



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(6): 1754-1757

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Received: 14-08-2020

Accepted: 02-10-2020

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Relative susceptibility of cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) populations to selected insecticides

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Abstract

Laboratory bioassay was conducted to observe the susceptibility level of field collected population of cotton leafhopper *Amrasca biguttula biguttula* (Ishida) against imidacloprid, thiamethoxam, acephate and fipronil. The results revealed that higher LC₅₀ values were recorded for acephate (316.04 ppm) and fipronil (42.01 ppm). However imidacloprid (24.91 ppm) and thiamethoxam (30.67 ppm) were showed lower LC₅₀ values and were highly effective in managing the leafhopper population as compared to that of other insecticides. Resistance ratios (RRs) were 4.67 fold for acephate, 3.15 fold for fipronil, 3.12 thiamethoxam and 2.06 folds for imidacloprid in field collected population of *A. biguttula biguttula* compared to the susceptible laboratory reared population.

Keywords: Cotton leafhopper, *Amrasca biguttula biguttula*, susceptibility level

Introduction

Cotton (*Gossypium* spp.) popularly referred to as "whitegold" or the "king of fibres" is one of the world's important commercial fibre crops and a major source of raw material for India's domestic textile industry. Cotton production is limited by both abiotic and biotic factors, among which damage caused by insect pests are of paramount importance. Worldwide, 1326 species of insects from sowing to maturity were harboured in cotton crops (Hargreaves, 1948)^[5] and 162 species were recorded in India on cotton crop (Sundramurthy and Chitra, 1992)^[17]. Approximately 134 arthropod species, including 54 species of pests, have been found to be associated with cotton crop (Bal and Dhawan, 2008)^[2]. The cotton crop insect pest complex is commonly divided into three groups, namely sucking pests (leafhopper, whitefly, thrips, aphid and mealybug), foliage feeders (tobacco caterpillar) and bollworms (American bollworm, spotted bollworm, spiny bollworm and pink bollworm). Throughout the cropping season, these cause damage to different plant parts at various growth stages. The adoption of transgenic *Bt* cotton has not only changed the cultivation profile, but also the pest scenario. Owing to the introduction of *Bt* cotton, the pest status of bollworm complex has declined (Dhawan *et al.*, 2011)^[4].

While genetically engineered *Bt* cotton offers a successful bollworm complex management option, sucking pests still pose a major threat to *Bt* cotton cultivation (Shera *et al.*, 2013)^[14]. Damage caused by sucking pests (leafhopper, thrips, whitefly, mirid bug, mealybug and dusky cotton bugs) account for about 22.85 per cent yield reduction (Satpute *et al.*, 1990)^[13]. Cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) (Hemiptera: Cicadellidae) is a devastating pest causing both quantitative and qualitative losses. With a pale green body, the adults are elongated and wedge-shaped. On both of its apical margins, its forewings have black spots and two black spots on the vertex of the head. With sideways movements, the adults are very active, but easy to hop (Jayarao *et al.*, 2015)^[7]. Both nymphs and adults of leafhopper suck the sap from ventral surface of the leaves and toxic saliva is injected into the tissues and cause phytotoxic symptoms known as "hopper burn", as the infestation progresses, the leaves turn pale at the margins first and subsequently become crinkled, curled, reddened and show browning symptoms leading to complete desiccation of the plants. Under high infestation, plants get stunted in case of susceptible varieties (Hormechan *et al.*, 2001)^[6]. It is a polyphagous insect pest of cotton, brinjal, okra and other economically important crops in Asia (Mohan and Nandini, 2011)^[10].

The leafhopper was a serious pest during vegetative phase in earlier period, but during the present context it is a serious pest during reproductive phase also, prevailing upto 120 days after sowing (Balakrishnan *et al.*, 2007) [3].

