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CROSS RESISTANCE PATTERN FOR EMAMECTIN BENZOATE AND SYNTHETIC PYRETHROIDS IN DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE)

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ABSTRACT

Three pyrethroids (deltamethrin, bifenthrin and ג-cyhalothrin) and four new chemistry insecticides were tested against second instar larvae of *Plutella xylostella* under laboratory condition. Based on their LC_{50} values, deltamethrin was most toxic than ג-cyhalothrin and bifenthrin and among new chemistry insecticides, emamectin benzoate was the most toxic followed by acetamiprid, diafenthiuron and imidacloprid. Selection of two subpopulations for five generations with gradual toxic exposures of deltamethrin and emamectin showed decrease in survival rate. However, there was no drastic increase in LC_{50} values for both selected subpopulations than that of unselected subpopulation. Such high cross-resistance between emamectin benzoate with deltamethrin and ג-cyhalothrin suggest their wise use against this important insect pest. Rotational use of insecticides with different mode of action against *P. xylostella* might help avoiding development of resistance.
Keywords: *Plutella xylostella*, cross-resistance, pyrethroids, emamectin, toxicity

INTRODUCTION

Insecticide resistance is a genetically acquired attribute allowing an organism to combat higher doses of insecticide than susceptible ones (Gorman, 2009). The genetic make-up of *Plutella xylostella* has allowed developing insecticide resistance to almost all major classes of insecticides (Fahmy et al., 1991). Insecticide resistance may develop either by genetic selection, direct exposure to insecticides or cross-resistance resulting from selection by insecticides belonging to different groups (Oppenoorth, 1985). Insecticide resistance in *P. xylostella* is widespread in South East Asia due to its greater disperse ability, multivoltine nature and frequent insecticide application per crop season (Mangagro and Edelson, 1990).

High rate of resistance has been reported against fenvalerate, quinalphos, cypermethrin and deltamethrin in *P. xylostella* strain collected from area where pyrethroids were mostly used at heavy dose rate (Saxena et al., 1989). *P. xylostella* is most obnoxious widely distributed insect pest of brassicaceous crop. It was the first insect pest to become DDT resistant and had shown resistance to almost all synthetic insecticides used in field that resulted in failure of economical production of crucifers (Capinera, 2001). Worldwide efforts are being undertaken to develop integrated pest management (IPM) programs based on utilization of its natural enemies. A combination of entomopathogens and parasitoids against *P. xylostella* in integrated pest management program has also been suggested (Sarfaraz et al., 2005).

P. xylostella is considered be the most injurious insect pest of cruciferous plants with cosmopolitan distribution (Shankar et al., 1996). It causes up to 90% loss in crucifers and may be responsible for 52% of yield loss in cabbages (Kumar et al., 1983; Verkerk and Wright, 1996). Cruciferous family includes cauliflowers, broccoli, rapeseed, cabbages, collards, brussels and sprouts (Capinera, 2001). Host range of *P. xylostella* is limited to crucifers who contain glucosides and mustard oil (Gupta and Thorsteinson, 1960). It is a persistent insect pest of crucifers all over the world and numbers of species have been reported in the USA (Kfir, 1998). In Indo-Pak subcontinent, it was first recorded in 1914 on crucifer vegetables (Fletcher, 1914; Ghouri, 1960) as a minor cruciferous pest, however, later in 1992, Abro et al. found it as an important cruciferous insect pest in Pakistan.

Cross resistance development of different insecticides is very important in their selection for effective pest control. Incorporation of emamectin in pest control program for *P. xylostella* as new chemistry insecticide made the present hypothesis to observe whether their

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exist some cross resistance between synthetic pyrethroids and new chemistry insecticides.

MATERIALS AND METHODS

To check the cross resistance between synthetic pyrethroids and new chemistry insecticides against *P. xylostella* field population, bioassays were performed in the Toxicology Laboratory, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi during 2011-12 under controlled environmental conditions.

Field Collection and insect population standardization

Collection of field population of *P. xylostella* was made from cauliflower field crop under insecticide exposure of Taxila. 200-300 larvae were collected randomly from cauliflower plants by hand picking and kept in plastic jars of 1kg (40-50 larvae/jar) with fresh leaves of cauliflower as diet. The jar was closed with a piece of muslin cloth and kept in laboratory for further rearing at 25±2ºC, 60±10% RH and 14hr photophase (Rafiq, 2005). Pupae were collected with the help of forceps and placed in separate plastic petri dishes. These pupae were observed daily for adult emergence which were shifted to rectangular rearing cages made up of iron rod (12"x6"x9") and covered with muslin cloth. Cotton balls soaked with 10-15% honey solution were placed in the rearing cage on alternate day replacement. These adults were provided with 2-3 potted plants of radish (30-40 days old) per cage. These potted plants were replaced on daily basis to collect eggs laid and then were put into new cages labeled with date of eggs collection to be used in bioassays at required stage.

