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Assessment of the insecticide resistance build up on cotton leafhopper *Amrasca bigutulla bigutulla* (Ishida)

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Keywords

Leafhopper,
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Abstract

The Bt cotton is affording protection against lepidopteron insect however sucking insect pests was key pest, among them leafhopper, *Amarasca biguttula biguttula* Ishida (Homoptera: Cicadellidae) is serious problem throughout the crop period.Development of resistance in insect pests to insecticides is a natural phenomenon. Realizing the significance of assessing resistance development to insecticides, the bioassay was tested two insecticidal group were neonicotinoid *viz*., imidacloprid 17.8 SL, thiamethoxam25 WG, acetamiprid 20 SP and organophosphate insecticides *viz*., acephate 75 SP and monocrotophos 36 WSL which was found neonicotinoids at moderate level and organophosphate to low level resistance against cotton leafhopper. The resistance ratio for imidacloprid, thiamethoxamand acetamiprid was 108.68, 78.24 and 25.96 fold, respectively. In organophosphate insecticides, monocrotophosand acephatewas 29.04 fold and 9.29 fold, respectively.

Introduction

Today cotton ecosystem is dominated by Bt cotton in season 2011-12 by way of acreage under Bt cultivation has been more than 91 per cent of the total acreage of around 121.91 lakh ha that effect rapidly change in pest dynamics. Bt cotton is affording protection against bollworms but susceptible to sucking pests. Thus, the use of commonly used insecticides against sucking pests in cotton has been expected to increase in the days to day and shaped problem like resistance, residual and resurgen.

Among the sucking pest, leafhopper (Jassids) *Amarasca biguttula biguttula* Ishida (Homoptera: Cicadellidae) is a key pest whose incidence and damage occurs throughout the cotton crop growth period. Judicious use of insecticide and among organophosphat and neonicotinoid was a highly consumptional group in cotton and also used aginst cotton leafhopper due to the effective at very low doses and relatively safer than conventional systemic insecticides. Leafhopper gote selection pressure to development of resistance where control measure was failure of control leafhopper on cotton.There are several instances of failure reported at label claim doses of the products and farmers was using higher doses than label claim or recommended for the control of leafhopper due to reduce sensitivity at label claim doses.

Hence,considering the possibility of development of resistance in cotton leafhopper,an investigation was thus planned with an objective to determination of the insecticide resistance build up on cotton leafhopper population of south Gujarat.

Materials and Methods

The bioassay was carried out in the laboratory of Main Cotton Research Station, Suart at an average room temperature of 27.19 ± 5.38 ºC and average relative humidity of 60.63 ± 2.40 per cent during September to October 2013. The commonly used neonicotinoid insecticides *viz.*, imidacloprid 17.8 SL, thiamethoxam 25 WG, acetamiprid 20 SP and two organophosphate insecticides *viz*., acephate 75 SP and monocrotophos 36 WSL were utilized for LDP assay against leafhopper. LDP assays were carried out for each insecticides with six concentrations (1,10, 50, 100, 1000 and 200 ppm) in distilled water with three replications. Insecticide solutions at diiferent concentrations were prepared by serial dilution techniques in the glass jars (height-15 cm, diameter-13 cm) just prior to experimentation and labelled accordingly.The first sampling for field populations of leafhopper was carried out during second week of September, 2013 at initial build up of sufficient population pressure of leafhopper from RCH 2 Bt sown during June 2013 at Main Cotton Research Station,

NAU, Surat when the field was unsprayed. The second sampling was performed at the reasonable population pressure in second week of October, 2013, wherein field populations have already been exposed to insecticides thrice (dimethoate, imidacloprid and acetamiprid at 8-10 days interval) before plucking the infested leaves. Infested leaves were plucked and collected in the special plastic bucket (height-26cm, diameter-30 cm) having 40 mesh wire net fitted window and the mouth of the bucket covered with muslin cloth and tied with rubber band. The 40 mesh wire net fitted window and muslin cloth provided sufficient aeration. Such 12-15 bucket full of infested leaves were brought to the laboartory. The young nymphs of the leafhopper were carefully aspirated with the help of aspirator in the plastic specimen tubes (height-7.5cm, diameter-2.5cm) having perforated lid by examining the each field collected infested leaves with the illuminated magnifying lense (10x) brought to the laboratory. Such 21 plastic specimen tubes having 50 nymphs were prepared for each insecticide for exposing to various test concentrations.

