RESEARCH

OVICIDAL EFFECT OF SOME INSECTICIDES ON THE DIAMONDBACK MOTH, *Plutella xylostella* (L.) (LEPIDOPTERA: YPONOMEUTIDAE)

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ABSTRACT

The diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), is a serious threat to Brassica vegetables in Iran, including Tehran province. The ovicidal effects of different classes of insecticides on *P. xylostella* were investigated, using three fixed doses (based on commercial formulations). At the lowest concentration (500 mg L^{-1}), the mortality effect of hexaflumuron and pyridalyl was higher than the other insecticides examined. Fipronil, hexaflumuron and spinosad and pyridalyl, however, showed high toxic effects at the median dose (1000 mg L^{-1}). On the other hand, at high concentration (2000 mg L^{-1}), all insecticides except lufenuron and indoxacarb (EC formulation) caused more than 85% mortality. Overall, these findings indicate that hexaflumuron, spinosad and fipronil, with low active ingredients and high mortality, could be the best choices for controlling the *P. xylostella* in the egg stage.

Key words: diamondback moth, *Plutella xylostella*, insecticides, ovicidal effect.

The diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), is the most destructive pest of brassicaceae in the world and a serious problem in Iran. Its global importance is reflected by the fact that almost US$1 billion is spent annually on its control (Talekar and Shelton, 1993; Mahmoudvand *et al*., 2009). Although chemical control is the most conventional method against the diamondback moth, resistance to insecticides has developed in *P. xylostella* (Sun *et al*., 1986; Shelton *et al*., 1991).

Insect growth regulators (IGRs) are a unique class of insecticides with selective effects on various life stages of some orders of insects. They interrupt molting (juvenile hormone or ecdysone mimics) and cuticle formation (chitin synthesis inhibitors) and affect the insect endocrine system (Dhadialla *et al*., 1998; Hoffmann and Lorenz, 1998). Chitin synthesis inhibitors (CSI), a group of IGRs, are benzoylphenylurea (BPU) with high specificity, low mammalian toxicity and rapid degradation in the environment. These compounds are effective on immature stages of insects (Mian and Mulla, 1982; Reynolds, 1987). Hexaflumuron (1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea), which is a CSI, has been used to control a wide range of agricultural pests. It has ingestion, contact and ovicidal toxicity (Sbragia *et al*., 1983; Su and La Fage, 1987). Lufenuron ((*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea), another IGR, is employed to control the lepidopteran and coleopteran larvae on vegetable and cotton crops (Mossan *et al*., 1995). Fipronil ((5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile) has a unique mode of action and interferes with the passage of chloride ions through the gamma-aminobutyric acid (GABA) regulated chloride channel (Cole *et al*., 1993). Spinosad (mixture of 50-95% of (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4 -tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-*as*-indaceno[3,2-*d*]oxacyclododecine-7,15-dione and 50-5% of (2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*S*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-*as*-indaceno[3,2-*d*]oxacyclododecine-7,15-dione), is a

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selective insecticide based on a fermentation product of the soil bacterium actinomycete (*Saccharopolyspora spinosa*), which was discovered in the 1980s (Mertz and Yao, 1990; Miles and Dutton 2000; Crouse *et al*., 2001). Pyridalyl (2,6-dichloro-4-(3,3-dichloroallyloxy) phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy] propyl ether), which is a new synthetic insecticide, was discovered at Sumitomo Chemical Co. Ltd., Japan. This insecticide has contact and ingestion toxic effects and is excellent for controlling lepidopteran and thysanopteran pests (Sakamoto *et al*., 2003; Saito *et al*., 2004). Indoxacarb (methyl(*S*)-*N*-[7-chloro-2,3,4a,5-tetrahydro-4a-(methoxycarbonyl)indeno[1,2-*e*][1,3,4]oxadiazin-2-ylcarbonyl]-4-(trifluoromethoxy)carbanilate or methyl (*S*)-7-chloro-2,3,4a,5-tetrahydro-2-[methoxycarbonyl(4-trifluoromethoxyphenyl)carbamoyl]indeno[1,2-*e*][1,3,4] oxadiazine-4a-carboxylate) has a good toxic effect as an oxadiazine insecticide against lepidopteran pests with no effect on non-target insects. This insecticide causes the lepidopteran larvae to stop feeding. It also results in irreversible convulsions and severe paralysis (Wing *et al*., 1998; Dinter and Wiles, 2000). Indoxacarb affects the target insects by blocking the sodium channels of nerve cells (Meier *et al*., 1992).

Brassica vegetables are important vegetable crops grown throughout Tehran province and other areas of Iran. *P. xylostella* occurs annually throughout Tehran fields, wherever brassicaceous crops are grown, and causes substantial crop losses, especially during outbreak years. To our knowledge, no insecticides for diamondback moth control in Iran have been investigated. This is the first study to show the toxicity of insecticides on *P. xylostella* eggs. In the field, insecticides are usually applied in their commercial formulation. Thus, the aim of this research was to compare the ovicidal property of some insecticides (spinosad, indoxacarb EC, indoxacarb SC, pyridalyl, fipronil, lufenuron and hexaflumuron) at three fixed commercial formulations (500, 1000, and 2000 mg L^{-1}) on the diamondback moth through a leaf dip bioassay.