The pesticides usage on cotton to control pests is both extensive and intensive. The reason for the development of insecticide resistance and resurgence of sucking pests is because of indiscriminate use of insecticides (Rohini *et al.*, 2012) [11]. Introduction of *Bt* cotton in India in 2002, enabled reduction of insecticide sprays for bollworms, however, this indirectly caused resurgence of sucking pests, specially leafhoppers (Kranthi, 2007) [8]. The cotton leafhopper developed resistance to the insecticidal groups like neonicotinoids and organophosphates (Kshirsagar *et al.*, 2012) [9].

The cotton leafhopper, *A. biguttula biguttula* showed resistance to the recommended organophosphate insecticides *viz.*, malathion, dimethoate, oxydemeton methyl and phosphamidon (Singh and Jaglan, 2005) [16]. Resistance in leafhopper population to organophosphates has also been reported by Sagar *et al.* (2013) [12]. Of late, a new group of insecticides, *i.e.*, neonicotinoids consisting of imidacloprid, Thiamethoxam and acetamiprid, were found to be more effective than traditional insecticides against cotton leafhoppers. However, in the recent days field level failure of neonicotinoid insecticides in controlling leafhopper populations in Andhra Pradesh was observed. The continuous use of neonicotinoids has probably led to development of resistance. Another key factor is that, in the market *Bt* cotton seeds available are imidacloprid treated ones which is giving an impetus for cotton leafhopper to develop resistance against insecticides (Kshirsagar *et al.*, 2012) [9]. In this context, the present investigation was carried out to monitor the levels of insecticidal resistance in field and laboratory reared populations of cotton leafhopper *A. biguttula biguttula*.

Materials and Methods

Studies comprising of insecticide resistance status of cotton leafhopper, *A. biguttula biguttula* was carried during 2018-19. The methodologies followed and techniques adopted for carrying out these studies are presented hereunder.

Collection and transportation of the pest population

The study pertaining to the insecticide resistance in *A. biguttula biguttula* was undertaken during 2018-19. The populations of cotton leafhopper (nymphs) were collected from cotton field in farm campus of National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru during early morning hours in cloth bags along with leaves and brought to the laboratory immediately and used for bioassay. The susceptible laboratory population of *A. biguttula biguttula* was initially collected from Attur farm, ICAR-

NBAIR, Bengaluru and maintained in wooden cages containing okra plants under nethouse conditions continuously without exposing them to insecticides since 2018. Bioassay for the susceptible laboratory population of *A. biguttula biguttula* was conducted at the farm campus of ICAR-NBAIR, Bengaluru.

Test insecticides

The insecticides commonly used for the management of sucking pests in cotton ecosystem were used to estimate the insecticide resistance levels in *A. biguttula biguttula*. The test insecticides included insecticides commonly recommended in cotton for sucking pest management namely, two neonicotinoids (*viz.*, imidacloprid 17.8% SL, thiamethoxam 25% WG), one organophosphate (acephate 75% SP) and one phenyl pyrazole (fipronil 5% SC) (Table 1).

Bioassay

Bioassay studies were conducted according to the standard *Bemisia tabaci* susceptibility test, insecticide resistance action committee (IRAC) method No. 8 developed and recommended by the committee. Insect breeding dishes were selected to conduct bioassay studies. Uncontaminated fresh cotton leaves were plucked from the field and cleaned with a wet cotton swab. The leaf petiole was cut to a length of approximately 4 to 5 cm, the petiole of the test leaf was passed through a centrifuge tube containing 10 per cent sucrose solution to maintain the turgidity of the cotton leaf and to allow the leafhopper nymphs to feed on the treated leaf. The test concentration of insecticides was prepared by using distilled water and the leaves were dipped in the insecticide solution for 10 seconds by holding the petiole of leaf. Then the leaves were kept for drying in the laboratory for approximately 5 minutes. The treated leaf was placed in an insect breeding dish after which 10 nymphs were released per cup and triplicate was maintained for each concentration. A control, treated with distilled water alone, was maintained during each time of experimentation. Observations were recorded 24 and 48 h after treatment. Moribund leafhopper nymphs which did not respond to probing were considered as dead. Percentage mortality at each concentration of the test insecticide and the controls were computed and corrected per cent mortality was calculated by Abbot's formula (Abbot, 1925) [1]. The corrected mortality data for each test insecticide from each location were subjected to probit analysis by using SPSS probit analysis software (version 23.0) for calculating LC₅₀ values. Later, the Resistance Ratio for each insecticide was calculated by using the following formula:

$$\text{Resistance Ratio (RR)} = \frac{\text{LC}_{50} \text{ of field collected leafhopper population}}{\text{LC}_{50} \text{ of susceptible laboratory reared population}}$$

Table 1: Insecticides selected for present study and their chemical group and mode of action

Insecticide	Chemical group	Mode of action
Acephate 75% SP	Organophosphate	Acetylcholinesterase(AChE) inhibitors
Fipronil 5% SC	Phenyl pyrozoles	GABA-gated chloride channel blockers
Imidacloprid 17.8% SL	Neonicotenoid	Nicotinic acetylcholine receptor competitive modulators
Thiamethoxam 25% WG	Neonicotenoid	Nicotinic acetylcholine receptor competitive modulators

Results and Discussion

Log dose probit assay was carried out for acephate 75% SP, fipronil 5% SC, imidacloprid 17.8% SL and thiamethoxam 25% WG across field collected and laboratory reared

population of cotton leafhopper. The results are presented insecticide-wise in the Tables 2 to 5. Resistance ratio was calculated for each insecticide by comparing the LC₅₀ of field collected population to LC₅₀ of the susceptible laboratory

reared population of *A. biguttula biguttula*.

Acephate 75% SP

LC₅₀ values of acephate 75% SP to field collected and laboratory maintained populations of cotton leafhopper are furnished in the Table 2. The LC₅₀ values for field collected and laboratory reared population were 316.04 and 67.61 ppm. The relative resistance (RR) ratio for field collected population was 4.67 folds as against the susceptible laboratory reared population.

Fipronil 5% SC

LC₅₀ values of fipronil 5% SC in field and laboratory maintained populations of cotton leafhopper are presented in Table 3. The toxicity of fipronil to field and laboratory maintained populations of *A. biguttula biguttula* were 42.01 and 13.30 ppm, respectively. The relative resistance (RR) ratio for field collected population was 3.15 folds as against the susceptible laboratory reared population.

Imidacloprid 17.8% SL

LC₅₀ values of imidacloprid 17.8% SL are presented in the Table 4 for field collected and laboratory reared populations of cotton leafhopper. The population collected from field recorded 2.06 folds resistance with LC₅₀ value of 24.91 ppm as compared to laboratory reared susceptible population (12.06 ppm).

Thiamethoxam 25% WG

LC₅₀ values of thiamethoxam 25% WG for field collected and laboratory reared populations of cotton leafhopper are furnished in Table 5. The toxicity of thiamethoxam to field and laboratory maintained populations of *A. biguttula biguttula* were 30.67 and 9.83 ppm, respectively. The relative resistance (RR) ratio for field collected population was 3.12 folds as against the susceptible laboratory reared population. The LC₅₀ values against acephate, fipronil, imidacloprid and thiamethoxam indicated that there was a shift in susceptibility of field population of *A. biguttula biguttula* as compared to

susceptible laboratory reared population. The resistance ratio of field collected population was 4.67 folds against acephate, 3.15 folds against fipronil, 2.06 folds against imidacloprid and 3.12 folds against thiamethoxam. Among the insecticides tested against *A. biguttula biguttula*, acephate showed highest LC₅₀ value and imidacloprid recorded the least; it was evident that *A. biguttula biguttula* was less sensitive to acephate and more sensitive to imidacloprid as compared to fipronil and thiamethoxam.

