CHARACTERIZTION OF *Cry IIa* TRANSGENIC CHICKPEA LINES AND THEIR INTERACTION WITH NATURAL ENEMIES OF *Helicoverpa armigera* (Hubner).

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M.Sc. (Ag.)

THESIS SUBMITTED TO
PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN AGRICULTURE

(ENTOMOLOGY)

CHAIRPERSON: Dr. T. RAMESH BABU

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COLLEGE OF AGRICULTURE
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RAJENDRANAGAR, HYDERABAD - 500 030.

2015

ACKNOWLEDGEMENTS

It is by the lavish and boundless blessing of the Almighty that I have been able to complete my studies successfully hitherto and present this humble piece of work for which I am eternally indebted.

Fervently and modestly, I extol the genuine cooperation, inspiration, advice and affection offered to me by the Chairman of my Advisory Committee ***Dr. T. Ramesh Babu,*** *Dean of Agiculture, College of Agriculture, Rajendranagar, Hyderabad, right from the initiation of the work to ship shaping of the manuscript. The present work bears at every stage of his impression of his counsel and concrete suggestions, careful reasoned criticism and meticulous attention to details. It was indeed a rare privilege for me to work wider his emending inspiration and indominable spirit.*

I am greatly beholden beyond words to express my deep sense of gratitude and a great privilege to work under the able and highly exceptional guidance of co chairman ***Dr. H.C. Sharma,*** *Principal Scientist, Entomology, ICRISAT, Patancheru, Hyderabad, for his brilliant counsel, constructive suggestions, indefatigable guidance, evincive criticism and inspiring encouragement to embellish the present study.*

I sincerely extend my gratitude to ***Dr. D. Sridevi****, Associate Professor, Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad and member of my advisory committee for her valuable suggestions during the course of investigation.*

I owe my effusive thanks to ***Dr. K. Radhika,*** *Associate Professor, Department of Genetics and Plant breeding, College of Agriculture, Rajendranagar and Member of my Advisory Committee for her valuable suggestions and affectionate encouragement during the course of investigation.*

It gives me great pleasure to express my deep sense of gratitude to ***Dr****.* ***P. Narayan Reddy,*** *Professor, Department of Plant Pathology, College of Agriculture, Rajendranagar for his cooperation and kindness during my study.*

I sincerely extend my profound gratitude and appreciation to ***Dr. S.R. Koteswara Rao, Dr. T. Uma Maheswari and Dr. K. Vijaya Lakshmi, Dr. C. Narendar Reddy*** *and* ***Dr. S.M.A.S. Rahman****, Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad.*

I express my heartful love and affection from the inner core of heart to my beloved mother ***Smt. P.S.M. Kezia****, for her blessings, inspiration, encouragement and moral support throughout my educational career. It remains less even if I say a lot about her.*

*I owe much to my loving grandma **Smt. Sulochana Mithra,** brother, Sisters and Aunties **Vijaya, Vinaya, Sandhya**, Uncle **Deen Dayal** for their everlasting affection, co-operation and encouragement in my life. They have been my source of confidence and motivation all through my life. It remains less even if I say a lot about them.*

*I express my heartful love and affection from the inner core of heart to **Suneel** for his inspiration, encouragement and moral support throughout my educational career.*

*My sincere thanks are due to **Mr. Rajendar, Mr. Suraj Sharma, Mr. Rashid, Mr. Akbar, Mr. Naga Raja, Mr. Venkateswarulu, Mr. Madhusudhan Reddy, Mr. Narayana Chandra, Mr. Venkateswar Rao, Mr. Harindranath, Mr. Raja Rao, Mr. Vittal Reddy, Mr. Ramulu, Mr. Ramana** and **Mrs. Ponamma** and all other staff of ICRISAT for their cooperation and help.*

*It is a pleasure to acknowledge the affection and inspiration rendered by my friends, **Shaku, Suneetha, Gowthami, Sowji, Madhavi.***

*I affectionately acknowledge the help received from juniors **Pavani, Vishveshwar, Satya, Jyosthsna, Nagendra, Deepa, Naganjali.***

*The financial assistance rendered by the **Professor Jayashankar Telangana State Agricultural University** and **ICRISAT** is gratefully acknowledged.*

I convey my wholehearted thanks to many of my well-wishers and to other friends requesting their forgiveness for not mentioning them here by name.

(Shaila. O)

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
ANOVA	:	Analysis of variance
C.V.	:	Coefficient of varience
^{0}C	:	degree centigrade
cm	:	centimeter
Conc.	:	concentration
CRD	:	Completely Randomized Design
d.f.	:	Degrees of Freedom
DR	:	Damage rating
et al.	:	and others
Fig.	:	Figure
g	:	gram
hr	:	hour
HPLC	:	High Performance Liquid Chromatography
i.e.	:	that is
kg	:	kilogram
l	:	Litre
L:D	:	Light:Dark
m.ha	:	million hectares
mg	:	milligram
ml	:	millilitre
mm	:	millimetre
m.t	:	million tones
Min	:	Minute
Mm	:	Millimolar
M	:	Molar
meq	:	Milliequlents
NS	:	Non significant
nm	:	Nano meter
RH	:	relative humidity
SEm	:	standard error of mean
t	:	tone
viz.	:	Namely
Wt	:	Weight
µg	:	Microgram
µl	:	Micro liter
/	:	per
@	:	at the rate of
<	:	Less than
>	:	Greater than
Vol.	:	Volume

Name of the author	:	**SHAILA. O**
Title of the thesis	:	**"CHARACTERIZATION OF *Cry IIa* TRANSGENIC CHICKPEA LINES AND THEIR INTERACTION WITH NATURAL ENEMIES OF *Helicoverpa armigera* (Hubner)."**
Degree	:	**DOCTOR OF PHILOSOPHY**
Faculty	:	**AGRICULTURE**
Department	:	**ENTOMOLOGY**
Chairperson	:	**Dr. T. RAMESH BABU**
University	:	**PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY**
Year of submission	:	**2015**

ABSTRACT

The present research on the "Characterization of *Cry IIa* transgenic chickpea lines and their interaction with natural enemies of *Helicoverpa armigera* (Hubner)" was carried out under laboratory and field conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2011 – 14.

The transgenic plants suffered significantly lower leaf damage as compared to the non-transgenic plants. The larval survival and weight gained by *H. armigera* larvae after 5 days was significantly reduced on transgenic lines as compared to that on non-transgenic chickpeas during October and November plantings 2011-12 and 2012-13. The transgenic lines BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 exhibited significantly lower leaf damage rating, larval survival and mean larval weight under laboratory conditions.

In glasshouse conditions, BS5A.1(T2) 18-1P1 suffered significantly lower leaf damage and mean larval weight was also reduced but the larval survival of *H. armigera* was significantly reduced on BS5A.2(T2) 19-2P1. Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera.* BS5A.2(T2) 19-2P1 had the highest dry matter weight, pod weight, seed weight and number of seeds formed as compared to the other transgenic and non-transgenic chickpea lines under infested and un-infested conditions.

The neonate larvae fed on artificial diet with BS5A.2(T2) 19-2P1 leaf powder exhibited lowest larval survival, larval weights at 5 and 10 days after initiation (DAI) and pupal weights as compared to insects reared on diets with leaf powder of non-transgenic plants and showed maximum resistance to *H. armigera*. The survival and development of third-instar *H. armigera* during 2012-13 was significantly reduced in insects reared on diets with leaf powder of transgenic chickpea BS5A.1(T2) 18-1P1 as against those reared on non-transgenic chickpeas and showed high levels of resistance to third-instar larvae of *H. armigera*.

Maximum amount of protein was recorded in ICC 506EB and among the transgenic lines, the protein content was highest in BS5A.1(T2) 18-2P1. The amounts of carbohydrates were significantly higher in the leaves of ICC 506EB as compared to that on transgenic lines.

The highest amount of lipids were recorded in BS5A.2(T2) 19-3P1 than in BS5A.2(T2) 19-3P2. There were no significant differences in phenol and tannin contents between the transgenic and non transgenic chickpea lines.

Significantly higher amounts of oxalic acid were recorded in BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 than in BS5A.2(T2) 19-2P1. Highest malic acid content was recorded on BS5A.1(T2) 18-1P1 and lowest on BS5A.2(T2) 19-3P2. Among the non-transgenics, the maximum amount of oxalic acid was observed in ICC 506EB, followed by Semsen. Oxalic acid content was positively correlated with larval survival and larval weight. A significant and negative association was observed between the amounts of the malic acid and leaf feeding, larval survival and larval weight.

Chlorogenic acid, gentisic acid, ferulic acid, naringin, naringenin and quercetin had a positive but non-significant correlation with resistance to *H. armigera*. There was a positive and significant association between 3,4 dihydroxy flavone, genistein, formononetin and biochanin A with leaf damage, larval survival and larval weight.

The amount of CryIIa protein was highest in the fresh leaf samples, followed by green pod wall, green seeds, dry pod wall, dry seeds and dry stems. In dry roots the protein concentration was quite low whereas in soil samples, it was below detectable levels. The CryIIa protein content was significantly higher in larvae fed on BS5A.2 (T2) 19-2P1 and BS5A.1 (T2) 18-1P1.

The CryIIa protein in *Bt* fed aphids, coccinellid grubs and *Bt* fed *Campoletis chlorideae* larvae was almost nil. Hence, the amount of CryIIa protein transferred from leaves to the non-target insects and natural enemies was negligible. The correlation co-efficient of CryIIa protein in fresh leaf, green pod wall, green seeds, dry pod wall, dry seeds, dry stems, dry roots and *H. armigera* larvae with leaf damage, larval survival and larval weight was negative and significant.

During November 2011-12 planting, among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on leaves of BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1. Among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on leaves of BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 as compared to that on other transgenic lines during November 2012-13 planting.

Survival and development of *C. chlorideae* wasps obtained from *H. armigera* larvae fed on diets with transgenic BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P1 leaf powder was better as compared to that on BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P2 lines during 2012-13. No CryIIa protein was detected in the *C. chlorideae* larvae, the negative effects of transgenic chickpeas on survival and development of *C. chlorideae* were due to the early mortality of *H. armigera* as a result the parasitoids failed to complete the development on such larvae. The survival and development of *C. chlorideae* was poorer when reared on *H. armigera* larvae fed on fresh leaf samples than the artificial diets intoxicated with transgenic chickpea leaf powders.

The survival and development of coccinellids was reduced when fed on diets with 0.1% of BS5A.2 (T2) 19-3P1 and BS5A.2 (T2) 19-3P2 leaf powder, but not on diets with BS5A.1(T2) 18-2P1 leaf powder. The direct effects of transgenic chickpeas on survival and development of lady bird beetles being 0.02% < 0.05% < 0.1%, respectively.

There were no significant effects on survival and development of coccinellid grubs when fed on aphids reared on diets with 0.02% and 0.1% leaf powder of transgenic chickpeas. The survival and development was slightly affected on diets with BS5A.2(T2) 19-3P2 leaf powder. The coccinellids fed on diets with 0.05% BS5A.2(T2) 19-3P1 leaf powder showed a marginal reduction in survival and development as compared to that on other transgenic lines during 2012-13.

The survival and development of coccinellids was slightly affected when fed on diets with BS5A.2(T2) 19-3P2 leaf powder as compared to that on other transgenic lines. In diets with 0.1%, the survival and development was affected adversely when the coccinellid grubs were fed on diets with BS5A.2(T2) 19-3P1 leaf powder during 2013-14. Though there was no detection of CryIIa protein in *Bt* fed aphids and coccinellids, but there were adverse effects observed on survival and development.

Chapter I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop, grown in an area of 8.21 m ha, with a total production of 7.48 m tonnes globally (FAOSTAT, 2011). The crop is largely grown by subsistence farmers in rain-fed areas (>70 per cent), which are less fertile and poor in moisture retention capacity. Although, India produces about 75 per cent of the chickpea, the production is inadequate to meet the demand of the domestic market. India imports about 1,85,000 metric tons of chickpea valued at US$ 94 m (FAOSTAT, 2011) The demand for chickpea is projected to double from 7 to 14 m tonnes by 2020. In the next 10 years the net import of chickpea will be close to 1.5 m tonnes to meet the domestic requirements. It is even more important for India, as the country's production accounts for 67 per cent of the global chickpea production, and chickpea constitutes about 40 per cent of India's total pulse production. It is a source of high quality protein for the poor people in many developing countries, including India. Chickpea yields are quite low, and have remained almost stagnant for the past 2 to 3 decades. It is valued for its nutritive seeds with high protein content (25.3–28.9 per cent).

Chickpea yields are low (400–600 kg/ha), because of several biotic and abiotic constraints, of which the pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) is the most important constraint in chickpea production (Manjunath *et al*., 1989). It is a major pest of chickpea in Asia, Africa, Australia and the Mediterranean region. Pod borers alone cause 25 to 40 per cent of the crop loss amounting US $ 325 million in chickpea (ICRISAT, 1992). *Helicoverpa* females lay eggs on leaves, flowers and young pods. The larvae feed on the young leaves of chickpea and the young seedlings may be destroyed completely, particularly under tropical climates in southern India. Larger larvae bore into the pods and consume the developing seeds inside the pod. The losses due to *H. armigera* magnify under drought conditions. In addition to chickpea, *H. armigera* also damages several other crops such as cereals, pulses, cotton, vegetables, fruit crops and forest trees. It causes an estimated loss of US $ 2 billion annually, despite the use of US $ 500 million worth of insecticides to control this pest worldwide (Sharma, 2005a).

In order to protect the crop from *H. armigera* damage, various pest management practices have been adopted by the Indian farmers. Efforts are being made to develop *H. armigera* resistant varieties by conventional breeding methods as well as modern biotechnological tools to develop transgenic chickpea varieties with resistance to this pest.

The conventional control measures are largely based on insecticides. With the development of resistance to insecticides in *H. armigera* populations (Kranti *et al*., 2002), there has been a renewed interest in developing alternative methods of pest control, of which host plant resistance to *H. armigera* is an important component.

Genetically modified plants expressing *Bt* δ-endotoxin genes have been developed for resistance to insect pests, and some of them have been deployed successfully on a commercial scale for pest management (Sharma *et al.*, 2006). Transgenic cotton and maize with resistance to lepidopteran insects have been released for cultivation in several countries, and were grown on more than 100 m ha worldwide in 2012. India ranks first in the world having 11.1 m ha area under *Bt*-cotton in 2011 (>90% of total cotton area in India), followed by China and USA (James, 2011).

Genetic transformation as a means to enhance crop resistance or tolerance to biotic constraints has shown considerable potential to achieve a more effective control of target insect pests for sustainable food production (Sharma *et al*., 2001). The δ-endotoxin genes from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) have been deployed in several crops for pest management (James, 2007). Efforts are underway to develop chickpea plants with *Bt* δ-endotoxin genes for resistance to *H. armigera* (Ramakrishna *et al*., 2005; Acharjee *et al*., 2010). However, concerns have been expressed that the trichome exudates in chickpea leaves and pods, which are highly acidic in nature (pH 2.0 – 3.5), may have a negative influence on the biological activity of *Bt* sprayed on chickpea (Bhagwat *et al.*, 1995) or toxin proteins expressed in transgenic chickpea (Devi *et al.*, 2011 and 2013).

Cotton is the first transgenic crop commercialized in India is hybrids carrying *cry1Ac* gene of *Bt* for resistance to *H. armigera*. The cotton industry in India has highly benefited immensely with the introduction of *Bt* cotton in 2002. Cotton production in India before 2002-2003 was about 2.55 to 2.75 mt, but over the past five years, cotton yield has increased by 50 per cent. In 2006, five new events, Bollgard II, Event I, GFM Cry1A, BNLA 601 and Event 9124, of *Bt* cotton expressing *cry1Ac, cry1Ab, cry2Ab* and *cry1Ac* either alone or in combination were approved for release in India (GEAC, 2009). Therefore, applying genetic engineering technologies to develop *Bt* chickpeas using bacterial '*cry'* genes could be appropriate to protect the crop from damage by *H. armigera*.

Pyramiding two or more *Bt* genes such as *cry1Ac* and *cry2A* in chickpea could be a preferred option to delay the evolution of insects with resistance to *Bt* due to different modes of action for these two genes. However, reports suggest that baseline frequency of *cry2Ab* resistance gene within populations of *H. armigera* (Mahon *et al.*, 2007) is substantially higher

than expected. Expressing *cry1Ac* gene in combination with *cry1F* gene may be effective to delay insect resistance because of their additive effect against *H. armigera* (Ibargutxi *et al.,* 2008). Moreover, use of hybrid Cry proteins such as Cry1Ab - Cry1Ac also conferred resistance to lepidopteran pest, *Spodoptera exigua* (de Maagd *et al*., 2000). Hybrid *Bt* protein containing domain I and II from Cry1Ba and domain II for Cry1IA was found to be effective against potato tuber moth**,** *Phthorimaea operculella* (Zeller) and Colorado beetle**,** *Leptinotarsa decemlineata* (Say) (Naimov *et al*., 2003). Development of transgenic plants expressing vegetative insecticidal proteins has also been found to be more effective against many lepidopteran pests, including *H. armigera*. In case of maize, it was found that *Vip3A* in combination with *cry1Ab* provided complete resistance to *Helicoverpa zea* (Boddie) under field condition (Burkness *et al.,* 2010). Transgenic chickpea stacked with *Bt* genes such as *cry1A* along with *Vip3A* or hybrid *Bt* protein in combination with *Vip3A*, could be a suitable combination for pest management.

The first successful genetic transformation of nuclear genome of chickpea was reported in 1997 using the *cry1Ac* gene (Kar *et al*., 1997). Subsequently, various research groups within India initiated genetic transformation of chickpea using *cry1Ac* gene and reported the generation of transgenic chickpeas (Sanyal *et al*., 2005 and Mehrotra *et al*., 2011). A second gene, *cry2Aa*, was also introduced in chickpea to facilitate gene pyramiding with the existing *cry1Ac* lines (Acharjee *et al*., 2010). Mehrotra *et al*. (2011) generated pyramided *cry1Ac* and *cry1Ab* chickpea.

The impact of genetically engineered insect-resistant crops on non-target organisms including biological control agents is one of the most widely discussed ecological effects. Natural enemies are of major concern as they often play an important role in regulation of pest populations, and are therefore of economic value. There is a concern that the insecticidal proteins expressed in transgenic plants may either effect the natural enemies directly (toxic effect) or indirectly (change in the prey or host-quality or abundance) (Romeis *et al*., 2006). The parasitoid, *Campoletis chlorideae* (Uchida) and the coccinellid predator, *Cheilomenes sexmaculatus* (L.) are important natural enemies of pod borer, *H. armigera* in grain legumes. It is therefore important to assess the non-target effects of *Bt* toxins to natural enemies of insect pests in different crops.

Several studies have reported the direct and indirect effects of transgene products and the transgenic plants on the beneficial insects (Dutton *et al.*, 2003; Lovei and Arpia 2005; Sharma *et al.,* 2007, 2008 and Dhillon *et al.,* 2008). The *Bt* toxins are not transported to the phloem in some crops, and therefore, insect pests such as corn leaf aphid, *Rhopalosiphum*

maidis (Fitch.) and the natural enemies feeding on it are not directly affected by the *Bt* toxins (Head *et al.,* 2001 and Dutton *et al.,* 2002). The cotton aphid, *Aphis gossypii* Glover, is insensitive to *Bt* toxins, but trace amounts of *Bt* toxins were detected in the aphids when fed on *Bt* cotton (Zhang *et al.,* 2006a). Presence of Cry IAc toxin in phloem sap from *Bt*-oilseed rape and in *Myzus persicae* Sulzer has indicated the importance of having an estimate of the effects of expected amounts of *Bt* toxins in the diets of non-target organisms predating on aphids fed on the transgenic crops (Burgio *et al.*, 2007). Moreover, some *Bt* isolates such as INS 2.13, HFZ 24.8 and GU 9.1 exhibit different levels of toxicity (LC_{50} values of 62, 328 and 114 ng/ml, respectively to the cotton aphid, *A. gossypii* (Malik and Sheikh, 2006).

The parasitic wasp, *Campoletis chlorideae* Uchida (Ichneumonidae: Hymenoptera), parasitizes several insect species. (Yan *et al.,* 2006; Dhillon and Sharma, 2007). The information on its parasitism potential, development and survival on different insect and crop hosts is scanty. However, under natural conditions, *H. armigera* is the most preferred host of *C. chlorideae* on a number of crops, *viz.,* cotton, groundnut, chickpea, pigeonpea, sorghum and pearl millet (Patel and Patel 1972; Bhatnagar *et al.,* 1982 and Kumar *et al.,* 1994). The introduction of transgenic crops has raised concerns regarding their impact on natural enemies (Sharma and Ortiz, 2000).

There is little information on the possible effects of transgenic crops on the generalist predators and host specific parasitoids in the tropics (Sharma *et al*., 2007). The cropping systems in tropics are quite diverse, and consist of several crops that serve as alternate and collateral hosts of the major pest, *H. armigera*, and other non-target insect pests, because of the multiplicity of crops and cropping systems, the performance and interactions of transgenic crops in different agro-ecosystems are likely to be quite complex. Also the issue of insecticide abuse and their adverse effects on insect diversity, pest resurgence and natural enemies is a major concern. Therefore, it is important to generate such information to take decisions about the impact of insecticide applications, *Bt*-transgenic crops and the crop genotypes on the relative abundance of target and non-target insect pests and their natural enemies. The present studies were undertaken to evaluate the effectiveness of transgenic chickpea lines to control, *H. armigera* and their interactions with natural enemies of *H. armigera* with the following objectives:

1) Phenotyping of *cry IIa* transgenic chickpea lines for resistance to pod borer, *H. armigera.*

2) Molecular and biochemical characterization of *cry IIa* transgenic chickpea lines for transgene expression.

3) Effect of *cry IIa* transgenic chickpea lines on the natural enemies of *H. armigera*.

Chapter II

REVIEW OF LITERATURE

The legume pod borer *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) is one of the most important constraints to crop production globally.It is a polyphagous pest and attacks more than 200 plant species including cotton, chickpea, pigeonpea, tomato, maize, sorghum, sunflower and a range of vegetables, fruit crops and tree species (Manjunath *et al*., 1989 and Fitt, 1991).

Host plant resistance is one of the most effective methods of pest control. However, the levels of resistance to pod borer in the cultivated germplasm is low to moderate (Sharma *et al.,* 2005a). Therefore transgenic plants with genes from *Bacillus thuringiensis* have been developed as an effective weapon for pest management (James, 2011). However, large scale development of transgenic plants may have some direct and indirect effects against the beneficial natural enemies of the crop pests (Sharma *et al.,* 2011). There is a need to asses the biosafety of transgenic plants to the nontarget natural enemies in the ecosystem.

2.1 Transgenic chickpea for insect resistance

Senthil *et al*. (2004) reported 5.1 per cent transformation frequency in chickpea. Southern blot analysis and histochemical and leaf painting demonstrated integration and expression of the transgenes I, the initial transformants, and two generations of progeny.

Sarmah *et al.* (2004) developed transgenic plants using a *crylAc* gene. The progeny did not confer resistance to pod borer. They reconstructed the *Bt* toxin genes (*crylAc* and *cry2Aa)* for expression in green tissues (using *Arabidopsis* SSU gene promoter and a tobacco SSU gene terminator) and inserted them into twin binary cassettes for transformation. Western blot analysis of six independent plants confirmed expression of *cry2Aa* gene in 5 out of 6 plants. These results suggested that genetic engineering of crops is an effective method for the production in pod borer-resistant chickpea plants.

Sanyal *et al.,* 2005 standardized a protocol for *Agrobacterium*-mediated gene transfer in chickpea from cotyledonary node explants with *crylAc* gene driven by CaMV 35s promoter and *nptII* gene for Kanamycin resistance. The regeneration and transformation frequency was recorded as 1.12 per cent.

Shivani *et al.* (2007) developed transgenic chickpea by introducing *crylAc* gene through particle bombardment method using epicotyl explants. These transgenic plants showed moderate protection and mortality for *Helicoverpa armigera* and *Spodoptera litura* (F) larvae as compared to control plants with the transfomation frequency of 18 per cent.

2.1.1. Phenotyping of Cry toxins in transgenic chickpea for resistance to *H. armigera*

Kar *et al*. (1997) reported transgenic plant generation using two strains of chickpea ICCV- 1 and ICCV- 6. Insect feeding assay indicated that the expression level of *cryIA(c)* gene was inhibitory to the development of the feeding larvae of *H. armigera*. The larvae which fed on normal plants (7.23 and 0.064 mg) attained their body weight about four times higher than transgenic plants (1.98 and 0.045 mg) signifying higher rate of growth.

Romeis *et al*. (2004) gave an overview on the available insecticidal genes that could be deployed to increase insect resistance in chickpea. Prior to commercialization, transgenic crops need to be assessed for their effects on the environment including the possible impact on non-target arthropods, many of which are important for biological pest control.

Insect feeding bioassay on transformed chickpea plants (T_0 and T_1) with larvae of pod borer insect *H. armigera* showed high levels of toxicity to insects and protection of transgenic plants. Transformed chickpea plants expressing soluble Cry1Ac protein above 10 ng mg^{-1} showed 80–85% protection and high mortality (>80%) of insects, while plants expressing between 5 and 10 ng mg^{-1} resulted in early pupation, significant loss in weight (45–55%) and moderate mortality of insects. Expression of truncated native *Bt cry1Ac* gene in chickpea has shown effective resistance in transgenic plants to the major pod borer insects (Sanyal *et al.*, 2005).

Lawo *et al*. (2008) experimentally proved that there was a high correlation between feeding damage caused by *H. armigera* larvae on the transgenic chickpea leaves and the weight of faeces they excreted. For the susceptible *H. armigera* strain, leaf damage was significantly higher for control leaves than for transgenic chickpea leaves after 24 h of feeding ($P<0.001$). The difference in feeding activities on the two plant types was also evident in the faeces weight measurements ($P<0.001$). No significant difference was observed in the number of leaflets damaged per leaf provided to the two strains on either plant type during 24 h (means for the susceptible strain, 8.1 to 9.3 leaflets damaged/leaf; Cry2A-resistant strain, 8.7 to 9.1 leaflets damaged/leaf).

Acharjee *et al.* (2010) tested transgenic chickpea, BS2A, BS5A and BS6H in insect bioassays using neonate *H. armigera* and compared to the non-transgenic ‘‘parents’’ cv ICCV 89314 and cv Semsen. There was significantly greater larval mortality among the larvae fed on transgenic leaves than those fed on controls and significant differences in mortality between all transgenic lines. Larval mortality was highest on the BS6H transgenic line whereas almost all larvae died during the assays.

Mehrotra *et al*. (2011) studied the expression of *Bt*-toxin in T_0 and T_1 transgenic chickpea plants and toxicity to *H. armigera* larvae. Insect bioassay performed with transgenic plants showed relatively higher toxicity for plants expressing Cry1Ac protein as compared to Cry1Ab to *H. armigera*. Pyramided transgenic plants with moderate expression levels (15–20 ng mg^{-1} of TSP) showed high-level of resistance and protection against *H. armigera* as compared to high level expression of a single toxin. These results have shown the significance of pyramiding and co-expression of two Cry toxins for efficient protection against lepidopteran pests of chickpea.

2.1.2. Efficacy of transgenic crops against pod borer, *H. armigera*

Benedict *et al.* (1993) evaluated transgenic cotton plants (*Btk* lines) carrying Cry1Ab delta endotoxin genes from *Bt* for resistance to *H. armigera*. The mean per cent injury was observed to be 2.3 in flowers and 1.1 in capsules as compared to 23 and 12 per cent in Coker 213, respectively.

Fitt *et al.* (1994) monitored the mortality of first instar *H. armigera* on experimental lines of transgenic cotton expressing Cry1Ab protein. No survival of larvae in young transgenic cotton was observed compared to survival in matured cotton.

Continuous feeding of *Bt* cotton was reported to cause 80-85 per cent mortality of first instar (Wang and Xia, 1997), 76-81 per cent mortality of second instar and 100 per cent mortality of 1-4 instar of *H. armigera* larvae (Zhao *et al*., 1998a; Cui and Xia, 2000 and Zhao *et al*., 2000a).

Interaction studies between *H. armigera* and transgenic cotton recorded low population population of *H. armigera* on *Bt* cotton compared to non- *Bt* transgenic cotton in China (Zhao *et al*., 1998b).

Zhao *et al*. (1999) studied the temporal and spatial variation of insecticidal activity of *Bt* cotton and the response of the cotton bollworm, *H. armigera* to the selection by *Bt* cotton and found that insecticidal effect of flowers of *Bt* cotton was lowest compared to leaves, squares and bolls.

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 of adult 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, respectively.

It was reported that *Bt* cotton was highly resistant to *H. armigera* and the larval population was significantly lower in *Bt* cotton than in non *Bt* cottons in China. And all the larvae of *H. armigera* from first to fourth instar died when fed continuously with transgenic *Bt* cotton plant parts, but 37.9 - 85.6 per cent of fifth instar and 63.4 - 96.5 per cent of fifth and sixth instar larvae survived, respectively. The pupation of the fifth instar larvae decreased by 48.2-87.5 per cent when fed continuously on *Bt* plant parts (Cui and Xia, 2000).

Olsen and Daly (2000) found that, transgenic cotton plants expressing the Cry 1Ac gene from *Bt* were less toxic to first instar *H. armigera* larvae after the plant started producing fruiting bodies.

Zhao *et al.* (2000b) reported continuous feeding of first to fourth instar of *H. armigera* larvae on *Bt* cotton leaves and squares caused no pupation, whereas on flowers, third and fourth instars were able to pupate and emerge partially.

Chakrabarti *et al*. (2000) conducted insect bioassays on the leaves of transgenic potato which showed considerable protection against the larvae of *H. armigera* in terms of leaf area consumed and larval weight reduction. Double-antibody quantitative sandwich ELISA analysis demonstrated high levels of Cry1Ab protein expression in transgenic plants.

Wu *et al.* (2003) observed the larval development of *H. armigera* on various structures of *Bt* and Non-*Bt* cotton. They found that the percentage of larvae reaching second instar after four days were 84.44 per cent and 0.0 per cent (terminal leaves), 68.34 per cent and 0.0 per cent (squares) on DP5415 (non-*Bt*) and NuCOTN 33B (*Bt*), respectively, which, in the conventional cotton was significantly higher than in *Bt* cotton. In flower-boll-stage of cotton, there also was a significant difference in larval development between DP5415 and NuCOTN 33B. The percentage of larvae growing to second instar after four days on DP5415 ranged from 21.33 per cent on young bolls to 82.67 per cent on terminal leaves. Percentage of larvae reaching second instar on flower-boll-stage NuCOTN 33B were 0,3.33, 8.33 and 46.66 per cent for squres, bolls, flowers and terminals, respectively.

Murugan *et al*. (2003) reported 92.8, 66.7 and 51.7 per cent mortality of *H. armigera* during first, second and third instar stages, respectively on *Bt* cultivars and observed that mortality of the larvae decreased as the larval instars advanced. The first instar *H. armigera*

larvae did not survive on young *Bt* cotton compared to matured *Bt* cotton (40-60 %) (Wu *et al*., 2003; Fitt *et al*., 1994).

Shudong *et al*. (2003) conducted bioassay which showed that the weights of sixth instar larvae and pupae from the colony reared on the *Bt* cotton were 25.6% and 18.2% less, respectively, compared to those raised on routine cotton. Cotton bollworms that fed on *Bt* cotton grew slower and their generation duration was at least 17 days longer than those that fed on non-*Bt* cotton. This resulted in reduced damage to transgenic *Bt* cotton plants.

Bird and Akhurst (2004) reported that the larval survival till pupation of resistant *H. armigera* strain on *Bt* cotton was 54 per cent lower than that on non *Bt* cotton. The maximum mortality of neonate larvae was 82.62 and 83.99 per cent on MECH 162 *Bt* and 184 *Bt*, respectively.

Srinivasa Rao (2004) found that none of the first and second instar larvae of *H. armigera* survived when fed on *Bt* cotton flower buds. However, few of third and many fourth and fifth instar larvae successfully transformed into adults.

Zhang *et al*. (2004) reported that significantly greater larval survival and higher consumption of *H. armigera* larvae were observed on non-transgenic cotton than on the transgenic *Bt* or CpTI-*Bt* cotton. In addition, significantly more neonates were found away from the leaf discs, lower consumption and higher mortality were achieved in the choice test with two transgenic cotton leaves than in the choice tests containing non-transgenic cotton leaves. Leaves and buds were examined in choice tests of fourth instars, it appeared that fourth instars were found in equal numbers on transgenic and non-transgenic cotton.

Vennila *et al*. (2006) studied the survival of *H. armigera* and *S. litura* on commercial transgenic cotton cultivar MECH 162 *Bt* and its conventional counter part non-*Bt* MECH 162 in laboratory using food material from field grown plants. Larval mortalities of 58.7 and 43.5 per cent and 7.69 and 5.88 per cent in respect of *Bt and* non-*Bt* cotton were observed for *H.armigera* and *S. litura,* respectively. Slow growth rate induced by the action of *Bt* insecticidal protein led to more number of days to mortality in *H.armigera* over non *Bt* cotton. Larval development period between *Bt* and non *Bt* cultivars was insignificant for *S. litura.* Survival index for *H.armigera* and *S. litura* on *Bt* and non *Bt* cotton was 20 and 47.7 per cent and 84.6 and 82.3 per cent, respectively.

Sharma and Pamapathy (2006) reported there were no significant differences in oviposition between the transgenic and the non transgenic cultivars under protected and unprotected conditions. The larval numbers were significantly lower on the transgenic hybrids during the 2004 rainy season under high infestation, but the differences in larval density between the transgenic and non-transgenic hybrids during 2002 and 2003 seasons under low levels of infestation were quite small.

The mean mortality of *H. armigera* neonates to top leaves of MECH 162, MECH 184 and RCH 2 were 90.0, 91.7 and 90.0%, respectively. The order of susceptibility of different larval instars to *Bt* cotton hybrids were neonates > second instar> third instar. The efficacy of different plant parts was in the order of top leaves > middle leaves > squares > bolls. Among the *Bt* hybrids, MECH 184 was highly effective, followed by MECH 184, MECH 162 and RCH 2 (Shanmugam *et al.* 2006).

Swamy *et al*. (2007) evaluated the usefulness of detached leaf assay to assess the efficacy of transgenic pigeonpea (ICPL 88039 and ICPL 87) plants carrying *cry1Ab* and *SBTI* genes for resistance to *H. armigera*. The levels of *cry1Ab* or *SBTI* proteins in the transgenic pigeonpea plants were not sufficient to cause significant deterrent effects on leaf feeding, larval survival, and larval weight of *H. armigera*. However, detached leaf assay was found to be quite useful for evaluation of transgenic pigeonpea plants for resistance to *H. armigera*.

Basavaraja *et al*. (2008) studied the effect of *Bt* cotton top leaves on consumption-utilization indices of *H. armigera* and *S. litura* larvae. There was a significant reduction in consumption index (CI), growth rate (GR), efficiency of conversion of ingested food (ECI), approximate digestibility or assimilation efficiency (AD/AE) and efficiency of conversion of digested food (ECD) in *Bt* genotypes at 70 and 100 days of crop age. At 130 days of crop age, no significant effect of *Bt* was observed on *H. armigera* larvae. The fourth instar larvae of *S. litura* were used to study the various consumption-utilization indices on leaves at an interval of 75, 105 and 135 days of crop age. There was no significant reduction in CI, GR, ECI, AD/AE and ECD in *Bt* genotypes at 75, 105 and 135 days of crop age.

In the transgenic cotton plant, larval populations of cotton bollworm, *H. armigera* was significantly reduced as compared to untreated varieties. Plant damage analyses upon maturity revealed significantly higher levels of sound bolls in transgenic cotton plants. Seed cotton yields and lint quality were also higher for the transgenic cotton than for untreated

convention varieties. The transgenic variety was always statistically equivalent or superior to the treated conventional one (Hema *et al*, 2009).

Arshad *et al.* (2009) studied that significant effects of *Bt* cotton on the per cent cumulative mortalities of all instars compared with non *Bt* cotton. However, a significant higher mortality (100%) was observed in neonates fed on *Bt* cotton leaves than those fed on *Bt* flower-bolls (93%). There was a marked difference in larval development period between *Bt* cotton (27.75 days) and on non *Bt* cotton (16.68 days) flower-bolls. Pupal weight was significantly higher for larvae fed on non *Bt* cotton compared with *Bt* cotton plant parts (leaves & flowers-bolls).

Hallad *et al*. (2011) carried out quantitative bioassays at 80 and 110 days after sowing (DAS) by leaf disc feeding method for *H. armigera* and *S. litura* using five different *Bt* cotton event genotypes for characterization of resistance to early and late instars. The highest second instar mortality (93.1 and 79.2 %) was found at 80 and 110 DAS in Tulasi 4BG-II (MON-15985). The mortality of third and fourth instar was 91.1 and 87.1 per cent at 80 DAS, respectively, for *H. armigera.* The highest mortality (80.3 and 71.3 %) for second instar larvae of *S. litura* at 80 and 110 in Tulasi 4BG-II and the mortality of third and fourth instar was 72.6 and 64.2 in Tulasi 4BG-II. Whereas Tulasi 4BG-I was recorded least mortality (7.9 and 5.8 %) of second instar at 80 and 110 DAS. As the larvae gained growth advancement the bioactivity of toxins was found to be reduced.

Arshad *et al.* (2011) reported that there was no significant difference in egg laid by the cotton bollworm, *H. armigera* between *Bt* (IRFH-901) and non *Bt* cotton. However, larval densities were significantly reduced in *Bt* cotton as compared to conventional non *Bt* cotton. No insecticide application was needed to control this pest in *Bt* cotton. The results indicated that transgenic *Bt* cotton played a significant role in reducing the pesticide application for the control of *H. armigera*.

Significant adverse effect of *Bt* on *H. armigera* was observed at 60, 90 and 100 days of crop age in top leaves, middle leaves, squares and bolls. The minimum per cent survival of larvae in transgenic *Bt* hybrid was observed at 60 days of crop age in top leaves (16.67-20.00%), middle leaves (13.33-20.00%), squares (26.67-36.67%) and bolls (30.00-36.67%). Similar trend was followed at 90 and 100 days of crop age. The effect of *Bt* at 120 and 140 days of crop age was non-significant on larval survival in comparison to non *Bt* and local hybrid HHH 223 (Basavaraja *et al*, 2011).

Basavaraja *et al*. (2012) studied the oppositional response of *H. armigera* to transgenic *Bt* and non *Bt* cotton hybrids. There was no significant variation found between *Bt* and non *Bt* genotypes. The total number of eggs laid on *Bt* and non *Bt* hybrids ranged from 344 to 361 eggs/2 twigs/4 females, respectively.

Mogali *et al*. (2012) performed leaf assays on cotton bollworm, *H. armigera*. Survival bioassay on detached leaf bits showed significant increase in larval mortality ranging from 72-76 per cent. There was significant increase in the final body weight of the larvae fed on negative control (111.55%), than the larvae fed on leaf bits of transgenic plants (56.36%).

2.1.3. Effect of Cry proteins on the survival and development of pod borer, *H. armigera*

Chandra *et al*. (1999) studied the effect of WT- and M-Cry1Ac proteins on neonate larvae of *H. armigera*. The M-Cry1Ac was eight times more effective than the WT-Cry1Ac in terms of larval mortality. The experiment was repeated with third instar larvae and essentially similar results were obtained. The mutant toxin was shown to be more toxic to the larvae of *H. armigera* than the wild type toxin.

Olsen and Daly (2000) developed two bioassay methods (leaf mush, leaf disk) to test if the physiological state of the plants explained changes in toxicity and a third method (diet incorporation) was developed to quantify the toxicity of *Bt* leaves when mixed in chickpea diet. Cry1Ac protein was less toxic to *H. armigera* larvae when the protein was mixed with leaves from fruiting versus presquare 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-toxin interactions in fruiting cotton reduced the toxicity of the Cry1Ac protein.

Chandrashekar and Gujar (2004) reported that *H. armigera*, evolved 31 fold resistances to selection pressure of *Bt* endotoxin CrylAc within six generations. The CrylAc selected larvae of *H. armigera* showed cross-resistance to CrylAa and CrylAb both in terms of mortality and growth reduction.

The toxicity and larval growth inhibition of 11 insecticidal proteins of *Bt* against neonate larvae of *H. armigera*, by a whole-diet contamination method, the most active toxins were Cry1Ac4 and Cry2Aa1, with LC_{50} values of 3.5 and 6.3 g/ml, respectively, at the concentrations tested, Cry1Ac4, Cry2Aa1, Cry9Ca, Cry1Fa1, Cry1Ab3, Cry2Ab2, Cry1Da, and Cry1Ja1, produced a signifcant growth inhibition, whereas Cry1Aa3, Cry1Ca2, and Cry1Ea had no effect (Avilla *et al*., 2005).

Kranthi *et al.* (2005) indicated that bioassay on *H. armigera*, utilizing *Bt*-cotton seed as a source of CrylAc toxin. The CrylAc content in seeds was found to be 1.77± 0.23 µg/g and the variability between individual seeds and seed lots was minimal. Bioassays on *H. armigera* using *Bt* seeds stored at room temperature for 2 years showed that there was no significant reduction in bio-activity of the toxin present in the seeds.

The baseline susceptibility of the larvae of *H. armigera* to Cry1Ac and other toxins carried out in many countries provided a basis for monitoring resistance. They opined that there is no evidence of development of field-level resistance in *H. armigera* leading to the failure of *Bt* cotton crop anywhere in the world, despite the fact that *Bt* cotton was grown on the largest ever area of 12.1 million hectares in 2006 and its cumulative cultivation over the last 11 years has surpassed the annual cotton area in the world. Nevertheless, the *Bt* resistance management has become a necessity to sustain *Bt* cotton and other transgenic crops in view of potential of the target insects to evolve Cry toxin resistance (Gujar *et al*., 2007).

Anilkumar *et al*. (2009) conducted bioassays to ascertain if Cry1Ac toxin-resistant *H. zea* population showed higher survival rates on field-cultivated *Bt* cotton squares (= flower buds) collected at prebloom-bloom than susceptible *H. zea.* The results showed that Cry1Ac toxin-resistant *H. zea* could not complete larval development on Cry1Ac-expressing *Bt* cotton, despite being more than 150-fold resistant to Cry1Ac toxin and were able to survive until pupation on Cry1Ac toxin concentrations greater than present in *Bt* cotton squares. Since mortality observed for Cry1Ac-resistant *H. zea* on *Bt* cotton was higher than expected, diet incorporation bioassays with Cry1Ac toxin alone, and with gossypol and 4 per cent cotton powder in the presence and absence of Cry1Ac showed Cry1Ac toxin was significantly more lethal to susceptible *H. zea* than to resistant *H. zea*, but no difference in susceptibility to gossypol was observed between strains. However, combinations of Cry1Ac with gossypol or cotton powder were synergistic against resistant, but not against susceptible *H. zea.* Gossypol concentrations in individual larvae showed no significant differences between insect strains or between larvae fed gossypol alone vs. those fed gossypol plus Cry1Ac.

Devi *et al*. (2011) depicted that biological activity of *Bt* (Biolep ®) on four chickpea genotypes with different levels of resistance to *H. armigera* under field conditions, and by incorporating lyophilized leaf and pod tissue into the artificial diet with and without *Bt*, there was no survival of *H. armigera* larvae in chickpea plants sprayed with 0.1, 0.2 and 0.5% *Bt*, there was a significant reduction in larval survival, larval and pupal weights and fecundity,

and prolongation of larval and pupal periods in chickpea plots sprayed with *Bt* (0.05%) as compared to the unsprayed plots. Biological activity of *Bt* was lower on artificial diets with leaf or pod powder of chickpea genotypes, larval survival, larval and pupal weights, pupation and adult emergence were significantly lower on diets with leaf or pod powder of the *H. armigera* resistant genotypes than on the susceptible check. Chickpea genotypes with resistance to *H. armigera* acted in concert with *Bt* to cause adverse effects on the survival and development of this insect.

At the highest concentration, Cry1Ac and Cry1Ca shortened 48.1 and 48.9 % of *H. armigera* female lifespan, and 43.5 and 38.5 % of *S. exigua* female lifespan, and they reduced 37.8 and 40.3 %, and 50.5 and 47.4 % of *H. armigera* and *S. exigua* male lifespans respectively. *Bt* toxins negatively affected copulation, exposure to 500 mg/ml of Cry1Ac and Cry1Ca greatly reduced 50.0 and 46.8 %, and 58.7 and 57.3 % spermatophore acceptance by *H. armigera* and *S. exigua* females, respectively. In contrast, both Cry1Ac and Cry1Ca did not negatively influence the egg hatchability (Zhang *et al*, 2013).

2.2. Molecular and biochemical characterization of transgenic crops

2.2.1 Molecular characterization

2.2.1.1. Detection of Cry protein in transgenic crops

The mean Cry1Ac levels declined significantly from 57.1 to 6.7 μg g^{-1}, in fruiting structures of cotton and from 163.4 to 34.5 μg g^{-1}, in leaves at 53 and 116 DAP, respectively, and terminal foliar concentrations were always greater than those found in fruit (Greenplate, 1999). The transgenic cotton plants expressing the Cry1Ac gene were less toxic to *H. armigera* after the fruiting stage compared to early stages of crop growth. Further, the interference of condensed tannins with Cry1Ac toxicity especially increased tannin content over the season and was also responsible for the decrease in toxicity (Olsen and Daly, 2000).

Adamczyk *et al.* (2001) observed that there was difference in the amount of delta-endotoxin present in various plant parts which were correlated with the larval survival of the bollworms throughout the growing season. Kranthi (2002) observed the expression tendency of Cry 1Ac in MECH-*Bt* cotton hybrids and stated that the earliest decline was in MECH-12 with toxin levels falling from 23 μg at 75 DAS to 1-2 μg by 85 DAS, where as in MECH-162 and MECH-184 the expression levels declined by 95^{th} day and 120^{th} day after sowing, respectively. The green tissue had the highest concentration of toxin followed by yellowish green and whitish yellow tissues. The expression levels of Cry 1Ac were high at 70 DAS

which steadily declined at 100 and 130 DAS among the different plant parts of *Bt* cotton cultivars. The mean Cry 1Ac levels in the leaves were 26.03, 23.01 and 7.09 μg g^{-1} in MECH-12 *Bt*, MECH-162 and MECH-184 *Bt*, respectively at 70 DAS and declined thereafter (Abel and Adamczyk, 2004).

Cry 1Ac concentrations in the squares were 17.04, 14.92 and 18.08 μg g^{-1} in MECH-12 *Bt*, MECH-162 and MECH-184 *Bt*, respectively at 70 DAS and declined therafter. Cry 1Ac levels were comparatively high in the seeds of green bolls with 41.62 and 16.67 and 18.77 μg/g in MECH-12 *Bt*, MECH-162 and MECH-184 *Bt*, respectively, at 70 DAS and declined thereafter (Srinivasa Rao, 2004). Zhang *et al.* (2004) found that the amount of *Bt* in different plant parts was high in NuCoTN 33B (79.7-139.0 ng g^{-1} fresh wt) than in GK-12.

2.2.1.2. Bio-safety of transgenic crops to natural enemies

2.2.1.2. 1 Detection of Cry protein in insect pests and their natural enemies

The corn leaf aphid, *Rhopalosiphum maidis*, feeding on diet solutions containing Cry1Ab protein, the level of the protein in the aphid was 250–500 times less than the original levels in the diet, whereas no Cry1Ab was detected by ELISA in aphids feeding on transgenic *Bt*-corn plants, for the lepidopteran insects, *Ostrinia nubilalis*, *Helicoverpa zea*, and *Agrotis ipsilon*, levels of Cry1Ab in larvae varied significantly with feeding treatment. When feeding for 24 h on artificial diets containing 20 and 100 ppm of Cry1Ab, the level of Cry1Ab in the larvae was about 57 and 142 times lower, respectively, than the original protein level in the diet for *O. nubilalis*, 20 and 34 times lower for *H. zea*, and 10 to 14 times lower for *A. ipsilon* (Head *et al*, 2001)

Vojtech *et al*. (2005) showed an ELISA and confirmed that *Spodoptera littoralis* larvae ingested high amounts of Cry1Ab toxin while feeding on *Bt* maize, no toxin was found in *S. littoralis* and *Cotesia marginiventris* adults. Thus the toxin was not accumulating in the trophic levels and infact appeared to be excreted. The results suggested that the effects on *C. marginiventris* when developing in susceptible *S. littoralis* larvae are indirect (host mediated). Mon810 *Bt* maize plants contained a mean concentration of 1.597 μg Cry1Ab toxin per gram fresh weight of plant tissue. *S. littoralis* larvae feeding on such plants contained between 0.595 and 0.645 μg Cry1Ab toxin per gram of fresh bodyweight. In *S. littoralis* pupae and *C. marginiventris* cocoon silk only traces of Cry1Ab were detected.

Adult *S. littoralis*, *C. marginiventris* cocoons (including pupae) and adult parasitoids, contained no detectable amount of Cry1Ab toxin.

Pont and Nentwig (2005) reported that *Porcellio scaber* feeding on N4640 *Bt* corn leaves digests a mean of 61.19/16.8 per cent of the *Bt*-protein they ingested, while *P. scaber* feeding on Max88 *Bt* corn leaves digested 80.59/14.4 per cent, which was significantly more (PB/0.05). The bioassays indicated that the *Bt*-protein excreted in the faeces was still insecticidally active. The study suggested that a part of the *Bt* protein taken up by primary decomposers is not digested and is released in its active form into the soils.

Zhang *et al*. (2006a) reported the trace amounts of *Bt* toxins (6.0 ng g^{-1} fresh mass [FM] in GK-12, 4.0 ng g^{-1} FM in NuCOTN 33B) detected in *A. gossypii* feeding on *Bt* cotton cultivars. *Bt* toxin was detected in ladybirds preying on *Bt*-fed aphids, and its quantity increased as the predatory period extended (5d-20d), small amounts of *Bt* toxin was also found in newly hatched, unfed coccinellid larvae when their parents fed on NuCOTN 33B-reared aphids (15.0 ng g^{-1} FM), but not when the parents were fed on GK-12D reared prey. These results indicate that *Bt* toxin expressed in transgenic cotton cultivars can be transmitted to a higher trophic level through a non-target pest insect and may alter the biology and behavior of a predatory ladybird.

Obrist *et al*. (2006) evaluated the uptake of Cry1Ab toxin by larvae of the green lacewing, *Chrysoperla carnea* after consuming two *Bt* maize-fed herbivores (*Tetranychus urticae* and *S. littoralis* by means of an immunological test (ELISA) and the activity of the Cry1Ab toxin following ingestion by the herbivores. ELISA confirmed the ingestion of *Bt* toxin by *C. carnea* larvae when fed with either of the two prey species. Feeding bioassays using the target pest showed that the biological activity of the Cry1Ab toxin is maintained after ingestion by both herbivore species. The purified Cry1Ab protein was more toxic to *O. nubilalis* compared to the plant-derived Cry1Ab toxin when applied at equal concentrations according to ELISA measurements.

Burgio *et al*. (2007) indicated that Cry toxin was detected in aphid samples, with a mean concentration in the positive samples of 2.0 ± 0.8 ppb. The majority (87.3%) of corn flea beetles, *Chaetocnema pulicaria*, screened positive for Cry1Ab proteins. The average recorded concentration of *Bt* endotoxin within *C. pulicaria* was 2.43 ± 0.13 μg Cry1Ab per g fresh weight (n=71). The screening of predators from the field indicated that natural enemies from three orders (Araneae, Coleoptera and Heteroptera) contained Cry1Ab endotoxins above the threshold of 0.5 μg Cry1Ab per g fresh weight.

Harwood *et al.* (2007) studied the detection of *Bt* endotoxins using a post-mortem enzyme-linked immunosorbent assay and examined the uptake of Cry1Ab-endotoxins by predatory coccinellids and the importance of anthesis to this trophic pathway. This was most evident in *Coleomegilla maculata*, with 12.8 per cent of 775 individuals testing positive for Cry1Ab-endotoxins. Presence of endotoxins in gut samples was not confined to periods around anthesis, but coccinellid adults tested positive two weeks before and upto ten weeks after pollen was shed, suggesting tri-trophic linkages in their food chain facilitates the transfer of endotoxins into higher-order predators. This contrasts with adult *C. maculata* entering overwintering sites where *Bt*-endotoxins were not detected in gut samples, indicating low levels of persistence of Cry1Ab-endotoxins within coccinellid predators.

Torres and Ruberson (2008) quantified Cry1Ac toxin in the cotton plants, the pests and predators, and the effects of continuous feeding on *S. exigua* larvae fed either *Bt* or non-*Bt* cotton on life history traits of *Podisus maculiventris*. All three herbivores were able to convey Cry1Ac toxin to their respective predators. Among the herbivores, *T. urticae* exhibited 16.8 times more toxin in their bodies than that expressed in *Bt*-cotton plant, followed by *S. exigua* (1.05 times), and *Frankliniella occidentalis* immatures and adults (0.63 and 0.73 times, respectively).

Chen *et al*. (2008a) conducted experiments to detect Cry1C toxin using ELISA in *Pieris rapae* pupae after older larvae fed on *cry1C* broccoli. However, no Cry1C toxin was detected in newly emerged *Pteromalus puparum* adults developing in *Bt*-fed hosts. Only a trace amount of toxin was detected from entire *P. puparum* pupae dissected from the *Bt* plant-fed host. Moreover, no negative effect was found on the progeny of *P. puparum* developing from the *Bt* plant-fed host when subsequently supplied with a healthy host, *P. rapae* pupae.

Chen *et al*. (2009) reported the bioaccumulation of *Bt* insecticidal toxins expressed in *Bt* plants using ELISA and evaluated the transfer of Cry1Ab toxin in a food chain of *Bt* rice (KMD1 and KMD2), the target insect, *Cnaphalocrocis medinalis*, and its predator, *Pirata subpiraticus*. Cry1Ab was detected in *C. medinalis* and *P. subpiraticus*. However, the concentration of Cry1Ab detected from *C. medinalis* and *P. subpiraticus* did not increase as feeding or preying time increased.

Dhillon and Sharma (2010) studied tritrophic interactions between *Bt* (administered as spray), chickpea genotypes, and the parasitoid, *Campoletis chlorideae*. The ELISA test detected >5 ppb of *Bt* toxins in *Bt*-sprayed chickpea genotypes, and the *H. armigera* larvae fed on them. However, no *Bt* toxins were detected in the larvae, cocoons and adults of *C.*

chlorideae reared on *Bt*-intoxicated *H. armigera* larvae, or in adult parasitoids fed on *Bt*-contaminated honey.

Stephensa *et al*. (2012) reported that of *Bt* proteins passed from the plant to the predator via the aphid using ELISA. This is the first report of negative impact of Cry3Bb *Bt*-maize on carabid activity-densities in the field and one of the first mechanistic examples of a negative indirect tritrophic level impact of a Cry3Bb *Bt*-maize on a coccinellid.

Murenga *et al*. (2012) reported the mean concentration of *Bt* δ-endotoxins as 4.93 and 4.63 µg/g in Events 216 and 223 (two public lines of *Bt* maize), respectively. As expected, F_1 generations of all the crosses had similar concentrations whereas the F_2 generations showed a spread of concentrations. These findings implied that genotypes with a higher mean concentration of *Bt* δ-endotoxins also have a lower level of plant damage traits expression.

Zhang *et al*. (2012) explained the effects of transgenic *Bt* cotton on *Aphis gossypii.* The *Bt* protein was detected by ELISA in the *Bt* cotton leaves, and the content varied significantly at different growth stages and trace amounts were detected in some of the *Bt*-fed aphids, and the honeydew of the *Bt*-fed aphids contained over 10 ng/g *Bt* protein. These results indicated that although trace amounts of the *Bt* protein were ingested, the *Bt* cotton had no significant negative impacts on *A. gossypii* in either short or long term. The *Bt* protein content in the leaves varied significantly at different growth stages. The leaves at the 4 true-leaf stage contained the highest average concentration of *Bt* protein (86.03 ng/g), the 2-cotyledon stage contained a moderate level (44.84 ng/g), and the lowest level was found at the boll stage (8.81 ng/g). The *Bt* protein could only be detected in one-third of the aphid samples with body weights over 20 mg.

2.2.2. Biochemical characterization

Extracts of a number of trees foliage have been shown to inhibit the growth of *B. thuringiensis* on artificial media (Smirnoff and Hutchinsun, 1965) and sweet gum was one such tree (Maksymiuk, 1970). Tannins have been shown to inactivate insecticidal crystal proteins of *B. thuringiensis* (Luthy *et al*., 1985ab) and tannin chemistry has been implicated in variation in the susceptible host.

Tannin is an important constituent of many plants, reacted strongly with the proteinaceous insecticidal metabolite of *B. thuringiensis*, solutions of a commercial tannin preparation stopped the activity of dissolved crystal protein and activated δ-endotoxin, Intact crystals lost their activity only partially in the presence of tannin. Interaction between host plant tannins and δ-endotoxin might be a major factor where the field efficacy of *B.*

thuringiensis preparations is unexpectedly low (Luthy *et al*., 1985b). The effectiveness of *B. thuringiensis* is greater on insect pests adapted to high tannin content (with a gut pH of 8.0 to 9.5). Thus, insect pathogens can be more effective in a pest management program if antibiosis factors of host resistance are compatible with the insect pests.

The susceptibility of gypsy moth, *Lymantria dispar* to the gypsy moth nuclear polyhedrosis virus was significantly altered when larvae were fed with virus in conjunction with diets containing different nutrients and plant allelochemicals. Larvae consuming virus on diets with additional sucrose, surfactants, or gallic acid showed no significant changes in mortality rates. Larvae consuming virus on diets with additional casein or salts, in diets made more acidic with HCl, or on diets containing hydrolysable or condensed tannins showed up to four fold significant decreases in susceptibility to gypsy moth virus (Keating *et al*., 1989).

Chhabra *et al*. (1990) studied sources of resistance in cultivars of *H. armigera* in field trials in India during 1976-80. The results were examined in relation to the biochemistry of the cultivars of chickpea tested. A high percentage of crude fibre, non-reducing sugars and low percentage of starch appeared to be related to the low incidence of the pest in cultivar GL645, while a high percentage of cellulose, hemicellulose and lignin in the pod wall are thought to inhibit pod damage.

Sivamani *et al*. (1992) conducted bioassays with *B. thuringiensis* var. *galleriae* Berliner δ-endotoxin and plant phenolics on *H. armigera* and reported the presence of plant phenolics with *Bt* var. *galleriae* endotoxin not only reduced feeding potential and weight gain by the larvae, but also enhanced the LC_{50} value of the toxin, indicating the effect of phytochemicals from resistant crop plants on the biocidal activity of *B. thuringiensis* strains in laboratory conditions.

Bhagwat *et al*. (1995) screened forty desi (local) early maturity chickpea genotypes for resistance to gram pod borer, *H. armigera*, under natural field conditions. ICC 506 exhibited 8 per cent pod damage and harboured 10 larvae on 10 plants and was designated as least susceptible. Whereas, ICC 14665 showed 41.8% pod damage and 26 larvae on 10 plants and categorized as most susceptible. A low amount of acidity in the leaf exudates (21.1 and 41.9 meq./100 g) of genotype (ICC 14665) was found to be associated with susceptibility to *H. armigera*, 60 and 75 days afer sowing. However, such a trend was not evident 90 days after sowing.

Yoshida et al. (1995) noted that mechanisms of resistance to *H. armigera* in chickpea. Inhibition of larval growth occurred in a feeding test using the leaves of chickpea genotypes,

which had previously been identified as having resistance to *H. armigera*. A feeding test using unwashed and washed leaves revealed that the substance responsible for the growth inhibition was water soluble and present on the surface of the leaves. Acid components of the leaf exudate were analyzed by high-performance liquid chromatography (HPLC). Oxalic acid and malic acid were detected as major components in all four genotypes that were analyzed. Genotypes resistant to *H. armigera* accumulated more oxalic acid on the leaves than susceptible genotypes. Oxalic acid showed significant growth inhibition on *H. armigera* larvae when included in a semi-artificial diet. The 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. Malic acid had no effect on larval growth.

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

Yoshida *et al*. **(**1997) studied the effects of malic acid and oxalic acid on oviposition of *H. armigera.* Malic acid stimulated oviposition at a concentration of 0.6 μmol cm^{-1} but inhibited it at 3.4 μmol cm^{-1}. Oxalic acid showed neither stimulation nor inhibition of oviposition at 0.25-1.7 μmol cm^{-1}. However, there was a significant negative correlation between pod damage and oxalic acid levels. Oxalic acid, which had been reported to have an antibiotic effect on *H. armigera* larvae, had an important role in resistance to this pest in chickpea.

Studies have been conducted to assess the level of tannins in the host plants (*Tectona grandis* and *Mellingtonia hortensis*) of teak defoliator, *Hyblaea puera* cram, and its influence on the neem seed kernel extract (NSKE) and bacterial pesticides, *B. thuringiensis kurstaki* (*Btk*). Higher morality of fourth-instar larvae was evident after the treatment of neem seed

kernel extract and *Btk* on the *Mellingtonia* reared larvae than the teak leaves reared larvae. Higher tannin content was evident in the leaves of teak and teak leaves reared larvae. Tannin level was comparatively lower in the *Mellingtonia* and insects reared on it. This suggested that the higher tannin level in the teak leaves facilitate the larvae to sequester the tannin and accumulate it in the body. Hence, tannin here has a protective role in insects and helps them to resist against the biopesticides such as neem and *Bt* toxins. The higher mortality of larvae of *H. puera* fed on *M. hortensis* with neem and *Bt* toxins treatment further suggested the less availability of tannin in the host leaves and susceptible against the biopesticides (Murugan and Babu, 1998).

Berbehenn and Martin (1994) and Berbehenn *et al*. (1996) have stated that tannins can enter the haemolymph of the insect through the peritrophic membrane of the gut. The peritrophic envelope of insects are capable of connecting tannins by attaching to the carbohydrates (e.g. chitin) of the envelopes, hydrolysable tannins of oak are well known as phenolics, which can negatively influence the growth of the gypsy moth (Rossitter *et al*., 1988). Dunning *et al*. (2002) reported on the feeding behavior of the generalist migratory grasshopper, on two species of oak with different tannin levels.

The interaction among white spruce, *Picea glauca*, purified acetone tannin extracts, *B. thuringiensis* subsp. *kurstski* Cry1A(c) δ-edotoxin strain HD-73 (*Btk*), and spruce budworm, *Choristoneura fumiferana* on larval survival, growth, and development were investigated over the whole larval feeding period by using artificial diet supplemented with *Btk* toxins and foliar tannin extracts. At high *Btk* concentration (1.72 μg/ml of diet), tannin antagonized *Btk* potency against spruce budworm by lowering *Btk*-related larval mortality from 83 to 43 per cent while at at moderate concentration tannin did not affect *Btk* potency. Host tree tannins antagonized not only the lethal effects of *Btk* toxin but also sublethal *Btk*-related impacts in terms of larval development, pupal weight, relative consumption rate, and growth rate (Bauce *et al*., 2006).

Saini and Dhawan (2010) had estimated the content of tannins and total phenols and a correlation was established between these two biochemical factors with toxins (Cry1Ac and Cry2Ab) and mortality of *H. armigera* and *S. litura*. Per cent tannin in leaves, squares and bolls was maximum in MRC 7031 and minimum in MRC 7017. It was maximum at 180-day-old and minimum at 60-day-old crop. Among plant parts, it was maximum in leaves followed by bolls and squares. Total phenols (mg/g dry weight) were maximum in Bollgard RCH 134 and minimum four in Tulsi. However, among plant parts, it was maximum in leaves followed

by squares and bolls. A positive correlation was observed between mortality of *H. armigera* and *S. litura* with Cry toxins and total phenols while negative with tannins. Similarly, Cry toxins showed negative correlation with tannins and positive with total phenols.

The biological activity of *Bt* towards *H. armigera* on chickpea genotypes with different amounts of organic acids, significantly lower leaf feeding, larval survival and larval weights were observed on ICC 506EB, followed by C 235, and ICCV 10 with an increase in *Bt* concentrations. Antifeedant effects of acid exudates reduced food consumption and hence might reduce the efficacy of *Bt* sprays on insect-resistant chickpea genotypes or *Bt*-transgenic chickpeas, although the combined effect of plant resistance based on organic acids, and *Bt* had a greater effect on survival and development of *H. armigera* than *Bt* alone. The influence of organic acids (oxalic acid and malic acid) present in the trichome exudates of chickpea on the biological activity and binding of *Bt* δ-endotoxin Cry1Ac to brush border membrane vesicles (BBMV) of the pod borer, *H. armigera*. Oxalic and malic acid in combination at concentrations present in chickpea leaves did not influence the biological activity of *Bt* toxin Cry1Ac towards *H. armigera* larvae, amounts of Cry1Ac protein in the midgut of insects reared on diets with organic acids were similar to those reared on artificial diet without the organic acids (Devi *et al*. 2011).

Narayanamma *et al.* (2013) characterized a diverse array of chickpea genotypes for organic acid profiles in the leaf exudates that are associated with resistance to *H. armigera*. Chickpea leaf exudates contained five major organic acids, which were identified as malic acid, oxalic acid, acetic acid, citric acid, and fumaric acid. The high performance liquid chromatography (HPLC) profiles of the leaf exudates of nine chickpea genotypes showed that amounts of malic acid were negatively correlated with leaf feeding by *H. armigera* larvae at flowering and maturity, and with pod damage. Oxalic acid showed a negative association with leaf damage in detached leaf assay, while the amounts of acetic acid were negatively correlated with larval weight, and damage rating at flowering and maturity. Citric acid levels were negatively associated with damage rating at flowering.

2.3. Effect of transgenic crops on natural enemies of *Helicoverpa armigera*

2.3.1. Effect of transgenic crops on the natural enemies

Schuler *et al*. (2001) studied the direct and indirect effects of *Bt* plants on a parasitoid of *P. xylostella* using Cry1Ac expressing transgenic oilseed rape (Canola), although *Cotesia*

plutellae larvae were forced to develop in *Bt*-treated susceptible hosts inevitably died with their hosts, behavioural factors are likely to limit the impact of this effect on field populations. *C. plutellae* mortality in susceptible hosts was not due to the direct toxic effect of Cry1Ac, but due to premature host mortality since *C. plutellae* larvae developed normally in *Bt*-resistant hosts on *Bt* plants. Adult *C. plutellae* females were highly attracted to *Bt* plants damaged by *Bt*-resistant hosts.

The parasitoid laid on an average 13.40 ± 3.02 and 42.00 ± 2.21 eggs after single mating and throughout its life span, respectively. The egg-larval and pupal period was 13.5 ± 0.45 and 7.0 ± 0.44 days, respectively. The emergence rate varied from 78.3 to 85.2 per cent. The sex ratio of male: female in mated progeny was 1: 3. Adult longevity increased when provided with food source, field release of 1-2 day old parasitoid with 15000 adults/ha (sex ratio 1:3) in chickpea showed encouraging results, wherein significant reduction in pest population and pod damage and increase in yield was obtained (Gupta *et al*, 2004).

Yang *et al*. (2005) reported the transgenic cotton suppressed the growth and development of the larvae parasitized or unparasitized by the wasps. The cocoon formation and cocoon weight of the two wasps parasitizing the larvae reared on transgenic cotton declined greatly. For *M. mediator*, the cocoon formation and cocoon weight decreased by 26.1 per cent and 1.0 mg, respectively; for *C. chlorideae*, the reductions were 17.9 per cent and 5.1 mg, respectively. The larvae of the two wasps developing in the haemocoel of *H. armigera* larvae reared on transgenic cotton exhibited delayed development and, in some cases, abnormal development. The total haemolymph protein content of the larvae fed on transgenic cotton was lower than that of the control.

The larval duration of the parasitoid was delayed, and the pupal weight, body weight of the newly emerged adult and adult longevity decreased significantly when the host's larvae fed on diet containing protoxin Cry1Ac at the concentrations of 0.5-8.0 μg/g in all time or from 12 h before parasitism till pupation of the parasitoid. Compared with the control, the larval weight, pupal weight and pupation rate of *H. armigera* decreased significantly when the larvae fed on diet containing cry1Ac (at 4.0 mg/g). It is concluded that feeding on diet containing *Bt* insecticidal protein, both strains of the cotton bollworm will have significantly negative effects on development of the parasitoid, *M. mediator* (Liu *et al*, 2004).

Liu *et al*. (2005) reported that the *H. armigera* larvae, in first, second and third instar could not survive when fed on transgenic cotton leaves. Consequently, *C. chlorideae* larvae

could not complete their development if parasitizing on such hosts, after *H. armigera* larvae were reared on transgenic cotton leaves for 12-48 hours, they were parasitized by *C. chlorideae* females. The results showed that the body weights of larvae of the parasitoids were significantly reduced when parasitized hosts fed on transgenic cotton leaves compared to those fed on traditional cotton. Duration of egg and larvae stage were significantly prolonged, pupal and adult weight of *C. chloridae* was decreased when the host larvae fed on transgenic cotton leaves longer than 48 h. The development duration of *C. chlorideae* pupae on the hosts fed on transgenic cotton leaves in each treatment was not significantly different from those of control. The longevity of parasitoid females and males fed with a solution containing Cry1Ac toxin was not significantly different with that of the control.

Zhang *et al*. (2006a) reported the life history parameters in two generations of endoparasitoid *C. chlorideae* using *Bt* resistant *H. armigera* larvae feeding on *Bt* toxin Cry1Ac. *C. chlorideae* pupae developed faster in *Bt* treatment than non-*Bt* treatment. The shortened pupal stage, body length of adult male decreased. However, survival, pupal mortality and adult longevity of *C. chlorideae* were almost unaffected in *Bt*-resistant *H. armigera* larvae feeding on *Bt*-toxin and suggested that there is very limited effect on the life history parameters in two generations of *C. chlorideae* parasitizing *Bt* fed *H. armigera* larvae, but both generations of *C. chlorideae* are affected when *Bt*-resistant *H. armigera* larvae fed on *Bt* toxin for different durations.

Zhang *et al*. (2006b) repoted that after ingesting *Bt*-treated resistant *H. armigera* larvae in the third and fourth instar, the body mass and body length of adult *P. japonica* decreased and larval survival and development in these two instars, pupal mortality, fecundity and adult longevity of *P. japonica* were not affected in both the generations. The results suggested that ingesting *Bt*-toxin Cry1Ac-treated pests in advanced larval stage might have no significant effect on the fitness of predator *P. japonica*.

The *H. armigera* larvae fed on artificial diet impregnated with Cry1Ab and Cry1Ac at LC_{50} and ED_{50} levels before and after parasitisation resulted in a significant reduction in cocoon formation and adult emergence of *C. chlorideae*. Larval period of the parasitoid was prolonged by two days when fed on *Bt*-intoxicated larvae, no adverse effects were observed on female fecundity. The observed effects appeared to be indirect in nature, because no *Bt* proteins were detected in the *C. chlorideae* larvae, cocoons, or adults fed on Cry1Ab or Cry1Ac-treated *H. armigera* larvae. The effects of *Bt* toxin proteins on *C. chlorideae* were

due to early mortality of *H. armigera* larvae, i.e., before completion of parasitoid larval development. The effects of transgenic cottons with *CryIAc* gene from *Bt* on the natural enemies of cotton boll worm, *H. armigera* and observed that there were no differences in coccinellid numbers between the transgenic and non transgenic. The number of *chrysopid* larvae was greater on the varieties Aravinda, L 604 and Mech 184 under both protected and unprotected conditions. However, the survival and development of *C. chloridae* was also poor when *H. armigera* larvae were fed on the leaves of *Bt* cotton hybrid Mech 184. The effects of transgenic cottons with cry1Ac gene from *Bt* on the natural enemies of cotton boll worm, *H. armigera*. There was no apparent effect of transgenic cotton on the relative abundance of predatory spiders (*Clubiona* sp. and *Neoscona* sp.), coccinellid (*Cheilomenes sexmaculatus*), and the chrysopid (*Chrysoperla carnea*). However, the abundance of spiders, coccinellids, and chrysopids was quite low in insecticide protected plots towards end of the cropping season. There was a significant reduction in cocoon formation and adult emergence of the ichneumonid parasitoid, *C. chlorideae* reared on *H. armigera* larvae fed on the leaves of transgenic cottons before and after parasitization. However, no *Bt* toxins were detected in *H. armigera* larvae and the parasitoid cocoons. Reduction in cocoon formation was because of early mortality of the *H. armigera* larvae due to *Bt* toxins in the leaves of transgenic cotton. There was a slight reduction in adult weight and fecundity, and prolongation of the larval period when the parasitoid was raised on *H. armigera* larvae fed on the leaves of transgenic cotton before and after parasitization. Survival and development of *C. chlorideae* was also poor when *H. armigera* larvae were fed on the leaves of cotton hybrid Mech 184. The adverse effects of transgenic cotton on survival and development of *C. chlorideae* were largely due to early mortality and possibly poor nutritional quality of *H. armigera* larvae due to toxic effects of the transgene (Sharma *et al*., 2007).

Dhillon and Sharma (2007) observed the parasitism by *C. chlorideae* females was least with reduction in cocoon formation and adult emergence on *H. armigera* larvae released on chickpea. Host insects also had significant effect on the development and survival of *C. chlorideae*. The larval period of *C. chlorideae* was prolonged by 2 - 3 days on *S. exigua*, *Mythimna separata* and *Achaea janata* when compared with *H. armigera*, *Helicoverpa assulta* and *S. litura*. Maximum cocoon formation and adult emergence were recorded on *H. armigera* (82.4 and 70.5 %, respectively) than on other insect hosts. The study had important implications on development and survival of *C. chlorideae* on alternate insect hosts on non-transgenic crop plants, when there is paucity of *H. armigera* larvae on transgenic crops expressing *Bt*-toxins.

Sharma *et al*. (2008) reported that there was a significant reduction in cocoon formation and adult emergence of *C. chlorideae* when *H. armigera* larvae fed on artificial diet impregnated with CryIAb and CryIAc at LC_{50} and ED_{50} levels before and after parasitisation. Larval period of the parasitoid was prolonged by 2 days and no adverse effects were observed on female fecundity. The observed effects appeared to be indirect in nature, because no *Bt* protein were detected in *C. chlorideae* larvae, cocoons, or adults fed on Cry IAb or Cry IAc treated *H. armigera* larvae.

The growth and survival of the parasitoid were normal when the host larvae were fed with sublethal doses or subjected to short time exposure to lethal doses of *Btk* HD-1. However, the parasitoid offsprings developed slowly and pupal as well as adult period, adult weight and adult emergence rate were reduced significantly if the parasitoid was developing inside a severely *Bt* intoxicated host larvae. There were no evident differences in longevity of parasitoid adults that were fed on honey solution containing different concentrations of *Btk* HD-1 as compared to adults fed only on honey solution. This indicates no direct adverse effect of *Btk* HD-1 on *C. chlorideae*. The gravid female parasitoid did not discriminate *Btk* HD-1 intoxicated and normal *H. armigera* larvae for oviposition (Mohan *et al.*, 2008).

Ding *et al*. (2009) reported that the developmental period of *Microplitis mediator* offspring's eggs and larvae were significantly delayed and pupal and adult weight were significantly less compared to the control when the female parasitoids parasitized *H. armigera* larvae that fed on diet containing 1, 2, 4 and 8 µg g^{-1} of Cry1Ac. Cry1Ac was detected in larvae and hemolymph of *H. armigera*, but not in the larvae of *M. mediator* and significant effects on several fitness parameters of the F_1 *M. mediator* developed from *H. armigera* fed Cry1Ac intoxicated diet most likely were host-quality mediated rather than direct effects of Cry1Ac.

Dhillon and Sharma (2009a) reported that there were no adverse effects of *Bt* toxins on *Cheilomenes sexmaculatus* when the larvae were reared on *Aphis craccivora* fed on different concentrations of CryIAb or CryIAc in the artificial diet, a significant and positive correlation was observed between the presence of *Bt* toxins in aphids and coccinellid larvae and adults ($r = 0.53$ to 0.86). The results suggested that a direct exposure to *Bt* toxins expressed in transgenic plants or predation on *H. armigera* on *Bt* transgenic plants have little effect on the activity and abundance of the ladybird, *C. sexmaculatus*.

There was a significant influence of host size on development and survival of the parasitoid. *Bt* toxins were detected in *H. armigera* larvae fed on *Bt*–sprayed chickpea, but not in *C. chlorideae* reared on *H. armigera* larvae fed on *Bt*-treated chickpeas, and in the parasitoid adults fed on honey intoxicated with 0.05 per cent *Bt.* The adverse effects of *Bt* on the parasitoid were largely through early mortality of *H. armigera* larvae or poor quality of the host (Dhillon and Sharma, 2010).

