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The bioactivity of *Ruta chalepensis* and *Peganum harmala* extracts on the migratory locust *Locusta migratoria* (Orthoptera: Acrididae)

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ABSTRACT

The effects of two medicinal plants which occur in the Tunisia flora, the Rutaceae Ruta chalepensis and the Zygophyllaceae Peganum harmala were observed in the laboratory conditions on L. migratoria. Newly emerged fifth instar nymphs and adults were exposed to various concentrations of the crude methanolic extract administered by ingestion. Results showed that treated larvae manifested a decrease in their food consumption and nutrient utilization, due probably to antifeedant effect of these plants. The treatment applied to adult females during the pre-ovipositional phase provokes a significant adverse influence on their reproductive potential resulting in a significant reduction of both fecundity and fertility, a delay of the first oviposition and a significant decrease in the number of eggs per ootheca.

Keywords: Locusts, plant extracts, antifeedant, reproduction.

INTRODUCTION

Locusts are one of the world's most destructive agricultural pests. The characteristic that makes locusts such devastating pests is the ability of migrating swarms to appear without warning in previously uninfested areas and rapidly damage pastures and crops. Locusts alternate between periods of low numbers (recessions) and very high numbers (plagues) and Uvarov [1] proposed the phase theory to explain the origin and disappearance of plagues. These insects are characterized by the pronounced ability to exhibit a continuum of forms between the extreme solitarious and gregarious phases. Solitary locusts live separately, the hoppers (nymphs) do not move together and the adults usually fly individually at night. Gregarious hoppers move in marching bands and the adults move together in cohesive day-flying swarms. Large hopper bands can cover several hectar and large swarms can cover hundreds of km^2; gregarious phases develop in response to a combination of crowding, pheromones, and host-plant substances. In the gregarious phase, migratory locusts cause substantial damage to crops and grazing and are responsible for enormous losses in agriculture in many regions of the world [2, 3]. The desert locusts, *Schistocerca gregaria*, consume approximately their own weight of fresh vegetation each day. Swarms often contain 50 million individuals per km^2 so that even a moderate swarm measuring 10 km^2 could consume about 1000 tons of fresh vegetation daily during migration [4]. The control of the migratory locust has been largely based on the use of broad-spectrum chemical pesticides, which can damage human health, agro-ecosystems (loss of beneficial insects and increase of insecticide-resistance), and the wider environment (effects on non-target species, groundwater, landscapes and communities) [5]. According to Wiktelius *et al.* [6] several of the chemical pesticides used for locusts control are highly or moderately toxic to other invertebrates, both terrestrial and aquatic. An increasing awareness of the negative impact of synthetic insecticides has led to the search of new environment friendly

methods of locust control. Botanicals are a promising source of pest control compounds. Today over 2000 species of plants are known to possess some insecticidal activity [7]. The most successful compound in this field is the azadiractin (active ingredients extracted from the neem tree Azadirachtaindica). It has many anti-insect properties, including antifeedant, antioviposition and growth-regulating [8]. In addition, nicotine from *Nicotiana tobaccum*, pyrethroids from *Chrysanthemum cinerariaefolium* and rotenoids from the roots of leguminous plants, *Lanchocarpous* spp. being good examples of natural compounds used long ago to control agricultural pests. The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms. They are also biodegradable and harmless to the environment [9]. In this paper, we study the effect of two medicinal plants which occur in the Tunisia flora, the Rutaceae *Ruta chalepensis* and the Zygophyllaceae *Peganum harmala* in the development and the reproductive potential of *L. migratoria*.

MATERIALS AND METHODS

Locust

Insects used for in this study came from a gregarious stock, which had been reared in breeding cages measuring 50 cm^3 and containing a few hundred specimens. The temperature was kept at 30 ± 1°C and a light/dark cycle of 12/12 h was used. Insects were fed with fresh sorghum leaves supplemented with wheat bran. The substratum for oviposition was composed of 2/3 peat and 1/3 sand.

Plants and treatments

The plant species originated in Center of Tunisia (Kairouan) and were collected during spring seasons (March-April). The samples, consisting of the aerial part of each plant, were dried in the shade and then ground into a fine powder. The extraction was carried out by macerating the powder for 24 hours in methanol 80%, followed by filtration and evaporation at 40°C. The dried extract was kept at 4°C until its use for treating insects. The crude methanolic extract of *R. chalepensis* (*ME-Rc*) and *P. harmala* (*ME-Ph*) was applied to the surface of sorghum leaves which were subsequently offered as a mono-specific diet for larvae adults of the migratory locust. Control locusts were fed on untreated sorghum leaves.

