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Insecticide Susceptibility Monitoring In Leafhopper *A. biguttula biguttula* (Ishida) On Cotton

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ABSTRACT

Insecticide-bioassays were carried out to assess the susceptibility of field-collected cotton leafhopper from Hisar, Sirsa and Bhiwani Districts of Haryana state to commercial formulations of imidacloprid 17.8 SL, thiamethoxam 25 WG and flonicamid 50 WG, by using leaf dip bioassay method. The observations on the mortality of leafhopper nymphs were recorded at 24 and 48 h after release. The corrected mortality data of each test insecticide of each location was subjected to probit analysis and further for calculation of LC_{50} and LC_{90} values to determine the comparative susceptibility of collected leafhopper populations. Based on intrinsic toxicity, the order of susceptibility to imidacloprid 17.8 SL was found to be Bhiwani population> Hisar Population> Sirsa population with corresponding LC_{50} values were 24.74, 29.83 and 34.60 ppm, respectively. Similarly, the order of susceptibility to thiamethoxam 25 WG and flonicamid 50 WG followed similar trend i.e., Bhiwani population> Hisar population> Sirsa population with the corresponding LC_{50} were 20.73, 26.27 and 29.60 ppm; 7.23, 10.09 and 12.17 ppm, respectively. Among different insecticides, flonicamid 50 WG with LC_{50} values ranging from 7.23 to 12.17 ppm showed maximum toxicity followed by thiamethoxam 25 WG (20.73 to 29.60 ppm) while imidacloprid 17.8 SL was found to be least toxic insecticide with LC_{50} ranging from 24.74 to 34.60 ppm.

Keywords Bioassay, Flonicamid, LC_{50}, Insecticides, Toxicity.

INTRODUCTION

Cotton (*Gossypium* spp.) is one of the most important cash crop of India, grown mainly for its lint and seed. It also offers the cotton textile industry with essential raw materials. Cotton, *Gossypium hirsutum* L., an important industrial crop cultivated in tropical as well as sub-tropical regions of more than seventy countries all over the world. It is grown in an area of more than 38 million hectares (m ha) in the world, of which approximately 24% is covered in India. China, India, USA, Pakistan, Uzbekistan, Argentina, Australia, Greece, Brazil, Mexico and Turkey are the major producers of cotton and contribute about 85%

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to the global cotton production. India is the leading country in terms of area under cotton cultivation with 38% of world cotton area and raw cotton production in the world (Anonymous 2022). The major cotton-growing states of India are Gujarat, Telangana, Maharashtra, Punjab, Haryana, Rajasthan, Madhya Pradesh, Andhra Pradesh, Karnataka and Tamil Nadu. In India, it is cultivated over an area of 12.35 m ha with production and productivity of 34.06 million bales and 468.87 kg ha^{-1} respectively (Kiruthika *et al.* 2022). Among Indian states, Haryana is the 6^{th} largest cotton-producing state with an area of 0.74 m ha, production of 1.82 million bales and productivity of 419 kg ha^{-1} (Anonymous 2021).

Even though cotton is one of India's most important cash crops, still there are many constraints in its production. The major limiting factor is damage due to insect pests. There was a check to the bollworms population after the introduction of Bt cotton, but the sucking pest problem remain as such. Moreover, in many parts of India, the population of sucking pests increased gradually reaching economic injury level (Saleem *et al.* 2018). In recent few years, among sucking pests of cotton, leafhopper, *A. biguttula biguttula* (Ishida), emerged as an important insect pest in cotton causing both quantitative and qualitative losses. Both the nymph and adult are damage causing stage. During the early stages of the crop growth, they suck the cell sap from the lower surface of the leaves causing specking symptoms, crinkling, distortion of leaves and reddening all along the sides of leaves with downward curling which results in production of typical "hopper burn" symptoms. Although it is an early phase pest, it occurs throughout the season serving as one of the major constraint in crop production.

Bt cotton is affording protection against bollworms but susceptible to sucking pests. Therefore, in order to combat the problem of sucking pests in cotton, use of commonly used insecticides has been expected to increase in days to day and creating issues including pests resistance, resurgence and secondary outbreak. Among the various measures used by farmers to control the cotton leafhopper, insecticides form the first line of defense in spite of their drawbacks. Several potent insecticides have been recommended for managing the sucking pests, but the continuous and indiscriminate use of insecticides has resulted in the development of resistance in insects to insecticides, resurgence, secondary pest outbreaks, disruption of the natural enemy complex, loss in biodiversity and environmental pollution (Raven and Wagner 2021). The cotton leafhopper has developed resistance to commonly used insecticides including neonicotinoids (Sagar and Balikai 2014). Extensive use of these novel compounds inevitably involves the risk of the development of new types of resistance. 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). 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 (Arunkumara *et al.* 2020).