Lawo *et al*. (2010) experimentally proved *Chrysoperla carnea* is negatively affected when fed *Bt*-susceptible but not Cry1Ac resistant *H. armigera* larvae that had fed *Bt*-transgenic cotton expressing *Cry1Ac*. In case of the Cry1Ac resistant *H. armigera* strain, feeding on *Bt* cotton resulted in a reduced glycogen content in the caterpillars. The predators, however, appeared to compensate for the reduced carbohydrate content of the prey by increasing biomass uptake which caused an excess intake of the other analyzed nutritional compounds. This study clearly proves that nutritional prey-quality factors other than *Bt* protein underlie the observed negative effects when *C. carnea* larvae are fed with *Bt* cotton-fed prey, possible factors were an altered sugar composition or fitness costs associated with the excess intake of other nutrients.

Dhillon and Sharma (2011) experimentally proved the influence of mating behaviour and abundance of the insect host on fecundity and sex-ratio of the parasitoid, *C. chlorideae*. There was no significant influence in number of matings and abundance of the insect host on cocoon formation, adult emergence and larval and puapal periods of *C. chlorideae*. However, fecundity and female longevity were significantly influenced by mating and abundance of the insect host. There was a significant and positive correlation (r =0.84) between longevity and fecundity of *C. chlorideae* females. The unmated *C. chlorideae* females produced only males, nearly 20 per cent of the females that had mated twice were male biased.

Salama *et al.* (2013) assessed prey consumption and development parameters of *Chrysoperla carnea* and observed no obvious differences in the development of *C. carnea* that preyed on *H. armigera* fed on transgenic tomato plants as compared to the control. This shows that transgenic tomato plants can be safely used as an efficient tool for the biocontrol of *H. armigera* with no effect on its predator, *C. carnea*.

Bahar *et al.* (2013) studied the effect of cotton aphids, *Aphis gossypii* as an alternative prey on the predation of *H. armigera* larvae by green lacewing larvae, *Mallada signatus*. The presence of *H. armigera* larvae alone, without the predator, caused a 24 per cent reduction in

the numbers of aphids on conventional, but not on *Bt* cotton plants. The combination of *Bt* cotton and lacewing larvae caused a 96.6 per cent removal of early-stage *H. armigera* larvae, a statistically significant increase over the addition of the proportions (91.6%) removed by each factor measured separately, providing evidence of synergism. The study suggests that the presence of aphids as alternative prey would not necessarily disrupt the predation by green lacewing on larvae of *H. armigera*, especially on *Bt* cotton.

2.3.2. Effect of *Cry* genes in transgenic crops on the natural enemies of lepidopteran pests

Chilcutt *et al*. (1997) tested the direct and indirect effects of the bacterium, *Bacillus thuringiensis* (*Bt*) on adult wasp longevity and oviposition behaviour, all parasitoids were observed to make oviposition attempts in both untreated and treated larvae. There was no effect of *Bt* treatment on parasitoid oviposition. The mean number of ovipositions in treated larvae (4.3±0.3) was not significantly different from untreated larvae (4.7±0.2).

Hilbeck *et al*. (1999) studied the prey-mediated effects of artificial diet containing *Bt* proteins on immature *Chrysoperla carnea*. The highest mortality (78%) was reported in comparision to control (26%) and delayed development of immature *C. carnea* raised on Cry1Ab toxin 100 μg g^{-1} diet–fed prey may have been confounded with an increased intoxication of *S. littoralis* larvae, prey-mediated total mortality of Cry1Ab protoxin-exposed chrysopid larvae was intermediate (46–62%) to Cry1Ab toxin exposed (55–78%) and Cry2A protoxin (47%) exposed *C. carnea*. Total development time of *C. carnea* was not consistent and significantly affected by the *Bt*-treatments except at the highest Cry1Ab toxin concentration, at all other *B. thuringiensis* protein concentrations *S. littoralis* was not lethally affected.

Erb *et al*. (2001) explained parasitoid-pathogen interactions in gypsy moth, *Lymantria dispar*, *Bt* and *Compsilura concinnata*. Gypsy moths were minimally affected by sublethal doses of *Bt* development of fourth instar was delayed, and male pupal mass reduced. *Compsilura concinnata* preferentially attacked and had higher superparasitism on non-infected hosts than on *Bt*-treated larvae. Exposure of gypsy moth to both sublethal doses of *Bt* and parasitoids reduced percentage parasitism and host larval survivorship. Parasitoids in *Bt*-treated, superparasitized gypsy moths had shorter larval development times and smaller pupal masses than parasitoids in untreated larvae, while parasitoids in singly parasitized larvae had larger pupal masses than those in superparasitized larvae. Timing of *Bt* infection

relative to parasitism is a factor in gypsy moth mortality, but not in parasitoid potential fecundity.

Hilbeck (2001) reported prey mediated effects of transgenic *Bt*-corn causing significantly higher mortality of *C. carnea* larvae. In choice feeding trials where *C. carnea* could choose between *Spodoptera littoralis* fed transgenic *Bt*-corn and *S. littoralis* fed non-transgenic corn, larger instars showed a significant preference for *S. littoralis* fed non-transgenic corn while this was not the case when the choice was between *Bt* and isogenic corn fed aphids.

Schuler *et al*. (2004) investigated the effects of Cry1Ac-expressing transgenic oilseed rape on the solitary braconid endoparasitoid *Cotesia plutellae. Bt* oilseed rape caused 100 per cent mortality in *Bt*-susceptible *P. xylostella* strain but no mortality in *Bt*-resistant *P. xylostella* strain NO QA. There was no statistically significant difference in the mean time from egg to emergence from hosts, the mean dry weight of females on *Bt* leaves compared to females on wild type leaves. Higher proportion of males emerged from hosts fed wildtype leaves than those fed *Bt* leaves. About 80 to 90 per cent of parasitoid adults successfully emerged from their pupal cocoons on *Bt* plants and the proportion of females amongst the parasitoid progeny was the same on *Bt* plants and wildtype plants or higher on *Bt* plants.

Vojtech *et al*. (2005) showed that *Spodoptera littoralis* larvae are negatively affected by *Bt* maize (Mon810, Monsanto) in terms of developmental time and survival and observed the highest mortality in the first larval stage which is in confirmation with other studies.The cocoons of *Cotesia marginiventris* were smaller and developmental time is longer and *C. marginiventris* suffered greater mortality when parasitizing caterpillars feeding on *Bt* maize.

Liu *et al*. (2011) reported the ecological implications on biological control of insecticidal transgenic plants. Parasitism rate and development of *Diadegma insulare* were not significantly different when different genotypes (*Bt*-resistant or susceptible) of insect host larvae fed on non-*Bt* broccoli plants. The parasitism rate, developmental period, pupal and adult weights of *D. insulare* that had developed on *Bt* broccoli-fed Cry1Ac-resistant *P. xylostella* larvae were not significantly different from those that developed on non-*Bt* broccoli-fed larvae. The life parameters of the subsequent generation of *D. insulare* from *P. xylostella* reared on *Bt* broccoli were not significantly different from those from non-*Bt* broccoli.

Ebrahimi *et al.* (2012) studied the effects of *Bt* on immature stages of *Diadegma insulare*, Ichneumonidae within larvae of diamond back moth, *Plutella xylostella*, parasitoid's adult mortality at field rate of *Bt* was not significantly different from that of control and the results showed that *Bt* kills *D. insulare* larvae indirectly by killing susceptible hosts.

Marzban (2012) evaluated interactions among *Chilo suppressalis*, its larval parasitoid *Trichogramma brassicae* and insect-resistant transgenic rice lines. The results showed that all neonates and second larvae of stem borer were dead regardless of being fed rotationally or permanently on *Bt* rice, but 18 and 28 per cent of the third and fourth instar larvae could complete development and turned to adults, respectively, when fed rotationally.

2.3.3. Effect of transgenic crops on the natural enemies of various crop pests

Hilbeck *et al*. (1998) studied the effects of Cry1Ab toxin on developmental time and mortality of *Chrysoperla carnea* larvae, mortality was higher in *C. carnea* fed the CryIAb-treated diet compared to the control and no or only small differences in developmental time were observed in *C. carnea* fed CryIAb-treated (100 μg/ml of diet) and untreated diets.

The adverse tri-trophic interactions involving a lectin-expressing transgenic crop, a target pest aphid and a beneficial aphidophagous predator. The results demonstrate no acute toxicity due to the transgenic plants and expression of a lectin gene for insect resistance in a transgenic potato line can cause adverse effects to a predatory ladybird via aphids in its food chain Birch *et al*. (1999).

Daly *et al*. (2005) investigated field populations of non-target arthropods in transgenic corn with the MON 810 event expressing the Cry1Ab endotoxin from *Bacillus thuringiensis* variety *kurstaki* (*Bt*), compared with those in conventional, near isogenic corn. The only insect whose numbers were strongly affected by the *Bt* corn was the corn earworm, *Helicoverpa zea*, a target insect. There were no consistent significant differences in nontarget phytophagous and predaceous arthropods in the visual counts and pitfall traps between *Bt* and non-*Bt* corn. The results indicate that transgenic *Bt* fed corn containing the MON 810 event did not have an adverse effect on populations of nontarget phytophagous or predaceous arthropods.

Zhu *et al*. **(**2006) studied the effects of transgenic cotton containing Cry1Ac toxin on the survival, development and fecundity of a predatory lady beetle, *Propylaea japonica*, through a food chain using cotton aphid, *Aphis gossypii* as an herbivorous prey, no significant differences were observed in total survival from hatching to adult, or in larval and pupal durations of *P. japonica* supplied with aphids fed on either transgenic or non-transgenic cotton. Similarly, no significant differences in longevity, reproduction, weight, or fatty acid contents of adult beetles were detected.

Mellet and Schoeman (2007) studied the effect of *Bt*-cotton on aphid, whitefly, chrysopid and coccinellid populations. The cultivation of *Bt*-cotton had no effect on aphid, whitefly, chrysopid or coccinellid abundance, positive density dependent interactions occurred between aphids and coccinellids which were not influenced by *Bt*-cotton, a significant relationship between whitefly and coccinellid abundance, *i.e*. predator-prey reaction, occurred in the control and sprayed non-*Bt* cotton fields but was absent from the *Bt*-cotton fields.

Chen *et al*. (2007) reported the impacts of rice type *Bt*/non-*Bt* on the population density of three plant hoppers, *Sogatella furcifera, Nilaparvata lugens* and *Laodelphax striatellus*, and the natural enemy, *Cyrtorhinus lividipennis*. Both in *Bt* rice and non-*Bt* rice plots, *S. furcifera* was the predominant species of planthoppers and no consistent effects of *Bt* rice and *Bt* rice×sampling date interaction on population dynamics of the predominant planthopper species, *S. furcifera*, and the predator, *C. lividipennis*, were observed throughout the sampling period. This field study indicates that, in comparison with non-*Bt* rice, *Bt* rice did not lead to higher planthopper populations and did not negatively affect the predator *C. lividipennis*.

Wang *et al*. (2007) studied the effects of transgenic *Bt* maize pollen expressing the Cry1Ab protein of *Bt* as a food source on *Trichogramma ostriniae* on longevity, progeny production and offspring sex ratio, females fed on suspension of pollen of *Bt* maize or non-*Bt* maize in water lived significantly longer than those fed on water alone, no significant differences in longevity, number of parasitized eggs, offspring emerged and the offspring sex ratio were observed between the females feeding on pollen of *Bt* maize and non-*Bt* maize. *Bt* maize pollen did not adversely affect *T. ostriniae*.

Ying *et al*. (2008) studied tri-trophic impacts of transgenic *Bt* cotton GK12 and NuCOTN 99B using a predator, the great lacewing *Chrysopa pallens*, and its prey, the cotton aphid *Aphis gossypii*. When fed GK12-originated aphid prey, pupal body mass of *C.*

pallens was significantly higher than that of the control, more females emerged, and these females laid significantly more eggs. These results indicate that *C. pallens* is sensitive to aphid prey from different cotton cultivars. Transgenic *Bt* cotton GK12-originated aphid prey has no adverse impact on survival, development, and fecundity of *C. pallens*.

Dhillon and Sharma (2009b) studied the effects of *Bt* cotton on non-target insect pests, generalist predators, arthropod diversity and toxin flow through different trophic levels under insecticide protected and unprotected conditions. The populations of major non-target insect pests (leafhoppers, whiteflies, ash weevils, aphids, dusky and red cotton bug, and green bug) and the generalist predators (ladybirds, chrysopids, and spiders) did not differ significantly between the *Bt* and non-*Bt* cottons, while their numbers were lower in insecticide protected than under unprotected conditions, except for aphids and whiteflies. Although, *Bt* toxin was detected in some insect species, no significant differences were observed in their abundance on *Bt* and non-*Bt* cottons.

Schmidt *et al*. (2009) reported there was significant higher mortality of *Adalia bipunctata* larvae fed with the lepidopteran-active Cry1Ab toxin even at the lowest concentration (5 µg/ml) than the control. At a concentration of 25 µg/ml, coleopteran-active Cry3Bb resulted in a marginally significant higher mortality compared to the control. This revealed slight decline in mortality at the highest concentration of 50 µg/ml and at concentrations between 10 and 100 µg/ml revealed no significant effects on development time and body mass of newly emerged adults.

Dhillon and Sharma (2009a) studied development, survival, and fecundity of field and laboratory strains of the *H. armigera* larval endoparasitoid, *Campoletis chlorideae* at different temperatures and suggested, the *C. chlorideae* adults stored at 18 ^{0}C could be used for parasitism, while the immature stages should be reared at 27 ^{0}C for mass production of the parasitoid for biological control of *H. armigera.*

In the study conducted by Yu *et al*. (2011), the results indicated that transgenic rice expressing *cry1Ab/cry1Ac*, *cry2A* and *cry1C* had no significant adverse effects on the population dynamics of three planthoppers (*Nilaparvata lugens, Sogatella furcifera* and *Laodelphax striatellus*) and their predators *Cyrtorhinus lividipennis, Pirata subpiraticus* and *Theridium octomaculatum*).

Digilio *et al.* (2012) reported no significant differences between performance of *Macrosiphum euphorbiae* on genetically modified tomato plants (line UC82*Bt*) with respect to their near-isogenic control line (line UC82). Similarly, no significant differences were found on the longevity and prey consumption of *M. caliginosus* when fed aphids reared on UC82*Bt* or on UC82. The genetic modification did not affect the attractiveness of uninfested tomato plants toward *A. ervi*. It is therefore concluded that Cry3Bb-expressing tomato plants did not show any acute adverse effects on the biological parameters of the non-target herbivore *M. euphorbiae* or its natural enemies, *M. caliginosus* and *A. ervi*.

Dhillon *et al.* (2012) studied the efficacy of *Bt* cotton for the management of bollworms, their effects on non-target insects. *H. armigera* and *Earias vittella* damage was significantly lower in *Bt*-cotton than in non-*Bt* cotton, while no significant differences were observed in egg-laying by *H. armigera*. The populations of major nontarget sucking insect pests such as *Amrasca biguttula biguttula*, *Bemisia tabaci*, *Aphis gossypii*, *Oxycarenus laetus*, *Dysdercus koenigii*, and *Nezara viridula*; and the generalist predators, *Cheilomenes sexmaculatus*, *Chrysopa* spp., and spiders did not differ significantly between *Bt* and non-*Bt* cottons. Bollworm damage was lower and seed cotton yields higher in *Bt* than in non-*Bt* cottons and concluded that *Bt* cotton hybrids are effective for the management of bollworms and yield more, and do not have any adverse effects on the abundance of generalist predators.

Chapter III

MATERIAL AND METHODS

Studies on the "Characterization of *Cry IIa* transgenic chickpea lines and their interaction with natural enemies of *Helicoverpa armigera* (Hubner)" were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2011-14. The materials utilized in conducting these experiments are elucidated below.

3.1 Phenotyping of *Cry IIa* transgenic chickpea lines for resistance to pod borer, *H. armigera*

3.1.1. Experimental material:

Six transgenic and two non transgenic chickpea lines were evaluated for resistance to *H. armigera* (Table 3.1). The plants were grown under greenhouse conditions (27 ± 5^0 C and 65 - 90% RH). The seeds were sown in a sterilized mixture of black soil (Vertisols), sand and farmyard manure (2:1:1) filled in medium sized plastic pots (30 cm in diameter, 30 cm in depth). The seeds were sown 5 cm below the soil surface and watered immediately and thereafter as and when required. Three plants with uniform growth were retained in each pot at 10 days after seedling emergence. Diammonium phosphate granules (DAP) were applied at 15 days after seedling emergence @ 20 g per pot. The experiment was laid out in a completely randomized design (CRD) with three replications.

3.1.2. Insect Culture:

Larvae of *H. armigera* used in the bioassays were obtained from a laboratory culture maintained at ICRISAT. The larvae were reared on chickpea based artificial diet (Armes *et al*., 1992) under laboratory conditions at 27^0C. 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 introduced into the laboratory culture to avoid contamination with the nuclear polyhedrosis virus, bacteria, or fungi. The neonates of *H. armigera* 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 is 3.5 cm in diameter, 2 cm in depth) to avoid cannibalism. Each cell well had sufficient quantity of diet (7 ml) to support larval development until pupation. The pupae were removed from cell wells, sterilized with

2% sodium hypochlorite solution, and kept in groups of 50 in plastic jars containing vermiculite. Upon emergence, ten pairs of adults were released inside an oviposition cage (30 x 30 x 30 cm), and provided with 10% sucrose or honey solution on a cotton swab as food (Plate 1). 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% sodium hypochlorite solution. The liners were dried under fan and then placed inside the plastic cups with artificial diet. After egg hatching, the larvae were moved to the artificial diet, and the liners were removed after 4 days. Neonate larvae were used for bioassays using diet impregnation assay (Sharma *et al*., 2005a).

3.1.3. Laboratory evaluation

The six transgenic chickpea lines, BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-1P2, BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P1, BS5A.2(T2) 19-3P2 and two non transgenic chickpea lines, ICC506 EB (Resistant check) and Semsen (Control) were sown in greenhouse during the post rainy seasons of 2011-12 and 2012-13 (Plate 2). The plants were used for the bioassays in the laboratory under uniform environmental conditions (27 ± 2^0 C, 65-75 % RH, and a photoperiod of 12:12 h. (Light : Dark) and evaluated for resistance to *H. armigera* using detached leaf assay against the neonate and second-instar larvae of *H. armigera*. Bioassays were conducted at the vegetative [30 days after emergence (DAE)] and flowering stages (45 DAE).

3.1.4. Detached leaf assay

The chickpea plants grown in the greenhouse were bioassayed under controlled conditions in the laboratory [27 ± 2^0 C temperature; 65 - 75% RH, and photoperiod of 12:12 h. (Light : Dark)]. Terminal branches of chickpea (three to four fully expanded leaves/bud) were placed into plastic cups (4.5 x 11.5 cm diameter) in solidified agar-agar (3%) (Sharma *et al*., 2005b). Agar-agar (3%) was boiled, and 10 ml solution was poured into a 250 ml plastic cup kept in a slanting manner (Plate 3). The solidified agar-agar served as a substratum for holding the chickpea branches and maintains the leaf turgid for 4-5 days. The terminal branches were cut with scissors and immediately placed in slanting manner in the agar-agar medium. Care was taken to see that the chickpea branches did not touch the inner walls of the cup. Ten neonates of *H. armigera* were released on the chickpea leaves in each cup, and then covered with a lid to keep the chickpea terminals in a turgid condition.

3.1.5. Cage screening

Each genotype was infested with neonate *H. armigera* at 30 DAE. Twenty neonates were released on the terminal branches of three plants in each pot using a camel hair brush.

The plants were covered with a wire framed cylindrical cage (25 cm in diameter and 25 cm in height) (Plate 4). The lower margin of the cage was pushed to a depth of 3 cm in the soil and covered with nylon bag of similar dimensions to prevent any escape of the larvae. There were three replications for each genotype. The experiment was monitored daily, and terminated when >80% of the leaf area was consumed in the control plants. The larvae were removed from the plants, placed individually in small plastic cups, and weighed after 4 h. The plants were then rated visually for the extent of leaf damage on a 1 to 9 damage rating scale (1 = <10% leaf area damaged; 2, 11-20%; 3, 21-30%; 4, 31-40%; 5, 41-50%; 6, 51-60%; 7, 61-70%; 8, 71-80%; and 9, >80% leaf area damaged). Data were recorded on leaf area damaged (visual damage rating), larval survival and larval weights.

The detached leaf assay and cage screening experiments were conducted in a completely randomized design (CRD) with three replications for each genotype.

3.1.6. Artificial diet for rearing *H. armigera*

For preparing the chickpea based diet for insect culture, all the ingredients (Armes *et al.,* 1992) (Table 2) were weighed separately. The ingredients of Fraction A and water (W1) were mixed thoroughly in a large bowl of 2 L capacity by using a hand mixer. The yeast was mixed with water (W2) in a saucepan on a hot plate and heated to boiling point, agar-agar was added and boiled and then again agar was mixed with other ingredients (Fraction A) in a plastic bowl and stirred until even consistency was obtained. This semi-cooled diet was poured into trays or 250 ml plastic cups (0.5 cm thin layer) placed on a level surface and allowed to cool under a laminar flow for one hour. Plastic cups were covered with a lids, whereas the, trays were wrapped with polythene sheet to avoid contamination. Nearly 300 ml diet was sufficient to rear 30-neonate larvae upto pupation.

3.1.7. Survival and development of *H. armigera* on artificial diet with lyophilized leaf powder of different transgenic chickpea lines

To study the effectiveness of transgenic chickpea against *H. armigera*, freeze-dried lyophilized powder of leaves and pods of chickpea genotypes were incorporated into the artificial diet.

Terminal branches with tender green leaves of six transgenic chickpea lines, BS5A.1(T2) 18-1 P1, BS5A.1(T2) 18-2 P1, BS5A.2(T2) 19-1 P2, BS5A.2(T2) 19-2 P1, BS5A.2(T2) 19-3 P1, BS5A.2(T2) 19-3 P2 and two non-transgenic chickpea lines, ICC 506 (Resistant check) and Semsen (Control) were collected from glasshouse. The leaves and pods

were frozen at -20^0 C and lyophilized (Plate 5). The lyophilized leaves and pods were powdered in a blender to obtain a fine powder (<80 μm). To study the effects of transgenic and non-transgenic chickpea lines against *H. armigera*, lyophilized leaf and pod powder of six transgenic and two non-transgenic chickpea lines was incorporated into the artificial diet (Table 3.3). There were three replications for each genotype in a CRD, 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 14.1. The treatment means were compared by DMRT to know the significance of differences among the transgenic and non transgenic chickpea lines.

3.2 Molecular and biochemical characterization of *Cry IIa* transgenic chickpea lines for nutritional equivalence

3.2.1 Estimation of biochemical constituents

3.2.1.1 Proteins

Sixty mg of the dried test sample was macerated in 10 ml of cold TCA (10%) for 30 min, kept at 4ºC for 24 h, and then centrifuged (Osborne, 1962). The supernatant was discarded and the resultant pellet was re-suspended in 5 per cent TCA (10 ml) and heated on a water bath at 80ºC for 30 min. The sample was cooled and re-centrifugated, and each time the supernatant was discarded. Finally pellet was washed with distilled water, centrifuged and the residue dissolved in 1N NaOH (10 ml), and left overnight at room temperature.

Total protein content was estimated in an aliquot of 1 ml sample extract, using the protocol of Lowry *et al.* (1951). A stock solution (1 mg/ml) of Bovine Serum Albumin (Sigma Chemicals Manufacturing Limited, St. Louis, Missouri, USA) was prepared in 1N NaOH, from which 0.1 to 0.9 ml of aliquots were dispensed in a series of test tubes. The volume was made up to 1 ml by adding distilled water. To each test sample, 5 ml freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1N NaOH and 1 ml of 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate) was added at room temperature and left undisturbed for a period of 10 min.

Subsequently, 0.5 ml of Folin-Ciocalteau reagent (prepared by diluting the reagent with distilled water in 1:2 ratio just before use) was added to each sample. The optical density (OD) of each sample was measured at 750 nm after 30 min in a spectrophotometer (Hitachi,

Tokyo, Japan, U 2900). Three replicates of each sample were taken and their mean values were used to prepare the standard curve. The total protein content in each sample was calculated from the standard curve for Bovine Serum Albumin (BSA). Three replicates were examined for each treatment.

3.2.1.2. Carbohydrates

The dried sample (50 mg each) was macerated in a grinder with 20 ml of ethanol and left for 12 h. The samples were then centrifuged at 1200 rpm for 15 min, the supernatants were removed and concentrated on a water-bath. The volume of aqueous concentrates was made up to 50 ml with distilled water (Extract A) and processed further by following the method of Loomis and Shull (1937) to estimate total soluble sugars.

Residual pellet obtained by centrifugation was suspended in a mixture of 5 ml of 52 per cent perchloric acid and 6.5 ml of distilled water, shaken vigorously (5 min) and centrifuged at 2500 rpm. This step was repeated three times and the supernatants were collected and pooled. The volume was made up to 100 ml with distilled water (Extract B). An aliquot of 1 ml was used to estimate starch content (McCready *et al*., 1950).

One ml aliquot of the test sample from Extracts A and B were used for quantifying total carbohydrates using phenol-sulphuric acid method (Dubois, 1951). A standard curve was prepared using glucose. A stock solution of glucose (100 µg/ml) was prepared in distilled water, of which 0.1 to 0.9 ml aliquots were transferred to a series of test tubes and the volume made up to 1 ml with distilled water. To each of these, 1 ml of 5 per cent aqueous phenol was added quickly in an ice chest and shaken gently and then 5 ml of concentrated H_2SO_4 was added by agitating the test tube. The test tubes were kept in a water-bath (26º–30ºC) for 20 min and the optical density (ODs) of the yellow orange color thus developed was recorded at 490 nm in a spectrophotometer after setting the instrument for 100 per cent transmission against the blank. Four replicates of each sample were run and the mean values calculated. A regression was computed between known concentrations and their respective OD (based on Beer's Lamberts Law). The concentration (mg/g dry weight) of total soluble sugars was estimated from the standard curve for glucose. Three replicates of each sample were taken and their mean values recorded. The carbohydrate content in terms of glucose equivalent and the conversion factor (0.9) were used to convert values of glucose to starch in each case.

Standards with different concentrations (i.e., 25, 50, 75, 100 and 125 μg of glucose) were prepared from the working standard, and their absorbance was read by taking 1 ml aliquots.

Total soluble sugars were calculated by using the formula:

$$\frac{\text{Conc. of standard}}{\text{Absorbance of standard}} \times \text{Absorbance of 1 ml extract} \times \frac{1}{10{,}00{,}000} \times \frac{3\text{ ml}}{0.1\text{g}} \times 100$$

3.2.1.3. Lipids

One g of each of the dried and milled test sample was macerated in 10 ml distilled water (Jayaraman, 1981). To this, 30 ml of chloroform : methanol (2:1 v/v) was added and mixed thoroughly. The mixture was left overnight at room temperature; 20 ml each of chloroform and distilled water was added to the sample and centrifuged. Of the three layers, a clear lower layer of chloroform containing lipids was collected in a pre-weighted beaker. The solvent was allowed to evaporate and the beaker was re-weighed and the amount of lipids were recorded and expressed as total lipids/g of the dried sample.

3.2.1.4. Phenols

Dried and milled test samples (200 mg) were homogenized in 80% ethanol (10 ml) for 2 h and left over night at room temperature. The samples were centrifuged and the supernatants were collected individually and the volume of each was made up to 40 ml with 80 per cent ethanol.

Total phenol content was estimated by following the protocol described by Bray and Thorpe (1954). A standard curve of caffeic acid (phenol) was prepared. A stock solution (100 μg/ml) of caffeic acid was prepared in 80 per cent ethanol, from which 0.1 to 0.9 ml aliquots were transferred into a series of test-tubes and the volume was made up to 1 ml with 80 per cent ethanol. To each of these tubes, 1 ml of Folin–Ciocalteau reagent (1: 2 ratio) with 2 ml of 20 per cent Na_2CO_3 solution was added and the contents mixed vigorously. The samples were incubated in boiling water bath for 1 min, cooled and diluted to 25 ml with distilled water. The optical density (OD) was recorded at 750 nm using a spectrophotometer against a blank.

Three replicates were taken for each concentration, and the average OD was plotted against the respective concentrations to prepare the standard curve. Each test sample was processed in a similar manner. Total amount of phenols was estimated from (with reference to caffeic acid) the standard curve.

The standard curve was prepared by plotting the average absorbance readings of the duplicate determinations of catechin concentrations and the catechin equivalents (CE) calculated by using the following formula.

$$CE\ (\%) = \frac{\text{mg catechin/ml}}{\text{Vol. of extract taken}} \times \frac{\text{Volume made up}}{\text{Wt. of sample}} \times 100$$

3.2.1.5. Tannins

The amounts of condensed tannins present in the leaves of chickpea were estimated by Vanillin – hydrochloride assay (Price *et al*., 1978). The following reagents were used in the present study.

3.2.1.5.1. Reagents

1. Vanillin-hydrochloride reagent:

Mixture of equal volumes of 8% hydrochloric acid in methanol and 4% vanillin in methanol.

2. 8% concentrated HCl in methanol (8 ml of HCl add to 92 ml of methanol).

3. 4% Vanillin in methanol (4 g of Vanillin brought to 96 ml of methanol).

4. Mixed 2 and 3 in equal volumes just before use.

3.2.1.5.2. Standard solution

A stock solution was prepared by dissolving 1 mg of catechin in 1 ml of methanol. The stock solution was diluted ten times (10 times dilution: 1 ml stock + 9 ml of methanol) and 10 ml to 100 ml (100 µg/ml).

Chickpea leaves were collected from the field at 30 DAE and placed in paper bags. These were initially shade-dried and kept in an oven at 50^0C for complete drying. These samples were ground to a fine powder in a blender from which 0.5 g of leaf powder was taken in 25 ml methanol. It was mixed by swirling occasionally and the sample kept at room temperature for 24 h, and centrifuged for 20 min at 4500 rpm.

From the above extract, 1 ml aliquot was pipetted out into a test tube to which freshly prepared vanillin – hydrochloride reagent was added slowly. An individual blank was

prepared for each extract by adding 5 ml of vanillin – hydrochloride to 1 ml aliquot. These tubes were incubated in the water bath for 20 min. The absorbance was recorded at 500 nm against the reagent blank in a Spectrophotometer. Standard curve was prepared by plotting the average absorbance readings of the duplicate determinations of catechin concentrations. The catechin equivalents were calculated by using the formula.

$$\text{Catechin Equivalents (\%)} = \frac{\text{(mg catechin/ ml)}}{\text{Volume of extract taken}} \times \frac{\text{Volume made up}}{\text{Weight of sample}} \times 100$$

3.2.1.6. Organic acids

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) and Narayanamma* et al. *(2013).

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

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

3.2.1.6.1. 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 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 (Plate 6).

3.2.1.6.2. 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, 6.805 g of KH_2PO_4 was weighed and transferred in a 2 L conical flask and mixed with 1 L of millipore water until KH_2PO_4 was completely dissolved. Then added 4 ml of H_3PO_4 and the volume made up 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). The samples (20 µl) were chromatographed singly on a Waters Atlantis C_{18} column (4.6 × 250 mm) with 5-µm pore size (A Waters HPLC 2695 separations module (alliance) system consisting of a PCM 11 reciprocating piston pump and a 2996 photodiode array detector in the range of 210 to 400 nm was used in a isocratic solvent system (25 Mm KH_2PO_4)). 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 areas 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 the 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.

3.2.1.7. Flavanoids

For estimation of flavanoids, 100 mg of leaf sample was weighed and homogenized in 10 ml of 80% MeOH with mortar and pestle. The homogenized samples were centrifuged at 4000 rpm for 10 min and supernatant was collected. Later, 3 volumes of hexane were added to the supernatant volume for partition in separation funnel and methanol phase was collected. This process was repeated thrice and the methanol phase was collected, and concentrated to 2 ml in a roto-vapor and the concentrated sample was filtered through 0.22 µm membrane filter and injected into HPLC (Plate 6).

3.2.2 Molecular characterization of *Cry IIa* transgenic chickpea lines for transgene expression using enzyme linked immunosorbent assay (ELISA)

3.2.2.1. Materials

Cry2A ELISA test kit (EnviroLogic Inc., Portland, ME, USA) was used for the quantitative detection of Cry2A toxin in different plant parts of transgenic chickpea. Antibody coated microtiter plates, peroxidase enzyme conjugate, TMB substrate, PBST wash buffer, and 3 M sulfuric acid (stop solution) were required along with a blender, air tight container, paper towels, distilled water, micropipettes, and sterile micropipette tips.

3.2.2.2. Sample preparation

Twenty mg of leaf sample was weighed and homogenized using pestle in 0.5 ml extraction buffer, mixed well and incubated for 30 - 60 minutes at room temperature, allowed the particle to settle, and used the supernatant for ELISA test.

3.2.2.3. Procedure

One hundred µl of negative control and calibrators were added to the wells, followed by 100 µl of sample extract to each well. To the blank well, 100 µl extraction buffer was added. The plate was covered and incubated for 15 min at room temperature, then 100 µl enzyme conjugate was added in each well and the plate covered and incubated for 60 min at room temperature. The wells were aspirated and washed 4 times with 300 µl of washing solution, followed by addition of 100 µl substrate to each well. The plate was covered and incubated for 30 min at room temperature. Finally, the reaction was terminated by adding 100 µl stop solution, and measured the optical density of the test wells on a plate reader at 450 nm (Plate 7).

3.3. Effect of *cryIIa* transgenic chickpea lines on the natural enemies of *Helicoverpa armigera*

3.3.1. Rearing of larval parasitoid, *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae)

The cocoons of *Campoletis chlorideae* were collected from chickpea fields and kept individually in glass tubes (2 cm in diameter × 10 cm in long) and plugged with cotton wool, until adult emergence. Adult female wasps were easily distinguishable from the males by the presence of a prominent ovipositor on the posterior end of the abdomen. Twenty pairs of adults were released in a cage (10 cm diameter x 20 cm in length, and closed with plastic cap lid having 60 wire mesh, and a cotton swab with 10% sucrose solution). Immediately after mating, the females along with the males were transferred to another cage. Single mated 5 -

10 days old female wasp was transferred to a transparent plastic vial (15 ml capacity) kept in an inverted position on a petri dish. Single *H. armigera* larva (3-day old / late second or early third instar, nearly 1 cm in length) was offered to a female wasp for oviposition. The females which showed efficient parasitisation were selected for further studies on non target effects of transgenic chickpea lines towards the parasitoid, *C. chlorideae*. In general, *C. chlorideae* females took 1–2 min for parasitizing a larva. Using this technique, 80-100 larvae were parasitized using 3 - 4 females. The parasitized *H. armigera* larvae were removed and placed on chickpea based artificial diet in a transparent glass tube (2 cm diameter, 10 cm long) plugged with cotton until adult emergence. Newly emerged adult wasps (≤ 24 h) were kept in cages with other virgin wasps. Most of the females mated immediately after release into the cages, and were kept separately for use in experiments. The culture was maintained at 27 ± 2^0C, 65 – 75% RH, 12 h photoperiod (Plate 8).

3.3.1.1. Observations

Parasitized *H. armigera* larvae were checked every day and observations were recorded on larval mortality, cocoon formation, days to cocoon formation (egg+larval period), pupal period, adult emergence, adult weight, sex ratio, and fecundity of the *C. chlorideae* females from different treatments. For the fecundity test, three randomly selected *C. chlorideae* adult pairs obtained from each treatment (including control) were released inside a cage (30 × 30 × 30 cm), and allowed to mate for 3 days. The adults were provided with 10% honey solution in a cotton swab as a food source. After 3 days, each female was provided with *H. armigera* larvae up to their daily parasitization capacity. Parasitization of *H. armigera* larvae with these females continued till they died. Total number of *H. armigera* larvae parasitized by a female in its lifetime was recorded as fecundity/female.

3.3.1.2. Detection of *Bt* proteins in *Helicoverpa armigera* and *Campoletis chlorideae*

After feeding the *H. armigera* larvae on *Bt* proteins transgenic plants, 5-6 specimens of each of the host larvae, parasitoid larvae, cocoons, or freshly emerged adults were collected and crushed together to detect the *Bt* proteins in the insect body using a double sandwich enzyme-linked immunosorbent assay (ELISA) kit (EnviroLogic Inc., Portland, ME, USA). The *C. chlorideae* larvae were collected from the live *H. armigera* larvae.

The *H. armigera* larvae showing symptoms of parasitization were dissected, and the parasitoid larvae were collected in eppendorf tubes when they were ready to emerge from the host larvae for pupation. The host/ parasitoid samples (whole body) were crushed together as one sample in phosphate-buffered saline (PBS) in the ratio of 1:10 (insect sample : buffer) in

Eppendorf tubes in a plastic pestle. The test samples were then centrifuged at 11, 269 g for 2–3 min, and 100 µl of supernatant was loaded in the test wells of ELISA plate pre-loaded with 100 µl peroxydase enzyme conjugate. The negative and positive controls, and 0.5, 2.5, and 5.0 ppb *Bt* standards were run along with the test samples for the comparison of ELISA results. The ELISA plate was incubated for 2 h in a moist paper towel fitted in a plastic box. After 2 h of incubation, the test wells were thoroughly washed with PBS buffer giving 5–6 flip washings, and kept the test wells filled with PBS buffer for 1 min at the end. After washing, the test wells were again loaded with 100 µl TMB substrate. The wells showing a deep blue color indicated the presence of the toxin. After 15 min of incubation, 50 µl of 2M sulphuric acid was added, and observations were recorded on an ELISA plate reader at 450 nm.

3.3.1.3. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using GenStat, version 14.1. The treatment means were compared by least significant differences (LSD) at $P \leq 0.05$. The figures presented in the tables are means across replications with F-probability and LSD values.

3.3.2. Rearing of generalist predator, *Cheilomenes sexmaculatus* (L.) (Coleoptera: Coccinellidae)

3.3.2.1. Insect culture

Cultures of the aphid, *Aphis craccivora* (Koch); and the predatory coccinellid, *C. sexmaulatus* were maintained on cowpea, *Vigna unguiculata* (L.) Walp. plants in a nylon net-house under ambient conditions (Plate 10). The aphids and coccinellids were obtained from *Glaricidia maculata* (Kunth.) Walp. growing at the ICRISAT farm. The *C. sexmaulatus* eggs were obtained from the net house-reared coccinellids as and when needed**.** The coccinellid eggs were removed from the oviposition substrate (to avoid fungus development and resultant larval mortality), and transferred on to a carbon paper in a plastic cup. The neonate *C. sexmaulatus* larvae from these plastic cups were used in the experiments (Plate 9).

3.3.2.2. Feeding *C. sexmaulatus* larvae on sucrose solution

The neonate *C. sexmaulatus* larvae were fed on one of the following food sources: (i) Pure 2M sucrose solution, (ii) 2M sucrose solution containing *Cry1Ia* transgenic leaf powder (0.02%, 0.05% and 0.1%), (iii) water, and (iv) no food. The survival of *C. sexmaulatus* larvae was recorded daily to assess whether the predator larvae had actually fed on sucrose solution

or sucrose solution containing *Cry1Ia* transgenic leaf powder. The experiment was conducted twice with 15 replications each, thus forming a total of 30 replicates for each treatment in a CRD. Ingestion of *Cry1Ia* protein by the coccinellid grubs was confirmed by ELISA (EnviroLogic Inc., Portland, ME, USA). ELISA test was carried out to detect the *Cry1Ia* in 2M sucrose mixed *Cry1Ia* transgenic leaf powder (0.02%, 0.05% and 0.1%) as described earlier.

3.3.2.3. Direct effects of *CryIIa* transgenic chickpea lines on survival and development of *C. sexmaculatus*

CryIIa transgenic leaf powder was dissolved in a 2M sucrose solution at the concentrations of 0.02%, 0.05% and 0.1% to assess the direct effects. Neonate *C. sexmaculatus* larvae were fed on: (i) pure 2M sucrose solution (sucrose and aphids), (ii) 2M sucrose solution containing *CryIIa* (*CryIIa* aphids) or *CryIIa* (*CryIIa* aphids) at 0.1% on alternate days. The *C. sexmaculatus* larvae were provided ad libitum *A. craccivora* (mixed stages) after every 24 h of feeding on one of the above foods till pupation. One set of *C. sexmaculatus* larvae were fed on *A. craccivora* only. The neonate *C. sexmaculatus* larvae were kept individually in bioassay cups (3.3 cm in diameter, 3.5 cm in depth), and fed on above mentioned foods in the insectary at 26 ± 8^0C, 80-95% RH, and a 12-h photoperiod. The experiment was conducted twice with 15 replications each, thus, forming a total of 30 replicates for each treatment in a CRD. Observations were recorded on larval and pupal periods, larval survival, weights of male and female larvae, adult emergence, and weights of male and female adults of *C. sexmaculatus*.

3.3.2.4. Indirect effects of *CryIIa* transgenic chickpea lines on survival and development of *C. sexmaculatus*

Indirect effects of *CryIIa* transgenic chickpea lines on *C. sexmaculatus* were measured through *A. craccivora* fed on 0.02%, 0.05% and 0.1% concentrations of *CryIIa* transgenic chickpea leaf powder in the artificial diet (Febvey *et al.,* 1999). The aphids were reared on artificial diets amended with different (0.02%, 0.05% and 0.1%) amounts of *CryIIa* transgenic leaf powders in aphid feeding apparatus (Plate 10). One set of aphids was also reared on control artificial diet. The *C. sexmaculatus* larvae were provided ad libitum *A. craccivora* (mixed stages) reared on different concentrations of transgenic leaf powders, until pupation. The experiment was conducted twice with 30 replicates each, thus forming a total of 60 replicates for each treatment in a CRD. Observations were recorded on larval and pupal

periods, larval survival, weights of male and female larvae, adult emergence, and weights of male and female adults of *C. sexmaculatus*.

3.3.2.5. Preparation of artificial diet for aphids

Diet was prepared in 1,000 ml volumes by adding the correct amount of amino acids (Table 3.4), vitamins, and minerals to a flask filled to two thirds of the total desired volume with an 845 mM solution of sucrose. The pH of the solution was then adjusted to 7.5 with KOH, and the final volume made upto 1,000 ml. The diet was then filter-sterilized by passing it through a 0.45 m Millipore filter, divided into 20-ml aliquots, and stored at 20 ^{0}C for no longer than 3 months. Distilled de-ionized water was used in all solutions.

3.3.2.6. Statistical analysis

All the experiments were conducted twice. Longevities of *C. sexmaculatus* larvae on different foods were analysed using analysis of variance (ANOVA) in a completely randomised design (CRD). Larval, pupal periods, larval survival, weights of larvae and adults, and adult emergence of *C. sexmaculatus* on different concentrations CryIIa transgenic chickpea leaf powder were analysed using ANOVA in a CRD.

Table 3.1: Transgenic Chickpea lines evaluated for resistance to *H. armigera*.

Transgenic lines	
1	BS5A.1(T2) 18-1 P1 (Early)
2	BS5A.1(T2) 18-2 P1 (Early)
3	BS5A.2(T2) 19-1 P2 (Early)
4	BS5A.2(T2) 19-2 P1 (Early)
5	BS5A.2(T2) 19-3 P1 (Early)
6	BS5A.2(T2) 19-3 P2 (Medium)
Non transgenic lines	
7	ICC 506 (Resistant check)
8	Semsen (Control)

Table 3.2: Composition of artificial diet for rearing *H. armigera* larvae.

Ingredients	Quantity
Chickpea flour	75 g
L-ascorbic acid	1.175 g
Sorbic acid	0.75 g
Methyl-4-hydroxy benzoate	1.25 g
Aureomycin	2.875 g
Yeast	12 g
Formaldehyde (40%)	1.0 ml
Vitamin stock solution	2.5 ml
Water (W1)	112.5 ml
Agar-agar solution	
Agar-agar	4.325 g
Water (W2)	200 ml

Table 3.3: Composition of artificial diet used for rearing *H. armigera* larvae with lyophilized leaf/pod powder.

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

Table 4: Amino acid composition (in millimolar) in the artificial diet of aphids.

S.no	Amino acids	mmol l^{-1}
1	Alanine	5.4
2	Arginine	3.5
3	Asparagine	179.0
4	Aspartic acid	11.9
5	Cysteine	1.6
6	Glutamic acid	4.9
7	Glutamine	5.9
8	Glycine	1.7
9	Histidine	1.7
10	Isoleucine	4.0
11	Leucine	3.8
12	Lysine	3.8
13	Methionine	0.8
14	Phenylalanine	3.0
15	Proline	4.3
16	Serine	10.4
17	Threonine	6.8
18	Tryptophan	1.4
19	Tyrosine	1.1
20	Valine	5.9

Chapter IV

RESULTS AND DISCUSSION

The results of the present investigations on, **"Characterization of *Cry IIa* transgenic chickpea lines and their interaction with natural enemies of *Helicoverpa armigera* (Hubner)"** are presented hereunder. The experiments were conducted in the glasshouse and under laboratory conditions at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2011-14.

4.1 Phenotyping of *Cry IIa* transgenic chickpea lines for resistance to pod borer, *H. armigera*

4.1.1. Response of transgenic chickpea lines against *H. armigera* under laboratory conditions

In the first planting during October 2011-12, the transgenic plants suffered significantly lower leaf damage rating (DR: 1.3 to 3.2) compared to the non-transgenics, Semsen (DR: 7.8) and ICC 506EB (DR: 5.3). Among the transgenic plants tested, BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P2 suffered greater leaf damage than the other lines (DR: 3.2 and 2.7, respectively) (Plate 11). The larval survival was significantly lower on transgenic plants (30.5 - 46.1%) compared to that on Semsen (83.8%) and the resistant check, ICC 506EB (74.1%). The weight gained by *H. armigera* larvae after 5 days was lower on transgenic lines BS5A.1(T2) 18-1P1 (0.6 mg larva^{-1}), BS5A.2(T2) 19-2P1 (0.8 mg larva^{-1}), BS5A.2(T2) 19-1 P2 (0.8 mg larva^{-1}), BS5A.2(T2) 19-3P1 (1.1 mg), BS5A.1(T2) 18-2P1 (1.2 mg larva^{-1}) and BS5A.2(T2 larva^{-1}) 19-3P2 (1.4 mg larva^{-1}) than on non-transgenic lines, Semsen (5.4 mg larva^{-1}) and ICC 506EB (3.8 mg larva^{-1}) (Table 4.1, Fig 1).

The leaf damage rating during October 2012-13 was greater on Semsen (DR: 4.6) and ICC 506EB (DR: 3.9) than on transgenic lines (DR: 1.0 to 1.6). The larval survival was significantly greater on non-transgenic lines, Semsen and ICC 506EB (73.8 and 77.7%, respectively) than on the transgenics. Significantly lower larval weight of *H. armigera* were recorded on BS5A2(T2) 19-2P1 (0.1 mg larva^{-1}) as compared to that on non-transgenics, Semsen (3.0 mg larva^{-1}) and the resistant check, ICC 506EB (2.4 mg larva^{-1}). The larval weight on the transgenic lines ranged between 0.3 to 0.6 mg larva^{-1} (Table 4.1, Fig 2).

Based on analysis of pooled data for 2011-12 and 2012-13 October sowings, significantly lower leaf damage was recorded on the transgenic lines (DR 1.3-2.3) as compared to that on the non-transgenic lines [Semsen (DR: 6.2) and ICC 506EB (DR: 4.6)].

The larval survival on Semsen and ICC 506EB was significantly greater (78.8% and 75.9%, respectively) as compared to that on the transgenic lines (25.2% - 38.0%) (Table 4.1).

The transgenic line BS5A.2(T2) 19-2P1 exhibited high levels of resistance to *H. armigera* (DR: 1.0), larval survival (10.5%) and mean larval weight (0.1 mg larva^{-1}) during the October planting 2012-13. Among the transgenic lines, BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-3P2 recorded significantly greater leaf damage (DR: 3.2 and 2.7) and the *H. armigera* larvae gained significantly more larval weight (1.2 and 1.43 mg larva^{-1}) during 2011-12 October planting (Table 4.1).

Similar observations have earlier been made by Law*o et al*. (2008), who reported that leaf damage by *H. armigera* was significantly higher on the non-transgenic controls than on the *Bt* chickpea leaves. Kar *et al.* (1997) reported that larvae fed on transgenic chickpea plants attained significantly lower body weight as compared to larvae fed on non-transgenic plants. Transformed chickpea plants have shown high mortality (> 80.0%) of *H. armigera* larvae (Sanyal *et al*. 2005).

BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 recorded significantly lower leaf damage rating (DR: 1.0) as compared to non-transgenic chickpea plants, Semsen (DR: 7.2) and ICC 506EB (DR: 3.3) during November 2011-12 planting. The leaf damage in the transgenic lines was significantly lower (DR: 1.2-1.6) than on the non-transgenics. The larval survival was significantly lower on BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 (21.6 and 24.4%, respectively) as compared to that on Semsen and ICC 506EB (75.0 and 72.7%, respectively). The larval survival was 33.3% on BS5A.1(T2) 18-2P1, 38.8% on BS5A.1(T2) 18-1P1, 39.3% on BS5A.2(T2) 19-3P2 and 48.3% on BS5A.2(T2) 19-3P1. The weight gained by the *H. armiger*a larvae after feeding on transgenic plants BS5A.2(T2) 19-1P2 (0.3 mg larva^{-1}) and BS5A.2(T2) 19-2P1 (0.3 mg larva^{-1}) was significantly lower than that on the non-transgenic plants ICC 506EB (4.4 mg larva^{-1}) and Semsen (3.7 mg larva^{-1}) (Table 4.2, Fig 3).

Leaf damage rating during November 2012-13 planting, was higher on Semsen (DR: 7.5), and ICC 506EB (DR: 4.3) than on the transgenic lines (DR: 1.2 to 2.3). The larval survival on non-transgenic lines ICC 506EB (62.2%) and Semsen (50.5%) was significantly higher than on the transgenic lines (10.0-30.0%). Significantly lower weight of *H. armigera* larve was recorded on BS5A2(T2) 19-1P2 (1.0 mg larva^{-1}) and BS5A2(T2) 19-2P1 (1.0 mg larva^{-1}) as compared to that on the resistant check, ICC 506EB (3.0 mg larva^{-1}) and Semsen (2.8 mg larva^{-1}). The weight gained by the *H. armigera* larvae on other transgenic lines ranged between 1.1 to 1.3 mg larva^{-1} (Table 4.2, Fig 4).

Similar trend in leaf damage, larval survival and larval weight was observed with pooled analysis of data during November 2011-12 and 2012-13 plantings. Transgenic lines BS5A.2(T2) 19-1P2 (DR: 1.0) and BS5A.2(T2) 19-2P1 (DR: 1.0) recorded significantly lower leaf damage as compared to that on Semsen (DR: 5.0) and ICC 506EB (DR: 3.1). Larval survival was significantly higher on ICC 506EB (67.5%) and Semsen (62.7%) as compared to BS5A.2(T2) 19-1P2 (15.8%) and BS5A.2(T2) 19-2P1 (18.6%). The larval survival on other transgenic lines ranged between 26.3-39.1%. The weight gain by *H. armigera* larvae fed on ICC 506EB (3.5 mg larva^{-1}) and Semsen (2.9 mg larva^{-1}) was significantly greater than the larvae fed on transgenic line BS5A.2(T2) 19-2P1 (0.7 mg larva^{-1}). The mean larval weight on other transgenic lines ranged between 0.7-1.0 mg larva^{-1} (Table 4.2).

Across the seasons (2011-12 and 2012-13), the transgenic chickpea lines BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 showed high levels of resistance to *H. armigera.* The weight gain by the *H. armigera* larvae was significantly greater on ICC 506EB (4.4 mg larva^{-1}) than on the transgenic lines. During 2012-13 November planting, leaf damage rating was significantly greater on Semsen (DR: 7.5) with larval survival of 50.5%, and mean larval weight of 2.8 mg larva^{-1}. ICC 506EB recorded low leaf damage (DR: 4.3), larval survival 62.2% and larval weight 3.0 mg larva^{-1} (Table 4.2).

The present results confirmed the observations made by Acharjee *et al.* (2010), who reported significantly greater larval mortality of the *H. armigera* larvae fed on transgenic leaves (BS2A, BS5A and BS6H) than the larvae fed on control (Semsen and ICCV89314). Mogali *et al.* (2012) reported significantly lower leaf damage on *Bt* cotton leaves due to feeding by *H. armigera* as compared to the wild type. There was a significant increase in final body weight of the larvae fed on –ve control (111.5%) as compared to the larvae fed on transgenic plants (56.3%).

4.1.2 Response of transgenic chickpea lines to damage by *H. armigera* under glasshouse conditions

During 2011-2012 December sowing, leaf damage was significantly greater on ICC 506 EB (DR: 8.0) and Semsen (DR: 7.8) as compared to that on BS5A.2(T2) 19-2P1 (DR: 1.6). Among the transgenic lines tested, BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P2 suffered greater leaf damage (DR: 4.1, 4.4 and 4.3, respectively) than other lines tested (Plate 12). Larval survival was significantly greater on Semsen (75.7%) and ICC 506EB (72.3%) as compared to that on the transgenic plants of BS5A.2(T2) 19-3P1 (35.0%). Among the transgenic chickpea lines tested, significantly greater larval survival was

recorded on BS5A.1(T2) 18-1P1 (52.3%) than on BS5A.2(T2) 19-3P1. The weight gain by the larvae (3.4 mg larva^{-1}) on *Bt* transgenic plants was significantly lower as compared to that on Semsen (12.7 mg larva^{-1}) and ICC 506EB (11.2 mg larva^{-1}). The weight gain by *H. armigera* larvae on other transgenic lines ranged from 5.1 to 8.7 mg larva^{-1}, with significantly greater weight gain on BS5A.2(T2) 19-3P2 (8.7 mg larva^{-1}) (Table 4.3, Fig 5).

The transgenic line BS5A.1(T2) 18-1P1 recorded significantly lower leaf damage rating (DR: 2.2), followed by BS5A.1(T2) 18-2P1 (DR: 2.5), BS5A.2(T2) 19-1P2 (DR: 3.2), BS5A.2(T2) 19-2P1 (DR: 3.7), BS5A.2(T2) 19-3P1 (DR: 3.7) and BS5A.2(T2) 19-3P2 (DR: 4.3) as compared to Semsen (DR: 7.7) and ICC 506EB (DR: 5.5) during 2012-13. The Larval survival on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 was significantly lower (37.6%) as compared to that on ICC 506EB (79.3%) and Semsen (70.2%). Larval survival on other transgenic lines ranged from 40.0 to 48.1%. Weight gain by the *H. armigera* larvae was significantly lower on BS5A.1(T2) 18-1P1 (2.9 mg larva^{-1}) as compared to ICC 506EB (17.0 mg larva^{-1}) and Semsen (13.6 mg larva^{-1}) (Table 4.3 and Fig 6).

Pooled analysis indicated that the transgenic line BS5A.1(T2) 18-1P1 suffered significantly lower leaf damage (DR: 2.4) as compared to Semsen (DR: 7.8) and ICC 506EB (DR: 6.7). The larval survival was significantly greater on ICC 506EB (75.8%) and Semsen (72.9%) than on the transgenic chickpea lines tested. The weight gain by the *H. armigera* larvae was significantly lower on BS5A.1(T2) 18-1P1 (3.4 mg larva^{-1}) as compared to that on ICC 506EB (14.1 mg larva^{-1}) and Semsen (13.1 mg larva^{-1}) (Table 4.3).

Similar observations on lower consumption of *Bt* cotton leaves by *H. armigera* larvae and higher mortality in choice tests has been reported by Zhang *et al*. (2004). Cotton bollworms fed on *Bt* cotton grew slower than those fed on non-*Bt* cotton, and also recorded less damage on transgenic *Bt* cotton plants (Shudong *et al*. 2003). The larval population was significantly lower on the transgenic hybrids as compared to the non-transgenic commercial cultivars of cotton (Sharma and Pampathy, 2006).

4.1.3 Grain yield of transgenic chickpea lines to damage by *H. armigera*

During December 2011-12 planting, there were significant differences in dry matter, pod weight, seed weight and the seed set between the transgenic and non-transgenic chickpea lines when infested with *H. armigera* larvae for 10 days. The weight of dry matter (5.0 to 6.5 g/3 plants) was significantly greater in BS5A.2(T2) 19-1P2 (6.5 g/3 plants) than Semsen (3.3 g/3 plants) and ICC 506EB (3.5 g/3 plants) The pod weight was also significantly greater in BS5A.2 (T2) 19-2P1 (2.6 g/3 plants), followed by BS5A.2 (T2) 19-3P1 (2.3 g/3 plants), BS5A.2 (T2) 19-3P2 (1.8 g/3 plants), BS5A.1 (T2) 18-1P1 (1.7 g/3 plants), BS5A.2 (T2) 19-

1P2 (1.6 g/3 plants) and ICC506 EB (1.3 g/3 plants) than Semsen (0.6 g/3 plants) (Table 4.4 and Fig 7).

Higher seed weight was recorded on BS5A.2(T2) 19-3P1 (2.1 g/3 plants) and BS5A.2(T2) 19-2P1 (2.0 g/3 plants) compared to Semsen (0.5 g/3 plants) and ICC 506EB (0.9 g/3 plants). The seed set in transgenic plants was higher than on non-transgenic plants. The number of seeds formed in BS5A.1(T2) 18-1P1(16) and BS5A.1(T2) 18-2P1 (14) were significantly more as compared to that on Semsen (2) and ICC 506EB (7) (Table 4.4).

During December 2012-13 planting, significantly higher dry matter weight was recorded in BS5A.2(T2) 19-2P1 (6.8 g/3 plants), and BS5A.1(T2) 18-2P1 (6.7 g/3 plants), BS5A.2(T2) 19-3P1 (6.7 g/3 plants), BS5A.2(T2) 19-3P2 (6.5 g/3 plants), BS5A.1(T2) 18-1P1 (6.2 g/3 plants) and BS5A.2(T2) 19-1P2 (5.2 g/3 plants) than in non-transgenic Semsen (3.6 g/3 plants) and ICC 506EB (4.0 g/3 plants). The pod weight was significantly higher in BS5A.2(T2) 19-2P1 (4.1 g/3 plants) as compared to that on ICC 506EB (1.2 g/3 plants) and Semsen (1.3 g/3 plants). The seed weight was significantly higher in BS5A.2(T2) 19-2P1 (3.5 g/3 plants) as compared to Semsen (0.9 g/3 plants) and ICC 506EB (1.0 g/3 plants). Similarly, number of seeds formed in BS5A.2(T2) 19-2P1 (26) were more compared to Semsen (3) and ICC 506EB (6) (Table 4.4 and Fig 8).

Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera.* Since leaf feeding damage was less in transgenic chickpea plants, the dry matter weight, pod weight, seed weight and number of seeds formed were significantly more than on non-transgenic chickpea plants. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas. During 2012-13 planting, BS5A.2(T2) 19-2P1 had the highest dry matter weight (6.8 g/3 plants), pod weight (4.1 g/3 plants), seed weight (3.5 g/3 plants) and number of seeds formed (26) as compared to the other transgenic and non-transgenic chickpea lines.

Based on pooled data, the grain yield was significantly greater in transgenic chickpea lines as compared to non-transgenic lines. The dry matter weight was significantly higher in BS5A.2(T2) 19-3P1 (6.5 g/3 plants), BS5A.1(T2) 18-2P1 (6.4 g/3 plants), BS5A.1(T2) 18-1P1 (6.0 g/3 plants), BS5A.2(T2) 19-1P2 (5.9 g/3 plants) and BS5A.2(T2) 19-1P2 (5.9 g/3 plants) and BS5A.2(T2) 19-3P2 (5.8 g/3 plants) than in Semsen (3.4 g/3 plants) and ICC 506EB (3.7 g/3 plants). The weight of the pods in transgenic chickpea lines ranged from 3.3 to 1.6 g/3 plants as compared to 1.0 g/3 plants in Semsen and 1.3 g/3 plants ICC 506EB. Seed weight was significantly greater in BS5A.2(T2) 19-2P1 (2.7 g/3 plants) compared to Semsen (0.7 g/3 plants) and ICC 506EB (1.0 g/3 plants). Number of seeds formed was highest in

BS5A.1(T2) 18-1P1 (18), followed by 17 in BS5A.2(T2) 19-2P1 and BS5A.2(T2) 19-1P2, 15 IN BS5A.2(T2) 18-2P1, 14 BS5A.2(T2) 19-3P1 and 11 BS5A.2(T2) 19-3P2 (Table 4.4).

4.1.4 Grain yield of transgenic and non-transgenic chickpea lines under un-infested conditions

In un-infested plants of transgenic and non-transgenic chickpeas during December 2011-12 planting, the dry matter weight was significantly higher in Semsen (9.3 g/3 plants) as compared to BS5A.1(T2) 18-2P1 (4.2 g/3 plants) and the dry matter weight in transgenic chickpeas ranged from 4.2 to 6.4 g/3 plants. The pod weight was significantly greater in BS5A.2(T2) 19-2P1 (3.3 g/3 plants), BS5A.2(T2) 19-1P2 (3.3 g/3 plants), BS5A.2(T2) 19-3P1 (3.0 g/3 plants), BS5A.1(T2) 18-2P1 (2.7 g/3 plants), BS5A.1(T2) 18-1P1 (2.6 g/3 plants), ICC 506EB (2.4 g/3 plants) and BS5A.2(T2) 19-3P2 (2.2 g/3 plants) as compared to Semsen (0.1 g/3) (Table 4.5 and Fig 9).

The seed weight was highest in BS5A.2(T2) 19-2P1 (2.6 g/3 plants) and lowest in Semsen (0.9 g/3 plants). In other transgenic plants, the seed weight ranged between 2.2-2.4 g/3 plants. The number of seeds formed (3 plants^{-1}) in BS5A.2(T2) 19-2P1 was highest (43), while the lowest was in Semsen (2). In other transgenic and non-transgenic plants, the seeds formed ranged from 16 to 38 (Table 4.5).

During December 2012-13 planting, similar trend was observed in dry matter weight, which was significantly higher in Semsen (8.5 g/3 plants) than in BS5A.1(T2) 18-2P1 (4.2 g/3 plants). In other transgenic plants, the dry matter weight ranged from 4.2 to 6.1 g/3 plants. Pod weight of the was significantly higher in BS5A.2(T2) 19-2P1 (5.2 g/3 plants) as compared to Semsen (3.5 g/3 plants) and ICC 506EB (3.7 g/3 plants), while in other transgenic plants, the pod weight ranged from 2.9 to 5.2 g/3 plants. Among transgenics plants, the seed weight was highest in BS5A.2(T2) 19-2P1 (5.3 g/3 plants) and lowest in BS5A.1(T2) 18-1P1 (2.3 g/3 plants). Whereas in non-transgenics, the seed weight was 6.0 g/3 plants in Semsen and 3.6 g/3 plants in ICC 506EB. Maximum number of seeds were formed in BS5A.2(T2) 19-2P1 (64), followed by BS5A.2(T2) 19-1P2 (53), BS5A.2(T2) 19-3P2 (53), BS5A.1(T2) 18-2P1 (47), ICC 506EB (44), and BS5A.1(T2) 18-1P1 (38). Minimum of 6 seeds were formed in Semsen (Table 4.5 and Fig 10).

Based on pooled data analysis, the dry matter weight was highest in Semsen (8.9 g/3 plants), but the pod weight (0.2 g/3 plants), seed weight (0.3 g/3 plants) and number of seeds formed (4) were significantly lower as compared to the other transgenic and non-transgenic chickpea lines tested. Among all the transgenic plants tested, BS5A.2(T2) 19-2 P1 had the

highest pod weight (4.2 g/3 plants), seed weight (3.9 g/3 plants) and the number of seeds formed (53) (Table 4.5).

4.1.5 Survival and development of the neonate larvae of *H. armigera* on artificial diet with lyophilized leaf powder of transgenic chickpea lines

Significant differences were observed in survival and development of the pod borer, *H. armigera* larvae reared on artificial diets with lyophilized leaf powders of transgenic and non-transgenic chickpea lines during 2011-2012. There were significant differences in larval survival between the *H. armigera* larvae reared on diets with leaf powders of transgenic and non-transgenic chickpeas. The larval survival was 10.0% on BS5A.2(T2) 19-3P2 and 12.0% on BS5A.2(T2) 19-2P1, which was significantly lower than that on Semsen (74.0%) and ICC 506EB (61.0%) (Table 4.6).

There were significant differences in larval and pupal weights between the larvae reared on diets with leaf powders of transgenic and non-transgenic lines. Larval weight at 5 DAI (days after initiation of the experiment) was lower on BS5A.2(T2) 19-2P1 (0.8 mg larva^{-1}), BS5A.2(T2) 19-3P2 (0.8 mg larva^{-1}), BS5A.1(T2) 18-2P1 (1.3 mg larva^{-1}), BS5A.1(T2) 18-1P1 (2.1 mg larva^{-1}), BS5A.2(T2) 19-3P1 (2.7 mg larva^{-1}) and BS5A.2(T2) 19-1P2 (4.5 mg larva^{-1}) as compared to that on non-transgenic Semsen (31.9 mg larva^{-1}) and ICC 506EB (28.8 mg larva^{-1}) (Table 4.6).

At 10 DAI, the weight of *H. armigera* larvae reared on diet with transgenic chickpea leaf powder ranged from 3.3 to 101.4 mg larva^{-1} as compared to 438.0 mg larva^{-1} on Semsen and 347.9 mg larva^{-1} ICC 506EB. Lower pupal weight was recorded in larvae reared on diets with leaf powder of transgenic line, BS5A.2(T2) 19-3P2 (20.5 mg pupa^{-1}), BS5A.1(T2) 18-2P1 (45.0 mg pupa^{-1}), BS5A.2(T2) 19-3P1 (45.2 mg pupa^{-1}), BS5A.2(T2) 19-2P1 (47.8 mg pupa^{-1}), BS5A.2(T2) 19-1P2 (63.8 mg pupa^{-1}) and BS5A.1(T2) 18-1P1 (65.6 mg pupa^{-1}) as compared to the larvae reared on Semsen (526.2 mg pupa^{-1}) and ICC 506 EB (523.8 mg pupa^{-1}) (Table 4.6).

Longer larval period of 23.5 days was observed in larvae reared on BS5A.1(T2) 18-1P1, as compared to ICC 506EB and Semsen (15.5 and 16.5 days, respectively). Longest pupal period was recorded in larvae reared on BS5A.1(T2) 18-2P1 (13.0 days) whereas the shortest pupal period was recorded on ICC 506 EB (8.5 days) (Table 4.6) .

There were significant differences in pupation between the larvae reared on diets with leaf powder of transgenic and non-transgenic lines. Pupation was significantly lower on BS5A.2(T2) 19-3P2 (5.0%) as compared to that on Semsen (34.0%) and ICC 506EB (31.0%). There was no adult emergence on BS5A.1(T2) 18-2P, but 1.0 - 3.0% adult

emergence was recorded on BS5A.2(T2) 19-3P1, BS5A.1(T2) 19-1P2, BS5A.2(T2) 19-3P2, BS5A.1(T2) 18-1P1, BS5A.2(T2) 19-2P1 compared to 19.0% on ICC 506EB and 12.0% Semsen (Table 4.6).

Adult longevity was lowest in larvae reared on diets with leaf powder of BS5A.2(T2) 19-2P1 (0.5 days), followed by those reared on BS5A.1(T2) 18-1P1 (2 days). Longer adult survival was recorded in insects reared on diets with leaf powder of ICC 506EB (8.0 days) and Semsen (6.0 days) than the insects reared on diets with leaf powder of transgenic chickpeas. Female longevity was significantly reduced in insects reared on diets with leaf powder of BS5A.2(T2) 19-3P1 (0.5 days), BS5A.2(T2) 19-1P2 (1.5 days), BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P2 (2.0 days) and BS5A.1(T2) 18-1P1 (4.5 days). Longer female survival was recorded in insects reared on diets with leaf powder of ICC 506EB (5.0 days) and Semsen (6.0 days) than on transgenic chickpeas. There were significant differences in fecundity between the larvae reared on diets with transgenic and non-transgenic chickpea leaf powders. No eggs were laid by the insects reared on diets with leaf powder of transgenic lines. Highest fecundity was recorded in insects reared on diets with leaf powder of Semsen (200.0 eggs female^{-1}), followed by ICC 506 EB (95.0 eggs female^{-1}). The survival and development of *H. armigera* was significantly better in insects reared on standard artificial diet as compared to those reared on diets with lyophilised leaf powders of transgenic or non transgenic chickpeas (Table 4.6 and Fig 11).

During 2012-13, there were significant differences in survival and development of neonate larvae of *H. armigera* larvae reared on artificial diets with lyophilized leaf powder of transgenic and non-transgenic chickpea lines. Lower larval survival was recorded in larvae reared diets with leaf powder of BS5A.2(T2) 19-2P1 (7.0%) as compared to the insects reared on diets with leaf powder of ICC 506EB (45.0%) and Semsen (39.0%). Larval survival among the transgenics ranged between 7.0 to 25.0%. The larval weights at 5 DAI were significantly lower (0.6 mg larva^{-1}) in the larvae reared on the diets containing leaf powder of BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P1 (9.5 mg larva^{-1}), BS5A.2(T2) 19-1P2 (14.6 mg larva^{-1}), BS5A.1(T2) 18-1P1 (19.7 mg larva^{-1}), BS5A.2(T2) 19-3P2 (25.4 mg larva^{-1}) and BS5A.1(T2) 18-2P1 (27.5 mg larva^{-1}) as compared to insects reared on diets with leaf powder of Semsen (162.5 mg larva^{-1}) and ICC 506EB (231.1 mg larva^{-1}). The mean larval weight at 10 DAI was significantly lower in larvae reared on diets with leaf powder of BS5A.2(T2) 19-2P1 (14.0 mg larva^{-1}) as compared to insects reared on diets with leaf powder of ICC 506EB (1366.1 mg larva^{-1}) and Semsen (1189.6 mg larva^{-1}) (Table 4.7).

There were significant differences in the pupal weights of *H. armigera* reared on diets with transgenic and non-transgenic leaf powders. The pupal weights were significantly lower in larvae reared on diets with leaf powder of BS5A.2(T2) 19-2P1 (7.0 mg pupa^{-1}) as compared to non-transgenics, ICC 506EB and Semsen (466.0 and 423.5 mg pupa^{-1}, respectively). The larval period was 15.0 days in insects reared on diets with transgenic leaf powder as compared to 16.5 days on Semsen and 12.5 days on ICC 506EB. Longer pupal period was recorded in the larvae reared on BS5A.2(T2) 19-1P2 (17.0 days) as compared to non-transgenics, Semsen and ICC 506EB (8.5 days and 9.5 days, respectively) (Table 4.7).

Pupation was reduced in insects reared on diets with leaf powder of BS5A.2(T2) 19-2P1 (2.0%) compared to ICC 506EB (29.0%) and Semsen (26.0%). Similarly, adult emergence was also reduced in insects reared on diets with BS5A.2(T2) 19-2 P1 leaf powder (1.0%) as compared to the insects reared on diets with leaf powder of ICC 506EB (20.0%) and Semsen (17.0%). Male longevity was significantly greater in insects reared on diets with leaf powder of ICC 506EB (9.5 days) as compared to 0.5 days on BS5A.2(T2) 19-1P2. Shorter female longevity was recorded on BS5A.2(T2) 19-1P2 (3.0 days) and BS5A.2(T2) 19-3P2 (3.0 days) as compared larvae reared on ICC 506EB (10.5 days). Significantly lower fecundity was recorded in insects reared on diets with leaf powder of BS5A.2(T2) 19-1P2 (32.5 eggs female^{-1}) as compared to that on ICC 506EB (332.5 eggs female^{-1}) and Semsen (187.5 eggs female^{-1}). The survival and development of *H. armigera* was better in insects reared on the standard artificial diet compared to those reared on diets with lyophilised leaf powders of transgenic and non transgenic chickpeas (Table 4.7 and Fig 12).

Based on the pooled data analysis, there were significant differences in the survival and development of *H. armigera* reared on artificial diets with lyophilized leaf powders of transgenic and non-transgenic chickpeas. Larval survival was significantly lower (9.5%) in insects reared on diets with leaf powder of BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P2 (13.5%), BS5A.2(T2) 19-3P1 (17.0%), BS5A.2(T2) 19-1P2 (21.5%) BS5A.2(T2) 18-1P1 (22.0%) and BS5A.2(T2) 18-2P1 (22.0%) than on Semsen (56.5%) and ICC 506EB (53.0%). The mean larval weight at 5 DAI was significantly lower in insects reared on diets with leaf powder of BS5A.2(T2) 19-2P1 (0.73 mg larva^{-1}) as compared to 129.9 mg larva^{-1} in ICC 506EB and 97.2 mg larva^{-1} in Semsen. Similarly, mean larval weight at 10 DAI was lower in insects reared on diets with BS5A.2(T2) 19-2P1 leaf powder (8.6 mg larva^{-1}) as compared to the insects reared on diet with ICC 506EB leaf powder (857.0 mg larva^{-1}) (Table 4.8).

Pupal weights were lower in insects reared on diets with BS5A.2(T2) 19-2P1 leaf powder (27.4 mg pupa^{-1}) as compared to that on ICC 506EB (494.9 mg pupa^{-1}). Longest

larval period was recorded in BS5A.2(T2) 18-1P1 (26.2 days) and the shortest on ICC 506EB (14.0 days). The pupal period was prolonged by 5 days in larvae reared on diets with BS5A.2(T2) 19-1P2 leaf powder (14.7 days) as compared to those with ICC 506EB leaf powder (9.0 days). Pupation was greater (30.0%) in insects reared on diets with Semsen and ICC 506EB leaf powder compared to that on BS5A.2(T2) 19-2P1 (5.5%). The adult emergence was lower on BS5A.2(T2) 19-2P1 (2.0%) than on ICC 506EB (19.5%) (Table 4.8).

Longevity of adult males was higher on ICC 506EB (8.7 days) than on BS5A.2(T2) 19-2P1 (0.2 days) and BS5A.2(T2) 19-1P2 (0.5 days). Shortest female longevity was recorded in insects reared on diets with BS5A.2(T2) 19-3P1 (0.2 days) and BS5A.2(T2) 19-2P1 (1.0 days) leaf powder, and longest on Semsen (7.0 days) and ICC 506EB (7.7 days). There were significant differences in fecundity between the insects reared on diet with transgenic and non-transgenic chickpea leaf powder. No eggs were laid by the insects reared on diets with BS5A.1(T2) 18-1P1, BS5A.2(T2) 19-2P1 and BS5A.2 (T2) 19-3P1 leaf powder. Lower fecundity was recorded in insects reared on BS5A.2(T2) 19-1P2 (16.2 eggs female^{-1}) as compared to that on ICC 506EB (213.7 egg female^{-1}) and Semsen (194.1 eggs female^{-1}). The survival and development of, *H. armigera* was better in insects reared on the standard artificial diet compared to those reared on diets with lyophilized leaf powders of transgenic and non-transgenic chickpeas (Table 4.8 and Fig 13).

During 2012-13, the larvae fed on diet with BS5A.2(T2) 19-2P1 leaf powder exhibited lowest larval survival, larval weights at 5 and 10 DAI and pupal weights as compared to insects reared on diets with leaf powder of non-transgenic plants. Insects reared on diet with BS5A.2(T2) 19-2P1 leaf powder showed maximum resistance to *H. armigera*.

4.1.6 Survival and development of third-instar larvae of *H. armigera* on artificial diet containing lyophilized leaf powder of transgenic chickpea

There were significant differences in survival and development of third-instar larvae of *H. armigera* reared on artificial diets with lyophilized leaf powders of transgenic and non-transgenic chickpea. During 2011-2012, the larval survival was significantly higher in larvae reared on diets with leaf powders of non-transgenic chickpea, ICC 506EB (85.0%) as compared to those reared on diet with leaf powder of transgenic lines BS5A.1(T2) 18-2P1 (27.0%), BS5A.2(T2) 19-2P1 (33.0%), BS5A.1(T2) 18-1P1 (42.0%), BS5A.2(T2) 19-3P2 (48.0%), BS5A.2(T2) 19-3P1 (52.0%) and BS5A.1(T2) 19-1P2 (60.0%). Similarly, the mean larval weights at 5 and 10 DAI were significantly lower in larvae reared on diets with leaf powder of BS5A.1(T2) 18-2P1 (8.1 and 277.9 mg larva^{-1}) than on ICC 506EB (112.9 and

1696.1 mg larva^{-1}, respectively) and Semsen (63.56 and 1217.1 mg larva^{-1}, respectively). The pupal weights of the larvae fed on diets with leaf powder of ICC 506EB and Semsen were significantly greater (1365.9 and 703.2 mg pupa^{-1}, respectively) than the larvae reared on diets with leaf powder of BS5A.1(T2) 18-2P1 (123.1 mg pupa^{-1}) (Table 4.9).

