Evaluation of *Bt* cotton hybrids against *Earias vittella* (Fabricius) under laboratory conditions

BY

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CERTIFICATE-I

This is to certify that this thesis entitled, "**Evaluation of *Bt* cotton hybrids against *Earias vittella* (Fabricius) under laboratory conditions**" submitted for the degree of **Master of Science** in the subject of **Entomology** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms. Vadde Anoosha, Admn. No. 2010A31M,** under my supervision and that no part of the thesis has been submitted by her for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that this thesis entitled, "**Evaluation of *Bt* cotton hybrids against *Earias vittella* (Fabricius) under laboratory conditions**" submitted by **Ms. Vadde Anoosha, Admn. No, 2010A31M** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Entomology** has been approved by the Student's Advisory Committee after an oral examination on the same.

MAJOR ADVISOR

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Hisar **(Vadde Anoosha)**

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CHAPTER-I

INTRODUCTION

Cotton (*Gossypium* spp.) is an important commercial fibre crop in India. It ranks third in global cotton production after the United States and China; occupying an area of 9.0 m ha (25% of global area) with a production of 4.9 million bales (16% of global cotton production). Cotton provides livelihood to more than 60 million people in India by way of support in agriculture, processing and by its use in textiles and contributes around 30% to the gross domestic product of Indian agriculture.

Cotton being a long duration crop of hot and humid weather with succulent leaves and soft and nutritious looking fruiting bodies, it is attacked by around 1326 species of insects worldwide (Hargreaves, 1948). Among these, bollworms and sucking pests are important and have been reported to cause serious quantitative and qualitative losses (Dhaliwal *et al*., 2006). Cotton accounts for the consumption of 50% of total pesticide usage in the country worth about Rs. 1600 million (Dhaliwal *et al.,* 2004). Among the bollworms, American bollworm, *Helicoverpa armigera* (Hubner), Pink bollworm, *Pectinophora gossypiella* (Saunders) and Spotted bollworm *Earias* spp., collectively have a damage potential of 60 to 80% (Vennila *et al*., 2004). During the decade prior to 2002, cotton production in India was in constant crisis due to insecticide resistant bollworms.

Though cultural practices, resistant traits (Sadras, 1995) and beneficial fauna activity (Wilson *et al*., 1994) make it possible to reduce insect pest damage, yet the economic sustainability of the cotton crop is not realised.

The main thrust of Indian growers has so far been towards the repeated application of synthetic pesticides to control these pests. The three R's (Resistance, Resurgence and Residue) are the potential problems associated with indiscriminate use of insecticides in cotton agro-ecosystem and have made cotton cultivation non profitable. To reduce excessive and indiscriminate use of pesticides the scientists developed new technology of genetically modified plants approved by the Genetic Engineering Approval Committee, (GEAC) Govt. of India.

Transgenic *Bt* cotton technology is probably one of the most exciting advances in cotton pest management in recent times. This technology not only reduces pest damage but also increases yields (Qaim and Zilberman, 2003). *Bt* cotton is one of the first crop protection products obtained from biotechnological research. *Bt* cotton contains one or more foreign genes from the soil dwelling bacterium, *Bacillus thuringiensis*. It is a gram positive, aerobic, endospore forming soil bacterium. It produces proteinaceous crystalline parasporal body known as crystal during sporulation, which possesses insecticidal properties (Bulla *et al*., 1977).

The *Bt* cotton is under cultivation in India since 2002 whereas in Haryana it was introduced in the year 2005. Although in USA *Bt* cotton was released for commercial cultivation in 1996.

In 2010-11, the area under cotton cultivation was 111.42 lakh ha with production of 339 lakh bales in India which was estimated to reach 121.91 lakh ha with production of 345 lakh bales during 2011-12. In Haryana, during 2010-11 the area under cotton cultivation was 4.92 lakh ha with production of 14 lakh bales which was estimated to reach 6.05 lakh ha with the production of 17 lakh bales during 2011-12.

The primary pest of cotton crop, being targeted by transgenic technology in India, is the American bollworm, *Helicoverpa armigera* (Hubner) (Arms *et al*., 1992). But recently some other lepidopterous pests like *Spodoptera litura* (Fabricius) were found to be not affected by *Bt* toxin from cry1Ac gene. Therefore, another gene (cry2Ab) coding for a different toxin was introduced into current *Bt* cotton containing cry1Ac (Bollgard) to develop Bollgard II improving its efficacy against American bollworm and other lepidopteran pests (Stewart *et al*., 2001).

Spotted bollworm, *E. vittella* is an important component of the lepidopteran pest complex of cotton in India. It is generally an early to mid-season pest that causes damage by attacking tender terminal shoots, boring into the stem, feeding on squares and green bolls. As a result, the quality and quantity of cotton is adversely affected. The repeated applications of insecticides do not provide satisfactory control of this pest due to its internal feeding habit and overlapping generations during crop season.

Baseline toxicity of cry1A toxins on seven field populations of *E. vittella* revealed that almost all populations tested were extremely susceptible to all three cry1A toxins (Kranthi *et al*., 1999). Likewise, laboratory assays indicated that dual toxin *Bt* cultivars were more toxic to bollworms, *Helicoverpa zea*, fall armyworm, *Spodoptera frugiperda* and beet armyworm *Spodoptera exigua,* than single toxin cultivars (Stewart *et al.,* 2001).

Of late, the cultivation of *Bt* cotton, especially that of BGII is gained so much momentum that it has reached almost all the corners of the country & the same is true for the state of Haryana. However, information with respect to the age of the plant, up to which the endotoxin produced by *Bt* genes, remain effective is still not available as far as *E. vittella* is concerned. Keeping this in view, it was proposed to evaluate the effect of *Bt* and BGII on the development of *E. vittella* on some popular commercial hybrids with the following objectives:

1. To study the effect of squares and bolls of *Bt* cotton hybrids on the biological parameters of *E. vittella* at different intervals during the crop growth.
2. To study the effect of *Bt* cotton hybrids on the consumption-utilization indices of *E. vittella*.
3. To study the effect of *Bt* cotton hybrids on the ovipositional preference of *E. vittella.*

CHAPTER-II

REVIEW OF LITERATURE

Transgenic *Bt* cotton technology is one of the most exciting advances in cotton pest management. *Bacillus thuringiensis* (*Bt)* which is a soil bacterium produces a crystal protein (delta-endotoxin) known to be toxic to *Helicoverpa armigera*, *Pectinophora gossypiella*, *Earias vittella and Earias insulana* (Jeff Whitworth *et al*., 2010). The government of India permitted the commercialization of *Bt* cotton technology during 2002-03 crop season. Bollgard II cotton (Cry1Ac and Cry2Ab) was commercially released in India in 2005-06.

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The primary pest of cotton crop, being targeted by transgenic technology in India is the American bollworm, *Helicoverpa armigera* (Hubner) (Arms *et al*., 1992). But recently some other lepidopterous pest like *Spodoptera litura* (Fabricius) was found to be not affected by *Bt* toxin from Cry1Ac gene. Therefore, another gene (Cry2Ab) coding for a different toxin was introduced into current *Bt* cotton containing Cry1Ac (Bollgard) to develop Bollgard II, There by improving its efficacy against American bollworm and other lepidopteran pests (Stewart *et al*., 2001). Relevant literature has been reviewed and presented as under:

2.1 Effect of *Bt* cotton on biological parameters of bollworms

The performances of certain released *Bt* cotton hybrids *viz.,* MECH 162 *Bt*, MECH 184 *Bt* and RCH2 *Bt* under laboratory conditions were evaluated for their effect on *Earias vittella,* neonates and third instar larvae on squares and bolls (Kumar and Indirapriyadarshini, 2010). They reported that on square basis, at 65 DAS, RCH2 *Bt* recorded higher percentage of mortality (96%) followed by MECH 162 *Bt* (93.33%) and MECH 184 *Bt* (89.33%) as compared to their non *Bt* counterparts and check varieties MCU 7 and SVPR3. Both on square and boll basis, the highest mortality was recorded in RCH2 *Bt* (93.67 and 29.37%) followed by MECH 162 *Bt* and MECH 184 *Bt*.

The laboratory performances of certain released *Bt* cotton hybrids for their effect on *Spodoptera litura* were evaluated in comparison with non *Bt* cotton plant parts by Govindan *et al.* (2010). Four plant parts *viz.,* top fully opened young leaves, middle leaves, squares and young green bolls were tested, and it was observed that in RCH 2 the order of mortality was in the sequence of fully opened young leaves > squares > middle leaves > young green bolls when observed at 168 hours after the treatments.

Mahon and Olsen (2009) conducted the experiment on Bollgard II cotton along with conventional cotton against *Helicoverpa armigera* (Hubner) larvae and found that survival of larvae was limited on Bollgard II but larval survival increased as plant got aged. Larvae survived on Bollgard II grew more slowly and produced smaller pupae that yielded adults with reduced longevity and fecundity.

The dynamics of Cry1Ac expression in leaves was high followed by petals, sepals and squares. Further parts of fruiting structures *viz*., ovary, boll rind, and raw seed also have shown a significant expression (Santhosh, 2008). The bio-efficacy of pink bollworm neonate larvae fed with flowers was 72.15 and 60.70% at 70 and 85 DAS, respectively. The bioassay with tiny bolls indicated that mortality of PBW larvae declined from 89.53% at 100 DAS to 28.55% at 160 DAS. Decline in expression of Cry1Ac protein was evident through ELISA quantification and bioassay studies

Eleven genotypes including 5 *Bt* and their corresponding non *Bt* hybrids with one local variety HHH 233 of cotton were evaluated against *S. litura* for various biological parameters (larval weight, larval period, larval survival, pupal period, pupal weight, pupal survival and adult emergence) by Basavaraja *et al.* (2008a). There were no significant differences between biological parameters at 80,120 and 140 days of crop age as observed on *Bt* and non *Bt* hybrids. Basavaraja *et al.* (2008b) also evaluated these genotypes against *H. armigera*. There were significant differences between biological parameters at 65,95 and 105 days of crop age but there were no significant differences between biological parameters at 125 and 145 days of crop age.