The cotton hybrid cv. RCH 2 non Bt was grown in isolated condition in pots under caged condition and the top leaves with long petioles of 75-90 days old plants were selected by slanting cut. The cut end of the petioles were wrapped immediately with cotton swab moisten with 10 per cent sucrose solution and sealed in plastic vial or tube (2 ml size) with parafilm. Such 150 leaves were kept ready for experimentation. The IRAC leaf dip method was used for the bioassay study of each insecticide. Three leaves (medium to large size) were dipped for 30 seconds in insecticidal solutions of each concentration prepared for each insecticides. In control treatment, leaves were dipped in distil water only. Each twigs with 2-3 leaves after dipping were allowed to air dry for 10 to 15 minutes and placed individually in the plastic specimen jar. Fifty (50) young nymphs aspirated previously in plastic specimen tube was released in each jar by gently opening the mouth towards treated leaf inside the plastic jar and the jar was covered with double layer muslin cloth immediately after release and tied with rubber band and closed with lid. Such 21 sets for each of the insecticide was used. Observations on mortality of nymphs of leafhopper were recorded up to 72 hours at one day interval. Similar procedure followed for second sampling and bioassay study during second week of October, 2013. At every 24 hour observations, the number of dead nymphs at the bottom of the jar were counted. Moribund nymphs not responding to disturbance by '0' no. camel hair brush was considered as dead. At the end of 72 hours, the number of live and dead nymphs were conted and the data so obtained for each concentrations including control were subjected to log dose probit analysis POLO software furnished through Central institute for cotton research (CICR) Nagpur. The LC_{50} and LC_{90} values of each insecticides so obtained for bioassay studies on leafhopper populations collected initially from unsprayed field and later from sprayed fields were compared. The LC_{50} (ppm) of each test insecticide against nymphs of leafhopper was taken into consideration for assessing the variability in their response. The resistance ratio between leafhopper populations collected from the treated and untreated fields of Main cotton research station (MCRS) Surat were determined for each insecticides using the formula as under

$$RR = \frac{LC_{50} \text{ of test insecticide against nymphs from sprayed fields}}{LC_{50} \text{ of test insecticide against nymphs from unsprayed fields}}$$

Results and Discussion

The data on LC_{50} (ppm) for tested insecticides to insecticide unexposed (early in the season) field populations of leafhopper (young nymphs) at seventy two hours after treatments are presented in Table 1. Amongst neonicotinoid insecticides, the LC_{50} (ppm) for imidacloprid 17.8 SL was 0.053 ppm with fiducial limit of 0.000 to 0.410 ppm. It was 0.051 ppm for thiamethoxam with fiducial limit of 0.0000 to 0.781 ppm and 0.055 ppm for acetamiprid 20 SP with fiducial limit of 0.0000 to 0.0897 ppm in nymphs of leafhopper populations (unexposed). In organophosphates, the LC_{50} value was 0.014 ppm for acephate 75 SP with fiducial limit of 0.0000 to 0.276 ppm whereas it was 0.156 ppm with fiducial limit of 0.0006 to 0.753 ppm for monocrotophos. The data revealed that there was not much variation with respect to insecticides belonged to neonicotinoid group when initial unexposed populations were used for bioassay studies. However, for organophosphate group, there was much variation in both the tested insecticides.