The larval period was prolonged by 4 days in insects reared on diets with BS5A.2 (T2) 19-1P2 leaf powder as compared to that on Semsen (14.5 days) and ICC 506EB (14.5 days). The pupal period was longer in insects reared on diet with leaf powder of BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-2P1 and BS5A.2(T2) 19-3P1 (15.0 days) than on ICC 506EB (11.0 days) and Semsen (11.5 days). Pupation was significantly lower (17.0 to 49.0%) in insects reared on diets with leaf powder of transgenic lines as against 77.0 and 51.0% pupation on non-transgenic chickpea ICC 506EB and Semsen, respectively (Table 4.9).

Adult emergence was significantly lower on BS5A.1(T2) 18-2P1, (13.0%) as compared to that on ICC 506EB (72.0%) and Semsen (48.0%). Male longevity was shorter on BS5A.1(T2) 18-2P1 (5.5 days) than on ICC 506EB (15.1 days) and Semsen (13.1 days). There were no significant differences in female longevity between the larvae reared on transgenic and non-transgenic diets. Female longevity was lower on BS5A.2(T2) 19-2P1 (5.5 days) as compared to that on BS5A.1(T2) 18-2P1 (8.5 days). Fecundity of the females reared on diets with leaf powder of transgenic chickpea ranged between 572.0 to 804.5 egg female^{-1}, highest fecundity was recorded in insects reared on diets with leaf powder of ICC 506EB and Semsen (1076.0 and 848.5 eggs female^{-1}, respectively). The survival and development of *H. armigera* was significantly better when reared on standard artificial diet as compared to those reared on diets with lyophilised leaf powders of transgenic and non-transgenic chickpeas The survival and development of third-instar *H. armigera* larvae was significantly lower on BS5A.1(T2) 18-2P1 than on other transgenic chickpeas. Maximum survival was recorded in larvae reared on diets with leaf powder of ICC 506EB and Semsen. (Table 4.9 and Fig 14).

During 2012-13, similar trend was observed for survival and development of third instar larvae of *H. armigera* reared on diets with leaf powder of transgenic and non-transgenic chickpeas. Lower larval survival was recorded in insects reared on diets with leaf powder of BS5A.1(T2) 18-1P1 (29.0%) as compared to that on ICC 506EB and Semsen (84.0 and 82.0%, respectively). Mean larval weights were significantly greater in larvae reared on diets with leaf powder of ICC 506EB and Semsen at 5 and 10 DAI (51.3 and 1305.6; 45.5 and 1348.8 mg larva^{-1}, respectively) as compared to the larvae fed on diets with leaf powder of transgenic chickpea line BS5A.1(T2) 18-1P1 (9.1 and 159.8 mg larva^{-1}, respectively) (Table 4.10).

Significantly lower pupal weight was recorded in *H. armigera* larvae reared on diets BS5A.1(T2) 18-2P1(98.5 mg pupa^{-1}) as compared to that of ICC 506EB (974.0 mg pupa^{-1}) and Semsen (952.6 mg pupa^{-1}). Maximum larval period was recorded in insects reared on BS5A.1(T2) 18-1P1 (16.5 days) and minimum on BS5A.2(T2) 19-3P2 (12.5 days). The larval period in insects reared on non-transgenic lines (ICC 506EB and Semsen) was recorded as 14.5 and 13.5 days, respectively. The pupal period was significantly longer (15.0 days) on BS5A.2(T2) 19-2P1 as compared to that on ICC 506EB and Semsen (11.0 and 11.5 days, respectively) (Table 4.10).

Pupation and adult emergence were significantly reduced in larvae reared on BS5A.1(T2) 18-1P1 (15.0 and 9.0%, respectively) as compared to those reared on ICC 506EB (77.0 and 65.0%, respectively). The female longevity was significantly higher in insects reared on diets with leaf powder of ICC 506EB (11.5 days) and Semsen (11.0 days) as compared to those reared on diets with leaf powder of transgenic lines (9.5 to 10.5 days). Male longevity was significantly longer in insects reared on diets with leaf powder of Semsen (10.0 days) than on BS5A.1(T2) 18-1P1(7.5 days). Eggs laid by the females were significantly reduced in insects reared on diets with leaf powder of BS5A.1(T2) 19-3P2 (545.0 eggs female^{-1}) as compared to those reared on ICC 506EB and Semsen (992.5 and 800.0 eggs female^{-1}) The survival and development of the pod borer, *H. armigera* larvae was significantly better when reared on the standard artificial diet compared to those reared on diets with lyophilized leaf powder of transgenic and non transgenic chickpeas. (Table 4.10 and Fig 15).

Compared to the first season 2011-12, the survival and development of third-instar *H. armigera* during 2012-13 was significantly reduced in insects reared on diets with leaf powder of transgenic chickpea BS5A.1(T2) 18-1P1 as against those reared on non-transgenic chickpea ICC 506EB. During 2012-13, BS5A.1(T2) 18-1P1 showed high levels of resistance to *H. armigera*. The survival and development of *H. armigera* neonate larvae reared on diets with leaf powder of transgenic chickpea was very poor as compared to those reared on non-transgenic chickpea. From the present study, it is clear that the survival and development of *H. armigera* larvae was significantly lower on transgenic chickpea diets as compared to those reared on non-transgenic chickpea diets.

Based on the pooled data, the survival and development of third-instar larvae of *H. armigera* reared on diets with leaf powder of non-transgenic chickpeas was greater as compared to those reared on transgenic chickpea lines. Larval survival was lowest in insects reared on diets with leaf powder of BS5A.1(T2) 18-1P1 (35.5%), larval weight at 5 DAI was

lowest on BS5A.1(T2) 18-2P1 (9.8 mg larva^{-1}) and the larval weight at 10 DAI was lowest on BS5A.1(T2) 18-1P1 (231.7 mg larva^{-1}). Pupal weight was lower on BS5A.1(T2) 18-2P1 (110.8 mg pupa^{-1}). Larval period was longer on BS5A.2(T2) 19-1P2 (17.0 days), and longest pupal period was recorded on BS5A.2(T2) 19-2P1 (15.0 days). Pupation and adult emergence was reduced on BS5A.1(T2) 18-2P1 (23.0 and 14.5%, respectively), and female longevity on BS5A.1(T2) 18-2P1 (7.5 days), and male longevity was lower on BS5A.2(T2) 18-1P1 (6.7 days). Eggs laid by the females was reduced in insects reared on diets with leaf powder of BS5A.2(T2) 19-1P2 (563.2 eggs female^{-1}) as compared to those reared on ICC 506EB (1034.2 eggs female^{-1}). The survival and development of *H. armigera* was significantly better when reared on the standard artificial diet compared to those reared on diets with lyophilised leaf powders of transgenic and non-transgenic chickpeas (Table 4.11 and Fig 16).

Similar observations have earlier been made by Khalique *et al.* (2003), who recorded reduced pupation, adult emergence and fecundity, inconsistent increase in pre-oviposition period, and prolongation of generation *H. armigera* fed on spore-δ-endotoxin complex of indigenous strain HD-695 (8500 IU mg^{-1}) of *Bt- var kurstaki.* Devi *et al*. (2011) also observed a significant reduction in larval survival, larval and pupal weights and fecundity, and prolongation of larval and pupal periods in chickpea plants sprayed with *Bt* (0.05%) as compared to unsprayed plots. Larval survival, larval and pupal weights, pupation and adult emergence were significantly lower on diets with leaf or pod powder of the *H. armigera* resistant genotypes than on the susceptible ones.

Zhang *et al*. (2013) studied the efficacy of Cry1Ac and Cry1Ca on lifespan and reproductive performance of *H. armigera* and *Spodoptera exigua* adults. Cry1Ac and Cry1Ca affected the life span of both males and females of *H. armigera* and *S. exigua*. Moreover, exposure of females to 500 mg/ml of Cry1Ac and Cry1Ca significantly affected the fecundity in *H. armigera* and *S. exigua*.

Continuous feeding on *Bt* cotton was resulted in 80-85 per cent mortality of first-instar (Wang and Xia, 1997) and 100 per cent mortality of one to fourth-instars of *H. armigera* larvae (Zhao *et al.*, 1998a; Cui and Xia, 1999; Zhao *et al*., 2000a. No bollworms larvae survived when fed with transgenic cotton line R93-4, with first to fourth instar to pupation, however, fifth-instar larvae fed on bollgard cotton survived to pupate (Zhao *et al.*, 1998b).

4.2 Molecular and biochemical characterization of *Cry IIa* transgenic chickpeas

4.2.1 Biochemical profile of different transgenic chickpea lines

There were no significant differences in the protein content between the transgenic and non-transgenic chickpea lines. Protein content was highest in the leaves of BS5A.2(T2) 19-1P2 (5.8 mg/g dw), followed by 5.5 mg/g in Semsen, 5.3 mg/g in BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-3P1, 5.2 mg/g in BS5A.1(T2) 18-1P1, BS5A.2(T2) 19-3P2, 4.9 mg/g in BS5A.2(T2) 19-2P1 and 4.8 mg/g in ICC 506EB. Highest amounts of carbohydrates were recorded in the leaves of ICC 506EB (55.0%), whereas the leaves of Semsen (24.3%) had the lowest amount of carbohydrates. The amount of carbohydrates ranged from 34.0 to 49.3% in transgenic chickpea lines. Among the transgenic chickpea lines tested, the amount of carbohydrates was significantly greater in the leaves of BS5A.2(T2) 19-3P1 (49.3%) than in BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-1P2 (34.0%) (Table 4.12).

There were no significant differences in lipid content between the transgenic and non transgenic chickpea lines. Among the transgenics, BS5A.2(T2) 19-2P1 leaves had the highest lipid content (16.4%), followed by 13.9% in BS5A.1(T2) 18-1P1, 11.9% in BS5A.2(T2) 19-3P1, 10.6% in BS5A.1(T2) 18-2P1 and 8.8% in BS5A.2(T2) 19-3P2. The lowest lipid content was detected in BS5A.2(T2) 19-1P2 (7.8%). Among the non-transgenic chickpea lines, Semsen and ICC 506EB had 13.7 and 11.5% lipid content, respectively (Table 4.12).
There were no significant differences in phenol content in the leaves between the transgenic and non-transgenic chickpeas. Phenol content (mg/g dw) of leaves was highest in BS5A.2(T2) 19-2P1 and BS5A.2(T2) 19-3P2 (1.2 mg/g), while the leaves of BS5A.2(T2) 19-3P1 had the lowest phenol content (0.9 mg/g). Leaves of BS5A.2(T2) 19-3P2 had the highest tannin content (3.2 mg/g), followed by 2.2 mg/g in BS5A.1(T2) 18-1P1, 2.1 mg/g in BS5A.2(T2) 19-2P1, 1.6 mg/g in BS5A.2(T2) 19-1P2, and 1.2 mg/g in BS5A.2(T2) 19-3P1. Tannin content was lowest in BS5A.1(T2) 18-2P1 (0.5 mg/g). Amounts of tannins in Semsen and ICC 506EB were 0.8 and 1.0 mg/g, respectively (Table 4.12 and Fig 17).

During 2012-13, the protein content was significantly higher in ICC 506EB (7.2 mg/g) than in Semsen (4.5 mg/g). Among the transgenic chickpea lines tested, the maximum amount of protein was observed in BS5A.2(T2) 19-2P1 (6.4 mg/g) and BS5A.1(T2) 18-1P1 had the lowest protein content (5.2 mg/g). There were no significant differences in carbohydrate content in the leaves between the transgenic and non transgenic chickpeas. The amounts of carbohydrates were highest (38.8%) in the leaves of BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P1. The leaves of BS5A.2(T2) 19-3P2 had the lowest (28.1%) of

carbohydrates. Highest amounts of lipids (29.4%) were recorded in BS5A.2(T2) 19-3P1, followed by BS5A.1(T2) 18-2P1 (16.7%), BS5A.1(T2) 18-1P1 (16.6%), BS5A.2(T2) 19-1P2 (14.0%), BS5A.2(T2) 19-2P1 (8.2%) and BS5A.2(T2) 19-3P2 (7.0%). The lipid content in Semsen and ICC 506EB was 20.1 and 13.7%, respectively. There were no significant differences in phenol content between the transgenic and non-transgenic chickpeas. Highest phenol content was recorded in BS5A.2(T2) 19-3P1 (1.2 mg/g) and the lowest in BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P2 and BS5A.1(T2) 18-1P1 (0.9 mg/g). Transgenic and non-transgenic chickpea lines differed significantly in tannin content in the leaves. BS5A.2(T2) 18-2P1 had the highest (2.0 mg/g), while BS5A.2(T2) 19-3P2 had lowest tannins (1.1 mg/g) (Table 4.12 and Fig 18).

Based on the pooled data analysis, there were no significant differences in protein content between the transgenic and non-transgenic chickpeas. Among the transgenic lines, the protein content was highest in BS5A.1(T2) 18-2P1 (5.8 mg/g). Maximum amount of protein was recorded in ICC 506EB (6.0 mg/g). The amounts of carbohydrates were significantly higher in the leaves of ICC 506EB (44.9%), followed by BS5A.2(T2) 19-3P1 (43.6%), BS5A.1(T2) 18-2P1 (41.7%) BS5A.1(T2) 19-2P1 (34.6%), BS5A.1(T2) 18-1P1 (34.5%), BS5A.2(T2) 19-1P2 (32.5%) and BS5A.2(T2) 19-3P2 (32.0%). Lowest carbohydrate content was recorded in Semsen (28.5%). There were no significant differences in lipid content between the leaves of transgenic and non transgenic chickpea lines. However, the amount of lipids were higher in BS5A.2(T2) 19-3P1 (20.6%) than in BS5A.2(T2) 19-3P2 (7.9%). There were no significant differences in phenol and tannin contents between the transgenic and non transgenic chickpea lines. The phenol content ranged from 1.0 mg/g to 1.1 mg/g and the tannins from 1.2 to 2.1 mg/g (Table 4.12).

4.2.1.2 Correlation between resistance/susceptibility to pod borer and the amounts of biochemical composition of chickpea lines (2011-12 and 2012-13)

During 2011-12, the protein content was negatively correlated with larval survival (r = -0.25), larval weight (r = -0.27) and leaf damage rating (r = -0.45). Significant positive correlation was observed between carbohydrate content and leaf damage (r = 0.4). Negative, but non-significant relationship of phenols was observed with leaf damage (r = -0.24), larval survival (r = -0.27) and larval weight (r = -0.17). There was a negative significant association of tannins with leaf feeding damage (r = -0.41), larval survival (r = -0. 40) and larval weight (r = -0.42) (Table 4.13).

During 2012-13, the correlation co-efficients between the protein content and damage rating (r = 0.31) was positive but non-significant and there was a negative association with

the larval survival (r = -0.23), larval weight (r = -0.29). Amounts of carbohydrates were positively correlated with leaf damage (r = 0.25), larval survival (r = 0.23) and larval weight (r = 0.22). There was a negative and significant association of the phenols with larval survival (r = -0.40). However, a negative but non-significant correlation was observed with leaf damage (r = -0.33) and larval weight (r = -0.23). Association between tannins and leaf damage (r = -0.47), larval survival (r= -0.45) and larval weight (r = -0.43) was found to be negative and significant (Table 4.13).

These results are in accordance with the earlier reports, wherein tannins have been shown to inactivate insecticidal crystal proteins of *B. thuringiensis* (Luthy *et al*., 1985a). Tannin chemistry has been implicated in variation in host plant resistance to insects. Tannins, an important constituent of many plants, reacts strongly with the proteinaceous insecticidal proteins of *B. thuringiensis*. Commercial tannin preparation inhibits the activity of activated δ-endotoxin. Interaction between host plant tannins and δ-endotoxins might be one of the factors affecting the field efficacy of *B. thuringiensis* preparations or of *Bt*-transgenic crops (Luthy *et al*., 1985b).

The effectiveness of *B. thuringiensis* is greater on insect pests adapted to high tannin content (with a gut pH of 8.0 to 9.5). Therefore, insect pathogens can be more effective in a pest management program if antibiosis factors of host resistance are compatible with the insect pests. Sivamani *et al*. (1992) conducted bioassays with *B. thuringiensis* var. *galleriae* Berliner δ-endotoxin and plant phenolics on *H. armigera* and reported that the presence of plant phenolics with not only reduced the feeding potential and weight gain by the larvae, but also enhanced the LC_{50} value of the toxin, indicating the effect of phytochemicals from resistant crop plants on the biocidal activity of *B. thuringiensis* under laboratory conditions.

At high *Btk* concentration (1.72 µg/ml of diet), tannins antagonized *Btk* potency against spruce budworm, *Choristoneura* sps by lowering *Btk*-related larval mortality from 83 to 43%, though at moderate concentration, tannin did not affect *Btk* potency. Host tree tannins antagonized not only the lethal effects of *Btk* toxin, but also sublethal *Btk*-related impacts in terms of larval development, pupal weight, relative consumption rate and growth rate (Bauce *et al*., 2006). Saini and Dhawan (2010) observed a positive correlation between mortality of *H. armigera* and *S. litura* with Cry toxins and total phenols, but a negative correlation with tannins. *Chrysoperla carnea* is negatively affected when fed with *Bt*-susceptible but not Cry1Ac resistant *H. armigera* larvae that were fed on *Bt*-transgenic cotton expressing Cry1Ac. In the case of Cry1Ac resistant *H. armigera* strain, feeding on *Bt* cotton resulted in reduced glycogen content in the caterpillars. The predators, however, appeared to

compensate for the reduced carbohydrate content of the prey by increasing biomass uptake which caused an excess intake of the other analyzed nutritional compounds. Nutritional prey-quality factors other than *Bt* protein may be responsible for the observed negative effects when *C. carnea* larvae were fed with *Bt* cotton-fed prey. Possible factors were an altered sugar composition or fitness costs associated with the excess intake of other nutrients (Lawo *et al*. 2010).

4.2.2 HPLC profiles of acid exudates of different transgenic chickpea lines

The HPLC analysis of leaf samples for acid exudates at 30 DAE indicated that the transgenic chickpea and non-transgenic chickpea lines had two common peaks for oxalic acid and malic acid, during 2011-12. Similarly, during 2012-13, two common peaks (oxalic and malic acid) were observed for all the transgenic and non-transgenic chickpea lines (Table 4.14).

4.2.2.1 Amounts of organic acids on fresh weight basis

During 2011-12, there were no significant differences in amounts of organic acids between the transgenic and non-transgenic chickpea lines. Maximum amount of oxalic acid was recorded on non-transgenic ICC 506EB (2.5 mg/g) and lowest on BS5A.2(T2) 19-2P1 (0.8 mg/g). Among the transgenics, highest amount of oxalic acid was recorded on the leaf surface of BS5A.2(T2) 19-3P2 (1.5 mg/g). High amounts of malic acid were observed in BS5A.1(T2) 18-1P1 (2.8 mg/g), ICC 506EB (2.7 mg/g), BS5A.2(T2) 19-3P1 (2.5 mg/g), BS5A.1(T2) 18-2P1 (2.4 mg/g), BS5A.2(T2) 19-1P2 (2.3 mg/g), BS5A.2(T2) 19-3P2 (2.2 mg/g) and BS5A.2(T2) 19-2P1 (2.1 mg/g) and lowest on Semsen (0.4 mg/g) (Table 4.15 and Fig. 19).

During 2012-13, among the transgenic chickpea lines, the amounts of oxalic acid and malic acid were highest on BS5A.2(T2) 18-1P1 (1.2 and 1.8 mg/g, respectively) and lowest on BS5A.2(T2) 19-3P2 (0.5 and 0.9 mg/g, respectively). Among the non-transgenics, maximum amounts of oxalic acid and malic acid were observed on ICC 506EB (2.0 mg/g and 2.9 mg/g), followed by Semsen (0.7 and 0.2 mg/g, respectively) (Table 4.15 and Fig 20).

Based on the pooled data, significantly higher amounts of oxalic acid were recorded in BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 (1.1 mg/g) than in BS5A.2(T2) 19-2P1 (0.8 mg/g). Highest malic acid content was recorded on BS5A.1(T2) 18-1P1 (2.3 mg/g) and lowest on BS5A.2(T2) 19-3P2 (1.5 mg/g). Among the non-transgenics, the maximum amount of oxalic acid was observed in ICC 506EB (2.2 mg/g), followed by Semsen (0.9 mg/g) (Table 4.15).

4.2.2.2 Correlation between resistance/susceptibility to pod borer and the amount of organic acids

During 2011-12, oxalic acid content was positively correlated with larval survival (r = 0.63) and larval weight (r = 0.60). A significant and negative association was observed between the amounts of the malic acid and leaf feeding (r = - 0.83), larval survival (r = - 0.93) and larval weight (r = - 0.95) (Table 4.16).

During 2012-13, there was a positive and significant correlation between the oxalic acid and mean larval weight (r = 0.56). However, a positive non-significant relationship was observed with leaf damage (r = 0.19) and larval survival (r = 0.47). Further, the amounts of malic acid had positive non-significant correlation with leaf damage (r = 0.18), larval survival (r = 0.23) and larval weight (r = 0.27) (Table 4.16).

Oxalic acid and malic acid were detected as major components in the leaf surface exudates of transgenic and non-transgenic lines (Yoshida *et al*., 1995). Bhagwat *et al*. (1995) reported a low amounts of acids in the leaf exudates (21.1 and 4.9 meq./100 gm) of genotypes (ICC 14665). Narayanamma *et al.* (2013) characterized a diverse array of chickpea genotype for organic acid profiles in the leaf exudates. Chickpea leaf exudates contained malic acid, oxalic acid, acetic acid, citric acid and fumaric acid.

4.2.3 HPLC profiles of flavonoids in transgenic and non-transgenic chickpea lines

During 2011-12, the HPLC analysis of leaf samples (dry weight basis) for flavonoids indicated that the transgenic and non-transgenic chickpea lines had 13 peaks *viz*., chlorgenic acid (peak 1), genstisic acid (peak 2), phloretic acid (peak 3), ferulic acid (peak 4), umbelliferone (peak 5), naringin (peak 6), 3,4 dihydroxy flavone (peak 7), quercetin (peak 8), cinnamic acid (peak 9), naringenin (peak 10), genstein (peak 11), formononetin (peak 12) and biochanin A (peak 13).

In BS5A.1(T2) 18-2P1 and Semsen, there were no peaks observed. Peak 2 (genstisic acid) peak 6 (naringin) were not observed in BS5A.2(T2) 19-3P1, while peak 2 (genstisic acid) was absent in BS5A.2(T2) 19-3P2. In ICC 506EB, peak 1(chlorgenic acid), peak 2 (genstisic acid), peak 3 (chlorogenic acid), peak 6 (naringin), peak 8 (quercetin), peak 9 (cinnamic acid), peak 10 (naringenin) were not observed, while in BS5A.2(T2) 19-2P1, peak 9 (cinnamic acid) was absent (Table 4.17).

During 2012-13, the HPLC analysis of leaf samples (dry weight basis) for flavonoids indicated that there were 11 peaks in transgenic and non-transgenic chickpea lines (chlorogenic acid, gentisic acid, phloretic acid, ferulic acid, naringin, 3,4 dihydroxy flavone, quercetin , naringenin, genistein, formononetin and biochanin A). In BS5A.1(T2) 18-1P1,

peak 5 (naringin) was not observed, in BS5A.2 (T2) 19-1P2, peak 8 (naringenin) was not identified, while in BS5A.2 (T2) 19-3P1, peak 11 (biochanin A) was not observed. In Semsen Peak 5 (naringin) was not identified, whereas in ICC 506EB peak 7 (quercetin) and peak 8 (naringenin) were not observed (Table 4.18).

4.2.3.1 Amounts of flavonoids in transgenic and non-transgenic chickpeas

During 2011-12, in BS5A.2(T2) 19-1P2, the amount of chlorogenic acid (1.6 mg/g) was significantly greater as compared to BS5A.2(T2) 19-3P1 (0.4 mg/g). and not detected in BS5A.1(T2) 18-2 P1, Semsen and ICC 506EB. Gentisic acid content was maximum in BS5A.1(T2) 18-1P1 (3.9 mg/g), followed by BS5A.2 (T2) 19-2P1 (3.4 mg/g), and in BS5A.2 (T2) 19-1P2, it was recorded as lowest (1.3 mg/g). In other lines tested, the gentisic acid content was not detected. Highest amount of phloretic acid was recorded in BS5A.2(T2) 19-2P1 (20.5 mg/g), followed by BS5A.2(T2) 19-3P1 (19.1 mg/g), BS5A.1(T2) 18-1P1 (10.3 mg/g) and BS5A.2(T2) 19-1P2 (10.2 mg/g). Lowest amount of phloretic acid was recorded in BS5A.2(T2) 19-3P2 (8.4 mg/g) and it was not detected in BS5A.1(T2) 18-2P1, Semsen and ICC 506EB. The highest amount of ferulic acid was recorded in BS5A.1(T2) 18-1P1 (26.0 mg/g). In the other transgenic chickpea plants, it ranged from 1.0–2.6 mg/g. Least amount of ferulic acid was recorded in ICC 506EB (0.1 mg/g), but was not detected in BS5A.1(T2) 18-2P1 and Semsen (Table 4.19).

Significantly higher amount of umbellifrone was recorded in the leaves of BS5A.2(T2) 19-3P2 (1.8 mg/g) as compared to BS5A.2(T2) 19-1P2 (0.5 mg/g). No umbelliferone content was recorded in BS5A.2(T2) 18-2P1, Semsen and ICC 506EB. Naringin content was higher in BS5A.2(T2) 19-2P1 (19.4 mg/g) as compared to BS5A.2 (T2) 19-3P2 (9.7 mg/g). The amount of 3,4 dihydroxy flavone was significantly higher in BS5A.2(T2) 19-3P2 (0.9 mg/g) as compared to BS5A.2(T2) 19-1P2 (0.2 mg/g) and BS5A.2(T2) 19-2P1 (0.2 mg/g) and nil in BS5A.1(T2) 18-2P1, Semsen and ICC 506EB (Table 4.19).

Quercetin content was highest in BS5A.1(T2) 18-1P1 (1.7 mg/g), and least in BS5A.2(T2) 19-2P1 (1.0 mg/g) and nil in BS5A.1(T2) 18-2P1, Semsen and ICC 506EB. In the leaves of BS5A.2(T2) 19-1P2 cinnamic acid content was highest (1.3 mg/g), and lowest in BS5A.2(T2) 19-3P2 (0.1 mg/g). Highest amount of naringenin was recorded in BS5A.1(T2) 18-1P1(25.0 mg/g) and the lowest in BS5A.2(T2) 19-3P2 (0.6 mg/g). Naringenin content in the other transgenic chickpea lines ranged between 1.0-5.2mg/g. In BS5A.1(T2) 18-2P1, Semsen and ICC 506EB, naringenin was not detected (Table 4.19).

Highest amount of genistein was recorded in BS5A.1(T2) 18-1P1 (2.5 mg/g) and lowest in ICC 506EB (0.6 mg/g) and it was not detected in BS5A.1(T2) 18-2P1 and Semsen. The amount of formononetin was highest in BS5A.2(T2) 19-1P2 (0.4 mg/g), followed by BS5A.2(T2) 19-3P1 (0.1 mg/g) and BS5A.2 (T2) 19-3P2 (0.1 mg/g). Biochanin A content was significantly higher in BS5A.1(T2) 18-1P1(0.8 mg/g) as compared to ICC 506EB (0.2 mg/g) (Table 4.19).

Highest amount of gentisic (3.9 mg/g), ferulic acid (2.6 mg/g) and biochanin A (0.8 mg/g) was recorded in the leaves of BS5A.1(T2) 18-1P1 and highest amount of chlorgenic was recorded in BS5A.2(T2) 19-1P2 (1.6 mg/g). In the leaves of BS5A.2(T2) 19-3P1, maximum amounts of umbelliferone (1.8 mg/g) and 3,4 dihydroxy flavone (0.9 mg/g) were recorded. Flavonoids were below detectable limits in BS5A.1(T2) 18-2P1 and Semsen (Table 4.19).

During 2012-13, chlorogenic acid content was significantly greater in BS5A.2(T2) 19-2P1 (1.3 mg/g) as compared to BS5A.1(T2) 18-1P1 (0.2 mg/g). Gentisic acid content was highest in BS5A.2(T2) 19-2P1 (3.7 mg/g), followed by BS5A.1(T2) 18-2P1 (3.3 mg/g), BS5A.2(T2) 19-3P2 (3.5 mg/g) and ICC 506EB (3.5 mg/g), Semsen (3.1 mg/g), BS5A.1(T2) 18-1P1 (2.6 mg/g) while in BS5A.2 (T2) 19-1P2 (1.3 mg/g) had the lowest amount of gentisic acid. Maximum amount of phloretic acid was recorded in BS5A.1(T2) 18-2P1(12.5 mg/g) and least in BS5A.2(T2) 19-3P2 (5.4 mg/g). The amount of ferulic acid was greater in Semsen (2.1 mg/g) than in BS5A.2(T2) 19-1P2 and ICC 506EB (0.5 mg/g) (Table 4.20).

Naringin content was highest in ICC 506EB (30.3 mg/g), followed by BS5A.2(T2) 19-3P2 (20.7 mg/g), BS5A.2(T2) 19-1P2 (20.2 mg/g), BS5A.2(T2) 19-3P1 (13.2 mg/g) and BS5A.2(T2) 19-2P1 (12.5 mg/g). The amount of narignin was lowest in BS5A.1(T2) 18-2P1 (12.1 mg/g), but below detectable limits in BS5A.1(T2) 18-1P1 and Semsen. Maximum amounts of 3,4 dihydroxy flavone (2.9 mg/g), quercetin (2.5 mg/g), naringenin (4.7 mg/g), formononetin (2.9 mg/g) and biochanin A (6.8 mg/g) were recoeded in Semsen (Table 4.20).

Among the transgenic lines tested, the amount of 3,4 dihydroxy flavone was maximum in BS5A.2(T2) 19-3P2 (0.9 mg/g). Amount of quercetin was significantly higher in BS5A.1(T2) 18-2P2 (1.7 mg/g) as compared to BS5A.1(T2) 18-1P1 (0.5 mg/g). Narigenin content was highest in BS5A.2(T2) 19-2P1 (1.9 mg/g) and lowest amount in BS5A.2(T2) 19-3P1 (0.6 mg/g) and was nil in BS5A.2(T2) 19-1P2, and ICC 506EB. In the leaves of BS5A.2(T2) 19-3P2 genistein content was highest (2.5 mg/g) whereas in BS5A.1(T2) 18-1P1 (0.3 mg/g) had the lowest amounts. In BS5A.2(T2) 19-3P2 (0.4 mg/g) had the highest amount of formononetin, while lowest amount was recorded in BS5A.2(T2) 19-3P1 (0.1 mg/g).

Maximum amount of biochanin A was recorded in BS5A.1(T2) 18-2P1 and BS5A.1(T2) 18-1P1 (0.8 mg/g) and least was in BS5A.2(T2) 19-1P2 (0.5 mg/g) while nil in BS5A.2(T2) 19-3P1 (Table 4.20).

4.2.3.2 Correlation between resistance/susceptibility to pod borer and the amount of flavonoids

During 2011-12, there was a positive and significant correlation between formononetin and biochanin A and 3,4 dihydroxy flavones and genistein with leaf damage, larval survival and larval weights. Correlations between the ferulic acid, quercetin, cinnamic acid, naringin, gentisic acid and naringenin and leaf damage, larval survival and larval weight were negative but non-significant. Chlorogenic acid, phloretic acid and umbelliferone amounts were negatively correlated with leaf damage, larval survival and larval weight (Table 4.21).

During 2012-13, chlorogenic acid, gentisic acid, ferulic acid, naringin, naringenin and quercetin had a positive but non-significant correlation with resistance to *H. armigera*. There was a positive and significant association between 3,4 dihydroxy flavone, genistein, formononetin and biochanin A with leaf damage, larval survival and larval weight. (Table 4.22).

4.2.3.3 Detection of Cry IIa protein in transgenic chickpea lines using ELISA

4.2.3.3.1 Cry IIa content in fresh leaves, green pod coat and green seeds

The Cry IIa content during 2011-12 was highest (75.0 ppb) in the fresh leaf samples of BS5A.2(T2) 19-1P2, BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P1, followed by 73.3 ppb in BS5A.2(T2) 19-3P2 and 57.6 ppb in BS5A.1(T2) 18-1P1 and BS5A.1 (T2) 18-2P1. During 2012-13, CryIIa protein in fresh leaves ranged between 51.6-72.0 ppb. The CryIIa protein concentration in fresh leaves was higher in BS5A.2(T2) 19-1P2, BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P1 and BS5A.2(T2) 19-3P2 (Table 4.23 and Fig 21).

The amounts of CryIIa proteins present in green pod wall in different transgenic plants were 73.0 ppb in BS5A.2(T2) 19-1P2, 71.0 ppb in BS5A.2(T2) 19-2P1, 70.0 ppb in BS5A.2(T2) 19-3P1 and 60.6 ppb in BS5A.2(T2) 19-3P2, 57.6 ppb in BS5A.1(T2) 18-1P1 and 54.3 ppb in BS5A.1(T2) 18-2P1. The amounts of CryIIa protein were maximum in the seeds of BS5A.2(T2) 19-3P1 (74.3 ppb), followed by 73.6 ppb in BS5A.2(T2) 19-2P1, 69.6 ppb in BS5A.2(T2) 19-1P2, 62.6 ppb in BS5A.2(T2) 19-3P2, 54.3 ppb in BS5A.1(T2) 18-2P1 and 53.3 ppb in BS5A.1(T2) 18-1P1. Similarly the CryIIa protein content was high in

dry pod wall (50.6 –70.6 ppb), dry seeds (53.6–70.0 ppb) and dry stems (49.3-70.0ppb) (Table 4.23).

4.2.3.3.2 CryIIa content in Dry root and Soil

The concentration of the CryIIa protein in dry roots ranged between 3.2-6.9 ppb. CryIIa protein was not detected in the soil samples after uprooting the transgenic and non-transgenics plants (Table 4.23 and Fig 22).

4.2.3.3 CryIIa content in *H. armigera* larvae fed on transgenic chickpea

During 2011-12, concentration of CryIIa protein was significantly high in *H. armigera* larvae fed on leaves of BS5A.2(T2) 19-1P2 (54.0 ppb) and BS5A.2(T2) 19-3P1 (52.3 ppb). The amount of protein in the larvae fed on BS5A.1(T2) 18-2P1 was 42.3 ppb. The protein concentration was significantly lowest in larvae fed on BS5A.2(T2) 19-3P2 (13.0 ppb), followed by BS5A.2(T2) 19-2P1 (17.0 ppb) and BS5A.1 (T2) 18-1P1 (19.0 ppb) (Table 4.23). During 2012-13, the CryIIa protein content was significantly higher in larvae fed on BS5A.2 (T2) 19-2P1 and BS5A.1 (T2) 18-1P1 (41.6 and 37.0 ppb, respectively) (Table 4.23).

4.2.3.3.4 CryIIa content in the aphids, *A. craccivora* fed on transgenic chickpea leaf powder mixed in 2M sucrose solution and in artificial diets

CryIIa protein content in *A. craccivora* fed on 0.05% of transgenic chickpea leaf powder in 2Mof sucrose solution was very low (0.9 - 1.7 ppb). CryIIa protein content in the aphids fed on artificial diet with 0.05% of leaf powder of transgenic chickpea lines ranged between 1.0 - 1.6 ppb (Table 4.23).

4.2.3.3.5 CryIIa content in coccinellid grubs fed on transgenic leaf powder

A negligible amounts of CryIIa protein were detected in the grubs fed on leaf powder of transgenic chickpea lines (1.1 to 2.4 ppb) (Table 4.23).

4.2.3.3.6 CryIIa content in *Campoletis chlorideae* larvae reared on *H. armigera* fed on transgenic chickpeas

The amount of CryIIa protein in *C. chlorideae* larvae reared on *H. armigera* fed larvae fed on transgenic chickpeas was very low (1.0 - 1.7 ppb) (Table 4.23 and Fig 23).

The CryIIa protein was high in fresh leaves in season I varied from 57.6 to 75.0 ppb and in season II, it ranged from 51.6 to 72.0, green pod coat (54.3 to 73.0 ppb), and green seeds (53.3 -74.3 ppb), dry pod wall (50.6 –70.6 ppb), dry seeds (53.6 –70.0 ppb) and dry stems (49.3 - 70.0 ppb). In dry roots the protein concentration was quite low (3.2 to 6.9 ppb) whereas in soil samples, it was below detectable levels (0.0 to 0.2 ppb). The amount of protein transferred from fresh leaves to *H. armigera* larvae ranged from 13.0 - 54.0 ppb in

season I, and 15.0 - 41.6 ppb in season II. The CryIIa protein in *Bt* fed aphids, coccinellid grubs and *Bt* fed *C. chlorideae* larvae was almost nil. Hence, the amount of CryIIa protein transferred from leaves to the non-target insects and natural enemies were negligible.

4.2.3.3.8 Correlation between resistance/susceptibility to *H. armigera* and the amounts of CryIIa protein

In both the seasons (2011-12 and 2012-13), the correlation co-efficient of CryIIa protein in fresh leaf, green pod wall, green seeds, dry pod wall, dry seeds, dry stems, dry roots and *H. armigera* larvae with leaf damage, larval survival and larval weight was negative and significant (Table 4.24 and 4.25).

Difference in amounts of δ-endotoxin present in various plant parts which was correlated with larval survival of the bollworms throughout the growing season (Adamczyk *et al.* 2001). The green tissue had the highest concentration of toxin, followed by yellow green and white yellow tissues (Abel and Adamczyk, 2004). Zhang *et al.* (2004) found that the amount of *Bt* toxin in different plant parts was high in NuCoTN 33B (79.7-139.0 ng/g fresh wt) than in GK-12. ELISA teat confirmed that *Spodoptera littoralis* larvae ingest high amounts of Cry1A(b) toxin while feeding on *Bt*-maize and no toxin was found in parasitoid of *S. littoralis*, *Cotesia marginiventris* adults (Vojtech *et al.,* 2005). Head *et al.* (2001) did not detect Cry1Ab by ELISA in aphids feeding on transgenic *Bt*-Corn plants. For the lepidopteran insects, *Ostrinia nubilalis, H. zea and Agrotis ipsilon*, the levels of Cry1Ab in the larvae varied with feeding treatments. Dhillon and Sharma (2010) reported >5 ppb of CryI Ac protein on *Bt*-sprayed chickpeas. However, no *Bt* toxins were detected in the larvae, cocoons and adults of *C. chlorideae* reared on *Bt*-intoxicated *H. armigera* larvae or in adult parasitoids fed on *Bt*-contaminated honey.

4.3 Effect of *Cry IIa* transgenic chickpea lines on the natural enemies of *H. armigera*

4.3.1 Effect of transgenic chickpea on the survival and development of the parasitoid, *Campoletis chlorideae*

During 2011-12 October planting, there were significant differences in survival and development the parasitoid, *C. chlorideae* when reared on *H. armigera* fed on transgenic and non-transgenic chickpea leaves. The larval period of *C. chlorideae* was prolonged in parasitoids reared on *H. armigera* fed on BS5A.1(T2) 18-1P1 (14.0 days) as compared to those reared on *H. armigera* fed on ICC 506EB and Semsen (8.8 and 8.3 days, respectively) (Table 4.26).

Among the transgenic lines tested, the larval period of *C. chlorideae* ranged from 9.3 to 10.4 days, however, there were no significant differences in pupal period of *C. chlorideae* reared on *H. armigera* fed on transgenic and non-transgenic chickpea (3.0 - 6.0 days). There was a prolongation of the post-embryonic development period (19.6 days) in *C. chlorideae* when reared on host larvae fed on the leaves of BS5A.1(T2) 18-1P1 as compared to those reared on other transgenic and non-transgenic chickpeas (11.8 to 16.4 days) (Table 4.26).

Cocoon formation was significantly lower when the parasitoid was reared on *H. armigera* fed on the leaves of BS5A.1(T2) 18-1P1 (16.0%), BS5A.2(T2) 19-3P1 (18.6%), BS5A.2(T2) 19-1P2 (22.6%), BS5A.2(T2) 19-3P2 (26.6%), BS5A.2(T2) 19-2P1 (29.3%) and BS5A.2(T2) 18-2P1 (33.3%) than those reared on Semsen (70.6%) and ICC 506EB (61.3%). Adult emergence was significantly reduced on BS5A.2(T2) 19-1P2 (3.8%) as compared to that on ICC 506EB and Semsen (39.8 and 41.5%, respectively) (Table 4.26).

Adult longevity of *C. chlorideae* was shorter in the parasitoids reared on *H. armigera* fed on transgenic chickpea lines compared to those fed on non-transgenic chickpeas. No males emerged from parasitoids reared on *H. armigera* fed on BS5A.2(T2) 19-1P2. Male longevity was shorter in parasitoids reared on *H. armigera* fed on BS5A.1(T2) 18-1P1 (3.6 days) as compared to those fed on Semsen and ICC 506EB (8.0 and 7.3 days, respectively). Female longevity was also shorter in insects reared on *H. armigera* larvae fed on BS5A.2(T2) 19-1P2 (3.0 days), BS5A.1(T2) 18-1P1 (4.0 days), BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P1 (16.3 days), BS5A.2(T2) 19-3P2 (9.3 days), BS5A.2(T2) 19-2P1 (10.6 days) and Semsen (10.0 days) as compared to that on ICC 506EB (17.0 days) (Table 4.26).

There were no significant differences in adult weights of *C. chlorideae* reared on *H. armigera* fed on transgenic and non-transgenic chickpea leaves. There were no males of *C. chlorideae* among the adults that emerged from *H. armigera* larvae fed on BS5A.2(T2) 19-1P2. Male adult weight was significantly reduced in insects reared on BS5A.1(T2) 18-1P1 (1.1 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (2.2 and 2.0 mg adult^{-1}, respectively). Weight of the adult females of *C. chlorideae* was also significantly lower (0.8 mg adult^{-1}) when reared on *H. armigera* larvae fed on the leaves of BS5A.1(T2) 18-1P1 as compared to those reared on Semsen and ICC 506EB (3.4 and 3.0 mg adult^{-1}, respectively). Number of males (0.0–2.0) and females (0.3-2.6) emerged from the cocoons were significantly reduced on transgenics as compared to that on non-transgenic chickpeas Semsen (5.0 and 6.0, respectively) and ICC 506EB (4.6 and 5.6, respectively). Eggs laid by the females that emerged from *H. armigera* larvae fed on the leaves of transgenic chickpea lines were significantly lower (6.6 to 81.0 eggs female^{-1}) than those reared on non-transgenic

chickpeas, Semsen and ICC 506EB (105.0 and 121.0 eggs female^{-1},) (Table 4.26 and Fig 24).

Among the transgenic lines tested, during October 2011-12 planting, a significant increase in egg+larval period post embryonic development period and reduction in cocoon formation, adult emergence, adult longevity, adult weights, sex ratio and fecundity was recorded in *C. chlorideae* reared on *H. armigera* fed on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-1P2.

During 2012-13 October planting, the egg+larval periods of *C. chlorideae* were significantly longer when *H. armigera* larvae were fed on the leaves of BS5A.1(T2) 18-1P1 (13.3 days) and BS5A.2(T2) 19-3P2 (13.3 days) as compared to those reared on *H. armigera* fed on Semsen and ICC 506EB (9.6 and 9.3 days, respectively). The egg+larval periods of parasitoids reared on *H. armigera* fed on other transgenic lines ranged from 11.6 to 12.6 days. The pupal period was prolonged by 5.6 - 8.0 days as compared that on Semsen and ICC 506EB (4.6 and 5.3 days, respectively). The post-embryonic development period of *C. chlorideae* was prolonged when the *H. armigera* were fed on the leaves of transgenic lines BS5A.2(T2) 19-2P1 (20.3 days) and BS5A.1(T2) 18-1P1 (19.6 days) as compared to that on ICC 506EB and Semsen (14.6 and 14.3 days, respectively) (Table 4.27).

Cocoon formation was significantly lower when the parasitoid was reared on *H. armigera* larvae fed on the leaves of transgenic plants BS5A.2(T2) 19-2P1 (21.1%) and BS5A.1(T2) 18-1P1 (23.3%) as compared to that on non-transgenic, ICC 506EB and Semsen (75.5% and 73.3%, respectively). The adult emergence was lowest when parasitoid was reared on host larvae fed on the leaves of BS5A.1(T2) 18-2P1 (11.1%), followed by BS5A.1(T2) 18-1P1 (12.2%), BS5A.2(T2) 19-1P2 (13.3%), BS5A.2(T2) 19-3P2 (15.5%), BS5A.2 (T2) 19-2P1 (17.7%) and BS5A.2(T2) 19-3P1 (26.6%) as compared to that on ICC 506EB and Semsen (65.5 and 50.0%, respectively). The early mortality of *H. armigera* was largely responsible for poor cocoon formation when the parasitoid was raised on *H. armigera* larvae fed on the leaves of transgenic plants (Table 4.27).

There were no significant differences in adult longevity between the parasitoids reared on *H. armigera* fed on transgenic and non-transgenic chickpea plants. The male adult longevity of *C. chlorideae* was slightly longer on BS5A.2(T2) 19-1P2 (11.6 days) and ICC 506EB (11.6 days) as compared to that on BS5A.2(T2) 19-3P1 (6.0 days). Female adult longevity was significantly higher in parasitoids *H. armigera* larvae fed on the leaves of BS5A.1(T2) 18-2P1 (23.3 days) as compared to that on BS5A.2(T2) 19-3P2 (15.3 days). There were no significant differences between the weights of the adult parasitoids reared on

H. armigera larvae fed on transgenic and non-transgenic chickpea plants. The male adult weight ranged from 2.6 to 3.4 mg adult^{-1}. The female adult weight was significantly reduced on BS5A.2(T2) 19-3P2 (1.9 mg adult^{-1}) as compared to Semsen and ICC 506EB (4.5 mg adult^{-1}) (Table 4.27).

Number of males (1.6-3.6) and females (1.6-4.3) emerged out of cocoons formed from the host larvae when fed on transgenic lines were significantly as compared to that on non-transgenic plants Semsen (7.0 and 7.3, respectively) and ICC 506EB (12.6 and 10.3, respectively). Eggs laid by the *C. chlorideae* females reared on *H. armigera* larvae fed on transgenic chickpea lines was significantly lower (6.6–50.6 eggs female^{-1}) than those reared on the non-transgenic chickpea plants, Semsen and ICC 506EB (145.0 and 131.0 eggs female^{-1}, respectively). Among transgenic lines tested, the survival and development of *C. chlorideae* was significantly lower when reared on BS5A.2(T2) 19-1P2 than on the non-transgenic control. However, the survival and development of parasitoids was better when reared on *H. armigera* larvae fed on BS5A.2(T2) 19-3P1 and BS5A.2(T2) 19-2P1 (Table 4.27 and Fig 25).

There was a prolongation of egg+larval period, pupal period and reduction in adult longevity, weights and sex ratio, and increase in cocoon formation of *C. chlorideae* reared on *H. armigera* fed on BS5A.2(T2) 19-3P1 and BS5A.2(T2) 19-3P2.

Based on the pooled data analysis, larval period of the parasitoids was significantly prolonged in *C. chlorideae* reared on *H. armigera* fed on BS5A.1(T2) 18-1P1 (13.1 days) as compared to that on non-transgenics, ICC 506EB and Semsen (9.0 days). There was no significant effect of transgenic plants on the pupal period of *C. chlorideae* (4.0 - 6.5 days). The post-embryonic development period was significantly prolonged on BS5A.1(T2) 18-1P1 (19.6 days) as compared to those reared on *H. armigera* fed on the leaves of Semsen and ICC 506EB (14.3 and 14.7 days, respectively). Cocoon formation was significantly lower in *C. chlorideae* reared on *H. armigera* fed on transgenic chickpea lines (19.6 to 33.8%) as compared to those fed on non-transgenic chickpea plants, Semsen and ICC 506EB (72.0 and 68.4%, respectively) (Table 4.28).

The adult emergence was significantly lower in *C. chlorideae* when reared on *H. armigera* larvae fed on BS5A.2(T2) 19-1P2 (7.3%), BS5A.1(T2) 18-1P1(8.1%), BS5A.1(T2) 18-2P1 (11.5%), BS5A.2(T2) 19-3P2 (14.4%), BS5A.2(T2) 19-3P1 (17.3%) as compared to that on ICC 506EB and Semsen (53.4 and 47.0%, respectively). Male adult longevity was shorter in parasitoid reared on *H. armigera* larvae fed on transgenic plants (6.0 – 8.6 days) as compared to those reared on non-transgenic Semsen and ICC 506EB (9.0 and

9.5 days, respectively). The shortest longevity of *C. chlorideae* females was recorded in insects reared on *H. armigera* fed on BS5A.2(T2) 19-1P2 (7.3 days) while the longest survival was recorded on non-transgenic plants (16.3 days) (Table 4.28).

There were no significant differences in male adult weights between the parasitoids reared on *H. armigera* larvae fed on transgenic and non transgenic plants (1.4 to 2.7 mg adult^{-1}). Female adult weight was significantly lower on BS5A.1(T2) 18-1P1 (1.5 mg adult^{-1}) as compared to that on Semsen (3.9 mg adult^{-1}) and ICC 506EB (3.7 mg adult^{-1}). Number of males (1.1-2.3) and females (1.0-2.6) emerged from the host larvae fed on transgenics were significantly reduced as compared to that on Semsen (6.0 and 6.6, respectively) and ICC 506EB (8.6 and 8.0, respectively). The eggs laid by the females when reared on *H. armigera* fed on transgenic plants were significantly reduced (6.6–62.6 egg female^{-1}) as compared to the wasps reared on *H. armigera* fed on non-transgenic plants, ICC 506EB and Semsen (126.6 and 125.2 egg female^{-1}, respectively) (Table 4.28 and Fig 26).

In general, reduced survival and prolonged development of the parasitic wasps was recorded when reared on *H. armigera* larvae fed on BS5A.1 (T2) 18-1P1 and BS5A.2 (T2) 19-1P2.

4.3.1.1 Effect of transgenic chickpea lines on survival and development of the parasitoid, *Campoletis chlorideae* (November, 2011-12 and 2012-13)

There were no significant differences in larval period, pupal period and post embryonic development period of *C. chlorideae* reared on *H. armigera* larvae fed on transgenic and non-transgenic plants. Longest larval period was recorded on BS5A.1(T2) 18-1P1 (12.3 days) and the shortest on BS5A.2 (T2) 19-2P1 (6.3 days). Pupal period was prolonged on BS5A.2(T2) 19-1P2 (6.0 days) as compared to that on on BS5A.1(T2) 18-2P1 (3.0 days). Post embryonic development period was also prolonged on BS5A.1(T2) 18-1P1 (17.6 days) as compared to BS5A.1(T2) 18-2P1 (11.3 days) (Table 4.29).

Cocoon formation was significantly lower in *C. chlorideae* reared on *H. armigera* larvae fed on leaves of BS5A.2(T2) 19-3P2 (3.3%), BS5A.1(T2) 18-1P1 (10.6%), BS5A.2(T2) 19-1P2 (14.9%), BS5A.2(T2) 19-2P1 (17.1%) and BS5A.2(T2) 19-3P1 (17.6%) as compared to those reared *H. armigera* larvae fed on ICC 506EB and Semsen (47.2 and 43.8%, respectively). Among the transgenic plants tested, the cocoon formation was highest in *C. chlorideae* reared on *H. armigera* fed on BS5A.1(T2) 18-2P1 (23.8%). Adult emergence was poor on BS5A.2(T2) 19-3P2 (2.3%) as compared to those fed on non-transgenic plants ICC 506EB and Semsen (39.3 and 32.0%, respectively). There were no significant differences in male and female adult longevity. However, male adult longevity

was longer on BS5A.2(T2) 19-3P2 (11.6 days) and as compared to that on Semsen (4.6 days) and ICC 506EB (5.3 days) and longest female longevity was recorded in the parasitoids reared on *H. armigera* larvae fed on BS5A.2(T2) 19-3P1 (22.8 days), while shortest was on BS5A.1(T2) 18-2P1 (0.6 days) and Semsen (1.3 days) (Table 4.29).

No significant differences were observed in weight of the adults (male, 1.3 to 2.8 mg adult^{-1} and female, 2.1 to 3.0 mg adult^{-1}). Number of males (0.6-2.0) and females (1.0-2.3) emerged out of cocoons developed on *H. armigera* larvae fed on the transgenic plants were significantly lower as compared to that on non-transgenic chickpea plants Semsen (3.6 and 4.3, respectively) and ICC 506EB (5.3 and 4.6, respectively). Eggs laying by the females was significantly reduced in *C. chlorideae* wasps obtained from *H. armigera* larvae fed on the transgenic plants BS5A.2(T2) 19-2P1 (15.0 eggs female^{-1}), BS5A.2(T2) 19-3P2 (25.0 eggs female^{-1}), BS5A.1(T2) 18-1P1 and BS5A.1(T2) 18-2P1 (33.3 eggs female^{-1}), BS5A.2(T2) 19-3P1 (45.0 eggs female^{-1}) and BS5A.2(T2) 19-1P2 (75.0 eggs female^{-1}) than on non-transgenic chickpea plants Semsen and ICC 506EB (102.3 and 91.6 eggs female^{-1}, respectively). Among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 (Table 4.29 and Fig 27).

During November 2012-13 planting, larval period of the parasitoid was significantly prolonged when reared on *H. armigera* fed on transgenic chickpea lines BS5A.1(T2) 18-2P1 (12.3 days) and BS5A.2 (T2) 19-3P1 (12.0 days) than on Semsen and ICC 506EB (8.3 and 8.0 days, respectively). There were no significant differences between pupal period. The post-embryonic development period was prolonged in parasitoids obtained from *H. armigera* larvae fed on BS5A.1(T2) 18-2P1 (20.3 days) as compared to those reared on ICC 506EB (14.6 days) and Semsen (15.6 days) (Table 4.30).

Cocoon formation and adult emergence were significantly reduced in the parasitoids reared on *H. armigera* larvae fed on the leaves of transgenic plants, BS5A.1(T2) 18-2P1 (25.5 and 12.2%, respectively) as compared to those fed on non-transgenic plants, ICC 506EB (68.8 and 54.4%, respecively) and Semsen (57.7 and 42.2%, respectively). Among the transgenic lines, *C. chlorideae* wasps obtained from BS5A.1(T2) 18-1P1 had the highest cocoon formation (48.8%) and adult emergence (26.6%). The longevity and weights of the adult parasitoids were not affected by transgenic chickpea plants. Number of males (1.3-4.0) and females (2.3-4.0) emerged from *H. armigera* larvae fed on the leaves of transgenic plants were significantly reduced when compared to those fed on non-transgenic plants, Semsen (6.6 and 6.0, respectively) and ICC 506EB (9.3 to 7.0, respectively) (Table 4.30).

There were no eggs laying by the females obtained from *H. armigera* fed on the leaves of transgenic chickpea, BS5A.1(T2) 18-2P1 and BS5A.2 (T2) 19-3P1 (0.0 eggs female^{-1}) as compared to that on Semsen and ICC 506EB (111.6 and 105.0 eggs female^{-1}, respectively) and on other transgenics, (Table 4.30 and Fig 28).

Among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 as compared to that on other transgenic lines.

Based on the pooled data analysis (2011-12 and 2012-13), the larval period was prolonged in *C. chlorideae* wasps reared on *H. armigera* larvae fed on BS5A.2(T2) 19-3P1 (11.3 days) as compared to that on ICC 506EB and Semsen (9.1 and 8.8 days, respectively). Among the transgenic lines, larval period ranged from 8.1 to 11.1 days, and there were no significant differences in pupal period (4.8 - 6.9 days), and post-embryonic development period (15.0-18.0) when reared on *H. armigera* larvae fed on transgenic and non-transgenic chickpea plants (Table 4.31).

Cocoon formation and adults emergence were significantly reduced in *C. chlorideae* reared on *H. armigera* larvae fed on BS5A.2(T2) 19-3P2 (17.2 and 10.6%, respectively) than on non-transgenic ICC 506EB (58.0 and 46.8%, respectively) and Semsen (50.8 and 37.1%, respectively). Male longevity was longer in *C. chlorideae* reared on *H. armigera* larvae fed on transgenics BS5A.2(T2) 19-3P2 (11.3 days) as compared to ICC 506EB and Semsen (7.5 and 6.5 days, respectively). Female longevity ranged from 8.5 to 19.7 days. The male and female adult weights did not differ significantly. Number of males (1.3-3.0) and females (1.5-2.8) emerged from *H. armigera* larvae fed on the leaves of transgenic plants were significantly reduced when compared to those fed on non-transgenic plants, Semsen (5.1 and 5.1, respectively) and ICC 506EB (7.3 and 5.8, respectively). There was a significant reduction in fecundity of the female wasps obtained from *H. armigera* fed on transgenic chickpea plants of BS5A.1(T2) 18-2P1 (16.6 eggs female^{-1}) as compared to those fed on Semsen and ICC 506EB (107.0 and 98.3 egg female^{-1}, respectively) (Table 4.31 and Fig 29).

Among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-3P1 as compared to the other lines tested.

4.3.1.2 Survival and development of *C. chlorideae* reared on *H. armigera* fed on artificial diets with lyophilized leaf powder of different transgenic and non-transgenic chickpea

During 2011-12, the egg+larval period was significantly longer in *C. chlorideae* reared on *H. armigera* larvae fed on diets with transgenic leaf powder BS5A.1(T2) 18-1P1 (15.6 days), BS5A.1(T2) 18-2P1 (15.0 days), BS5A.2(T2) 19-1P2 (14.0 days), BS5A.2(T2) 19-2P1(12.6 days), BS5A.2(T2) 19-3P1 (11.0 days) and BS5A.2 (T2) 19-3P2 (10.0 days) as compared to that on Semsen and ICC 506EB (8.6 and 9.3 days, respectively). There were no significant differences in the pupal period of parasitoids reared on *H. armigera* fed on diets with transgenic and non-transgenic chickpeas (7.0 to 11.0 days). The post-embryonic development period was longer when *C. chlorideae* were reared on the *H. armigera* fed on diets with transgenic chickpea leaf powder (18.0 to 26.6 days) as compared to those reared on non-transgenic Semsen and ICC 506EB (15.6 and 18.0 days, respectively) (Table 4.32).

Cocoon formation of *C. chlorideae* was significantly reduced in when reared on *H. armigera* fed on diets with transgenic chickpea leaf powder BS5A.2(T2) 19-3P2 (54.4%) as compared to non-transgenic chickpea leaf powder ICC 506EB and Semsen (91.1 and 88.8%, respectively). Among the transgenic chickpeas, cocoon formation and adult emergence was better on BS5A.1(T2) 18-2P1 (80.0 and 63.3%, respectively) and BS5A.1(T2) 18-1P1 (65.5 and 52.2%, respectively). Adult emergence was significantly reduced on BS5A.2(T2) 19-1P2 (21.1%) as compared to that on Semsen and ICC 506EB (61.1 and 63.3%, respectively) (Table 4.32).

There was no negative effects of transgenic chickpeas on male longevity, and adult weights of the parasitoids. The male longevity was ranged from 8.0 to 11.3 days. The female longevity longest on BS5A.2(T2) 19-2P1 (24.0 days), and the shortest on BS5A.2(T2) 19-3P1 (9.3 days) (Table 4.32).

Number of males (4.0-9.6) emerged from *H. armigera* larvae fed on the leaves of transgenic plants were significantly lower as compared to that on non-transgenic plants, Semsen (12.0) and ICC 506EB (14.0), and the female parasitoids emerged from *H. armigera* larvae fed on the leaves of transgenic plants BS5A.1(T2) 18-2P1 (9.3) and BS5A.1(T2) 18-1P1 (7.6) as compared to that on Semsen (6.3) and ICC 506EB (5.0). Eggs laid by the females were significantly reduced when *C. chlorideae* was reared on *H. armigera* larvae fed on transgenic chickpea lines BS5A.2(T2) 19-1P2 (24.6 eggs female^{-1}) and BS5A.2(T2) 19-3P1 (30.6 eggs female^{-1}) as compared those fed on non-transgenic chickpeas, ICC 506EB and Semsen (152.3 and 119.3 eggs female^{-1}, respectively). Among the transgenic chickpea lines,

higher fecundity was recorded on BS5A.1(T2) 18-1P1 (71.0 eggs female^{-1}) and BS5A.1(T2) 18-2P1 (53.3 eggs female^{-1}) (Table 4.32 and Fig 30).

In general, the survival and development of parasitoids were affected when reared on *H. armigera* larvae fed on diets with transgenic BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 leaf powder as compared to that on other transgenics lines during 2011-12.

During 2012-13, significant reduction in survival and development of *C. chlorideae* wasps reared on *H. armigera* fed on diets with leaf powder of different transgenic lines. The egg+larval period, pupal, and post-embryonic development periods were significantly reduced in insects reared on transgenics lines, BS5A.1(T2) 18-2P1 (12.0, 9.3 and 21.3 days, respectively) as compared to the non-transgenic Semsen (9.0,6.0 and 15.0 days, respectively) and ICC 506EB (8.3, 7.0 and 15.3 days, respectively) (Table 4.33).

Cocoon formation and adult emergence were significantly reduced in *C. chlorideae* reared on *H. armigera* larvae fed on diets with transgenic leaf powder of BS5A.2(T2) 19-1P2 (28.8 and 21.1%, respectively) and BS5A.2(T2) 19-3P2 (37.7 and 22.2%, respectively) as compared to that on Semsen (76.6% and 58.8%, respectively) and ICC 506EB (56.6 and 32.2%, respectively). There was a significant difference in male longevity, the males of *C. chlorideae* survived for longer period when reared on *H. armigera* larvae fed on diets with transgenic leaf powder BS5A.2(T2) 19-2P1 (10.3 days) than on BS5A.2(T2) 19-3P2 (3.3 days). There were no significant differences in female longevity ranged from 8.6 to 19.3 days between the *C. chlorideae* reared on *H. armigera* larvae fed on diets with leaf powder of transgenic and non-transgenic chickpeas. The male adult weights were significantly reduced in *C. chlorideae* reared on *H. armigera* larvae fed on diets with transgenic BS5A.2(T2) 19-2P1 leaf powder (1.8 mg adult^{-1}) as compared to that on non-transgenic chickpeas Semsen and ICC 506EB (1.9 and 3.0 mg adult^{-1}, respectively). Female weights were greater in *C. chlorideae* reared on *H. armigera* larvae fed on diets with transgenic BS5A.1(T2) 18-2P1 leaf powder (4.0 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (3.0 and 3.1 mg adult^{-1}, respectively) (Table 4.33).

Number of males (3.3-5.0) and females (2.6-4.0) emerged from *H. armigera* larvae fed on the leaves of transgenic plants were significantly lower as compared to that on non-transgenic plants, Semsen (9.0 and 5.3, respectively) and ICC 506EB (5.3 and 4.3, respectively). Eggs laid by the females were lowest in parasitoids reared on *H. armigera* fed on diets with transgenic chickpea leaf powder BS5A.2(T2) 19-1P2 (20.0 eggs female^{-1}), BS5A.2(T2) 19-3P2 (26.6 eggs female^{-1}), BS5A.2(T2) 19-2P1 (48.3 eggs female $^{-1}$), BS5A.1(T2) 18-2P1 (50.0 eggs female^{-1}), BS5A.2(T2) 19-3P1 (70.0 eggs female $^{-1}$) and

BS5A.1(T2) 18-1P1 (83.3 eggs female $^{-1}$) as compared to that on Semsen and ICC 506EB (163.3 and 112.3 eggs female^{-1}, respectively) (Table 4.33 and Fig 31).

Survival and development of *C. chlorideae* wasps obtained from *H. armigera* larvae fed on diets with transgenic BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P1 leaf powder was better as compared to that on BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P2.

Based on pooled data analysis (2011-12 and 2012-13), the egg+larval period was significantly extended when *C. chlorideae* developed in *H. armigera* fed on diets with BS5A.1(T2) 18-2P1 leaf powder (13.5 days) as compared to that on Semsen and ICC 506EB (8.8 days). The larval period on other transgenic lines ranged from 10.1 to 12.6 days. The pupal periods ranged from 6.5 to 9.3 days but there were no significant differences between the lines tested. The post embryonic development period was significantly prolonged in *C. chlorideae* reared on *H. armigera* fed on diets with transgenic chickpea leaf powder (18.8 to 22.8 days) as compared to non-transgenic plants of Semsen and ICC 506EB (15.3 and 16.6 days, respectively). Cocoon formation and adult emergence were significantly reduced in *C. chlorideae* reared on host larvae fed on diets with transgenic chickpea BS5A.2(T2) 19-1P2 leaf powder (44.4 and 21.1%, respectively) and BS5A.2(T2) 19-3P2 (46.1 and 23.8%, respectively) as compared to Semsen (82.7 and 60.0%, respectively) and ICC 506EB (73.8 and 47.7%, respectively). Among the transgenic chickpea lines tested, highest cocoon formation and adult emergence were recorded in *C. chlorideae* reared on *H. armigera* fed on diets with BS5A.1(T2) 18-2P1 leaf powder (66.1 and 46.6%, respectively) (Table 4.34).

Adult longevity of both males (5.6-9.6 days) and the females (11.5-19.7 dats) of *C. chlorideae* was not affected when reared on *H. armigera* fed on diets with transgenic and non-transgenic chickpea leaf powder. The male adult weight was significantly reduced on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 (2.3 mg adult^{-1}) as compared to that on ICC 506EB (3.2 mg). There were no significant differences in female adult weight. Number of males (4.1-7.3) and females (2.1-6.6) emerged from *H. armigera* larvae fed on the leaves of transgenic plants were significantly lower as compared to that on non-transgenic plants, Semsen (10.5 and 7.5, respectively) and ICC 506EB (9.6 and 8.6, respectively). Fecundity of the parasitoids was reduced in *C. chlorideae* reared on *H. armigera* fed on diets with transgenic chickpea leaf powder BS5A.2(T2) 19-1P2 (22.3 eggs female^{-1}) and BS5A.2(T2) 19-3P2 (39.6 egg female^{-1}) as compared to that on Semsen and ICC 506EB (141.3 and 132.3 eggs female^{-1}, respectively). Among the transgenic lines tested, fecundity was significantly

greater in the wasps reared on BS5A.1(T2) 18-1P1 (77.1 eggs female^{-1}) and BS5A.1(T2) 18-2P1 (51.6 eggs female^{-1}) than on other transgenic lines tested (Table 4.34 and Fig 32).

Across the seasons (November, 2011-12 and 2012-13), survival and development of *C. chlorideae* was better in insects reared on *H. armigera* larvae fed on diets with leaf powder of BS5A.1(T2) 18-1P1 and BS5A.1(T2) 18-2P2 than on other chickpea lines. The survival and development of *C. chlorideae* was affected when reared on *H. armigera* larvae fed on fresh leaves of transgenic lines than those reared on *H. armigera* larvae fed on diets with transgenic chickpea leaf powder.

No CryIIa protein was detected in the *C. chlorideae* larvae, the negative effects of transgenic chickpeas on survival and development of *C. chlorideae* were due to the early mortality of *H. armigera* as a result the parasitoids failed to complete the development on such larvae. The survival and development of *C. chlorideae* was poorer when reared on *H. armigera* larvae fed on fresh leaf samples than the artificial diets intoxicated with transgenic chickpea leaf powders.

Similar results were reported by Sharma *et al*. (2007), who reported poor survival and development of *C. chloridae* obtained from *H. armigera* larvae fed on the leaves of *Bt* cotton hybrid Mech 184. When *H. armigera* larvae were fed on artificial diet impregnated with Cry1Ab and Cry1Ac at LC_{50} and ED_{50} levels before and after parasitisation, there was a significant reduction in cocoon formation and adult emergence of *C. chlorideae*. Larval period of the parasitoid was prolonged by 2 days when fed on *Bt*-intoxicated larvae. No adverse effects were observed on female fecundity.

Yang *et al*. (2005) observed reduced cocoon formation and cocoon weight in parasitic wasps reared on *H. armigera* fed on diets made with transgenic cotton for *Microplitis mediator*, the cocoon formation and cocoon weight was reduced by 26.1% and 1 mg, respectively where for *C. chlorideae*, the reduction was 17.9% and 5.1 mg, respectively and larvae of the two wasps developing in the haemocoel of *H. armigera* larvae reared on transgenic cotton exhibited delayed development and, in some cases, abnormal development. The body weight of the larvae of the parasitoids was significantly reduced when obtained from hosts fed on transgenic cotton leaves compared to those fed on traditional cotton. Duration of egg and larval period was significantly prolonged, whereas pupal and adult weights of *C. chloridae* decreased when the host larvae were fed on transgenic cotton leaves for more than 48 h.

The development duration of *C. chlorideae* pupae on the hosts fed with transgenic cotton leaves was not significantly different than those on the controls. The longevity of female and male parasitoids fed on a solution containing Cry1Ac toxin did not differ significantly with that of the control (Liu *et al.*, 2005). Zhang *et al*. (2006b) observed shortened pupal stage and reduced body length of adult male. Survival, pupal mortality, and adult longevity of *C. chlorideae* were unaffected in *Bt*-resistant *H. armigera* larvae fed on *Bt*-toxin, suggesting that there is very limited effect on the life history parameters in two generations *C. chlorideae* parasitizing *Bt*–*Bt H. armigera* larvae. In both the generations, *C. chlorideae* was affected when *Bt*-resistant *H. armigera* larvae were fed on *Bt* toxin for different durations.

Maximum cocoon formation and adult emergence were recorded on *H. armigera* (82.4% and 70.5%, respectively) than on other insect hosts. When the *H. armigera* larvae were fed on artificial diet impregnated with *CryIAb* and *CryIAc* at LC_{50} and ED_{50} levels before and after parasitisation. There was a significant reduction in cocoon formation and adult emergence. Larval period of the parasitoid was prolonged by 2 days when *Bt*–intoxicated larvae and no adverse effects were observed on female fecundity (Sharma *et al*., 2008). Mohan and Sushil (2008) reported that no larvae survived in diets with a lethal dose of *Btk* HD-1 (LC_{70} and LC_{90}). The growth and survival of the parasitoid were normal when the host larvae were fed with sublethal doses or subjected to short time exposure to lethal doses of *Btk* HD-1. However, the parasitoid offsprings developed slowly, and pupal as well as adult periods, adult weights and adult emergence rate were reduced significantly if the parasitoid was developing inside a severely *Bt* intoxicated host larvae. There was a significant influence of host size on development and survival of the parasitoid. *Bt* toxins were detected in *H. armigera* larvae fed on *Bt*–sprayed chickpea, but not in *C. chlorideae* reared on *H. armigera* larvae fed on *Bt*-sprayed chickpeas, and in the parasitoid adults fed on honey intoxicated with 0.05% *Bt* (Dhillon and Sharma, 2010).

4.3.2. Direct effect of CryIIa transgenic chickpea on coccinellid, *Cheilomenus sexmaculatus*

During 2012-13, survival of coccinellid, *C. sexmaculatus* grubs was significantly reduced when fed on 0.02% of transgenic chickpea leaf powder in 2M sucrose solution. The larval survival was significanlty lower when fed on diets with leaf powder of transgenic chickpea lines (56.6 to 70.0%) as compared to that on non-transgenic chickpeas, ICC 506EB

and Semsen (83.3 and 80.0%, respectively). There were no significant differences in larval (6.0 - 7.3 days), pupal period (3.0 - 5.3 days), and mean grub weight (10.7 - 20.7 mg grub^{-1}) when fed on transgenic and non-transgenic chickpea intoxicated diets.

Pupation and adult emergence were significantly reduced in coccinellids fed on diets intoxicated with transgenic BS5A.2(T2) 19-3P1 leaf powder (30.0 and 23.3%, respectively), as compared to that on non-transgenics chickpeas ICC 506EB (63.3 and 50.0%, respectively) and Semsen (56.6 and 40.0%, respectively). Among the transgenic lines, pupation and adult emergence were significantly greater on BS5A.1(T2) 18-1P1 (40.0 and 33.3%, respectively) and BS5A.2(T2) 19-1P2 (46.6 and 36.6%, respectively) than on the other transgenic lines tested. The weight of males was significantly reduced in coccinellids fed on diets intoxicated with transgenic BS5A.2(T2) 19-2P1 leaf powder (4.0 mg adult^{-1}) as compared to that on ICC 506EB (8.6 mg adult^{-1}). There were no significant differences in weights of *C. sexmaculatus* females fed on transgenic and non-transgenic leaf powder intoxicated diets (5.7-9.6 mg adult^{-1}). The survival and development of coccinellid grubs was significantly higher when fed on 0.02% diet intoxicated with BS5A.2(T2) 19-3P1 leaf powder as compared to that on other transgenic lines (Table 4.35 and Fig 33).

At 0.05% concentration, larval survival was significantly reduced in coccinellids fed on diets intoxicated with transgenic BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P2 (46.6%) leaf powder as compared to that on Semsen and ICC 506EB (80.0%). Among the transgenic lines, highest larval survival was recorded on BS5A.1(T2) 18-1P1 (60.0%). The larval period was prolonged on transgenic lines (8.3 to 9.3 days) as compared to that on non-transgenic chickpeas (6.6 and 7.3 days, respectively) (Table 4.35).

The mean grub weight was drastically reduced when fed on diets with 0.05% transgenic BS5A.1(T2) 18-2P1 leaf powder (1.7 mg grub^{-1}) as compared to that on non-transgenic diets, ICC 506EB and Semsen (9.5 and 9.9 mg grub^{-1}, respectively). The pupal period was prolonged in coccinellids fed on diets intoxicated with transgenic leaf powder (3.6 -5.0 days) as compared to that on non-transgenics (3.0 days). Pupation was significantly reduced when coccinellid grubs fed on diets with BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P2 (30.0%) leaf powder as compared to that on ICC 506EB and Semsen (63.3%). Adult emergence was significantly reduced in coccinellids fed on diets intoxicated with transgenic leaf powder BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P2 (10.0%) as compared to that on Semsen and ICC 506EB (40.0%). Among the transgenic lines tested, the

pupation and adult emergence were highest on BS5A.1(T2) 18-1P1 (46.6 and 36.6%, respectively) (Table 4.35).

The adult weight of males were slightly reduced on transgenics (5.5 to 6.3 mg adult^{-1}) as compared to that on non-transgenic chickpeas ICC 506EB and Semsen (7.9 and 8.0 mg adult^{-1}, respectively). There were no significant differences in adult female weight (7.7 to 9.7 mg adult^{-1}) (Table 4.35 and Fig 33).

In diets having 0.02% and 0.05% leaf powder, the survival and development of coccinellids was greater when developed on BS5A.1(T2) 18-1P1 intoxicated diet as compared to that on the other transgenic lines tested.

The larval survival was significantly reduced when fed on diets with of 0.1% transgenic chickpea leaf powder (26.6 to 53.3%) as compared to that on non-transgenic chickpeas, Semsen and ICC 506EB (80.0 and 83.3%, respectively). Larval period was significantly prolonged in coccinellids reared on diets intoxicated with transgenic leaf powder BS5A.2(T2) 19-2P1 (10.6 days) as compared to that on non-transgenic chickpeas, Semsen and ICC 506EB (7.0 and 6.6 days). The mean grub weight was significantly reduced on BS5A.1(T2) 18-1P1 (1.9 mg grub^{-1}) as compared to that on Semsen and ICC 506EB (9.7 and 11.6 mg grub^{-1}, respectively) (Table 4.35).

There were no significant differences in pupal period of *C. sexmaculatus* reared on diets intoxicated with transgenic and non transgenic leaf powder (2.6 to 4.6 days). Pupation was significantly reduced in *C. sexmaculatus* reared on diets intoxicated with transgenic chickpea BS5A.2(T2) 19-1P2 leaf powder (13.3%) as compared to that on Semsen and ICC 506EB (53.3 and 66.6%, respectively). Among the transgenic lines tested, pupation was highest on BS5A.1(T2) 18-2P1 (33.3%). Similarly, adult emergence was significantly reduced in *C. sexmaculatus* reared on diets intoxicated with transgenic BS5A.1(T2) 18-1P1 leaf powder (3.3%) as compared to that on Semsen and ICC 506EB (36.6 and 43.3%, respectively). Among the transgenic lines tested, highest adult emergence was recorded on BS5A.2(T2) 19-3P2 (20.0%). The male adult weight was not affected (1.5–7.5 mg adult^{-1}), but the female adult weight was significantly reduced on BS5A.1(T2) 18-1P1 (2.1 mg adult^{-1}) as compared to that on ICC 506EB and Semsen (9.7 and 9.8 mg adult^{-1}, respectively) (Table 4.35 and Fig 33).

The survival and development of coccinellid was significantly affected when fed on diets intoxicated with BS5A.1(T2) 18-1P1 leaf powder but better survival was recorded on BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P2. In general, the direct effects on coccinellids

were greater when fed on 0.1% *Bt* intoxicated diet, followed by diets with 0.05% and 0.02% *Bt*.