The variation in the larval mortality to Cry 1Ac and Cry 1 Ac+Cry 2 Ab was evident through bioassay as the RCH-2 BG-II cotton genotype provided an additional advantage of controlling *S. litura* apart from enhanced mortality of other bollworms from 80 to 120 DAS. The larval mortality, on an average of *H. armigera* (94.38%), *E. vittella* (96.96%), *P. gossypiella* (88.92%) and *S. litura* (77.66%) was recorded in RCH-2 BG-II as compared to RCH-2 *Bt* (79.85, 86.77, 75.28 and 8.76 per cent, respectively) (Onkaramurthy, 2008).

Larvae of first four instars of *Trichoplusia ni* (Hubner) died when they were fed on the terminal leaves of BGII plants at 50 days after planting (DAP) (Li *et al.,* 2007). However, 51.3 per cent of fifth instar larvae that fed on 50 DAP *Bt* cotton leaves, pupated and 87.1 per cent of the pupae successfully developed into adults. Smaller pupae developed from larvae fed on BGII cotton. Further, it was concluded that leaves of 120 DAP *Bt* cotton were less toxic to *Trichoplusia ni* larvae. When the first instar larvae continuously fed on 120 DAP *Bt* cotton leaves, only 75.9, 60.6, 56.4, and 38.4 per cent of larvae survived to second, third, fourth, and fifth instar, respectively and 20.9 per cent pupated and 17.9 per cent successfully became adults. However, it took the surviving first instars 37.1 days to become adults, which was 7.2 and 8.9 days longer than those fed on 50 and 120 DAP non *Bt* respectively.

Mean mortality of *E. vittella* neonates to *Bt* cotton top leaves, middle leaves, squares and bolls were observed in the range of 93.38 to 96.66, 93.33 to 96.66, 83.33 to 93.33 and 76.66 to 80.00%, respectively (Shanmugam *et al.* 2006). The effectiveness of *Bt* cotton hybrids was more at 60 DAS, and decreased at 90 and 120 DAS. The order of susceptibility of different larval instars to *Bt* cotton hybrids was neonates > second instar > third instar. The efficacy of different plant parts was in the order of top leaves > middle leaves > squares > bolls.

In RCH-2 *Bt* with Cry1Ac, maximum mortality (>90%) of *H. armigera* and *E. vittella* from 60 to 80 DAS was noticed. Mortality of *P. gossypiella* larvae was 85.35 per cent at 80 DAS (Udikeri, 2006). Mortality of *E. vittella*, *H. armigera* and *P. gossypiella* neonates was maximum (>99%) between 40 and 80 DAS in RCH-2 BG-II with Cry1Ac+Cry2Ab genes and was effective against *S. litura*

Increased concentration of Cry1Ac and Cry 2 Ab in the diet *of Helicoverpa zea* (Boddie) and *Heliothis virescens* (Fabricius) resulted in high mortality and longer developmental period (Gore *et al.,* 2005). Cry1Ac toxin level decreased as the crop matured and was very low or undetectable in squares (Kranthi *et al.,* 2005). The laboratory study indicated that transgenic *Bt* cotton was highly toxic to *E. vittella* causing 100% mortality when larvae were fed on flower buds of *Bt* cotton (Abro *et al.,* 2004).

Chitkowski *et al.* (2003) reported that larval populations of *H. zea* and *S. frugiperda* were significantly lower in Bollgard II than in Bollgard (*Bt*) and conventional cotton and the mortality of both *S. frugiperda* and *S. exigua* was significantly greater on Bollgard II plant material.

Bioassay of transgenic *Bt* cotton against *H. armigera* under laboratory conditions revealed that *Bt* cotton hybrids *viz*., MECH 12 *Bt*, MECH 162 *Bt* and MECH 184 *Bt* were found more toxic to first instar larvae (92.8%) than third instar larvae (51.7%) when fed with leaves, squares and bolls Murugan *et al*., (2003).

When the lepidopteron larvae first fed on different parts of BGII cotton plant and then transferred to diet containing *Bt* toxin resulted in low mortality (Stewart *et al.,* 2001).

Laboratory assays of several lepidopteran larvae fed with fresh plant tissues indicated that dual toxin *Bt* cultivars expressing both Cry1Ac and Cry2Ab endotoxins of *Bt* were more toxic to bollworms, *Helicoverpa zea*, fall armyworm, *Spodoptera frugiperda* and beet armyworm *Spodoptera exigua,* than single toxin cultivars expressing Cry1Ac (Stewart *et al.,* 2001). In general Cry protein expression in bolls is lower than in squares or leaves (Greenplate e*t al.,* 1998, Adamzyck and Doulgas*,* 2001).

Baseline toxicity of Cry1A toxins on seven field populations of *E. vittella,* determined through log dose probit analysis, revealed that almost all populations tested were extremely susceptible to all the three Cry1A toxins. The LC_{50} obtained from the data were 0.59, 0.47 and 0.88 ng/sq cm, for Cry1Aa, Cry1Ab and Cry1Ac, respectively (Kranthi *et al.,* 1999).

There is a temporal and spatial variation in Cry1Ac protein levels in *Bt* cotton (Luttrell and Mink, 1999).

Survival and development of fall armyworm *S. furgiperda* on leaves and bolls of variety DP5415 and variety NuCOTN 33B were examined by Adamzyck *et al.* (1998) and reported non-significant differences in larval survival between the 2 varieties at 2,4,6,8,10 and 12 days after exposure and also no significant differences were observed between number of larvae that pupated and emerged as adult.

Bt cotton which express Cry 1 Ac caused 100% and 75 to 90% mortality in susceptible *H. virescens* and *H. zea* correspondingly (Mahaffey *et al*., 1995).

2.2 Effect of *Bt* cotton on Consumption – Utilization indices of larvae

Somashekara *et al.* (2011) evaluated laboratory performance of certain released *Bt* and BG-II cotton hybrids for their effect against food consumption utilization indices (CI, ECI, AD/AE, ECD) of *Earias vittella*. The *Bt* cotton hybrids were namely RCH-2 *Bt*, RCH-2 BG-II, Bunny *Bt* and Bunny BG-II and respective non *Bt* hybrids of RCH-2 and Bunny. There were significant differences between parameters observed on different *Bt* hybrids. The food utilization efficiency of *E. vittella* on squares of *Bt* cotton genotypes was greatly reduced compared to non *Bt* genotypes. The consumption index (CI) was maximum in conventional non *Bt* hybrids of Bunny (0.89) and RCH -2 (0.87). In *Bt* hybrids, CI was significantly less than non *Bt* hybrids i.e. RCH-2 (0.36) and Bunny (0.40). BG-II hybrids RCH-2 (0.17) and Bunny (0.19) recorded minimum CI. Similar trend was followed in growth rate (GR), efficiency of conversion of ingested food (ECI), approximate digestibility (AD) and efficiency of conversion of digested food (ECD).

Basavaraja *et al*. (2008b) studied the effect of *Bt* cotton fruiting bodies on consumption utilization indices of fourth instar larvae of *Helicoverpa armigera* (Hubner) under laboratory conditions and revealed that there was significant reduction in consumption index, growth rate, efficiency of conversion of ingested food, approximate digestibility and efficiency of conversion of digested food in *Bt* genotypes at 70 and 100 days of crop age, but not at 130 days.

Basavaraja (2007) studied various consumption-utilization indices of fourth instar larvae of *Spodoptera litura* (Fabricius) at an interval of 75, 105, 135 days of crop age on top and middle leaves of transgenic *Bt* cotton (Cry1Ac). There was no significant reduction in CI, GR, ECI, AD/AE and ECD of larvae on *Bt* genotypes. At 75 days of crop age the CI ranged from 1.29 to 1.39. GR ranged from 0.22 to 0.24 mg/day, ECI ranged from 17.49 to 19.60 per cent. AD/AE and ECD was ranged from 68.23 to 75.06 and 23.53 to 28.43 per cent, respectively on *Bt* and non *Bt* genotypes.

Chen FaJun *et al*. (2005) stated that the efficacy of conversion of ingested food and efficacy of digested food of bollworm were significantly reduced when fed with transgenic *Bt* cotton, but there was no significant CO_2 or CO_2 × cotton cultivar interaction. Approximate digestibility of larvae reared on transgenic cotton grown on elevated CO_2 was higher compared to that of larvae fed non-transgenic cotton grown at ambient CO_2.

Zhang *et al.* (2004) observed food consumption of neonates of *Helicoverpa armigera* (Hubner) in choice test with both transgenic and non-transgenic cotton leaves and reported that significantly greater proportion of larvae were observed with higher consumption on non-transgenic cotton than on transgenic *Bt* cotton.

Murugan *et al.* (2003) reported that the growth of fourth or fifth instar larvae of *H. armigera* on transgenic *Bt* cotton was severely hindered over 72 hours feeding on *Bt* cotton as compared to non *Bt* cotton as counterparts under laboratory conditions.

Gujar and Mohan (2000) reported that exposure of *H. armigera* to low doses of *Bt* toxin resulted in the prolonged larval and pupal periods and reduced the larval and pupal weight.

Berdegue *et al.* (1996) studied the effect of Cry1Ac toxin on feeding behavior of *Spodoptera exigua* (Hubner) in USA and revealed that the larvae significantly avoided the *Bt* treated diet in choice test and third instars consumed more non treated than treated diet.

2.3 Effect of *Bt* cotton on ovipositional preference of Bollworms

Basavaraja *et al*. (2012) examined the impact of transgenic cotton on ovipositional preference of *H. armigera* on twigs of *Bt* and non *Bt* genotypes bearing leaves, flowers, squares and bolls at 80 days of crop age under laboratory conditions and revealed that no significant variation was found between *Bt* and non *Bt* genotypes under choice conditions. The total number of eggs laid on *Bt* and non *Bt* hybrids ranged from 344 to 361 eggs/ 2 twigs/4 females

Mohite and Uthamasamy (2005) studied the ovipositional preference of *H. armigera* on wild species of *Gossypium* under laboratory conditions and revealed that wild species of *Gossypium* were less preferred for oviposition compared to checks under choice situation. Under no choice test conditions, relatively more eggs were laid per plant on the different wild genotypes than under choice conditions.

Vennila *et al*. (2004) stated that there was no particular pattern of egg laying by *H. armigera* between *Bt* and non *Bt* hybrids under field conditions.