A consolidated report on insecticide resistance indicated the development of insecticide resistance in *Amrasca devastans* for organophosphorus compounds (Maha Lakshmi and Prasad 2020). Due to arbitrary use of systemic insecticides, their efficacy has been lost leading to the buildup of resistance to these insecticides and selection pressure has increased (Kshirsagar *et al.* 2012). In recent past, the sucking pest damage has increased and leafhopper damage is more due to the development of resistance to chloronicotinyl insecticides (Dhawan 2012). 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. Several factors including continuous and indiscriminate insecticide usage, cross-resistance, wrong policies concerning seed treatment with neonicotinoids and role of mixed function oxidases are playing a major role in the development of insecticide resistance in cotton leafhopper. This necessitates the need to assess and monitor the responses of the target population to insecticides to enable the timely use of alternative control measures including rotation of different insecticides, limiting their applications or the use of synergists. In light of this, the current research was designed to examine the level of insecticide resistance and the relative toxicity of different insecticides against this pest.

Table 1. Details of insecticides used in bioassay study.

Insecticide	Chemical group	Formulation
Imidacloprid	Chloronicotinyls	17.8 SL
Thiamethoxam	Thianicotinyls	25 WG
Flonicamid	Pyridinecarboxamides	50 WG

MATERIALS AND METHODS

The susceptibility of insecticides against cotton leafhopper populations of major cotton growing districts namely, Hisar, Sirsa and Bhiwani of Haryana state was studied at Department of Entomology, CCS HAU, Hisar during *kharif* 2019. The seeds of American cotton var Ganganagar Ageti were sown at weekly intervals in the earthen pots having a mixture of soil and well rotten farmyard manure to ensure continuous supply of cotton plants at different time intervals and fresh cotton leaves to conduct the bioassays. As the germination progressed, the seedlings were covered with a nylon net to prevent the entry of other insects and natural enemies. These plants were maintained without giving exposure to insecticide applications. After that the nymphal population of leafhopper was collected from major cotton growing districts of Haryana viz., Hisar, Sirsa, and Bhiwani from cotton growing farmers' fields in the month of July, 2019. The leafhoppers were collected with the help of an aspirator without damaging them and transferred to fine wire mesh cages (15 cm × 15 cm × 15 cm) having fresh cotton leaves (petiole wrapped in moist cotton) and brought to Cotton Research area, Deptt of Genetics and Plant Breeding, CCS HAU, Hisar. Each district collection of test insects constituted the composite collection from 4-5 cotton farmers' fields. The field-collected populations of *A. biguttula biguttula* from different districts were released on the potted unsprayed cotton plants covered with nylon net and maintained separately in screen house to make an assured and continuous supply of leafhopper required for bioassay. Commercial formulations of test insecticides namely, imidacloprid, thiamethoxam and flonicamid were procured from the market. The details of the test insecticides are given below (Table 1). The desired insecticide concentrations were prepared in distilled water at the time of bioassay.

The leaf dip bioassay method adopted by Preetha *et al.* (2014) with some modifications was used. The fresh and uncontaminated medium size leaves (Ganganagar Ageti) were selected and brought to the laboratory. The petiole of leaves was cut to a length of approximately 5 cm. The leaves were dipped in respective insecticide concentrations for five seconds by holding the petiole end using forceps, and then leaves were shade-dried at room temperature. The control was maintained by dipping the leaves in distilled water alone. The petiole of each shade-dried leaf was dipped in 1.0 ml glass vial containing water in order to maintain the turgidity of leaves and to prevent leaves from drying. The mouth of the vial was first sealed with cotton and then wrapped by parafilm to prevent water leakage. Afterward, the leaves were transferred to the Petri plate (15 cm dia) individually with proper labeling of insecticide concentrations. The uniform size nymphs (third instar) were collected from potted plants and released into the Petri plate with the help of fine camel hair brush gently. A total of ten nymphs per Petri plates were released and six replications for each treatment were maintained. The Petri plates were covered with muslin cloth for proper aeration and tied with the help of a rubber band. At the initial stage, bracketing or preliminary range-finding tests was done to arrive at the required concentrations of insecticides for drawing concentration-log mortality response curve. The observations on the mortality of leafhopper nymphs were recorded at 24 and 48 h after release. The moribund leafhopper nymphs which failed to respond with a gentle touch of fine hairbrush were considered as dead. Percentage of mortality for each concentration of test insecticide and control were computed and corrected per cent mortality was calculated by using Abbott's correction (Abbott 1925) for mortality in the control if any.

