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RESISTANCE IN *SPODOPTERA LITURA* (F.) TO INSECTICIDES AND DETOXIFICATION ENZYMES

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ABSTRACT

Standard leaf dip bioassay experiments were conducted to study the insecticide resistance in *Spodoptera litura* (F.) in soybean. Chlorantraniliprole was the most toxic while, cypermethrin and organophosphates (profenophos and triazophos) recorded least toxicity against *S. litura* populations. Field population of *S. litura* showed the highest resistance to cypermethrin with resistance ratios (RRs) ranging from 244 to 376. The field populations collected from three locations of Southern Rajasthan exhibited low resistance to newer insecticides, chlorantraniliprole and spinetoram with Lab-SS strain. All the enzyme activities were significantly higher in the whole larval extracts and midgut extracts of *S. litura* larvae when compared to the Lab-SS strain. Analysis of the carboxyl esterase enzyme in the native PAGE assay revealed that the electrophoretic profiles showed presence of different band regions (EST 1 to EST 7) with esterase activity in the field populations along with the laboratory susceptible strain. The susceptibility of three field populations to different group of insecticides along with the profile of detoxification enzymes indicate the need to formulate a region-specific insecticide resistance management (IRM).

Key words: *Spodoptera litura*, carboxylesterase, detoxification enzymes, insecticide resistance, electrophoresis, resistance ratio, chlorantraniliprole, cypermethrin, organophosphates

Larvae from the *Spodoptera* genus are distributed worldwide and gaining importance due to their polyphagous nature and causing significant losses in economically important crops. *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is commonly known as the common cutworm and one of the most important polyphagous pests of soybean. Larvae feed on the foliage, resulting in complete defoliation and in case of severe infestation, complete devastation of soybean crop occurs during its reproductive stage (Chattopadhyay et al., 2019). In recent years, the tobacco caterpillar has become a serious pest on soybean in some parts of India (Dhaliwal et al., 2010; Chattopadhyay et al., 2019). Indiscriminate use of different groups of insecticides for the control of *S. litura* led to the development of multiple types of resistance (Armes et al., 1997; Kranthi et al., 2001; Ahmad et al., 2007a,b). The extensive use of conventional insecticides belonging to organochlorines, carbamates, organophosphates (OPs) and pyrethroids) and other newer group of chemicals like fipronil, avermectins, indoxacarb, spinosad, emamectin benzoate, chlorantraniliprole and insect growth regulators (Ahmad et al., 2008) have been reported to shown resistance in *S. litura* from different countries viz., China, Pakistan and India.

Metabolic detoxification mechanism with elevated enzyme activities is the main cause of insect pests showing insecticide resistance against a different group of insecticides. Increased level of detoxification enzymes like cytochrome P450 (P450), carboxy/ cholinesterase (CCE), and glutathione S-transferase (GST) is linked to insecticide resistance (Sreelakshmi et al., 2019). There have been reports on poor bioefficacy of the conventional insecticides as well as newer chemicals against *S. litura* in soybean crop and this led to the outbreak of this pest on soybean in various districts in Southern Rajasthan. Therefore, the present study was undertaken to determine the information on resistance in *S. litura* against the new chemistry insecticides and conventional insecticides.

MATERIALS AND METHODS

Susceptible strain (Lab-SS) of *S. litura* obtained from National Bureau of Agriculturally Insect Resources (NBAIR), Bengaluru kept and reared in the laboratory without exposure to insecticides. Insecticide bioassays were performed during 2017 at Agricultural Research Station (ARS), Banswara, Rajasthan to get the data on mortality for different group of insecticides. The field populations of *S. litura* were collected from