Ghuguskar *et al.* (2001) conducted the field evaluation of *hirsutum* and *arboreum* cotton genotypes for ovipositional preference of *H. armigera* were conducted separately at regional station, NARP, Yavatonal. Among hirsutum genotypes AKA 9131 and AKH 9119 recorded least number of eggs followed by AKH8263, AKH8931, AKH8362 and AKH8708. Among arboretum hybrids, AKDH 3 recorded significantly less number of eggs than both checks AKA8401 and AKA 5.

CHAPTER-III

MATERIAL AND METHODS

Six cotton hybrids as mentioned below were evaluated against *Earias vittella* (Fabricius) in the present investigations.

Various materials and methods used are described as under

3.1 Cotton hybrids used for evaluation

Non *Bt*	**Bollgard I**	**Bollgard II**
BIO 6488 non *Bt*	BIO 6488 *Bt*	BIO 6488 BGII
RCH 134 non *Bt*	RCH 134 *Bt*	RCH 134 BGII

All the hybrids were sown in the 2nd fortnight of May 2011 by dibbling method keeping spacing of 67.5 cm x 60 cm (plant to plant, row to row) at the Research Farm, Department of Entomology, CCS Haryana Agricultural University, Hisar, India. The experiment was laid out in randomized block design (RBD), replicated thrice with a plot size of 7m x 4m. Thinning of plants was undertaken after one month of sowing to maintain proper plant population. All other cultural practices like fertilizer application, weeding, hoeing, irrigation, etc., were adopted as per the recommendations made in Package and Practice of Kharif crops (Anonymous, 2011).

3.2 Rearing of spotted bollworm, *Earias vittella* (Fabricius) under laboratory conditions

Larvae of *E. vittella* were collected from okra fields of the Research Farm of CCS Haryana Agricultural University, Hisar, India, for mass multiplication. Larvae were reared in glass jars (20 cm x 15 cm) on okra fruits. Pupae were transferred into glass jars for adult emergence. Adults emerging from such pupae were transferred to separate jars for egg laying. Cotton swabs, dipped in 16 per cent sugar solution were provided as food for adults. Zig zag folded paper strips were provided as oviposition substrate. Neonates of *E. vittella* larvae were transferred to young okra fruits with the help of wet camel hair brush and kept in petri plates (10 cm diameter) and the food was changed on alternate days. Like this *E. vittella* mass culture was maintained and used for various experiments.

3.3 Effect of transgenic *Bt* cotton squares and bolls of plants of different ages on some biological parameters of *Earias vittella* (Fabricius) larvae

In this experiment studies on the larval survival (%) of *E. vittella* on squares and bolls of *Bt* and non *Bt* hybrids and other biological parameters *viz*., larval period (days), larval weight (mg), pupal period (days), pupal weight (mg) and adult emergence (%) were undertaken.

For working out larval survival, two plant parts *viz*; squares and bolls of *Bt* and non *Bt* hybrids of cotton, were collected randomly, from crops at 60, 90, 105 and 125 days after sowing and brought to the laboratory. One (square/boll) each were put in separate petri plates for feeding to first instar larvae of *E. vittella* (10 larvae/plant part/hybrid) in a thrice replicated experiment, for five days. The food was changed on alternate days.

Likewise studies on the effect of *Bt* and non *Bt* hybrids of cotton on other biological parameters of *E. vittella* were conducted by using third instar larvae as test insect. Squares and bolls of crops of 60, 90, 105 and 125 days after sowing were collected from the respective plots and each of these were kept in Petri plates. In this experiment each replication consisted of 10 larvae.

The larval period was worked out by adding the larval period of first two instars to the period when larvae moulted to enter fourth instar. Further, larval weight (mg), larval survival (%), pupal weight (mg), pupal period (days) and adult emergence (%) were also worked out.

3.4 Effect of transgenic *Bt* cotton squares and bolls of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

The experiment was conducted in battery jars (20 cm x 15 cm) in which the respective foods (squares/bolls) were kept. Two plant parts *viz*., squares and bolls, collected from 70, 100 and 120 days after sowing of crop, were fed to the fourth instar larvae of *E. vittella*.

The experiment was replicated three times. In this experiment each replication consisted of 10 larvae. These larvae were starved for about four hours and weighed individually before releasing them on the squares and bolls in the battery jars.

The fresh weight of the food in each jar was also recorded before the larvae were released on it. After 48 hours uneaten food material, larvae and faecal matter were separated and weighed. The weight gained (mg) by the larvae after feeding and mean weight (mg) of larvae during feeding period (mg) were also worked out.

The data so generated was utilized to compute consumption-utilization indices *viz*., Consumption Index (CI), Growth Rate (GR), Efficiency of Conversion of Ingested food (ECI), Approximate Digestibility/Assimilation Efficiency (AD/AE) and Efficiency of Conversion of Digested food (ECD) with the help of following formulae (Waldbauer 1968).

Formulae used

Consumption Index (CI)

Growth Rate (GR)

Efficiency of Conversion of Ingested food (ECI)

Approximate Digestibility/ Assimilation Efficiency (AD/AE)

Efficiency of Conversion of Digested food

3.5 Ovipositional preference of *Earias vittella* (Fabricius) on different parts of *Bt* cotton plants of age 80 days after sowing

The experiment was conducted in a wooden cage (40cm x 40 cm x 40 cm) with wire mesh sides. About 45 cm twigs bearing leaves, squares, flowers and bolls were plucked from the respective plants of age 80 DAS and brought to laboratory. One such twig, the cut end of which was dipped in water, was placed in every cage. In each cage four pairs of newly emerged adults in 1:1 ratio were released for 2 days. The experiment was replicated 3 times. Cotton swab, dipped in 16 per cent sugar solution was provided as food for adults. The observations on the number of eggs laid on different parts of twig were recorded as per the method adopted by Sharma and Agarwal (1983).

CHAPTER-IV

RESULTS

Evaluation of *Bt* cotton hybrids against spotted bollworm, *Earias vittella* (Fabricius) was conducted at CCS Haryana Agricultural University, Hisar, India. The research was carried out under laboratory conditions. Results obtained from various experiments are presented here in this chapter.

4.1 Effect of transgenic *Bt* cotton squares and bolls of plants of different ages on some biological parameters of *Earias vittella* (Fabricius) larvae

4.1.1 Effect of transgenic *Bt* cotton squares of plants of different ages on larval survival (first instar) of *Earias vittella* (Fabricius)

The data pertaining to this experiment, carried out on the survival of first instar larvae of *E. vittella* on squares (Table 1), revealed that the larval survival varied significantly among the *Bt* and non-*Bt* hybrids.

The larval survival on squares of crop of age 60 days after sowing ranged from 0.00 to 1.33 per cent on *Bt* and BGII hybrids and 93.33 to 96.66 per cent on non *Bt* hybrids (Table 1). Among the hybrids none of the larvae survived on RCH 134 BGII and BIO 6488 BGII. On *Bt* hybrids the larval survival was significantly less than on non-*Bt* hybrids i.e. RCH 134 (0.66%) followed by BIO 6488 (1.33%). Larval survival on BGII hybrids BIO 6488 and RCH 134 was recorded to be the minimum; however the differences between *Bt* and BGII hybrids were statistically at par with each other. At 90 days after sowing of crop, results followed similar trends as in case of 60 DAS of crop. Lowest larval survival (1.33%) was recorded on BIO 6488 BGII hybrid followed by RCH 134 BGII (1.66%), while the highest larval survival (96.66%) was recorded on BIO 6488 non-*Bt*. RCH 134 BGII and BIO 6488 BGII were statistically at par with each other. The larval survival was maximum on non-*Bt* hybrids of BIO 6488 (96.66%) and RCH 134 (93.33%). On *Bt* hybrids the larval survival was significantly less than non-*Bt* hybrids i.e. RCH 134 (2.33%) followed by BIO 6488 (2.66%) and were statistically at par with each other. There was no significant difference between *Bt* and BGII hybrids.

At 105 days after sowing of crop the lowest larval survival (6.66%) was recorded on both BGII hybrids RCH 134 and BIO 6488 (Table 1) and there were statistically at par with each other, while highest larval survival (83.33%) was recorded on BIO 6488 non-*Bt*. In *Bt* hybrids the larval survival was significantly less

Table 1: Effect of *Bt* cotton squares and bolls of plants of different ages on larval survival (first instar) of *Earias vittella* (Fabricius)

Hybrids	Survival (%) on squares				Survival (%) on bolls			
	60 DAS*	**90 DAS**	**105 DAS**	**125 DAS**	**60 DAS**	**90 DAS**	**105 DAS**	**125 DAS**
RCH 134 *Bt*	0.66(5.73)**	2.33(8.90)	16.66(24.24)	46.66(43.06)	0.33(5.04)	1.66(4.05)	6.66(13.95)	40.00(39.13)
RCH 134 BGII	0.00(4.05)	1.66(7.22)	6.66(11.67)	30.00(32.98)	0.00(4.05)	1.00(9.00)	3.33(9.00)	23.33(28.76)
RCH 134 non *Bt*	96.66 (81.28)	93.33(76.65)	80.00(64.28)	73.33(59.18)	86.66(69.26)	83.33(81.28)	86.66(69.26)	80.00(63.90)
BIO 6488 *Bt*	1.33(6.78)	2.66(9.46	10.00(16.62)	33.33(35.20)	0.66(5.73)	2.00(4.05)	6.66(13.95)	23.33(28.76)
BIO 6488 BG II	0.00(4.05)	1.33(6.78)	6.66(13.95)	26.66(30.98)	0.00(4.05)	0.66(9.00)	3.33(9.00)	16.66(23.84)
BIO 6488 non *Bt*	93.33(78.53)	96.66(81.28)	83.30(67.03)	66.66(54.76)	86.66(69.26)	86.66(76.65)	86.66(69.26)	80.00(63.90)
SEm(±)	(3.79)	(3.55)	(5.44)	(3.13)	(1.77)	(3.91)	(4.34)	(3.296)
CD(p=0.05)	(11.80)	(11.07)	(16.96)	(9.76)	(5.53)	(12.19)	(13.52)	(10.27)

*DAS-days after sowing.
**Figures in parentheses are n+0.5 arc sine values.

than non-*Bt* hybrids i.e. BIO 6488 (10.00%) followed by RCH 134 (16.66%) and were statistically at par with each other. There was no significant difference between *Bt* and BGII hybrids. At 125 days after sowing of crop larval survival ranged from 66.66 to 73.33 per cent on non-*Bt*, 33.33 to 46.66 per cent on *Bt* hybrids and 26.66 to 30.00 per cent on BGII hybrids. There was no significant difference between *Bt* and BGII hybrids. The lowest larval survival (26.66%) was on BIO 6488 BGII, while highest larval survival (73.33%) was recorded on RCH 134 non-*Bt*.

