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Effects of Aqueous Extracts of Seeds of *Peganum harmala* L. (zygophyllaceae) on 5th Stage Larvae *Locusta migratoria cinerascens* (Fabricius, 1781) (*Orthoptera*: Oedipodinae)

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Abstract: The study has for objective the determination of the efficiency of the aqueous extracts from seeds of *Peganum harmala* L. on the mortality of the larvas of 5th stage and on the fertility of the female adults of *Locusta migratoria cinerascens*. For that purpose, a breeding of locusts was realized in the conditions of laboratories. At hatching, the larvae are fed daily basis lawn *Stenotaphrum americanum* and a protein supplement of wheat bran. The extraction of the aqueous extract of the seeds of *P. harmala* is done after maceration in the ethanol, under magnetic stirring using a rotavapor. To determine larval mortality L5, two modes of treatment have been made, one by contact and another by ingestion, using for both treatment 4 doses in a geometric progression, 0.03 mg/mL, 0.06 mg/mL, 0.12 mg/mL and 24 mg/mL. The results showed that the mortality for the doses of 0.12 mg/mL and 0.24 mg/mL, reaches respectively 40% and 60% on the 3rd day, as well for the treatment by contact as by ingestion. But the LD50 for ingestion treatment is lower. It is 0.19 mg/mL contrary to that of the contact treatment (0.19 mg/mL). The larvae that survived the treatment by ingestion, have suffered morphological changes as well as physiological which consist of a deformation of the wings, delayed of the larval molt, of 6 days, blocking the fledging, the change of the pigmentation as well as an extension of the preoviposition. Fertility was also affected and females lay only twice, a small number of eggs, unlike untreated females which come to lay 3 times with an average of 62.7 eggs/female at first spawning against 50 eggs for the females treated.

Key words: *Locusta migratoria cinerascens*, aqueous extracts, *Peganum harmala*, lethal dose, fecundity.

1. Introduction

Locusts as harmful insects occupy a very important place among agricultural pests. It is a heterogeneous group that includes both the locust and the grasshopper. The majority of crop pests are located in the African continent. The subspecies *L. migratoria cinerascens*, wide distribution Mediterranean is present in Europe (France, Italy, Spain, Yugoslavia, Greece) and North Africa (Morocco, Algeria, Tunisia) [1]. In Algeria, it is characteristic of coastal areas and plains of the Tellien Atlas as well as in the south of the Saharan Atlas, including Tamanrasset and Adrar which offer a permanent habitat conducive to maintaining and the dispersion of the locusts, whether in remission or invasion period, due to its favorable climatic ecological conditions [2-4]. Indeed, *L. cinerascens migratoria* has the ability to be in two phases, the one solitary and the other one gregarious. These are the larval bands of the gregarious phase which are formidable biography aggressors and which causes considerable damage to farmers because of their large polyphagia [5]. In fact, since its signaling in 1991 and 1994, in the perimeters of irrigated wheat of Zaouiet-Kounta (Adrar) [2] and in the region of Touat (Adrar) [6], in the central Sahara Algerian, it has

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become a pest potential concern. A great many plants are then likely to be attacked, they are timber as the banana and date palm [7-10].

Currently chemical control against insects in general, and locusts in particular uses an arsenal of active materials equally effective as the other. It remains, in effect, the only solution to cope with this scourge, in case of invasion, despite the catastrophic consequences on the environment and the fragile ecosystems of desert regions or semi desert. Of this fact, several scientists were interested in the alternative solutions to substitute the pesticides organic of synthesis by bio pesticides of vegetable origin, biodegradable, not polluting and respectful of the environment [11-15].

Indeed, the use of plants as a source of pesticides is reported by an abundant literature [16-18]. Of by secondary compounds (alkaloids, carbenolides and glucosinolates terpenes) that they contain, many plants are now known to posses insecticidal properties. Their toxic action (contact and inhalation), their repellency, their anti-palatability, as well as their adverse effects on the reproductive potential, growth, development, and longevity [19] insects have been repeatedly proven. Regarding Algeria, the use of plants rich in allelochimiques molecules as a means of protection of cultures remains an area of low or not exploited even if the problems posed by the insecticides are always of current events. The recourse to plants as a source of pesticides is necessary, especially since the country has a very rich botanical heritage but unfortunately very little explored. It is this lack of data on the Algerian plants with insecticidal character which incited to us to opt for the choice of *P. harmala* as biopesticide because of its high toxicity.