4.3.3. Direct effects of *CryIIa* transgenic chickpea on coccinellid, *Cheilomenus sexmaculatus*

During 2013-14, there were significant effects of *Cry IIa* transgenic chickpeas on survival and development of coccinellids fed on diets with different concentrations (0.02, 0.05, and 0.1%) of transgenic and non-transgenic chickpea leaf powder. When the coccinellid grubs were fed on 0.02% of leaf powder intoxicated diets, the larval survival was significantly lower on BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-2P1 (43.3%) as compared to that on Semsen and ICC 506EB (80.0 and 73.3%, respectively) (Table 4.36).

There were significant differences in larval period of coccinellids and longest larval period was recorded in insects reared on diets with BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-2P (17.6 days) leaf powder while the shortest period was on BS5A.2(T2) 19-3P2 and ICC 506EB (6.0 days). The mean grub weight was significantly reduced in *C. sexmaculatus* reared on diets intoxicated with transgenic BS5A.2 (T2) 19-2P1 (8.6 mg grub^{-1}), BS5A.1(T2) 18-1P1 (9.0 mg grub^{-1}), BS5A.1(T2) 18-2P1 (10.3 mg grub^{-1}), BS5A.2(T2) 19-1P2 (10.7 mg grub^{-1}), BS5A.2(T2) 19-3P1 (12.1 mg grub^{-1}) and BS5A.2(T2) 19-3P2 (12.1 mg grub^{-1}) leaf powder as compared to that on non-transgenic plants, Semsen and ICC 506EB (26.5 and 25.0 mg grub^{-1}, respectively). The pupal period was prolonged in insects reared on BS5A.1(T2) 18-1P1 (5 days) as compared to that on Semsen and ICC 506EB (3.3 days) (Table 4.36).

There was a significant reduction in pupation and adult emergence in coccinellids fed on diets with BS5A.2(T2) 19-2P1 (30.0 and 16.6%, respectively) and BS5A.1(T2) 18-2P1 (30.0 and 20.0%, respectively) leaf powder as compared to that on ICC 506EB (66.6 and 60.0%, respectively) and Semsen (66.6 and 56.6%, respectively). The male adult weight was significantly reduced on BS5A.1(T2) 18-1P1 (4.5 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (9.1 and 8.5 mg adult^{-1}, respectively). Similarly, a slight reduction in female weight was observed on BS5A.2(T2) 19-2P1 (7.9 mg adult^{-1}), and highest weights were recorded on ICC 506EB (10.0 mg adult^{-1}). Among the transgenic lines, the survival and development was least affected when the grubs were fed on diets intoxicated with BS5A.2(T2) 19-1P2 leaf powder as compared to that on BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-2P1 (Table 4.36 and Fig 34).

In diets with 0.05% leaf powder, the survival of coccinellid grubs was significantly lower on BS5A.2(T2) 19-1P2 (33.3%), BS5A.2(T2) 19-2P1 and BS5A.2(T2) 19-3P1 (36.6%), BS5A.2(T2) 19-3P2 (46.6%), BS5A.1(T2) 18-2P1 (53.3%) and BS5A.1(T2) 18-1P1 (63.3%)

as compared to that on Semsen and ICC 506EB (83.3 and 80.0%, respectively). The larval period was prolonged on BS5A.2(T2) 19-1P2 (9.3 days) as compared to that on ICC 506EB and Semsen (5.0 and 6.0 days, respectively). The mean grub weight of the grubs was significantly reduced when fed on diets intoxicated with transgenic chickpea leaf powder (3.9 to 9.1 mg grub^{-1}) as compared to that on non-transgenic chickpeas (21.8 and 22.9 mg grub^{-1}, respectively). There were no significant differences in pupal period. Pupation and adult emergence were significantly reduced in coccinellids fed on diets intoxicated with BS5A.2(T2) 19-2P1 leaf powder (6.6 and 3.3%, respectively) as compared to those fed on diets with Semsen (70.0 and 60.0%, respectively) and ICC 506EB (70.0 and 56.6%, respectively) leaf powder. Male adult weight was significantly reduced when the coccinellid grubs were fed on BS5A.2(T2) 19-2P1 leaf powder intoxicated diets (1.8 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (9.0 and 8.9 mg adult^{-1}). There were no significant effects on the weight of adult females (7.2 to 9.9 mg adult^{-1}) (Table 4.36 and Fig 34).

Among the transgenic lines tested, coccinellids were least affected when fed on BS5A.1(T2) 18-1P1 and BS5A.1(T2) 18-2P1 intoxicated leaf powder diets as compared compared to those fed on BS5A.2(T2) 19-2P1.

In the diet 0.1% leaf powder concentration, larval survival was lowest in *C. sexmaculatus* fed on diets with BS5A.1(T2) 18-2P1 (26.6%) leaf powder. On other diets the larval survival ranged from 33.3 to 43.3% as compared to 80.0% on ICC 506EB and 76.6% on Semsen. The larval period was prolonged when coccinellid grubs were fed on diets intoxicated with transgenic chickpeas (6.6 to 8.6 days) as compared to that on ICC 506EB and Semsen (5.3 and 5.6 days, respectively) (Table 4.36).

The mean grub weight was drastically reduced when the coccinellids were fed on BS5A.1(T2) 18-1P1 (1.7 mg grub^{-1}), BS5A.2(T2) 18-2P1 (2.2 mg grub^{-1}), BS5A.2(T2) 19-3P1 (2.5 mg grub^{-1}), BS5A.2(T2) 19-1P2 (3.2 mg grub^{-1}), BS5A.2(T2) 19-2P1 (3.3mg grub^{-1}) and BS5A.2(T2) 19-3P2 (4.1 mg grub^{-1}) as compared to Semsen and ICC 506EB (19.7 and 24.0 mg grub^{-1}, respectively). There were no significant differences in pupal periods of coccinellids (2.6 to 4.6 days). Pupation and adult emergence were significantly reduced when the grubs were fed on the diets with BS5A.1(T2) 18-2P1 leaf powder (6.6 and 0.0%, respectively) as compared to that on ICC 506EB (66.6 and 53.3%, respectively) and Semsen (60.0 and 53.3%, respectively) (Table 4.36).

Since there was no adult emergence was observed on BS5A.1(T2) 18-2P1, the adult weight was recorded as zero (0.0 mg adult^{-1}). The male adult weight was significantly

reduced on BS5A.1(T2) 18-1P1 (1.8 mg adult^{-1}) and BS5A.2(T2) 19-3P1 (2.1 mg adult^{-1}) as compared to that on ICC 506EB and Semsen (7.6 and 6.8 mg adult^{-1}, respectively). The female adult weights ranged from 0.0 to 5.5 mg adult^{-1} when coccinellids were fed on diets intoxicated with transgenic leaf powder as compared to that on non-transgenic chickpeas (8.5 and 8.2 mg, respectively). The survival and development of the coccinellids were significantly reduced when fed on diet with 0.1% transgenic chickpea BS5A.1(T2) 18-2P1 leaf powder as compared to that on BS5A.2(T2) 19-2P1 (Table 4.36 and Fig 34).

Based on the pooled data analysis, there were significant differences in survival and development of coccinellid grubs when fed on diets intoxicated with 0.02% transgenic chickpea leaf powder as compared to those fed on diets with leaf powder of non-transgenic chickpeas. The larval survival was reduced on diets with BS5A.2(T2) 19-2P1 leaf powder (50.0%) as compared to that on Semsen and ICC5O6 EB (80.0 and 78.3%, respectively). There was a slight prolongation in the larval period when fed on diets intoxicated with transgenic chickpea leaf powder (6.0 to 7.5 days) as compared to Semsen and ICC 506EB (6.6 and 6.1 days, respectively). Mean grub weight was significantly reduced when fed on diets with BS5A.2(T2) 19-1P2 leaf powder (10.7 mg grub^{-1}) as compared to that on Semsen and ICC 506EB (22.9 and 18.4 mg grub^{-1}). The pupal period was significantly prolonged on BS5A.2(T2) 19-2P1 (9.0 days) as compared to that on Semsen and ICC 506EB (3.1 days) (Table 4.37).

Pupation and adult emergence were significantly reduced on BS5A.2(T2) 19-2P1 (33.3 and 21.6%, respectively) and BS5A.1(T2) 18-2P1 (35.0 and 21.6%, respectively) as compared to that on ICC 506EB (65.0 and 55.0%, respectively) and Semsen (61.6 and 48.3%, respectively). Among the transgenic lines, pupation and adult emergence were highest when fed on diets with BS5A.2(T2) 19-1P2 leaf powder (45.0 and 40.0%, respectively). The weight of the males was slightly reduced when reared on diets with transgenic chickpea leaf powder (5.1-7.9 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (8.7 and 8.5 mg adult^{-1}, respectively). There were no significant differences in female adult weights ranged from 8.0 to 9.3 mg adult^{-1}. The survival and development were significantly reduced when reared on diets with 0.02% of BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-2P1 leaf powder as compared to that on BS5A.2(T2) 19-1P2 (Table 4.37 and Fig 35).

At 0.05% concentration, the survival of grubs was significantly lower on diets with BS5A.2(T2) 19-1P2 (43.3%) leaf powder as compared to that on non-transgenic chickpeas (80.0 to 81.6%). The larval period was prolonged in grubs fed on diets with BS5A.2(T2) 19-1P2 leaf powder (9.3 days) as compared to that on Semsen and ICC 506EB (6.6 and 5.8 days,

respectively). The mean grub weight was significantly reduced in grubs fed on diets with transgenic leaf powder (2.9 to 5.9 mg grub^{-1}) as compared to that on non-transgenic Semsen and ICC 506EB (15.8 and 16.2 mg, respectively). The pupal period was prolonged (4.0-4.6 days) when coccinellids were fed on diets with transgenic leaf powder as compared to that on non-transgenic Semsen and ICC 506EB (3.0 and 3.1 days, respectively) (Table 4.37).

Pupation was significantly lower when the grubs were fed on diets with BS5A.2(T2) 19-2P1 leaf powder (18.3%) as compared to that on Semsen and ICC5O6 EB (66.6%). Adult emergence was also significantly reduced in *C. sexmaculatus* grubs fed on diets with BS5A.2(T2) 19-2P1 leaf powder (11.6%) as compared to that on Semsen and ICC 506EB (50.0 and 48.3%, respectively). The male and female weights were reduced in coccinellids fed on diets with BS5A.2(T2) 19-2P1 leaf powder (3.9 and 5.7 mg adult^{-1}, respectively) as compared to that on Semsen (8.5 and 9.8 mg adult^{-1}, respectively) and ICC 506EB (8.4 and 8.5 mg adult^{-1}, respectively). At 0.05% concetration, the survival and development of coccinellids were adversely affected when fed on diets with BS5A.2(T2) 19-2P1 leaf powder (Table 4.37 and Fig 35).

There were significant differences in the survival and development of coccinellid grubs fed on diets with 0.1% transgenic and non-transgenic chickpea leaf powder. The Larval survival was significantly reduced when fed on diets intoxicated with BS5A.2(T2) 19-3P2 (45.0%), BS5A.2(T2) 19-3P1 (50.0%), BS5A.1(T2) 18-1P1 (53.3%), BS5A.2(T2) 19-1P2 (53.3%), BS5A.1(T2) 18-2P1 (60.0%), BS5A.2(T2) 19-2P1 (70.0%) as compared to that on Semsen and ICC506 EB (71.6 and 75.0%, respectively). There was no significant effect on larval period. The weight of the grubs was significantly reduced in coccinellids fed on diets intoxicated with transgenic leaf powder (4.7 to 8.8 mg grub^{-1}, respectively) as compared to that on Semsen and ICC 506EB (23.5 and 21.5 mg grub^{-1}). There were no significant differences in pupal period (3.3 to 4.0 days). Pupation and adult emergence were significantly reduced on BS5A.2(T2) 19-3P2 (33.3 and 11.6%, rfespectively) as compared to that on Semsen (60.0 and 45.0%, respectively) and ICC 506EB (61.6 and 48.3%, respectively). The male adult weight was significantly reduced on BS5A.1(T2) 18-1P1 (6.2 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (13.2 and 10.8 mg adult^{-1}) but there were no significant effects on female adult weights (9.0-10.6 mg adult^{-1}) (Table 4.37 and Fig 35).

The survival and development of coccinellids was reduced when fed on diets with 0.1% of BS5A.2 (T2) 19-3P1 and BS5A.2 (T2) 19-3P2 leaf powder, but not on diets with BS5A.1(T2) 18-2P1 leaf powder. Based on the pooled data analysis, the direct effects of

transgenic chickpeas on survival and development of lady bird beetles were 0.02% < 0.05% < 0.1%.

Based on the earlier studies, the CryIAb has been detected in the phloem sap of *Bt*-oilseed rape and the aphids, *Myzus persicae* feeding on the *Bt*-oil seed rape plants (Burgio *et.al.* 2007). *Bt* toxins have also been detected in the coccinellid, *Propylaea japonica* larvae and the prey, *A. gossypii* when reared on *Bt* cottons (Zhang *et.al.*2006c). There was a significant and positive correlation between *Bt* detection in aphids, and survival of coccinellids larvae and adults. The amounts of *Bt* toxins detected in coccinellid grubs were higher as compared to the aphids, suggesting that coccinellid larvae accumulated *Bt* toxins in their gut (Haider *et al.*, 1986).

4.3.4 Indirect effect of *CryIIa* transgenic chickpea lines on survival and development of *C. sexmaculatus* reared on *A. craccivora* fed on diets with chickpea leaf powder

During 2012-13, there were no significant differences in survival and development of the coccinellids fed on aphids that were reared diets intoxicated with 0.02% leaf powder of plants. The larval survival ranged from 70.0 to 76.6%. The larval period was prolonged in coccinellids fed on aphids reared on diets intoxicated with transgenic leaf powder (8.0 to 9.0 days) as compared to that on Semsen and ICC506 EB (6.6 and 7.0 days, respectively). The mean grub weight was significantly reduced in coccinellid reared on aphids fed on diets intoxicated with transgenic chickpea leaf powder (2.7 to 8.9 mg grub^{-1}) as compared to that on ICC506 EB and Semsen (11.2 and 6.9 mg, respectively). The pupal period was significantly prolonged when *C. sexmaculatus* grubs were reared on aphids fed on diets intoxicated with BS5A.2(T2) 19-1P2 leaf powder (4.3 days) as compared to that on Semsen and ICC506 EB (3.0 days). There were no significant differences in the pupation (36.6-73.3%) and adult emergence (30.0-63.3%) of coccinellids grubs when reared on aphids fed on diets intoxicated with transgenic and non-transgenic chickpea leaf powder (Table 4.38).

There was a slight reduction in adult weights of the males of coccinellids when fed on diets with transgenic and non-transgenic chickpea leaf powder. Highest male adult weight was recorded on diets with ICC506 EB leaf powder (9.3 mg adult^{-1}) as compared to that on BS5A.2(T2) 19-3P2 (5.3 mg adult^{-1}). However, the female adult weights were unaffected (7.0-9.4 mg adult^{-1}) (Table 4.38 and Fig 36).

In diets with 0.05% leaf powder, there were no significant differences in the larval survival (43.3-60.0%), larval period (5.0-6.3 days), pupal period (3.0-4.0 days), pupation (36.6-50.0%), adult emergence (16.6-30.0%) and adult weights [male (5.2-7.5 mg adult^{-1}) and

female (9.6-13.7 mg adult^{-1})] in coccinellids fed on diets with transgenic and non transgenic leaf powder. However, mean grub weight was significantly reduced on diets with BS5A.1T2) 18-1P1 and BS5A.2(T2) 19-2P1 (3.8 mg grub^{-1}) leaf powder as compared to that on Semsen (5.2 mg grub^{-1}) and ICC 506EB (7.9 mg grub^{-1}) (Table 4.38 and Fig 36).

In diets with 0.1% leaf powder there were no significant differences in survival and development of coccinellid grubs when fed on aphids reared on diets with transgenic and non-transgenic chickpea leaf powder. There were no significant differences in larval survival (46.6-70.0%), larval period (4.6-5.6 days), pupal period (3.3-3.6 days), pupation (43.3-56.6%) and adult emergence (23.3-46.6%). The mean grub weight was significantly reduced in diets with BS5A.2(T2) 19-3P2 (5.0 mg grub^{-1}) leaf powder as compared to that on Semsen and ICC 506EB (18.7 and 17.8 mg grub^{-1}, respectively). The male adult weight was significantly reduced on diets with BS5A.1T2) 18-1P1 (6.0 mg adult^{-1}) leaf powder as compared to that on Semsen and ICC 506EB (17.3 and 12.6 mg adult^{-1}, respectively). The female adult weights were greater on diets with BS5A.2(T2) 19-1P2 (11.7 mg adult^{-1}) leaf powder as compared to that on Semsen and ICC 506EB (8.5 and 9.0 mg adult^{-1}, respectively) (Table 4.38 and Fig 36).

In general, there were no significant effects on survival and development of coccinellid grubs when fed on aphids reared on diets with 0.02% and 0.1% leaf powder of transgenic chickpeas. The survival and development was slightly affected on diets with BS5A.2(T2) 19-3P2 leaf powder. The coccinellids fed on diets with 0.05% BS5A.2(T2) 19-3P1 leaf powder showed a marginal reduction in survival and development as compared to that on other transgenic lines.

4.3.5 Indirect effect of *CryIIa* transgenic chickpea lines on survival and development of *C. sexmaculatus* reared on *A. craccivora* fed on diets with chickpea leaf powder

During 2013-14, in diets with 0.02% leaf powder, there was a slight reduction in larval survival in coccinellid grubs reared on aphids fed on diets with transgenic and non transgenic leaf powder. Larval survival was significantly reduced on diets with BS5A.2(T2) 19-1P2 (60.0%) leaf powder as compared to that on Semsen and ICC 506EB (83.3%). Larval period was prolonged in coccinellids reared on aphids fed on diets intoxicated with BS5A.2(T2) 19-3P2 (7.3 days) leaf powder as compared to that on Semsen and ICC 506EB (5.3 days). The mean grub weight was also significantly reduced on diets with BS5A.2(T2) 19-3P2 (2.2 mg grub^{-1}) leaf powder as compared to that on Semsen and ICC 506EB (12.1 and 12.3 mg grub^{-1}, respectively).There was no effect on pupal period. Pupation was significantly reduced in

coccinellid grubs reared on aphids fed on diets with transgenic BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 leaf powder (43.3%) as compared to that on Semsen and ICC 506EB (63.3 and 76.6%, respectively). Adult emergence ranged from 36.6% to 56.6%. Male adult weight was significantly reduced on diets with BS5A.1(T2) 18-2P1 leaf powder (6.5 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (9.9 and 10.5 mg adult^{-1}, respectively). Female weight was also significantly reduced on diets with BS5A.1(T2) 18-1P1 leaf powder (8.0 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (10.3 and 12.2 mg adult^{-1}, respectively) (Table 4.39 and Fig 37).

In diets with 0.05% leaf powder, there were no significant differences in larval survival (46.6-56.6%). The larval period increased marginally in coccinellid grubs reared on aphids fed on diets with BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P2 leaf powder (7.0 days) as compared to that on non-transgenic chickpea Semsen and ICC 506EB (6.0 days). There were significant differences in mean grub weight of coccinellid grubs reared on aphids fed on diets with BS5A.1(T2) 18-1P1 leaf powder (5.3 mg grub^{-1}) as compared to that on ICC 506EB (12.5 mg). There were no significant differences in pupal period (3.6 to 4.6 days), pupation (30.0-53.3%) and adult emergence (23.3-40.0%). The maximum male weight was recorded in grubs reared on diets with BS5A.2(T2) 19-2P1 leaf powder (9.0 mg adult^{-1}), and the lowest on BS5A.1(T2) 18-1P1 (6.5 mg adult^{-1}). The female adult weight was significantly reduced on diets with BS5A.2(T2) 19-2P1 leaf powder (7.0 mg adult^{-1}) as compared to that on Semsen and ICC5O6 EB (12.0 and 11.7 mg adult^{-1}, respectively). The survival and development of coccinellids were slightly affected when fed on diets with BS5A.2(T2) 19-3P2 leaf powder as compared to that on other transgenic lines (Table 4.39 and Fig 37).

In diet with 0.1% leaf powder, there were significant differences in survival and development of coccinellids reared on aphids fed on diets with transgenic and non-transgenic chickpea leaf powder. The larval survival was significantly reduced in coccinellids reared on aphids fed on diets with leaf powder of BS5A.2(T2) 19-3P1 (40.0%), BS5A.2(T2) 19-3P2 (43.3%), BS5A.1(T2) 18-1P1(46.6%), BS5A.2(T2) 19-1P2 (46.6%), BS5A.1(T2) 18-2P1 (53.3%) and BS5A.2(T2) 19-2P1 (70.0%) as compared to that on Semsen and ICC 506EB (76.6 and 80.0%, respectively). There were no significant differences in larval period (5.3 to 6.6 days). The mean grub weight was significantly reduced in coccinellid grubs reared on aphids fed on diets with BS5A.2(T2) 19-3P1 leaf powder (3.7 mg grub^{-1}) as compared to that on Semsen and ICC 506EB (18.4 and 15.3 mg grub^{-1}, respectively). There was a slight reduction in pupal period of coccinellids reared on aphids fed on diets with BS5A.1(T2) 18-

2P1 leaf powder (3.0 days) as compared to that on Semsen and ICC 506EB (3.3 days). Significant reduction in pupation and adult emergence were recorded on diets with BS5A.2(T2) 19-3P1(20.0 and 10.0%, respectively) leaf powder as compared to that on ICC 506EB (66.0 and 56.6%, respectively) and Semsen (63.3 and 53.3%, respectively) No significant differences in the male (6.5-9.1 mg adult^{-1}) and female adult weights (9.3-11.5 mg adult^{-1}) was recorded between the transgenic and non-transgenic chickpeas (Table 4.39 and Fig 37).

In diets with 0.1%, the survival and development was affected adversely when the coccinellid grubs were fed diets with BS5A.2(T2) 19-3P1 leaf powder.

Based on the pooled data analysis, at 0.02%, there were no significant differences in survival and development of coccinellid grubs. Larval survival ranged from 70.0 to 83.3%. Significant prolongation of larval period was observed on diets with BS5A.2(T2) 19-3P2 leaf powder (8.1 days) as compared to that on Semsen and ICC506 (6.0 and 6.1 days, respectively). The weight gain by the grubs was significantly reduced when coccinellid grubs were reared on aphids that fed on diets with BS5A.2(T2) 19-2P1 leaf powder (4.5 mg grub^{-1}) as compared to that on Semsen and ICC 506EB (14.5 and 16.7 mg grub^{-1}, respectively). Pupal period was prolonged on diets with BS5A.2(T2) 19-1P2 (4.5 days) leaf powder as compared to Semsen and ICC 506EB (3.1 and 3.0 days, respectively). Pupation was also reduced significantly in coccinellids reared on aphids fed on diets with BS5A.2(T2) 19-3P2 leaf powder (43.3%) as compared to that on Semsen and ICC 506EB (65.0 and 75.0%, respectively). There were no significant differences in adult emergence (46.6 to 60.0%). The male adult weight was reduced significantly when reared on diets with BS5A.2(T2) 19-3P2 leaf powder (6.5 mg adult^{-1}) compared to that on Semsen and ICC 506EB (8.2 and 9.9 mg adult^{-1}, respectively). There was a slight reduction in female weights on diets BS5A.2(T2) 19-2P1 and BS5A.2(T2) 19-3P2 leaf powder (7.9 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (9.5 and 10.8 mg adult^{-1}, respectively) (Table 4.40 and Fig 38).

In diets with 0.05% leaf powder, there were no significant effects on larval survival (48.3-61.6%). The larval period was prolonged in coccinellids grubs reared on aphids fed on diets with transgenic BS5A.1(T2) 18-2P1 leaf powder (6.8 days) as compared to that on Semsen and ICC 506EB (5.8 and 5.5 days, respectively). Similarly, the mean grub weight was significantly reduced on diets with BS5A.1(T2) 18-1P1 leaf powder (4.6 mg grub^{-1}) as compared to that on Semsen and ICC 506EB (7.1 and 12.7 mg grub^{-1}, respectively). There were no significant differences in pupal period (3.3-4.1 days). Pupation was significantly higher on diets with BS5A.2(T2) 19-3P1 leaf powder (51.6%) as compared to that on Semsen

and ICC 506EB (46.6 and 48.3%, respectively). Similarly, significant differences were recorded in adult emergence (20.0-33.3%). Effects of *Bt* transgenic chickpeas on male adult weights were non-significant (6.7-7.8 mg adult^{-1}), but there was a slight increase in female adult weight on diets with BS5A.1(T2) 18-2P1 leaf powder (11.5 mg adult^{-1}) but a slight reduction was observed on BS5A.1(T2) 18-1P1 (8.9 mg adult^{-1}) (Table 4.40 and Fig 38).

In diets with 0.1% leaf powder, significantly lower larval survival was recorded on BS5A.2(T2) 19-3P2 (45.0%) as compared to that on Semsen (71.6%) and ICC5O6 EB (75.0%). There was no significant difference in larval (5.3-6.1 days) and pupal period (3.1-4.0 days). But there was a significant reduction in mean grub weight was observed in coccinellid grubs reared on aphids fed on diets with BS5A.2(T2) 19-3P2 leaf powder (4.7 mg grub^{-1}) as compared to that on Semsen and ICC506 (13.5 and 11.5 mg grub^{-1}). Pupation and adult emergence were reduced on diets with BS5A.2(T2) 19-3P2 leaf powder (33.3 and 21.6%, respectively) as compared to that on Semsen (60.0 and 45.0%, respectively) and ICC5O6 EB (61.6 and 48.3%, respectively). The male adult weight was significantly reduced when coccinellids reared on aphids fed on diets with BS5A.1(T2) 18-1P1 leaf powder (6.2 mg adult^{-1}) as compared to that on Semsen and ICC 506EB (13.2 and 10.8 mg adult^{-1}, respectively) but there was no significant differences in the female adult weights (9.0 to 10.6 mg adult^{-1}) (Table 4.40 and Fig 38).

The survival and development of coccinellid grubs were slightly affected when reared on aphids fed on diets with different concentrations (0.02%, 0.05% and 0.1%) of transgenic chickpea leaf powder.

Based on the earlier studies, no adverse effects of *Bt*-transgenic crops have been reported on the development, survival, and development of lady bird beetles, *C. maculata*, *Hippodamia convergens* (Guerin-Meneville), and *P. japonica* through their aphid preys on *Bt*-transgenic crops (Dogan *et al.,* 1996; Duan *et al.,* 2002; Lundgren and Weidenmann 2002; Zhu *et al*., 2006). *Chrysoperla carnea* larvae were also not affected on *Bt*-maize reared aphids (Lozzia., 1998; Dutton *et al.,* 2002), or through the *Bt*-maize reared spider mites, *Tetranychus urticae* (Koch), even though the spider mites had much more amounts of Cry1 Ab toxin than the lepidopteran larvae (Dutton *et al.* 2002). However, poor prey quality and Cry1 Ac toxin mediated negative effects have been observed on the predatory beetle, *P. japonica* when fed on young *S. litura* larvae fed on *Bt*-transgenic cotton (Zhang *et al.,* 2006a). Such negative effects of *Bt* toxins on the coccinellid, *C. sexmaculatus* were also observed when fed on young *H. armigera* larvae reared only on *Bt*-ammended artificial diet (Sharma, H.C, unpublished data), indicating that the adverse effects of *Bt* toxins to the

coccinellids might depend on the processing of the *Bt* toxins and the quality of the insect host. Hilbeck et al., 1998 observed some adverse effects of the *Bt*-fed lepidopteran, *Spodoptera littoralis* (Boisduval) larvae on the chrysopid, *C. carnea*. Under natural conditions in the field, no significant differences were observed in the abundance of coccinellid beetles between *Bt*-transgenic and non-transgenic cottons (Sharma *et al.,* 2007). Dhillon and Sharma (2009a) reported that there were no adverse effects of *Bt* toxins on *C. sexmaculatus* when the larvae were reared on *A. craccivora* fed on different concentrations of CryIAb or CryIAc in the artificial diet, a significant and positive correlation was observed between the presence of *Bt* toxins in aphids and coccinellid larvae and adults. The results suggested that a direct exposure to *Bt* toxins expressed in transgenic plants or predation on *H. armigera* on *Bt* transgenic plants have little effect on the activity and abundance of the ladybird, *C. sexmaculatus*.

FIGURES

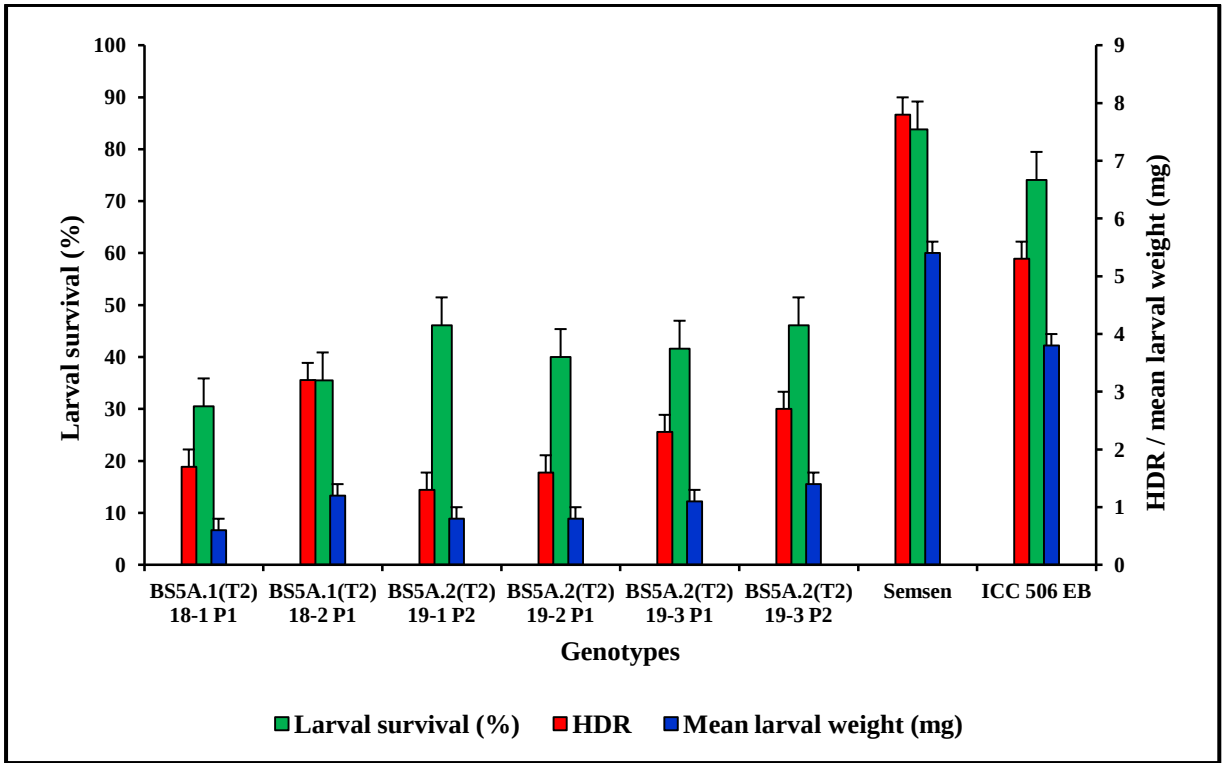


Figure 1: Evaluation of transgenic chickpea lines for resistance to *Helicoverpa armigera* under greenhouse conditions (October planting 2011-2012)

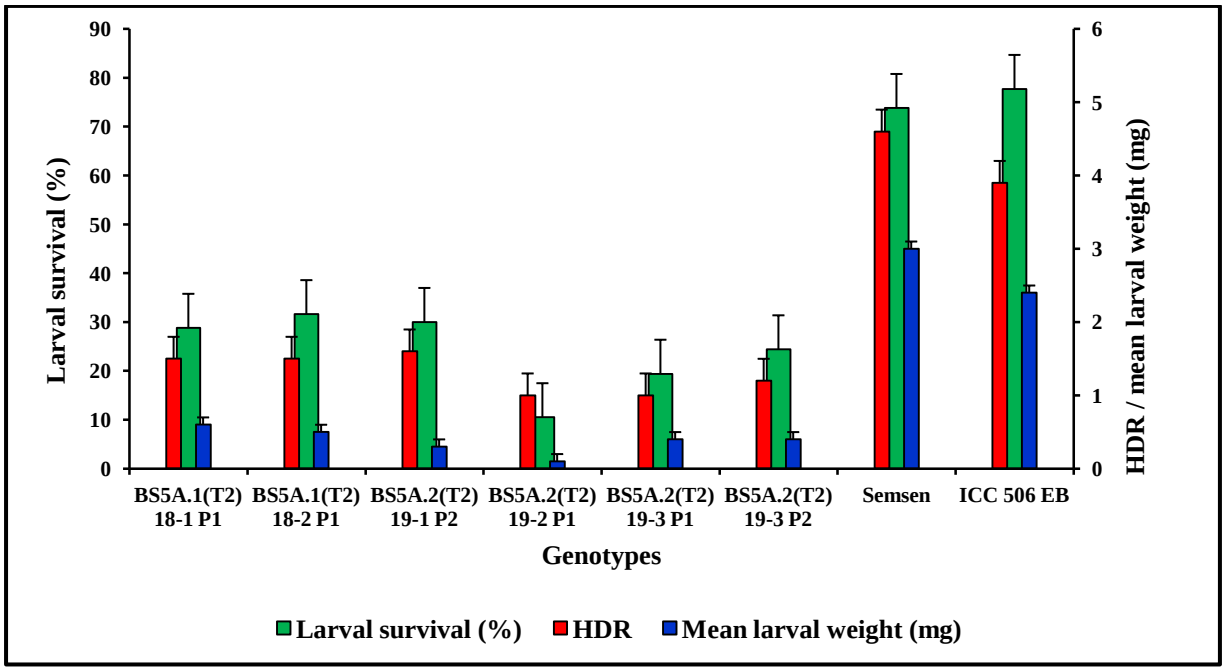


Figure 2: Evaluation of transgenic chickpea lines for resistance to *H. armigera* under green house conditions (October planting 2012-2013)

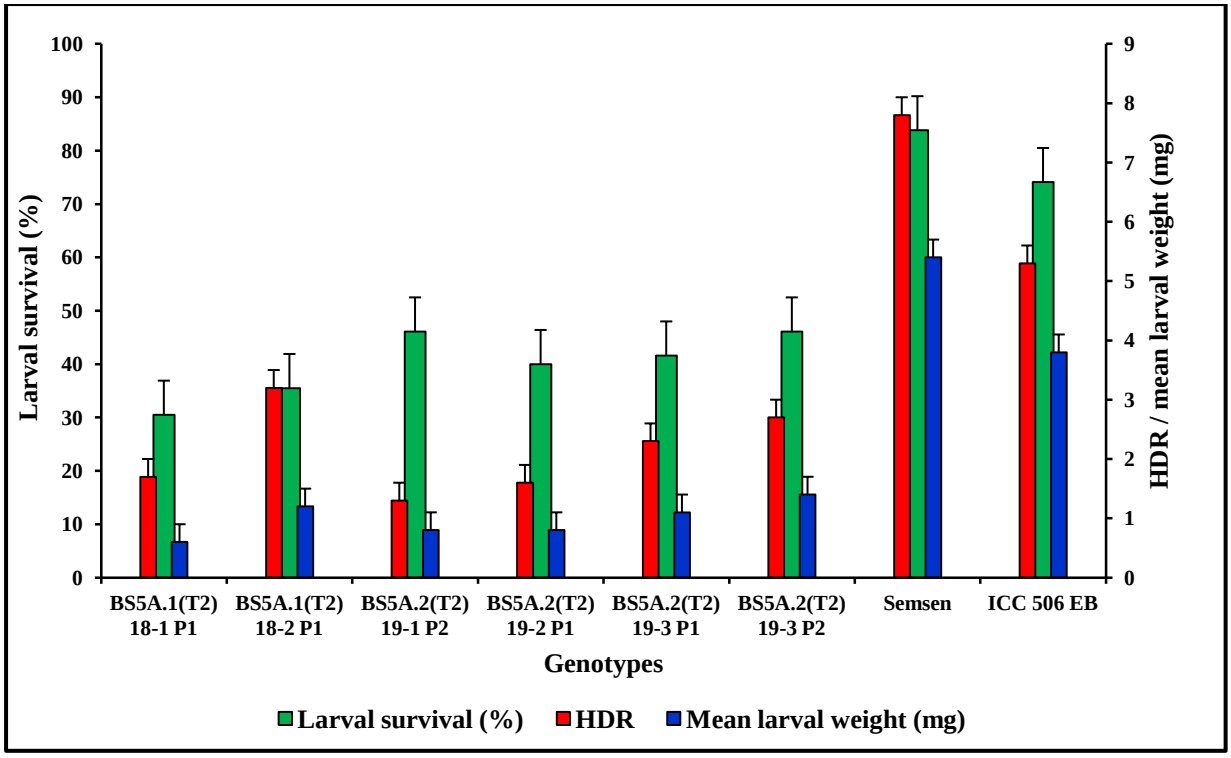


Figure 3: Evaluation of transgenic chickpea lines for resistance to *H. armigera* under greenhouse conditions (November planting 2011-2012)

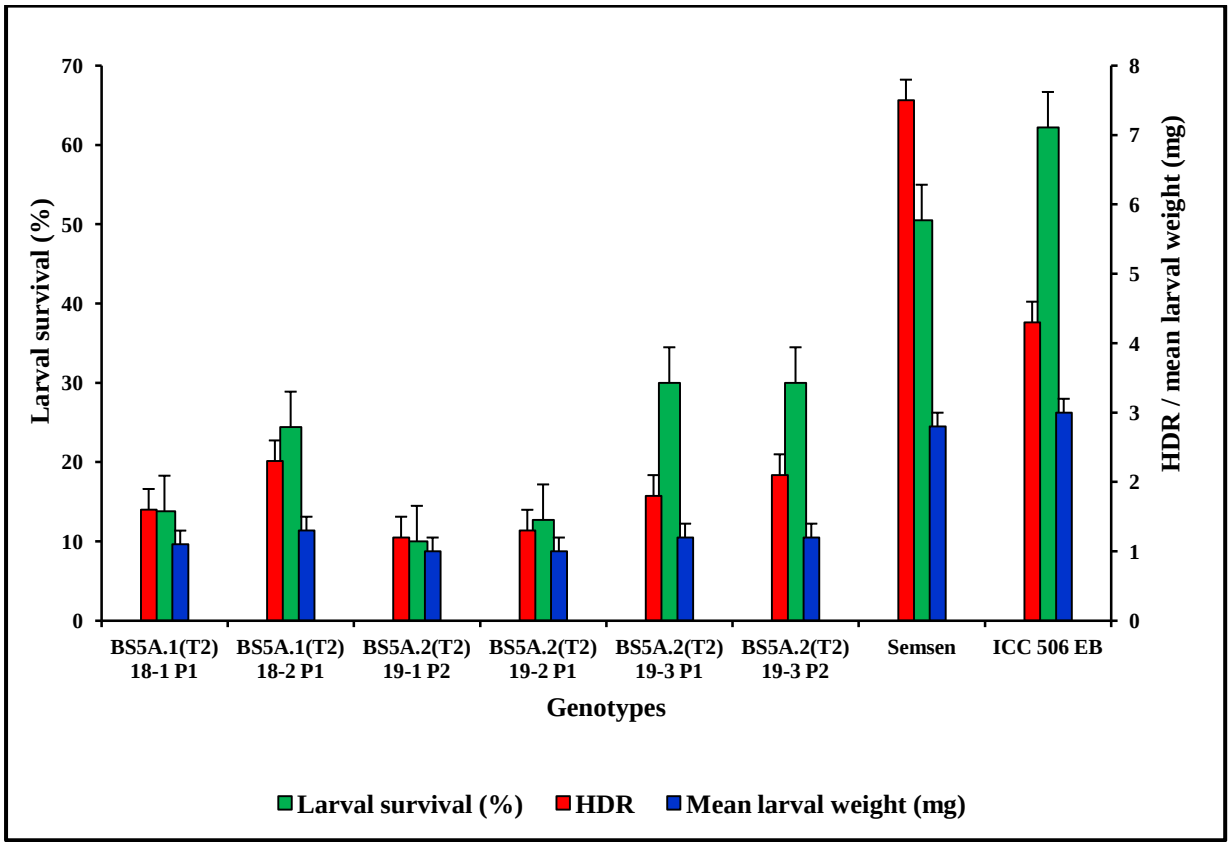


Figure 4: Evaluation of transgenic chickpea lines for resistance to *H. armigera* under greenhouse conditions (November planting 2012-2013)

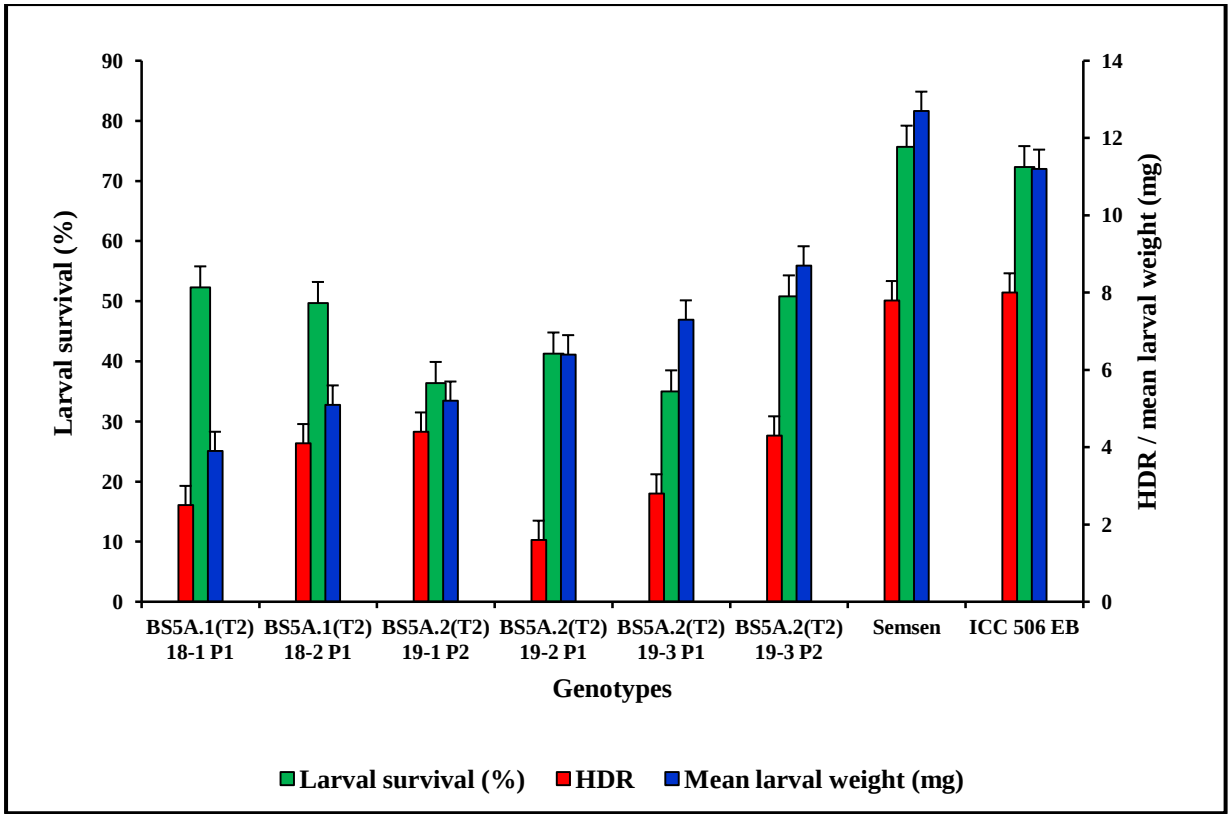


Figure 5: Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique (2011-2012)

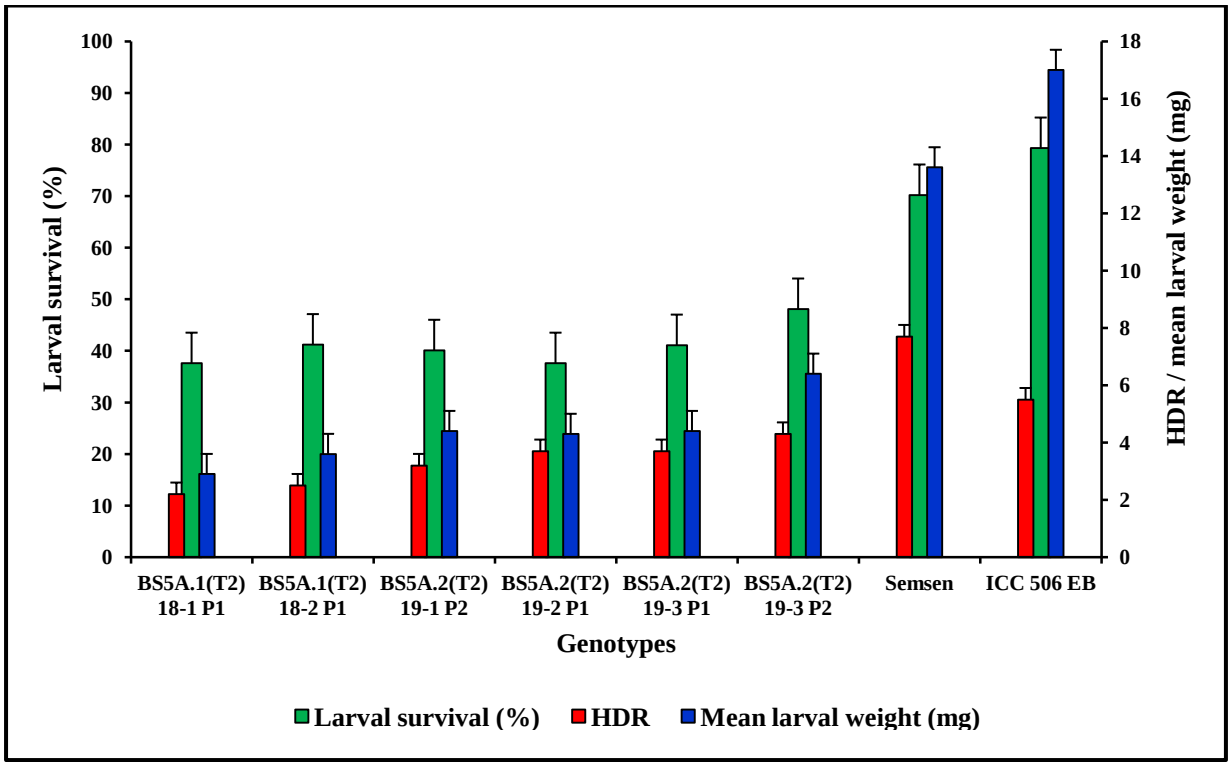


Figure 6: Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique (2012-2013)

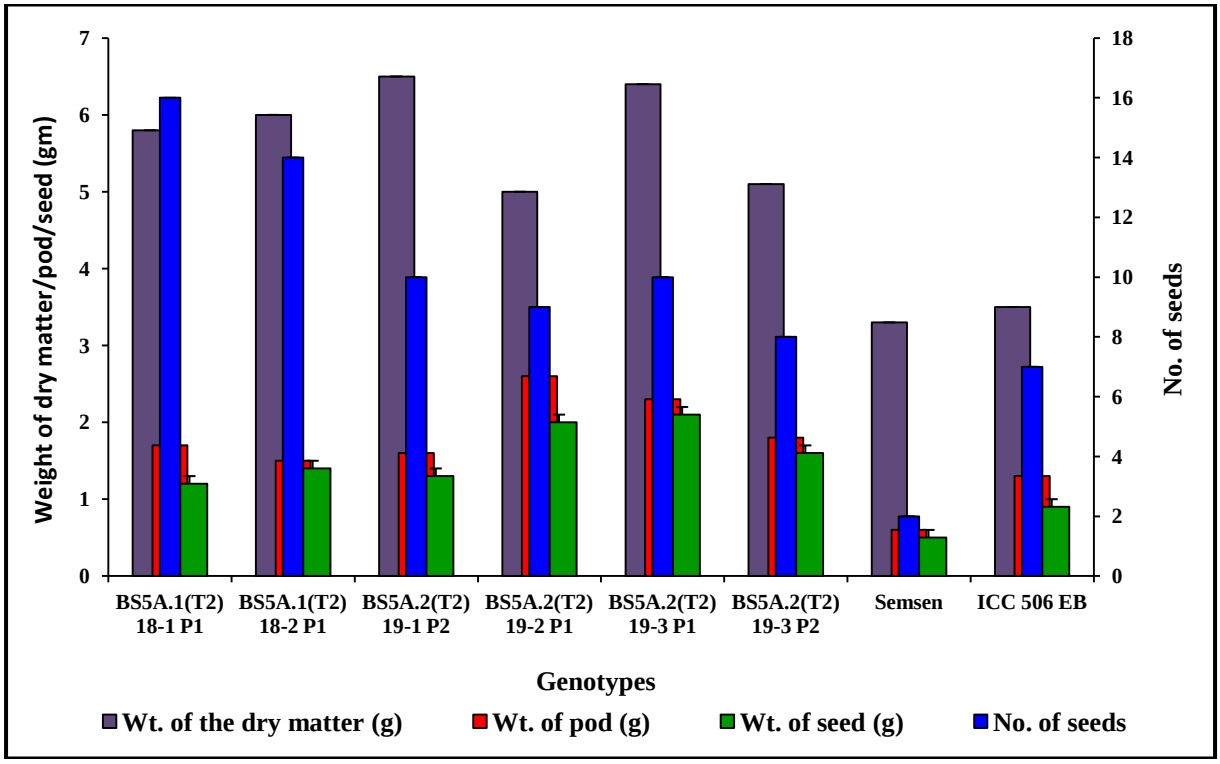


Figure 7: Agrnomic performance of transgenic chickpea lines (g/3 plants) with resistance to *H. armigera* under greenhouse conditions using cage technique (2011-2012)

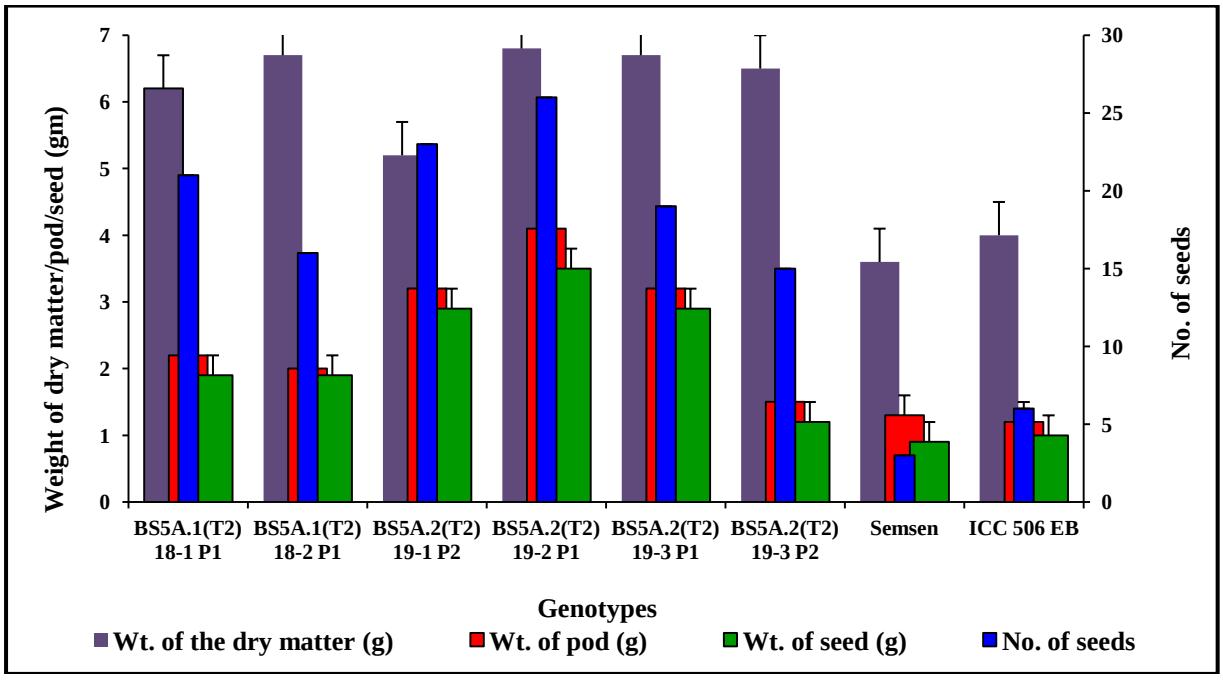


Figure 8: Agrnomic performance of transgenic chickpea lines (g/3 plants) with resistance to *H. armigera* under greenhouse conditions using cage technique (2012-2013)

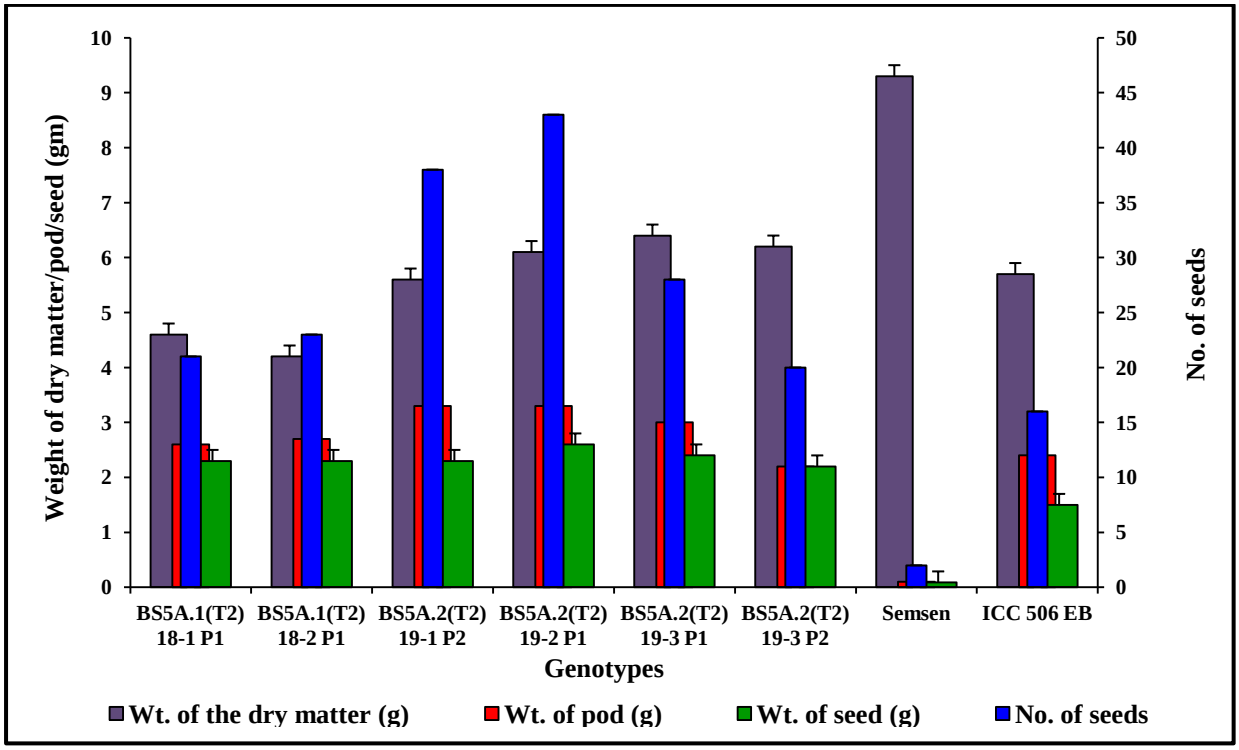


Figure 9: Agronomic performance of transgenic chickpea lines in un infested plants (g/3 plants) under green house conditions (2011-2012)

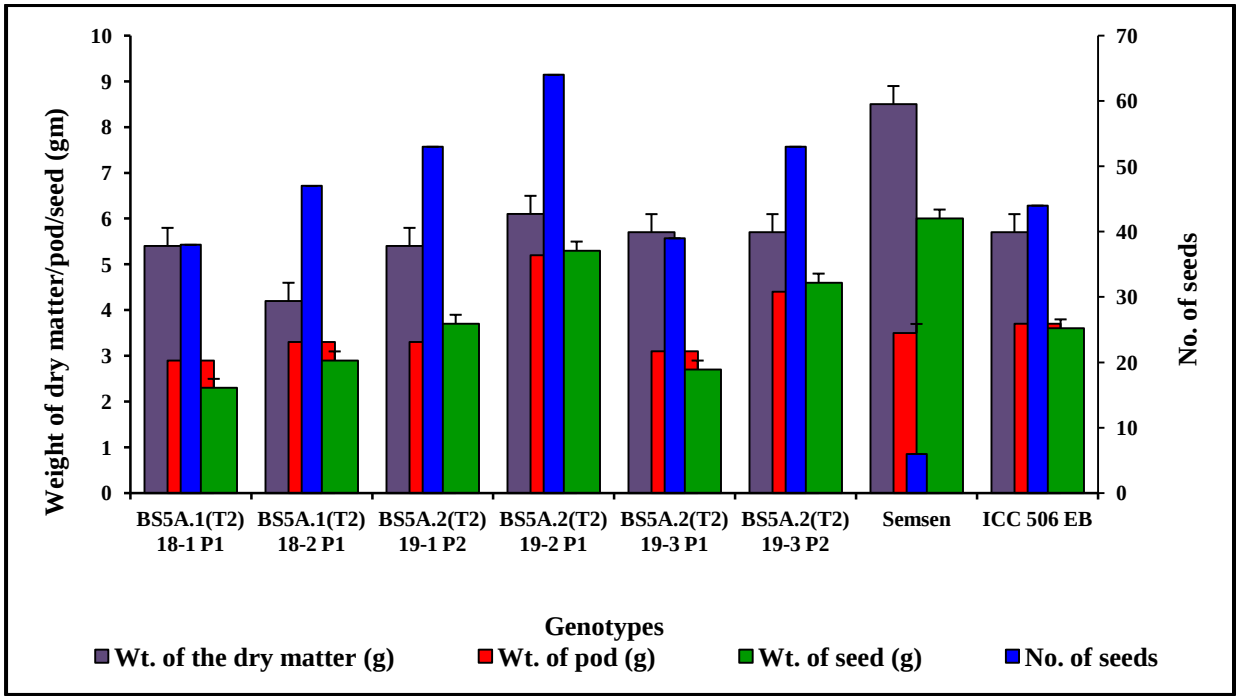
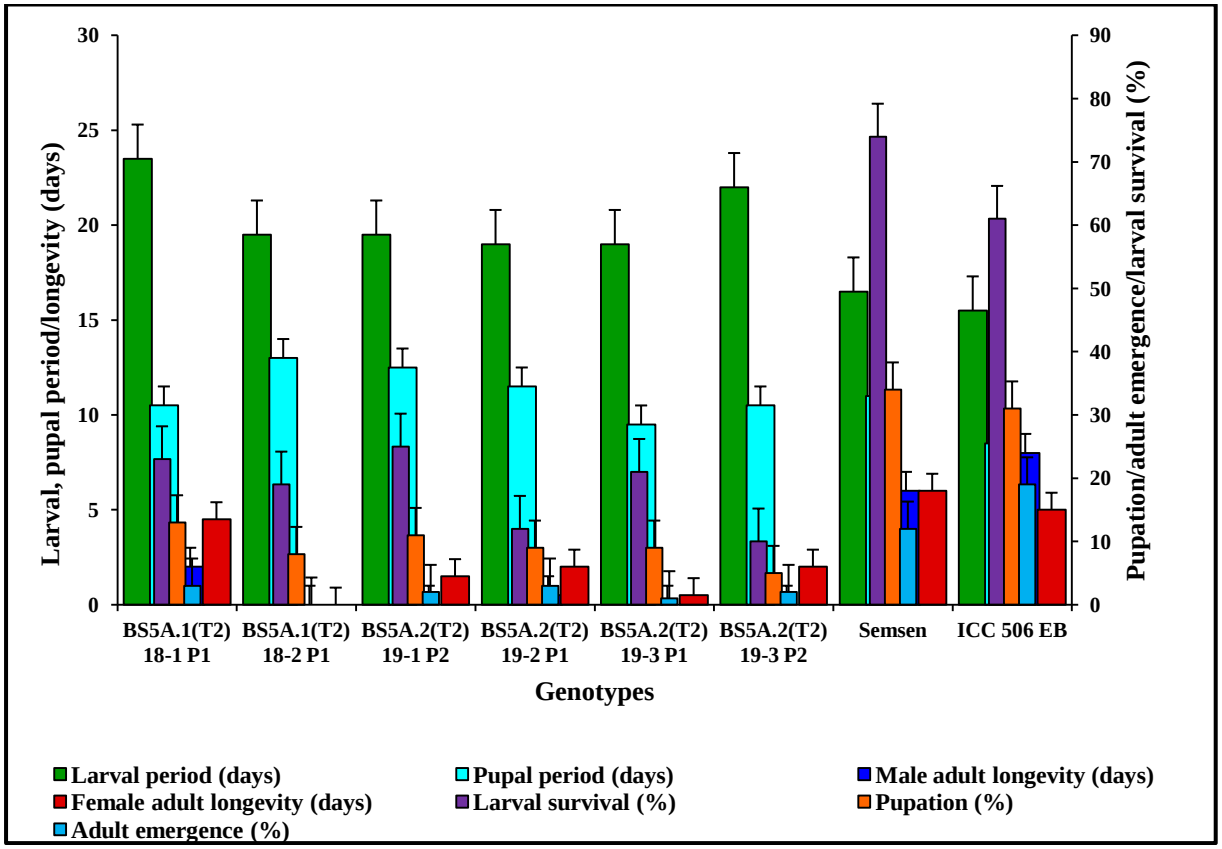


Figure 10: Agronomic performance of transgenic chickpea lines in un infested plants (g/3 plants) under green house conditions (2012-2013)

a)



b)

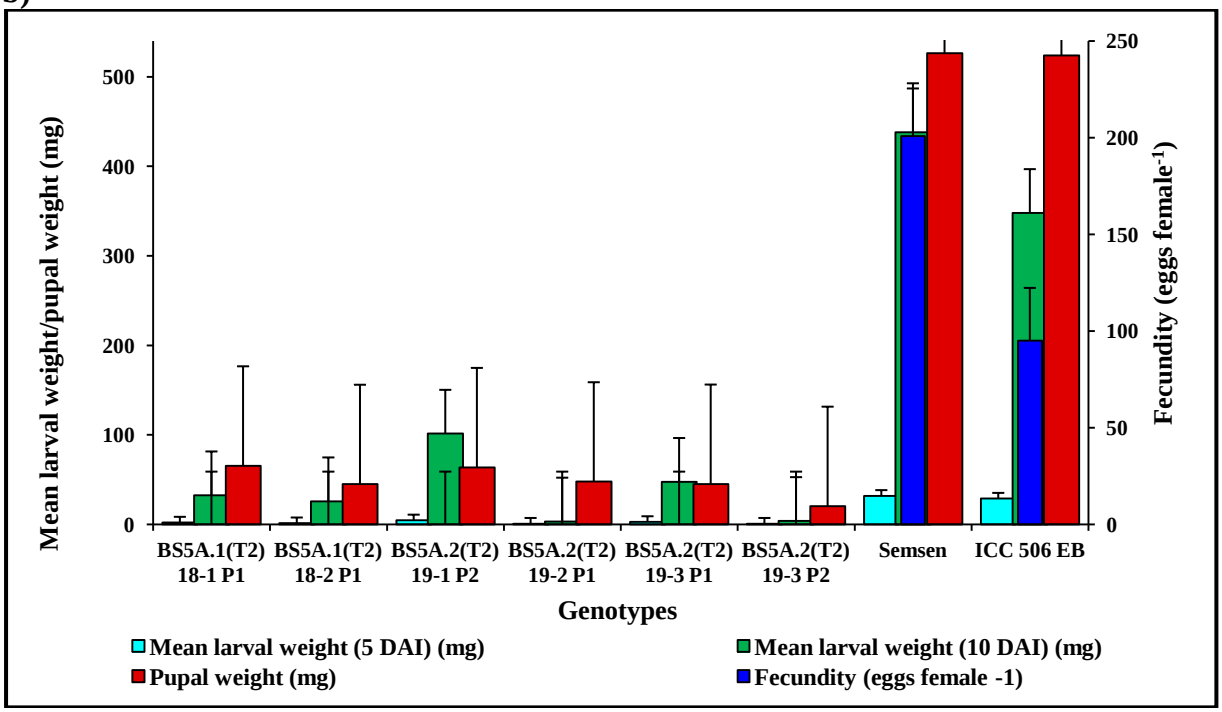
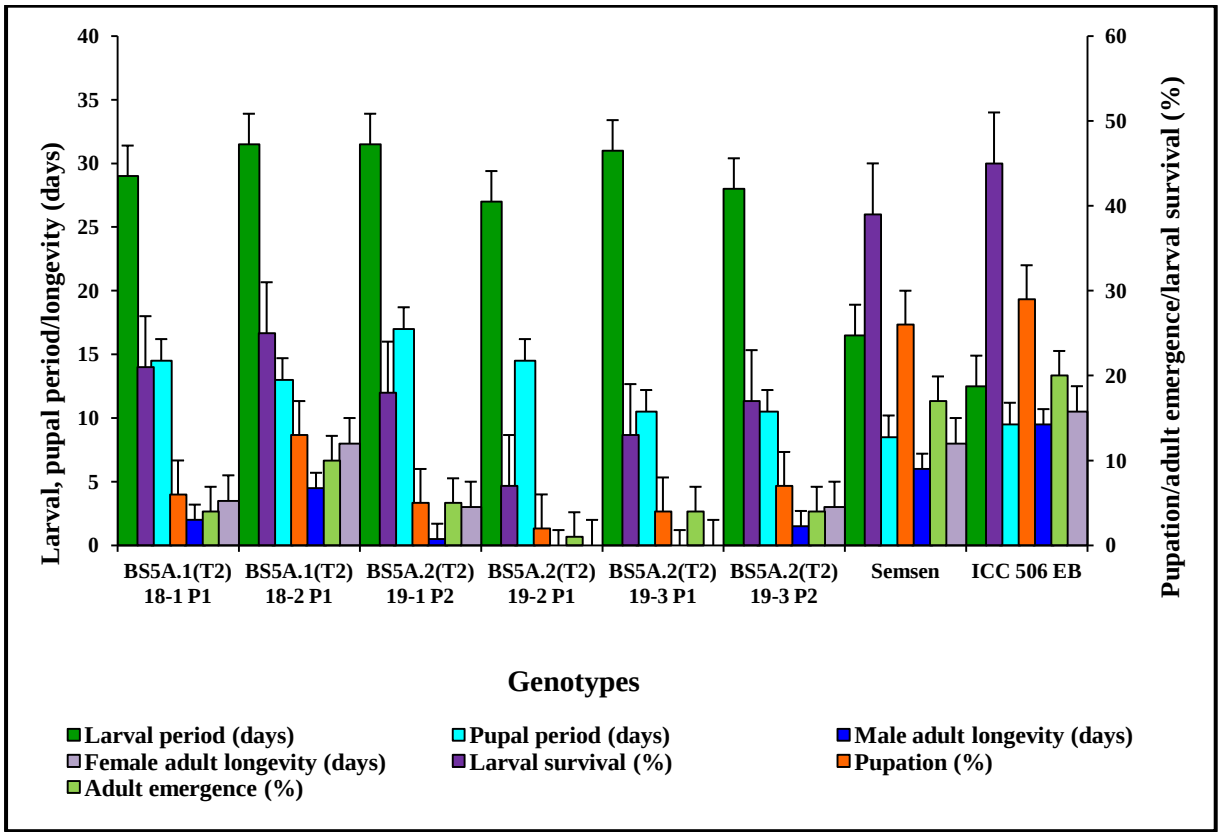


Figure 11: Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (2011-2012).

a)



b)

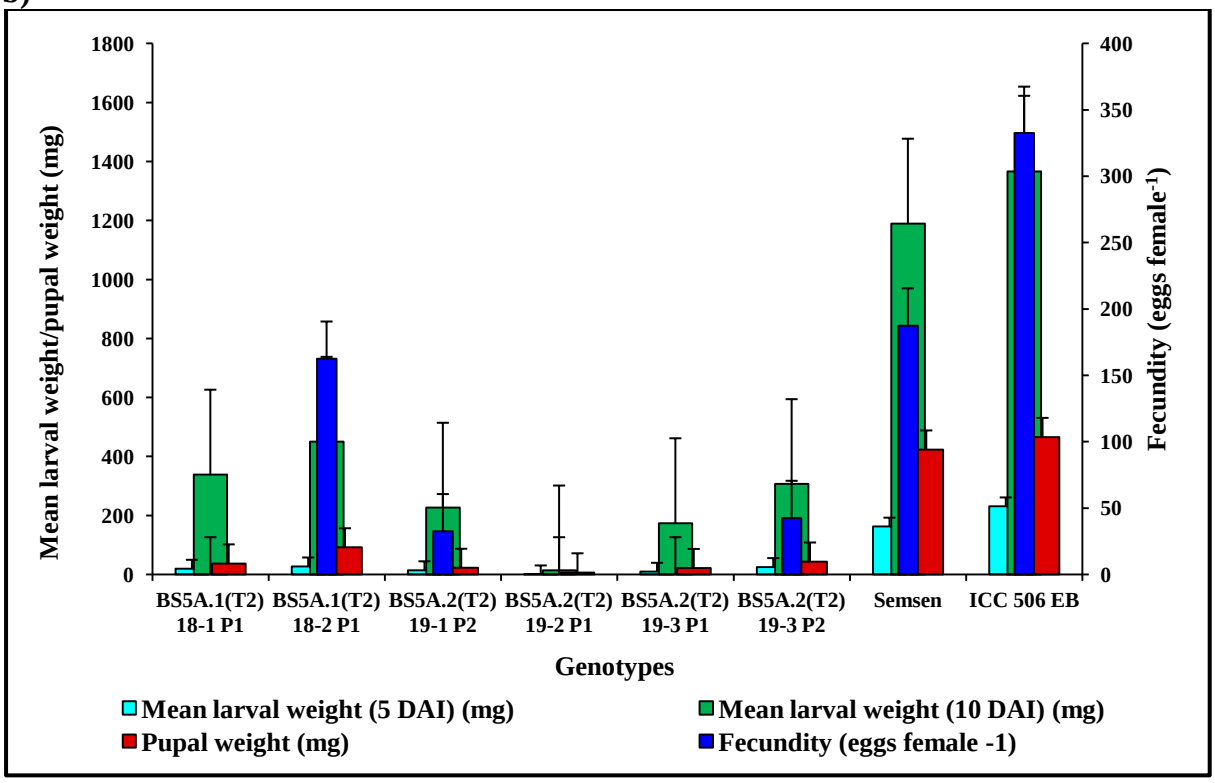
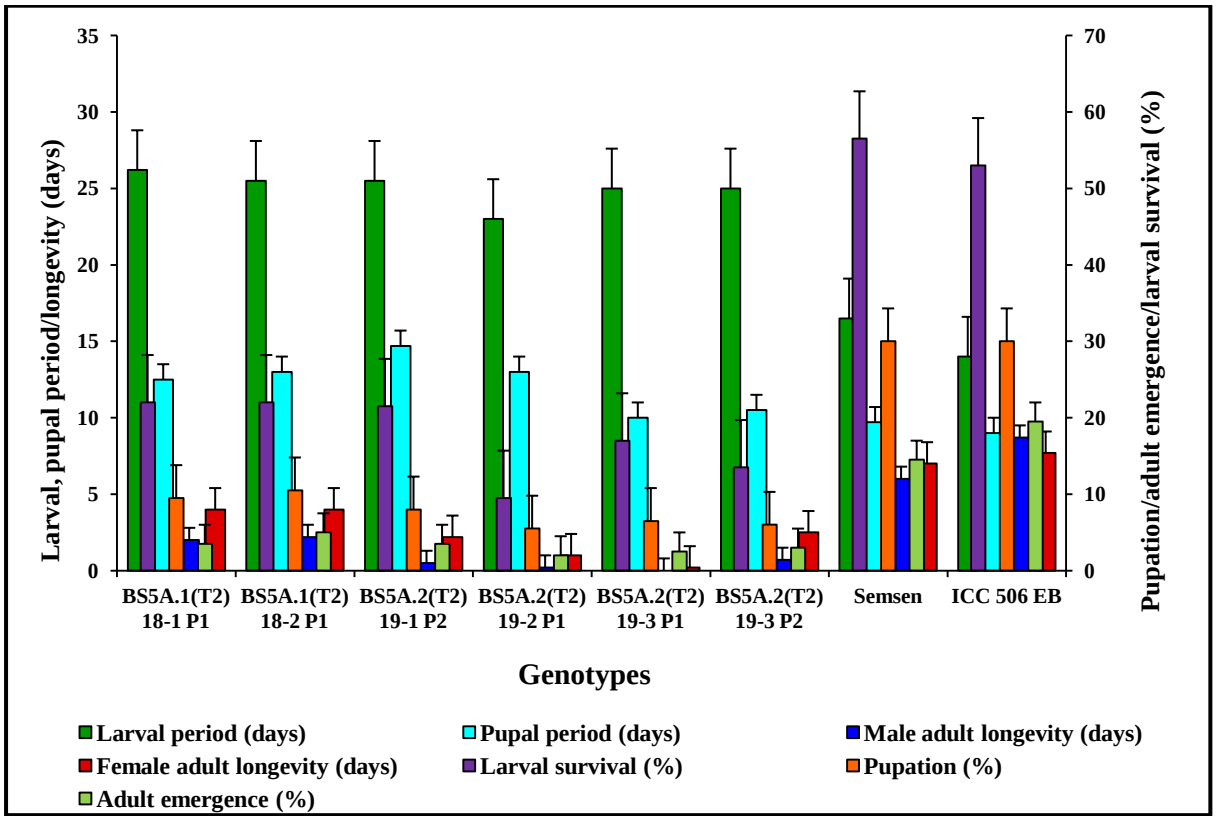


Figure 12: Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (2012-13)

a)



b)

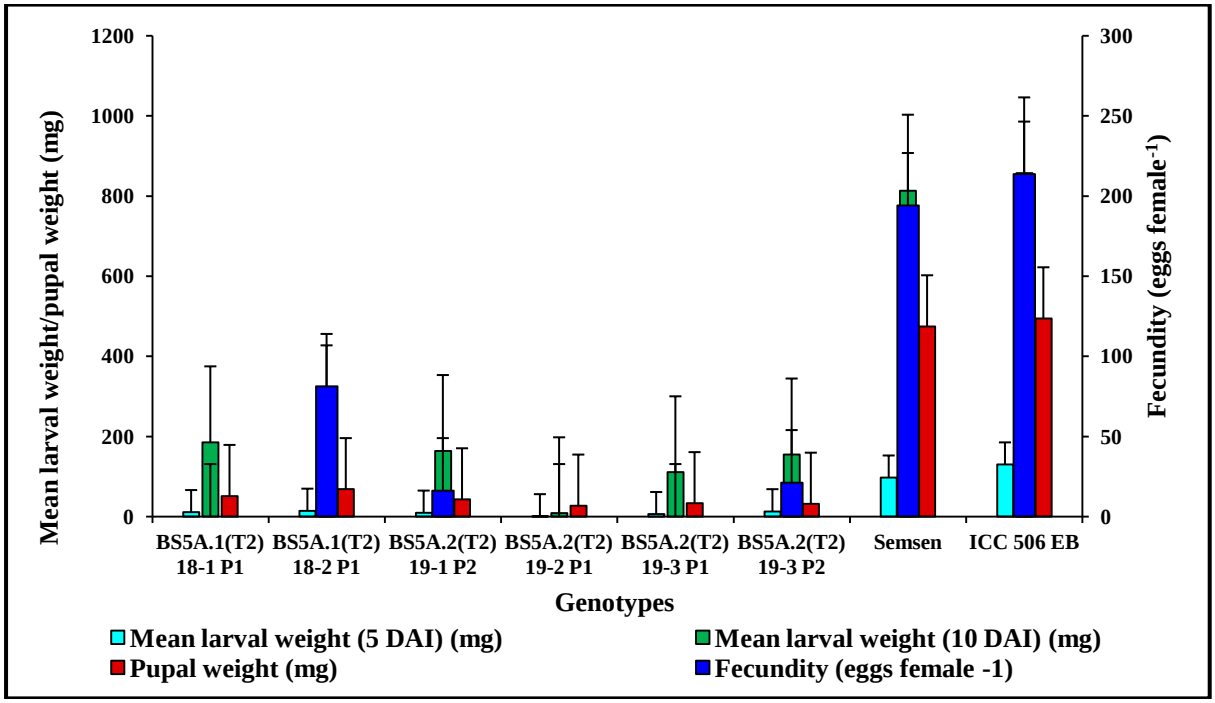


Figure 13: Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (2011- 2013, Pooled)