4.1.2 Effect of transgenic *Bt* cotton bolls of plants of different ages on larval survival (first instar) of *Earias vittella* (Fabricius)

The data with respect to this experiment carried out on the survival of first instar larvae of *E. vittella* on bolls revealed that the larval survival varied significantly among the hybrids (Table 1).

At 60 days after sowing of crop (Table 1) the larval survival ranged from 0.00 to 0.66 per cent on *Bt* and BGII hybrids and 86.66 per cent on non *Bt* hybrids. Among the hybrids the lowest survival (0.00%) was recorded on RCH 134 BGII and BIO 6488 BGII. In *Bt* hybrids the larval survival was significantly less than non-*Bt* hybrids i.e. RCH 134 (0.33%) followed by BIO 6488 (0.66%). There was no significant difference between *Bt* and BGII hybrids. At 90 DAS lowest larval survival (0.66%) was recorded on BIO 6488 BGII followed by RCH 134 BGII (1.00%), while highest larval survival (86.66%) was recorded on BIO 6488 non-*Bt*. Larval survival on RCH 134 BGII and BIO 6488 BGII were statistically at par with each other. The larval survival was maximum on non-*Bt* hybrids of BIO 6488 (86.66%) and RCH 134 (83.33%). On *Bt* hybrids the larval survival was significantly less than non-*Bt* hybrids i.e. RCH 134 (1.66%) followed by BIO 6488 (2.00%) and were statistically at par with each other. There was no significant difference between the larval survival on *Bt* and BGII hybrids.

At 105 DAS (Table 1) the lowest larval survival (3.33%) was recorded on both BGII hybrids of RCH 134 and BIO 6488 was recorded and these were statistically at par with each other, while highest larval survival (86.66%) was recorded on BIO 6488 non-*Bt*. The larval survival was maximum on non-*Bt* hybrids of BIO 6488 and RCH 134 (86.66%). On *Bt* hybrids the larval survival was significantly less than non-*Bt* hybrids i.e. BIO 6488 and RCH 134 (6.66%) and these were statistically at par with each other. There was no significant difference between *Bt* and BGII hybrids. At 125 DAS, larval survival was 80.00 per cent on both non-*Bt*, 23.33 to 40.00 per cent in *Bt* hybrids and 16.66-23.33 per cent on BGII hybrids. There was significant difference between *Bt* and BGII hybrids. The lowest larval survival (16.66%) was recorded on BIO 6488 BGII, while highest larval survival (80.00%) was recorded on RCH 134 non-*Bt* and BIO 6488 non-*Bt*.

4.1.3 Effect of transgenic *Bt* cotton squares of plants of different ages on some biological parameters of third instar larvae of *Earias vittella* (Fabricius)

At 60 days after sowing of crop the larval period on squares ranged from 12.50 to 14.16 days on *Bt* and BGII hybrids and 8.50 to 9.16 days on non *Bt* hybrids (Table 2). On *Bt* hybrids the larval period was significantly more than non-*Bt* hybrids i.e. RCH 134 (12.50 days) followed by BIO 6488 (13.50 days). Maximum larval period was recorded on BGII hybrids RCH 134 (13.16 days) and BIO 6488 (14.16 days). Larval weight ranged from 29.32 to 35.41 mg on *Bt* and BGII hybrids and 62.32 to 63.12 mg on non-*Bt* hybrids. Among the hybrids the minimum larval weight (29.32 mg) was recorded on BIO 6488 *Bt*, while maximum (63.12 mg) on RCH 134 non-*Bt* hybrid. Larval survival ranged from 39.68 to 40.01 per cent on *Bt* and BGII hybrids and 73.32 to 76.68 per cent on non-*Bt* hybrids. Lowest larval survival (39.68%) was recorded on RCH 134 BGII followed by BIO 6488 BGII (40.01%), while highest (76.68%) on BIO 6488 non-*Bt* followed by (73.32%) on RCH 134 non-*Bt* hybrids.

Pupal period ranged from 11.33 to 13.33 days on *Bt* and BGII hybrids and 7.66 to 8.33 days on non-*Bt* hybrids (Table 3). Among the hybrids the maximum pupal period (13.33 days) was recorded on BGII hybrids of BIO 6488 and RCH 134, while minimum (7.66 days) on RCH 134 non-*Bt* followed by BIO 6488 non-*Bt* hybrids(8.33 days). The pupal period was significantly more on *Bt* hybrids as compared to on non-*Bt* hybrids. Pupal weight ranged from 23.53 to 28.29 mg on *Bt* and BGII hybrids and 50.29 to 54.31 mg on non-*Bt* hybrids. Among the hybrids, the minimum pupal weight (23.53 mg) was recorded on BIO 6488 *Bt,* while maximum (54.31 mg) on RCH 134 non-*Bt* hybrid. Adult emergence ranged from 23.33 to 26.68 per cent on *Bt* and BGII hybrids and 56.68 to 63.32 per cent on non-*Bt* hybrids. The lowest adult emergence (23.33%) was recorded on BIO 6488 *Bt,* while highest (63.32%) on BIO 6488 non-*Bt* hybrid.

At 90 days after sowing of crop, the variations on various biological parameters *viz.,* larval period (8.16-15.16 days), larval weight (30.33-63.43 mg), larval survival (36.67-73.33 %), pupal period (8.5-12.5 days), pupal weight (25.65-53.32 mg) and adult emergence (26.68-60.00%) on different *Bt*, BGII and non-*Bt* hybrids varied significantly (Table2,3). The maximum larval (15.16 days) and pupal period (12.5 days) were recorded on *Bt* hybrids. The minimum larval weight (30.33 mg) and pupal weight (25.65 mg) were recorded on *Bt* hybrids. The lowest larval

Table 2: **Effect of transgenic *Bt* cotton squares of plants of different ages on some biological parameters of third instar larvae of *Earias vittella* (Fabricius)**

Hybrids												
	Larval period (days)				**Larval weight (mg)**				**Larval survival (%)**			
	60 DAS*	**90 DAS**	**105 DAS**	**125 DAS**	**60 DAS**	**90 DAS**	**105 DAS**	**125 DAS**	**60 DAS**	**90 DAS**	**105 DAS**	**125 DAS**
RCH 134 *Bt*	12.50	14.66	14.33	10.66	32.53	33.52	34.49	35.62	43.33(41.15)**	46.68(43.08)	53.32(46.89)	73.32(58.88)
RCH 134 BGII	13.16	13.66	15.33	12.00	35.41	36.38	37.48	39.23	39.68(37.26)	36.68(37.26)	50.01(44.99)	70.00(56.77)
RCH 134 non *Bt*	8.50	8.16	8.66	9.66	63.12	64.12	65.66	65.62	73.32(58.88)	73.33(58.89)	73.32(58.88)	76.68(61.10)
BIO 6488 *Bt*	13.50	15.16	14.66	10.33	29.32	30.33	32.64	33.33	43.33(41.15)	43.33(41.15)	50.01(44.99)	70.01(56.77)
BIO 6488 BGII	14.16	14.00	12.66	11.33	30.51	31.62	33.48	33.67	40.01(39.22)	36.67(37.26)	46.67(43.07)	73.32(58.88)
BIO 6488 non *Bt*	9.16	8.66	8.33	8.16	62.32	63.43	64.22	65.32	76.68(61.10)	73.33(58.88)	76.68(61.10)	76.68(61.10)
SEm(±)	0.57	1.03	1.02	0.74	0.03	0.03	0.03	0.03	(0.03)	(0.02)	(0.03)	(0.03)
CD(p=0.05)	1.79	3.21	3.20	2.31	0.12	0.09	0.10	0.10	(0.06)	(0.06)	(0.08)	(0.08)

*DAS - days after sowing.
**Figures in parentheses are arc sine values.

Table 3: Effect of transgenic *Bt* cotton squares of plants of different ages on some biological parameters of *Earias vittella* (Fabricius) (pupae and adult)

Hybrids	Pupal period (days)				Pupal weight (mg)				Adult emergence (%)			
	60 DAS*	90 DAS	105 DAS	125 DAS	60 DAS	90 DAS	105 DAS	125 DAS	60 DAS	90 DAS	105 DAS	125 DAS
RCH 134 *Bt*	11.33	11.83	12.33	8.83	28.29	29.42	30.13	31.32	26.68(31.09)**	30.01(33.20)	33.33(35.25)	60.01(50.75)
RCH 134 BGII	13.33	11.66	12.16	9.16	26.91	30.34	31.23	33.66	26.66(31.08)	26.68(31.09)	26.67(31.09)	56.68(48.82)
RCH 134 non *Bt*	7.66	8.66	8.16	8.33	54.31	53.32	55.12	56.55	56.68(48.82)	53.33(46.89)	56.67(48.82)	63.32(52.70)
BIO 6488 *Bt*	12.66	12.16	10.33	8.66	23.53	25.65	26.33	27.32	23.33(28.87)	30.00(33.20)	26.68(31.09)	60.06(50.75)
BIO 6488 BGII	13.33	12.5	11.5	8.83	24.91	25.96	26.78	27.36	26.68(31.09)	26.68(31.09)	26.68(31.09)	66.68(54.72)
BIO 6488 non *Bt*	8.33	8.50	8.33	7.83	50.29	52.55	53.66	54.21	63.32(52.71)	60.00(50.75)	63.32(52.71)	66.67(54.72)
SEm(±)	0.70	0.89	0.91	0.76	0.03	0.02	0.03	0.03	(0.02)	(0.02)	(0.02)	(0.02)
CD(p=0.05)	2.20	2.78	2.86	N.S.	0.10	0.09	0.10	0.10	(0.07)	(0.07)	(0.07)	(0.07)

* DAS – days after sowing.
**Figures in parentheses are arc sine values.

survival (36.67 %) and adult emergence (26.68 %) were recorded on *Bt* hybrids. The trend was similar to that of 60 days after sowing (Table2, 3).