Insecticide Bioassays

Three pyrethroids namely ג-cyhalothrin (Karate® 2.5EC, Syngenta), bifenthrin (Talstar® 10EC, FMC), deltamethrin (Battalion® 2.5EC, 4B Pesticides) and four new chemistry insecticides as imidacloprid (Confidor® 20SL, BayerCrop Science), acetamiprid (Mospilon® 20SP, DuPont), emamectin benzoate (Proclaim®1.9EC, Syngenta) and diafenthiuron (Polo® 50SC, Syngenta) were used. Serial concentration solutions (6-8 levels) were prepared to check the insecticide toxicity of each insecticide using leaf dip method (no choice method).

Plastic petri dishes having diameter of 5cm were used for toxicity bioassays. These petri dishes were lined with moist filter paper. Fresh leaves of cauliflower were cut into desired shape using 5cm diameter leaf cutter. Eight leaves were dipped in each serial solution for 5-10 seconds and left to dry in fume hood for half an hour on tissue paper away from direct sunlight. These treated leaf discs were placed in petri dishes with adaxial side upward. Petri dishes were marked with the concentration of the leaf placed in it. Five second instar larvae were released in each petri dish using camel hair brush. In case of control, the leaves were dipped in water only. Larval mortality was recorded after 48 and 72 hours and mortality as end point was recorded.

Selection of population with two insecticides

After performing initial bioassays with insecticides, two insecticides deltamethrin and emamectin were used for selection of *P. xylostella* population. About 400-500, second instar larvae for each insecticide were used in selection at LC_{50} level. The concentration was increased in later generation for enhancement of resistance to these two insecticides. This selection was performed for five generations and then selected populations for these two insecticides were tested for all the individual insecticides under study. The bioassays results obtained were used to find out the possibilities of cross-resistance among these insecticides.

Data analysis

LC_{50}, LC_{90} and their fiducial limits were calculated using POLO-PC (Russell et al., 1977). Data of selected populations after desired selection level was compared through correlation for presence or absence of cross-resistance (Travis and Rick, 2000).

RESULTS AND DISCUSSION

Emamectin benzoate was significantly more toxic followed by deltamethrin, ג-cyhalothrin, bifenthrin, acetamiprid, diafenthiuron and imidacloprid with LC_{50s} of 0.37, 9.34, 11.1, 13.9, 24.7, 25.5 and 27.9 observed at 48hr, respectively. High cross-resistance existed between emamectin benzoate, deltamethrin and ג-cyhalothrin (table 1).

Toxicity of pyrethroids and new chemistry insecticides to a field population

After 48 hours observation, deltamethrin was the most effective insecticide with LC_{50} (ug/ml) of 9.34 followed by ג- cyhalothrin and bifenthrin (13.9, 11.1), respectively. All tested pyrethroids were 3-5 times more toxic after 72 hour (Table 1). In new chemistry insecticides, emamectin benzoate with LC_{50} of 0.37 after 48 hrs was significantly more toxic than others. Diafenthiuron and imidacloprid were the least toxic against *P. xylostella* with LC_{50} of 25.5 and 27.9, respectively. However, acetamiprid with LC_{50} of 24.7 was more toxic than diafenthiuron and imidacloprid. Comparison ratio revealed that these new chemistry insecticides were 2.46-4.7 times most effective after 72 hours (Table 1).

Toxicity of pyrethroids and new chemistry insecticides to Delta-Sel and Ema-Sel subpopulations

Delta-selected strain of *P. xylostella* was tested to pyrethroids i.e., ג-cyhalothrin, bifenthrin and deltamethrin. As compared with unselected strain it was observed that delta-Sel strain showed high resistance to deltamethrin and ג-cyhalothrin and low to bifenthrin. Selection of a field population with deltamethrin not only resulted in high resistance to deltamethrin but also moderate resistance to ג-cyhalothrin (Tables 2). This Delta-Sel strain was moderately resistance to emamectin benzoate as Ema-Sel strain showed moderate resistance to ג-cyhalothrin and deltamethrin. However, rate of survival decreased with increase in generation from G1 to G5 for the deltamethrin more profoundly than that for emamectin (Table 3).

Pair-wise comparison Delta-Sel and Ema-Sel populations of *Plutella xylostella*

Pair-wise comparison of LC_{50}s of insecticides tested for delta-Sel population of *P. xylostella* showed positive correlation with all tested insecticides. The three pyrethroids showed high correlation with emamectin and moderate to ג-cyhalothrin (Table 4). Ema-Sel population was also highly correlated with deltamethrin and moderate with bifenthrin and ג-cyhalothrin, respectively indicating presence of cross-resistance with them (Table 4).