soybean crops located in ARS, Banswara (ARSB) and farmers field at Banswara (FFB), Pratapgarh (FFP) districts at Southern Rajasthan, India from 2017-2019 during *Kharif* season. Approximately 100-200 third to fifth instar larvae were collected from soybean crop and reared on natural diet (castor leaves). The culture was maintained in the insect rearing room at ARS, Banswara at a temperature of 25± 1^{0}C, 60± 5% RH and a 14:10 h light: dark cycle. Commercial formulations of pesticides viz., Emamectin benzoate (Proclaim 5% SG; Syngenta India Limited); Chlorantraniliprole (Coragen 18.5% SC; Dupont); Indoxacarb (Avaunt15.8 EC; DuPont); Thiodicarb (Larvin 75% WP; Bayer Crop Science); Cypermethrin (Cymbush 25% EC; Bayer Crop Science); Profenophos Carina 50EC; PI Industries; Triazophos (Josh 40% EC; UPL); Spinetoram (Delegate 11.7% SC; Dow Agrosciences); Novaluron (Rimon 10% EC; Crystal) were used for the bioassay.

The standard leaf dip bioassay (Sayyed et al., 2000; Ahmad et al., 2007b) was used to determine the dose-response for insecticides from different groups in *S. litura*. Various concentrations of insecticides were prepared using distilled water containing 0.1% Triton X-100 and leaf discs (8/5 cm) of castor was dipped into the test insecticides for 15 seconds with gentle agitation. Control discs were treated with water containing 0.1% Triton X-100. The castor leaf discs treated with insecticide or water (control) were dried at room temperature for 1 hr. Treated leaves were then transferred to individual Petri dishes (8/5 cm in diam.) which were lined with moistened with Whatman filter paper. Three third-instar larvae of *S. litura* from F1 generation laboratory cultures were released onto each leaf disc and maintained at 27± 1^{o}C, 60-70% RH with a 14:10 hr light: dark cycle. At least thirty 3rd instar larvae were exposed to each dose for at least six concentrations of each insecticide. Mortalities after 48 or 72 hr of exposure were recorded. The 3rd to 5th instar larvae of *S. litura* were separated and starved for three hr to remove food particles. The crude and the microsomal preparation were prepared according to Mohan and Gujar, 2003; Ramya et al., 2016). The total activities of P450s, ESTs, and GSTs were determined by using crude enzyme preparation. The QuickStart Bradford assay kit of Biorad was used for the estimation of protein content as per Bradford (1976). The cytochrome P450 assay was carried out as per the procedure of Mohan and Gujar (2003) and Kranti (2005). Carboxylesterase activity was determined by the method of Kranti (2005). The GSH-S-transferase enzyme activity in the gut samples of *S. litura* was measured by the method of Mohan and Gujar (2003) and Kranti (2005). The acetylcholinesterase enzyme activity was measured by the method of Kranti (2005). Gel electrophoresis was carried out on Native PAGE with 10% resolving gel for the separation of esterase enzymes of *S.litura* along with the Lab-Susceptible populations as per the procedure of Kranti (2005). Bioassay data of three replicates were pooled and subjected to Abbotts' formula (Abbott, 1925) and analyzed using Indostat Services, Hyderabad.

