

## Larvicidal and Oviposition Deterrent Effects of the Jimsonweed (*Datura stramonium* L.) Extracts on the Diamondback Moth, *Plutella xylostella* (L.)

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### ABSTRACT

The importance of the diamondback moth, *Plutella xylostella* (L.), has dramatically risen mainly due to the overuse of broad-spectrum insecticides, which has resulted in increased selection for pesticide resistance, destruction of natural enemies, and pest resurgence. These issues accompanied by increasing insecticide residue on crops have created an urgent need for more sustainable strategies for *P. xylostella* management. The present study examined the efficacy of extracts from different parts of the jimsonweed, *Datura stramonium* L., as larvicide and oviposition deterrent on *P. xylostella* larvae and adults, respectively. Bioassays revealed higher toxicity (LC<sub>50</sub> and LC<sub>90</sub>) of flower (82.3 and 1,475.4 mg L<sup>-1</sup>), seed (146.8 and 4,828.7 mg L<sup>-1</sup>) and root (165.3 and 3,493.8 mg L<sup>-1</sup>) extracts of *D. stramonium* compared with leaf (526.6 and 29,352.1 mg L<sup>-1</sup>) and stem (841.4 and 136,248.1 mg L<sup>-1</sup>) extracts. In addition, flower extract showed the greatest toxicity between the extracts based on LC<sub>50</sub> dose ratio (0.561, 0.498, 0.156 and 0.098) and LC<sub>90</sub> dose ratio (0.306, 0.422, 0.050 and 0.011) when compared with seed, root, leaf and stem extract, respectively. Furthermore, oviposition deterrence tests using sublethal doses exhibited that the highest oviposition inhibition was provoked by flower extract of *D. stramonium* (63.0%) compared with root (46.0%), seed (43.8%), stem (33.7%), and leaf (28.3%) extracts. Results indicated that jimsonweed extracts could be used as an effective pesticide and oviposition deterrent against *P. xylostella*. Possibilities for application of *D. stramonium* extracts in pest management and for developing a botanical insecticide or an oviposition deterrent in context of sustainable pest management are discussed.

**Keywords:** Botanical pesticides, Cruciferae, Pest management, Plant extract.

### INTRODUCTION

Crucifers are a morphologically diverse group of crop plants belonging to the mustard family (Cruciferae or Brassicaceae), a large family with over 3700 species in 338 genera ("List of Plants", 2015). Crucifers have great economic value worldwide as leafy, stored and processed vegetables (*e.g.*, cabbage, cauliflower, broccoli, Brussels sprouts, turnip and radish), condiment source (*e.g.*, mustard), source of edible and industrial oil (*e.g.*, rapeseed), animal fodder, green manure, ornamental species (*e.g.*,

sweysson), and annual wildflowers and weeds that blanket so many landscapes in spring and summer (Schmidt and Bancroft, 2011; Gupta, 2017).

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), has become the most economically important insect pest of cruciferous plants worldwide (Zalucki *et al.*, 2012; Furlong *et al.*, 2013). The importance of *P. xylostella* has dramatically risen mainly due to the overuse of broad-spectrum insecticides, which has resulted in increased selection pressure for resistance (Talekar and Shelton, 1993). *Plutella*

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*xylostella* is now one of the few insect species that has developed field resistance to all major classes of insecticides, including selective insecticides such as *Bacillus thuringiensis*-based products, indoxacarb, avermectins, spinosad, benzoylureas, and chlorantraniliprole (Furlong *et al.*, 2013; “Arthropod Pesticide Resistance Database”, 2019). In addition, the destruction of natural enemies through the overuse of persistent broad-spectrum insecticides has led to pest resurgence (Furlong *et al.*, 2004).

The pesticide resistance and pest resurgence issues accompanied by increasing insecticide residue on crops (Verkerk and Wright, 1997) has created an urgent need for more sustainable strategies for *P. xylostella* management. In this regard, botanical insecticides and deterrents are one of the most promising candidates (Isman, 2006). With a survey on the literature, it can be seen that many plant extracts have been tested against *P. xylostella*, from which highly effective plant extracts belong to 35 plant species from 24 families (Table 1). These effective plant extracts influence *P. xylostella* larvae (as larvicide or antifeedant) or adults (as oviposition deterrence), which can be combined with other sustainable pest management efforts, such as biological control using parasitoids and microbial insecticides in a pest management program (Jafary *et al.*, 2016; Karimzadeh and Besharatnejad, 2017a, b).

The jimsonweed, *Datura stramonium* L. (Angiosperms, Solanaceae), also called thorn apple or devil's snare, is an annual herbaceous plant, growing to a height of 1-2 m, with green stems sometimes tinged with purple, simple alternate leaves with toothed to lobed margins, large white or violet trumpet-shaped flowers, and large spiny capsule fruits. The plant is possibly native to Central America, and is considered an invasive species throughout much of the Northern Hemisphere (Jimsonweed, 2015). The leaves of jimsonweed are a well known source of tropane alkaloids, mainly hyoscyamine (generally the most abundant) and scopolamine (oxygenated analog of

hyoscyamine), which have anticholinergic activities (Miraldi *et al.*, 2001; Friedman, 2004). Hyoscyamine (*i.e.*, the levorotatory stereoisomer of atropine) tends to racemize, forming atropine (*i.e.*, racemic hyoscyamine) during acid-base extraction (Lanfranchi *et al.*, 2010). The production of hyoscyamine and scopolamine significantly vary in different plant parts and life stages. For example, the maximum content of these alkaloids has been found in the stems and leaves of young plant (Miraldi *et al.*, 2001). The seeds of jimsonweed, which contaminate grain and animal feed, also contain atropine and scopolamine whose production substantially vary with the plant geographical location (Friedman and Levin, 1989; Friedman, 2004).

