Interaction between host plant resistance and biological activity of *Bacillus thuringiensis* in managing the pod borer *Helicoverpa armigera* in chickpea

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Crop Protection

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Abstract

The legume pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) has developed high levels of resistance to conventional insecticides, and therefore, efforts are being made to develop transgenic chickpea expressing toxin genes from the bacterium, *Bacillus thuringiensis* (*Bt*) for controlling this pest. However, there is an apprehension that acid exudates in chickpea might interfere with biological activity of *Bt.* Therefore, we studied the biological activity of *Bt* (Biolep^{R}) on four chickpea genotypes with different levels of resistance to *H. armigera* under field conditions, and by incorporating lyophilized lead and pod tissue into the artificial diet with and without *Bt.* The pH of the acid exudates varied from 2.1 to 2.90, and malic and oxalic acid were the major components of the acid exudates in different chickpea genotypes. There was no survival of *H. armigera* larvae in chickpea plants spayed with 0.1, 0.2 and 0.5% of *Bt.* There was a significant reduction in larval survival, larval and pupal weights and fecundity, and prolongation of larval and pupal periods in chickpea plots sprayed with *Bt* (0.05%) as compared to the unsprayed plants. Biological activity of *Bt* was lower on artificial diets with leaf or pod

powder of chickpea genotypes, which might be because of a low intake of *Bt* toxins due to antifeedant effects of acid exudates in the chickpea or reduction in biological activity of *Bt* due to the interaction of biochemical constituents in chickpea with the *Bt* toxins. Larval survival, larval and pupal weights, pupation and adult emergence were significantly lower on diets with leaf or pod powder of the *H. armigera*-resistant genotypes than on the susceptible check. Chickpea genotypes with resistance to *H. armigera* acted in concert with *Bt* to cause adverse effects on the survival and development of this insect. The results suggested that development of transgenic chickpeas expressing toxin genes form *Bt* will be quite effective for controlling of the pod borer, *H. armigera.*

Keywords: Chickpea, *Helicoverpa armigera, Bacillus thuringiensis*, host plant resistance, acid exudates, transgenics, pest management

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1. Introduction

The legume pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera), is one of the most important constraints to crop production globally and is widely distributed in Asia, Africa, Australia and the Mediterranean Europe (Sharma, 2005). It is a polyphagous pest and attacks more than 200 plant species including cotton, chickpea, pigeonpea, tomato, maize, sorghum, sunflower and a range of vegetables, fruit crops and tree species (Manjunath et al., 1989; Fitt, 1991). In India, it has been recorded from over 20 crops and 180 wild hosts (Manjunath et al., 1989). *Helicoverpa armigera* causes an estimated loss of US$325 million in chickpea (ICRISAT, 1992) and over US$5 billion on different crops worldwide, despite application of pesticides costing over US$2 billion annually (Sharma, 2005). Insecticides have been widely used for controlling this pest on different crops, but undesirable side effects of synthetic insecticides, including development of resistance, have necessitated a shift to more eco-friendly approaches for controlling this pest (McCaffery et al., 1989; Kranthi et al., 2002). Several chickpea genotypes with low to moderate levels of resistance have been identified in the past (Lateef,

1985; Sharma et al., 2007). However, the levels of resistance are unstable across seasons and locations because of variable population density and climatic conditions, and therefore, there is a need to improve the level of resistance to this pest in chickpea.

Genetic transformation as a means to enhance crop resistance or tolerance to biotic constraints has shown considerable potential to achieve a more effective control of target insect pests for sustainable food production (Sharma et al., 2002, 2004; Sharma, 2009). The δ-endotoxin genes from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) have been deployed in several crops for pest management (James, 2007). Efforts are underway to develop chickpea plants with *Bt* δ-endotoxin genes for resistance to *H. armigera* (Ramakrishna et al., 2005; Sharma et al., 2005; Sanyal et al., 2005). However concerns have been expressed that the trichome exudates in chickpea leaves and the pods, which are highly acidic in nature (pH 2.0 – 3.5) (Bhagwat et al., 1995), may have a negative influence on the biological activity of *Bt* sprayed on chickpea or toxin proteins expressed in transgenic chickpea.

Acidic exudates in chickpea have been reported to influence the biological activity of *H. armigera* nucleopolyhedrosis virus (HaNPV) (Rabindra et al., 1992; Bhagwat, 2001). The activity of *Bt* δ-endotoxins increases with an increase in pH from 8 to 10, but declines at a pH more than 10 (Behle et al., 1997). The acid exudates from chickpea are highly acidic in nature (Bhagwat et al., 1995), and this might influence the biological activity of *Bt t*oxins towards *H. armigera*. Food consumption by the third-instar larvae of *Spodoptera litura* (Fab.) decreases gradually on *Bt* treated food when exposed to increasing the pH from 6 to 10 (Somasekhar and Krishnayya, 2004). Because of the possible effect of pH on the biological activity of *Bt*, the present studies were undertaken to examine the interaction between chickpea genotypes with different levels of resistance to *H. armigera* and a commercial formulation of *Bt.* The focus of our studies was to determine if combination of varying levels of host plant resistance will significantly affect the performance of *Bt* against *H. armigera*. The results of this study will be useful for developing an integrated pest management program in chickpea, and in designing appropriate strategies for deployment of *Bt* genes in transgenic chickpea for controlling *H. armigera*.

2. Materials and Methods

2.1. Test material

Four chickpea genotypes with different levels of resistance to *H. armigera* (Sharma et al., 2007) (ICC 506EB - resistant, ICCV 10 - resistant, C 235 - moderately susceptible and L 550 - susceptible) were used to study the interaction of chickpea genotypes with *Bt* under field and laboratory conditions. The test genotypes were grown in the field during the 2005/07 post-rainy season at the research farm of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The plot size was 2 x 2.4 m^{-2} (four rows of 2 m length and planted at 60 x 10 cm row-to-row and plant-to-plant spacing). Diammonium phosphate (100 kg ha^{-1}) was applied as a basal dressing before sowing. The seeds were planted with a four cone planter and the field was irrigated immediately after planting and at monthly intervals thereafter. Normal agronomic practices were followed for raising the crop.

