SCIENTIFIC NOTE

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TOXIC AND REPELLENT EFFECT OF HARMAL (*Peganum harmala* L.) ACETONIC EXTRACT ON SEVERAL APHIDS AND *Tribolium castaneum* (HERBST)

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To reduce the dependence on the sometimes unwise use of synthetic pesticides in fruit and vegetable plantations, the toxicity and repellence of *Peganum harmala* L. (Zygophyllaceae) acetonic seed extract was assayed against several insect pests. For contact toxicity, 3- to 4-d-old individuals of *Aphis fabae* Scopoli, *A. gossypii* Glover, *A. nerii* Boyer de Fonscolombe, and *Myzus persicae* (Sulzer) were included, as well as 1- to 7-d-old adult *Tribolium castaneum* (Herbst). Repellent effect experiments were conducted on adult, 1- to 2- and 3- to 4-d old *M. persicae* individuals. At 60 mg mL^{-1}, the topical bioassay mortality percentage was significantly higher in *A. gossypii* than in *A. fabae* and *A. nerii* after 12-72 h. Mortality of the treatments on *M. persicae* was 87.1% and 90.0% after 24 and 48 h, respectively, and significantly higher than *A. fabae* and *A. nerii* during this period. At 60 mg mL^{-1}, the mortality of *T. castaneum* was much lower than that of the aphid species. The highest repellent index (over 72%) was observed on 1- to 2-d-old *M. persicae* individuals.

Key words: *Peganum harmala*, topical bioassay, aphid species, *Tribolium castaneum*, *Aphis fabae*, *Aphis gossypii*, *Aphis nerii*, *Myzus persicae*.

The widespread use of synthetic pesticides has led to several adverse effects such as food, soil, ground water, and air contamination with toxic residues, which have side effects on non-target insects and other organisms (Bughio and Wilkins, 2004). To overcome these problems, it is necessary to seek safe, convenient, environmental, and low-cost alternative pest control methods. Considerable efforts have focused on plant-derived materials that are potentially useful as commercial insecticides. Plant derivatives are less toxic or nontoxic to mammals, other vertebrates, and invertebrates. Plant products have several uses in insect control. These products have also been studied for acute toxicity, antifeedant, or repellent, attractant, and fumigant effects, as well as inhibiting reproduction of many pest species (Cox, 2004; Kubo, 2006). The structural diversity and bioactivity of plant alkaloids, such as in *Peganum harmala* L. (Zygophyllaceae), makes them one of the most important groups of natural origin substances.

Peganum harmala is a perennial glabrous herb that grows in semi-arid conditions, steppe areas, and sandy soils. It has frequently been used in traditional medicine and as an abortive agent (Lamchouri *et al.*, 2002). In Iran, dried capsules –mixed with other ingredients– are burnt to produce scented smoke that is used to purify the air and the mind, but it is mostly used as a charm against “the evil eye” (Frison *et al.*, 2008).

Peganum harmala is a rich source of b-carboline and quinazoline alkaloids (Kartal *et al.*, 2003). The possible use of *P. harmala* in modern phyto-indole entheogen preparations is correlated to its b-carboline content: harmine, harmaline, and tetrahydroharmine (THH), collectively known as harmala alkaloids, which are mostly found in the seeds and roots. Harmine and harmaline are competitive and reversible inhibitors of monoamine oxidase type-A (MAO-A) enzymes, whereas THH is believed to inhibit serotonin uptake (Buckholtz and Boggan, 1977; Kim *et al.*, 1997). Regarding its toxicity against different insects, Abbassi *et al.* (2003) found the toxic effect of *P. harmala* on the survival, feeding, behavior, and reproduction of the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae), under laboratory conditions. Jbilou *et al.* (2006) 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* Herbst (Coleoptera: Tenebrionidae) after a period of time.

