani and Hermas, 2008), *Pieris rapae* (Lepidoptera. Pyralidae) (Anqing *et al.,* 2003), *Plodia punctella* (Lepidoptera. Pyralidae) (Rharrab*et al.,* 2007), *Bactrocera oleae* (Diptera.Tephritidae) (Rehman *et al.*, 2009), *Culex pipiens* (Diptera.Culicidae) (Zeng *et al.*, 2010), *Aphis gossypii* (Hemiptera. Aphididae) (Salari*et al.*, 2012) and *Tribolium castaneum* (Coleoptera.Tenebrionidae) (Dastagir and Hussain, 2013).

Perfection bibliography made during this study show that only (Tahrouch *et al.*, 2002; Selim *et al.*, 2013; Dastagir *et al.*, 2014 *)* studies, are interested for *Peganum harmala* essential oils and their biochemical compositions while the studies that investigate their insecticidal activities are rare. It is in this contest that our study is organized whose the objective is to evaluate ovicidal, larvicidal and adulticidal activities of *Peganum harmala* essential oils against *Ectomyelois ceratoniae*.

Materials and methods

Animal material

Animal material is represented by individual's (eggs, larvae and adults) of *Ectomyelois ceratoniae* collected from rearing colony initiated in the laboratory of national institute of plant protection, Biskra, Algeria.

Insect rearing

Infested dates collected from palm garden were conducted in boxe cages, placed in rearing room under following conditions: (temperature of 27± 2C, relative humidity of 65±10%) and photoperiod (16: 8) (L: D) (Al Izzi *et al.*, 1987). *Ectomyelois ceratoniae* was reared on an artificial diet based on wheat bran (60%), sucrose (12%), salt mixture (2%), yeast (1.3%), lysine (1.23%), methyl paraben (0.13%), vitamin C (0.67%), aureomycine (0.67%), glycerine (150 ml) and distilled water (150 ml) (Mediouni Ben Jemma and Dhouibi, 2007).

Plant material

Plant material is represented by *Peganum harmala* leaves. In Algeria, this plant is common in steppe, Northern, Southern Sahara and central Sahara Mountains (Ozenda, 1991).The plant was collected from Itel River (El Meghaier, Algeria) during March 2014.

Essential oils extraction

Hydro distillation involves immersing a primary material in a water bath, the whole is brought to boiling and the operation is generally conducted at atmospheric pressure (Hernandez Ochoa, 2005). While all parts of this plant are toxic, only leaves are used in our study and subjected to hydro distillation using a modified Clevenger type apparatus. *Peganum harmala* leaves are richer in volatile compounds then the seeds and stems (Tahrouch *et al.*, 2002).

Topic toxicity

Ovicidal and larvicidal activities of essential oils are carried out by topical application. For eggs, 195 eggs (24 hours old) were divided in to 3 petri dishes at rate of 65 eggs per dish and pulverized by essential oils. The control is pulverized by distilled water. Eggs hatched are daily counting under binocular loupe. For the fourth instar larvae (L_4), 60 larvae are divided in to 3 petri dishes at rate of 20 larvae per dish and pulverized by essential oils when the control is pulverized by distilled water. Mortality was recorded daily until death of all treated larvae, if appropriate up, to the next instar larvae pass.

Fumigant toxicity

This test consist to impregnated a piece of cotton by essential oils which attached to the lid of bottles (500 ml capacity), served as fumigant chambers. 20 adults (24 hours old) are introduced in this bottles which is closed and placed under rearing conditions. Three repetitions are performed with non-treated adults, keep in the same conditions,served as a control (cotton impregnated in distilled water), the test is followed until death of all treated adults.

Statistical analyses

To assess the Impact of essential oils against eggs, the data are subjected to Chi Square (☐2) test, and to determine effectiveness of essential oils on forth instar larvae and adults, data underwent to one way ANOVA using XL stat 2015 program. The lethal time 50 (LT_{50}), this designed a median effective time to cause mortality of 50% individuals exposed to a dose or concentration, is calculated from regression equation of probits corresponding corrected mortality according to the logarithms of treatment time (Ramade, 2007), using Schneider- oreli, 1947 formula (Xu,2004) and probits table.

Schneider-Orelli Formula: MC = [M2 – M1/ 100 – M1] × 100

Where: MC: corrected mortality (%)
M2: mortality in the treatment(%)
M1: mortality in the control(%).

Results

Topic toxicity

The results of toxic effect of *P. harmala* essential oils against *E. ceratoniae* hatching rate (Table 1), show that only 05.65% of treated eggs were successful to hatch. The *P. harmala* essential oils inhibited hatching of 94.35% of treated eggs whose 13.33% have incomplete embryogenesis and 81.02% have infertile eggs appearance.

Table 1. Hatching rate (%) recorded on control and treated eggs by *P. harmala* essential oils.

control		Treated eggs			☐2	P
Hatch	No hatch	Hatch	No Hatch	incomplete embryogenesis		
85.20±3.52	14.80±3.53	5.64±1.77	81.02±3.20	13.33±2.35	263.73	<0.0001

Hatching rate of 87.35% was obtained for the control. Chi Square(☐2) analysis indicate that *P. harmala* essential oils showed significant difference in treated eggs hatching rate compared to control hatching rate (☐2= 263.73 and P<0.0001). For forth instar larvae (L_4) treated by essential oils, mortality of 56,66 % was recorded at 5 days (Table 2). For control, *P. harmala* essential oils achieved no mortality. Analysis of variance showed that *P. harmala* essential oils have a significant effect on larvae (L_4) mortality with F = 710.52 and P<0.0001(Table 2).