MATERIALS AND METHODS

Insect rearing

An initial *P. xylostella* colony was collected in August 2008 from cauliflower (*Brassica oleracea* var. *botrytis* L.) fields of Shahre-Rey in the south of Tehran, Iran. For the egg-laying, about 500 adults of the *P. xylostella* were placed in a plastic cage (50 × 30 × 30 cm) and eggs were transferred to cauliflower leaves as food material to continue their development. The insect stock was maintained at 25 ± 1 °C and 65 ± 5% relative humidity under a 16:8 (L:D) photoperiod in a growth chamber.

Insecticides

The commercially formulated insecticides used in the present study were spinosad (Tracer 240 SC [AI], DowAgrosciences, Indianapolis, USA) indoxacarb (Avaunt, 15% EC and 150 SC [AI], DuPont, Cernay, France), pyridalyl (Sumipolo, 50% EC [AI], Sumitomo Chemical, Shikoku, Japan), fipronil (Agenda, 2.5% EC [AI], Agrochemicals, North West Maharashtra, India), lufenuron (Match 50% EC [AI], Syngenta India Limited, Mumbai, India) and hexaflumuron (Consult, 50% EC [AI], Dow Agrosciences, Indianapolis, USA).

Ovicidal assessment test

A leaf dip method was used for all bioassay experiments (Tabashnik and Cushing, 1987). Cabbage (*Brassica oleracea* var. *botrytis* L.) leaf disks containing 10 eggs (< 10 h old) of *P. xylostella* were dipped in three concentrations (500, 1000 and 2000 mg L^{-1}) of commercial formulations of the aforementioned insecticides, including 0.02% Tween-80 for 30 s. In control group, the leaf disks were dipped in water containing 0.02% Tween-80. The treated leaf disks were dried at room temperature for 2 h. Then, the leaf disks were placed in Petri dishes (8 cm in diameter, 1.5 cm depth). The treated eggs were then checked daily until all larvae hatched or the eggs died. Each treatment had four replicates.

Data analysis

Data obtained were subjected to one-way ANOVA ($P < 0.05$) after checking for normality. Means were compared by Duncan's Multiple Range Test, admitting significant differences at $P < 0.05$. SAS software was used for all analyses (SAS Institute, 1997).

RESULTS

Tables 1-3 show the effects on *P. xylostella* of different insecticides at different doses. At 500 mg L^{-1}, the toxic effects of all insecticides, except pyridalyl, were lower than 50%. The efficacy of pyridalyl and hexaflumuron were 65.12 and 47.5%, respectively, and these insecticides were better than the other insecticides in the lowest applied dose. There was no significant difference between control and fipronil, indoxacarb (both formulations) and lufenorun ($P < 0.0001$, $F = 15.19$, df = 7.24) (Table 1). At the median concentration (1000 mg L^{-1}), all insecticides, except indoxacarb (EC), significantly affected the hatchability of diamondback moths. Similar to the last dose, treatment with pyridalyl caused the highest and excellent egg mortality (97.72%) ($P < 0.0001$, $F = 41.39$, df = 7.24) (Table 2). Table 3 shows the ovicidal effects at the highest tested dose, 2000 mg L^{-1} (commercial formulation), of the aforementioned insecticides on eggs

Table 1. Ovicidal effect of different insecticides on *Plutella xylostella* eggs exposed to 500 mg L^{-1} (commercial formulation).

Treatment	Active ingredient	Ovicidal
	mg L^{-1}	%
Control		1.98c
Fipronil	12.5	6.94 ± 4.16c
Hexaflumuron	50	47.50 ± 5.50ab
Indoxacarb (SC)	75	10.44 ± 3.56c
Indoxacarb (EC)	75	1.92 ± 1.92c
Spinosad	120	33.75 ± 12.14b
Pyridalyl	250	65.12 ± 5.60a
Lufenuron	250	4.58 ± 2.66c
P		P < 0.0001
		15.19
		7.24

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

Table 2. Ovicidal effect of different insecticides on *Plutella xylostella* eggs exposed to 1000 mg L^{-1} (commercial formulation).

Treatment	Active ingredient	Ovicidal
	mg L^{-1}	%
Control		1.98e
Fipronil	25	69.63 ± 6.44b
Hexaflumuron	100	65.00 ± 2.88b
Indoxacarb (SC)	150	29.85 ± 2.04c
Indoxacarb (EC)	150	10.70 ± 5.88de
Spinosad	240	77.75 ± 1.44b
Pyridalyl	500	97.72 ± 2.27a
Lufenuron	500	20.17 ± 8.51cd
P		P < 0.0001
		41.39
		7.24

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

of *P. xylostella*. All eggs were killed by pyridalyl, spinosad and hexaflumuron and the mortality of eggs was enhanced as the concentrations increased. Indoxacarb (SC) and fipronil also had a high ovicidal effect on the reduction of egg hatching. Indoxacarb (EC) at 2000 mg L^{-1} had no significant ovicidal effect compared to the control group ($P < 0.0001$, $F = 12.07$, df = 7.24) (Table 3).