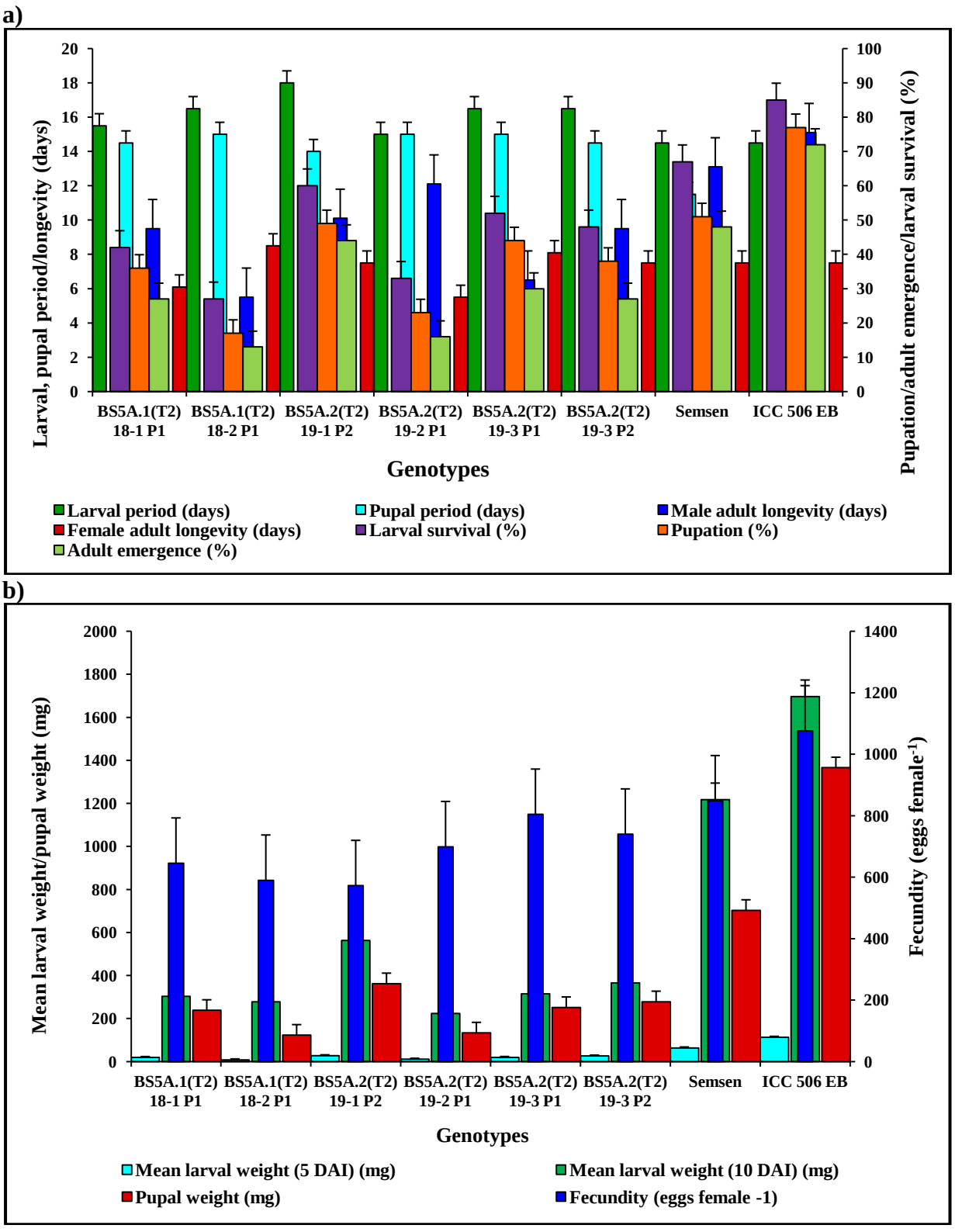
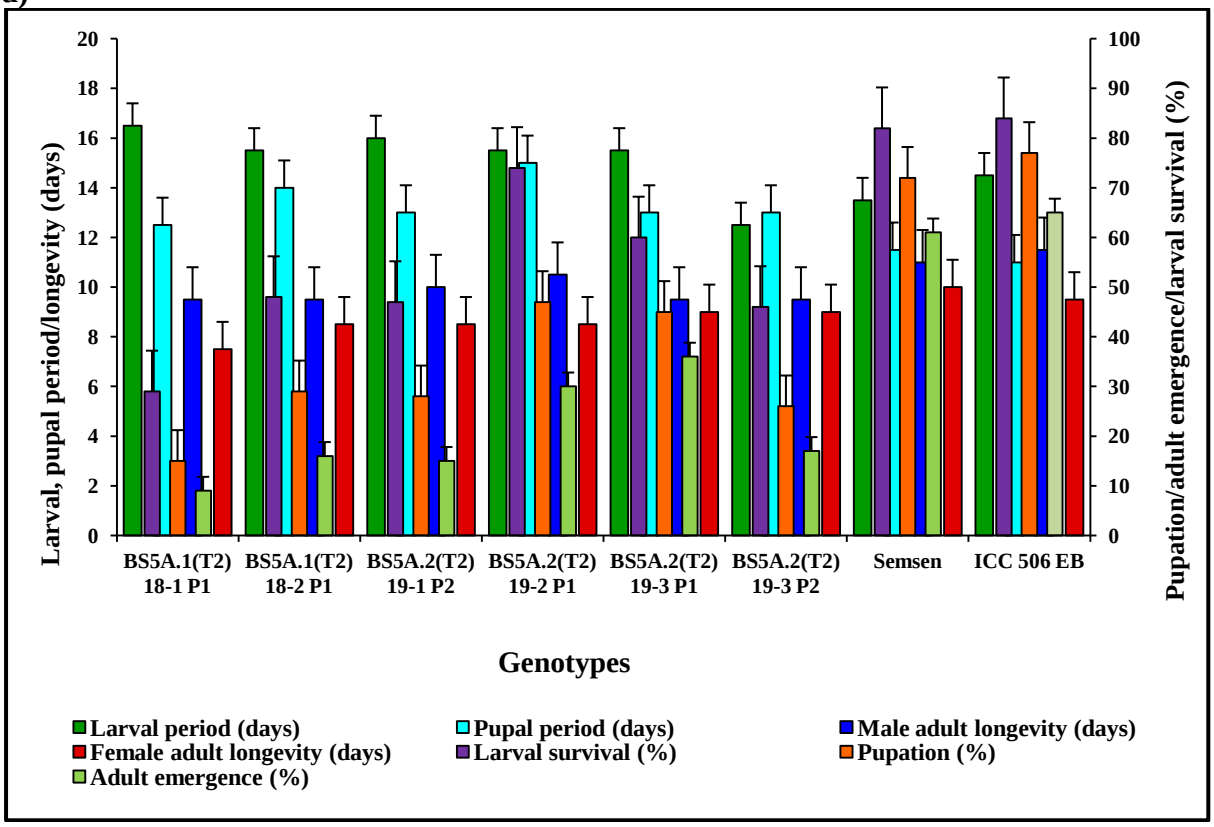


Figure 14: Survival and development of third-instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (2011-12)

a)



b)

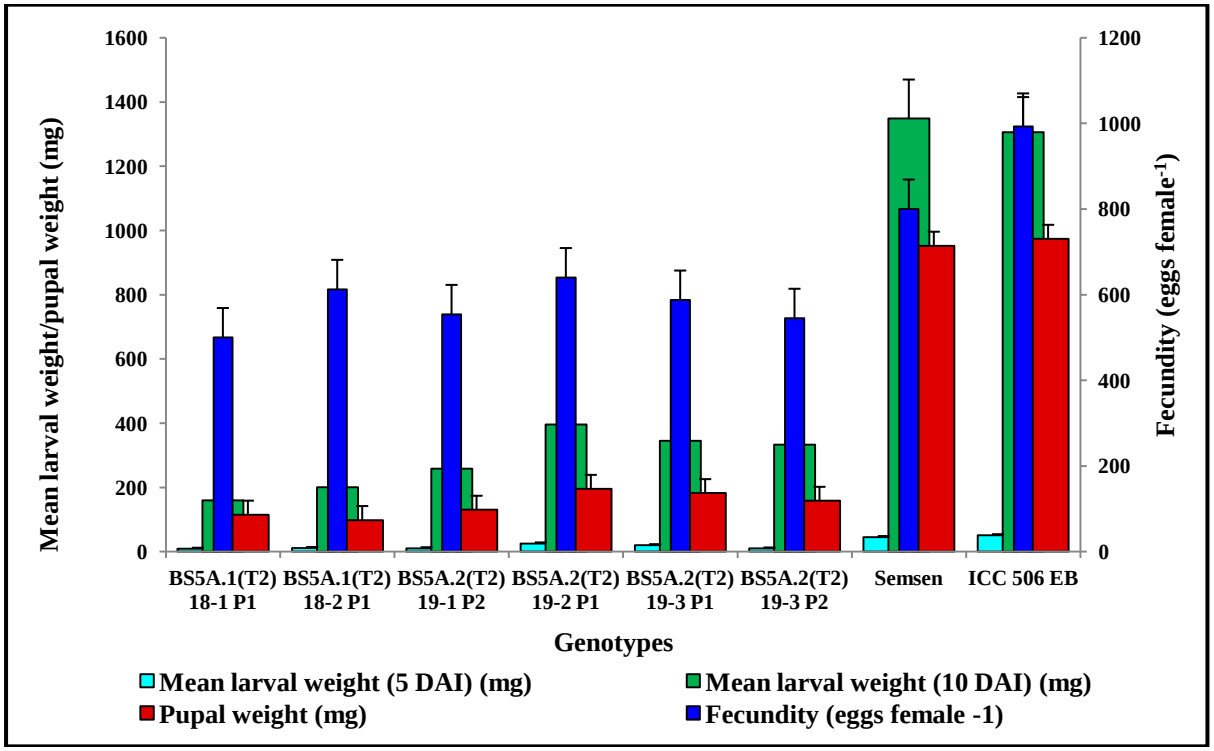
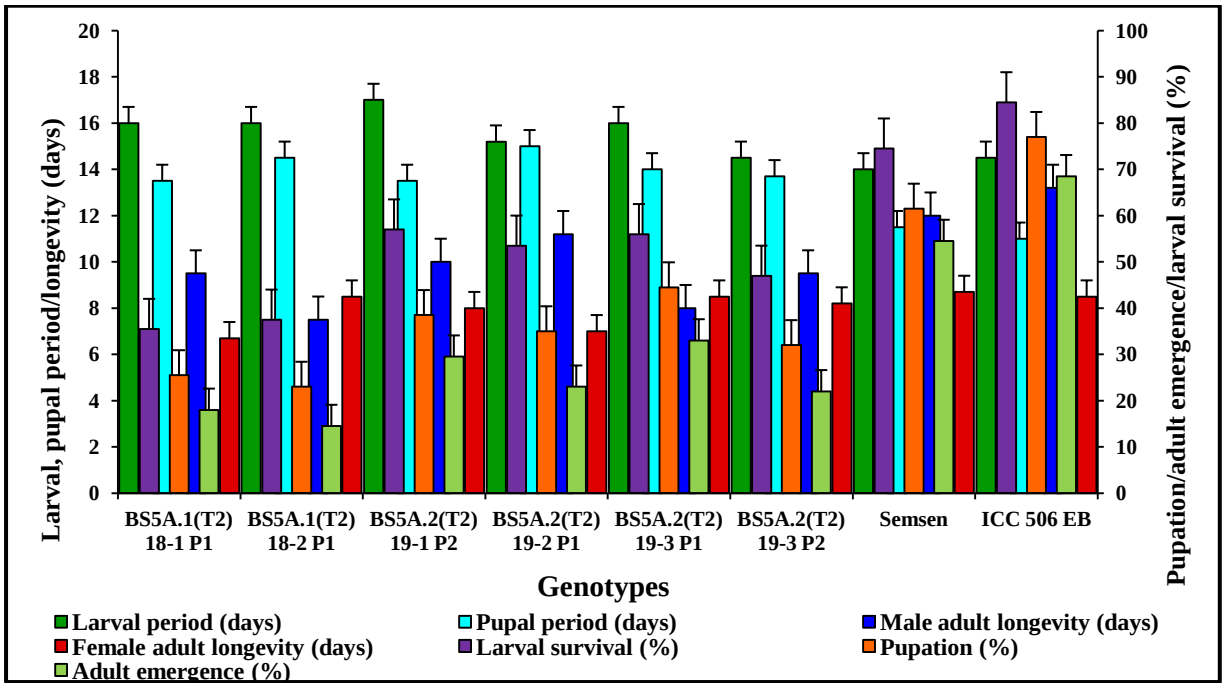


Figure 15: Survival and development of third-instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (2012-13)

a)



b)

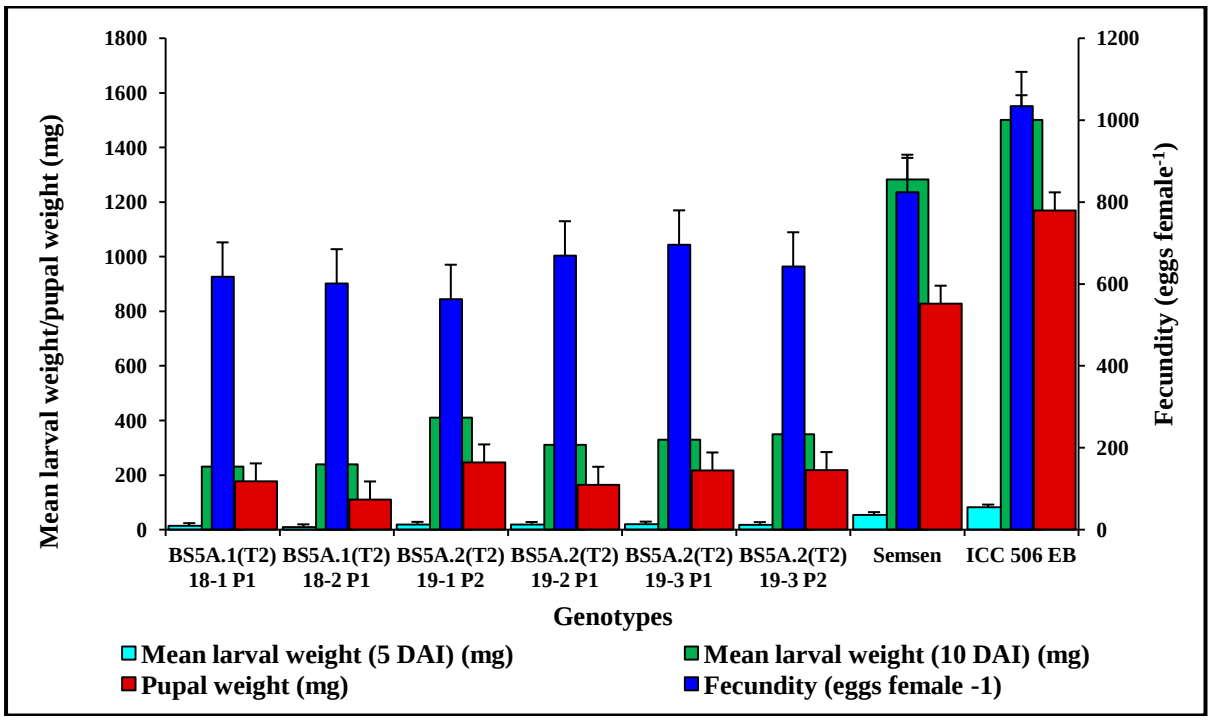


Figure 16: Survival and development of third-instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (2011-2013 Pooled)

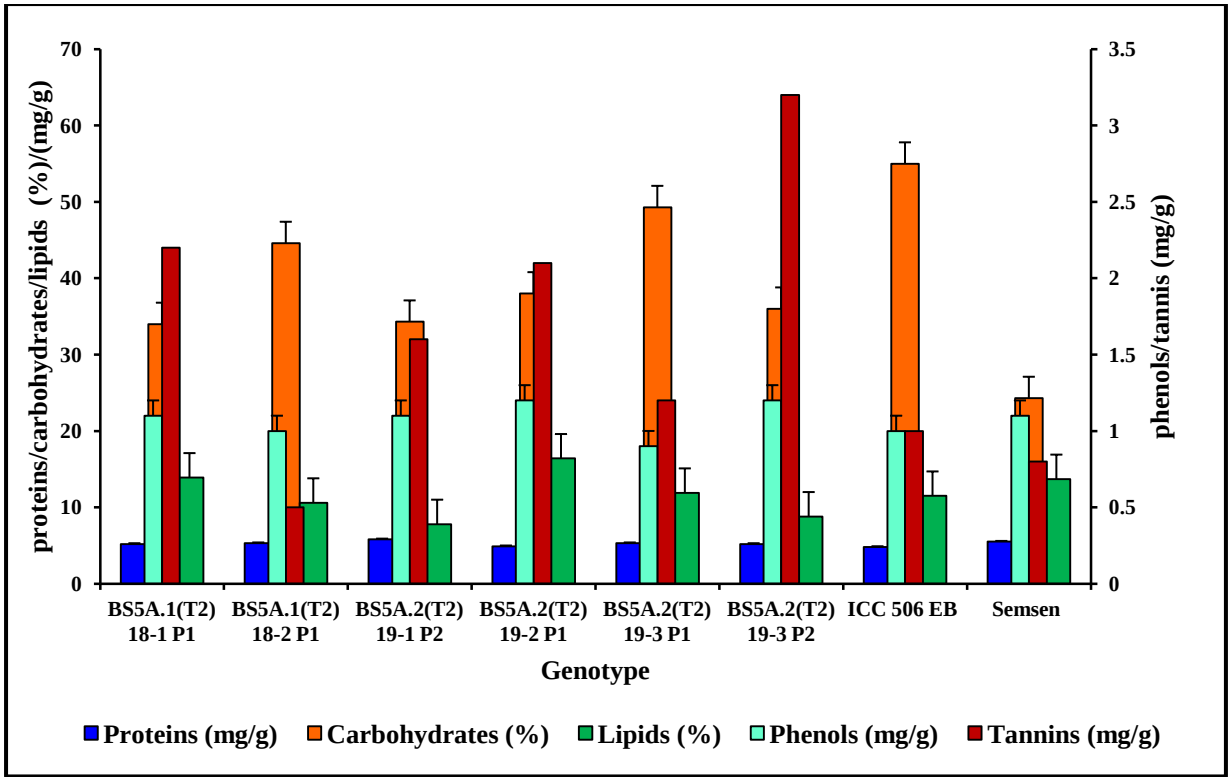


Figure 17: Biochemical profile of different transgenic chickpea lines (dry wt basis) (2011-12)

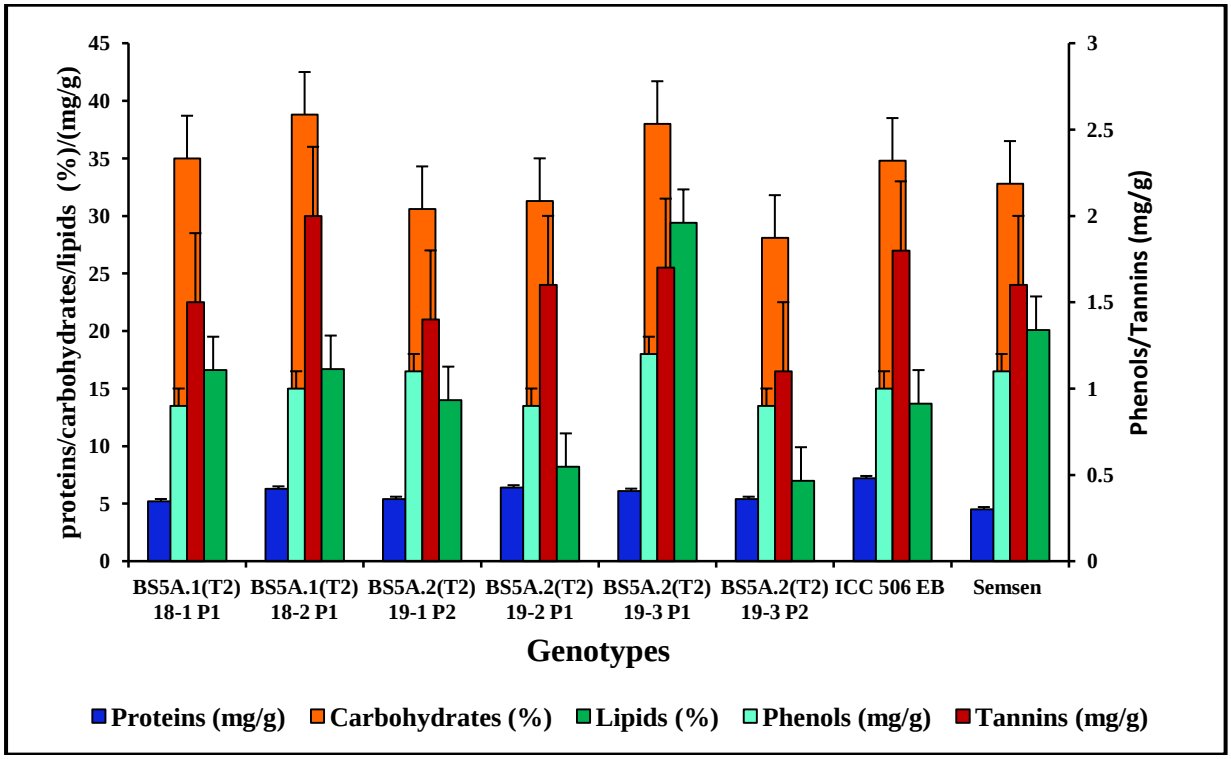


Figure 18: Biochemical profile of different transgenic chickpea lines (dry wt basis) (2012-13)

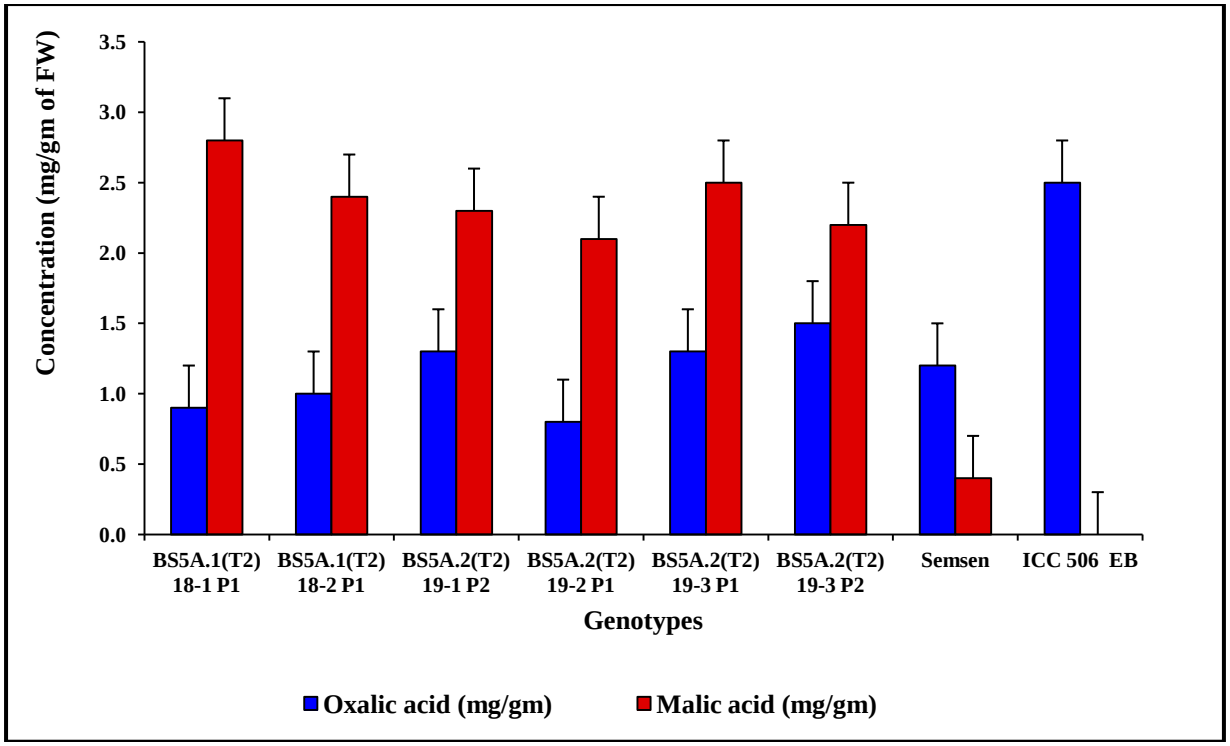


Figure 19: Concentration of organic acids (on fresh weight basis) present on the leaf surface of transgenic chickpea lines 2011-2012)

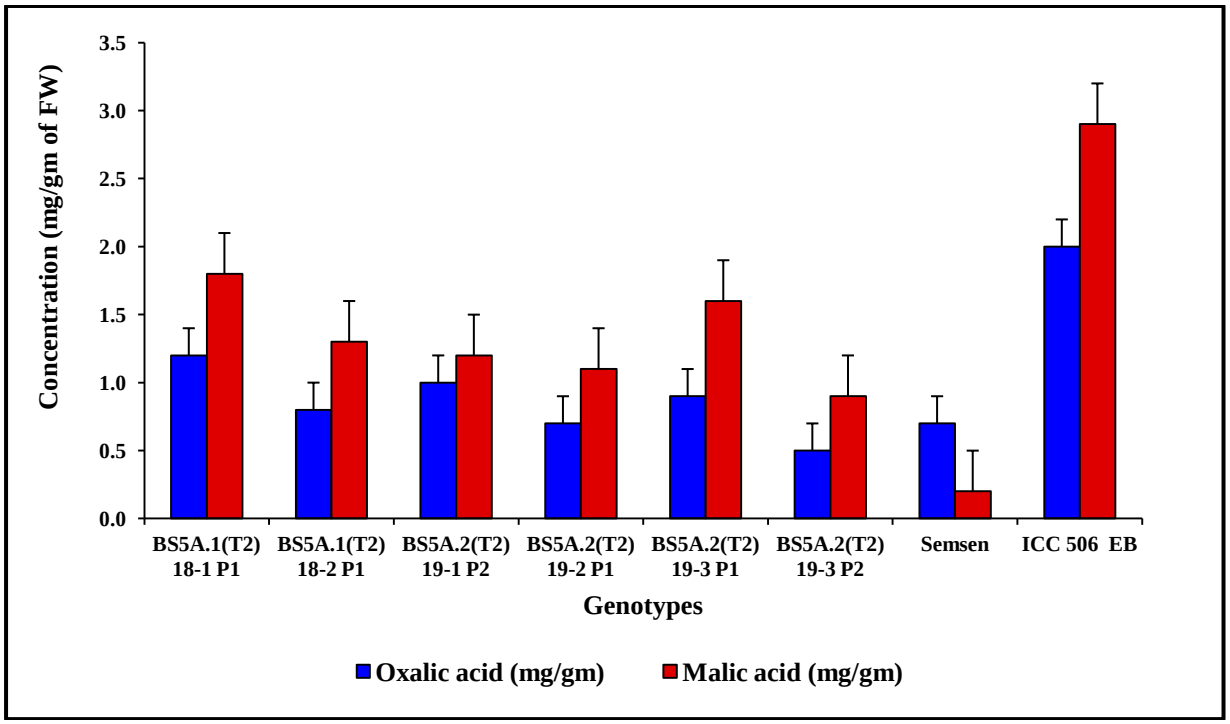


Figure 20: Concentration of organic acids (on fresh weight basis) present on the leaf surface of transgenic chickpea lines 2012-2013)

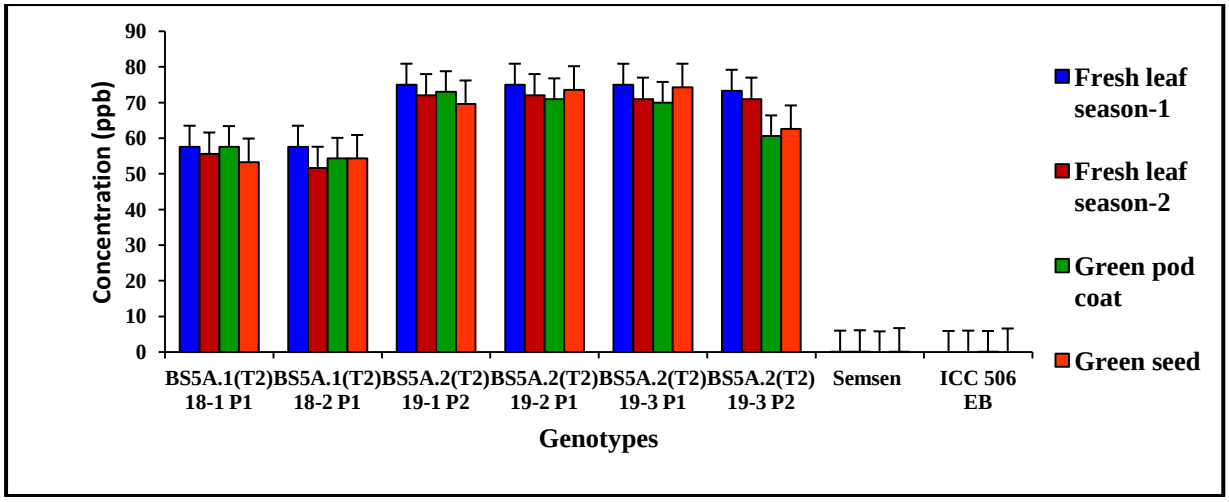


Figure 21: Amount of Cry IIa protein (ppb) in different green plant parts of transgenic chickpea lines

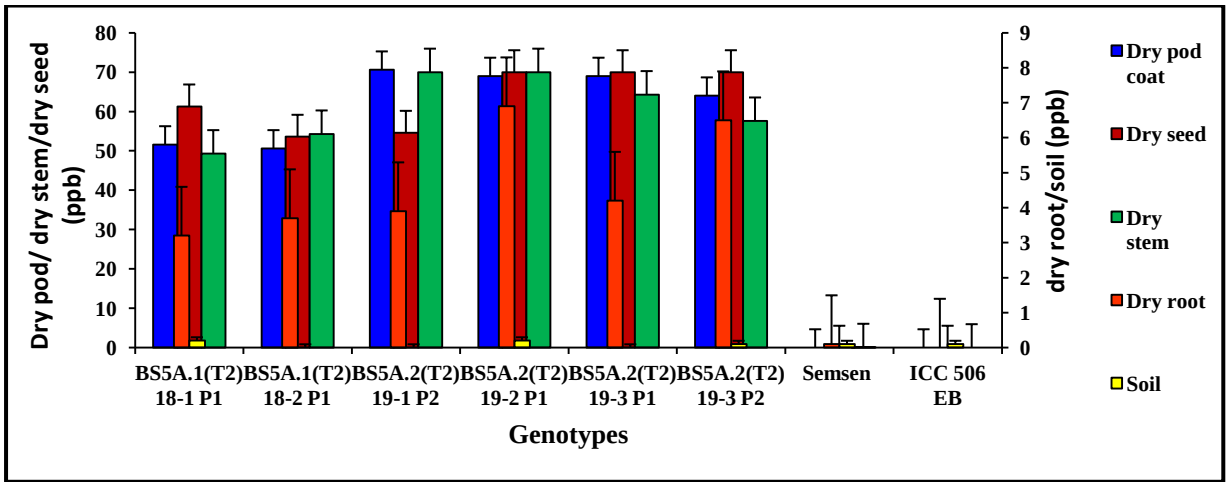


Figure 22: Amount of Cry IIa protein (ppb) in different dry plant parts of transgenic Chickpea lines

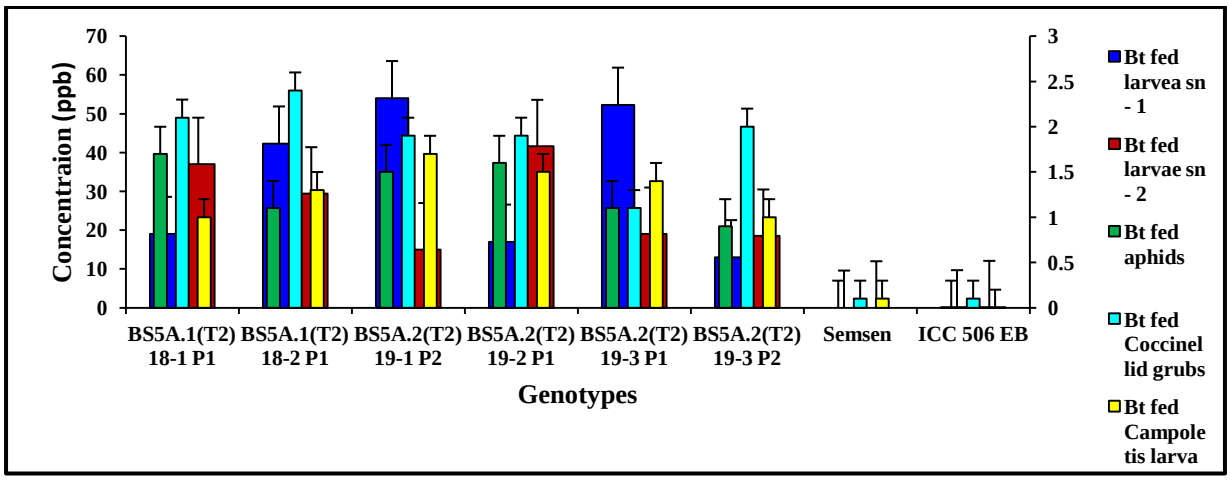


Figure 23: Amount of Cry IIa protein (ppb) in *Bt* fed *H. armigera* larvae, aphids and natural enemies

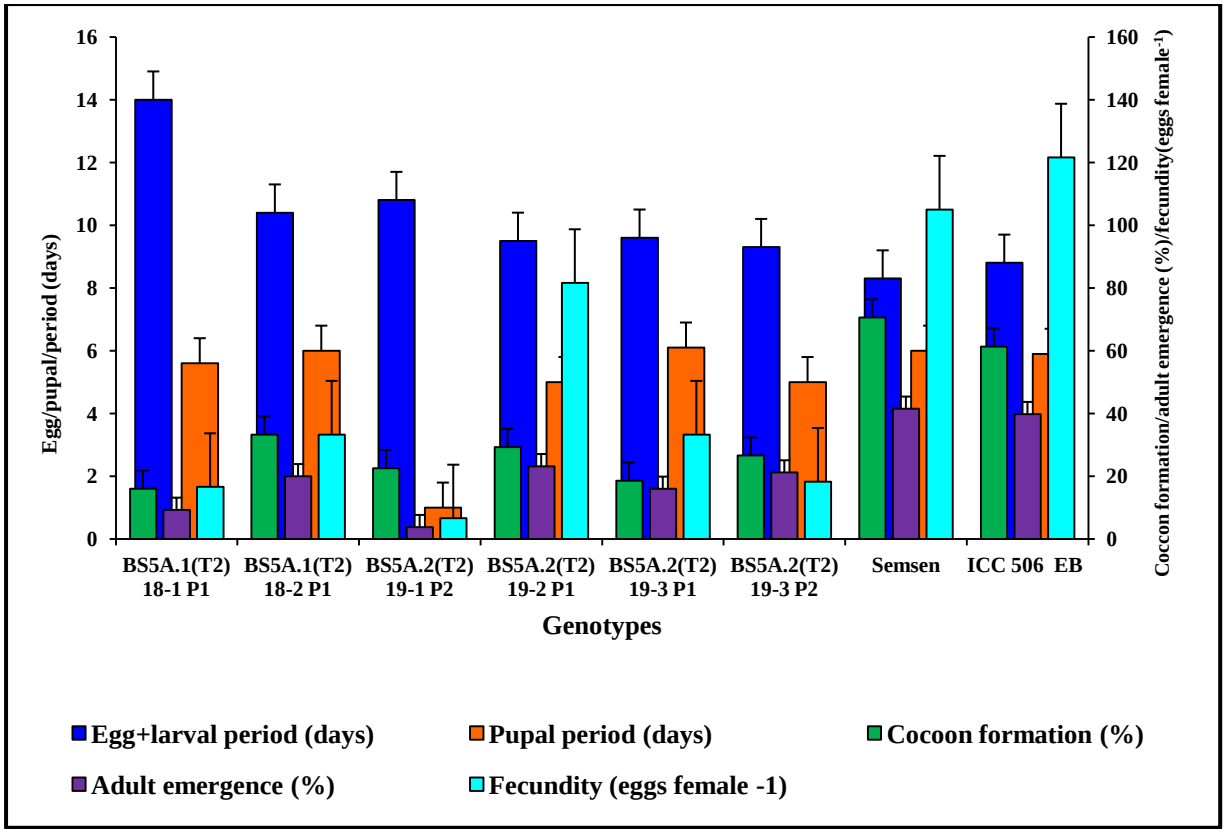


Figure 24: Biology of *Campoletis chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (October 2011-2012)

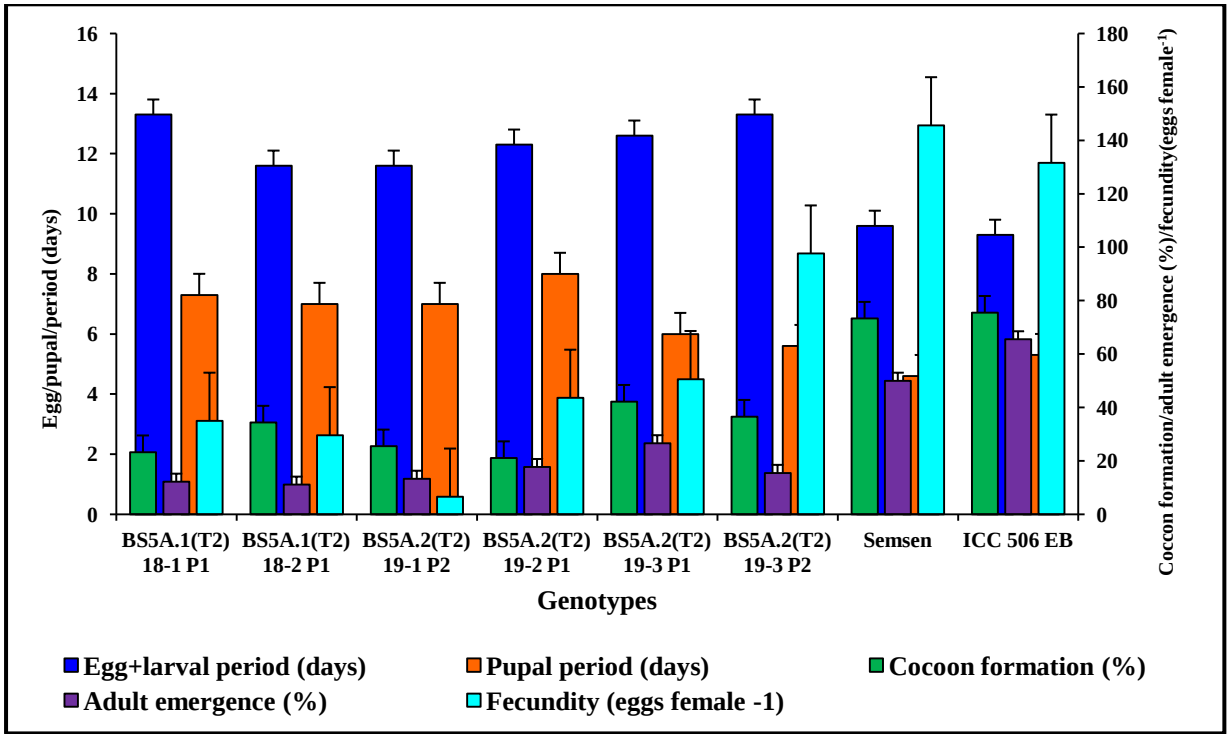


Figure 25: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (October 2012-2013)

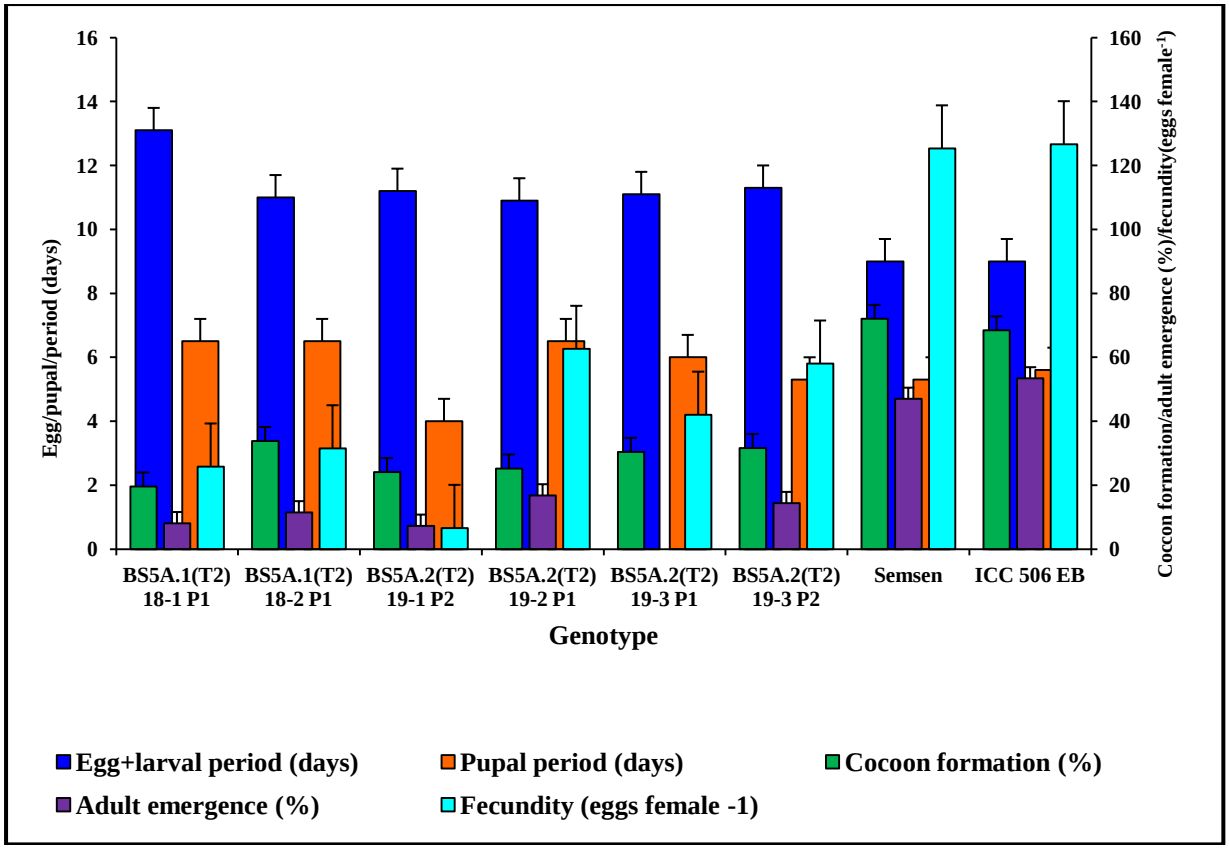


Figure 26: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (October 2011-2012 and 2012-2013) (Pooled analysis).

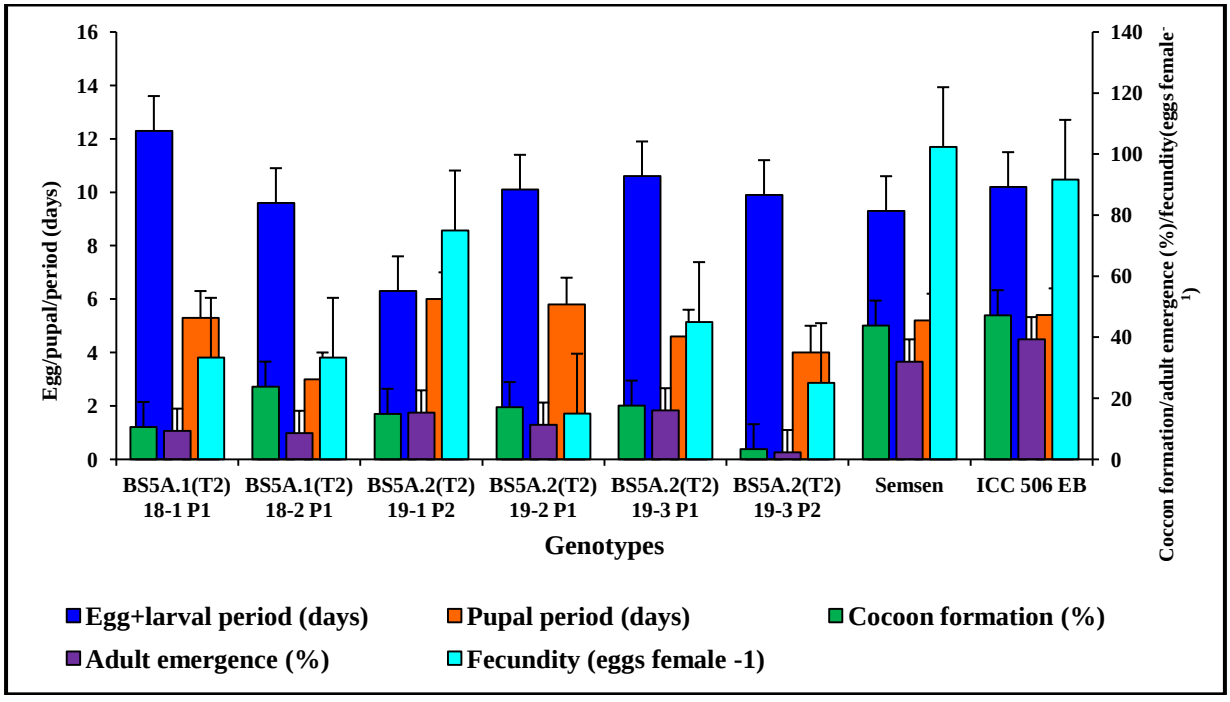


Figure 27: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (November 2011-2012)

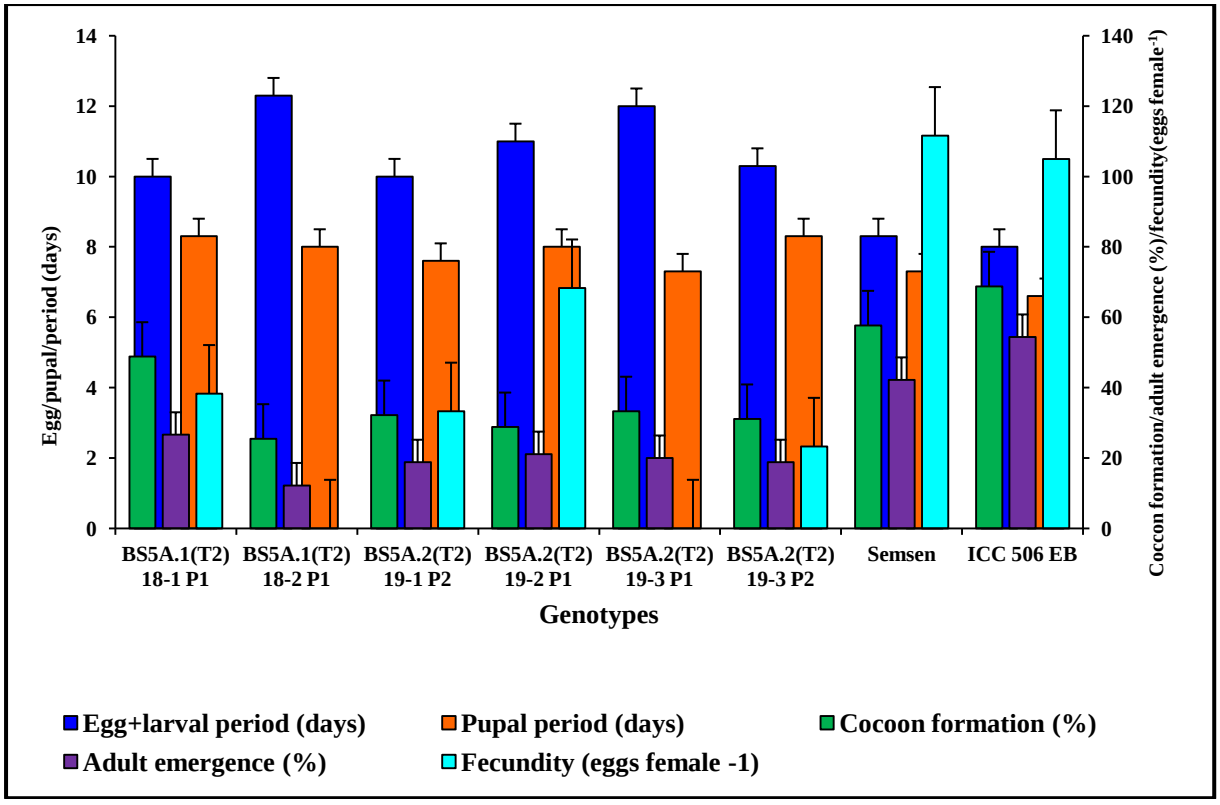


Figure 28: Biology of *C. chloridea* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (November 2012-2013)

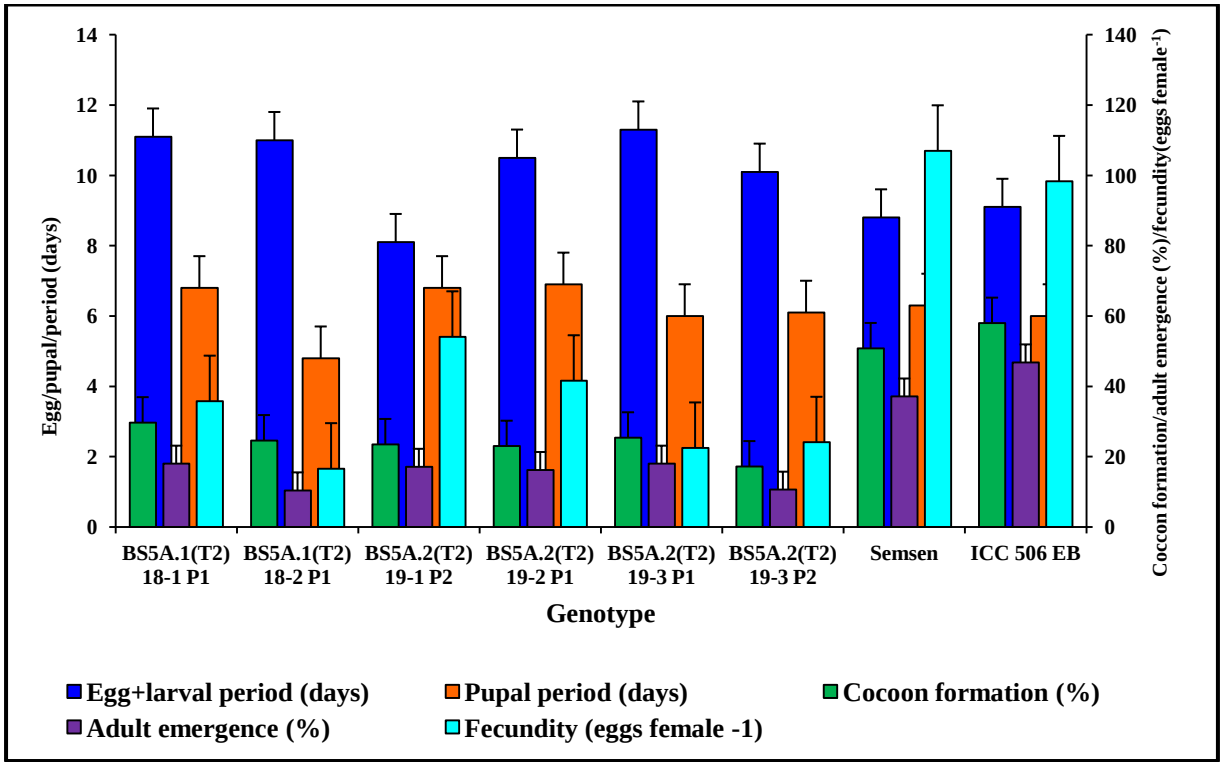


Figure 29: Biology of *C.chloridea* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (2012 and 2013, November, pooled)

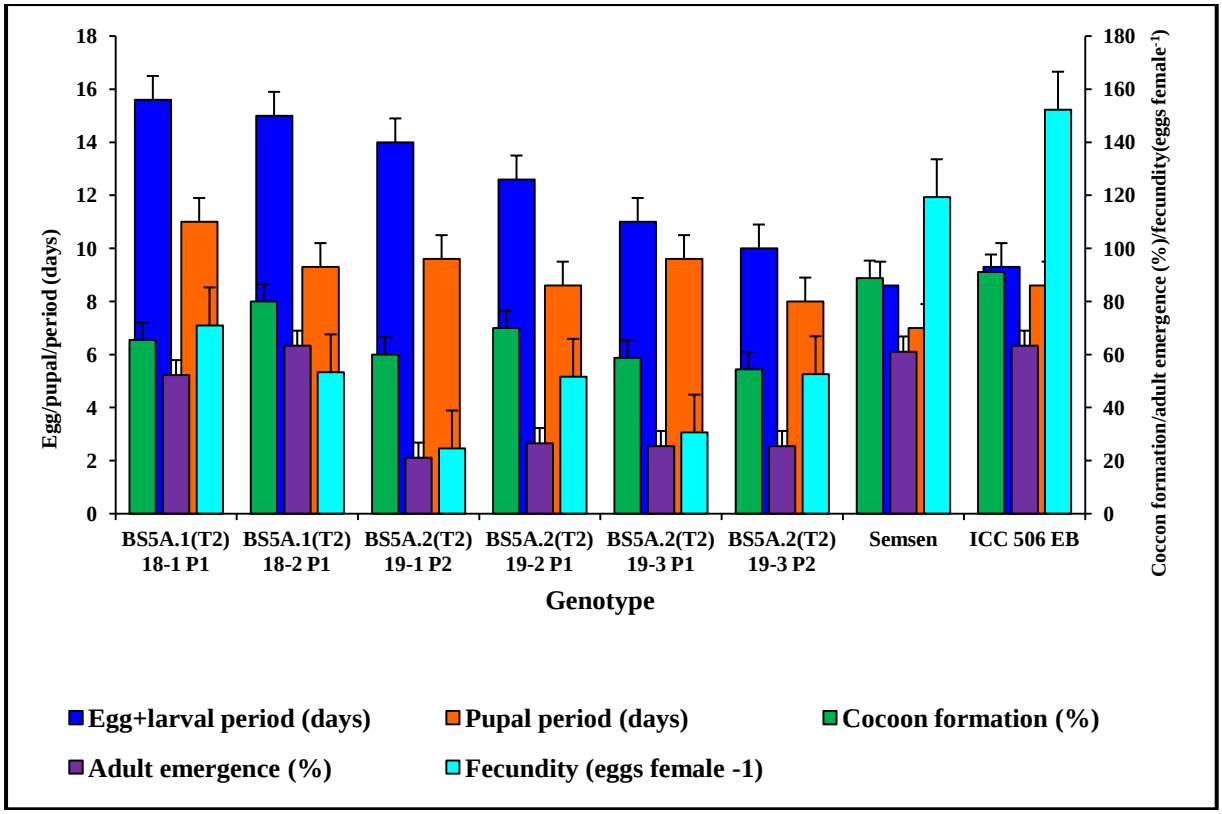


Figure 30: Biology of *C. chlorideae* parasitizing *H. armigera* fed on diets with lyophilized leaf powders of different transgenic chickpea lines (2011-2012)

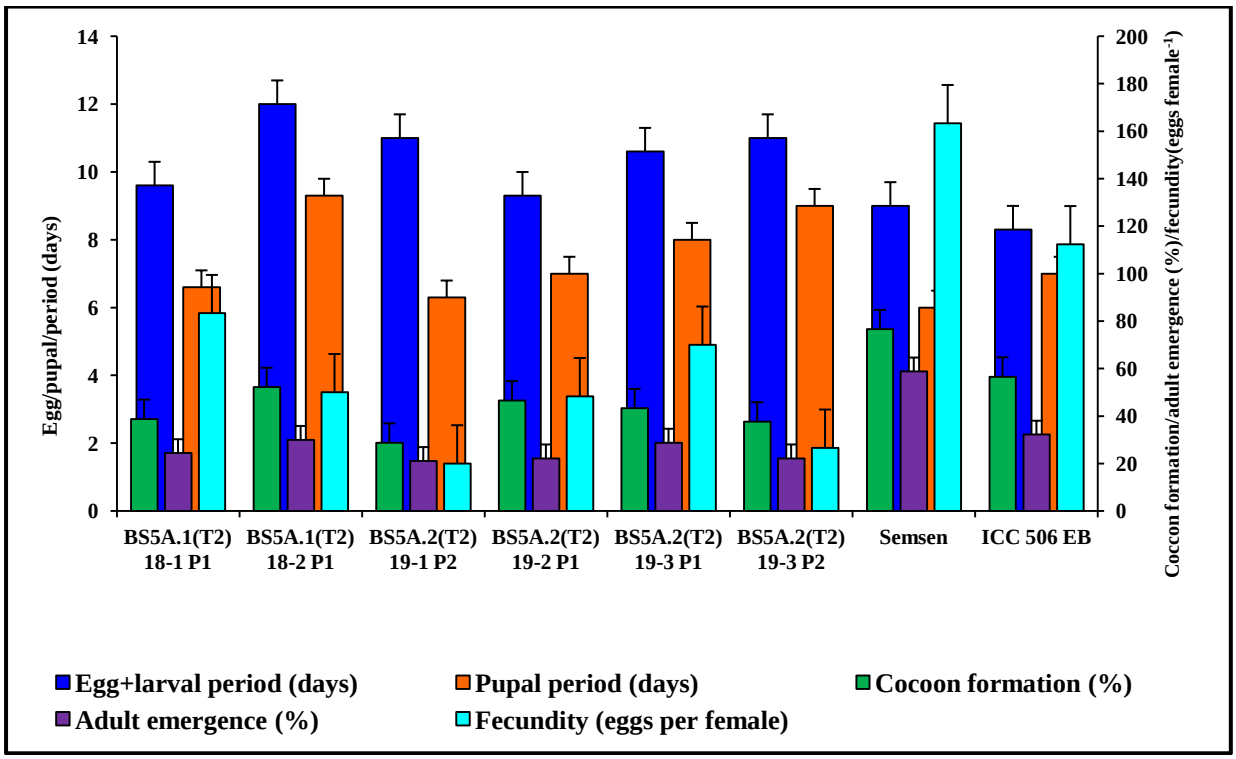


Figure 31: Biology of *C. chlorideae* parasitizing *H. armigera* fed on diets with lyophilized leaf powders of different transgenic chickpea lines (2012-2013)

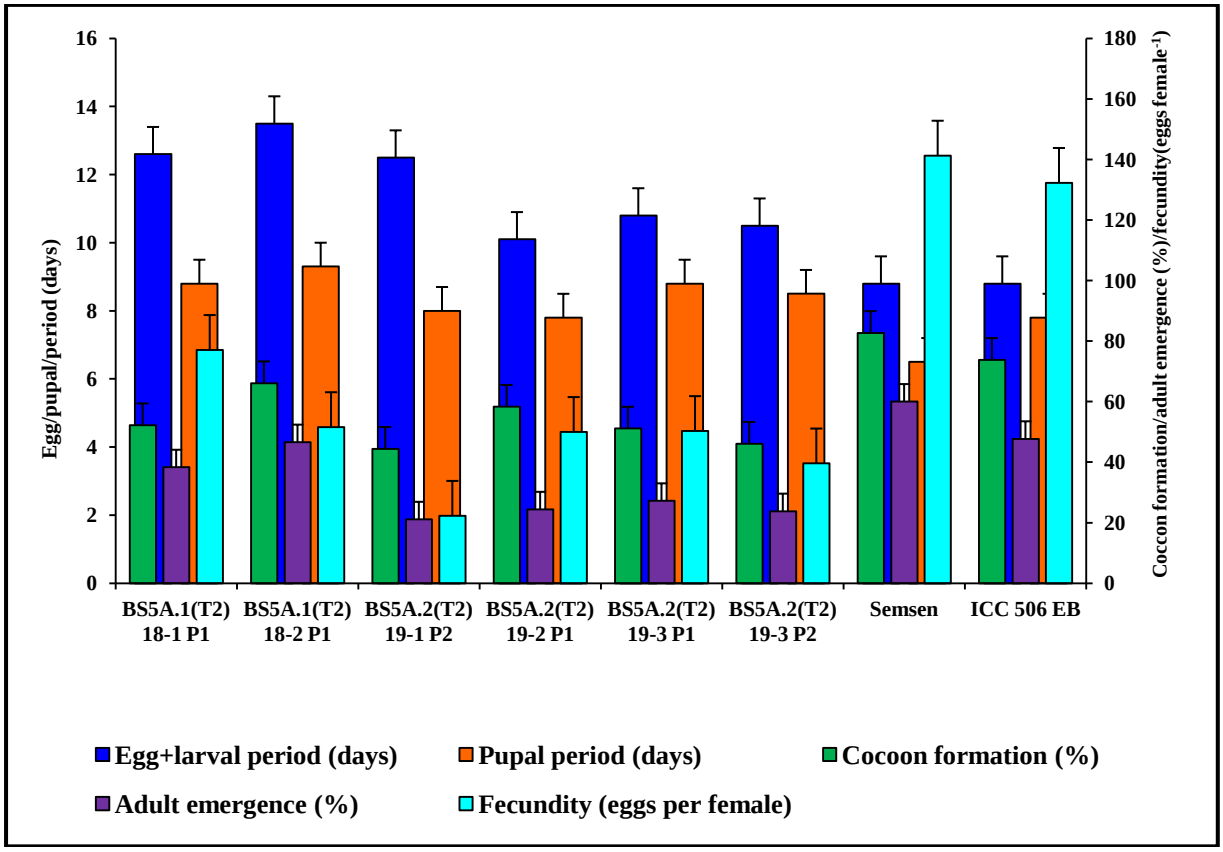
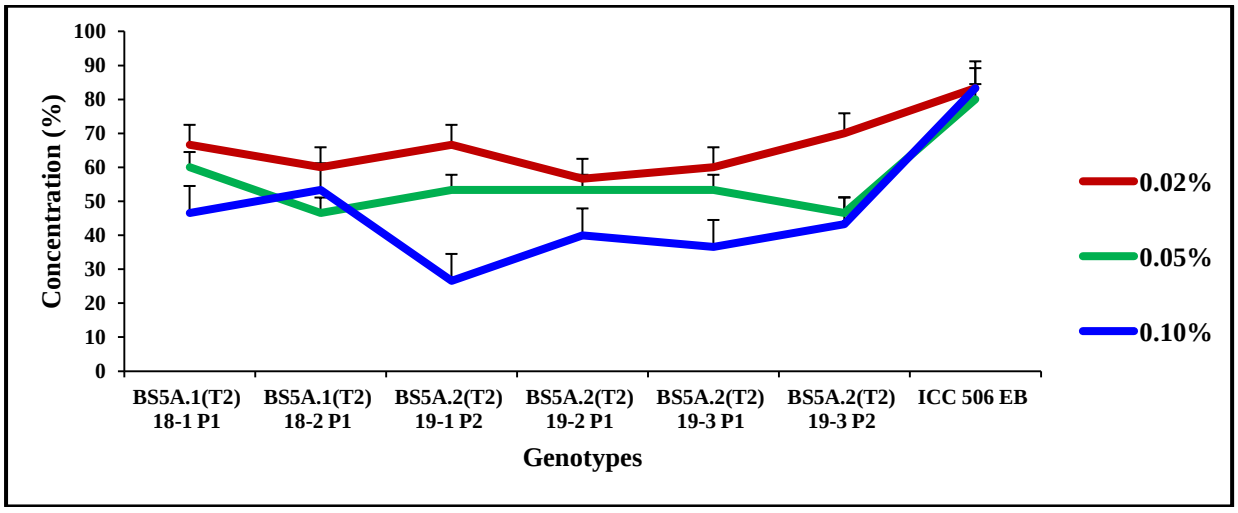
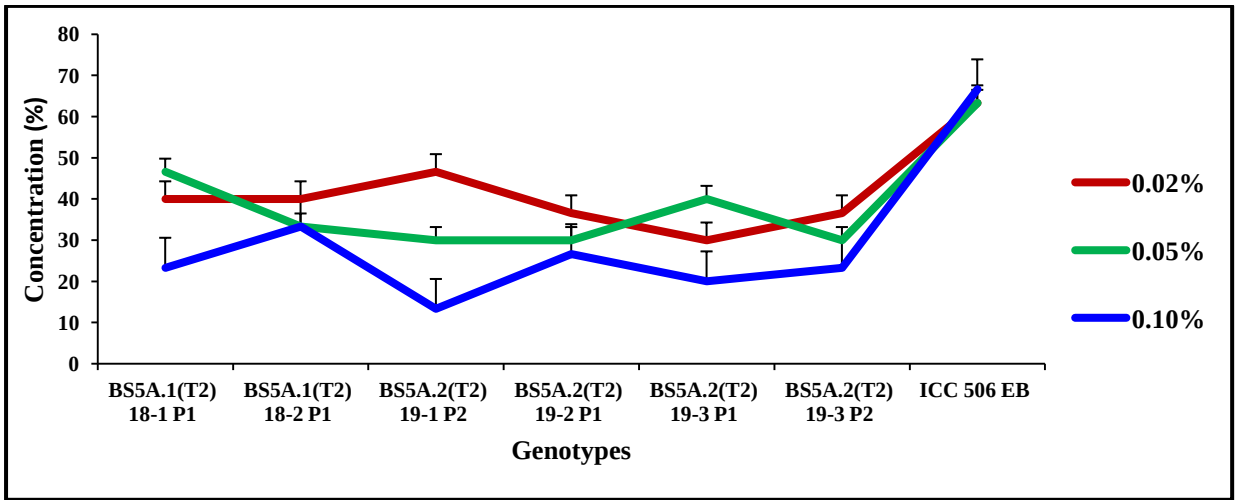


Figure 32: Biology of *C. chlorideae* parasitizing *H. armigera* fed on diets with lyophilized leaf powders of different transgenic chickpea lines (2011-12 and 2012-2013) (pooled analysis)

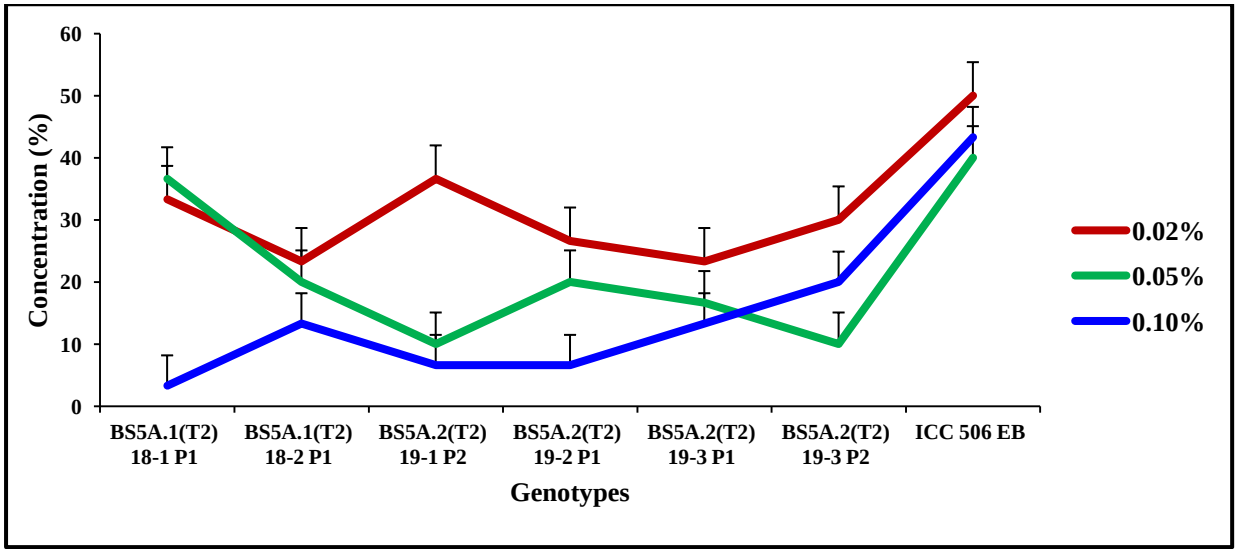
a) Larval survival (%)



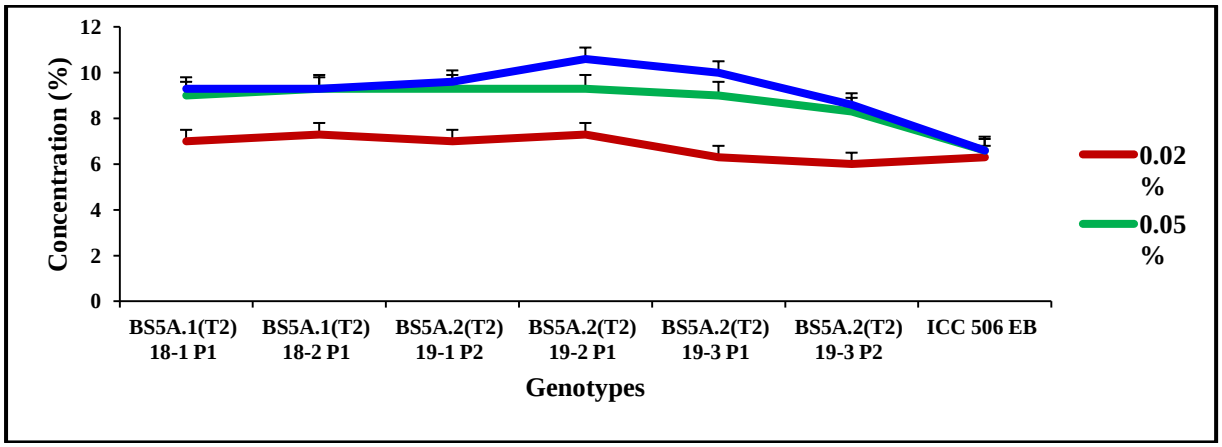
b) Pupation (%)



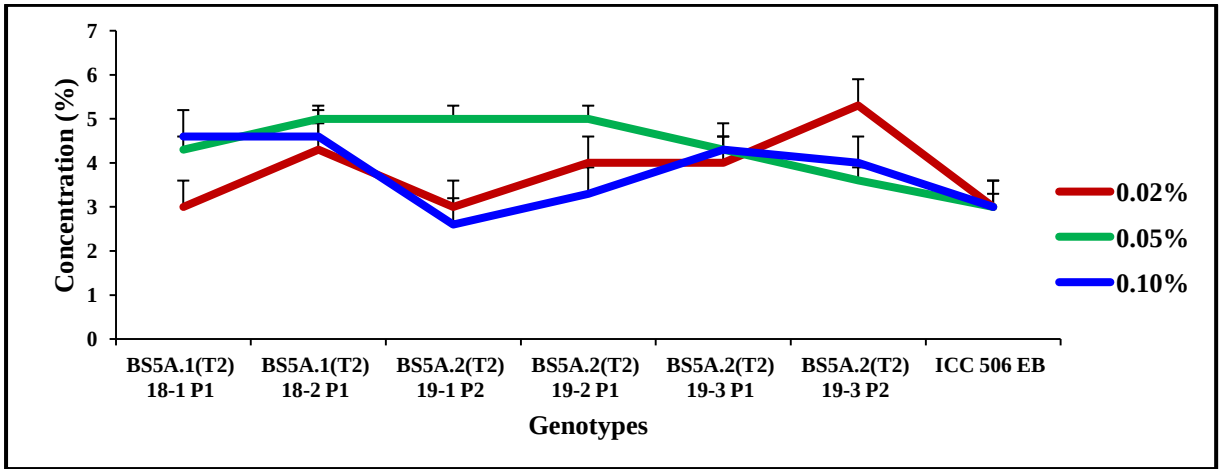
c) Adult emergence (%)



d) Larval period (days)



e) Pupal period (days)



f) Mean grub weight (mg)

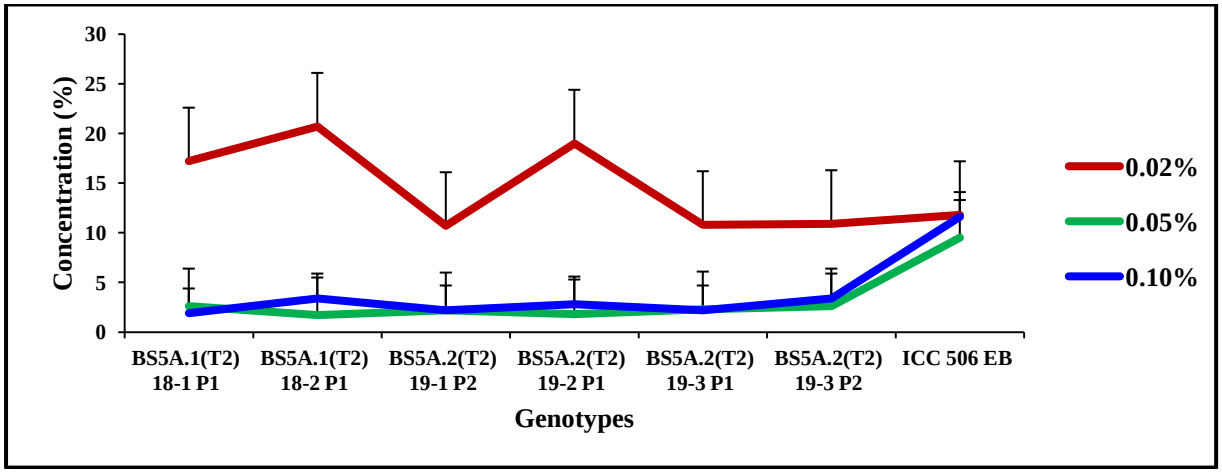
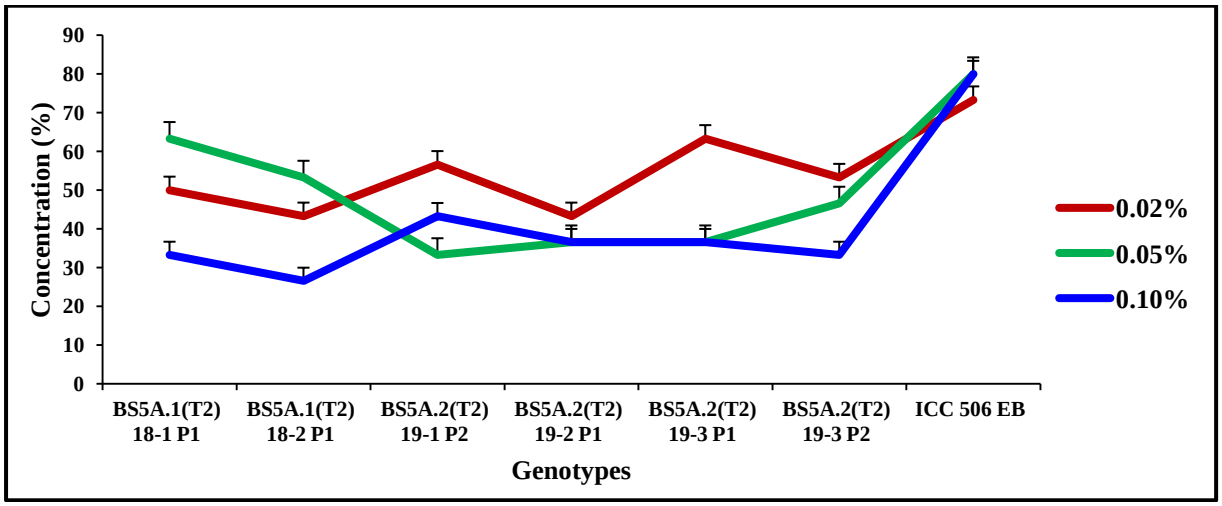
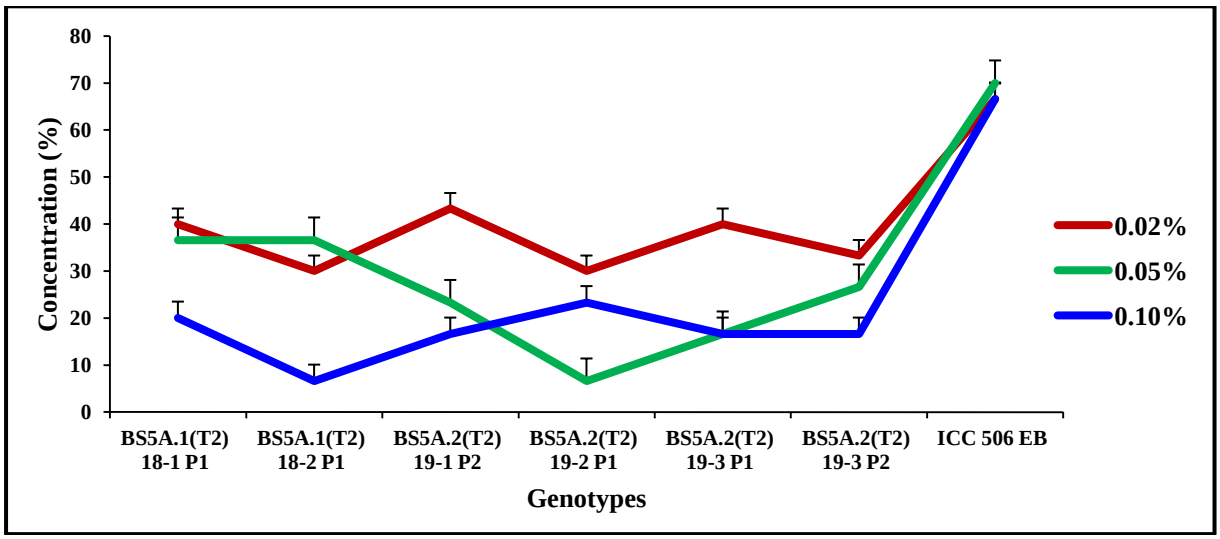


