Bulletin of Insectology ***63*** *(2): 259-263, 2010*
ISSN 1721-8861

Insecticidal activity of *Peganum harmala* seed extract against the diamondback moth, *Plutella xylostella*

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Abstract

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera Plutellidae) is a key pest in cruciferous plants. Ethanol extract of *Peganum harmala* L. (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, *P. xylostella.* A mortality of 66 and 100% was found in the third instar larvae that had fed for two days on the cabbage leaves treated with the ethanol extract at concentrations of 30 and 40 mg/ml, respectively. Significant dose response was observed on larval and pupal weight; pupal and adult emergence rate. Significant differences were also observed on oviposition. Percentage of egg hatching was reduced significantly in 30 and 40 mg/ml but not in 10 and 20 mg/ml concentrations. Obtained results showed that ethanol extract of *P. harmala* had a good insecticidal activity on *P. xylostella*.

Key words: *Plutella xylostella*, diamondback moth, seed extract, *Peganum harmala*, harmal, insecticidal effects.

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera Plutellidae), is the most serious insect pest of cruciferous crops throughout the world (Sarfraz *et al*., 2006). It is a major pest of this family in Tehran province of Iran, too (Mahmoudvand *et al.*, 2009). The level of infestation varies according to plant type, locality and the level of natural enemies. If no control measures are undertaken, this insect can cause up to 100% crop loss (Shelton *et al*., 1993). Chemical control of *P. xylostella* has become less effective because of its quick development of resistance to almost all groups of insecticides, including organochlorines, organophosphates, carbamates, pyrethroids, insect growth regulators, abamectins, pyrazoles, oxadiazines, neonicotinoids, spinosad and indoxacarb (Abdel-Razek *et al*., 2006; Charleston *et al*., 2005; Qian *et al*., 2008). The indiscriminate use of synthetic insecticides has given rise to many ecological problems, including toxic residues, harm to mammals and the accumulation of harmful residues in the environment (Shelton *et al*., 1993; Khan *et al*., 2005). Therefore, the development of techniques that would provide more efficient *P. xylostella* control without serious effects on the environment is required. Among current alternative methods aiming at decreasing the use of synthetic insecticides, botanicals have been suggested as alternative sources for insect control because many products are selective to insect pests and have no or little harmful effects on non-target organisms and the environment. Furthermore, they are easily available and less expensive than synthetic insecticides (Regnault-Roger, 1997; Schmutterer, 1997; Liang *et al*., 2003; Charleston *et al*., 2005). *Peganum harmala* L. (Zygophyllaceae), commonly known as "harmal", is claimed to be an important medicinal plant in traditional Iranian medicine. It has been used traditionally as an emmenagogue and an abortifacient agent in the Middle East and North Africa (Lamchouri *et al*., 2002; Zargari, 1998). It has been confirmed that harmal extract is a rich resource of β-carboline alkaloids including harmol, harmine and harmaline. It has quinazoline derivatives: vasicine and vasicinone, too. The unripe seeds have less alkaloids compared to the ripe ones. These compounds are responsible of the toxicity of this extract (Li, 1996; Kamel *et al.*, 1970; Kartal *et al*., 2003). β-carboline alkaloids are reported from *Banisteriopsis caapi* (Spruce ex Griseb.) C.V.Morton (Malpighiaceae) and *P. harmala*. These compounds exhibited psychoactive actions (Callaway *et al*., 2005). Susceptibility of some animals such as camel (El-Bahri and Chemli, 1991), cattle (Bailey, 1979), donkeys (Bailey and Danin, 1981), sheep and horse (Bailey, 1986) to this plant have reported so far. Abbassi *et al*. (2003) found the toxic effect of harmal on survival, feeding, behavior and reproduction of desert locust, *Schistocerca gregaria* (Forskal) under laboratory conditions.

However, there have been no studies on the effect of this plant against the diamondback moth, *P. xylostella*. In this study, we investigated some biological parameters such as larval mortality, larval and pupal weight, pupation rate and adult emergence of the diamondback moth after treating the 3rd instar larvae of this major pest by ethanol extract of *P. harmala*. Also, oviposition deterrence of harmal and effect of that on egg hatching percentage of the diamondback moth was studied.

