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STATUS OF INSECTICIDE RESISTANCE IN LEAFHOPPER, *AMRASCA BIGUTTULA BIGUTTULA* (ISHIDA) ON COTTON

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ABSTRACT
Laboratory studies were carried out to investigate the development of resistance towards recommended insecticides in cotton leafhopper, *Amrasca biguttula biguttula* collected from four different locations *viz*., Ludhiana, Muktsar, Mansa and Abohar of Punjab. Five insecticides *viz*., imidacloprid, acetamiprid, dimethoate, monocrotophos and triazophos were tested. Among different populations, Abohar population was found to be the least susceptible with LC_{50} values ranging from 0.0020-0.0496 per cent whereas Ludhiana population was found to be more susceptible with LC_{50} values ranging from 0.0012-0.0255 per cent to all the insecticides tested. Among different insecticides, imidacloprid with LC_{50} values ranging from 0.0012 to 0.0020 per cent showed maximum toxicity followed by acetamiprid, dimethoate and monocrotophos while triazophos was found to be least effective insecticide with LC_{50} values ranging from 0.0255 to 0.0496 per cent. Small differences in the susceptibility from the baseline LC_{50} values against all the tested insecticides indicated that there are no serious levels of resistance (2.12-5.11 x) in *A. biguttula biguttula* with respect to these insecticides in Punjab.

INTRODUCTION

Cotton is one of the most important cash crops of India. It is grown for its lint and seed. In India, cotton was cultivated on an area of 11.70 million ha with a production of 29.00 million bales of seed cotton during 2013-14. Average productivity of cotton in India is 540 kg lint per ha, which is low when compared to world average of 766 kg lint per ha (AICCIP, 2013-14). The major limiting factor in its production is damage due to insect pests. After the introduction of *Bt* cotton, there was a check to the bollworm complex, but the sucking pest population increased gradually reaching economic injury level in many parts of India (Mohan and Nandini, 2011). Among the sucking pests, the cotton leafhopper, *A. biguttula biguttula*, is an important sucking pest causing both quantitative and qualitative losses. Though it is an early phase pest, it occurs all throughout the season serving as one of the limiting factors in crop production. Cotton leafhopper nymphs as well as adults suck sap from the leaves and damages the phloem tubes causing the distortion of leaves resulting into a condition known as 'hopper burn'. Severe infestation on cotton results in shedding of leaves, squares and young bolls which leads to significant yield losses (Narayanan and Singh, 1994, Memon and Chang, 2005). Inspite of repeated use of insecticides, it is becoming difficult to manage this pest. Though control failure may be due to many factors, one of the major factors is the development of resistance to insecticides (Jeya Pradeepa and Regupathy, 2002). The indiscriminate use of insecticides has resulted in the development of resistance in insects to insecticides and resurgence of sucking pests (Rohini *et al*. 2012). Insecticide resistance is the development of an ability in a strain of insects to tolerate doses of toxicant which would prove lethal to majority of individuals in a normal population of same species. This pest was found to have developed resistance to various insecticides *viz*., malathion, dimethoate, oxydemeton methyl and phosphamidon (Singh and Jaglan, 2005). Resistance in leafhopper population against organophosphates has also been reported by Sagar *et al*. (2013). Although various insecticides have been recommended for the control of the pest but the pest problem is aggravated due to control failures in many areas in Punjab (Dhawan and Simwat, 2002). Newer insecticides belonging to neonicotinoid group *viz*., imidacloprid, thiamethoxam and acetamiprid have been introduced which were found to be quite effective at very low doses and are relatively safer than conventional systemic insecticides (Chalam *et al*., 2003). Neonicotinoids have historically given very good control of leafhopper, in recent past, field level failure of neonicotinoids was noticed in the leafhopper population of Andhra Pradesh (AICCIP, 2008-09). As neonicotinoids along with other insecticides have been recommended in Punjab to control sucking pests of cotton, there was a need to assess the level of resistance developed by this pest against these insecticides. Keeping this in view, present studies were planned to know the status of insecticide resistance and to determine the relative toxicity of different insecticides against this pest.