In Algeria, *P. Harmala* with plant wide geographical distribution occupies mainly the northern Sahara and the Algerian highlands. It is used in traditional medicine in Algeria and the Maghreb, in internal and external use to treat different disorders, but it is not consumed by the animals they are cattle or sheep. All parts of the plant (root, stem, leaf and seed) are characterized by high toxicity linked to its richness in alkaloids indoliques [20], which are becoming much more significant during the phase of ripening of the seed [21]. This is why we considered it useful to study the effect of aqueous extracts from seeds of *P. harmala* on some physiological parameters (mortality, larval molt, fertility and pigmentation) and morphological changes on larvae of the 5th stage of *L. migratoria cinerascens*.

2. Materials and Methods

2. 1 Breeding of the Locust

Breeding was performed in rectangular cages with wooden stand, dimensions 119 cm × 44 cm × 36 cm, they are provided with nest boxes containing moist sand in which egg pods are introduced. At hatching, the larvae are fed daily based turf, *Stenotaphrum americanum* (Poaceae), and a protein supplement of wheat bran. Eggs and larvae are subject to the same experimental conditions: a temperature of 31 °C ± 2 °C, a photoperiod of 12 h and a relative humidity of 40% ± 5%.

2.2 Extraction of the Aqueous Extract of the Seeds

The seeds are dried for several days before being ground using a coffee grinder. 10 mg of the ground are removed, then soaked in 50 mL of ethanol for 2 h with magnetic stirring using a rotary evaporator. After removal of alcohol, doses in geometric progression, are obtained by simple dilution: $d_1 = 0.03$ mg/mL, $d_2 = 0.06$ mg/mL, $d_3 = 0.12$ mg/mL and $d_4 = 0.24$ mg/mL).

2.3 Treatment of L5 Larvae

Two processing modes, each with four replicates and one control, were conducted, by contact and ingestion one by one. Spraying of the aqueous extract is made on crickets or food, according to the mode of treatment. It was performed on 10 larvae (L5), in

cages of dimensions 51 cm × 49 cm × 71 cm, containing always *S. americanum*. Lots of insect controls (10 larvae) were treated with distilled water. The counting of larval mortality was done for 3 consecutive days (24 h, 48 h and 72 h) and 10th day. The processing conditions are maintained as in the case of livestock.

2.4 Determination of Fertility Treatment after Ingestion

The determination of fertility was performed taking into account 10 untreated females and 5 females survived treatmentby ingestion. They were isolated in two separate cages, the same size and under the same conditions as above. Fertility is determined by counting the number of eggs given after each egg. For reasons that are outside our control, we did not consider individuals who have not survived the treatment contact in determining the fertility of *L. migratoria cinerascens.*

2.5 Method of Analysis Results

To estimate the LD_{50}, lethal dose from which we obtain 50% mortality, corrected mortalities were transformed into probit, a dose in decimal logarithm, that establish the equations of regression lines. The results are also treated statistically by analysis of variance (XLSTAT version 6.0, ANOVA).

3. Results and Discussion

3.1 Treatment Effect of Contact on Larval Mortality

Like many plants, *P. harmala* has great potential insecticides with respect to *L. migratoria cinerascens.* Its toxic effect lethality caused more or less important depending on the mode of penetration of the aqueous extract and doses. The toxicity of the extract is even higher than the doses are important both for the test of contact and ingestion, although the biopesticide effect of the latter is more important. However, insect mortality decreases with time and it does not exceed 10% on day 10 for doses $d_1 = 0.03$ mg/mL and $d_2 = 0.06$ mg/mL (Table 1). This is likely due to the volatility of certain components of the aqueous extract. This characteristic should be checked since Ref. [22] showed a toxicity of 100% at day 16 of treatment. In the same way, the extract of *Calotropis procera*, rich in alkaloids, caused a mortality of 100% on the desert locust after 15-day treatment (*Schistocerca gregaria*). In the case, although the doses $d_3 = 0.12$ mg/mL and $d_4 = 0.24$ mg/mL gave respective deaths, 40% and 60%, the third day, they are 20%, day 10 or cumulative mortality for both doses is 60% and 80% (Table 1).

