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Effect of insecticides on locomotory behaviour and enzyme activity of *Spodoptera litura* (Fabricius) populations from Punjab

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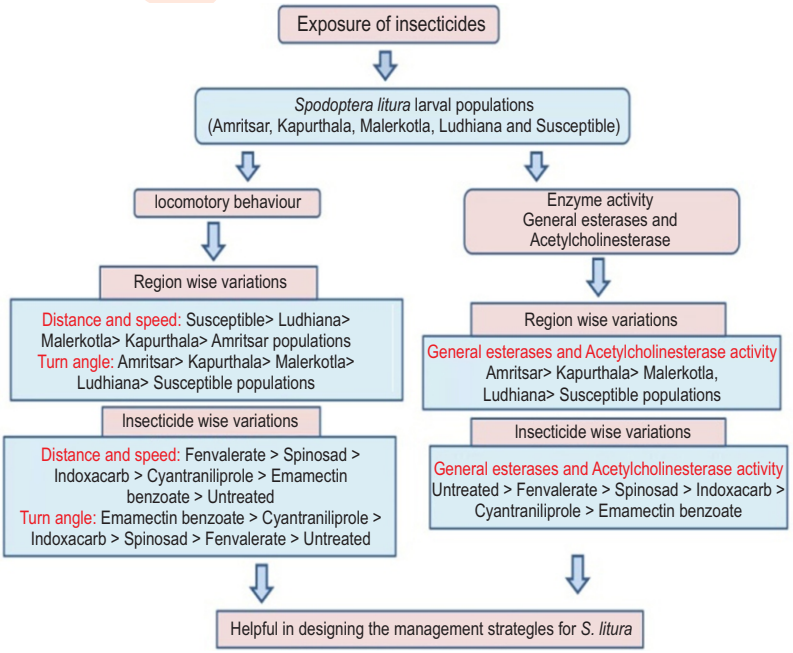
Abstract

Aim: The variations in locomotory behaviour and enzyme activity, *i.e.,* acetylcholinesterase and general esterases of *Spodoptera litura* Fabricius larval populations collected from different regions of Punjab, *i.e.,* Amritsar, Kapurthala and Ludhiana were investigated in the laboratory prior exposure to different insecticides.

Methodology: The test larvae were exposed to sublethal doses of different insecticides via surface and food treatment. For locomotory behaviour, they were allowed to move on treated surface and their movements were recorded with an automated video tracking system, Ethovision while on other hand food treatment was opted for observing the influence on enzyme activity.

Results: The pronounced variations were observed in the locomotor behaviour of *S. litura* upon surface exposure of different insecticides. The larva travelled more distance with higher speed and larger turn angle on insecticide-treated surfaces than the untreated ones, while on comparison of different larval populations, the Amritsar population larvae travelled less distance with low speed and maximum turnings. The decrease in acetylcholinesterase and general esterase activity was recorded in treated larvae than untreated ones. The influence of emamectin benzoate was recorded more on locomotion and enzyme activity of *S. litura* as compared to the remaining insecticides and the Amritsar population was least affected.



Interpretation: The sublethal doses of insecticides influenced the overall behavioural physiology of *S. litura* and this biological parameter could be useful in designing the management strategies for *S. litura*.

Key words: Acetylcholinesterase, Esterases, Insecticides, Locomotory behaviour, *S. litura*