Chloronicotynyls (imidacloprid, acetamiprid and thiamethoxam) of neonicotinoid group which are selectively more effective against sucking pests and less toxic to beneficial insects as compared to all the conventional insecticides have been added to sustainable pest management (Kranthi, 2007) [18].

The per cent mortality of leafhopper nymphs was more in thiamethoxam (50.67 %) followed by imidacloprid (46.67 %) and clothianidin (37.33 %) (Shreevani *et al.*, 2014) [15]. The insecticides *viz.*, imidacloprid, acetamiprid, clothianidin, acephate, dimethoate, monocrotophos, oxydematon methyl, buprofenzin and fipronil showed least per cent mortality. Irrespective of all the locations, dinotefuran and flonicamide showed maximum per cent mortality, followed by thiamethoxam and were highly effective in managing the leafhopper *A. biguttula biguttula* population as compared to the other insecticides (Vimala *et al.*, 2016) [18]. Insecticide resistance monitoring study against, *A. biguttula biguttula* revealed moderate to high levels of resistance in field collected leafhopper population against the neonicotinoids tested, *viz.*, imidacloprid and acetamiprid with LC₅₀ values of 374.47 ppm and 420.36 ppm, respectively as compared to dimethoate (137.15 ppm) which was found to be the most effective insecticide among the insecticides tested. The resistance ratio for imidacloprid, acetamiprid and dimethoate was 23.41, 19.08 and 5.21 fold, respectively when compared to the susceptible strain of cotton leafhopper (Kshirsagar *et al.*, 2012) [9]. These findings of earlier workers are similar to the results of the present study.

Table 2: Relative susceptibility of *A. biguttula biguttula* populations against acephate 75% SP

Location	No. of insects used	LC ₅₀ (ppm)	Slope± SE	Fiducial limits		χ ² Value	Degrees of freedom	Resistance ratio
				Lower	Upper			
Field population	210	316.04	1.83±0.33	201.21	429.22	2.55	4	4.67
Laboratory population	210	67.61	1.74±0.33	39.37	94.50	2.06	4	-

Table 3: Relative susceptibility of *A. biguttula biguttula* populations against fipronil 5% SC

Location	No. of insects used	LC ₅₀	Slope± SE	Fiducial limits		χ ² Value	Degrees of freedom	Resistance ratio
				Lower	Upper			
Field population	210	42.01	1.84±0.29	30.96	55.89	1.20	4	3.15
Laboratory population	210	13.30	1.44±0.27	9.27	19.37	0.17	4	-

Table 4: Relative susceptibility of *A. biguttula biguttula* populations against imidacloprid 17.8% SL

Location	No. of insects used	LC ₅₀	Slope± SE	Fiducial limits		χ ² Value	Degrees of freedom	Resistance ratio
				Lower	Upper			
Field population	210	24.91	1.78±0.31	16.53	33.58	2.97	4	2.06
Laboratory population	210	12.06	1.56 ±0.28	8.57	16.91	0.63	4	-

Table 5: Relative susceptibility of *A. biguttula biguttula* populations against thiamethoxam 25% WG

Location	No. of insects used	LC ₅₀	Slope± SE	F. limits		χ ² Value	Degrees of freedom	Resistance ratio
				Lower	Upper			
Field population	210	30.67	1.45±0.28	19.38	43.31	1.76	4	3.12
Laboratory population	210	9.83	1.31±0.27	2.69	14.39	0.09	4	-

Conclusion

The high resistance ratio recorded against acephate, fipronil and thiamethoxam for the field collected population. Among the insecticides tested against *A. biguttula biguttula*, acephate showed highest LC₅₀ value and imidacloprid recorded the least; it was evident that *A. biguttula biguttula* was less sensitive to acephate and more sensitive to imidacloprid as compared to fipronil and thiamethoxam. Thus, amongst the neonicotinoid insecticides, imidacloprid and thiamethoxam can be used in rotation basis to suppress the population of leafhopper in cotton ecosystem.

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