At 105 days after sowing of crop variations in various biological parameters *viz*., larval period (8.33-15.33 days), larval weight (32.64-65.66 mg), larval survival (46.67-76.68 %), pupal period (8.16-12.33 days), pupal weight (26.33-55.12 mg) and adult emergence (26.67-63.32 %) on different *Bt*, BGII and non-*Bt* hybrids was similar to that of crop at 90 days after sowing (Table2, 3). At 125 days after sowing of crop variations in various biological parameters *viz*., larval period (8.16-11.33 days), larval weight (33.33-65.62 mg), larval survival (70.00-76.68 %), pupal period (7.83-9.16 days), pupal weight (27.32-56.55 mg) and adult emergence (56.68-66.68 %) on different *Bt*, BGII and non-*Bt* hybrids were not as high as observed from squares from plants 105 days after sowing.

4.1.4 Effect of transgenic *Bt* cotton bolls of plants of different ages on some biological parameters of third instar larvae of *Earias vittella* (Fabricius)

At 60 days of crop age the larval period on bolls ranged from 11.50 to 14.33 days on *Bt* and BGII hybrids and 7.66 to 8.16 days on non *Bt* hybrids (Table 4). The larval period was significantly more on *Bt* hybrids than on non-*Bt* hybrids i.e. RCH 134 (11.5 days) followed by BIO 6488 (11.83 days). Maximum larval period was recorded on BGII hybrids of RCH 134 (12.00 days) and BIO 6488 (14.33 days). Larval weight ranged from 31.66 to 38.67 mg on *Bt* and BGII hybrids and 67.12 to 67.66 mg on non-*Bt* hybrids. Lower larval weight was recorded on BIO 6488 *Bt* (31.66 mg) followed by BIO 6488 BGII (32.31 mg), RCH 134 *Bt* (36.57 mg) and RCH 134 BGII (38.67 mg), respectively. Larval survival ranged from 36.67 to 46.68 per cent on *Bt* and BGII hybrids and 73.34 to 76.68 per cent on non-*Bt* hybrids. Lowest larval survival (36.67 %) was recorded on BIO 6488 BGII followed by RCH 134 BGII (36.68 %).

Pupal period ranged from 10.33 to 12.83 days on *Bt* and BGII hybrids and 6.83 to 8.00 days on non *Bt* hybrids (Table 5). The pupal period was significantly more on *Bt* hybrids than on non-*Bt* hybrids. Maximum pupal period was recorded on BGII hybrids RCH 134(12.33 days) and BIO 6488 (12.83 days). Pupal weight ranged from 26.21 to 30.30 mg on *Bt* and BGII hybrids and 56.31 to 58.40 mg on non-*Bt* hybrids. Lower pupal weight was noticed on BIO 6488 *Bt* (26.21 mg) followed by BIO 6488 BGII (28.40 mg), RCH 134 *Bt* (30.10 mg) and RCH 134 BGII (30.30 mg), respectively. Adult emergence ranged from 23.34 to 33.32 per cent on *Bt* and BGII hybrids and 59.99 to 60.01 per cent on non-*Bt* hybrids. The lowest adult emergence (23.34%) was recorded on BIO 6488 *Bt*, while highest (60.01%) on RCH 134 non-*Bt* hybrid.

Table 4: Effect of transgenic *Bt* cotton bolls of plants of different ages on some biological parameters of third instar larvae of *Earias vittella* (Fabricius)

Hybrids	Larval period (days)				Larval weight (mg)				Larval survival (%)			
	60 DAS*	90 DAS	105 DAS	125 DAS	60 DAS	90 DAS	105 DAS	125 DAS	60 DAS	90 DAS	105 DAS	125 DAS
RCH 134 *Bt*	11.5	13.66	13.66	9.33	36.57	27.72	34.80	54.32	46.68(43.08)**	43.34(41.16)	56.66(44.81)	73.31(58.87)
RCH 134 BGII	12.00	13.00	14.33	11.66	38.67	31.90	39.16	54.52	36.68(37.26)	40.00(39.22)	50.00(44.99)	53.33(45.71)
RCH 134 non *Bt*	7.66	7.33	7.33	8.66	67.12	50.12	55.32	56.79	76.68(61.10)	76.66(61.09)	76.67(61.10)	76.65(61.08)
BIO 6488 *Bt*	11.83	15.16	11.33	9.33	31.66	33.53	33.69	51.90	39.80(39.10)	46.68(43.08)	56.64(48.80)	73.33(58.89)
BIO 6488 BGII	14.33	13.00	13.66	12.00	32.31	27.26	33.46	52.91	36.67(37.26)	43.33(41.15)	53.33(46.89)	76.67(61.10)
BIO 6488 non *Bt*	8.16	7.50	7.50	7.33	67.66	51.27	55.00	55.02	73.34(58.89)	80.00(63.41)	83.33(65.88)	76.67(61.09)
SEm(±)	1.05	0.07	0.81	1.07	0.03	0.07	0.04	0.04	(0.05)	(0.03)	(0.03)	(6.29)
CD(p=0.05)	3.29	0.21	2.53	N.S.	0.12	0.21	0.12	0.15	(0.16)	(0.09)	(0.08)	(N.S.)

*DAS – days after sowing.
* *Figures in parentheses are arc sine values.

Table 5: Effect of transgenic *Bt* cotton bolls of plants of different ages on some biological parameters of *Earias vittella* (Fabricius) (pupae and adult)

Hybrids	Pupal period (days)				Pupal weight (mg)				Adult emergence (%)			
	60 DAS*	90 DAS	105 DAS	125 DAS	60 DAS	90 DAS	105 DAS	125 DAS	60 DAS	90 DAS	105 DAS	125 DAS
RCH 134 *Bt*	10.33	10.83	12.33	7.66	30.10	20.10	24.30	42.8	33.32(35.24)**	33.33(35.24)	46.66(43.06)	66.65(54.70)
RCH 134 BGII	12.33	10.33	11.66	8.66	30.30	21.20	26.21	43.10	26.68(31.08)	23.34(28.87)	36.67(37.25)	64.33(53.31)
RCH 134 non *Bt*	6.83	7.50	7.00	7.66	58.40	45.80	41.10	46.21	60.01(50.75)	60.00(50.74)	70.00(56.76)	73.32(58.88)
BIO 6488 *Bt*	12.00	12.00	9.33	7.33	26.21	26.50	25.99	40.30	23.34(28.87)	33.33(35.24)	43.32(41.14)	66.66(54.71)
BIO 6488 BGII	12.83	12.33	11.33	7.66	28.40	20.30	25.20	42.00	26.65(31.06)	33.33(35.25)	40.00(39.21)	69.99(56.76)
BIO 6488 non *Bt*	8.00	7.33	6.83	6.50	56.31	47.60	43.40	45.89	59.99(50.74)	63.34(52.71)	66.66(54.71)	70.00(56.76)
SEm(±)	0.87	1.12	0.99	0.77	0.04	0.04	0.04	0.03	(0.03)	(0.02)	(0.02)	(0.24)
CD(p=0.05)	2.73	3.50	3.10	N.S.	0.13	0.12	0.13	0.10	(0.09)	(0.08)	(0.08)	(0.76)

*DAS – days after sowing.
**Figures in parentheses are arc sine values.

At 90 days after sowing, variations on various biological parameters *viz.,* larval period (7.33-15.16 days), larval weight (27.26-51.27 mg), larval survival (40.00-80.00 %), pupal period (7.33-12.33 days), pupal weight (20.10-47.60 mg) and adult emergence (23.34-63.34 %) on different *Bt*, BGII and non-*Bt* hybrids varied significantly. The maximum larval (15.16 days) and pupal (12.33 days) periods were recorded on *Bt* hybrids. The minimum larval (27.26 mg) and pupal (20.10 mg) weights were recorded on non-*Bt* hybrids. The lowest larval survival (40.00 %) and adult emergence (23.34 %) were recorded on *Bt* hybrids similar to that of 60 days after sowing (Table 4, 5).

At 105 days after sowing, variations on various biological parameters *viz*., larval period (7.33-14.33 days), larval weight (33.6-55.32 mg), larval survival (50.00-83.33 %), pupal period (6.83-12.33 days), pupal weight (24.30-43.40 mg) and adult emergence (36.67-70.00 %) on different *Bt,* BGII and non-*Bt* hybrids were similar to that of crop 90 days after sowing (Table 4, 5). At 125 days after sowing variations on various biological parameters *viz*., larval period (87.33-12.00 days), larval weight (51.90-56.79 mg), larval survival (73.31-76.67 %), pupal period (6.5-8.66 days), pupal weight (40.30-46.21 mg) and adult emergence (64.33-73.32 %) on different *Bt,* BGII and non-*Bt* hybrids were statistically significant.

4.2 Effect of transgenic *Bt* cotton plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

4.2.1 Effect of transgenic *Bt* cotton squares of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

The data of the experiment carried out on consumption utilization indices of *E. vittella* fourth instar larvae on squares of *Bt*, BGII and non-*Bt* hybrids are presented in Table 6 and 7.

At 70 days after sowing of crop the consumption index (CI) of larvae ranged from 0.13 to 0.35 on *Bt* and BGII hybrids and 0.81 to 0.83 on non *Bt* hybrids (Table 6). The consumption index (CI) was maximum on non-*Bt* hybrids RCH 134 (0.83) and BIO 6488 (0.81). On *Bt* hybrids the CI was significantly less than non-*Bt* hybrids. BGII hybrids BIO 6488 (0.13) and RCH 134 (0.14) recorded minimum CI. Growth rate of larvae ranged from 0.11 to 0.18 mg/day on *Bt* and BGII hybrids and 0.22 to 0.25 mg/day on non-*Bt* hybrids. Among the hybrids the lowest growth rate (0.11 mg/day) was recorded on RCH 134 BGII, while highest (0.25 mg/day) on RCH 134 non-*Bt*. Lower growth rate was noticed on RCH 134 BGII (0.11 mg/day) followed by BIO 6488 BGII (0.13 mg/day), RCH 134 *Bt* (0.15 mg/day) and BIO 6488 *Bt* (0.18 mg/day) respectively.