MATERIALS AND METHODS

The study on insecticide resistance in *A. biguttula biguttula* was undertaken during 2014-15 in the Department of Entomology, PAU, Ludhiana. The populations of cotton leafhopper adults were collected from cotton fields of various cotton growing areas of Punjab *viz*., Ludhiana, Muktsar, Mansa

and Abohar and were reared on potted cotton plants in screen houses. Five formulated insecticides used for bioassay were imidacloprid 17.8 SL, triazophos 40 EC, dimethoate 30 EC, monocrotophos 36 SL and acetamiprid 20 SP. The adult leafhoppers were taken from the culture maintained for the treatment and were exposed to graded concentrations of these insecticides. The bioassay method followed was leaf disc dip bioassay as described by Ahmad *et al*. (1999). Cotton leaf discs (5 cm diameter) were cut and dipped into test solutions for 15 s with gentle agitation and were air dried. The treated discs were then placed into glass containers (5 cm diameter). Twenty adults were released at each concentration in five replications along with an untreated control. After releasing insects, glass containers were covered with muslin and were placed in incubator at a constant temperature of 25 ± 2°C. Observations on mortality of leafhoppers were recorded after 48 hours. Moribund leafhopper adults which did not respond to probing were considered as dead. The mortality data of each of test insecticide of each location was subjected to probit analysis using the POLOPLUS programme (LeOra Software, 2003) based on calculations given by Finney (1971). The resistance ratio for each insecticide and location was calculated using the formula given below:

$$RR = \frac{LC_{50} \text{ of particular location leafhopper population}}{LC_{50} \text{ of susceptible leafhopper population}}$$

RESULTS AND DISCUSSION

Log dose probit assay was carried out for imidacloprid, acetamiprid, dimethoate, monocrotophos and triazophos across four different geographic populations of *A. biguttula biguttula* of major cotton growing areas of Punjab. The LC_{50} values and resistance ratios of different insecticides with respect to populations of Ludhiana, Mansa, Muktsar and Abohar are presented in table1. Among different insecticides tested, imidacloprid was found to be most effective insecticide. The LC_{50} values worked out for imidacloprid were 0.0012, 0.0017, 0.0019 and 0.0020 per cent, respectively against pest populations collected from Ludhiana, Mansa, Muktsar and Abohar areas and the relative resistance folds as compared to susceptible population were found to be 2.40, 3.40, 3.80 and 4.00, respectively. The lower LC_{50} values of imidacloprid in the present study as compared to other conventional insecticides indicated its highest toxicity towards the leafhopper populations. Similarly, Jeya Pradeepa and Regupathy (2002) reported imidacloprid as highly toxic to leafhopper with LC_{50} value as 0.0005 per cent as compared to acephate, dimethoate and methyl demeton. Highest toxicity of imidacloprid against leafhopper has also been reported by Shinde *et al*. (2011). Shreevani *et al*. (2012) reported imidacloprid as second most toxic insecticide against leafhopper after thiamethoxam. The LC_{50} value of imidacloprid worked out was 0.006 per cent which is slightly higher than the present studies. The higher effectiveness of imidacloprid against leafhopper has also been confirmed by Kalyan *et al*. (2012) and Bharpoda *et al*. (2014). Acetamiprid was found to be the next best treatment against leafhopper populations. The respective LC_{50} values of acetamiprid for the pest populations from Ludhiana, Mansa, Muktsar and Abohar areas were 0.0029, 0.0038, 0.0041 and 0.0051 per cent and the resistance ratios were as 1.32, 1.73, 1.86 and 2.32. Effectiveness of acetamiprid against cotton leafhopper has also been confirmed by Preetha *et al*. (2014). Kshirsagar *et al*. (2012) observed LC_{50} value of acetamiprid as 0.0420 per cent in field populations of cotton leafhopper. However, the LC_{50} value of acetamiprid against the laboratory reared susceptible strain of cotton leafhopper was reported to be 0.0022 per cent, which is near about to the values obtained in present studies. Similarly, acetamiprid was found to be effective against leafhopper populations after dinotefuran, clothianidin and thiamethoxam as observed by Mandal *et al*. (2013). The overall mean per cent reduction of leafhopper was recorded in dinotefuran 40 g a.i. per ha (89.18%) followed by clothianidin @ 40 g a.i. per ha (85.74%), thiamethoxam @ 50 g a.i. per ha (80.48%) and acetamiprid @ 40 g a.i. per ha (78.73%). Abbas *et al*. (2012) reported neonicotinoids *viz*., imidacloprid, thiamethoxam and acetamiprid to be highly effective against cotton leafhopper. Acetamiprid @ 125 ml per acre was found to be quite satisfactory in reducing the leafhopper population with more than 80 per cent mortality of leafhopper after imidacloprid and thiamethoxam. Among the organophosphates tested, dimethoate proved to be more effective as compared to monocrotophos and triazophos. For dimethoate, the LC_{50} values were observed to be 0.0032, 0.0055, 0.0065 and 0.0111 per cent, respectively against pest populations from Ludhiana, Mansa, Muktsar and Abohar areas with resistance ratios as 1.23, 2.50, 2.12 and 4.27, respectively. These results are in agreement with the findings of Sagar *et al*. (2013) who reported dimethoate to be more toxic than monocrotophos against leafhopper under laboratory conditions with LC_{50} values of dimethoate ranging from 0.0066 to 0.0127 per cent. Similar results of susceptibility of this pest to dimethoate with LC_{50} values as 0.0154 per cent to 0.0041 per cent for first and seventh generation, respectively were obtained by Jeya Pradeepa and Regupathy (2002).Kshirsagar *et al*. (2012) also reported dimethoate as highly effective against leafhopper under laboratory conditions. The LC_{50} value of dimethoate against the susceptible strain was observed as 0.0026 per cent while LC_{50} value for field population was 0.0137 per cent.

