ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 367-371: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

INSECTICIDE RESISTANCE MONITORING IN *AMRASCA BIGUTTULA BIGUTTULA* (ISHIDA) ON COTTON

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KEYWORDS

Insecticide resistance
A. biguttula biguttula
Imidacloprid
Flonicamid
Acephate
GST

Proceedings of National Conference on Harmony with Nature in Context of Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

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AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

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ABSTRACT

Insecticidal bioassay and insect biochemical assay were carried out during 2015-2016 to monitor insecticide resistance of organophosphates and neonicotinoids against cotton leafhopper, *Amrasca biguttula biguttula*. populations from 5 different talukas of Akola district. Leafhopper collected from Akot location recorded higher LC_{50} value i.e. 152.66 ppm and 110.36 ppm to imidacloprid and dimethoate insecticides respectively, whereas highest LC_{50} of 120.29 ppm was observed in Akola location, in case acephate insecticide. Newer molecule, flonicamid recorded higher LC_{50} (35.15 ppm) in Patur location population. Relatively more Glutathion S Transferase activity of 156.29 μmoles/min/ml of protein corresponding to higher LC_{50} value was recorded in Akola location leafhopper population followed by Akot (155.21 μmoles/min/ml of protein) and Patur (153.76 μmoles/min/ml of protein) as against 104.19 μmoles/min/ml of protein in susceptible strain, indicating the role of enzyme in detoxification of insecticides. *Increase in GST* activity in resistant strain over susceptible was 1.5, 1.5, 1.4, 1.5, 1.4 fold in *Akola, Patur, Murtizapur, Akot and Balapur talukas respectively*, suggesting the positive correlation between the detoxifying enzymes activity and the insecticide resistance level.

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INTRODUCTION

Cotton (*Gossypium spp*.) popularly known as "White Gold" is a major commercial crop designed as "King of Fibres" and has a global significance which is grown for its lint and seed. It provides 65 per cent raw material to industry and contributed one third of total exchange earning of India (Mayee and Rao, 2002). Hence after the introduction of Bt cotton in 2002, there was a check only to bollworm complex, but not to sucking pest population (Mohan and Nandini, 2011).

Among the sucking pest complex of Bt cotton, the cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) (Homoptera:Cicadellidae) is an alarming pest causing both quantitative and qualitative losses. Pesticides are key component for the management of crop pests. But continuous, injudicious and indiscriminate use of insecticides by farmers resulted in resistance development, resurgence of pests and destruction of natural enemies and pollution in environment. Most of the farmers are now experiencing that the recommended doses of largely used insecticides could not give the expected control of sucking pests (Patil *et al*., 2014). In spite of repeated use of insecticides, it is becoming difficult to manage this pest and control failures have been experience by cotton growers at times. Though control failure may be due to many factors, one of the major factors is development of resistance to insecticides (Pradeepa and Regupathy, 2002). Sood *et al*. (2006) reported that the insect is also fastly acuiring resistance even to neonicotinoid insecticide imidacloprid and suggested its restricted use. The development of resistance against insecticides by insects is either due to pre-adaptation or post adaptation process. In pre-adaptation, the genetic differences are already present in the insect population, while in post adaptation, the resistance is physiological by the presence of detoxifying enzyme which breakdown the toxicant much faster (Srivastava and Saxena, 2000). Thus, considering the possibility of development of resistance in cotton leafhoppers, the present investigation was undertaken.

MATERIALS AND METHODS

The present investigation was undertaken during 2015-2016 in the Department of Agricultural Entomology, PGI, PDKV Akola. Population of cotton leafhoppers was collected from the sprayed Bt cotton farmers fields from the different talukas of Akola district for monitoring insecticide resistance. The nymphal stages collected from the Bt cotton fields were considered for conducting bioassays. These populations were exposed to graded concentration of test insecticides viz., imidacloprid 17.8% SL, acephate 75 SP, dimethoate 30 EC, flonicamid 50 WG. The susceptible population was developed by maintaining leafhoppers on plants under cages without selection pressure of any insecticides. Bioassay was conducted as per method given by Ahmad *et al*. (1999). Initially six concentrations for each formulated insecticide were prepared by serial dilution method in distilled water. Cotton leaves were dipped into the test solutions with gentle agitation and allowed to dry for some time. Then petioles of treated leaves were fixed in agar gel (3%) in

plastic cup. The nymphs were transferred on leaves into plastic cup by fine camel hairbrush. Minimum 300 leafhoppers were used for each set of bioassay (60/plastic cup) and four such sets for each insecticide were maintained. The same numbers of leafhoppers were kept on untreated leaves at ambient room temperature in plastic cups and the mortality was assessed daily till fifth day after the treatment. Moribund leafhoppers not responding to probing were considered as dead. The values of median lethal concentration (LC_{50}) for each insecticide were worked out using probit analysis by Finney (1977) and by computer software SAS 9.2. Similarly, the LC_{50} values of these four insecticides against the susceptible population of cotton leafhoppers were calculated and compared with the LC_{50} values of field population to know the level of resistance as per method used by Kshirsagar et al. (2012).