Antibacterial and antifungal activities of the extract of different parts (including leaves, stems, roots, flowers, fruits and seeds) of jimsonweed have been reported in literature (Eftekhari *et al.*, 2005; Usha *et al.*, 2009; Iranbakhsh *et al.*, 2010). Such activities have been found to be related to atropine (Iranbakhsh *et al.*, 2010). There are also reports on the toxicity, repellency, and oviposition deterrent and antifeedant activity of *D. stramonium* leaf and seed extracts on mites and insects such as two-spotted spider mite (*Tetranychus urticae* Koch), European red mite (*Panonychus ulmi* (Koch)), red flour beetle (*Tribolium castaneum* (Herbst)) and mosquitos (*Aedes aegypti* (L.), and *Culex quinquefasciatus* Say) (Pascual-Villalobos and Robledo 1998; Kumral *et al.*, 2010, 2013; Swathi *et al.*, 2010). Scopolamine and atropine extracted from the flowers and leaves of *D. stramonium* have also shown allelopathic effects on Johnsongrass, *Sorghum halepense* (Butnariu 2012). The present study aimed to examine the jimsonweed extracts toxicity and oviposition deterrence on a model lepidopteran pest. The specific objective was to determine and compare the efficacy of the extracts from different parts of *D. stramonium* as larvicide and deterrent on *P. xylostella* larvae and adults, respectively.

**Table 1.** Highly effective plant extracts tested against *P. xylostella* as larvicide, antifeedant and oviposition deterrent.

Plant		The part extracted	Solvent <sup>a</sup>	Effect <sup>b</sup>	Reference
Family	Scientific name				
Acanthaceae	<i>Andrographis paniculata</i>	Leaf and stem	E, A	A, O	Hermawan <i>et al.</i> (1993, 1994)
Amaranthaceae	<i>Gomphrena globosa</i>	Seed	E	R	Dadang and Ohsawa (2001)
Anacardiaceae	<i>Rhus chinensis</i>	Gall	M	A	Kweon <i>et al.</i> (1994)
Annonaceae	<i>Annona squamosa</i>	Seed	E	L	Leatemia and Isman (2004a,b)
Aristolochiaceae	<i>Asarum sieboldii</i>	Leaf	M	A	Kweon <i>et al.</i> (1994)
Asteraceae	<i>Ageratum conyzoides</i>	Leaf	W	R	Amoabeng <i>et al.</i> (2013)
	<i>Chromolaena odorata</i>	Leaf	W	R	Amoabeng <i>et al.</i> (2013)
	<i>Chrysanthemum cinerariaefolium</i>	Flower	E	L	Stein and Klingauf (1990)
	<i>Chrysanthemum morifolium</i>	Leaf	M	O	Liu <i>et al.</i> (2006)
	<i>Synedrella nodiflora</i>	Leaf	W	R	Amoabeng <i>et al.</i> (2013)
Berberidaceae	<i>Jeffersonia dubia</i>	Root	M	A	Kweon <i>et al.</i> (1994)
Boraginaceae	<i>Lithospermum erythrorhizon</i>	Stem	M	A	Kweon <i>et al.</i> (1994)
Campanulaceae	<i>Platycodon grandiflorum</i>	Root	M	A	Kweon <i>et al.</i> (1994)
	<i>Codonopsis pilosula</i>	Root	M	A	Kweon <i>et al.</i> (1994)
Compositae	<i>Spilanthes acmella</i>	Seed	M, H	L	Sharma <i>et al.</i> (2012)
Cucurbitaceae	<i>Momordica charantia</i>	Leaf	E	A	Bing <i>et al.</i> (2008)
Cyperaceae	<i>Cyperus rotundus</i>	Tuber	M	L	Dadang <i>et al.</i> (1996)
Euphorbiaceae	<i>Ricinus communis</i>	Seed	E	A	Stein and Klingauf (1990)
		Seed kernel	W	L	Kodjo <i>et al.</i> (2011)
		Leaf	W	R	Amoabeng <i>et al.</i> (2013)
		Leaf	E	L	Roni <i>et al.</i> (2015)
Hypneaceae	<i>Hypnea musciformis</i>	Leaf	E	L	Roni <i>et al.</i> (2015)
Lauraceae	<i>Persea americana</i>	Leaf	E	A	Stein and Klingauf (1990)
Melanthiaceae	<i>Veratrum nigrum</i>	Root, rhizome	Ea, E, A	L	Vanichpakorn <i>et al.</i> (2010)
Meliaceae	<i>Azadirachta indica</i>	Neemix 4.5®	W	A	Charleston <i>et al.</i> (2005)
	<i>Melia azedarach</i>	Leaf	W	O	Charleston <i>et al.</i> (2005)
	<i>Melia volkensii</i>	Seed	M	A	Akhtar and Isman (2004a,b)
Monimiaceae	<i>Hortonia angustifolia</i>	Root	M	L, A	Hewage <i>et al.</i> (1997)
Ranunculaceae	<i>Coptis chinensis</i>	Root	M	L, A	Kweon <i>et al.</i> (1994)
Rutaceae	<i>Pleiospermium alatum</i>	Stem, bark	H	L	Hewage <i>et al.</i> (1997)
	<i>Severinia buxifolia</i>	Root bark	E	A	Wu <i>et al.</i> (1997)
Solanaceae	<i>Nicotiana tabacum</i>	Leaf	W	R	Amoabeng <i>et al.</i> (2013)
	<i>Cestrum auranticum</i>	Leaf	M	L, A	Hewage <i>et al.</i> (1997)
Verbenaceae	<i>Clerodendrum inerme</i>	Leaf	E	R	Yankanchi and Patil (2009)
	<i>Vitex negundo</i>	Leaf	E	R	Yankanchi and Patil (2009)
Zingiberaceae	<i>Alpinia galanga</i>	Rhizome	M	L, A	Dadang <i>et al.</i> (1998)
		Rhizome	E	R	Dadang and Ohsawa (2001)
		Tuber	M	A	Hewage <i>et al.</i> (1997)
Zygophyllaceae	<i>Peganum harmala</i>	Seed	E	L, O	Abbasipour <i>et al.</i> (2010)