Phytochemical screening

The extracts were subjected to phytochemical tests for plant secondary metabolites, flavonoids, alkaloids, tannins, coumarins, steroids and saponins using standard qualitative methods described by Harbone [10] and Trease and Evans [11].

Feeding assay

The effects of *ME-Rc* and *ME-Ph* on the development and food consumption of *L. migratoria* were investigated by exposing freshly emerged (0-1 day old) 5^{th} instar larvae to fresh *S. vulgare* leaves treated by three different concentrations (0.5%, 1% and 2%), denoted respectively as C_1, C_2 and C_3. The freshly emerged larvae (n = 10 for each concentration) were weighed and kept in individual 2-L plastic boxes. Insects were starved for 8 hours prior to the assay to standardize their state of hunger. Every morning, definite quantities of fresh *S. vulgare* leaves were provided to the insects and aliquots of the same food were kept in the same conditions to calibrate the water lost from the food provided. Uneaten food was separated from the faeces and weighed. In order to study the utilisation of the ingested food, the approximate digestibility (AD) index was calculated using the formula given in Waldbauer (1968): AD= (wt. of food ingested - wt. of faeces)/(wt. of food ingested)*100. This value is an index for the extent at which the ingested food is actually digested.

Reproductive potential

Newly emerged males and females (0-12h post-emergence) were treated and immediately paired in individual 2-L plastic boxes containing food and placed under the same conditions described above for mass rearing. The fecundity (number of eggs deposited by the female though its lifespan) and the fertility rate ((number of hatched eggs per ootheca)/(number of deposited eggs per ootheca)x100) were recorded. The time of first oviposition (TFOp) and the number of eggs per ootheca (NE/Ot) were recorded for each pair.

Statistical analysis

Results are expressed as means ± standard deviation (SD). To identify significant effects of the treatments on the variables measured, data were submitted to analysis of variance (ANOVA) using SPSS (Version 15.0). The significance between control and treated series was made by Student-Newman-Keuls (*SNK*) test at the 5% level.

RESULTS

Phytochemical screening

The result of the phytochemical screening (Table 1) reveals that flavonoids, alkaloids, coumarins and steroids were positive in both *R. chalepensis* and *P. harmala* extracts. Also, catechic tannins were detected in both extracts

whereas gallic tannins were detected only in the extract of *P. harmala*. The preliminary phytochemical analysis also showed that the presence of saponins in *R. chalepensis* is uncertain.

Table 1. Preliminary phytochemical screening of *R. chalepensis* and *P. harmala* methanolic extract.

	R. chalepensis	*P. harmala*
Flavonoids	+++	++
Catechic tannins	+++	+++
Gallic tannins	-	+++
Alkaloids	+++	+++
Coumarins	+++	++
Steroids	+++	++
Saponins	±	+++

-: Negative result; +++: Positive results; ++: Moderately positive, ±: Doubtful reaction

Feeding activity

Both the plant extracts, *ME-Rc* and *ME-Ph*, reduced the amount of food ingested by *L. migratoria* larvae. At both extracts, a reduction in food intake ($P<0.05$) was observed during the assay. Indeed, the analysis of variance showed a significant difference among treatments from the second day with *ME-Rc* and *ME-Ph*. The amounts of food ingested calculated 5 day after treatment on C_3-treated larvae were 1.14 ± 0.14 and 1.28 ± 0.32 g/larva respectively to *ME-Rc* and *ME-Ph*. However, we measured in control group of the same age the quantity of 2.1 ± 0.43 (Fig. 1).

