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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Received: July 17, 2012 / Accepted: September 19, 2012 / Published: February 28, 2013.

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 larvae 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 day, 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*, widespread 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 maintenance and the dispersion of the locusts, whether in remission or invasion periods, 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 othergregarious. These are the larval bands of the gregarious phase which are formidable biological aggressors and which cause 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 biopesticides of vegetable origin, biodegradable, non 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 secondary compounds (alkaloids, cardenolides and glucosinolates terpenes) that they contain, many plants are now known to possess insecticidal properties. Their toxic action (contact and inhalation), their repellency, their antipalatability, 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 allelochemicals 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 plant that the insecticidal character which incited us to opt for the choice of P. harmala as biopesticide because of its high toxicity.

In Algeria, P. Harmala with plant wid 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 a 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 base 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: $d_1 = 0.03 \text{ mg/mL}$, $d_2 = 0.06 \text{ mg/mL}$, $d_3 = 0.12 \text{ mg/mL}$ and $d_4 = 0.24 \text{ mg/mL}$.

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
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cages of dimensions 51 cm × 49 cm × 71 cm, containing always S. americanum. Lots of insects 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 treatment by ingestion. They were isolated in two separate cages, the same size and under the same conditions as a bove. 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 survived the treatment contact in determining the fertility of L. migratoria cinerascens.

2.5 Method of Analysis Results

To estimate the LD50, lethal dose from which we obtain 50% mortality, corrected mortalities were transformed into probit, a dose in decimal logarithm, that estimates 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 im portant. However, insect mortality decreases with time and it does not exceed 10% on day 10 for doses d1 = 0.03 mg/mL and d2 = 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 d3 = 0.12 mg/mL and d4 = 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 LD50 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>
<thead>
<tr>
<th>Doses (g/mL)</th>
<th>Percentage of corrected mortality at 3 days</th>
<th>Percent mortality the 10th day</th>
<th>Cumulative mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>d1</td>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>d2</td>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>d3</td>
<td>40</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>d4</td>
<td>60</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Witness</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 Mortalities in probits of the larvae L5 of L. migratoria cinerascens after treatment by contact.
(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 low 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.](image1)

![Fig. 2 Regression of mortality in probit (treatment by ingestion).](image2)

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

<table>
<thead>
<tr>
<th>Doses (g/mL)</th>
<th>Percentage of corrected mortality at 3 days</th>
<th>Percentage mortality the 10th day</th>
<th>Cumulative mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_1$</td>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>$d_2$</td>
<td>30</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>$d_3$</td>
<td>60</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>$d_4$</td>
<td>80</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Witness 0</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

$y = 1.27x + 2.1$

$R^2 = 0.89$

$DL_{50} = 2.28 = 0.19$ mg/mL

$y = 1.91x + 1.21$

$R^2 = 0.98$

$DL_{50} = 1.98 = 0.095$ mg/mL
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Table 3  Fecundity of females of L. migratoria cinerascens before and after treatment (n = 10).

<table>
<thead>
<tr>
<th></th>
<th>First spawning</th>
<th>Second spawning</th>
<th>Third spawning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated females</td>
<td>62.7</td>
<td>49.3</td>
<td>32.7</td>
</tr>
<tr>
<td>Treated females</td>
<td>50</td>
<td>31</td>
<td>0</td>
</tr>
</tbody>
</table>

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 on 4 days after fledging), a delay of 6 days the larval molt (12 days for untreated individuals and 18 days for individuals treated), the locking of a day by the molting imaginal and an extension of the preoviposition (10 days for females 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 were died with out being able to lay eggs. In contrast, among 10 untreated females, nine have laid eggs once, emitting on average 62.7 eggs. Four have lain twice by producing 49.3 eggs/female, three 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 molting 6 to 8 days, and a change in pigmentation. The latter becomes brownish at the legs, pro notum and abdomen. The results are consistent with those of [22] who observed the same phenomenon on S. gregaria. Thus, the effect of antipalatable P. harmala resulting in decreased weight of insects, decreased 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 the molting stages of the plant and especially in the seeds of which the alkaloid levels rise 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 potentialities 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 against economic factor as chemical crop protection might fit into the context of alternative and complementary strategy in defense of plants.

References

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