Pakistan Entomologist

Journal homepage: www.pakentomol.com

EFFECT OF INSECTICIDES ON SUSCEPTIBILITY AND ESTERASE ACTIVITY OF *SPODOPTERA LITURA* (NOCTUIDAE:LEPIDOPTERA)

Sana Barkat[1], Asim Gulzar[1*], Muhammad Tariq[1], Muhammad Javaid Asad[2], Raja Tahir Mahmood[2], Asad Ali[3] and Kaleem Tariq[3]

[1]Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi
[2]Department of Biochemistry, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi
[3]Department of Agriculture, Abdul Wali Khan University, Mardan

ARTICLE INFORMATION

Received: January 16, 2016
Received in revised form: May 25, 2016
Accepted: June 05, 2016

***Corresponding Author**:
Asim Gulzar

E-mail: asim@uaar.edu.pk

ABSTRACT

Spodoptera litura is the major pest of economically important crops and many vegetables. It has more than 120 host plant species and is the major pest of cauliflower, cotton, strawberry, tomato, apple and many other crops. Many insecticides have been used for the management of *S. litura* but the continuous use of insecticide leads to the development of resistance against these insecticides. *S. litura* has developed resistance against the major insecticides including pyrethroids and carbamates. Present study was conducted to determine the susceptibility and esterase activity of the 3rd and 5th instar larva of *Spodoptera litura* against three insecticides. Esterase is one of the enzymes which cause the detoxification of the insecticides leading to the resistance development. Three different concentrations i.e. LC_{40}, LC_{50} and LC_{60} of each insecticide were used. Esterase activity against each insecticide was determined after 30 min, 2, 24 and 48 hrs. The results revealed that highest activity was found against fenvelrate and lowest activity was found against flubendamide in 3rd and 5th instar of *S.litura*. On the basis of toxicity and enzyme activity among all the tested insecticides, flubendamide found to be very effective against *S.litura* and can be used for the control of this pest.

Keywords: Insecticide, Esterase, *Spodoptera litura*

INTRODUCTION

Spodoptera litura is the major pest of economically important crops and many vegetables in South Asian countries (Qin *et al.*, 2004; Mallikarjuna *et al*., 2004. Moist and warm field conditions are important for its multiplication and development (Dhir *et al*., 1992). It is notorious leaf feeding caterpillar and has ability to travel long distance, therefore it is difficult to control it in case of outbreak situation (Sayyed *et al*., 2008). It has more than 120 host plant species and is the major pest of cauliflower, cotton, strawberry, tomato, apple and many other crops (Rao *et al.,* 1993). Many insecticides have been used for the management of *S. litura* but the continuous use of insecticide lead to development of resistance against these insecticides (Zhai and Robinson, 1992). *S. litura* had developed resistance against the major insecticides including pyrethroids and carbamates (Ahmad *et al*., 2007).

There are number of factors responsible for the development of resistance against the major insecticides in *S. litura*. Insecticide resistance involves three major mechanisms i.e., enhance detoxification, decreased penetration and target site insensitivity (Ahmad and Mccaffery, 1999). Enzyme detoxification is important mechanism against the synthetic insecticides. These enzymes include esterases and cytochrome, P450, monooxygenases enzymes (Ahmad *et al*., 2007). The detoxification mechanism of enzyme is divided into metabolic transformation and reaction (Visetson, 1991). It is reported that enzymes are accumulated in intestine and adipose cells. When insects are exposed to chemicals they may change their behavior and decreases the chemical effect within the insect body and this behavior is called behavior avoidance. Insects have several detoxification and behavior avoidance system. Insects use detoxification enzymes to decrease the toxicity of poison (Visetson and Milne, 2001).

Esterase is large group of hydrolase, which hydrolyze non ester and ester compound, and play a vital role in conferring detoxification of insecticides in arthropods species and insect (Mouches *et al*., 1986). It has two types, arylesterases which does not inhibit by the organophosphate and the second type is

Cite this article as: Barkat, S., A. Gulzar, M. Tariq, M.J. Asad, R.T. Mahmood, A. Ali and K. Tariq, 2016. Effect of insecticides on susceptibility and esterase activity of *Spodoptera litura* (Noctuidae: Lepidoptera). Pak. Entomol., 38(1):33-38.

carboxylesterase cholinesterase and aliesterase which are inhibited by organophosphate due to the irreversible phosphorylation of active serine site (Dauterman, 1985). The main objective of the present study was to determine the susceptibility and activities of esterase enzymes of *S. litura* under the application of different insecticides for better results.

