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RELATIVE TOXICITY OF DIFFERENT INSECTICIDES AGAINST LARVAE OF *PLUTELLA XYLOSTELLA* (LINNAEUS) IN PUNJAB

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ABSTRACT : *Plutella xylostella* (Linnaeus) populations were collected from different regions of Punjab *i.e.* Amritsar, Kapurthala, and Ludhiana. Toxicity of some commonly used insecticides i.e. spinosad 48 SC, quinalphos 25 EC, fenvalerate 20 EC, flubendamide 480 SC, chlorantraniliprole 18.5 SL against third instar larvae of *P. xylostella* was determined by using the standard 'Leaf-disc dip' method of bioassay. LC_{50} values for these test-insecticides for Amritsar population were 0.00541, 3.66899, 4.12462, 0.00062 and 0.00037 per cent, respectively. For Kapurthala population LC_{50} values were 0.00514, 3.48554, 3.94417, 0.00054 and 0.000321 per cent, respectively. The LC_{50} values for Ludhiana populations against third instar larvae of *P. xylostella* were 0.00486, 3.30209, 3.76072, 0.00050 and 0.000275 per cent, respectively. Based on these LC_{50} values, the order of toxicity of the insecticides against *P. xylostella* was found to be chlorantraniliprole > flubendiamide > spinosad > quinalphos > fenvalerate for all the three populations. Perusal of data revealed that chlorantraniliprole was found to be the most toxic to all the three populations of *P. xylostella* with LC_{50} values ranging from 0.000275 per cent for Ludhiana, 0.000321 per cent for Kapurthala and 0.00037 per cent for Amritsar. The maximum level of resistance in *P. xylostella* was observed for fenvalerate followed by quinalphos, spinosad and flubendiamide. This assessment of changing insecticide resistance is essential for its management.

Key words : Insecticide-resistance, *Plutella xylostella,* toxicity.

INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera : Plutellidae) is considered the most destructive and one of the most important pests of cole crops in Punjab, India and rest of the world where it has posed very serious threat to the crops causing over US $ 4-5 billion worth damage globally per annum and has become very challenging to control (Talekar and Shelton, 1993; Zalucki *et al*, 2012). It became the first crop pest in the world to develop resistance to and ranks among the top 20 most resistant insect species reported so far (Mota-Sanchez *et al,* 2002). This exceptional pest status is due to the diversity and abundance of host plants, lack or disruption of its natural enemies, short generation time, its high reproductive potential, up to 20 generations per year and its proven ability to rapidly evolve resistance to insecticides (Shelton, 2004). Presently, it is known to have developed resistance to more than 46 insecticides from all groups including new generation insecticides such as neonicotinoids, avermectins, macrocyclic lactones, IGRs and Bt formulations etc. (Singh *et al*, 2005; Oliveira *et al*, 2011). It also has the distinction of being the first insect to develop resistance in the field to *Bt* (Tabashnik *et al*, 1987). *P. xylostella* is one of the most economically important insect pests throughout the world distributed in areas of different climatic conditions including tropical, subtropical and temperate zones and has the ability to migrate among different climatic zones (Chapman *et al*, 2002). Outbreaks of DBM in South-East Asia sometimes cause more than 90% crop loss (Verkerk and Wright, 1996). In India, it was first reported in 1914 (Fletcher, 1914) and is most destructive pest of national importance on cabbage causing 50-80% annual loss in marketable yield (Ayalew, 2006) and annual losses of about US $ 16.0 million (Mohan and Gujar, 2003).

In India, DBM is the most devastating pest of cole crops in states of Punjab, Haryana, Himachal Pradesh, Delhi, Uttar Pradesh, Bihar, Andhra Pradesh, Tamil Nadu, Maharashtra and Karnatka (Uthamasamy *et al*, 2010). As the diamondback moth is a weak flier, it acquires local importance depending upon cropping systems and agroecological conditions (Mohan and Gujar, 2002). DBM has posed a serious threat to the cultivation of cauliflower and cabbage, especially early and late season crops in Punjab. Consequently farmers intensively spray cabbage and cauliflower with insecticides either singly or in mixtures throughout the growing season of the crop (Chandi *et al*, 2011). This practice may lead to worsening the problems of insecticide resistance by increased selection pressure on the pest. This study aimed to assess the current status of insecticide resistance to five insecticides including new chemistry in DBM populations.