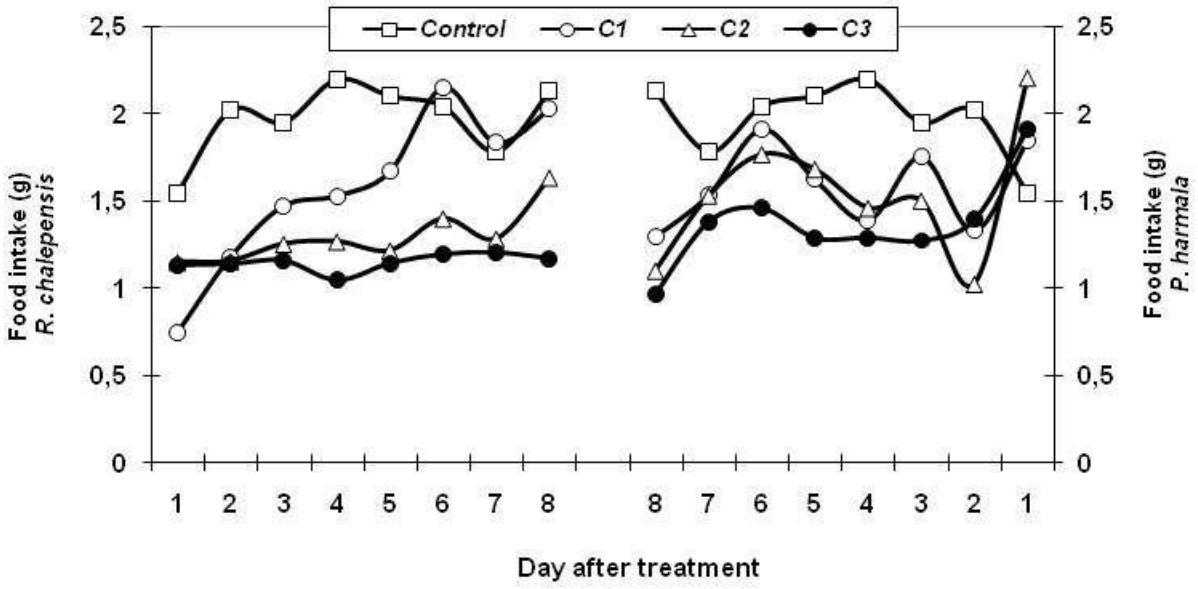

Fig. 1. Effect of *R. chalepensis* and *P. harmala* methanolic extract on daily food intake (fresh weight) of *L. migratoria* fifth instar nymphs (n = 10).

The mean fresh weight of food consumed by larvae during the fifth instar was measured and reported in figure 2. Obtained results showed a significant adverse influence of plants extract on the feeding activity of *L. migratoria* larvae in a dose-dependent manner. Larval food consumption was progressively severely reduced during the fifth instar as dose increased, reaching a 42.14 and 30.97% reduction respectively to *ME-Rc* and *ME-Ph* applied at the highest concentration compared to control group (Fig. 2).

Approximate digestibility

A reduction in the AD was observed in larvae whose food was treated with *ME-Rc* and *ME-Ph*. The two extracts evoked a significant decline ($P<0.05$) in the AD during the fifth instar compared to the untreated controls. The analysis of variance with the dose as classification criteria showed a significant difference among treatments and the *SNK*-test gives heterogeneous groups represented by different letters in figure 3. This indicated that tested insects were able to digest untreated food more efficiently than those impregnated with *ME-Rc* and *ME-Ph*. Results also revealed that methanolic extract derived from *P. harmala* plant (*ME-Ph*) provoked an important reduction in the AD compared to *ME-Rc* (Fig. 3). Indeed, the AD recorded in treated larvae with the highest concentration (C_3= 2%) are 46.92±7.83% and 39.8 ± 10.44% respectively to *ME-Rc* and *ME-Ph* whereas it reached 93.85±2.91% in untreated controls during the fifth instar giving an inhibition of 50.01 and 57.6 % respectively to *ME-Rc* and *ME-Ph* (Fig. 3).