Although aphids are major vectors of viral diseases and serious pests for many cultivated plants worldwide, there is little knowledge in the literature about the effect of a plant extract such as *P. harmala* against aphids. Salari *et al.* (2010) found the toxic effect of *Otostegia persica* (Burmeister) Boissduval (Labiatae) acetonic

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extract on some aphid species. Several authors have tested some plant-derived compounds such as aphid repellents. Gutiérrez *et al*. (1997) tested bisabolene, farnesol, and geraniol in assays using apterous *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and leaf discs embedded in agar.

This study bioassayed the toxicity of *P. harmala* seed extract on several aphids and on the red flour beetle *T. castaneum*; it also determined the repellent effect of this plant against *M. persicae*.

MATERIALS AND METHODS

Plant extract preparation

Plant material (800 g), collected in Iran's Kerman province (30°19'51.33" N, 56°55'41.72" E, 1776 m.a.s.l.) in August 2010, was dried at room temperature under shade conditions for 1 wk until crisp. Then, 50 g of dried seeds were ground and powdered with an electric stainless steel blender for 5 min and placed in a 500 mL Erlenmeyer flask with 90 mL 99.9% acetone for extraction. The mixture was filtered with Whatman N° 91 filter paper, and the residues were soaked and shaken again in 10 mL acetone for 2 h (total 50 g seeds:100 mL acetone). The solution was filtered again over a new filter, and the first and second filtrates were mixed and concentrated in 15 cm diameter Petri dishes under a laboratory hood for 24 h to obtain 3.55 g dry residue (total 7.1% yield percentage) that was stored at 4 °C. A specific volume of this dry residue was used in distilled water and dimethyl sulfoxide (DMSO) to make different concentrations for each test.

Insect rearing

The insects in this study were the *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis nerii* Boyer de Fonscolombe, and *M. persicae* (Hemiptera: Aphididae) aphids, and the red flour beetle *T. castaneum*.

The aphid species colony in this study came from Shahid Bahonar University field infestation and, after identification, a stock culture was maintained on broad beans *Vicia faba* L. cv. Aquadulce. They were placed on freshly excised 4.5 cm diameter broad bean leaf discs set in 5.5 cm diameter plastic Petri dishes with a 0.5 cm thick layer of 0.7% agar gel, with a meshed hole in the lid to allow air exchange, and were reared in a growth chamber at 25 ± 1 °C, 60 ± 10% relative humidity, and 16 h of artificial light at an intensity of about 4000 lux. The relatively susceptible of 3- to 4-d-old *A. fabae*, *A. gossypii*, *A. nerii,* and *M. persicae* individuals were employed for the experiments.

Tribolium castaneum was reared on wheat flour at room temperature. for the experiments, 1- to 7-d-old adult insects were used.

Bioassays with plant extracts

Topical application. Direct contact toxicity was tested by topically applying the *P. harmala* acetonic seed extract with a micropipette on the top of the abdomen of the relatively susceptible 3- to 4-d-old *A. fabae*, *A. gossypii*, *A. nerii,* and *M. persicae* individuals, as well as on the thorax of 1- to 7-d-old adult *T. castaneum*. A concentration of 60 mg mL^{-1} for aphids and two concentrations of 60 and 120 mg mL^{-1} for the red flour beetle were applied. The experiments were carried out under bioassay laboratory conditions at 25 ± 1 °C, 60 ± 10% relative humidity, and 16 h of artificial light at an intensity of about 4000 lux. Only distilled water and dimethyl sulfoxide (DMSO) were applied in the control treatments. Each treatment had 20 replicates and each replicate included 10 insects. Aphid mortality was determined after 12, 24, 48, and 72 h, and after 2, 12, 24, 48, and 72 h from the beginning of exposure for *T. castaneum*. Insects were considered dead when no leg or antennal movements were observed.