The biocidal action of *P. harmala* concerns not only the migratory locust, but also other zoological groups where its insecticidal activity by contact of the black bean aphid (Aphis fabae) causes toxicity by 30%. While by ingestion, mortality was 70% [23]. It also showed that the aqueous extract of *P. harmala* has a nematicidal ranging from 60% to 95%, the only direct contact *in vitro*, similar to that of an ematicide business (Vydate) against *Meloidogyne* spp (root-knot nematodes) [13]. Also, *Acacia gummifera Acacia gummifera* (Fabaceae) and *Tagetes patula* L. (Asteraceae) have a toxic power of 84% and 82% against nematodes because of their relatively high content of flavonoids [24], substances that also exist in *P. harmala*.

The calculation of LD_{50} gave a value of 0.19 mg/mL and the correlation between mortality and dose($r = 0.94$) (Fig. 1). Similarly, the analysis of variance

Table 1 Mortalities in probits of the larvae L5 of *L. migratoria cinerascens* after treatment by contact.

Doses (g/mL)	Percentage of corrected mortality at 3 days	Percent mortality the 10th day	Cumulative mortality
d_1	20	10	30
d_2	20	10	30
d_3	40	20	60
d_4	60	20	80
Witness	0	0	0

(ANOVA) revealed a significant difference between doses at $P < 0.05$ ($F = 3.78$, $df = 1.19$, $P = 0.026$). Consequently, mortality is even more important that the dose is high.

3.2 Treatment Effect of Ingestion on Larval Mortality

As for the contact treatment, the doses of 0.03 mg/mL and 0.06 mg/mL caused dlow mortality at 3 days (20% and 30%). It reached 60% and 80% always on 3rd day, for doses of 0.12 mg/mL and 0.24 mg/mL. Again, mortality at day 10 does not exceed 10% whatever the dose, while cumulative mortality was 70% and 90% (Table 2).

The calculation of LD_{50} gave a value of 0.095 mg/mL. The correlation coefficient is close to 1 (r = 0.99), and indicates a strong correlation between mortality and dose (Fig. 2). The analysis of variance(ANOVA) showed a significant difference

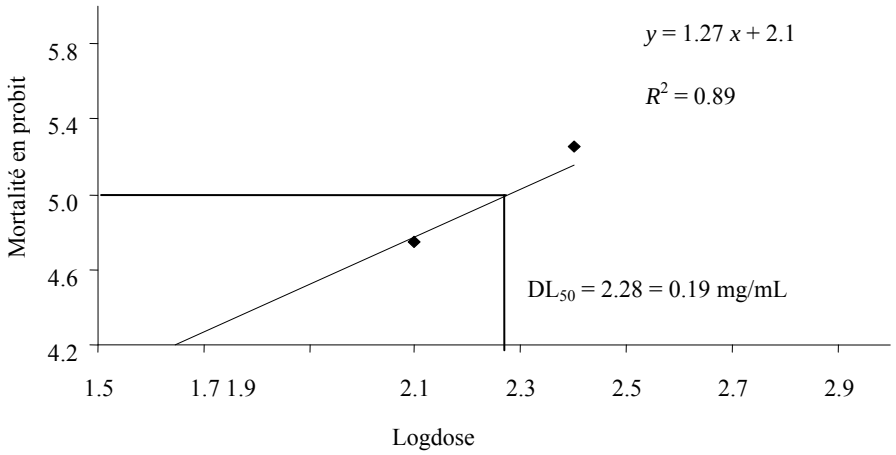

Fig. 1 Regression of mortality in probit treatment by contact.