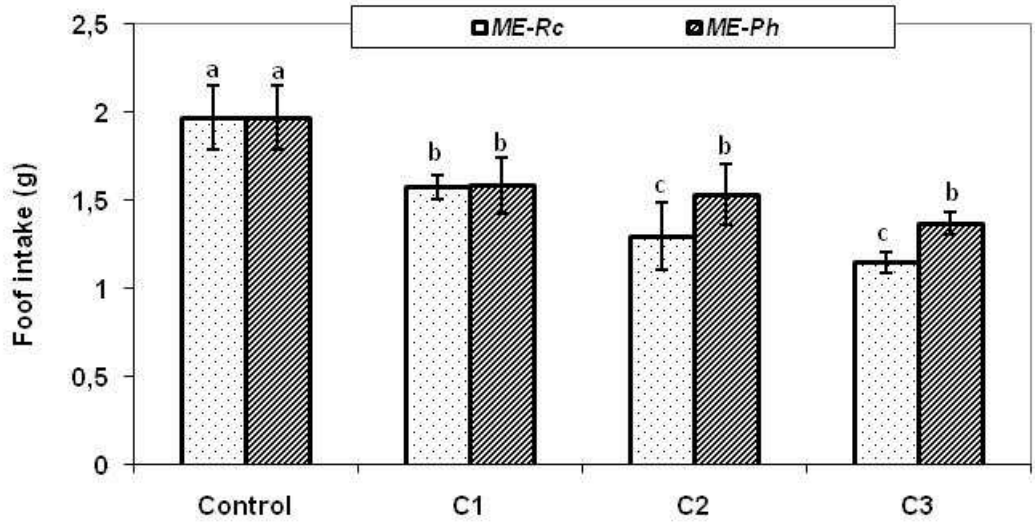

Fig. 2. Effect of *R. chalepensis* and *P. harmala* methanolic extract on the mean fresh weight of consumed food during the fifth instar larvae (n = 10).

Means followed by the same letters are not significantly different (P<0.05). (Bar = Standard Deviation).

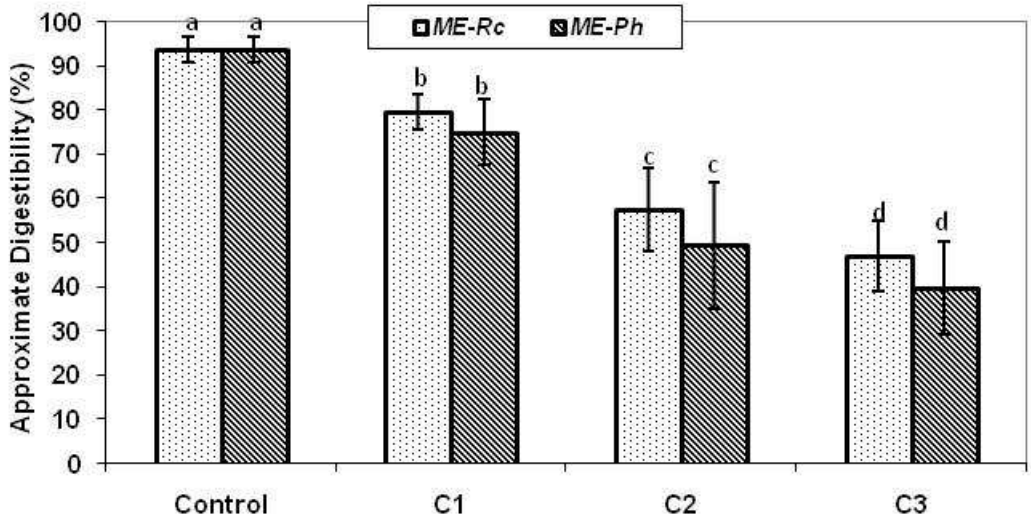

Fig. 3. Effect of *R. chalepensis* and *P. harmala* methanolic extract on the approximate digestibility of *L. migratoria* fifth instar nymphs (n = 10).

Means followed by the same letters are not significantly different (P<0.05). (Bar = Standard Deviation).

Other effects

Toxicity of plant extracts was also demonstrated by a lengthening of time spent in the fifth instar, reduction in larvae mobility and severe morphological disturbances especially during the imaginal molt. We noted exuviations difficulties causing larval mortality and increased percentage of abnormal imagos. These difficulties were due to the persistence of the larval cuticle and the impossibility to reject the old integuments. Surviving insects showed deformities of the antennae, legs, wings and eyes.

Reproductive potential

Data reported in table 2 showed a significant adverse influence of *ME-Rc* and *ME-Ph* on the reproductive potential of *L. migratoria*. Treatment significantly ($P<0.05$) influenced the duration of adult pre-oviposition periods. For each extract, statistical analysis with the dose as classification factor showed a significant difference among treatments and the *SNK*-test gives heterogeneous groups represented by different letters in table 2. In treated females, sexual maturity was reached on day 22±1.73 and 18±2.64 respectively to *ME-Rc* and *ME-Ph* applied at the highest concentration. However, untreated control females were needed only 12.33 ±2.51days to produce the first ootheca (table 2). The perturbation of reproduction events observed in the treated females also appeared with a significant

decrease ($P<0.05$) in the number of eggs per ootheca (NE/Ot). Indeed, as illustrated in table 2, we noted the number of 42.66±6.02 eggs/ootheca in control groups whereas we counted only 22.66±5.68 and 27±5.66 eggs/ootheca respectively to *ME-Rc* and *ME-Ph* treated female at the highest concentration.