Table 2. Corrected mortality (C. M) recorded on larvae (L_4) treated by *P. harmala* essential oils.

Time (Day)	C. M (%) ± SD	df	F	P
1	40.00±8.66			
2	50.00±5.00			
3	53.33±2.88	28	710.52	<0.0001
4	53.33±2.88			
5	56.66±2.88			

Fumigant toxicity

Given the results obtained, it appears that *E. ceratoniae* adults are sensitive to *P. harmala* essential oils, this sensitivity reflected by 100% mortality obtained at 5 days after treatment, whereas at the same period, 20% of mortality is achieved for the control. Analysis of variance showed that *P. harmala* essential oils have a significant effect on adults mortality with F = 15.46 and P= 0.002 (Table 3).

Table 3. Corrected mortality (C. M) recorded on adults treated by *P. harmala*essential oils.

Time (Day)	C. M (%) ± SD	df	F	P
1	00±0.00	28	15.46	0.002
2	88.88±5.00			
3	87.5±5.00			
4	87.5±5.00			
5	100±0.00			

Probits analysis showed a significant correlation (R^2 = 0.96 and P = 0.007) between mortality recorded on L_4 and the exposure time. The LT_{50} is 2.57 days, while for adults, no significant correlation is recorded (R^2 = 0.46 and P = 0.319) between mortality and the exposure time. The LT_{50} is 1.45 days (Table 4).

Discussion

Our study showed that *P. harmala* essential oils demonstrated toxic effects against eggs, larvae (L_4) and adults of *E. ceratoniae*. Our results are similar to results recorded by Bachrouch *et al.* (2010), which show that *Pistachia lentiscus* essential oils achieved 57.1% mortality for *E. ceratoniae* eggs. Itaoua Apoyolo *et al.* (2003), reported that essential oils of *Chenopodium ambrosioides* and *Eucalyptus citrodora* have ovicidal activity against *Caryedon serratus*, the embryonic mortality exceed 70%. Furthermore Ketoh *et al.* (1998) and Namador *et al.*(2010), have recorded hatching rate of 06.25 % for *Callosobruchus maculatus* eggs fumigated by *Cymbopogon nardus* essential oils against 90% recorded for the control. Eggs affected have their ovular content dissolved but cuticles remains intact.

Larvae or internal forms of this pest, developed inter date, is in the fact, the most damaging stage as it destroys dates quality and at the same time, they are protected by date pericarp, this later raison explain the paucity of toxicological studies against larval stages. Study of Mehaoua (2014), show that azadiractin achieved 75.29% mortality of *E. ceratoniae* first instar larvae, the lethal time 50 (LT_{50}) is 5 days. The treatment of forth instar larvae of *Culex pipiens* by *Thymus vulgaris* essential oils, recorded 100% mortality after 24 hours(El Khal *et al.*, 2015). *P. harmala* essential oils induced 100% mortality of fifth instar larvae after 8 min 30´ with LT_{50} of 6 min 12´(Kemassi *et al.*, 2014).

About adulticidal activity, our results are comparable to those of Haouel *et al.* (2010) who have shown that *Eucalyptus camaldulansis* and *E. rudis* essential oils achieved 100% mortality of *E. ceratoniae* adults after 24 hours of treatment. Our results are agree with those of Mediouni Ben djemaa *et al.* (2009), who reported that *P. lentiscus* reveal 100% mortality of *E. ceratoniae* adults after 2 days of treatment.

The *P. harmala* essential oils have power acridicidal activity against desert locust adults with LT_{50} of 19 min 21´(Kemassi, 2008). So; *P. harmala* essential oils are more efficient against *Schistocerca gregaria* then *E. ceratoniae* adults. In the same contest Pyrovi *et al.* (2011), demonstrated that *Ferula assafoetida* essential oils reduced infestation rate of pomegranate fruits by *E. ceratoniae*, this reduction can be explained either by repulsive effect of essential oils or by disruption of adults reproductive behaviors who can´t detected their bridge site or even by combination of these tow effects (Goldansaz *et al.*, 2012).

The essential oils proved toxic effect against different development stages of *E. ceratoniae*. Essential oils toxicity is related to their compositions in oxygenated monoterpenes, compounds that possess insecticidal activity against various insect species (Papachristod and Stamopoulosd, 2002). The richness of *P. harmala* essential oils by oxygenated monoterpenes proven by Dastagir *et al.* (2014) may explain their insecticidal effect against *E. ceratoniae*.

Table 4. Toxicological parameters of *P. harmala* essential oils according to exposure time.

Time (days)	Regression Equation	R^2	LT_{50} (days)	F	P
Larvae L_4	Y= 0.56X + 4.77	0.96	2.57	43.47	0.007
Adults	Y= 5.03X + 4.19	0.46	1.45	1.72	0.319

The examination of eggs, larvae and adults corpses under binocular loupe showed that there are no cuticle lesions in all treated stages, which proves that *P. harmala* essential oils have fumigated effect. Ketoh *et al.* (1998) reported that made that essential oils from plant are effective both against eggs, larvae and adults is thought that they act via respiratory system. The exact mode of action mechanism of essential oils via respiratory system remains unknown (Tripathi *et al.*, 2009).

Conclusion

The treatment of different developed stages of *E. ceratoniae* by *P. harmala* essential oils under controlled conditions revealed their insecticidal properties. This toxic effect due to the presence of one or many actives compounds in such oils. So the identification of compounds responsible of this activities and toxicological studies to determine their mode of action are necessary to confirm their performances as bio insecticide alternative to chemical control.

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