$$\text{Corrected per cent mortality} \quad \frac{T - C}{100 - C} \times 100$$

Where, T- per cent mortality in treatment
C - per cent mortality in control

The experiment was repeated whenever the mortality in the control was greater than 20%. The corrected mortality data of each test insecticide of each location was subjected to probit analysis using OPSTAT software to estimate the log concentration-mortality regression and further for calculation

Table 2. Comparative intrinsic toxicity of imidacloprid to nymphs of *A. biguttula biguttula* populations from selected districts.

District	LC_{50} (ppm)	Fiducial limits (ppm) Lower	Upper	LC_{90} (ppm)	Fiducial limits (ppm) Lower	Upper	Slope	Regression equation
Sirsa	34.60	24.98	47.92	177.87	128.41	246.36	1.79	y = 2.27+1.787x
Hisar	29.83	21.42	41.55	158.99	114.15	221.45	1.76	y = 2.40+1.764x
Bhiwani	24.74	18.07	33.89	117.74	85.97	161.25	1.91	y = 2.33+1.905x

of LC_{50} and LC_{90} values to determine the comparative susceptibility of collected leafhopper populations (Finney 1971).

RESULTS AND DISCUSSION

The response of different populations of *A. biguttula biguttula* nymphs collected from major cotton growing areas of Haryana, to tested insecticides using leaf dip bioassay method was recorded. The nymphal populations collected from Sirsa, Hisar and Bhiwani districts were exposed to graded concentrations of test insecticides. viz, imidacloprid, thiamethoxam and flonicamid and data on mortality was recorded. Based on LC_{50} values the intrinsic toxicity of insecticides to nymphs of *A. biguttula* biguttula populations from selected districts were evaluated.

The data on the LC_{50} values of imidacloprid to three selected populations of *A. biguttula biguttula* are presented in Table 2. The results indicated that there were marked differences in LC_{50} values among the different location populations. A comparison of LC_{50} and LC_{90} values of imidacloprid revealed that the Sirsa population recorded the maximum values (34.60 and 177.87 ppm, respectively) followed by the population from Hisar (29.83 and 158.99 ppm, respectively). Lowest LC_{50} and LC_{90} values were obtained in the population of Bhiwani (24.74 and 117.74 ppm, respectively). Present findings of LC_{50} values of imidacloprid (24.74-34.60 ppm) are in contradictory with the results of Shreevani *et al.* (2012) who reported lower LC_{50} value of imidacloprid (0.022 ppm) on leafhopper and Kalyana (2004) who also reported lower LC_{50} value of imidacloprid (0.0004 to 0.0005%) in field collected leafhopper populations from different locations viz., Ludhiana, Hoshiarpur, Faridkot and Mansa Districts of Punjab. The reason attributed might be due to the continuous increase in number of sprays and repeated use of insecticides year after year in cotton crop resulted in increased LC_{50} values in the present studies. Similarly the present findings of LC_{50} values of imidacloprid are contradictory with the results of Kapasi *et al.* (2018) who reported the higher LC_{50} value of 161.31 ppm and 174.48 ppm for imidacloprid against leafhopper during 2014-15 and 2015-16, respectively and Sagar *et al.* (2013) also reported higher LC_{50} (75.21 ppm and 85.75 ppm) during 2011-12 and 2012-13, respectively.

The LC_{50} values of thiamethoxam to three selected populations of *A. biguttula biguttula* are presented in Table 3. It was observed that there was a marked difference in LC_{50} values among the populations of the different location. The LC_{50} values of thiamethoxam to three populations of *A. biguttula biguttula* varied from 20.73 ppm (Bhiwani) to 29.60 ppm (Sirsa). Higher LC_{50} and LC90 values were noticed in leafhopper population of Sirsa (29.60 and152.45 ppm, respectively) followed by Hisar (26.27 and 130.72 ppm, respectively), while lowest was observed in population from Bhiwani District (20.73 and 104.52 ppm, respectively). many reports were available on development of resistance in leafhoppers

Table 3. Comparative intrinsic toxicity of thiamethoxam to nymphs of *A. biguttula biguttula* populations from selected districts.

