INTERACTION OF ACID EXUDATES IN CHICKPEA WITH BIOLOGICAL ACTIVITY OF CRY TOXINS FROM *Bacillus thuringiensis* BERLINER AGAINST *Helicoverpa armigera* (HUBNER)

Report submitted to
Agricultural College, Bapatla
Acharya N G Ranga Agricultural University
Rajendranagar, Hyderabad, Andhra Pradesh, India

By
V. SUREKHA DEVI

International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324
Andhra Pradesh, India
icrisat@cgiar.org

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To whom It May Concern

It is to certify that **Ms. V. Surekha Devi,** who was accepted as research Scholar at ICRISAT, Patancheru, has satisfactorily completed the assigned experiments as part of the project titled **"Interaction of acid exudates in chickpea with biological activity of cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)"** under my supervision for her Ph.D. in Entomology to Acharya N G Ranga Agricultural University. The work has been done during November 2005 to June 2009 and was satisfactory. I appreciate her performance in the laboratory.

H C Sharma
Principal Scientist (Entomology)

Headquarters: Patancheru 502 324, Andhra Pradesh, India
Tel +91 40 30713071 **Fax** +91 40 30713074 +91 40 30713075 **Email** icrisat@cgiar.org
Liaison Office: CG Centers Block, NASC Complex, Dev Prakash Shastri Marg, New Delhi 110 012, India
Tel +91 11 32472306 to 08 **Fax** +91 11 25841294

ICRISAT-Niamey (Regional hub WCA)
BP 12404
Niamey, Niger (Via Paris)
Tel +227 20 722626, 20 722529
Fax +227 20 734329
Email icrisatne@cgiar.org

ICRISAT-Bamako
BP 320
Bamako
Mali
Tel +223 20 223378
Fax +223 20 228683
Email icrisat-w-mali@cgiar.org

ICRISAT-Bulawayo
Matopos Research Station
PO Box 776,
Bulawayo, Zimbabwe
Tel +263 83 8311 to 15
Fax +263 83 8253, 8307
Email icrisatzw@cgiar.org

ICRISAT-Lilongwe
Chitedze Agricultural Research Station
PO Box 1096
Lilongwe, Malawi
Tel +265 1 707297, 071, 057, 067
Fax +265 1 707298
Email icrisat-malawi@cgiar.org

ICRISAT-Maputo
c/o MAE, Av. das FPLM No 2698
Caixa Postal 1906
Maputo, Mozambique
Tel +258 21 461657
Fax +258 21 461581
Email icrisatmoz@panintra.net

ABSTRACT

Title	:	**Interaction of acid exudates in chickpea with biological activity of Cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)**
Name	:	**V. SUREKHA DEVI**
Institute	:	**Acharya N G Ranga Agricultural University**
Supervisor	:	**Dr. H.C. Sharma**
Submitted	:	**June 2009**

The *H. armigera* egg and larval numbers before and after sprays of *B. thuringiensis* (*Bt*) formulation Biolep® were low on the chickpea genotypes ICC 506 and ICCV 10 across *Bt* concentrations as compared to those on L 550 and C 235 during vegetative and flowering stages. Leaf feeding and pod damage were lowest on ICC 506, followed by ICCV 10 across *Bt* concentrations as compared to that on L 550 and C 235. During the 2005-06 post-rainy season and first planting during the 2006-07 post-rainy season, highest grain yield was recorded in case of ICCV 10. In the second planting, the genotypes ICCV 10 and ICC 506 recorded the highest total grain yield. There were no significant differences between the genotypes and *Bt* concentrations for grain yield.

Significantly lower leaf damage was recorded on ICC 506 across *Bt* concentrations in detached leaf assay under laboratory conditions. Larval survival and larval weights were lower in ICC 506, ICCV 10, and C 235 across *Bt* concentrations as compared to that of L 550. Survival and development of *H. armigera* on ICC 506, ICCV 10, and C 235 sprayed with different concentrations of *Bt* differed significantly with that of the unsprayed plants of these genotypes. At higher concentrations of *Bt* (0.1, 0.2, and 0.5%), there was no survival of the *H. armigera* larvae. At lower concentrations of *Bt* (0.05%), the larval survival and larval and pupal weights were lower on the sprayed (0.05%) plants than those on the unsprayed plants of different chickpea genotypes. Larval and pupal periods were prolonged on plants sprayed with *Bt*. There was a significant reduction in fecundity of *H. armigera* reared on chickpea plants sprayed with *Bt*.

At the vegetative and flowering stages, ICC 506 had the highest amounts of oxalic acid on dry weight and leaf area basis. At the podding stage, ICCV 10 had the highest amount of oxalic acid on dry weight basis. On dry weight basis, ICCV 10 had the highest amount of malic acid at the vegetative, flowering, and podding stages. On leaf area basis, ICCV 10 had the highest amount of malic acid at the vegetative stage, whereas C 235 had the highest amount of malic acid at the flowering and podding stages. Fumaric and citric acids were recorded at the podding stage only. The genotype C 235 had the highest amounts of fumaric and citric acids. There was no citric acid in ICC 506.

The survival and development of *H. armigera* larvae reared on artificial diet with leaf/pod powder of different chickpea genotypes and *Bt* were significantly lower as compared to that on the standard artificial diet, and the diets without *Bt*. Larval survival, larval and pupal weight, and pupation and adult emergence were lower on the resistant genotypes than on the susceptible ones, and the standard artificial diet. Oxalic and malic acids in the artificial diet increased the biological activity of *Bt* toxins on the *H. armigera*, and resulted in reduced larval weight, prolonged development, and reduced longevity and fecundity.

The food, midgut, and faecal matter samples of larvae fed on diet with different amounts of organic acids and *Bt* indicated the conversion of protoxin to toxin, and binding to the brush border membrane vesicles (BBMV) of midgut. Due to the conversion of protoxin to toxin and binding to the BBMV, the amount of *Bt* toxin in the midgut samples was greater as compared to that in the food samples. There were no significant differences in the amounts of *Bt* protein present in the midgut samples of the larvae fed on diet with different amounts of oxalic and malic acids and *Bt* toxin, indicating that the organic acids did not influence the conversion of protoxin to toxin. The amounts of protein in the BBMV preparations ranged from 0.131 to 0.326 mg g^{-1}. The amount of protein estimated from the BBMV of larvae fed on diets with *Bt* was higher as compared to the amounts in the BBMV of the larvae fed on diet without *Bt*, indicating the binding of the *Bt* protein to the BBMV, which resulted in increased protein content in the BBMV.

The insecticidal activity of *Bt* endotoxins depends on the amounts of food ingested by the target insects. The organic acids (oxalic and malic acids) also act as antifeedents, and therefore, may reduce the effect of *Bt* as less amounts of food will be consumed by the larvae. However, the amounts of oxalic and malic acids impregnated in to the diet did not effect the conversion of protoxin to toxin and binding to the BBMV, and thus the effectiveness of *Bt* toxins.

Declaration by the Student

I, **V. SUREKHA DEVI,** here by declare that the thesis entitled "**Interaction of acid exudates in chickpea with biological activity of Cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)"** submitted to Acharya N G Ranga Agricultural University for the degree of **DOCTOR OF PHILOSOPHY in Agriculture** is a result of original research work done by me. I also declare that the material contained in this thesis or part thereof has not been published earlier in any manner.

Date: 3-2-2010

V. Surekha Devi

(V. SUREKHA DEVI)

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3-2-2010
Date: ~~[illegible]~~

V Surekha Devi
(V. SUREKHA DEVI)

List of Symbols, Abbreviations, and Acronyms

$	:	Dollar
%	:	per cent
@	:	at the rate of
<	:	less than
≤	:	less than or equal to
>	:	greater than
^{0}C	:	degrees centigrade
10^{-1} plants	:	per 10 plants
5^{-1} plants	:	per 5 plants
ANOVA	:	Analysis of variance
BBMV	:	Brush Border Membrane Vesicles
BSA	:	Bovine Serum Albumin
Bt	:	*Bacillus thuringiensis*
Cm	:	Centimeter
CRD	:	completely Randomized Design
Cry	:	Crystal
cv.	:	Cultivar
EC	:	Emulsifiable concentration
EC_{50}	:	Effective concentration for 50% mortality
ED_{50}	:	Effective dose for 50% mortality
EGTA	:	Ethylene Glycol Tetra Acetic acid
ELISA	:	Enzyme linked immuno sorbant assay
et al.	:	and others
FAO	:	Food and Agricultural Organization
female^{-1}	:	per female
Fig.	:	Figure
G	:	Gram
g^1	:	Gyrations
H	:	Hour
Ha	:	Hectare
ha^{-1}	:	per hectare
HaNPV	:	*Helicoverpa armigera* Nuclear Polyhedrosis Virus
HCl	:	Hydrochloric acid
HPLC	:	High Performance Liquid Chromatography
HV	:	High Voltage
i.e	:	that is
ICP	:	Insecticidal Crystal Proteins

ICRISAT	:	International Crops Research Institute for Semi Arid Tropics
Imu ml^{-1}	:	International units per milliliter
kDa	:	kilo Dalton
kg	:	Kilogram
kg ha^{-1}	:	kilograms per hectare
L	:	Litre
L ha^{-1}	:	litres per hectare
L: D	:	light: dark
LC_{50}	:	Lethal concentration for 50% mortality
LC_{99}	:	Lethal concentration for 99% mortality
LD_{50}	:	Lethal dose for 50% mortality
LE ha^{-1}	:	Larval equivalents per hectare
LSD	:	least significance difference
m	:	meter
M	:	molar
m^2	:	square meter
m^{-2}	:	per square meter
meq	:	milliequivalents
MET	:	Mannitol, EGTA, Tris-HCl
mg	:	milligram
mg 100^{-1} g	:	milligrams per 100 grams
mg 100^{-1} ml	:	milligrams per 100 milliliter
mg g^{-1}	:	milligrams per gram
mg L^{-1}	:	milligrams per litre
mg ml^{-1}	:	milligrams per milliliter
min	:	minute
ml	:	milliliter
ml ha^{-1}	:	milliliters per hectare
ml min^{-1}	:	milliliters per minute
ml^{-1}	:	per milliliter
mM	:	milli molar
mm	:	millimeter
N	:	normal
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide
ng	:	nanogram

ng g^{-1}	:	nanograms per gram
ng ml^{-1}	:	nanograms per milliliter
nM	:	nano molar
nm	:	nanometer
nS	:	nano siemen
NS	:	non-significant
PAGE	:	Poly Acrylamide Gel Electrophorosis
PBST	:	Phosphate Buffered Saline ~~Tween 20~~
pH	:	Negative logarithm of Hydrogen ion (-log $[H^+]$)
pmol mg^{-1}	:	pico moles per milligram
ppm	:	parts per million
PVDF	:	Polyvinylidene diflouride
q ha^{-1}	:	quintals per hectare
RH	:	relative humidity
rpm	:	rotations per minute
SDS	:	Sodium Dodecyl Sulphate
SE	:	Standard Error
SP	:	Soluble powder
TMB	:	tetramethylbenzidine
Tris	:	N-Tris (hydroxymethyl) aminomethane
US	:	United States
viz.,	:	namely
WG	:	Wettable grade
WP	:	Wettable powder
α	:	alpha
β	:	beta
δ	:	delta
µg	:	microgram
µg cm^{-2}	:	micrograms per square centimeter
µg ml^{-1}	:	micrograms per milliliter
µg $µl^{-1}$	:	micrograms per microliter
µl	:	microliter
µm	:	micrometer
µmol cm^{-2}	:	micromoles per square centimeter

CHAPTER-I

Introduction

CHAPTER – I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world after dry beans and peas (FAO, 2003). It is widely grown in South and West Asia, North and Eastern Africa, Australia, Mexico, and North America. While this pulse crop is an important source of dietary protein for human consumption, it is also important for the management of soil fertility due to its nitrogen fixing ability (Maiti, 2001). It is a cool season legume that has been widely adapted, and most of the crop is grown under rainfed conditions, though upto 20 per cent of chickpea crop may receive irrigation. In 2005, the worldwide area under chickpea was 10.2 million ha, the total grain production being 8.76 million tonnes. India is the largest producer and consumer of chickpea, and accounts for 65.1 per cent of area and 62.45 per cent of the total production (FAOSTAT, 2005).

Chickpea accounts for 41.79 per cent of the total pulse production in the country (CMIE, 2007b). Area under chickpea in 2007 was 7.71 million ha. The total grain production was 5.60 million tonnes, with an average grain yield of 726 kg ha^{-1} (CMIE, 2007a). In Andhra Pradesh, the area under chickpea was 0.421 million ha, with a grain production of 0.417 million tonnes, the grain yield being 990 kg ha^{-1} (CMIE, 2007a).

Chickpea yield has remained stagnant over the past two decades, and several biotic and abiotic factors constrain chickpea production, of which the pod borer, *Helicoverpa armigera* (Hubner) is most important constraint worldwide.

The legume pod borer, *H. armigera* 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 300 plant species (Arora *et al.,* 2005) including cotton, chickpea, pigeonpea, groundnut, cowpea, tomato, peas, castor, maize, sorghum, sunflower, field bean, mungbean, tobacco, 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 (Sharma, 2005). The pod borer, *H. armigera* causes an estimated loss of US$ 927 million in chickpea and pigeonpea, and possibly over US$ 5 billion on different crops worldwide, despite application of pesticides costing over US$ 2 billion annually (ICRISAT, 1992; Sharma, 2005). The damage caused by this pest on chickpea may be upto 84.4 per cent, with an average of seven per cent (Lateef, 1992). Pod damage to the extent of 90 per cent has been reported in Northern India (Sehgal and Ujagir, 1990).

So far, use of insecticides have been the major component for controlling this pest on different crops, but undesirable side effects of synthetic insecticides including development of resistance has necessiated a shift to more eco-friendly approaches for controlling this pest (Mc-Caffery *et al.*, 1989 and Kranthi *et al.*, 2002). Massive application of insecticides to control this pest has resulted in elimination of natural

enemies and resulted in environmental pollution, and therefore, there is a need to adopt integrated management practices, including host plant resistance to minimize the extent of losses due to this pest.

Several chickpea genotypes with low to moderate levels of resistance have been identified (Lateef, 1985). However, the levels of resistance are unstable across seasons and locations, and therefore, there is a need to develop transgenic chickpeas with high levels of resistance to *H. armigera.* Genetic transformation to enhance crop resistance or tolerance to biotic constraints has considerable potential to achieve more effective control of target insect pests and sustainable food production, particularly in the more vulnerable farming systems in the semi-arid tropics (Sharma *et al.,* 2002).

Novel genes, such as delta-endotoxins from the bacterium, *Bacillus thuringiensis* Berliner (*Bt*) need to be deployed through transgenic chickpea to make host plant resistance an effective weapon for the control of *H. armigera. In vitro* regeneration and genetic transformation of chickpea has been carried out earlier (Kar *et al.*, 1996), and efforts are continuing at ICRISAT to produce chickpea plants with *cry*1Ab and *cry*1Ac genes from *Bt* for controlling *H. armigera*. Chickpea cultivars ICCV 1 and ICCV 6, transformed with *cry* 1Ac gene, have been found to inhibit the development and feeding of *H. armigera* (Kar *et al.,* 1997). However, there is an apprehension that the acid exudates in chickpea leaves and pods may influence the effectiveness of *Bt* toxins produced in the transgenic plants.

Justin *et al.* (1989) observed an increase in susceptibility of *H. armigera* and *Spodoptera litura* (Fab.) to insecticides when the larvae were treated with *B. thuringiensis*. High larval mortality of *H. armigera* due to nuclear polyhedrosis virus (HaNPV) was observed when reared on *Helicoverpa*-susceptible chickpea varieties than on resistant ones (Rabindra *et al.*, 1992). Maximum reduction in larval numbers and reduced pod damage, and high grain yield were recorded when HaNPV was applied on susceptible chickpea genotype than on the resistant ones (Bhagwat, 2001).

Liu *et al.* (2000) reported that reduced conversion of crystalline protoxin to toxin confers three-fold resistance in diamond back moth, *Plutella xylostella* L., and there were no significant differences in binding of Cry1C to brush border membrane vesicles in resistant and susceptible strains of *P. xylostella*. The activity of the delta-endotoxins also increases with an increase in pH from eight to ten, but declines at pH more than ten. Increase in the pH of the formulation above 11 results in a decline in larval mortality. However, the spore viability was not affected adversely by pH upto12.5 (Behle *et al.*, 1997). Reduction in food consumption of third-instar larvae of *S. litura* increased gradually both in pure culture and formulation of *Bt* when it was exposed to increasing pH from six to ten (Somasekhar and Krishnayya, 2004). Because of the profound effect of pH on the biological activity of *Bt*, it is important to study the interaction of acid exudates in chickpea with biological activity of Cry toxins from *B. thuringiensis* against *H. armigera* to develop appropriate strategies for development of transgenic chickpeas with *Bt* genes for the management of this pest. Therefore, the present investigations were planned to study.

Objectives of the investigation

1. Effectiveness of *B. thuringiensis* against *H. armigera* on resistant and susceptible genotypes of chickpea,
2. Interaction of acid exudates (malic and oxalic acid) with biological activity of *Bt* toxins against *H. armigera*, and
3. Effect of acid exudates on protoxin-toxin conversion and binding to the brush border membrane of the gut of *H. armigera* larvae.

CHAPTER-II

Review of literature

CHAPTER – II

REVIEW OF LITERATURE

The literature pertaining to genotypic resistance to *Helicoverpa armigera* (Hubner) and its interaction with other components of managing this pest has been reviewed and presented in the following pages.

The females of *H. armigera* start laying eggs some hours after dusk, initially alternating with feeding, and later becoming the predominant activity until soon after midnight. The eggs are laid singly, late in the evening, mostly after 21 h to midnight. On the host plants, the eggs are laid on the lower surface of the leaves along the midrib, when the plants are still very small (Jayaraj, 1982).

Mullick and Singh (2001) evaluated the effect of larval food, *i.e.*, leaves and flower buds of four leguminous plants *viz.*, chickpea, pigeonpea, blackgram, and cowpea on the pre-oviposition period, fecundity, and longevity of *H. armigera* females. Pre-oviposition period of females reared on chickpea leaves was significantly shorter compared to those reared on leaves of other host plants. The fecundity of females fed on cowpea and pigeonpea leaves during the larval stages was statistically not different. Leaves of different test plants did not influence longevity of females. The fecundity indices of females reared on cowpea (56.21) and pigeonpea leaves (44.73) were

statistically similar, but significantly greater than those reared on black gram (39.38) and chickpea (37.89) leaves. No significant differences were observed in the pre-oviposition period of females fed on flower buds of different leguminous plants during the larval stages.

2.1 RELATIVE SUSCEPTIBILITY OF CHICKPEA GENOTYPES FOR RESISTANCE TO THE POD BORER, *H. armigera*

Chhabra and Kooner (1980) screened 332 germplasm entries, the cultivars GL 645, P 1324-II, P1697, P 6292-I, Dulia 6-28, GGP chaffa, and selection 418 exhibited low pod borer damage, ranging from zero to five per cent relative to 36 per cent in susceptible cultivar.

Borikar *et al.* (1982) screened 18 chickpea genotypes (comprising of 12 mutants, 3 strains, and 3 varieties), and reported that the strain 2-52-2, and the mutant Pinnate showed low damage and at the same time gave higher yields showing resistance to the pest.

Dias *et al.* (1983) screened 49 varieties of chickpea to determine their susceptibility to *H. armigera* and reported that the pod damage was lower in H 75-58, ICCC 18, Kanpur local, Gonola II local, and Mirzapur local.

Lateef (1985) evaluated a large number of genotypes for resistance to *H. armigera*, and recorded 58 larvae per five plants on ICC 506 compared to 103 larvae on Annigeri, 99 on ICC 10619, 202 on ICC 3137, 112 on ICC 7320-11-1, and 147 on ICC 8835.

Chhabra *et al.* (1990) screened seven cultivars (GL 645, Dulia 6-28, GGP chaffa, P 1324-II, P 1697, P 6292, and selection 418) along with standard varieties (G 130, and

L 550) and infestor, seven cultivars recorded less than five per cent pod damage as against 16.1 and 36.0 per cent in standard varieties and infestor, respectively.

Prasad *et al.* (1990) recorded 13.1 per cent pod damage in ICC 5819, 23.1 per cent in BG 275, 33.6 per cent in ICC 1931, and 52.8 per cent in BG 276.

Kotikal and Panchabavi (1992) screened eight genotypes and observed lowest pod damage in ICCX 790197-3PLB 3PLB-BPLB, which was on a par with that of ICCX 790197-23 PLB2EB, ICC 506, ICCX 780286-5 PLB 2PLB 2EB, ICCX 73008-8-1-1, and P-BP, but differed significantly from Annegeri and Bheema. The genotype ICC 506 (2.08) recorded lower damage rating as compared to Annegeri (8.33).

Chauhan and Dahiya (1994) evaluated 35 chickpea genotypes along with four checks (H 208, C 235, H 75-35, and H 82-2). The genotypes BG 372, BG 390, GNG 469, PDE 2-1, and PDE 3-2 recorded low pod damage and better yields. The genotype H 82-2 inspite of high pod damage (21.08%) gave higher grain yield (23.5 q ha^{-1}), which was greater than other genotypes, this might be due to its tolerance to the pod borer.

Deshmukh *et al.* (1996a) reported lower pod borer damage in Phule G 87207 (Vishal), as compared to Phule G 12 (12.45 %), and Phule G 5 (14.74%).

Deshmukh *et al.* (1996b) in an another experiment reported that wilt resistant cultivar Vijay (Phule G 81-1-1) recorded less pod borer damage (9.58%) as compared to Phule G 12 (12.45%), and Phule G 5 (14.74%).

Reddy *et al.* (1996) evaluated fourteen chickpea varieties against pod borer, *H. armigera* and reported that Pusa 261, BG 374, and BG 386 were the least susceptible varieties.

Yelshetty *et al.* (1996) screened 17 chickpea genotypes out of which the genotype BJ 256 recorded significantly lower pod damage (3.93%).

Olla and Saini (1999) evaluated eight chickpea genotypes in the laboratory for feeding preference by the fifth instar *H. armigera* larvae and observed that H 92-67 and H 91-47 were the most resistant, while H 86-18, HK 89-96, and HK 89-131 were highly susceptible. However, C 235 and L 550 showed moderate level of resistance.

Singh and Yadav (1999a) used three parameters, the number of larvae, number of pods, percentage pod damage, and grain yield to select for resistance to *H. armigera* and screened seventy desi chickpea genotypes under normal sown conditions. The genotypes DHG 84-11, P 240, BG 79, and DHG 88-20 proved less susceptible or more tolerant, whereas GNG 752, GCP 89, G 211, and KBG 2 were highly susceptible in comparison to P 256, BR 77, C 235, and C 243. Under late sown conditions (Singh and Yadav, 1999b) the genotypes DHG 84-11, ICC 29, DHG 86-38, DHG 88-20, SG 90-55, KBG 1, IH 83-83, NP 37, DHG 87-54, GNG 669, and SG 89-11 were more tolerant or as good as common cultivars *viz.*, P 240, P 256, C 235, and BR 77.

Gumber *et al.* (2000) evaluated 62 chickpea germplasm accessions and six approved cultivars for resistance to pod borer, and the accessions ICC 93512, ICC 93515, and ICC 93212 were the most promising with higher seed yield and low pod borer damage. The pod borer damage was positively correlated to the total number of pods and pod length.

2.1.1 Effect of different chickpea genotypes on development and survival of *H. armigera* (antibiosis)

Antibiosis is the adverse effect of a plant on development and survival of insects (Painter, 1958). Antibiosis is expressed in terms of larval mortality, decreased larval and pupal weights, prolonged larval and pupal development, failure to pupate, and reduced fecundity and egg viability (Yoshida *et al.,* 1995).

Chickpea varieties differ in their susceptibility to *H. armigera* due to differences in oviposition preference and antibiosis (Singh and Sharma, 1970). Studies on antibiosis to *H. armigera* in chickpea have earlier been reported by Jayaraj (1982), Srivastava and Srivastava (1989, 1990), Cowgill and Lateef (1996), Yoshida *et al.* (1995, 1997), and Sharma *et al.* (2005c).

Rembold (1981) reported that malic acid acts as a deterrent to the *H. armigera* larvae, and pod borer resistant lines have more amounts of malic acid than the susceptible lines. Rembold and Winter (1982) found that the threshold for low pod borer damage is 250 mg malate ml^{-1} of exudates.

Lateef (1985) suggested that the amount of acid exudates on leaves is an useful index for distinguishing relatively resistant genotypes from the susceptible ones.

Srivastava and Srivastava (1989) screened eight desi chickpea genotypes, and reported that low levels of acidity in the leaf extracts of different genotypes were associated with susceptibility to *H. armigera* in ICC 3137, K 850, and ICC 1403.

Rembold *et al.* (1990) suggested that chickpea exudates can be used to select for resistance to *H. armigera*, the main components being malate and oxalate, which are present in variable amounts in different genotypes of chickpea.

Srivastava and Srivastava (1990) assessed the antibiotic effect of chickpea genotypes in terms of larval survival, larval and pupal weights, egg viability, adult longevity, fecundity, and Howe's growth index.

Chhabra *et al.* (1993) reported that chemical components such as malic acid, sugars, crude fibre, cellulose, and lignin were responsible for low incidence of *H. armigera* in desi 3108, GL 1002, and LCG 3580.

Bhagwat *et al.* (1995) observed that low acidity of the leaf exudates and malic acid content were associated with the susceptibility of ICC 14665 to *H. armigera* at 60 and 75 days after sowing. However, this trend was not apparent at 90 days after sowing.

Patnaik and Senapati (1995) studied the influence of acidity on *H. armigera* incidence in 13 desi early-maturing chickpea cultivars. The egg and larval counts were negatively correlated with concentrations of acid exudates in the leaf extracts. Low density of eggs (0.7 to 1.6 eggs 10^{-1} plants) and larvae (3.0 to 4.0 larvae 10^{-1} plants) were associated with high acidity (24.2 to 25.3 meq), while the cultivars with low acid content (13.5 to 15.1 meq) harboured more eggs (> 2.7 eggs 10^{-1} plants) and larvae (> 5.9 larvae 10^{-1} plants). However, resistance expressed by resistant cultivars such as PDE 3-3, PDE 7-3, and ICC 506 was attributed to factors other than the acidity, while that of PDE 7-2 appeared to be due to high acidity.

Yoshida *et al.* (1995) reported that genotypes resistant to *H. armigera* accumulated more oxalic acid on the leaves than the susceptible genotypes. Oxalic acid showed significant growth inhibition of *H. armigera* larvae when incorporated into a semi-artificial diet. The effective accumulation of oxalic acid is considered to be one of the mechanisms of *H. armigera* resistance in chickpea. Inhibition of larval growth by

oxalic acid was not caused by antifeedant effects, but was more likely attributable to antibiosis.

Cowgill and Lateef (1996) screened five short-medium duration desi, and five medium-long duration kabuli chickpea genotypes for antibiosis to *H. armigera*. Significant variation among the desi genotypes was found for pupal weight and larval survival.

Patnaik (1996) reported adverse effects on growth and development of *H. armigera* on the resistant cultivars ICC 506 and PDE 5-2.

Yoshida *et al.* (1997) investigated the effects of malic and oxalic acids on oviposition of *H. armigera*. Malic acid stimulated oviposition at a concentration of 0.6 μmol cm^{-2} but inhibited at 3.4 μmol cm^{-2}. Oxalic acid showed neither stimulation nor inhibition of oviposition at 0.25 to 1.7 μmol cm^{-2}. Malic acid on the leaves stimulated oviposition during the vegetative and flowering stages (0.1 to 0.7 μmol cm^{-2}). During the podding stage, there was no significant association between egg density or pod damage and malic acid levels. However, there was a significant and negative correlation between pod damage and oxalic acid levels.

Singh (1999) studied the effects of powdered seed materials of chickpea, soybean, and maize incorporated into artificial diet on the growth, food consumption, and feeding preferences of *H. armigera* larvae. Food consumption and growth of final-instar larvae were minimal on maize diet. The nutritive value of the soybean diet was higher, but the consumption rate was the highest on chickpea diet as compared to other test diets.

Ghodeswar *et al.* (2003) reported that the grain and pod shell tissues of chickpea cultivars tolerant to *H. armigera* contained significantly higher amounts of total

phenolics, chlorogenic acid, silica, malic acid, and had higher activities of polyphenol oxidase (catechol oxidase) and peroxidase than the susceptible cultivars at 70 days after sowing. The susceptible cultivars had higher crude protein and sugars.

Sreelatha (2003) recorded lower larval and pupal weights and prolonged larval and pupal periods on leaves, pods, and artificial diet impregnated with lyophilized leaves and pods of resistant genotypes (ICC 12475, ICC 12476, ICC 12477, ICC 12478, ICC 12479, ICC 14876, ICC 12490, ICC 12491, and ICC 12495) as compared to that of the susceptible genotypes (ICC 12426, ICC 3137, ICC 4973, and ICC 4962).

Narayanamma *et al.* (2007) evaluated a set of diverse chickpea genotypes with different levels of resistance to *H. armigera*, and their F_1 hybrids for oviposition non-preference, antibiosis, and tolerance. The genotypes ICC 12476, ICC 12477, ICC 12478, ICC 12479, and ICC 506EB were not preferred for oviposition as compared to ICCC 37. Antibiosis expressed in terms of low larval weights was observed in insects reared on ICC 12476, ICC 12478, ICC 506EB and weight gain by the third-instar larvae was also low on ICC 12476, ICC 12477, ICC 12478, ICC 12479, and ICC 506EB at podding stage. Non-preference for oviposition and antibiosis were also expressed in F_1 hybrids based on ICC 12476, ICC 12477, ICC 12478, ICC 12479, and ICC 506EB indicating that ovipositional non-preference and antibiosis is indicated by the parent genotype.

Narayanamma *et al.* (2008) compared the survival and development of *H. armigera* larvae on nine chickpea genotypes using two food substrates, and recorded that the larval and pupal weights, and larval survival were greater in larvae reared on artificial diet containing lyophilized leaf or pod powder of different chickpea genotypes as compared to that on fresh leaves/ pods. Reduced larval and pupal weights, and

prolonged larval period was observed when the insects were reared on the fresh leaves/ pods of ICC 12476, ICC 12477, ICC 12478, and ICC 12479. Larval survival, pupation and adult emergence were lower, when the insects were reared on the fresh leaves/pods, and artificial diets with leaf/pod powders of ICC 12476, ICC 12477, ICC 12478, and ICC 506EB as compared to ICCC 37 and ICC 4918. Larval survival and development were also adversely affected on F_1 hybrids based on ICC 12476, ICC 12477, ICC 12478, and ICC 506EB, suggesting that antibiosis mechanism of resistance is transferred to the progeny from the resistant parents.

2.1.2 Effect of *Bt* sprays on pod damage and grain yield of chickpea

Kulkarni and Amonkar (1988) evaluated purified spores and crystals of three isolates (ISPC-1, ISPC-4 and ISPC-7) of *B. thuringiensis* against second instar larvae of *H. armigera*, and observed that the spores alone gave 10 to 20 per cent mortality, whereas crystals gave 100 per cent mortality. Reduction in larval numbers was recorded in *Bt* treated plots, but there were no significant differences in grain yield between *Bt* treated and untreated control plots.

Wanjari *et al.* (1998) evaluated HaNPV (nuclear polyhedrosis virus), a commercial formulation of *B. thuringiensis* subsp. *kurstaki* (Dipel), neem seed extract, and endosulfan either alone or in combination against *H. armigera* in chickpea. All the treatments were effective in reducing larval populations as well as pod damage, and resulted in greater grain yields compared to the untreated control. Plots treated with *B. thuringiensis* subsp. *kurstaki*, and alternated with endosulfan were effective for controlling *H. armigera*.

Kulat *et al.* (1999) evaluated a number of treatments against *H. armigera* on chickpea. Endosulfan (0.07%) was the most effective in reducing the larval population and increasing grain yield, followed by *B. thuringiensis* subsp. *kurstaki* (176×10^2 spores ml^{-1}) at 750 ml ha^{-1} and *H. armigera* nuclear polyhedrosis virus (NPV) at 250 to 750 larval equivalents (LE) ha^{-1}.

Singh *et al.* (1999) evaluated the efficacy of Dipel 8L (1 L ha^{-1}) (*B. thuringiensis* subsp. k*urstaki)*, Delfin WG (1 kg ha^{-1}) (*B. thuringiensis*), and NPV (250 LE ha^{-1}) alone and in combination with endosulfan (35 EC) for their effectiveness against *H. armigera* The biopesticides in combination with endosulfan, were more effective reducing pod damage by *H. armigera*, with an average of 4.21, 5.65, and 6.66 per cent pod damage, and 49.7, 47.2, and 46.7 per cent increase in grain yield in plots treated with Delfin, Dipel, and NPV, respectively.

Balasubramanian *et al.* (2002) compared the efficacy of *B. thuringiensis* var. *galleriae* (Spicturin) with cartap against *H. armigera*. There was a significant reduction in larval population, pod damage, and increase in grain yield of chickpea in plots treated with Spicturin (2 L ha^{-1}) followed by cartap 50% SP (2.5 kg ha^{-1}).

Mandal *et al.* (2003) determined the efficacy of three *B. thuringiensis* formulations (Biolep, Bioasp, and Dipel) and nuclear polyhedrosis virus (Virin) for controlling *H. armigera* on chickpea. Plots treated with biopesticides resulted in higher grain yield, and benefit-cost ratio, and lower number of larvae, and pod damage compared to the untreated control plots. Among the biopesticides tested, Biolep treated plots had the lowest number of larvae per ten plants, and pod damage, and resulted in the

highest grain yield, whereas the plots treated with Dipel gave the highest benefit-cost ratio.

Bhojne *et al.* (2004) conducted experiments to compare the efficacy of *B. thuringiensis* var. *kurstaki* (HD-1) and var. *morrisoni* (HD-12), against second-instar larvae of *H. armigera*. Strain HD-1 was more toxic than HD-12. Strain *kurstaki* caused 79.38 per cent mortality at 0.00963 per cent, while the strain *morrisoni* caused 89.66 per cent mortality at 0.0576 per cent concentration. The LC_{50} value of HD-1 was 0.0055 per cent, whereas that of HD-12 was 0.02 per cent.

Singh and Ali (2005) evaluated the efficacy of *H. armigera* nuclear polyhedrosis virus (HaNPV) at 250, 350, and 450 LE ha^{-1}, *B. thuringiensis* (*Bt*) at one per cent, neem seed kernel extract (NSKE) at five per cent, and endosulfan at 0.07 per cent against *H. armigera* infesting chickpea cv. K 850. The first spray was given when the larval population was above the economic threshold level of three larvae m^{-2}, and the second spray at ten days thereafter. Maximum larval mortality was recorded in plots treated with endosulfan (85%), followed by those treated with *Bt* formulation (80%), and Ha NPV at 450 LE ha^{-1} (75%). NSKE was the least effective treatment. The highest grain yield was obtained in endosulfan treated plots (25 q ha^{-1}), followed by plots treated with 1 per cent *Bt* (24 q ha^{-1}), and Ha NPV at 450 LE ha^{-1} (23.66 q ha^{-1}).

2.2 INTERACTION OF CHICKPEA GENOTYPES WITH BIOLOGICAL ACTIVITY OF *Bt* TOXINS AGAINST *H. armigera*

2.2.1 Influence of transgenic chickpea plants on survival and development of *H. armigera*

Kar *et al.* (1997) transferred two strains of chickpea, ICCV 1 and ICCV 6 with *Bt* toxin gene, *cry 1Ac* gene. Insect feeding assay indicated that the expression level of the *cry1Ac* gene was inhibitory to the development of the larvae of *H. armigera*.

Since the literature pertaining to influence of transgenic chickpea plants on survival and development of *H. armigera* was meager, literature pertaining to influence of other transgenic plants on survival and development of *H. armigera* was also reviewed.

Cui and Xia (1999) studied the effects of transgenic *Bt* cotton on development and reproduction of *H. armigera* and reported that the first- to fourth-instar larvae fed with transgenic *Bt* cotton continuously were dead. The survival of fifth- and sixth-instar larvae was 37.9 to 85.6 and 63.4 to 96.5 per cent, respectively as compared to the control. The rate of emergence decreased by 66.7 to 100 per cent, number of eggs decreased by 50.1 to 69.7 per cent and the rate of hatching by 80.6 to 87.8 per cent.

Li and Wang (1999) studied the resistance of transgenic *Bt* cotton cultivars (R 93-4, 33-B, 9501, Huadai, and GK 12) to second-instar larvae of *H. armigera* reared on young leaves, apical shoot tips, and immature bolls. Insect mortality on bolls was 77.04 per cent for cv. 33-B, 77.55 per cent for cv. GK 12, 88.00 per cent for cv. 9501, 89.00 per cent for cv. Huadai, 29.41 per cent for cv. R 93-4, and 3.70 per cent for control (cv. Chun aizao). Mortality on leaves was 81.08 per cent for cv. 33-B, 71.62 per cent for cv. 9501,

53.00 per cent for cv. Huadai, 59.00 per cent for GK 12, 14.67 per cent for R 93-4, and zero per cent for control. The mortality on shoot tips was 36.54 per cent for R 93-4, whereas it ranged between 96.51 to 98.75 per cent for other cultivars, and 3.70 per cent for the control. The larval weight and duration of each instar were significantly greater on the control and R 93-4.

Zhou *et al.* (2001) recorded that *Bt* transgenic cotton exhibited antifeedant activity on the third-, fourth-, and fifth-intsar larvae of *H. armigera*. The antifeedant activity and the activity of midgut digestive enzymes were the lowest in fifth-instar larvae.

Murugan *et al.* (2003) evaluated the efficacy of *Bt* cotton cultivars MECH 12, MECH 162, and MECH 184 on the early instars of *H. armigera* and recorded 92.8, 66.7, and 51.7 per cent mortality at first-, second-, and third-instar stages, respectively. The *Bt* cotton feeding reduced the final weight and relative weight gain of each instar compared to non-transgenic fed larvae. The fourth-, and fifth-instar growth was severely hindered even though considerable quantities of plant tissues were consumed.

Wang *et al.* (2003) reported that the larval pupation rate, eclosion rate, pupal weight, body length, and feeding duration decreased, and the spin drooping time increased when the larvae were fed on transgenic *Bt* cotton.