Materials and methods

Insect rearing

P. xylostella population (larval stage), was collected from cauliflower crops of Shahre-Rey at the south of Tehran, Iran. For egg laying, about 500 adults of diamondback moth (DBM) were used in a plastic cage (50×30×30 cm). For egg laying, leaves of cauliflower, *Brassica oleracea* L. var. *botrytis* (Brassicaceae) were used and eggs were transferred to leaves of mentioned

plant to continue their development. Insect stock was maintained at 25 ± 1 °C and 65 ± 5% relative humidity (RH) under a 16L:8D photoperiod in a growth chamber.

Plant material extraction

The seeds of plant were collected from garden of medicinal plants of Shahed University and were ground into powder. In each extraction, fifty grams of powdered seeds were extracted by maceration in 200 ml of 99.8% ethanol at room temperature for 2 days. After filtering through a Buchner funnel, the filtrates were concentrated to dryness by a rotary evaporator under low pressure. Seed extract was stored in a refrigerator at temperature of 4 °C.

Bioassay

A leaf dipping bioassay method described by Tabashnik and Cushing (1987) was adapted to evaluate the insecticidal activity of harmal seed extract against *P. xylostella* larvae. Cabbage leaves were washed with distilled water and dried for about 2 hour. Four concentrations 10, 20, 30 and 40 mg/ml of the seed ethanol extract were prepared with ethanol. Ethanol was used for the control. Two cabbage leaf disks (3 cm diameter) were cut from fully expanded cabbage leaves grown in a greenhouse. The disks were dipped for 30 seconds in the test solutions and control. After air-drying at the room temperature, each two disks were then placed in a plastic cup (5.5 cm in diameter, 3 cm in depth). Twenty third instar larvae were starved for 2 h and then released into the plastic cup. Treatments were replicated five times. The cups were placed in a growth chamber at 25 ± 1 °C, 65 ± 5% RH and 16L:8D cycle. Mortalities were recorded 48 h after treatment. Larvae were considered dead if they did not move when prodded with fine brush. Live larvae were transferred to untreated fresh cabbage leaves to continue their growth and development. The cabbage leaves were replaced with fresh ones when needed. After two days, 25 larvae from each treatment were randomly chosen and weighted individually. After pupation, pupae were placed individually in Petri dishes (8 cm in diameter) until adults emerged. Also, 25 individuals of 2 day-old pupae of each treatment were randomly selected and weighed individually.

Oviposition deterrence and egg hatching

For this study, five pairs of adults for each concentration and control were selected and placed in a plastic cage (14×11×5 cm). For each cage, a leaf cabbage disk (5 cm in diameter) dipped in four concentrations (10, 20, 30, 40 mg/ml) and a control for 30 s. Each concentration, replicated three times. Sugar solution (10%) was provided for moth adults feeding.

The number of laid eggs was recorded after 48 h and oviposition deterrence was calculated with the following formula (Pascual-Villalobos and Robledo, 1998). Two hundred eggs were selected from each concentration and effect of harmal extract on percentage of egg hatching was estimated:

$$\text{Oviposition deterrence} = \left[1 - \frac{NE_t}{NE_c}\right] \times 100$$

Where NE_t is the number of eggs in treatment and NE_c is the number of eggs in control.

Statistical analysis

Experimental data were subjected to one-way ANOVA at 0.05 significance level using SAS software Ver. 6.12 (SAS Institute, 1997). Means were then compared by Tukey's Studentized Range Test at $P < 0.05$. Data of ovipositon deterrence were subjected to one-way ANOVA ($P < 0.05$) using software SPSS Ver. 16.

Results

Larvicidal activity

The larvicidal activity of different concentrations of seed extract of harmal against the third instar larvae of *P. xylostella* differed significantly ($F = 96.95$, $P < 0.0001$, df = 4, 20) among the tested concentrations after 48 h (table 1). A mortality of 66 and 100% was obtained from the ethanol extract of harmal at concentrations of 30 and 40 mg/ml, respectively. Concentrations of 10 and 20 mg/ml showed little larvicidal activity (< 20% mortality) that was not significantly different from the control.

Larval and pupal weights, pupation rate and adult emergence

The results of larval and pupal weights of treated groups and control are shown in figures 1 and 2. Means of larval weight in control, 10, 20 and 30 mg/ml concentrations were 4.46, 4.32, 2.99 and 2.33 mg, respectively. The comparison of means showed that there were significant differences between 20 and 30 mg/ml treatments ($F = 42.15$, $P < 0.0001$, df = 3, 96). All larvae that were treated with 40 mg/ml concentration were dead (figure 1). Mean of pupal weight in control, 10, 20 mg/ml concentrations were 4.09, 3.77 and 2.95 mg, respectively. Comparison of means showed that there is no significant difference between control and 10 mg/ml concentration, but 20 mg/ml concentration caused significant reduction in pupal weight compared to control. In concentrations (30 and 40 mg/ml) all larvae failed to develop into pupae ($F = 17.09$, $P < 0.0001$, df = 2, 72) (figure 2). The pupation rate treated with different concentration was significantly lower than control. Pupal

Table 1. Percentage of larval mortality (Mean ± SE) of *P. xylostella* treated with different concentrations of harmal extract.

