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EFFECT OF INSECTICIDES ON SUSCEPTIBILITY LEVEL AND DETOXIFYING ENZYMES IN COTTON LEAFHOPPER *AMRASCA (SUNDAPTERYX) BIGUTTULA* (ISHIDA)

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ABSTRACT

The present study evaluated the relative susceptibility of insecticides viz., imidacloprid, thiamethoxam, thiacloprid, flonicamid, clothianidin, diafenthiuron, spiromesifen, thiodicarb and chlorpyriphos against field collected population of *Amrasca (S.) biguttula.* Out of nine insecticides, maximum susceptibility was observed with thiamethoxam. The descending order of susceptibility was observed as thiamethoxam> thiacloprid> diafenthiuron> spiromesifen> imidacloprid> clothianidin> flonicamid> thiodicarb> chlorpyriphos. Based on the relative toxicity value it was observed that the insecticides such as chlorpyriphos, thiodicarb, flonicamid and clothianidin were 14.04, 12.01, 9.43 and 9.41x, respectively less toxic as compared to thiamethoxam. The detoxification enzyme assay revealed that the activity of esterase was high in thiamethoxam and thiacloprid exposed leafhopper, while cytochrome p450 activity was high in spiromesifen, thiamethoxam and thiacloprid exposed ones. Elevated level of esterase and cytochrome p450 in the insecticide exposed leafhoppers indicates the probability of insecticide resistance development.

Key words: Cotton, *Amrasca* (*Sundapteryx*) *biguttula*, insecticides, resistance, susceptibility, detoxifying enzymes, cytochrome p450, esterases

Cotton (*Gossypium* sp.) is an important cash crop and also known as "white gold" grown in more than 83 countries across the world. Introduction of *Bt* cotton to control the bollworm complex resulted in the disruption of pest complex in cotton ecosystem. The minor sucking pests have attained major pest status in many parts of India (Mohan and Nandini, 2011). Among the sap sucking pests, the leafhopper also called Indian jassid, *Amrasca* (*Sundapteryx*) *biguttula* (Ishida) is major pest of cotton in India, Pakistan, Bangladesh, China, and North Africa (Murugesan et al., 2009; Saeed et al., 2015; Kranthi, 2017). Both adults and nymphs suck the sap from leaves and inject toxic saliva resulting in 'hopper burn' symptoms, which ultimately result in the loss of plant vigour and significant yield losses up to 50% (Atakan, 2009).

Indiscriminate use of insecticides has led to development of insecticide resistance in leafhoppers. The cotton leafhopper had been found to be resistant to conventional groups such as cyclodienes, organophosphates, and pyrethroids (Santhini and Uthamasamy, 1997; Chalam and Subbaratnam, 1999; Chalam et al., 2001). Also, the fact that the Bt cotton seeds sold in the market are imidacloprid treated adds to the development of resistance (Kshirsagar et al., 2012). In Punjab, Rajwinder and Kang (2015) observed the no serious levels of resistance to imidacloprid, dimethoate, monocrotophos, triazophos and acetamiprid in cotton leafhopper. Continuous and indiscriminate use of organophosphates and neonicotinoids has probably led to development of resistance (Sagar and Balikai, 2014). Substantial misuse of insecticides resulted in the development of resistance to organophosphates (Rajwinder and Kang, 2015) and neonicotinoids (Shreevani et al., 2012). The studies pertaining to susceptibility status of leafhopper to conventional and newer molecules are available. However, very less studies are available on the dynamics of detoxification enzymes towards in insecticide resistant cotton leafhopper populations. Hence, the present study to find out the susceptibility status of cotton leafhopper to most commonly used insecticides and the level of detoxification enzymes present in the insecticide exposed leafhoppers.

MATERIALS AND METHODS

The leafhopper samples were collected from the experimental farms and the toxicity assay of insecticides was carried out under laboratory condition (27 ± 2^0C, 70% RH), at the Insectary, ICAR-CICR, RS, Coimbatore. The selection of the insecticide was based on the recommendations of Central Insecticide Board

and Registration Committee (CIBRC), Government of India and famers' practice. Neonicotinoids (imidacloprid 17.80%SL, thiamethoxam 25%WG, clothionidin 50%WDG and thiacloprid 21.7% w/w); organophosphate (chlorpyriphos 20%EC); carbamates (thiodicarb 75%WP); tetronic acids (spiromesifen 22.9%SC); pyridine carboxamide (flonicamid 50%WG); and insect growth regulator (diafenthiuron 50%WP) were included in the bioassay studies. Commercial formulations of insecticides were diluted to obtain the desired concentrations. Preliminary range finding tests were carried out to fix the test concentrations, which cause 20 to 80% mortality to the leafhoppers.