Sharma and Pampapathy (2006) evaluated the effectiveness of transgenic cottons with *Bt cry1Ac* gene along with non-transgenic commercial cultivars for the management of *H. armigera* and recorded low larval numbers, low boll damage and greater seed yield on transgenic hybrids than that of non-transgenic counterparts.

2.2.2 The HPLC profiles of leaf exudates and their relevance of expression of resistance to insect population

Yoshida *et al.* (1995) reported that acid components of the leaf exudates were analyzed by high performance liquid chromatography, the oxalic and malic acids were detected as major acid components in the leaf exudates of chickpea genotypes. Fumaric and citric acid were also detected, but as minor components at less than one per cent that of the major acids. The concentrations of oxalic acid was consistently higher in resistant (ICC 506 and ICCL 86102) than in susceptible (Annegeri and ICCX 730266-3-4) genotypes at both vegetative and flowering stages. Malic acid concentration did not appear to be related with resistance status. Oxalic acid when included in a semi-artificial diet, inhibition of larval growth and prolongation of larval period were observed, where as malic acid had no significant effect.

Broils *et al.* (1998) used high performance liquid chromatography (HPLC) for identification of active constituents of *Hypericum perforatum* L. using a wide pore RP-18 column and a water-methanle-acetonitrile-phosphoric acid mobile phase system. The identification of flavonoids, naphodianthrone and phloroglucinol constituents was performed using combined HPLC-diode array detection (DAD) analysis, HPLC-thermospray, and HPLC-electrospray mass spectrometry. Chlorogenic acid, quercetin, quercitrin, isoquercitrin, rutin, hyperoside, 13,II8-biapigenin, pseudohypericin, hypericin, hyperforin, and adhyperforin were separated by an aqueous phosphoric acid-acetonitrile-methanol gradient within 50 min.

Narayanamma (2005) reported that the malic acid content estimated through HPLC was significantly and negatively correlated with damage rating and pod damage,

whereas oxalic acid was negatively and significantly correlated with damage rating. Acetic acid showed a negative correlation with larval weight and damage rating, whereas citric acid showed negative and significant correlation with damage rating.

2.2.3 Effect of *B. thuringiensis* on growth and behaviour of *H. armigera*

Dulmage and Martinez (1973) reported that, development time of *Heliothis virescens* (F.) increased, pupal weights decreased, and the number of larvae able to complete the cycle, fertility, and fecundity reduced in insects emerging from larvae reared in the presence of toxin.

Khalique *et al.* (1989) evaluated Bactospeine WP (*B. thuringiensis* subsp. *thuringiensis*) and the standard strain HD-1-S-1980 (*B. thuringiensis* subsp. *kurstaki*) against first- and third-instar larvae of *H. armigera* in the laboratory at 26^0 C and 65 to 80 per cent RH. The LC_{50} of *B. t. thuringiensis* for first- and third-instar larvae were 63.52 and 177.60 Imu ml^{-1}, respectively, after seven days exposure. The LC_{50} for *B. t. kurstaki* with first-and third-instar larvae were 56.16 and 126.40 Imu ml^{-1}.

Gould and Anderson (1991) evaluated the effects of commercial formulation (Dipel 2x) and purified HD-73 delta endotoxin of *B. thuringiensis* var. *kurstaki* on the growth, behaviour, and fitness of susceptible and toxin adapted strains of *H. virescens* by incorporation of the materials into artificial diets. Both strains avoided moderate to high concentrations of the toxins. At the lowest concentration of HD-73, neither strain avoided the diet, while at the lowest concentration of Dipel, only the susceptible strain avoided the diet.

Gould *et al.* (1991) examined the effects of *B. thuringiensis* spore/crystal mixtures and purified endotoxins on growth and behaviour of *H. virescens* using diet incorporation

assays. The young larvae avoided the formulations and endotoxins that did not cause any mortality or severe inhibition of growth, while the fourth- and fifth-instar larvae avoided formulations and endotoxin containing diets when the concentrations used inhibited the larval growth.

Chandra *et al.* (1999) tested the effectiveness of *B. thuringiensis* (*Bt*) based products Biolep, Dipel, and Biobit (*B. t.* subsp. *kurstaki*) against third-instar larvae of *H. armigera* under environmentally controlled conditions. The LC_{50} values for Biobit, Biolep, and Dipel were 0.114, 0.211, and 0.213 per cent, respectively, with an exposure period of 48 h. For the post exposure period (until pupation), the LC_{50} values were 0.087, 0.186, and 0.159 per cent. All the concentrations tested had an adverse effect on growth and development of *H. armigera*. Increased larval mortality, larval period, growth inhibition, and decreased pupation, pupal weight, and adult emergence were recorded with increased doses of *Bt*.

Gujar and Mohan (2000) reported that the Cry1Ab endotoxin of *B. thuringiensis* subsp. *kurstaki* was 16-fold less toxic to neonate larvae of *H. armigera* than the HD-1 endotoxin. Neither preparation caused any mortality in second- and third-instar larvae, nor inhibited growth and development at low concentrations. The EC_{50} of Cry1Ab for inhibition of larval growth over a seven-day period was 0.93 ppm for the second-instar and 3.45 ppm for the third-instar. HD-1 had an EC_{50} of 0.23 ppm for the third-instar and was more active than Cry1Ab.

Gujar *et al.* (2000) studied the effect of *B. thuringiensis* formulations/strains on the growth and development of *H. armigera in vitro*. Biobit was the most toxic to the neonates, followed by Biolep, HD-1, HD-73, and Dipel. Toxicity of these formulations

decreased considerably against five-day old larvae. HD-1 showed the highest toxicity to the five-day-old larvae, followed by Biobit and Biolep. Larval growth was considerably reduced in HD-1 and other formulations, except for HD-73, where it was at par with control. *Bt* affected last-instar larval growth and development, pupation, and adult emergence.

Zhang *et al.* (2000a) reported that *B. thuringiensis* was highly deterrent to feeding by *H. armigera* larvae when incorporated into diets, even at non-lethal dosages. As the dosage of *Bt* increased, the deterrence increased.

Gujar *et al.* (2001) investigated the effect of sublethal concentrations of *B. thuringiensis* on the first-, third-, fourth-, and fifth-instar larvae of *H. armigera* on food consumption, digestion, utilization, and insect development till adult emergence. Young larvae surviving *B. thuringiensis* during the first- and third-instars had prolonged larval development (by 2 or 3 days), but did not consume more food and showed higher digestibility of food as compared to the control, which was compensated by reduced ability to utilize the digested food into body matter, whereas fifth-instar larvae surviving *B. thuringiensis* consumed less food, showed less absorption efficiency of digested food, but compensated by increase in utilization of ingested and digested food into body matter. The moths emerging form *B. thuringiensis* treated larvae had a sex ratio favouring females, and laid lower number of fertile eggs than those from the untreated diet.

The baseline toxicity of Cry1A delta-endotoxins (derived from *B. thruringiensis*) on field populations of *H. armigera*, were determined through Log dose probit analysis by Kranthi *et al.* (2001). The Cry1Ac was found to be the most toxic, followed by Cry1Aa, and Cry1Ab. The LC_{50} values ranged from 0.07 to 0.99 $\mu g\ ml^{-1}$ (14-fold) for

Cry1Aa, 0.69 to 9.94 μg ml^{-1} (14-fold) for Cry1Ab, and 0.01 to 0.67 μg ml^{-1} of diet (67-fold) for Cry1Ac. The LC_{50} values deduced from the cumulative Log dose probit response of the data pooled from all assays were 0.62 μg ml^{-1} for Cry1Aa, 4.43 μg ml^{-1} for Cry1Ab, and 0.100 μg ml^{-1} of diet for Cry1Ac. The respective LC_{50} values represented the baseline susceptibility indices for resistance monitoring through the conventional Log dose probit assays. The LC_{99} values derived from the cumulative data were 515 μg ml^{-1} for Cry1Aa, 13385 μg ml^{-1} for Cry1Ab, and 75 μg ml^{-1} of diet for Cry1Ac. These values represent the diagnostic doses for routine monitoring of resistance to respective toxins through discriminating dose assays after introduction of *Bt* transgenic crops.

Chandrashekar *et al.* (2005) studied baseline susceptibility of larvae of *H. armigera*, to *B. thuringiensis* var. *kurstaki* (*B. thuringiensis* subsp. *kurstaki)* by diet incorporation method. Ninety-six hour median lethal concentrations (LC_{50}) of *Bt* var. *kurstaki* strains and parasporal crystal toxins varied widely for neonate larvae of different populations. Insect populations from nine locations in India showed differences in their susceptibility to *Bt* var. *kurstaki* strains, and individual Cry toxins, *i.e.*, Cry1Aa 10.5, Cry1Ab 12.8, Cry1Ac 16.2, HD-1 14.1, and HD-73. Insect populations obtained from pigeonpea crops at Navsari from December 2000 to January 2001, and at Delhi from October 1998 to November 2000 showed temporal variation in their susceptibility to *Bt* var. *kurstaki* HD-1 and HD-73. Temporal variation in insect susceptibility was correlated with temperature. Insect acclimatization to pre-treatment temperature influenced the susceptibility of the F_1 generation to *Bt* var. *kurstaki*. An increase in ambient temperature (approximately 10^0 C) increased the susceptibility to *Bt* var. *kurstaki* HD-73 by 7.5-fold.

2.2.4 Effect of plant allelochemicals on toxicity of *B. thuringiensis* to *H. armigera*

Sivamani *et al.* (1992) observed that plant phenolics enhanced the activity of *B. thuringiensis* var. *gallariae* endotoxin to the larvae of *H. armigera*. Plant phenolics in combination with *B. thuringiensis* var. *gallariae* endotoxin not only reduced the feeding potential and weight gain by the larvae, but also enhanced the LC_{50} value of the toxin.

The role of tannic acid [tannins] in increasing the effectiveness of *B. thuringiensis* subsp. *kurstaki* (HD-1) against *H. armigera* was examined in bioassays on semisynthetic diet by Wang *et al.* (1997). Concentrations of *B. thuringiensis* (0.00, 0.005, 0.01, 0.015, 0.02, and 0.025% wet weight) were incorporated into the diet containing 0.025 per cent tannins and into tannin-free diet. LD_{50} of *B. thuringiensis* with tannin was 0.006 per cent and without tannin was 0.011 per cent. Both *B. thuringiensis* and tannin retarded growth of *H. armigera* significantly, but there was no synergistic effect between them. Choice tests showed that *B. thuringiensis* deterred feeding of the fifth-instar larvae of *H. armigera*, but tannins had no such effect. Experiments on colony growth of *B. thuringiensis* on NBA media containing tannin (0, 1, 3, 6, 9, 12, 15, 18, and 21 mg 100^{-1} ml) demonstrated that tannin reduced colony growth of *B. thuringiensis* and inhibited sporulation above 15 mg 100^{-1} ml.

Ahmed *et al.* (1998) assessed dosage-mortality response of neonate larvae of *H. armigera* due to feeding on HD-I-S-1980 and malic acid alone or combinations of both incorporated into the artificial diet. Significant synergistic interactions were observed in most of the combinations of HD-I-S-1980 with malic acid (from 1 to 4% concentrations). Slopes of regressions upto four per cent malic acid + HD-I-S-1980 combinations were steeper than HD-I-S-1980 alone. The LC_{50}, of HD-I-S-1980 decreased

with one, two, and four per cent malic acid. Potency of HD-I-S-1980 was increased by 0.4, 2.7, and 12.89 folds in one, two, and four per cent malic acid combinations, respectively. The dosage-mortality responses of the noctuid, *H. armigera* to combination treatments of the US reference standard (HD-I-S-1980) with malic acid enhanced the effectiveness of the bacterium.

Olsen and Daly (2000) reported that the Cry1Ac protein was less toxic to *H. armigera* larvae when the protein was mixed with leaves from fruiting versus pre-square conventional cotton. Differences in LC_{50} varied from 2.4- to 726-fold, depending on the source of toxin and conventional plant material. These results suggest that plant-toxins in fruiting cotton reduced the toxicity of Cry1Ac protein.

Zhang *et al.* (2000b) observed that there were no significant differences in larval mortality of *H. armigera* when a sublethal dose of *B. thuringiensis* var. *kurstaki* HD-1 crystal was supplemented with soybean trypsin inhibitor (STI) in the artificial diet, but supplementing a non-lethal dose of crystal with STI in the diet led to a pronounced reduction of larval growth. The results of substrate-gel electrophoresis demonstrated that the proteases in the *H. armigera* midgut fluid were responsible for degradation of protein. Retention time of toxins in the larval midgut was extended and synergism between insecticidal crystal protein and STI inhibited the larval growth.

The literature pertaining to the effect of plant allelochemials on toxicity of *B. thuringiensis* to other insects were also reviewed hereunder.

Krischik *et al.* (1988) studied the role of plant allelochemicals on biological activity of *B. thuringiensis* var. *kurstaki* on the larvae of *Manduca sexta* (L.). Concentrations of nicotine and rutin reflecting the levels found in tobacco and tomato

plants were incorporated into synthetic diet containing 0.001 per cent *B. thuringiensis* and *Bt* free diet, and observed that survivorship of *M. sexta* larvae increased at higher levels of nicotine, but higher concentrations of rutin did not increase larval survivorship.

Brewer and Anderson (1990) reported that the phenolic compounds in sunflower such as cinnamic or coumaric acids in combination with *B. thuringiensis* in artificial diet reduced the growth of sunflower moth, *Homoeosoma electellum* (Hulst) as compared to that of the larvae fed on diets containing *B. thuringiensis* alone.

Lord and Undeen (1990) reported that the mortality of *Aedes aegypti* L. larvae treated with *B. thuringiensis* var. *kurstaki* was reduced by tannic acid at concentrations as low as 0.25 mM (425 mg L^{-1}). The LC_{50} for *B. thuringiensis* var. *israelensis* was 45 ng ml^{-1} in deionized water and 233 ng ml^{-1} in 1.5 mM of tannic acid.

Ludlum *et al.* (1991) explored the effects of quinone alkylation in tomato plant on toxicity of *B. thuringiensis* subsp. *kurstaki* on the larvae of *Heliothis (Helioverpa) zea* (Boddie). When *Btk* incubated with phytochemicals, chlorogenic acid and polyphenol oxidase (which form orthoquinones in damaged plant tissue) it was more toxic than the larvae treated with *Btk* (not incubated with phytochemicals). Digestability experiments suggested that alkylation enhanced the solubilization and/or proteolysis of *Bt* crystal protein *in vivo.*

Murray *et al.* (1993) examined the effects of citrus limonoids (antifeedants) used alone and in sequence with a *B. thuringiensis* var. *sandiego* delta endotoxin against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and observed that larval developmental time was significantly prolonged among the larvae treated with limonoid

or endotoxin, the most delayed development occurred among the larvae treated with the endotoxin followed by limonoid.

Navon *et al.* (1993) investigated the potential interactions among a plant produced allelochemical, a phytophagous insect, and an endotoxin produced by *B. thuringiensis* using purified condensed tannins from the cotton. Purified condensed tannins from cotton fed to neonate *H. virescens* reduced feeding and mortality caused by insecticidal crystals of *B. thuringiensis*. In fifth instars, tannins reduced relative growth rate, and consumption rate, but antagonized the effects of the crystal δ-endotoxin. Tannins adversely affected feeding after ingestion. These results suggested that insect control tactics that employ δ-endotoxins in microbial insecticides and transgenic cotton plants may not be compatible when used in conjunction with plants containing high tannin concentrations.

The effect of host plant on the effectiveness of thuricide, a commercial formulation of *B. thuringiensis* subsp. *kurstaki*, towards gypsy moth *Lymantria dispar* L. was examined by Appel and Schultz (1994). Thuricide was administered to second and fourth instar larvae of gypsy moths on foliage from red oak, chestnut oak and quaking aspen. Oaks were two to five times more inhibitory of thuricide than aspen. Among the oaks red oak was more inhibitory than chestnut oak. Mortality was correlated negatively with concentration of total phenolics, gallotannins, and protein binding activity in leaves. Purified tannins from the oaks were highly inhibitory, indicating that leaf tannins are primarily responsible for reducing the effectiveness of thuricide.

Morris *et al.* (1994) reported increased mortality of bertha armyworm, *Memestra configurata* Walker, when sub-lethal concentrations of caffeine and related compounds were combined with sub-lethal concentrations of *B. thuringiensis*.

Gibson *et al.* (1995) identified tannic acid as an expensive additive that increased the efficacy of sub-lethal concentrations of *B. thuringiensis* subsp. *kurstaki*. Development of cabbage looper, *Trichoplusia ni* (Hubner) larva was affected in a concentration dependent manner by 2.5 to 500 ppm of tannic acid.

2.3 EFFECT OF ACID EXUDATES ON PROTOXIN-TOXIN CONVERSION AND BINDING TO THE BRUSH BORDER MEMBRANE OF THE GUT OF *H. armigera* LARVAE

After ingestion of *Bt* toxins by the insect larvae, the crystalline inclusions are solubulized in the alkaline gut environment and the Cry proteins are proteolytically converted into smaller toxic polypeptides (Hofte and Whiteley, 1989; Gill *et al.*, 1992). These polypeptides bind to specific binding receptors located in the midgut brush border membrane. After the insertion into membrane, they make low selective ion pores (Lorence *et al.*, 1995). The resulting permeability produces ionic and water flow, that leads to midgut cell swelling and eventual cell lysis (Schnepf *et al.*, 1998). The intoxicated insects stop feeding and eventually die.

Dissolution and activation of parasporal crystals in larval midgut are the first two steps in its insecticidal process. Each kind of crystal protein has a specific protease inhibitor, while each kind of protease in larval midgut has an optimal pH. The pH of the environment effects the solubility of the crystal, and activation of protoxin by proteases (Aronson *et al.*, 1991; Dai and Gill, 1993).

Parenti *et al.* (1995) measured the activities of three related *B. thuringiensis* delta-endotoxins (Cry1Aa, Cry1Ab, and Cry1Ac) as inhibitors of K^+-dependent amino acid transport into membrane vesicles from the anterior and posterior portions of *Bombyx*

mori L. larval midgut, and indicated that Cry1Aa toxin specifically inhibited K^+/leucine symport in the midgut, the interaction between cotransporters and toxin was affected by the pH of the medium, and the K^+/leucine cotransporter or a strictly associated protein served as a membrane receptor for Cry1Aa delta-endotoxin in the *B. mori* larval midgut.

Denolf (1999) reviewed the information on molecular characterization of *B. thuringiensis* delta-endotoxin receptors in the insect midgut. He suggested that reduced binding to its receptor may be causative of delta-endotoxin resistance. In order to guarantee the future effectiveness of *B. thuringiensis* insecticidal crystal proteins (ICPs), several efforts were directed at characterization of the ICP-receptor interaction. Three different midgut epithelial membrane components involved in the binding of ICPs have been identified as aminopeptidase N molecules, cadherin-like proteins, and glycolipids.

2.3.1 Effect of pH on the toxicity of *B. thuringiensis*

Venugopal *et al.* (1992) used circular dichroism spectroscopy to examine the secondary structures of the protoxin and toxin molecules at different levels of pH to determine if there are detectable conformational changes associated with pH-dependent functional properties. At pH ten, where toxic activity is approximately maximal, both the protoxin and toxin molecules assumed a conformation approximately 26 per cent α-helix and 45 per cent β-structure. As the pH increased above ten, where the insecticidal activity decreases, the magnitude of circular dichroism decreased for protoxin and the α-helix contents of both protoxin and toxin molecules. The net secondary structure did not change significantly at pH values below ten. Significant conformational differences were observed between the secondary structure of protoxin and toxin molecules at different pH

values. The pH dependent changes in secondary structure of the protoxin and toxin can be correlated with the effects of pH on the insecticidal activity of these proteins.

The activity of the delta-endotoxins increases with an increase in pH from eight to ten and then declines at pH greater than ten. Increase in the pH of the formulation above 11 results in a decline in larval mortality. However, the spore viability was not affected adversely by pH upto 12.5 (Behle *et al.*, 1997).

Tran *et al.* (2001) examined the effect of pH (6.5 to 10.5) on the pore forming ability of two *B. thuringiensis* toxins, Cry1Ac and Cry1C, with midgut brush border membrane vesicles isolated from the tobacco hornworm *M. sexta,* and a light scattering assay. The results indicated that pores formed by Cry1Ac were significantly smaller at pH 6.5 than under alkaline conditions, whereas the pore forming ability of Cry1C decreases at pH > 8.5. The reduced activity of Cry1C at high pH correlates well with the fact that its toxicity for *M. sexta* is considerably weaker than that of Cry1Aa, Cry1Ab, and Cry1Ac and suggested that although pH can influence toxin activity, additional factors also modulate toxin potency in the insect midgut.

Reduction in food consumption of third-instar larvae of *Spodoptera litura* (Fab.) increased gradually both in pure culture and formulation of *Bt* when it was exposed to increasing pH from six to ten (Somasekhar and Krishnayya, 2004).

2.3.2 Effect of midgut juice on the conversion of protoxin to toxin

Pang and Gringorten (1998) reported that the amount of 60 to 65 kDa toxin protein produced when *B. thuringiensis* subsp. *kurstaki* (HD-1) and *sotto* δ-endotoxins were activated from their protoxins in 50 per cent *Choristoneura fumiferana* (Clem.) gut juice was much less than that produced in one per cent gut juice. In bioassays against

B. mori larvae, the toxicity of activation mixtures with 50 per cent *C. fumiferana* gut juice was less than the activation mixtures with one per cent gut juice, suggesting that *B. thuringiensis* subsp. *kurstaki* (HD-1) is more toxic to *B. mori* than to *C. fumiferana* larvae, indicating enhancement of *B. thuringiensis* δ-endotoxin insecticidal activity by protease inhibitors may be a result of reduced toxin degradation in the insect midgut.

Shao *et al.* (1998) reported that *B. mori* was more sensitive to the protoxins of *B. thuringiensis* subsp. *kurstaki* HD-1 than *H. armigera*. SDS-PAGE analysis showed that a large amount of activated toxin was obtained from protoxin by *B. mori* gut juice, while little yield was obtained by gut juice of *H. armigera*. Further degradation of activated toxin was observed in *H. armigera* midgut juice. Influence of pH on the proteolytic activity of midgut juice was significant, but there was no obvious effect of pH on degradation of activated toxin.

Lightwood *et al.* (2000) investigated the role of proteolytic processing in determining the potency of the *B. thuringiensis* Cry1Ac δ-endotoxin towards *Pieris brassicae* L. and *Memestra brassicae* (L.). Differential processing of membrane bound Cry1Ac was also observed in qualitative binding experiments performed with brush border membrane vesicles from the two insects, and in midguts isolated from toxin treated insects.

Rukmini *et al.* (2000) reported that conversion of δ-endotoxins of *B. thuringiensis* to active toxins is mediated by trypsin, insect midgut, and bacterial proteases, these proteases also play an important role in influencing host range of toxin, and in development of resistance to the toxin protein.

Miranda *et al.* (2001) analyzed the *in vitro* and *in vivo* activation of Cry1Ab, and observed considerable differences in the processing of Cry1Ab by *M. sexta* and *Spodoptera frugiperda* (J.E. Smith) midgut proteases as compared to trypsin. Pore formation assays showed that midgut juice produced a more active toxin than trypsin, and suggested that the cleavage in domain II may be involved in toxin inactivation, and that the 30 kDa fragments are stable intermediates in the degradation pathway.

Wagner and Schnetter (2002) investigated the successive *in vitro* degradation of the scarab-specific insecticidal Cry8C toxin of *B. thuringiensis japonensis* (BTJ) in the midgut juice of the BTJ-resistant European cockchafer, *Melolontha melolontha* L. In an initial step, the crystalline 130 kDa protoxin was dissolved in a chaotropic reducing environment and subsequently activated to a toxic 65 kDa fragment by endogenous proteinases of the bacterium. Proteolytic assays with partially purified midgut juice proteinases of *M. melolontha* revealed that mainly tyrpsin like enzymes are responsible for inactivation of Cry8C by degrading it to fragments less than 10 kDa.

2.3.3 Binding of toxins to the brush border membrane vesicles (BBMVs)

Hofmann *et al.* (1988a) examined the binding of ^{125}I-toxin to the brush border membrane vesicles (BBMVs) prepared from midgut epithelium of *P. brassicae* and small intestine of rat. The toxin bound specifically to insect vesicles. Vesicles of *P. brassicae* possess two individual binding sites for iodinated toxin with dissociation constants of 46 nM and 490 nM, the Hill coefficients of both sites were approximately one, and the binding capacities were 0.2 and 30 pmol mg^{-1} vesicle protein for the high and low-affinity sites, respectively.

Hofmann *et al.* (1988b) performed binding studies with two ^{125}I-labeled *B. thuringiensis* δ-endotoxins on BBMVs prepared from the larval midgut of the tobacco hornworm, *M. sexta* or the cabbage butterfly, *P. brassicae*. One Bt2-protoxin (130 kDa) from *B. thuringiensis* subsp. *berliner* binds saturably with high affinity to BBMV from the midgut of both species. Other δ-endotoxin Bt4412-protoxin form *B. thuringiensis* subsp. *thuringiensis*, which is highly toxic to *P. brassicae* but not for *M. sexta*, shows high affinity saturable binding to *P. brassicae* vesicles, but not to *M. sexta* vesicles.

Ferre *et al.* (1991) studied biochemical mechanisms of resistance to *B. thuringiensis* crystal proteins in a field population of diamondback moth, *Plutella xylostella* (L.) with a reduced susceptibility to the insecticidal sprays. The toxicity and binding characteristics of three crystal proteins (Cry1Ab, Cry1B, and Cry1C) were compared between the field population and a laboratory strain. The field population proved resistant (>200 fold) to Cry1Ab, one of the crystal proteins in the insecticidal formulation. Binding assays showed that the two strains differed in a membrane receptor that recognizes Cry1Ab, this crystal protein did not bind to the brush border membrane of the midgut epithelial cells of the field population, either because of strongly reduced binding affinity or because of complete absence of the receptor molecule. Both strains proved fully susceptible to the Cry1B and Cry1C crystal proteins, which were not present in the *B. thuringiensis* formulation used in the field.

Oddou *et al.* (1991) investigated the specificity of *B. thuringiensis* var. *kurstaki* strain HD-1 ICPs in ligand blot experiments with three activated Cry1A toxins, Cry1Aa and Cry1Ab toxins bind to the same 170 kDa protein, but at two different binding sites, whereas Cry1Ac binds two proteins of molecular masses 140 and 120 kDa.

Carroll and Ellar (1993) studied changes in the membrane permeability of *M. sexta* midgut BBMVs after addition of *B. thuringiensis* δ-endotoxin using osmotic swelling experiments, volume changes being monitored as the change in 900 light scattering. The permeability change was relatively non-selective with cations, anions, and neutral solutes, all traversing the membrane to an increased extent in the presence of Cry1Ac, whereas the Cry1B toxin had no effect on BBMV permeability.

Martin and Wolfersberger (1995) reported that *B. thuringiensis* δ-endotoxin and *M. sexta* larval midgut BBMVs act synergistically to cause very large increases in the conductance of planar lipid bilayers, at pH 9.6 the toxin dependent increase in bilayer conductance was 13 nS.

Aranda *et al.* (1996) conducted experiments on binding of different *B. thuringiensis* ICPs to the midgut epithelium of *S. frugiperda* with midgut tissue sections and isolated brush border membrane vesicles. Results show that ICPs interact with microvilli of epithelial cells of *S. frugiperda* in two different ways. The first is typical of highly toxic proteins such as Cry1Ac and Cry1D. Interaction was saturable and specific. Some nontoxic proteins such as Cry1Ab interacted non-specifically.

Fiuza *et al.* (1996) conducted experiment on binding of *B. thuringiensis* Cryl toxins (Cry1Aa, Cry1Ac, and Cry1Ba) to the midgut BBMVs of *Chilo suppressalis* (Walker). Direct binding experiments showed that Cry1Aa and Cry1Ba recognized a single class of binding sites with different affinities, whereas Cry1Aa recognized two classes of binding sites, one with a high affinity and low concentration, and the other with a lower affinity. Higher concentration and competition experiments showed that Cry1Ac

and Cry1Ba shared a binding site in the *C. suppressalis* midgut membrane, and that site was also the low affinity binding site for Cry1Aa.

Carroll *et al.* (1997) examined the membrane permeabilising activity of Cry1Ac using BBMVs prepared from the anterior and posterior sub-regions of the *M. sexta* midgut, and found that there were two mechanisms by which Cry1Ac permeabilises the *M. sexta* midgut membrane: a N- acetyl-D-galactosamine (GalNAc) sensitive mechanism restricted to the posterior midgut region, probably involving aminopeptidase N binding, and a previously undetected mechanism found in both the posterior and anterior regions.

Kwa *et al.* (1998) studied the effect of Cry1C on several insect culture cell lines by toxicity assays, ligand blotting, and toxin binding, and observed clear differences in sensitivity toward Cry1C between the insect cell lines. The *S. frugiperda* cell line Sf9 was most sensitive, the *Spodoptera exigua* (Hubner) cell lines SeUCR and SelZD2109 were moderately sensitive, whereas *M. brassicae* (Mb0503) and *Drosophila melanogaster* (Meigen) (Dm1) cells were least sensitive.

Liu *et al.* (2000) reported that binding assays with midgut BBMVs prepared from whole larvae of *P. xylostella* showed no significant differences between resistant and susceptible strains in binding of radioactively labeled Cry1C, and suggested that reduced conversion of Cry1C protoxin to toxin is a minor mechanism of resistance to Cry1C.

Hua *et al.* (2001) ascertained whether Cry1F, Cry9C, or Cry9E recognize the Cry1Ab binding site in *Ostrinia nubilalis* (Hubner) brush border by three approaches (an optical biosensor technology based on surface plasmon resonance, a radioligand approach and lligand blot analysis), and speculated that isoforms of aminopeptidase and cadherin in the brush border membrane serve as Cry1Ab, Cry1Ac, and Cry1F binding proteins.

Alcantara *et al.* (2004) examined receptor-binding steps in the molecular mode of action of five δ-endotoxins (Cry1Ab, Cry1Ac, Cry1C, Cry2A, and Cry9C) from *B. thuringiensis* to find toxins with different receptor sites in the midgut of stripped stem borer (SSB), *C. suppressalis*, and yellow stem borer (YSB), *Scirpophaga incertulas* (Walker). Homologous competition binding among the Cry toxins did not affect toxin binding affinity to both insect midgut BBMVs. Heterologous competition binding assays suggested that Cry1Ab and Cry1Ac compete for the same binding sites in SSB and YSB, whereas other toxins bind with weak (Cry1C, Cry2A) or no affinity (Cry9C) to Cry1Ab and Cry1Ac binding sites.

Liao *et al.* (2005) undertook a study of binding sites for some Cry proteins in the BBMV of *H. armigera* and *Helicoverpa punctigera* (Wallengren), the binding affinity for Cry1Ac was higher than Cry1Ab, matching their relative toxicities, and Cry1Ac and Cry1Ab were shared at least one binding site. However, Cry2Aa did not compete with Cry1Ac for binding.

CHAPTER-III

Materials & Methods

CHAPTER-III

MATERIALS AND METHODS

The laboratory and field studies were conducted at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2005-2007, to study the **"Interaction of acid exudates in chickpea with biological activity of Cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)"**. Materials utilized in conducting the experiments and various methods employed during the course of these investigations are given hereunder:

3.1 EFFECTIVENESS OF *Bacillus thuringiensis* AGAINST *Helicoverpa armigera* ON RESISTANT AND SUSCEPTIBLE GENOTYPES OF CHICKPEA

The effectiveness of *B. thuringiensis* against *H. armigera* was studied on four chickpea genotypes (ICC 506EB-resistant, ICCV 10-moderately resistant, C 235-moderately susceptible, and L 550–susceptible) under field conditions.

3.1.1 Lay out of the experiment

During 2005-06 post rainy season the selected chickpea genotypes *viz.*, L 550, C 235, ICCV 10, and ICC 506 were sown on 25th November (Plate 1), while the sowing was undertaken on 25th October (Plate 2) and 4th December (Plate 3), during the 2006-07 post rainy season. The experiment was laid out in a factorial design having four chickpea

Plate 1: Chickpea genotypes to study the interaction of *Bt* toxins with organic acids (ICRISAT, Patancheru, 2005-06post-rainy season)

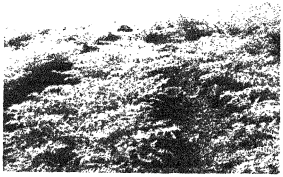


Plate 2: Chickpea genotypes to study the interaction of *Bt* toxins with organic acids (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting)

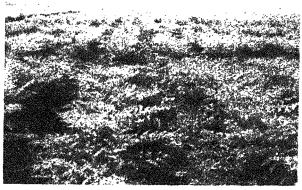


Plate 3: Chickpea genotypes to study the interaction of *Bt* toxins with organic acids (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting)

genotypes as the main treatments, and five concentrations of *Bt* formulation (Biolep®) as the sub treatments. There were three replications for each genotypes x *Bt* spray concentrations. Plot size was 2 x 2.4 m^2 (four rows of 2 m long, and planted at 60 x 10 cm, row-to-row and plant-to-plant spacing). The fertilizer (diammonium phosphate @ 100 kg ha^{-1}) was applied before sowing. The seeds were planted with a 4-lane planter, and the field was irrigated immediately after planting, and at monthly intervals thereafter. Normal agronomic practices were followed for raising the crop. There was no insecticide application in the experimental plots.

3.1.2 *Bt* sprays

Biolep®, a commercial formulation of *B. thuringiensis* var. *kurstaki* (strain z-52, serotype H-3a, 3b), was obtained from Biotech International Ltd, New Delhi, India. Four concentrations (0.05, 0.1, 0.2, and 0.5%) of the *Bt* formulation were prepared in water and the spraying was carried with a knapsack sprayer. Plots sprayed with water only served as a control. The sprays were applied in order of increasing concentrations of *Bt*.

First spray was given at 30 days after emergence (DAE), which represented the vegetative stage. Sprays at the flowering stage were applied twice, first spray at 55 DAE which covered the flowering stage of L 550, ICCV 10, and ICC 506, and second spray at 12 to 14 days later which covered the flowering stage of C 235. Spraying at the podding stage was also carried out twice, the first spray at 75 DAE, which cover the podding stage of L 550, ICCV 10, and ICC 506, and second spray at 12 days later, which covered podding stage of C 235.

3.1.3 Observations

Data were recorded on egg and larval numbers before and after spraying on five plants tagged at random in each treatment during the vegetative, flowering, and podding stages. Post-treatment counts were recorded at 24, 48, and 72 h after spraying. The pod borer, *H. armigera* damage to the pods was evaluated visually on 1 to 9 rating scale at maturity (1 = <10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, and 9 = >80% pods damaged) (Sharma *et al.*, 2005b). At harvest, data were also recorded on proportion of pods damaged by counting the total number of pods and pods damaged by *H. armigera* in five plants picked up at random from each plot.

Five plants selected at random were harvested individually and threshed to record the grain yield. Total grain yield was also recorded for each plot, and the grain yield of the five plants sampled from each plot was added to estimate net yield per plot. Grain yield (kg ha^{-1}) for each treatment combination was estimated from net plot yield.

3.1.4 Statistical analysis

The data were subjected to analysis of variance by using GENSTAT version 10.1. Data on egg and larval counts, damage rating, pod borer damage (%), and grain yield were analysed by factorial analysis. Duncan's new multiple range test (DMRT) was used to judge the significance of differences among the genotypes and *Bt* concentrations tested.

3.1.5 Evaluation of biological activity of *B. thuringiensis* towards *H. armigera* on different chickpea genotypes under laboratory conditions (detached leaf assay)

The plants grown in the field and sprayed with *Bt* (as described above) were also bioassayed under controlled conditions in the laboratory [27 ± 2^0 C, 65 to 75% RH and a photoperiod of 12: 12 h (L: D)] using detached leaf assay (Sharma *et al.*, 2005a). Plastic cups of 4.5 x 11.5 cm diameter were used for detached leaf assay. Agar-agar (3 %) was boiled and poured into the plastic cups kept in a slanting manner. Nearly 10 ml of agar-agar was poured into each cup. The solidified agar-agar served as a substratum for holding a chickpea terminal branch with 3 to 4 fully expanded leaves and a terminal bud in a slanting manner (Plate 4). Care was taken to see that the chickpea branches did not touch the inner walls of the cup. Ten neonate *H. armigera* larvae were released on the chickpea leaves in each cup, and then covered with a lid immediately. This system kept the chickpea terminals in turgid condition for one week.

The experiment was conducted in a completely randomized design (CRD), and there were five replications. The experiment was terminated when more than 80 per cent of the leaf area was consumed in the susceptible control or when there were maximum differences between the resistant and susceptible checks (generally 5 to 6 days after releasing the larvae on the leaves). Detached leaf bioassay was conducted with both *Bt* sprayed (Plate 5) and unsprayed plants at the vegetative and flowering stages. The data were recorded on leaf damage score, larval survival, and mean larval weight.

Leaf feeding by *H. armigera* larvae was evaluated visually on a 1 to 9 scale (1= <10%, and 9= >80% leaf area damaged). The number of larvae that survived after the feeding period was recorded, and the larvae were then placed in 25 ml plastic cups

individually. The weights of larvae were recorded at 4 h after separating them from the food. The data were expressed as percentage of larval survival and mean weight of the larvae in each treatment.

Data on percentage of larval survival, mean larval weight, and leaf damage rating were subjected using factorial design using GENSTAT version 10.1. The significance of differences between the treatments was judged by F-test, while the treatment means were compared by the least significant difference at $P \leq 0.05$.