District	LC_{50} (ppm)	Fiducial limits (ppm) Lower	Upper	LC_{90} (ppm)	Fiducial limits (ppm) Lower	Upper	Slope	Regression equation
Sirsa	29.60	21.37	41.01	152.45	110.05	211.18	1.78	y = 2.38+1.78x
Hisar	26.27	19.05	36.21	130.72	94.82	180.20	1.83	y = 2.39+1.83x
Bhiwani	20.73	14.96	28.74	104.52	75.41	144.87	1.80	y = 2.60+1.80x

against neonicotinoids. Kalra *et al.* (2001) reported the toxicity of various insecticides viz., malathion, oxydemeton methyl, phosphamidon, dimethoate, thiamethoxam, endosulfan and monocrotophos with their corresponding LC_{50} values 1.097, 0.126, 0.112, 0.178, 0.000447, 0.0063 and 0.063%, respectively to *A. biguttula biguttula* on okra at Hisar. But in present study the LC_{50} value of thiamethoxam from Hisar population was reported to be 26.27 ppm indicating six times increase in LC_{50} value. The most probable reason for increase in LC_{50} value might be due to over, indiscriminate and continuous use of the same insecticides year after year. The present reasoning for increased LC_{50} values is in agreement with Kranthi (2007), who reported that overuse of any of these chloronicotinyls (imidacloprid and thiamethoxam) of neonicotinoids can lead to the development of pest resistance to the insecticides with scant regard for the principles of insecticide resistance management. It might also be due to development of cross-resistance to neonicotinoids by the leafhopper population. Preetha *et al.* (2014) reported that the level of resistance was 6.67 to 15.38 for imidacloprid, 3.33 to 15.09 for thiamethoxam and 5.00 to 20.00 for acetamiprid in different places of Tamilnadu. Similarly, Chaudhari *et al.* (2015) reported very high resistance ratios such as 108.68, 78.24 and 25.96 fold for imidacloprid, thiamethoxam and acetamiprid, respectively when compared to 29.04 and 9.29 folds for monocrotophos and acephate, respectively from Surat.

On the basis of LC_{50} values in present study, thiamethoxam (20.73-29.60 ppm) was found to be more toxic against cotton leafhopper than imidacloprid (24.74-34.60 ppm). The present findings are in line with the earlier findings of Shreevani *et al.* (2014) who reported that thiamethoxam to be the most toxic insecticide to third nymphal instar of *A.biguttula biguttula* with LC_{50} value of 0.001% than imidacloprid with corresponding LC_{50} value of 0.007%. Also, the similar results were reported by Halappa and Patil (2016) i.e., thiamethoxam as a highly toxic insecticide to leafhopper with a LC_{50} value range of 26.89 to 142.00 ppm and imidacloprid as the least toxic with a higher LC_{50} value range of 54.91 to 201.36 ppm among the neonicotinoid groups of insecticides evaluated. Furthermore, the higher LC_{50} values for imidacloprid compared to thiamethoxam against leafhopper might be due to the fact that mainly imidacloprid is used for seed treatment in cotton and thiamethoxam is being used after imidacloprid for seed dressing as well as spray and also for foliar application imidacloprid is more preferred by farmers than thiamethoxam, which appeared to be resulting in more and constant exposer of same insecticides and further lead to increase in selection pressure on cotton leafhopper to this insecticide. However, the present findings are in contrast with the results of Phulse and Udikeri (2017) who reported imidacloprid as more toxic to cotton leafhopper with LC_{50} value ranging from 150-250 ppm than thiamethoxam with LC_{50} values of 160-260 ppm. Similarly, on the basis of LC_{50} values, imidacloprid showed high toxicity (24.11-202.47 ppm and 27.37-226.37 ppm) against *A. biguttula biguttula* than thiamethoxam (87.63-193.04 ppm and 31.92-209.70 ppm) during 2011-12 and 2012-13, respectively (Sagar *et al.* 2013). The present study also revealed that the LC_{50} values for imidacloprid 17.8% SL and thiamethoxam 25% WG are in close proximity to the recommended dose. The reasons attributed to higher LC_{50} of imidacloprid and thiamethoxam are might be due to the fact that in India Bt cotton seeds are available for sale as imidacloprid treated and there is recommendation of the use of neonicotinoids group of insecticides as foliar sprays against the sucking pests of cotton which results in heavy selection pressure of these neonicotinoids against cotton leafhopper resulting in declining of susceptibility to neonicotinoids. Also, Dhawan (2012) reported that the reason for a low level of susceptibil-

Table 4. Comparative intrinsic toxicity of flonicamid to nymphs of *A. biguttula biguttula* populations from selected districts.