Generating base line susceptible data for leafhopper is crucial requiring continuous rearing and maintaining the unexposed populations over several generations. While studying toxicity of various insecticides to leafhopper, Kalra *et al*. (2001) found LC_{50} of imidacloprid as 0.00447 and for thiamethoxam it was 0.063. Pradeepa and Regupathy (2002) generated baseline susceptible data for cotton leafhopper by exposing leafhopper for bioassay from F1 to F7 generations which showed LD_{50} varied from 114.79 to 46.02 ppm for acephate. They reported LC_{50} of imidacloprid as 0.000457 ppm in F1 generation and could not estimated data in F7 generation of susceptible populations owing to high sensitive response. They considered LC_{95} for acephate as 850 ppm as discriminating dose for monitoring the field populations for their resistance to insecticide due to lack of previous base line data. The LC_{50} for imidacloprid was reported to be in the range of 0.00040 to 0.00050 per cent in field collected leafhopper populations from different locations *viz*., Ludhina, Hoshiarpur, Faridkot and Mansa districts of Punjab. (Sunadaram, 2004, unpublished).Shreevani *et al.* (2012) reported the LD_{50} value for the thiamethoxam 25 WDG, imidacloprid 17.8 SL and clothianidin 50 WDG as 0.001, 0.007 and 0.041 ppm, respectively at 24 hours after spray. While resistance monitoring across India, Kranthi *et al.* (2014) found that the leafhopper populations collected from Bhatinda, Punjab was most susceptible to imidacloprid with $LC_{50}$of 0.02 ppm and whereas field populations of Hisar, Haryana was most susceptible to thiamethoxam with LC_{50} of 0.01 ppm than different locations across India. The present investigation of LC_{50} of imidacloprid as 0.053 ppm and of thiamethoxam as

Table 1 : LC_{50} of insecticides in young nymphs of leafhopper populations collected from unsprayed fields (72 hours after treatment)

S.N.	Insecticide	df	LC_{50}	FL	LC_{90}	FL	X^2	g	Heterogeneity	slope
Neonicotinoid insecticides										
1	Imidacloprid 17.8 SL	4	0.053	0.000 - 0.410	59.363	20.420 - 282.065	3.717	0.194	0.93	0.420± 0.094
2	Thiamethoxam 25 WG	4	0.051	0.0000 - 0.781	76.844	15.278 - 23326.492	4.250	0.428	1.06	0.403 ± 0.092
3	Acetamiprid 20 SP	4	0.055	0.0000 - 0.897	94.679	17.656 - 4875.086	4.410	0.457	1.10	0.396 ± 0.092
Orgenophosphate insecticides										
4	Acephate 75 SP	4	0.014	0.0000 - 0.276	339.906	78.361 - 10519	1.677	0.3	0.42	0.292 ± 0.082
5	Monocrotophos 36 WSL	4	0.156	0.0006- 0.753	79.452	31.037 - 326.769	2.473	0.149	0.62	0.473± 0.093

Table 2 : LC_{50} of insecticides in young nymphs of leafhopper populations collected from sprayed fields (72 hours after treatment)

S.N.	Insecticide	df	LC_{50}	FL	LC_{90}	FL	X^2	g	Heterogeneity	slope	RR*
Neonicotinoid insecticides											
1	Imidacloprid 17.8 SL	4	5.76	0.11 - 28.078	2364.90	347.457 - 1047383.15	7.208	0.352	1.8	0.491± 0.078	108.68
2	Thiamethoxam 25 WG	4	3.99	0.23 - 21.571	3603.06	405.139 - 20627174.98	7.796	0.422	1.95	0.434 ± 0.073	78.24
3	Acetamiprid 20 SP	4	1.43	0.28 - 3.803	447.18	174.378 - 2067.13	3.328	0.097	0.83	0.513 ± 0.081	25.99
Orgenophosphate insecticides											
4	Acephate 75 SP	4	0.13	0.001 - 1.010	1012.13	232.925 - 26824.43	2.053	0.216	0.51	0.491± 0.078	9.29
5	Monocrotophos 36 WSL	4	4.53	0.16 - 25.784	3903.71	399.887 - 95375900.7	8.713	0.465	2.18	0.434 ± 0.073	29.04

df: degree of freedom, LC; Lethal concentration, RR: Resistance ratio, FL: Fiducial limit, X^2:Chiqe square,* Resistance ratio estimated by dividing LC_{50} (ppm) of test insecticide against leafhopper populations collected from sprayed fields to that of unsprayed fields.

0.051 ppm revealed that the leafhopper populations collected even in the initial season from unsprayed fields showed resistance build up in comparisons to above earlier reports of LC_{50} in susceptible populations.