<sup>a</sup> A= Acetone; E= Ethanol; Ea= Ethyl acetate; H= N-Hexane; M= Methanol; W= Water. <sup>b</sup> A= Antifeeding (including larval deterrence); L= Larvicidal; O= Oviposition deterrence/Inhibition (including repellency); R= Reduction of larval density.

## MATERIALS AND METHODS

### Plant-Insect Rearing Protocols

*Brassica rapa* L. var. *pekinensis* (Chinese cabbage) cv. Hero (Takii Seed, Kyoto, Japan) were grown under glasshouse conditions (25±5°C; L:D 16:8 h) without the application of any pesticide (Karimzadeh *et al.*, 2004,

2013). In particular, the seeds of Chinese cabbage were sown individually in cells of 96-cell seed starting trays containing peat moss. At 2-leaf stage, seedlings were individually transferred to plastic pots (12 cm dia.) containing a mixture of sterilized soil (loam), leaf mold, manure, peat moss and humic acid (in a volume ratio of 30:10:10:3:1). A supplementary fertilizer (NPK 20:20:20 plus micronutrients) was used to speed up plant growth.



A field population of *P. xylostella* was originally collected from the common cabbage fields of Isfahan Province (central Iran). Cultures of *P. xylostella* were maintained on 4-week-old Chinese cabbage in ventilated oviposition cages (40×40×40 cm) under a standard constant environment (25±2°C; 70±5% RH; L:D 16:8 h) as described previously (Karimzadeh *et al.*, 2004, 2013). In particular, insect cultures were initially started with rearing larvae and pupae collected from field. Newly emerged adults were then mated and fed on honey solution (20%) for 24 h, before releasing into oviposition cages containing fresh Chinese cabbages. Usually, a population of 100-200 *P. xylostella* adults with a sex ratio of 1:1 was kept in each oviposition cage to provide a sufficient gene pool from diverse individuals of the species. Fresh Chinese cabbages were kept in oviposition cages for 24 hours, after which the plants with enough *P. xylostella* eggs were transferred to separate cages until eggs developed as early 3<sup>rd</sup> instar larvae, which were used for bioassays. In addition, a portion of larvae was allowed to pupate. Pupae were then transferred to new Petri dishes (10 cm dia.) until their emergence. Newly emerged offspring adults were used either for oviposition deterrence experiments or for the culture maintenance.

### Plant and Extract Preparation

The specimens of *D. stramonium* were collected from different fields of Mobarakeh County (32° 20' 47" N, 51° 30' 16" E, 1,673 m; Isfahan Province, central Iran) from June to September 2015. For the purpose of solid/liquid extraction, plant materials from different parts, including flower (just petal), seed, leaf, stem and root, of *D. stramonium* were washed thoroughly, and shadow dried at room temperature. Plant materials were then crushed and finely powdered using a blender. The samples (20 g) of powdered plant materials were extracted with MeOH (100 mL) in a shaker for 24 hours, filtered (i.e., purification) twice with a Whatman filter

paper No. 201, and evaporate (i.e. solvent separation) to dryness at room temperature (i.e. concentration) to get a solidified crude extracts. The crude extracts were finally stored in dark airtight containers at a cool place (4°C) until performance of experiments (Kim *et al.*, 2003).

### Dose-Response Bioassays

To prepare a stock solution, the extracts were diluted in distilled water. To be highly accurate in dose-mortality regression (one of the most frequently used statistical technique in toxicology), dose selection must include extreme doses (Robertson *et al.*, 1984, 2017), which imply doses that cause 10 and 90% mortalities in the present study. To determine the concentrations of main tests, preliminary experiments were performed using 30 *P. xylostella* larvae for each one of the dilutions of 0 (control), 30, 100, 300, 1,000, 3,000, 10,000 mg L<sup>-1</sup>. Preliminary LC<sub>10</sub> and LC<sub>90</sub> (i.e., extreme doses) were then estimated from preliminary experiments, and used as the lowest and highest concentrations of main tests. To perform the bioassay (i.e., main tests), the leaf discs (4.8 cm dia.) of the 4-week-old Chinese cabbage were immersed in the test solutions (five concentrations with logarithmic intervals between extreme doses, including 15-2,700, 10-32,000, 10-13,000, 10-9,000 and 10-2,000 mg L<sup>-1</sup> for root, stem, leaf, seed and flower extracts, respectively) for 10 seconds, and then kept on a corrugated sheet of aluminum foil with the adaxial leaf surface uppermost for 2 hours at room temperature to dry up. Control leaf discs were immersed in distilled water. The leaf discs were then transferred to individual plastic Petri dishes (5 cm dia.) containing a moistened filter paper. Ten early 3<sup>rd</sup> instar larvae of *P. xylostella* were then placed on each leaf disc. The leaf discs were replaced every 24 h with fresh, untreated ones. All the concentrations as well as the control were replicated eight times, and mortality was recorded after 48 hours. All the tests were performed under the standard environment

conditions ( $25\pm 2^\circ\text{C}$ ;  $70\pm 5\%$  RH; L:D 16:8 h; Karimzadeh and Sayyed, 2011; Jafary *et al.*, 2016). In all the experiments, the institutional and national guidelines for the care and use of laboratory animals (Institutional Animal Care and Use Committee Guidebook) were followed.