Table 6: Effect of transgenic *Bt* cotton squares of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

Hybrids	Consumption- Index (CI)			Growth Rate (GR) (mg/day)		
	70 DAS*	100 DAS	120 DAS	70 DAS	100 DAS	120 DAS
RCH 134 *Bt*	0.34	0.33	0.46	0.15	0.16	0.18
RCH 134 BGII	0.14	0.15	0.21	0.11	0.12	0.12
RCH 134 non *Bt*	0.83	0.82	0.84	0.25	0.25	0.25
BIO 6488 *Bt*	0.35	0.36	0.48	0.18	0.19	0.20
BIO 6488 BGII	0.13	0.14	0.23	0.13	0.13	0.13
BIO 6488 non *Bt*	0.81	0.8	0.83	0.22	0.21	0.21
SEm(±)	0.01	0.02	0.03	0.01	0.02	0.03
CD(p=0.05)	0.04	0.06	0.11	0.03	0.07	N.S.

*DAS- days after sowing

Table 7: Effect of transgenic *Bt* cotton squares of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

Hybrids	ECI (%)			AD/AE (%)			ECD (%)		
	70 DAS*	100 DAS	120 DAS	70 DAS	100 DAS	120 DAS	70 DAS	100 DAS	120 DAS
RCH 134 *Bt*	18.13(25.19)**	18.36(25.37)	22.45(28.28)	38.32(38.23)	38.78(38.50)	39.22(38.76)	28.43(32.21)	28.36(32.16)	29.32(32.77)
RCH 134 BGII	11.93(20.20)	12.02(20.28)	14.19(22.12)	22.43(28.26)	23.05(28.68)	25.68(30.04)	13.76(21.77)	13.57(21.61)	13.69(21.71)
RCH 134 non *Bt*	30.36(33.42)	31.40(34.07)	30.34(33.41)	58.66(49.97)	59.09(50.22)	60.22(50.88)	55.36(48.06)	55.19(47.96)	55.70(48.26)
BIO 6488 *Bt*	18.86(25.73)	18.98(25.82)	19.03(25.85)	39.41(38.87)	39.93(39.18)	39.78(39.09)	28.36(32.16)	27.70(31.75)	28.08(31.99)
BIO 6488 BGII	13.18(21.28)	12.98(21.11)	12.26(20.49)	23.52(29.00)	25.12(30.07)	26.99(31.29)	15.14(22.89)	15.27(23.00)	15.37(23.07)
BIO 6488 non *Bt*	32.13(34.52)	33.24(35.20)	30.08(33.25)	59.13(50.24)	59.35(50.37)	59.98(50.74)	55.72(48.27)	68.21(55.66)	68.81(56.03)
SEm(±)	(0.02)	(0.03)	(0.04)	(0.02)	(0.02)	(0.16)	(0.03)	(0.03)	(0.12)
CD(p=0.05)	(0.05)	(0.09)	(0.12)	(0.07)	(0.07)	(0.49)	(0.08)	(0.08)	(0.37)

*DAS- days after sowing.
** Figures in parentheses are arc sine values.
ECI= Efficiency of Conversion of Ingested food
AD/AE= Approximate Digestibility/Assimilation Efficiency
ECD= Efficiency of Conversion of Digested food

Efficiency of conversion of ingested food (ECI) ranged from 11.93 to 18.86 per cent on *Bt* and BGII hybrids and 30.36 to 32.13 per cent on non-*Bt* hybrids (Table 7). It was highest on BIO 6488 non-*Bt* (32.13%) and RCH 134 non-*Bt* (30.36%). Lowest ECI was observed on BGII hybrids RCH 134 (11.93%) and BIO 6488 (13.18%). Further, Approximate digestibility or Assimilation efficiency (AD/AE) ranged from 22.43 to 39.41 per cent on *Bt* and BGII hybrids and 58.66 to 59.13 per cent on non-*Bt* hybrids. AD/AE was found to be highly suppressed on BGII hybrids (22.43% and 23.52%) followed by *Bt* hybrids (38.32% and 39.41%) and higher in case of non-*B*t hybrids (58.66% and 59.13%).Efficiency of conversion of digested food (ECD) ranged from 13.76 to 28.43 per cent on *Bt* and BGII hybrids and 55.36 to 55.72 per cent on non-*Bt* hybrids. Among the hybrids the lowest ECD (13.76%) was recorded on RCH 134 BGII, while highest (55.72%) on BIO 6488 non-*Bt*.

At 100 days after sowing of crop the variations on various consumption and utilization indices *viz.,* CI, GR, ECI, AD/AE and ECD on *Bt*, BGII and non-*Bt* hybrids were significantly different (Table 6, 7) and the trend was similar to that on crop age of 70 days after sowing.

At 120 days of crop age the consumption index (CI) ranged from 0.21 to 0.48 on *Bt* and BGII hybrids and 0.83 to 0.84 on non *Bt* hybrids (Table 6). On *Bt* hybrids the CI was significantly less than non-*Bt* hybrids. BGII hybrids RCH 134 (0.21) and BIO 6488 (0.23) recorded minimum CI. Growth rate of larvae ranged from 0.12 to 0.20 mg/day on *Bt* and BGII hybrids and 0.20 to 0.25 mg/day on non-*Bt* hybrids. Lower growth rate was noticed on RCH 134 BGII (0.12 mg/day) followed by BIO 6488 BGII (0.13 mg/day), RCH 134 *Bt* (0.18 mg/day) and BIO 6488 *Bt* (0.20 mg/day), respectively.

Efficiency of conversion of ingested food (ECI) ranged from 12.26 to 22.45 per cent on *Bt* hybrids and 30.08 to 30.34 per cent on non-*Bt* hybrids (Table 7). Further, Approximate digestibility or Assimilation efficiency (AD/AE) ranged from 25.68 to 39.78 per cent on *Bt* and BGII hybrids and 59.98 to 60.22 per cent on non-*Bt* hybrids. It was found to be highly suppressed on BGII hybrids (25.68% and 26.99%) followed by *Bt* hybrids (39.22% and 39.78%) and higher in case of non-*Bt* hybrids (59.98% and 60.22%). Efficiency of conversion of digested food (ECD) ranged from 13.69 to 29.32 per cent on *Bt* and BGII hybrids and 55.70 to 68.81 per cent on non-*Bt* hybrids. Among the hybrids the lowest ECD (13.69%) was recorded on RCH 134 BGII, while highest (68.81%) on BIO 6488 non-*Bt*.

4.2.2 Effect of transgenic *Bt* cotton bolls of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

The data of the experiment carried out on consumption utilization indices of *E. vittella* fourth instar larvae on bolls of *Bt*, BGII and non-*Bt* hybrids are summarised in Table 8 and 9.

At 70 days after sowing of crop the consumption index (CI) ranged from 1.39 to 1.51 on *Bt* and BGII hybrids and 1.95 to 2.21 on non *Bt* hybrids (Table 8). On *Bt* hybrids the CI was significantly less than non-*Bt* hybrids. BGII hybrids BIO 6488 (1.39) and RCH 134 (1.49) recorded minimum CI. Growth rate of larvae ranged from 0.28 to 0.32 mg/day on *Bt* and BGII hybrids and 0.42 to 0.47 mg/day on non-*Bt* hybrids. Lower growth rate was recorded on BIO 6488 BGII (0.28 mg/day) followed by RCH 134 *Bt*, RCH 134 BGII (0.30 mg/day), and BIO 6488 *Bt* (0.32 mg/day), respectively.

Efficiency of conversion of ingested food (ECI) ranged from 12.93 to 14.24 per cent on *Bt* and BGII hybrids and 22.46 to 22.54 per cent on non-*Bt* hybrids (Table 9). Further, Approximate digestibility or Assimilation efficiency (AD/AE) ranged from 30.42 to 36.73 per cent on *Bt* and BGII hybrids and 63.53 to 63.57 per cent on non-*Bt* hybrids. It was found to be highly suppressed on BGII hybrids (30.42% and 30.72%) followed by *Bt* hybrids (36.54% and 36.73%) and higher in case of conventional non-*B*t hybrids (63.53% and 63.57%). Efficiency of conversion of digested food (ECD) ranged from 17.14 to 24.36 per cent on *Bt* and BGII hybrids and 57.03 to 57.30 per cent on non-*Bt* hybrids. Among the hybrids the lowest ECD (17.14%) was recorded on RCH 134 BGII, while highest (57.30%) on BIO 6488 non-*Bt*.

At 100 days after sowing the variations on various consumption and utilization indices *viz.,* CI, GR, ECI, AD/AE and ECD on *Bt*, BGII and non-*Bt* hybrids were significant (Table 8, 9) and the trend was similar to that on crop of age 70 days after sowing.

At 120 days of crop age the consumption index (CI) ranged from 1.61 to 1.80 on *Bt* and BGII hybrids and 1.99 to 2.22 on non *Bt* hybrids (Table 8). BGII hybrids of RCH 134 (1.58) recorded minimum CI. Growth rate of larvae ranged from 0.31 to 0.39 mg/day on *Bt* and BGII hybrids and 0.44 to 0.48 mg/day on non-*Bt* hybrids. Lower growth rate was recorded on RCH 134 *Bt* (0.31 mg/day) followed by BIO 6488 BGII (0.32 mg/day), BIO 6488 *Bt* (0.36 mg/day), and RCH 134 BGII (0.39 mg/day), respectively.