MATERIALS AND METHODS

Field collection and rearing of *Spodoptera litura*

The larvae of *S. litura* were collected from cauliflower field crops of National Agriculture Research Centre (NARC). Larvae were reared according to the method of Smith (1996). Larvae were placed in six-holed Petri dishes and artificial diet was provided to each larvae. Diet was changed after 3-4 days. The pupae were kept in separate boxes till the adult emergence. Adults were transferred to the plastic jars and provided 10 % sugar solution as food. Egg batches were collected daily and were transferred to the new six-holed Petri dishes.

Insecticides

Three different insecticides, Flubendiamide (Belt®48SC, Bayer Crop Sciences Pvt Pakistan), Fenvalerate (Fencur) and Deltamethrin (Decis 25 EC, Bayer Crop Sciences Pvt Pakistan) were used to evaluate the esterase activity of *S. litura.*

Toxicity bioassays

Leaf dip Bioassays were conducted with the 3rd and 5th instar larvae of *S. litura* (Sayyed *et al.*, 2008). Test solutions were prepared in distilled water with Triton X-100 (50 µg/ml). Leaf discs (4.8 cm dia.) were immersed in a test solution for 10 s, allowed to dry at room temperature and placed in Petri dishes (5 cm dia.) containing a moistened filter paper. Five larvae were placed in each dish, and each treatment was replicated 7 times. The mortality was assessed after 48 h. Three different concentrations *e.i*. LC_{40}, LC_{50} and LC_{60} were used to measure the esterase enzyme activity of *S. litura.*

Sample preparation

For esterase extraction, *S. litura* treated with LC_{40}, LC_{50} and LC_{60} of insecticides were ground in 3 ml of ice-cold sodium phosphate buffer (0.04 M, pH 7.0) in a tissue grinder. The homogenates were centrifuged at 10,000 rpm for 15 min at 4 ºC. The resulting supernatants were used as the enzyme source. *S. litura* place on leaves treated with water were used as control. The samples were taken after 30 min, 2h, 24 h and 48h.

Esterase assay

Esterase assay was performed by mixing 1 mL of enzyme stock solution. (10 µl of supernatant of *S. litura*) homogenate in 990 µl phosphate buffer (40 mM, pH 6.8) into 5 mL of substrate soln. (30mM α-naphtyhl acetate). Mixture was incubated at 30ºC for 20 min and then 1 mL of staining solution (Fast blue BS salt (1%), phosphate buffer (0.04 M) and SDS 5% w/v) was added and again incubated for 20 min. After that absorbance was taken spectrophotometrically at 590 nm to measure esterase activity.

Enzyme Activity

Enzyme activity was measured as follows.

$$\text{Enzyme Activity} = \frac{\text{Absorbance} \times \text{Standard factor} \times \text{DF}}{20}$$

Statistical analysis

LC_{40}, LC_{50} and LC_{60} values of the insecticide were determined with R version 2.9.0 (R Development Core Team, 2009).

RESULTS

Susceptibility of insecticide against *S. litura*

The susceptibility of three insecticides was evaluated against 3rd and 5th instars larvae of *S. litura*. The higher LC values were found for deltamethrin against 3rd instar larva of *S. litura* and lower LC value was observed for flubendamide (Table 1). Lethal concentration values (LC_{40}, LC_{50}, and LC_{60}) were also determined for 5th instar larvae (Table 2).

Esterase activity of *S. litura* larvae after application of deltamethrin

The mean esterase activity in 3rd and 5th instar larvae of *S. litura* after the application of deltamethrin was given in table3. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{40} in 3rd instar was 97.433, 340.333, 264.666 and 252.667 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{40} in 5th instar was 494.333, 442.56, 562.633 and 376.666 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{50} in 3rd instar was 391.933, 322.333, 254.667 and 248.332 IU/ml/min respectively . The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{50} in 5th instar was 472.6667, 432.333, 562.3 and 345 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{60} in 3rd instar was 253.233, 311.666, 241.66 and 253.333 IU/ml/min. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{60} in 5th instar was 435.6, 439.66, 548.53 and 613 IU/ml/min respectively. Highest mean esterase activity (562.6333 IU/ml/min) was recorded against 5th instar at LC_{40} after 24 hrs. While lowest mean esterase activity (97.433 IU/ml/min) was recorded with LC_{40}after 30 min.