Table 2 Mortalities in probits of the larvae L5 of *L. migratoria cinerascens* after treatment by ingestion.

Doses (g/mL)	Percentage of corrected mortality at 3 days	Percentage mortality the 10th day	cumulative mortality
d_1	20 10		30
d_2	30 10		40
d_3	60 10		70
d_4	80 10		90
Witness 0		10	10

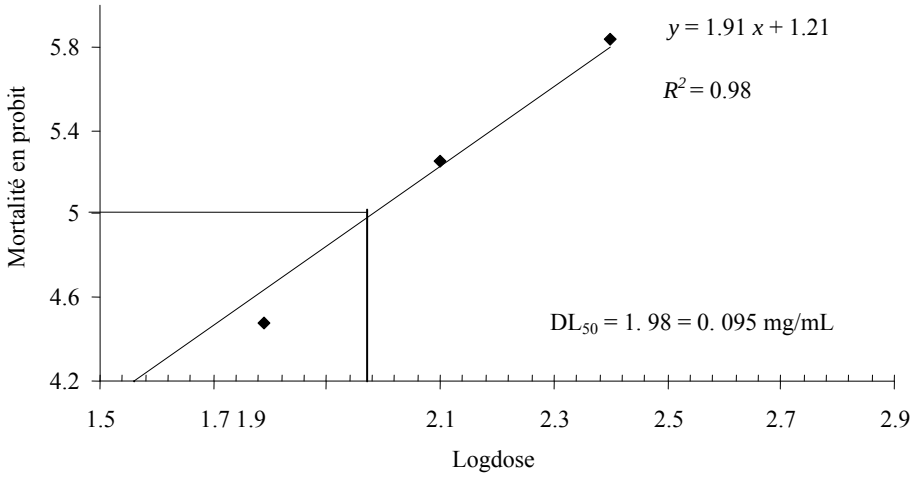

Fig. 2 Regression of mortality in probit (treatment by ingestion).

Table 3 Fecundity of females of *L. migratoria cinerascens* before and after treatment (n = 10).

	First spawning	Second spawning	Third spawning
Untreated females	62.7	49.3	32.7
Treated females	50	31	0

between doses at $P < 0.05$ ($F = 3.57$, $df = 1.19$, $Pr = 0.027$). And mortality is even more important that the dose is high.

3.3 Effects of Both Treatments on the Physiology and Morphology

The larvae, which survived the treatment with oral intake, underwent both morphological and physiological changes which lead to a deformation of the wings of certain individuals (2 females and 1 male, died one day after fledging), a delay of 6 days the larval molt (12 days for untreated individuals and 18 days for individuals treated), blocking fledging (4 females and 2 males), change in pigmentation (3 females brownish died two days after molting imaginal) and an extension of the preoviposition (10 days for femal es treated and 6 days for untreated females).

Fecundity was also altered. Among five females survived to treatment effect of Ingestion, three have produced an average of 50 eggs/female, one of them has laid twice by producing 31 eggs while the other two are died without being able to lay eggs. In contrast, among 10 untreated females, nine have laid eggs once, emitting on average 62.7 eggs. 4 have lain twice by producing 49.3 eggs/female, 3 have lain three times by producing 32.7 eggs/female. Some females lay three times as well. The number of eggs produced progressively decreases from first to third oviposition (Table 3).

Moreover, *P. harmala* also causes physiological disturbances of the insect in this case a delay of larval moulting 6 to 8 days, and a change in pigmentation. The latter becomes brownish at the legs, pronotum and abdomen. The results are consistent with those of [22] who observed the same phenomenon on *S. gregaria*. The effect of antipalatable *P. harmala* resulting in decreased weight of insects, delayed sexual maturity and reduced fertility, which is particularly marked after treatment by ingestion [11-25]. Other plants such as *Mentha spicata* L. and *Origanum glandulosum* L. (Lamiaceae) had the same effect on the fecundity and *Callosobruchus maculatus* L. (Coleoptera) [26].

