Egypt. Acad. J. Biolog. Sci., 3 (1): 51- 57 (2011) **F. Toxicology &Pest control**
Email: egyptianacademic@yahoo.com **ISSN: 2090 - 0791**
Received: 21 / 3 / 2011 **www.eajbs.eg.net**

Pesticide detoxifying mechanism in field population of *Spodoptera litura* (Lepidoptera: noctuidae) from South India

Muthusamy[1], Shivakumar[2], Karthi[2], Ramkumar[2]
Department of Biotechnology,
Periyar University,
Salem, Tamilnadu.
India – 636011
Corresponding Author: skentomol@gmail.com

ABSTRACT

The army worm *Spodoptera litura* (fabricius) is one of the most damaging pests of ground nut and castor in India. We determine the susceptibility of *S. litura* reared on a castor leaves, to three insecticides (Temephos, Dichlorvos, and lambda-cyhalothrin) under laboratory conditions, associated with the enzyme activities of acetylcholinesterase (AChE), carboxylesterase (CarE), glutathione S-transferase (GST) and glutathione S- reductase (GSH) in this larvae. Among treatments Dichlorvos showed high mortality then lambda cyhalothrin and Temephos in whole body of the insect. The AChE activities of *Spodoptera litura* treated with lambda cyhalothrin, Temephos at 10ppm were almost 1.5 fold high. The CarE activity at 10ppm was almost 3 fold high. The GSH and GST activity were low then control (0.2 ± 0.4, 1.5 µM mg protein^{-1} min^{-}1). The present study suggests that esterase and acetylcholine esterase may play a role in detoxification of synthetic pyrethroid and organophosphates in *Spodoptera litura* from South India.

Keywords: Esterases, Synthetic pyrethroid, organophosphates, Army worm, detoxifying enzyme.

INTRODUCTION

Spodoptera litura (fabricius) (Lepidoptera: Noctuidae) is a polyphagous insect pest (Holloway, 1989). It is an indigenous pest of a variety of crops in South Asia and was found to cause more than 26-100% yield loss in groundnut (Dhir *et al*., 1992). It is variously known as Indian Leaf worm and tobacco cutworm (Rao *et al*., 1993). The control of this pest has depended on application of chemical insecticides; as a result which may develop resistance (Armes *et al*., 1997; Kranthi *et al*., 2002) and subsequent control failures. Insecticide resistance in lepidopteran population involves mainly two mechanisms, enhanced detoxification, and target site insensitivity. In many cases detoxification enhancement causes metabolic resistance and involves three major groups of enzymes like esterases, glutathione complex and cytochrome P450 (Hemingway, 2004).

Various detoxifying enzymes confer insecticide resistance to insects. In insect AChE is the major target for organophosphate (OP) and carbamates insecticides, which inhibit enzyme activity by covalently phosphorylating or carbamylating the serine residues with in active site (Corbett, 1974). Resistance to organophosphate and carbamates pesticides has been reported in *Helicoverpa armigera* and the army worm *Spodoptera litura* in India (Kranthi *et al*., 2001).

Insecticide detoxifying enzyme, glutathione s- transferase (GST) belongs to a protein family involved in detoxification of xenobiotics, protection from oxidative damage and intracellular transport of hormones, endogenous metabolites and exogenous chemicals.

Insect GSTs are primarily to confer resistance to organophosphorous and pyrethroid insecticides (Vontas *et al*., 2002).

Carboxylesterase (CarE) is the cytoplasmic enzyme which plays an important role in neutralizing xenobiotics (Tang *et al*., 1990). These esterases detoxify organophosphate (OP) and carbamates (CB) pesticides and synthetic pyrethroid (SPs) by two main ways, hydrolysis of the ester bond and binding of the pesticide (OP) to the active site of CarE (Crow *et al*., 2007).

The goal of this present study is to understand the detoxification mechanism of *Spodoptera litura* in field population from South India.