3.2 INTERACTION OF CHICKPEA GENOTYPES WITH BIOLOGICAL ACTIVITY OF *Bt* TOXINS AGAINST *H. armigera*

3.2.1 Insect culture

Larvae of *H. armigera* used in bioassays were obtained from a laboratory culture maintained at ICRISAT, Patancheru, Andhra Pradesh, India. Larvae were reared on chickpea based artificial diet (Armes *et al.*, 1992) at 27^0 C. The laboratory culture was supplemented with field-collected population every six months to maintain the heterogeneity of the laboratory culture. Field collected larvae of *H. armigera* were reared in the laboratory on the natural host for one generation before being introgressed into the laboratory culture to avoid contamination with the nuclear polyhedrosis virus, bacteria, or fungi. The *H. armigera* neonates were reared in groups of 200 to 250 in 200 ml plastic cups (having 2 to 3 mm layer of artificial diet on the bottom and sides) for five days. After five days, the larvae were transferred individually to six cell well plates (each cell well 3-5 cm in diameter, 2 cm in depth) to avoid cannibalism. Each cell well had sufficient amount of diet (7 ml) to support larval development until pupation. The pupae were removed from cell wells, sterilized with 2 per cent sodium hypochlorite solution,

and kept in groups of 50 in plastic jars containing vermiculite. Upon emergence, ten pairs of adults were released inside an oviposition cage (30 x 30 x 30 cm). Adults were provided with 10 per cent sucrose or honey solution on a cotton swab for feeding. Diaper liners, which have a rough surface, were hung inside the cage as an oviposition substrate. The liners were removed daily and the eggs sterilized in 2 per cent sodium hypochlorite solution. The liners were dried under fan and then placed inside the plastic cups with artificial diet. After egg hatching, the larvae moved to the artificial diet, and the liners were removed after 4 days. Neonate larvae were used for bioassays using diet impregnation assay experiments (Sharma *et al.*, 2005a).

Artificial diet for rearing *H. armigera* and for bioassay was prepared as follows:

1. Mix measured quantities (Table 1) of chickpea flour, ascorbic acid, sorbic acid, methyl-4-hydroxy benzoate, aureomycin, yeast in a bowl, add 112.5 ml of warm water, and mix thoroughly using a blender.
2. Add 1.0 ml of formaldehyde and 2.5 ml of vitamin stock solution (Table 2) and mix well.
3. Add 4.325 g of agar-agar to 200 ml of water in a separate container and boil for 5 min.
4. Add the agar-agar solution to the other diet ingredients, and mix thoroughly in a blender to get an even consistency.
5. Pour the diet into small plastic cups and allow to cool under a laminar flow for 1 to 2 h.
6. Around 300 ml of diet was used to rear 30-neonate larvae upto pupation.

Table 1: Composition of artificial diet for *H. armigera* larvae

Ingrediants	**Quantity**
Chickpea flour (g)	75
L-ascorbic acid (g)	1.175
Sorbic acid (g)	0.75
Methyl-4-hydroxy benzoate (g)	1.25
Aureomycin (g)	2.875
Yeast (g)	12
Formaldehyde (40%) (ml)	1.0
Vitamin stock solution (ml)	2.5
Water (ml)	112.5
Agar-agar solution	
Agar-agar (g)	4.325
Water (ml)	200

Table 2: Composition of vitamin stock solution (500 ml)

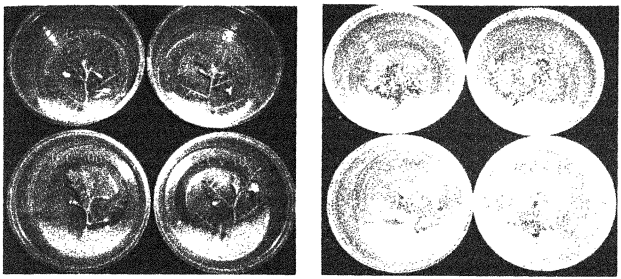
Ingrediants	**Quantity**
Nicotinic acid (g)	1.528
Calcium pantothenate (g)	1.528
Riboflavin (g)	0.764
Aneurine hydrochloride (g)	0.382
Pyridoxine hydrochloride (g)	0.382
Folic acid (g)	0.382
D-Biotin (g)	0.305
Cyano cobalamine (g)	0.003
Water (ml)	500

3.2.2 Survival and development of *H. armigera* on different chickpea genotypes

Neonate larvae of *H. armigera* were fed on leaves of four genotypes (L 550, C 235, ICCV 10, and ICC 506EB) for seven days, and then on pods upto pupation. Larvae were fed on fresh leaves individually in plastic cups (11 cm diameter and 13 cm in height) at 27^0 C under photoperiod of 12: 12 h (L: D) using the detached leaf assay. The food was changed daily. Larval weights were recorded on tenth day after release of larvae, while the pupal weights were recorded one day after pupation. The experiment was conducted in a CRD, and there were three replications for each treatment. Data were recorded on larval and pupal periods, pupation and adult emergence (%), adult longevity (male and female), and fecundity. Three pairs of adults were released in a cage to record the data on fecundity (Plate 6).

Survival and development of larvae was also studied on different chickpea genotypes sprayed with *Bt* (0.05, 0.1, 0.2, and 0.5%). The larvae were reared upto pupation on *Bt* sprayed leaves. Food was changed every three days, and each time, fresh *Bt* sprayed leaves were given as a food. Data were recorded on larval and pupal weights, larval and pupal periods, pupation and adult emergence (%), adult longevity, and fecundity.

The data were subjected to analysis of variance by using GENSTAT version 10.1 using factorial design. The significance of differences between the treatments was measured by F-test, whereas, the treatment means were compared by using the LSD at $P \leq 0.05$.



Before feeding

After feeding

Plate 4: Detached leaf assay for unsprayed chickpea genotypes

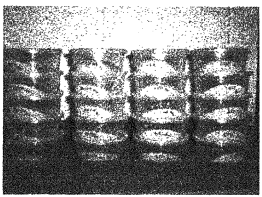


Plate 5: Detached leaf assay for sprayed chickpea genotypes

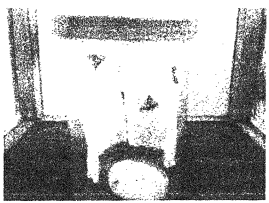


Plate 6: Adults released into jar for egg laying

Data on survival and development of larvae on sprayed chickpea genotypes were subjected to factorial analysis, and the treatment means compared by DMRT to know the significance of differences among the treatments.

3.2.3 Survival and development of *H. armigera* on artificial diet with lyophilized leaf and pod powder of different chickpea genotypes and *Bt* toxins

To study the interaction of acid exudates of chickpea with the biological activity of *Bt* toxins against *H. armigera*, freeze-dried powder of leaves and pods of chickpea genotypes were incorporated into the artificial diet along with *Bt*.

Chickpea branches with tender green leaves and tender green pods with developing seeds of four genotypes of chickpea (L 550, C 235, ICCV 10, and ICC 506EB) were collected from pesticide-free plots. The leaves and pods were frozen at -20^0 C and lyophilized (Plate 7). The lyophilized leaves and pods were powdered in a blender to obtain a fine powder (<80 μm).

To study the interaction between chickpea genotypes and *Bt* toxins, lyophilized leaf and pod powder of four genotypes were incorporated into the artificial diet (Table 3) along with *Bt* at ED_{50}. There were three replications for each genotype, and 10 neonates were released on the artificial diet. The larvae were reared individually in six cell well plates, and kept at 27^0 C. Data were recorded on larval and pupal weights, larval and pupal periods, pupation and adult emergence (%), adult longevity, and fecundity.

Data were subjected to analysis of variance by using GENSTAT version 10.1. Data were analysed by factorial analysis. The treatment means were compared by DMRT to know the significance of differences among the genotypes, and the *Bt* treatments tested.

Table 3: Composition of artificial diet for *H. armigera* larvae having lyophilized leaf / pod powder

Ingrediants	**Quantity**
Chickpea flour (g)	55
Lyophilized leaf/ pod powder (g)	20
L-ascorbic acid (g)	1.175
Sorbic acid (g)	0.75
Methyl-4-hydroxy benzoate (g)	1.25
Aureomycin (g)	2.875
Yeast (g)	12
Formaldehyde (40%) (ml)	1.0
Vitamin stock solution (ml)	2.5
Water (ml)	112.5
Agar-agar solution	
Agar-agar (g)	4.325
Water (ml)	200

3.2.4 Survival and development of *H. armigera* on artificial diet with organic acids and *Bt* toxins

The interaction of organic acids present in the leaf exudates of chickpea with the biological activity of *Bt* toxins (at ED_{50} level) was studied by incorporating the organic acids (oxalic and malic acids) into the artificial diet at levels estimated through HPLC. The experiment was conducted in a CRD with three replications, and there were 10 neonate larvae per replication. The neonate larvae were reared individually in six cell well plates at 27^0 C. Data were recorded on larval and pupal weights, larval and pupal periods, pupation and adult emergence (%), adult longevity, and fecundity. Data were statistically analysed as described above.

3.2.5 Estimation of organic acids in chickpea leaf exudates of different chickpea genotypes

A standard protocol for collection and analysis of organic acids from chickpea leaf exudates was followed, with a slight modification of the method used by Yoshida *et al.* (1997).

Standards: Oxalic acid, malic acid, fumaric acid, and citric acid.

Reagents: Potassium phosphate (KH_2PO_4), phosphoric acid (H_3PO_4), and millipore water.

Materials: Forceps, scissors, pipette, centrifuge tubes, and HPLC (Plate 8).

Procedure

Preparation of standards and sample collection

Two replicates of each standard organic acid were prepared by mixing 2 to 10 mg of standard organic acid in 10 ml of water to get concentrations of 200 to 1000 ppm. The

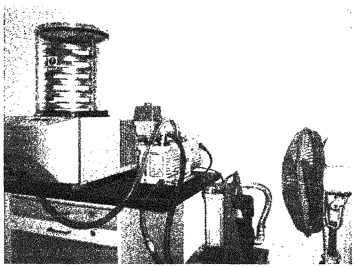


Plate 7: Lyophilizer for freeze-drying of leaf and/or pod samples

Plate 8: High performance liquid chromatography (HPLC) for estimation of organic acids

chickpea leaf samples were collected early in the morning (before 9 am) in 25 ml centrifuge tubes containing 5 ml double distilled water/millipore water. The tubes were labelled for each genotypes, and weight of the tube and water was recorded (initial weight). First fully expanded leaf from three plants was excised with scissors and placed in the respective tubes containing double distilled millipore water for 10 to 15 min. The weight of tube with water and the leaves was recorded (final weight). Based on the initial and final weights, the fresh weights of the leaves were recorded. After extraction of the exudates, the leaves were removed from the tubes and placed on a filter paper for 1 h to remove the excess water. Later, the leaf area was measured using a leaf area meter. The dry weight of the leaves was recorded by placing the leaf samples in an oven at 45^0 C for three days.

The leaf exudates extracted in water were filtered through 45 µm hydrophilic PVDF millipore millex-HV filters using a 5 ml luer lock syringes. Approximately 3 ml sample solution was taken in 5 ml luer lock syringe from the centrifuge tubes. The needle was removed from the syringe and attached to millipore filter to dispense 1.5 ml of the filtrate into the HPLC vials. There were three replicates for each sample.

Quantification of organic acids in leaf exudates of chickpea by high performance liquid chromatography (HPLC)

For preparing 2 L of 25 mM KH_2PO_4 of pH 2.5 with H_3PO_4, weighed 6.805 g of KH_2PO_4 in a 2 L conical flask and mixed it with 1 L of millipore water until KH_2PO_4 was completely dissolved. Then added 4 ml of H_3PO_4 and made up the volume to 1.8 L, adjusted the pH to 2.5 by adding drop-by-drop H_3PO_4, and finally made up the volume to 2 L.

After priming, the mobile phase was run for 1 h. The vials containing leaf exudates of different chickpea genotypes were arranged in a carousel. Analysis was carried out by using Atlantis dC-18 column (4.6 x 250 mm, 5 µm). Chromatographic separation was done using mobile phase with a flow rate 0.8 ml min^{-1}, and the injected volume was 20 µl with 20 min run time per sample.

Based on the standards, retention time and peak area of different organic acids present in the samples were identified and quantified. From the known concentrations of the standards, linear curve was plotted against concentration on X-axis and absorbance on Y-axis. From the linearity curve, unknown concentrations of different organic acids from the samples were plotted and the amounts estimated. Amounts of organic acids present in a sample were expressed in mg g^{-1} fresh or dry weight or µg cm^{-2} leaf area.

Estimation of *Bt* toxin proteins

Shao *et al.* (1998) method was slightly modified to prepare protoxin from the commercial *Bt* formulation.

- Ten grams (2.5 x 4) of commercial *Bt* formulation (Biolep) was taken into centrifuge tubes and washed with one molar NaCl (10 ml each time), and centrifuged at 4000 rpm for 5 min.
- Then washed twice with deionised water, and centrifuged at 4000 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.
- Centrifuged the contents at 4000 rpm for 20 min, supernatant collected and pH adjusted to 4.4 with 2 M acetic acid.

- Then centrifuged the contents at 4000 rpm for 20 min. Protoxin precipitate was collected and dialyzed against water (dialyzed overnight, and water changed 3 times).

Amount of protein present in the precipitate was estimated by Lowry *et al.* (1951) method as described below.

Reagents

1. **Solution A:** Two per cent sodium carbonate (anhydrous) in 0.1 N NaOH.
2. **Solution B:** 0.5 per cent copper sulphate ($CuSO_4$. $5H_2O$) in 1 per cent sodium potassium tartrate (freshly prepared).
3. **Solution C (alkaline copper solution):** Mix 50 ml of solution A with 1 ml of solution B, prior to use.
4. **Folin-Ciocalteu Reagent (FCR):** Dilute the commercial reagent (2 N) with an equal volume of water on the day of use.
5. **Stock standard protein solution:** Dissolve 50 mg of BSA in 50 ml of water.
6. **Working standard solution:** Dilute 10 ml of the stock solution to 50 ml with water to obtain protein at a concentration of 200 $\mu g\ ml^{-1}$.

Procedure

1. Pipette out 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the working standard solution into a series of test tubes.
2. Pipette out 0.1 and 0.2 ml of the sample extract into two other test tubes.
3. Make up the volume to 1.0 ml with distilled water in all the tubes. The tube with 1.0 ml of distilled water served as a blank.
4. Add 5 ml of solution C, mix well, and incubate at room temperature for 10 min.

5. Add 0.5 ml of FCR, mix well immediately, and incubate at room temperature in dark for 30 min.
6. Read the absorbance at 660 nm against the blank.

Draw a standard graph, calculate the amount of protein in the sample, and express the results as mg g^{-1} or mg 100^{-1} g sample or percentage.

3.3 EFFECT OF ACID EXUDATES ON PROTOXIN-TOXIN CONVERSION AND BINDING TO THE BRUSH BORDER MEMBRANE OF THE GUT OF *H. armigera*

3.3.1 Effect of acid exudates on *Bt* protoxin - toxin conversion

Acid exudates present in the chickpea genotypes estimated through HPLC, were mixed into the artificial diet along with Cry toxin and fed to third-instar larvae of *H. armigera*. Sample of food offered for larvae at the time of larval release was also collected to estimate the amounts of Cry toxins in the diet. After three days of feeding, faecal matter and larvae were collected, and midguts were taken out by dissecting the larvae (Plate 9) and stored in deep freeze at -20^{0}C. The amounts of toxin present in the food, larval midguts, and faecal matter were estimated through ELISA.

3.3.1.1 Enzyme linked immunosorbent assay (ELISA)

Materials used for ELISA were:

Antibody coated microtiter plates, peroxidase enzyme conjugate, TMB substrate, PBST wash buffer, and 3 M sulfuric acid (stop solution).

To carryout the ELISA test, we also used a blender, air tight container, paper towels, distilled water, micropipettes, and sterile micropipette tips.

PBST wash buffer consisted of sodium chloride (8.0 g), sodium phosphate, dibasic (anhydrous) (1.15g), potassium phosphate, monobasic (anhydrous) (0.2 g), potassium chloride (0.2 g), Tween-20 (0.5 g), and distilled water (1000 ml). All the above were dissolved in distilled water and pH was adjusted to 7.4.

Procedure

1. Added 100 µl of enzyme conjugate per well.
2. Following loading added 100 µl of each sample into the appropriate test wells of the ELISA plate.
3. Added 100 µl of each positive and negative controls, into the appropriate test well.
4. Placed the plate inside a humid box and incubated for two hours at room temperature or overnight in the refrigerator (4^0C).
5. When the incubation with the sample was complete, washed the plate (while squeezing the long sides of the frame to hold the strips in place). Used a quick flipping motion to empty the contents of the wells into a sink or waste container.
6. Filled all the wells to overflowing with 1x PBST wash buffer, then quickly emptied them, and again repeated the process six to seven times.
7. Filled each well with 1x PBST wash buffer, and allowed to sit for at least one min.
8. Emptied the wells with a quick flipping motion, and then held the frame upside down and tapped firmly on a folded paper towel to remove the remaining drops of buffer from the wells.

9. Added 100 µl of the TMB substrate solution into each well of the plate. Set plate aside and waited for color development.
10. Color developed between 5 to 15 min. Measured the optical density of the test wells on a plate reader at 650 nm.
11. Wells in which a blue color developed indicated positive results, and the ones that remained clear or had very light blue indicated negative results.
12. At the end of 15 min incubation with TMB substrate, added 50 µl of 3 M sulfuric acid to each test well.
13. Measured the optical density of the test wells on a plate reader at 450 nm.

3.3.2 Effect of acid exudates on binding of *Bt* toxins to brush border membrane vesicles of the midgut of *H. armigera* larvae

To study the effect of acid exudates on binding of Cry toxins to the brush border membrane vesicles, acid exudates (estimated through HPLC) and Cry toxin were mixed in the artificial diet and fed to third-instar larvae of *H. armigera*. After three days of feeding, the larval midguts were isolated from the larvae, and brush border membrane vesicles (BBMV) were prepared and examined for the amount of Cry toxin binding to BBMV.

Preparation of BBMV

BBMV were prepared according to the protocol used by Wolfersberger *et al.* (1987).

Reagents:

Magnesium Chloride ($MgCl_2$) – 24 mM

MET-Buffer (pH 7.5): Mannitol (0.3 mM), EGTA (5 mM), and Tris HCl (17 mM)

Procedure

1. Larvae were chilled on ice for 15 min, dissected, and midguts gently pulled out.
2. Each midgut was opened by a longitudinal cut and rinsed free of peritrophic membrane and gut contents, using an ice cold MET-buffer.
3. The isolated midguts were blotted and weighed.
4. Midguts were placed either in a vial with a small amount of MET-buffer (Plate 10), frozen quickly by immersing the vial in liquid nitrogen and stored at -80^0C or used immediately for BBMV preparation.
5. Placed the isolated midguts in an electric blender with nine times their weight of ice cold MET-buffer.
6. Blended the mixture for two one-minute periods at medium speed (setting 5) separated by one minute cooling interval (alternately the homogenate was prepared using a glass and Teflon homogenizer, nine strokes at 3000 rpm).
7. Added an equal volume of cold 24 mM $MgCl_2$ to midgut homogenate, blended the resulting mixture thoroughly and allowed to stand on ice for 15 min.
8. Centrifuged the contents at 4,500 rpm (approx. $2,500g^1$) for 15 min at 4^0C, the supernatant transferred to another tube, and centrifuged at 16,000 rpm (approx. $30,000g^1$) for 30 min at 4^0C.
9. The pellet was suspended in 0.5x homogenate volume of ice cold MET- buffer treated with 24 mM $MgCl_2$, and centrifuged at 4,500 rpm, and the supernatant again centrifuged at 16,000 rpm.

10. The final pellet constituted the BBMV (Plate 11). The pellet was suspended in cold half strength MET-buffer and distributed in 50 to 200 µl aliquots, which were immediately frozen and stored at -80^0 C until use.

The concentration of protein in the brush border membrane vesicles preparations was estimated by using Lowry *et al.* (1951) method.

Estimation of protein in 96 well plates by Lowry's method

Reagents

1. **Solution A:** Two per cent sodium carbonate (anhydrous) in 0.1 N NaOH
2. **Solution B:** 0.5 per cent copper sulphate ($CuSO_4$. $5H_2O$) in one per cent sodium potassium tartrate (freshly prepared)
3. **Solution C (alkaline copper solution):** Mix 50 ml of solution A with 1 ml of solution B prior to use.
4. **Folin-Ciocalteu Reagent (FCR):** Dilute the commercial reagent (2 N) with an equal volume of water on the day of use.
5. **Stock standard protein solution:** Dissolve 50 mg of BSA in 50 ml of water.
6. **Working standard solution:** Dilute 10 ml of the stock solution to 50 ml with water to obtain protein at a concentration of 200 µg ml^{-1}.

Procedure

1. Pipetted out 0.0, 10.0, 18.4, 27.6, 36.0, and 46.0 µl of the working standard solution into series of wells.
2. Made up the volume to 46.0 µl with distilled water in all the wells. A well with 46.0 µl of water served as a blank.
3. Pipetted out 46.0 µl of the sample extract into the other wells.

Plate 9: Collection of midgut from the larvae (for ELISA test)

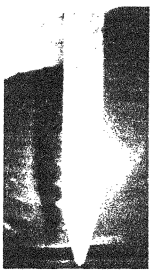


Plate 10: Midguts collected in MET-buffer for BBMV preparation

Plate 11: BBMV precipitated at the bottom (in the circle) of the tube

4. Added 231.0 µl of solution C, mixed well and incubated at room temperature for 10 min. Added 23.0 µl of FCR, mixed well immediately and incubated at room temperature in dark for 30 min.
5. Read the absorbance at 630 nm against the blank using micro-plate reader.

CHAPTER-IV

Results

CHAPTER – IV

RESULTS

The result of the present investigations on **"Interaction of acid exudates in chickpea with biological activity of Cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)"** are presented hereunder. The experiments were conducted in the field and laboratory at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2005-07.

4.1 EFFECTIVENESS OF *B. thuringiensis* AGAINST *H. armigera* ON RESISTANT AND SUCEPTIBLE GENOTYPES OF CHICKPEA

The effect of *Bt* sprays against *H. armigera* on resistant and susceptible genotypes of chickpea was studied under field conditions at ICRISAT, Patancheru, Andhra Pradesh, during 2005-06 and 2006-07 post-rainy seasons.

4.1.1 Egg and larval numbers

4.1.1.1 Vegetative stage

During the 2005-06 post-rainy season, there were no significant differences in egg numbers before spraying between the genotypes tested. Numerically low oviposition was recorded on ICC 506 as compared to that on L 550 and C 235 (Table 4). At 24 h after spray, the interaction effect between the genotypes × *Bt* concentrations was non-

Table 4: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Eggs 5^{-1}plants before spray									
C 235	22.3	29.3	13.7	17.3	20.7	**20.7**	Concentration	0.295	2.73	NS
ICC 506	13.7	12.0	14.0	9.3	19.3	**13.7**	Genotype	0.236	2.44	NS
ICCV 10	24.7	20.0	21.7	18.7	8.7	**18.7**	Concentration	0.405	5.46	NS
L 550	29.3	15.3	19.0	18.0	10.0	**18.3**	× Genotype			
Mean	**22.5**	**19.2**	**17.1**	**15.8**	**14.7**					
	Eggs 5^{-1}plants at 24 h after spray									
C 235	8.7	14.0	18.7	13.3	14.7	**13.9**^{c}	Concentration	0.627	1.43	NS
ICC 506	7.7	9.3	6.0	10.3	11.7	**9.0**^{ab}	Genotype	0.006	1.28	3.66*
ICC V10	3.7	5.3	8.3	10.0	9.0	**7.3**^{ab}	Concentration	0.401	2.85	NS
L 550	14.3	9.7	7.3	9.0	11.3	**10.3**^{bc}	× Genotype			
Mean	**8.6**	**9.6**	**10.1**	**10.7**	**11.7**					
	Eggs 5^{-1}plants at 72 h after spray									
C 235	29.0^{d}	14.0^{b}	9.3^{ab}	8.3^{ab}	10.3^{abc}	**14.2**	Concentration	<0.001	1.59	4.56*
ICC 506	6.7^{ab}	5.7^{ab}	5.0^{ab}	3.0^{a}	5.3^{ab}	**5.1**	Genotype	<0.001	1.43	4.08*
ICCV 10	19.3^{c}	4.3^{a}	6.7^{ab}	4.3^{a}	5.3^{ab}	**8.0**	Concentration	0.039	3.19	9.13*
L 550	31.0^{d}	6.0^{ab}	7.3^{ab}	4.0^{a}	3.7^{a}	**10.4**	× Genotype			
Mean	**21.5**	**7.5**	**7.1**	**4.9**	**6.2**					

*LSD at P≤ 0.05 %.

significant, but the differences between the genotypes were significant. Fewer eggs were recorded on ICCV 10 and ICC 506 as compared to those on C 235 and L 550. At 72 h after spray, there were significant differences among the genotypes and *Bt* concentrations tested. The interaction effects were also significant. In general, the egg counts were significantly lower in plots of ICC 506 and ICCV 10 across *Bt* concentrations. The *Bt* sprayed plots had lower egg counts than those on the unsprayed plots of different chickpea genotypes.

There were no significant differences in larval numbers before spraying between the genotypes tested. Numerically lower numbers of larvae were recorded on ICCV 10 and ICC 506 as compared to that on L 550 and C 235 (Table 5). At 24 h after spray, the interaction effects between the genotypes × *Bt* concentrations were non-significant, but the differences between *Bt* concentrations were significant. Fewer larvae were recorded in plots sprayed with 0.5 per cent *Bt* as compared to the unsprayed plots of different chickpea genotypes. At 72 h after spray, the interaction effects were significant. Lower larval density was recorded in plots sprayed with *Bt* as compared to the unsprayed plots of different chickpea genotypes. The larval numbers were significantly lower in plots of ICC 506 and ICCV 10 across *Bt* concentrations than on the other two genotypes tested.

During the 2006-07 post-rainy season October planting, there were no significant differences in egg numbers before spraying, and at 24 and 48 h after spraying between the genotypes. Low oviposition was recorded on ICC 506 as compared to that on ICCV 10, L 550, and C 235 (Table 6). At 72 h after spraying, the interaction effects between the genotypes × *Bt* concentrations, and the differences between *Bt* concentrations were significant. The egg counts were significantly lower on ICC 506

Table 5: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Larvae 5^{-1} plants before spray									
C 235	5.3	9.3	14.3	15.0	14.3	**11.7**	Concentration	0.584	1.59	NS
ICC 506	5.0	7.0	7.0	12.7	12.0	**8.7**	Genotype	0.114	1.42	NS
ICCV 10	10.0	6.3	4.0	8.3	5.3	**6.8**	Concentration	0.435	3.18	NS
L 550	9.7	11.7	12.7	8.0	8.7	**10.1**	× Genotype			
Mean	**7.5**	**8.6**	**9.5**	**11.0**	**10.1**					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	12.0	3.0	5.3	9.3	5.0	**6.9**	Concentration	0.003	1.00	2.87*
ICC 506	6.7	6.7	4.0	4.7	2.7	**4.9**	Genotype	0.238	0.90	NS
ICCV 10	10.7	9.3	2.0	8.0	4.0	**6.8**	Concentration	0.306	2.00	NS
L 550	10.0	7.3	8.7	6.0	5.0	**7.4**	× Genotype			
Mean	**9.8^{b}**	**6.6^{a}**	**5.0^{a}**	**7.0^{ab}**	**4.2^{a}**					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	27.0^{g}	6.3^{cd}	3.7^{abcd}	2.0^{abc}	0.0^{a}	**7.8**	Concentration	<0.001	0.86	2.46*
ICC 506	12.0^{e}	5.7^{bcd}	3.7^{abcd}	0.7^{ab}	0.0^{a}	**4.4**	Genotype	<0.001	0.77	2.20*
ICCV 10	18.0^{f}	7.0^{d}	4.0^{abcd}	0.7^{ab}	0.0^{a}	**5.9**	Concentration	<0.001	1.72	4.92*
L 550	37.3^{h}	8.3^{de}	5.3^{bcd}	1.0^{ab}	0.0^{a}	**10.4**	× Genotype			
Mean	**23.6**	**6.8**	**4.2**	**1.1**	**0.0**					

*LSD at P≤ 0.05 %.

Table 6: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Eggs 5^{-1} plants before spray									
C 235	0.3	1.3	3.3	2.7	2.0	**1.9**	Concentration	0.264	0.49	NS
ICC 506	0.0	1.0	0.0	2.3	0.0	**0.7**	Genotype	0.162	0.44	NS
ICCV 10	0.3	1.0	0.3	0.7	1.3	**0.7**	Concentration	0.836	0.98	NS
L 550	0.0	1.0	0.7	1.3	1.7	**0.9**	× Genotype			
Mean	**0.2**	**1.1**	**1.1**	**1.7**	**1.2**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	1.0	2.3	1.0	1.3	0.0	**1.1**	Concentration	0.943	0.32	NS
ICC 506	0.0	0.0	0.0	0.3	0.3	**0.1**	Genotype	0.075	0.29	NS
ICCV 10	0.0	0.0	0.7	0.7	0.0	**0.3**	Concentration	0.547	0.64	NS
L 550	0.0	0.0	0.3	0.0	1.3	**0.3**	× Genotype			
Mean	**0.2**	**0.6**	**0.5**	**0.6**	**0.4**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.3	0.7	0.0	0.7	0.0	**0.3**	Concentration	0.258	0.29	NS
ICC 506	0.0	0.7	0.0	0.3	0.0	**0.2**	Genotype	0.527	0.26	NS
ICCV 10	0.0	0.0	1.7	1.7	0.3	**0.7**	Concentration	0.691	0.58	NS
L 550	0.3	0.0	0.0	1.3	0.3	**0.4**	× Genotype			
Mean	**0.2**	**0.3**	**0.4**	**1.0**	**0.2**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}	**0.4**	Concentration	0.032	0.36	1.02*
ICC 506	0.0^{a}	0.0^{a}	0.0^{a}	0.7^{ab}	0.0^{a}	**0.1**	Genotype	0.313	0.32	NS
ICCV 10	1.0^{ab}	0.0^{a}	0.0^{a}	2.7^{bc}	0.0^{a}	**0.7**	Concentration	0.046	0.71	2.05*
L 550	0.7^{ab}	0.0^{a}	0.0^{a}	0.3^{a}	3.7^{c}	**0.9**	×Genotype			
Mean	**0.4**	**0.0**	**0.0**	**0.9**	**1.42**					

*LSD at P≤ 0.05 %.

across *Bt* concentrations than on the other genotypes tested. The differences in larval numbers before spraying between the genotypes and *Bt* concentrations were significant. Fewer larvae were recorded on ICC 506 as compared to that on other genotypes. Larval counts were significantly lower in *Bt* sprayed plots as compared to the unsprayed plots (Table 7). At 24 and 48 h after spraying, the interaction effects were non-significant, but the differences between the genotypes and *Bt* concentrations were significant. Larval numbers were lower on ICC 506 as compared to other genotypes. At 72 h after spraying, the interaction effects were non-significant, but the differences between *Bt* concentrations were significant. The *Bt* sprayed plots had fewer larvae than those in the unsprayed plots of different chickpea genotypes.

During the 2006-07 post-rainy season December planting, there were no significant differences between the genotypes in egg numbers before spraying, and at 24, 48, and 72 h after spraying (Table 8). The differences in larval numbers between the genotypes and *Bt* concentrations were significant (Table 9). Fewer larvae were recorded on ICC 506. Larval abundance was lower in plots sprayed with *Bt* as compared to the unsprayed plots of different chickpea genotypes. At 24 h after spray, there were no significant interaction effects, but the differences between the genotypes as well as between *Bt* concentrations were significant. Larval numbers were lower on ICC 506 and in plots sprayed with *Bt* as compared to the unsprayed plots. At 48 h after spray, there were significant differences across the genotypes and *Bt* concentrations. The interaction effects were also significant. In general, the larval numbers were significantly lower in plots of ICC 506 and ICCV 10 across *Bt* concentrations. At 72 h after spray, there were significant differences across the genotypes and *Bt* concentrations. The interaction effects

Table 7: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Larvae 5^{-1} plants before spray									
C 235	8.0	7.7	7.3	8.0	7.3	7.7^{ab}	Concentration	0.038	0.73	2.08*
ICC 506	5.0	9.3	8.0	5.3	4.3	6.4^{a}	Genotype	<0.001	0.65	1.86*
ICCV 10	9.3	12.7	6.3	6.7	6.3	8.3^{b}	Concentration			
L 550	11.3	10.7	10.3	11.0	9.0	10.5^{c}	× Genotype	0.354	1.45	NS
Mean	8.4^{ab}	10.1^{b}	8.0^{ab}	7.7^{a}	6.7^{a}					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	9.0	5.0	7.0	7.7	4.7	6.7^{b}	Concentration	0.010	0.74	2.12*
ICC 506	4.3	6.3	5.7	5.7	1.7	4.7^{a}	Genotype	0.002	0.66	1.89*
ICCV 10	7.3	10.7	4.7	5.0	4.7	6.5^{ab}	Concentration	0.334	1.48	NS
L 550	10.0	10.7	8.0	8.3	6.7	8.7^{c}	× Genotype			
Mean	7.7^{b}	8.2^{b}	6.3^{ab}	6.7^{b}	4.4^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	6.3	5.7	6.0	3.7	1.7	4.7^{a}	Concentration	<0.001	0.46	1.32*
ICC 506	3.7	6.0	4.7	3.0	1.3	3.7^{a}	Genotype	<0.001	0.41	1.18*
ICCV 10	6.7	6.7	3.7	2.3	3.0	4.5^{a}	Concentration	0.078	0.92	NS
L 550	9.7	9.7	6.0	4.3	1.7	6.3^{b}	× Genotype			
Mean	6.6^{d}	7.0^{d}	5.1^{c}	3.3^{b}	1.9^{a}					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	3.7	5.0	7.7	2.0	1.3	**3.9**	Concentration	<0.001	0.63	1.80*
ICC 506	4.3	4.7	3.0	1.7	1.0	**2.9**	Genotype	0.156	0.56	NS
ICCV 10	5.3	5.7	4.0	3.0	2.0	**4.0**	Concentration	0.062	1.26	NS
L 550	10.3	6.3	3.0	2.7	1.7	**4.8**	× Genotype			
Mean	5.9^{b}	5.4^{b}	4.4^{b}	2.3^{a}	1.5^{a}					

*LSD at $P \leq 0.05$ %.

Table 8: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Eggs 5^{-1} plants before spray									
C 235	2.7	3.3	2.7	1.7	0.0	**2.1**	**Concentration**	0.053	0.84	**NS**
ICC 506	0.7	0.0	4.7	2.3	1.3	**1.8**	**Genotype**	0.707	0.75	**NS**
ICCV 10	0.7	0.0	5.0	0.0	0.0	**1.1**	**Concentration**	0.855	1.69	**NS**
L 550	2.7	1.7	3.7	3.7	0.0	**2.3**	**× Genotype**			
Mean	**1.7**	**1.2**	**4.0**	**1.9**	**0.3**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	0.0	2.0	2.3	0.0	2.0	**1.3**	**Concentration**	0.022	0.88	**2.53***
ICC 506	2.0	0.3	6.7	1.7	2.0	**2.5**	**Genotype**	0.282	0.79	**NS**
ICCV 10	0.7	1.7	5.0	0.0	0.7	**1.6**	**Concentration**	0.979	1.77	**NS**
L 550	2.3	3.3	5.7	2.0	3.0	**3.3**	**× Genotype**			
Mean	**1.2[a]**	**1.8[a]**	**4.9[b]**	**0.9[a]**	**1.9[a]**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.0	8.0	6.0	0.3	3.0	**3.5**	**Concentration**	0.796	1.28	**NS**
ICC 506	0.7	1.0	0.7	5.7	3.0	**2.2**	**Genotype**	0.825	1.15	**NS**
ICCV 10	3.0	0.7	5.3	1.7	1.7	**2.5**	**Concentration**	0.161	2.56	**NS**
L 550	7.0	7.0	0.0	0.0	2.7	**3.3**	**× Genotype**			
Mean	**2.7**	**4.2**	**3.0**	**1.9**	**2.6**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	2.3	3.7	3.7	2.0	4.7	**3.3**	**Concentration**	0.926	1.47	**NS**
ICC 506	0.0	0.7	0.0	5.3	4.7	**2.1**	**Genotype**	0.912	1.32	**NS**
ICCV 10	2.0	1.7	6.0	1.0	0.0	**2.1**	**Concentration**	0.900	2.95	**NS**
L 550	3.7	1.0	4.0	3.3	2.0	**2.8**	**× Genotype**			
Mean	**2.0**	**1.7**	**2.1**	**2.9**	**2.8**					

*LSD at P≤ 0.05 %.

Table 9: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Larvae 5^{-1}plants before spray									
C 235	15.7	11.7	7.7	10.3	5.3	10.1^{bc}	Concentration	<0.001	1.07	3.05*
ICC 506	7.7	4.7	7.0	6.7	5.7	6.9^{a}	Genotype	0.001	0.95	2.73*
ICCV 10	11.3	8.3	7.7	7.7	6.0	8.2^{ab}	Concentration	0.326	2.13	NS
L 550	19.3	12.0	15.3	7.3	8.7	12.5^{c}	× Genotype			
Mean	13.5^{c}	9.9^{b}	9.4^{ab}	8.0^{ab}	6.4^{a}					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	12.0	5.3	5.3	3.0	5.3	6.2^{a}	Concentration	<0.001	0.83	2.38*
ICC 506	7.7	6.3	4.7	6.3	3.3	5.7^{a}	Genotype	0.001	0.74	2.13*
ICCV 10	10.7	7.0	7.0	6.0	5.0	7.1^{a}	Concentration	0.090	1.67	NS
L 550	16.3	10.7	13.3	4.7	4.3	9.9^{b}	× Genotype			
Mean	11.7^{d}	7.3^{bc}	7.6^{c}	5.0^{ab}	4.5^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	9.0^{d}	5.0^{abcd}	3.3^{abc}	5.3^{abcd}	3.3^{abc}	5.2	Concentration	<0.001	0.77	2.22*
ICC 506	6.3^{cd}	5.7^{bcd}	2.7^{abc}	3.7^{abc}	1.0^{a}	3.9	Genotype	0.012	0.69	1.98*
ICCV 10	5.3^{abcd}	3.3^{abc}	4.3^{abc}	2.7^{abc}	1.3^{ab}	3.4	Concentration	0.004	1.55	4.43*
L 550	17.7^{e}	6.0^{cd}	6.3^{cd}	1.3^{ab}	1.3^{ab}	6.5	× Genotype			
Mean	9.6	5.0	4.2	3.3	1.7					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	3.7^{abcdef}	4.0^{bcdef}	2.0^{abc}	1.7^{abc}	1.7^{abc}	2.6	Concentration	<0.001	0.61	1.73*
ICC 506	5.0^{cdef}	7.3^{fg}	2.7^{abcd}	2.3^{abc}	0.7^{ab}	3.6	Genotype	0.013	0.54	1.55*
ICCV 10	3.7^{abcdef}	2.0^{abc}	3.0^{abcde}	2.7^{abcd}	1.3^{ab}	2.5	Concentration	0.049	1.21	3.47*
L 550	9.7^{g}	6.0^{def}	6.3^{efg}	2.0^{abc}	0.3^{a}	4.9	× Genotype			
Mean	5.5	4.8	3.5	2.2	1.0					

*LSD at P≤ 0.05 %.

were also significant. The *Bt* sprayed plots had lower larval abundance than in the unsprayed plots of different chickpea genotypes.