The plots of the four chickpea genotypes were sprayed with a commercial formulation of *Bt* (Biolep®, Biotech International Ltd., India) (0.05, 0.1, 0.2 and 0.5%) using a Knapdack sprayer (100 L ha^{-1}) at the vegetative stage (30 days after seedling emergence) with a Knapsack sprayer (ZENOAH, Japan). Unsprayed plots served as untreated controls. There were three replications for each genotype x *Bt* treatment in randomized complete block design. A polyethylene plastic sheet was held downwind to prevent spray drift to the adjoining plots. The leaves of the treated and untreated chickpea genotypes were collected 4 h after spraying for feeding to the *H. armigera* larvae in the laboratory.

2.2. Insect culture

The larvae of *H. armigera* used in the bioassays were obtained from the laboratory culture maintained at ICRISAT, Patancheru, Andhra Pradesh, India. Larvae were reared on chickpea based artificial diet (Armes et al., 1992) at 27 $\pm$ 1 $^{\circ}$C and 12 h photoperiod. The neonates were reared for 5 days in groups of 200 to 250 in 200 ml plastic cups containing a 2 to 3 mm layer of artificial diet on the bottom

and sides of the cup. Thereafter, the larvae were transferred individually to six cell-well plates (each cell-well was 3.5 cm in diameter and 2 cm in depth) to avoid cannibalism. Each cell-well had a sufficient amount of diet (7 ml) to support larval development until pupation. The pupae were removed from cell-wells, sterilized with 2% sodium hypochlorite solution and kept in groups of 50 in plastic jars containing Vermiculite. Upon emergence, 10 pairs of adults were released inside an oviposition cage (30 x 30 x 30 cm). Adults were provided with 10% sucrose or honey solution on a cotton swab for feeding. Diaper liners, which have a rough surface, were provided as a substrate for egg laying. The liners were removed daily and the eggs sterilized in 2% sodium hypochlorite solution. The liners with eggs were dried under a table fan and then placed inside the plastic cups with artificial diet. The liners were removed after 4 days. Freshly emerged neonate larvae were used for bioassays using the diet impregnation assay (Narayanamma et al., 2008).

2.3. Acidity of the leaf exudates and estimation of organic acids using high performance liquid chromatography

Since acid exudates were thought to influence the biological activity of the *Bt*, we measured the pH of the leaf extracts of the genotypes tested at the vegetative stage and also assessed the amounts of the organic acids (mainly oxalic and malic acids) in the leaf exudates using high performance liquid chromatography (HPLC). The acidity (pH) of the leaf exudates was measured by extracting 10 g of the terminal leaf tissue in 10 ml of distilled water in a test tube for 2 – 3 min. The leaves were then removed form the water and the volume made up to 10 ml. The pH of the extract was measured using a pH meter.

The amounts of different organic acids were estimated in the leaf samples collected at different stages of plant growth using HPLC. The terminal branches (with 3 – 4 leaves, 5 cm long) were excised from the plants in the field and placed in 10 ml test tubes. The test tubes were placed in an ice box and brought to the laboratory. The fresh weight of the samples was recorded on a Mettler balance. The samples were then Vortexed in 5 ml of milli Q water for 1 min in a test tube. The leaves were removed from the test tube and oven dried at 55 $^{\circ}$C for 3 days to record their dry weight. The leaf extracts were

filtered through 0.2 µm pore size PVDF (polyvinylidene difluoride) membrane filters and injected into HPLC [Waters, C18 column, 5 µm (4.6 x 250 mm)]. Mobile phase was potassium phosphate buffer 25 mM, pH 2.5. After equilibration of column with the mobile phase, 20 µl of processed sample was injected into HPLC system (automated program). The flow rate was 1 ml/min; run time 20 min and the compounds were detected at 215 nm using a photodiode detector. The peaks for oxalic and malic acids were identified by spiking the samples with the respective acids. The amounts of organic acids (oxalic and malic acids) were quantified from the standard curves prepared from the pure compounds.

2.4. Preparation of Bt δ-endotoxin proteins

The method reported by Shao et al. (1998) was slightly modified to prepare protoxin from the commercial *Bt* formulation. Ten grams (2.5 g x 4 tubes) of commercial *Bt* formulation (Biolep^{R}) was placed in centrifuge tubes and washed with one molar NaCl (10 ml each time) and centrifuged at 4,000 rpm for 5 min and then washed twice with deionised water and centrifuged at 4,000 rpm for 5 min. The sediment was dissolved in 2 per cent β-mercaptoethanol-NaOH buffer (2 ml of β-mercaptoethanol in 100 ml of water. The pH was adjusted to 10.7 with NaOH solution) and kept for 2 h on a stirrer at room temperature. The contents were centrifuged at 4000 rpm for 20 min, supernatant collected and pH adjusted to 4.4 with 2 M acetic acid. The contents were centrifuged at 4,000 rpm for 20 min. The protoxin precipitate was collected and dialyzed against water (dialyzed overnight and water changed 3 times). The amount of protein present in the precipitates were estimated by the method of Lowry et al. (1951). This protoxin preparation was used in the experiments.