Repellent effect experiment. The dual choice test was conducted to measure the repellent effect of the *P. harmala* extract on adults of relatively susceptible 3- to 4-d-old and 1- to 2-d-old *M. persicae* individuals. In each experiment, 20 insects were released in the middle of withered broad bean leaf discs that were placed on top of cotton tying tape (12 cm) linkage in two round plastic Petri dishes (5.5 cm diameter), one as a treatment and the other as a control. The round plastic Petri dishes, with freshly excised broad bean leaf discs (4.5 cm diameter), were filled with a 0.5 cm thick layer of 0.7% agar gel. In the treatments, only a minute quantity of pure extract was applied with a micropipette at the end of its cotton tying tape and on the leaf disc, while only distilled water was applied in the control. The round plastic Petri dishes were positioned in a Plexiglas cage (15 × 7.5 × 4.5 cm) during a trial. Experiments were replicated 10 times. After 48 h, the number of insects was counted in the treated (T) and control (C) Petri dishes. The repellent index (RI) was calculated by the following formula: RI = [(C-T)/C+T)] × 100 (Pascual-Villalobos and Robledo, 1998). Positive and negative values indicate repellent and attractant effects, respectively.

Statistical analysis

Mortality data were adjusted for mortality in the control by Abbott's (Abbott, 1925) correction, and was calculated as: M_a (%) = [(M_t - M_c)/(100 - M_c)] × 100 where M_a is corrected mortality (%), M_t is mortality in the treatment (%), and M_c is mortality in the control (%). Mortality and repellency data were subjected to a one-way ANOVA and followed by the Tukey and Fisher tests (StatPlus 4.9, 2007, Croydon, UK), respectively.

RESULTS

Topical bioassay

The effect of the *P. harmala* acetonic seed extract among aphid species after 12-72 h is shown in Table 1. This extract was highly toxic against *A. gossypii* since it was able to kill 95% after 72 h. At 60 mg mL^{-1}, mortality after 12-72 h was significantly higher ($P \leq 0.05$) in *A. gossypii* than *A. fabae* and *A. nerii*. Mortality of *A. gossypii* was 88.0% after 12 h, while it was 67.7% and 57.0% in *A. fabae* and *A. nerii*, respectively. Mortality of *M. persicae* was 87.1% and 90.0% after 24 and 48 h, respectively, and significantly higher ($P \leq 0.05$) than *A. fabae* and *A. nerii*. The mortality of *A. nerii* treatments after 72 h was 80%, which was significantly higher ($P \leq 0.05$) than after 12 h.

Significant differences in *T. castaneum* mortality to the *P. harmala* extract occurred between 60 and 120 mg mL^{-1} after 2-72 h ($P \leq 0.05$), although it did not differ significantly in each time exposure period (Figure 1). At 120 mg mL^{-1}, mortality was 27.5% after 72 h, and 8.0% at 60 mg mL^{-1}. At this concentration, *T. castaneum* mortality was much less than the four aphid species.

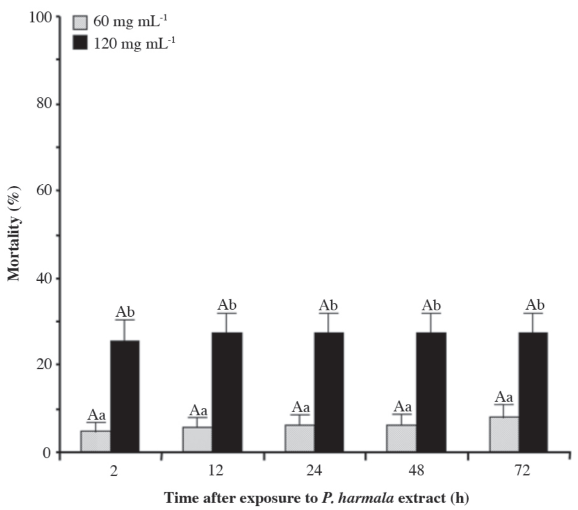
Dual choice bioassay

The results of the dual choice bioassay are shown in Figure 2 expressed as repellent index (RI) of the extracts at 48 h after application on different developmental stages of *M. persicae*. The repellent effect of *P. harmala* extract was with high RI on 1- to 2-d-old *M. persicae* individuals (RI = 72.8%), while it was 53.4% and 52.6% against adult and relatively susceptible 3- to 4-d-old *M. persicae* individuals, respectively, with intermediate RI. There were no significant repellent effects between developmental stages and adult treatments.

