Detoxifying enzyme studies on cotton leafhopper, *Amrasca biguttula biguttula* (Ishida), resistance to neonicotinoid insecticides in field populations in Karnataka, India

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Abstract: The cotton leafhopper (*Amrasca biguttula biguttula* Ishida) is considered to be an alarming insect pest causing both quantitative and qualitative loss in cotton. *In situ* bioassay studies were done and the role of detoxifying enzymes in conferring resistance to neonicotinoid groups of insecticides in low (MUD), medium (DVG), high (HVR) and very high (GLB) pesticide usage areas of Karnataka were determined. Bioassay studies showed that imidacloprid, thiamethoxam, acetamiprid, thiacloprid and clothianidin registered varying levels of resistance for all the locations studied. The resistance ratio was high in imidacloprid (3.35, 8.57, 9.15 and 12.27 fold respectively) and the lowest in dinoferuran (1.86, 5.13, 6.71 and 9.88 fold respectively). Furthermore, the enzyme activity ratio (glutathione-S-transferase) was relatively greater, and corresponded to the higher LC_{50} values of neonicotinoids for very high, high, medium and low pesticide usage areas. Our study suggested that the higher activity of the detoxifying enzyme in the resistance population of cotton leafhopper apparently has a significant role in endowing resistance to neonicotinoid groups of insecticides. However, this study recommends using neonicotinoids in cotton growing areas with caution.

Key words: *Amrasca biguttula biguttula*, bioassay, glutathione-S-transferase, neonicotinoids, resistance

Introduction

Cotton (*Gossypium* spp.) popularly known as "white gold" or "king of fibers" is one of the most important commercial fibre crops of global significance and a major source of raw material for the domestic textile industry in India. Cotton and textile exports account for nearly one-third of total foreign exchange earnings of India. It also provides a means of livelihood for millions of farmers and workers involved in the cotton industry, from growing and processing to trading (Mayee *et al.* 2004). India has the unique distinction of being the only country in the world to cultivate all four cultivable *Gossypium* species *viz.*, old world cotton *G. arboreum* L., *G. herbaceum* L. and new world cotton *G. barbadense* L. and *G. hirsutum* L. as well as hybrids. The American cotton, *G. hirsutum* represents 90% of the hybrid cotton genotypes grown in India (Kohel *et al.* 2001; Hong-Bin *et al*. 2008).

Cotton is cultivated on about 35.71 M ha across the world and on about 12.6 M ha in the country. India ranks first in terms of cultivated area, occupying 32% of the global cotton area followed by China and contributes 21% of the global cotton produce (36.10 mln bales), ranking second to China. The domestic consumption of cotton in India was about 40 mln bales during 2014–2015 (Anonymous 2015). The productivity of cotton in India is about 537 kg · ha^{-1}, whereas, Brazil holds the highest productivity level of 2,027 kg · ha^{-1} among the major cotton growing countries. The major cotton growing states in India are Maharashtra (7.36 M ha), which account for 37% of the area followed by Gujarat (3.06 M ha), Andhra Pradesh (5.79 M ha) Punjab, Haryana and Rajasthan (1.50 M ha), Madhya Pradesh (0.76 M ha), Karnataka (0.70 M ha) and Tamil Nadu (0.57 M ha) (Anonymous 2015).

The crop can be ravaged by several insect pests causing drastic reductions in yield. In many cotton growing areas of the world, the major limiting factor in its production is damage due to insect pests (Bennett *et al.* 2004). The pest spectrum of cotton is quite complex. About 1,326 insects and mites all over the world (Hargreaves 1948) and about 162 in India have been recorded as pests of cotton, among them 15 are production constraints. In the early stages of the crop, sucking pests like aphids [*Aphis gossypii* (Glover)], leafhopper [*Amrasca biguttula biguttula* (Ishida)], thrips [*Thrips tabaci* (Hood)] and whitefly [*Bemisia tabaci* (Gennadius)] and in later stages, the bollworm complex cause significant damage to the crop. The extent of losses caused by sucking pests and bollworms has been worked out to be 12 and 44%, respectively (Dhawan *et al.* 1988).