2.5. Survival and development of H. armigera on different chickpea genotypes sprayed with different concentrations of Bt

Survival and development of *H. armigera* larvae were studied on four chickpea genotypes sprayed with a commercial formulation of *Bt* (Biolep®, Biotech International Ltd., India) (0.05, 0.1, 0.2

and 0.5%). The larvae were reared up to pupation on leaves obtained from freshly sprayed chickpea plants in the field. Ten neonate larvae were fed on terminal branches picked up at random from each plot, by using the detached leaf assay (Sharma et al. 2005) and there were 30 larvae for each treatment. After five days, when the larvae enter third-instar and become cannibalistic, the larvae were reared in individually in small plastic cups (20 ml capacity). Food was changed every three days and fresh *Bt* sprayed leaves (4h after spraying) were given as a food each time. Data were recorded on larval weights at 5 and 10 days after initiation (DAI) of the experiment using a microbalance (Mettler AE 200^{R}). For this purpose, larvae were removed from the rearing cups, cleaned and kept in 15 ml glass vials for 2 h, weighed and then placed back on the respective diets. The pupal weights were recorded 1 day after pupation. Pupae from each replication were placed in a 1 L plastic jar containing moist Vermiculite. Pupation and adult emergence were computed in relation to number of neonate larvae released in each replication. Data were also recorded on larval and pupal periods. The adults were collected with an aspirator from the jars and three pairs of adults emerging on the same day on a particular genotype were placed inside an oviposition cage (30 × 30 ×30 cm) and provided with diaper liners for oviposition to record data on fecundity of insects reared on different chickpea genotypes treated with different concentrations of the *Bt* formulation. The adults were provided with 10% sucrose solution on a cotton swab as a food.

2.6. Survival and development of H. armigera on artificial diet containing lyophilized leaf or pod powder of different chickpea genotypes and Bt δ-endotoxin proteins

To assess the interaction of organic acids in the trichome exudates of chickpea leaves or pods with biological activity of *Bt* δ-endotoxin proteins towards *H. armigera*, chickpea terminals (having four fully expanded leaves) at the flowering stage and young pods at the milk stage (12 0 15 days after flowering) were collected and stored at -20 $^{\circ}$C. The leaves and pods from each treatment were freeze-dried separately, powdered in a blender to obtain a fine powder (<80 μm) and used in artificial diet incorporation assay (Narayanamma et al., 2008) to assess the interaction of organic acids in the leaf and pod exudates with biological activity of *Bt* towards *H. armigera*.

To assess the interaction between chickpea genotypes and *Bt* δ-endotoxin proteins, lyophilized leaf or pod powder of the chickpea genotypes (20 g of freeze-dried leaf or pod powder of each genotype as a replacement for chickpea flour of the susceptible chickpea variety, KAK 2) were incorporated into the artificial diet (having ingredients sufficient for 300 ml artificial diet) for rearing *H. armigera* (Armes et al., 1992) along with *Bt* toxin Cry1Ac at the ED_{50} (27.3 ng ml^{-1}) level (effective dose to reduce the weight of the larvae by 50%) (Sharma et al., 2008). Seven ml of diet was poured into each cell-well in a six cell-well plate and then neonate larvae were released individually into the cell-wells. There were three replications for each genotype and each replication had 10 neonate larvae (N = 30 for each treatment). Data were recorded on larval weights at 5 and 10 days after initiation of the experiment, pupal weights, larval and pupal periods, pupation, adult emergence, adult longevity and fecundity as described above.

2.7. Statistical analysis

Data were subjected to factorial analysis by using GENSTAT version 10.1. The treatment means were compared by DMRT to know the significance of differences among the genotypes, *Bt* concentrations and the interaction effects, if any, at $P \leq 0.05$. The treatment (*Bt* concentrations) x genotype means were compared for significance of differences when the interaction effects were non-significant. When *Bt* treatment x genotype interaction effect were significant, the values for each genotype under different *Bt* treatments were compared for significance of differences.

3. Results

3.1. Acidity of the leaf exudates and amounts of organic acids at different stages of plant growth

Amounts of malic acid increased progressively from vegetative to the podding stage (Table 1). ICCV 10 had the highest amounts of malic acid during the vegetative, flowering and podding stages, followed by C 235; while ICC 506EB had the least amounts of malic acid, except during the vegetative stage. The amounts of oxalic acid increased from 5.31 mg g^{-1} during the vegetative stage to 12.80 mg g^{-1} during the flowering stage, but declined to 8.72 mg g^{-1} during the podding stage. Amounts of oxalic acid were

maximum in ICCV 506EB during the vegetative and flowering stages closely followed by ICCV 10 (except during the flowering stage); while C 235 had the least amounts of oxalic acid.

3.2. Survival and development of H. armigera *on different chickpea genotypes sprayed with Bt*

There were significant differences in survival and development of the pod borer, *H. armigera* on the *Bt*-sprayed and unsprayed plots of different chickpea genotypes (Table 2). The interaction effects between the genotypes × *Bt* concentrations for larval weight at 10 DAI were significant. There was no larval survival on the four chickpea genotypes sprayed with 0.5% *Bt* and on C 235 sprayed with 0.2% *Bt*. Lowest larval weight (5.5 mg) was recorded in the larvae reared on ICC 506EB sprayed with 0.2% *Bt*, followed by those reared on ICCV 10 sprayed with 0.1% *Bt* (13.6 mg), suggesting that chickpea genotypes with resistance to *H. armigera* act in concert with *Bt* to cause adverse effects on the survival and development of this insect. Only the *H. armigera* larvae reared on unsprayed leaves and the leaves sprayed with 0.05% *Bt* were able to pupate. The pupal weights were lower in larvae reared on ICC 506EB (180.2 mg) and ICCV 10 (208.5 mg) sprayed with 0.05% *Bt* as compared to those reared on the unsprayed leaves of L 550 (262.3 mg) (Table 3).

