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Impact of Different Insecticides on Locomotory Behaviour of *Plutella xylostella* (Linnaeus)

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A B S T R A C T

Locomotory behaviour of different populations of Punjab collected from three districts (Amritsar, Kapurthala and Ludhiana) was studied with the help of Ethovision. Larvae from different populations travelled more distance when allowed to walk on fenvalerate-treated (496.556 cm) surface for five minutes followed by quinalphos-treated (383.889 cm), spinosad-treated (331.111 cm), flubendiamide-treated (260.222 cm) and chlorantraniliprole-treated (203.556 cm) surfaces. The trend was observed to be same for the speed and opposite for the turn angle. The maximum distance on fenvalrate-treated surface travelled was recorded for the larval populations collected from Ludhiana district (661.33 cm/5 min) followed by Kapurthala district (470.00 cm/5 min) and Amritsar district (358.33cm/5 min). The minimum distance travelled was recorded on the untreated wax-coated paper (78.333 cm). Consequently, speed of larval populations for fenvalrate was also more for Ludhiana district (2.204 cm/s) as compared to those taken from fields of Kapurthala district (1.566 cm/s) followed by Amritsar district (1.194 cm/s). The turn angle of larval population from Amritsar district (264.33^0) was significantly higher as compared to those from Kapurthala (104.33^0) followed by Ludhiana district (35.33^0). Hence, certain pronounced behavioural differences were registered in locomotion of different populations of Punjab and this knowledge would help to find management solutions to *Plutella xylostella*.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) is ubiquitous and poses pernicious effect on the cole crops in Punjab, India and rest of the world causing over US $ 1 billion worth damage globally per annum and has become very challenging to control (Talekar and Shelton 1993; Khaliq *et al.*, 2007). In Southeast Asia, more than 90% crop losses are the result of damage by the *P. xylostella* (Verkerk and Wright, 1996). It was first reported in 1914 that 50-80 % annual loss in marketable yield and annual losses of about US $ 16.0 million was caused by this pest in India (Mohan and Gujar, 2003). This exceptional pest status is due to the universal distribution and abundance of host plants, lack or disruption of its natural enemies, its high reproductive potential up to 20 generations per year, the high selection pressure with insecticides and its proven ability to rapidly evolve resistance to insecticides (Shelton 2004, Zhou *et al.*, 2011). The mechanism of insecticide resistance to insects can be divided into three broad categories. The first category is biochemical mechanisms which include alteration in insecticide detoxification and sensitivity to target sites. The second category is physiological mechanisms, which include alteration in insecticide penetration, transport, storage and excretion. The third category is behavioural mechanisms. To manage insecticide resistance in any insect, comprehensive knowledge of its behavioural physiology and biochemistry is of paramount importance. Insects have evolved a variety of physiological and behavioural responses to various toxins in natural and managed ecosystems (Jallow and Hoy 2005, Li *et al.*, 2007). Some insects are capable of changing their behaviour in response to their sensory perception of insecticides (Desneux *et al.*, 2007). In the past, though much work has been done on the biochemical aspects of resistance but very less attention has been paid to understand the role of behaviour especially locomotory behaviour in the development of resistance in a target species. In fact, it plays a crucial role in resistance development. Role of behavioural response in the evolution of insecticide resistance deserves great attention. Mechanism of behavioural resistance is the least studied and the least understood in pest management though its role in the insecticide resistance may be as important as of biochemical mechanisms. Most bioassays are designed to find differences in survival that depend on the insect's physical ability to tolerate more of the toxicant. Conversely, behavioural mechanisms are usually undetectable with most bioassays used to monitor resistance. The role of behaviour of an insect is crucial to understanding of insect adaptation to toxins especially the locomotory behaviour. In fact, this understanding is important in preventing development of insecticide resistance in pest populations. There also exists a knowledge gap as regards comparative study of locomotory behaviour in different populations of *P. xylostella.* This knowledge may help to find management solution to the diamondback moth. All this has necessitated the need of a comprehensive study of locomotory behavioural physiology of *P. xylostella* to determine variations between different populations of *P. xylostella* as regards various parameters of locomotion.