Table 8: **Effect of transgenic *Bt* cotton bolls of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)**

Hybrids	**Consumption- Index (CI)**			**Growth Rate (GR) (mg/day)**		
	70 DAS*	**100 DAS**	**120 DAS**	**70 DAS**	**100 DAS**	**120 DAS**
RCH 134 *Bt*	1.48	1.47	1.61	0.30	0.27	0.31
RCH 134 BGII	1.49	1.49	1.58	0.30	0.32	0.39
RCH 134 non *Bt*	1.95	1.95	1.99	0.42	0.43	0.44
BIO 6488 *Bt*	1.51	1.49	1.80	0.32	0.31	0.36
BIO 6488 BGII	1.39	1.40	1.63	0.28	0.29	0.32
BIO 6488 non *Bt*	2.21	2.18	2.22	0.47	0.49	0.48
SEm(±)	0.03	0.04	0.03	0.03	0.03	0.03
CD(p=0.05)	0.09	0.13	0.09	0.11	0.11	0.11

* DAS- days after sowing

Table 9: Effect of transgenic *Bt* cotton bolls of plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius)

Hybrids	ECI (%)			AD/AE (%)			ECD (%)		
	70 DAS*	100 DAS	120 DAS	70 DAS	100 DAS	120 DAS	70 DAS	100 DAS	120 DAS
RCH 134 *Bt*	12.93(21.07)**	12.91(21.05)	14.24(22.17)	36.73(37.29)	38.03(38.06)	38.33(38.24)	24.36(29.56)	24.48(29.65)	25.24(30.15)
RCH 134 BGII	13.50(21.55)	13.48(21.53)	14.39(22.29)	30.42(33.46)	30.82(33.71)	30.90(33.76)	17.14(24.45)	17.33(24.59)	17.93(25.04)
RCH 134 non *Bt*	22.46(28.28)	22.50(28.30)	23.14(28.74)	63.53(52.83)	64.34(53.32)	64.46(53.38)	57.03(49.02)	58.24(49.73)	59.60(50.51)
BIO 6488 *Bt*	14.01(21.98)	14.01(21.98)	16.20(23.72)	36.54(37.18)	40.01(39.22)	41.24(39.94)	23.16(28.76)	25.31(30.19)	33.01(35.06)
BIO 6488 BGII	14.24(22.16)	14.26(22.18)	15.36(23.07)	30.72(33.65)	32.42(34.70)	33.42(35.31)	18.60(25.54)	19.26(26.02)	19.78(26.40)
BIO 6488 non *Bt*	22.54(28.34)	22.56(28.35)	23.26(28.83)	63.57(52.86)	64.22(53.24)	64.64(53.49)	57.30(49.18)	58.53(49.89)	58.88(50.10)
SEm(±)	(0.05)	(0.03)	(0.03)	(0.05)	(0.03)	(0.04)	(0.02)	(0.03)	(0.03)
CD(p=0.05)	(0.17)	(0.10)	(0.08)	(0.16)	(0.09)	(0.12)	(0.07)	(0.08)	(0.10)

*DAS-days after sowing.
* *Figures in parentheses are arc sine values.
ECI= Efficiency of Conversion of Ingested food
AD/AE= Approximate Digestibility/Assimilation Efficiency
ECD= Efficiency of Conversion of Digested food

Efficiency of conversion of ingested food (ECI) ranged from 14.24 to 16.20 per cent on *Bt* and BGII hybrids and 23.14 to 23.26 per cent on non-*Bt* hybrids (Table 9). Further, Approximate digestibility or Assimilation efficiency (AD/AE) ranged from 30.90 to 41.24 per cent on *Bt* and BGII hybrids and 64.46 to 64.64 per cent on non-*Bt* hybrids. It was found to be highly suppressed on BGII hybrids (30.90% and 33.42%) followed by *Bt* hybrids (38.33% and 41.24%) and higher in case of conventional non-*B*t hybrids (64.46% and 64.64%). Efficiency of conversion of digested food (ECD) ranged from 17.93 to 33.01 per cent on *Bt* and BGII hybrids and 58.88 to 59.60 per cent on non-*Bt* hybrids. Among the hybrids the lowest ECD (17.93%) was recorded on RCH 134 BGII, while highest (59.60%) on RCH 134 non-*Bt*.

4.3 Ovipositional preference of *Earias vittella* (Fabricius) on different parts of *Bt* cotton plants of age 80 days after sowing

The data on ovipositional preference of *E. vittella* at 80 days of crop age under choice condition between *Bt,* BGII and non *Bt* twigs revealed there was no significant variation among *Bt,* BGII and non *Bt* hybrids (Table 10). The number of eggs laid on leaves ranged from 197 to 229 in *Bt* and BGII hybrids and 246 on non *Bt* hybrids. The number of eggs laid on squares ranged from 133 to 159 on *Bt* and BGII hybrids and 150 to 157 on non *Bt* hybrids. The number of eggs laid on flowers ranged from 44 to 59 in *Bt* and BGII hybrids and 64 to 78 on non *Bt* hybrids. Similarly, number of eggs laid on bolls ranged from 24 to 27 in *Bt* and BGII hybrids and 27 in non *Bt* hybrids. The total number of eggs laid on *Bt*, BGII and non *Bt* hybrids ranged from 405 to 531 eggs/1 twig/4 females. However, maximum number of eggs (531) was laid on RCH 134 non *Bt* hybrid. It was also noticeable that maximum number of eggs was laid on leaves followed by squares, flowers and bolls. Therefore from the present studies it may be concluded that *E. vittella* females are not able to distinguish between *Bt*, BGII and non *Bt* hybrids.

Table 10: Ovipositional preference of *Earias vittella* (Fabricius) on different parts of *Bt* cotton plants of age 80 days after sowing

Hybrids	Average number of eggs laid/1 twig/4 females after 48 hours				
	Leaves	Squares	Flowers	Bolls	Total
RCH 134 *Bt*	220.33(14.85)*	159.00(12.63)	59.00(7.72)	26.66(5.26)	473.33(21.75)
RCH 134 BG II	229.00(15.11)	136.00(11.68)	44.33(6.72)	27.33(5.31)	432.33(20.77)
RCH 134 non *Bt*	246.00(15.69)	150.66(12.31)	64.33(8.09)	27.33(5.32)	531.66(23.08)
BIO 6488 *Bt*	209.66(14.49)	148(12.19)	52.33(7.28)	24.66(5.06)	434.66(20.84)
BIO 6488 BGII	197.33(14.04)	133.33(11.59)	47.00(6.86)	26.66(5.25)	405.33(20.11)
BIO 6488 non *Bt*	246.33(15.71)	157.66(12.59)	78.66(8.90)	27.33(5.31)	517.00(22.76)
SEm(±)	(0.71)	(0.40)	(0.56)	(0.20)	(0.76)
CD(p=0.05)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)

*Figures in parentheses are square root transformed values.

CHAPTER-V

DISCUSSION

Results achieved under various experiments have been discussed here under in the light of available information

5. Effect of transgenic *Bt* cotton squares and bolls of plants of different ages on some biological parameters of *Earias vittella* (Fabricius) larvae.

5.1.1 Effect of transgenic *Bt* cotton squares and bolls of plants of different ages on larval survival (first instar) of *Earias vittella* (Fabricius).

In the present investigation, for working out larval survival, two plant parts *viz*., squares and bolls of *Bt,* BGII and non *Bt* hybrids of cotton, were collected randomly, from the plants at 60, 90, 105 and 125 days after sowing and brought to the laboratory. One (square/boll) each was put in separate petri plates for feeding to first instar larvae of *E. vittella* (10 larvae/plant/hybrid) in a thrice replicated experiment, for five days. The food was changed on alternate days. Observations recorded till fifth day.

At 60 days after sowing of crop the larval survival ranged from 0.00 to 1.33 per cent on *Bt* and BGII hybrids and 93.33 to 96.66 per cent on non *Bt* hybrids. Among the hybrids none of the larvae survived on RCH 134 BGII and BIO 6488 BGII. On *Bt* hybrids the larval survival was significantly less than non-*Bt* hybrids i.e. RCH 134 (0.66%) followed by BIO 6488 (1.33%); however the differences between *Bt* and BGII hybrids were at par with each other. At 90 and 105DAS of crop age similar trends were observed. At 125 DAS larval survival ranged from 66.66 to 73.33 per cent on non *Bt*, 33.33 to 46.66 per cent on *Bt* hybrids, 26.66 to 30.00 per cent on BGII hybrids. There was no significant difference between *Bt* and BGII hybrids. Kumar and Indirapriyadarshini (2010) also reported higher percentage of mortality of *E. vittella* on *Bt* hybrids than their non *Bt* counterparts on both square and boll basis. Shanmugam *et al*. (2006) also observed similar pattern on *Bt* cotton against *E. vittella* and revealed that neonate mortality was more at 60 DAS and decreased at 90 and 120 DAS. Similarly, sSomasekhara *et al*. (2011) studied the impact of *Bt* and BGII hybrids against *E. vittella* neonate mortality and reported that BGII hybrids recorded cent per cent mortality at 60 DAS. Mahon and Olsen (2009) observed impact of Bollgard II cotton against *H. armigera* and revealed that survival of larvae was increased with the increase of plant age, hence, in conformity with the present findings.

5.1.2 Effect of transgenic *Bt* cotton squares and bolls of plants of different ages on some biological parameters of *Earias vittella* (Fabricius) larvae

Third instar larvae of *E. vittella* were used to record various biological parameters. Squares and bolls of plants of 60, 90, 105 and 125 days after sowing were collected from the respective plots and each was kept in petri plates. It is a thrice replicated experiment (10 larvae/replication). The larval period (days), larval weight (mg), larval survival (%), pupal weight (mg), pupal period (days) and adult emergence (%) were observed.

The larval period of *E. vittella* on non *Bt* hybrids remained normal at 60 days after sowing of crop (DAS). On *Bt* and BGII hybrids larval period (12.50-13.50 days) increased significantly as compared with non *Bt* at same stage. Increase in larval period on transgenic cotton was also reported by Wu *et al*. (2009) against *Spodoptera exigua* (Hubner) while feeding on transgenic cotton. Similarly, Li *et al*. (2007) revealed that when larvae of cabbage looper, *Trichoplusia ni* (Hubner) fed with *Bt* cotton (Cry 1 Ac and Cry 2 Ab), an increased larval period was observed. Mahon and Olsen (2009) and Basavaraja, (2007) studied *Helicoverpa spp.* on transgenic *Bt* cotton and observed similar results. Observations on bolls also gave almost similar results as in case of squares. The larval period on non *Bt* hybrids had no effect and remained normal as on squares. Increased larval period (11.50-14.33 days) was observed on *Bt* and BGII hybrids. In case of non *Bt* hybrids no effect was observed on larval weight of *E. vittella.* Low larval weight (29.32-38.67 mg) was observed on *Bt* and BGII hybrids. On both squares and bolls observations remained almost similar. Murugan *et al.* (2003) also reported that feeding *Bt* cotton to *Helicoverpa armigera* (Hubner) reduced weight of each instar of larvae. Basavaraja (2007) also recorded results similar to the present findings.