None of the larvae survived on C 235 sprayed with 0.05% *Bt*. The larval period was prolonged by more than 2 days in the larvae reared on the unsprayed leaves of ICC 506EB and ICCV 10 (21.9 and 21.0 days, respectively) and in larvae reared on ICC 506EB sprayed with 0.05% *Bt* (20.6 days). Pupal period was also prolonged on ICC 506EB sprayed with 0.05% *Bt* (14.3 days) as compared to that on L 550 (9.1 days). The pupation was significantly lower in insects reared on ICCV 10 (10%), L 550 (16%) and ICC 506EB (26%) sprayed with 0.05% *Bt* as compared to those reared on the unsprayed leaves of L 550 (76%) (Table 3).

Adult emergence was lower in insects reared on ICCV 10 sprayed with 0.05% *Bt* (60%) and unsprayed ICC 506EB (63.8%) as compared to unsprayed L 550 (84.1%) (Table 4). The *H. armigera* females survived for 2.8 to 6.4 days when the larvae were reared on L 550, ICCV 10 and ICC 506EB sprayed with 0.05% *Bt* as compared to 13.3 days in insects reared on unsprayed ICCV 10. The males

survived for 5.0 to 6.4 days when reared on L 550 and ICC 506EB sprayed with 0.05% *Bt* compared to 16.0 days in insects reared on unsprayed leaves of L 550. Fecundity was significantly lower in insects reared on ICC 506EB (166 eggs female^{-1}) and L 550 (232 eggs female^{-1}) sprayed with 0.05% *Bt* as compared to those reared on unsprayed L 550 (1,226 eggs female^{-1}). The interaction effects between the chickpea genotypes × *Bt* (0.05% concentration) for adult emergence were significant.

3.3. Survival and development of H. armigera *on artificial diet with lyophilized leaf powder of different chickpea genotypes and Bt δ-endotoxin proteins*

There were significant differences in larval weights at 5 DAI between the genotypes and the *Bt* δ-endotoxin protein treatments (Table 5) and the interaction effects were non-significant. The larval weights were lower in larvae reared on artificial diets with leaf powder of ICC 506EB (13.3 mg), followed by ICCV 10 (14.7 mg). Larval weights were also lower in larvae reared on diets with *Bt* δ-endotoxin proteins (11.8 mg) as compared to the larvae reared on diets without *Bt* δ-endotoxin proteins (23.2 mg). At 10 DAI, the interaction effects between the genotypes × *Bt* δ-endotoxin proteins were significant. The larvae reared on the standard artificial diet with *Bt* had lowest larval weights (132.9 mg), followed by those reared on a diet with ICCV 10 leaf powder + *Bt* δ-endotoxin proteins (158.1 mg). Larval weight was highest in the larvae reared on the standard artificial diet without *Bt* δ-endotoxin proteins (411.3 mg), followed by the larvae reared on diets with leaf powders of L 550 (399.5 mg) and ICC 506EB (382.9 mg). The interaction effects for pupal weight between the genotypes × *Bt* δ-endotoxin proteins were significant. Pupal weights were lowest in insects reared on standard artificial diet with *Bt* (309.8 mg), followed by those reared on diets with leaf powders of L 550 + *Bt* (316.3 mg) and ICCV 10 + *Bt* (319.7 mg).

The interaction effects for larval and pupal periods, pupation, adult emergence and fecundity were significant (Tables 6 and 7). Larval period increased by more than 2 days in larvae reared on the standard artificial diet with *Bt* (17.5 days), followed by those reared on diets with leaf powder of ICC 506EB (17.1 days) (Table 6). Pupal period was shorter in insects reared on standard artificial diet (14.8

days) as compared to those reared on diets with ICCV 10 leaf powder + *Bt* δ-endotoxin proteins (17.5 days), ICC 506EB leaf powder (16.2 days) and ICCV 10 leaf powder (15.9 days). Lowest pupation was recorded on standard artificial diet with *Bt* (70.0%), followed by insects reared on diets with leaf powder of ICC 506EB (86.7%). Adult emergence was lowest on the standard artificial diet with *Bt* (40.0%), followed by insects reared on diets with leaf powder of ICC 506EB (53.3%). More than 90% adult emergence was recorded on the standard artificial diet without *Bt* δ-endotoxin proteins (93.3%). The interaction effects for adult longevity were non-significant (Table 7). Lowest fecundity was recorded in insects reared on diets with C 235 leaf powder + *Bt* δ-endotoxin proteins (447 eggs female^{-1}), followed by the insects reared on diets with leaf powders of ICC 506EB (653 eggs female^{-1}), ICCV 10 (670 eggs female^{-1}) and ICCV 10 + *Bt* δ-endotoxin proteins (699 eggs female^{-1}). The insects reared on the standard artificial diet without *Bt* δ-endotoxin proteins laid 1,760 eggs female^{-1}.

3.4. Survival and development of H. armigera *on artificial diets having lyophilized pod powder of different chickpea genotypes and Bt*

Larval weights were lower in insects reared on diets with pod powders of ICCV 10 + *Bt* δ-endotoxin protein (5.5 mg) and ICC 506EB + *Bt* δ-endotoxin proteins (5.9 mg) as compared to the larvae reared on standard artificial diet without *Bt* (15.4 mg) (Table 8). The interaction effects for larval weights at 5 DAI were significant. At 10 DAI, larval weights were lower in insects reared on the diets with *Bt* δ-endotoxin proteins (147.2 mg) as compared to the insects reared on diets without *Bt* (368.8 mg). Lowest larval weight (230.6 mg) was recorded in insects reared on diets with pod powders of ICC 506EB, followed by C 235 (235.8 mg) and ICCV 10 (249.8 mg). Pupal weight was lowest in insects reared on diets with pod powder of L 550 + *Bt* δ-endotoxin proteins (281.6 mg) and ICC 506EB + *Bt* δ-endotoxin proteins (286.1 mg).