The current study was designed to investigate effects of different insecticides on locomotory behavior of insect pest *P. xyllostella* that were collected from Amritsar, Kapurthala and Ludhiana districts of Punjab.

Materials and Methods

Rearing of the test-insect

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° C and 65 per cent relative humidity (RH) in Insect Physiology Laboratory of Department of Entomology, Punjab Agricultural University, 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 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.

Test- insecticides

The following insecticides were used in various experiments:

Spinosad (48 SC)
Quinalphos (Ekalux 25 EC)
Fenvalerate (Sumicidin 20 EC)
Flubendiamide (480 SC)
Chlorantraniliprole (18.5 SL)

Locomotory behaviour of the larvae of *Plutella xylostella*

Locomotor behavior is a parameter which is crucial for food seeking, avoiding predators, migration and reproduction. Locomotor activity of *P. xylostella* was quantifiable with the aid of modern video tracking system *i.e.* Ethovision which enable researchers to study behaviour in a reliable and consistent way and over longer time periods than if they were using manual recording. Ethovision is an integrated system comprising of various software and hardware components which first detects an object (larva) and then transforms into a series of parameters quantifying the behaviour of the object. In this system, video camera observes the movement of an object and passes images of the object to the computer.

The larvae from different populations were allowed to move on different concentrations of insecticide-treated and untreated surfaces. Larval movements for these insects were recorded on treated and untreated surfaces. Test-insecticides (conc.= LC_{50}) were applied uniformly with help of an atomizer and allowed to dry for three hours and then a single third instar larva was released on insecticide-treated and untreated surfaces in a Petri dish (10 cm diameter). Larval movements for all the populations were recorded on treated and untreated surfaces and its behaviour was videotaped for 5 minutes. The experiment was replicated thrice for all the populations. Observations were recorded for distance travelled by the test-larvae per unit time and path shape *i.e.* distance travelled, speed, turn angle.

Statistical analysis

The data were subjected to analysis by Completely Randomised Design (CRD) and factorial design described by Gomez and Gomez (1984) using the statistical software package (CPCS1).

Results and Discussion

Larvae of *P. xylostella* from the fields of Ludhiana district travelled more distance on insecticide-treated or untreated surface and consequently their speed was also more as compared to those taken from fields of Kapurthala district followed by Amritsar district. The composite mean values of distance travelled by the larvae from Ludhiana, Kapurthala and Amritsar district in 5 minutes were 413.111, 263.278 and 200.444 cm, respectively which differed highly significantly and so was their speed *i.e.* 0.674, 0.901 and 1.450 cm/s, respectively. The distance travelled of the larvae from Ludhiana district was maximum when allowed to walk on fenvalerate-treated surface (661.333 cm), followed by those moved on quinalphos -treated (521.000 cm), spinosad-treated (470.667 cm), flubendiamide-treated (406.667

cm) and chlorantraniliprole-treated surfaces (343.333 cm) in 5 minutes, all the values were significantly different from each other. The minimum travelled-distance was recorded for the larvae which were allowed to walk on the untreated wax-coated paper *i.e.* 75.667 cm in 5 minutes. The distance travelled by the larvae from Kapurthala district was maximum when allowed to walk on fenvalerate-treated surface (470.000 cm), followed by those moved on quinalphos -treated (363.667 cm), spinosad-treated (304.000 cm), flubendiamide-treated (205.000 cm) and chlorantraniliprole-treated surfaces (153.667 cm) in 5 minutes, all the values were significantly different from each other. The minimum distance travelled was recorded for the larvae which were allowed to walk on the untreated wax-coated paper *i.e.* 83.333 cm in 5 minutes. Similar trend was also observed for the larval populations from Amritsar district where distance travelled recorded for larvae which moved on fenvalerate-treated surface was (358.333 cm), followed by those moved on quinalphos -treated (267.000 cm), spinosad-treated (218.667 cm), flubendiamide-treated (169.000 cm) and chlorantraniliprole-treated surfaces (113.667 cm) in 5 minutes, all the values were significantly different from each other. The minimum distance travelled was recorded for the larvae which were allowed to walk on the untreated wax-coated paper *i.e.* 76.000 cm in 5 minutes (Table 1, Figure 1).