Esterase activity of *S. litura* larvae after the application of fenvelrate

The mean esterase activity in 3rd and 5th instar larvae of *S. litura* after the application of fenvelrate was given in table3.The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{40} in 3rd instar was 241.66 , 421.6667, 246.66 and 251.6667 IU/ml/min. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{40} in 5th instar was 217.333, 409.333, 262.333 and 244 IU/ml/min. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{50} in 3rd instar was 413.88, 425.66, 252 and 216.66 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{50} in 5th instar was 275.33, 401.667, 275.333 and 210 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{60} in 3rd instar was 164.667, 412.667, 240.333 and 257.333 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs

application of LC_{60} in 5th instar was 331.33, 374.33, 331.33 and 187.66 IU/ml/min respectively. The highest mean esterase activity (834 IU/ml/min) was recorded in 5th instar with LC_{50} after 30 minutes and lowest mean esterase activity (237 IU/ml/min) was recorded in 3rd instar larvae with LC_{40} after 30 min.

Esterase activity of *S. litura* larvae after the application of flubendamide

The mean esterase activity in 3rd and 5th instar larvae of *S. litura* after the application of flubendamide was given in table 3. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{40} in 3rd instar was 241.66, 425.5, 246.67 and 151.3 IU/ml/min. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{40} in 5th instar was 217.33, 249.33, 262.33 and 244 IU/ml/min. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{50} in 3rd instar was 413.88, 164.16, 252.1 and 116.2 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{50} in 5th instar was 275, 401, 301 and 210 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{60} in 3rd instar was 164.12, 251.61, 240.3 and 157.31 IU/ml/min respectively. The mean esterase activity after 30 min, 2 hr, 24 hrs and 48 hrs application of LC_{60} in 5th instar was 331.3, 374.2, 331.5 and 187.61 IU/ml/min respectively. Highest esterase activity (413 IU/ml/min) was recorded in 3rd instar with LC_{50} after 30 min and lowest esterase activity (164 IU/ml/min) was recorded in 3rd with LC_{60} after 30 min.

Table 1

Lethal concentration values of Deltamethirn, Flubendamide and Fenvelrate against 3rd instar larvae of *S. litura.*

Insecticides	LC (ppm)			Slope± SE	N
	LC_{40} (95%CI)	LC_{50} (95% CI)	LC_{60} (95% CI)		
Deltamethrin	91 (57.4-209.7)	188 (105-757.5)	390 (180-925)	0.803±0.234	300
Flubendamid	19 (10.9-27)	31 (21-48)	53 (36- 96)	1.142±0.279	300
Fenvelrate	95 (52-162)	17 (104- 371)	306 (177- 1000)	1.002±177.9	300

Table 2

Lethal concentration values of Deltamethirn, Flubendamide and Fenvelrate against 5th instar larvae of *S. litura.*

Insecticides	LC (ppm)			Slope± SE	N
	LC_{40} (95%CI)	LC_{50} (95% CI)	LC_{60} (95% CI)		
Deltamethrin	236 (173.5-559.3)	348 (127.9-229.3)	511 (298-969)	1.515±0.507	300
Flubendamid	31 (18.52-50.43)	54 (35.35-111.9)	95 (65-290.9)	1.052±0.308	300
Fenvelrate	188.73 (110.8-446.6)	329.808 (187-1323)	576.35 (289-4310.)	1.045±0.353	300

Table 3

Esterase activity in 3rd and 5th instar larvae of *S. litura* after the application of Deltamethrin .

Time	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
30 min	238	427	97.43	494.33	491.93	472.66	253.23	435.6
2 h	301	447	440.33	442.56	322.33	432.33	253.23	439.66
24 h	292	506	264.66	562.63	254.66	562.3	241.66	435.6
48 h	231	307	252.66	376.66	248.33	345	253.33	613

Table 4

Esterase activity in 3rd and 5th instar larvae of *S. litura* after the application of Flubendamide.

Time	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
30 min	238	427	41.66	217.33	313.88	275	164.12	331.31
2 h	301	447	325.5	249.33	164.16	401	251.61	374.2
24 h	292	506	246.67	262.33	252.1	301	240.3	331.25
48 h	231	307	151.3	244	116.2	210	157.31	187.61

Table 5

Esterase activity in 3rd and 5th instar larvae of *S. litura* after the application of Fenvelrate.