The genetically modified cotton, popularly known as 'Bt-cotton', was released for commercial cultivation in India in March, 2002. Since then, it has played a major role in effectively protecting the crop from bollworms, especially the American bollworm, *Helicoverpa armigera* (Hübner), thus preventing yield losses (Benedict 1996; Jenkins

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et al. 1997; Perlak *et al.* 2001). Currently, about 90% of the total cultivated area in the country is under Bt-cotton. The biggest gain from technology is in the form of reduced insecticide usage from 46% in 2001 to less than 26% after 2006 and 21% during the years 2009 and 2011 (Rohini *et al.* 2012).

Among the sucking pests of economic importance, leafhopper and whitefly cause both qualitative and quantitative losses in cotton (Dhaliwal *et al.* 2006). Leafhopper is a regular and key pest not only in India but also in Pakistan, Bangladesh, Thailand, Australia and other South East Asian countries (Singh and Agarwal 1988). This pest is prevalent in cotton from the vegetative to reproductive phases of the crop. The loss in seed cotton yield due to leafhopper alone accounted to 390 kg · ha^{-1} (Pandi 1997) and a 11.60% reduction in cotton yield (Dhawan *et al.* 1988). It is beyond doubt that Bt-cotton has played a major role in effectively protecting the crop from bollworms. However with the introduction of Bt-cotton hybrids, the pest scenario of cotton crop has changed. The decline of the bollworm complex coincides with an increased incidence of sucking pests, especially the leafhopper scourge. Both nymph and adult leafhoppers suck the sap from the under surface of the leaf during the early stages of the crop causing specking symptoms, crinkling, distortion of leaves and reddening all along the sides of leaves with downward curling leading to the production of typical "hopper burn" symptoms (Painter 1951; Uthamasamy 1985), affecting the growth by shedding of leaves, squares and young bolls with significant yield losses. After the introduction of transgenic cotton, chemical control is primarily targeted against sucking pest complexes. Among the various strategies adopted by farmers, insecticides form the most popular defense in suppressing the sucking pests in spite of many drawbacks (Preetha *et al.* 2013). Indiscriminate use of insecticides in the cotton ecosystem has resulted in the development of resistance, resurgence, pest outbreaks, effects on non targets, loss in biodiversity and environmental pollution (Dhaliwal and Arora 2001).

The cotton leafhopper, *A. biguttula biguttula* was found to have developed resistance to the recommended organophosphate insecticides *viz*., metasystox, dimethoate and phosphamidon in India (Santhini and Uthamasamy 1998; Chalam and Subbaratnam 1999; Chalam *et al.* 2001; Praveen 2003). Of late, a new group of insecticides *viz.*, neonicotinoids consisting of imidacloprid, thiamethaxam and acetamiprid were found to be more effective against cotton leafhoppers than conventional insecticides. These neonicotinoids occupy almost 20% of insecticides on the world market. Currently, neonicotinoids are the leading insecticides in the world and many companies are focusing on increasing their market share by developing new neonicotinoid compounds. Neonicotinoids have become an important class of insecticides and act as a competitive inhibitor on nicotinic acetylcholine receptors in the central nervous system (Bai *et al.* 1991; Chao *et al.* 1997). Due to their unique action mechanism, neonicotinoids have strong insecticidal toxicity and relatively little toxicity to nontargets (Xie 1998). Presently neonicotinoid insecticides are classified mainly into three generations based on their chemical structure: I generation – neonicotinoids having a heterocycle of chloropyridine (acetamiprid, thiacloprid, nitenpyram and imidacloprid), II generation – neonicotinoids having a heterocycle of chlorinated thiazole (thiamethoxam and clothianidin) and III generation – neonicotinoids having a heterocycle of tetrahydrofuran (dinotefuran) (Yang *et al.* 2007). To counter the development of resistance in *A. biguttula biguttula* to neonicotinoid groups of insecticides, it is necessary to monitor the level of resistance in the field populations of *A. biguttula biguttula*. In the past few years, imidacloprid has been a major neonicotinoid insecticide for controlling *A. biguttula biguttula* and other sucking pests in India. However, resistance to imidacloprid has been recorded in many target insects, especially *A. biguttula biguttula* (Kshirsagar *et al.* 2012) and cotton aphid, *A. gossypii* (Zhang and Zhang 1999; Wang *et al.* 2001). Furthermore, imidacloprid resistant insects have shown cross resistance to other groups of insecticides, including other neonicotinoid insecticides (Liu *et al.* 1993). Recently failure of neonicotinoids in the field was noticed in the leafhopper populations of Andhra Pradesh and Maharashtra (Anonymous 2013). The continuous use of neonicotinoids has probably led to the development of resistance. Also, the fact that imidacloprid treated Bt-cotton seeds are available on the market is giving an impetus for cotton leafhopper to develop resistance to insecticides (Kshirsagar *et al.* 2012; Anonymous 2013).