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Introduction

The tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is a polyphagous, globally important serious crop pest distributed widely from tropical to temperate regions, throughout the Middle East, East Asia, Pacific Islands and Oceania (Bhati, 2020; Chandi *et al.,* 2022). The wider host range of this pest can be considered as an important evolutionary strategy to ensure its survival in nature (Ullah *et al.,* 2016). Variations in the plant host species have been recorded by various authors that range from 120 to a maximum of 389 species (CABI, 2018; Shekhawat *et al.,* 2018). This includes crops like cotton, maize, sorghum, soybean, castor, groundnut, tomato, okra, potato, chilli, cucumber, pumpkin, cabbage, pigeon pea, green gram, tobacco, linseed and mustard, weeds and ornamental plants (Bhati, 2020; Sarwar *et al.,* 2021). Larvae of *S. litura* feed on various parts of a plant comprising leaves, flowers and fruits, causing leaf skeletalization in the early growth stage of the crop and severe defoliation in later stages overall leading to a reduction in the photosynthetic capacity of plants (Ullah *et al.,* 2016; Bhati, 2020). The development of insecticide resistance in this pest has led to sporadic outbreaks and severe crop damage and yield losses (Fu *et al.,* 2015; Wang *et al.,* 2018). Locomotory behaviour parameters are considered as important and sensitive biomarkers to evaluate the effects of sublethal doses of insecticides on insects (Tooming *et al.,* 2017).

Among various parameters of locomotion, the distance travelled and speed gives the best expression about the level of toxicity caused by insecticides towards an insect (Tooming *et al.,* 2014; Tooming *et al.,* 2017). The condition of physiological, anatomical, neurological and metabolic systems of an insect is often reflected through its locomotion. It also governs other important behaviours which are necessary for the survival and reproduction of an organism such as foraging, migration, predation, selection of habitat, behavioural thermoregulation, inter and intraspecific interactions (Augusiak *et al.,* 2016; Tooming *et al.,* 2017). The variations in locomotory behaviour of insects eventually disrupt the standard imperatives of behaviour and physiology, which are important for overall fitness and survival of an insect from individual to population level (Merivee *et al.,* 2015). The metabolic rates of any insect pest have a strong association with activities of various enzymes such as esterases and acetylcholinesterase. The insecticide resistance in insect pests is often related to change in activities of metabolic enzymes (Gong *et al.,* 2013). The studies on change in locomotory behaviour and enzymatic activities in *S. litura* prior exposure to insecticides are lacking with respect to different regions of Punjab. Thus, the present study was undertaken to study the effect of insecticides on locomotory behaviour and enzyme activity (acetylcholinesterase and esterase) in *S. litura*.

Materials and Methods

Test insect and its rearing: The populations of *S. litura* were collected from cauliflower/cabbage fields of different regions of Punjab, *i.e.,* Amritsar, Malerkotla, Kapurthala and Ludhiana and brought to laboratory in perforated polythene bags. The insects were reared on cauliflower leaves in a B.O.D incubator maintained at 26±1ºC and 65±5% relative humidity. To develop *S. litura* susceptible population, the rearing was done for twenty generations.

Test insecticides: The commercial formulations of emamectin benzoate 5 SG, spinosad 45 SC, indoxacarb 14.5 SC, cyantraniliprole 10.26 OD and fenvalerate 20 EC were purchased from local pesticide dealer. The basis of selection was according to package of practice for vegetables, Punjab Agricultural University, Ludhiana. The LC_{50} values of different insecticides were used as treatment against different larval populations. The difference in LC_{50} values (Table 1) of same insecticide for different region is due to variations in insecticide use pattern in different regions of Punjab (Chandi *et al.,* 2019).

Locomotory behaviour: The larvae from different populations (Amritsar, Malerkotla, Kapurthala and Ludhiana) were allowed to move on wax paper (modified after Head *et al.,* 1995 a; b). Test-insecticides (conc.= LC_{50}) was applied uniformly with help of an atomizer and allowed to dry for 3 hr and then a single third instar larva was released on treated wax paper contained in a Petri dish and then videotaped with computerized video tracking system, Ethovision (Noldus Information technology, Wageningen, The Netherlands). Observations were recorded for distance travelled by the test-larvae per unit time and path shape, *i.e.,* distance travelled, speed, turn angle.