### Oviposition Deterrence

The sublethal concentrations ( $\text{LC}_{25}$ ; Table 3) were used to examine the effects of jimsonweed extracts on oviposition deterrence of *P. xylostella* females. Each extract was spread on the internal surface of a 175-mL plastic cup. Control cups were treated with distilled water. The treated cups were air dried at room temperature for 2 h, and covered with a piece of netting cloth to prevent the test insects from escaping. A pair (male and female) of newly emerged *P. xylostella* adults were introduced to each cup and fed with honey solution (20%). Each treatment was replicated ten times and kept under the standard environment conditions ( $25\pm 2^\circ\text{C}$ ;  $70 \pm 5\%$  RH; L:D 16:8 h). The number of eggs laid by the *P. xylostella* female was recorded daily for 7 days (Rafiei-Karahroodi *et al.*, 2011).

### Statistical Analyses

Generalized Linear Models (GLMs) with the family Poisson and the family binomial were applied to the data. In particular, the dose-response data were analyzed using logistic regression (binomial errors), where regression lines were fitted to dose-mortality data on a log (of concentration)-logit (of mortality) scale, and the estimated Lethal Concentrations (LC) and associated 95% Confidence Intervals (CI) were then calculated from the estimated linear regression parameters (Crawley, 2013). Because of non-parallel regression lines of different extracts, toxicity ratio test (lethal dose ratios) was used to compare LCs, instead of overlapping CIs, based on the

statistical power of ratio test and its better type I error rates (Wheeler *et al.*, 2006). The number of eggs laid was analyzed using log-linear models (Poisson errors). In case of over-dispersion, the model was refitted using quasi-Poisson rather than Poisson errors. To achieve the minimal adequate model, non-significant terms were removed through model simplification, in which the original and simplified models were compared by an F test. The statistical significance of minimal adequate model was then expressed as Z-values (i.e., standard normal deviates) or t-value (in case of overdispersion) (Crawley, 2013; Karimzadeh *et al.*, 2013; Saadat *et al.*, 2014; Jafary *et al.*, 2016). Oviposition Deterrence (OD) was calculated using the following formula (Rafiei-Karahroodi *et al.*, 2011):

$$OD(\%) = 100\left(1 - \frac{N_t}{N_c}\right) \quad (1)$$

Where,  $N_t$  and  $N_c$  denote the Number of eggs in the treatments and control, respectively. ODs were then analyzed using one-way ANOVA after arcsine transformation,  $\sin^{-1} \sqrt{0.01 \times OD}$ . Pairwise comparisons were performed using Tukey's Honestly Significant Difference (Karimzadeh and Wright, 2008; Crawley, 2013). All statistical analyses were completed in R 3.5.3 (R Development Core Team).

## RESULTS

### Dose-Response Bioassays

Logistic regression showed a significant (Z-value= 8.887, 9.121, 8.590, 8.736 and 8.499;  $P < 0.001$ ) linear relationship between the extract concentrations (as logarithm) and mortality (as logit) of *P. xylostella* larvae for all the extracts (Table 2). There was no significant difference between the Lethal Concentration of 10% ( $\text{LC}_{10}$ ) of the different extracts (ranged between 4.5 and 9.4  $\text{mg L}^{-1}$ ; Table 3). Higher lethal concentrations, however, showed significant differences between the extracts. The Lethal

**Table 2.** Logistic regression of dose-response (*Datura stramonium* extracts)-(*Plutella xylostella* larval mortality) data.

Extract	Fitted model <sup>a</sup>				
	a (SE)	b (SE)	Z value <sup>b</sup>	P value <	Adjusted R <sup>2</sup>
Flower	-3.35735 (0.41135)	0.76125 (0.08566)	8.887	0.001	0.825
Seed	-3.13806 (0.37797)	0.62899 (0.06896)	9.121	0.001	0.834
Root	-3.67853 (0.44954)	0.72018 (0.08384)	8.590	0.001	0.946
Leaf	-3.42456 (0.39777)	0.54649 (0.06256)	8.736	0.001	0.979
Stem	-2.90902 (0.34642)	0.43192 (0.05082)	8.499	0.001	0.847

<sup>a</sup>  $y = \frac{e^{a+bx}}{1+e^{a+bx}}$ , where x and y represent natural logarithm of concentration (mg L<sup>-1</sup>) and the proportion of dead larvae, respectively.

<sup>b</sup> The standard normal deviate.