4. Conclusion

It is known that toxins in *P. harmala* are harmane, harmaline, harmine and harmol (harmalol), harmaline which is most toxic to the extent that it contains 2/3 of alkaloids [27]. The process is the toxicity to the wealth of indole alkaloids that act through harmine and harmaline, substances present in all phenological stages of the plant and especially in the seeds of which the alkaloid levels rises sharply in summer (3-4%) during the phase of fruit ripening [25]. The harmine and harmaline are responsible for the toxicity of the aqueous extract face to face the Locust and act by ingestion through the digestive tract, which deserves further study.

In the present study, the authors have tried to emphasize the poyrntialities agro phytosanitary of *P. harmala*, a plant widespread in Algeria, which could be a source of natural insecticide could replace chemical inputs that have partly contributed to the pollution of the biosphere. The involvement of aqueous extracts of this plant in the fight economic factor as chemical crop protection might fit into the context of alternative and complementary strategy indefense of plants.

References

[1] H. Hamdi, Bio-ecological study of the locust settlements of fixed dunes of the Algerian coast, Master Thesis, Agronomic National Institute, Algiers, 1989. (in French)

[2] M.D. Ould El Hadj, Bio-ecology of the locusts and the

grasshoppers in three areas of study in the Sahara, Master Thesis, Agrono mic National In stitute, Algi ers, 1991. (in French)

[3] G.B. Popov , J.F . Duran ton, J. G igault, Ecological stud y of lo cust biotop es *Schistocerca gregaria* (Forskål, 1775) in nor th-western Afric a, Im plementation and des cription of eco logically homogeneous, in The Locust C ollection, Cirad-Prifas Press, Montpell ier, France, 1991 , p. 744. (in French)

[4] A. Drias, Repor t on the lo cust s ituation in th e wilaya of Adrar (Alger ia), Nation al Instit ute of Plan t Pr otection INPV, 1994, pp. 10. (in French)

[5] W.M. Blaney , C. Winstanley, Food selection behaviour in Lo custa m igratoria, in: P roceedings of the 5 th International S ymposium on In sect Plant R elationships, Wageningen, the Netherlands, 1982, pp. 365-366.

[6] L. Benf ekih, A. F oucart, D. Pe tit, Ce ntral Sa haran populations o f *Locusta migratoria cinerascens* (Orthoptera: Acrididae) in ir rigated perimeters: is it a recent co lonisation ev ent?, Annales de la s ociété entomologique de Fran ce 47 (1-2) (2011) 147 -153. (in French)

[7] B.T. K abassina, Faunistic comparison of Cae lifera in Caïd Gacem station and in and various bioclimatic stages of Togo , Eng ineer Thesis, Agro nomic Nat ional Institute Algiers, 1990, pp. 144.

[8] J.F. Duranton, M. Launois , M.H. Launois-Lu ong, M. Lecoq, Manual locust survey in dr y tropical zone, Grouping Stud y and Research for the Dev elopment of Tropical Agriculture, 1982, pp. 1496.

[9] M.A. Launo is-Luong, M. Lecoq, Vade Mecu m of the Sahelian grass hoppers, in Operational A cridology, Montpellier, 1989, pp. 125.

[10] M. Launois, Locust monitor ing and fighting men-locusts in the Sahel, Sécheresse 2 (7) (1996) 83-85.

[11] J. Auger, S. Du gravaot, A. Naudin, A. Abo-Ghalia, D. Pierre, E . Thibaut, Use of Al lium al lelochemicals as insecticides, Bulletin 25 (13) (2002) 295-306

[12] K. Abbassi, L. Mergaoui, Z. Atay-Kadiri, A. St ambouli, S. Ghaout, Biological activities of the leaves of *Peganum harmala* (Zygophyllaceae) in flo wering on m ortality and reproductive activity in the locust, baetica Zoologica 16 (2005) 3-46.

[13] N. El Allagui, S. Tahrou ch, M. Bourij ate, A. Hatimi, Action of different plan t extr acts on th e mortality of root-knot n ematodes Meloi dogyne spp, Acta Botanica Gallica 154 (4) (2007) 503-509.