Similarly, the speed of the larval population from Ludhiana district was maximum when allowed to walk on fenvalerate-treated surface (2.204 cm/s), followed by those moved on quainalphos-treated (1.736 cm/s), spinosad-treated (1.568 cm/s), flubendiamide-treated (1.355 cm/s) and chlorantraniliprole-treated surfaces (1.144 cm/s) all the values were significantly differed from each other. The minimum distance travelled was recorded for the larvae which were allowed to walk on the untreated wax-coated paper *i.e.* 0.694 cm/s. The speed of the larval population from Kapurthala district was maximum when allowed to walk on fenvalerate-treated surface (1.566 cm/s), followed by those moved on quainalphos-treated (1.212 cm/s), spinosad-treated (1.013 cm/s), flubendiamide-treated (0.683 cm/s) and chlorantraniliprole-treated surfaces (0.512 cm/s) all the values were significantly differed from each other. The minimum distance travelled was recorded for the larvae which were allowed to walk on the untreated wax-coated paper *i.e.* 0.420 cm/s. Similar trend was also observed for the larval populations from Amritsar district where speed for larvae which moved on fenvalerate-treated surface (1.194 cm/s), followed by those moved on quainalphos-treated (0.890 cm/s), spinosad-treated (0.728 cm/s), flubendiamide-treated (0.563 cm/s) and chlorantraniliprole-treated surfaces (0.378 cm/s) all the values were significantly differed from each other. The minimum distance travelled was recorded for the larvae which were allowed to walk on the untreated wax-coated paper *i.e.* 0.288 cm/s (Table 2, Figure 1).

Turn angle has also been studied for all the populations of *P. xylostella*. The turn angle of larval population from Amritsar district was significantly higher as compared to those from Kapurthala followed by Ludhiana district. The composite mean values of turn angle of larvae from Ludhiana, Kapurthala and Amritsar district were 77.389˚, 140.222˚ and 256.944˚ respectively which differed significantly. The turn angle of larval population from Ludhiana district was maximum when allowed to walk on chlorantraniliprole-treated surface (111.000˚), followed by those moved on flubendiamide - treated (104.667˚), spinosad-treated (94.000˚), quinalphos-treated (88.000˚) and fenvalerate-treated surfaces (35.333˚) all the values were significantly differed from each other.

Table.1 Distance travelled by third instar larvae of *P. xylostella* on insecticide-treated wax paper

Treatment	**Mean* Distance travelled (cm) in 5 minutes**			
	Amritsar	**Kapurthala**	**Ludhiana**	**Composite mean**
Chlorantraniliprole	113.667	153.667	343.333	203.556
Flubendiamide	169.000	205.000	406.667	260.222
Spinosad	218.667	304.000	470.667	331.111
Quinalphos	267.000	363.667	521.000	383.889
Fenvalrate	358.333	470.000	661.333	496.556
Untreated	76.000	83.333	75.667	78.333
Composite mean	200.444	263.278	413.111	
C.D (p=0.05) Population (P)= 2.246 Insecticide (I)= 3.176 Interaction (I×P)= 5.501				

*Based on three replications, each comprising a single larva

Table.2 Speed of third instar larvae of *P. xylostella* on insecticide-treated wax paper