**Table 3.** Toxicity of different *Datura stramonium* extracts on *Plutella xylostella* larvae.

Extract	Lethal concentration (95% CI) in mg L <sup>-1</sup> (n= 8)			
	LC <sub>10</sub>	LC <sub>25</sub>	LC <sub>50</sub>	LC <sub>90</sub>
Flower	4.6 (2.2-9.8) a	19.4 (11.7-32.4) a	82.3 (55.8-121.4) a	1475.4 (707.6-3076.2) a
Seed	4.5 (1.8-10.9) a	25.6 (13.9-47.1) ab	146.8 (91.1-236.4) ab	4828.7 (1993.2-11698.2) b
Root	7.8 (3.5-17.3) a	36.0 (21.3-60.8) abc	165.3 (110.5-247.3) b	3493.8 (1549.5-7877.8) ab
Leaf	9.4 (3.6-25.1) a	70.5 (36.8-135.1) c	526.6 (306.2-905.8) c	29352.1 (9557.5-90143.6) c
Stem	5.2 (1.5-17.9) a	66.1 (29.7-147.2) bc	841.4 (433.6-1633.0) c	136248.1 (31948.3-581050.1) c

<sup>a-c</sup> Values marked with different letters within columns are significantly (P < 0.05) different (based on ratio test; see Table 4).

Concentration of 25% (LC<sub>25</sub>) of the flower extract (19.4 mg L<sup>-1</sup>) was significantly smaller than stem (66.1 mg L<sup>-1</sup>) and leaf (70.5 mg L<sup>-1</sup>) extracts (Table 3). LC<sub>25</sub> of the second best toxicant (the seed extract; 25.6 mg L<sup>-1</sup>) was significantly smaller than leaf extract only. LC<sub>25</sub> of the root extract (36.0 mg L<sup>-1</sup>) did not show any significant difference with other tested plant extracts. This means that based on the effects of sublethal concentrations (LC<sub>10</sub>s and LC<sub>25</sub>s), the flower and seed extracts can be considered as the most effective extracts against *P. xylostella* larvae, respectively.

Based on the median Lethal Concentrations (LC<sub>50</sub>s; Table 3), the lethal dose ratios, and their confidence intervals (Table 4), the flower extract showed the highest toxicity against *P. xylostella* larvae. LC<sub>50</sub> of the flower extract (82.3 mg L<sup>-1</sup>) was significantly smaller than LC<sub>50</sub> of root extract (165.3 mg L<sup>-1</sup>), which in turn, was significantly smaller than LC<sub>50</sub> of the leaf (526.6 mg L<sup>-1</sup>) and stem (841.4 mg L<sup>-1</sup>) extracts. The second highest

toxicity was observed for the seed extract (with an LC<sub>50</sub> of 146.8 mg L<sup>-1</sup>), which showed no significant difference with higher (flower extract) and lower (root extract) categories. This means that based on the effects of the median Lethal Concentrations (LC<sub>50</sub>s), the flower, seed, and root extracts can be considered as the most effective extracts against *P. xylostella* larvae, respectively.

This trend was somewhat different when LC<sub>90</sub>s were compared. Again, the flower extract showed the highest toxicity against *P. xylostella* larvae. The leaf and stem extracts also exhibited the lowest toxicity against *P. xylostella* larvae. The root and seed extracts, however, showed different toxicity at higher doses. LC<sub>90</sub> of the flower extract (1,475.4 mg L<sup>-1</sup>) was significantly smaller than LC<sub>90</sub> of seed extract (4828.7 mg L<sup>-1</sup>), which, in turn, was significantly smaller than LC<sub>90</sub> of the leaf (29,352.1 mg L<sup>-1</sup>) and stem (136,248.1 mg L<sup>-1</sup>) extracts (Tables 3 and 4). The second highest toxicity was observed for the root extract (with an LC<sub>90</sub> of 3,493.8 mg L<sup>-1</sup>),

**Table 4.** Relative toxicity ratios of different *Datura stramonium* extracts on *Plutella xylostella* larvae

		Lethal dose ratio (95% CI) <sup>a</sup>			
LC <sub>10</sub>		Seed	Flower	Stem	Root
	Flower	0.972 (0.302-3.134)			
	Stem	0.859 (0.187-3.947)	0.883 (0.207-3.763)		
	Root	0.571 (0.173-1.887)	0.587 (0.196-1.759)	0.664 (0.153-2.890)	
	Leaf	0.472 (0.126-1.776)	0.486 (0.141-1.673)	0.550 (0.114-2.660)	0.828 (0.235-2.920)
LC <sub>25</sub>		Flower	Seed	Root	Stem
	Seed	0.759 (0.343-1.682)			
	Root	0.541 (0.260-1.124)	0.712 (0.318-1.592)		
	Stem	0.294 (0.114-0.759)	0.387 (0.142-1.058)	0.544 (0.209-1.416)	
	Leaf	0.276 (0.121-0.629)	0.363 (0.149-0.884)	0.510 (0.221-1.176)	0.937 (0.335-2.627)
LC <sub>50</sub>		Flower	Seed	Root	Leaf
	Seed	0.561 (0.303-1.037)			
	Root	0.498 (0.285-0.871)	0.888 (0.476-1.657)		
	Leaf	0.156 (0.080-0.304)	0.279 (0.135-0.574)	0.314 (0.160-0.617)	
	Stem	0.098 (0.045-0.211)	0.174 (0.077-0.395)	0.196 (0.090-0.427)	0.626 (0.266-1.474)
LC <sub>90</sub>		Flower	Root	Seed	Leaf
	Root	0.422 (0.141-1.263)			
	Seed	0.306 (0.097-0.965)	0.724 (0.218-2.406)		
	Leaf	0.050 (0.013-0.192)	0.119 (0.030-0.476)	0.165 (0.039-0.687)	
	Stem	0.011 (0.002-0.055)	0.026 (0.005-0.135)	0.035 (0.006-0.194)	0.215 (0.034-1.348)

<sup>a</sup> If the 95% confidence interval of the ratio includes 1, then the lethal doses are not significantly different.