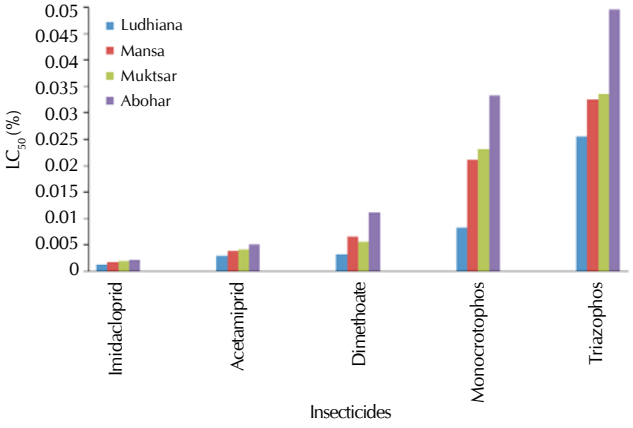

Figure 1: Toxicity of different insecticides against *Amrasca biguttula biguttula* in Punjab

Table 1: Comparative toxicity and resistance ratio of various insecticides against *Amrasca biguttula biguttula* in cotton growing districts of Punjab

Insecticides	Location	LC_{50} (%)	LC_{90} (%)	Fiducial limits at 95% CL	Slope	χ^2	d.f.	RR
Imidacloprid 17.8 SL	MAN	0.0017	0.0104	0.0012-0.0029	1.650 ± 0.247	1.9318	6	3.4
	MKT	0.0019	0.0129	0.0012-0.0034	1.559 ± 0.264	1.5946	6	3.8
	ABR	0.002	0.0096	0.0013-0.0034	1.886 ± 0.353	0.8182	6	4
	LDH	0.0012	0.0061	0.0008-0.0019	1.821 ± 0.310	1.1744	6	2.4
Acetamiprid	MAN	0.0038	0.0403	0.0023-0.0072	1.245 ± 0.192	1.5128	7	1.73
20 SP	MKT	0.0041	0.0487	0.0028-0.0071	1.189 ± 0.164	2.9195	7	1.86
	ABR	0.0051	0.0509	0.0036-0.0086	1.283 ± 0.169	2.3823	7	2.32
	LDH	0.0029	0.026	0.0019-0.0051	1.339 ± 0.191	2.7692	7	1.32
Dimethoate	MAN	0.0065	0.0478	0.0041-0.0115	1.473 ± 0.234	1.8384	7	2.5
30 EC	MKT	0.0055	0.0463	0.0041-0.0092	1.390 ± 0.175	1.8597	7	2.12
	ABR	0.0111	0.0928	0.0081-0.0184	1.388 ± 0.175	1.8553	7	4.27
	LDH	0.0032	0.0239	0.0021-0.0057	1.472 ± 0.234	1.7914	7	1.23
Monocrotophos 36 SL	MAN	0.021	0.1976	0.0120-0.0323	1.317 ± 0.223	1.3708	7	2.63
	MKT	0.0232	0.2803	0.0155-0.0402	1.184 ± 0.166	1.4743	7	2.9
	ABR	0.0333	0.3985	0.0229-0.0577	1.188 ± 0.162	1.7297	7	4.16
	LDH	0.0083	0.0996	0.0057-0.0144	1.188 ± 0.162	1.7297	7	1.04
Triazophos	MAN	0.0325	0.2784	0.0236-0.0539	1.373 ± 0.174	1.4468	7	3.35
40 EC	MKT	0.0336	0.2826	0.0245-0.0557	1.386 ± 0.175	1.4038	7	3.46
	ABR	0.0496	0.3555	0.0309-0.0896	1.499 ± 0.257	1.7392	6	5.11
	LDH	0.0255	0.1721	0.0147-0.0470	1.546 ± 0.296	2.8175	6	2.63