RESULTS AND DISCUSSION

The results revealed that chlorantraniliprole was the most toxic while the synthetic pyrethroid, cypermethrin and organophosphates (profenophos and triazophos) recorded least toxicity to *S.litura* populations collected from all the three locations (Table 1). The indiscriminate use of these compounds in the region is the cause for the development of resistance. Karuppaiah et al. (2017) revealed that chlorantraniliprole 18.5% SC @ 1-4 ppm (LC_{50}) and emamectin benzoate 5% SG @ 1-3ppm (LC_{50}) was found highly effective. Chlorantraniliprole was the most toxic insecticide against jute hairy caterpillar too (Selvaraj et al., 2015). The field population of *S. litura* showed highest resistance to cypermethrin with resistance ratios (RRs) ranging from 244 to 376. Ruttanaphan et al. (2018) also reported that the field populations from Thailand exhibited resistance to cypermethrin. The resistance to chlorantraniliprole and spinetoram was the lowest in the ARSB and FFP population, respectively. Dhawan et al. (2007), Bhatnagar et al. (2013), Kaur et al. (2007) and Karuppaiah et al. (2017) also reported higher toxicity of chlorantraniliprole and emamectin benzoate. The carbamate, thiodicarb and oxadiazine, indoxacarb evaluated in the present study, had low to moderate levels of resistance and RR values ranged from 10- to 40-fold and 19- to 25-fold, respectively. Tong et al. (2013) recorded low to medium levels of resistance to indoxacarb. Profenophos and triazophos showed RR values from 43 to 172 compared to the Lab-SS strain. Tong et al. (2013) reported a high level of 50 fold resistance in populations from China. In India, Armes et al. (1997) and Kranti et al. (2002) reported resistance to organophosphates. In the case of insect growth regulator, novaluron, populations revealed high to very high-level of resistance and reported RR values ranging from 52- (FFB) to 106-fold (ARSB).

Detoxification enzyme activities, viz., GSH-S-transferase (GST), cytochrome P450 (MFO),

Table 1. Toxicity of insecticides against field populations of *S. litura*

Insecticides	Location	LC50 (PPM)	Fiducial limit (95% CI)		χ^2	Slope (± SE)	RR	Resistance level
			Lower	Upper				
Chlorantraniliprole	FFB	0.78	0.47	1.29	5.49	1.31± 0.14	8.66	Very low
	ARSB	0.36	0.21	0.63	2.93	1.28± 0.14	4.00	Very low
	FFP	1.07	0.64	1.79	0.58	1.06± 0.17	11.88	Low
	Lab-SS	0.09	0.05	0.16	2.10	1.20± 0.15	1.00	-
Indoxacarb	FFB	2.12	1.21	3.73	4.33	0.98± 0.18	21.2	Moderate
	ARSB	1.92	0.98	3.76	6.32	0.78± 0.23	19.2	Low
	FFP	2.57	1.35	4.88	2.40	0.85± 0.21	25.70	Moderate
	Lab-SS	0.10	0.06	0.16	6.40	1.14± 0.16	1.0	-
Thiodicarb	FFB	6.04	2.97	12.27	1.48	0.76± 0.24	35.53	Moderate
	ARSB	6.90	3.61	13.15	3.60	0.81± 0.22	40.58	Moderate
	FFP	1.71	0.77	3.79	6.16	0.89± 0.20	10.05	Low
	Lab-SS	0.17	0.22	0.64	2.07	0.99± 0.18	1.00	-
Emamectin Benzoate	FFB	1.41	0.78	2.56	0.67	1.07± 0.17	17.62	Low
	ARSB	1.09	0.52	2.29	2.79	0.92± 0.19	13.62	Low
	FFP	2.52	1.33	4.76	1.84	0.89± 0.20	31.50	Moderate
	Lab-SS	0.08	0.04	0.17	1.28	0.79± 0.23	1.00	-
Cypermethrin	FFB	15.04	7.24	17.19	5.23	1.47± 0.14	376	Very high
	ARSB	11.16	7.24	17.19	5.23	1.47± 0.12	279	Very high
	FFP	9.76	5.96	14.73	4.53	1.44± 0.12	244	Very high
	Lab-SS	0.04	0.02	0.08	0.24	0.77± 0.11	1.00	-
Spinetoram	FFB	1.06	0.52	2.15	2.81	0.79± 0.10	8.83	Low
	ARSB	1.19	0.64	2.23	4.06	1.01± 0.18	9.91	Low
	FFP	0.71	0.36	1.37	1.36	1.04± 0.17	5.91	Low
	Lab-SS	0.12	0.08	0.18	6.84	1.49± 0.12	1.00	-
Novaluron	FFB	3.64	1.88	7.07	6.47	1.01± 0.18	52	High
	ARSB	7.47	4.24	13.16	3.54	1.01± 0.18	106.71	Very high
	FFP	4.61	2.47	8.60	6.01	0.98± 0.18	65.85	High
	Lab-SS	0.07	0.03	0.15	0.60	0.87± 0.21	1.00	-
Profenophos	FFB	40.52	29.06	56.50	1.88	1.57± 0.11	109.51	Very high
	ARSB	16.04	9.83	26.17	1.42	1.24± 0.14	43.35	Moderate
	FFP	63.93	40.65	100.54	4.27	1.60± 0.11	172.78	Very high
	Lab-SS	0.37	0.22	0.64	2.07	1.00± 0.18	1.00	-
Triazophos	FFB	52.50	31.48	87.56	8.43	1.03± 0.17	78.35	High
	ARSB	41.91	21.46	81.83	10.25	1.10± 0.16	62.55	High
	FFP	85.33	52.00	140.05	3.05	1.27± 0.14	127.35	Very high
	Lab-SS	0.67	0.34	1.31	6.23	0.83± 0.22	1.00	-