Time	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
30 min	238	427	237.813	816.10	349	834.83	297.6	753.72
2 h	301	447	395	697.6	293.3	691	425	688
24 h	292	506	325.24	683	314.6	672.6	297.6	753.72
48 h	231	307	365	637.6	376	625.3	326	372

DISCUSSION

The results of the present study revealed that the flubendamide is more toxic to the 3rd and 5th instar larvae of *S. litura* than deltamethrin and fenvelrate at LC_{40}, LC_{50} and LC_{60}. The toxicity of the insecticide depends upon the larval instars and the chemical nature of the insecticides. Toxicity of different insecticide was evaluated against different insect pests by many researchers. Sheikh *et al*. (2012) conducted experiment on 4th instar larvae of S. *litura* to evaluate the effect of chlropyrifos and LC_{50} values were reported 17.50 ppm. In another study dichlorovos was used against 3rd instar larvae of *S. litura* and LC_{50} values recorded were 9.04 ppm (Muthusamy *et al*., 2011). Extract of *A. annua* used against 3rd instar larvae and adult of *S. litura* showed LC_{50} of 48 ppm and 19.14ppm respectively (Shekari *et al*., 2008). Deltamethrin showed LC_{50} value of 25ppm on 3rd instar larvae of army worm *(*Cho *et al*., 1999).

The ineffectiveness of insecticide in controlling insect pests and subsequently the development of insecticide resistance are due to the action of enzymes which are either insensitive to the insecticide or able to degrade it to less toxic metabolites (Biddinger, *et al.,* 1996). There is a relationship between the increase of insecticide resistance and the activity of detoxification enzymes (Xin-Ju and Hui-Min, 2011). Esterases are large and diverse group of hydrolases that hydrolyze numerous substrates (Walker and Mackness, 1983). Esterase is one of the enzymes which cause the detoxification of the insecticides leading to the resistance development against the insecticides. The level of the esterase is directly related to the resistance against the insecticides (Huang and Han, 2007). Increased esterase activity is a major mechanism of insecticide insensitivity or even resistance in many insect species (Zhou, *et al*., 2002).

In the present study, esterase activities in 3rd and 5th instar larvae of *S. litura,* treated with three insecticides at LC_{40}, LC_{50} and LC_{60} was measured after 30 min, 2h, 24 h and 48 h. The result revealed that highest activity was found against fenvelrate and lowest activity was found against flubendamide in 3rd and 5th instar of *S.litura*. Previously, Xue *et al*., (2010) evaluated the susceptibility of four host plants againts larvae of *S. litura* and also reported that the glutathione S-transferase activities of larvae were highest when they fed on tobacco, followed by Chinese cabbage and cowpea, and the lowest activities were observed when larvae fed on sweet potato. The extract of *A. annua* affects the biochemical properties of the elm leaf beetles (Shekari *et al*., 2008). Su *et al*. (2012) reported that esterase activities of most of the field populations were higher than the susceptible populations of *S. litura.* The larvae of *S. litura* treated with indoxacarb for 24 hour showed alpha esterase activities of 517 and 945 IU/ml/min in 2nd and 4th instars respectively. The beta esterase activity was 786 and 450 IU/ml/mint in 2nd and 4th instar respectively (Gamil *et al*., 2011).

Conclusion/recommendations

Esterase enzyme activity responded towards the insecticide application. Enzyme activity fluctuated up and down from normal count due to destruction caused by application of insecticides. Higher activity indicates resistance against insecticides and decrease esterase activity shows susceptibility against insecticides. It is concluded that based on toxicity and enzyme activity among all the tested insecticides, flubendamide found to be very effective against *S. litura* and can be used for the control of this pest.

REFERENCES

Ahmad, M., A. R. Maaaffery, 1999. Penetration and metabolism of trans cypermethrin in a susceptible and a pyrethroid resistant strain of *Helicoverpa armigera*. Pestic. Biochem., 65: 6-14.

Ahmad, M., M. I. Arif and M. Ahmad, 2007. Occurrence of insecticides resistance in field population of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Protect., 26(6): 809-817.

Biddinger, D.J., L.A. Hull and B.A. Mcpheron, 1996. Cross resistance and synergism in azinphosmethyl resistant and susceptible strains of tufted apple bud moth (Lepidoptera: Tortricidae) to variousinsect growth regulators and avarmectin. J. Econ. Entomol., 89: 274-287

Cho, J.R., Y.J. Kim, J.J. Kim, H.S. Kim, J.K. Yoo and J.O. Lee, 1999. Electrophoretic pattern of larval esterases in field and laboratory selected strains of the tobacco cutworm, *Spodoptera litura* Fabricus. J. Asia-Pacific. Entomol., 2(1): 39-44.

Dhir, B.C., H.K. Mohapatra and B. Snapati, 1992. Assesment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F). Indian J. Pl. Prot., 20: 215-217.

Dauterman, W.C., 1985. Insect metabolism extra microsomal. In: Kerkut, G.A. and Gilbert L.I., eds. Comprehensive Insect Physiology Biochemistry Pharmacology, Pergamon Press, Oxford, 12: 713-730.