Therefore, insecticide resistance is a serious threat to cotton growers and it is imperative to test the level of resistance in order to design and implement successful insecticide resistance management strategies. This highlights the need to assess and monitor the responses to insecticides of the target population to enable the timely use of alternative control measures, such as rotation of different insecticides, reduction in the number of applications or the use of synergists (McAuslane *et al.* 1993; Stansly *et al*. 1997). Therefore, in order to assess a sustainable resistance management programme it is essential to survey the insecticide resistance levels in cotton-growing areas (Nibouche 1994). Thus, in the present study we collected leafhopper populations from different cotton growing areas of Karnataka and identified the susceptible population by bioassay studies, coupled with an estimation of detoxifying enzyme. Glutathione-S-transferase (GST) which is used for detecting the level of resistance developed by the leafhopper to neonicotinoids.

Materials and Methods

The experiment on the toxicity of neonicotinoid insecticides to cotton leafhopper, *A. bigittula biguttula* was carried out at the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad (UASD), Karnataka, India during 2013–2014. The leafhopper populations were collected from low (Mundgod; MUD), medium (Davanagere; DVG), high (Haveri; HVR) and very high (Gulbarga; GLB) pesticide usage areas of Karnataka.

Culturing of test insects

Susceptible *A. biguttula biguttula* required for bioassay were mass cultured under caged conditions. Bhendi were

sown at weekly intervals in 10 × 10 m² areas and maintained without exposure to any insecticides. As germination progressed, the seedlings were covered with nylon cages of mesh size 0.15 × 0.15 cm to prevent the escape of leafhoppers. The caged bhendi plants, about 20–25 days old, were inoculated with the field collected leafhopper adults and the leafhoppers were cultured continuously for six generations to get a homozygous and relatively susceptible population to work out the resistance ratio (*RR*).

Insecticides used

The neonicotinoid compounds used for bioassay studies are given in Table 1. Commercial formulations of insecticides were diluted with analytical grade acetone to obtain the desired concentrations. Preliminary range-finding tests were carried out to fix the test concentrations, which cause 20 to 80% mortality to the leafhoppers.