Resistance level classified as none (RF= 1), very low (RF = 2–10), low (RF= 11–20), moderate (RF= 21–50), high (RF=51–100) and very high (RF=> 100) (Ahmad et al. 2007)

carboxylesterase (CarE) and acetylcholinesterase (AChE) of *S.litura* are presented in Fig. 1-4. All the enzyme activities were significantly higher in the whole larval extracts and midgut extracts of larvae collected from all three locations when compared to the Lab-SS strain. The whole larval extracts and midgut extracts from the field populations (FFB, ARSB, FFC and FFP) had significantly higher carboxylesterase enzyme activity. Cui et al. (2015) demonstrated that an increased amount of carboxylesterase is a major factor that induces insecticide resistance. Sreelakshmi et al. (2019) also reported that the resistance field populations of *S.litura* exhibited a two to three-fold increase in carboxylesterase (CarE) enzyme activity. Field populations of *S.litura* showed increased GSH-S-transferase enzyme activity in whole larval extracts and midgut extracts which ranged from 5.3 to 13.8 fold and 5.0 to 11.47 fold. In the case of cytochrome P450 the midgut extracts were studied, and o-demethylase activity of FFP and FFC field populations had increased enzyme activity with the 19-26 folds with that of the Lab-SS strain. Similarly, Sreelakshmi et al. (2019) observed the variations in the specific activity profiles of glutathion-S-transferase (GST) in the field populations and the laboratory susceptible strains. Resistant populations exhibited the higher MFO specific