Leaf dip method (IRAC method No. 001) according to Nauen and Elbert (2003) was followed with slight modification for bioassay. Fresh tender cotton leaves (variety LRA 5166) with petioles free from any insect infestation and without any pre-exposure to insecticides were used. The leaves were washed thoroughly with running tap water and shade dried on blotting paper. Individual leaves were dipped into the desired concentration of insecticides with cut portions of petioles wrapped with wet cotton inside micro centrifuge tubes. Each treatment included five replicates and, in each replication, ten leafhopper adults were exposed. The bioassays were conducted in insect rearing chamber with the temperature, photoperiod, and RH conditions as mentioned earlier. Insect mortality was recorded at 24 hr after treatment. The leafhoppers were considered dead, if no coordinated movement or deficient response to external stimulus (i.e. when gently probed with a fine paintbrush) was observed under the light microscope. Mortality was estimated by counting the total number of dead and live insects. The survived adults of leafhopper in each treatment were transferred to -20^{0}C for further study on detoxification enzyme assay.

The survived adults exposed to insecticides were used for assessing the activity of detoxifying enzymes such as carboxylesterase (COE) and cytochrome p450 which are commonly implicated against organophosphates (OPs)/ carbamates/ nenonicotinoids in insects. Total COE activity was estimated using 1-naphthylacetate as substrate (Stumpf and Nauen, 2002) and the activity was measured at 450 nm continuously for 10 min at 27°C a SPECTRA maxplus384 absorbance microplate reader (Molecular Devices) and expressed in micromoles of napthol formed/ min/ µg protein. Cytochrome p450 activity was estimated and expressed in terms of general oxidase, which is an indirect measure of cytochrome p450 by heme-peroxidation using 3,3',5,5'-tetramethyl-benzidine dihydronchloride as a substrate (Brogdon et al. 1997). Absorbance was read at 620 nm against blanks (wells containing all reaction components except enzyme source) in a SPECTRA maxplus384 absorbance microplate reader (Molecular Devices) after the 5 min incubation. A standard curve for heme peroxidase activity was prepared using different concentrations of cytochrome C. Cytochrome p450 (general oxidase) activity obtained from plate reading was expressed as equivalent units (EU) of cytochrome p450/ milligram of protein by using the standard curve of cytochrome C and they were expressed as µg/ ml/ min. Protein content was estimated to compute specific activity of detoxification enzymes. Standard protocol given by Bradford, (1976) was followed for the estimation of total protein content in *A (S). biguttula*.

Necessary corrections were made with respect to natural mortality in the control using Abbott's formula (Abbott, 1925) and then the data was subjected to probit analysis as per Finney (1971). The LC_{50} and LC_{90} values, 95% confidence limits, standard errors, the slopes of the regression lines and χ^2 significance tests, were estimated by probit analysis using PoloPlus 2.0 software (LeOra Software, California, United States). The relative toxicity (RT) of tested insecticide to leafhopper was calculated by keeping the most toxic insecticide as unit i.e (1.00). Enzyme activity ratio (EAR) was calculated by comparing the enzyme activity in (insecticide exposed) / control (insecticide unexposed).

RESULTS AND DISCUSSION

The acute toxicity assay revealed that of the nine insecticides evaluated against *A. biguttula,* thiamethoxam was found to be more toxic (n=210, 3.06 mg ai^{-}L) followed by thiacloprid (n=226, 3.06 mg ai-L). The descending order of susceptibility is thiamethoxam> thiacloprid> diafenthiuron> spiromesifen> imidacloprid> clothianidin> flonicamid> thiodicarb> chlorpyrifos. The relative toxicity (RT) value reveals that the insecticides such as chlorpyrifos, thiodicarb, flonicamid and clothianidin were 14.04, 12.01, 9.43 and 9.41 times less toxic respectively as compared to thiamethoxam (Table 1).