MATERIALS AND METHODS

Insects

Spodoptera litura populations used in this study were obtained from groundnut field at Salem district, Tamil Nadu (India) during 2009-2010; the larvae were periodically collected, with no contact with any kind of insecticide. Samples were brought to the laboratory, caster leaves were replaced on alternate days as a food source. Larvae was maintained at 26±1°C and 55±10 %RH with 16:8(L: D) photoperiod.

Bioassay

Bioassays were performed on 3rd instars larvae using the standard topical application procedure followed (Robertson and Preisler, 1992). Three pesticides were used namely: Temephos 65% EC (emulsifiable concentrate), Dichlorvos 50% EC, and λ-cyhalothrin 5% EC purchased from commercial suppliers in Tamilnadu. Different concentration of pesticide was prepared and control with water. After treatment the larvae was released in 9mm diameter Petri plate. Six third instar larvae were released and each treatment as replicated three times. Observations for mortality were recorded for 24hours. Larvae were considered dead if unable to move in a coordinated way when prodded with a fine haired brush. The corrected mortality was calculated by using (Abbott's, 1925) formula data were subjected to probit analysis as described by (Finney, 1971).

Sample preparation

Intoxicated insects from selected bioassay experiments were weighed, were rinsed with acetone (2 X 5ml) to remove surface residues, the whole larval homogenate was prepared by grinding twenty 3rd instar larvae in ice- cold 50mM sodium phosphate buffer (pH 7.5). The homogenate was centrifuged at 10, 000 rpm for 20 min at 4ºC. The supernatant were stored at -20ºC and used as enzyme source. The protein content of the enzyme extract was estimated by using the (Lowry *et al*., 1951) method, using bovine serum albumin (BSA) as a standard protein to construct the standard curve.

Enzyme assays

Carboxylesterase Assay

Carboxylesterase activity was measured by the method of (Kranthi, 2005). 100µl of enzyme solution from untreated control (water) and treated larvae were added to the tubes containing (100µl 0.3 mM α- napthyl acetate as a substrate, 4.8ml of 40mM PB pH 6.8) was added to the test tubes and incubated in dark for 20 minutes at room temperature. After gentle shaking, 1ml of staining solution (1% fast blue BB salt in phosphate buffer [40mM pH 6.8] with 5% sodium dodecyl sulphate (SDS) was added to each tube and incubated at 20ºC for 30 minutes the absorbance was recorded at 590 nm. The enzyme activity was calculated from α- napthol standard curve. Each sample was measured in triplicate to minimize error.

Glutathione S-transferase Assay

Activity of Glutathione S-transferase (GST) was carried using the method of (Habig *et al*., 1974). Fifty micro liters of 50mM (CDNB) and 150µl of reduced glutathione (GSH) were added to 2.79 ml phosphate buffer

(40mM pH 6.8). Ten microliters of enzyme stock was then added. The mixture were gently shaken and incubated for 2-3 minutes at 20ºC and then transferred in the sample cuvette slot of a UV spectrophotometer. The change in absorbance was measured at 340nm up to 5 min and the enzyme the enzyme activity in terms of µmol of CDNB conjugated min^{-1} mg of enzyme $protein^{-1}$ was calculated using the extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Acetylcholinesterase Assay

Acetylcholine esterase (AChE) activity was measured using acetylcholine-iodide as a substrate according to (Ellman *et al*., 1961).Two hundred micro liters of enzyme stock and 100µl of (0.075 M acetylthiocholine-iodide), 240µl of 0.1M phosphate buffer (pH 7.4) were added and incubated for 15 min at 27ºC, and then 500µl of 0.1M eserine was added and mixed. The change in absorbance was measured at 412 nm.

Glutathione S-reductase Assay

The activity of GSH was measured as per the method (Beutlar *et al*., 1963) with slight modification. Thirty microliters of enzyme stock and1.7 ml of phosphate buffer (pH 7.4) were added then the tubes were incubated for 3min at room temperature. After gentle shaking 100µl of 10mM 5, 5 dithiobis 2-nitrobenzoic acid (DTNB), 50µl of 50mM reduced glutathione, 50µl of 4.3mM Nicotinamide adenine diphosphate (NADPH) was added then the mixture was Incubate for 2-3 minutes at 20ºC. The change in absorbance was measured at 412 nm.