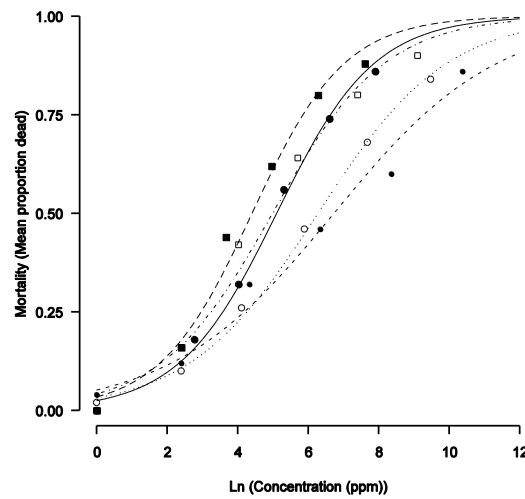
which showed no significant difference with higher (flower extract) and lower (seed extract) categories. This means that based on the effects of the 90% Lethal Concentrations (LC<sub>90</sub>s), the flower, root and seed extracts can be considered as the most effective extracts against *P. xylostella* larvae, respectively.

More detailed explanations of different extract toxicities can be observed using the dose-response curves (Figure 1). It is clear that in whole range of doses, except doses smaller than LC<sub>10</sub>, flower extract is the most toxic extract. The next best (more toxic) extracts are seed and root extracts, with the stronger effect of seed extract in lower doses and the stronger effect of root extract in higher doses. Indeed, for concentrations lower than 403.4 mg L<sup>-1</sup> (i.e., a natural logarithm of concentration equal to 6 in x-axis of Figure 1), seed extract caused more mortalities compared with root extract. However, for concentrations equal or higher than 403.4 mg L<sup>-1</sup>, root extract caused more mortalities compared with seed extract.

Finally, leaf and stem extract come last, with a better performance (more toxic) of leaf extract in doses higher than LC<sub>25</sub>. More exactly, for concentrations lower than 90.0 mg L<sup>-1</sup> (i.e. a natural logarithm of concentration equal to 4.5 in x-axis of Figure 1), stem extract caused more mortalities compared with leaf extract. However, for concentrations equal or higher than 90.0 mg/L, leaf extract caused more mortalities compared with stem extract.

### Oviposition Deterrence

When the sublethal concentrations (LC<sub>25</sub>) were used, log-linear models showed significant ( $t_5 = -4.282, P < 0.001$ ) differences between treatments for *P. xylostella* female fecundity. The mean number of eggs laid by a *P. xylostella* female on control cup (144.2±10.8) was significantly greater than that on stem extract-treated cup (95.6±11.8), which was in turn greater than that on flower



**Figure 1.** Dose-response curves (of fitted logistic regression) of the diamondback moth (*Plutella xylostella*) larvae subjected to jimsonweed (*Datura stramonium*) extracts. Long-dashed (filled square), dash-dot (empty square), solid (big filled circle), dotted (empty circle) and dashed (small filled circle) lines (points) represent the extract of flower, seed, root, leaf and stem of *D. stramonium*, respectively.

extract-treated cup ( $53.4 \pm 5.1$ ; Figure 2). Except leaf extract ( $103.4 \pm 12.1$  eggs cup<sup>-1</sup>), all other extracts showed significant differences with the control. However, flower extract had a stronger effect (in term of oviposition inhibition) compared with stem extract; seed ( $81.0 \pm 15.3$  eggs cup<sup>-1</sup>) and root ( $77.8 \pm 7.8$  eggs cup<sup>-1</sup>) extracts were intermediate of these two groups. In other words, the flower extract, followed by root and seed extracts, can be considered as the most effective oviposition inhibitors against *P. xylostella* adults, respectively.

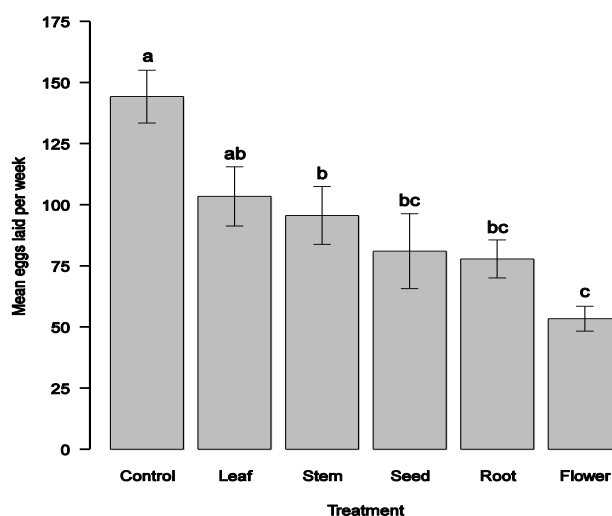
When the data were analyzed using one-way ANOVA, oviposition deterrence of *P. xylostella* females was significantly ( $F_{4,45} = 3.654$ ,  $P < 0.05$ ) different between treatments. Oviposition deterrent effect of flower extract ( $63.0 \pm 3.1\%$ ) was significantly greater than stem ( $33.7 \pm 7.3\%$ ) and leaf ( $28.3 \pm 7.5\%$ ) extracts (Figure 3). Oviposition deterrent effect of root ( $46.0 \pm 4.9\%$ ) and seed ( $43.8 \pm 9.5\%$ ) extracts were intermediate of these two groups. Again, this indicates that the flower extract has the strongest oviposition deterrent effect against *P. xylostella* adults, and root and seed extracts

can be considered as the second most effective *P. xylostella* oviposition inhibitors.