Insecticide exposure influences the level of esterase present in field population. As compared to control (unexposed to insecticide), the esterase activity increased due to insecticide exposure except for diafenthiuron. The activity was maximum in leafhopper exposed to thiamethoxam (21.614 uM napthol/ min/ mg protein) followed by imidacloprid (17.586 uM napthol/ min/ mg

Table 1. Relative toxicity of insecticides and the level of detoxifying enzymes in field populations of *A (S.) biguttula* in cotton

Insecticide	n	Slope	LC_{50} mg ai·L	Fiducial limit		RT*	LC_{99}	Enzyme activity			
				Min	Max			Esterase (uM napthol / min / mg protein)	EAR**	Mixed function oxidase (nM cyto / min / mg protein)	EAR
Imidacloprid	210	0.980+-0.172	13.07	3.85	27.84	4.28	26.57	17.586	1.22	81.47	0.93
Thiamethoxam	210	0.665+-0.124	3.06	1.26	6.012	1.00	25.95	21.614	1.50	117.30	1.34
Thiocloprid	210	0.411+-0.079	4.44	1.16	13.21	1.45	58.25	17.046	1.18	100.52	1.15
Flonicamid	210	0.541+-0.114	28.87	12.33	74.45	9.43	167.35	16.309	1.13	83.84	0.96
Clothionidin	210	0.653+-0.128	28.82	11.4	57.07	9.41	163.84	17.213	1.19	36.67	0.42
Diafenthiuron	210	0.862+-0.129	5.35	3.01	8.75	1.75	86.38	14.272	0.99	85.60	0.98
Spiromesifen	210	0.593+-0.111	5.61	2.32	12.07	1.83	81.55	16.507	1.14	118.17	1.35
Thiodicarb	210	0.914+-0.146	36.73	20.51	60.75	12.01	192.86	16.091	1.11	110.79	1.26
Chlorpyriphos	210	0.600+-0.138	42.55	19.04	97.42	14.04	283.95	16.504	1.14	98.23	1.12
Control	-	-	-	-	-	-	-	14.443	1.00	87.62	1.00

*Relative toxicity (RT) LC_{50} of test insecticide / LC_{50} of most toxic insecticide; **Enzyme activity ratio (EAR) = enzyme activity in field population (insecticide exposed)/ enzyme activity in field population (insecticide unexposed)

protein) and clothianidin ((17.213 uM napthol/ min/ mg protein). Based on the EAR it was observed that, all the insecticides were influenced by the level of esterase in the leafhopper. Similarly, insecticide exposure significantly influences the level of cytochrome p450. The activity was high in spiromesifen, thiamethoxam and thiacloprid exposed leafhopper. The elevated level of cytochrome p450 in the insecticide exposed leafhopper implies the probability of development of insecticide resistance. The values of enzyme activity ratios (EAR) for both the enzymes suggest the tolerance of leafhopper. Further, when compared to control, the EAR values were >1, which indicates role of detoxification enzymes in resistant development. The neonicotinoid insecticide, thiamethoxam was found to be more toxic followed by thiacloprid and imidacloprid. Application of neonicotinoids, imidacloprid and acetamiprid (Patel et al., 2017) and thiamethoxam (Rekha et al., 2017; Sesha MahaLakshmi and Prasad, 2020) reduced the leafhopper incidence. Next to thiamethoxam, thiacloprid, diafenthiuron and flonicamid were also found toxic. Application of diafenthiuron, flonicamid and fipronil in cotton reduced the incidence and enhanced the yield (Vimala et al., 2016; Kalyan et al., 2017). In the present study RT values reveal that chlorpyriphos, thiodicarb, flonicamid and clothianidin were 14.04, 12.01, 9.43 and 9.41x less toxic as compared to thiamethoxam.

India has a long history of insecticide resistance development in sucking pests of cotton including leafhopper (Santhini and Uthamasamy, 1997; 2011; Kshirsagar et al., 2012; Sagar and Balikai 2014; Rekha et al., 2017; Sesha Maha Lakshmi and Prasad, 2020). Metabolic resistance through detoxification enzymes is the most common phenomenon reported to occur in several species of insects showing resistance to insecticides (Devorshak and Roe, 1998; Li et al., 2007). Measurement of enzymatic activities of detoxification enzymes has been effectively used to gauge the level of tolerance to insecticides belonging to OP, carbamates and neonicotinoids in several species of insects and natural enemies (Saha et al., 2012; Srinivasa Murthy et al., 2014). Insecticide exposure significantly influences the level of mixed function oxidase in leafhopper. The MFOs activity was high in spiromesifen, thiamethoxam and thiacloprid exposed leafhopper. The elevated level of MFOs in the insecticide exposed leafhopper indicates the probability of development of insecticide resistance. Sagar et al., 2013 found relatively more MFOs activity in leafhoppers treated with organophosphates (monocrotophos, acephate, oxydemeton methyl and dimethoate) in major cotton growing districts of Karnataka, which indicated the role of MFOs in detoxification of insecticides.

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