Statistical analysis

The percentage of larval mortality was corrected and the data's were subjected to probit analysis. Variation in the activities of AChE, CarE, GST and GSH in each treatment and control were analyzed using Analysis of Variance (ANOVA) with Bonferronii multiple comparison tests.

RESULTS

Bioassay:

The toxicity of *Spodoptera litura* to organophosphate and synthetic pyrethroid is given in (Table 1). Among tested Dichlorvos shows high mortality (75%) at 9 ppm. Synthetic pyrethroid showed maximum mortality at 10ppm. Whereas Temephos at 31.6ppm.

Table 1: LD_{50} Values of 3rd Instar *Spodoptera litura* exposed to organophosphate and synthetic pyrethroid, after 24 hours observation.

Insecticide	LD_{50} value (in ppm)
Dichlorovos	9.045963
λ-Cyhalothrin	10

Care activity:

Esterase activity was 3 fold higher in λ-cyhalothrin treatment as compared to dichlorvos treatment (500 µM mg $protein^{-1}$ min^{-1}) at 10ppm, whereas at 100ppm the significant reduction was recorded in control and other treatments (Fig 1).

GST activity:

GST activity was 2 fold less in λ-cyhalothrin treatment as compared to dichlorvos (Fig 2). However in both treatments the activity decreased (1.3 µM mg $protein^{-1}$ min^{-1}) as compare to (2.4 µM mg $protein^{-1}$ min^{-1}) control.

AChE activity:

The activity of AChE was significantly increased at 10ppm in λ-cyhalothrin (1.5 µM mg $protein^{-1}$ min^{-1}) treatment. But the activity was decreased in both treatments at 100ppm as compared to control (Fig 3).

GSH activity

GSH activity was increased in Dichlorvos treatment at both concentrations 10ppm, 100ppm (0.2 ± 0.4 µM mg $protein^{-1}$ min^{-1}), however the activity decreased in all treatments as compared to control (Fig 4).

Figures: Detoxification enzyme profile of 3rd instar *Spodoptera litura* larvae exposed to organophosphate and synthetic parathyroid insecticides.

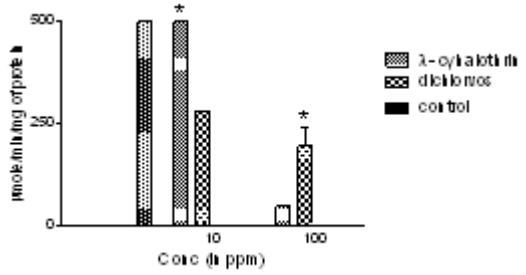

Fig. 1: Esterase activity of 3rd instar *Spodoptera litura* exposed to organophosphate and synthetic parathyroid. Mean (±SD) values are expressed based on One Way ANOVA (Bonferronii post-hoc test). Significant difference among treatments represented by *(P<0.05) with respect to control.

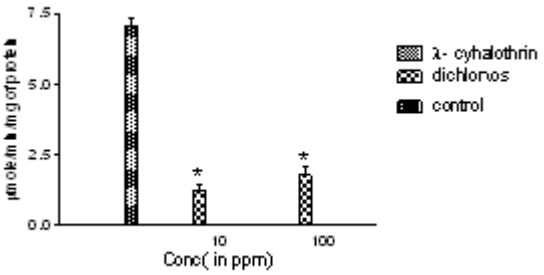

Fig. 2: GST activity of 3rd instar *Spodoptera litura* exposed to organophosphate and synthetic pyrethroid. Mean (±SD) values are expressed based on One Way ANOVA (Bonferronii post-hoc test). Significant difference among treatments represented by *(P<0.05) with respect to control.