4.1.1.2 Flowering stage

During the 2005-06 post-rainy season, there were no significant differences in egg numbers before spraying, and at 24, 48, and 72 h after spraying between the genotypes tested. Numerically low oviposition was recorded on ICC 506 (Table 10). There were no significant interaction effects between the genotypes × *Bt* concentrations in terms of larval numbers before spraying, but the differences between the genotypes and *Bt* concentrations were significant. Larval numbers were lower on ICC 506 and ICCV 10 as compared to those on C 235 and L 550 (Table 11). At 24 h after spraying, the interaction effects were non-significant, but the differences between the genotypes were significant. Fewer larvae were recorded on ICC 506 and ICCV 10 as compared to those on C 235 and L 550. At 48 h after spray, there were no significant interaction effects, but the differences between the genotypes and between *Bt* concentrations were significant. Lower larval numbers were recorded on ICC 506 and ICCV 10 as compared to those on C 235 and L 550. At 72 h after spray, the differences between the genotypes and between *Bt* concentrations were significant, and fewer larvae were recorded in ICC 506 plots sprayed with *Bt* than the unsprayed plots of different chickpea genotypes.

During the 2006-07 post-rainy season October planting, there were no significant interaction effects for egg numbers before the first spray, but differences were significant among the genotypes tested. Egg numbers were lower on ICC 506 and ICCV 10 as compared to those on C 235 and L 550 (Table 12). At 24 and 48 h after spray, the interaction effects were non-significant, but the differences between the genotypes were

Table 10: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Eggs 5^{-1} plants before spray									
C 235	1.0	1.7	0.3	4.7	0.7	**1.7**^{b}	**Concentration**	0.453	0.60	**NS**
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**^{a}	**Genotype**	0.041	0.54	1.54*
ICCV 10	2.3	2.0	2.3	0.3	1.3	**1.7**^{b}	**Concentration**	0.488	1.20	**NS**
L 550	2.0	1.7	0.7	4.3	2.0	**2.1**^{b}	**× Genotype**			
Mean	**1.3**	**1.3**	**0.8**	**2.3**	**1.0**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	2.0	2.0	0.3	1.3	0.0	**1.1**	**Concentration**	0.072	0.35	**NS**
ICC 506	0.0	0.7	0.7	0.0	0.0	**0.3**	**Genotype**	0.149	0.31	**NS**
ICCV 10	0.3	0.7	0.0	0.0	0.0	**0.2**	**Concentration**	0.311	0.69	**NS**
L 550	3.0	0.0	0.0	0.0	0.0	**0.6**	**× Genotype**			
Mean	**1.3**	**0.8**	**0.3**	**0.3**	**0.0**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	5.0	1.7	0.3	1.3	0.0	**1.7**	**Concentration**	<0.001	0.57	1.64*
ICC 506	0.7	0.3	0.0	0.0	0.0	**0.2**	**Genotype**	0.238	0.51	**NS**
ICCV 10	3.3	0.3	0.3	0.0	0.0	**0.8**	**Concentration**	0.884	1.15	**NS**
L550	4.3	1.7	0.0	0.0	0.0	**1.2**	**× Genotype**			
Mean	**3.3**^{b}	**1.0**^{a}	**0.2**^{a}	**0.3**^{a}	**0.0**^{a}					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.0	0.0	1.0	0.0	0.0	**0.2**	**Concentration**	0.191	0.18	**NS**
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	**Genotype**	0.517	0.16	**NS**
ICCV 10	0.3	0.3	1.0	0.0	0.0	**0.3**	**Concentration**	0.609	0.37	**NS**
L 550	0.0	0.0	0.3	1.0	0.0	**0.3**	**× Genotype**			
Mean	**0.1**	**0.1**	**0.6**	**0.2**	**0.00**					

*LSD at P≤ 0.05 %.

Table 11: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
Larvae 5^{-1} plants before spray										
C 235	6.0	3.7	7.3	9.7	10.3	**7.4^{b}**	**Concentration**	0.003	0.88	2.53*
ICC 506	0.7	1.3	2.0	0.7	3.3	**1.6^{a}**	**Genotype**	<0.001	0.79	2.26*
ICCV 10	3.3	2.7	8.3	5.7	6.0	**5.2^{b}**	**Concentration × Genotype**	0.300	1.76	NS
L 550	7.7	9.7	8.3	14.3	15.7	**11.1^{c}**				
Mean	**4.4^{a}**	**4.3^{a}**	**6.5^{ab}**	**7.6^{b}**	**8.8^{b}**					
Larvae 5^{-1} plants at 24 h after spray										
C 235	3.7	4.0	3.7	4.3	4.7	**4.1^{cd}**	**Concentration**	0.856	0.59	NS
ICC 506	1.3	1.0	1.0	2.3	1.7	**1.5^{ab}**	**Genotype**	<0.001	0.52	1.50*
ICCV 10	3.0	2.0	3.3	3.3	2.7	**2.9^{bc}**	**Concentration × Genotype**	0.984	1.17	NS
L 550	6.3	5.0	5.0	5.3	3.7	**5.1^{d}**				
Mean	**3.6**	**3.0**	**3.3**	**3.8**	**3.2**					
Larvae 5^{-1} plants at 48 h after spray										
C 235	6.3	5.0	4.7	1.7	2.7	**4.1^{c}**	**Concentration**	<0.001	0.51	1.47*
ICC 506	1.0	1.7	0.7	0.3	0.3	**0.8^{a}**	**Genotype**	<0.001	0.46	1.32*
ICCV 10	3.7	1.7	5.7	0.3	0.0	**2.3^{b}**	**Concentration × Genotype**	0.083	1.03	NS
L 550	6.7	5.3	9.3	3.3	2.0	**5.3^{c}**				
Mean	**4.4^{bc}**	**3.4^{b}**	**5.1^{c}**	**1.4^{a}**	**1.3^{a}**					
Larvae 5^{-1} plants at 72 h after spray										
C 235	5.3	4.0	1.7	1.0	0.0	**2.4^{b}**	**Concentration**	0.002	0.74	2.11*
ICC 506	0.3	1.0	0.7	0.0	0.3	**0.5^{a}**	**Genotype**	0.004	0.66	1.89*
ICCV 10	3.0	2.7	4.7	0.7	1.7	**2.5^{b}**	**Concentration × Genotype**	0.277	1.48	NS
L 550	8.7	5.7	5.3	0.7	0.3	**4.1^{b}**				
Mean	**4.3^{b}**	**3.3^{b}**	**3.1^{b}**	**0.6^{a}**	**0.6^{a}**					

*LSD at P≤ 0.05 %.

Table 12: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting first spray)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Eggs 5^{-1} plants before spray									
C 235	3.7	6.0	5.7	10.0	9.0	**6.9^{b}**	**Concentration**	0.684	1.27	**NS**
ICC 506	0.0	2.3	0.7	0.0	1.3	**0.9^{a}**	**Genotype**	0.005	1.13	3.25*
ICCV 10	1.3	4.3	4.0	1.7	3.0	**2.9^{a}**	**Concentration**	0.953	2.54	**NS**
L 550	2.7	2.7	2.7	0.7	4.7	**2.7^{a}**	**× Genotype**			
Mean	**1.9**	**3.8**	**3.3**	**3.1**	**4.5**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	6.3	1.7	6.0	6.7	1.7	**4.5^{b}**	**Concentration**	0.910	0.88	**NS**
ICC 506	0.0	1.3	1.3	1.3	1.7	**1.1^{a}**	**Genotype**	0.039	0.79	2.25*
ICCV 10	1.7	2.0	3.0	2.0	3.0	**2.3^{ab}**	**Concentration**	0.530	1.76	**NS**
L 550	3.7	4.3	2.7	0.7	2.3	**2.7^{ab}**	**× Genotype**			
Mean	**2.9**	**2.3**	**3.3**	**2.7**	**2.2**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	4.3	7.0	4.7	9.3	1.0	**5.3^{c}**	**Concentration**	0.349	0.90	**NS**
ICC 506	0.0	0.3	1.7	0.7	0.3	**0.6^{a}**	**Genotype**	0.001	0.80	2.29*
ICCV 10	1.7	4.3	2.3	0.7	3.3	**2.5^{ab}**	**Concentration**	0.258	1.79	**NS**
L 550	3.0	3.3	2.3	7.3	5.0	**4.2^{bc}**	**× Genotype**			
Mean	**2.3**	**3.7**	**2.7**	**4.5**	**2.4**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	2.0	4.3	5.0	8.3	5.7	**5.1**	**Concentration**	0.004	1.18	3.37*
ICC 506	4.0	4.3	1.7	6.7	6.0	**4.5**	**Genotype**	0.672	1.05	**NS**
ICCV 10	3.7	0.7	2.0	7.3	2.7	**3.3**	**Concentration**	0.841	2.35	**NS**
L 550	3.7	1.0	2.0	12.0	3.3	**4.4**	**× Genotype**			
Mean	**3.3^{a}**	**2.6^{a}**	**2.7^{a}**	**8.6^{b}**	**4.4^{a}**					

*LSD at P≤ 0.05 %.

significant. Similar results were observed at 72 h after spray. The differences in larval numbers before spraying between the genotypes were significant, lower numbers of larvae were recorded on ICC 506 and ICCV 10 as compared to those on C 235 and L 550 (Table 13). At 24, 48, and 72 h after spray, the interaction effects were non-significant, but the differences between the genotypes and *Bt* concentrations were significant. Relatively lower numbers of larvae were recorded on ICC 506 and ICCV 10 as compared to those on C 235 and L 550.

There were no significant differences between the genotypes for egg numbers before the second spraying, and at 24, 48, and 72 h after spraying (Table 14). The interaction effects between the genotypes × *Bt* concentrations were non-significant for larval numbers before spraying and 24 h after spraying, but the differences between the genotypes and *Bt* concentrations were significant. Fewer larvae were recorded on ICC 506 and ICCV 10 as compared to those on C 235 and L 550 (Table 15). At 48 and 72 h after spray, there were significant differences between the genotypes and *Bt* concentrations tested, and the interaction effects were also significant. Larval numbers were significantly lower in plots of ICC 506 and ICCV 10 across *Bt* concentrations.

During the 2006-07 post-rainy season December planting, there were no significant differences between the genotypes tested for egg numbers before the first spraying, and at 24, 48, and 72 h after spraying (Table 16). The interaction effects were non-significant for larval numbers before the first spraying, and at 24 and 48 h after spraying, but the differences between the genotypes were significant. Significantly lower numbers of larvae were recorded on ICC 506 than on the other genotypes tested (Table 17). At 72 h after spray, the interaction effects were non-significant, but the

Table 13: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting first spray)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.05					
	Larvae 5^{-1} plants before spray									
C 235	13.7	11.7	14.7	14.3	8.3	12.5^{c}	Concentration	0.482	1.20	NS
ICC 506	3.0	5.3	4.0	2.7	1.7	3.3^{a}	Genotype	<0.001	1.71	3.08*
ICCV 10	7.7	7.7	7.0	5.7	6.0	6.8^{b}	Concentration	0.739	2.41	NS
L 550	12.7	15.0	20.0	17.3	17.3	16.5^{d}	× Genotype			
Mean	9.3	9.9	11.4	10.0	8.3					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	14.0	7.3	4.3	6.3	4.3	7.3^{b}	Concentration	<0.001	0.87	2.49*
ICC 506	3.0	4.0	1.3	1.7	1.3	2.3^{a}	Genotype	<0.001	0.78	2.23*
ICCV 10	6.7	3.0	2.3	3.0	2.7	3.5^{a}	Concentration	0.623	1.74	NS
L 550	13.0	12.3	9.3	9.3	7.7	10.3^{c}	× Genotype			
Mean	9.2^{c}	6.7^{b}	4.3^{ab}	5.1^{ab}	4.0^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	15.0	9.3	3.0	9.3	0.7	7.5^{b}	Concentration	<0.001	1.20	3.45*
ICC 506	7.0	3.7	1.7	0.0	0.3	2.5^{a}	Genotype	<0.001	1.08	3.08*
ICCV 10	6.0	1.3	3.3	2.7	3.0	3.3^{a}	Concentration	0.494	2.41	NS
L 550	14.0	9.0	8.7	7.3	4.7	8.7^{b}	× Genotype			
Mean	10.5^{c}	5.8^{b}	4.2^{ab}	4.8^{ab}	2.2^{a}					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	20.0	5.3	9.0	9.3	3.7	9.5^{bc}	Concentration	<0.001	2.11	6.05*
ICC 506	16.7	4.7	2.0	0.0	0.3	4.7^{ab}	Genotype	0.009	1.89	5.41*
ICCV 10	5.7	4.3	5.3	2.3	2.0	3.9^{a}	Concentration	0.412	4.23	NS
L 550	30.7	13.3	9.7	6.7	1.7	12.4^{c}	× Genotype			
Mean	18.3^{b}	6.9^{a}	6.5^{a}	4.6^{a}	1.9^{a}					

*LSD at P≤ 0.05 %.

Table 14: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting second spray)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Eggs 5^{-1} plants before spray									
C 235	0.0	0.7	2.3	0.0	0.0	**0.6**	**Concentration**	0.134	0.30	**NS**
ICC 506	0.0	0.0	0.0	0.0	0.7	**0.1**	**Genotype**	0.443	0.27	**NS**
ICCV 10	0.0	0.0	1.7	0.0	0.0	**0.3**	**Concentration**	0.587	0.61	**NS**
L 550	0.0	0.0	0.0	0.0	0.0	**0.0**	**× Genotype**			
Mean	**0.0**	**0.2**	**1.0**	**0.0**	**0.2**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	1.7	3.7	0.0	0.0	3.0	**1.7**	**Concentration**	0.088	0.46	**NS**
ICC 506	0.0	0.0	0.0	0.7	0.0	**0.1**	**Genotype**	0.089	0.41	**NS**
ICCV 10	1.3	0.0	0.0	0.0	2.3	**0.7**	**Concentration**	0.378	0.93	**NS**
L 550	0.7	0.0	0.0	1.0	2.0	**0.7**	**× Genotype**			
Mean	**0.9**	**0.9**	**0.0**	**0.4**	**1.8**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.0	1.0	0.3	0.7	1.7	**0.7**	**Concentration**	0.541	0.35	**NS**
ICC 506	0.0	0.0	0.7	0.0	0.0	**0.1**	**Genotype**	0.584	0.32	**NS**
ICCV 10	0.7	0.0	0.7	0.0	0.0	**0.3**	**Concentration**	0.756	0.71	**NS**
L 550	0.0	0.0	0.0	0.0	2.0	**0.4**	**× Genotype**			
Mean	**0.2**	**0.2**	**0.4**	**0.2**	**0.9**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.0	0.3	0.0	3.3	2.3	**1.2**	**Concentration**	0.575	0.44	**NS**
ICC 506	0.0	0.0	0.0	0.0	1.0	**0.2**	**Genotype**	0.279	0.39	**NS**
ICCV 10	1.0	0.0	2.7	1.3	0.0	**1.0**	**Concentration**	0.101	0.88	**NS**
L 550	2.3	1.3	0.0	1.0	0.7	**1.1**	**× Genotype**			
Mean	**0.8**	**0.4**	**0.7**	**1.4**	**1.0**					

LSD at P≤ 0.05 %.

Table 15: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting second spray)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Larvae 5^{-1} plants before spray									
C 235	16.3	17.0	11.3	12.3	7.7	12.9^{b}	Concentration	0.019	1.16	3.31*
ICC 506	10.3	5.0	5.0	4.3	4.3	5.8^{a}	Genotype	<0.001	1.03	2.96*
ICCV 10	13.7	5.0	5.3	8.7	6.7	7.9^{a}	Concentration	0.203	2.31	NS
L 550	12.7	10.3	16.3	9.3	12.0	12.1^{b}	× Genotype			
Mean	13.3^{b}	9.4^{a}	9.5^{a}	8.7^{a}	7.7^{a}					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	16.0	6.7	4.3	5.0	3.0	7.0^{b}	Concentration	<0.001	0.91	2.61*
ICC 506	11.3	1.7	1.0	0.7	2.7	3.5^{a}	Genotype	<0.001	0.82	2.34*
ICCV 10	9.3	3.7	3.0	3.3	1.3	4.1^{a}	Concentration	0.245	1.83	NS
L 550	21.7	8.7	5.0	6.3	2.7	8.9^{b}	× Genotype			
Mean	14.6^{c}	5.2^{b}	3.3^{ab}	3.8^{ab}	2.4^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	16.0^{h}	8.3^{g}	6.7^{efg}	5.3^{cdefg}	0.3^{a}	7.3	Concentration	<0.001	0.67	1.92*
ICC 506	6.3^{ef}	4.0^{abcde}	3.3^{abcde}	1.0^{ab}	0.7^{a}	3.1	Genotype	<0.001	0.60	1.72*
ICCV 10	7.7^{fg}	6.3^{efg}	4.7^{bcdefg}	5.3^{cdefg}	1.7^{abc}	5.1	Concentration	0.001	1.34	3.85*
L 550	19.0^{h}	5.7^{defg}	8.3^{g}	4.7^{bcdefg}	2.3^{abcd}	8.0	× Genotype			
Mean	12.3	6.1	5.7	4.1	1.30					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	16.3^{f}	8.3^{de}	6.3^{bcde}	4.7^{abcd}	0.3^{a}	7.2	Concentration	<0.001	0.84	2.40*
ICC 506	4.3^{abcd}	4.7^{abcd}	3.3^{abc}	1.7^{ab}	1.3^{a}	3.1	Genotype	<0.001	0.75	2.15*
ICCV 10	7.0^{cde}	4.3^{abcd}	4.0^{abcd}	6.3^{cde}	2.0^{ab}	4.7	Concentration	0.020	1.68	4.81*
L 550	17.3^{f}	10.7^{e}	9.7^{e}	10.3^{e}	2.7^{abc}	10.1	× Genotype			
Mean	11.3	7.0	5.8	5.7	1.6					

*LSD at P≤ 0.05 %.

Table 16: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting first spray)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Eggs 5^{-1} plants before spray									
C 235	1.0	2.3	0.7	3.0	0.0	**1.4**	**Concentration**	0.364	0.67	**NS**
ICC 506	1.0	0.7	1.0	1.0	0.0	**0.7**	**Genotype**	0.256	0.60	**NS**
ICCV 10	1.0	0.7	0.0	0.0	0.0	**0.3**	**Concentration**	0.607	1.34	**NS**
L 550	1.3	3.7	4.7	0.0	0.0	**1.9**	**× Genotype**			
Mean	1.8	1.8	1.6	**1.0**	**0.0**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	0.0	1.3	0.0	0.7	0.0	**0.4**	**Concentration**	0.595	0.47	**NS**
ICC 506	0.0	0.0	0.0	0.00	0.0	**0.0**	**Genotype**	0.344	0.42	**NS**
ICCV 10	0.0	0.0	3.0	0.3	0.0	**0.7**	**Concentration**	0.383	0.94	**NS**
L 550	2.7	2.0	0.7	0.0	0.0	**1.1**	**×Genotype**			
Mean	**0.7**	**0.8**	**0.9**	**0.3**	**0.0**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.0	2.0	0.0	4.7	0.7	**1.5**	**Concentration**	0.604	0.61	**NS**
ICC 506	0.3	1.0	1.3	1.0	1.3	**1.0**	**Genotype**	0.504	0.54	**NS**
ICCV 10	0.0	0.0	0.0	0.0	2.0	**0.4**	**Concentration**	0.484	1.22	**NS**
L 550	0.0	1.3	1.3	0.0	0.0	**0.5**	**×Genotype**			
Mean	**0.1**	**1.1**	**0.7**	**1.4**	**1.0**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.7	0.0	5.0	0.0	0.0	**1.1**	**Concentration**	0.511	0.63	**NS**
ICC 506	0.3	0.0	0.0	0.0	0.3	**0.1**	**Genotype**	0.497	0.56	**NS**
ICCV 10	2.3	0.0	0.0	0.0	0.0	**0.5**	**Concentration**	0.505	1.26	**NS**
L 550	0.0	0.0	0.0	0.0	0.0	**0.0**	**×Genotype**			
Mean	**0.8**	**0.0**	**1.3**	**0.0**	**0.1**					

LSD at P≤ 0.05 %.

Table 17: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting first spray)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Larvae 5^{-1} plants before spray									
C 235	6.7	13.7	9.3	9.3	13.7	10.5^{bc}	Concentration	0.077	1.26	NS
ICC 506	4.0	6.0	7.7	4.3	5.3	5.5^{a}	Genotype	<0.001	1.13	3.22*
ICCV 10	7.3	6.3	6.3	6.3	10.7	7.4^{ab}	Concentration ×Genotype	0.722	2.52	NS
L 550	9.0	16.0	17.0	9.7	14.0	13.1^{c}				
Mean	6.7	10.5	10.1	7.4	10.9					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	5.7	10.3	6.0	5.0	6.7	6.7^{bc}	Concentration	0.851	0.76	NS
ICC 506	2.3	2.0	2.0	3.3	2.3	2.4^{a}	Genotype	<0.001	0.67	1.93*
ICCV 10	6.0	4.3	6.3	7.3	6.0	6.0^{b}	Concentration ×Genotype	0.572	1.51	NS
L 550	7.3	9.0	7.7	7.3	9.3	8.1^{c}				
Mean	5.3	6.4	5.5	5.7	6.1					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	4.7	9.0	6.7	8.0	3.3	6.3^{bc}	Concentration	0.194	0.79	NS
ICC 506	1.7	3.3	6.0	2.7	2.0	3.1^{a}	Genotype	<0.001	0.71	2.03*
ICCV 10	7.0	4.3	4.3	5.3	5.7	5.3^{b}	Concentration ×Genotype	0.227	1.59	NS
L 550	8.7	5.7	10.7	9.3	6.0	8.1^{c}				
Mean	5.5	5.6	6.9	6.3	4.3					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	6.7	6.0	5.0	6.3	1.3	5.1^{bc}	Concentration	<0.001	0.57	1.62*
ICC 506	3.0	1.7	3.0	1.7	0.7	2.0^{a}	Genotype	<0.001	0.51	1.45*
ICCV 10	5.3	5.7	5.3	4.0	3.7	4.8^{b}	Concentration ×Genotype	0.191	1.13	NS
L 550	5.7	7.7	10.3	5.7	3.0	6.5^{c}				
Mean	5.2^{b}	5.3^{b}	5.9^{b}	4.4^{b}	2.2^{a}					

*LSD at P≤ 0.05 %.

differences between the genotypes and *Bt* concentrations were significant. Fewer larvae were recorded on ICC 506 and in plots sprayed with *Bt* as compared to the unsprayed plots.

During the 2006-07 post-rainy season December planting, there were no significant differences between the genotypes tested for egg numbers before the second spray, and at 24, 48, and 72 h after spraying (Table 18). However, the differences between *Bt* concentrations for larval numbers were significant (Table 19). At 24 and 48 h after spray, there were no significant differences between the genotypes tested. Numerically lower larval density was recorded on ICC 506 than those on the other genotypes tested. At 72 h after spray, the differences between the genotypes and *Bt* concentrations were significant. Larval density was low on ICC 506 across *Bt* concentrations.

4.1.1.3 Podding stage

During the 2005-06 post-rainy season, there were no significant differences between the genotypes tested for egg numbers before spraying, and at 48 and 72 h after spraying (Table 20). At 48 h after spray, there were significant differences between *Bt* concentrations, and the interaction effects were also significant. There were no eggs on ICC 506, ICCV 10, and C235. Differences between the genotypes and *Bt* concentrations for larval numbers were significant before spraying. Lower numbers of larvae were recorded on C 235 and ICC 506 as compared to L 550 and ICCV 10 (Table 21). The differences between the genotypes and *Bt* concentrations were significant at 24, 48, and 72 h after spray. The interaction effects were significant at 72 h after spray. Larval

Table 18: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting second spray)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Eggs 5^{-1} plants before spray									
C 235	2.3	1.7	0.7	2.0	4.7	**2.3**	Concentration	0.117	0.56	NS
ICC 506	3.3	0.0	0.0	0.0	0.7	**0.8**	Genotype	0.083	0.50	NS
ICCV 10	2.0	0.0	1.3	3.3	4.0	**2.1**	Concentration ×Genotype	0.329	1.12	NS
L 550	0.7	1.3	1.0	1.0	0.7	**0.9**				
Mean	**2.1**	**0.7**	**0.7**	**1.6**	**2.5**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	2.0	1.3	0.0	2.0	0.7	**1.2**	Concentration	0.696	0.77	NS
ICC 506	0.7	0.0	0.0	0.0	2.3	**0.6**	Genotype	0.339	0.69	NS
ICCV 10	0.0	2.0	2.0	0.0	0.0	**0.8**	Concentration ×Genotype	0.728	1.55	NS
L 550	0.7	4.0	0.0	2.7	4.0	**2.3**				
Mean	**0.8**	**1.8**	**0.5**	**1.2**	**1.7**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.0	2.0	0.7	1.3	1.7	**1.1**	Concentration	0.860	0.98	NS
ICC 506	0.0	3.7	3.3	1.0	0.0	**1.6**	Genotype	0.696	0.88	NS
ICCV 10	2.3	0.0	7.0	2.0	1.7	**2.6**	Concentration ×Genotype	0.425	1.97	NS
L 550	3.3	0.0	0.0	2.0	3.3	**1.7**				
Mean	**1.4**	**1.4**	**2.7**	**1.6**	**1.7**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	1.7	0.0	1.0	0.3	1.7	**0.9**	Concentration	0.032	0.76	2.17*
ICC 506	0.0	0.0	0.0	2.3	2.3	**0.9**	Genotype	0.082	0.68	NS
ICCV 10	0.3	0.3	2.0	1.3	10.0	**2.8**	Concentration ×Genotype	0.090	1.52	NS
L 550	0.3	0.3	0.7	0.7	0.0	**0.4**				
Mean	**0.6**[a]	**0.2**[a]	**0.9**[a]	**1.2**[a]	**3.5**[b]					

*LSD at P≤ 0.05 %.

Table 19: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting second spray)

Genotype	*Bt* concentration (%)							Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50	Mean				
	Larvae 5^{-1} plants before spray									
C 235	2.7	3.7	6.0	6.0	4.7	**4.6**	Concentration	<0.001	0.73	2.09*
ICC 506	3.7	4.3	7.0	5.7	3.7	**4.9**	Genotype	0.149	0.65	NS
ICCV 10	2.7	6.7	5.3	9.7	5.0	**5.9**	Concentration ×Genotype	0.593	1.46	NS
L 550	3.3	8.3	7.7	9.7	3.7	**6.5**				
Mean	**3.1**^{a}	**5.7**^{bc}	**6.5**^{c}	**7.7**^{c}	**4.2**^{ab}					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	2.7	4.3	3.3	1.7	2.3	**2.9**	Concentration	0.057	0.58	NS
ICC 506	1.0	2.0	3.3	2.7	0.7	**1.9**	Genotype	0.112	0.51	NS
ICCV 10	3.3	4.0	4.3	4.0	1.0	**3.3**	Concentration ×Genotype	0.706	1.15	NS
L 550	3.3	5.7	2.3	4.7	2.3	**3.7**				
Mean	**2.6**	**4.0**	**3.3**	**3.3**	**1.6**					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	1.3	2.3	4.3	3.0	0.7	**2.3**	Concentration	0.063	0.59	NS
ICC 506	2.3	2.3	2.7	1.7	0.7	**1.9**	Genotype	0.104	0.53	NS
ICCV 10	2.3	3.0	4.7	4.3	2.7	**3.4**	Concentration ×Genotype	0.864	1.19	NS
L 550	3.3	5.7	3.3	3.7	1.7	**3.5**				
Mean	**2.3**	**3.3**	**3.7**	**3.2**	**1.4**					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	0.3	2.3	3.3	4.7	2.0	**2.5**^{b}	Concentration	0.026	0.44	1.26*
ICC 506	0.0	1.7	0.3	2.0	0.3	**0.9**^{a}	Genotype	0.012	0.39	1.13*
ICCV 10	1.3	3.7	2.3	2.3	1.3	**2.2**^{b}	Concentration ×Genotype	0.205	0.88	NS
L 550	3.3	4.3	2.0	1.7	1.7	**2.6**^{b}				
Mean	**1.2**^{a}	**3.0**^{b}	**2.0**^{ab}	**2.7**^{b}	**1.3**^{a}					

*LSD at P≤ 0.05 %.

Table 20: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Eggs 5^{-1} plants before spray									
C 235	0.0	1.0	0.0	0.0	0.0	**0.2**	Concentration	0.134	0.28	NS
ICC 506	0.0	0.0	0.0	0.0	1.7	**0.3**	Genotype	0.490	0.25	NS
ICCV 10	0.0	0.0	0.0	0.7	2.0	**0.5**	Concentration	0.451	0.55	NS
L 550	0.0	0.0	0.0	0.0	0.0	**0.0**	× Genotype			
Mean	**0.0**	**0.2**	**0.0**	**0.2**	**0.9**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.0**	Concentration	0.030	0.06	0.18*
ICC 506	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.0**	Genotype	0.042	0.06	0.16*
ICCV 10	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.0**	Concentration	0.005	0.13	0.37*
L 550	1.0^{b}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.2**	× Genotype			
Mean	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.3	0.0	0.0	0.0	0.0	**0.1**	Concentration	0.680	0.12	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.592	0.11	NS
ICCV 10	0.0	0.0	0.7	0.0	0.0	**0.1**	Concentration	0.498	0.24	NS
L 550	0.3	0.7	0.0	0.0	0.0	**0.2**	× Genotype			
Mean	**0.2**	**0.2**	**0.2**	**0.0**	**0.0**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.3	0.0	0.0	0.0	0.0	**0.1**	Concentration	0.461	0.19	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.455	0.17	NS
ICCV 10	0.0	0.0	0.0	0.0	0.0	**0.0**	Concentration	0.424	0.38	NS
L 550	0.0	0.0	0.0	1.7	0.0	**0.3**	× Genotype			
Mean	**0.1**	**0.0**	**0.0**	**0.4**	**0.0**					

*LSD at $P \leq 0.05$ %.

Table 21: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Larvae 5^{-1} plants before spray									
C 235	1.3	1.7	2.7	2.3	5.0	**2.6^{a}**	Concentration	0.013	0.67	1.91*
ICC 506	2.7	1.3	4.3	3.7	3.0	**3.0^{a}**	Genotype	<0.001	0.60	1.71*
ICCV 10	4.3	5.7	9.3	7.7	10.0	**7.4^{c}**	Concentration × Genotype	0.521	1.33	NS
L 550	4.7	5.0	3.3	6.3	7.3	**5.3^{b}**				
Mean	**3.2^{a}**	**3.4^{a}**	**4.9^{ab}**	**5.0^{ab}**	**6.3^{b}**					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	1.7	0.0	1.0	0.3	1.3	**0.9^{ab}**	Concentration	0.005	0.45	1.28*
ICC 506	2.0	0.0	0.3	0.0	0.3	**0.5^{a}**	Genotype	0.004	0.40	1.15*
ICCV 10	4.3	3.3	0.7	0.0	1.0	**1.9^{bc}**	Concentration × Genotype	0.463	0.90	NS
L 550	3.7	3.7	2.7	1.3	1.3	**2.5^{c}**				
Mean	**2.9^{c}**	**1.7^{bc}**	**1.2^{ab}**	**0.4^{a}**	**1.0^{ab}**					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	0.7	0.0	1.0	0.3	0.7	**0.5^{a}**	Concentration	<0.001	0.35	0.99*
ICC 506	3.0	0.3	0.0	0.0	0.0	**0.7^{a}**	Genotype	0.001	0.31	0.89*
ICCV 10	4.3	2.3	0.7	0.0	1.0	**1.7^{b}**	Concentration × Genotype	0.257	0.69	NS
L 550	3.7	2.0	1.7	1.7	1.7	**2.1^{b}**				
Mean	**2.9^{b}**	**1.2^{a}**	**0.8^{a}**	**0.5^{a}**	**0.8^{a}**					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	1.0^{abcd}	0.7^{abc}	0.3^{ab}	0.0^{a}	0.0^{a}	**0.4**	Concentration	<0.001	0.20	0.58*
ICC 506	1.7^{cd}	0.3^{ab}	0.0^{a}	0.0^{a}	0.7^{abc}	**0.5**	Genotype	<0.001	0.18	0.52*
ICCV 10	1.3^{bcd}	2.0d	1.7^{cd}	0.7^{abc}	0.7^{abc}	**1.3**	Concentration × Genotype	<0.011	0.40	1.16*
L 550	3.7^{e}	1.0^{abcd}	1.0^{abcd}	1.0^{abcd}	0.0^{a}	**1.3**				
Mean	**1.9**	**1.0**	**0.7**	**0.4**	**0.3**					

*LSD at P≤ 0.05 %.

numbers were lower in the plots sprayed with *Bt* as compared to the unsprayed plots of different genotypes.

In the October planting during the 2006-07 post-rainy season, there were no significant differences between the genotypes in egg numbers before the first spraying, and at 24, 48, and 72 h after spraying (Table 22). There were no significant interaction effects for larval numbers before spraying, but the differences between the genotypes were significant. There were very few larvae on ICC 506, C 235, and ICCV 10 as compared to that on L 550 (Table 23). At 24, 48, and 72 h after spray, the interaction effects were non-significant, but the differences between the genotypes and *Bt* concentrations were significant. Larval numbers were low on ICC 506 and ICCV 10 as compared to that on C 235 and L 550. There were no eggs on the genotypes across *Bt* concentrations before and after second spray. The interaction effects for larval numbers before second spray, and after spraying at different intervals were non-significant, but the differences between the genotypes were significant (Table 24). At 24, 48, and 72 h after spray, the differences between the genotypes and *Bt* concentrations were significant. There were no larvae on the ICC 506 across *Bt* concentrations.

In the December planting, before first spray, there were no significant differences between the genotypes for egg numbers, and at 24, 48, and 72 h after spraying (Table 25). The interaction effects between the genotypes × *Bt* concentrations for larval numbers were significant. Larval numbers were low on ICC 506, ICCV 10, and C 235 across *Bt* concentrations as compared to that on L 550 (Table 26). At 24 h after spray, the interaction effects were non-significant. The genotypes ICC 506, ICCV 10, and C 235 had lower larval numbers as compared to L 550. At 48 h after spray, the interaction

Table 22: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting first spray)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Eggs 5^{-1} plants before spray									
C 235	3.0	0.0	0.0	0.0	0.3	**0.7**	Concentration	0.154	0.29	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.256	0.26	NS
ICCV 10	0.0	0.0	0.0	0.0	0.3	**0.1**	Concentration	0.067	0.58	NS
L 550	0.0	0.0	0.0	0.0	2.0	**0.4**	× Genotype			
Mean	**0.7**	**0.0**	**0.0**	**0.0**	**0.7**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	0.7	0.0	0.7	0.0	1.3	**0.5**	Concentration	0.361	0.46	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.301	0.41	NS
ICCV 10	1.0	0.0	0.0	0.0	0.0	**0.2**	Concentration	0.787	0.93	NS
L 550	2.7	0.0	2.7	0.0	0.0	**1.1**	× Genotype			
Mean	**1.1**	**0.0**	**0.8**	**0.0**	**0.2**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	0.0	0.0	0.0	0.0	0.0	**0.0**	Concentration	0.420	0.11	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.403	0.10	NS
ICCV 10	0.0	0.0	1.0	0.0	0.0	**0.2**	Concentration	0.467	0.22	NS
L 550	0.0	0.0	0.0	0.0	0.0	**0.0**	× Genotype			
Mean	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.0	0.0	0.0	0.0	0.0	**0.0**	Concentration	0.420	0.07	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.403	0.07	NS
ICCV 10	0.0	0.0	0.0	0.0	0.0	**0.0**	Concentration	0.467	0.15	NS
L 550	0.0	0.0	0.0	0.7	0.0	**0.1**	× Genotype			
Mean	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**					

LSD at P≤ 0.05 %.

Table 23: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting first spray)

Genotype	*Bt* concentration (%)									
	0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Larvae 5^{-1} plants before spray									
C 235	7.7	6.3	8.0	4.3	5.7	**6.4**^{a}	Concentration	0.429	1.05	NS
ICC 506	7.3	5.7	7.0	3.3	2.7	**5.2**^{a}	Genotype	0.005	0.94	2.69*
ICCV 10	7.0	7.3	6.0	6.3	6.7	**6.7**^{a}	Concentration	0.930	2.10	NS
L 550	10.0	9.3	10.7	7.7	12.7	**10.1**^{b}	× Genotype			
Mean	**8.0**	**7.2**	**7.9**	**5.4**	**6.9**					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	12.3	14.3	11.0	3.7	5.0	**9.3**^{b}	Concentration	0.002	1.04	2.99*
ICC 506	10.0	6.0	6.7	3.7	4.0	**6.1**^{a}	Genotype	0.031	0.93	2.67*
ICCV 10	8.3	8.3	6.7	6.7	4.7	**6.9**^{ab}	Concentration	0.590	2.09	NS
L 550	11.3	9.3	11.3	8.7	6.7	**9.5**^{b}	× Genotype			
Mean	**10.5**^{b}	**9.5**^{b}	**8.9**^{b}	**5.7**^{a}	**5.1**^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	9.3	11.3	8.0	5.3	3.0	**7.4**^{b}	Concentration	<0.001	0.77	2.20*
ICC 506	11.3	4.7	4.7	3.7	1.3	**5.1**^{a}	Genotype	0.010	0.69	1.97*
ICCV 10	9.0	9.7	8.7	5.7	4.0	**7.4**^{b}	Concentration	0.101	1.54	NS
L 550	8.3	10.7	13.3	6.0	4.3	**8.5**^{b}	× Genotype			
Mean	**9.5**^{b}	**9.1**^{b}	**8.7**^{b}	**5.2**^{a}	**3.2**^{a}					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	7.0	9.7	5.0	4.0	1.0	**5.3**	Concentration	<0.001	1.11	3.18*
ICC 506	6.3	7.3	4.7	2.7	1.3	**4.5**	Genotype	0.341	0.99	NS
ICCV 10	9.0	7.7	10.0	5.3	0.7	**6.5**	Concentration	0.911	2.22	NS
L 550	10.0	7.3	10.3	4.0	2.0	**6.7**	× Genotype			
Mean	**8.1**^{b}	**8.0**^{b}	**7.5**^{b}	**4.0**^{a}	**1.3**^{a}					

*LSD at P≤ 0.05 %.