[14] L.M. Idrissi Hassani, J. Hermas, Effects of diet in *Peganum harmala* L. (Zy gophyllaceae) on the dige stive t ract of th e desert loc ust *Schistocerca gregaria* For sk, (Orthoptera, Acrididae) Zoologica baetica 19 (2008) 71-84.

[15] A. Ben zara, E ffectiveness of aqu eous ex tracts from *Peganum harmala* (Z ygophylacee) on l arvae L5 of th e desert loc ust, *Schistocerca gregaria* (Forsskål, 1775 (Orthoptera, Ac rididae), in : Pro ceedings of th e seven th Congress of Plant Protection A ssociation, R abat, 2010 , pp. 469- 474.

[16] C. R egnault-Roger, A. Ha mraoui, M. Holema n, E. Théron, R. Pinel, Insecticidal effect of essential oils from mediterranean p lants upon A. obtectus Say (Co leoptera, Bruchidae), a pest of kidn ey bean (*Phaseolus vulgaris* L.), Journal of Chemical Eco logy 19 (6) (1993) 1231-1242. (in French)

[17] M. Rahim , Biol ogical a ctivity of az adirac h t in-enriched neem kern el ex tra cts against *Ryzopertha Jominiea* (F.) (Co!eopteru: Bo strychidae) in stored wheat, Journal o f Stored Products Research 34 (2-3) (1998) 123-128.

[18] N.E.S. Lale, A . Mustapha, Poten tial of Com bining neem (Azadirachta in dica A . J uss) s eed oil with varietal resistance for the ma nagement of th e cowpea bruch id, *Callosobruchus maculatus* (F.), Journal of Stored Product Research 36 (2000) 215-222.

[19] S.S. Duffey , M.J. Stout , A ntinutritive an d toxic components of p lant defense ag ainst insects, Archives of Insect Biochemistry Physiology 32 (1996) 3-37.

[20] C. Munir, M. Idrees Zaïdi, N. Ahmad; A. Ur-Rahman, An easy ra pid me tal me diated me thod of isolation harmine and harmaline from *Peganum harmala*, Fitoterapia 66 (1) (1995) 73-76.

[21] N. Ben Salah, M. Amamou, Z. Jerbi, F. Ben Salah, M. Yacoub, Aspects cliniques , pharmacolog iques et toxicologiques du surdosage par une plante médicinale: le Harmel, Essaydali scientifique 21 (1986) 13-18

[22] K. Abbassi, L. Mergaouib, Z. Atay-Kadiri, A. Stambouli, S. Ghaout, Activité b iologique de l'extrait de g raines de *Peganum harmala* sur le c riquet pè lerin (*Schistocerca gregaria* Forskål 1775) , Journal of Orthoptera Research 12 (1) (2003) 71-78. (in French)

[23] S. Guerrida, Ev aluation d e l' activité s ystémique de tro is végétaux et d' un insectic ide sur puceron, Magister . Thesis, INA d'Alger, 2009. (in French)

[24] M.A. Siddiqui, M.M. Alam, Co ntrol of p arasit nematode by Tagete tenuifolia, Revue Nematology 11 (1988) 3.

[25] R. Jbilou, F. Sayah, E ffects of *peganum harmala* (Zygophyllaceae) seed extracts on the develop ment of *Tribolium castaneum* (Coleo ptera: Tenebrionidae), International Jo urnal of Trop ical Insect Scien ce 27 (3-4) (2007) 199-209.

[26] O. Khalfi-Habes, Evaluation du potentiel biocide et étude de l'influence de la composition des huiles essentielles de quelques plantes algériennes su r *Rhyzopertha dominica* (F.) (Coleop tera: Bos trychidae) e t *Callosobruchus maculatus* (F.), Doctorat.Thesis, INA d'Alg er, 2 008. (in French)

[27] R. Pa ris, H. M oyse, M atière Médicale, Ma sson Pre ss, Paris, 1981, p. 292. (in French)