Bioassay

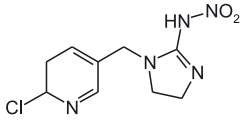
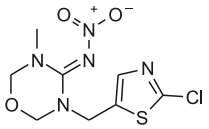
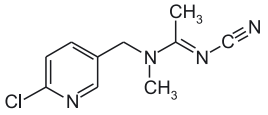
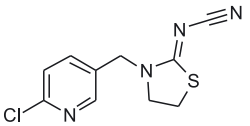
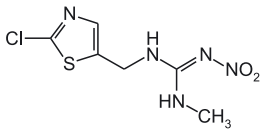
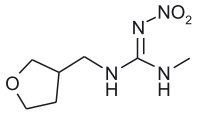
Bioassay studies were conducted according to the standard *Bemesia tabaci* susceptibility test, Insecticide Resistance Action Committee (IRAC) method No. 8 developed and recommended by the IRAC. Plastic cups (1 lit capacity) were selected to conduct bioassay studies. The uncontaminated fresh cotton leaves (DCH-32) plucked from the cotton field were selected and cleaned with the wet cotton swab. The leaf petiole was cut to a length of approximately 4–5 cm, the petiole of the test leaf was passed through a centrifuge tube containing 10% 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 acetone and the leaves were dipped in the insecticide solution for 10 sec by holding the leaf of the petiole. Then the leaves were kept for drying in the laboratory, approximately 5–10 min. The treated leaf was placed in a plastic cup and 10 nymphs from laboratory and field collected populations were released per cup and the cups were covered with muslin. A control, treated with acetone alone, was maintained at each time of experimentation. Observations were recorded 24, 48 and 72 h after treatment. Moribund leafhopper nymphs which did not respond to probing were considered as dead. Percentages of mortality for each concentration of test insecticide and the controls were computed and corrected percent mortality was calculated by Abbot's formula (Abbot 1925).

$$\text{Corrected percent mortality} = \frac{T - C}{100 - C} \times 100$$

where: T – percent mortality in treatment; C – percent mortality in control.

The corrected mortality data of each test insecticide of each location (low, medium, high and very high pesticide usage areas) were subjected to probit analysis using SPSS probit analysis software (version 16.0) used for calculat-

Table 1. Neonicotinoids used for bioassay studies

Compound	Chemical name	% Active ingredient	Structure
Imidacloprid	(EZ)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	17.8 SL	
Thiamethoxam	(EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine	25 WG	
Acetamiprid	(E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine	20 SP	
Thiacloprid	(Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2-ylidenecyanamide	21.7 SC	
Clothianidin	1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	50 WG	
Dinotefuran	2-methyl-1-nitro-3-[(tetrahydro-3-furanyl)methyl]guanidine	20 SG	

ing of LC_{50} and LC_{90} values. Later, the *RR* for each insecticide was calculated using the formula given below:

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

Biochemical basis of insecticide resistance

The biochemical basis of insecticide resistance in cotton leafhoppers was determined by estimating the GSTs enzyme according to standard protocol (Habig *et al.* 1974). Randomly 50–60 *A. biguttula biguttula* nymphs weighing 0.15 g were collected from the field. A whole body homogenate of nymphs was prepared by using 2 ml sodium phosphate (SPB) buffer [0.1 mM pH 7.0 containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), propylthiouracil (PTU) and phenylmethylsulfonyl fluoride (PMSF) each]. The homogenate was centrifuged at 5,000 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 an enzyme source. The actual quantification of GSTs was done by following standard protocol given by Jakoby (1978). Later, the enzymatic activity ratio (*EAR*) for GSTs enzyme for a leafhopper population was calculated using the formula:

$$EAR = \frac{\text{Enzymatic activity in leafhopper population of particular location}}{\text{Enzymatic activity in leafhopper population maintained in the laboratory}}$$

Results and Discussion

Bioassay studies

Using preliminary data from farmers about pesticide usage patterns (based on the number of sprays), cotton crops were categorized as low (Mundgod), medium (Davanagere), high (Haveri) and very high (Gulbarga) pesticide usage areas of Karnataka. At the very high pesticide usage area (Gulbarga), dinotefuran was found to be highly toxic to leafhopper with the least LC_{50} value of 19.76 ppm followed by clothianidin (68.96 ppm), thiacloprid (96.75 ppm), thiamethoxam (142.00 ppm) and acetamiprid (119.86 ppm). Imidacloprid was found to be the least toxic to the leafhopper with a higher LC_{50} value of 201.36 ppm. The order of toxicity of neonicotinoid groups of insecticides to leafhopper was as follows: dinotefuran > clothianidin > thiacloprid > acetamiprid > thiamethoxam > imidacloprid (Table 2). At the high pesticide usage area (Haveri), dinotefuran was found to be highly toxic with the least LC_{50} value of 13.41 ppm while imidacloprid was the least toxic with a LC_{50} value of 150.11 ppm. The order of toxicity followed the same trend as Gulbarga. In Davanagere (medium pesticide usage area), dinotefuran emerged as highly toxic to leafhopper with a LC_{50} value of 10.65 ppm and imidacloprid was found to be the least toxic to leafhopper with a higher LC_{50} value of 140.69 ppm among the neonicotinoid groups of insecticides evaluated against cotton leafhopper. Even low pesticide usage areas showed the same order of toxicity to different neonicotinoids with maximum toxicity in dinotefuran with a LC_{50} of 3.72 ppm and the least toxicity in imidacloprid with a LC_{50} of 54.91 ppm (Table 2).

