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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