The resistance ratio was calculated by using following formula

$$RR = \frac{\text{LC50 of resistant strain (RS)}}{\text{LC50 of susceptible strain (SS)}}$$

Biochemical assay

Randomly 50-60 nymphs of A. b. b. weighing 0.15 g were collected from the fields of Bt cotton treated with systemic insecticides. Then, the whole body homogenate of nymphs were prepared by using 2 ml sodium phosphate (SPB) buffer (0.1 mM pH 7.0 containing 0.1 mM EDTA, PTU and PMSF each). The homogenate, thus, obtained was centrifuged at 5000 rpm for 10 min. Solid debris and cellular material was discarded. The resultant supernatant was stored at -20°C by making aliquots and used as enzyme source. The actual quantification of Carboxylesterase was carried out by following the standard protocol given by Kranthi (2005).

RESULTS AND DISCUSSION

During 2015-16, the log dose probit (LDP) assays were carried out to determine the resistance profile of cotton leaf hoppers population against conventional and newer insecticides.

The population of *A. biguttula biguttula* collected from Akola location revealed that, flonicamid registered lowest LC_{50} value of 32.08 ppm and had corresponding fiducial limit of 17.75-50.65 (Table 1). It was followed by dimethoate and acephate with LC_{50} of 102.49 ppm (53.99-169.21) and 120.29 ppm (77.74-180.65), respectively. The least effective insecticide was imidacloprid with median lethal concentration of 143.35 ppm and a fiducial limit of 77.05-238.07 ppm against *A. biguttula biguttula* population. The results of bioassay of *A. biguttula biguttula* population collected from Akola location revealed that cotton leaf hopper developed high resistance to acephate followed by imidacloprid, dimethoate and flonicamid.

The population of *A. biguttula biguttula collected from Patur location was presented in Table 2. The results showed that,* flonicamid registered lowest LC_{50} value of 35.15 ppm and had corresponding fiducial limit of 15.92-65.88 followed by dimethoate and acephate with LC_{50} of 98.94 ppm (51.68-163.27) and 102.52 ppm (49.34-180.95), respectively. The least effective insecticide was imidacloprid with median lethal concentration of 149.11 ppm and a fiducial limit of 82.24-242.20 ppm against *A. biguttula biguttula* population.

The results of bioassay of *A. biguttula biguttula* population collected from Patur location revealed that cotton leaf hopper developed high resistance to acephate followed by imidacloprid, flonicamid and dimethoate.

The population of *A. biguttula biguttula collected from* Murtizapur location was presented in Table 3. The results

Table 1: Resistance profile of cotton leaf hoppers population collected from Akola location against conventional and newer insecticides

Insecticide	Numberexposed	LC_{50} (ppm)	Fiducial limit at 50%	LC_{90} (ppm)	Slope	X2	RR(fold)
Imidacloprid	300	143.35	77.05-238.07	1856.97	1.15±0.15	5.63	3.4
Dimethoate	300	102.49	53.99-169.21	1035.54	1.27±0.18	1.87	3.3
Acephate	300	120.29	77.74-180.65	479.90	2.17±0.37	6.35	4.5
Flonicamid	300	32.08	17.75-50.65	224.49	1.51±0.23	1.72	3.1

tabular value at 0.05 level – 9.48; (RR = Resistance ratio / Resistance factor)

Table 2: Resistance profile of cotton leaf hoppers population collected from Patur location against conventional and newer insecticides

Insecticide	Number exposed	LC_{50} (ppm)	Fiducial limit at 50%	LC_{90} (ppm)	Slope	X2	RR (fold)
Imidacloprid	300	149.11	82.24-242.20	1581.75	1.24±0.17	5.84	3.6
Dimethoate	300	98.94	51.68-163.27	937.16	1.31±0.19	2.63	3.2
Acephate	300	102.52	49.34-180.95	1589.71	1.07±0.14	5.07	3.8
Flonicamid	300	35.15	15.92-65.88	925.06	0.90±0.11	5.50	3.4

tabular value at 0.05 level – 9.48; (RR = Resistance ratio / Resistance factor)

Table 3: Resistance profile of cotton leaf hoppers population collected from Murtizapur location against conventional and newer insecticides