Table 1. Mortality of aphid species after exposure to *Peganum harmala* acetonic seed extract (60 mg mL^{-1}).

Time after treatment (h)	Mortality (%) (Mean ± SE)			
	Aphis fabae	*Aphis gossypii*	*Aphis nerii*	*Myzus persicae*
12	67.7 ± 5.39Abc	88.0 ± 3.37Aa	57.0 ± 5.28Ac	81.4 ± 5.33Aab
24	67.7 ± 5.39Ab	91.0 ± 3.06Aa	65.0 ± 5.00ABb	87.1 ± 3.38Aa
48	71.1 ± 5.65Ab	94.0 ± 2.10Aa	73.0 ± 5.08ABb	90.0 ± 2.77Aa
72	71.1 ± 5.65Ab	95.0 ± 1.98Aa	80.0 ± 4.35Bb	90.0 ± 2.77Aab

Means in columns followed by different lower case letters indicate significant differences between the different biotests within the same time period. Means in columns followed by different upper case letters indicate significant differences between the time periods within the same biotests at ($P \leq 0.05$) (one-way ANOVA).



Bars with different lower case letters indicate significant differences between the different concentrations within the same time periods. Bars with different upper case letters indicate significant differences between the time periods within the same concentrations at ($P \leq 0.05$) (one-way ANOVA).

Figure 1. Mortality of two *Peganum harmala* acetonic seed extract concentrations on *Tribolium castaneum* after 2-72 h.

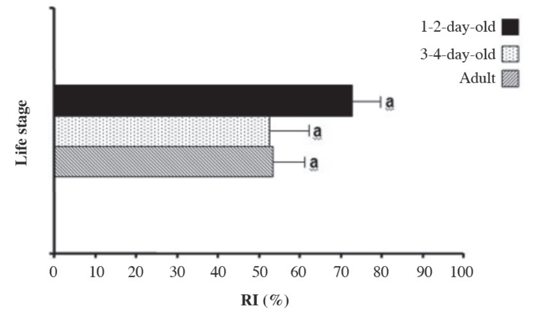


Figure 2. Repellent index (RI) of *Peganum harmala* acetonic seed extract against different developmental stages of *Myzus persicae* as determined in a dual choice bioassay after 48 h.

DISCUSSION

The most important finding of our study is the demonstrated toxicity of the seed extract on different aphid species. Comparing total mortality percentages of *P. harmala* acetonic seed extract on different treatments gives a good insight about its bioactivity. The *P. harmala* acetonic seed extract had a knockdown effect, which resulted in the sudden death of the treated insects, except *A. nerii*, after the extract was applied. At first, this extract caused moderate toxicity on *A. nerii*, but after a period of time, mortality increased remarkably, while it caused the highest mortality in *A. gossypii* after it was applied. The differences in insect mortality to the extract are perhaps related to its penetration and detoxification mechanisms. In the case of the aphid species, there seems to be greater activity against the more susceptible and smaller bulk of *A. gossypii*. Results revealed the insecticidal effect of *P. harmala* on different pests. Similar observations have been reported for other plant extract effects on several insects. The bioactivity of the harmala plant was shown by Abbassi *et al*. (2003), who reported that the alkaloids extracted by ethanol from *P. harmala* leaves caused significant mortality of the desert locust, *S. gregaria,* reduced female fecundity, as well as hatching rate when compared to the untreated control. Compared with our data, the *Eichhornia crassipes* (Pontederiaceae) extract delayed the development of the 4^{th} instar larva of *T. castaneum* and provoked its mortality (Rani and Jamil, 1989).