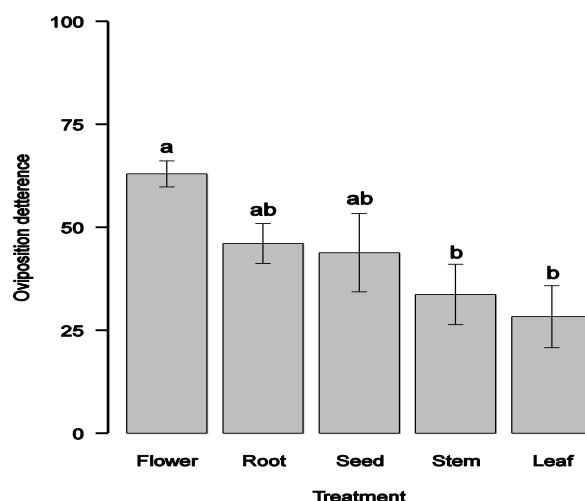
## DISCUSSION

Here, using standard experiments with adequate sample size, careful dose selection, and sound experimental design, we indicated that jimsonweed extracts may be used as an effective botanical pesticide and deterrent against the diamondback moth. Based on  $LC_{50s}$  and  $LC_{90s}$ , it was indicated that flower, seed, and root extracts of *D. stramonium* were highly toxic against *P. xylostella* larvae. In addition, it was clear that flower was the most toxic part of jimsonweed against diamondback moth. Based on the number of egg load influenced by  $LC_{25s}$ , it was also shown that flower, root, and seed extracts of *D. stramonium* act as oviposition inhibitors for *P. xylostella* adults. Furthermore, it was distinct that flower extract had the highest oviposition deterrent effect on diamondback moth.





**Figure 2.** The effects of sublethal concentrations ( $LC_{25}$ ) of jimsonweed (*Datura stramonium*) extracts on the fecundity of the diamondback moth (*Plutella xylostella*) adults.



**Figure 3.** Oviposition deterrence of the diamondback moth (*Plutella xylostella*) adults by sublethal concentrations ( $LC_{25}$ ) of jimsonweed (*Datura stramonium*) extracts.

Previous studies have shown controversial results about the alkaloid contents of jimsonweed. Some studies have revealed that the most abundant tropane alkaloids in jimsonweed are hyoscyamine and scopolamine (Miraldi *et al.*, 2001; Lanfranchi *et al.*, 2010). It has been pointed out that these alkaloids can significantly vary with plant part, plant life stage, and geographical location. For instance, Miraldi *et al.* (2001) found the highest content of atropine and

scopolamine in stems of young plants, and lack of these alkaloids in stem and root of adult plants. A completely different profile of alkaloids was found in jimsonweed extracts, when Berkov *et al.* (2006) compared different varieties of *D. stramonium* [var. *stramonium* (S), var. *tatula* (T) and var. *godronii* (G)] from Bulgaria and Egypt (only S): alkaloid patterns of the roots, leaves and seeds of all varieties grown in Bulgaria were similar but different from the variety grown in Egypt.



Apart from qualitative patterns of alkaloids, quantitative differences were considerable between different varieties, such that hyoscyamine was the main alkaloid in leaves of all varieties, seeds of Bulgarian varieties, and root of G. The main alkaloid in roots of Bulgarian S and T, however, was 3,6-Ditigloyloxy-7-hydroxytropine.

Alternatively, tropine was the main alkaloid in root and seed of Egyptian S. After these considerations, Berkov *et al.* (2006) concluded that jimsonweed alkaloid pattern was influenced more strongly by the environmental factors than genetic ones. Therefore, further GC-MS studies are necessary to determine the exact patterns of alkaloids in different parts of Iranian jimsonweed.

Field experiments for determining efficacy of pesticides are usually designed on the basis of LC<sub>90</sub> and two lower and higher logarithmic doses (Robertson *et al.*, 2017). Given this, the flower and root extracts of *D. stramonium* might be more appropriate candidates for developing a botanical insecticide. However, it is well known that root is the main site of tropane alkaloid synthesis (Cromwell, 1943; Oksman-Caldentey and Arroo, 2000), and that the amount of the extract that can be obtained from the roots of *D. stramonium* would be much more compared with the flower. The latter makes the root extract of *D. stramonium* most suitable candidate for applied experiments of developing a new, safe insecticide against *P. xylostella*. In this regard, side effects of *D. stramonium* extracts on natural enemies and pollinators must be taken into consideration (Kumral *et al.*, 2013), which remains for a future work.

The present study also showed that the egg load of *P. xylostella* females could be highly influenced by sublethal dose of flower, root, and seed extracts of *D. stramonium*. Limited studies have shown *D. stramonium* oviposition deterrent activity against mites (*T. urticae* by leaf and seed extracts) and insects (*A. aegypti* and *C. quinquefasciatus* by leaf extract) (Kumral *et al.*, 2010; Swathi *et al.*, 2010). The mechanism of such oviposition inhibition, however, is unknown

and requires further investigations. In the present study, the flower, root, and seed extracts of *D. stramonium* showed oviposition deterrent activity against *P. xylostella*, but the oviposition deterrence of diamondback moth females by the flower extract of jimsonweed was considerable (63%) which can be of interest of future applied studies. It is noteworthy to mention that out of 35 plant species whose extracts are highly effective on *P. xylostella* (reported at Table 1), only four species (*Andrographis paniculata*, *Chrysanthemum morifolium*, *Melia azedarach*, and *Peganum harmala*) have exhibited oviposition deterrence or inhibition against *P. xylostella* adults (Hermawan *et al.*, 1994; Liu *et al.*, 2006; Charleston *et al.*, 2005; Abbasipour *et al.*, 2010). This is indeed the first report of oviposition inhibition of a flower extract on *P. xylostella* adults. Further laboratory and field experiment are necessary to determine applied aspects of the oviposition deterrent activities of *D. stramonium* flower extract as a safe semiochemical for ecological pest management programs in organic production of cruciferous crops.