Resistance ratio

Among the neonicotinoids, the LC_{50} value of susceptible laboratory populations was too low in dinotefuran (2.00 ppm) followed by clothianidin (5.20 ppm) and thiacloprid (9.38 ppm) when maintained up to six generations under caged conditions without any selection pressure. Imidacloprid, thiamethoxam and acetamiprid recorded higher LC_{50} values of 16.41 ppm, 12.56 ppm and 11.78 ppm, respectively (Table 2). A higher resistance ratio was recorded for clothianidin at the very high pesticide usage area (Gulbarga) (13.26), followed by imidacloprid (12.27) and thiamethoxam (11.20). A similar trend was observed in the medium pesticide usage area (Davanagere), whereas, in the high pesticide usage area (Haveri) a higher resistant ratio was recorded in thiamethoxam (9.57) followed by imidacloprid (9.15). Thiacloprid recorded a lower resistant ratio of 5.92 and dinotefuran, 6.71. However, in the low pesticide usage area (Mundgod) the resistance ratio for all the test insecticides varied from 1.12 to 3.35 ppm and was the highest with imidacloprid (3.35) and the lowest with thiacloprid (1.12) (Table 2).

Table 2. Development of resistance by *Amrasca biguttula biguttula* to neonicotinoid groups of insecticides at low, medium, high and very high pesticide usage areas of Karnataka

Insecticides	LC_{50} values [ppm]					*RR* [in folds]			
	laboratory susceptible population	low	medium	high	very high	low	medium	high	very high
Dinotefuran 20 SG	2.00	3.72	10.25	13.41	19.76	1.86	5.13	6.71	9.88
Clothianidin 50 WG	5.20	7.16	41.21	45.30	68.26	1.37	7.93	8.71	13.26
Thiacloprid 21.7 SC	9.38	10.51	51.57	55.56	96.75	1.12	5.50	5.92	10.31
Acetamiprid 20 SP	11.78	14.72	83.59	102.76	119.86	1.25	7.10	8.72	10.18
Thiamethoxam 25 WG	12.56	26.89	97.21	120.19	142.00	2.14	7.74	9.57	11.30
Imidacloprid 17.8 SL	16.41	54.91	140.69	150.11	201.36	3.35	8.57	9.15	12.27

low – Mundgod; medium – Davanagere; high – Haveri; very high – Gulbarga; *RR* – resistance ratio (LC_{50} values of particular location/LC_{50} value of laboratory susceptible population)