P. xylostella is well-known for its short life cycle, high disperseability and prominent role in resistance development to almost all insecticide groups such as organophosphates, carbamates, pyrethroids and biological products (Baker and Kovaliske, 1999; Capinera, 2001; Huang and Wu, 2003). It is accountable for billions of dollar losses worldwide and continuous threat for crucifer crops as they depreciate the quality and quantity of these vegetables (Abro et al., 1994; Vervek and Wright, 1996). Almost all kinds of pest management techniques including chemical, biological, genetic, bio-pesticide have been tested and integrated to manage this pest effectively (Perera et al., 2000; Patcharaporn et al., 2010).

Crucifer growing areas of Taxila from where the population of *P. xylostella* was collected during March-April, 2011 were not under abundant insecticide application and only one or two insecticides were used before collection. The LC_{50} values of the tested insecticides revealed that emamectin benzoate was significantly more toxic than other tested insecticides. These results are in conformity with Kao and Cheng et al. (2001) found emamectin the most effective against susceptible and resistant populations of *P. xylostella*. ג-cyhalothrin was four times less toxic than emamectin benzoate. However, ג-cyhalothrin was more toxic than deltamethrin, bifenthrin and diafenthiuron whereas acetamiprid and imidacloprid were the least effective insecticides. An unselected population of *P. xylostella* collected from Cameron showed high susceptibility to ג-cyhalothrin and deltamethrin (Sayyed and Crickmore, 2007). Insecticide resistance in *P. xylostella* is widespread in South East Asian countries due to dispersal ability of its moths, multiple generations in a year and frequent insecticide application for its management in field (Mangagro and Edelson, 1990).

Different insecticides in managing cabbage pests previously revealed that pyrethroids consistently provided effective control against lepidopteran insect pests (Andaloro et al., 1993). It may develop either by genetic selection, direct exposure to insecticides or cross-resistance resulting from selection by other insecticides belonging to different groups (Oppenoorth, 1985). Branco and Gatehouse (1997) tested the level of resistance of deltamethrin, cartap and methamidophos against three populations of DBM in laboratory indicating 4-47 fold resistance to deltamethrin and 2-9 fold to methamidophos and no

resistance to cartap when compared with susceptible strain. *P. xylostella*, in Florida, collected from cabbage showed high resistance to pyrethroids i.e., ג-cyhalothrin, permethrin and cypermethrin (Yu and Nguyen, 1992).

Schuler et al. (1998) found that resistant strain of *P. xylostella* selected with fenvalerate was strongly resistant to range of pyrethroids. According to their results high resistance ratios were obtained with deltamethrin and fenvalerate. Dhumale et al. (2009) reported that increased resistance ratio was observed in strains of *P. xylostella* which are under constant selection pressure in consecutive generations in respect of deltamethrin, fenvalerate and cypermethrin. Cross resistance of *P. xylostella* selected with phenthoate against various insecticides showed high resistance to chlorpyrifos and parathion whereas moderate resistance was shown to organophosphates and minimum level of cross resistance to most neonicotinoids, chlorpyrifos, pyrethroids and carbamates (Park et al., 2004). A field population of *P. xylostella* collected from Pakistan showed high resistance to deltamethrin but no or little resistance to spinosad, indoxacarb, abamectin and fipronil (Sayyed et al., 2005). They further reported that delta-selected strain showed no cross-resistance to fipronil, spinosad and indoxacarb (Sayyed et al., 2005).

A program of rotating selected insecticides for controlling resistant DBM has been practiced in several countries and gained successful results which proved that some insecticides are useful in rotation to lower down the resistance problems (Cheng et al., 1996). In the present cross-resistance study, the delta-selected strain showed moderate resistance to emamectin benzoate and ג-cyhalothrin. Whereas, no or very little cross-resistance to bifenthrin. High rate of resistance against deltamethrin in areas where pyrethroids were used at heavy doses against P. xylostella in India was observed previously (Saxena et al., 1989). The ema-selected strain showed moderate resistance to ג-cyhalothrin and deltamethrin, however, no or very low cross-resistance to bifenthrin. Change in resistance in different organophosphates and pyrethroids is suggested to be due to multiple resistance mechanisms with comparatively higher toxicity of new chemistry insecticides than pyrethroids against *P. xylostella* (Khaliq et al., 2007). Presence of cross resistance in similar geographic population of *P. xylostella* shows development of resistance in new chemistry insecticides which might be due to this multiple resistance mechanisms selection. High use of new chemistry insecticides such as emamectin might influence possible cross resistance, however, structural and type of pyrethroid may have variation in resistance which showed variable toxicity levels for different pyrethroids (Huang and Wu, 2003; Sayyed et al., 2005; Khaliq et al., 2007).