Table 24: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting second spray)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Larvae 5^{-1} plants before spray									
C 235	3.7	4.0	3.3	2.3	2.3	**3.1**^{b}	Concentration	0.729	0.45	NS
ICC 506	0.7	0.0	0.7	0.0	0.0	**0.3**^{a}	Genotype	<0.001	0.40	1.15*
ICCV 10	2.0	0.3	1.0	0.0	1.3	**0.9**^{a}	Concentration	0.739	0.90	NS
L 550	1.0	2.7	3.3	2.7	2.3	**2.4**^{b}	× Genotype			
Mean	**1.8**	**1.7**	**2.1**	**1.2**	**1.5**					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	3.0	1.3	1.0	0.7	0.0	**1.2**^{b}	Concentration	0.019	0.28	0.81*
ICC 506	0.0	0.0	0.3	0.0	0.0	**0.1**^{a}	Genotype	0.013	0.25	0.72*
ICCV 10	2.0	0.7	0.3	0.0	0.0	**0.6**^{ab}	Concentration	0.327	0.56	NS
L 550	1.0	1.3	1.7	0.3	1.0	**1.1**^{b}	× Genotype			
Mean	**1.5**^{b}	**0.8**^{ab}	**0.8**^{ab}	**0.3**^{a}	**0.3**^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	2.3	1.7	0.7	1.0	0.0	**1.1**^{b}	Concentration	0.005	0.24	0.69*
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**^{a}	Genotype	<0.001	0.22	0.62*
ICCV 10	0.3	0.0	0.3	0.3	0.0	**0.2**^{a}	Concentration	0.072	0.48	NS
L 550	2.7	3.0	2.3	0.7	0.3	**1.8**^{c}	× Genotype			
Mean	**1.3**^{c}	**1.2**^{bc}	**0.8**^{bc}	**0.5**^{ab}	**0.1**^{a}					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	0.3	1.0	0.7	0.3	0.0	**0.5**	Concentration	0.167	0.14	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.016	0.13	0.36*
ICCV 10	0.0	0.0	0.3	0.0	0.0	**0.1**	Concentration	0.341	0.28	NS
L 550	1.0	1.0	0.0	0.3	0.0	**0.5**	× Genotype			
Mean	**0.3**^{ab}	**0.5**^{b}	**0.3**^{ab}	**0.2**^{ab}	**0.0**^{b}					

*LSD at $P \leq 0.05$ %.

Table 25: Effect of *B. thuringiensis* sprays on oviposition by *H. armigera* females on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting first spray)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Eggs 5^{-1} plants before spray									
C 235	1.0	1.0	0.0	1.3	0.0	**0.7**	Concentration	0.131	0.22	NS
ICC 506	0.7	0.0	0.0	0.0	0.0	**0.1**	Genotype	0.249	0.20	NS
ICCV 10	1.3	0.0	0.0	0.0	0.0	**0.3**	Concentration	0.556	0.45	NS
L 550	0.0	0.0	0.0	0.7	0.3	**0.2**	×Genotype			
Mean	**0.7**	**0.2**	**0.0**	**0.5**	**0.1**					
	Eggs 5^{-1} plants at 24 h after spray									
C 235	0.7	0.7	0.0	0.7	0.0	**0.4**	Concentration	0.542	0.24	NS
ICC 506	0.3	0.0	0.0	0.0	0.0	**0.1**	Genotype	0.589	0.22	NS
ICCV 10	0.0	0.7	0.0	0.0	1.0	**0.3**	Concentration	0.867	0.49	NS
L 550	0.7	1.0	0.0	0.7	0.0	**0.5**	×Genotype			
Mean	**0.4**	**0.6**	**0.0**	**0.3**	**0.2**					
	Eggs 5^{-1} plants at 48 h after spray									
C 235	1.3	0.0	0.3	0.0	0.0	**0.3**	Concentration	0.433	0.32	NS
ICC 506	0.3	0.0	0.0	0.0	0.0	**0.1**	Genotype	0.787	0.29	NS
ICCV 10	0.0	0.0	0.3	0.0	0.7	**0.2**	Concentration	0.560	0.65	NS
L 550	0.0	0.0	2.3	0.0	0.0	**0.5**	×Genotype			
Mean	**0.4**	**0.0**	**0.7**	**0.0**	**0.2**					
	Eggs 5^{-1} plants at 72 h after spray									
C 235	0.0	0.0	0.0	0.0	0.0	**0.0**	Concentration	0.455	0.17	NS
ICC 506	0.0	0.0	0.0	0.0	0.0	**0.0**	Genotype	0.123	0.15	NS
ICCV 10	1.7	0.0	0.0	0.3	0.3	**0.5**	Concentration	0.137	0.34	NS
L 550	0.0	1.0	0.0	0.0	0.0	**0.2**	×Genotype			
Mean	**0.4**	**0.2**	**0.0**	**0.1**	**0.1**					

LSD at P≤ 0.05 %.

Table 26: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting first spray)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
Larvae 5^{-1} plants before spray										
C 235	1.0^{abc}	2.0^{abcde}	6.0^{g}	4.0^{efg}	1.0^{abc}	**2.8**	**Concentration**	<0.001	0.45	1.29*
ICC 506	0.7^{ab}	1.7^{abcd}	3.0^{bcdef}	3.3^{cdef}	0.0^{a}	**1.7**	**Genotype**	<0.001	0.40	1.16*
ICCV 10	1.3^{abcd}	3.0^{bcdef}	3.0^{bcdef}	6.0^{g}	0.7^{ab}	**2.8**	**Concentration**	0.017	0.90	2.59*
L 550	6.0^{g}	8.3^{h}	4.7^{fg}	5.7^{fg}	3.7^{d}	**5.7**	**×Genotype**			
Mean	**2.2**	**3.7**	**4.2**	**4.7**	**1.3**					
Larvae 5^{-1} plants at 24 h after spray										
C 235	1.3	1.3	3.7	2.3	0.3	**1.8**^{a}	**Concentration**	0.030	0.60	1.71*
ICC 506	0.0	0.7	1.3	1.3	0.0	**0.7**^{a}	**Genotype**	0.001	0.53	1.53*
ICCV 10	1.0	3.0	3.3	2.0	0.3	**1.9**^{a}	**Concentration**	0.464	1.19	NS
L 550	6.7	5.0	4.0	3.3	0.7	**3.9**^{b}	**×Genotype**			
Mean	**2.2**^{b}	**2.5**^{b}	**3.1**^{b}	**2.2**^{b}	**0.3**^{a}					
Larvae 5^{-1} plants at 48 h after spray										
C 235	0.7^{ab}	3.0^{de}	2.7^{cde}	2.3^{bcde}	0.3^{a}	**1.8**	**Concentration**	<0.001	0.32	0.91*
ICC 506	0.7^{ab}	1.7^{abcde}	1.3^{abcd}	0.7^{ab}	0.0^{a}	**0.9**	**Genotype**	<0.001	0.28	0.81*
ICCV 10	1.3^{abcd}	3.3^{e}	3.3^{e}	1.7^{abcde}	1.0^{abc}	**2.1**	**Concentration**	0.034	0.63	1.82*
L 550	3.3^{e}	5.3^{f}	6.3^{f}	1.7^{abcde}	0.3^{a}	**3.4**	**×Genotype**			
Mean	**1.5**	**3.3**	**3.4**	**1.6**	**0.4**					
Larvae 5^{-1} plants at 72 h after spray										
C 235	2.0	2.0	3.3	3.3	2.3	**2.6**^{a}	**Concentration**	0.006	0.62	1.77*
ICC 506	2.7	1.7	2.3	1.7	0.0	**1.7**^{a}	**Genotype**	0.007	0.55	1.58*
ICCV 10	3.0	4.3	5.3	2.0	0.7	**3.1**^{ab}	**Concentration**	0.474	1.24	NS
L 550	7.0	5.0	6.7	2.7	1.3	**4.5**^{b}	**×Genotype**			
Mean	**3.7**^{bc}	**3.2**^{bc}	**4.4**^{c}	**2.4**^{ab}	**1.1**^{a}					

*LSD at P≤ 0.05 %.

effects were significant and very few larvae were recorded on ICC 506 across *Bt* concentrations. At 72 h after spray, the interaction effects were non-significant, but the differences between the genotypes and *Bt* concentrations were significant.

During 2006-07 post-rainy season December planting, there were no eggs on the genotypes across *Bt* concentrations before and after spray. The interaction effects for larval numbers before spraying, and at 24, 48, and 72 h after spraying were non-significant. However, differences between the genotypes and *Bt* concentrations were significant. The genotypes ICC 506, ICCV 10, and C 235 had lower numbers of *H. armigera* larvae as compared to that on L 550 (Table 27).

4.1.2 Damage rating (DR)

During the 2005-06 post-rainy season, there were significant differences between the genotypes and *Bt* concentrations (Table 28). The damage rating was significantly lower (DR 1.60 to 1.70) on ICC 506 and ICCV 10 as compared to C 235 and L 550 (DR 2.83 to 4.13). In the October planting during the 2006-07 post-rainy season, the differences between the genotypes and *Bt* concentrations were significant. The interaction effects were also significant (Table 29). The damage rating across *Bt* concentrations was lower on ICC 506 (1.40) and ICCV 10 (1.97) as compared to that on L 550 (4.03) and C 235 (5.10). In the December planting, the interaction effects were non-significant (Table 30). The damage rating was lower on ICC 506 (2.67) and ICCV 10 (3.53) as compared to that on L 550 (4.20) and C 235 (5.60).

4.1.3 Pod damage (%)

During the 2005-06 post-rainy season, the differences between the genotypes and *Bt* concentrations were significant (Table 28). The pod damage was lower in ICC 506

Table 27: Effect of *B. thuringiensis* sprays on the abundance of *H. armigera* larvae on four chickpea genotypes at the podding stage (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting second spray)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Larvae 5^{-1} plants before spray									
C 235	5.0	4.7	3.7	4.0	3.0	4.1^{ab}	Concentration	0.029	0.92	2.65*
ICC 506	3.3	4.3	2.7	1.0	0.7	2.4^{a}	Genotype	<0.001	0.83	2.37*
ICCV 10	4.3	5.7	8.0	3.3	3.0	4.9^{b}	Concentration ×Genotype	0.591	1.85	NS
L 550	14.3	10.3	8.3	7.0	5.3	9.1^{c}				
Mean	6.7^{c}	6.2^{bc}	5.7^{bc}	3.8^{ab}	3.0^{a}					
	Larvae 5^{-1} plants at 24 h after spray									
C 235	4.3	2.3	2.7	2.7	0.3	2.5^{ab}	Concentration	<0.001	0.63	1.79*
ICC 506	2.3	2.0	1.7	1.0	0.0	1.4^{a}	Genotype	<0.001	0.56	1.60*
ICCV 10	7.0	4.3	2.0	1.7	0.7	3.1^{b}	Concentration ×Genotype	0.177	1.25	NS
L 550	11.0	6.7	5.3	2.3	0.7	5.2^{c}				
Mean	6.2^{d}	3.8^{c}	2.9^{bc}	1.9^{ab}	0.4^{a}					
	Larvae 5^{-1} plants at 48 h after spray									
C 235	5.0	3.0	3.0	1.7	0.0	2.5^{b}	Concentration	<0.001	0.58	1.66*
ICC 506	2.3	1.3	0.7	0.3	0.0	0.9^{a}	Genotype	<0.001	0.52	1.49*
ICCV 10	4.0	2.7	1.3	0.7	0.7	1.9^{ab}	Concentration ×Genotype	0.720	1.16	NS
L 550	8.0	6.7	4.7	3.3	0.3	4.6^{c}				
Mean	4.8^{d}	3.4^{cd}	2.4^{bc}	1.5^{ab}	0.2^{a}					
	Larvae 5^{-1} plants at 72 h after spray									
C 235	3.3	0.3	2.3	0.7	0.7	1.5^{a}	Concentration	<0.001	0.68	1.95*
ICC 506	1.7	0.7	0.0	0.0	0.0	0.5^{a}	Genotype	<0.001	0.61	1.75*
ICCV 10	6.0	1.3	1.0	0.3	0.0	1.7^{a}	Concentration ×Genotype	0.225	1 36	NS
L 550	10.7	6.3	6.0	2.3	0.7	5.2^{b}				
Mean	5.4	2.2^{a}	2.3^{b}	0.8^{a}	0.3^{a}					

*LSD at P≤ 0.05 %.

Table 28: Effect of *B. thuringiensis* sprays on damage rating and pod damage by *H. armigera* on four chickpea genotypes (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
	Damage rating**									
C 235	4 0	3 2	2 7	2 3	2 0	**2.8^{b}**	Concentration	<0 001	0 20	0 57*
ICC 506	1 3	2 0	2 0	1 3	1 3	**1.6^{a}**	Genotype	<0 001	0 18	0 51*
ICCV 10	2 3	2 2	1 7	1 0	1 3	**1.7^{a}**	Concentration	0 157	0 40	NS
L 550	5 7	4 0	4 0	4 0	3 0	**4.1^{c}**	× Genotype			
Mean	**3.3^{d}**	**2.8^{cd}**	**2.6^{bc}**	**2.2^{ab}**	**1.9^{a}**					
	Pod damage (%)									
C 235	3 79 (11 10)	5 45 (13 48)	4 31 (11 80)	5 81 (13 73)	2 68 (9 36)	**4.41^{b} (11.89)**	Concentration	0 019	0 86	2 47*
ICC 506	0 99 (5 75)	1 40 (6 65)	0 42 (3 62)	1 68 (7 20)	2 63 (8 98)	**1.42^{a} (6.44)**	Genotype	<0 001	0 77	2 21*
ICCV 10	9 77 (17 97)	11 16 (19 49)	6 37 (14 61)	7 56 (15 15)	2 46 (8 98)	**7.47^{c} (15.24)**	Concentration	0 070	1 72	NS
L 550	7 17 (15 24)	6 04 (14 22)	4 19 (11 47)	4 55 (12 10)	4 35 (11 09)	**5.26^{b} (12.82)**	× Genotype			
Mean	**5.43^{bc} (12.52)**	**6.01^{c} (13.46)**	**3.82^{ab} (10.37)**	**4.90^{bc} (12.04)**	**3.03^{a} (9.60)**					

Figures in parenthesis are angular transformed values *LSD at P≤ 0 05 %

** Damage rating (1 = <10 %, 2 = 11 to 20 %, 3 = 21 to 30 %, 4 = 31 to 40 %, 5 = 41 to 50 %, 6 = 51 to 60 %, 7 = 61 to 70 %, 8 = 71 to 80 %, and 9 = >80 % pods damaged)

(1.42%) as compared to that on C 235 (4.41%), L 550 (5.26%), and ICCV 10 (7.47%). In the October planting during 2006-07 post-rainy season, the differences between the genotypes and *Bt* concentrations were significant, and the interaction effects were also significant (Table 29). The pod damage was lower in the *Bt* sprayed plots as compared to that in the unsprayed plots of different chickpea genotypes. In the December planting, differences between the genotypes and *Bt* concentrations were significant (Table 30). Pod damage was lower in ICC 506 (7.38%) as compared to that on C 235 (14.68%), ICCV 10 (15.14%), and L 550 (15.23%).

4.1.4 Grain yield

During the 2005-06 post-rainy season, the interaction effects were significant (Table 31), the genotype ICCV 10 recorded the highest yield plant^{-1} (14.91 g) across *Bt* concentrations. In the October planting during the 2006-07 post-rainy season, grain yield plant^{-1} was the highest in *Bt* sprayed plots as compared to that in the unsprayed plots of different chickpea genotypes (Table 32). In the December planting, the differences for grain yield per plant between the genotypes were significant (Table 33). The genotypes L 550 and ICCV 10 recorded the highest yield plant^{-1} (40.48 to 36.72 g) as compared to ICC 506 (33.13 g) and C 235 (27.25 g). The interaction effects were non-significant.

During the 2005-06 post-rainy season, the genotypes C 235 (1289.2 kg ha^{-1}) and ICCV 10 (1270.6 kg ha^{-1}) yielded more as compared to L 550 (1018.2 kg ha^{-1}) and ICC 506 (1060.4 kg ha^{-1}) (Table 31). During the 2006-07 post-rainy season, the differences between the genotypes and *Bt* concentrations were significant (Table 32 & 33). The *Bt* sprayed plots recorded higher grain yield as compared to the unsprayed plots of different chickpea genotypes. Grain yield was higher in ICCV 10 and

Table 29: Effect of *B. thuringiensis* sprays on damage rating and pod damage by *H. armigera* on four chickpea genotypes (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
				Damage rating**						
C 235	6.3^{h}	5.8^{gh}	5.7^{fgh}	4.3^{de}	3.3^{cd}	**5.1**	Concentration	<0.001	0.21	0.61*
ICC 506	2.0^{ab}	2.0^{ab}	1.0^{a}	1.0^{a}	1.0^{a}	**1.4**	Genotype	<0.001	0.19	0.54*
ICCV 10	3.3^{cd}	2.8^{bc}	1.0^{a}	1.7^{ab}	1.0^{a}	**2.0**	Concentration × Genotype	0.025	0.43	1.22*
L 550	5.0^{efg}	5.7^{fgh}	4.5^{def}	3.3^{cd}	1.7^{ab}	**4.0**				
Mean	**4.2**	**4.1**	**3.0**	**2.6**	**1.7**					
				Pod damage (%)						
C 235	20.53^{bcd} (26.91)	19.65^{bcd} (26.18)	16.35^{abc} (23.81)	17.12^{abc} (24.39)	16.75^{abc} (24.03)	**18.08 (25.07)**	Concentration	<0.001	1.19	3.42*
ICC 506	23.91^{cde} (29.16)	39.44^{f} (38.58)	18.96^{abcd} (25.78)	12.11^{ab} (20.19)	11.00^{a} (19.24)	**21.08 (26.59)**	Genotype	0.003	1.07	3.06*
ICCV 10	35.72^{f} (36.64)	31.83^{ef} (34.29)	27.91^{def} (31.90)	16.10^{abc} (23.51)	19.46^{ab} (25.98)	**26.20 (30.46)**	Concentration × Genotype	0.022	2.39	6.84*
L 550	23.49^{cde} (28.68)	31.88^{ef} (34.19)	28.27^{def} (32.12)	23.96^{cde} (29.24)	16.05^{abc} (23.43)	**24.73 (29.53)**				
Mean	**25.91 (30.35)**	**30.70 (33.31)**	**22.87 (28.40)**	**17.32 (24.33)**	**15.81 (23.17)**					

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %.

** Damage rating (1 = <10 %, 2 = 11 to 20 %, 3 = 21 to 30 %, 4 = 31 to 40 %, 5 = 41 to 50 %, 6 = 51 to 60 %, 7 = 61 to 70 %, 8 = 71 to 80 %, and 9 = >80 % pods damaged).

Table 30: Effect of *B. thuringiensis* sprays on damage rating and pod damage by *H. armigera* on four chickpea genotypes (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting)

Genotype	*Bt* concentration (%) 0.00	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
				Damage rating**						
C 235	5.7	7.3	5.7	5.7	3.7	**5.6^{c}**	Concentration	0.004	0.37	1.05*
ICC 506	1.7	4.0	2.3	2.7	2.7	**2.7^{a}**	Genotype	<0.001	0.33	0.94*
ICCV 10	3.0	4.7	4.0	3.3	2.7	**3.5^{ab}**	Concentration × Genotype	0.738	0.73	NS
L 550	4.7	4.7	4.7	4.0	3.0	**4.2^{b}**				
Mean	**3.7^{ab}**	**5.2^{c}**	**4.2^{bc}**	**3.9^{ab}**	**3.0^{a}**					
				Pod damage (%)						
C 235	14.71 (22.35)	16.29 (23.75)	15.43 (22.12)	18.35 (25.35)	8.61 (16.96)	**14.68^{b} (22.11)**	Concentration	0.019	1.36	3.90*
ICC 506	2.88 (9.57)	9.67 (17.78)	10.81 (18.74)	8.37 (16.44)	5.18 (12.97)	**7.38^{a} (15.10)**	Genotype	<0.001	1.22	3.49*
ICCV 10	9.27 (17.29)	19.69 (26.17)	14.80 (21.83)	18.74 (25.49)	13.20 3 (20.96)	**15.14^{b} (22.35)**	Concentration × Genotype	0.227	2.72	NS
L 550	18.08 (25.01)	10.01 (17.58)	19.90 (26.33)	18.38 (25.23)	9.77 (18.15)	**15.23^{b} (22.46)**				
Mean	**11.24^{ab} (18.55)**	**13.92^{bc} (21.32)**	**15.23^{bc} (22.25)**	**15.96^{c} (23.13)**	**9.19^{a} (17.26)**					

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %.

** Damage rating (1 = <10 %, 2 = 11 to 20 %, 3 = 21 to 30 %, 4 = 31 to 40 %, 5 = 41 to 50 %, 6 = 51 to 60 %, 7 = 61 to 70 %, 8 = 71 to 80 %, and 9 = >80 % pods damaged).

Table 31: Effect of *B. thuringiensis* sprays on grain yield of four chickpea genotypes (ICRISAT, Patancheru, 2005-06 post-rainy season)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Yield plant^{-1} (g)									
C 235	11.37^{bcde}	10.18^{cde}	11.43^{bcde}	19.07^{a}	15.57^{ab}	**13.53**	**Concentration**	0.180	0.98	**NS**
ICC 506	10.77^{bcde}	9.66^{de}	16.29^{ab}	12.61^{bcde}	10.04^{cde}	**11.87**	**Genotype**	0.035	0.88	2.52*
ICCV 10	15.52^{abc}	16.29^{ab}	11.18^{bcde}	15.99^{ab}	15.57^{ab}	**14.91**	**Concentration**			
L 550	14.73^{abcd}	8.17^{e}	13.85^{abcd}	10.81^{bcde}	10.09^{cde}	**11.53**	**× Genotype**	0.026	1.96	5.63*
Mean	**13.10**	**11.08**	**13.19**	**14.62**	**12.82**					
	Yield (kg ha^{-1})									
C235	1115.0^{bcde}	1092^{bcde}	1185.0^{bcd}	1672.0^{a}	1382.0^{ab}	**1289.2**	**Concentration**	0.098	57.10	163.50*
ICC506	1237.0^{bcde}	1018.0^{cde}	1016.0^{cde}	860.0^{e}	1171.0^{bcde}	**1060.4**	**Genotype**	<0.001	51.10	146.30*
ICCV10	1254.0^{bcde}	1399.0^{ab}	1035.0^{cde}	1283.0^{bc}	1382.0^{ab}	**1270.6**	**Concentration**			
L550	958.0^{de}	963.0^{de}	974.0^{de}	1162.0^{bcde}	1034.0^{cde}	**1018.2**	**× Genotype**	0.043	114.20	327.10*
Mean	**1141.0**	**1118.0**	**1052.0**	**1244.3**	**1242.3**					

*LSD at P≤ 0.05 %.

Table 32: Effect of *B. thuringiensis* sprays on grain yield of four chickpea genotypes (ICRISAT, Patancheru, 2006-07 post-rainy season, October planting)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Yield plant^{-1} (g)									
C 235	11.67	12.57	11.13	15.12	17.25	**13.55^{b}**	**Concentration**	0.011	1.36	3.90*
ICC 506	13.75	16.40	18.15	18.99	16.83	**16.82^{b}**	**Genotype**	0.002	1.22	3.49*
ICCV 10	15.32	19.96	19.41	28.83	20.06	**20.72^{a}**	**Concentration**			
L 550	11.89	13.30	15.48	14.06	23.49	**15.64^{b}**	**× Genotype**	0.288	2.73	**NS**
Mean	**13.16^{c}**	**15.56^{bc}**	**16.04^{abc}**	**19.25^{ab}**	**19.41^{a}**					
	Yield (kg ha^{-1})									
C 235	928.0	1282.0	1217.0	1561.0	1726.0	**1342.8^{c}**	**Concentration**	<0.001	65.90	188.80*
ICC 506	732.0	1440.0	1561.0	2165.0	1879.0	**1555.4^{b}**	**Genotype**	<0.001	59.00	168.80*
ICCV 10	1939.0	2159.0	2535.0	2497.0	2619.0	**2349.8^{a}**	**Concentration**			
L 550	1004.0	1248.0	1429.0	1548.0	1699.0	**1385.6^{bc}**	**× Genotype**	0.121	131.90	**NS**
Mean	**1150.8^{b}**	**1532.3^{b}**	**1685.5^{b}**	**1942.8^{a}**	**1980.8^{a}**					

*LSD at P≤ 0.05 %.

Table 33: Effect of *B. thuringiensis* sprays on grain yield of four chickpea genotypes (ICRISAT, Patancheru, 2006-07 post-rainy season, December planting)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Yield plant^{-1}									
C 235	39.87	22.33	24.93	25.53	23.60	**27.25^{c}**	Concentration	0.064	1.98	NS
ICC 506	34.53	35.07	35.80	34.40	25.87	**33.13^{b}**	Genotype	<0.001	1.77	5.08*
ICCV 10	36.20	33.40	45.33	36.73	31.93	**36.72^{ab}**	Concentration			
L 550	42.40	43.80	35.33	43.33	37.53	**40.48^{a}**	× Genotype	0.165	3.97	NS
Mean	**38.25**	**33.65**	**35.35**	**35.00**	**29.73**					
	Yield (kg ha^{-1})									
C 235	2249.0	2563.0	1991.0	2128.0	1986.0	**2183.4^{c}**	Concentration	<0.001	94.50	270.50*
ICC 506	2951.0	2625.0	2483.0	2197.0	2470.0	**2545.2^{b}**	Genotype	<0.001	84.50	241.90*
ICCV 10	3256.0	3276.0	2791.0	2693.0	2278.0	**2858.8^{a}**	Concentration			
L 550	2322.0	2708.0	2142.0	2177.0	1821.0	**2234.0^{c}**	× Genotype	0.488	188.90	NS
Mean	**2694.5^{a}**	**2793.0^{a}**	**2351.8^{b}**	**2298.8^{b}**	**2138.8^{b}**					

* LSD at P≤ 0.05 %.

ICC 506 (2858.8 and 2545.2 kg ha^{-1}) as compared to C 235 (2183.4 kg ha^{-1}) and L 550 (2234.0 kg ha^{-1}) across *Bt* concentrations during the 2006-07 post-rainy season in the December planting.

4.1.5 Evaluation of biological activity of *B. thuringiensis* against *H. armigera* on different chickpea genotypes under laboratory conditions (detached leaf assay)

At the vegetative stage during the 2006-07 post-rainy season, the interaction effects were significant for leaf damage (Table 34). The genotype ICC 506 recorded lower leaf damage rating (2.56), followed by ICCV 10 (3.12) as compared to that on L 550 (4.68) and C 235 (3.68). The leaf damage rating was the lowest in the chickpea genotypes sprayed with *Bt* as compared to the unsprayed genotypes. At the flowering stage after first spray during the 2006-07 post-rainy season, the interaction effects for leaf damage were significant (Table 35). Leaf damage rating was low on ICC 506 (2.64) and C 235 (3.08) as compared to that on ICCV 10 (3.80) and L 550 (3.72). The chickpea genotypes sprayed with 0.05 per cent *Bt* recorded the highest leaf damage as compared to the unsprayed plots and other *Bt* sprayed plots of different chickpea genotypes. After second spray during the 2006-07 post-rainy season, the interaction effects for leaf damage between the genotypes × *Bt* concentrations were significant (Table 36). Leaf damage was low on ICC 506 (3.20) and C 235 (3.34) as compared to that on L 550 (4.16) and ICCV 10 (3.78). The chickpea genotypes sprayed with *Bt* recorded lower leaf damage as compared to the unsprayed genotypes (6.10).

During the 2006-07 post-rainy season at the vegetative stage, the interaction effects were significant for larval survival (Table 34). Larval survival was the lowest in the larvae fed on ICCV 10 (65.60%), C 235 (68.40%), and ICC 506 (68.80%) as

compared to those fed on L 550 (82.00%). During the 2006-07 post-rainy season at the flowering stage after first spray, there were significant interaction effects for larval survival (Table 35). The larval survival was the lowest in the larvae reared on plant material from the plots treated with 0.5 per cent *Bt* (27.50%), followed by those treated with 0.2 per cent *Bt* (52.00%). During the 2006-07 post-rainy season at the flowering stage after second spray, the interaction effects between the genotypes × *Bt* concentrations were significant for larval survival (Table 36). The genotypes C 235 (42.00%), ICCV 10 (46.40%), and ICC 506 (49.60%) recorded the lowest larval survival as compared to that on L 550 (55.20%). The chickpea genotypes sprayed with *Bt* had lower larval survival as compared to the unsprayed genotypes (64.50%).

At the vegetative stage during the 2006-07 post-rainy season, the interaction effects were significant for larval weight (Table 34). Larval weight was lower in the larvae fed on ICC 506 (16.18 mg) as compared to those fed on L 550 (34.20 mg), C 235 (28.43 mg), and ICCV 10 (24.26 mg). At the flowering stage after first spray during the 2006-07 post-rainy season, there were significant interaction effects for larval weight (Table 35). The larval weight was lower in the larvae fed on the chickpea genotypes treated with 0.5 per cent *Bt* (5.10 mg), followed by those treated with 0.2 per cent *Bt* (13.60 mg). At the flowering stage after second spray during the 2006-07 post-rainy season, the interaction effects between the genotypes × *Bt* concentrations were significant for larval weight (Table 36). Larval weights were lower in the larvae fed on C 235 (21.20 mg) and ICC 506 (22.60 mg) as compared to those fed on L 550 (30.90 mg) and ICCV 10 (27.00 mg). The larvae fed on plants from sprayed plots weighed lower as compared to those reared on unsprayed genotypes (46.40 mg).

Table 34: Survival of neonate larvae of *H. armigera* on four chickpea genotypes sprayed with *Bt* at the vegetative stage (ICRISAT, Patancheru, 2006-07 post-rainy season)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Leaf damage rating**									
C 235	6.20^{ij}	4.60^{fgh}	4.00^{efg}	2.20^{abc}	1.40^{ab}	**3.68**	**Concentration**	<0.001	0.23	0.65*
ICC 506	4.00^{efg}	2.60^{bcd}	3.00^{cde}	2.20^{abc}	1.00^{a}	**2.56**	**Genotype**	<0.001	0.21	0.58*
ICCV 10	5.20^{ghi}	5.00^{ghi}	3.20^{cde}	1.20^{a}	1.00^{a}	**3.12**	**Concentration**	0.024	0.46	1.30*
L 550	7.00^{j}	5.80^{hij}	4.00^{efg}	3.00^{cde}	3.60^{def}	**4.68**	**× Genotype**			
Mean	**5.60**	**4.50**	**3.55**	**2.15**	**1.75**					
	Larval survival (%)									
C 235	92.00^{g} (77.30)	80.00^{efg} (64.40)	82.00^{efg} (65.80)	44.00^{ab} (41.30)	44.00^{ab} (40.90)	**68.40 (57.90)**	**Concentration**	<0.001	2.59	7.29*
ICC 506	84.00^{efg} (67.00)	68.00^{cde} (56.20)	80.00^{efg} (64.40)	82.00^{efg} (67.70)	30.00^{a} (33.10)	**68.80 (57.70)**	**Genotype**	<0.001	2.31	6.52*
ICCV 10	88.00^{fg} (72.00)	80.00^{efg} (65.40)	78.00^{def} (62.40)	48.00^{abc} (43.80)	32.00^{ab} (34.20)	**65.60 (55.60)**	**Concentration**	0.050	5.17	14.58*
L 550	92.00^{g} (77.30)	92.00^{g} (77.30)	90.00^{fg} (76.00)	80.00^{efg} (66.50)	56.00^{bcd} (48.50)	**82.00 (69.10)**	**× Genotype**			
Mean	**89.00 (73.40)**	**80.50 (65.80)**	**82.50 (67.20)**	**63.50 (54.80)**	**40.50 (39.20)**					
	Larval weight (5 DAI) (mg)									
C 235	50.76^{gh}	42.28^{fg}	30.22^{def}	7.60^{abc}	11.30^{abc}	**28.43**	**Concentration**	<0.001	2.22	6.25*
ICC 506	30.12^{def}	14.88^{bc}	18.92^{cd}	14.64^{abc}	2.32^{a}	**16.18**	**Genotype**	<0.001	1.98	5.59*
ICCV 10	56.12^{h}	31.46^{ef}	18.48^{cd}	10.08^{abc}	5.16^{ab}	**24.26**	**Concentration**	0.011	4.43	12.49*
L 550	61.38^{h}	40.26^{fg}	33.80^{f}	16.52^{bc}	19.04^{cde}	**34.20**	**× Genotype**			
Mean	**49.59**	**32.22**	**25.36**	**12.21**	**9.46**					

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %. DAI- Days after initiation of experiment.

** Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged).

Table 35: Survival of neonate larvae of *H. armigera* on four chickpea genotypes sprayed with *Bt* at the flowering stage first spray (ICRISAT, Patancheru, 2006-07 post-rainy season)

Genotype	*Bt* concentration (%)								
	0.00	**0.05**	**0.10**	**0.20**	**0.50**	**Mean**		**Fp**	**SE±**	**LSD**
	Leaf damage rating**									
C 235	4.20^{efg}	4.00^{def}	3.40^{cde}	2.00^{abc}	1.80^{ab}	**3.08**	**Concentration**	<0.001	0.25	0.70*
ICC 506	1.80^{ab}	4.00^{def}	3.20^{bcde}	2.60^{abcd}	1.60^{a}	**2.64**	**Genotype**	<0.001	0.22	0.63*
ICCV 10	3.60^{de}	5.80^{h}	5.60^{gh}	2.60^{abcd}	1.40^{a}	**3.80**	**Concentration**			
L 550	5.40^{fgh}	5.20^{fgh}	3.60^{de}	3.20^{bcde}	1.20^{a}	**3.72**	**× Genotype**	0.001	0.50	1.41*
Mean	**3.75**	**4.75**	**3.95**	**2.60**	**1.50**					
	Larval survival (%)									
C 235	60.00^{cd} (51.00)	58.00^{cd} (49.70)	54.00^{bcd} (47.20)	50.00^{bcd} (44.90)	42.00^{abc} (39.70)	**52.80 (46.50)**	**Concentration**	<0.001	2.47	6.95*
ICC 506	34.00^{ab} (35.00)	70.00^{de} (57.50)	52.00^{bcd} (46.20)	56.00^{bcd} (48.50)	26.00^{a} (30.40)	**47.60 (43.50)**	**Genotype**	0.445	2.21	**NS**
ICCV 10	60.00^{cd} (51.00)	72.00^{de} (58.70)	76.00^{e} (66.50)	42.00^{abc} (39.70)	20.00^{a} (26.60)	**54.00 (48.50)**	**Concentration**			
L 550	64.00^{cde} (53.40)	62.00^{cd} (52.40)	60.00^{cd} (51.50)	60.00^{cd} (50.90)	22.00^{a} (27.20)	**53.60 (47.10)**	**× Genotype**	0.019	4.94	13.91*
Mean	**54.50 (47.60)**	**65.50 (54.60)**	**60.50 (52.80)**	**52.00 (46.00)**	**27.50 (31.00)**					
	Larval weight (5 DAI) (mg)									
C 235	22.60^{fghi}	22.20^{efghgi}	21.70^{efghi}	15.80^{bcdefg}	10.40^{abcdef}	**18.50**	**Concentration**	<0.001	2.43	6.85*
ICC 506	7.30^{abcd}	30.00^{hij}	23.60^{fghi}	$13.20^{abcdefg}$	5.40^{abc}	**15.90**	**Genotype**	0.135	2.18	**NS**
ICCV 10	25.60^{ghi}	40.30^{j}	31.60^{ij}	8.70^{abcde}	1.70^{a}	**21.60**	**Concentration**			
L 550	38.70^{j}	33.30^{ij}	21.00^{defghi}	16.80^{cdefgh}	2.70^{ab}	**22.50**	**× Genotype**	0.010	4.87	13.70*
Mean	**23.60**	**31.50**	**24.50**	**13.60**	**5.10**					

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %. DAI- Days after initiation of experiment.
**Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged).