Larval period was shorter by 1.6 days in insects reared on diets without *Bt* δ-endotoxin proteins (15.1 days) as compared to those reared on diets with *Bt* δ-endotoxin proteins (16.7 days) (Table 9). Pupal period was also shorter (11.7 days) in insects reared on diets without *Bt* as compared to those reared on

diets with *Bt* (12.3 days). The interaction effects for pupation between the genotypes × *Bt* δ-endotoxin protein were significant. Pupation was lower in insects reared on the standard artificial diet with *Bt* (66.7%), followed by those reared on diets with pod powder of C 235 + *Bt* δ-endotoxin proteins (76.7%) and ICC 506EB (83.3%).

The interaction effects were significant between the genotypes × *Bt* δ-endotoxin proteins for adult longevity (Table 10). Female longevity was shortest in insects reared on diets with pod powder of C 235 + *Bt* δ-endotoxin proteins (9.7 days), followed by the insects reared on diets with pod powder of ICCV 10 (9.9 days) and C 235 (11.0 days). Male longevity was shorter in insects reared on diets with pod powder of ICCV 10 (8.1 days), followed by those reared on diets with pod powder of ICC 506EB (8.4 days), C 235 + *Bt* δ-endotoxin proteins (8.6 days) and ICC 506EB + *Bt* δ-endotoxin proteins (8.7 days). The differences in fecundity between the genotypes, *Bt* δ-endotoxin proteins and the interaction effects were significant (Table 10). Fecundity was lowest in insects reared on standard artificial diet with *Bt* (333.2 eggs female^{-1}), followed by the insects reared on diets with pod powder of ICCV 10 + *Bt* δ-endotoxin proteins (434.3 eggs female^{-1}), L 550 + *Bt* δ-endotoxin proteins (447.7 eggs female^{-1}) and C 235 + *Bt* δ-endotoxin proteins (494.3 eggs female^{-1}).

4. Discussion

Chickpea genotypes with low to moderate levels of resistance to *H. armigera* have been identified earlier, but the expression of resistance varies with growth stage, seasons and locations (Sharma et al., 2007). Therefore, there is a need to augment host plant resistance with other methods of controlling *H. armigera*, including the use of biopesticides, natural plant products and synthetic insecticides (Sharma, 2005). Genetic engineering of chickpea with δ–endotoxin genes from the *Bt* can also be exploited to make host plant resistance an effective tool for controlling *H. armigera* (Sharma et al., 2004). Efforts are underway to develop chickpea plants expressing *Bt cry* genes for conferring resistance to *H. armigera* (Ramakrishna et al., 2005; Sanyal et al., 2005).

Bt sprays have been found to be effective for *H. armigera* control on chickpea (Balasubramanian et al., 2002; Mandal et al., 2003; Bhojne et al., 2004; Singh and Ali, 2005). There were significant differences in the survival and development of *H. armigera* larvae on *Bt*-sprayed and unsprayed chickpeas. Pupation and adult emergence were significantly lower in insects reared on ICCV 10, L 550 and ICC 506EB sprayed with 0.05% *Bt* as compared to the insects reared on unsprayed plants of L 550. Fecundity was also reduced significantly in insects reared on ICC 506EB and L 550 sprayed with 0.05% *Bt* as compared to those reared on unsprayed leaves of L 550. The results suggested that ICC 506EB and ICCV 10, with low to moderate levels of resistance to *H. armigera*, acted in concert with *Bt*, and these genotypes also had high amounts of oxalic and/or malic acid in the leaf exudates.

When the lyophilized leaves of different chickpea genotypes were incorporated into the artificial diet, the weights of *H. armigera* larvae were lower in insects reared on diets with chickpea leaf or pod powder + *Bt* δ-endotoxin proteins as compared to the diets without *Bt* δ-endotoxin proteins. Pupal weights were also lower in insects reared on artificial diet with *Bt* and on diets with leaf powders of L 550 and ICCV 10 along with *Bt* δ-endotoxin proteins. Lowest pupation and adult emergence were recorded on artificial diet with *Bt* δ-endotoxin proteins, followed by the insects reared on diets with leaf powder of ICC 506EB. Fecundity was lower in insects reared on diets with leaf or pod powder of different chickpea genotypes and *Bt* δ-endotoxin proteins.

The biological activity of *Bt* δ-endotoxin proteins was greater in artificial diet without chickpea leaf or pod powder, suggesting that chemical components in the chickpea leaves and pods had a negative effect on the biological activity of *Bt*. However, *Bt* incorporated into artificial diet has also been shown to act as a feeding deterrent to the larvae of *H. armigera* (Zhang et al., 2000) and results in reduced survival of *H. armigera* (Chandra et al., 1999). The pH of the acid exudates varied from 2.1 to 2.90 and ICCV 10 had greater amounts of malic acid than C 235; while the amounts of oxalic acid were greater in ICC 506EB than in C 235. The reduced effectiveness of *Bt* δ-endotoxins might not be entirely due to acidic exudates as ICC 506EB and ICCV 10, which showed maximum amounts of oxalic and malic acids, respectively, also resulted in adverse effects on development and survival of *H. armigera*. However, C

235 also resulted on adverse effects on *H. armigera* in combination with *Bt*, although it had low amounts of oxalic acids, but considerably high amounts of malic acid in the leaf exudates. Expression of resistance to pod borer, *H. armigera* is influenced by the pH and amounts of malic and oxalic acids in the leaf exudates (Bhagwat et al., 1995; Yoshida et al., 1995, 1997) and reduced larval and pupal weights and prolonged larval and pupal periods have been observed in insects reared on leaves, pods and in artificial diets impregnated with lyophilized leaves and pods of *H. armigera*-resistant genotypes of chickpea as compared to those of the susceptible ones (Sreelatha, 2003; Narayanamma et al., 2008). Reduced biological activity of *Bt* in artificial diets with leaf or pod powder of chickpea genotypes might be due to interaction of *Bt* proteins with biochemical constituents in chickpea, in addition to reduced feeding because of antifeedant effect of acid exudates towards the *H. armigera*. The results suggested that *H. armigera* – resistant genotypes with high amounts of acidic exudates, acted in concert with *Bt*, and therefore, it will be desirable to use *Bt* sprays on *H. armigera*-resistant genotypes for integrated management and that transgenic chickpeas expressing toxin genes form *Bt* could be developed for controlling the pod borer, *H. armigera.*

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Table 1

Amounts of malic and oxalic acids in four chickpea genotypes (mg g^{-1} dry weight) at different stages of plant growth (ICRISAT, Patancheru, India).