The present study indicated that jimsonweed extracts have high capability as an efficient larvicide and oviposition deterrent against *P. xylostella*. The diamondback moth, however, is a model insect that successfully develops resistance to any synthetic insecticides (Talekar and Shelton, 1993; Furlong *et al.*, 2013; "Arthropod Pesticide Resistance Database", 2019). Phytochemicals such as plant extracts also are not immune to development of resistance by *P. xylostella* (Rattan, 2010). However, development of such a resistance in *P. xylostella* may occur more slowly due to existence of numerous compounds in plant extracts and their complex mode of action (Vollinger, 1987). In addition, a combination of plant extracts with other non-chemical tactics such as host-plant resistance, biological control, trap cropping, and application of botanical/microbial bio-pesticides (Afiunizadeh and Karimzadeh, 2015; Karimzadeh and Besharatnejad, 2017a,

b; Daniarzadeh *et al.*, 2014; Jafary *et al.*, 2016) can postpone or deter development of insecticide resistance. On the other hand, there is no report of resistance to oviposition deterrence of plant extracts that make such compounds excellent candidate for developing a safe and efficient tool for ecological pest management. To develop and implement pest management strategies that are environmentally-safe and cost-effective, a total system approach (Lewis *et al.*, 1997; Thomas, 1999) must be considered that includes multi-trophic interactions between the target pest, its host crop, and other related organisms and the environment. In this regard, it is necessary to investigate compatibility of phytochemicals with the environment, wildlife, and human health.

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### اثرات لاروکشی و بازدارندگی تخم‌گذاری عصاره‌های داتوره (*Datura stramonium* L.) روی بید کلم (*Plutella xylostella* L.)

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#### چکیده

اهمیت بید کلم، (*Plutella xylostella* L.) به‌طور شگرفی افزایش یافته است که عمدتاً به‌علت استفاده بیش از حد از حشره‌کش‌های وسیع‌الطیف است که به ایجاد مقاومت به آفت‌کش‌ها، نابودی دشمنان طبیعی و طغیان مجدد آفت منجر شده است. این مشکلات به همراه افزایش بقایای حشره‌کش‌ها در محصولات کشاورزی، نیاز فوری به راهبردهای پایدار مدیریت بید کلم را شدت بخشیده است. در مطالعه حاضر، کارایی عصاره‌های قسمت‌های مختلف علف هرز داتوره، *Datura stramonium* L. به‌عنوان لاروکش و بازدارنده تخم‌گذاری به‌ترتیب روی لاروها و حشرات کامل بید کلم بررسی شد. آزمون‌های زیست‌سنجی نشان از سمیت (LC<sub>50</sub> و LC<sub>90</sub>) بالاتر عصاره‌های گل (۸۲/۳ و ۱۴۷۵/۴ میلی‌گرم بر لیتر)، بذر (۱۴۶/۸ و ۴۸۲۸/۷ میلی‌گرم بر لیتر) و ریشه (۱۶۵/۳ و ۳۴۹۳/۸ میلی‌گرم بر لیتر) داتوره در مقایسه با عصاره‌های برگ (۵۲۶/۶ و ۲۹۳۵۲/۱ میلی‌گرم بر لیتر) و ساقه (۸۴۱/۴ و ۱۳۶۲۴/۱ میلی‌گرم بر لیتر) داشت. به‌علاوه، عصاره گل بیشترین سمیت را بین تمام عصاره‌ها نشان داد که در مقایسه با عصاره‌های بذر، ریشه، برگ و ساقه به‌ترتیب نسبت دوز LC<sub>50</sub> (۰/۵۶۱، ۰/۴۹۸، ۰/۱۵۶ و ۰/۰۹۸) و نسبت دوز LC<sub>90</sub> (۰/۳۰۶، ۰/۴۲۲، ۰/۰۵۰ و ۰/۰۱۱) کمتری نشان داد. علاوه بر این، آزمون‌های بازدارندگی تخم‌گذاری با استفاده از دوزهای زیرکشنده نشان داد که بیشترین بازدارندگی تخم‌گذاری بر اثر عصاره گل داتوره (۰/۶۳/۰) در مقایسه با عصاره‌های ریشه (۰/۴۶/۰)، بذر (۰/۴۳/۸)، ساقه (۰/۳۳/۷) و برگ (۰/۲۸/۳) ایجاد شده است. در مطالعه حاضر نشان داده شد که عصاره‌های داتوره می‌توانند به‌عنوان آفت‌کش و بازدارنده تخم‌گذاری موثر بر علیه بید کلم به کار روند. امکان کاربرد عصاره‌های داتوره در مدیریت آفت و امکان توسعه آن‌ها به‌عنوان یک حشره‌کش گیاهی یا بازدارنده تخم‌گذاری در زمینه مدیریت پایدار آفات بحث شده است.