	Concentrations of seed extract (mg/ml)				
	Control	10	20	30	40
Larval mortality (%)	6 ± 4 c	15 ± 5.70 c	16 ± 4.58 c	66 ± 4 b	100 a

Means marked with the different letters within the same row are significantly different ($P < 0.05$; Tukey).

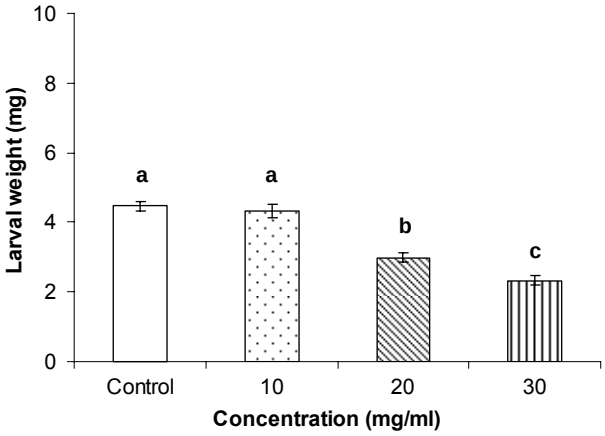

Figure 1. Mean Larval weight (mg ± SE) of *P. xylostella* treated with different concentrations of *P. harmala* seed extract. Means marked with the different letters are significantly different ($P < 0.05$; Tukey) ($F = 42.15$, $P < 0.0001$, $df = 3, 96$).

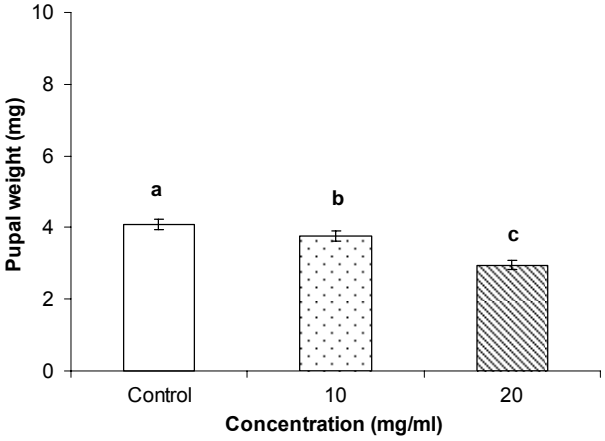

Figure 2. Pupal weight (Mean ± SE) of *P. xylostella* (mg) in control and different concentrations of *P. harmala* seed extract. Means marked with the different letters are significantly different ($P < 0.05$; Tukey) ($F = 17.09$, $P < 0.0001$, $df = 2, 72$).

rate decreased with increasing the concentration of the plant extract ($F = 244.55$, $P < 0.0001$, $df = 4, 20$) (table 2).

Adult emergence of *P. xylostella* was significantly affected by seed extract. Comparison of means showed that 10 and 20 mg/ml concentrations are not significant with control ($F = 1.81$, $P = 0.2054$, $df = 2, 12$) (table 3).

Oviposition deterrence and egg hatching

Results showed that different concentrations of seed extract of harmal caused significant oviposition deterrence in *P. xylostella*. The highest detergency was seen at 40 mg/ml concentration with 93% reduction and the lowest deterrence in 10 mg/ml with 39% reduction ($F = 23.91$, $P < 0.0001$, $df = 4, 10$) (table 4).

Percentage of egg hatching of *P. xylostell*a was reduced significantly in 30 and 40 mg/ml compared with control but not in 10 and 20 mg/ml concentrations ($F = 16.66$, $P = 0.0002$, $df = 4, 10$) (table 5).

Discussion and conclusion

Results of this study demonstrated that the ethanol seed extract of harmal was effective on pupal and larval weight, pupation rate, adult emergence and larval mortality of *P. xylostella* larvae. Also, extract of this plant had a good effect on oviposition deterrence of the diamondback moth, too. Naturally, with increasing the

Table 2. Percentage of pupal rate (Mean ± SE) of *P. xylostella* treated with different concentrations of harmal extract.