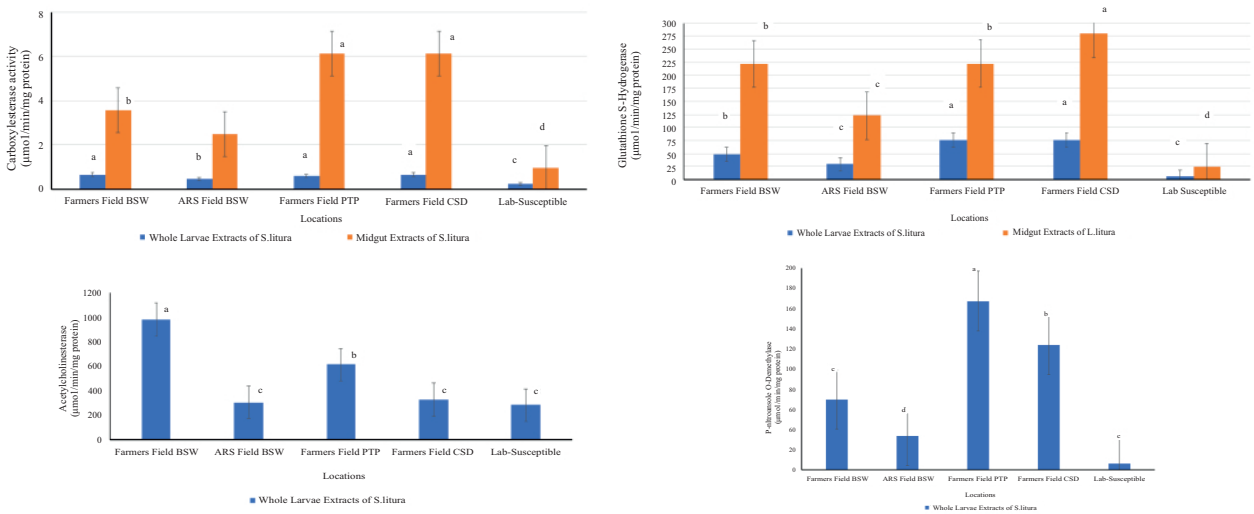

Fig. 1-4. GSH-S-transferase (GST), cytochrome P450 (MFO), carboxylesterase (CarE) and acetylcholinesterase (AChE) activity in *S.litura*

activity when compared to the laboratory susceptible strains in the studies by Huang and Han (2007) and Karuppaiah et al. (2017). This high expression level of MFO specific activity may be the reason for the development of resistance in synthetic pyrethroid. Similarly, MFO activities were found to be higher in the field populations (Sreelakshmi et al., 2019).

Among the different detoxification enzymes studied, the acetylcholinesterase activity was found highest in whole larval extracts of all the field populations of *S. litura*. Sreelakshmi et al. (2019) observed that the field populations of *S.litura* exhibited the 4- to 16- fold increase in specific activity of acetylcholine esterase (AChE). In the present study, a high amount of GST activity was observed in *S. litura* whereas the total enzyme activity is varying with the results of Karuppaiah et al. (2017). Analysis of the carboxyl esterase enzyme in the native PAGE assay revealed that the electrophoretic profiles showed the presence of different band regions (EST 1 to EST 7) (Fig. 5) with esterase activity in the field populations of *S. litura* along with the laboratory susceptible strain. The results demonstrated that the five populations

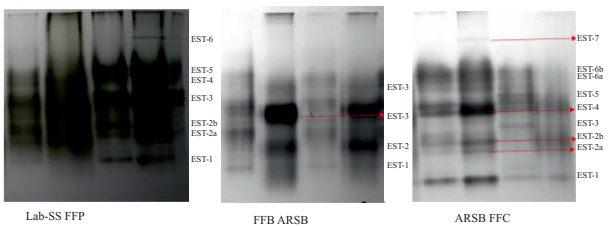

Fig. 5. Carboxyl esterase (CarE) in the native PAGE assay-, electrophoretic profiles showing band regions (EST 1 to EST 7)

and Lab SS of *S. litura* shared some of the similar esterase band patterns. One or two prominent esterase bands were observed in all the field populations except Chottisadri populations whereas, one faint esterase band was present in FFB and FFC populations. Esterase bands 5, 6, and 7 were not visible in the Lab-SS strain of *S. litura*. Ramya et al. (2016) analyzed the electrophoretic profiles of four bands (Est-1, Est-2, Est-3 and Est-4) with CarE activity in the field populations of diamond backmoth and one (Est-2) of these bands in the laboratory susceptible strain.

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REFERENCES

Abbott M S. 1925. A method of computing effectiveness of an insecticide. Journal of Economic Entomology 18: 265-26.

Ahmad M, Sayyed A H, Saleem M A. Ahmad M. 2008. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. Crop Protection 27: 1367-1372.

Ahmad M, Arif M I. Ahmad M. 2007a. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Protection 26: 809-817.

Ahmad M, Sayyed A H, Crickmore N. Saleem M A. 2007b. Genetics and mechanism of resistance to deltamethrin in a field population of *Spodoptera litura* (Lepidoptera: Noctuidae). Pest Management Science 63: 1002-1010.