Further, the data on LC_{50} (ppm) for tested insecticides to insecticide exposed (late in the season) field populations of leafhopper (young nymphs) at seventy two hours after treatments presented in Table 2 revealed the increased values of LC_{50}. Amongst neonicotinoid insecticides, the LC_{50} (ppm) for imidacloprid 17.8 SL was 5.76 ppm with fiducial limit of 0.11 to 28.078 ppm. It was 3.99 ppm for thiamethoxam with fiducial limit of 0.23 to 21.571 ppm and 1.43 ppm for acetamiprid 20 SP with fiducial limit of 0.28 to 3.803 ppm in nymphs of leafhopper populations (exposed). The LC_{50} values for organophosphates was 0.13 ppm for acephate 75 SP with fiducial limit of 0.001-1.010 ppm whereas it was 4.53 ppm with fiducial limit of 0.16 to 25.784 ppm for monocrotophos. The data revealed that there was much variability in toxicity response of leafhopper with respect to insecticides *viz*., imidacloprid and thiamethoxam in neonicotinoid group and both the organophosphate tested. The ineffectiveness of recommended dose in the field conditions or failures of the control of target pests have also been reported by several workers in the recent decade. Santhini and Uthasamy (1998) studied the susceptibility of cotton leafhopper to different insecticides at recommended dose and reported the variable response across locations (Coimbatore, Annur and Udumalpet) giving 26.67 to 33.3 per cent mortality of field collected first generation of third instar nymphs of leafhopper which indicated that the tolerance of same insecticide to leafhopper varied across locations. Reduced effectiveness of insecticides (methyl-o-demeton and dimethoate) at recommended dose has also been reported by Vidyasekaran *et al.* (1989) in Andhra Pradesh and Patel and Yadav (1995) in Gujarat. Kalra *et al.* (2001) found ineffectiveness of fenvalerate even at 30 times the normal concentration (0.15 per cent) indicating development of resistance in leafhopper to fenvalerate used widely in cotton. The data on present investigations on LC_{50} and LC_{90} for different insecticides for field populations collected from sprayed fields clearly showed resistance to all five insecticides under study. This showed temporal variability owing to exposure to same group insecticides when used frequently repeatedly.

In the absence of base line susceptible data of tested insecticides against leafhopper infesting cotton, resistance ratios were estimated from the variability in LC_{50} (ppm) of respective insecticides in the field populations during early (unexposed to insecticides) and late infestations (already exposed to insecticides) in cotton. The resistance ratio was 108.68 folds against imidacloprid in leafhopper populations collected from sprayed fields whereas it was 78.24 and 25.96 fold for thiamethoxam and acetamiprid 20 SP, respectively (Table 2). In organophosphate insecticides, 29.04 fold resistance was found in monocrotophos 36 WSL as against 9.29 fold in acephate 75 SP.Resistance to imidacloprid has also been reportedKshirsagar *et al.* (2012) found moderate to high level of resistance against imidacloprid (23.41 fold) and acetamiprid (19.08 fold) compared to dimethoate (5.21 fold).

Further they reported that the mechanism of resistance to these neonicotinoids linked with higher glutathion-s-transferase (GST) activity (10.89 fold), being more in field collected strain (0.147 nM-1min-1mg-1 with absorbance value of 0.066nm) than susceptible populations (0.0135 nM-1min-1mg-1 proteins. In Gujarat also, field populations of leafhopper were found to develop resistance against imidacloprid and thiamethoxam where used extensively showing resistance of 5 to 800 fold against imidacloprid and 20 to 3200 fold against thiamethoxam in four districts of Saurashtra during 2009-10 when LC_{50} compared to most susceptible strain of leafhopper at Bhatinda, Punjab in absence of base line susceptibility data (Kranthi *et al.*, 2014, accepted for publication).The present investigations on variability in LC_{50} of tested insecticides to early season unsprayed populations and late season sprayed populations in absence of base line data as well as comparing the earlier reports of LC_{50} of insecticides estimated by above referred authors clearly indicated the built up of resistance in the leafhopper populations, especially in imidacloprid and thiamethoxam amongst neonicotinoids at moderate level and monocrotophos and acephate to low level.

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