Genotype	Malic acid (mg g^{-1} dry weight)			Oxalic acid (mg g^{-1} dry weight)		
	Vegetative stage	Flowering stage	Podding stage	Vegetative stage	Flowering stage	Podding stage
C 235	7.52[ab]	33.51[c]	73.45c	2.19[a]	7.80[a]	6.67[a]
ICC 506EB	5.99[a]	8.03a	37.82[a]	10.20[c]	17.70[d]	6.04[a]
ICCV 10	12.55[b]	37.71[c]	86.78[d]	5.42[b]	10.05[b]	13.07[b]
L 550	3.60[a]	18.42[ab]	52.54[b]	3.44_{ab}	13.59[c]	9.09[ab]
Mean	7.42	24.40	62.60	5.31	12.28	8.72
Fprob	0.05	0.001	0.109	0.001	0.01	0.05
SE (df = 3, 6)	1.49	4.25	12.28	1.16	0.48	1.41
LSD (P = 0.05)	5.15	14.72	NS	4.00	1.65	4.89

Figures followed by the same letter within a column do not differ significantly at $P \leq 0.05$.

Table 2

Weights of *H. armigera* larvae (at10 days after infestation) and pupae on four chickpea genotypes sprayed with different concentrations of *Bt* (ICRISAT, Patancheru, 2006-07 post-rainy season)*

Genotype	Larval weight 10 DAI (mg)						Pupal weight (mg)	
	Bt concentrations (%)							
	0.0**	0.05	0.1	0.2	0.5	Mean	0.0	0.05
C 235	80.7	36.9^{e}	17.7^{bc}	-	-	13.6	256.2^{d}	0
ICC 506EB	45.5	65.0^{f}	26.1^{cd}	5.5^{a}	-	24.2	235.2^{c}	180.2^{a}
ICCV 10	53.9	30.7^{de}	13.6^{ab}	17.9^{bc}	-	15.6	253.0^{d}	208.5^{b}
L 550	113	61.8^{f}	29.3^{de}	18.2^{bc}	-	27.3	262.3^{d}	234.7^{c}
Mean	73.2	48.6	21.7	10.4	-	-	251.7	155.8
For comparing				Fprob		LSD (P = 0.05)	Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 6)				<0.001		4.31	<0.001	5.97
Genotypes (df = 2, 6)				<0.001		4.31	<0.001	5.97
Bt concentrations x Génotypes (df = 2, 6)				<0.001		8.62	<0.001	11.93

*There were 30 larvae in each treatment.

DAI = Days after infestation.

**Control values were not included in analysis of variance.

– = There was no larval survival.

Figures followed by the same letter within a column do not differ significantly at $P \leq 0.05$.

Table 3

Development and survival of *H. armigera* larvae reared on four chickpea genotypes sprayed with *Bt* (ICRISAT, Patancheru, 2006-07 post-rainy season)*

Genotype	*Bt* concentration (%)							
	Larval period (days)		Pupal period (days)		Pupation (%)		Adult emergence (%)	
	0.0	0.05	0.0	0.05	0.0	0.05	0.0	0.05
C 235	20.5[c]	-	13.1[b]	-	64.0[f]	-	81.2[a]	-
ICC 506EB	21.9[d]	20.6[c]	13.6[b]	14.3[b]	34.0[d]	26.0[c]	63.8[a]	76.7[a]
ICCV 10	21.0[c]	18.6[a]	12.9[b]	9.8[a]	42.0[e]	10.0[a]	76.0[a]	60.0[a]
L 550	18.9[a]	20.2[b]	12.8[b]	9.1[a]	76.0[g]	16.0[b]	84.1[b]	70.0[a]
Mean	20.6	14.9	13.1	8.3	54	13	76.2	51.7
For comparing	Fprob	LSD (P = 0.05)	Fprb	LSD (P = 0.05)	Fprob	LSD (P = 0.05)	Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 6)	<0.001	0.13	<0.001	1.58	<0.001	0.93	<0.001	10.75
Genotypes(df = 3, 6)	<0.001	0.13	<0.001	1.58	<0.001	0.93	0.009	10.75
Bt concentrations x Genotypes (df = 3, 6)	<0.001	0.27	<0.001	3.17	<0.001	1.86	<0.001	21.5

*There were 30 larvae in each treatment.

- = There was no larval survival.

Figures followed by the same letter within a column do not differ significantly at $P \leq 0.05$.