Estimation of general esterases activity: The fourth instar larvae from different populations of *S. litura* were treated with LC_{50} dose of different insecticides. A known and weighed amount of insect whole larvae (fourth instar) from different *S. litura* populations were homogenized in pestle mortar in a pre-chilled 0.04 mM phosphate buffer (pH 6.8) containing 0.01 % Triton X-100 @ 500 µl buffer for one insect. The extract was centrifuged at 10,000 rpm for 20 min at 4º C. The supernatant thus obtained was used as enzyme extract for the determining the enzyme activity. The Van Asperen (1962) method was followed for estimation of enzyme activity.

Estimation of acetylcholinesterase activity: Fourth instar larvae treated with different insecticides (LC_{50}) were homogenized in 4-5 ml of 0.05 mM phosphate buffer, pH 7.2 containing 0.5 % Triton X-100 and 2 mM EDTA. The samples were then centrifuged at 10,000 rpm at 4º C for 20 min and the supernatant was used as an enzyme source. The enzyme activity was determined according to Ellman *et al.* (1961) method.

Statistical analyses: The data were subjected to analyses by Completely Randomized Design (CRD) using the statistical software package (CPCS1).

Results and Discussion

S. litura larva from susceptible populations travelled more distance on insecticide-treated and untreated surface

Table 1: LC_{50} values of various insecticides with respect to different *S. litura* populations of Punjab

Treatments	LC_{50} (%)				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Fenvalerate	0.0023150	0.0034530	0.003898	0.004228	0.005622
Spinosad	0.0012240	0.0015580	0.0019580	0.0021680	0.002914
Indoxacarb	0.0000599	0.0000738	0.0000838	0.0000967	0.000142
Cyantraniliprole	0.0000479	0.0000542	0.0000642	0.0000707	0.000113
Emamectin benzoate	0.0000086	0.0000091	0.0000115	0.0000126	0.000020

The LC_{50} concentrations of different insecticides were prepared by using normality equation *i.e.,* N1 V1 = N2 V2; Where, N1 - Concentration of commercial insecticide; V1 - Volume of insecticide; N2 – The required concentration of insecticide; V2 - Volume of solvent required

Table 2: Effect of insecticides on distance travelled by different populations of *S. litura*

Treatments	Mean distance travelled (cm) in 5 min				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Fenvalerate	302.24	292.38	272.56	246.18	220.77
Spinosad	290.34	275.21	260.40	231.34	202.61
Indoxacarb	278.78	264.66	253.97	222.53	188.34
Cyantraniliprole	258.33	251.32	236.55	214.79	155.26
Emamectin benzoate	220.23	208.55	183.71	163.38	132.74
Untreated	111.17	101.38	95.34	88.12	78.56

Populations F (4,25)= 1.4913 (*NS); Insecticides F (5,24)= 17.8671 (*NS); *NS indicate values are non-significant as P>0.05

Table 3: Effect of insecticides on the speed of different populations of *S. litura*

Treatments	Mean speed (cm s^{-1})				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Fenvalerate	1.15	1.10	1.02	0.94	0.88
Spinosad	1.08	1.01	0.88	0.80	0.72
Indoxacarb	0.95	0.90	0.76	0.68	0.52
Cyantraniliprole	0.86	0.78	0.64	0.55	0.40
Emamectin benzoate	0.72	0.65	0.52	0.42	0.31
Untreated	0.55	0.47	0.40	0.30	0.20

Populations F (4,25)= 2.4906 (*NS); Insecticides F (5,24)= 11.5568 (*S); *NS indicate values are non-significant as P>0.05; *S indicate values are significant as P<0.05