Figure 33: Direct effect of *Cry IIa* transgenic chickpea lines on *Cheilomenes sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%) (2012-2013)

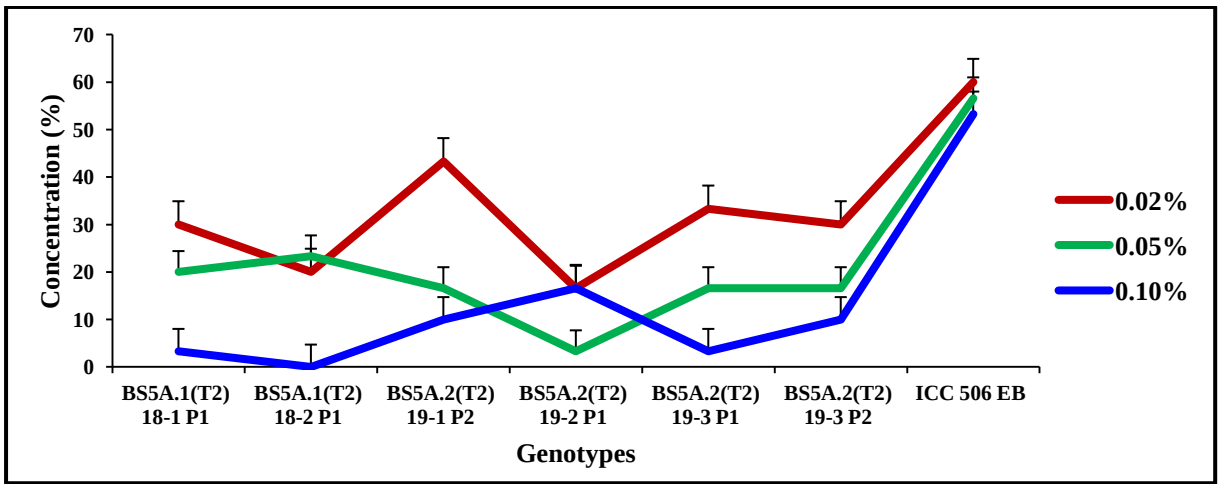
a) Larval survival (%)



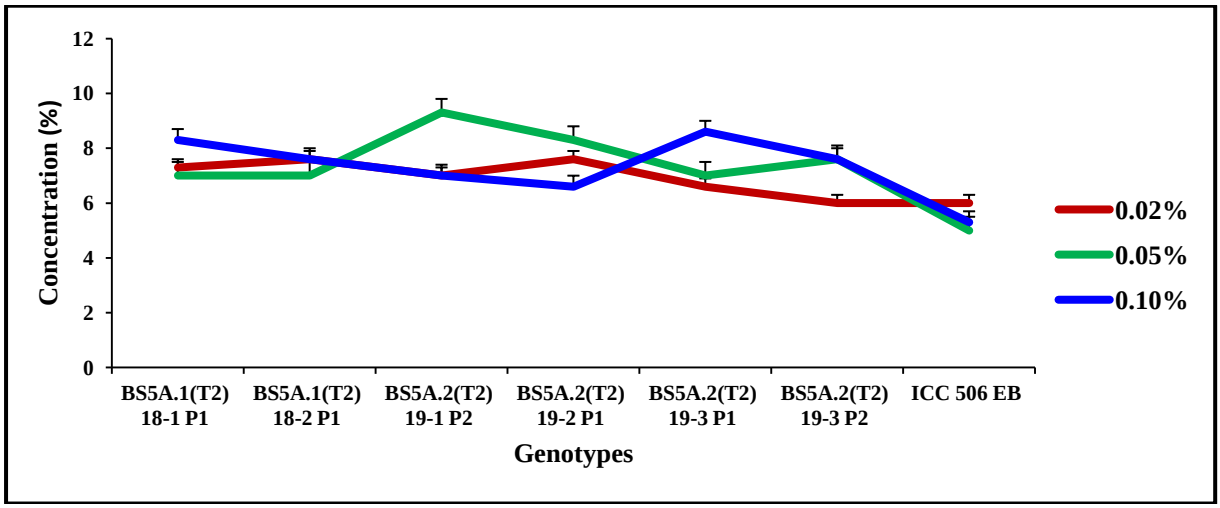
b) Pupation (%)



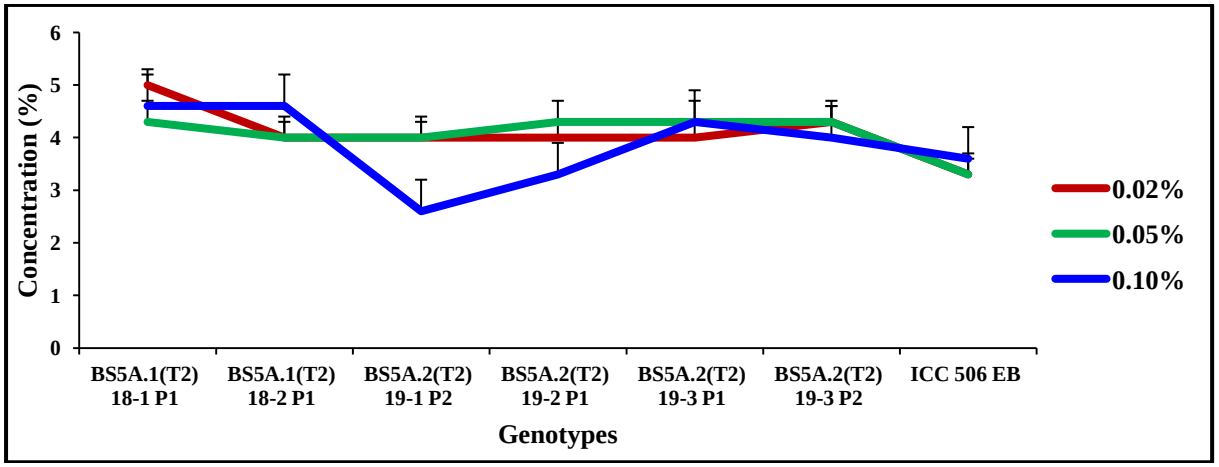
c) Adult emergence (%)



d) Larval period (days)



e) Pupal period (days)



f) Mean grub weight (mg)

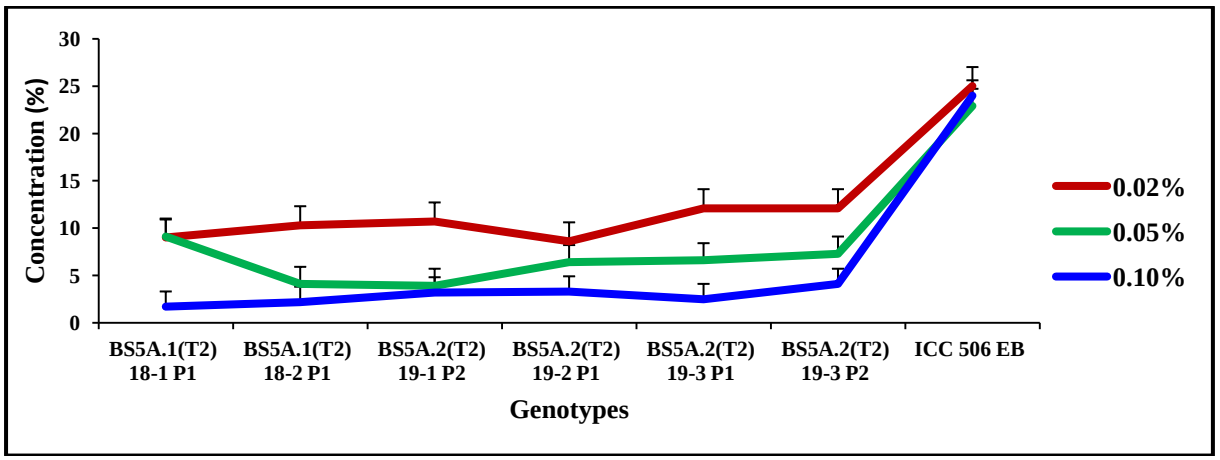
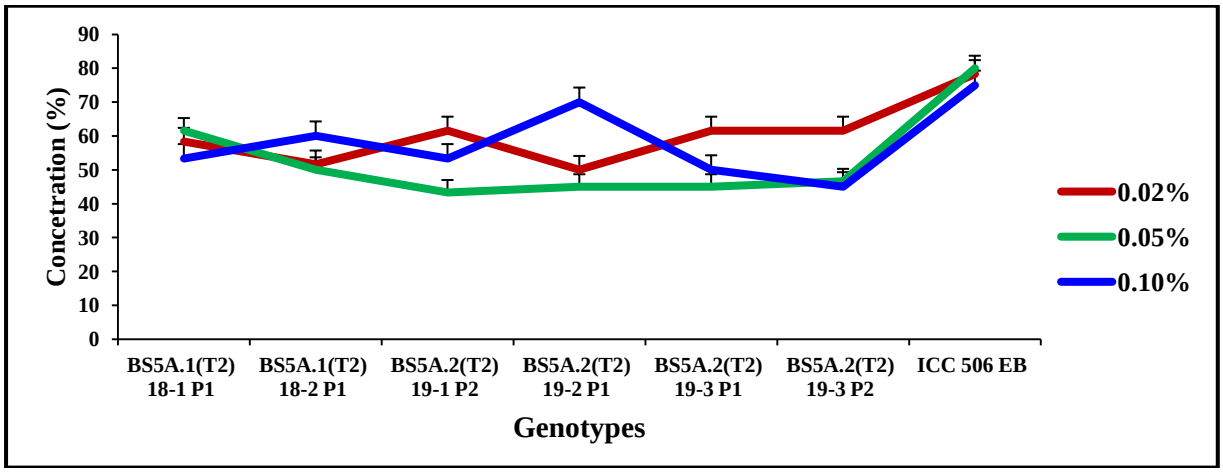
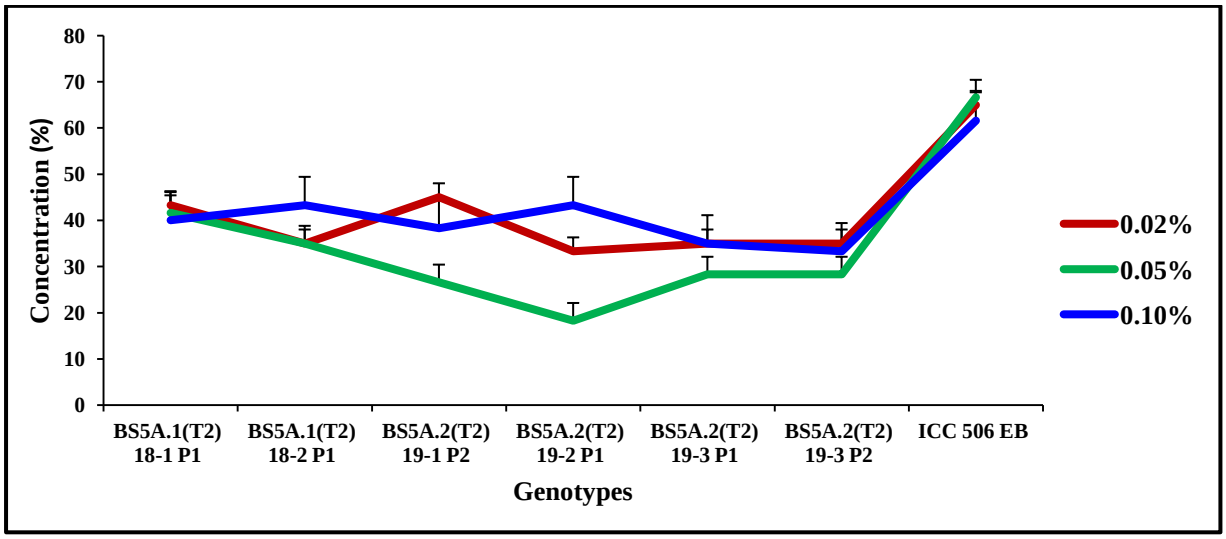


Figure 34: Direct effect of *Cry IIa* transgenic chickpea lines on *C. sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%) (2013-2014)

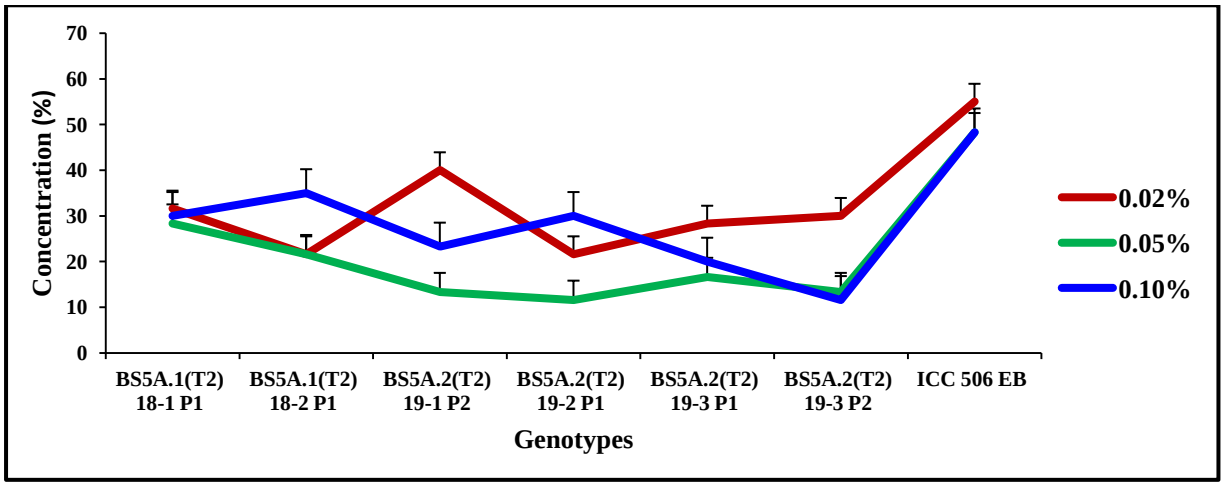
a) Larval survival (%)



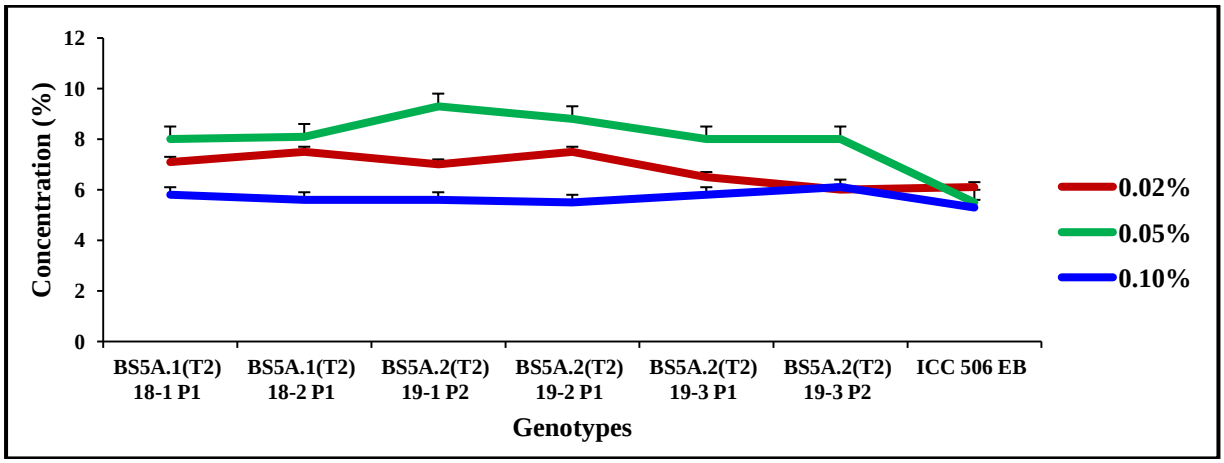
b) Pupation (%)



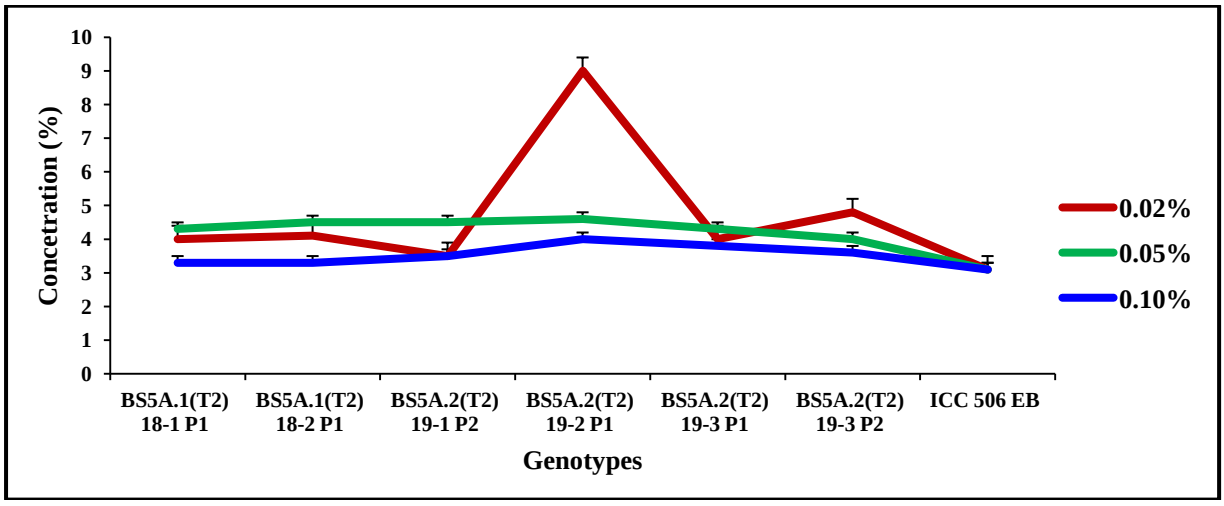
c) Adult emergence (%)



d) Larval period (days)



e) Pupal period (days)



f) Mean grub weight (mg)

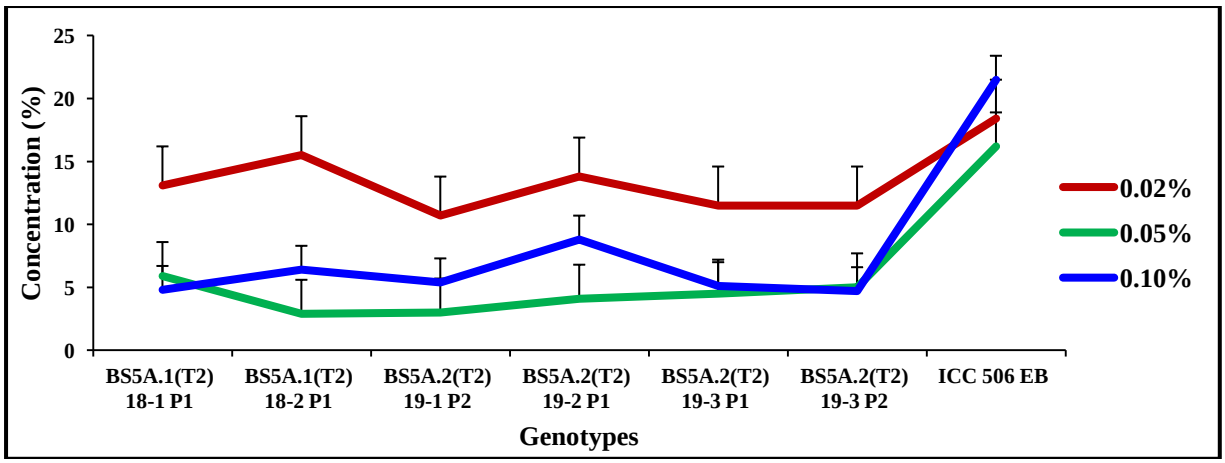
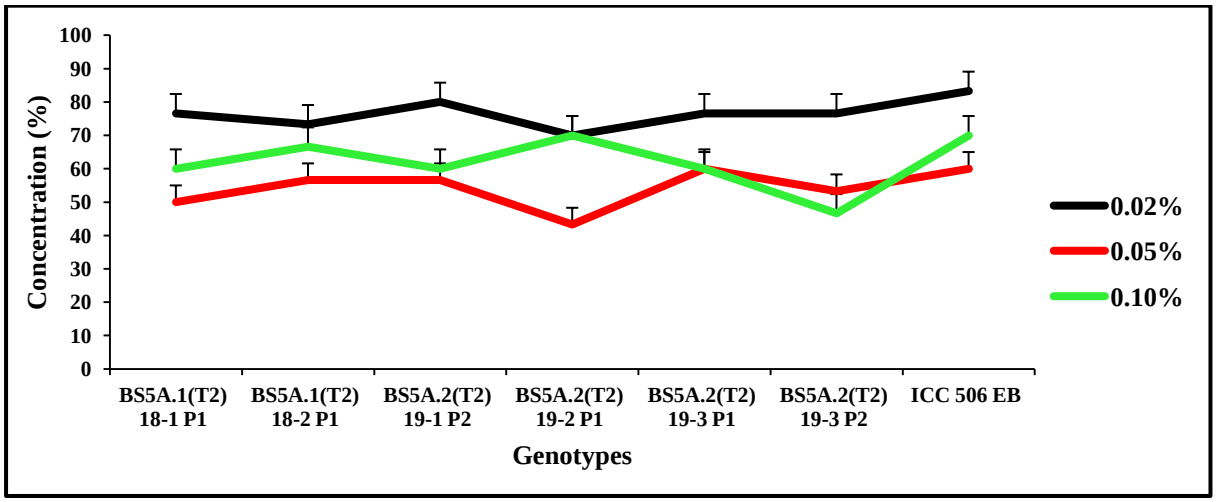
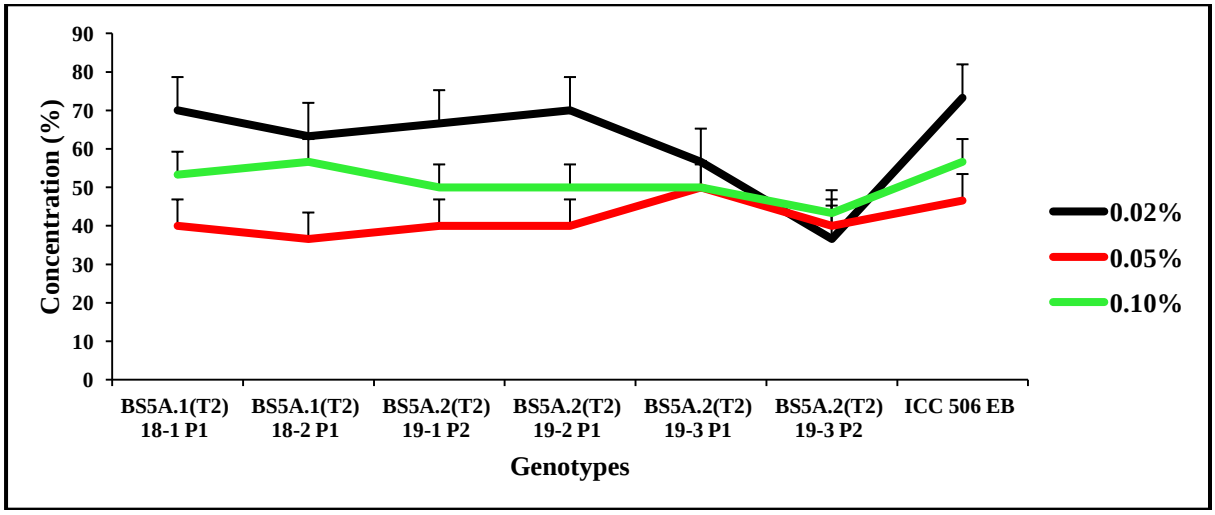


Figure 35: Direct effect of *Cry IIa* transgenic chickpea lines on *Cheilomenes sexmaculat* at different concentrations (0.02%, 0.05% and 0.1%) (2012-2013 and 2013-14) (pooled)

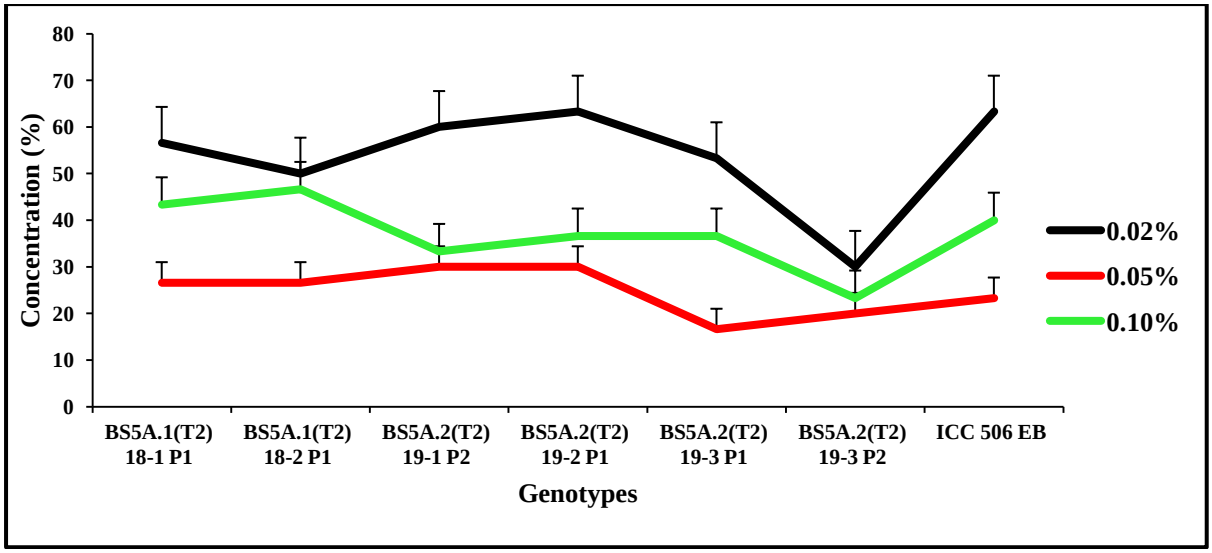
a) Larval survival (%)



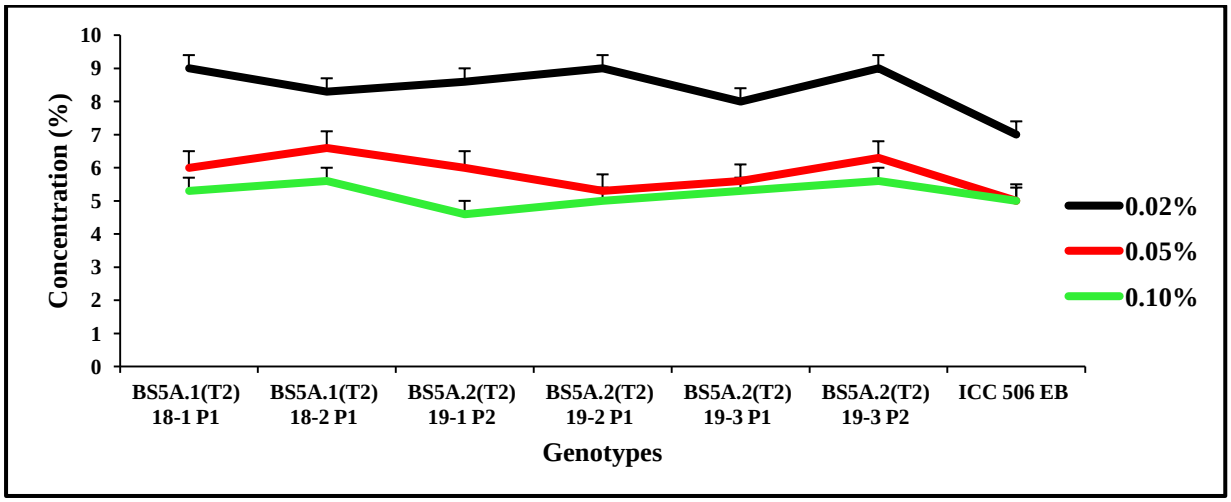
b) Pupation (%)



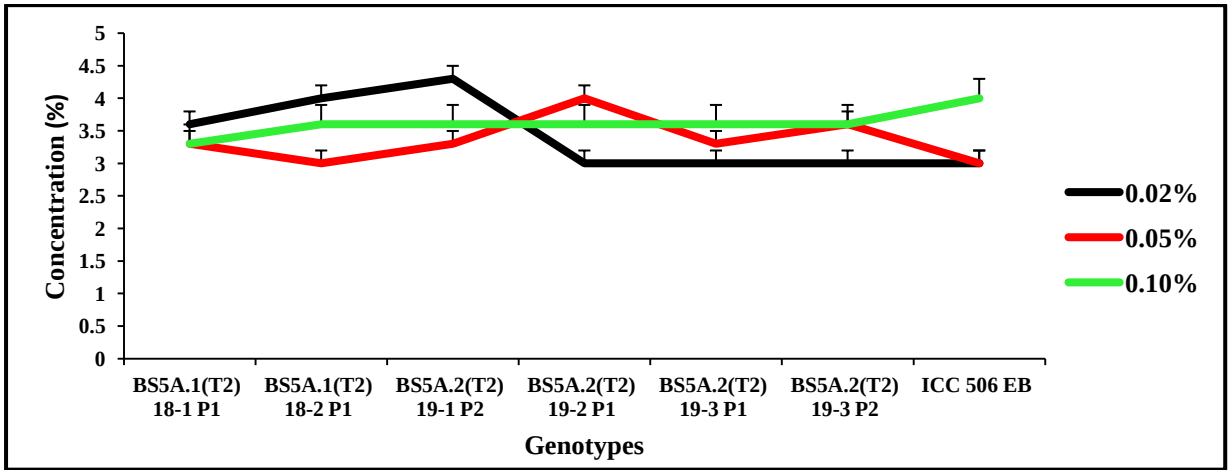
c) Adult emergence (%)



d) Larval period (days)



e) Pupal period (days)



f) Mean grub weight (mg)

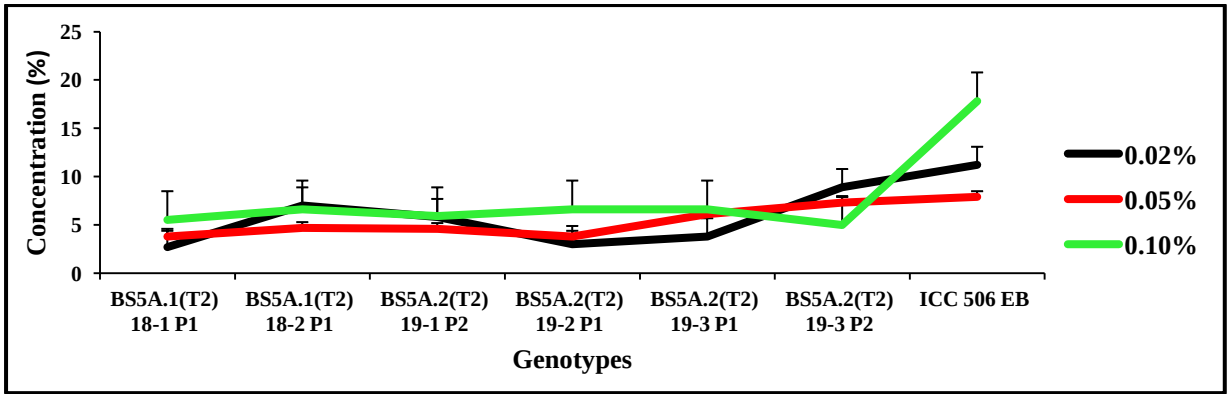
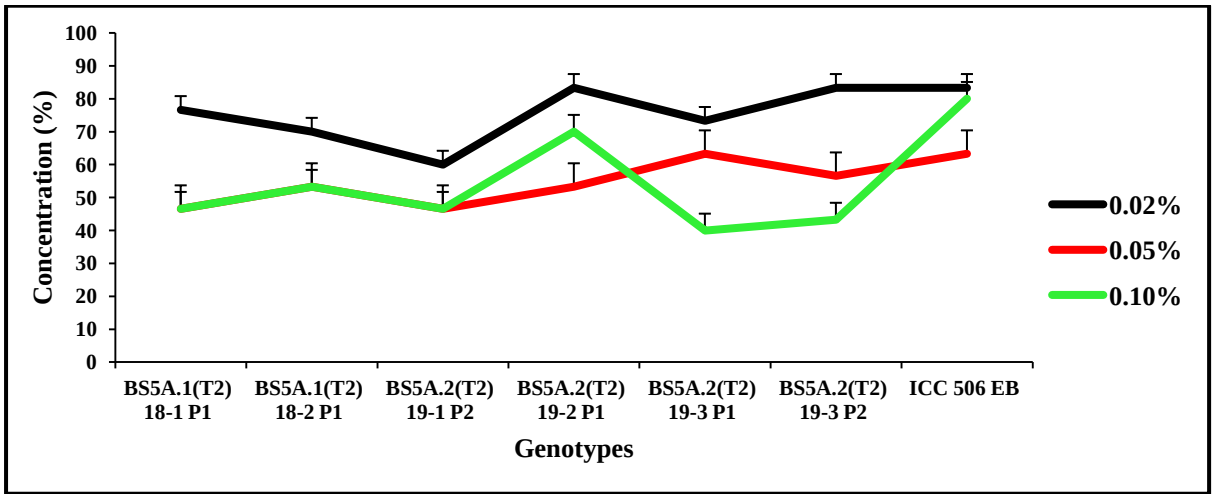
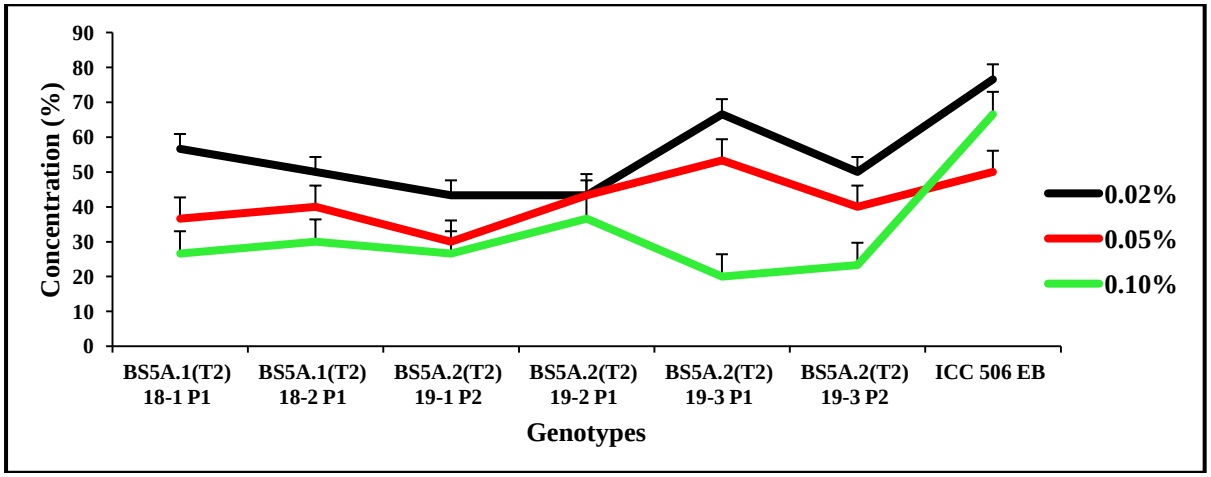


Figure 36: Indirect effect of *Cry IIa* transgenic chickpea lines on different biological parameters of the coccinellid, *C. sexmaculatus* reared on *Bt* intoxicated artificial diet fed *Aphis craccivora* (2012-2013)

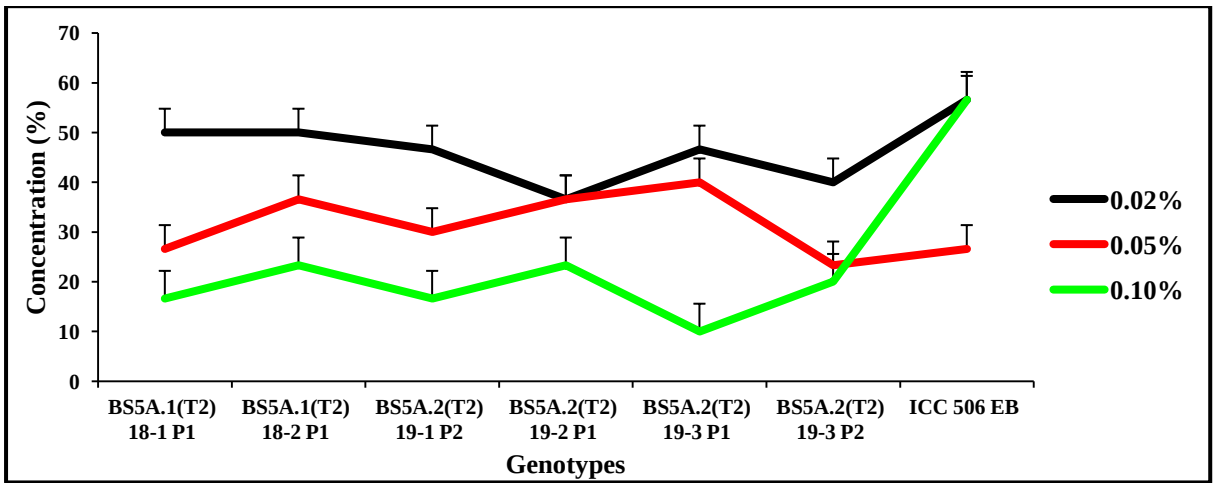
a) Larval survival (%)



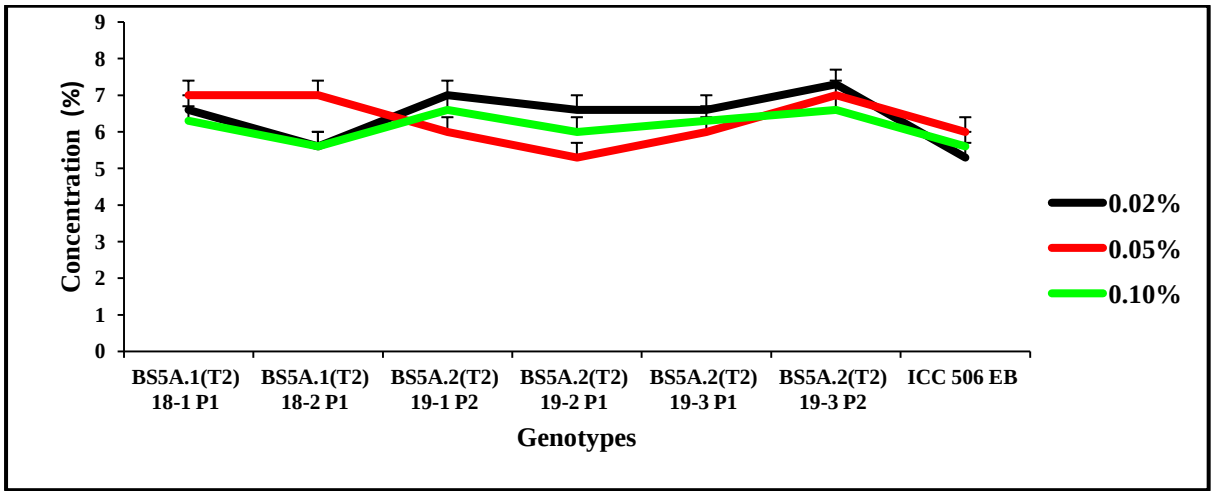
b) Pupation (%)



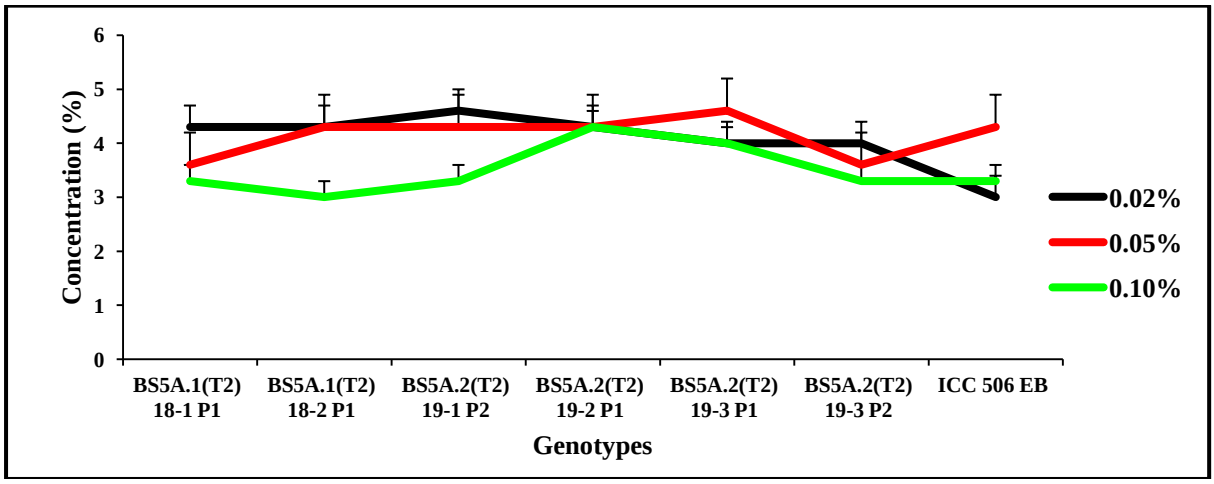
c) Adult emergence (%)



d) Larval period (days)



e) Pupal period (days)



f) Mean grub weight (mg)

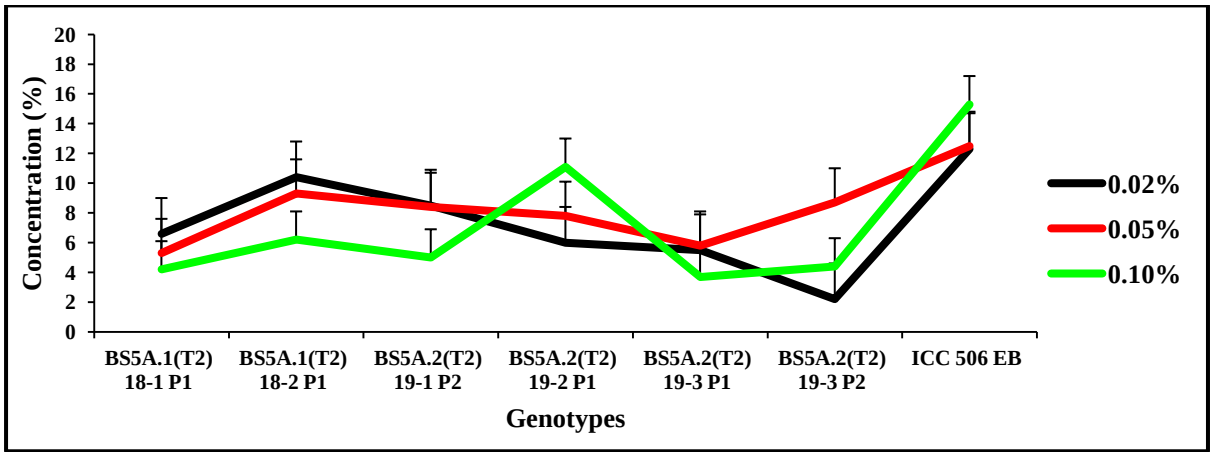
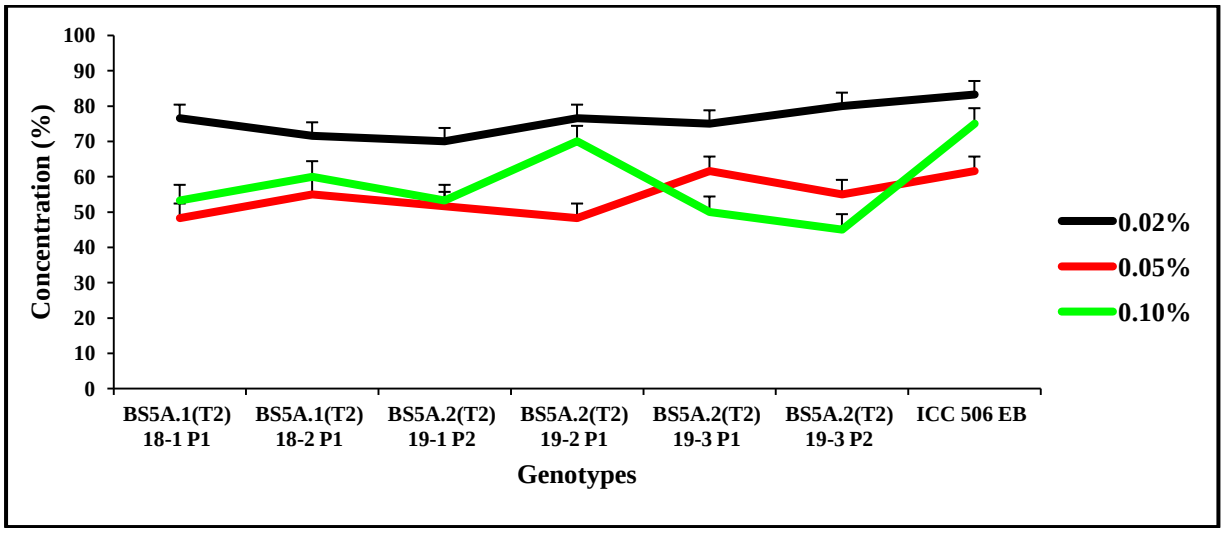
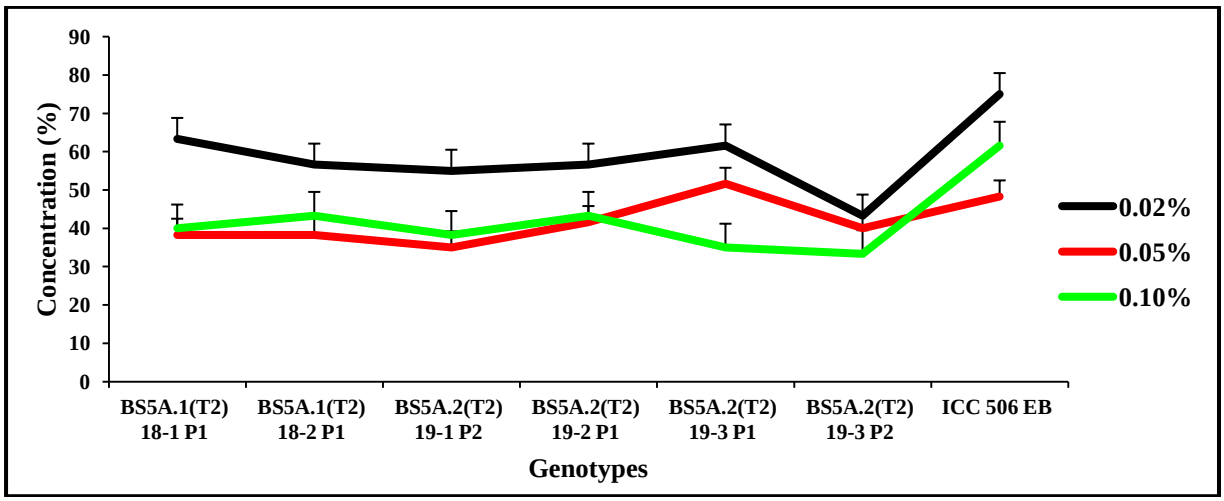


Figure 37: Indirect effect of *Cry IIa* transgenic chickpea lines on different biological parameters of the coccinellid, *C. sexmaculatus* reared on *Bt* intoxicated artificial diet fed *A. craccivora* (2013-2014)

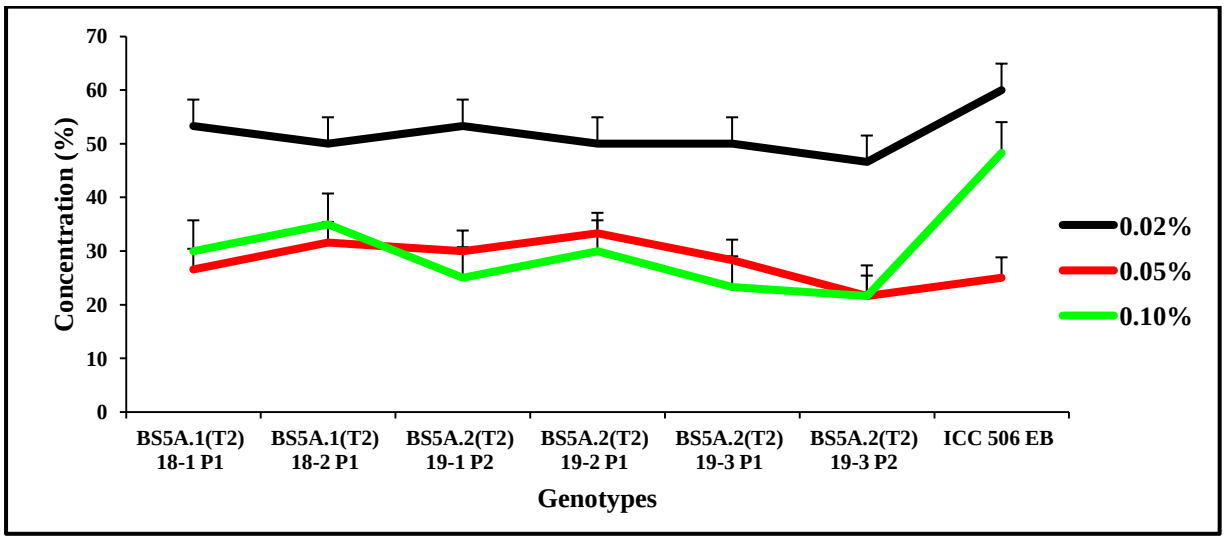
a) Larval survival (%)



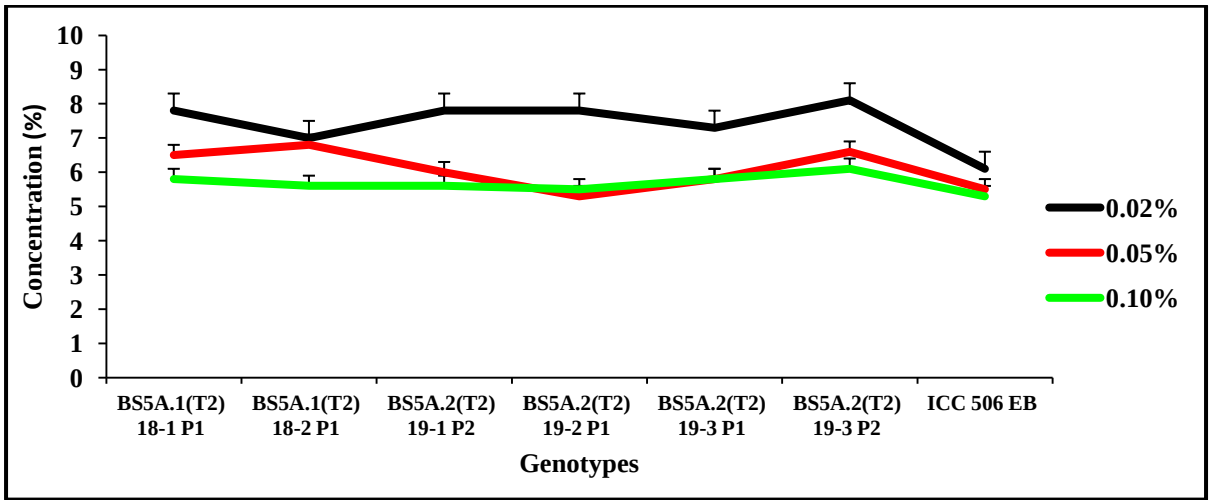
b) Pupation (%)



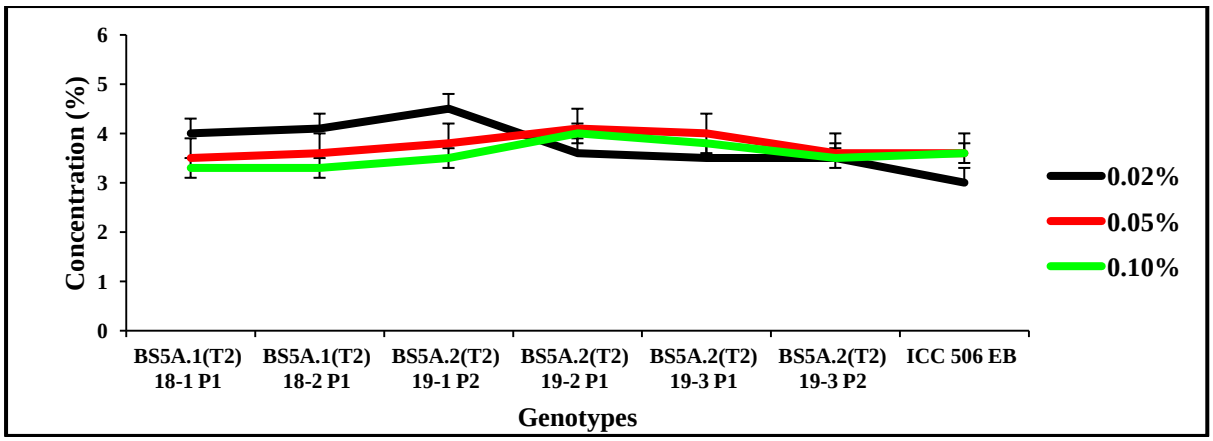
c) Adult emergence (%)



d) Larval period (days)



e) Pupal period (days)



f) Mean grub weight (mg)

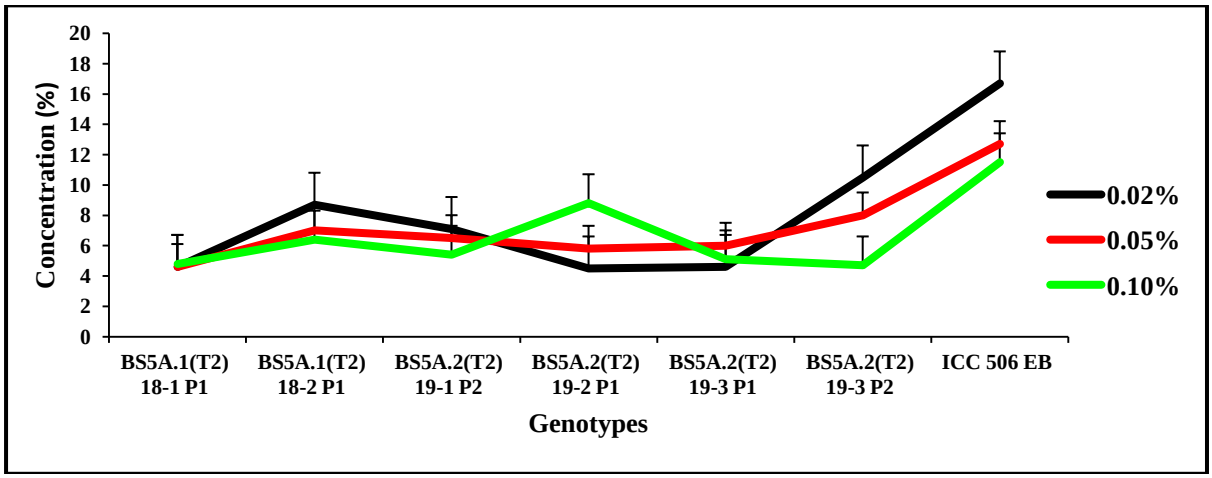


Figure 38: Indirect effect of *Cry IIa* transgenic chickpea lines on different biological parameters of the coccinellid, *C. sexmaculatus* reared on *Bt* intoxicated artificial diet fed *A. craccivora* (2012-2014) (pooled)

PLATES

Black stage eggs/neonate larvae of *H. armigera* on cotton liners.

H. armigera neonate larvae in chickpea based artificial diet

Rearing of *H. armigera* larvae on chickpea artificial diet in multi cell well plates.

H. armigera pupae on vermiculite in pupation jars

H. armigera adults (a) Male (b) Female

Oviposition cage

Plate1: Rearing of *H. armigera* under laboratory conditions

Plate 2: Transgenic chickpea plants grown under glass house conditions

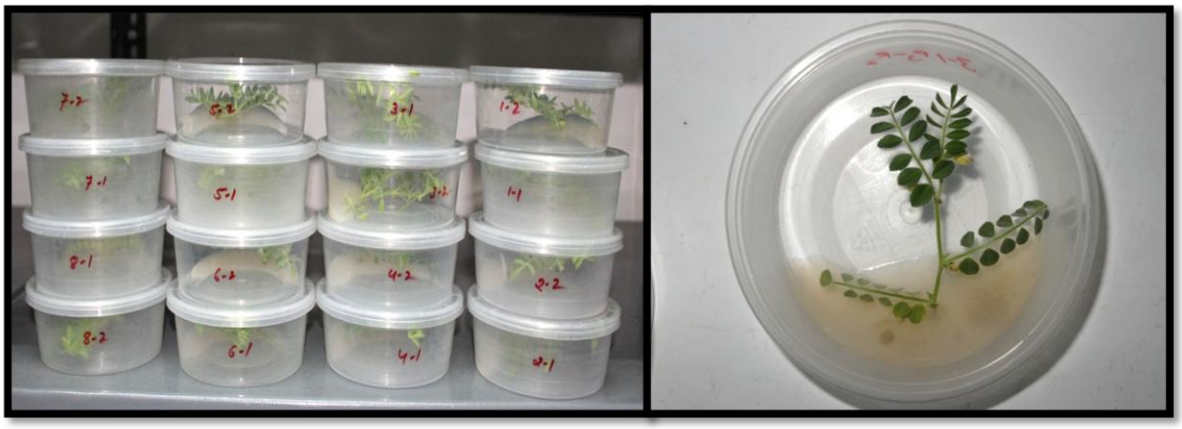


Plate 3: Detached leaf assay

Plate 4: Cage screening method to screen chickpea genotypes for resistance to *H. armigera*

Plate 5: Lyophilizer

Plate 6: High performance liquid chromatography (HPLC)

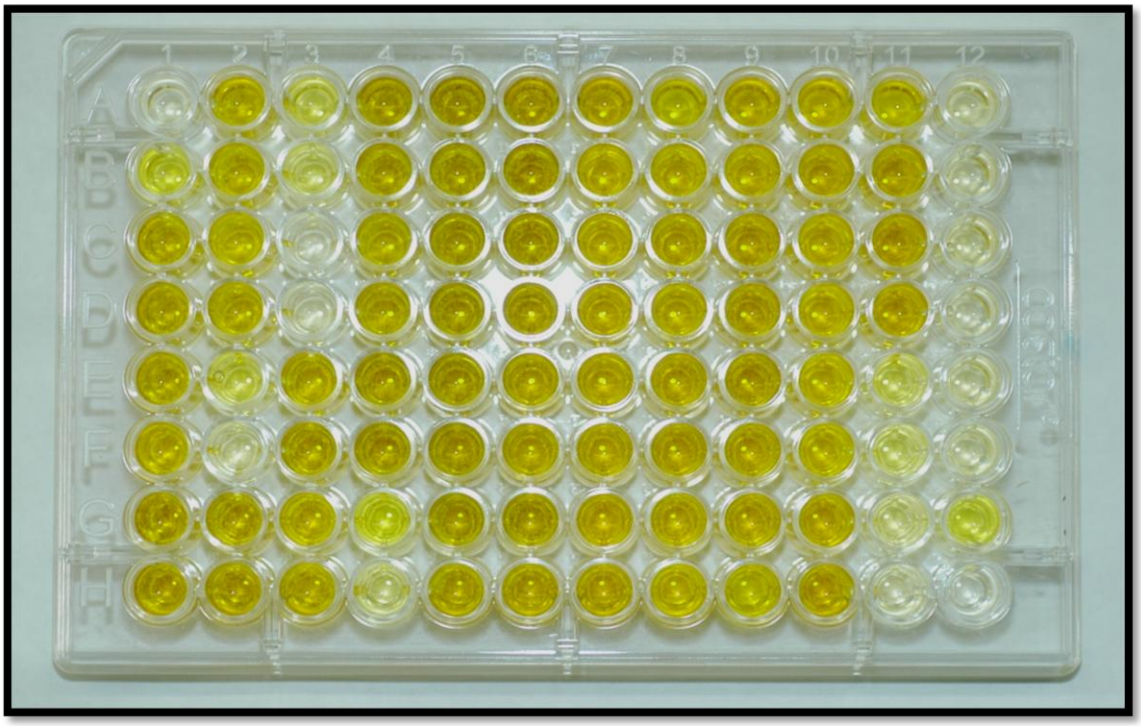


Plate 7: Detection of Cry 2A protein by ELISA

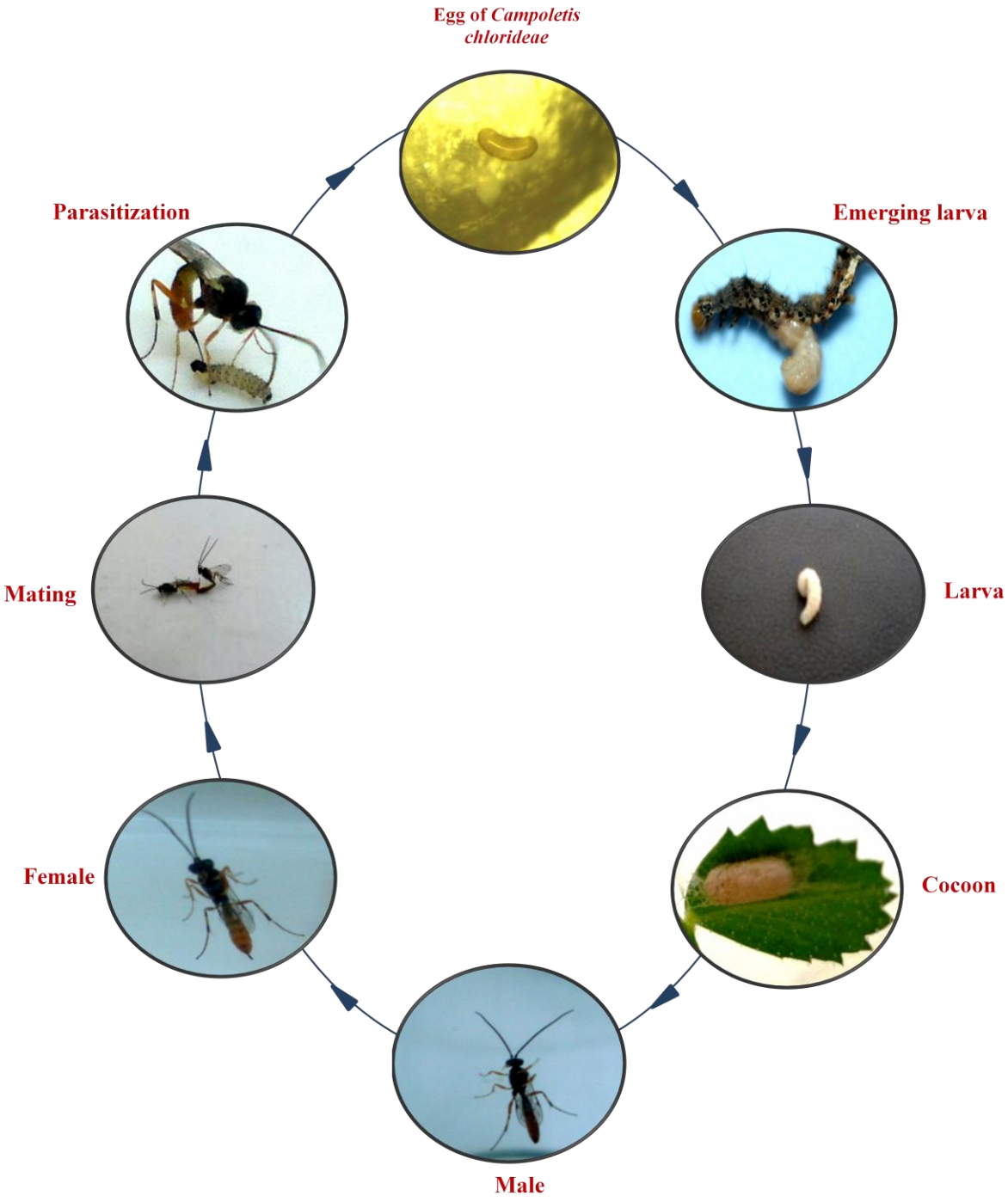


Plate 8: Life cycle of *Campoletes chlorideae* (Uchida)

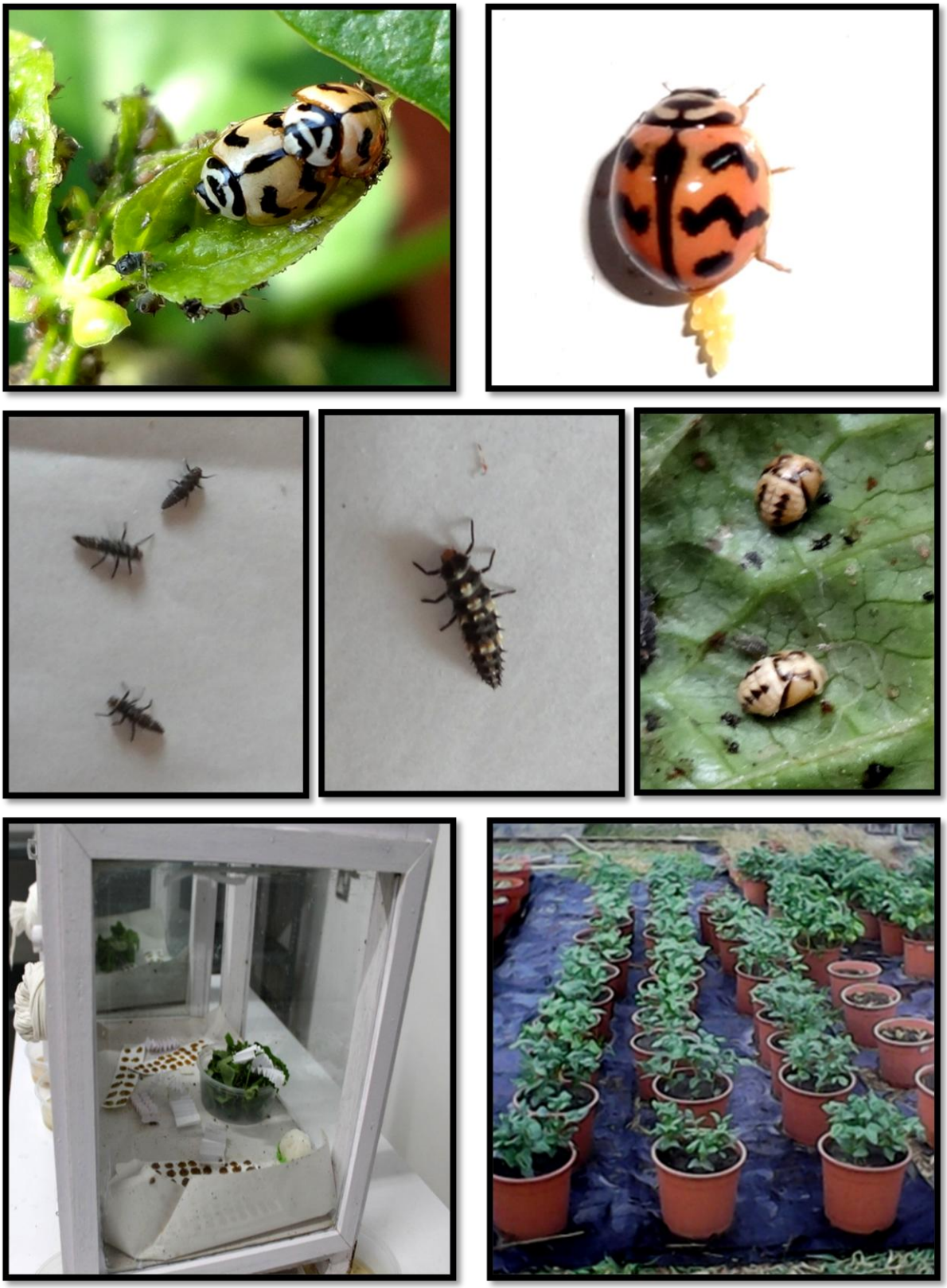


Plate 9: Rearing of *Cheilomenes sexmaculatus*

Plate 10: Aphid feeding apparatus

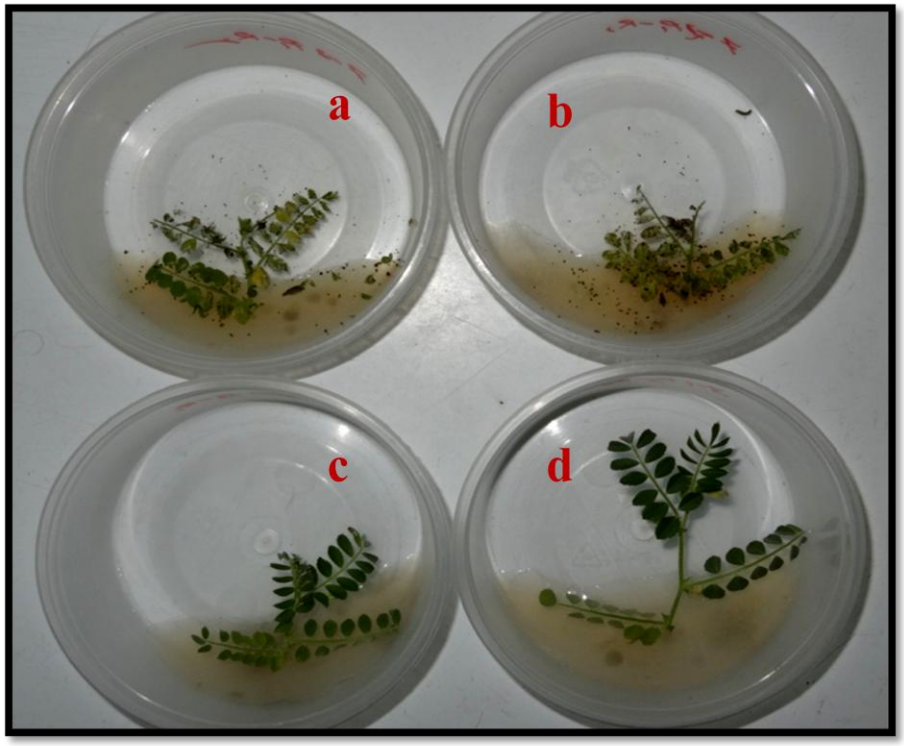


Plate 11: Detached leaf assay of (a) & (b) non-transgenic chickpea and (c) & (d) transgenic chickpea for resistance to *H. armigera.*

Plate 12: *H. armigera* leaf damage under glass house using cage technique

Table 4.1: Evaluation of transgenic chickpea lines for resistance to *Helicoverpa armigera* under greenhouse conditions (ICRISAT, Patancheru 2011-2013)

Genotype	October , 2011-12			October, 2012-13			Pooled analysis		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	1.7[ab]	30.5[a] (33.3)	0.6[a]	1.5[a]	28.8[a] (32.1)	0.6[a]	1.6[a]	29.7[a] (32.7)	0.6[a]
BS5A.1(T2) 18-2 P1	3.2[c]	35.5[a] (36.5)	1.2[a]	1.5[a]	31.6[a] (33.8)	0.5[a]	2.3[a]	33.61[a] (35.2)	0.8[a]
BS5A.2(T2) 19-1 P2	1.3[a]	46.1[a] (42.7)	0.8[a]	1.6[a]	30.0[a] (32.8)	0.3[a]	1.5[a]	38.0[a] (37.7)	0.6[a]
BS5A.2(T2) 19-2 P1	1.6[ab]	40.0[a] (38.9)	0.8[a]	1.0[a]	10.5[a] (17.0)	0.1[a]	1.3[a]	25.2[a] (27.9)	0.4[a]
BS5A.2(T2) 19-3 P1	2.3[abc]	41.6[a] (40.0)	1.1[a]	1.0[a]	19.4[a] (24.3)	0.4[a]	1.6[a]	30.5[a] (32.1)	0.7[a]
BS5A.2(T2) 19-3 P2	2.7[bc]	46.1[a] (42.7)	1.4[a]	1.2[a]	24.4[a] (29.4)	0.4[a]	1.9[a]	35.2[a] (36.1)	0.9[a]
Semsen (Control)	7.8[e]	83.8[b] (66.5)	5.4[c]	4.6[b]	73.8[b] (59.4)	3.0[c]	6.2[c]	78.8[b] (63.0)	4.2[c]
ICC 506 EB (Resistant check)	5.3[d]	74.1[b] (59.6)	3.8[b]	3.9[b]	77.7[b] (61.8)	2.4[b]	4.6[b]	75.9[b] (60.7)	3.1[b]
Mean	3.2	49.7	1.9	2.0	37.1	1.0	2.6	43.4	1.4
SE ±	0.3	5.4	0.2	0.3	7.0	0.1	0.4	5.4	0.3
Fp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Vr	33.8	12.0	35.6	12.4	12.3	48.7	16.9	15.2	22.2
LSD (P 0.05)	1.1*	16.5*	0.8*	1.1*	21.5*	0.4*	1.2*	15.6*	0.8*
CV (%)	20.3	19.0	26.5	33.1	33.1	26.8	39.7	30.8	50.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Figures in parenthesis are Angular transformed values, HDR[1]-Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged)

Table 4.2: Evaluation of transgenic chickpea lines for resistance to *H. armigera* under greenhouse conditions (ICRISAT, Patancheru 2011-2013).

Genotype	November, 2011-12			November, 2012-13			Pooled analysis		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	1.4[a]	38.8[ab] (38.4)	0.8[a]	1.6[ab]	13.8[a] (20.1)	1.1[a]	1.3[a]	26.3[ab] (29.3)	1.0[a]
BS5A.1(T2) 18-2 P1	1.5[a]	33.3[ab] (34.9)	0.9[a]	2.3[b]	24.4[ab] (29.3)	1.3[a]	1.4[a]	28.8[ab] (32.1)	0.9[a]
BS5A.2(T2) 19-1 P2	1.0[a]	21.6[a] (27.5)	0.3[a]	1.2[a]	10.0[a] (16.4)	1.0[a]	1.0[a]	15.8[a] (21.9)	0.2[a]
BS5A.2(T2) 19-2 P1	1.0[a]	24.4[a] (29.4)	0.3[a]	1.3[ab]	12.7[a] (19.9)	1.0[a]	1.0[a]	18.6[a] (24.7)	0.7[a]
BS5A.2(T2) 19-3 P1	1.2[a]	48.3[b] (44.0)	0.7[a]	1.8[ab]	30.0[b] (33.0)	1.2[a]	1.3[a]	39.1[b] (38.5)	0.7[a]
BS5A.2(T2) 19-3 P2	1.6[a]	39.3[ab] (38.7)	1.2[a]	2.1[ab]	30.0[b] (33.1)	1.2[a]	1.5[a]	34.6[b] (35.9)	1.0[a]
Semsen (Control)	7.2[c]	75.0[c] (61.1)	3.7[b]	7.5[d]	50.5[c] (45.3)	2.8[b]	5.0[c]	62.7[c] (53.2)	2.9[b]
ICC 506 EB (Resistant check)	3.3[b]	72.7[c] (58.6)	4.4[b]	4.3[d]	62.2[c] (52.1)	3.0[b]	3.1[b]	67.5[c] (55.4)	3.5[b]
Mean	2.3	44.2	1.6	2.8	29.2	1.6	1.9	36.7	1.4
SE ±	0.3	6.4	0.3	0.3	4.5	0.2	0.4	5.1	0.2
Fp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Vr	35.0	9.8	22.1	37.6	17.1	12.6	12.5	14.1	15.5
LSD (P 0.05)	1.08*	19.5*	1.0*	1.0*	13.6*	0.7*	1.1*	14.6*	0.8
CV (%)	26.8	25.2	36.5	30.7	26.7	25.3	50.3	34.0	51.4

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Figures in parenthesis are Angular transformed values, HDR[1]-Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged).

Table 4.3: Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique (ICRISAT, Patancheru 2011-2013).