The cotton leafhopper, *A. biguttula biguttula* is an alarming pest causing both quantitative and qualitative losses in cotton. It has developed resistance against most of the commonly used insecticides (including neonicotinoids) due to indiscriminate usage for the past decade to manage sucking pests in cotton. The resistance development by the leafhopper to neonicotinoid groups of insecticides at very high (Gulbarga), high (Haveri), medium (Davanagere) and low (Mundgod) pesticide usage areas of Karnataka revealed that imidacloprid recorded a higher level of resistance development for very high (LC_{50} = = 201.36 ppm and *RR* = 12.27-fold) to low (LC_{50} = = 54.94 ppm and *RR* = 3.35-fold) pesticide usage areas followed by thiamethoxam (LC_{50} = 142.0 ppm, *RR* = 11.30-fold and LC_{50} = 26.89 ppm, *RR* = 2.14-fold), acetamiprid (LC_{50} = 119.86 ppm, *RR* = 10.18-fold and LC_{50} = 14.72 ppm, *RR* = 1.25-fold), thiacloprid (LC_{50} = 96.75 ppm, *RR* = 10.31-fold and LC_{50} = 10.51 ppm, *RR* = 1.12-fold), clothianidin (LC_{50} = 68.26 ppm, *RR* = 13.26-fold and LC_{50} = 7.16 ppm, *RR* = 1.37-fold) and the least was dinoferuran (LC_{50} = = 19.76 ppm, *RR* = 9.88-fold and LC_{50} = 3.72 ppm, *RR* = 1.86-fold) (Table 2).

The present findings clearly indicated that all the neonicotinoid insecticides showed considerable variation in resistance development in leafhopper even though they have similar modes of action. It might be evident that, according to structural analysis, acetamiprid, thiacloprid, nitenpyram and imidacloprid are classified as the first generation of neonicotinoid compounds with a heterocycle of chloropyridine. Thiamethoxam and clothianidin are classified as the second generation with a heterocycle of chlorinated thiazole and dinotefuran is classified as the third generation with a heterocycle of tetrahydrofuran (Yang *et al.* 2007) and extensive insecticide usage pattern. Kranthi (2007) opined that overuse of neonicotinoid groups of insecticides *viz.*, imidacloprid, acetamiprid and thiamethoxam with scant regard for the principles of insecticide resistance management can lead to the development of resistance to the insecticides. In the present study a faster development of resistance was noticed in newer neonicotinoids such as clothianidin, thiacloprid and dinotefuran. This might be caused by cross resistance between different groups of neonicotinoids due to the presence of similar active groups and modes of action. The present study corroborates with many other researchers (Horowitz *et al.* 2004; Kshirsagar *et al.* 2012; Sagar *et al.* 2013).

Biochemical basis of insecticide resistance in cotton leafhopper populations

Glutathione S-transferases (GSTs) are the major family of detoxification enzymes. They catalyze the conjugation of the tripeptide glutathione to electrophilic centers of lipophilic compounds, thereby increasing their solubility and aiding excretion from the cell. They possess a wide range of substrate specificities, including endogenous substrates, such as reactive unsaturated carbonyls, reactive DNA bases, epoxides and organic hydroperoxides produced *in vivo* as the breakdown products of macromolecules during periods of oxidative stress (Hayes and Pulford 1995). Thus GSTs play a vital role in protecting tissues against oxidative damage and stress. The GSTs in insects are primarily of interest because of their role in insecticide resistance. They are involved in the Odealkylation or O-dearylation of organophosphorus insecticides (Hayes and Wolf 1988) as a secondary mechanism in the detoxification of organophosphate metabolites (Hemingway *et al.* 1991) and in the dehydrochlorination of organochlorines (Clark and Shamaan 1984). In leafhopper, MFO's (mixed function oxidases), GSTs and carboxylesterase play a predominant role in imparting resistance to insecticides (Regupathy and Ayyasamy 2004; Kshirsagar *et al.* 2012; Sagar *et al.* 2013). Similarly, GSTs and carboxylesterase are important in creating resistance in aphids (Ibrahim *et al.* 2016).

The bioassay studies are also supported by the presence of detoxifying enzymes GST activity in the laboratory populations of *A. biguttula biguttula* collected from different locations and subjected to biochemical analysis of insecticide resistance. The results revealed that the detoxifying enzyme GSTs activity was highest in the cotton leafhopper population of Gulbarga (very high pesticide usage) (0.241 nM · min^{-1} · mg^{-1}) followed by Haveri (high pesticide usage) (0.190 nM · min^{-1} · mg^{-1}), Davanagere (medium pesticide usage) (0.150 nM · min^{-1} · mg^{-1}) and Mundgod (low pesticide usage area) (0.031 nM · min^{-1} · · mg^{-1}) (Table 3). The enzymatic activity ratio was worked out by comparing GSTs activity in field collected leafhopper populations and laboratory maintained susceptible strains. The enzymatic activity ratio in the leafhopper population of Gulbarga area recorded the highest GST activity ratio (11.36) followed by Haveri (8.97), Davanagere (7.08) and Mundgod (1.47) (Table 3).