Insecticide	Number exposed	LC_{50}(ppm)	Fiducial limit at 50%	LC_{90} (ppm)	Slope	X2	RR (fold)
Imidacloprid	300	139.90	75.28-233.73	2080.60	1.09±0.14	5.19	3.4
Dimethoate	300	107.60	55.35-180.52	1185.68	1.22±0.17	1.83	3.5
Acephate	300	118.79	72.90-175.84	484.26	2.10±0.37	6.47	4.4
Flonicamid	300	32.89	14.24-57.98	850.84	0.90±0.11	3.89	3.2

tabular value at 0.05 level – 9.48; (RR = Resistance ratio/ resistance factor)

Table 4: Resistance profile of cotton leaf hoppers population collected from Akot location against conventional and newer insecticides

Insecticide	Number exposed	LC_{50} (ppm)	Fiducial limit at 50%	LC_{90} (ppm)	Slope	X2	RR (fold)
Imidacloprid	300	152.66	80.84-256.95	2245.36	1.09 ± 0.14	3.25	3.7
Dimethoate	300	110.36	60.48-186.22	1161.90	1.26 ± 0.18	1.76	3.6
Acephate	300	109.27	57.65-182.98	1432.55	1.14 ± 0.15	1.93	4.0
Flonicamid	300	31.78	14.41-59.56	844.36	0.89 ± 0.10	4.35	3.0

tabular value at 0.05 level – 9.48; (RR = Resistance ratio / Resistance factor)

Table 5: Resistance profile of cotton leaf hoppers population collected from Balapur location against conventional and newer insecticides

Insecticide	Number exposed	LC_{50} (ppm)	Fiducial limit at 50 %	LC_{90} (ppm)	Slope	X2	RR (fold)
Imidacloprid	300	140.62	77.42-228.93	1532.71	1.23 ± 0.16	5.06	3.4
Dimethoate	300	102.75	56.13-163.43	771.33	1.46 ± 0.22	2.14	3.4
Acephate	300	111.28	56.42- 179.25	1561.24	1.15 ± 0.15	2.12	4.1
Flonicamid	300	29.95	13.61-56.17	432.30	0.89 ± 0.11	4.81	2.9

tabular value at 0.05 level – 9.48; (RR = Resistance ratio / resistance factor)

Table 6: Resistance profile of field susceptible cotton leaf hoppers, *A. biguttula biguttula* population

Insecticide	Number exposed	LC_{50} (ppm)	Fiducial limit at 50%	LC_{90} (ppm)	Slope	X2
Imidacloprid	300	41.23	19.12-75.65	957.87	0.93 ± 0.12	3.00
Dimethoate	300	30.16	13.65-57.07	883.21	0.87 ± 0.10	4.00
Acephate	300	26.74	11.78-51.28	786.04	0.87 ± 0.10	3.16
Flonicamid	300	10.30	5.23-17.30	107.37	1.25 ± 0.18	2.32

tabular value at 0.05 level – 9.48; (RR = Resistance ratio / resistance factor)

Table 7: Detoxifying enzymes activity in field susceptible and resistant population of *A. bigutulla bigutulla* collected from different *talukas* of Akola district

Location	GST activity μmoles /min/ml of protein	Fold increase in GST activity	LC_{50} of Imidacloprid	LC_{50} of Acephate	LC_{50} of Dimethoate	LC_{50} of Flonicamid
Akola	156.29	1.5	143.35	120.29	102.49	32.08
Patur	153.76	1.5	149.11	102.52	98.94	35.15
Murtizapur	149.52	1.4	139.90	118.79	107.60	32.89
Akot	155.21	1.5	152.66	109.27	110.36	31.78
Balapur	152.71	1.4	140.62	111.28	102.75	29.95
Susceptible strain	104.19	-	41.23	30.16	26.74	10.30

showed that, flonicamid registered lowest LC_{50} value of 32.89 ppm and had corresponding fiducial limit of 14.24-57.98 ppm followed by dimethoate and acephate with LC_{50} of 107.60 ppm (55.35-180.52) and 118.79 ppm (72.90-175.84), respectively. The least effective insecticide was imidacloprid with median lethal concentration of 139.90 ppm and fiducial limit of 75.28-233.73 ppm against *A. biguttula biguttula* population. The results of bioassay of *A. biguttula biguttula* population collected from Murtizapur location revealed that cotton leaf hopper developed high resistance to acephate followed by imidacloprid, dimethoate and flonicamid.

The population of *A. biguttula biguttula collected from Akot location revealed that,* flonicamid registered lowest LC_{50} value of 31.78 ppm and had corresponding fiducial limit of 14.41-59.56 ppm (Table 4). It was followed by acephate and dimethoate with LC_{50} of 109.27 ppm (57.65-182.98) and 110.36 ppm (60.48-186.22), respectively. The least effective insecticide was imidacloprid with median lethal concentration of 152.66 ppm and a fiducial limit of 80.84-256.95 ppm against *A. biguttula biguttula* population. The results of bioassay of *A. biguttula biguttula* population collected from Akot location revealed that cotton leaf hopper developed high resistance to acephate followed by imidacloprid, dimethoate and flonicamid.