Treatment	**Mean* Speed (cm/s)**			
	Amritsar	**Kapurthala**	**Ludhiana**	**Composite mean**
Chlorantraniliprole	0.378	0.512	1.144	0.678
Flubendiamide	0.563	0.683	1.355	0.867
Spinosad	0.728	1.013	1.568	1.103
Quinalphos	0.890	1.212	1.736	1.279
Fenvalrate	1.194	1.566	2.204	1.655
Untreated	0.288	0.420	0.694	0.467
Composite mean	0.674	0.901	1.450	
C.D (p=0.05) Population (P)= 0.082 Insecticide= 0.116 Interaction (I×P)= 0.200				

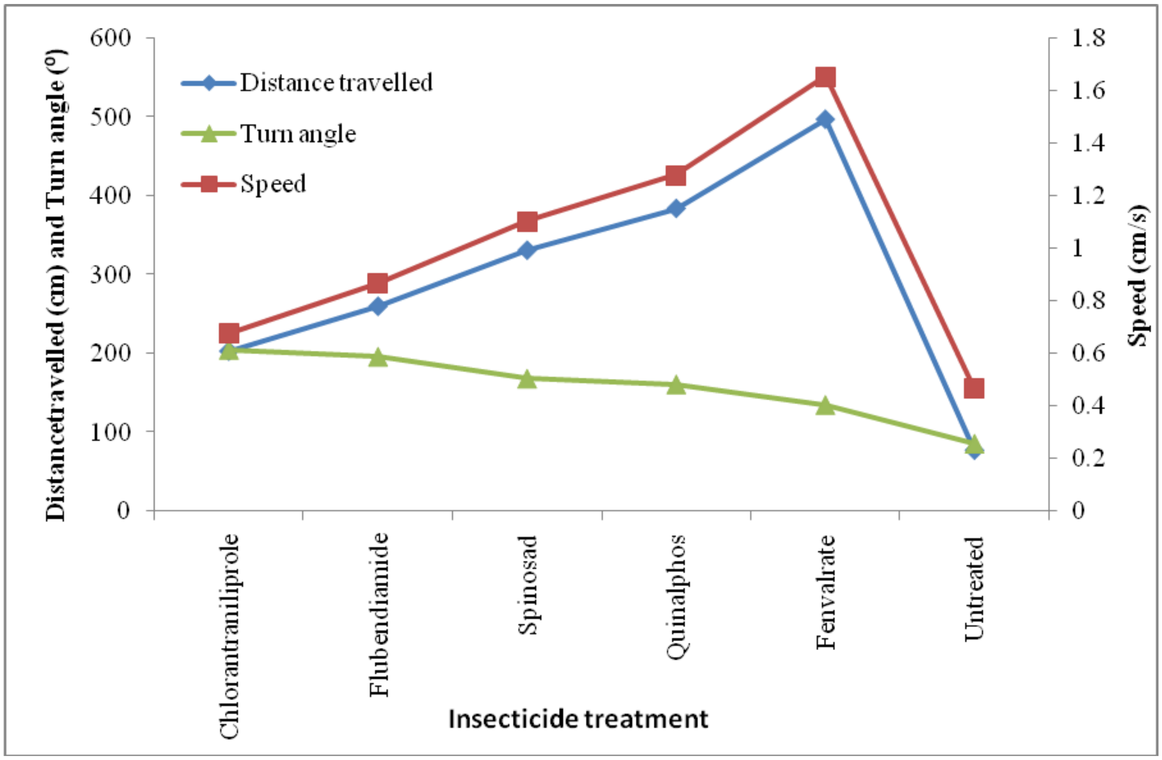
*Based on three replications, each comprising a single larva

Table.3 Turn Angle of third instar larvae of *P. xylostella* on insecticide-treated wax paper

Treatment	**Mean turn angle (°)**			
	Amritsar	**Kapurthala**	**Ludhiana**	**Composite mean**
Chlorantraniliprole	299.000	203.333	111.000	204.444
Flubendiamide	292.000	190.333	104.667	195.667
Spinosad	288.000	121.667	94.000	167.889
Quinalphos	275.667	118.667	88.000	160.778
Fenvalrate	264.333	104.333	35.333	134.667
Untreated	122.667	103.000	31.333	85.667
Composite mean	256.944	140.222	77.389	
C.D (p=0.05) Population (P) = 2.622 Insecticide (I) = 3.707 Interaction (I×P) = 6.421				