Gamil, W.E., F.M. Mariy., L.A. Yoseef and S.M. Halim, 2011. Effect of indoxacarb on some biological and biochemical aspects of *Spodoptera littoralis* larvae (Boisd). Agric. Sci., 52(2): 121-126

Huang, S. and Z. Han, 2007. Mechanism for multiple resistances in field population of common cutworm *Spodoptera litura* (F) in China. Pestic. Biochem. Physiol., 87: 14-90.

Mallikarjuna, N., K.R. Kranthi, D.R. Jadhav, S. Kranthi and S. Chandra, 2004. Influence of foliar chemical compound on the development of *Spodoptera litura* (Fab). In interspecific derivatives of groundnut. J. Appl. Entomol., 128: 321-328.

Mouches, C., N. Pasteur, J.B. Berge, O. Hyrien, M. Raymond, B.R.D. Saint Vincent. M.D. Silvestri and G.P. Georghiou, 1986. Amplification of an esterase gene is responsible for insecticide resistance in Californian *Culex* mosquito. Sci. Rev., 233: 778-780.

Muthusamy, S. Kumar, Karthi and R. Kumar, 2011. Pesticide detoxifying mechanism in field population of *Spodoptera litura* (Lepidoptera: Noctuidae) from South India. Egypt. Acad. J. Biolog. Sci., 3(1): 51-57.

Qin, H., Z. Ye, S. Huang, J. Ding and R. Lou, 2004. The correlations of the different host plants with preference level, life duration and survival rate of *Spodoptera litura* Fabricius. Chinese J. Ecol. Agric., 12: 217-233.

Rao, G.V.R., J.A. Wightman and R.D.V. Rao, 1993. World review of the natural enemies and diseases of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Insect

Sci. App., 14: 273-284.

Sayyed, A.H., M. Ahmad and M.A. Saleem, 2008. Cross resistance and genetic of resistance to Indoxacarb in *Spodoptera litura*. (Lepidoptera: Noctuidae). J. Econ. Entomol., 101: 472-479.

Sheikh, E.T. A.A., 2012. Comparative toxicity and biochemistry of organophosphate and pyrethroid compound on both laboratory and field strain of the cotton leafworm, *Spodoptera littoralis* (Boisd). Physiol. Molec. Boil., 4: 141-151.

Shekari, M., J.J. Sendi., K. Etebari., A. Zibaee and K. Shadparvar, 2008. Effect of *Artemisia annua* (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca Luteola* (Coleoptera: Chrysomelliadae). Pest. Biochem. Physiol., 91:66-74.

Smith, C.N., 1996. Insect colonization and mass production. Academic press, London, pp.479-486.

Su, J., T. Li and J. Li, 2012. Susceptibility of field population of *Spodoptera litura* (Fabricious) (Lepidoptera: Noctuidae) in china to chlorantraniliprole and the activities of detoxification enzyme. Crop Protect., 42: 217-222.

Visetson, S., 1991. Insecticide resistance mechanism in the red rust flour beetle (*Tribolium castaneum* Burst). Ph.D. Thesis. Uni. of Sydney, Australia.

Visetson and M. Milne, 2001. Effect of root extract from derris (*Derris elliptica Benth*) on mortality and detoxification enzyme level in diamond back moth larvae (*Plutellaxylostella* linn). Kasetsart. J. Nat. Sci., 35: 157-163.

Walker, C.H. and M.I. Mackness, 1983. Esterases: problems of identification and classification. Biochem. Pharma., 32: 3265-3269.

Xin-Ju, G and S. Hui-Min, 2011. Resistance selection with fenpropathrin and the change of detoxification enzyme activities in *Tetranychus urticae* Koch (Acari: Tetranychidae). Acta Entomologica Sin., 54 (1): 64-69.

Xue, M., Y.H. Pang., Q.L. Li and T.X. Liu, 2010. Effect of four host plants on the susceptibility of *Spodoptera litura* (Lepidoptera: Noctuidae) larvae to five insecticides and activities of detoxification esterases. Pest Manag. Sci., 66: 1273-1279.

Zhai, J. and W. H. Robinson, 1992. Measuring cypermethrin resistance in German cockroach. J. Econ. Entomol., 85: 348-351.

Zhou, X., M.E. Scharf, S. Parimi, L.J. Meinke, R.J. Wright, L.D. Chandler and B.D. Siegfried, 2002. Diagnostic assays based on esterasemediated resistance mechanisms in western corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol., 95: 1261-266