Table 2. Effect of *R. chalepensis* and *P. harmala* methanolic extract, applied on newly emerged adults of *L. migratoria* (n=15), on reproductive potential parameters (mean ± SD).

	R. chalepensis		*P. harmala*	
	TFOp	NE/Ot	TFOp	NE/Ot
Control	12.33±2.51^{a}	42.66±6.02^{a}	12.33±2.51^{a}	42.66±6.02^{a}
C_1	23±7.21^{b}	28.33±7.63^{b}	16.33±2.3^{ab}	34±4^{ab}
C_2	24.33±4.16^{b}	26.66±4.5^{b}	21±1^{b}	28.6±3.05^{b}
C_3	22±1.73^{b}	22.66±5.68^{b}	18±2.64^{b}	27±5.56^{b}

** TFOp: time of first oviposition NE/Ot: number of eggs per ootheca*
** Different letters in the same column denote significant differences (SNK-test, P<0.05)*

Treatment of newly emerged females with the two plant extracts resulted in a significant decrease ($P<0.05$) of both fecundity and fertility. A significant difference was observed between control and treated females during the first gonadotrophic cycle (Fig. 4). Fecundity was reduced to 21.4 ± 4.96 and 27.96 ± 5.27% in females treated with the highest concentration of *ME-Rc* and *ME-Ph*, respectively (Fig. 4). The treatments also significantly reduced fertility which reached 40.09 ± 9.12 and 13.36 ± 1.05% in the case of C_3-treated females with *ME-Rc* and *ME-Ph*, respectively, even after extending the incubation period. In control individuals, hatching success was 73.32 ± 6.75 and 66.6 ± 11.1% respectively to the *ME-Rc* and *ME-Ph* (Fig. 4).

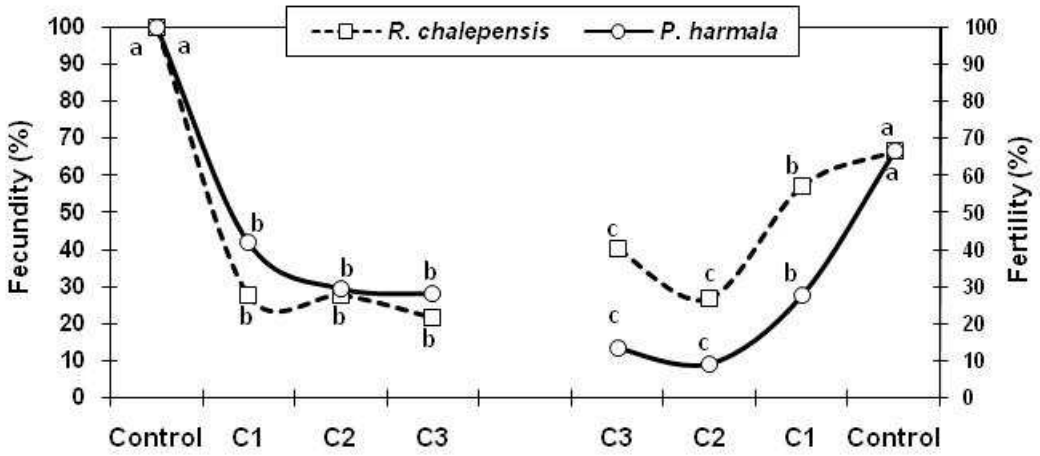

Fig. 4. Effect of *R. chalepensis* and *P. harmala* methanolic extract, applied on newly emerged adults of *L. migratoria* (n=15), on fecundity and fertility.
Means followed by the same letters are not significantly different (P<0.05). (Bar = Standard Deviation).