Present study indicated presence of cross resistance among all tested insecticides. High level of cross-resistance was observed among emamectin benzoate, deltamethrin and ג-cyhalothrin suggesting that these should not be used in rotation. Bifenthrin, on the other hand, showed very low cross resistance and can be used as alternate to deltamethrin or ג-cyhalothrin. The rotational use of insecticides with different mode of actions like use of organophosphates, carbamates may also be other alternates in avoiding development of field resistant *P. xylostella* populations.

Table 1. Toxicity of pyrethroids and new chemistry insecticides against second instar larvae of *Plutella xylostella* using leaf-dip bioassay under controlled laboratory conditions

Insecticide	Time (hr)	LC_{50}	FL at 95%	LC_{90}	FL at 95%	Slope±SE	CR
Deltamethrin	48	9.34	5.55-16.5	187.5	70.6-1536	0.9±0.19	25.2
	72	5.29	3.19-8.05	67.3	34.3-238	1.16±0.2	18.9
ג-cyhalothrin	48	11.1	6.3-22.4	312	95.4-5158	0.9±0.19	30
	72	4.29	2.49-6.52	48.7	26.5-147	1.2±0.2	15.3
Bifenthrin	48	13.9	6.8-76.2	3780	312-754	0.5±0.16	36.6
	72	5.2	2.54-10.2	588	113-8015	0.6±0.16	18.6
Emamectin	48	0.37	0.23-0.59	6.08	2.78-28.1	1.05±0.19	1
	72	0.28	0.17-0.44	4.29	2.11-16.4	1.08±0.19	1
Acetamiprid	48	24.7	12.1-151	3031	332-3932	0.6±0.17	66.8
	72	13.4	7.06-49.9	1986	241-2191	0.6±0.17	47.9
Diafenthiuron	48	25.5	10.2-249	3157	293-5961	0.6±0.19	68.9
	72	10.2	4.47-30.7	931	149-4221	0.6±0.18	36.4
Imidacloprid	48	27.9	12.3-203	2532	289-5511	0.7±0.19	75.4
	72	10.3	4.13-39.7	1618	187-7568	0.6±0.18	36.8

LC = lethal concentration levels at 50 and 90 percent
FL = fiducial limits; SE = standard error; CR = comparative ratio

Table 2: Increase in tolerance to insecticides selection for *Plutella xylostella* in selected insecticides after five generation toxicity stress

Insecticide	Population	LC_{50}	CR
deltamethrin	Unsel	9.34	25.2
	Del-Sel	22.1	43.3
	Emma-Sel	17.6	14.7
ג-cyhalothrin	Unsel	11.1	30.0
	Del-Sel	15.9	31.2
	Emma-Sel	16.8	14.0
Bifenthrin	Unsel	13.9	37.6
	Del-Sel	16.4	32.2
	Emma-Sel	17.4	14.5
Emamectin	Unsel	0.37	1.00
	Del-Sel	0.51	1.00
	Emma-Sel	1.20	1.00

LC = lethal concentration levels at 50 and 90 percent
FL = fiducial limits; SE = standard error; CR = comparative ratio
Unsel = unselected; Del-Sel = deltamethrin selected strain; Ema-Sel = emamectin selected strain

Table 3: Rate of selection of deltamethrin and emamectin to Plutella xylostella for five generations under laboratory condition

Insecticide	Generation	Concentration level	% mortality	% survival
Deltamethrin	1	LC_{50}	49	51
	2	LC_{60}	55.3	44.7
	3	LC_{70}	59.5	40.5
	4	LC_{80}	65.2	34.8
	5	LC_{90}	74.8	25.2
Emamectin	1	LC_{50}	49.7	50.3
	2	LC_{60}	56.7	43.3
	3	LC_{70}	64.5	35.5
	4	LC_{80}	66.7	33.3
	5	LC_{90}	76.5	23.5

Table 4: Pair wise comparison of LC_{50}s of insecticides tested for delta-selected and ema-selected populations of *Plutella xylostella*

Delta-Sel Strain	ƛ-Cyhalothrin	Bifenthrin	Deltamethrin
bifenthrin	0.98		
deltamethrin	0.89	0.77	
emamectin	0.97	0.89	0.98
Ema-Sel Strain			
bifenthrin	0.97		
deltamethrin	0.98	0.90	
emamectin	0.71	0.54	0.82

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