The population of *A. biguttula biguttula* collected from Balapur location revealed that, flonicamid registered lowest LC_{50} value of 29.95 ppm and had corresponding fiducial limit of 13.61-56.17 ppm (Table 5). It was followed by dimethoate and acephate with LC_{50} of 102.72 ppm (56.13-163.43) and 111.28 ppm (56.42-179.25), respectively. The least effective insecticide was imidacloprid with median lethal concentration of 140.62 ppm and a fiducial limit of 77.42-228.93 ppm against *A. biguttula biguttula* population. The results of bioassay of *A. biguttula biguttula* population collected from Balapur location revealed that cotton leaf hopper developed high resistance to acephate followed by imidacloprid, dimethoate and flonicamid.

The susceptible population of *A. biguttula biguttula* collected from Akola location revealed that, flonicamid registered lowest LC_{50} value of 10.30 ppm and had corresponding fiducial limit of 5.23-17.30 ppm (Table 6). It was followed by acephate and dimethoate with LC_{50} of 26.74 ppm (11.78-51.28) and 30.16

ppm (13.65-57.07), respectively. The least effective insecticide was imidacloprid with median lethal concentration of 41.23 ppm and a fiducial limit of 19.12-75.65 ppm against *A. biguttula biguttula* population.

Kshirsagar *et al.,* 2012 conducted bioassay studies against cotton leaf hoppers and revealed that resistance ratio for imidacloprid, acetamiprid and dimethoate was 23.41, 19.08 and 5.21 fold, respectively are in conformity with present work. The present findings are in line with Rajwinder Kumar Sandhu and Kang (2015) who reported that the resistance ratio of imidacloprid insecticide varied from 2.4 (Ludhiana) to 4.0 (Abohar) indicating that leafhopper population was found to be upto 4.0 fold resistant when compared with the susceptible strain. Sagar et al. (2013) reported that the LC_{50} and LC_{90} value along with resistance ratio to acephate was highest in leafhopper population in major cotton growing districts of Karnataka. The LC_{50} value ranges from 183.34 ppm to 78.14 ppm. Also the LC_{50} values for dimethoate against the cotton leafhopper population of major cotton growing districts of Karnataka during 2011-2012 and 2012-2013 varied from 127.23 ppm (Dharwad) to 66.47 ppm (Mysore) and 139.72 ppm (Dharwad) to 72.17 ppm (Mysore). While relative resistance ratio at LC_{50} in comparison with susceptible strain varied from 1.91 to 1.16 fold and 1.93 to 1.15 fold. These findings are in supportive with present findings. Prasada Rao (2012) who mentioned that after the introduction of Bt cotton, leafhopper has became a major constraint and reported that resistance monitoring studies conducted in Andhra Pradesh during the 2013-2014 revealed the leaf hopper population developed 35 fold resistance against imidacloprid.

Detoxifying enzymes activity in field susceptible and resistant population of *A. biguttula biguttula*.

The data regarding detoxifying enzymes (Glutathion S transferase) activity in *A. biguttula biguttula* collected from different five *talukas* of Akola district is presented in table no.7 and described as follows.

The data pertaining to Glutathion S transferase (GST) was observed to be high in resistant strain of leaf hopper as 156.29, 153.76, 149.52, 155.21, 152.71 μmoles/min/ml of protein for Akola, Patur, Murtizapur, Akot and Balapur *talukas* respectively than the field susceptible population having low titers of GST 104.19 μmoles/min/ml of protein.

Increase in GST activity in resistant strain over susceptible was 1.5, 1.5, 1.4, 1.5, 1.4 fold in *Akola, Patur, Murtizapur, Akot and Balapur talukas respectively*, which suggested the positive correlation between the detoxifying enzymes activity and the insecticide resistance level.

Kshirsagar et al. (2012) reported that GST activity in the susceptible strain of cotton leaf hopper was observed to be 0.0135 nM/min/mg proteins. Thus, the fold increase in the GST activity in the field population of cotton leafhopper collected during the month of August, 2010 was 10.89 fold. Relatively more glutathione S transferase values corresponding to the higher LD_{50} values of neonicotinoids indicating the role of GST in imparting resistance in cotton leafhoppers against the imidacloprid and acetamiprid. These results are in conformity with the present work. Present findings are also in consistent with work of Chen et al. (2015) who reported that cotton aphid which shows resistance to imidacloprid show obvious cross resistance to other neonicotinoids insecticides. Also Chen reported that imidacloprid resistance and cross resistance mainly result from metabolic detoxification of esterase and cytochrome P450. The increase in activities of esterases and cytochrome P450 had been reported to be one of most important mechanism for imidacloprid resistance in many insects.

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