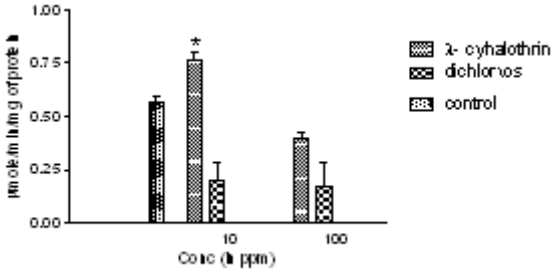

Fig. 3: AChE activity of 3rd instar *Spodoptera litura* exposed to organophosphate and synthetic pyrethroid. Mean (±SD) AChE values are expressed based on One Way ANOVA (Bonferronii post-hoc test). Significant difference among treatments represented by *(P<0.05) with respect to control.

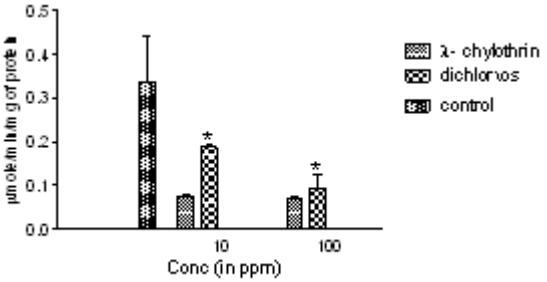

Fig. 4: GSH activity of 3rd instar *Spodoptera litura* exposed to organophosphate and synthetic pyrethroid. Mean (±SD) values are expressed based on One Way ANOVA (Bonferronii post-hoc test). Significant difference among treatments represented by *(P<0.05) with respect to control.

DISCUSSION

Insecticide resistance in lepidopteran is a major concern throughout the world. Several species of lepidopteran viz *Helicoverpa armigera*, *Spodoptera litura,* and *Plutella xylostella* have been reported to have developed resistance to several classes of insecticides (Denholm *et al*., 1998). In the present study dichlorvos was found to be more toxic as compared to temephos and lambda-cyhalothrin. Similar observations were done by (Hassan *et al*., 2009). Bioefficacy of insecticides on *Spodoptera exigua* showed that Bifenethrin was highly effective as compared to other classes of insecticides (Jian-Long and Toscano 2005).

Carboxylesterases are important hydrolyses for the detoxification of various endogenous and exogenous substances. This large family of enzymes can be characterized based on their electrophoresis nobilities or inhibitor and

substrate specificities (Dauterman, 1985; Soderlund, 1997). Esterase activity in treated samples at lower pesticide concentration and control samples showed an increased activity. The activity was decreased high dose. This indicates that xenobiotic elicit increased activity of esterases at lower concentrations in *Spodoptera litura*. In supporting this suggestion (Yang *et al*., 2004; Gao *et al*., 1998; Xu *et al*., 1999) reported a high esterase activity is normally correlated with development of resistance in insects. Glutathione S-transferase activity was higher in control as compared to treatments, showing that organophosphate and synthetic pyrethroid interfere with GST mediated detoxification at low and high concentrations. GSH being a free radical scavenger is known to be an important component of pesticide detoxification in several insects (Buyukguzel, 2009).

However Decreased GSH content indicates that organophosphate and synthetic pyrethroid may consume GSH through detoxification reactions of glutathione-dependent enzymes (Jovanovic-Galovic *et al*. 2004).

Acetylcholinesterase is a key enzyme in the insect nervous system, terminating neurotransmission by the hydrolysis of the neurotransmitter acetylcholine. AChE is the target-site of inhibition by organophosphate and carbamate insecticides, and if this hydrolysis does not take place, build-up of acetylcholine occurs and leads to repeated firing of neurons and ultimately death of the insect (McCaffery, 1999; Gunning and Moores, 2001).

Most of the organophosphate induces excitotoxicity by phosphorylating the serine of active site in the AChE blocking the hydrolysis of the neurotransmitter acetylcholine (Hirvonen et al.1993).These metabolically activated intermediates inhibit not only AChE but also impair enzymatic and non enzymatic activity that actually functions to detoxify contaminants (Malik & Summer 1982). AChE activity was higher in pyrethroid treatment as compared to control and organophosphates; this suggests an increased expression level of AChE enzyme in response to pesticide exposure showed tolerance and increased AChE activity, target site insensitivity which seems to be dominant mechanisms conferring resistance in lepidopteran pest (Gao, 1992). The present study shows that esterase and acetylcholine esterase enzymes may play an important role in detoxification of synthetic pyrethriods and organophosphates in *Spodoptera litura*. Further studies are carrying out to understand the gene expression patterns in field population.