Table 36: Survival of neonate larvae of *H. armigera* on four chickpea genotypes sprayed with *Bt* at flowering stage second spray (ICRISAT, Patancheru, 2006-07 post-rainy season)

Genotype	*Bt* concentration (%)					Mean		Fp	SE±	LSD
	0.00	0.05	0.10	0.20	0.50					
	Leaf damage rating**									
C 235	5.40^{i}	4.20^{fgh}	3.10^{def}	3.00^{de}	1.00^{a}	**3.34**	**Concentration**	<0.001	0.20	0.57*
ICC 506	5.00^{hi}	3.60^{defg}	2.80^{cd}	2.80^{cd}	1.80^{abc}	**3.20**	**Genotype**	0.001	0.18	0.51*
ICCV 10	7.00^{j}	4.60^{ghi}	3.20^{def}	2.60^{bcd}	1.50^{ab}	**3.78**	**Concentration**			
L 550	7.00^{j}	5.40^{i}	4.00^{efgh}	3.40^{def}	1.00^{a}	**4.16**	**× Genotype**	0.046	0.41	1.14*
Mean	**6.10**	**4.45**	**3.28**	**2.95**	**1.33**					
	Larval survival (%)									
C 235	52.00^{cde} (46.20)	50.00^{cde} (45.00)	34.00^{bc} (35.40)	50.00^{cde} (44.90)	24.00^{ab} (29.10)	**42.00 (40.10)**	**Concentration**	<0.001	2.34	6.58*
ICC 506	56.00^{cde} (48.50)	54.00^{cde} (47.30)	54.00^{cde} (47.30)	40.00^{bcd} (39.00)	44.00^{bcd} (42.00)	**49.60 (44.80)**	**Genotype**	0.015	2.09	5.89*
ICCV 10	60.00^{de} (50.90)	48.00^{cde} (43.80)	48.00^{cde} (43.20)	42.00^{bcd} (40.20)	34.00^{bc} (35.40)	**46.40 (42.70)**	**Concentration**			
L 550	90.00^{f} (78.50)	68.00^{e} (55.80)	60.00^{de} (51.50)	46.00^{bcd} (42.50)	12.00^{a} (20.10)	**55.20 (49.70)**	**× Genotype**	<0.001	4.67	13.16*
Mean	**64.50 (56.00)**	**55.00 (48.00)**	**49.00 (44.40)**	**44.50 (41.60)**	**28.50 (31.60)**					
	Larval weight (5 DAI) (mg)									
C 235	53.10^{gh}	20.00^{bcd}	15.00^{abc}	14.20^{abc}	3.90^{a}	**21.20**	**Concentration**	<0.001	2.51	7.07*
ICC 506	26.40^{cde}	25.00^{bcde}	28.00^{cde}	19.60^{bcd}	14.20^{abc}	**22.60**	**Genotype**	0.014	2.24	6.32*
ICCV 10	44.00^{fg}	37.60^{ef}	25.70^{cde}	16.40^{abc}	11.30^{ab}	**27.00**	**Concentration**			
L 550	62.00^{gh}	33.50^{def}	31.00^{def}	25.20^{bcde}	2.50^{a}	**30.90**	**× Genotype**	0.002	5.02	14.14*
Mean	**46.40**	**29.00**	**24.90**	**18.90**	**8.00**					

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %. DAI- Days after initiation of experiment.
**Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged).

4.2 INTERACTION OF ACID EXUDATES (MALIC AND OXALIC ACID) WITH BIOLOGICAL ACTIVITY OF *Bt* TOXINS AGAINST *H. armigera*

4.2.1 Survival and development of *H. armigera* on different chickpea genotypes

There were significant differences in survival and development of the pod borer, *H. armigera* on different chickpea genotypes (Table 37).

The larval weights at 10 days after initiation of experiment (DAI) were significantly lower (45.49 to 53.92 mg) in the larvae reared on leaves and pods of ICC 506 and ICCV 10 as compared to those larvae reared on the leaves and pods of C 235 and L 550 (80.72 to 113.04 mg). The pupal weights were significantly lower (235.2 mg) in larvae reared on ICC 506 as compared on those reared on L 550 (262.3 mg). The larval period was prolonged by more than two days on ICC 506 and ICCV 10 (21.85 and 21.02) as compared to that on L 550 (18.93). Differences in pupal period were non-significant. Pupation was lower on ICC 506 (34.00%) and ICCV 10 (42.00%) as compared to that on C 235 (64.00%) and L 550 (76.00%). Similarly, adult emergence was also lower on ICC 506 (63.75%) as compared to that on L 550 (84.09%). The interaction effects were significant for fecundity. Fecundity was lower on ICC 506 (533.2 eggs female^{-1}) and ICCV 10 (726.0 eggs female^{-1}) as compared to that on C 235 (897.0 eggs female^{-1}) and L 550 (1226.2 eggs female^{-1}).

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

There were significant differences in survival and development of the pod borer, *H. armigera* on the unsprayed and *Bt* sprayed chickpea genotypes (Table 38).

Table 37: Survival and development of neonate larvae of *H. armigera* on four chickpea genotypes (ICRISAT, Patancheru, 2006-07 post-rainy season)

Genotype	Larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult longevity		Fecundity (Eggs female^{-1})
							Male (days)	Female (days)	
C 235	80.72^{b}	256.20^{b}	20.47^{b}	13.13	64.00 (53.23)^{b}	81.24 (64.51)^{b}	15.20	12.20	897.00^{c}
ICC 506	45.49^{a}	235.20^{a}	21.85^{a}	13.62	34.00 (35.32)^{a}	63.75 (53.29)^{a}	12.87	11.17	533.20^{a}
ICCV 10	53.92^{a}	253.00^{b}	21.02^{ab}	12.93	42.00 (40.38)^{a}	76.00 (60.69)^{b}	13.80	13.30	726.00^{b}
L 550	113.04^{c}	262.30^{b}	18.93^{c}	12.82	76.00 (61.20)^{c}	84.09 (66.70)^{b}	15.96	11.88	1226.20^{d}
Fp	<0.001	0.022	<0.001	0.351	<0.001	0.006	0.103	0.246	<0.001
SE±	3.45	5.41	0.31	0.33	2.12	2.26	0.86	0.71	31.50
LSD	10.62*	16.66*	0.95*	**NS**	6.52*	6.98*	**NS**	**NS**	97.20*
CV (%)	10.50	4.80	3.30	5.60	10.00	8.30	13.30	13.00	8.30

DAI = Days after initiation of experiment. *LSD at P≤ 0.05 %.
Figures in parenthesis are angular transformed values.

4.2.2.1 Larval and pupal weights

The interaction effects between the genotypes × *Bt* concentrations for larval weight at 10 DAI were significant (Table 38). There was no larval survival in larvae fed on plant material from plots sprayed with 0.5 per cent *Bt*. The lowest larval weight (5.51 mg) was recorded in the insects reared on ICC 506 sprayed with 0.2 per cent *Bt*, followed by those reared on ICCV 10.

The interaction effects between the genotypes × *Bt* concentrations were significant for pupal weight. Pupal weights were lower in the larvae reared on ICC 506 (180.20 mg) and ICCV 10 (208.50 mg) sprayed with 0.05 per cent *Bt* as compared to those reared on the unsprayed L 550 (262.30 mg). None of the larvae survived on plant material treated with more than 0.05 per cent *Bt*.

4.2.2.2 Larval and pupal periods

The interaction effects were significant for larval and pupal periods (Table 38). The larval period was prolonged by more than two days in insects reared on plant material from the unsprayed plots of ICC 506 and ICCV 10 (21.85 and 21.02) as compared to the larvae reared on the unsprayed L 550 (18.93). Pupal period was longer on ICC 506 sprayed with 0.05 per cent *Bt* (14.27 days) as compared to that on L 550 (9.10 days) and ICCV 10 (9.80 days) sprayed with 0.05 per cent *Bt*.

4.2.2.3 Pupation and adult emergence (%)

The pupation was lower on ICCV 10 (10.00%), L 550 (16.00%), and ICC 506 (26.00%) sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550 (76.00%).

Table 38: Survival and development of neonate larvae of *H. armigera* on four chickpea genotypes sprayed with *Bt* (ICRISAT, Patancheru, 2006-07 post-rainy season)

Genotype	*Bt* concentration (%) 0.00**	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
Larval weight (10 DAI) (mg)										
C235	80.72	36.86^{e}	17.67^{bc}	-	-	13.63	Concentration	<0.001	1.52	4.31*
ICC506	45.49	65.04^{f}	26.10^{cd}	5.51^{a}	-	24.16	Genotype	<0.001	1.52	4.31*
ICCV10	53.92	30.70^{de}	13.60^{ab}	17.90^{bc}	-	15.55	Concentration	<0.001	3.05	8.62*
L550	113.04	61.76^{f}	29.26^{de}	18.16^{bc}	-	27.30	× Genotype			
Mean	--	48.59	21.66	10.39	-					
Pupal weight (mg)										
C235	256.20	-	-	-	-	-	Concentration	<0.001	2.11	5.97*
ICC506	235.20	180.20^{a}	-	-	-	45.10	Genotype	<0.001	2.11	5.97*
ICCV10	253.00	208.50^{b}	-	-	-	52.10	Concentration	<0.001	4.22	11.93*
L550	262.30	234.70^{c}	-	-	-	58.70	× Genotype			
Mean	-	155.80	-	-	-					
Larval period (days)										
C235	20.47	-	-	-	-	-	Concentration	<0.001	0.05	0.13*
ICC506	21.85	20.63^{c}	-	-	-	5.16	Genotype	<0.001	0.05	0.13*
ICCV10	21.02	18.60^{a}	-	-	-	4.65	Concentration	<0.001	0.09	0.27*
L550	18.93	20.20^{b}	-	-	-	5.05	× Genotype			
Mean	-	14.86	-	-	-					
Pupal period (days)										
C235	13.13	-	-	-	-	-	Concentration	<0.001	0.56	1.58*
ICC506	13.62	14.27^{b}	-	-	-	3.57	Genotype	<0.001	0.56	1.58*
ICCV10	12.93	9.80^{a}	-	-	-	2.45	Concentration	<0.001	1.12	3.17*
L550	12.82	9.10^{a}	-	-	-	2.28	× Genotype			
Mean	-	8.29	-	-	-					
Pupation (%)										
C235	64.00 (53.23)	-	-	-	-	-	Concentration	<0.001	0.3	0.93*
ICC506	34.00 (35.32)	26.00^{c} (30.55)	.	.	.	6.50 (7.64)	Genotype	<0.001	0.33	0.93*
ICCV10	42.00 (40.38)	10.00^{a} (18.44)	.	-	.	2.50 (4.61)	Concentration	<0.001	0.66	1.86*
L550	76.00 (61.20)	16.00^{b} (23.31)	.	.	.	4.00 (5.83)	× Genotype			
Mean	-	13.00 (18.08)	0.00	0.00	0.00					

**Control (0.00) values were given only for the comparison (analysis was not done). DAI= Days after initiation of experiment. Figures in the parenthesis are angular transformed values. *LSD at P≤ 0.05 %.

The interaction effects between the genotypes × *Bt* concentrations for adult emergence were significant (Table 39). Adult emergence was lower on ICCV 10 (60.00%), L 550 (70.00%), and ICC 506 (76.70%) sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550 (84.09%).

4.2.2.5 Adult longevity and fecundity

The interaction effects for adult longevity were significant (Table 39). Female longevity was shorter on L 550 (2.80 days), ICCV 10 (4.00 days), and ICC 506 (6.40 days) sprayed with 0.05 per cent *Bt* as compared to that on unsprayed ICCV 10 (13.30 days). No males survived on ICCV 10 sprayed with 0.05 per cent *Bt*. The shortest male survival was recorded on L 550 (5.00 days) and ICC 506 (6.40 days) sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550 (15.96 days) and C 235 (15.20 days).

The interaction effects for fecundity were non-significant, but the differences between *Bt* concentrations were significant (Table 39). The fecundity was lower on ICC 506 (166.00 eggs female^{-1}) and L 550 (232.00 eggs female^{-1}) sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550 (1226.20 eggs female^{-1}).

4.2.3 The HPLC profiles of acid exudates in chickpea

The HPLC analysis of leaf samples at the vegetative and flowering stages and of pod samples revealed the following results.

4.2.3.1 Vegetative stage

The genotypes ICC 506, ICCV 10, C 235, and L 550 had first three peaks (Peak 1, 2, and 3), oxalic, and malic acid as common peaks (Table 40). However, the genotypes

Table 39: Adult emergence, longevity, and fecundity of insects of *H. armigera* on four chickpea genotypes sprayed with *Bt* (ICRISAT, Patancheru, 2006-07 post-rainy season)

Genotype	*Bt* concentration (%)									
	0.00**	0.05	0.10	0.20	0.50	Mean		Fp	SE±	LSD
Adult emergence (%)										
C235	81.24 (64.51)	-	-	-	-	-	Concentration	<0.001	3.80	10.75*
ICC506	63.75 (53.29)	76.70[a] (66.90)	-	-	-	19.20 (16.70)	Genotype	0.009	3.80	10.75*
ICCV10	76.00 (60.69)	60.00[a] (54.00)	-	-	-	15.00 (13.50)	Concentration	<0.001	7.60	21.50*
L550	84.09 (66.70)	70.00[a] (63.00)	-	-	-	17.50 (15.80)	× Genotype			
Mean	-	51.70 (46.00)	0.00	0.00	0.00					
Female longevity (days)										
C235	12.20	-	-	-	-	-	Concentration	<0.001	0.36	1.02*
ICC506	11.17	6.40[b]	-	-	-	1.60	Genotype	0.024	0.36	1.02*
ICCV10	13.30	4.00[a]	-	-	-	1.00	Concentration	0.002	0.72	2.04*
L550	11.88	2.80[a]	-	-	-	0.70	× Genotype			
Mean	-	3.30	-	-	-					
Male longevity (days)										
C235	15.20	-	-	-	-	-	Concentration	<0.001	0.34	0.95*
ICC506	12.87	6.40[a]	-	-	-	1.60	Genotype	<0.001	0.34	0.95*
ICCV10	13.80	-	-	-	-	0.00	Concentration	<0.001	0.67	1.90*
L550	15.96	5.00[a]	-	-	-	1.25	× Genotype			
Mean	-	0.00	-	-	-					
Fecundity (eggs female^{-1})										
C235	897.00	-	-	-	-	-	Concentration	0.002	20.90	59.10*
ICC506	533.20	166.00	-	-	-	42.00	Genotype	0.125	20.90	NS
ICCV10	726.00	-	-	-	-	-	Concentration	0.056	41.80	NS
L550	1226.20	232.00	-	-	-	58.00	× Genotype			
Mean	-	100.00[a]	-	-	-					

** Control (0.00) values were given only for the comparison (analysis was not done). Figures in parenthesis are angular transformed. *LSD at P≤ 0.05 %.

ICC 506 and L 550 had an extra peak at peak 10, ICCV 10 had an extra peak at peak 5, whereas C 235 had three extra peaks 4, 8, and 9 (Fig 1; Table 40).

Oxalic acid peak had 73.95 per cent area in ICC 506, 33.90 per cent in ICCV 10, 20.62 per cent in C 235, and 67.78 per cent in L 550. Malic acid comprised of 2.48 per cent peak area in ICC 506, 10.24 per cent in ICCV 10, 7.84 per cent in C 235, and 5.22 per cent in L 550 (Table 40).

4.2.3.2 Flowering stage

The peaks 1, 2, and 3, oxalic acid, malic acid, and peak 13 were common for the genotypes ICC 506, ICCV 10, C 235, and L 550. The peak 4 was present in C 235 only, peak 7 was present in ICCV 10 and C 235, and peak 8 was present in ICC 506, C 235, and L 550. The peaks 9, 10, and 12 were present in ICCV 10 only. Peak 11 was present in ICC 506 and C 235 (Fig 2; Table 41).

Oxalic acid comprised of 82.42 per cent peak area in ICC 506, 61.91 per cent in ICCV 10, 48.26 per cent in C 235, and 77.93 per cent in L 550. Peak area for malic acid was 3.92 per cent in ICC 506, 22.09 per cent in ICCV 10, 18.92 per cent in C 235, and 8.05 per cent in L 550 (Table 41).

4.2.3.3 Podding stage

The peaks 2, 3, and 4, oxalic acid, malic acid, and fumaric acid were common for all the genotypes. The genotype ICC 506 had an extra peak 1. Peak 6 was present in ICC 506, ICCV 10, and C 235. The genotypes C 235 and L 550 had peaks 7 and 8. Peak 9 was present in C 235 only. The citric acid was present in ICCV 10, C 235, and L 550 (Fig 3; Table 42).

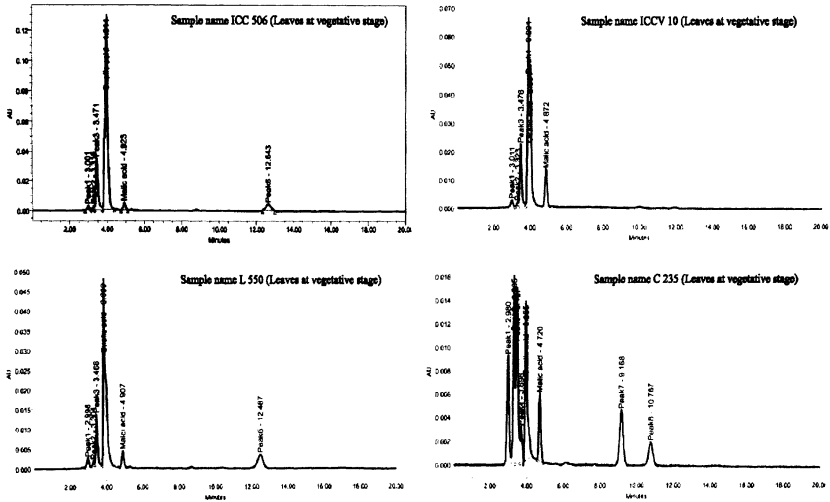


Fig 1: HPLC fingerprints of organic acids in four chickpea genotypes at the vegetative stage

Table 40: HPLC fingerprints of organic acids in four chickpea genotypes at the vegetative stage (ICRISAT, Patancheru, India)

Peaks	Peak area (%)			
	ICC 506	ICCV 10	C 235	L 550
Peak 1	1.30	1.71	12.71	2.71
Peak 2	0.87	0.71	18.82	1.21
Peak 3	16.62	16.16	20.86	14.67
Peak 4	-	-	4.17	-
Peak 5	-	37.28	-	-
Oxalic acid	73.95	33.90	20.62	67.78
Malic acid	2.48	10.24	7.84	5.22
Peak 8	-	-	10.81	-
Peak 9	-	-	4.18	-
Peak 10	4.78	-	-	8.41

Table 41: HPLC fingerprints of organic acids in four chickpea genotypes at the flowering stage (ICRISAT, Patancheru, India)

Peaks	Peak area (%)			
	ICC 506	ICCV 10	C 235	L 550
Peak 1	0.81	1.14	1.78	1.03
Peak 2	0.51	0.76	1.31	0.75
Peak 3	2.94	10.88	14.40	10.47
Peak 4	-	-	1.11	-
Oxalic acid	82.42	61.91	48.26	77.93
Malic acid	3.92	22.09	18.92	8.05
Peak 7	-	0.40	0.60	-
Peak 8	0.11	-	0.85	0.18
Peak 9	-	0.12	-	-
Peak 10	-	0.47	-	-
Peak 11	0.20	-	0.72	-
Peak 12	-	0.94	-	-
Peak 13	9.10	1.28	12.05	1.60

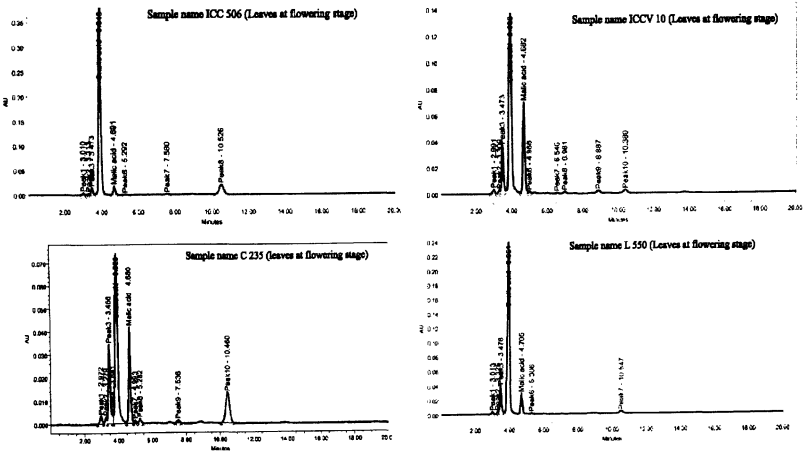


Fig 2: HPLC fingerprints of organic acids in four chickpea genotypes at the flowering stage

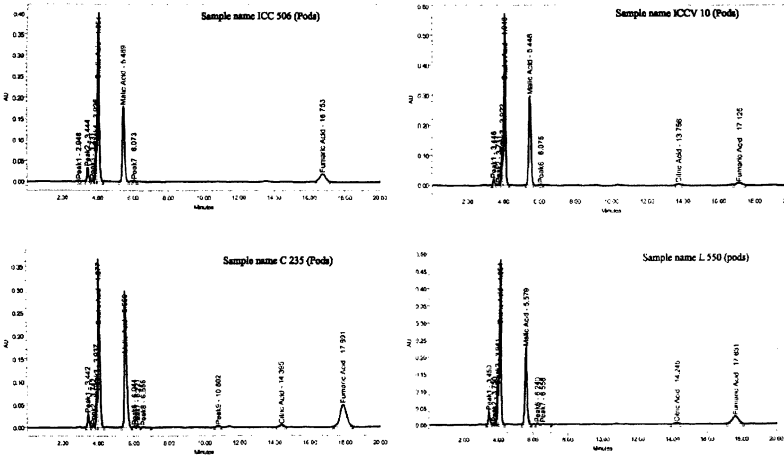


Fig 3: HPLC fingerprints of organic acids in the four chickpea genotypes at the podding stage

Oxalic acid comprised of 50.19 per cent peak area in ICC 506, 52.22 per cent in ICCV 10, 33.81 per cent in C 235, and 47.52 per cent in L 550. Peak area for malic acid was 28.29 per cent in ICC 506, 35.57 per cent in ICCV 10, 36.67 per cent in C 235, and 29.42 per cent in L 550 (Table 42).

4.2.3.3 Amounts of organic acids in four chickpea genotypes on dry weight basis (mg g^{-1})

During the vegetative stage, ICC 506 had the highest (10.20 mg g^{-1}) amounts of oxalic acid, followed by ICCV 10 (5.42 mg g^{-1}), whereas C 235 had the lowest (2.19 mg g^{-1}) amounts of oxalic acid, followed by L 550 (3.44 mg g^{-1}). The genotype ICCV 10 had the highest amounts of malic acid (12.55 mg g^{-1}), followed by C 235 (7.52 mg g^{-1}) and ICC 506 (5.99 mg g^{-1}), whereas L 550 recorded the lowest amounts of malic acid (3.60 mg g^{-1}) (Table 43; Graph 1).

During the flowering stage, ICC 506 had the highest (17.70 mg g^{-1}) amounts of oxalic acid, followed by L 550 (13.59 mg g^{-1}), and ICCV 10 (10.05 mg g^{-1}). The oxalic acid amount was lowest in C 235 (7.80 mg g^{-1}). The genotype ICCV 10 had the highest (37.71 mg g^{-1}) amounts of malic acid, followed by C 235 (33.51 mg g^{-1}), and L 550 (18.42 mg g^{-1}). Malic acid amount was lowest in ICC 506 (8.03 mg g^{-1}) (Table 43; Graph 2).

During the podding stage, ICCV 10 had the highest (13.07 mg g^{-1}) amounts of oxalic acid, followed by L 550 (9.09 mg g^{-1}). The amounts of oxalic acid were lowest in ICC 506 (6.04 mg g^{-1}), followed by C 235 (6.67 mg g^{-1}). The genotype ICCV 10 had the highest amounts of malic acid (86.78 mg g^{-1}), followed by C 235 (73.45 mg g^{-1}) and L 550 (52.54 mg g^{-1}). Lowest amount was recorded in ICC 506 (37.82 mg g^{-1}) (Table 43).

The genotype C 235 had the highest amounts of fumaric acid (43.38 mg g^{-1}), followed by ICC 506 (15.00 mg g^{-1}). Lowest amounts of fumaric acid were recorded in L 550 (6.33 mg g^{-1}), and ICCV 10 (7.00 mg g^{-1}). Highest amounts of citric acid were recorded in C 235 (1.59 mg g^{-1}), followed by ICCV 10 (1.16 mg g^{-1}), and L 550 (1.00 mg g^{-1}). There was no citric acid in ICC 506 (Table 43; Graph 3).

4.2.3.4 Amounts of organic acids in four chickpea genotypes on leaf area/pod surface area basis (μg cm^{-2})

During the vegetative stage, ICC 506 recorded the highest (38.10 μg cm^{-2}) amounts of oxalic acid, followed by ICCV 10 (22.60 μg cm^{-2}). The amounts of oxalic acid were lowest in C 235 (9.60 μg cm^{-2}), followed by L 550 (14.20 μg cm^{-2}). The genotype ICCV 10 had the highest amounts of malic acid (52.40 μg cm^{-2}), followed by C 235 (33.20 μg cm^{-2}), and ICC 506 (22.80 μg cm^{-2}). The genotype L 550 recorded the lowest amounts of malic acid (14.90 μg cm^{-2}) (Table 44; Graph 4).

During the flowering stage, ICC 506 had the highest (49.40 μg cm^{-2}) amounts of oxalic acid, followed by L 550 (45.50 μg cm^{-2}), and C 235 (39.30 μg cm^{-2}). The oxalic acid amount was lowest in ICCV 10 (33.60 μg cm^{-2}). The amounts of malic acid were highest in C 235 (170.40 μg cm^{-2}), followed by ICCV 10 (124.80 μg cm^{-2}), and L 550 (60.70 μg cm^{-2}). Malic acid amount was lowest in ICC 506 (22.60 μg cm^{-2}) (Table 44; Graph 5).

During the podding stage, ICC 506 recorded the highest (308.00 μg cm^{-2}) amounts of oxalic acid, followed by C 235 (287.40 μg cm^{-2}). The amounts of oxalic acid were lowest in L 550 (210.00 μg cm^{-2}), and ICCV 10 (253.70 μg cm^{-2}). The genotype C 235 had the highest (3150.80 μg cm^{-2}) amounts of malic acid, followed by ICC 506

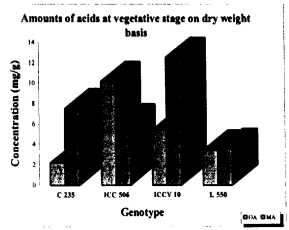
Table 42: HPLC fingerprints of organic acids in four chickpea genotypes at the podding stage (ICRISAT, Patancheru, India)

Peaks	Peak area (%)			
	ICC 506	ICCV 10	C 235	L 550
Peak 1	0.43	-	-	-
Peak 2	4.17	1.67	2.81	3.78
Peak 3	0.58	0.32	0.46	0.57
Peak 4	6.79	7.09	5.82	8.69
Oxalic acid	50.19	52.22	33.81	47.52
Malic acid	28.29	35.57	36.67	29.42
Peak 6	0.71	0.24	0.35	-
Peak 7	-	-	0.48	0.38
Peak 8	-	-	0.28	0.35
Peak 9	-	-	0.44	-
Citric acid	-	0.69	0.99	0.72
Fumaric acid	8.83	2.21	17.89	8.58

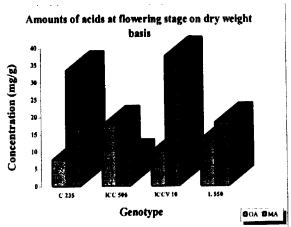
Table 43: Amounts of organic acids in four chickpea genotypes on dry weight basis (ICRISAT, Patancheru, India)

Genotype	Amounts of organic acids (mg g^{-1})			
	Oxalic acid	Malic acid	Fumaric acid	Citric acid
Vegetative stage				
C 235	2.19	7.52	--	--
ICC 506	10.20	5.99	--	--
ICCV 10	5.42	12.55	--	--
L 550	3.44	3.60	--	--
Flowering stage				
C 235	7.80	33.51	--	--
ICC 506	17.70	8.03	--	--
ICCV 10	10.05	37.71	--	--
L 550	13.59	18.42	--	--
Podding stage				
C 235	6.67	73.45	43.38	1.59
ICC 506	6.04	37.82	15.00	0.00
ICCV 10	13.07	86.78	7.00	1.16
L 550	9.09	52.54	6.33	1.00

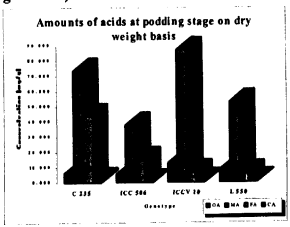
Graph 1: Amounts of organic acids in four chickpea genotypes at the vegetative stage (on dry weight basis)



Graph 2: Amounts of organic acids in four chickpea genotypes at the flowering stage (on dry weight basis)



Graph 3: Amounts of organic acids in four chickpea genotypes at the podding stage (on dry weight basis)



(1925.10 μg cm^{-2}) and ICCV 10 (1686.00 μg cm^{-2}). Lowest amount of malic acid was recorded in L 550 (1232.60 μg cm^{-2}). The genotype C 235 had the highest amounts of fumaric acid (1844.10 μg cm^{-2}), followed by ICC 506 (759.10 μg cm^{-2}). Lowest amounts of fumaric acid were recorded in ICCV 10 (135.60 μg cm^{-2}), and L 550 (148.10 μg cm^{-2}). Highest amounts of citric acid were recorded in C 235 (69.24 μg cm^{-2}), followed by L 550 (23.27 μg cm^{-2}), and ICCV 10 (22.17 μg cm^{-2}). There was no citric acid in ICC 506 (Table 44; Graph 6).

4.2.4 ED_{50} value for *Bt* toxin

The ED_{50} value for *Bt* formulation was estimated by Probit analysis (Table 45). The ED_{50} for *Bt* formulation was 4.147x 10^{-5} mg ml^{-1} of diet. The *Bt* formulation contains five to eight per cent of Cry toxin. From the ED_{50} value of *Bt* formulation, the ED_{50} for Cry toxin was calculated and it was 27.3 ηg ml^{-1}.

4.2.5 Amount of protein in protoxin preparation

The amount of protein present in the protoxin prepared from the *Bt* formulation Biolep® was estimated through Lowry's method (Table 46). The amount of protein present in the protoxin was 51.995 μg μl^{-1}.

4.2.6 Survival and development of *H. armigera* on artificial diet with lyophilized leaf and pod powder of different chickpea genotypes and *Bt* toxin

4.2.6.1 Leaf powder diet

There were significant differences in larval weight at five DAI between the genotypes and *Bt* treatments (Table 47). The interaction effects were non-significant. The larval weight was lower in larvae reared on diets with ICC 506 leaf powder (13.25 mg), followed by ICCV 10 (14.73 mg). Similarly, larval weights were lower in larvae fed on

Table 44: Amounts of organic acids present in four chickpea genotypes on leaf/ pod surface area basis (ICRISAT, Patancheru, India)

Genotype	Amounts of organic acids (μg cm^{-2})			
	Oxalic acid	Malic acid	Fumaric acid	Citric acid
		Vegetative stage		
C 235	9.60	33.20	--	--
ICC 506	38.10	22.80	--	--
ICCV 10	22.60	52.40	--	--
L 550	14.20	14.90	--	--
		Flowering stage		
C 235	39.30	170.40	--	--
ICC 506	49.40	22.60	--	--
ICCV 10	33.60	124.80	--	--
L 550	45.50	60.70	--	--
		Podding stage		
C 235	287.40	3150.80	1844.10	69.24
ICC 506	308.00	1925.10	759.10	0.00
ICCV 10	253.70	1686.00	135.60	22.17
L 550	210.00	1232.60	148.10	23.27

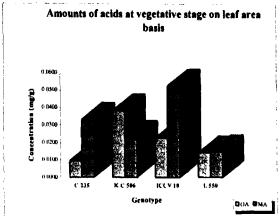
Table 45: Effective dosage (ED_{50}) of *B. thuringiensis* required to result in 50 per cent reduction in weight of neonate larvae of *H. armigera* (ICRISAT, Patancheru, India)

Component	ED_{50} (Fudicial limits)	Heterogeneity	SE ± of slope	Regression equation
Biolep (Bt formulation)	4.147×10^{-5} (3.015×10^{-5} to 5.618×10^{-5})	12.354	6.411 $\times 10^{-2}$	14.122 ± 2.0818 ×

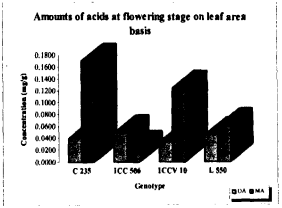
Table 46: Amount of *Bt* protein in protoxin preparation (estimated by Lowry method)

Component	OD Value	Protein (μg μl^{-1})
Protoxin	0.875	51.995

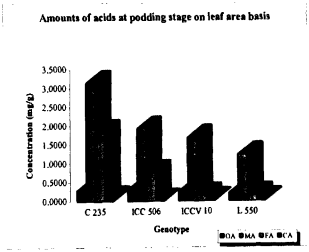
Graph 4: Amounts of organic acids in four chickpea genotypes at the vegetative stage (on leaf area basis)



Graph 5: Amounts of organic acids in four chickpea genotypes at the flowering stage (on leaf area basis)



Graph 6: Amounts of organic acids in four chickpea genotypes at the podding stage (on pod surface area basis)



diets with *Bt* (11.77 mg) as compared to those fed on diets without *Bt* (23.24 mg). At 10 DAI, the differences between the genotypes and *Bt* treatments were significant (Table 47). Larvae reared on standard artificial diet with *Bt* had the lowest larval weights (132.9 mg) (Plate 12), followed by those reared on artificial diet with ICCV 10 leaf powder + 8.19 µg of *Bt* (Cry) toxin (158.1 mg) (Plate 13). Larval weights were highest in the larvae reared on the standard artificial diet without *Bt* (411.3 mg), followed by those reared on diets with L 550 leaf powder (399.5 mg) (Plate 14), ICC 506 leaf powder (382.9 mg) (Plate 15), ICC 506 leaf powder + 8.19 µg of *Bt* toxin (324.3 mg), C 235 leaf powder (308.8 mg) (Plate16), C 235 leaf powder + 8.19 µg of *Bt* toxin (259.6 mg), and ICCV 10 leaf powder (223.0 mg). There were significant interaction effects for pupal weights between the genotypes × *Bt* treatments (Table 47). Pupal weights were lowest on the standard artificial diet with *Bt* (309.8 mg), followed by diets with L 550 leaf powder + 8.19 µg of *Bt* toxin (316.3 mg), ICCV 10 leaf powder + 8.19 µg of *Bt* toxin (319.7 mg), ICC 506 leaf powder + 8.19 µg of *Bt* toxin (323.3 mg), ICCV 10 leaf powder (326.6 mg), C 235 leaf powder + 8.19 µg of *Bt* toxin (327.1 mg), and ICC 506 leaf powder (327.3 mg). The interaction effects were non-significant.

The interaction effects for larval and pupal periods, pupation and adult emergence, and fecundity were significant between the genotypes × *Bt* treatments (Table 48 & 49). Larval period increased by more than two days in the larvae reared on the standard artificial diet with 8.19 µg of *Bt* toxin (17.46 days), followed by those reared on ICC 506 leaf powder (17.12 days) (Table 48). Differences in pupal period between the genotypes were significant. The interaction effects were also significant (Table 48). Pupal period was shorter on the standard artificial diet (14.76 days) as compared to that on diets

Table 47: Survival and development of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of four chickpea genotypes and *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	23.52	15.71	**19.62**^{b}	308.8^{def}	259.6^{cde}	**284.2**	331.9^{bc}	327.1^{abc}	**329.5**
ICC 506	19.36	7.15	**13.25**^{a}	382.9^{fgh}	324.3^{efg}	**353.6**	327.3^{abc}	323.3^{abc}	**325.3**
ICCV 10	20.34	9.12	**14.73**^{a}	223.0^{bc}	158.1^{ab}	**190.5**	326.6^{abc}	319.7^{ab}	**323.2**
L 550	25.39	14.31	**19.85**^{b}	399.5^{gh}	234.8^{bcd}	**317.1**	343.1^{c}	316.3^{ab}	**329.7**
Standard artificial diet	27.56	12.54	**20.05**^{b}	411.3^{h}	132.9^{a}	**272.1**	364.3^{d}	309.8^{a}	**337.1**
Mean	**23.24**^{b}	**11.77**^{a}		**345.1**	**221.9**		**338.6**	**319.3**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
***Bt* treatment**	<0.001	0.78	3.68*	<0.001	12.25	36.40*	<0.001	2.97	8.82*
Genotype	0.002	1.24	2.33*	<0.001	19.37	57.56*	0.31	4.69	**NS**
***Bt* treatment × Genotype**	0.392	1.75	**NS**	0.002	27.40	81.40*	0.005	6.64	19.72*

DAI – Days after initiation of experiment. *LSD at P≤ 0.05 %.

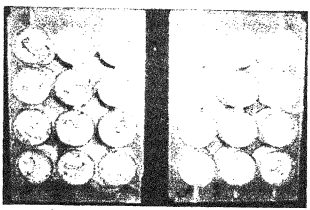


Plate 12: Larval growth on standard artificial diet with out *Bt* (A) and with *Bt* (B)

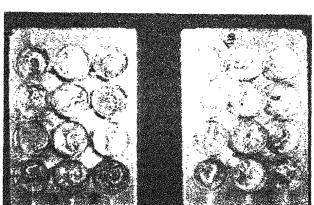


Plate 13: Larval growth on ICCV 10 leaf powder diet with out *Bt* (A) and with *Bt* (B)

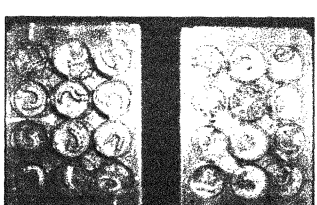


Plate 14: Larval growth on L 550 leaf powder diet with out *Bt* (A) and with *Bt* (B)

with ICCV 10 leaf powder + 8.19 µg of *Bt* toxin (17.48 days), ICC 506 leaf powder (16.21 days), and ICCV 10 leaf powder (15.92 days). Overall, pupal period was shorter on the standard artificial diet compared to that on diets with leaf powder of different chickpea genotypes. Lowest pupation was recorded on the standard artificial diet with 8.19 µg of *Bt* toxin (70.00%), followed by diets with ICC 506 leaf powder (86.70%). There was a 100 per cent pupation in the insects reared on L 550 leaf powder and the standard artificial diet (Table 48). The differences in adult emergence between the chickpea genotypes and *Bt* treatments were also significant (Table 48). Adult emergence was lowest on the standard artificial diet with 8.19 µg of *Bt* toxin (40.00%), followed by diets with ICC 506 leaf powder (53.33%). More than 90 per cent adult emergence was recorded on the standard artificial diet without *Bt* (93.33%) and L 550 leaf powder (90.00%).

The interaction effects for adult longevity were non-significant (Table 49). Lowest fecundity was recorded in insects reared on diets with C 235 leaf powder + 8.19 µg of *Bt* toxin (447eggs female^{-1}), followed by diets with ICC 506 leaf powder (653 eggs female^{-1}), ICCV 10 leaf powder (670 eggs female^{-1}), and ICCV 10 leaf powder + 8.19 µg of *Bt* toxin (699 eggs female^{-1}). Overall, the fecundity was highest in insects reared on the standard artificial diet (1760 eggs female^{-1}) as compared to those reared on diets with leaf powders of different chickpea genotypes.