MATERIAL AND METHODS

Nearly a month old seedlings of cauliflower (*Brassica oleracea* var. *botrytis*) were transplanted in the field at regular intervals so as to ensure continuous supply of food for the test-insect. Any insect pest infestation on the plants was checked manually without using any insecticide. Leaves of only these plants were used for rearing of *P. xylostella* and for experimentation. The larvae of *P. xylostella* were collected from the fields of cabbage and cauliflower from Amritsar, Kapurthala and Ludhiana districts of Punjab State. Culture of *P. xylostella* was maintained on the cauliflower leaves kept in glass jars (10 × 15 cm) placed in an incubator at 27^0 C and 65 per cent relative humidity (RH) in Insect Physiology Laboratory of Department of Entomology, Punjab Agricultural University and Ludhiana. Each jar was covered with a piece of *dasuti* cloth and fastened with rubber bands around its rim. Food was changed daily till the onset of pupation. The leaf portions bearing pupae were transferred into other glass jars and those remaining attached to the walls of the jars were allowed to emerge as adults as such. The emerging adults were sexed on the basis of the differences in the brightness of diamond patterns on their forewings. The males are dark brown in colour with a pattern of three consecutive white diamond shaped spots on its back. The female moth is tan coloured and its diamond patterns are less distinct as compared to the males. Moreover, tip of abdomen was pointed in females, whereas it was slanting in males. Also, size of the females was bigger than that of the males. The emerging adults were sexed and transferred into new jars for mating and oviposition on the same day. A piece of cauliflower leaf placed in each jar acted as stimulant for egg laying. A cotton swab dipped in 10 per cent honey solution was hung from top of the *dasuti* cloth covering the mouth of the jar which provided food to the adults. The leaf with eggs laid upon was removed daily and replaced with a new one to facilitate further oviposition.

The standard 'Leaf-disc dip' method of bioassay was employed to determine LC_{50} values of the test-insecticides against third instar larvae of *P. xylostella.* Different concentrations of the test-insecticides i.e Spinosad 48 SC, Quinalphos 25 EC, Fenvalerate 20 EC, Flubendiamide 480 SC, Chlorantraniliprole 18.5 SL were prepared by serial dilutions with distilled water. Leaf-discs (4.8 cm diameter) were cut from the centre of the middle leaves of cauliflower. Each disc was dipped in a concentration of the insecticide for 20 seconds and then allowed to dry at room temperature for about one hour by hanging it with the help of a clip. The leaf-discs were then shifted to plastic Petri plates (5 cm diameter). Ten test-larvae (third instar larvae) were then transferred to each Petri plate containing a treated leaf-disc. Preliminary bioassay was carried to determine the range of toxic levels of the test-insecticides. Based on this, at least six serial dilutions were prepared to work out LC_{50} with three replications. The larvae were allowed to feed on treated leaf-discs for 48 hours. Experiment was conducted with three replications and ten larvae per replication. The observations for mortality were recorded after 24 and 48 hours of exposure of larvae to the treated leaf discs. The log concentration-mortality regression was worked out by log probit technique (Finney, 1971) employing the computer programme POLO (Robertson *et al*, 1980).

RESULTS AND DISCUSSION

The LC_{50} values for test-insecticides (Spinosad 48 SC, Quinalphos 25 EC, Fenvalerate 20 EC, Flubendiamide 480 SC, Chlorantraniliprole 18.5 SL) against third instar larvae of *P. xylostella* have been worked out and the values for these insecticides for Amritsar population were 0.00541, 3.66899, 4.12462, 0.00062 and 0.00037 per cent, respectively. For Kapurthala population LC_{50} values were 0.00514, 3.48554, 3.94417, 0.00054 and 0.000321 per cent, respectively (table 2). The LC_{50} values for Ludhiana populations against third instar larvae of *P. xylostella* were 0.00486, 3.30209, 3.76072, 0.00050 and 0.000275 per cent, respectively (table 3). Based on these LC_{50} values, the order of toxicity of the insecticides against *P. xylostella* was found to be Chlorantraniliprole > Flubendiamide > Spinosad > Quinalphos > Fenvalerate for all the three populations. It is clear from the data that Chlorantraniliprole was found to be the most toxic to all the three populations of *P. xylostella* with LC_{50} values ranging from 0.000275 per cent for Ludhiana, 0.000321 per cent for Kapurthala and 0.00037 per cent for Amritsar. The maximum level of resistance in *P. xylostella* was observed for Fenvalerate followed by Quinalphos, Spinosad and Flubendiamide.