ACKNOWLEDGEMENTS

The authors acknowledge the instrument and infrastructural facilities has been provided by Department of Biotechnology, Periyar University, Salem- India.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 18: 265-267.

Armes, N. J.; Wightman, J. A.; Jadhav, D. R.; and Ranga Rao, G. V. (1997). Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. Pesticide Science. 50: 240-248.

Beutler, E.; Duron, O.; and Kelley, B. M. (1963). Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine. 61**:** 882-888.

Corbett, J. R. (1974). The Biochemical Mode of Action of Pesticides; Academic Press. London, UK, pp.102-130.

Crow, J. A.; Potter, P. M.; Borazjani, A.; and Ross, M. K. (2007). Hydrolysis of pyrethroid by human and rate tissues: examination of intestinal, liver and serum carboxylestersaes.

Toxicology & Applied Pharmacology. 221: 1-12.

Dauterman, W. C. (1985). Insect metabolism: extramicrosomal. In: *Comprehensive* Insect Physiology, Biochemistry and Pharmacology (Eds. GA., Kerkut LI., Gilbert); Pergamon, Oxford, UK, 12: 713-730.

Denholm, I.; Pickett, J. A.; and Devonshire, A. L. (1998)**.** Insecticide resistance: from mechanisms to management. Philosophical Transactions of Royal Society Biological Sci., 353: 1673-1795.

Dhir, B. C.; Mohapatra, H. K.; and Senapati, B. (1992). Assessment of crop loss in ground nut due to tobacco caterpillar, *Spodoptera litura* (F.). Indian J. Plant Protectection. 20: 215–217.

Ellman, G. L.; Courthy, K. D.; Andres, V.; and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochemical Pharmacology. 7**:**115-121.

Ender Buyukguze, (2009). Evidence of Oxidative and Antioxidative Responses by *Galleria mellonella* larvae to Malathion. J. Economic Entomology. 102:152-159.

Finney, D. J. (1971). Probit analysis: A statistical treatment of the sigmoid response curve; *Cambridge university press*. London, New York: Melbourne, pp. 333.

Gao, X. W.; Zhao, Y.; Wang, X.; Dong, X. L.; and Zheng, B. Z. (1998). Induction of carboxylesterase in *Helicoverpa armigera* by insecticides and plant allelochemicals. Acta Entomologica Sinica. 41: 5-11.

Gao, X. W. (1992). Effects of host plant on Carboxylesterase activity in cotton aphid *Aphis gossypii* Glov. Acta Entomologica Sinica. 3:267-272.

Gunning, R. V.; and Moores, G. D. (2001). Insensitive acetylcholinesterase as sites for resistance to organophosphates and carbamates in insects: insensitive acetylcholinesterase confers resistance in Lepidoptera; In: Biochemical Sites of Insecticide Action and Resistance. (Ed. I. Ishaaya), Springer- Verlag, Berlin, Heidelberg, pp.221-238.

Habig, W.H.; Pabst, M. J.; and Jakoby, W. B. (1974). Glutathione S-transferase: The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry. 249: 7130-7139.

Hassan, F.; Dahi Yasser, A.; Sayed Nehad, M.; Barkey Mona, F.; and Abd Aziz. (2009). Toxicological and biochemical studies of Methylamine Avermactin, a new type of bioinsecticide against the cotton leaf worm, *Spodoptera littoralis*. J. Biological Sci., 2: 103-116.

Hemingway, J.; Hawkes, N. J.; McCarroll, L.; and Ranson, H. (2004**).** The molecular basis of insecticide resistance in mosquitoes. Insect Biochemistry & Molecular Biology. 34: 653-665.

Hirvonen, M.; Paljarvi, R.; and Savolainen, K. M. (1993). Malaoxon-induced neurotoxicity in old rats: alterations in cerebral inositol lipid signalling, brain tissue calcium levels and early neuronal injury. Toxicology. 79: 157-167.