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

Bhatnagar B, Kumar Y. Karnatak A K. 2013. Evaluation of toxicity of indoxacarb and flubendiamide against *Spodoptera litura* Fab. Bioinfolet 10: 394-396.

Bradford M M. 1976. A rapid and sensitive a method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Chattopadhyay N, Balasubramaniam R, Attri S D, Kamaljeet R, Gracy J, Khedikar S. Karmakar C. 2019. Forecasting of incidence of *Spodoptera litura* (Tobacco caterpillar) in soybean and cotton using statistical and synoptic approach. Journal of Agricultural Meteorology 21: 68-75.

Cui F, Xia-Li M, Chang H. J, Mao Y, Zhang H Y, Xia L U, Guo-Yan S, Lin-Lang M, Liu L. Ling Qiao C. 2015. Carboxylesterase mediated insecticide resistance: quantitative increase induces broader metabolic resistance than qualitative change. Pesticide Biochemistry and Physiology 2: 88-96.

Dhaliwal G S, Jindal V. Dhawan A. K. 2010. Insect Pest Problems and Crop Losses: Changing Trends. Indian Journal of Ecology 37: 1-7.

Dhawan A K, Kaur A. Kumar R. 2007. Relative toxicity of new insecticides against *Spodoptera litura*. Annals of Plant Protection Sciences 15: 238-239.

Huang S. Han Z. 2007. Mechanisms for multiple resistance in field populations of common cutworm, *Spodoptera litura* (Fabricius) in China. Pesticide Biochemistry and Physiology 87: 14-22.

Karuppaiah K, Srivastava C, Padaria J C. Subramanian S. 2017. Quantitative changes of the carboxylesterase associated with pyrethroid susceptibility in *Spodoptera litura* (Lepidoptera: Noctuidae). African Entomology 25: 175-182.

Kaur A, Kang B K. Singh B. 2007. Toxicity of different insecticides against *Spodoptera litura* (Fabricius) in Punjab, India. Pesticide Research Journal 19: 47-50.

Kranti K R. 2005. Insecticide resistance – monitoring, mechanisms and management manual. CICR, Nagpur, India, and ICAC, Washington, U.S.A.

Kranthi K R, Jadhav D R, Wanjari R R, Ali S S. Russell D. 2001. Carbamate and organophosphate resistance in cotton pests in India, 1995 to 1999. Bulletin of Entomological Research 91: 37-46.

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

Mohan M. Gujar G T. 2003. Local variation in susceptibility of the diamond backmoth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. Crop Protection 22: 495-504.

Ramya S L, Venkatesan T, Murthy K S, Jalali S K. Abraham V. 2016. Field-evolved insecticide resistance and biochemical validation of enzyme a ctivities in diamondback moth, *Plutella xylostella.* Indian Journal of Plant Protection 44: 9-15.

Ruttanaphan T, Pluempanupat W. Bullangpot V. 2018. Cypermethrin resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) from three locations in Thailand and detoxification enzyme activities. Agriculture Natural Resources 52: 484-488.

Sayyed A H, Haward R, Herrero S, Ferre J. Wright D J. 2000. Genetic and biochemical approach for characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a field population of the diamondback moth, *Plutella xylostella*. Applied Environmental Biology 66: 1509-1516.

Selvaraj K, Ramesh Babu V, Gotyal B S. Satpathy S. 2015. Toxicity and bioefficacy of individual and combination of diversified insecticides against jute hairy caterpillar, *Spilarctia obliqua*. Journal of Environmental Biology 36: 1409-1414.

Sreelakshmi P, Thomas Biju Mathew, Umamaheswaran K. Josephrajkumar A. 2019. Modulation in activity profiles in insecticide-resistant population of tobacco caterpillar, *Spodoptera litura* (Fabricius). Current Science 116: 664-669.

Tong H, Qi S, Xiaomao Z. Lianyang B. 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. Journal of Pest Science 86: 599-609.

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