Table 4

Longevity and fecundity of *H. armigera* reared on four chickpea genotypes with sprayed with *Bt* (ICRISAT, Patancheru, 2006-07 post-rainy season)*

Genotype	*Bt* Concentrations (%)					
	Female longevity (days)		Male longevity (days)		Fecundity (eggs female^{-1})	
	0.0	0.05	0.0	0.05	0.0	0.05
C 235	12.2	-	15.2^{d}	-	897	-
ICC 506EB	11.2	6.4^{b}	12.9^{c}	6.4^{b}	533	166
ICCV 10	13.3	4.0^{a}	13.8^{c}	-	726	-
L 550	11.9	2.8^{a}	16.0^{d}	5.0^{a}	1226	232
Mean	12.2	3.3	14.5	2.9	846^{b}	100.0^{a}
For comparing	Fprob	LSD (P = 0.05)	Fprob	LSD (P = 0.05)	Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 6)	<0.001	1.02	<0.001	0.95	0.002	59.1
Genotypes (df = 3, 6)	0.024	1.02	<0.001	0.95	0.125	NS
Bt concentrations x Genotypes (df = 3, 6)	0.002	2.04	<0.001	1.9	0.056	NS

*There were 30 larvae in each treatment.

- = No larval survival.

Figures followed by the same letter within a column do not differ significantly at $P \leq 0.05$.

Table 5

Larval and pupal weights of *H. armigera* reared on artificial diets with lyophilized leaf powder of four chickpea genotypes with and without *Bt* (ICRISAT, Patancheru, 2006-07)*

Genotype	Larval weight (5 DAI) (mg)			Larval weight (10 DAI) (mg)			Pupal weight (mg)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	23.5	15.7	19.6^{b}	308.8^{def}	259.6^{cde}	284.2	331.9^{bc}	327.1^{abc}	329.5
ICC 506EB	19.4	7.2	13.3^{a}	382.9^{fgh}	324.3^{efg}	353.6	327.3^{abc}	323.3^{ab}	325.3
ICCV 10	20.4	9.1	14.7^{a}	223.0^{bc}	158.1^{ab}	190.5	326.6^{abc}	319.7^{ab}	323.2
L 550	25.4	14.3	19.9^{b}	399.5^{gh}	234.8^{bcd}	317.1	343.1^{c}	316.3^{ab}	329.7
Standard artificial diet	27.6	12.5	20.1^{b}	411.3^{h}	132.9^{a}	272.1	364.3^{d}	309.8^{a}	337.1
Mean	23.2^{b}	11.8^{a}	17.5	345.1	221.9	283.5	338.6	319.3	329.0
For comparing		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 8)		<0.001	3.69		<0.001	36.40		<0.001	8.82
Genotypes (df = 4, 8)		0.002	2.33		<0.001	57.56		0.31	NS
Bt concentrations x Genotypes (df = 4, 8)		0.392	NS		0.002	81.40		0.005	19.72

*There were 30 larvae in each treatment.

DAI = Days after initiation of experiment.

Figures followed by the same letter in a column do not differ significantly at $P \leq 0.05$.

Table 6

Post-embryonic development, and pupation and adult emergence of *H. armigera* reared on artificial diet with lyophilized leaf powder of four chickpea genotypes with and without *Bt* (ICRISAT, Patancheru, 2006-07)*

Genotype	Larval period (days)			Pupal period (days)			Pupation (%)			Adult emergence (%)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	16.0^{abcd}	16.1^{abcd}	16.0	14.9^{ab}	15.8^{ab}	15.4	96.7^{b}	90.0^{b}	93.3	86.7^{de}	60.0^{bc}	73.3
ICC 506EB	17.1^{cd}	15.8^{abc}	16.4	16.2^{bc}	14.7^{a}	15.5	86.7^{ab}	93.3^{b}	90.0	53.3^{ab}	73.3^{cd}	63.3
ICCV 10	16.7^{bcd}	15.1^{ab}	15.9	15.9^{ab}	17.5^{c}	16.7	96.7^{b}	90.0^{b}	93.3	86.7^{de}	66.7^{bc}	76.7
L 550	14.7^{a}	16.1^{abcd}	15.4	15.2^{ab}	15.2^{ab}	15.2	100.0^{b}	96.7^{b}	98.3	90.0^{de}	76.7^{cde}	83.3
Standard artificial diet	14.8^{a}	17.5^{d}	16.1	14.8^{a}	15.6^{ab}	15.2	100.0^{b}	70.0^{a}	85.0	93.3^{e}	40.0^{a}	66.7
Mean	15.9	16.1	16.0	15.4	15.8	15.6	96.0	88.0	92.0	82.0	63.3	72.7
For comparing		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0 .05)
Bt concentrations (df = 1, 8)		0.478	NS		0.228	NS		0.021	6.65		<0.001	8.71
Genotypes (df = 4, 8)		0.471	NS		0.017	0.94		0.149	NS		0.049	13.77
Bt concentrations x Genotypes (df = 4, 8)		0.004	1.60		0.028	1.32		0.025	14.87		<0.001	19.47

*There were 30 larvae in each treatment.

Figures followed by the same letter in a column do not differ significantly at $P \leq 0.05$.

Table 7

Longevity and fecundity of *H. armigera* reared on artificial diet with lyophilized leaf powder of four chickpea genotypes and *Bt* (ICRISAT, Patancheru, 2006-07)*

Genotype	Female longevity (days)			Male longevity (days)			Fecundity (eggs female $^{-1}$)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	15.6	17.3	16.5^{b}	20.9	19.2	20.1	805^{bcd}	447^{a}	626
ICC 506EB	17.4	16.3	16.9^{b}	18.3	20.1	19.2	653^{b}	853^{cd}	753
ICCV 10	19.0	18.1	18.5^{b}	17.8	17.9	17.6	670^{b}	699^{bc}	684
L 550	16.3	18.6	17.4^{b}	18.3	18.2	18.2	831^{bcd}	797^{bcd}	814
Standard artificial diet	13.8	14.2	14.0^{a}	15.7	14.2	14.9	1760^{e}	934^{d}	1347
Mean	16.4	16.9	16.7	18.2	17.9	18.1	944	746	845
For comparing (df = 1, 8)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)
Bt concentrations (df = 4, 8)		0.488	NS		0.804	NS		<0.001	79.50
Genotypes		0.005	2.16		0.069	NS		<0.001	125.80
Bt concentrations x Genotypes (df = 4, 8)		0.408	NS		0.841	NS		<0.001	177.90

*There were 30 larvae in each treatment.