4.2.6.2 Survival and development of *H. armigera* on artificial diet with lyophilized pod powder of different chickpea genotypes and *Bt* toxins

The interaction effects were significant for larval weights at five DAI (Table 50). Lower larval weight was recorded in insects reared on diets with ICCV 10 pod powder +

Table 48: Post-embryonic development, pupation, and adult emergence of *H. armigera* reared on artificial diet with lyophilized leaf powder of four chickpea genotypes and *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	15.98^{abcd}	16.06^{abcd}	**16.02**	14.94^{ab}	15.77^{ab}	**15.35**	96.70^{bc} (83.90)	90.00^{abc} (75.00)	**93.33 (79.40)**	86.70^{cd} (68.90)	60.00^{ab} (50.90)	**73.33 (59.90)**
ICC 506	17.12^{cd}	15.77^{abc}	**16.44**	16.21^{bc}	14.71^{a}	**15.46**	86.70^{ab} (68.90)	93.33^{bc} (81.10)	**90.00 (75.00)**	53.33^{ab} (47.00)	73.33^{bc} (59.00)	**63.33 (53.00)**
ICCV 10	16.68^{bcd}	15.09^{ab}	**15.89**	15.92^{ab}	17.48^{c}	**16.70**	96.70^{bc} (83.90)	90.00^{abc} (75.00)	**93.33 (79.40)**	86.70^{cd} (68.90)	66.70^{abc} (54.80)	**76.70 (61.80)**
L 550	14.73^{a}	16.14^{abcd}	**15.44**	15.24^{ab}	15.22^{ab}	**15.23**	100.00^{c} (90.00)	96.70^{bc} (83.90)	**98.33 (86.90)**	90.00^{d} (78.90)	76.70^{bc} (61.70)	**83.33 (70.33)**
Standard artificial diet	14.77^{a}	17.46^{d}	**16.11**	14.76^{a}	15.64^{ab}	**15.20**	100.00^{c} (90.00)	70.00^{a} (57.80)	**85.00 (73.90)**	93.33^{d} (81.10)	40.00^{a} (38.90)	**66.70 (60.00)**
	15.86	**16.10**		**15.41**	**15.77**		**96.00 (83.33)**	**88.00 (74.60)**		**82.00 (69.00)**	**63.33 (53.00)**	
	Fp	SE±	LSD	Fp	SE±	LSD	Fp	SE±	LSD	Fp	SE±	LSD
***Bt* treatment**	0.478	0.24	**NS**	0.228	0.20	**NS**	0.031	2.65	7.88*	<0.001	2.46	7.31*
Genotype	0.471	0.38	**NS**	0.017	0.32	0.94*	0.245	4.19	**NS**	0.076	3.89	**NS**
***Bt* treatment × Genotype**	0.004	0.54	1.60*	0.028	0.45	1.32*	0.026	5.93	17.62*	0.003	5.50	16.35*

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %.

Table 49: Longevity and fecundity of insects 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	15.64	17.33	**16.49^{b}**	20.93	19.21	**20.07**	805^{bcde}	447^{a}	**626**
ICC 506	17.44	16.33	**16.89^{b}**	18.28	20.08	**19.18**	653^{b}	853de	**753**
ICCV 10	18.97	18.10	**18.54^{b}**	17.82	17.93	**17.88**	670^{bc}	699^{bcd}	**684**
L 550	16.32	18.56	**17.44^{b}**	18.26	18.22	**18.24**	831^{cde}	797^{bcde}	**814**
Standard artificial diet	13.81	14.17	**13.99^{a}**	15.67	14.17	**14.92**	1760^{f}	934^{e}	**1347**
Mean	**16.44**	**16.90**		**18.19**	**17.92**		**944**	**746**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
***Bt* treatment**	0.488	0.46	**NS**	0.804	0.76	**NS**	<0.001	26.80	79.50*
Genotype	0.005	0.73	2.16*	0.069	1.21	**NS**	<0.001	42.30	125.80*
***Bt* treatment × Genotype**	0.408	1.03	**NS**	0.841	1.71	**NS**	<0.001	59.90	177.90*

*LSD at P≤ 0.05 %.

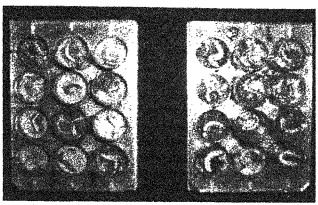
8.19 µg of *Bt* toxin (5.54 mg) and ICC 506 pod powder + 8.19 µg of *Bt* toxin (5.93 mg) as compared to the larvae reared on diets with ICC 506 pod powder (15.96 mg), standard artificial diet without *Bt* (15.37 mg), and L 550 pod powder (14.72 mg). The interaction effects for larval weight at 10 DAI were non-significant, but the differences between the genotypes and *Bt* treatments were significant (Table 50). Larval weights were lower in insects reared on the diets with *Bt* (147.2 mg) as compared to the diets without *Bt* (368.8 mg). Lowest weight (230.6 mg) was recorded in the larvae reared on diets with ICC 506 pod powder (Plate 17), followed by C 235 pod powder (235.8 mg) (Plate 18), and ICCV 10 pod powder (249.8 mg) (Plate 19), and highest in those reared on the standard artificial diet without *Bt* (297.3 mg) and L 550 pod powder (276.3 mg) (Plate 20). The interaction effects for pupal weights were significant (Table 50). Pupal weight was lowest in insects reared on diets with L 550 pod powder + 8.19 µg of *Bt* toxin (281.6 mg), followed by ICC 506 pod powder + 8.19 µg of *Bt* toxin (286.1 mg), and C 235 pod powder (287.4 mg).

Differences between the genotypes and *Bt* treatments for larval period were significant (Table 51). Larval period was shortest on diets without *Bt* (15.09 days) as compared to that on diets with *Bt* (16.68 days). Larval period was shorter in insects reared on diets with L 550 pod powder (15.30 days), standard artificial diet without *Bt* (15.60 days), and ICCV 10 pod powder (15.80 days) as compared to the insects reared on diets with ICC 506 (16.46 days) and C 235 (16.27 days) pod powders. The differences in pupal period between the genotypes and *Bt* treatments were significant. Pupal period (11.71 days) was shorter on diets without *Bt* as compared to insects reared on diets with *Bt* (12.27 days). Pupal period was shorter on ICC 506 (10.90 days) as compared to that

Table 50: Survival and development of *H. armigera* larvae reared on artificial diet with lyophilized pod powder of four chickpea genotypes and *Bt* (ICRISAT, Patancheru, 2006-07)

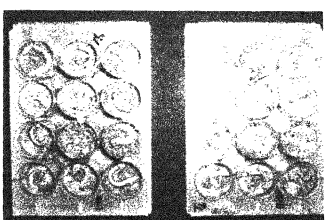
Genotype	Larval weight (5 DAI) (mg)			Larval weight (10 DAI) (mg)			Pupal weight (mg)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
C 235	14.15^{de}	10.07^{b}	**12.11**	332.6	139.0	**235.8**^{a}	287.4^{a}	293.1^{a}	**290.2**
ICC 506	15.96^{e}	5.93^{a}	**10.95**	324.1	137.1	**230.6**^{a}	309.8^{b}	286.1^{a}	**298.0**
ICCV 10	13.56^{cd}	5.54^{a}	**9.55**	374.7	125.0	**249.8**^{ab}	319.5^{bc}	292.0^{a}	**305.7**
L 550	14.72^{de}	10.92^{b}	**12.82**	400.6	152.1	**276.3**^{bc}	325.2^{c}	281.6^{a}	**303.4**
Standard artificial diet	15.37^{de}	11.73^{bc}	**13.55**	412.1	182.9	**297.3**^{c}	283.3^{a}	311.3^{bc}	**297.3**
Mean	**14.75**	**8.84**		**368.8**^{b}	**147.2**^{a}		**310.6**	**287.2**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
***Bt* treatment**	<0.001	0.29	0.87*	<0.001	6.45	19.15*	<0.001	2.24	6.66*
Genotype	<0.001	0.46	1.37*	<0.001	10.19	30.28*	0.052	3.55	10.54*
***Bt* treatment × Genotype**	<0.001	0.65	1.94*	0.118	14.41	**NS**	0.002	5.02	14.90*

DAI – Days after initiation of experiment. *LSD at P≤ 0.05 %.



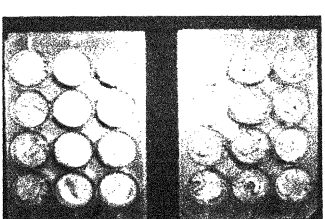
A B

Plate 15: Larval growth on ICC 506 leaf powder diet with out *Bt* (A) and with *Bt* (B)



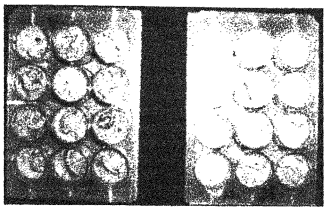
A B

Plate 16: Larval growth on C 235 leaf powder diet with out *Bt* (A) and with *Bt* (B)



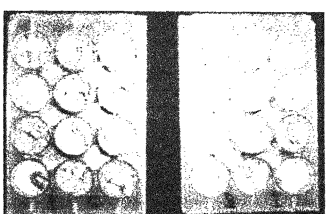
A B

Plate 17: Larval growth on ICC 506 pod powder diet with out *Bt* (A) and with *Bt* (B)



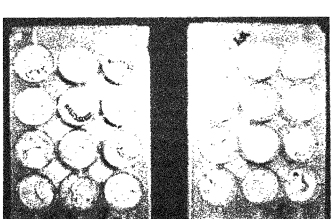
A B

Plate 18: Larval growth on C 235 pod powder diet with out *Bt* (A) and with *Bt* (B)



A B

Plate 19: Larval growth on ICCV 10 pod powder diet with out *Bt* (A) and with *Bt* (B)



A B

Plate 20: Larval growth on L 550 pod powder diet with *Bt* (A) and with *Bt* (B)

on the standard artificial diet without *Bt* (12.09 days), ICCV 10 (12.15 days), C 235 (12.24 days), and L 550 (12.56 days). The interaction effects between the genotypes × *Bt* treatments and the differences between *Bt* treatments were significant for pupation (Table 51). Lowest pupation was recorded in the larvae reared on the standard artificial diet with 8.19 µg of *Bt* toxin (66.70%), C 235 pod powder + 8.19 µg of *Bt* toxin (76.70%), ICC 506 pod powder (83.30%), ICCV 10 pod powder + 8.19 µg of *Bt* toxin (86.70%), and L 550 pod powder + 8.19 µg of *Bt* toxin (86.70%). Overall, pupation was lower in larvae reared on diets with *Bt* (82.70%) as compared to the larvae reared on diets without *Bt* (91.30%). There were no significant differences in the adult emergence (Table 51).

There were significant interaction effects between the genotypes × *Bt* treatments for longevity of adults (Table 52). Female longevity was lowest on diets with C 235 pod powder + 8.19 µg of *Bt* toxin (9.67 days), followed by ICCV 10 pod powder + 8.19 µg of *Bt* toxin (9.89 days), C 235 pod powder (11.00 days), and standard artificial diet with 8.19 µg of *Bt* toxin (11.00 days). Shortest male longevity was recorded in insects reared on diets with ICCV 10 pod powder (8.08 days), followed by ICC 506 pod powder (8.36 days), C 235 pod powder + 8.19 µg of *Bt* toxin (8.61 days), ICC 506 pod powder + 8.19 µg of *Bt* toxin (8.67 days), C 235 pod powder (8.87 days). The differences between the genotypes, *Bt* treatments, and the interaction effects for fecundity were significant (Table 52). Fecundity was lowest in insects reared on the standard artificial diet with 8.19 µg of *Bt* toxin (333.2 eggs female^{-1}), followed by ICCV 10 pod powder + 8.19 µg of *Bt* toxin (434.3 eggs female^{-1}), L 550 pod powder + 8.19 µg of *Bt* toxin (447.7 eggs female^{-1}), C 235 pod powder + 8.19 µg of *Bt* toxin (494.3 eggs female^{-1}), ICC 506 pod

Table 51: 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)

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	15.21	17.34	**16.27**^{bc}	11.99	12.49	**12.24**^{b}	90.00^{abcd} (75.00)	76.70^{ab} (61.20)	**83.30 (68.10)**	70.00 (57.70)	63.33 (52.90)	**66.70 (55.33)**
ICC 506	16.16	16.76	**16.46**^{c}	10.32	11.47	**10.90**^{a}	83.30^{abc} (66.60)	96.70^{cd} (83.90)	**90.00 (75.20)**	73.33 (59.00)	83.33 (70.80)	**78.33 (64.90)**
ICCV 10	14.83	16.77	**15.80**^{ab}	12.09	12.21	**12.15**^{b}	93.30^{bcd} (77.70)	86.70^{abcd} (72.80)	**90.00 (75.20)**	83.33 (66.10)	80.00 (63.90)	**81.70 (65.00)**
L 550	14.31	16.29	**15.30**^{a}	12.28	12.84	**12.56**^{b}	90.00^{bcd} (78.90)	86.70^{abc} (68.90)	**88.30 (73.90)**	83.33 (66.66)	63.33 (53.20)	**73.33 (59.90)**
Standard artificial diet	14.94	16.27	**15.60**^{a}	11.85	12.34	**12.09**^{b}	100.00^{d} (90.00)	66.70^{a} (55.10)	**83.30 (72.50)**	80.00 (63.44)	40.00 (39.10)	**60.00 (51.33)**
Mean	**15.09**^{a}	**16.68**^{b}		**11.71**^{a}	**12.27**^{b}		**91.30 (77.70)**	**82.70 (68.40)**		**78.00 (62.60)**	**66.00 (56.00)**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
***Bt* treatment**	<0.001	0.12	0.36*	0.004	0.12	0.36*	0.043	3.02	8.98*	0.090	2.61	**NS**
Genotype	0.003	0.19	0.57*	<0.001	0.19	0.57*	0.818	4.78	**NS**	0.120	4.13	**NS**
***Bt* treatment × Genotype**	0.064	0.27	**NS**	0.47	0.27	**NS**	0.02	6.75	20.07*	0.068	5.84	**NS**

Figures in parenthesis are angular transformed values. *LSD at P≤ 0.05 %.

Table 52: Longevity and fecundity of insects of *H. armigera* reared on artificial diet with lyophilized pod 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	11.00^{ab}	9.67^{a}	**10.33**	8.87^{b}	8.61^{ab}	**8.739**	687.0^{e}	494.3^{bc}	**590.7**
ICC 506	13.00^{cd}	12.16^{bc}	**12.58**	8.36^{ab}	8.67^{b}	**8.511**	574.0^{cd}	554.0^{cd}	**564.0**
ICCV 10	9.89^{a}	13.67^{cd}	**11.78**	8.08^{a}	10.22^{c}	**9.153**	623.0^{de}	434.3^{b}	**528.7**
L 550	12.62^{cd}	14.00^{d}	**13.31**	10.23^{c}	12.00^{e}	**11.12**	692.0^{e}	447.7^{b}	**569.8**
Standard artificial diet	12.17^{bc}	11.00^{ab}	**11.58**	10.83^{d}	9.83^{c}	**10.33**	921.3^{f}	333.2^{a}	**627.3**
Mean	**11.74**	**12.10**		**9.27**	**9.867**		**699.5**	**452.7**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
Bt treatment	0.306	0.24	NS	<0.001	0.08	0.25*	<0.001	12.61	37.47*
Genotype	<0.001	0.38	0.72*	<0.001	0.13	0.39*	0.034	19.94	59.25*
Bt treatment × Genotype	<0.001	0.54	1.61*	<0.001	0.18	0.55*	<0.001	28.20	83.79*

*LSD at P≤ 0.05 %.

powder + 8.19 µg of *Bt* toxin (554.0 eggs female^{-1}), and ICC 506 pod powder (574.0 eggs female^{-1}).

4.2.7 Survival and development of *H. armigera* on artificial diet with malic and oxalic acids along with *Bt* toxins

4.2.7.1 Larval and pupal weights

The interaction effects for larval weights between organic acids and *Bt* toxins were significant (Table 53). Larval weight at five DAI was lowest (8.71 mg) in the larvae reared on diets with 1.33 g oxalic acid (OA) + 2.83 g malic acid (MA) + 8.19 µg of *Bt* toxin, followed by the larvae reared on the diet having 1.33 g OA + 2.83 g MA (8.98 mg), 0.59 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (9.16 mg). Maximum larval weight (22.92 mg) was recorded in insects reared on the standard artificial diet without *Bt*, followed by those reared on diet with 0.60 g MA (20.67 mg).

There were significant differences in larval weights between organic acid amounts and *Bt* at 10 DAI. The interaction effects were also significant (Table 53). The larval weight was lowest (101.50 mg) in diets with 0.59 g OA + 2.51 g MA + 8.19 µg of *Bt* toxin, followed by those reared on a diets with 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (116.80 mg), and 2.83 g MA (139.10 mg) (Plate 21). Larval weight was highest (289.00 mg) on the standard artificial diet without *Bt* (Plate 21), followed by 260.50 mg in diets with 1.33 g OA + 0.60 g MA (plate 21). Lowest pupal weight (201.00 mg) was recorded in insects reared on diets with 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin, followed by those reared on diets with 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (204.10 mg). Highest pupal weight (286.80 mg) was recorded in insects reared on diets with 1.33 g OA.

Table 53: Survival and development of *H. armigera* larvae reared on artificial diet with different amounts of organic acids (oxalic and malic acid) and *Bt* (ICRISAT, Patancheru, 2006-07)

Amount of acids (g/ 300 ml of diet)	Larval weight (5DAI) (mg)			Larval weight (10DAI) (mg)			Pupal weight (mg)		
	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean	Without *Bt*	With *Bt*	Mean
0.59 g OA	15.05^{efg}	17.84^{b}	**16.45**	201.90^{fg}	187.40^{ef}	**194.70**	250.80^{fghij}	277.90^{klm}	**264.40**
1.33 g OA	18.89^{b}	17.97^{b}	**18.43**	221.40^{hi}	144.90^{bc}	**183.10**	286.80^{m}	264.70^{ijkl}	**275.70**
0.60 g MA	20.67^{j}	16.21^{fg}	**18.44**	240.20^{j}	225.90^{hij}	**233.10**	286.40^{m}	280.60^{lm}	**283.50**
2.83 g MA	18.09^{b}	16.31^{g}	**17.20**	231.30^{ijk}	139.10^{b}	**185.20**	259.60^{hijk}	257.30^{ghijk}	**258.50**
0.59 g OA + 0.60 g MA	19.52^{ij}	14.91^{ef}	**17.22**	246.40^{kl}	229.00^{ij}	**137.70**	269.90^{jkl}	267.20^{ijklm}	**268.50**
0.59 g OA + 2.83 g MA	10.76^{c}	9.16^{ab}	**9.96**	188.10^{ef}	150.60^{bcd}	**169.40**	233.40^{cdef}	236.20^{def}	**234.80**
1.33 g OA + 2.83 g MA	8.98^{ab}	8.71^{a}	**8.85**	139.70^{b}	116.80^{a}	**128.20**	213.20^{abc}	204.10^{ab}	**208.70**
1.33 g OA + 0.60 g MA	15.54^{efg}	14.31^{e}	**14.92**	260.50^{l}	156.90^{cd}	**208.70**	237.50^{defg}	241.90^{efgh}	**239.70**
0.75 g OA + 2.83 g MA	10.09^{bc}	12.75^{d}	**11.42**	221.80^{hi}	179.20^{e}	**200.50**	214.00^{abc}	217.30^{abcd}	**215.60**
1.02 g OA + 1.38 g MA	12.38^{d}	12.73^{d}	**12.56**	191.60^{ef}	161.80^{d}	**176.70**	224.40^{bcde}	201.00^{a}	**212.70**
0.59 g OA + 2.51 g MA	18.09^{b}	18.29^{hi}	**18.19**	219.00^{hi}	101.50^{a}	**160.20**	213.50^{abc}	247.50^{fghi}	**230.50**
Standard artificial diet	22.92^{k}	12.89^{d}	**17.91**	289.00^{m}	210.60^{gh}	**249.80**	285.90^{m}	265.90^{ijklm}	**275.90**
Mean	**15.91**	**14.34**		**220.90**	**167.00**		**248.00**	**246.80**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
***Bt* treatment**	<0.001	0.13	0.38*	<0.001	1.66	4.73*	0.701	2.12	**NS**
Amount of acids	<0.001	0.33	0.94*	<0.001	4.07	11.58*	<0.001	5.19	14.79*
Amount of acids × *Bt* treatment	<0.001	0.47	1.33*	<0.001	5.76	16.38*	0.005	7.35	20.91*

OA = Oxalic acid; MA = Malic acid. *LSD at P< 0.05 %.

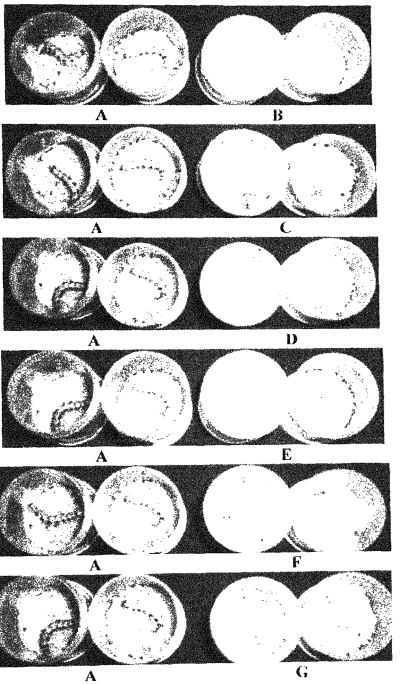


Plate 21: Larval growth on standard artificial diet and combination of oxalic and malic acid diets

(A) Standard artificial diet (B) 1.33 g of oxalic acid diet (C) 2.83 g of malic acid diet
(D) 1.33 g of oxalic acid + 0.60 g of malic acid diet (as in ICC 506)
(E) 0.75 g of oxalic acid + 2.83 g of malic acid diet (as in ICCV 10)
(F) 0.59 g of oxalic acid + 2.51 g of malic acid diet (as in C 235)
(G) 1.02 g of oxalic acid + 1.38 g of malic acid diet (as in L 550)

4.2.7.2 Larval and pupal periods

The interaction effects were significant for larval period (Table 54). Larval period was longer (22.05 days) in insects reared on diets with 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin, followed by those reared on diets with 0.59 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (20.99 days), 0.75 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (20.31 days), 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (20.22 days), and 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin (19.40 days). The interaction effects for the pupal period were significant (Table 54). Shortest pupal period (10.00 days) was recorded in diets with 0.75 g OA + 2.83 g MA, followed by diets with 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin (11.67 days) and 0.59 g OA + 2.51 g MA (11.67 days). Longest (14.00 days) pupal period was recorded in insects reared on diet with 1.33 g OA + 2.83 g MA.

4.2.7.3 Pupation and adult emergence

There were significant differences in pupation between diets with different amounts of organic acids and *Bt*. The interaction effects were also significant (Table 54). Pupation was lowest (43.33%) in diets with 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin, followed by 53.33 per cent pupation in diets with 0.59 g OA + 2.51 g MA, 56.67 per cent in diets with 1.33 g OA + 0.60 g MA. Pupation was highest (96.67%) in diets with 0.75 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin, followed by 93.33 per cent pupation in diets with 0.59 g OA + 2.51 g MA + 8.19 µg of *Bt* toxin and 0.59 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin. There was 90.00 per cent pupation on standard artificial diet without *Bt*.

The interaction effects between the organic acid amounts × *Bt* treatments were significant for adult emergence (Table 54). Adult emergence was lowest (5.56%) in diets

Table 54: Post-embryonic development, pupation, and adult emergence of *H. armigera* reared on artificial diet with different amounts of organic acids (oxalic and malic acid) and *Bt* (ICRISAT, Patancheru, 2006-07)

Amount of acids (g/ 300 ml of diet)	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
0.59 g OA	16.39^{abc}	16.67^{bcd}	**16.53**	12.17^{bc}	13.13^{efgh}	**12.65**	60.00^{abc} (50.77)	90.00^{fgh} (71.56)	**75.00** (**61.17**)	61.11^{ijk} (51.51)	55.56^{hijk} (48.22)	**58.34** (**49.86**)
1.33 g OA	16.67^{bcd}	16.74^{cd}	**16.70**	13.14^{efgh}	13.13^{efgh}	**13.14**	56.67^{ab} (48.85)	93.33^{hi} (77.71)	**75.00** (**63.28**)	64.45^{ijk} (53.43)	57.04^{hijk} (49.07)	**60.74** (**51.25**)
0.60 g MA	16.78^{cd}	15.73^{a}	**16.25**	12.10^{bc}	12.96^{defg}	**12.53**	76.67^{cdef} (61.22)	90.00^{ghi} (75.00)	**83.33** (**68.11**)	47.62^{ghij} (43.62)	85.09^{lm} (67.58)	**66.36** (**55.60**)
2.83 g MA	16.42^{abc}	15.88^{ab}	**16.15**	12.43^{cde}	13.30^{fghi}	**12.86**	80.00^{def} (63.93)	86.67^{fgh} (68.85)	**83.33** (**66.39**)	62.10^{ijk} (52.03)	72.69^{klm} (58.67)	**67.40** (**55.35**)
0.59 g OA + 0.60 g MA	16.87^{cd}	20.99^{j}	**18.93**	12.27^{bcd}	12.61^{cdef}	**12.44**	66.67^{bcd} (54.78)	90.00^{ghi} (75.00)	**78.33** (**64.89**)	65.08^{ijk} (53.84)	66.39^{jkl} (54.60)	**65.74** (**54.22**)
0.59 g OA + 2.83 g MA	18.57^{fg}	18.89^{gh}	**18.73**	12.56^{cde}	13.67^{ghi}	**13.11**	76.67^{cdef} (61.22)	93.33^{hi} (77.71)	**85.00** (**69.47**)	34.52^{defgh} (35.95)	25.18^{bcdef} (29.97)	**29.85** (**32.96**)
1.33 g OA + 2.83 g MA	16.61^{bc}	22.05^{j}	**19.33**	14.00^{f}	12.50^{cde}	**13.25**	66.67^{bcd} (54.78)	43.33^{a} (41.15)	**55.00** (**47.97**)	5.56^{a} (8.04)	16.67^{abc} (20.00)	**11.11** (**14.02**)
1.33 g OA + 0.60 g MA	18.49^{fg}	20.22^{ij}	**19.36**	13.33^{fghi}	13.33^{fghi}	**13.33**	56.67^{ab} (48.85)	83.33^{efg} (66.15)	**70.00** (**57.50**)	17.78^{bcde} (24.93)	36.11^{efgh} (36.92)	**26.95** (**30.93**)
0.75 g OA + 2.83 g MA	16.62^{bc}	20.31^{ij}	**18.46**	10.00^{a}	13.00^{defg}	**11.50**	60.00^{abc} (50.77)	96.67^{i} (83.38)	**78.33** (**67.31**)	5.56^{a} (8.04)	6.67^{a} (8.85)	**6.11** (**8.45**)
1.02 g OA + 1.38 g MA	18.02^{ef}	19.40^{hi}	**18.71**	12.83^{cdef}	11.67^{b}	**12.25**	76.67^{cdef} (61.22)	66.67^{bcd} (54.78)	**71.67** (**58.00**)	42.96^{fgh} (40.93)	15.08^{bcd} (22.85)	**29.02** (**31.89**)
0.59 g OA + 2.51 g MA	18.06^{ef}	16.79^{cd}	**17.42**	11.67^{b}	13.67^{ghi}	**12.67**	53.33^{ab} (46.92)	93.33^{hi} (77.71)	**73.33** (**62.32**)	12.22^{ab} (16.89)	30.36^{cdefg} (33.36)	**21.29** (**25.13**)
Standard artificial diet	17.44^{de}	17.46^{de}	**17.46**	13.74^{hi}	13.00^{defg}	**13.37**	90.00^{fgh} (71.56)	70.00^{bcde} (56.79)	**80.00** (**64.18**)	85.19^{m} (71.26)	61.90^{ijk} (51.94)	**73.55** (**61.60**)
Mean	**17.24**	**18.43**		**12.52**	**13.00**		**68.33** (**56.24**)	**83.06** (**68.86**)		**42.01** (**38.37**)	**44.06** (**40.17**)	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE**	**LSD**
***Bt* treatment**	<0.001	0.08	0.23*	<0.001	0.07	0.21*	<0.001	1.12	3.18*	0.354	1.36	**NS**
Amount of acids	<0.001	0.20	0.57*	<0.001	0.18	0.51*	<0.001	2.74	7.79*	<0.001	3.3	9.46*
Amount of acids × *Bt* treatment	<0.001	0.28	0.80*	<0.001	0.26	0.73*	<0.001	3.87	11.02*	<0.001	4.70	13.38*

Figures in the parenthesis are angular transformed values. OA = Oxalic acid; MA = Malic acid. *LSD at P≤ 0.05 %.

with 1.33 g OA + 2.83 g MA and 0.75 g OA + 2.83 g MA, followed by 6.67 per cent adult emergence in 0.75 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin diet, and 12.22 per cent adult emergence in 0.59 g OA + 2.51 g MA diet. Adult emergence was highest 85.19 per cent in the standard artificial diet without *Bt*, followed by 85.09 per cent adult emergence in diet with 0.60 g MA + 8.19 µg of *Bt* toxin.

4.2.7.5 Adult longevity and fecundity

The interaction effects for the organic acid amounts × *Bt* treatments were significant for adult longevity (Table 55). There were no females in insects reared on diets with 1.33 g OA + 0.60 g MA or 1.33 g OA + 2.83 g MA. Female longevity was shorter (1.33 days) in diets with 0.75 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin, followed by diets with 0.59 g OA + 0.60 g MA (3.67 days). Longest survival of females (10.33 days) was recorded in diet with 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin, followed by the insects reared on 0.59 g OA + 2.83 g MA (9.17 days) and the standard artificial diet without *Bt* (9.03 days).

No males were recorded on diets with 1.33 g OA + 2.83 g MA, 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin, 0.75 g OA + 2.83 g MA, 0.75 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin, 0.59 g OA + 2.51 g MA, and 0.59 g OA + 2.51 g MA + 8.19 µg of *Bt* toxin. Shortest survival (1.33 days) was recorded in insects reared on the diets with 1.02 g OA +1.38 g MA + 8.19 µg of *Bt* toxin, followed by those reared on diets with 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (2.33 days) (Table 55). Longest survival (10.33 days) was recorded in insects reared on diet with 0.59 g OA + 2.83 g MA, followed by insects reared on diets with 0.60 g OA (9.56 days), 2.83 g MA (8.99 days), 0.59 g OA + 0.60 g MA (8.71 days).

There were significant differences between the organic acid amounts × *Bt* treatments for fecundity per female (Table 55). Fecundity was lowest (207 eggs female^{-1}) in diet with 0.59 g OA + 2.83 g MA, followed by the insects reared on diet with 1.33 g OA (290 eggs female^{-1}), and 0.59 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (368 eggs female^{-1}). Highest fecundity was recorded in insects reared on the standard artificial diet without *Bt* (1267 eggs female^{-1}), followed by insects reared on the diets with 0.60 g MA diet (850 eggs female^{-1}), 2.83 g MA + 8.19 µg of *Bt* toxin (845 eggs female^{-1}), and 0.60 g MA + 8.19 µg of *Bt* toxin (795 eggs female^{-1}).

4.3 EFFECT OF ACID EXUDATES ON PROTOXIN-TOXIN CONVERSION AND BINDING TO THE BRUSH BORDER MEMBRANE OF THE GUT OF *H. armigera*

4.3.1 Effect of acid exudates on protoxin to toxin conversion

4.3.1.1 Enzyme linked immunosorbent assay (ELISA)

The amounts of Cry toxin present in the food, midgut, and faecal matter were estimated through ELISA. The optical density (OD) values of food, midgut, and faecal matter of larvae reared on the standard artificial diet, and diets with different amounts of organic acids were low (Plate 22), and nearer to the OD values of negative control (0.003). The OD values of food, midgut, and faecal matter of larvae reared on diets with *Bt* toxins were high, showing the presence of *Bt* toxins in these samples. The amounts of Cry toxin protein present in the food ranged from 1.017 to 1.534 ηg g^{-1}. In the midgut, it ranged from 1.080 to 3.241 ηg g^{-1}, and in faecal matter from 0.397 to 1.145 ηg g^{-1} (Table 56).

Table 55: Longevity and fecundity of *H. armigera* reared on artificial diet with different amounts of organic acids (oxalic and malic acid) and *Bt* (ICRISAT, Patancheru, 2006-07)

Amount of acids (g/ 300 ml of diet)	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
0.59 g OA	6 50^{defgh}	6 33^{defg}	**6.42**	5 78^{def}	5 78^{def}	**5.78**	513de	552de	**532**
1.33 g OA	7 00^{defghi}	4 67^{cde}	**5.83**	7 28^{efg}	5 50de	**6.39**	290^{ab}	557^{e}	**423**
0.60 g MA	7 56^{fghij}	6 40^{defg}	**6.98**	9 56^{hi}	7 44^{efh}	**8.50**	850^{h}	795^{gh}	**822**
2.83 g MA	7 50^{fghij}	5 67^{cdefg}	**6.58**	8 99^{ghi}	6 39^{ef}	**7.69**	718^{fg}	845^{h}	**782**
0.59 g OA + 0.60 g MA	3 67^{bc}	5 00^{cdef}	**4.33**	8 71^{ghi}	5 33de	**7.02**	450^{cd}	368^{bc}	**409**
0.59 g OA + 2.83 g MA	9 17^{ijk}	5 83^{cdefg}	**7.50**	10 33^{i}	7 33^{efg}	**8.83**	468^{cde}	207^{a}	**337**
1.33 g OA + 2.83 g MA	0 00^{a}	7 00^{defghi}	**3.50**	0 00^{a}	2 33^{bc}	**1.17**	-	-	-
1.33 g OA + 0.60 g MA	0 00^{a}	10 33^{k}	**5.17**	5 67de	0 00^{a}	**2.83**	-	-	-
0.75 g OA + 2.83 g MA	6 00^{cdefg}	1 33^{ab}	**3.67**	0 00^{a}	0 00^{a}	**0.00**	-	-	-
1.02 g OA + 1.38 g MA	8 00^{ghijk}	4 50^{cd}	**6.25**	3 67^{cd}	1 33^{ab}	**2.50**	-	-	-
0.59 g OA + 2.51 g MA	10 00^{ijk}	7 17^{efghi}	**6.92**	0 00^{a}	0 00^{a}	**3.00**	-	-	-
Standard artificial Diet	9 03^{hijk}	6 00^{cdefg}	**7.51**	7 96^{fgh}	5 33de	**6.65**	1267^{i}	665^{f}	**966**
Mean	**6.20**	**5.85**		**5.66**	**3.90**		**380**	**332**	
	Fp	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**	**Fp**	**SE±**	**LSD**
***Bt* treatment**	<0 001	0 64	1 83*	<0 001	0 55	1 56*	<0 001	25 60	72 87*
Amount of acids	0 353	0 26	**NS**	<0 001	0 22	0 63*	0 002	10 45	29 75*
Amount of acids × *Bt* treatment	<0 001	0 91	2 59*	0 001	0 77	2 20*	<0 001	36 20	103 06*

OA = Oxalic acid, MA = Malic acid *LSD at P≤ 0 05 %

The *Bt* toxin present in the food was highest in the diet with 0.59 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (1.534 ηg g^{-1}), followed by diets with 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin (1.323 ηg g^{-1}), 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (1.156 ηg g^{-1}). Lowest Cry toxin was recorded in diets with 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (1.017 ηg g^{-1}), followed by diets with 0.59 g OA + 2.83 g MA (1.021 ηg g^{-1}), the standard artificial diet with 8.19 µg of *Bt* toxin (1.059 ηg g^{-1}), and the diet with 0.59 g OA + 2.51 g MA + 8.19 µg of *Bt* toxin (1.083 ηg g^{-1}) (Table 56).

The amounts of *Bt* toxin present in the midgut of larvae were highest in larvae reared on diets with 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (3.241 ηg g^{-1}), followed by diets with 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin (3.164 ηg g^{-1}), and 0.59 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (2.701 ηg g^{-1}) (Table 56). Lowest *Bt* toxin protein was recorded in insects reared on diets with 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (1.080 ηg g^{-1}), followed by 0.59 g OA + 2.83 g MA (1.466 ηg g^{-1}), 0.59 g OA + 2.51 g MA + 8.19 µg of *Bt* toxin (1.059 ηg g^{-1}).

The amounts of Cry toxin were highest in the faecal matter of insects reared on diet with 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin (1.145 ηg g^{-1}), followed by insects reared on 0.59 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (0.992 ηg g^{-1}), and 1.33 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (0.694 ηg g^{-1}). Amounts of *Bt* toxin were lowest in faecal matter of insects reared on diet with 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (0.397 ηg g^{-1}), followed by those reared on the diets with 0.75 g OA + 2.83 g MA + 8.19 µg of *Bt* toxin (0.463 ηg g^{-1}), 0.59 g OA + 2.51 g MA + 8.19 µg of *Bt* toxin (0.543 ηg g^{-1}), and the insects reared on the standard artificial diet with 8.19 µg of *Bt* toxin (0.544 ηg g^{-1}) (Table 56).