Genotype	2011-2012			2012-2013			Pooled analysis		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	2.5^{ab}	52.3^{b} (46.5)	3.9^{a}	2.2^{a}	37.6^{a} (37.5)	2.9^{a}	2.4^{a}	44.9^{a} (41.9)	3.4^{a}
BS5A.1(T2) 18-2 P1	4.1^{b}	49.7^{b} (44.8)	5.1^{ab}	2.5^{a}	41.2^{a} (39.8)	3.6^{a}	3.3^{ab}	45.4^{a} (42.3)	4.3^{a}
BS5A.2(T2) 19-1 P2	4.4^{b}	36.4^{a} (37.1)	5.2^{ab}	3.2^{ab}	40.1^{a} (39.2)	4.4^{ab}	3.8^{ab}	38.2^{a} (38.2)	4.8^{ab}
BS5A.2(T2) 19-2 P1	1.6^{a}	41.3^{ab} (40.0)	6.4^{bc}	3.7^{ab}	37.6^{a} (37.8)	4.3^{ab}	2.7^{a}	39.4^{a} (38.9)	5.4^{ab}
BS5A.2(T2) 19-3 P1	2.8^{ab}	35.0^{a} (36.2)	7.3^{cd}	3.7^{ab}	41.1^{a} (39.9)	4.4^{ab}	3.2^{ab}	38.1^{a} (38.1)	5.8^{ab}
BS5A.2(T2) 19-3 P2	4.3^{b}	50.8^{b} (45.4)	8.7^{d}	4.3^{bc}	48.1^{a} (43.9)	6.4^{b}	4.3^{b}	49.4^{a} (44.6)	7.5^{b}
Semsen (Control)	7.8^{c}	75.7^{c} (60.5)	12.7^{e}	7.7^{d}	70.2^{b} (56.9)	13.6^{c}	7.8^{c}	72.9^{b} (58.7)	13.1^{c}
ICC 506 EB (Resistant check)	8.0^{c}	72.3^{c} (58.2)	11.2^{e}	5.5^{c}	79.3^{b} (62.9)	17.0^{d}	6.7^{c}	75.8^{b} (60.6)	14.1^{c}
Mean	4.4	51.7	7.5	4.1	49.4	7.1	4.3	50.6	7.3
SE +	0.5	3.5	0.5	0.4	5.9	0.7	0.4	3.4	0.8
Fp	<0.001	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	<0.001	<0.001
Vr	19.2	18.0	30.0	16.7	7.4	51.9	15.2	19.0	21.7
LSD (P 0.05)	1.7*	11.9*	1.8*	1.4*	19.8*	2.4*	1.4*	10.2*	2.5*
CV (%)	16.9	9.8	10.5	14.9	17	14.5	23.2	13.8	23.8

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05

Figures in parenthesis are Angular transformed values. HDR[1]-Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged).

Table 4.4: Agrnomic performance of transgenic chickpea lines (g/3 plants) with resistance to *Helicoverpa armigera* under greenhouse condition using cage technique (ICRISAT, Patancheru 2011-2013).

Genotype	Infested (2011-2012)				Infested (2012-2013)				Pooled analysis			
	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds
BS5A.1(T2) 18-1 P1	5.8^{bc}	1.7^{b}	1.2^{bc}	16^{c}	6.2^{b}	2.2^{ab}	1.9^{ab}	21^{bcd}	6.0^{b}	1.9^{abc}	1.5^{ab}	18^{c}
BS5A.1(T2) 18-2 P1	6.0^{c}	1.5^{b}	1.4^{c}	14^{c}	6.7^{b}	2.0^{a}	1.9^{ab}	16^{b}	6.4^{b}	1.7^{ab}	1.6^{abc}	15^{bc}
BS5A.2(T2) 19-1 P2	6.5^{c}	1.6^{b}	1.3^{bc}	10^{b}	5.2^{ab}	3.2^{bc}	2.9^{bc}	23^{cd}	5.9^{b}	2.4^{bcd}	2.1^{bcd}	17^{c}
BS5A.2(T2) 19-2 P1	5.0^{b}	2.6^{c}	2.0^{d}	9^{b}	6.8^{b}	4.1^{c}	3.5^{c}	26^{d}	5.9^{b}	3.3^{d}	2.7^{d}	17^{c}
BS5A.2(T2) 19-3 P1	6.4^{c}	2.3^{c}	2.1^{d}	10^{b}	6.7^{b}	3.2^{bc}	2.9^{bc}	19^{bc}	6.5^{b}	2.7^{cd}	2.5^{cd}	14^{bc}
BS5A.2(T2) 19-3 P2	5.1^{b}	1.8^{b}	1.6^{c}	8^{b}	6.5^{b}	1.5^{a}	1.2^{a}	15^{b}	5.8^{b}	1.6^{ab}	1.4^{ab}	11^{bc}
Semsen (Control)	3.3^{a}	0.6^{a}	0.5^{a}	2^{a}	3.6^{a}	1.3^{a}	0.9^{a}	3^{a}	3.4^{a}	1.0^{a}	0.7^{a}	2^{a}
ICC 506 EB (Resistant check)	3.5^{a}	1.3^{b}	0.9^{ab}	7^{b}	4.0^{a}	1.2^{a}	1.0^{a}	6^{a}	3.7^{a}	1.3^{a}	1.0^{ab}	7^{ab}
Mean	5.2	1.7	1.4	0.0	5.	2.3	2.0	0.0	5.4	2.0	1.7	0.0
SE $\pm$	0.0	0.0	0.1	0.0	0.5	0.3	0.3	0.0	0.3	0.0	0.0	0.0
Fp	<0.001	<0.001	<0.001	<0.001	0.014	0.004	0.006	<0.001	<0.001	<0.001	<0.001	0.006
Vr	26.7	18.6	15.6	16.0	6.2	9.7	8.2	21.4	9.9	6.4	5.7	3.9
LSD (P 0.05)	0.7*	0.4*	0.4*	3.5*	1.7*	1.1*	1.1*	0.0*	1.1*	0.8*	0.8*	0.0*
CV (%)	6.4	11.3	12.9	16.2	12.9	19.9	23.4	15.3	13.7	30.1	33.7	44.7

*Figures followed by the same letter within a column are not significantly different at P$\leq$ 0.05.

Table 4.5: Agronomic performance of transgenic chickpea lines in un-infested plants (g/3 plants) under green house conditions (ICRISAT, Patancheru 2011-2013).

Genotype	2011-2012				2012-2013				Pooled analysis			
	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds
BS5A.1(T2) 18-1 P1	4.6^{a}	2.6^{bc}	2.3^{c}	21^{c}	5.4^{ab}	2.9^{b}	2.3^{b}	38^{b}	5.0^{b}	2.7^{b}	2.3^{b}	29^{b}
BS5A.1(T2) 18-2 P1	4.2^{a}	2.7^{bc}	2.3^{c}	23^{c}	4.2^{a}	3.3^{b}	2.9^{bc}	47^{cd}	4.2^{a}	3.0^{b}	2.6^{bc}	35^{bc}
BS5A.2(T2) 19-1 P2	5.6^{b}	3.3^{c}	2.3^{c}	38^{e}	5.4^{ab}	3.3^{b}	3.7^{d}	53^{d}	5.5^{bc}	3.3^{bc}	3.0^{bc}	45^{bc}
BS5A.2(T2) 19-2 P1	6.1^{bc}	3.3^{c}	2.6^{c}	43^{f}	6.1^{b}	5.2^{d}	5.0^{f}	64^{e}	6.1^{c}	4.2^{c}	3.9^{c}	53^{c}
BS5A.2(T2) 19-3 P1	6.4^{c}	3.0^{c}	2.4^{c}	28^{d}	5.7^{ab}	3.1^{b}	2.7^{b}	39^{bc}	6.1^{c}	3.0^{b}	2.5^{bc}	33^{bc}
BS5A.2(T2) 19-3 P2	6.2^{bc}	2.2^{b}	2.2^{c}	20^{bc}	5.7^{ab}	4.4^{cd}	4.6^{e}	53^{d}	5.9^{c}	3.3^{bc}	3.4^{bc}	36^{bc}
Semsen (Control)	9.3^{d}	0.1^{a}	0.9^{a}	2^{a}	8.5^{c}	3.5^{a}	2.0^{a}	6^{a}	8.9^{d}	0.2^{a}	0.3^{a}	4^{a}
ICC 506 EB (Resistant check)	5.7^{b}	2.4^{bc}	1.5^{b}	16^{b}	5.7^{ab}	3.7^{bc}	3.6^{cd}	44.0^{bcd}	5.7^{bc}	3.0^{b}	2.5^{bc}	30^{b}
Mean	6.0	2.5	2.0	23.6	5.8	3.3	3.2	0.0	5.96	2.90	2.62	0.03
SE +	0.2	0.2	0.2	0.0	0.4	0.2	0.2	0.0	0.26	0.36	0.48	0.01
Fp	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001
Vr	58.5	17.2	17.1	86.2	6.6	33.8	49.7	42.5	28.00	11.00	5.00	5.000
LSD (P 0.05)	0.6*	0.8*	0.2*	0.0*	1.5*	0.8*	0.6*	0.0*	0.7*	1.0*	1.4*	0.0*
CV (%)	4.7	13.9	14.2	8.1	11.4	10.4	9.0	8.8	8.6	24.5	36.6	37.9

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.6: Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (ICRISAT, Patancheru 2011-2012).

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Mean larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)
BS5A.1(T2) 18-1 P1	23.0[a] (28.5)	2.1[a]	32.6[a]	65.6[a]	23.5[c]	10.5[ab]
BS5A.1(T2) 18-2 P1	19.0[a] (25.6)	1.3[a]	25.9[a]	45.0[a]	19.5[abc]	13.0[b]
BS5A.2(T2) 19-1 P2	25.0[a] (29.7)	4.5[a]	101.4[a]	63.8[a]	19.5[abc]	12.5[b]
BS5A.2(T2) 19-2 P1	12.0[a] (20.0)	0.8[a]	3.3[a]	47.8[a]	19.0[abc]	11.5[ab]
BS5A.2(T2) 19-3 P1	21.0[a] (26.7)	2.7[a]	47.6[a]	45.2[a]	19.0[abc]	9.5[ab]
BS5A.2(T2) 19-3 P2	10.0[a] (18.3)	0.8[a]	3.9[a]	20.5[a]	22.0[bc]	10.5[ab]
Semsen (Control)	74.0[bc] (59.4)	31.9[b]	438.0[b]	526.2[b]	16.5[ab]	11.0[ab]
ICC 506 EB (Resistant check)	61.0[b] (51.3)	28.8[b]	347.9[b]	523.8[b]	15.5[ab]	8.5[a]
Artificial diet	88.0[c] (69.8)	62.6[c]	1056.0[c]	1870.1[c]	14.5[a]	10.0[ab]
Mean	37.0	15.1	229.0	356.0	18.7	10.7
SE +	5.2	6.4	48.9	111.0	1.8	1.0
Fp	<0.001	0.001	<0.001	<0.001	0.111	0.237
Vr	31.0	11.2	50.8	29.6	2.4	1.6
LSD (P 0.05)	17.1*	21.0*	48.9*	361.9*	**NS**	**NS**
CV (%)	20	60.6	30.3	44	14	14.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values. DAI- Days after initiation of experiment.

Table 4.6 (Conti.)

Genotype	Pupation (%)	Adult emergence (%)	Adult longevity (days)		Fecundity (eggs female $^{-1}$)
			Male	Female	
BS5A.1(T2) 18-1 P1	13.0^{a} (21.1)	3.0^{a} (9.8)	2.0^{a}	4.5^{bcd}	-
BS5A.1(T2) 18-2 P1	8.0^{a} (16.3)	0.0^{a} (0.0)	-	-	-
BS5A.2(T2) 19-1 P2	11.0^{a} (19.2)	2.0^{a} (5.7)	-	1.5^{ab}	-
BS5A.2(T2) 19-2 P1	9.0^{a} (17.4)	3.0^{a} (9.8)	0.5^{a}	2.0^{abc}	-
BS5A.2(T2) 19-3 P1	9.0^{a} (16.7)	1.0^{a} (4.0)	-	0.5^{a}	-
BS5A.2(T2) 19-3 P2	5.0^{a} (12.8)	2.0^{a} (5.7)	-	2.0^{abc}	-
Semsen (Control)	34.0^{b} (35.6)	12.0^{ab} (20.2)	6.0^{b}	6.0^{d}	200.8^{c}
ICC 506 EB (Resistant check)	31.0^{b} (33.6)	19.0^{b} (25.7)	8.0^{b}	5.0^{cd}	95.0^{a}
Artificial diet	76.0^{c} (60.9)	19.0^{b} (25.7)	9.5^{b}	6.5^{d}	325.0^{d}
Mean	21.8	9.8	2.8	3.1	69.0
SE +	4.3	4.3	1.0	0.9	27.3
Fp	<0.001	0.001	<0.001	0.01	<0.001
Vr	28.1	11.7	13.1	5.9	18.7
LSD (P 0.05)	14.0*	14.2*	3.4*	3.2*	89.2*
CV (%)	28.0	63.0	52.1	45.1	56.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.7: Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (ICRISAT, Patancheru 2012-13).

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Mean larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)
BS5A.1(T2) 18-1 P1	21.0^{ab} (27.2)	19.7^{a}	338.9^{ab}	37.3^{a}	29.0^{b}	14.5^{ab}
BS5A.1(T2) 18-2 P1	25.0^{abc} (30.0)	27.5^{a}	450.2^{abc}	91.9^{a}	31.5^{b}	13.0^{ab}
BS5A.2(T2) 19-1 P2	18.0^{a} (24.9)	14.6^{a}	226.8^{ab}	22.5^{a}	31.5^{b}	17.0^{b}
BS5A.2(T2) 19-2 P1	7.0^{a} (14.9)	0.6^{a}	14.0^{a}	7.0^{a}	27.0^{b}	14.5^{ab}
BS5A.2(T2) 19-3 P1	13.0^{a} (18.7)	9.5^{a}	174.2^{ab}	21.8^{a}	31.0^{b}	10.5^{a}
BS5A.2(T2) 19-3 P2	17.0^{a} (23.0)	25.4^{a}	306.8^{ab}	43.6^{a}	28.0^{b}	10.5^{a}
Semsen (Control)	39.0^{bcd} (38.6)	162.5^{b}	1189.6^{bcd}	423.5^{b}	16.5^{a}	8.5^{a}
ICC 506 EB (Resistant check)	45.0^{cd} (42.1)	231.1^{b}	1366.1^{cd}	466.0^{b}	12.5^{a}	9.5^{a}
Artificial diet	52.0^{d} (46.1)	356.7^{c}	1653.1^{d}	702.1^{c}	15.5^{a}	11.0^{ab}
Mean	26.3	94.2	639.0	202.0	24.7	12.1
SE +	6.0	29.8	287.2	64.5	2.4	1.7
Fp	0.008	<0.001	0.026	<0.001	0.002	0.114
Vr	6.5	18.1	4.3	16.0	9.4	2.4
LSD (P 0.05)	19.6*	97.3*	936.5*	210.4*	8.1*	**NS**
CV (%)	32.3	44.8	63.9	45.2	14.2	20.8

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values. DAI- Days after initiation of experiment.

Table 4.7 (Conti.)

Genotype	Pupation (%)	Adult emergence (%)	Adult longevity (days)		Fecundity (eggs female $^{-1}$)
			Male	Female	
BS5A.1(T2) 18-1 P1	6.0^{a} (13.9)	4.0^{a} (11.5)	2.0^{ab}	3.5^{abc}	-
BS5A.1(T2) 18-2 P1	13.0^{ab} (21.1)	10.0^{ab} (18.3)	4.5^{abc}	8.0^{bc}	162.5^{b}
BS5A.2(T2) 19-1 P2	5.0^{a} (12.8)	5.0^{a} (12.8)	0.5^{a}	3.0^{ab}	32.5^{a}
BS5A.2(T2) 19-2 P1	2.0^{a} (5.7)	1.0^{a} (4.0)	-	-	-
BS5A.2(T2) 19-3 P1	4.0^{a} (8.2)	4.0^{a} (8.2)	-	-	-
BS5A.2(T2) 19-3 P2	7.0^{a} (14.2)	4.0^{a} (8.2)	1.5^{a}	3.0^{ab}	42.5^{a}
Semsen (Control)	26.0^{bc} (30.5)	17.0^{b} (23.8)	6.0^{bcd}	8.0^{bc}	187.5^{b}
ICC 506 EB (Resistant check)	29.0^{cd} (32.4)	20.0^{b} (26.5)	9.5^{d}	10.5^{c}	332.5^{c}
Artificial diet	42.0^{d} (40.3)	36.0^{c} (36.8)	8.0^{cd}	8.5^{bc}	312.5^{c}
Mean	14.9	11.2	3.5	4.9	118.9
SE ±	4.0	2.9	1.2	2.0	28.0
Fp	<0.001	<0.001	0.004	0.042	<0.001
Vr	12.3	14.6	7.8	3.6	23.1
LSD (P 0.05)	13.0*	9.6*	4.1*	6.5*	91.4*
CV (%)	38.1	37.3	51.1	57.8	33.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.8: Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (ICRISAT, Patancheru 2011-2013) (Pooled analysis).

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Mean larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)
BS5A.1(T2) 18-1 P1	22.0[a] (27.8)	10.9[a]	185.7[a]	51.5[a]	26.2[c]	12.5[abc]
BS5A.1(T2) 18-2 P1	22.0[a] (27.8)	14.4[a]	238.1[a]	68.5[a]	25.5[c]	13.0[bc]
BS5A.2(T2) 19-1 P2	21.5[a] (27.3)	9.6[a]	164.0[a]	43.1[a]	25.5[c]	14.7[c]
BS5A.2(T2) 19-2 P1	9.5[a] (17.5)	0.7[a]	8.60	27.4[a]	23.0[bc]	13.0[bc]
BS5A.2(T2) 19-3 P1	17.0[a] (22.7)	6.1[a]	110.9[a]	33.5[a]	25.0[c]	10.0[ab]
BS5A.2(T2) 19-3 P2	13.5[a] (20.7)	13.1[a]	155.3[a]	32.1[a]	25.0[c]	10.5[ab]
Semsen (Control)	56.5[b] (49.0)	97.2[ab]	813.8[b]	474.9[b]	16.5[ab]	9.7[ab]
ICC 506 EB (Resistant check)	53.0[b] (46.7)	129.9[ab]	857.0[b]	494.9[b]	14.0[a]	9.0[a]
Artificial diet	70.0[b] (57.9)	209.6[b]	1354.6[b]	1286.1[c]	15.0[ab]	10.5[ab]
Mean	31.7	55.0	432.0	279.0	21.7	11.4
SE +	6.2	39.5	189.0	127.1	2.6	1.0
Fp	<0.001	0.007	<0.001	<0.001	0.006	0.014
Vr	12.3	3.5	5.9	11.1	3.6	3.0
LSD (P 0.05)	18.2*	114.7*	549.4*	369.5*	7.6*	3.1*
CV (%)	39.6	144.0	87.5	91.1	24.3	19.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values. DAI- Days after initiation of experiment.

Table 4.8 (Conti.)

Genotype	Pupation (%)	Adult emergence (%)	Adult longevity (days)		Fecundity (eggs female $^{-1}$)
			Male	Female	
BS5A.1(T2) 18-1 P1	9.5^{a} (17.5)	3.5^{a} (10.6)	2.0^{a}	4.0^{ab}	-
BS5A.1(T2) 18-2 P1	10.5^{a} (18.7)	5.0^{a} (9.1)	2.2^{a}	4.0^{ab}	81.2^{a}
BS5A.2(T2) 19-1 P2	8.0^{a} (16.0)	3.5^{a} (9.3)	0.5^{a}	2.2^{a}	16.2^{a}
BS5A.2(T2) 19-2 P1	5.5^{a} (11.6)	2.0^{a} (6.9)	0.2^{a}	1.0^{a}	-
BS5A.2(T2) 19-3 P1	6.5^{a} (12.4)	2.5^{a} (6.1)	-	0.2^{a}	-
BS5A.2(T2) 19-3 P2	6.0^{a} (13.5)	3.0^{a} (6.9)	0.7^{a}	2.5^{a}	21.2^{a}
Semsen (Control)	30.0^{b} (33.0)	14.5^{b} (22.0)	6.0^{b}	7.0^{b}	194.1^{b}
ICC 506 EB (Resistant check)	30.0^{b} (33.0)	19.5^{b} (26.1)	8.7^{c}	7.7^{b}	213.7^{b}
Artificial diet	59.0^{c} (50.6)	41.0^{c} (39.7)	8.7^{c}	7.5^{b}	318.7^{c}
Mean	18.3	10.5	3.2	4.0	94.0
SE ±	4.3	2.5	0.8	1.4	32.7
Fp	<0.001	<0.001	<0.001	0.003	<0.001
Vr	17.2	25.1	18.0	4.0	13.2
LSD (P 0.05)	12.69*	7.531*	2.486*	4.074*	94.9*
CV (%)	47.6	49.3	53.1	69.6	69.5

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.9: Survival and development of third-instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (ICRISAT, Patancheru 2011-12).

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Mean larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)
BS5A.1(T2) 18-1 P1	42.0^{abc} (40.3)	19.0^{abc}	303.5^{ab}	238.5^{ab}	15.5^{ab}	14.5^{b}
BS5A.1(T2) 18-2 P1	27.0^{a} (31.3)	8.1^{a}	277.9^{a}	123.1^{a}	16.5^{ab}	15.0^{b}
BS5A.2(T2) 19-1 P2	60.0^{d} (54.9)	27.0^{c}	563.0^{b}	362.3^{b}	18.0^{b}	14.0^{ab}
BS5A.2(T2) 19-2 P1	33.0^{ab} (35.0)	11.0^{ab}	224.2^{a}	133.5^{a}	15.0^{a}	15.0^{b}
BS5A.2(T2) 19-3 P1	52.0^{cd} (46.1)	19.2^{abc}	315.2^{ab}	251.7^{ab}	16.5^{ab}	15.0^{b}
BS5A.2(T2) 19-3 P2	48.0^{bc} (43.8)	25.7^{bc}	365.4^{ab}	278.4^{ab}	16.5^{ab}	14.5^{ab}
Semsen (Control)	67.0^{d} (54.9)	63.5^{d}	1217.1^{c}	703.2^{c}	14.5^{a}	11.5^{ab}
ICC 506 EB (Resistant check)	85.0^{e} (67.2)	112.9^{e}	1696.1^{d}	1365.9^{d}	14.5^{a}	11.0^{a}
Artificial diet	92.0^{e} (73.6)	129.8^{f}	1881.0^{d}	1212.9^{d}	14.5^{a}	13.5^{ab}
Mean	57.0	46.2	760.3	518.8	15.7	13.7
SE ±	4.9	4.4	77.3	48.8	0.7	0.9
Fp	<0.001	<0.001	<0.001	<0.001	0.087	0.124
Vr	20.4	105.0	72.4	92.6	2.7	2.3
LSD (P 0.05)	16.1*	14.5*	252.1*	159.3*	2.4*	**NS**
CV (%)	12.3	13.6	14.4	13.3	6.7	10.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values. DAI- Days after initiation of experiment.

Table 4.9 (Conti.)

Genotype	Pupation (%)	Adult emergence (%)	Adult longevity (days)		Fecundity (eggs female $^{-1}$)
			Male	Female	
BS5A.1(T2) 18-1 P1	36.0^{b} (36.8)	27.0^{ab} (31.2)	9.5^{abc}	6.1^{ab}	645.5^{a}
BS5A.1(T2) 18-2 P1	17.0^{a} (24.2)	13.0^{a} (21.0)	5.5^{a}	8.5^{b}	590.0^{a}
BS5A.2(T2) 19-1 P2	49.0^{bc} (44.4)	44.0^{cd} (41.5)	10.1^{abc}	7.5^{ab}	572.5^{a}
BS5A.2(T2) 19-2 P1	23.0^{a} (28.6)	16.0^{ab} (23.4)	12.1^{bc}	5.5^{a}	699.0^{a}
BS5A.2(T2) 19-3 P1	44.0^{bc} (41.4)	30.0^{bc} (32.9)	6.5^{ab}	8.1^{ab}	804.5^{ab}
BS5A.2(T2) 19-3 P2	38.0^{bc} (38.0)	27.0^{ab} (31.2)	9.5^{abc}	7.5^{ab}	740.0^{a}
Semsen (Control)	51.0^{c} (45.5)	48.0^{d} (43.8)	13.1^{c}	7.5^{ab}	848.5^{ab}
ICC 506 EB (Resistant check)	77.0^{d} (61.3)	72.0^{e} (58.0)	15.1^{c}	7.5^{ab}	1076.0^{ab}
Artificial diet	84.0^{d} (66.4)	78.0^{e} (62.0)	13.5^{c}	8.5^{b}	1295.5^{b}
Mean	46.6	39.4	10.5	7.3	808.0
SE ±	3.9	4.6	1.7	0.7	147.2
Fp	<0.001	<0.001	0.058	0.189	0.096
Vr	32.1	25.2	3.2	1.9	2.0
LSD (P 0.05)	12.8*	15.0*	5.7*	**NS**	479.9*
CV (%)	11.9	16.5	23.8	14.2	25.8

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.10: Survival and development of third-instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (ICRISAT, Patancheru 2012-13).

Genotype	Larval survival (%)	Mean larval weight(mg) (5 DAI)	Mean larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)
BS5A.1(T2) 18-1 P1	29.0[a] (32.5)	9.1[a]	159.8[a]	115.3[a]	16.5[b]	12.5[ab]
BS5A.1(T2) 18-2 P1	48.0[ab] (43.8)	11.5[a]	201.1[a]	98.5[a]	15.5[ab]	14.0[ab]
BS5A.2(T2) 19-1 P2	47.0[ab] (43.2)	10.7[a]	258.1[a]	130.6[a]	16.0[b]	13.0[ab]
BS5A.2(T2) 19-2 P1	74.0[bcd] (59.7)	25.7[b]	396.5[a]	195.60[a]	15.5[ab]	15.0[b]
BS5A.2(T2) 19-3 P1	60.0[bc] (50.7)	20.3[ab]	344.8[a]	182.3[a]	15.5[ab]	13.0[ab]
BS5A.2(T2) 19-3 P2	46.0[ab] (2.64)	10.0[a]	333.7[a]	158.4[a]	12.5[a]	13.0[ab]
Semsen (Control)	82.0[cd] (66.3)	45.5[c]	1348.8[b]	952.6[b]	13.5[ab]	11.5[ab]
ICC 506 EB (Resistant check)	84.0[cd] (66.8)	51.3[c]	1305.6[b]	974.0[b]	14.5[ab]	11.0[ab]
Artificial diet	92.0[d] (73.6)	69.2[d]	1438.6[b]	949.0[b]	12.5[a]	10.5[a]
Mean	62.4	28.2	643.0	417.0	14.6	12.6
SE ±	8.2	3.7	121.6	44.1	0.9	1.1
Fp	0.007	<0.001	<0.001	<0.001	0.098	0.253
Vr	6.8	34.3	20.2	85.0	2.6	1.6
LSD (P 0.05)	26.7*	12.2*	396.7*	143.9*	3.0*	**NS**
CV (%)	18.6	18.8	26.8	15.0	8.9	12.6

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values. DAI- Days after initiation of experiment.

Table 4.10 (Conti.)

Genotype	Pupation (%)	Adult emergence (%)	Adult longevity (days)		Fecundity (eggs female $^{-1}$)
			Male	Female	
BS5A.1(T2) 18-1 P1	15.0^{a} (22.6)	9.0^{a} (17.4)	9.5^{a}	7.5^{a}	500.0^{ab}
BS5A.1(T2) 18-2 P1	29.0^{ab} (32.5)	16.0^{a} (23.4)	9.5^{a}	8.5^{a}	612.5^{ab}
BS5A.2(T2) 19-1 P2	28.0^{ab} (31.8)	15.0^{a} (22.7)	10.0^{a}	8.5^{a}	554.0^{a}
BS5A.2(T2) 19-2 P1	47.0^{b} (43.2)	30.0^{b} (33.1)	10.5^{a}	8.5^{a}	640.0^{ab}
BS5A.2(T2) 19-3 P1	45.0^{b} (42.1)	36.0^{b} (36.8)	9.5^{a}	9.0^{a}	587.5^{ab}
BS5A.2(T2) 19-3 P2	26.0^{ab} (30.3)	17.0^{a} (24.1)	9.5^{a}	9.0^{a}	545.0^{a}
Semsen (Control)	72.0^{c} (58.2)	61.0^{c} (51.3)	11.0^{a}	10.0^{a}	800.0^{bc}
ICC 506 EB (Resistant check)	77.0^{c} (61.6)	65.0^{c} (53.7)	11.5^{a}	9.5^{a}	992.5^{c}
Artificial diet	80.0^{c} (63.4)	75.0^{d} (60.0)	12.5^{a}	9.5^{a}	958.5^{c}
Mean	46.6	36.0	10.3	8.8	698.0
SE ±	6.2	2.8	1.3	1.1	69.3
Fp	<0.001	<0.001	0.698	0.894	0.009
Vr	15.1	75.5	0.6	0.4	6.3
LSD (P 0.05)	20.4*	9.3*	**NS**	**NS**	226.0*
CV (%)	19.1	11.2	17.8	18.7	14.0

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.11: Survival and development of third instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines (ICRISAT, Patancheru 2011-2013) (Pooled analysis).

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Mean larval weight (mg) (10 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)
BS5A.1(T2) 18-1 P1	35.5^{a} (36.4)	14.0^{a}	231.7^{a}	176.9^{a}	16.0^{bc}	13.5^{bcd}
BS5A.1(T2) 18-2 P1	37.5^{ab} (37.5)	9.8^{a}	239.5^{a}	110.8^{a}	16.0^{bc}	14.5^{d}
BS5A.2(T2) 19-1 P2	57.0^{bc} (49.1)	18.8^{a}	410.5^{a}	246.4^{a}	17.0^{c}	13.5^{bcd}
BS5A.2(T2) 19-2 P1	53.5^{ab} (47.4)	18.4^{a}	310.4^{a}	164.5^{a}	15.2^{abc}	15.0^{d}
BS5A.2(T2) 19-3 P1	56.0^{abc} (48.4)	19.7^{a}	330.0^{a}	217.0^{a}	16.0^{bc}	14.0^{cd}
BS5A.2(T2) 19-3 P2	47.0^{ab} (43.2)	17.9^{a}	349.5^{a}	218.4^{a}	14.5^{ab}	13.7^{bcd}
Semsen (Control)	74.5^{cd} (60.6)	54.5^{b}	1282.9^{b}	827.9^{b}	14.0^{ab}	11.5^{ab}
ICC 506 EB (Resistant check)	84.5^{d} (67.0)	82.1^{c}	1500.8^{bc}	1169.9^{c}	14.5^{ab}	11.0^{a}
Artificial diet	92.0^{d} (73.6)	99.5^{c}	1659.8^{c}	1080.9^{c}	13.5^{a}	12.0^{abc}
Mean	59.7	37.2	701.6	468.0	15.1	13.1
SE +	6.5	9.4	90.5	65.6	0.7	0.7
Fp	<0.001	<0.001	<0.001	<0.001	0.029	0.01
Vr	9.2	12.5	43.1	42.8	2.6	3.2
LSD (P 0.05)	19.0*	27.3*	263.3*	190.7*	2.0*	2.2*
CV (%)	22	50.5	25.8	28	9.3	11.6

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values. DAI- Days after initiation of experiment.

Table 4.11 (Conti.)

Genotype	Pupation (%)	Adult emergence (%)	Adult longevity (days)		Fecundity (eggs female $^{-1}$)
			Male	Female	
BS5A.1(T2) 18-1 P1	25.5^{a} (29.7)	18.0^{ab} (24.3)	9.5^{abcd}	6.7^{a}	617.8^{a}
BS5A.1(T2) 18-2 P1	23.0^{a} (28.3)	14.5^{a} (22.2)	7.5^{a}	8.5^{a}	601.2^{a}
BS5A.2(T2) 19-1 P2	38.5^{ab} (38.1)	29.5^{bc} (32.1)	10.0^{abcd}	8.0^{a}	563.2^{a}
BS5A.2(T2) 19-2 P1	35.0^{ab} (35.9)	23.0^{abc} (28.2)	11.2^{bcde}	7.0^{a}	669.5^{a}
BS5A.2(T2) 19-3 P1	44.5^{b} (41.8)	33.0^{c} (34.8)	8.0^{ab}	8.5^{a}	696.0^{a}
BS5A.2(T2) 19-3 P2	32.0^{ab} (34.2)	22.0^{abc} (27.7)	9.5^{abc}	8.2^{a}	642.5^{a}
Semsen (Control)	61.5^{c} (51.9)	54.5^{d} (47.6)	12.0^{cde}	8.7^{a}	824.2^{ab}
ICC 506 EB (Resistant check)	77.0^{cd} (61.4)	68.5^{e} (55.9)	13.2^{e}	8.5^{a}	1034.2^{bc}
Artificial diet	82.0^{d} (64.9)	76.5^{e} (61.0)	13.0^{ce}	9.0^{a}	1127.0^{c}
Mean	46.6	37.7	10.4	8.1	753.0
SE +	5.4	4.6	1.0	0.7	83.7
Fp	<0.001	<0.001	0.006	0.449	<0.001
Vr	16.1	24.8	3.6	1.0	5.7
LSD (P 0.05)	15.8*	13.3*	3.1*	2.2*	243.4*
CV (%)	23.4	24.4	20.8	18.8	22.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.12: Biochemical profile of different transgenic chickpea lines (dry weight basis) (ICRISAT, Patancheru 2011-13).

Genotype	2011-2012					2012-2013					Pooled analysis				
	Proteins (mg/g)	Carbohydrates (%)	Lipids (%)	Phenols (mg/g)	Tannins (mg/g)	Proteins (mg/g)	Carbohydrates (%)	Lipids (%)	Phenols (mg/g)	Tannins (mg/g)	Proteins (mg/g)	Carbohydrates (%)	Lipids (%)	Phenols (mg/g)	Tannins (mg/g)
BS5A.1(T2) 18-1 P1	5.2^{ab}	34.0^{b}	13.9^{a}	1.1^{a}	2.2^{d}	5.2^{ab}	35.0^{a}	16.6^{ab}	0.9^{a}	1.5^{a}	5.2^{a}	34.5^{abc}	15.2^{ab}	1.0^{a}	1.9^{a}
BS5A.1(T2) 18-2 P1	5.3^{ab}	44.6^{cd}	10.6^{a}	1.0^{a}	0.5^{a}	6.3^{cd}	38.8^{a}	16.7^{ab}	1.0^{a}	2.0^{a}	5.8^{a}	41.7^{bcd}	13.6^{ab}	1.0^{a}	1.2^{a}
BS5A.2(T2) 19-1 P2	5.8^{b}	34.3^{b}	7.8^{a}	1.1^{a}	1.6^{c}	5.4^{bcd}	30.6^{a}	14.0^{ab}	1.1^{a}	1.4^{a}	5.6^{a}	32.5^{ab}	10.9^{a}	1.1^{a}	1.5^{a}
BS5A.2(T2) 19-2 P1	4.9^{a}	38.00^{bc}	16.4^{a}	1.2^{a}	2.1^{d}	6.4^{de}	31.3^{a}	8.2^{a}	0.9^{a}	1.6^{a}	5.6^{a}	34.6^{abc}	12.3^{ab}	1.1^{a}	1.8^{a}
BS5A.2(T2) 19-3 P1	5.3^{ab}	49.3^{de}	11.9^{a}	0.9^{a}	1.2^{b}	6.1^{bcd}	38.0^{a}	29.4^{c}	1.2^{a}	1.7^{a}	5.7^{a}	43.6^{cd}	20.6^{b}	1.0^{a}	1.4^{a}
BS5A.2(T2) 19-3 P2	5.2^{ab}	36.0^{bc}	8.8^{a}	1.2^{a}	3.2^{e}	5.4^{bc}	28.1^{a}	7.0^{a}	0.9^{a}	1.1^{a}	5.3^{a}	32.0^{a}	7.9^{a}	1.0^{a}	2.1^{a}
ICC 506 EB (Resistant check)	4.8^{a}	55.0^{e}	11.5^{a}	1.0^{a}	1.0^{b}	7.2^{e}	34.8^{a}	13.7^{ab}	1.0^{a}	1.8^{a}	6.0^{a}	44.9^{d}	12.6^{ab}	1.0^{a}	1.4^{a}
Semsen (Control)	5.5^{ab}	24.3^{a}	13.7^{a}	1.1^{a}	0.8^{b}	4.5^{a}	32.8^{a}	20.1^{b}	1.1^{a}	1.6^{a}	5.0^{a}	28.5^{a}	16.9^{ab}	1.1^{a}	1.2^{a}
Mean	5.2	39.5	11.8	1.1	1.6	5.8	33.7	15.7	1.0	1.6	5.5	36.5	13.8	1.0	1.6
SE ±	0.2	2.8	3.2	0.1	0.1	0.2	3.7	2.9	0.1	0.4	0.3	3.0	2.8	0.1	0.3
Fp	0.191	<0.001	0.641	0.695	<0.001	<0.001	0.502	0.003	0.80	0.901	0.422	0.002	0.101	1.00	0.318
Vr	1.6	11.5	0.7	0.6	60.5	9.0	0.9	5.7	0.5	0.3	1.0	3.9	1.8	0.1	1.2
LSD (P 0.05)	**NS**	8.69	**NS**	**NS**	0.3468	0.8426	**NS**	8.947	**NS**	**NS**	**NS**	8.684	**NS**	**NS**	**NS**
CV (%)	7.6	12.6	47.7	19.0	12.3	8.2	19.3	32.5	23.5	45.5	14.1	20.3	50.3	20.8	46.6

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.14 : HPLC fingerprints (area) of organic acids on leaf surface of transgenic chickpea lines (ICRISAT, Patancheru 2011-2013).

Genotype	2011-2012		2012-2013	
	Peak 1	Peak 2	Peak 1	Peak 2
	Oxalic acid (µV*sec)	Malic acid (µV*sec)	Oxalic acid (µV*sec)	Malic acid (µV*sec)
BS5A.1(T2) 18-1 P1	541334^{ab}	127666^{bc}	1157860^{b}	145349^{b}
BS5A.1(T2) 18-2 P1	510886^{ab}	103817^{bc}	898310^{ab}	121824^{b}
BS5A.2(T2) 19-1 P2	579302^{ab}	85062^{b}	847282^{ab}	79664^{ab}
BS5A.2(T2) 19-2 P1	502150^{a}	100117^{bc}	652908a	77532^{ab}
BS5A.2(T2) 19-3 P1	817376^{ab}	130085^{c}	779966^{a}	110829^{b}
BS5A.2(T2) 19-3 P2	1076296^{b}	124572^{bc}	805094^{a}	112528^{b}
Semsen (Control)	672385^{ab}	18891^{a}	615042^{a}	15646^{a}
ICC 506 EB (Resistant check)	1059318^{ab}	128734^{bc}	827882^{ab}	99784^{b}
Mean	719881	86276	823043	95305
SE +	157289.6	12180.4	97781.1	22627.7
Fp	0.148	<0.001	0.093	0.087
Vr	2.29	16.93	2.88	2.98
LSD (P 0.05)	**NS**	40732.4*	326987.7*	75668.9*
CV (%)	30.9	20.0	16.8	33.5

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.15: Concentration of organic acids (on fresh weight basis) present on the leaf surface of transgenic chickpea lines (ICRISAT, Patancheru 2011-2013)

Genotype	2011-2012		2012-2013		Pooled	
	Oxalic acid (mg/g)	Malic acid (mg/g)	Oxalic acid (mg/g)	Malic acid (mg/g)	Oxalic acid (mg/g)	Malic acid (mg/g)
BS5A.1(T2) 18-1 P1	0.9^{a}	2.8^{b}	1.2^{ab}	1.8^{bc}	1.0^{a}	2.3^{b}
BS5A.1(T2) 18-2 P1	1.0^{a}	2.4^{b}	0.8^{a}	1.3^{ab}	0.9^{a}	1.9^{b}
BS5A.2(T2) 19-1 P2	1.3^{abc}	2.3^{b}	1.0^{ab}	1.2^{ab}	1.1^{a}	1.7^{ab}
BS5A.2(T2) 19-2 P1	0.8^{a}	2.1^{b}	0.7^{a}	1.1^{ab}	0.8^{a}	1.6^{ab}
BS5A.2(T2) 19-3 P1	1.3^{ab}	2.5^{b}	0.9^{a}	1.6^{b}	1.1^{a}	2.1^{b}
BS5A.2(T2) 19-3 P2	1.5^{abcd}	2.2^{b}	0.5^{a}	0.9^{ab}	1.0^{a}	1.5^{ab}
Semsen (Control)	1.2^{a}	0.4^{a}	0.7^{a}	0.2^{a}	0.9^{a}	0.3^{a}
ICC 506 EB (Resistant check)	2.5^{bd}	2.7^{a}	2.0^{b}	2.9^{c}	2.2^{b}	2.6^{ab}
Mean	1.3	1.8	1.0	1.4	1.1	1.6
SE +	0.3	0.3	0.2	0.3	0.2	0.4
Fp	0.132	0.004	0.095	0.022	0.007	0.152
Vr	2.4	9.7	2.8	5.2	3.7	1.7
LSD (P 0.05)	**NS**	1.1*	0.9*	1.1*	0.6*	NS
CV (%)	35.4	25.5	37.7	34.8	40.0	55.8

*Figures followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Table 4.13: Correlation between resistance/susceptibility to pod borer, *H. armigera* and the amounts of biochemical components in transgenic chickpea (on dry weight basis) (ICRISAT, Patancheru 2011-13).

HDR	2011-12					2012-13				
	Proteins	**Carbohydrates**	**Lipids**	**Phenols**	**Tannins**	**Proteins**	**Carbohydrates**	**Lipids**	**Phenols**	**Tannins**
	-0.45*	0.40*	0.02	-0.24	-0.41*	0.31	0.25	0.05	-0.33	-0.47*
Larval survival (%)	-0.25	0.15	0.08	-0.27	-0.40*	-0.23	0.23	0.00	-0.40*	-0.45*
Mean larval wt. (mg)	-0.27	0.10	0.09	-0.17	-0.42*	-0.29	0.22	0.07	-0.23	-0.43*

,* Significant at P≤ 0.05 and 0.01, respectively

Table 4.16: Correlation between resistance/susceptibility to pod borer, *H. armigera* and the amounts organic acids in transgenic chickpea (on fresh weight basis) (ICRISAT, Patancheru 2011-13).

	2011-12		2012-13	
	Oxalic acid	**Malic acid**	**Oxalic acid**	**Malic acid**
HDR	0.32	-0.83**	0.19	0.18
Larval survival (%)	0.63**	-0.93**	0.47	0.23
Mean larval wt. (mg)	0.60*	-0.95**	0.56*	0.27

,* Significant at P≤ 0.05 and 0.01, respectively

Table 4.17: HPLC finger prints (area) of flavonoids in leaf samples of transgenic chickpea lines (on dry weight basis) (ICRISAT, Patancheru 2011-2012).

Genotype	Peak1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
	Chlorogenic acid (µV*sec)	Gentisic acid (µV*sec)	Phloretic acid (µV*sec)	Ferulic acid (µV*sec)	Umbelliferone (µV*sec)	Naringin (µV*sec)
BS5A.1(T2) 18-1 P1	329266^{ab}	372440^{b}	437575^{abc}	16131072^{a}	271966^{ab}	1701744^{ab}
BS5A.1(T2) 18-2 P1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
BS5A.2(T2) 19-1 P2	551442^{b}	125474^{a}	433084^{abc}	735058^{a}	142180^{ab}	1442113^{ab}
BS5A.2(T2) 19-2 P1	421452^{b}	323184^{b}	867355^{c}	665861^{a}	343448^{ab}	2559470^{b}
BS5A.2(T2) 19-3 P1	148518^{ab}	0^{a}	807677^{c}	1241720^{a}	334952^{ab}	0^{a}
BS5A.2(T2) 19-3 P2	394218^{ab}	0^{a}	593158^{ac}	1644224^{a}	500959^{b}	1283384^{ab}
Semsen (Control)	0^{a}	0^{a}	0^{ab}	0^{a}	0^{a}	0^{a}
ICC 506 EB (Resistant check)	0^{a}	0^{a}	0^{ab}	63298^{a}	84122^{a}	0^{a}
Mean	230612	102637	392356	2560154	209703	873339
SE ±	112818	47404.4	164726.1	5316741.2	110570.8	678247.8
Fp	0.048	0.003	0.028	0.463	0.107	0.161
Vr	3.84	11.1	4.76	1.08	2.69	2.2
LSD (P 0.05)	377272.5*	158524.2*	550857.4*	**NS**	**NS**	**NS**
CV (%)	69.2	65.3	59.4	293.7	74.6	109.8

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.17 (Conti.)

Genotype	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
	3, 4 Dihydroxy flavones (µV*sec)	Quercetin (µV*sec)	Cinnamic acid (µV*sec)	Naringenin (µV*sec)	Genistein (µV*sec)	Formononetin (µV*sec)	Biochanin A (µV*sec)
BS5A.1(T2) 18-1 P1	347334[a]	1623148[f]	158572[a]	5042944[a]	2862246[d]	256907[bc]	625598[b]
BS5A.1(T2) 18-2 P1	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
BS5A.2(T2) 19-1 P2	191440[a]	1116417d	1199464[b]	1067709[a]	1163205[abc]	317068[c]	437628[ab]
BS5A.2(T2) 19-2 P1	196366[a]	970904[b]	0[a]	209626[a]	2020932[cd]	262563[bc]	358566[ab]
BS5A.2(T2) 19-3 P1	648484[a]	1040136[c]	0[a]	229192[a]	1422276[bc]	85014[a]b	513928[ab]
BS5A.2(T2) 19-3 P2	787536[a]	1317268[e]	127563[a]	129458[a]	2280872[cd]	238864[bc]	270246[ab]
Semsen (Control)	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
ICC 506 EB (Resistant check)	303457[a]	0[a]	0[a]	0[a]	687841[ab]	109255[ab]	222427[ab]
Mean	309327	758484	185700	834866	1304671	158709	303549
SE +	267806.3	2822.9	76838.1	1861350.8	347850.5	50623.7	150915.9
Fp	0.442	<0.001	<0.001	0.57	0.005	0.014	0.152
Vr	1.1	54469.8	29.1	0.8	9.1	6.1	2.2
LSD (P 0.05)	**NS**	9439.9*	256952.8*	**NS**	1163239.9*	169289.8*	**NS**
CV (%)	122.4	0.5	58.5	315.3	37.7	45.1	70.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.18: HPLC finger prints (area) of flavonoids in leaf samples of transgenic chickpea lines (on dry weight basis) (ICRISAT, Patancheru 2012-2013).

Genotype	Peak1	Peak 2	Peak 3	Peak 4	Peak 5
	Chlorogenic acid (µV*sec)	Gentisic acid (µV*sec)	Phloretic acid (µV*sec)	Ferulic acid (µV*sec)	Naringin (µV*sec)
BS5A.1(T2) 18-1 P1	341620[a]	246850[a]	384178[a]	437546[a]	0[a]
BS5A.1(T2) 18-2 P1	139686[a]	368740[a]	528310[a]	1150268[ab]	1594698[ab]
BS5A.2(T2) 19-1 P2	263896[a]	126810[a]	484580[a]	337444[a]	2663882[ab]
BS5A.2(T2) 19-2 P1	442229[a]	375851[a]	663945[a]	829470[ab]	1650000[ab]
BS5A.2(T2) 19-3 P1	287856[a]	189262[a]	459612a	438298[a]	1750243[ab]
BS5A.2(T2) 19-3 P2	324675[a]	334284[a]	231320[a]	937321[ab]	2696740[ab]
Semsen (Control)	305630[a]	297672[a]	281908[a]	1355736[b]	0[a]
ICC 506 EB (Resistant check)	375348[a]	334730[a]	416121[a]	359800[a]	4002615[b]
Mean	310118	284275	431247	730735	1794772
SE +	124285.1	98619.6	187470.3	251127.3	1081227
Fp	0.806	0.599	0.785	0.13	0.28
Vr	0.51	0.82	0.54	2.45	1.58
LSD (P 0.05)	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	56.7	49.1	61.5	48.6	85.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.18 (Conti.)

Genotype	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
	3, 4 Dihydroxy flavones (µV*sec)	Quercetin (µV*sec)	Naringenin (µV*sec)	Genistein (µV*sec)	Formononetin (µV*sec)	Biochanin A (µV*sec)
BS5A.1(T2) 18-1 P1	85750[a]	466168[a]	181786[bc]	444424[a]	257604[ab]	671826[f]
BS5A.1(T2) 18-2 P1	618770[a]	1619896[a]	207626[c]	2082074[bc]	251340[ab]	663998[e]
BS5A.2(T2) 19-1 P2	534006[a]	453998[a]	0[a]	716590[a]	229824[ab]	450466[b]
BS5A.2(T2) 19-2 P1	248480[a]	1365238[a]	253814[c]	1409192[ab]	307628[b]	606301[d]
BS5A.2(T2) 19-3 P1	122678[a]	753750[a]	86438[ab]	1077256[ab]	128575[a]	0[a]
BS5A.2(T2) 19-3 P2	814960[a]	716384[a]	231976[c]	2913119[b]	371516[b]	595440[c]
Semsen (Control)	2580110[a]	2349121[a]	629969[d]	17232816[c]	2317562[c]	5286246[h]
ICC 506 EB (Resistant check)	1045459[a]	0[a]	0[a]	2738210[b]	401805[b]	972870[g]
Mean	756277	965570	198951	3576710	533232	1155893
SE ±	829938.7	1017055	30436.2	562610.3	48808.7	2278.1
Fp	0.525	0.769	<0.001	<0.001	<0.001	<0.001
Vr	0.95	0.56	43.5	98.75	221.24	1.04
LSD (P 0.05)	**NS**	**NS**	101781.0*	1881413.9*	163220.3*	7618.3*
CV (%)	155.2	149	21.6	22.2	12.9	0.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.19: Amount of flavonoids in transgenic chickpea lines (dry weight basis) (ICRISAT, Patancheru 2011-2012).

Genotype	Chlorogenic acid (mg/g)	Gentisic acid (mg/g)	Phloretic acid (mg/g)	Ferulic acid (mg/g)	Umbelliferone (mg/g)	Naringin (mg/g)
BS5A.1(T2) 18-1 P1	0.9^{ab}	3.9^{b}	10.3^{ab}	26.0^{a}	1.0^{ab}	12.9^{ab}
BS5A.1(T2) 18-2 P1	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
BS5A.2(T2) 19-1 P2	1.6^{b}	1.3^{a}	10.2^{ab}	1.1^{a}	0.5^{a}	10.9^{ab}
BS5A.2(T2) 19-2 P1	1.2^{b}	3.4^{b}	20.5^{b}	1.0^{a}	1.2^{ab}	19.4^{b}
BS5A.2(T2) 19-3 P1	0.4^{ab}	0.0^{a}	19.1^{b}	2.0^{a}	1.2^{ab}	0.0^{a}
BS5A.2(T2) 19-3 P2	1.1^{ab}	0.0^{a}	8.4^{ab}	2.6^{a}	1.8^{b}	9.7^{ab}
Semsen (Control)	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
ICC 506 EB (Resistant check)	0.0^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}
Mean	0.6	1.0	8.5	4.1	0.7	6.6
SE $\pm$	0.3	0.5	4.5	8.5	0.3	5.1
Fp	0.048	0.003	0.070	0.463	0.053	0.161
Vr	3.8	11.1	3.2	1.0	3.6	2.2
LSD (P 0.05)	1.1*	1.6*	15.3*	**NS**	1.2*	**NS**
CV (%)	69.2	65.3	75.3	293.7	70.9	109.8

*Figures followed by the same letter within a column are not significantly different at P$\leq$ 0.05.

Table 4.19 (Conti.)

Genotype	3, 4 Dihydroxy flavone (mg/g)	Quercetin (mg/g)	Cinnamic acid (mg/g)	Naringenin (mg/g)	Genistein (mg/g)	Formononetin (mg/g)	Biochanin A (mg/g)
BS5A.1(T2) 18-1 P1	0.4[a]	1.7[f]	0.1[a]	25.0[a]	2.5[d]	0.3[b]	0.8[b]
BS5A.1(T2) 18-2 P1	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
BS5A.2(T2) 19-1 P2	0.2[a]	1.1[d]	1.3[b]	5.2[a]	1.0[abc]	0.4[b]	0.5[ab]
BS5A.2(T2) 19-2 P1	0.2[a]	1.0[a]	0.0[a]	1.0[a]	1.7[cd]	0.3[b]	0.4[ab]
BS5A.2(T2) 19-3 P1	0.7[a]	1.1[a]	0.0[a]	1.1[a]	1.2[bc]	0.1[ab]	0.6[ab]
BS5A.2(T2) 19-3 P2	0.9[a]	1.4[e]	0.1[a]	0.6[a]	1.9[cd]	0.1[ab]	0.3[ab]
Semsen (Control)	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
ICC 506 EB (Resistant check)	0.3[a]	0.0[a]	0.0[a]	0.0[a]	0.6[ab]	0.1[ab]	0.2[ab]
Mean	0.3	0.8	0.2	4.1	1.1	0.1	0.3
SE +	0.3	0.0	0.1	9.2	0.3	0.1	0.1
Fp	0.442	<0.001	<0.001	0.57	0.005	0.066	0.152
Vr	1.1	54469.8	29.1	0.8	9.1	3.3	2.2
LSD (P 0.05)	**NS**	0.0*	0.2*	**NS**	1.0*	0.2*	**NS**
CV (%)	122.4	0.5	58.5	315.3	37.7	68.1	70.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.20: Amount of flavonoids in transgenic chickpea lines (dry weight basis) (ICRISAT, Patancheru 2012-2013).

Genotype	Chlorogenic acid (mg/g)	Gentisic acid (mg/g)	Phloretic acid (mg/g)	Ferulic acid (mg/g)	Naringin (mg/g)
BS5A.1(T2) 18-1 P1	1.0[a]	2.6[a]	9.1[a]	0.7[a]	0[a]
BS5A.1(T2) 18-2 P1	0.4[a]	3.9[a]	12.5[a]	1.8[ab]	12.1[ab]
BS5A.2(T2) 19-1 P2	0.7[a]	1.3[a]	11.4[a]	0.5[a]	20.2[ab]
BS5A.2(T2) 19-2 P1	1.3[a]	4.0[a]	8.3[a]	1.3[ab]	12.5[ab]
BS5A.2(T2) 19-3 P1	0.8[a]	2.0[a]	10.8[a]	0.7[a]	13.2[ab]
BS5A.2(T2) 19-3 P2	0.9[a]	3.5[a]	5.4[a]	1.5[ab]	20.7[ab]
Semsen (Control)	0.9[a]	3.1[a]	6.6[a]	2.1[b]	0[a]
ICC 506 EB	1.1[a]	3.5[a]	9.8[a]	0.5[a]	30.3[b]
Mean	0.9	3.0	9.2	1.1	13.6
SE +	0.3	1.0	4.0	0.4	8.2
Fp	0.806	0.599	0.906	0.130	0.280
Vr	0.5	0.8	0.3	2.4	1.5
LSD (P 0.05)	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	56.7	3.5	62.0	48.6	85.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.20 (Conti.)

Genotype	3, 4 Dihydroxy flavone (mg/g)	Quercetin (mg/g)	Naringenin (mg/g)	Genistein (mg/g)	Formononetin (mg/g)	Biochanin A (mg/g)
BS5A.1(T2) 18-1 P1	0.0[a]	0.5[a]	1.3[bc]	0.3[a]	0.3[ab]	0.8[f]
BS5A.1(T2) 18-2 P1	0.7[a]	1.7[a]	1.5[c]	1.8[ab]	0.3[ab]	0.8[e]
BS5A.2(T2) 19-1 P2	0.6[a]	0.4[a]	0.0[a]	0.6[a]	0.2[ab]	0.5[b]
BS5A.2(T2) 19-2 P1	0.2[a]	1.4[a]	1.9[c]	1.2[ab]	0.3[b]	0.7[d]
BS5A.2(T2) 19-3 P1	0.1[a]	0.8[a]	0.6[ab]	0.9[ab]	0.1[a]	0.0[a]
BS5A.2(T2) 19-3 P2	0.9[a]	0.7[a]	1.7[c]	2.5[b]	0.4[b]	0.7[c]
Semsen (Control)	2.9[a]	2.5[a]	4.7[d]	1.5[ab]	2.9[c]	6.8[h]
ICC 506 EB	1.2[a]	1.0[a]	0.0[a]	2.3[b]	0.5[b]	1.2[g]
Mean	0.8	1.0	1.5	1.1	0.6	1.4
SE ±	0.9	1.0	0.2	0.4	0.0	0.0
Fp	0.525	0.769	<.001	<.001	<.001	<.001
Vr	0.9	0.5	43.5	98.7	221.2	5.5
LSD (P 0.05)	**NS**	**NS**	0.7*	1.6*	0.2*	0.1*
CV (%)	155.2	149.0	21.6	22.2	12.9	0.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.

Table 4.21: Correlation between resistance/susceptibility to pod borer, *H. armigera* and, the amount of flavonoids in transgenic chickpea lines (on dry weight basis) (ICRISAT, Patancheru 2011-12).

	Chlorogenic acid	Gentisic acid	Phloretic acid	Ferulic acid	Umbelliferone	Naringin	3, 4 Dihydroxy flavone	Quercetin	Cinnamic acid	Naringenin	Genistein	Formononetin	Biochanin A
HDR	-0.57*	-0.30	-0.75**	-0.25	-0.58*	-0.56	0.89**	-0.09	-0.34	-0.20	0.89**	0.87**	0.90**
Larval survival (%)	-0.51*	-0.39	-0.66**	-0.30	-0.52*	-0.34	0.84**	-0.37	-0.33	-0.28	0.72**	0.69**	0.72**
Mean larval wt. (mg)	-0.54*	-0.31	-0.76**	-0.29	-0.60*	-0.33	0.83**	-0.38	-0.34	-0.27	0.72**	0.71**	0.74**

,* Significant at P≤ 0.05 and 0.01, respectively

Table 4.22: Correlation between resistance/susceptibility to pod borer, *H. armigera* and, the amount of flavonoids in transgenic chickpea lines (on dry weight basis) (ICRISAT, Patancheru 2012-13).

	Chlorogenic acid	Gentisic acid	Phloretic acid	Ferulic acid	Naringin	3, 4 Dihydroxy flavone	Quercetin	Naringenin	Genistein	Formononetin	Biochanin A
HDR	0.07	0.20	-0.39	0.46	0.19	0.94**	0.45	0.64	0.91**	0.90**	0.91**
Larval survival (%)	0.12	0.24	-0.28	0.19	0.13	0.71**	0.10	0.30	0.62**	0.59*	0.59*
Mean larval wt. (mg)	0.22	0.36	-0.29	0.19	0.13	0.72**	0.09	0.31	0.61*	0.60*	0.63**

,* Significant at P≤ 0.05 and 0.01, respectively**.**

Table 4.23: Amount of Cry IIa protein (ppb) in different plant parts of transgenic chickpea lines, *Bt* fed *H. armigera* larvae, aphids, and natural enemies (ICRISAT, Patancheru).

Genotype	Plant samples (ppb)									*Bt* fed *H. armigera* larvea		*Bt* fed aphids		*Bt* fed natural enemies	
	Fresh leaf S - 1	Fresh leaf S - 2	Green pod coat	Green seed	Dry pod coat	Dry seed	Dry stem	Dry root	Soil	*Bt* fed larvea S - 1	*Bt* fed larvae S - 2	Bt fed aphids	*Bt* in artifitial diet of aphids	*Bt* fed Coccinellid grubs	*Bt* fed *Campoletis* larva
BS5A.1(T2) 18-1 P1	57.6^{b}	55.6^{bc}	57.6^{b}	53.3^{b}	51.6^{bc}	61.3^{bc}	49.3^{b}	3.2^{a}	0.2^{a}	19.0^{ab}	37.0^{a}	1.7^{b}	1.6^{c}	2.1^{b}	1.0^{bc}
BS5A.1(T2) 18-2 P1	57.6^{b}	51.6^{b}	54.3^{b}	54.3^{b}	50.6^{b}	53.6^{b}	54.3^{bc}	3.7^{a}	0.0^{a}	42.3^{bc}	29.4^{a}	1.1^{ab}	1.2^{c}	2.4^{b}	1.3^{c}
BS5A.2(T2) 19-1 P2	75.0^{b}	72.0^{c}	73.0^{b}	69.6^{b}	70.6^{c}	54.6^{b}	70.0^{d}	3.9^{a}	0.0^{a}	54.0^{c}	15.0^{a}	1.5^{b}	1.2^{c}	1.9^{b}	1.7^{c}
BS5A.2(T2) 19-2 P1	75.0^{b}	72.0^{bc}	71.0^{b}	73.6^{b}	69.0^{c}	70.0^{c}	70.0^{cd}	6.9^{a}	0.2^{a}	17.0^{ab}	41.6^{a}	1.6^{b}	1.1^{bc}	1.9^{b}	1.5^{c}
BS5A.2(T2) 19-3 P1	75.0^{b}	71.0^{c}	70.0^{b}	74.3^{b}	69.0^{c}	70.0^{c}	64.3^{bcd}	4.2^{a}	0.0^{a}	52.3^{c}	19.0^{a}	1.1^{ab}	1.3^{c}	1.1^{a}	1.4^{c}
BS5A.2(T2) 19-3 P2	73.3^{b}	71.0^{bc}	60.6^{b}	62.6^{b}	64.0^{bc}	70.0^{c}	57.6^{bc}	6.5^{a}	0.1^{a}	13.0^{ab}	18.5^{a}	0.9^{ab}	1.0^{abc}	2.0^{b}	1.0^{bc}
Semsen (Control)	0.1^{a}	0.1^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{ab}	0.0^{a}	0.1^{ab}
ICC 506 EB (Resistant check)	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}
Mean	51.7	49.3	49.1	48.7	48.1	50.3	47.4	4.2	0.1	25.1	20.1	1.0	1.0	1.5	1.0
SE +	5.9	6.0	5.8	6.6	4.7	5.6	6.0	1.4	0.1	9.6	12.0	0.3	0.2	0.2	0.2
Fp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.31	0.58	0.01	0.27	0.07	0.03	0.001	0.01
Vr	30.1	26.4	25.0	21.3	41.0	29.0	25.7	1.3	0.8	5.0	1.6	3.3	4.9	12.3	6.2
LSD (P 0.05)	18.0*	18.3*	17.6*	20.0*	14.3*	16.9*	18.4*	**NS**	**NS**	29.1*	**NS**	1.2*	0.6*	0.7*	0.7*
CV (%)	20.0	21.3	20.5	23.5	17.1	19.3	22.2	61.4	133.3	66.3	85.1	52.4	26.8	20.1	31.9

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. S1-season 1(2011-12), S2-season 2 (2012-13)

Table 4.24: Correlation between resistance/susceptibility to pod borer, *H. armigera* and the amounts of Cry IIa protein in transgenic chickpea (ICRISAT, Patancheru 2011-12).

	Fresh leaf	Green pod coat	Green seed	Dry pod coat	Dry seed	Dry stem	Dry root	*Bt* fed larvae
HDR	-0.89**	-0.90**	-0.89**	-0.91**	-0.86**	-0.89**	-0.51*	-0.62**
Larval survival (%)	-0.92**	-0.94**	-0.92**	-0.94**	-0.89**	-0.93**	-0.57**	-0.63**
Mean larval wt. (mg)	-0.96**	-0.98**	-0.97**	-0.98**	-0.93**	-0.96**	-0.58**	-0.69**

,* Significant at P≤ 0.05 and 0.01, respectively

Table 4.25: Correlation between resistance/susceptibility to pod borer, *H. armigera* and the amounts of Cry IIa protein in transgenic chickpea (ICRISAT, Patancheru 2012-13).

	Fresh leaf	Green pod coat	Green seed	Dry pod coat	Dry seed	Dry stem	Dry root	*Bt* fed larvae
HDR	-0.91**	-0.92**	-0.92**	-0.93**	-0.89**	-0.91**	-0.55*8	-0.76**
Larval survival (%)	-0.89**	-0.92**	-0.89**	-0.91**	-0.82**	-0.94**	-0.59**	-0.81**
Mean larval wt. (mg)	-0.97**	-0.99**	-0.97**	-0.98**	-0.92**	-0.98**	-0.61**	-0.73**

,* Significant at P≤ 0.05 and 0.01, respectively

Table 4.26: Biology of *Campoletis chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (ICRISAT, Patancheru October 2011-2012)

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female^{-1})
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	14.0^{b}	5.6^{b}	19.6^{c}	16.0^{a} (23.1)	9.3^{ab} (4.0)	3.6^{ab}	4.0^{a}	1.1^{ab}	0.8^{a}	0.6^{a}	0.3^{a}	16.6^{a}
BS5A.1(T2) 18-2 P1	10.4^{a}	6.0^{b}	16.4^{bc}	33.3^{a} (35.0)	20.0^{bc} (12.0)	5.6^{b}	6.3^{a}	1.4^{ab}	1.5^{ab}	2.0^{a}	1.0^{ab}	33.3^{ab}
BS5A.2(T2) 19-1 P2	10.8^{a}	3.0^{a}	11.8^{a}	22.6^{a} (27.1)	3.8^{a} (1.3)	0.0^{a}	3.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	0.3^{a}	6.6^{a}
BS5A.2(T2) 19-2 P1	9.5^{a}	5.0^{b}	14.5^{ab}	29.3^{a} (32.6)	23.2^{c} (16.0)	4.3^{ab}	10.6^{ab}	1.8^{ab}	2.6^{ab}	1.3^{a}	2.6^{b}	81.6^{bc}
BS5A.2(T2) 19-3 P1	9.6^{a}	6.1^{b}	15.7^{b}	18.6^{a} (25.5)	16.0^{bc} (8.0)	6.0^{b}	6.3^{a}	1.7^{ab}	1.9^{ab}	1.0^{a}	1.0^{ab}	33.3^{ab}
BS5A.2(T2) 19-3 P2	9.3^{a}	5.0^{b}	14.3^{ab}	26.6^{a} (31.0)	21.2^{c} (13.3)	7.3^{b}	9.3^{ab}	1.6^{ab}	2.4^{ab}	1.3^{a}	2.0^{ab}	18.3^{a}
Semsen (Control)	8.3^{a}	6.0^{b}	14.7^{ab}	70.6^{b} (57.2)	41.5^{d} (44.0)	8.0^{b}	10.0^{ab}	2.2^{b}	3.4^{b}	5.0^{b}	6.0^{c}	105.0^{c}
ICC 506 EB (Resistant check)	8.8^{a}	5.9^{b}	14.7^{ab}	61.3^{b} (51.5)	39.8^{d} (41.3)	7.3^{b}	17.0^{b}	2.0^{b}	3.0^{ab}	4.6^{b}	5.6^{c}	121.6^{c}
Mean	10.1	5.1	15.2	34.8	17.5	5.2	8.3	1.5	2.1	2	2.3	52.1
SE +	0.9	0.8	1.1	5.8	3.9	1.6	2.8	0.5	0.6	0.6	0.6	17.1
Fp	0.029	0.011	0.018	<0.001	<0.001	0.078	0.067	0.251	0.173	<0.001	<0.001	0.001
Vr	3.2	4.1	3.7	12.0	16.8	2.3	2.5	1.4	1.7	8.6	13.2	6.6
LSD (P 0.05)	2.9*	2.5*	3.5*	17.6*	12.0*	5.1*	8.5*	**NS**	**NS**	1.8*	1.8*	52.1*
CV (%)	16.8	28.5	13.3	28.9	39.2	55.3	58.4	65.8	57	54.1	45.6	57.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.27: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (ICRISAT, Patancheru October 2012-2013).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female $^{-1}$)
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	13.3^{b}	7.3^{bc}	19.6^{b}	23.3^{a} (28.5)	12.2^{a} (20.1)	8.6^{a}	20.3^{a}	3.1^{a}	2.1^{ab}	2.0^{a}	1.6^{a}	35.0^{a}
BS5A.1(T2) 18-2 P1	11.6^{b}	7.0^{abc}	18.6^{b}	34.4^{a} (35.9)	11.1^{a} (19.1)	8.0^{a}	23.3^{a}	2.7^{a}	3.0^{abc}	1.6^{a}	1.6^{a}	29.6^{a}
BS5A.2(T2) 19-1 P2	11.6^{b}	7.0^{abc}	18.6^{b}	25.5^{a} (30.1)	13.3^{a} (21.3)	11.6^{a}	11.6^{a}	2.9^{a}	2.6^{abc}	2.3^{a}	1.6^{a}	6.6^{a}
BS5A.2(T2) 19-2 P1	12.3^{b}	8.0^{c}	20.3^{b}	21.1^{a} (27.2)	17.7^{ab} (24.8)	11.0^{a}	17.3^{a}	2.8^{a}	4.0^{bc}	2.6^{a}	2.6^{a}	43.6^{ab}
BS5A.2(T2) 19-3 P1	12.6^{b}	6.0^{abc}	18.6^{b}	42.2^{a} (40.3)	26.6^{b} (31.0)	6.0^{a}	16.6^{a}	2.9^{a}	4.1^{bc}	3.6^{a}	4.3^{ab}	50.6^{ab}
BS5A.2(T2) 19-3 P2	13.3^{b}	5.6^{abc}	19.0^{b}	36.6^{a} (37.2)	15.5^{a} (23.2)	10.0^{a}	15.3^{a}	2.6^{a}	1.9^{a}	3$^{.0}$	1.6^{a}	97.6^{bc}
Semsen (Control)	9.6^{a}	4.6^{a}	14.3^{a}	73.3^{b} (59.7)	50.0^{c} (45.0)	10.0^{a}	18.0^{a}	3.2^{a}	4.5^{c}	7.0^{b}	7.3^{bc}	145.6^{c}
ICC 506 EB (Resistant check)	9.3^{a}	5.3^{ab}	14.6^{b}	75.5^{b} (60.6)	65.5^{d} (54.0)	11.6^{a}	15.6^{a}	3.4^{a}	4.5^{c}	12.6^{c}	10.3^{c}	131.6^{c}
Mean	11.6	6.3	18.0	41.5	26.5	9.6	17.3	2.9	3.3	4.3	3.9	67.6
SE +	0.5	0.7	0.8	6.2	3.0	2.0	4.0	0.2	0.6	0.6	1.2	18.0
Fp	<0.001	0.069	0.001	<0.001	<0.001	0.537	0.644	0.339	0.039	<0.001	<0.001	<0.001
Vr	7.9	2.4	7.0	11.7	44.5	0.8	0.7	1.2	2.9	29.3	7.1	8.0
LSD (P 0.05)	1.5*	2.1*	2.5*	19.0*	9.2*	**NS**	**NS**	**NS**	1.8*	2.0*	3.7*	54.6*
CV (%)	7.5	19.5	8.1	26.2	19.9	37.5	40.5	14.1	31	27.4	54.2	46.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.28: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (ICRISAT, Patancheru October 2011-2012 and 2012-2013) (Pooled analysis).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female $^{-1}$)
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	13.1^{c}	6.5^{b}	19.6^{b}	19.6^{a} (25.8)	8.1^{a} (14.7)	6.1^{a}	12.1^{a}	2.1^{a}	1.5^{a}	1.3^{a}	1.0^{a}	25.8^{ab}
BS5A.1(T2) 18-2 P1	11.0^{acb}	6.5^{b}	17.5^{ab}	33.8^{a} (35.4)	11.5^{a} (19.6)	6.8^{a}	14.8^{a}	2.0^{a}	2.3^{abc}	1.8^{a}	1.3^{a}	31.5^{ab}
BS5A.2(T2) 19-1 P2	11.2^{abc}	4.0^{a}	15.2^{a}	24.1^{a} (28.6)	7.3^{a} (12.5)	5.8^{a}	7.3^{a}	1.4^{a}	1.8^{ab}	1.1^{a}	1.0^{a}	6.6^{a}
BS5A.2(T2) 19-2 P1	10.9^{abc}	6.5^{b}	17.4^{ab}	25.2^{a} (29.9)	16.8^{a} (24.0)	7.6^{a}	14.0^{a}	2.3^{a}	3.3^{bc}	2.0^{a}	2.6^{a}	62.6^{b}
BS5A.2(T2) 19-3 P1	11.1^{abc}	6.0^{b}	17.2^{ab}	30.4^{a} (32.9)	17.3^{a} (23.5)	6.0^{a}	11.5^{a}	2.3^{a}	3.0^{abc}	2.3^{a}	2.6^{a}	42.0^{ab}
BS5A.2(T2) 19-3 P2	11.3^{bc}	5.3^{ab}	16.6^{ab}	31.6^{a} (34.1)	14.4^{a} (22.2)	8.6^{a}	12.3^{a}	2.1^{a}	2.1^{abc}	2.1^{a}	1.8^{a}	58.0^{b}
Semsen (Control)	9.0^{a}	5.3^{ab}	14.3^{a}	72.0^{b} (58.5)	47.0^{b} (43.2)	9.0^{a}	14.0^{a}	2.7^{a}	3.9^{c}	6.0^{b}	6.6^{b}	125.3^{c}
ICC 506 EB (Resistant check)	9.0^{ab}	5.6^{ab}	14.7^{a}	68.4^{b} (56.1)	53.4^{b} (46.9)	9.5^{a}	16.3^{a}	2.7^{a}	3.7^{c}	8.6^{c}	8.0^{b}	126.6^{c}
Mean	10.8	5.7	16.6	38.2	22.0	7.4	12.8	2.2	2.7	3.1	3.1	59.8
SE ±	0.7	0.7	1.0	4.4	3.5	1.7	3.3	0.4	0.5	0.8	0.7	13.5
Fp	0.004	0.27	0.021	<0.001	<0.001	0.654	0.694	0.625	0.027	<0.001	<0.001	<0.001
Vr	3.6	1.3	2.7	20.5	25.6	0.7	0.6	0.7	2.6	9.5	11.8	10.8
LSD (P 0.05)	2.0*	**NS**	3.0*	12.8*	10.1*	**NS**	**NS**	**NS**	1.6*	2.4*	2.2*	38.6*
CV (%)	15.8	32.1	15.8	28.8	39.3	56.4	63.3	49.9	50.4	66.7	60.9	55.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.29: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (ICRISAT, Patancheru November 2011-2012).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female $^{-1}$)
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	12.3^{b}	5.3^{b}	17.6^{a}	10.6^{a} (18.5)	9.3^{ab} (17.2)	7.3^{ab}	12.6^{ab}	1.9^{a}	2.7^{a}	0.6^{ab}	1.6^{abcd}	33.3^{ab}
BS5A.1(T2) 18-2 P1	9.6^{ab}	3.0^{a}	11.3^{a}	23.8^{abc} (27.5)	8.6^{ab} (14.0)	7.6^{ab}	0.6^{a}	1.7^{a}	2.6^{a}	1.3^{ab}	1.0^{ab}	33.3^{ab}
BS5A.2(T2) 19-1 P2	6.3^{a}	6.0^{b}	12.3^{a}	14.9^{a} (21.6)	15.3^{abc} (22.7)	7.0^{ab}	9.4^{ab}	2.6^{a}	2.5^{a}	2.0^{ab}	2.0^{abcd}	75.0^{abc}
BS5A.2(T2) 19-2 P1	10.1^{ab}	5.8^{b}	15.9^{a}	17.1^{ab} (24.4)	11.3^{ab} (16.1)	8.6^{ab}	18.5^{ab}	1.3^{a}	2.6^{a}	2.0^{ab}	1.0^{abc}	15.0^{a}
BS5A.2(T2) 19-3 P1	10.6^{ab}	4.6^{ab}	15.3^{a}	17.6^{ab} (24.3)	16.0^{abc} (23.2)	8.3^{ab}	22.8^{b}	2.8^{a}	2.4^{a}	2.0^{ab}	2.3^{abcd}	45.0^{abc}
BS5A.2(T2) 19-3 P2	9.9^{ab}	4.0^{ab}	13.9^{a}	3.3^{a} (6.2)	2.3^{a} (5.1)	11.6^{b}	16.8^{ab}	2.7^{a}	3.0^{a}	0.3^{a}	0.3^{a}	25.0^{a}
Semsen (Control)	9.3^{ab}	5.2^{b}	14.6^{a}	43.8^{bc} (41.3)	32.0^{bc} (33.6)	4.6^{a}	1.3^{ab}	2.3^{a}	2.1^{a}	3.6^{bc}	4.3^{bc}	102.3^{c}
ICC 506 EB (Resistant check)	10.2^{ab}	5.4^{b}	15.6^{a}	47.2^{c} (43.3)	39.3^{c} (38.5)	5.3^{a}	12.0^{ab}	2.6^{a}	2.7^{a}	5.3^{c}	4.6^{d}	91.6^{bc}
Mean	9.8	4.7	14.6	22.3	16.8	7.5	11.8	2.2	2.6	2.1	2.1	52.6
SE +	1.3	1.0	1.9	8.2	7.3	1.5	6.4	0.6	0.4	0.9	0.9	19.6
Fp	0.245	0.178	0.387	0.022	0.043	0.141	0.246	0.689	0.9	0.04	0.067	0.05
Vr	1.5	1.7	1.1	3.5	2.8	1.9	1.5	0.6	0.3	2.9	2.5	2.7
LSD (P 0.05)	**NS**	**NS**	**NS**	25.0*	22.3*	**NS**	**NS**	**NS**	**NS**	2.8*	3.0*	59.5*
CV (%)	24.1	38.7	22.5	64.2	76.1	35.4	94.3	49.5	27.1	76	79.2	64.7

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.30: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines (ICRISAT, Patancheru November 2012-2013).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female $^{-1}$)
						Male	Female	Male	Female	Males	Females	
BS5A.1(T2) 18-1 P1	10.0^{b}	8.3^{a}	18.3^{b}	48.8^{abc} (44.3)	26.6^{ab} (30.7)	9.0^{ab}	11.3^{ab}	2.9^{b}	2.5^{abc}	4.0^{ab}	4.0^{ab}	38.3^{ab}
BS5A.1(T2) 18-2 P1	12.3^{c}	8.0^{a}	20.3^{c}	25.5^{a} (30.1)	12.2^{a} (20.1)	7.6^{ab}	16.3^{ab}	1.8^{ab}	2.8^{abc}	1.3^{a}	2.3^{a}	0.0^{a}
BS5A.2(T2) 19-1 P2	10.0^{b}	7.6^{a}	17.6^{b}	32.2^{ab} (33.0)	18.8^{a} (21.3)	7.0^{a}	13.6^{ab}	0.9^{a}	1.7^{a}	2.3^{a}	3.3^{ab}	33.3^{ab}
BS5A.2(T2) 19-2 P1	11.0^{bc}	8.0^{a}	19.0^{bc}	28.8^{ab} (32.4)	21.1^{a} (27.1)	10.6^{ab}	21.0^{b}	2.3^{ab}	2.1^{ab}	2.6^{a}	3.6^{ab}	68.3^{bc}
BS5A.2(T2) 19-3 P1	12.0^{c}	7.3^{a}	19.3^{bc}	33.3^{ab} (35.2)	20.0^{a} (26.1)	13.3^{b}	7.0^{a}	2.3^{ab}	2.6^{abc}	4.0^{ab}	2.0^{a}	0.0^{a}
BS5A.2(T2) 19-3 P2	10.3^{b}	8.3^{a}	18.6^{bc}	31.1^{ab} (32.7)	18.8^{a} (23.9)	11.0^{ab}	15.0^{ab}	1.5^{ab}	3.3^{bc}	3.0^{ab}	2.6^{ab}	23.3^{ab}
Semsen (Control)	8.3^{a}	7.3^{a}	15.6^{a}	57.7^{bc} (49.6)	42.2^{bc} (40.3)	8.3^{ab}	18.0^{b}	2.3^{ab}	2.6^{abc}	6.6^{bc}	6.0^{ab}	111.6^{c}
ICC 506 EB (Resistant check)	8.0^{a}	6.6^{a}	14.6^{a}	68.8^{c} (56.4)	54.4^{c} (47.5)	9.6^{ab}	14.0^{ab}	2.3^{ab}	3.9^{c}	9.3^{c}	7.0^{b}	105.0^{c}
Mean	10.2	7.7	17.9	40.8	26.8	9.5	14.5	2.0	2.7	4.1	3.8	47.5
SE +	0.5	0.5	0.5	9.3	6.4	1.3	3.2	0.4	0.4	1.1	1.3	13.8
Fp	<0.001	0.33	<0.001	0.047	0.006	0.303	0.179	0.12	0.111	0.005	0.158	<0.001
Vr	9.1	1.2	10.1	2.8	4.8	1.7	1.7	2.0	2.1	4.9	1.8	9.9
LSD (P 0.05)	1.5*	**NS**	1.8*	28.4*	19.6*	**NS**	**NS**	**NS**	**NS**	3.5*	**NS**	41.8*
CV (%)	8.6	11.5	5.8	39.8	41.9	32	38.2	36.3	29.5	48.7	58.5	50.4

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.31: Biology of *C. chlorideae* parasitizing *H. armigera* fed on leaves of transgenic chickpea lines November planting (ICRISAT, Patancheru 2011-12 and 2012-13) (pooled analysis).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female $^{-1}$)
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	11.1^{b}	6.8^{a}	18.0^{a}	29.7^{a} (31.4)	18.0^{a} (24.0)	8.1^{abc}	12.0^{a}	2.4^{a}	2.6^{ab}	2.3^{a}	2.8^{a}	35.8^{a}
BS5A.1(T2) 18-2 P1	11.0^{b}	4.8^{a}	15.8^{a}	24.6^{a} (28.8)	10.4^{a} (17.1)	7.6^{abc}	8.5^{a}	1.7^{a}	2.7^{ab}	1.3^{a}	1.6^{a}	16.6^{a}
BS5A.2(T2) 19-1 P2	8.1^{a}	6.8^{a}	15.0^{a}	23.5^{a} (27.3)	17.1^{a} (22.0)	7.0^{ab}	11.6^{a}	1.8^{a}	2.1^{a}	2.1^{a}	2.6^{ab}	54.1^{a}
BS5A.2(T2) 19-2 P1	10.5^{ab}	6.9^{a}	17.4^{a}	23.0^{a} (28.4)	16.2^{a} (21.6)	9.6^{abc}	19.7^{a}	1.8^{a}	2.3^{ab}	2.3^{a}	2.3^{a}	41.6^{a}
BS5A.2(T2) 19-3 P1	11.3^{b}	6.0^{a}	17.3^{a}	25.4^{a} (29.7)	18.0^{a} (24.7)	10.8^{bc}	14.9^{a}	2.5^{a}	2.5^{ab}	3.0^{ab}	2.1^{a}	22.5^{a}
BS5A.2(T2) 19-3 P2	10.1^{ab}	6.1^{a}	16.3^{a}	17.2^{a} (19.4)	10.6^{a} (14.5)	11.3^{c}	15.9^{a}	2.1^{a}	3.1^{b}	1.6^{a}	1.5^{a}	24.1^{a}
Semsen (Control)	8.8^{ab}	6.3^{a}	15.1^{a}	50.8^{b} (45.4)	37.1^{b} (36.9)	6.5^{a}	9.6^{a}	2.3^{a}	2.3^{ab}	5.1^{bc}	5.1^{bc}	107.0^{b}
ICC 506 EB (Resistant check)	9.1^{ab}	6.0^{a}	15.1^{a}	58.0^{b} (49.9)	46.8^{b} (43.0)	7.5^{ab}	13.0^{a}	2.4^{a}	3.3^{b}	7.3^{c}	5.8^{c}	98.3^{b}
Mean	10.0	6.2	16.2	31.6	21.8	8.5	13.2	2.1	2.6	3.1	3.0	50.0
SE +	0.8	0.9	1.3	7.2	5.1	1.2	4.0	0.4	0.3	0.9	0.8	12.9
Fp	0.078	0.809	0.647	0.002	<0.001	0.06	0.574	0.697	0.145	<0.001	0.004	<0.001
Vr	2.0	0.5	0.7	4.0	6.5	2.1	0.8	0.6	1.6	5.0	3.6	7.1
LSD (P 0.05)	2.4*	**NS**	**NS**	20.7*	14.6*	3.5*	**NS**	**NS**	**NS**	2.6*	2.4*	37.1*
CV (%)	20.5	36.8	20.8	56.2	57.5	35.1	74.4	45.8	28.6	70.3	68.1	63.5

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.32: Biology of *C. chlorideae* parasitizing *H. armigera* fed on diets with lyophilized leaf powders of different transgenic chickpea lines (ICRISAT, Patancheru 2011-2012).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female^{-1})
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	15.6^{d}	11.0^{b}	26.6^{d}	65.5^{ab} (54.5)	52.2^{b} (46.2)	10.0^{a}	14.0^{ab}	2.4^{a}	4.0^{a}	8.3^{abc}	7.6^{bc}	71.0^{a}
BS5A.1(T2) 18-2 P1	15.0^{d}	9.3^{ab}	24.3^{cd}	80.0^{bc} (63.8)	63.3^{b} (52.7)	9.3a	20.0^{bc}	3.0^{ab}	4.4^{a}	9.6^{abc}	9.3^{c}	53.3^{a}
BS5A.2(T2) 19-1 P2	14.0^{cd}	9.6^{ab}	23.6^{bcd}	60.0^{ab} (51.0)	21.1^{a} (25.5)	10.3^{a}	30.8^{d}	3.1^{ab}	2.5^{a}	4.6^{a}	1.6^{a}	24.6^{a}
BS5A.2(T2) 19-2 P1	12.6^{bcd}	8.6^{ab}	21.3^{abcd}	70.0^{abc} (57.3)	26.6^{a} (31.0)	8.6^{a}	24.0^{cd}	2.8^{ab}	2.2^{a}	6.6^{ab}	1.3^{a}	51.6^{a}
BS5A.2(T2) 19-3 P1	11.0^{abc}	9.6^{ab}	20.6^{abc}	58.8^{ab} (50.2)	25.5^{a} (30.2)	8.6^{a}	9.3^{a}	2.8^{ab}	3.5^{a}	4.0^{a}	3.6^{abc}	30.6^{a}
BS5A.2(T2) 19-3 P2	10.0^{ab}	8.0^{ab}	18.0^{ab}	54.4^{a} (47.5)	25.5^{a} (29.9)	8.0^{a}	14.0^{ab}	2.7^{ab}	4.3^{a}	4.3^{a}	3.3^{ab}	52.6^{a}
Semsen (Control)	8.6^{a}	7.0^{a}	15.67^{a}	88.8^{c} (70.8)	61.1^{b} (51.4)	9.0^{a}	18.3^{bc}	3.1^{ab}	3.3^{a}	12.0^{bc}	6.3^{abc}	119.3^{b}
ICC 506 EB (Resistant check)	9.3^{a}	8.6^{ab}	18.0^{ab}	91.1^{c} (75.7)	63.3^{b} (53.2)	11.3^{a}	19.6^{bc}	3.5^{b}	3.2^{a}	14.0^{c}	5.0^{abc}	152.3^{b}
Mean	12.0	9.0	21.0	71.1	42.3	9.4	18.7	2.9	3.4	7.9	4.7	69.5
SE ±	0.9	0.9	1.7	6.6	5.7	1.2	2.2	0.2	0.6	1.7	1.7	14.3
Fp	<0.001	0.189	0.01	0.008	<0.001	0.616	<0.001	0.249	0.242	0.01	0.05	<0.001
Vr	7.8	1.7	4.3	4.5	11.0	0.7	8.5	1.4	1.5	4.3	2.7	9.5
LSD (P 0.05)	2.8*	NS	5.4*	20.0*	17.5*	NS	6.9*	NS	NS	5.4*	5.1*	43.5*
CV (%)	13.7	17.8	14.8	16.1	23.6	22.5	21	15.1	32.8	39	61.8	35.8

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.33: Biology of *C. chlorideae* parasitizing *H. armigera* fed on diets with lyophilized leaf powders of different transgenic chickpea lines (ICRISAT, Patancheru 2012-2013).