Table 3. Glutathion-S-transferase (GST) activity in field collected populations of leafhopper at very high, high, medium and low pesticide usage areas of Karnataka

No.	District	GST activity* [nM · min^{-1} · mg^{-1}]	Increases in GST activity over susceptible population
1	GLB – very high pesticide usage area	0.241±0.010	11.36
2	HVR – high pesticide usage area	0.190±0.040	8.97
3	DVG – medium pesticide usage area	0.150±0.020	7.08
4	MUD – low pesticide usage area	0.031±0.002	1.47

*mean±standard deviation

GLB – Gulbarga; HVR – Haveri; DVG – Davanagere; MUD – Mundgod; GST activity in laboratory susceptible population is 0.021±0.010 [nM · min^{-1} · mg^{-1}]

The GSTs activity in cotton leafhoppers collected from field populations from very high, high, medium and low pesticide usage areas of Karnataka varied from 0.2410 nM · min^{-1} · mg^{-1} (very high pesticide usage area) to 0.0123 nM · min^{-1} · mg^{-1} (low pesticide usage area) (Table 3). The enzymatic activity ratio of different pesticide usage areas varied from 1.47 to 11.36 (very high pesticide usage area). Higher GSTs activity and enzymatic activity ratios were noticed in the leafhopper population of Gulbarga (very high), followed by Haveri (high) and Davanagere (medium) pesticide usage areas while, lower GSTs activity and enzymatic ratios were noticed in the low pesticide usage area (Mundgod). The increased GSTs activity in field collected leafhopper populations indicated the role of GSTs in the detoxification of insecticides resulting in resistance to the neonicotinoids, which is evident from an inventory of insecticide resistance results. Although little research has been done in this area, there are a few reports available which have some good points for discussion. In the present study, insecticide resistance results (LC_{50} values) corresponded with the results of GSTs activity. Therefore it is evident that the development of resistance to neonicotinoid insecticides is in line with the reports of Kshirsagar *et al.* (2012) who reported that relatively more GSTs values corresponding to the higher LC_{50} values of neonicotinoids indicated the role of GSTs in imparting resistance in cotton leafhoppers to imidacloprid and acetamiprid. Wen *et al.* (2009) also opined that the resistance of brown plant hopper to imidacloprid was to be attributed to detoxification caused by the enhancement of cytochrome P450 monooxygenases activity. Similarly, biochemical analysis of *Laodelphax striatellus* (Fallen) showed that the increase in cytochrome P450 monooxygenase and esterase plus acetylcholinesterase insensitivity may be involved in resistance to imidacloprid (Gao *et al.* 2008).

From the present findings it can be concluded, that the development of resistance to neonicotinoid groups of insecticides in the field populations of *A. biguttula biguttula* could be due to repeated and indiscriminate use of neonicotinoids and enhanced activity of the detoxifying enzymes i.e. GSTs. Furthermore, the enhanced activity of GST's, in the resistant populations might also elucidate the observed cross resistance against tested new groups of neonicotinoid insecticides (clothianidin, thiacloprid and dinotefuran). However, this study recommends that neonicotinoids be used with caution in cotton growing areas.

The present study suggests that to attain effective and sustainable leafhopper management, it is prudent to use all the possible ecological engineering and biorationals available for insecticide resistance management practices such as the use of resistant/tolerant genotypes (Bt-cotton or Non-Bt cotton), or intercropping with lucerne, groundnut and green gram to encourage natural enemy populations in the cotton ecosystem. It is also 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 adaption of Resistance Management Strategies (IRM) to delay the development of resistance to sucking pests.

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