The inferences of the present study, showing resistance by *P. xylostella* to the test-insecticides, are endorsed by the earlier findings where varying levels of resistance to these insecticides were reported. Joia and Udeaan (2001), Rafiq (2005) and Kang and Dhaliwal (2008) reported resistance ratio in *P. xylostella* ranging between 10.5 to 53.7 folds for different populations/ locations (Nirmal and Singh, 2004; Kang and Dhaliwal, 2008). Similarly, relative toxicity of quinalphos to *P. xylostella* also varied as reported by different workers. In a study conducted to monitor the insecticide resistance status in *P. xylostella,* Joia and Udeaan (1998) registered 40-128 fold resistance from different locations in Punjab and later in the year 2005, an increase in the resistance

Table 1 : Toxicity of insecticides against third instar larvae of *Plutella xyllostella* in Amritsar.

Insecticide	LC_{50}(%)	Fiducial limit		Slope
		Lower limit	Upper limit	
Spinosad	0.00541	0.00344	0.00846	2.392±0.583
Quinalphos	3.66899	2.32895	5.73508	2.392±0.583
Fenvalerate	4.12762	2.62007	6.4519	2.392±0.583
Flubendiamide	0.00062	0.00034	0.00103	1.944±0.529
Chlorantraniliprole	0.00037	0.00023	0.00057	2.392±0.583

Table 2 : Toxicity of insecticides against third instar larvae of *Plutella xyllostella* in Kapurthala.

Insecticide	LC_{50}(%)	Fiducial limit		Slope
		Lower limit	Upper limit	
Spinosad	0.00514	0.00326	0.00803	2.392±0.583
Quinalphos	3.48554	2.21250	5.44833	2.392±0.583
Fenvalerate	3.94417	2.50302	6.16521	2.392±0.583
Flubendiamide	0.00054	0.00030	0.00090	1.944±0.529
Chlorantraniliprole	0.000321	0.00204	0.00502	2.392±0.583

Table 3. Toxicity of insecticides against third instar larvae of *Plutella xyllostella* in Ludhiana

Insecticide	LC_{50}(%)	Fiducial limit		Slope
		Lower limit	Upper limit	
Spinosad	0.00486	0.00309	0.00760	2.392±0.583
Quinalphos	3.30209	2.09605	5.16157	2.392±0.583
Fenvalerate	3.76072	2.38717	5.87846	2.392±0.583
Flubendiamide	0.00050	0.00028	0.00084	1.944±0.529
Chlorantraniliprole	0.000275	0.00175	0.00430	2.392±0.583

level to 218 fold was registered (Joia *et al*, 2005). Gujar and Sohal (2010) observed Amritsar population of *P. xylostella* to be more tolerent to quinalphos (1.56 times) in comparison to Ludhiana population. Earlier, more than 39 fold resistance to quinalphos in *P. xylostella* was also reported from Taiwan (Cheng, 1986).

The maximum level of relative resistance ratio (515.86 fold) w.r.t. fenvalerate, recorded in the study is in line with the results of various previous studies. Chawla and Joia (1991) and Joia *et al*, 2005) registered 22 to > 2700 fold resistance in *P*. *xylostella* to fenvalerate from different populations and locations in Punjab. Nirmal and Singh (2004), Kang and Dhaliwal (2008) observed the resistance to fenvalerate from 245 to 4576 fold. Given to the trend of development of resistance, close monitoring of its toxicity status is needed since very high level of resistance to it in *P. xylostella* have been reported world wide. Arora *et al* (2003) reported LC_{50} value of 0.00033 % for spinosad indicating its higher toxicity to *P. xylostella*. Also, Singh *et al* (2012) worked out various toxicity values of spinosad for second instar larvae of *P. xylostella.* The LC_{30}, LC_{50} and LC_{70} values computed were 0.00010, 0.00057 and 0.00327 % respectively. Ali *et al* (2004) and Zhao *et al* (2006) also observed the spinosad resistance in multi-resistant field selected population of *P. xylostella* and reported 12 to > 6422 fold resistance.

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