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female^{-1})
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	9.6^{abc}	6.6^{ab}	16.3^{ab}	38.8^{a} (38.3)	24.4^{a} (29.4)	9.3^{bc}	12.3^{ab}	2.1^{a}	3.1^{ab}	4.6^{a}	2.6^{a}	83.3^{bc}
BS5A.1(T2) 18-2 P1	12.0^{c}	9.3^{c}	21.3^{f}	52.2^{ab} (46.2)	30.0^{a} (33.1)	8.3^{bc}	14.6^{ab}	2.1^{a}	4.0^{c}	5.0^{a}	4.0^{a}	50.0^{ab}
BS5A.2(T2) 19-1 P2	11.0^{bc}	6.3^{ab}	17.3^{abcd}	28.8^{a} (32.4)	21.1^{a} (27.2)	6.0^{ab}	8.6^{a}	2.0^{b}	3.8^{bc}	3.6^{a}	2.6^{a}	20.0^{a}
BS5A.2(T2) 19-2 P1	9.3^{ab}	7.0^{a}	16.3^{abc}	46.6^{a} (42.9)	22.2^{a} (27.5)	10.3^{c}	15.0^{ab}	1.8^{a}	3.4^{abc}	3.3^{a}	3.3^{a}	48.3^{ab}
BS5A.2(T2) 19-3 P1	10.6^{abc}	8.0^{bc}	18.6^{bde}	43.3^{a} (40.6)	28.8^{a} (31.6)	6.6^{abc}	13.6^{ab}	2.5^{ab}	2.8^{a}	4.3^{a}	4.3^{a}	70.0^{abc}
BS5A.2(T2) 19-3 P2	11.0^{bc}	9.0^{c}	20.0^{ef}	37.7^{a} (37.7)	22.2^{a} (27.5)	3.3^{a}	10.0^{a}	2.5^{ab}	2.9^{a}	4.3^{a}	2.3^{a}	26.6^{a}
Semsen (Control)	9.0^{ab}	6.0^{a}	15.0^{a}	76.6^{b} (61.4)	58.8^{a} (50.2)	8.0^{bc}	13.0^{ab}	1.9^{a}	3.0^{ab}	9.0^{b}	8.6^{b}	163.3^{d}
ICC 506 EB (Resistant check)	8.3^{a}	7.0^{ab}	15.3^{a}	56.6^{ab} (48.8)	32.2^{a} (34.5)	8.0^{bc}	19.3^{b}	3.0^{b}	3.1^{ab}	5.3^{a}	4.3^{a}	112.3^{c}
Mean	10.1	7.4	17.5	47.6	30.0	7.5	13.3	2.3	3.3	4.9	4.0	71.8
SE +	0.7	0.5	0.7	8.2	5.9	1.1	2.4	0.2	0.2	1.1	0.9	16.2
Fp	0.036	0.002	<0.001	0.033	0.009	0.022	0.158	0.01	0.042	0.081	0.007	<0.001
Vr	3.0	5.9	9.8	3.1	4.3	3.4	1.8	4.2	2.9	2.3	4.6	8.6
LSD (P 0.05)	2.1*	1.5*	2.1*	25.0*	17.9*	3.0*	**NS**	0.6*	0.7*	3.4*	2.88*	49.1*
CV (%)	12.1	11.8	7.1	30.0	34.1	26.8	31.3	15.2	13.5	39.9	40.3	39.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.34: Biology of *C. chlorideae* parasitizing *H. armigera* fed on diets with lyophilized leaf powders of different transgenic chickpea lines (ICRISAT, Patancheru 2011-12 and 2012-2013) (pooled analysis)

Genotype	Egg+larval period (days)	Pupal period (days)	Post embryonic development period (days)	Cocoon formation (%)	Adult emergence (%)	Adult longevity (days)		Wt of the adult (mg)		No. of adults emerged		Fecundity (eggs female^{-1})
						Male	Female	Male	Female	Male	Female	
BS5A.1(T2) 18-1 P1	12.6^{bc}	8.8^{b}	21.5^{c}	52.2^{ab} (46.4)	38.3^{ab} (37.8)	9.6^{b}	13.1^{a}	2.3^{a}	3.5^{ab}	6.5^{ab}	5.1^{abc}	77.1^{b}
BS5A.1(T2) 18-2 P1	13.5^{c}	9.3^{b}	22.8^{c}	66.1^{abc} (55.0)	46.6^{bc} (42.9)	8.8^{b}	17.3^{a}	2.5^{abc}	4.2^{b}	7.3^{ab}	6.6^{bc}	51.6^{ab}
BS5A.2(T2) 19-1 P2	12.5^{bc}	8.0^{ab}	20.5^{bc}	44.4^{a} (41.7)	21.1^{a} (26.5)	8.1^{ab}	19.7^{a}	3.0^{bc}	3.1^{ab}	4.1^{a}	2.1^{a}	22.3^{a}
BS5A.2(T2) 19-2 P1	10.1^{abc}	7.8^{ab}	18.8^{abc}	58.3^{ab} (50.1)	24.4^{a} (29.3)	9.5^{b}	19.5^{a}	2.3^{abc}	2.8^{a}	5.0^{a}	2.3^{a}	50.0^{ab}
BS5A.2(T2) 19-3 P1	10.8^{abc}	8.8^{b}	19.6^{abc}	51.1^{ab} (45.4)	27.2^{a} (30.9)	7.6^{ab}	11.5^{a}	2.6^{abc}	3.1^{ab}	4.1^{a}	4.0^{ab}	50.3^{ab}
BS5A.2(T2) 19-3 P2	10.5^{ab}	8.5^{ab}	19.0^{abc}	46.1^{a} (42.6)	23.8^{a} (28.7)	5.6^{a}	12.0^{a}	2.6^{abc}	3.6^{ab}	4.3^{a}	2.8^{a}	39.6^{a}
Semsen (Control)	8.8^{a}	6.5^{a}	15.3^{a}	82.7^{c} (66.1)	60.0^{c} (50.8)	8.5^{ab}	15.6^{a}	2.5^{abc}	3.2^{ab}	10.5^{b}	7.5^{c}	141.3^{c}
ICC 506 EB (Resistant check)	8.8^{a}	7.8^{ab}	16.6^{ab}	73.8^{bc} (62.2)	47.7^{bc} (43.9)	9.6^{b}	19.5^{a}	3.2^{c}	3.2^{ab}	9.6^{b}	8.6^{c}	132.3^{c}
Mean	11.0	8.2	19.2	59.4	36.2	8.4	16.0	2.6	3.3	6.4	4.4	70.6
SE +	0.8	0.7	1.4	7.2	5.8	0.9	2.6	0.2	0.3	1.3	1.0	11.5
Fp	0.002	0.18	0.013	0.005	<0.001	0.104	0.122	0.06	0.291	0.005	0.008	<0.001
Vr	4.2	1.5	3.0	3.5	5.8	1.8	1.7	2.1	1.2	3.5	3.2	14.2
LSD (P 0.05)	2.4*	**NS**	4.0*	20.8*	16.7*	**NS**	**NS**	0.6*	**NS**	3.8*	3.1*	33.1*
CV (%)	18.6	21	17.9	30.1	39.6	28.6	39.9	20	27.3	51.1	60.7	40.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.35: Direct effect of *Cry IIa* transgenic chickpea lines on *Cheilomenes sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%) (ICRISAT, Patancheru 2012-2013).

	0.02%							
Genotype	**Larval survival (%)**	**Larval period (days)**	**Mean grub weight (mg)**	**Pupal period (days)**	**Pupation (%)**	**Adult emergence (%)**	**Adult weight (mg)**	
							Male	**Female**
BS5A.1(T2) 18-1 P1	66.6^{abcd} (54.7)	7.0^{a}	17.2^{a}	3.0^{a}	40.0^{ab} (43.0)	33.3^{ab} (35.0)	5.6^{a}	7.6^{a}
BS5A.1(T2) 18-2 P1	60.0^{ab} (50.8)	7.3^{a}	20.7^{a}	4.3^{a}	40.0^{ab} (39.2)	23.3^{a} (28.7)	6.0^{a}	9.6^{a}
BS5A.2(T2) 19-1 P2	66.6^{abcd} (55.0)	7.0^{a}	10.7^{a}	3.0^{a}	46.6^{bc} (43.0)	36.6^{ab} (37.2)	4.9^{a}	5.7^{a}
BS5A.2(T2) 19-2 P1	56.6^{a} (48.9)	7.3^{a}	19.0^{a}	4.0^{a}	36.6^{ab} (36.9)	26.6^{a} (30.7)	4.0^{a}	8.0^{a}
BS5A.2(T2) 19-3 P1	60.0^{abc} (50.8)	6.3^{a}	10.8^{a}	4.0^{a}	30.0^{a} (33.2)	23.3^{a} (28.7)	8.0^{b}	8.6^{a}
BS5A.2(T2) 19-3 P2	70.0^{abcd} (57.0)	6.0^{a}	10.9^{a}	5.3^{a}	36.6^{a} (37.2)	30.0^{a} (33.0)	8.2^{b}	8.9^{a}
Semsen (Control)	80.0^{bd} (63.9)	6.6^{a}	19.4^{a}	3.0^{a}	56.6^{cd} (48.9)	40.0^{ab} (39.1)	8.2^{b}	7.8^{a}
ICC 506 EB (Resistant check)	83.3^{d} (66.1)	6.3^{a}	11.8^{a}	3.0^{a}	63.3^{d} (52.7)	50.0^{b} (44.9)	8.6^{b}	8.3^{a}
Mean	67.9	6.7	15.1	5.0	44.6	32.9	6.7	11.8
SE +	5.9	0.5	5.4	0.6	4.3	5.4	0.6	9.4
Fp	0.064	0.618	0.699	0.45	0.001	0.044	<0.001	0.445
Vr	2.5	0.7	0.6	1.0	6.5	2.8	8.1	1.0
LSD (P 0.05)	18.1*	**NS**	**NS**	**NS**	13.1*	16.3*	1.8*	**NS**
CV (%)	15.3	14.5	61.9	128.8	16.9	28.4	15.9	138.4

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values

Table 4.35 (Conti.)

Genotype	0.05%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	60.0[a] (50.8)	9.0[b]	2.6[a]	4.3[bc]	46.6[b] (43.0)	36.6[b] (37.2)	6.1[a]	8.2[ab]
BS5A.1(T2) 18-2 P1	46.6[a] (43.0)	9.3[b]	1.7[a]	5.0[c]	33.3[a] (35.2)	20.0[a] (26.5)	5.8[a]	8.4[abc]
BS5A.2(T2) 19-1 P2	53.3[a] (46.9)	9.3[b]	2.2[a]	5.0[c]	30.0[a] (33.2)	10.0[a] (15.0)	5.8[a]	8.3[abc]
BS5A.2(T2) 19-2 P1	53.3[a] (46.9)	9.3[b]	1.8[a]	5.0[c]	30.0[a] (33.0)	20.0[a] (26.0)	6.0[a]	8.5[abc]
BS5A.2(T2) 19-3 P1	53.3[a] (46.9)	9.0[b]	2.3[a]	4.3[bc]	40.0[ab] (39.2)	16.67[a] (23.8)	6.3[a]	7.7[a]
BS5A.2(T2) 19-3 P2	46.6[a] (43.0)	8.3[ab]	2.6[b]	3.6[ab]	30.0[a] (33.2)	10.0[a] (15.0)	5.5[a]	8.6[abc]
Semsen (Control)	80.0[b] (63.9)	7.3[ab]	9.9[a]	3.0[a]	63.3[c] (52.7)	40.0[b] (39.1)	8.0[b]	9.7[abc]
ICC EB 506 (Resistant check)	80.0[b] (63.9)	6.6[a]	9.5[a]	3.0[a]	63.3[c] (52.7)	40.0[b] (39.1)	7.9[b]	8.6[b]
Mean	59.2	8.5	6.6	4.1	42.1	24.2	6.4	8.5
SE ±	4.5	0.6	3.8	0.3	3.2	5.1	0.4	9.5
Fp	<0.001	0.092	0.018	0.001	<0.001	0.002	0.008	0.272
Vr	8.8	2.2	3.7	6.5	19.9	6.1	4.4	1.4
LSD (P 0.05)	13.84*	2.0*	11.5*	1.0*	9.7*	15.7*	1.3*	**NS**
CV (%)	13.4	13.8	99.5	13.9	13.2	37.1	12.3	131.8

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.35 (Conti.)

Genotype	0.1%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	46.6^{ab} (43.0)	9.3^{c}	1.9^{a}	4.6^{a}	23.3^{a} (28.7)	3.3^{a} (6.1)	1.5^{a}	2.1^{a}
BS5A.1(T2) 18-2 P1	53.3^{b} (46.9)	9.3^{c}	3.4^{a}	4.6^{a}	33.3^{a} (34.1)	13.3^{ab} (17.2)	3.9^{ab}	4.7^{ab}
BS5A.2(T2) 19-1 P2	26.6^{a} (30.2)	9.6^{c}	2.2^{a}	2.6^{a}	13.3^{a} (17.7)	6.6^{ab} (12.2)	3.6^{ab}	6.4^{bc}
BS5A.2(T2) 19-2 P1	40.0^{ab} (39.1)	10.6^{c}	2.8^{a}	3.3^{a}	26.6^{a} (31.0)	6.6^{ab} (12.2)	4.6^{ab}	7.2^{bc}
BS5A.2(T2) 19-3 P1	36.6^{ab} (36.9)	10.0^{c}	2.2^{a}	4.3^{a}	20.0^{a} (26.0)	13.3^{ab} (21.1)	6.5^{b}	6.4^{bc}
BS5A.2(T2) 19-3 P2	43.3^{ab} (41.0)	8.6^{bc}	3.4^{a}	4.0^{a}	23.3^{a} (28.0)	20.0^{b} (26.0)	7.1^{b}	6.8^{bc}
Semsen (Control)	80.0^{c} (63.9)	7.0^{ab}	9.7^{b}	3.0^{a}	53.3^{bc} (46.9)	36.6^{c} (37.2)	6.5^{b}	9.8^{c}
ICC 506 EB (Resistant check)	83.3^{c} (66.1)	6.6^{a}	11.6^{b}	3.6^{a}	66.6^{c} (54.7)	43.3^{c} (41.1)	7.5^{b}	9.7^{c}
Mean	51.2	8.9	4.6	3.7	32.5	17.9	5.1	6.6
SE ±	7.9	0.5	2.5	0.6	7.3	4.9	1.4	1.2
Fp	0.001	0.003	<0.001	0.29	0.002	<0.001	0.135	0.01
Vr	6.6	5.6	26.5	1.3	6.0	8.9	1.9	4.3
LSD (P 0.05)	23.9*	1.8*	2.2*	**NS**	22.4*	14.8*	**NS**	3.6*
CV (%)	26.7	11.6	27.1	29.4	39.4	47.4	49.8	31.4

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.36: Direct effect of *Cry IIa* transgenic chickpea lines on *Cheilomenes sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%) (ICRISAT, Patancheru 2013-2014).

Genotype	0.02%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	50.0^{ab} (45.0)	7.3^{b}	9.0^{a}	5.0^{b}	40.0^{ab} (39.2)	30.0^{abc} (33.2)	4.5^{a}	8.8^{abc}
BS5A.1(T2) 18-2 P1	43.3^{a} (41.1)	7.6^{b}	10.3^{a}	4.0^{ab}	30.0^{a} (33.2)	20.0^{ab} (26.5)	5.9^{b}	9.0^{abc}
BS5A.2(T2) 19-1 P2	56.6^{bc} (48.8)	7.0^{ab}	10.7^{a}	4.0^{ab}	43.3^{b} (41.1)	43.3^{cd} (41.1)	7.6^{cd}	9.1^{abc}
BS5A.2(T2) 19-2 P1	43.3^{a} (41.1)	7.6^{b}	8.6^{a}	4.0^{ab}	30.0^{a} (33.2)	16.6^{a} (19.9)	7.2^{c}	7.9^{a}
BS5A.2(T2) 19-3 P1	63.3^{cd} (52.7)	6.6^{ab}	12.1^{a}	4.0^{ab}	40.0^{ab} (39.1)	33.3^{bc} (35.2)	6.5^{bc}	8.7^{ab}
BS5A.2(T2) 19-3 P2	53.3^{abc} (46.9)	6.0^{a}	12.1^{a}	4.3^{ab}	33.3^{ab} (35.2)	30.0^{abc} (33.0)	7.5^{cd}	9.3^{bc}
Semsen (Control)	80.0^{e} (63.4)	6.6^{ab}	26.5^{b}	3.3^{a}	66.6^{c} (54.7)	56.6^{de} (48.8)	9.1^{e}	8.6^{ab}
ICC 506 EB (Resistant check)	73.3^{de} (59.0)	6.0^{a}	25.0^{b}	3.3^{a}	66.6^{c} (54.7)	60.0^{e} (50.8)	8.5^{de}	10.0^{c}
Mean	57.9	6.8	14.3	4.0	43.8	36.2	7.1	8.9
SE ±	3.5	0.3	2.0	0.3	3.3	4.9	0.3	0.3
Fp	<0.001	0.01	<0.001	0.08	<0.001	<0.001	<0.001	0.05
Vr	14.0	4.0	12.4	2.4	20.1	10.1	14.4	2.8
LSD (P 0.05)	10.8*	1.0*	6.1*	1.0*	10.1*	15.1*	1.1*	1.0*
CV (%)	10.7	8.3	24.6	14.9	13.2	23.8	9.4	6.9

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.36 (Conti.)

Genotype	0.05%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	63.3^{c} (50.8)	7.0^{bc}	9.1^{a}	4.3^{a}	36.6^{c} (37.1)	20.0^{b} (26.5)	6.7^{bc}	8.8^{b}
BS5A.1(T2) 18-2 P1	53.3^{bc} (46.9)	7.0^{bc}	4.1^{a}	4.0^{a}	36.6^{c} (37.2)	23.3^{b} (28.2)	6.9^{bc}	8.2^{b}
BS5A.2(T2) 19-1 P2	33.3^{a} (35.2)	9.3^{d}	3.9^{a}	4.0^{a}	23.3^{bc} (28.7)	16.6^{ab} (19.9)	4.6^{ab}	8.3^{b}
BS5A.2(T2) 19-2 P1	36.6^{a} (37.2)	8.3^{cd}	6.4^{a}	4.3^{a}	6.6^{a} (8.8)	3.3^{a} (6.1)	1.8^{a}	7.8^{ab}
BS5A.2(T2) 19-3 P1	36.6^{a} (37.2)	7.0^{bc}	6.6^{a}	4.3^{a}	16.6^{ab} (23.8)	16.6^{ab} (23.8)	6.8^{bc}	7.8^{ab}
BS5A.2(T2) 19-3 P2	46.6^{ab} (43.0)	7.6^{bcd}	7.3^{a}	4.3^{a}	26.6^{bc} (31.0)	16.6^{ab} (23.8)	7.2^{bc}	7.2^{ab}
Semsen (Control)	83.3^{d} (66.6)	6.0^{ab}	21.8^{b}	3.0^{a}	70.0^{d} (57.0)	60.0^{c} (50.8)	9.0^{c}	9.9^{b}
ICC 506 EB (Resistant check)	80.0^{d} (63.4)	5.0^{a}	22.9^{b}	3.3^{a}	70.0^{d} (56.7)	56.6^{c} (48.8)	8.9^{c}	8.4^{b}
Mean	54.2	7.1	10.2	3.9	35.8	26.7	6.5	7.3
SE +	4.3	0.5	1.8	0.4	4.8	4.4	1.2	1.5
Fp	<0.001	0.001	<0.001	0.21	<0.001	<0.001	0.02	0.11
Vr	20.0	5.8	16.6	1.6	23.5	20.7	3.7	2.1
LSD (P 0.05)	13.3*	1.6*	5.6*	**NS**	14.5*	13.5*	3.6*	**NS**
CV (%)	14.0	13.3	31.6	17.9	23.2	29.1	32.0	36.2

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.36 (Conti.)

Genotype	0.1%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	33.3^{ab} (35.2)	8.3^{de}	1.7^{a}	4.6^{a}	20.0^{b} (26.5)	3.3^{ab} (6.1)	1.8^{ab}	2.5^{ab}
BS5A.1(T2) 18-2 P1	26.6^{a} (31.0)	7.6^{cde}	2.2^{a}	4.6^{a}	6.6^{a} (12.2)	0.0^{a} (0.0)	0.0^{a}	0.0^{a}
BS5A.2(T2) 19-1 P2	43.3^{b} (41.1)	7.0^{bcd}	3.2^{a}	2.6^{a}	16.6^{ab} (23.8)	10.0^{ab} (18.4)	4.7^{abc}	5.1^{ab}
BS5A.2(T2) 19-2 P1	36.6^{ab} (37.2)	6.6^{abc}	3.3^{a}	3.3^{a}	23.3^{b} (28.7)	16.6^{b} (23.8)	4.3^{abc}	5.2^{ab}
BS5A.2(T2) 19-3 P1	36.6^{ab} (37.2)	8.6^{e}	2.5^{a}	4.3^{a}	16.6^{ab} (23.8)	3.3^{ab} (6.1)	2.1^{b}	2.5^{ab}
BS5A.2(T2) 19-3 P2	33.3^{ab} (35.2)	7.6^{cde}	4.1^{a}	4.0^{a}	16.6^{ab} (23.8)	10.0^{ab} (15.0)	5.0^{abc}	5.5^{ab}
Semsen (Control)	76.6^{c} (61.2)	5.6^{ab}	19.7^{b}	3.0^{a}	60.0^{c} (50.8)	53.3^{c} (46.9)	6.8^{bc}	8.5^{b}
ICC 506 EB (Resistant check)	80.0^{c} (63.9)	5.3^{a}	24.0^{b}	3.6^{a}	66.6^{c} (54.9)	53.3^{c} (47.0)	7.6^{c}	8.2^{b}
Mean	45.8	7.1	7.6	3.7	28.	18.8	4.0	4.7
SE ±	3.4	0.4	1.6	0.6	3.5	4.7	1.6	2.0
Fp	<0.001	0.001	<0.001	0.29	<0.001	<0.001	0.07	0.11
Vr	35.0	6.0	27.6	1.3	38.1	21.1	2.5	2.1
LSD (P 0.05)	10.5*	1.4*	5.1*	**NS**	10.8*	14.4*	4.9*	**NS**
CV (%)	13.1	11.8	38.4	29.4	22.0	44.1	69.7	74.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.37: Direct effect of *Cry IIa* transgenic chickpea lines on *Cheilomenes sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%) (ICRISAT, Patancheru 2012-2013 and 2013-14) (pooled analysis).

Genotype	0.02%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	58.3^{a} (49.8)	7.1^{cd}	13.1^{ab}	4.0^{a}	43.3^{bc} (41.1)	31.6^{ab} (34.1)	5.1^{a}	8.2^{a}
BS5A.1(T2) 18-2 P1	51.6^{a} (46.0)	7.5^{d}	15.5^{ab}	4.1^{a}	35.0^{ab} (36.2)	21.6^{a} (27.6)	5.9^{ab}	9.3^{a}
BS5A.2(T2) 19-1 P2	61.6^{a} (51.9)	7.0^{bcd}	10.7^{a}	3.5^{a}	45.0^{c} (42.1)	40.0^{bc} (39.1)	6.2^{ab}	8.4^{a}
BS5A.2(T2) 19-2 P1	50.0^{a} (45.0)	7.5^{d}	13.8^{ab}	9.0^{a}	33.3^{a} (35.0)	21.6^{a} (25.3)	5.6^{a}	8.0^{a}
BS5A.2(T2) 19-3 P1	61.6^{a} (51.8)	6.5^{abc}	11.5^{a}	4.0^{a}	35.0^{ab} (36.1)	28.3^{ab} (32.0)	7.2^{bc}	8.7^{a}
BS5A.2(T2) 19-3 P2	61.6^{a} (51.9)	6.0^{a}	11.5^{a}	4.8^{a}	35.0^{ab} (36.2)	30.0^{ab} (33.0)	7.9^{c}	9.1^{a}
Semsen (Control)	80.0^{b} (63.6)	6.6^{abcd}	22.9^{b}	3.1^{a}	61.6^{d} (51.8)	48.3^{cd} (44.0)	8.7^{c}	8.2^{a}
ICC 506 EB (Resistant check)	78.3^{b} (62.5)	6.1^{ab}	18.4^{b}	3.1^{a}	65.0^{d} (53.7)	55.0^{d} (47.8)	8.5^{c}	9.1^{a}
Mean	62.9	6.	14.7	4.4	44.2	34.6	6.9	8.4
SE ±	4.1	0.2	3.1	0.4	3.0	3.9	0.5	4.7
Fp	<0.001	0.001	0.001	0.001	<0.001	<0.001	<0.001	0.40
Vr	6.9	3.9	1.8	1.0	17.2	9.4	7.4	1.0
LSD (P 0.05)	11.9*	0.8*	**NS**	**NS**	8.6*	11.3*	1.4*	**NS**
CV (%)	16.3	10.4	51.8	102.5	16.8	28.0	17.7	111.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.37 (Conti.)

Genotype	0.05%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	61.6^{b} (50.8)	8.0^{bc}	5.9^{a}	4.3^{b}	41.6^{c} (40.1)	28.3^{b} (34.1)	6.4^{bc}	8.5^{a}
BS5A.1(T2) 18-2 P1	50.0^{a} (45.0)	8.1^{bc}	2.9^{a}	4.5^{b}	35.0^{bc} (36.2)	21.6^{ab} (27.6)	6.3^{bc}	8.3^{a}
BS5A.2(T2) 19-1 P2	43.3^{a} (41.0)	9.3^{c}	3.0^{a}	4.5^{b}	26.6^{ab} (31.0)	13.3^{a} (39.1)	5.2^{ab}	6.8^{a}
BS5A.2(T2) 19-2 P1	45.0^{a} (42.0)	8.8^{c}	4.1^{a}	4.6^{b}	18.3^{a} (20.9)	11.6^{a} (25.3)	3.9^{a}	5.7^{a}
BS5A.2(T2) 19-3 P1	45.0^{a} (42.0)	8.0^{bc}	4.5^{a}	4.3^{b}	28.3^{ab} (31.5)	16.6^{ab} (32.0)	6.6^{bc}	7.7^{a}
BS5A.2(T2) 19-3 P2	46.6^{a} (43.0)	8.0^{bc}	5.0^{a}	4.0^{b}	28.3^{ab} (32.1)	13.3^{a} (33.0)	6.3^{bc}	7.9^{a}
Semsen (Control)	81.6^{c} (65.2)	6.6^{ab}	15.8^{b}	3.0^{a}	66.6^{d} (54.8)	50.0^{c} (44.0)	8.5^{c}	9.8^{a}
ICC 506 EB (Resistant check)	80.0^{c} (63.6)	5.8^{a}	16.2^{b}	3.1^{a}	66.6^{d} (54.7)	48.3^{c} (47.8)	8.4^{c}	8.5^{b}
Mean	56.7	7.85	8.4	4.0	39.0	25.4	0.7	7.9
SE +	3.7	0.5	2.7	0.2	3.8	4.2	1.9	5.0
Fp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.23
Vr	17.8	4.6	4.8	5.3	22.5	13.2	4.7	1.4
LSD (P 0.05)	10.8*	1.5*	7.8*	0.7*	11.0*	12.2*	6.5*	**NS**
CV (%)	16.4	16.3	79.8	16.6	24.3	41.4	26.2	124.6

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.37 (Conti.)

Genotype	0.1%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	53.3^{ab} (46.9)	5.8^{a}	4.8^{a}	3.3^{ab}	40.0^{a} (38.9)	30.0^{bc} (32.5)	6.2^{a}	9.2^{a}
BS5A.1(T2) 18-2 P1	60.0^{bc} (51.0)	5.6^{a}	6.4^{a}	3.3^{ab}	43.3^{ab} (40.9)	35.0^{bcd} (35.8)	7.7^{a}	10.4^{a}
BS5A.2(T2) 19-1 P2	53.3^{ab} (46.9)	5.6^{a}	5.4^{a}	3.5^{ab}	38.3^{ab} (38.0)	23.3^{ab} (28.5)	8.3^{a}	10.6^{a}
BS5A.2(T2) 19-2 P1	70.0^{cd} (57.0)	5.5^{a}	8.8^{a}	4.0^{b}	43.3^{a} (41.0)	30.0^{bc} (32.6)	8.7^{a}	10.1^{a}
BS5A.2(T2) 19-3 P1	50.0^{ab} (45.0)	5.8^{a}	5.1^{a}	3.8^{ab}	35.0^{a} (35.7)	20.0^{ab} (18.0)	8.2^{a}	9.0^{a}
BS5A.2(T2) 19-3 P2	45.0^{a} (42.0)	6.1^{a}	4.7^{a}	3.6^{ab}	33.3^{a} (34.5)	11.6^{a} (26.0)	8.3^{a}	9.2^{a}
Semsen (Control)	71.6^{cd} (58.0)	5.5^{a}	23.5^{b}	3.5^{ab}	60.0^{b} (50.9)	45.0^{cd} (42.1)	13.2^{a}	9.7^{a}
ICC 506 EB (Resistant check)	75.0^{d} (60.4)	5.3^{a}	21.5^{b}	3.1^{a}	61.6^{b} (51.9)	48.3^{d} (43.8)	10.8^{b}	9.3^{a}
Mean	59.8	5.6	10.0	3.5	44.4	30.4	8.2	9.7
SE ±	4.3	0.3	1.9	0.2	6.1	5.2	2.3	0.5
Fp	<0.001	0.75	<0.001	0.14	0.01	<0.001	0.001	0.27
Vr	6.5	0.6	16.3	1.7	3.0	5.4	3.9	1.3
LSD (P 0.05)	12.5*	**NS**	5.5*	**NS**	17.6*	15.1*	6.8*	**NS**
CV (%)	17.9	14.3	47.1	14.8	34.0	42.5	57.1	13.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.38: Indirect effect of *Cry IIa* transgenic chickpea lines on different biological parameters of the coccinellid, *C. sexmaculatus* reared on *Bt* intoxicated artificial diet fed *Aphis craccivora* (ICRISAT, Patancheru 2012-2013).

Genotype	0.02%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	76.6[a] (61.7)	9.0[c]	2.7[a]	3.6[b]	70.0[b] (57.0)	56.6[a] (48.8)	6.1[ab]	8.5[ab]
BS5A.1(T2) 18-2 P1	73.3[a] (59.0)	8.3[c]	7.0[ab]	4.0[bc]	63.3[ab] (52.7)	50.0[a] (45.0)	6.8[ab]	7.8[ab]
BS5A.2(T2) 19-1 P2	80.0[a] (63.9)	8.6[c]	5.8[ab]	4.3[c]	66.6[b] (54.7)	60.0[a] (51.1)	5.9[ab]	8.6[ab]
BS5A.2(T2) 19-2 P1	70.0[a] (56.7)	9.0[c]	3.0[a]	3.0[a]	70.0[b] (56.7)	63.3[a] (53.0)	6.9[ab]	6.6[a]
BS5A.2(T2) 19-3 P1	76.6[a] (61.7)	8.0[bc]	3.8[a]	3.0[a]	56.6[ab] (48.8)	53.3[a] (46.9)	7.7[bc]	9.1[ab]
BS5A.2(T2) 19-3 P2	76.6[a] (61.7)	9.0[c]	8.9[ab]	3.0[a]	36.6[a] (32.0)	30.0[a] (32.5)	5.3[a]	7.0[ab]
Semsen (Control)	83.3[a] (66.1)	6.6[a]	6.9[ab]	3.0[a]	66.6[b] (54.7)	50.0[a] (45.0)	6.4[ab]	8.8[ab]
ICC 506 EB (Resistant check)	83.3[a] (70.0)	7.0[ab]	11.2[b]	3.0[a]	73.3[b] (59.7)	63.3[a] (53.0)	9.3[c]	9.4[b]
Mean	77.5	8.2	6.2	3.4	62.9	56.2	6.8	8.3
SE +	5.8	0.4	1.9	0.2	8.7	7.7	0.6	0.8
Fp	0.723	0.002	0.074	<0.001	0.162	0.818	0.011	0.251
Vr	0.6	6.3	2.4	11.6	1.8	0.5	4.2	1.5
LSD (P 0.05)	**NS**	1.1*	5.7*	0.4*	**NS**	**NS**	1.8*	**NS**
CV (%)	13.0	7.8	53.0	8.2	24.0	23.8	15.2	17.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values

Table 4.38 (Conti.)

Genotype	0.05%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	50.0[a] (45.0)	6.0[ab]	3.8[a]	3.3[ab]	40.0[a] (39.1)	26.6[ab] (31.0)	7.0[a]	9.6[a]
BS5A.1(T2) 18-2 P1	56.6[a] (48.8)	6.6[b]	4.7[ab]	3.0[a]	36.6[a] (37.2)	26.6[ab] (31.0)	7.5[a]	13.7[b]
BS5A.2(T2) 19-1 P2	56.6[a] (48.9)	6.0[ab]	4.6[ab]	3.3[ab]	40.0[a] (39.2)	30.0[b] (33.0)	6.8[a]	11.9[ab]
BS5A.2(T2) 19-2 P1	43.3[a] (41.1)	5.3[ab]	3.8[a]	4.0[b]	40.0[a] (39.0)	30.0[b] (33.0)	6.6[a]	10.9[a]
BS5A.2(T2) 19-3 P1	60.0[a] (50.7)	5.6[ab]	6.1[bc]	3.3[ab]	50.0[a] (44.9)	16.6[ab] (23.8)	6.7[a]	11.2[a]
BS5A.2(T2) 19-3 P2	53.3[a] (46.9)	6.3[ab]	7.3[c]	3.6[ab]	40.0[a] (39.2)	20.0[ab] (26.5)	6.7[a]	10.2[a]
Semsen (Control)	50.0[a] (45.0)	5.6[ab]	5.2[ab]	3.0[a]	43.3[a] (41.0)	13.3[a] (21.1)	5.2[a]	10.6[a]
ICC 506 EB (Resistant check)	60.0[a] (50.8)	5.0[a]	7.9[c]	3.0[a]	46.6[a] (43.0)	23.3[ab] (28.2)	6.9[a]	10.5[a]
Mean	53.8	5.8	5.5	3.3	42.1	23.3	6.7	11.1
SE +	5.0	0.5	0.6	0.2	6.9	4.4	0.8	0.7
Fp	0.304	0.320	0.002	0.118	0.894	0.1	0.757	0.044
Vr	1.3	1.3	5.9	2.0	0.3	1.94	0.59	2.8
LSD (P 0.05)	**NS**	**NS**	1.9*	**NS**	**NS**	**NS**	**NS**	**NS**
CV (%)	16.0	13.9	20.2	12.9	28.7	32.9	21.9	11.6

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.38 (Conti.)

Genotype	0.1%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	60.0^{ab} (50.8)	5.3^{a}	5.5^{a}	3.3^{ab}	53.3^{a} (46.9)	43.3^{ab} (41.1)	6.0^{a}	9.1^{ab}
BS5A.1(T2) 18-2 P1	66.6^{b} (55.0)	5.6^{a}	6.6^{a}	3.6^{ab}	56.6^{a} (48.9)	46.6^{b} (42.9)	8.6^{ab}	10.8^{bc}
BS5A.2(T2) 19-1 P2	60.0^{ab} (50.7)	4.6^{a}	5.9^{a}	3.6^{ab}	50.0^{a} (45.0)	33.3^{ab} (35.2)	9.4a^{bcd}	11.7^{c}
BS5A.2(T2) 19-2 P1	70.0^{b} (57.0)	5.0^{a}	6.6^{a}	3.6^{ab}	50.0^{a} (45.0)	36.6^{ab} (37.2)	8.8^{abc}	8.7^{a}
BS5A.2(T2) 19-3 P1	60.0^{ab} (50.8)	5.3^{a}	6.6^{a}	3.6^{ab}	50.0^{a} (45.0)	36.6^{ab} (37.2)	8.6^{ab}	8.4^{a}
BS5A.2(T2) 19-3 P2	46.6^{a} (43.0)	5.6^{a}	5.0^{a}	3.6^{ab}	43.3^{a} (41.0)	23.3^{a} (28.7)	8.8^{abc}	9.0^{ab}
Semsen (Control)	66.6^{b} (54.7)	5.6^{a}	18.7^{c}	3.0^{a}	56.6^{a} (48.8)	36.6^{ab} (37.2)	17.3^{bd}	8.5^{a}
ICC 506 EB (Resistant check)	70.0^{b} (57.0)	5.0^{a}	17.8^{b}	4.0^{b}	56.6^{a} (48.9)	40.0^{ab} (38.8)	12.6^{e}	9.0^{ab}
Mean	62.5	5.3	10.4	3.6	52.1	37.1	12.6	9.5
SE +	5.8	0.4	3.0	0.3	6.0	5.9	2.6	0.6
Fp	0.176	0.418	<.001	0.338	0.742	0.276	<.001	0.011
Vr	1.8	1.1	8.0	1.3	0.6	1.4	11.3	4.2
LSD (P 0.05)	**NS**	**NS**	9.1*	**NS**	**NS**	**NS**	7.9*	1.7*
CV (%)	16.1	11.8	50.3	12.7	20	27.5	36	10.9

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.39: Indirect effect of *Cry IIa* transgenic chickpea lines on different biological parameters of the coccinellid, *C. sexmaculatus* reared on *Bt* intoxicated artificial diet fed *Aphis craccivora* (ICRISAT, Patancheru 2013-2014).

Genotype	0.02%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	76.6^{b} (61.7)	6.6^{bc}	6.6^{a}	4.3^{bc}	56.6^{abc} (48.8)	50.0^{abc} (45.0)	8.4^{b}	8.0^{a}
BS5A.1(T2) 18-2 P1	70.0^{ab} (57.0)	5.6^{ab}	10.4^{a}	4.3^{bc}	50.0^{ab} (45.0)	50.0^{abc} (45.0)	6.5^{a}	8.4^{ab}
BS5A.2(T2) 19-1 P2	60.0^{a} (50.7)	7.0^{c}	8.5^{a}	4.6^{c}	43.3^{a} (41.1)	46.6^{abc} (42.9)	7.7^{ab}	8.7^{abc}
BS5A.2(T2) 19-2 P1	83.3^{b} (66.1)	6.6^{bc}	6.0^{a}	4.3^{bc}	43.3^{a} (41.1)	36.6^{a} (37.2)	7.0^{a}	9.3^{abc}
BS5A.2(T2) 19-3 P1	73.3^{ab} (59.0)	6.6^{bc}	5.5^{a}	4.0^{abc}	66.6^{cd} (54.9)	46.6^{abc} (43.0)	7.6^{ab}	10.1^{bc}
BS5A.2(T2) 19-3 P2	83.3^{b} (66.1)	7.3^{c}	2.2^{a}	4.0^{abc}	50.0^{ab} (45.0)	40.0^{ab} (39.2)	7.6^{ab}	8.9^{abc}
Semsen (Control)	83.3^{b} (66.1)	5.3^{a}	12.1^{b}	4.3^{ab}	63.3^{bcd} (52.7)	53.3^{bc} (46.9)	9.9^{c}	10.3^{c}
ICC 506 EB (Resistant check)	83.3^{b} (66.1)	5.3^{a}	12.3^{b}	3.0^{a}	76.6^{d} (61.2)	56.6^{c} (48.8)	10.5^{c}	12.2^{c}
Mean	76.7	6.3	8.7	4.0	56.2	47.5	8.2	9.5
SE +	4.2	0.4	2.4	0.4	4.3	4.8	0.4	0.6
Fp	0.011	0.008	<0.001	0.112	<0.001	0.143	<0.001	0.002
Vr	4.1	4.5	8.3	2.1	7.7	1.9	11.7	6.0
LSD (P 0.05)	12.7*	1.1*	7.2*	**NS**	13.0*	**NS**	1.2*	1.6*
CV (%)	9.5	10.0	35.1	16.8	13.2	17.4	8.6	10.0

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05. Figures in parenthesis are Angular transformed values.

Table 4.39 (Conti.)

Genotype	0.05%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	46.6[a] (43.0)	7.0[b]	5.3[a]	3.6[a]	36.6[ab] (37.2)	26.6[ab] (31.0)	6.5[a]	8.3[ab]
BS5A.1(T2) 18-2 P1	53.3[a] (46.9)	7.0[b]	9.3[a]	4.3[a]	40.0[ab] (39.2)	36.6[ab] (37.2)	7.0[ab]	9.3[abc]
BS5A.2(T2) 19-1 P2	46.6[a] (43.0)	6.0[ab]	8.4[a]	4.3[a]	30.0[a] (33.0)	30.0[ab] (33.0)	7.7[abc]	10.3[bcd]
BS5A.2(T2) 19-2 P1	53.3[a] (47.2)	5.3[a]	7.8[a]	4.3[a]	43.3[ab] (41.0)	36.6[ab] (37.1)	9.0[c]	7.0[a]
BS5A.2(T2) 19-3 P1	63.3[a] (52.7)	6.0[ab]	5.8[a]	4.6[a]	53.3[b] (46.9)	40.0[b] (39.1)	7.2[abc]	9.0[abc]
BS5A.2(T2) 19-3 P2	56.6[a] (48.8)	7.0[b]	8.7[a]	3.6[a]	40.0[ab] (39.2)	23.3[a] (28.7)	7.4[abc]	7.9[ab]
Semsen (Control)	60.0[a] (50.8)	6.0[ab]	9.1[a]	3.6[a]	50.0[ab] (45.0)	26.6[ab] (31.0)	8.4[bc]	12.0[d]
ICC 506 EB (Resistant check)	63.3[a] (53.0)	6.0[ab]	12.5[a]	4.3[a]	50.0[ab] (45.0)	26.6[ab] (31.0)	7.8[abc]	11.7[cd]
Mean	55.4	6.3	9.0	4.1	42.9	30.8	7.7	9.5
SE +	7.1	0.4	2.3	0.6	6.1	4.8	0.5	0.8
Fp	0.552	0.052	0.056	0.842	0.193	0.209	0.090	0.006
Vr	0.9	2.7	2.7	0.5	1.7	1.6	2.3	4.8
LSD (P 0.05)	**NS**	1.1*	6.9*	**NS**	**NS**	**NS**	1.6*	2.5*
CV (%)	22.3	10.5	43.8	24.3	24.5	26.9	12.1	14.9

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.39 (Conti.)

Genotype	0.1%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	46.6[a] (43.0)	6.3[a]	4.2[a]	3.3[ab]	26.6[a] (31.0)	16.6[a] (23.8)	6.5[a]	9.3[a]
BS5A.1(T2) 18-2 P1	53.3[a] (46.9)	5.6[a]	6.2[ab]	3.0[a]	30.0[a] (33.0)	23.3[a] (28.7)	6.9[a]	9.9[a]
BS5A.2(T2) 19-1 P2	46.6[a] (43.0)	6.6[a]	5.0[ab]	3.3[ab]	26.6[a] (31.0)	16.6[a] (23.8)	7.3[a]	9.4[a]
BS5A.2(T2) 19-2 P1	70.0[b] (57.0)	6.0[a]	11.1[b]	4.3[c]	36.6[a] (37.1)	23.3[a] (28.0)	8.6[a]	11.5[b]
BS5A.2(T2) 19-3 P1	40.0[a] (39.2)	6.3[a]	3.7[a]	4.0[bc]	20.0[a] (26.5)	10.0[a] (18.4)	7.8[a]	9.7[a]
BS5A.2(T2) 19-3 P2	43.3[a] (41.0)	6.6[a]	4.4[a]	3.3[ab]	23.3[a] (28.0)	20.0[a] (26.0)	7.8[a]	9.5[a]
Semsen (Control)	76.6[b] (61.2)	5.3[a]	18.4[c]	3.3[ab]	63.3[b] (53.0)	53.3[b] (47.0)	9.1[a]	10.9[ab]
ICC 506 EB (Resistant check)	80.0[b] (63.9)	5.6[a]	15.3[d]	3.3[ab]	66.6[b] (54.9)	56.6[b] (48.8)	9.1[a]	9.6[a]
Mean	57.1	6.1	9.8	3.5	36.7	27.5	7.9	10.0
SE +	5.1	0.4	1.9	0.3	6.4	5.6	0.8	0.5
Fp	<.001	0.224	<.001	0.072	<.001	<.001	0.208	0.194
Vr	10.0	1.6	17.1	2.5	8.2	9.9	1.6	2.3
LSD (P 0.05)	15.3*	**NS**	5.8*	0.8*	19.2*	16.8*	**NS**	**NS**
CV (%)	15.4	11.3	34.2	13.8	30.0	35.0	16.9	9.3

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.40: Indirect effect of *Cry IIa* transgenic chickpea lines on different biological parameters of the coccinellid, *C. sexmaculatus* reared on *Bt* intoxicated artificial diet fed *A. craccivora* (ICRISAT, Patancheru 2013 and 2014) (pooled analysis).

Genotype	0.02%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	76.6^{ab} (61.7)	7.8^{b}	4.6^{a}	4.0^{bc}	63.3^{bc} (52.9)	53.3^{a} (46.9)	7.3^{a}	8.2^{a}
BS5A.1(T2) 18-2 P1	71.6^{ab} (58.0)	7.0^{a}	8.7^{ab}	4.1^{bc}	56.6^{ab} (48.8)	50.0^{a} (45.0)	6.6^{a}	8.1^{a}
BS5A.2(T2) 19-1 P2	70.0^{a} (57.3)	7.8^{b}	7.1^{a}	4.5^{c}	55.0^{ab} (47.9)	53.3^{a} (47.0)	6.8^{a}	8.7^{a}
BS5A.2(T2) 19-2 P1	76.6^{ab} (61.4)	7.8^{b}	4.5^{a}	3.6^{ab}	56.6^{ab} (48.9)	50.0^{a} (45.1)	7.0^{a}	7.9^{a}
BS5A.2(T2) 19-3 P1	75.0^{ab} (60.3)	7.3^{ab}	4.6^{a}	3.5^{ab}	61.6^{bc} (51.9)	50.0^{a} (45.0)	7.6^{a}	9.6^{ab}
BS5A.2(T2) 19-3 P2	80.0^{ab} (63.9)	8.1^{b}	10.5^{abc}	3.5^{ab}	43.3^{a} (38.5)	46.6^{a} (43.0)	6.5^{a}	7.9^{a}
Semsen (Control)	83.3^{b} (66.1)	6.0^{a}	14.5^{bc}	3.1^{a}	65.0^{bc} (53.7)	51.6^{a} (45.9)	8.2^{a}	9.5^{ab}
ICC 506 EB (Resistant check)	83.3^{b} (68.1)	6.1^{a}	16.7^{c}	3.0^{a}	75.0^{c} (60.4)	60.0^{a} (50.9)	9.9^{b}	10.8^{b}
Mean	77.1	7.3	9.0	3.7	59.6	51.9	7.5	8.9
SE ±	3.8	0.5	2.1	0.3	5.5	4.9	0.5	0.6
Fp	0.147	0.022	<.001	0.003	0.02	0.708	<.001	0.022
Vr	1.7	2.7	5.0	3.8	2.8	0.7	4.5	2.7
LSD (P 0.05)	**NS**	1.4*	6.0*	0.7*	15.7*	**NS**	1.5*	1.7*
CV (%)	12.2	16.7	57.5	17.2	22.6	22.9	17.3	17

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.40 (Conti.)

Genotype	.05%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	48.3^{a} (44.0)	6.5^{bc}	4.6^{a}	3.5^{a}	38.3^{ab} (38.1)	26.6^{ab} (31.0)	6.7^{a}	8.9^{a}
BS5A.1(T2) 18-2 P1	55.0^{abc} (47.8)	6.8^{c}	7.0^{a}	3.6^{a}	38.3^{ab} (38.2)	31.6^{ab} (34.1)	7.2^{a}	11.5^{a}
BS5A.2(T2) 19-1 P2	51.6^{abc} (46.0)	6.0^{abc}	6.5^{a}	3.8^{a}	35.0^{a} (36.1)	30.0^{ab} (33.0)	7.3^{a}	11.1^{a}
BS5A.2(T2) 19-2 P1	48.3^{ab} (44.1)	5.3^{a}	5.8^{a}	4.1^{a}	41.6^{ab} (40.0)	33.3^{b} (35.0)	7.8^{a}	9.0^{a}
BS5A.2(T2) 19-3 P1	61.6^{ac} (51.7)	5.8^{ab}	6.0^{a}	4.0^{a}	51.6^{b} (45.9)	28.3^{ab} (31.5)	6.9^{a}	10.1^{a}
BS5A.2(T2) 19-3 P2	55.0^{abc} (47.8)	6.6^{bc}	8.0^{a}	3.6^{a}	40.0^{ab} (39.2)	21.6^{ab} (27.6)	7.0^{a}	9.0^{a}
Semsen (Control)	55.0^{abc} (47.9)	5.8^{ab}	7.1^{a}	3.3^{a}	46.6^{ab} (43.0)	20.0^{a} (26.0)	6.8^{a}	11.3^{a}
ICC 506 EB (Resistant check)	61.6^{abc} (51.9)	5.5^{a}	12.7^{b}	3.6^{a}	48.3^{ab} (44.0)	25.0^{ab} (29.6)	7.4^{a}	11.1^{a}
Mean	54.6	6.1	7.3	3.7	42.5	27.1	7.2	10.3
SE ±	4.1	0.3	1.5	0.4	4.2	3.8	0.6	0.8
Fp	0.168	0.005	0.020	0.781	0.093	0.18	0.933	0.064
Vr	1.6	3.5	2.8	0.6	1.9	1.6	0.3	2.1
LSD (P 0.05)	**NS**	0.8*	1.4*	**NS**	11.9*	**NS**	**NS**	2.2*
CV (%)	18.4	11.9	49.7	23.3	24.0	34.1	19.4	18.4

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Table 4.40 (Conti.)

Genotype	0.1%							
	Larval survival (%)	Larval period (days)	Mean grub weight (mg)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Adult weight (mg)	
							Male	Female
BS5A.1(T2) 18-1 P1	53.3^{ab} (46.9)	5.8^{a}	4.8^{a}	3.3^{ab}	40.0^{a} (38.9)	30.0^{ab} (32.5)	6.2^{a}	9.2^{a}
BS5A.1(T2) 18-2 P1	60.0^{bc} (51.0)	5.6^{a}	6.4^{a}	3.3^{ab}	43.3^{ab} (40.9)	35.0^{abc} (35.8)	7.7^{a}	10.4^{a}
BS5A.2(T2) 19-1 P2	53.3^{ab} (46.9)	5.6^{a}	5.4^{a}	3.5^{ab}	38.3^{a} (38.0)	25.0^{a} (29.5)	8.3^{a}	10.6^{a}
BS5A.2(T2) 19-2 P1	70.0^{cd} (57.0)	5.5^{a}	8.8^{a}	4.0^{b}	43.3^{ab} (41.0)	30.0^{ab} (32.6)	8.7^{a}	10.1^{a}
BS5A.2(T2) 19-3 P1	50.0^{ab} (45.0)	5.8^{a}	5.1^{a}	3.8^{ab}	35.0^{a} (35.7)	23.3^{a} (27.8)	8.2^{a}	9.0^{a}
BS5A.2(T2) 19-3 P2	45.0^{a} (42.0)	6.1^{a}	4.7^{a}	3.5^{ab}	33.3^{a} (34.5)	21.6^{a} (27.4)	8.3^{a}	9.2^{a}
Semsen (Control)	71.6^{cd} (58.0)	5.5^{a}	13.5^{a}	3.1^{a}	60 .0^{b} (50.9)	45.0^{bc} (42.1)	13.2^{b}	9.7^{a}
ICC 506 EB (Resistant check)	75.0^{d} (60.4)	5.3^{a}	11.5^{a}	3.6^{ab}	61.6^{b} (51.9)	48.3^{c} (43.8)	10.8^{b}	9.3^{a}
Mean	59.8	5.7	5.1	3.5	44.4	32.3	10.2	9.7
SE ±	4.4	0.3	1.9	0.2	6.2	5.7	2.4	0.5
Fp	<.001	0.746	<.001	0.138	0.012	0.012	0.002	0.271
Vr	6.5	0.6	16.4	1.7	3.1	3.0	4.0	1.3
LSD (P 0.05)	12.5*	**NS**	5.5*	**NS**	17.6*	16.2*	6.8*	**NS**
CV (%)	17.9	14.3	47.1	14.8	34.0	43	57.1	13.1

*Figures followed by the same letter within a column are not significantly different at P≤ 0.05.
Figures in parenthesis are Angular transformed values.

Chapter V
SUMMARY AND CONCLUSIONS

The studies on "Characterization of *Cry IIa* transgenic chickpea lines and their interaction with natural enemies of *Helicoverpa armigera* (Hubner)" were conducted in the glasshouse and under laboratory conditions at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2011-14.

The transgenic plants suffered significantly lower leaf damage as compared to the non-transgenic plants. The larval survival and weight gained by *H. armigera* larvae after 5 days was significantly reduced on transgenic lines as compared to that on non-transgenic chickpeas during October and November plantings of 2011-12 and 2012-13.

Among the transgenic plants tested, significantly lower leaf damage rating, larval survival and mean larval weight was observed on BS5A.2(T2) 19-2P1. Similarly, during November planting across the seasons (2011-12 and 2012-13), the transgenic lines BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 exhibited significantly lower leaf damage rating, larval survival and mean larval weight under laboratory conditions.

In glasshouse conditions, BS5A.1(T2) 18-1P1 suffered significantly lower leaf damage and mean larval weight was also reduced. The larval survival of *H. armigera* was significantly reduced on BS5A.2(T2) 19-2P1. Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera.* The dry matter weight, pod weight, seed weight and number of seeds formed were significantly more on transgenic lines as compared to that on non-transgenic chickpea plants. BS5A.2(T2) 19-2P1 had the highest dry matter weight, pod weight, seed weight and number of seeds formed as compared to the other transgenic and non-transgenic chickpea lines under infested and un-infested conditions.

From the present study, it is clear that the survival and development of *H. armigera* larvae was significantly lower on transgenic chickpea diets as compared to those reared on non-transgenic chickpea diets. During 2012-13, when the neonate larvae were fed on artificial diet with BS5A.2(T2) 19-2P1 leaf powder they exhibited lowest larval survival, larval weights at 5 and 10 DAI and pupal weights as compared to insects reared on diets with leaf powder of non-transgenic plants. Insects reared on diets with BS5A.2(T2) 19-2P1 leaf powder showed maximum resistance to *H. armigera*.

The survival and development of *H. armigera* was significantly better when reared on the standard artificial diet compared to those reared on diets with lyophilized leaf powders of

transgenic and non-transgenic chickpeas. Compared to the first season 2011-12, the survival and development of third-instar *H. armigera* during 2012-13 was significantly reduced in insects reared on diets with leaf powder of transgenic chickpea BS5A.1(T2) 18-1P1 as against those reared on non-transgenic chickpea ICC 506EB.

The transgenic line BS5A.1(T2) 18-1P1 showed high levels of resistance to third-instar larvae of *H. armigera*. The survival and development of *H. armigera* neonate larvae reared on diets with leaf powder of transgenic chickpea was very poor as compared to third-instar larvae.

Maximum amount of protein was recorded in ICC 506EB and among the transgenic lines, the protein content was highest in BS5A.1(T2) 18-2P1. The amounts of carbohydrates were significantly higher in the leaves of ICC 506EB as compared to that on transgenic lines. The highest amount of lipids were recorded in BS5A.2(T2) 19-3P1 than in BS5A.2(T2) 19-3P2. There were no significant differences in phenol and tannin contents between the transgenic and non transgenic chickpea lines.

The protein content was negatively correlated with larval survival, larval weight and leaf damage rating. Significant positive correlation was observed between carbohydrate content and leaf damage. Negative, non-significant relationship of phenols was observed with leaf damage, larval survival and larval weight. There was a negative significant association of tannins with leaf feeding damage, larval survival and larval weight.

Significantly higher amounts of oxalic acid were recorded in BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 than in BS5A.2(T2) 19-2P1. Highest malic acid content was recorded on BS5A.1(T2) 18-1P1 and lowest on BS5A.2(T2) 19-3P2.

Among the non-transgenics, the maximum amount of oxalic acid was observed in ICC 506EB, followed by Semsen. Oxalic acid content was positively correlated with larval survival and larval weight. A significant and negative association was observed between the amounts of the malic acid and leaf feeding, larval survival and larval weight.

Chlorogenic acid content was significantly greater in BS5A.2(T2) 19-2P1 as compared to BS5A.1(T2) 18-1P1. Gentisic acid content was highest in BS5A.2(T2) 19-2P1, while in BS5A.2 (T2) 19-1P2 had the lowest. Maximum amount of phloretic acid was recorded in BS5A.1(T2) 18-2P1 and least in BS5A.2(T2) 19-3P2. The amount of ferulic acid was greater in Semsen than in BS5A.2(T2) 19-1P2 and ICC 506EB.

Naringin content was highest in ICC 506EB, while it was lowest in BS5A.1(T2) 18-2P1, but below detectable limits in BS5A.1(T2) 18-1P1 and Semsen. Maximum amounts of

3,4 dihydroxy flavone, quercetin, naringenin, formononetin and biochanin A were recorded in Semsen.

Among the transgenic lines tested, the amount of 3,4 dihydroxy flavone was maximum in BS5A.2(T2) 19-3P2 and quercetin was significantly higher in BS5A.1(T2) 18-2P2 as compared to BS5A.1(T2) 18-1P1. Naringenin content was highest in BS5A.2(T2) 19-2P1 and lowest amount in BS5A.2(T2) 19-3P1 and was nil in BS5A.2(T2) 19-1P2, and ICC 506EB.

In the leaves of BS5A.2(T2) 19-3P2 genistein content was highest and in BS5A.1(T2) 18-1P1 had the lowest amounts. In BS5A.2(T2) 19-3P2 had the highest amount of formononetin, while lowest amount was recorded in BS5A.2(T2) 19-3P1. Maximum amount of biochanin A was recorded in BS5A.1(T2) 18-2P1 and BS5A.1(T2) 18-1P1 and least was in BS5A.2(T2) 19-1P2 while nil in BS5A.2(T2) 19-3P1.

Chlorogenic acid, gentisic acid, ferulic acid, naringin, naringenin and quercetin had a positive but non-significant correlation with resistance to *H. armigera*. There was a positive and significant association between 3,4 dihydroxy flavone, genistein, formononetin and biochanin A with leaf damage, larval survival and larval weight.

The amount of CryIIa protein was highest in the fresh leaf samples, followed by green pod wall, green seeds, dry pod wall, dry seeds and dry stems. In dry roots the protein concentration was quite low whereas in soil samples, it was below detectable levels. The CryIIa protein content was significantly higher in larvae fed on BS5A.2 (T2) 19-2P1 and BS5A.1 (T2) 18-1P1. The CryIIa protein in *Bt* fed aphids, coccinellid grubs and *Bt* fed *C. chlorideae* larvae was almost nil. Hence, the amount of CryIIa protein transferred from leaves to the non-target insects and natural enemies was negligible.

In both the seasons (2011-12 and 2012-13), the correlation co-efficient of CryIIa protein in fresh leaf, green pod wall, green seeds, dry pod wall, dry seeds, dry stems, dry roots and *H. armigera* larvae with leaf damage, larval survival and larval weight was negative and significant.

Among the transgenic lines tested, during October 2011-12 planting, a significant increase in egg+larval period, post embryonic development period and reduction in cocoon formation, adult emergence, adult longevity, adult weights, sex ratio and fecundity was recorded in *C. chlorideae* reared on *H. armigera* fed on leaves of BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-1P2. There was a prolongation of egg+larval period, pupal period and reduction in adult longevity, weights and sex ratio, and increase in cocoon formation of *C.*

chlorideae reared on *H. armigera* fed on leaves of BS5A.2(T2) 19-3P1 and BS5A.2(T2) 19-3P2 during October 2012-13 planting.

During November 2011-12 planting, among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on leaves of BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1. Among the transgenic lines tested, the survival and development of *C. chlorideae* was significantly better when reared on *H. armigera* fed on leaves of BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 as compared to that on other transgenic lines during November 2012-13 planting.

The survival and development of parasitoids were affected when reared on *H. armigera* larvae fed on diets with transgenic BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 leaf powder as compared to that on other transgenics lines during 2011-12. Survival and development of *C. chlorideae* wasps obtained from *H. armigera* larvae fed on diets with transgenic BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P1 leaf powder was better as compared to that on BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P2 lines during 2012-13.

No CryIIa protein was detected in the *C. chlorideae* larvae, the negative effects of transgenic chickpeas on survival and development of *C. chlorideae* were due to the early mortality of *H. armigera* as a result the parasitoids failed to complete the development on such larvae. The survival and development of *C. chlorideae* was poorer when reared on *H. armigera* larvae fed on fresh leaf samples than the artificial diets intoxicated with transgenic chickpea leaf powders.

In diets having 0.02% and 0.05% leaf powder, the survival and development of coccinellids was greater when reared on BS5A.1(T2) 18-1P1 intoxicated diet as compared to that on the other transgenic lines tested during 2012-13. The survival and development of coccinellid was significantly affected when fed on diets intoxicated with BS5A.1(T2) 18-1P1 leaf powder but better survival was recorded on BS5A.1(T2) 18-2P1 and BS5A.2(T2) 19-3P2. In general, the direct effects on coccinellids were greater when fed on 0.1% *Bt* intoxicated diet, followed by diets with 0.05% and 0.02% *Bt*.

Among the transgenic lines tested, coccinellids were least affected when fed on BS5A.1(T2) 18-1P1 and BS5A.1(T2) 18-2P1 intoxicated leaf powder diets as compared to those fed on BS5A.2(T2) 19-2P1. The survival and development of the coccinellids was significantly reduced when fed on diet with 0.1% transgenic chickpea BS5A.1(T2) 18-2P1 leaf powder as compared to that on BS5A.2(T2) 19-2P1 during 2013-14.

The survival and development of coccinellids was reduced when fed on diets with 0.1% of BS5A.2 (T2) 19-3P1 and BS5A.2 (T2) 19-3P2 leaf powder, but not on diets with BS5A.1(T2) 18-2P1 leaf powder, the direct effects of transgenic chickpeas on survival and development of lady bird beetles were 0.02% < 0.05% < 0.1%.

In general, there were no significant effects on survival and development of coccinellid grubs when fed on aphids reared on diets with 0.02% and 0.1% leaf powder of transgenic chickpeas. The survival and development was slightly affected on diets with BS5A.2(T2) 19-3P2 leaf powder. The coccinellids fed on diets with 0.05% BS5A.2(T2) 19-3P1 leaf powder showed a marginal reduction in survival and development as compared to that on other transgenic lines during 2012-13.

The survival and development of coccinellids were slightly affected when fed on diets with BS5A.2(T2) 19-3P2 leaf powder as compared to that on other transgenic lines. In diets with 0.1%, the survival and development was affected adversely when the coccinellid grubs were fed on diets with BS5A.2(T2) 19-3P1 leaf powder during 2013-14.

The survival and development of coccinellid grubs were slightly affected when reared on aphids fed on diets with different concentrations (0.02%, 0.05% and 0.1%) of transgenic chickpea leaf powder.

From the present studies it can be concluded that

- The transgenic plants suffered significantly lower leaf damage as compared to the non-transgenic plants.
- The larval survival and weight gained by *H. armigera* larvae after 5 days was significantly reduced on transgenic lines as compared to that on non-transgenic chickpeas.
- Significantly higher grain yield was recorded in transgenic plants when infested with *H. armigera* as compared to non-transgenic chickpeas.
- The survival and development of *H. armigera* larvae was significantly lower on transgenic chickpea diets as compared to those reared on non-transgenic chickpea diets.
- The neonates reared on diets with BS5A.2(T2) 19-2P1 and BS5A.1(T2) 18-1P1 leaf powder showed maximum resistance to *H. armigera* and BS5A.1(T2) 18-1P1 showed high levels of resistance to third-instar larvae of *H. armigera*.

- The survival and development of *H. armigera* neonate larvae reared on diets with leaf powder of transgenic chickpea was very poor as compared to third-instar larvae.
- The amount of proteins, carbohydrates, lipids, phenols and tannins were non-significant between the transgenic and non-transgenic chickpea lines.
- The protein content was negatively correlated with larval survival, larval weight and leaf damage rating.
- Significantly higher amounts of oxalic acid were recorded in BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 than in BS5A.2(T2) 19-2P1. Highest malic acid content was recorded on BS5A.1(T2) 18-1P1 and lowest on BS5A.2(T2) 19-3P2.
- Chlorogenic acid, gentisic acid, ferulic acid, naringin, naringenin and quercetin had a positive but non-significant correlation with resistance to *H. armigera*.
- There was a positive and significant association between 3,4 dihydroxy flavone, genistein, formononetin and biochanin A with leaf damage, larval survival and larval weight.
- The amount of CryIIa protein was highest in the fresh leaf samples, followed by green pod wall, green seeds, dry pod wall, dry seeds and dry stems.
- The amount of CryIIa protein transferred from leaves to the non-target insects and natural enemies were negligible.
- A significant increase in egg+larval period, post embryonic development period, pupal period and reduction in cocoon formation, adult emergence, adult longevity, adult weights and fecundity of *C. chlorideae* when reared on *H. armigera* fed on fresh leaves of transgenic lines.
- The survival and development of parasitoids were affected when reared on *H. armigera* larvae fed on diets with transgenic BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-3P1 leaf powder as compared to that on other transgenics lines.
- No CryIIa protein was detected in the *C. chlorideae* larvae, the negative effects of transgenic chickpeas on survival and development of *C. chlorideae* were due to the early mortality of *H. armigera* as a result the parasitoids failed to complete the development on such larvae.

- The survival and development of *C. chlorideae* was poorer when reared on *H. armigera* larvae fed on fresh leaf samples than the artificial diets intoxicated with transgenic chickpea leaf powders.

- The direct effects on coccinellids were greater when fed on 0.1% *Bt* intoxicated diet, followed by diets with 0.05% and 0.02% transgenic leaf powder.

- The coccinellids fed on diets with 0.05% BS5A.2(T2) 19-3P1 leaf powder showed a marginal reduction in survival and development as compared to that on other transgenic lines.

- The survival and development of coccinellid grubs were slightly affected when reared on aphids fed on diets with different concentrations (0.02%, 0.05% and 0.1%) of transgenic chickpea leaf powder.

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