ranging from 111.17 to 302.24 cm as compared to Ludhiana (101.38 to 292.38 cm), Malerkotla (95.34 to 272.56 cm), Kapurthala (88.12 to 246.18 cm) and Amritsar (78.56 to 220.77 cm) populations (Table 2). The speed of larvae was also high for susceptible population (0.55 to 1.15 cm s^{-1}) followed by Ludhiana (0.47 to 1.10 cm s^{-1}), Malerkotla (0.40 to 1.02 cm s^{-1}), Kapurthala (0.30 to 0.94 cm s^{-1}) and Amritsar (0.20 to 0.88 cm s^{-1}) populations (Table 3). The turn angle of larvae from Amritsar (70.34 to 120.45º) region population was higher as compared to those from Kapurthala (63.77 to 110.72º) followed by Malerkotla (54.16 to 103.55º), Ludhiana (42.45 to 95.52º) and susceptible (33.14 to 88.68º) populations (Table 4). The distance travelled by the *S. litura* larvae populations from all regions was maximum when allowed to walk on fenvalerate-treated (220.77 to 302.24 cm) surface, followed by those moved on spinosad -treated (202.61 to 290.34 cm), indoxacarb-treated (188.34 to 278.78 cm), cyantraniliprole–treated (155.26 to 258.33 cm), emamectin benzoate-treated (132.74 to 220.23 cm) surfaces and minimum travelled-distance was recorded on the untreated (78.56 to 111.17 cm) surface (Table 2).

The speed of the *S. litura* larvae populations from all regions was high when allowed to walk on fenvalerate-treated (0.88 to 1.15 cm s^{-1}) surface, followed by those moved on spinosad -treated (0.72 to 1.08 cm s^{-1}), indoxacarb-treated (0.52 to 0.95 cm s^{-1}), cyantraniliprole–treated (0.40 to 0.86 cm s^{-1}), emamectin

Table 4: Effect of insecticides on turn angle of different populations of *S. litura*

Treatments	Mean Turn Angle (°)				
	Amritsar	Kapurthala	Malerkotla	Ludhiana	Susceptible
Emamectin benzoate	120.45	110.72	103.55	95.52	88.68
Cyantraniliprole	112.88	100.34	90.21	83.38	75.44
Indoxacarb	102.32	91.45	81.94	74.66	67.34
Spinosad	94.56	80.31	70.67	63.73	56.18
Fenvalerate	82.85	72.45	64.92	51.68	45.38
Untreated	70.34	63.77	54.16	42.45	33.14

Populations F (4,25)= 3.4354 (*S); Insecticides F (5,24)= 8.5631 (*S); *S indicate values are significant as P<0.05

Table 5: Effect of insecticides on general esterases activity in different larval populations of *S. litura*

Treatments	Specific activity (µg of α-naphthol produced min^{-1} mg^{-1} protein)				
	Amritsar	Kapurthala	Malerkotla	Ludhiana	Susceptible
Emamectin benzoate	1.60±0.46	1.35±0.43	1.18±0.40	1.05±0.35	0.85±0.34
Cyantraniliprole	1.98±0.31	1.68±0.23	1.52±0.35	1.22±0.24	1.01±0.26
Indoxacarb	2.30±0.49	2.11±0.42	1.96±0.32	1.55±0.34	1.34±0.28
Spinosad	2.85±0.36	2.58±0.33	2.15±0.42	2.04±0.24	1.95±0.30
Fenvalerate	3.10±0.28	2.95±0.27	2.60±0.29	2.45±0.31	2.27±0.19
Untreated	4.90±0.19	4.55±0.23	3.82±0.25	3.60±0.23	3.41±0.27

Populations F (4, 25)= 0.8969 (*NS); Insecticides F (5, 24)= 29.8556 (*NS); *NS indicate values are non-significant as P>0.05

benzoate -treated (0.31 to 0.72 cm s^{-1}) surfaces and minimum speed was recorded on the untreated (0.20 to 0.55 cm s^{-1}) surface (Table 3). On the other hand turn angle was maximum on emamectin benzoate -treated (88.68 to 120.45º) surface followed by cyantraniliprole–treated (75.44 to 112.88º), indoxacarb-treated (67.34 to 102.32º), spinosad-treated (56.18 to 94.56º), fenvalerate -treated (45.38 to 82.85º) surface and untreated (33.14 to 70.34º) surface (Table 4). The activity of general esterases was maximum for Amritsar population of S. litura ranging from 1.60 to 4.90 µg of α-naphthol produced min^{-1} mg^{-1} protein followed by Kapurthala (1.35 to 4.55 µg of α-naphthol produced min^{-1} mg^{-1} protein), Malerkotla (1.18 to 3.82 µg of α-naphthol produced min^{-1} mg^{-1} protein), Ludhiana (1.05 to 3.60 µg of α-naphthol produced min^{-1} mg^{-1} protein) and susceptible (0.85 to 3.14 µg of α-naphthol produced min^{-1} mg^{-1} protein) populations.