*Based on three replications, each comprising a single larva

Fig.1 Locomotory behaviour of *P. xylostella* on insecticide treated surface



The minimum turn angle was recorded for the larvae which were allowed to walk on the untreated wax-coated paper (31.333˚). The turn angle of larval population from Kapurthala district was also maximum when allowed to walk on chlorantraniliprole-treated surface (203.333˚), followed by those moved on flubendiamide -treated (190.333˚), spinosad -treated (121.667˚), quinalphos-treated (118.667˚) and fenvalerate-treated surfaces (104.333˚) all the values were significantly differed from each other. The minimum turn angle was recorded for the larvae which were allowed to walk on the untreated wax-coated paper (103.000˚). Similar trend was also observed for the larval populations from Amritsar district where turn angle for larvae which moved on chlorantraniliprole-treated surface (299.000˚), followed by those moved on flubendiamide -treated (292.000˚), spinosad -treated (288.000˚), quinalphos-treated (275.667˚) and fenvalerate-treated surfaces (264.333˚) all the values were significantly differed from each other. The minimum turn angle was recorded for the larvae which were allowed to walk on the untreated wax-coated paper 122.667˚ (Table 3 and Figure 1).

Inferences of present study are corroborated by earlier findings of Adams *et al.,* (1992) wherein they also observed that *P. xylostella* larvae from susceptible population travelled more distance on permethrin-treated surface than that by resistant ones. It is hypothesized that this type of variation between the two populations might reflect difference in the insect's sensory perception of insecticides. Charlotte *et al.,* (1997) also observed the change in locomotor behavior of ground beetle, *Pterostichus cupreus* (Linnaeus) on application of dimethoate. The proportion of time spent in locomotor activity and average walking velocity was reduced, hence increase in turning and less distance covered in both sexes as compared to untreated beetles. This reduction in locomotor activity on the application of dimethoate was attended by a change in the pattern of movement. Thus, both male and female beetles responded with an increased turning rate (*p*, 0.01) and in the number of stops per distance covered (*p*, 0.01), resulting in a less continuous pattern of movement. Pereira *et al.*, (2009) also investigated that mobility parameters of the maize weevil, *Sitophilus zeamais* Motsch from different populations varied on the insecticide-treated surfaces. They indicated that inter-population variation might reflect differences in insects' sensory perception of insecticides and could lead to development of behavioural resistance if such differences are inheritable. Earlier, Brett and Ross (1985, 1986) too found that susceptible male german cockroach, *Blatella germanica* (L.) moved significantly faster than did resistant *B. germanica* in presence of propoxur. The observations of Head *et al.*, (1995 a,b) that speed of the larvae of *P. xylostella* was higher on the insecticide-treated surface than the untreated one, is also in line with the results of the present study where significantly more speed of the larvae (both from the susceptible and resistant population of *P. xylostella*) was recorded on the insecticide-treated surface in case of all the four test-insecticides *i.e.* endosulfan, quinalphos, fenvalerate and spinosad. The increased turn angle recorded for the resistant larvae in the study might be the result of avoidance behaviour. Head *et al.*, (1995) also noted such type of behaviour of *P. xylostella* larva in the form of increased turnings when insecticide was present.

Locomotory behaviour of different populations of *P. xylostella* from different regions of Punjab was studied and certain pronounced behavioural differences were registered in locomotion. It was observed that populations which were under high insecticide pressure (Amritsar population) travelled less distance with more turn angle in order to avoid insecticide than the populations from less insecticide pressure (Ludhiana population) which moved faster with less value of turn angle. So locomotory behavioural variations observed in different populations of Punjab could be helpful in its management solutions.

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