DISCUSSION

In order to identify both environmentally acceptable and effective locust-control products, we assessed in the present study the effects of *R. chalepensis* and *P. harmala* methanolic extracts on the feeding activity and the reproduction of *L. migratoria*. Analysis of the results revealed that tested plants significantly reduced food consumption and nutrient utilization in *L. migratoria* fifth instar nymphs. Results also showed that extracts derived from these plants provoked a significant adverse influence on the reproductive potential of *L. migratoria* adult females which resulted in a significant reduction of both fecundity and fertility and a delay of the first oviposition. Previous studies had documented the antifeedant properties of *R. chalepensis* and *P. harmala* against different orders of insects. Abbasi *et al.* [12] observed that *P. harmala* leaf extracts (at vegetative or fructification stages) involved a decrease in food intake, a loss of weight and a reduction of motricity in the female adults of *S. gregaria*. The alkaloids extracted from the leaves of *P. harmala* caused a significant decline in food intake and a decrease in adult weight compared to untreated controls [3]. The authors suggested that indole alkaloids were probably responsible for the inhibition of feeding behaviour in locusts treated with *P. harmala*. Our results concurred with the observations of Rehman *et al.* [13] , who evaluated the effects of ethanol extracts of *P. harmala* seeds on the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). They found that in choice tests, female *B. oleae* spent >99% of their time foraging on

untreated fruit rather than *P. harmala*-treated fruit. Similarly, Salari *et al.* [14] observed that *P. harmala* acetonic seed extract showed a strong repellent effect against *Myzus persicae* (Hemiptera: Aphididae). Jbilou *et al.* [15] found that methanol extracts from different medicinal plants, including *P. harmala* seeds, have insecticidal effects on the larvae and adults of the stored grain pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). The antifeedant effect of *R. chalepensis* on insects has been proven previously. *R. chalepensis* extract reduced the food consumption of *Hypsipyla grandella* (Lepidoptera: Pyralidae) under laboratory conditions [16]. Antifeedant effect by common rue extracts was also demonstrated for the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Coccinellidae) 4th instar larvae and adults [17]. Emam *et al.* [18] also reported the aqueous ethanolic extract of *R. chalepensis* leaves showed antifeedant activities against *S. littoralis* larvae. Jeon *et al.* [19] observed insecticidal activities of *R. chalepensis* using fumigant and contact toxicity methods against rice weevil, *Sitophilus oryaze* (Coleoptera: Curculionidae) adults. The feeding deterrency caused by *R. chalepensis* and *P. harmala* methanolic extracts, probably reflect the antifeeding effect of the secondary substances, especially the alkaloids, extracted from these plants. Indeed, *P. harmala* is a rich source of b-carboline and quinazoline alkaloids [20]. Quinoline alkaloids (arborinine, dictamnine, graveoline, kokusaginine and skimmianine) were also isolated from *Ruta* spp. [21]. The Antifeedant activity of alkaloids is already known from a number of toxic plants [22]. Indolic alkaloids extracted from leaves and seeds of *P. harmala* and the alkaloids occurring in leaves of *Calotropis procera* functioned as a feeding deterrent against *S. gregaria* [3]. Inhibition of feeding behavior by alkaloids could be the result of stimulation of a deterrent receptor, or blockage of the input from neurons that detect phagostimulatory compounds. Electrophysiological studies have shown that contact chemoreceptors on the tibia and tarsus of *S. americana* are stimulated by alkaloids and have demonstrated an association between the neuron activity and the antifeedant response [23, 24]. Therefore, it is possible that the tested plant alkaloids were detected by the alkaloid-sensitive neurons.

The results of the present study also showed that *R. chalepensis* and *P. harmala* methanolic extracts significantly reduced both fecundity and fertility of *L. migratoria*. Similarly, Abbassi *et al.* [3] reported that the alkaloids extracted from *P. harmala* leaves caused significant reduction in female fecundity and hatching rate in *S. gregaria* when compared to the untreated control. Ethanol extract of *P. harmala* seed have shown pronounced effect on larval mortality, larval and pupal weight, oviposition deterrence, percent pupation, egg hatching and adult emergence of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) [25]. Abbassi *et al.* [12] observed that leaf extracts (vegetative stage) caused a delay of sexual maturity in *S. gregaria* females and a decrease in both fecundity and hatching rate. The different perturbations of reproduction observed in the treated females are a consequence of the reduction of food intake observed previously. These changes probably result in inadequate energy reserves for egg-yolk formation, or an interference in endocrine metabolic processes involved in reproduction with the secondary substances.

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