MAN = Mansa, MKT = Muktsar, ABR = Abohar, LDH = Ludhiana and RR = Resistance ratio. Base line values (%): *imidacloprid = 0.0005, **acetamiprid = 0.0022, **dimethoate = 0.0026, ***monocrotophos = 0.0080 and ****triazophos = 0.0097; Source: *Jeya Pradeepa and Regupathy (2002), **Kshirsagar et al. (2012), ***Sagar et al. (2013) and ****Ahmad et al. (2010)

Efficacy of dimethoate against leafhopper populations has also been confirmed by Kalyan *et al*. (2012). The usage of dimethoate was reduced in cotton growing regions of Punjab in the recent past, because of this reason, dimethoate has shown more efficacy as compared to triazophos which is used regularly. These findings indicated that leafhopper population has become relatively susceptible to dimethoate and for insecticidal resistance management strategies, neonicotinoids can be rotated with dimethoate to delay the process of resistance development in cotton leafhopper. The corresponding LC_{50} values for monocrotophos were worked out as 0.0083, 0.0210, 0.0232 and 0.0333 per cent and the resistance ratios were as 1.04, 2.63, 2.90 and 4.16 for Ludhiana, Mansa, Muktsar and Abohar, respectively. The lowest LC_{50} value to monocrotophos was found in the leafhopper population from Ludhiana region followed by Mansa, Muktsar and Abohar. Ravikumar *et al*. (2003) also reported the higher LC_{50} values of monocrotophos (0.0251) as compared to other insecticides *viz*., imidacloprid (0.0008), thiamethoxam (0.0003) indicating this insecticide to be least effective against leafhopper populations. These results are in close confirmity to those obtained in the present findings. Whereas, Kalra *et al*. (2001) reported monocrotophos to be more toxic than dimethoate against leafhopper. The LC_{50} value of monocrotophos observed was 0.0630 per cent and was second most effective insecticide after thiamethoxam. Similarly, susceptibility of cotton leafhopper to monocrotophos with 95.09 per cent mortality of leafhopper population after 24 hours of spray was reported by Asi *et al*. (2008). Triazophos was found to be the least effective insecticide among all the tested insecticides. The LC_{50} values were computed to be 0.0255, 0.0325, 0.0336 and 0.0496 per cent against Ludhiana, Mansa, Muktsar and Abohar populations with resistance ratios as 2.63, 3.35, 3.46 and 5.11, respectively. Similar results have been observed by Dhawan and Brar (1995) during their studies on the efficacy of some insecticides *viz*., triazophos, acephate, quinalphos etc. on population buildup of sucking pests in cotton. Triazophos was found to be second least effective insecticide.The present findings are in agreement with the reports of Sreekanth and Reddy (2011) who reported triazophos 40 EC to be the least effective insecticide against cotton leafhopper with only 55.87 per cent mortality in cotton leafhopper population after 7 days of application. Kalyan *et al*. (2012) also observed triazophos as the least effective chemical for the control of this pest as compared to other insecticides *viz*., imidacloprid and dimethoate. Lower toxicity of triazophos as compared to thiamethoxam was also reported by Patil *et al*. (2014). In present study, the resistance ratio of this insecticide varied from 2.63 (Ludhiana) to 5.11 (Abohar) indicating that leafhopper population was found to be upto 5.11 folds resistant when compared with the susceptible strain. Triazophos is the most frequently used insecticide on cotton crop against sucking pests and intensive use of triazophos in all the cotton growing regions of Punjab has resulted in the decreased susceptibility of leafhopper populations against this insecticide. Based on LC_{50} values obtained, the order of toxicity of these insecticides against all the tested populations was found out to be imidacloprid > acetamiprid > dimethoate > monocrotophos > triazophos.

In conclusion, this study has shown that among various insecticides tested, imidacloprid with minimum LC_{50} values (0.0012-0.0020 %) proved to be highly toxic followed by acetamiprid and dimethoate (Fig. 1). The study also revealed small differences in susceptibility from baseline LC_{50} values for different populations tested against different insecticides suggesting that there are no serious levels of resistance (2.12-5.11 x) in *A. biguttula biguttula* with respect to these insecticides in Punjab and neonicotinoids can be used in rotation with other insecticidesto prolong the phenomenon of resistance development in cotton leafhopper.

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