The higher activity of general esterases was recorded in untreated populations (3.41 to 4.90 µg of α-naphthol produced/min/mg protein) as compared to fenvalerate-treated (2.27 to 3.10 µg of α-naphthol produced min^{-1} mg^{-1} protein), spinosad-treated (1.95 to 2.85 µg of α-naphthol produced min^{-1} mg^{-1} protein), indoxacarb-treated (1.34 to 2.30 µg of α-naphthol produced min^{-1} mg^{-1} protein), cyantraniliprole-treated (1.01 to 1.98 µg of α-naphthol produced min^{-1} mg^{-1} protein) and emamectin benzoate-treated (0.85 to 1.60 µg of α-naphthol produced min^{-1} mg^{-1} protein) populations (Table 5). The maximum activity of acetylcholinesterase was recorded for Amritsar population of *S. litura* ranging from 0.93 to 3.78 n moles min^{-1} mg^{-1} protein followed by Kapurthala (0.89 to 3.68 n moles min^{-1} mg^{-1} protein), Malerkotla (0.82 to 3.67 n moles min^{-1} mg^{-1} protein), Ludhiana (0.73 to 3.32 n moles min^{-1} mg^{-1} protein) and susceptible (0.44 to 2.27 n moles min^{-1} mg^{-1} protein) populations. The higher activity of acetylcholinesterase was observed in untreated populations (2.27 to 3.78 n moles min^{-1} mg^{-1} protein) as compared to fenvalerate-treated (2.17 to 2.72 n moles min^{-1} mg^{-1} protein), spinosad-treated (1.20 to 1.81 n moles min^{-1} mg^{-1} protein), indoxacarb-treated (1.01 to 1.48 n moles min^{-1} mg^{-1} protein), cyantraniliprole-treated (0.61 to 1.17 n moles min^{-1} mg^{-1} protein) and emamectin benzoate-treated (0.44 to 0.93 n moles min^{-1} mg^{-1} protein) populations (Table 6). The trend was similar for all populations in case of both enzymes and it is evident from the results that emamectin benzoate treatment led to decrease in the activity of esterases and acetylcholinesterase.

In the present study, it is indicated that larvae of *S. litura* populations travelled more distance with higher speed and turn angle on the insecticide-treated surface than on the untreated one. The larvae of Amritsar population walked less distance in low speed with high turn angle on treated surfaces as compared to the other larval populations', *i.e.,* Malerkotla, Kapurthala, Ludhiana and susceptible. The difference in LC_{50} values concerning different insecticides for respective *S. litura* populations, indicates that the larval population with higher LC_{50} is more resistant (Amritsar) as compared to other ones (Kapurthala, Malerkotla and Ludhiana). These results are collateral to earlier findings of different workers (Adams *et al.,* 1992; Head *et al.,* 1995b) who

Table 6: Effect of insecticides on acetylcholinesterase activity in different larval populations of *S. litura*

Treatments	Specific activity (n moles min^{-1} mg^{-1} protein)				
	Amritsar	Kapurthala	Malerkotla	Ludhiana	Susceptible
Emamectin benzoate	0.93±0.25	0.89±0.17	0.82±0.13	0.73±0.19	0.44±0.21
Cyantraniliprole	1.17±0.23	1.12±0.21	0.95±0.24	0.80±0.12	0.61±0.23
Indoxacarb	1.48±0.31	1.44±0.12	1.30±0.34	1.20±0.23	1.01±0.25
Spinosad	1.81±0.35	1.76±0.23	1.65±0.11	1.62±0.14	1.20±0.19
Fenvalerate	2.72±0.28	2.44±0.14	2.38±0.27	2.30±0.21	2.17±0.12
Untreated	3.78±0.20	3.68±0.20	3.67±0.23	3.32±0.25	2.27±0.17