District	LC_{50} (ppm)	Fiducial limits (ppm) Lower	Upper	LC_{90} (ppm)	Fiducial limits (ppm) Lower	Upper	Slope	Regression equation
Sirsa	12.17	8.69	17.04	65.01	46.44	91.02	1.72	y = 3.15+1.72x
Hisar	10.09	7.33	13.90	49.64	36.04	68.36	1.83	y = 3.19+1.83x
Bhiwani	7.23	5.43	9.63	28.93	21.73	38.52	2.09	y = 3.22+2.09x

Table 5. Susceptibility of *A. biguttula biguttula* populations of different districts to different insecticides.

District	LC_{50} (ppm) Imidacloprid	Thiamethoxam	Flonicamid
Sirsa	34.60 (1.00)	29.60 (1.00)	12.17 (1.00)
Hisar	29.83 (1.16)	26.27 (1.13)	10.09 (1.21)
Bhiwani	24.74 (1.40)	20.73 (1.43)	7.23 (1.68)

Figures in the parentheses are comparative susceptibility index.

ity to insecticides in sucking pests of Bt cotton was due to the wrong policy adopted by GEAC regarding seed treatment, which was not desired.

The LC_{50} and LC_{90} values of flonicamid to three populations of *A.biguttula biguttula* varied from 7.23 ppm (Bhiwani) to 12.17 (Sirsa) and 28.93 (Bhiwani) to 65.01 ppm (Sirsa), respectively (Table 4). The literature pertaining to the susceptibility of leafhopper population to flonicamid 50% WG is scanty. Present findings of LC_{50} values of flonicamid 50% WG are in contradictory with the results of Kapasi *et al.* (2018) who reported higher LC_{50} value of flonicamid against leafhopper population collected from Raichur i.e., 53.41 ppm and 61.33 ppm during 2014-15 and 2015-16, respectively and also with the findings of Thakare *et al.* (2016) who reported higher LC_{50} value of flonicamid ranging from 29.95 to 35.15 ppm against Amrasca biguttula biguttula populations.. The reason attributed to relatively lower LC_{50} values of flonicamid in present study might be due to different populations and the fact that this insecticide is being used by the farming community in cotton ecosystem from the last three-four years only. Results of the present study on flonicamid and thiamethoxam are contrary to the findings of Rekha *et al.* (2017) who studied the nymphal susceptibility of *A. biguttula biguttula* to insecticides and found that thiamethoxam 25 WDG as most toxic insecticide to okra leafhopper with LC_{50} value of 4.03 ppm followed by flonicamid 50 WG (4.50 ppm).

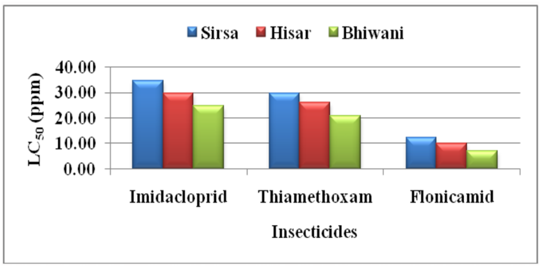

Fig. 1. Susceptibility of *A. biguttula biguttula* populations to different insecticides.

The susceptibility status of *Amrasca biguttula* biguttula populations collected from selected districts to test insecticides were evaluated by calculating the susceptibility index. Based on LC_{50} values, Sirsa district population were considered as resistant population and correspondingly susceptibility index was calculated for other district populations using Sirsa population as a reference. It was observed that susceptibility index for Hisar population to imidacloprid, thiamethoxam and flonicamid was found to be 1.21, 1.13 and 1.40 and, for Bhiwani population was 1.40, 1.43 and 1.68 respectively (Table 5 and Fig. 1).

CONCLUSION

The present study concludes that on the basis of intrinsic toxicity, the order of susceptibility to imidacloprid, thiamethoxam and flonicamid was found to be Bhiwani population> Hisar Population > Sirsa population. And on the basis of insecticides toxicity, flonicamid was found to be highly toxic followed by thiamethoxam and imidacloprid. So it is recommended that rational and sensible sequences of insecticides effective to target species and safe to non-targets be used in order to minimize selection pressure as well as rotation of insecticides with different modes of action and adoption of Resistance Management Strategies (IRM) to delay the development of resistance to cotton leafhopper can be prove effective.

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