Table 56: Amounts of *Bt* protein in the artificial diet (with different amounts of organic acids and *Bt* toxin for feeding to larvae of *H. armigera*), midgut and faecal matter of larvae fed on the diet with different amounts of organic acids and *Bt* (based on ELISA test)

Treatment	Diet		Larval midgut		Faecal matter	
	OD	Protein (ηg g^{-1})	OD	Protein (ηg g^{-1})	OD	Protein (ηg g^{-1})
0.59 g OA + 0.60 g MA	-0.006	-0.463	0.004	0.309	0.003	0.231
1.33 g OA + 2.83 g MA	0.003	0.174	0.004	0.309	0.004	0.258
1.33 g OA + 0.60 g MA	-0.004	-0.237	0.003	0.231	-0.004	-0.309
0.75 g OA + 2.83 g MA	-0.006	-0.340	0.001	0.077	0.002	0.154
0.59 g OA + 2.51 g MA	0.001	0.059	0.001	0.077	0.003	0.231
1.02 g OA + 1.38 g MA	0.001	0.059	0.003	0.231	0.000	0.000
0.59 g OA + 2.83 g MA	-0.004	-0.236	0.001	0.077	0.004	0.309
Standard artificial diet	0.000	0.000	0.001	0.077	0.004	0.305
Bt diet	0.019	1.059	0.028	2.160	0.007	0.544
0.59 g OA + 0.60 g MA + *Bt*	0.025	1.534	0.035	2.701	0.013	0.992
1.33 g OA + 2.83 g MA + *Bt*	0.020	1.156	0.014	1.080	0.009	0.694
1.33 g OA + 0.60 g MA + *Bt*	0.016	1.017	0.042	3.241	0.006	0.397
0.75 g OA + 2.83 g MA + *Bt*	0.018	1.152	0.021	1.620	0.006	0.463
0.59 g OA + 2.51 g MA + *Bt*	0.018	1.083	0.020	1.543	0.008	0.543
1.02 g OA + 1.38 g MA + *Bt*	0.023	1.323	0.041	3.164	0.017	1.145
0.59 g OA + 2.83 g MA + *Bt*	0.018	1.021	0.019	1.466	0.008	0.617
Positive control	1.495	-	-	-	-	-
Negetive control	0.003	-	-	-	-	-

OA =Oxalic acid; MA = Malic acid, OD = Optical density.

4.3.2 Effect of acid exudates on binding of toxins to brush border membrane vesicles (BBMV) of midgut of *H. armigera* larvae

The amount of protein present in the BBMV preparations was estimated through the Lowry's method of protein estimation (Plate 23) (Table 57). The amounts of protein present in the BBMV ranged from 0.131 to 0.326 mg g^{-1}. The amounts of protein estimated from the BBMV of larvae fed on diets with *Bt* were higher as compared to larvae fed on diets without *Bt*. The amount of protein present in the BBMV preparations from insects fed on standard artificial diet with 8.19 µg of *Bt* toxin was highest (0.326 mg g^{-1}), followed by the insects fed on diets with 1.33 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (0.287 mg g^{-1}), 0.59 g OA + 0.60 g MA + 8.19 µg of *Bt* toxin (0.265 mg g^{-1}), and 1.02 g OA + 1.38 g MA + 8.19 µg of *Bt* toxin (0.261 mg g^{-1}). The amount of protein present in the BBMV preparations made from the larvae fed on diets with 1.33 g OA + 0.60 g MA was lowest (0.131 mg g^{-1}), followed by insects reared on diets with 0.75 g OA + 2.83 g MA (0.144 mg g^{-1}), 0.59 g OA + 2.83 g MA (0.150 mg g^{-1}).

There was a positive correlation between the larval weight and amount of protein in the BBMV, when the larvae were reared on the diet with increasing amounts of oxalic acid and *Bt* (0.77), and increasing amounts of malic acid and *Bt* (0.85).

There was a decrease in the amount of protein with an increase in the amount of oxalic acid (Fig 4), and malic acid (Fig 5). However, an increase in the amounts of oxalic acid (Fig 6) or malic acid (Fig 7) also resulted in a decrease in larval weights, which may be due to antifeedant and/or antibiotic effects of these organic acids on the larvae of *H. armigera*.

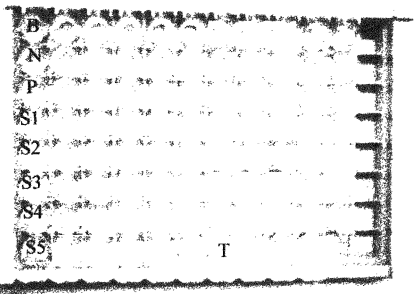


Plate 22: ELISA test results showing the presence of *Bt* toxin (yellow wells) (B-Blank, N-Negative control, P-Positive control, S1 to S5-Standards)

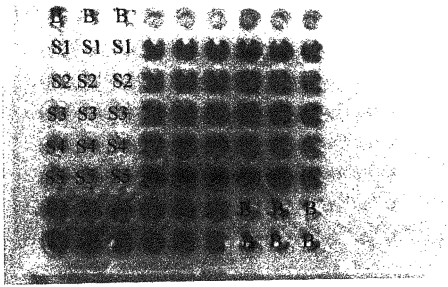


Plate 23: Protein estimation in BBMV preparations by 96 well plate by Lowry method (B-Blank, S1 to S5-Standards)

Table 57: Amounts of protein present in the brush border membrane vesicles (BBMV) of the larvae fed on artificial diet with different amounts of organic acids and *Bt*

Treatment	OD value		Protein (mg g^{-1})	
	Without *Bt*	With *Bt*	Without *Bt*	With *Bt*
0.59 g OA + 0.60 g MA	0.361	0.334	0.182	0.265
1.33 g OA + 2.83 g MA	0.474	0.467	0.166	0.195
1.33 g OA + 0.60 g MA	0.314	0.717	0.131	0.287
0.75 g OA + 2.83 g MA	0.422	0.393	0.144	0.222
0.59 g OA + 2.51 g MA	0.348	0.481	0.165	0.213
1.02 g OA + 1.38 g MA	0.312	0.317	0.153	0.261
0.59 g OA + 2.83 g MA	0.370	0.652	0.150	0.249
Standard artificial diet	0.694	0.355	0.209	0.326
Blank	0.000	-	-	-

OA =Oxalic acid; MA = Malic acid, OD = Optical density.

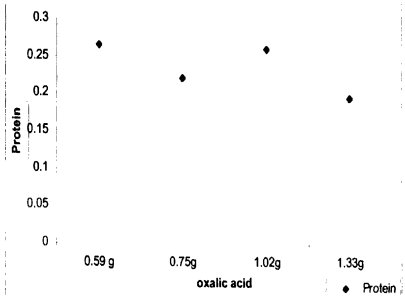


Fig 4: Amount of protein in the BBMV of larvae fed on the diets with increasing amounts of oxalic acid

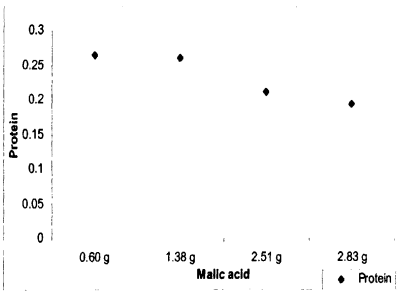


Fig 5: Amount of protein in the BBMV of larvae fed on the diets with increasing amounts of malic acid

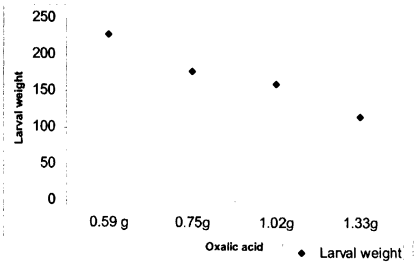


Fig 6: Weight of the larvae fed on the diets with increasing amounts of oxalic acid

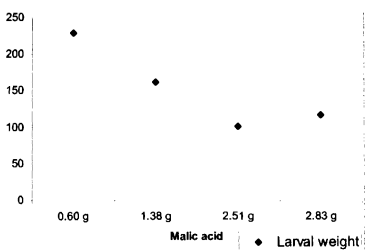


Fig 7: Weight of larvae fed on the diets with increasing amounts of malic acid

CHAPTER-V

Discussion

CHAPTER – V

DISSCUSSION

Chickpea is damaged by nearly 57 species of insects, of which pod borer *Helicoverpa armigera* (Hubner) is the most important pest in the semi-arid tropics. Insecticide application for controlling *H. armigera* is uneconomical under subsistence farming, and at times is largely beyond the means of resource poor farmers. Therefore, host plant resistance assumes a pivotal role in controlling *H. armigera* damage either alone or in combination with other methods of pest control. Several chickpea genotypes with low to moderate levels of resistance have been identified (Lateef, 1985). However, the levels of resistance are unstable across seasons and locations, and therefore, there is a need to develop transgenic chickpeas with high levels of resistance to *H. armigera*. However there is an apprehension that the acid exudates in chickpea leaves and pods may influence the effectiveness of *Bacillus thuringiensis* Berliner (*Bt*) toxins produced in the transgenic plants. Therefore, it is important to study the influence of acid exudates in chickpea on the biological activity of Cry toxins from *B. thuringiensis* against *H. armigera*.

The results of the present investigation on, **"Interaction of acid exudates in chickpea with biological activity of Cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)"** were discussed below.

5.1 EFFECTIVENESS OF *B. thuringiensis* AGAINST *H. armigera* ON RESISTANT AND SUCEPTIBLE GENOTYPES OF CHICKPEA

The egg and larval numbers were lower on ICC 506 and ICCV 10 across *Bt* concentrations as compared to those on L 550 and C 235 during the vegetative and flowering stages, before and after sprays. Low oviposition and larval numbers on ICC 506 have earlier been observed by Lateef (1985). Leaf feeding and pod damage were lowest on ICC 506, followed by ICCV 10 across *Bt* concentrations as compared to those on L 550 and C 235. Percentage pod damage by *H. armigera* larvae was lower in ICC 506 across *Bt* concentrations as compared to that on ICCV 10, C 235, and L550.

During 2005-06 post-rainy season and October planting during 2006-07 post-rainy season, highest grain yield was recorded in case of ICCV 10. There were no significant differences between the genotypes and *Bt* concentrations for grain yield plant^{-1} and total grain yield. In the December planting during 2006-07 post-rainy season, the genotypes ICCV 10 and ICC 506 recorded highest grain yield. Similar results were reported by Kulkarni and Amonkar (1988), who observed that *Bt* treated crop showed a reduction in larval population, but there were no significant effects on grain yield. Singh (1999) reported low pod damage and increase in grain yield when Delfin, Dipel, and NPV were used in combination with endosulfan. Balasubramanian *et al.* (2002) also observed a reduction in larval population and pod damage. Maximum grain yield was recorded in plots sprayed with Spicturin (2L ha^{-1}). Mandal *et al.* (2003) reported that

among the biopesticides tested, Biolep treated plots had the lowest number of larvae per ten plants, low pod damage, and highest grain yield.

5.1.1 Evaluation of biological activity of *B. thuringiensis* against *H. armigera* on different chickpea genotypes under laboratory conditions (detached leaf assay)

Detached leaf assay not only gives an idea of the relative feeding by the larvae on different genotypes but also provides useful information on antibiosis component of resistance interms of larval weight (Sharma *et al.,* 2005b). Chickpea varieties differ in their susceptibility to *H. armigera* due to differences in antibiosis (Singh and Sharma, 1970). Lateef (1985) suggested that amounts of acid exudates on leaves could be used as a criteria for distinguishing chickpea genotypes for resistance to *H. armigera*. Rembold (1981) recommended it as a marker to identify *H. armigera* resistant genotypes of chickpea. Low amounts of acidity in the leaf extracts of chickpea genotypes are associated with susceptibility to *H. armigera* (Yoshida *et al.,* 1997), and the larvae gain maximum weights on the susceptible genotypes as compared to that on the resistant ones (Srivastava and Srivastava, 1990).

Significantly lower leaf feeding was observed on the ICC 506, followed by ICCV 10. Larval survival and larval weights were lowest on ICC 506, followed by ICCV 10, suggesting that antibiosis is one component of resistance to *H. armigera* in chickpea. Leaf exudates play an important role in *H. armigera* resistance in chickpea (Rembold, 1981; Rembold and Winter, 1982; Srivastava and Srivastava, 1989; and Yoshida *et al.,* 1997), and may be responsible for antifeedant and/or antibiosis towards this insect.

During the vegetative stage, significantly lower leaf damage was recorded on ICC 506, followed by ICCV 10. Larval survival across *Bt* concentrations was lowest on ICCV 10, followed by C 235, and ICC 506, whereas larval weight was lowest of the larvae reared on ICC 506, followed by those reared on ICCV 10 and C 235. During the flowering stage, lowest leaf damage was recorded in ICC 506, followed by C 235 across *Bt* concentrations. Larval survival was low on C 235, followed by ICCV 10 and ICC 506, while larval weights were lower on C 235, followed by ICC 506 across *Bt* concentrations.

5.2 INTERACTION OF ACID EXUDATES (MALIC ACID AND OXALIC ACID) WITH BIOLOGICAL ACTIVITY OF *Bt* TOXINS AGAINST *H. armigera*

5.2.1 Survival and development of *H. armigera* on chickpea genotypes

Lowest larval and pupal weights were recorded on ICC 506, followed by ICCV 10. Larval period was longer on ICC 506 and ICCV 10 than on L 550 and C 235. Percentage pupation and adult emergence were lower on ICC 506 and ICCV 10 as compared to those on L 550 and C 235. Longevity of adults from insects reared on different chickpea genotypes did not differ significantly. Fecundity was lower in insects reared on ICC 506, followed by those reared on ICCV 10. Similar results have been reported by Srivastava and Srivastava (1989), Chhabra *et al.* (1993), Bhagwat *et al.* (1995), Patnaik and Senapati (1995), who indicated that low amounts of acidity of leaf exudates were associated with susceptibility to *H. armigera*. Cowgill and Lateef (1996) observed that insects reared on the leaves and pods of resistant lines weighed significantly lower than those reared on the susceptible genotypes.

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

There were significant differences in survival and development of the pod borer, *H. armigera* on the chickpea genotypes sprayed with *Bt*. Lowest larval survival was recorded in larvae reared on ICC 506 sprayed with 0.2 per cent *Bt*, followed by ICCV 10 sprayed with 0.2 per cent *Bt*, C 235 sprayed with 0.1 per cent *Bt*, ICCV 10 sprayed with 0.1 per cent *Bt*, and L 550 sprayed with 0.2 per cent *Bt*. There was no pupation in the larvae reared on C 235 sprayed with 0.05 per cent *Bt*. Pupal weights were lower in the larvae reared on ICC 506 and ICCV 10 sprayed with 0.05 per cent *Bt* as compared to those reared on the unsprayed L 550 (262.30 mg). The larval period was prolonged by more than two days in the larvae reared on the unsprayed ICC 506, ICCV 10, and ICC 506 sprayed with 0.05 per cent *Bt* as compared to the larvae reared on the unsprayed L 550. Pupal period was longer on ICC 506 sprayed with 0.05 per cent *Bt* as compared to that on L 550 and ICCV 10 sprayed with 0.05 per cent *Bt*. The pupation was lower on ICCV 10, L 550, and ICC 506 sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550. Adult emergence was lower on ICCV 10, L 550, and ICC 506 sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550. Female longevity was shorter on L 550, ICCV 10, and ICC 506 sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed ICCV 10. No males survived on ICCV 10 sprayed with 0.05 per cent *Bt*. Shortest male survival was recorded on L 550 and ICC 506 sprayed with 0.05 per cent *Bt* as compared to that on the unsprayed L 550 and C 235. The fecundity was lower on ICC 506 and L 550 sprayed with 0.05 per cent *Bt* as compared to that on the

unsprayed L 550. The eggs laid by these adults were infertile, as no larvae emerged from these eggs.

The survival and development of *H. armigera* on ICC 506, ICCV 10, and C 235 sprayed with different concentrations of *Bt* significantly differed with that on the unsprayed chickpeas. At higher concentrations of *Bt* (0.1, 0.2, and 0.5%), there was no survival of the *H. armigera* larvae. Only at lower concentrations of *Bt* (0.05%), larvae survived, but had lower larval and pupal weights, prolonged larval and pupal periods, and reduced fecundity.

5.2.3 The HPLC profiles of acid exudates in chickpea

Highest number of HPLC peaks (11) were observed in C 235 at the podding stage, and lowest (6) number of peaks were observed in ICC 506, ICCV 10, and L 550 at the vegetative stage, and L 550 at the podding stage. The genotype ICC 506 at the flowering stage had one major peak (83.42% area), and two major peaks at the vegetative and podding stages. ICCV 10 had four major peaks at the vegetative stage, three major peaks at the flowering stage, and two major peaks at the podding stage, while C 235 had five major peaks at the vegetative stage, four at the flowering stage, and three at the podding stage. L 550 had two major peaks at the vegetative, flowering, and podding stages. At the vegetative and flowering stages, ICC 506 had the highest amounts of oxalic acid on dry weight and leaf area basis. At the podding stage, ICCV 10 had the highest amount of oxalic acid on dry weight basis, whereas on leaf area basis, ICC 506 had the highest amount. On dry weight basis, ICCV 10 had the highest amount of malic acid at the vegetative, flowering, and podding stages. On leaf area basis, ICCV 10 had the highest amount of malic acid at the vegetative stage, whereas C 235 had the highest

amount of malic acid at the flowering and podding stages. Fumaric and citric acids were recorded at the podding stage only. The genotype C 235 had the highest amounts of fumaric and citric acids. There was no citric acid in ICC 506. Oxalic acid and malic acid have been reported to have an antibiotic effect on *H. armigera* larvae (Yoshida *et al.*, 1995), and it is possible that the antifeedant/ antibiotic properties of oxalic acid influence the contribution of oviposition non-preference to determine the size of the larval population and pod damage on a particular genotype (Yoshida *et al.*, 1997, Rembold, 1981, and Rembold and Winter, 1982).

5.2.4 Survival and development of *H. armigera* on artificial diets with lyophilized leaf and pod powder of different chickpea genotypes along with *Bt* toxins

5.2.4.1 Leaf powder diet

There were no significant differences in the larval weights of five day-old larvae, but the differences in ten-day-old larval weights were significant. Lowest larval weight was recorded in the larvae reared on the standard artificial diet with 8.19 µg of *Bt* toxin (132.9 mg), whereas highest larval weight was recorded in the larvae reared on the standard artificial diet without *Bt* (411.3 mg), followed by those reared on diets with L 550 leaf powder (399.5 mg). Pupal weight was lowest on the standard artificial diet with 8.19 µg of *Bt* toxin (309.8 mg), followed by diets with L 550 leaf powder + 8.19 µg of *Bt* toxin (316.3 mg). Highest pupal weight was recorded in insects reared on the standard artificial diet without *Bt* (364.3 mg), followed by those reared on diets with L 550 leaf powder (343.1 mg). Larval period increased by more than two days in larvae reared on the standard artificial diet with 8.19 µg of *Bt* toxin, followed by those reared on ICC 506 leaf powder. Pupal period was shorter on the standard artificial diet without *Bt*

as compared to insects reared on diets with ICCV 10 leaf powder + 8.19 µg of *Bt* toxin, ICC 506 leaf powder, and ICCV 10 leaf powder. Pupation and adult emergence were lowest on the standard artificial diet with 8.19 µg of *Bt* toxin, and on diets with ICC 506 leaf powder as compared to the insects reared on the standard artificial diet, and with L 550 leaf powder. There were no significant differences in the longevity of adults. Lowest fecundity was recorded in insects reared on diets with C 235 leaf powder + 8.19 µg of *Bt* toxin, followed by those reared on ICC 506 leaf powder, ICCV 10 leaf powder, and ICCV 10 leaf powder + 8.19 µg of *Bt* toxin.

5.2.4.2 Pod powder diet

Weights of five day-old larvae were lowest in insects reared on diets with ICCV 10 pod powder + 8.19 µg of *Bt* toxin, followed by those reared on ICC 506 pod powder + 8.19 µg of *Bt* toxin. There were no significant differences in the ten-day-old larvae for larval weights. Pupal weight was lowest in insects reared on diets with L 550 pod powder + 8.19 µg of *Bt* toxin (281.6 mg), followed by ICC 506 pod powder + 8.19 µg of *Bt* toxin, and C 235 pod powder. Highest pupal weight was recorded in insects reared on diets with L 550 and ICCV 10 pod powders. There were no significant differences in the larval and pupal periods. Lowest pupation was recorded on the standard artificial diet with 8.19 µg of *Bt* toxin, and highest on the standard artificial diet without *Bt*, and ICC 506 pod powder + 8.19 µg of *Bt* toxin. There were no significant differences in adult emergence, while female longevity was lowest on diets with C 235 pod powder + 8.19 µg of *Bt* toxin, and highest on L 550 pod powder + 8.19 µg of *Bt* toxin. Male longevity was lowest on ICCV 10 pod powder and highest on L 550 pod powder +

8.19 µg of *Bt* toxin. Fecundity was lowest on the standard artificial diet with 8.19 µg of *Bt* toxin, and highest on the standard artificial diet without *Bt* (921.3).

The survival and development of larvae reared on the artificial diet with leaf or pod powders of different chickpea genotypes with *Bt* toxins was significantly lower as compared to that on the standard artificial diet, and diets with leaf or pod powder of different chickpea genotypes without *Bt*. Larval survival, larval and pupal weights, and pupation and adult emergence were consistently lower on the resistant genotypes than on the susceptible ones, and the standard artificial diet. The results suggested that the components in the leaf or pod powders do not interfere with the toxicity of the *Bt*.

5.2.5 Survival and development of *H. armigera* on artificial diet with organic acids and *Bt* toxins

Weights of five day-old larvae were lowest in larvae reared on diets with 1.33 g of oxalic acid + 2.83 g of malic acid + 8.19 µg of *Bt* toxin, and highest in larvae reared on the standard artificial diet without *Bt*. Tenth day larval weight was lowest in diets with 0.59 g of oxalic acid + 2.51 g of malic acid + 8.19 µg of *Bt* toxin, and highest on the standard artificial diet without *Bt*. Pupal weights were lowest in insects reared on diets with 1.02 g of oxalic acid + 1.38 g of malic acid diet, and highest on the standard artificial diet without *Bt*. Shortest larval period was recorded in diets with 0.60 g of malic acid + 8.19 µg of *Bt* toxin, and highest on diets with 1.33 g of oxalic acid + 2.83 g of malic acid + 8.19 µg of *Bt* toxin. There were no significant differences in the pupal period. Pupation was lowest (43.33%) in diets with 1.33 g of oxalic acid + 8.19 µg of *Bt* toxin, and highest (96.67%) in diets with 0.75 g of oxalic acid + 2.83 g of malic acid + 8.19 µg of *Bt* toxin. Lowest adult emergence (5.56%) was recorded in diets with 1.33 g of

oxalic acid + 2.83 g of malic acid, and highest (85.19%) on the standard artificial diet without *Bt*. Longevity of adults and fecundity were adversely affected by oxalic acid, malic acid, and *Bt* in the standard artificial diet.

The present study indicated that both oxalic and malic acid increase the biological activity of *Bt* toxins to the *H. armigera*. The organic acids result in reduced larval weight, prolonged development, and reduced longevity and fecundity. Similar effects of *Bt* toxins have earlier been reported by several workers (Ludlum *et al.*, 1991, Sivamani *et al.*, 1992, Morris *et al.*, 1994, Gibson *et al.*, 1995, Wang *et al.*, 1997, Ahmed *et al.*, 1998, Zhang *et al.*, 2000a).

5.3 EFFECT OF ACID EXUDATES ON PROTOXIN-TOXIN CONVERSION AND BINDING TO THE BRUSH BORDER MEMBRANE OF THE GUT OF *H. armigera*

5.3.1 Effect of acid exudates on protoxin to toxin conversion

The food, midgut, and faecal matter samples of larvae fed on diet with different amounts of acids and *Bt* indicated the conversion of protoxin to toxin and binding to the BBMV of midgut. The amount of Cry toxin protein present was highest in the diets with 0.59 g of oxalic acid + 0.60 g of malic acid + 8.19 µg of *Bt* toxin, and lowest in diets with 1.33 g of oxalic acid + 0.60 g of malic acid + 8.19 µg of *Bt* toxin. In the midgut, the amount of protein present was highest in larvae fed on diets with 1.33 g of oxalic acid + 0.60 g of malic acid + 8.19 µg of *Bt* toxin, and lowest in larvae fed on diets with 1.33 g of oxalic acid + 2.83 g of malic acid + 8.19 µg of *Bt* toxin. The amount of protein present was highest in the faecal matter of larvae fed on diets with 1.02 g of oxalic acid + 1.38 g

of malic acid + 8.19 µg of *Bt* toxin, and lowest in larvae fed on diets with 1.33 g of oxalic acid + 0.60 g of malic acid + 8.19 µg of *Bt* toxin.

Due to the conversion of protoxin to toxin and binding to the BBMV in the midgut, the amount of Cry toxin in the midgut samples was higher as compared to that in the food samples. There were no significant differences in the amount of *Bt* protein present in the midgut samples of the larvae fed on diets with different amounts of oxalic and malic acids, and Cry toxin, indicating that the organic acids do not show any effect on conversion of protoxin to toxin. The amounts of acids present in the diet probably indirectly affected the amount of food taken by the larvae.

5.3.2 Estimation of proteins in the BBMV by Lowry's method

The amount of protein in the BBMV preparations ranged from 0.131 to 0.326 mg g^{-1}. The amount of protein estimated from the BBMV of larvae fed on diet with Cry toxin was highest as compared to the BBMV of larvae fed on diet without Cry toxin, indicating binding of the Cry protein to the BBMV increased the protein content in the BBMV. Due to the antibiotic effect of oxalic and malic acids and *Bt*, the larvae probably consumed less food, showed less absorption efficiency of the digested food, but compensated it by increased utilization of ingested and digested food into the body substance, and such interactions were common in insects and other animals. When the larvae utilized the digested food, the *Bt* toxins present in the midgut bind to the BBMV forming pores, increasing the permeability of gut. The insect gut and its contents influence the activity of microbial pathogens, and provide the targets for improvement of microbial pesticides. The insecticidal properties of the *Bt* endotoxins depend on ingestion by the target insects. The antifeedents present in chickpea leaves may interfere with the effectiveness of the

endotoxin as less amounts of food will be consumed by the larvae of *H. armigera*. The amounts of oxalic and malic acids present in leaves and pods of different chickpea genotypes impregnated in to diet did not effect the conversion of protoxin to toxin, and binding to the BBMV, and thus the effectiveness of *Bt* toxin. There was a positive correlation between the larval weight and amount of protein in the BBMV, when the larvae were reared on the diet with increasing amounts of oxalic acid and *Bt* (0.77), and with increasing amounts of malic acid and *Bt* (0.85). There was a decrease in amounts of *Bt* protein with an increase in amounts of organic acids. The increased amounts of organic acids resulted in a decrease in larval weights due to antifeedant and/or antibiotic effects of these compounds in the larvae of *H. armigera* (Yoshida *et al.*, 1995, 1997).

CHAPTER-VI

Summary

CHAPTER – VI

SUMMARY

The present research was taken up to study the "**Interaction of acid exudates in chickpea with biological activity of Cry toxins from *Bacillus thuringiensis* Berliner against *Helicoverpa armigera* (Hubner)".** These studies were carried out at the International Crops Research Institute for Semi-Arid Tropics (ICRSAT), Patancheru, Andhra Pradesh, India, during 2005-08. The effect of acid exudates on the biological activity of cry toxins from *B. thuringiensis* was studied both under laboratory and field conditions.

Number of *H. armigera* eggs and larvae before and after sprays of *B. thuringiensis* (*Bt*) formulation Biolep® were low on the chickpea genotypes ICC 506 and ICCV 10 across *Bt* concentrations as compared to those on L 550 and C 235 during vegetative and flowering stages. Leaf feeding and pod damage were lowest on ICC 506, followed by ICCV 10 across *Bt* concentrations as compared to that on L 550 and C 235. During the 2005-06 post-rainy season and first planting during the 2006-07 post-rainy season, highest grain yield was recorded in case of ICCV 10. In the second planting, the genotypes ICCV 10 and ICC 506 recorded the highest total grain yield. There were no significant differences between the genotypes and *Bt* concentrations for grain yield.

Significantly lower leaf damage was recorded on ICC 506 across *Bt* concentrations in detached leaf assay under laboratory conditions. Larval survival and larval weights were lower in ICC 506, ICCV 10, and C 235 across *Bt* concentrations as compared to that of L 550. Survival and development of *H. armigera* on ICC 506, ICCV 10, and C 235 sprayed with different concentrations of *Bt* differed significantly with that of the unsprayed plants of these genotypes. At higher concentrations of *Bt* (0.1, 0.2, and 0.5%), there was no survival of the *H. armigera* larvae. At lower concentrations of *Bt* (0.05%), the larval survival and larval and pupal weights were lower on the sprayed (0.05%) plants than those on the unsprayed plants of different chickpea genotypes. Larval and pupal periods were prolonged on plants sprayed with *Bt*. There was a significant reduction in fecundity of insects reared on chickpea plants sprayed with *Bt*.

At the vegetative and flowering stages, ICC 506 had the highest amounts of oxalic acid on dry weight and leaf area basis. At the podding stage, ICCV 10 had the highest amount of oxalic acid on dry weight basis. On dry weight basis, ICCV 10 had the highest amount of malic acid at the vegetative, flowering, and podding stages. On leaf area basis, ICCV 10 had the highest amount of malic acid at the vegetative stage, whereas C 235 had the highest amount of malic acid at the flowering stage and podding stages. Fumaric and citric acids were recorded at the podding stage only. The genotype C 235 had the highest amounts of fumaric and citric acids. There was no citric acid in ICC 506.

The survival and development of *H. armigera* larvae reared on artificial diet with leaf/pod powders of different chickpea genotypes and *Bt* were significantly lower as compared to that on the standard artificial diet, and the diets without *Bt*. Larval survival, larval and pupal weights, and pupation and adult emergence were lower on the resistant

genotypes than on the susceptible ones, and the standard artificial diet. Oxalic and malic acids in the artificial diet increased the biological activity of *Bt* toxins on the *H. armigera*, and resulted in reduced larval weight, prolonged development, and reduced longevity and fecundity.

The food, midgut, and faecal matter samples of larvae fed on diet with different amounts of organic acids and *Bt* indicated the conversion of protoxin to toxin, and binding to the brush border membrane vesicles (BBMV) of midgut. Due to the conversion of protoxin to toxin and binding to the BBMV, the amount of *Bt* toxin in the midgut samples was greater as compared to that in the food samples. There were no significant differences in the amounts of *Bt* protein present in the midgut samples of the larvae fed on diets with different amounts of oxalic and malic acids and *Bt* toxin, indicating that the organic acids did not influence the conversion of protoxin to toxin.

The amounts of protein in the BBMV preparations ranged from 0.131 to 0.326 mg g^{-1}. The amount of protein estimated from the BBMV of larvae fed on diets with *Bt* was higher as compared to the amounts in the BBMV of the larvae fed on diet without *Bt*, indicating the binding of the *Bt* protein to the BBMV, which resulted in increased protein content in the BBMV.

The insecticidal activity of *Bt* endotoxins depends on the amounts of food ingested by the target insects. The organic acids (oxalic and malic acids) also act as antifeedents, and therefore, may reduce the effect of *Bt* as less amounts of food will be consumed by the larvae. However, the amounts of oxalic and malic acids impregnated in to the diet did not effect the conversion of protoxin to toxin and binding to the BBMV, and thus the effectiveness of *Bt* toxins.

CONCLUSIONS

- The relative susceptibility of different chickpea genotypes (sprayed and unsprayed) in the field and in the detached leaf assay may be influenced by the relative importance of non-preference for feeding.
- The acid components in the leaf or pod powders do not interfere with the toxicity of the *Bt*.
- The organic acids, both oxalic and malic acid, increased the biological activity of *Bt* toxins on the *H. armigera*.
- The amounts of oxalic and malic acids present in leaves and pods of different chickpea genotypes did not influence the conversion of protoxin to toxin, and binding to the BBMV, and thus the effectiveness of *Bt* toxin.

FUTURE LINE OF WORK:

- Effect of organic acids on consumption and utilization of food, and the relevance of antifeedant/ antibiotic activity of organic acids for biological activity of *Bt* toxins and binding to brush border membrane vesicles.
- Factors influencing the protoxin-toxin conversion in *H. armigera*, including the midgut microflora.
- Effect of organic acids on stability/ degradation of *Bt* toxins in insect midgut.

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* Originals not seen

Note: The literature is cited as per the "Thesis Guidelines" prescribed by Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad.

Appendix

APPENDIX

Table 58: HPLC peak results of ICC 506 at the vegetative stage

Peak results of ICC 506					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.00	22059	1.30	2892
2	**Peak 2**	3.32	14792	0.87	2616
3	**Peak 3**	3.47	282008	16.62	34784
4	**Oxalic acid**	4.01	1254912	73.95	129251
5	**Malic acid**	4.92	42071	2.48	5081
6	**Peak 6**	12.64	81105	4.78	4151

Table 59: HPLC peak results of ICC 506 at the flowering stage

Peak results of ICC 506					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.01	28922	0.81	3262
2	**Peak 2**	3.31	18014	0.51	2641
3	**Peak 3**	3.47	104399	2.94	14306
4	**Oxalic acid**	3.95	2930322	82.42	379400
5	**Malic acid**	4.69	139386	3.92	17629
6	**Peak 6**	5.29	3836	0.11	480
7	**Peak 7**	7.58	6985	0.20	684
8	**Peak 8**	10.53	323467	9.10	19618

Table 60: HPLC peak results of ICC 506 at the podding stage

Peak results of ICC 506					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	2.95	23234	0.43	3067
2	**Peak 2**	3.44	223794	4.17	33546
3	**Peak 3**	3.73	31351	0.58	3143
4	**Peak 4**	3.93	364233	6.79	84808
5	**Oxalic acid**	4.05	2690669	50.19	403157
6	**Malic acid**	5.47	1516565	28.29	181344
7	**Peak 7**	6.07	37933	0.71	3538
8	**Fumaric acid**	16.75	473309	8.83	19326

Table 61: HPLC peak results of ICCV 10 at the vegetative stage

Peak results of ICCV 10					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.01	18092	1.71	2249
2	**Peak 2**	3.32	7472	0.71	1425
3	**Peak 3**	3.48	170657	16.16	22101
4	**Peak 4**	3.89	393582	37.28	66082
5	**Oxalic acid**	3.99	357901	33.90	48206
6	**Malic acid**	4.87	108164	10.24	13066

Table 62: HPLC peak results of ICCV 10 at the flowering stage

Peak results of ICCV 10					
S. No.	**Name**	RT	Area	% Area	Height
1	**Peak 1**	2.99	28641	1.14	3303
2	**Peak 2**	3.30	19026	0.76	2854
3	**Peak 3**	3.47	273013	10.88	37132
4	**Oxalic acid**	3.93	1553831	61.91	136149
5	**Malic acid**	4.68	554499	22.09	69518
6	**Peak 6**	4.99	10096	0.40	1279
7	**Peak 7**	6.55	3129	0.12	454
8	**Peak 8**	6.98	11811	0.47	1164
9	**Peak 9**	8.89	23683	0.94	1864
10	**Peak 10**	10.39	32138	1.28	2223

Table 63: HPLC peak results of ICCV 10 at the podding stage

Peak results of ICCV 10					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.45	118626	1.67	19191
2	**Peak 2**	3.73	22694	0.32	3206
3	**Peak 3**	3.92	503978	7.09	110602
4	**Oxalic acid**	4.05	3712498	52.22	571855
5	**Malic acid**	5.45	2529009	35.57	298692
6	**Peak 6**	6.08	16737	0.24	2465
7	**Citric acid**	13.76	49166	0.69	3334
8	**Fumaric acid**	17.13	156952	2.21	6956

Table 64: HPLC peak results of C 235 at the vegetative stage

Peak results of C 235					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	2.98	72077	12.71	9202
2	**Peak 2**	3.31	106736	18.82	15979
3	**Peak 3**	3.47	118318	20.86	14891
4	**Peak 4**	3.70	23643	4.17	3010
5	**Oxalic acid**	3.96	116930	20.62	13612
6	**Malic acid**	4.72	44455	7.84	5730
7	**Peak 7**	9.17	61290	10.81	4541
8	**Peak 8**	10.77	23681	4.18	1678

Table 65: HPLC peak results of C 235 at the flowering stage

Peak results of C 235					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	2.97	31698	1.78	3553
2	**Peak 2**	3.28	23412	1.31	3055
3	**Peak 3**	3.47	257101	14.40	34268
4	**Peak 4**	3.68	19833	1.11	4119
5	**Oxalic acid**	3.93	861809	48.26	74209
6	**Malic acid**	4.68	337834	18.92	41858
7	**Peak 7**	4.99	10695	0.60	1295
8	**Peak 8**	5.28	15179	0.85	1863
9	**Peak 9**	7.54	12897	0.72	1245
10	**Peak 10**	10.46	215179	12.05	13200

Table 66: HPLC peak results of C 235 at the podding stage

Peak results of C 235					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.44	202985	2.81	30294
2	**Peak 2**	3.74	32922	0.46	3783
3	**Peak 3**	3.94	420684	5.82	79811
4	**Oxalic acid**	4.08	2442342	33.81	368707
5	**Malic acid**	5.56	2648520	36.67	300924
6	**Peak 6**	6.04	25572	0.35	2806
7	**Peak 7**	6.22	34952	0.48	3377
8	**Peak 8**	6.56	19857	0.28	1631
9	**Peak 9**	10.80	31852	0.44	2550
10	**Citric acid**	14.40	71434	0.99	4257
11	**Fumaric acid**	17.89	1292032	17.89	49815

Table 67: HPLC peak results of L 550 at the vegetative stage

Peak results of L550					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.00	18102	2.71	2246
2	**Peak 2**	3.30	8069	1.21	1398
3	**Peak 3**	3.47	97901	14.67	13098
4	**Oxalic acid**	3.89	452231	67.78	47562
5	**Malic acid**	4.91	34795	5.22	4207
6	**Peak 6**	12.49	56088	8.41	3071

Table 68: HPLC peak results of L 550 at the flowering stage

Peak results of L 550					
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.01	28437	1.03	3380
2	**Peak 2**	3.33	20620	0.75	3212
3	**Peak 3**	3.48	288961	10.47	39188
4	**Oxalic acid**	3.95	2151031	77.93	237823
5	**Malic acid**	4.71	222089	8.05	28201
6	**Peak 6**	5.31	4887	0.18	704
7	**Peak 7**	10.55	44249	1.60	2972

Table 69: HPLC peak results of L 550 at the podding stage

	Peak results of L 550 R2				
S. No.	**Name**	**RT**	**Area**	**% Area**	**Height**
1	**Peak 1**	3.45	255841	3.78	38731
2	**Peak 2**	3.75	38350	0.57	4844
3	**Peak 3**	3.94	589218	8.69	119456
4	**Oxalic acid**	4.08	3220722	47.52	481108
5	**Malic acid**	5.58	1993683	29.42	228311
6	**Peak 6**	6.24	25635	0.38	2689
7	**Peak 7**	6.56	23483	0.35	1748
8	**Citric acid**	14.25	48921	0.72	2948
9	**Fumaric acid**	17.63	581142	8.58	22995