Populations F (4, 25)= 0.4461 (*NS); Insecticides F (5, 24)= 47.0016 (*NS); *NS indicate values are non-significant as P>0.05

also found similar pattern of observation in susceptible *Plutella xylostella* on insecticide treated surfaces. Chandi and Singh (2017) inferred that the susceptible larval population of *P. xylostella* travelled more distance with faster speed on an insecticide-treated surface (endosulfan, quinalphos, fenvalerate and spinosad) on comparison with resistant population, but on contrary, the turn angle was larger in case of resistant population of *P. xyostella* as compared to susceptible ones. Chandi and Kaur (2017) also found that the larvae of *P. xylostella* covered more distance with higher speed and greater turn angle on insecticide-treated surface (chlorantraniliprole, novaluron, thiodicarb, chlorpyriphos and deltamethrin). Similar observations were also recorded by Chandi (2019) and Chandi and Suri (2019), in case of different *S. litura* and *Helicoverpa armigera* larval populations from Punjab where larvae travelled less distance with lower speed and minimum turnings on untreated surface as compared to insecticide- treated surface. The turn angle was maximum with low speed and distance coverage in case of Amritsar population larvae for both insect pests irrespective of insecticide treated or untreated surface. It is inferred from observations that this type of variations between different insect-pest populations might be due to the difference in the insect's sensory perception for different insecticides exposure and increased turn angle could represent a type of avoidance behaviour of larvae (Adams *et al.,*1992; Chandi and Singh, 2017). The variations could be inheritable, which can lead to the behavioural resistance development (Pereira *et al.,* 2009). The more resistant population (Amritsar) showed the avoidance behaviour in presence of insecticides, which is absent in case of susceptible population of *S. litura*.

The estimation of general esterases and acetylcholinesterase activities is also a potent biochemical tool for monitoring resistance in Lepidopteran insect-pests along with behavioural parameters (Gujar and Sohal, 2010; Chandi and Singh, 2014). The decline in activity of both enzymes is recorded in case of treated *S. litura* larvae. The inhibition of enzymes was less in the Amritsar population in comparison to other populations, *i.e.,* Kapurthala, Malerkotla and Ludhiana. The observations indicate towards high resistance in Amritsar population as compared to other populations. Yu and Nguyen (1992) also observed that activity of esterases/acetylcholinesterase and other enzymes was 1.4 to 2.7 fold higher in field population of *P. xylostella* than the susceptible one. The increased levels of carboxylesterase (1.05 fold), acetylcholinesterase (1.4 fold) and GSH S-transferase (1.5 fold) was reported in the highly resistant population (Karsara) upon comparison with the susceptible population (Tikri) of *P. xylostella* near Varanasi by Singh (2002). The insecticide treatment (profenofos, lufenuron, emamectin benzoate, cyfluthrin, spinetoram) inhibited the acetylcholinesterase activity in laboratory and field strain of *Spodoptera littoralis* (Boisd.) larvae (Ismail, 2020). The higher activity of esterases and acetylcholinesterase indicates, the change in metabolism of resistant populations of *S. litura*, resulting in physiological resistance. The resistant *S. litura* populations can eliminate the insecticide from body by enhancement of detoxification enzymes activity. This change could be favourable for survival of these populations in presence of insecticides, which is lacking in susceptible population.

Hence, it is concluded that the development of resistance in *S. litura* populations could be a club of changes in overall behavioural physiology (locomotory behaviour and enzyme activity) of this insect-pest. The emamectin benzoate was found to be an effective insecticide, influencing the both locomotory and biochemical parameters of *S. litura*. This study could help to develop management strategies for *S. litura*.

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