	Concentrations of seed extract (mg/ml)				
	Control	10	20	30	40
Pupation (%)	89.16 ± 1.90 a	64.88 ± 2.06 b	42.11 ± 3.95 c	0 d	0 d

Means marked with the different letters within the same row are significantly different ($P < 0.05$; Tukey).

Table 3. Percentage of adult emergence (Mean ± SE) of *P. xylostella* treated with different concentrations of harmal extract.

	Concentrations of seed extract (mg/ml)				
	Control	10	20	30	40
Adult emergence (%)	92.94 ± 2.35 a	83.95 ± 4.78 a	82.42 ± 5.00 a	-	-

Means marked with the different letters within the same row are significantly different ($P < 0.05$; Tukey).

Table 4. Mean percentage (± SE) of oviposition deterrence of *P. harmala* extract on adult of *P. xylostella*.

	Concentrations of seed extract (mg/ml)			
	10	20	30	40
Oviposition deterrence (%)	0.39 ± 0.01 a	0.55 ± 0.04 ab	0.76 ± 0.16 bc	0.93 ± 0.06 c

Means marked with the different letters within the same row are significantly different ($P < 0.05$; Tukey).

Table 5. Mean percentage of egg hatching (± SE) of *P. xylostella* treated with different concentrations of *P. harmala.*

	Concentrations of seed extract (mg/ml)				
	Control	10	20	30	40
Egg hatch (%)	96.00 ± 1.76 a	91.00 ± 1.52 a	84.66 ± 3.38 ab	76.00 ± 2.30 bc	67.66 ± 4.25 c

Means marked with the different letters within the same row are significantly different (P < 0.05; Tukey).

concentrations, the extract effect was increased.

In previous studies, larvicidal activity and oviposition deterrence of many plant extracts on *P. xylostella* were investigated (Chen *et al*., 1996; Banaag *et al*., 1997; Jiwajinda *et al*., 2001; Lee *et al*., 2001; Pipithsangchan *et al*., 2004; Trindade *et al*., 2008). In the other hand, in some studies effects of alkaloids of harmal on lepidopteran insects were investigated. El-Gengaihi *et al*. (1997) studied effect of each alkaloid of harmal extract separately on cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera Noctuidae) and found that harmine alkaloid caused an increase in larval period and larval mortality. Larval mortality of their study accepted our results using different insect and indicated that this plant through ingestion can be effective on larvae of Lepidoptera. Also in other research, similar to our results, Shonouda *et al*. (2008) reported that the leaf extract and its fractions of *P. harmala* reduced the percentage of pupation in the cotton leaf worm, *S. littoralis*. This indicated that toxin alkaloids of harmal inhibit developing process to other stage (pupae) of lepidopteran larvae. In the other hand, in current study, adult emergence unlike investigation of Shonouda *et al*. (2008) didn't differ with control. In our study, seed extract of harmal had a significant effect on larval and pupal weight of the diamondback moth. Jbilou *et al*. (2006) found that methanol extracts harmal seeds had a significant decreasing on larval weight of stored grain pest, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) but adult emergence of pupae were similar to control. It is clear that feeding from treated meal is less than untreated that and obviously when nourishment was lower, weight was decreased. Abbassi *et al*. (2003) reported that the alkaloids extracted from harmal with ethanol caused significant mortality, reduction in fecundity of female and egg hatching in desert locust, *S. gregaria*. In this study, the percentage of egg hatching significantly decreased after treating the leaves by harmal. The results reported on desert locust by Abbassi *et al*. (2003), similar to ours obtained on diamondback moth, demonstrated the harmal ovicidal effect on different insect species. Rehman *et al*. (2009) reported olive fruit treated with 2% extract of harmal reduced oviposition in *Bactrocera oleae* (Rossi). According to obtained from oviposition inhibition of current study and Rehman *et al*. (2009) can judge seed extract of harmal is a good compound to prevention of egg laying.

In conclusion, seed extracts of harmal have been demonstrated to have a strong insecticidal activity against *P. xylostella* larvae. Thus, this plant has excellent potential to be utilized as a naturally occurring agent for *P. xylostella* control.

Acknowledgements

We thank section of Agricultural Entomology of Iranian Research Institute of Plant Protection, Tehran, Iran and section of Entomology at Department of Plant Protection of Shahed University, Tehran, Iran.

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Received April 23, 2010. Accepted October 25, 2010.