The insecticidal activity varied between insect species and exposure time. Our results showed that *P. harmala* acetonic extract was not very effective against *T. castaneum* after 3 d, while Jbilou *et al*. (2006) have reported that the methanol extract from *P. harmala* seeds had a high insecticidal effect on *T. castaneum* after 32 d. This difference was probably due to the slow action of some plant extracts on *Tribolium* genus. Athanassiou *et al*. (2005) have reported that NeemAzal was not very effective against *T. confusum* Duval (Col., Tenebrionidae) where adult mortality was low even after 14 d of exposure at 200 ppm.

The results of applying *P. harmala* extract on different *M. persicae* life stages showed that this extract was highly repellent on *M. persicae*. Repellent effects on *M. persicae* have been reported by several authors. Gutiérrez *et al*. (1997) tested bisabolene, farnesol, and geraniol as aphid repellents against *M. persicae* in assays using apterous aphids and leaf discs embedded in agar. All three compounds showed activity, but only bisabolene was further tested because the others proved phytotoxic to *Capsicum annuum* L. (Solanaceae) leaves. Apparently farnesol may also have been toxic to the aphids. Park *et al*. (2005) demonstrated that several monoterpenes repelled the mosquito *Culex pipiens pallens* Coquillett (Diptera: Culicidae) and the non-oxygenated monoterpene a-terpinene is more repellent than the DEET commercial repellent. The mugwort *Artemisia vulgaris* L. (Asteraceae) also contains volatile insect repellents against the yellow fever mosquito *Aedes aegypti* (L.) Diptera: Culicidae) (Hwang *et al*., 1985). Hori (1998) and Bruce *et al*. (2005) found linalool was effective, but the response may vary with species. All three studies found that linalool repelled *M. persicae*, but it was not effective on *Sitophilus avenae*.

CONCLUSIONS

The *P. harmala* acetonic seed extract is potent and could be useful for aphid pest management in greenhouse plants because it was able to kill the treated pest through contact and oral toxicity, and it also acted as a strong insect repellent. This study is a preliminary investigation and its purpose was to compare the effect of a single rate of *P. harmala* acetonic seed extract against several pests. More studies are needed to bioassay the activity of other concentrations and each identified compound against aphid species and other pests.

Efecto tóxico y repelente del extracto acetónico de Harmal (*Peganum harmala* L.) sobre varias especies de áfidos y *Tribolium castaneum* (Herbst). Para reducir la dependencia de los pesticidas sintéticos en plantaciones frutales y hortalizas, se realizó un ensayo para medir la toxicidad y repelencia de un extracto acetónico obtenido a partir de semillas de *Peganum harmala* L. (Zygophyllaceae) contra diferentes especies de plagas. Para evaluar la toxicidad del extracto al contacto con los insectos, se incluyeron individuos de 3-4 d de edad de *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis nerii* Boyer de Fonscolombe, y *Myzus persicae* (Sulzer), así como adultos 1-7 d de edad de *Tribolium castaneum* (Herbst). Experimentos para medir el efecto repelente se llevaron a cabo con individuos de 1-2 y 3-4 d de edad de *M. persicae*. En los resultados de los bioensayos tópicos el porcentaje de mortalidad fue significativamente mayor en la especie *A. gossypii* que en *A. fabae* y *A. nerii,* después de 12-72 h con una concentración de 60 mg mL^{-1}. La mortalidad de los tratamientos contra *M. persicae* fueron 87,1% y 90,0% después de 24 y 48 h, respectivamente, la cual fue significativamente mayor que la obtenida con *A. fabae* y *A. nerii* durante el mismo período de tiempo. Con la concentración de 60 mg mL^{-1}, la mortalidad de *T. castaneum* fue muy inferior a las obtenidas con las diferentes especies de áfidos. El mayor índice de repelencia (mayor a 72%) se observó en individuos de 1 a 2 d de edad de *M. persicae* entre los diferentes tratamientos.

Palabras clave: *Peganum harmala*, bioensayo tópico, áfidos, *Tribolium castaneum*, *Aphis fabae*, *Aphis gossypii*, *Aphis nerii*, *Myzus persicae*.

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