Figures followed by the same letter in a column do not differ significantly at $P \leq 0.05$.

Table 8

Development of *H. armigera* larvae reared on artificial diet with lyophilized pod powder of four chickpea genotypes and *Bt* (ICRISAT, Patancheru, 2006-07)*

Genotypes	Larval weight (5 DAI) (mg)			Larval weight (10 DAI) (mg)			Pupal weight (mg)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	14.2^{de}	10.1^{b}	12.1	332.6	139.0	235.8^{a}	287.4^{a}	293.1^{a}	290.2
ICC 506EB	16.0^{e}	5.9^{a}	10.9	324.1	137.1	230.6^{a}	309.8^{b}	286.1^{a}	298.0
ICCV 10	13.6^{cd}	5.5^{a}	9.6	374.7	125.0	249.8^{ab}	319.5^{bc}	292.0^{a}	305.7
L 550	14.7^{de}	10.9^{b}	12.8	400.6	152.1	276.3^{bc}	325.2^{c}	281.6^{a}	303.4
Standard artificial diet	15.4^{de}	11.7^{bc}	13.6	412.1	182.9	297.3^{c}	283.3^{a}	311.3^{bc}	297.3
Mean	14.8	8.8	11.8	368.8^{b}	147.2^{a}	258.0	310.6	287.2	298.9
For comparing		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 8)		<0.001	0.87		<0.001	19.15		<0.001	6.66
Genotypes (df = 4, 8)		<0.001	1.37		<0.001	30.28		0.052	10.54
Bt concentrations x Genotypes (df = 4, 8)		<0.001	1.94		0.118	NS		0.002	14.90

*There were 30 larvae in each treatment.

DAI = Days after initiation of experiment.

Figures followed by the same letter in a column do not differ significantly at $P \leq 0.05$.

Table 9

Post-embryonic development, pupation, and adult emergence of *H. armigera* reared on artificial diet with lyophilized pod powder of four chickpea genotypes and *Bt* (ICRISAT, Patancheru, 2006-07)*

Genotypes	Larval period (days)			Pupal period (days)			Pupation (%)			Adult emergence (%)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	15.2	17.3	16.3^{bc}	12.0	12.5	12.2^{b}	90.0^{bc}	76.7^{ab}	83.3	70.0^{b}	63.3^{ab}	66.7
ICC 506EB	16.2	16.8	16.5^{c}	10.3	11.5	10.9^{a}	83.3^{abc}	96.7^{c}	90.0	73.3^{b}	83.3^{b}	78.3
ICCV 10	14.8	16.8	15.8^{ab}	12.1	12.2	12.2^{b}	93.3^{bc}	86.7^{bc}	90.0	83.3^{b}	80.0^{b}	81.7
L 550	14.3	16.3	15.3^{a}	12.3	12.8	12.6^{b}	90.0^{bc}	86.7^{bc}	88.3	83.3^{b}	63.3^{ab}	73.3
Standard artificial diet	14.9	16.3	15.6^{a}	11.8	12.3	12.1^{b}	100.0^{c}	66.7^{a}	83.3	80.0^{b}	40.0^{a}	60.0
Mean	15.1^{a}	16.7^{b}	15.9	11.7^{a}	12.3^{b}	12.0	91.3	82.7	87.0	78.0	66.0	72.0
For comparing		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 8)		<0.001	0.36		0.004	0.36		0.045	8.46		0.028	16.62
Genotypes (df = 4, 8)		0.003	0.58		<0.001	0.57		0.684	NS		0.083	NS
Bt concentrations x Genotypes (df = 4, 8)		0.064	NS		0.47	NS		0.027	18.91		0.053	23.51

*There were 30 larvae in each treatment.

Figures followed by the same letter in a column do not differ significantly different at $P \leq 0.05$.

Table 10

Longevity and fecundity of *H. armigera* reared on artificial diet with lyophilized pod powder of four chickpea genotypes and *Bt* (ICRISAT, Patancheru, 2006-07)*

Genotypes	Female longevity (days)			Male longevity (days)			Fecundity (eggs female $^{-1}$)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	11.0^{ab}	9.7^{a}	10.3	8.9^{b}	8.6^{ab}	8.7	687.0^{e}	494.3^{bc}	590.7
ICC 506EB	13.0^{cd}	12.2^{bc}	12.6	8.4^{ab}	8.7^{b}	8.5	574.0^{cd}	554.0^{cd}	564.0
ICCV 10	9.9^{a}	13.7^{cd}	11.8	8.1^{a}	10.2^{c}	9.2	623.0^{de}	434.3^{b}	528.7
L 550	12.6^{cd}	14.0^{d}	13.3	10.2^{c}	12.0^{e}	11.1	692.0^{e}	447.7^{b}	569.8
Standard artificial diet	12.2^{bc}	11.0^{ab}	11.6	10.8^{d}	9.8^{c}	10.3	921.3^{f}	333.2^{a}	627.3
Mean	11.7	12.1	11.9	9.3	9.9	9.6	699.5	452.7	576.1
For comparing		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)		Fprob	LSD (P = 0.05)
Bt concentrations (df = 1, 8)		0.306	NS		<0.001	0.24		<0.001	37.47
Genotypes (df = 4, 8)		<0.001	0.72		<0.001	0.39		0.034	59.25
Bt concentrations x Genotypes (df = 4, 8)		<0.001	1.61		<0.001	0.55		<0.001	83.79

*There were 30 larvae in each treatment.

Figures followed by the same letter in a column do not differ significantly different at $P \leq 0.05$.