Table 3. Ovicidal effect of different insecticides on *Plutella xylostella* eggs exposed to 2000 mg L^{-1} (commercial formulation).

Treatment	Active ingredient	Ovicidal
	mg L^{-1}	%
Control		1.98c
Fipronil	50	90.17 ± 6.07a
Hexaflumuron	200	100a
Indoxacarb (SC)	300	86.02 ± 25.16a
Indoxacarb (EC)	300	10.44 ± 1.11c
Spinosad	480	100a
Pyridalyl	1000	100a
Lufenuron	1000	49.55 ± 4.66b
P		P < 0.0001
F		12.07
		7.24

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

DISCUSSION

The insecticides applied in the present study were effective on eggs of the diamondback moth. Since all tests were performed using the leaf dip method, the ovicidal effect indicated a contact toxicity of these insecticides. To the best of our knowledge, there are few studies regarding the effect of insecticides on eggs of lepidopteran pests and most reports have been focused on the control of larval stage, therefore, little information is available about the effect of pesticide residues on eggs of *P. xylostella*. Here we showed that pyridalyl has best contact toxicity on eggs of *P. xylostella* compared to the other tested insecticides (at the median dose). One possible explanation for this could be the high active ingredient (ai) of the aforementioned insecticides. The fipronil and hexaflumuron applied in this study contained 10 and 2.5% ai. Compared to other insecticides, they are more economical for controlling *P. xylostella* eggs. Concentrations applied for ovicidal effect are higher than those for the larvicidal bioassay. This fact was recorded by Liu *et al*. (2002). They reported that in the indoxacarb bioassay of *Trichoplusia ni* (Hubner) (Lep.: Noctuidae), the 50% lethal dose (LC_{50}) for the egg stage is higher than that for the larval stage.

In this study we also observed that spinosad had high toxicity on Lepidoptera eggs. This is supported by Boiteau and Noronha (2007), who treated the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) with spinosad. In the present study, although lufenuron had an ovicidal effect on *P. xylostella*, it was not as good as the effect of hexaflumuron, another IGR. This could be due to difference in their respective compounds. This is the first study to test the ovicidal effects of hexaflumuron on *P. xylostella* and the result is almost conclusive. Sáenz-de-Cabezón *et al*. (2006) showed that lufenuron has ovicidal activity on *Lobesia botrana* (Lepidoptera: Tortricidae) in contact treatment. El-Barkey *et al*. (2009) stated that hexaflumuron had ovicidal effects on *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), an important lepidopteran pest of cotton. Oouchi (2005) reported that an IGR, pyriproxyfen at 100 mg L^{-1} (AI) caused egg mortality of *P. xylostella* up to 89%. Here, pyridalyl showed a good ovicidal effect on *P. xylostella*. Isayama *et al*. (2005) reported that the efficacy of pyridalyl at 100 mg mL^{-1} on eggs of *Orius stringicollis* (Hemiptera: Anthocoridae) was lower than 20%. Vastrad *et al*. (2005) also estimated the ovicidal action of fipronil and lufenuron on *P. xylostella*. They observed that lufenuron and fipronil exert ovicidal effects on the diamondback moth. A notable aspect of this study was the strong difference between two formulations of indoxacarb (SC and EC).

The EC formulation in the 500 mg L^{-1} treatment was similar to the SC formulation, but the 1000 and 2000 mg L^{-1} groups had lower toxicity on eggs than SC with the same level of active ingredient. Liu *et al*. (2002) reported that indoxacarb does not have significant ovicidal effect on *P. xylostella*.

CONCLUSION

The results of this study indicated that the insecticides applied, except indoxacarb (EC), had good toxicity on the egg stage of the diamondback moth. Among the other insecticides examined, fipronil, spinosad, and hexaflumuron had the greatest efficacy on diamondback moth eggs in response to their active ingredient.

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RESUMEN

Efecto ovicida de algunos insecticidas sobre la polilla del repollo, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). La polilla del repollo, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), es un serio riesgo para especies Brassica en Irán, incluyendo la provincia de Teherán. Se investigó el efecto ovicida de algunos insecticidas pertenecientes a diferentes clases de *P. xylostella* usando tres dosis (basadas en formulaciones comerciales). A la menor concentración (500 mg L^{-1}) el efecto de mortalidad de hexaflumuron y piridalil fue mayor que las otras dosis examinadas. Fipronil, hexaflumuron, spinosad y piridalil, sin embargo, mostraron fuertes efectos tóxicos a la dosis media (1000 mg L^{-1}). Por otra parte, a alta concentración (2000 mg L^{-1}) todos los insecticidas excepto lufenuron e indoxacarb (formulación EC) causaron más de 85% mortalidad. Finalmente, estos hallazgos indican que hexaflumuron, spinosad y fipronil con baja concentración de ingredientes activos y alta mortalidad pueden ser la mejor elección para controlar *P. xylostella* en el estado de huevo.

Palabras clave: polilla del repollo, *Plutella xylostella* insecticidas, efecto ovicida.

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