Holloway, J. D. (1989). The moths of Borneo: family Noctuidae, trifinesubfamilies: Noctuinae, Heliothinae, Hadeninae, Acronictinae, Amphipyrinae, Agaristinae. Malayan National J., 42: 57-226.

Jian-Long, B.; Toscano. (2005). Toxicity of Selected Insecticides against Immature and Adult Stages of Beet Armyworm (Lepidoptera: Noctuidae). Journal of subtropical plant Science. 57: 1- 4.

Jovanovic-Galovic, A.; Blagojevic, D. P.; Grubor-Lajsic, G.; Worland, R.; and Spasic, M. B. (2004). Role of antioxidant defense during different

stages of preadult life cycle in European corn borer (*Ostrinia nubilalis,* Hubn.): diapauses and metamorphosis. Archives of Insect Biochemistry and Physiology. 55:79-89.

Kranthi, K. R. (2005). Insecticide resistance monitoring, mechanisms and management manual; Central Institute for Cotton Research. India, pp.78-82.

Kranthi, K. R.; Jadhav, D. R.; Kranthi, S.; Wanjari, R. R.; Ali, S. S.; and Russell, D. A. (2002). Insecticide resistance in five major insect pests of cotton in India. *Crop Protection*. 21: 449 - 460.

Kranthi, K. R.; Jadhav, D. R.; Wanjari, L. R.; Alis, S.S.; and Russel, D. (2001). Carbamate and organophosphate resistance in cotton pests in India 1995-1996. *Bulletin Entomological Research*. 91:37-46.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; and Randall, R. J. (1951**).** Protein measurement With the Folin-Phenol reagent. J. of Biological Chemistry. 193: 265-275.

Malik, J. K.; and summer, K. M. (1982). Toxicity and metabolism of Malathion and its impurities in isolated rat hepatocytes: role of glutathione. Toxicology and Applied Pharmacology. 66: 69-76.

McCaffery, A. R. (1999). Resistance to insecticides in *heliothine* Lepidoptera: a global view In: Insecticide Resistance: From Mechanisms to Management*;* (Eds. I. Denholm A., Pickett and AL., Devonshire), *CABI* Publishing London, pp.59-74.

Rao, G. V. R.; Wightman, J. A.; and Ranga Rao, D. V. (1993). World review of the natural enemies and diseases of *Spodoptera litura* (Lepidoptera:Noctuidae). Insect Science and Application. 14: 273-284.

Robertson, J. L.; and Preisler, H. K. (1992). Pesticide Bioassay with Arthropods. CRC Press, Boca Raton, Florida.

Soderlund, D. M. (1997). Molecular mechanisms of insecticide resistance; In: Molecular mechanisms of resistance to agrochemicals. (Vol. 13 Ed. V. Sjut), Spinger, Heidelberg, New York, pp.21-56.

Tang, Z. H.; Wood, R. J.; and Cammak, S. L. (1990). Acetyl cholinesterase activity in organ phosphorous and carbamate resistance and susceptible strains of the *Culex pipens* complex. Pesticide Biochemistry and Physiology. 37: 192-199.

Vontas, J. G.; Small, G. J.; Nikou, D. C.; Ranson, H.; and Hemigway, J. (2002). Purification, molecular cloning and hetrrologous expression of a glutathione S-transferase involved in insecticide resistance from the rice brown planthopper, *Nilaparvata* lugens. Biochemistry Journal. 362: 329-337.

Xu, X. S.; Han, Z. J.; and Wang, Y. C. (1999). Relationship between Carboxylesterase and Organophosphate resistance in *Helicoverpa armigera* (Hubner). Journal of Nanjing Agricultural University. 22: 41– 44.

Yang, Y.; Wu, Y.; Chen, S.; Devine, G. J.; Denholm, I.; Jewess, P.; and Moores, G D. (2004). The involvement of microsomal oxidases in pyrethroid resistance in *Helicoverpa armigera* from Asia. Insect Biochemistry and Molecular Biology. 34:763-773.