The *Bt* and BGII hybrids showed significant effect on survival of third instar larvae of *E. vittella*. The larval survival on non *Bt* hybrids (73.32-76.68 %) was higher than *Bt* and BGII hybrids (36.68-46.68%) on both squares and bolls. These studies reflected the effectiveness of *Bt* and BGII against *E. vittella* larvae. The pupal period of *E. vittella* on non *Bt* hybrids remained normal at 60 days after sowing of crop (DAS). On *Bt* and BGII hybrids increased pupal period (10.33-13.33 days) was recorded as compared with non *Bt* at this stage. The pupal weight was remained normal on non *Bt* hybrids and lowest pupal weight (23.53-30.30 mg) was recorded on *Bt* and BGII hybrids, it showed effectiveness of Cry 1 Ac and Cry 2 Ab against *E. vittella*. These observations followed similar trend for both squares and bolls and showed effectiveness against of *E. vittella.* The adult emergence from pupae of *Bt* and BGII hybrids (23.34-33.32 %) significantly differed from non *Bt* hybrids (56.68-63.32 %). Mahon and Olsen (2009) also reported that survivors of *H. armigera* larvae on Bollgard II genotypes produced smaller pupae and adults showed reduced longevity and fecundity. Similarly Li *et al.* (2007) reported that pupae developed from larvae of *T.ni* fed on *Bt* cotton (Cry1Ac and Cry2Ab) were smaller in size compared with non *Bt* and only 17.9 per cent larvae could become adults on *Bt* cotton. These findings are in accordance with the present results achieved. Adamzyck *et al.* (1998), Zhang *et al.* (2005) and Basavaraja *et al.* (2008b) also strongly favoured the present investigations.

At 90 days after sowing of crop, results followed similar trend as in case of 60 DAS. The larval period (13.66-15.16 days), larval weight (27.26-56.38 mg), larval survival (36.67-46.68 %), pupal period (11.66-15.16 days), pupal weight (25.65-33.53 mg) and adult emergence (23.34-33.33 %) on *Bt* and BGII hybrids showed significant variations. In case of *Bt* and BGII hybrids, larval weight, pupal weight on bolls was lower than squares but differences were marginal. Similar results reported by Basavaraja (2007) in the *Bt* cotton (Cry1Ac) which was effective against bollworm, *Helicoverpa armigera* (Hubner) till 100 days after sowing, The same was observed in the present studies.

At 105 DAS, the larval period (11.33-14.66 days) of *Bt* and BGII hybrids followed similar trend as in case of 60 and 90 DAS. The larval weight on non *Bt* hybrids remained unaffected as was observed at 60 and 90 DAS. There was reduction in the larval weight (32.64-39.68 mg) when observed on *Bt* and BGII hybrids. The above statement is also supported by studies carried out by Basavaraja (2007), Li *et al.,* (2007) and Mahon and Olsen (2009). Pupal period (10.33-12.33 days) of *Bt* and BGII hybrids followed similar trend as in case of 60 and 90 DAS. The pupal weight at 105 DAS, remained almost similar as on 60 and 90 DAS on *Bt* and non *Bt* hybrids but increased values in the larval weight was observed on BGII hybrids. An increase in larval survival (46.67-56.66 %) and adult emergence (46.66 %) was recorded. Basavaraja (2007) stated that pupal period was non significant on different *Bt* genotypes against *H. armigera* after 100 days.

At 125 DAS, the larval period (9.33-12.00 days) on *Bt* and BGII hybrids also decreased as compared with 60 and 90 and 105 DAS. An increase in the larval weight (33.33-54.52 mg) and pupal weight (27.32-43.10 mg) was observed. An enhanced larval survival (70.00-76.67 %) was also observed at 125 days after sowing of the crop on both squares and bolls of *Bt* and BGII genotypes. Similar observations were made by Li *et al.* (2007) who observed that first four larval instars of soybean looper (*T. ni*) died when fed with transgenic cotton (Cry1Ac and Cry2Ab) at 50 days after planting (DAP). When *Bt* cotton leaves were continuously fed to first instar larvae at 120 DAP, 75.9, 60.6, 56.4 and 38.4 per cent of larvae survived to second, third, fourth and fifth instars, respectively. Mahon and Olsen (2009) also reported that production of Cry1Ac was reduced as plant gets aged. Based on observations, it is concluded that though the survival of larvae increased on both *Bt* and BGII hybrids but was not comparable with the larval survival on non *Bt* hybrids. It is therefore, assumed that *Bt* toxin Cry 1 Ac and Cry 2 Ab might be present but at low level. According to Basavaraja, (2007) and Li *et al.,*(2007) the toxicity decreased as plant get aged. This finding supported the present study.

The adult emergence (56.68-69.99 %) was high on *Bt* and BGII hybrids at 120 DAS. Li *et al.,*(2007) also stated that at 120 days after planting (DAP), transgenic cotton became less toxic to soybean looper (*T.ni*) which resulted in increased pupa formation and adult emergence.

5.2 Effect of transgenic *Bt* cotton plants of different ages on consumption and utilization indices of fourth instar larvae of *Earias vittella* (Fabricius).

The experiment was conducted in battery jars (20 cm x 15 cm) in which the respective foods (squares/bolls) were kept. Two plant parts *viz*., squares and bolls, collected from 70, 100 and 120 days after sowing of crop, were fed to the fourth instar larvae of *E. vittella*.

In this experiment each replication consisted of 10 larvae and total of three replications were maintained. These larvae were starved for about four hours and weighed individually before releasing them on the squares, bolls in the battery jars. The fresh weight of the food in each jar was also recorded before the larvae were released on it. After 48 hours uneaten food material, larvae and faecal matter were separated and weighed.

Consumption – utilization indices *viz*., CI, GR, ECI, AD/AE and ECD of fourth instar larvae of *E. vittella* were calculated.

Consumption index (CI) on various hybrids differed significantly at 70 DAS of crop. This indicated that consumption by *E. vittella* was variable due to *Bt,* BGII and non *Bt* hybrids. These results are also supported by Berdegue *et al.* (1996) who found significant avoidance of *Bt* toxin treated diet by *S.exigua*. Basavaraja *et al.* (2008b) also made similar observations, who noticed significant reduction in consumption of *Bt* (Cry1Ac) cotton by *H. armigera*. The consumption index (CI) and growth rate (GR) of larvae in case of non *Bt* hybrids remained unaffected but growth of larvae and consumption index (0.13-1.51) was very less on *Bt* and BGII hybrids. The decreased growth rate (0.11-0.32) indicated effectiveness of *Bt* and BGII hybrids against *E. vittella.* The efficiency of conversion of ingested food (ECI) was about normal on non *Bt* hybrids but ECI on *Bt* and BGII hybrids (11.93-18.86%) remained significantly low at 70 DAS. The present findings are supported by Somasekhara *et al.* (2011), who observed greatly reduced food utilization efficiency of *Earias vittella* on squares of *Bt* and BGII cotton genotypes. Similar trend was followed in GR, ECI, AD/AE and ECD. Wu *et al.* (2009) also supported that growth rate remained lower on transgenic *Bt* cotton incase of *S. exigua*. Basavaraja *et al.* (2008b) studies are also in conformity with present findings. Prutz and Dentter (2005) also supported low growth rate and ECI of larvae of *H. armigera* on *Bt* treated maize. Approximate digestibility / Assimilation efficiency (AD/AE) was also low on *Bt* and BGII hybrids (22.43-39.41%) as compared with non *Bt* hybrids. These results are supported by Basavaraja *et al.*(2008b) who observed low AD/AE on *Bt* cotton against *H. armigera.* The efficiency of conversion of digested food (ECD) was not affected on non *Bt* genotypes but on *Bt* and BGII hybrids (13.76-28.43%) they turned out very low which further confirmed the effectiveness of *Bt* and BGII hybrids against *E. vittella* (Prutz and Dentter, 2005).

On *Bt* and BGII hybrids, Consumption index (0.14-1.49) at 100 DAS again observed significant against *E. vittella*. All the observations at 100 DAS were similar to earlier observations at 70 DAS. Growth rate on non *Bt* hybrids was unaffected and low growth rate (0.12-0.32) was observed on *Bt* and BGII hybrids at 100 DAS. The efficiency of conversion of ingested food (12.91-18.98 %) and efficiency of conversion of digested food (13.57-25.31 %) remained low for *Bt* and BGII hybrids at both the stages. The AD/AE (23.05-40.01 %) was observed similar to 70 DAS. Studies carried out by Basavaraja *et al.,* (2008b) and Wu *et al.,* (2009), Somashekara *et al.* (2011) supported the present investigations.

The effect of *Bt* and BGII hybrids was observed low at 120 DAS, as all the parameters like CI (0.21-1.80), GR (0.12-0.32), ECI (12.26-16.20%) and ECD (13.69-33.01%) showed increasing trends which were observed low at early stages. There was some increase in growth rate also when compared with 100 DAS observations. Basavaraja *et al.* (2008b) also observed that differences between parameters remained non significant at 130 days of crop age. Li *et al.* (2007) also noticed that leaves of *Bt* cotton at 120 days after planting (DAP) were less toxic to soybean looper, hence these studies are in accordance with present investigation. On *Bt* and BGII hybrids, efficiency of conversion ingested food and efficiency of conversion of digested food showed some increase compared with earlier observations at 70 and 100 DAS but these observations were still lower than the results achieved on non *Bt* genotypes. Basavaraja *et al.,* (2008b) also noticed that *Bt* (Cry1Ac) became ineffective against *H. armigera* at 130 days of crop age and support the results achieved during present study. It was observed that parameters were increasing at 120 DAS on BGII genotypes but their values were not comparable with values of *Bt* and non *Bt* genotypes. This indicates that *Bt* toxin (Cry2Ab) might still be present in the BGII genotypes. As no assay (ELISA) was carried to find the exact level of the toxin in the crop so it is difficult to justify level of toxin. Mahon and Olsen (2009) found that in BGII genotypes cotton Cry 2Ab was present at higher level in the plant throughout the growing season.

5.3 Ovipositional preference of *Earias vittella* (Fabricius) on different parts of *Bt* cotton plants of age 80 days after sowing

The ovipositional preference studies of females of *E. vittella* were carried out at 80 days after sowing of the crop. The experiment was conducted in a wooden cage (40cm x 40 cm x 40 cm). About 45 cm twigs bearing leaves, squares, flowers and bolls were plucked from the respective plants of age 80 days after sowing and brought to laboratory. One such twig, the cut end of which was dipped in water, was placed in every cage. In each age four pairs of newly emerged adults in 1:1 ratio were released in each cage for 2 days. The observations on the number of eggs laid on different parts of twig were recorded. The experiment was replicated 3 times.

The differences between eggs laid on different hybrids were found non significant at 80 days after sowing of crop. The eggs were laid randomly on all twigs without any discrimination of the hybrids used. Differences between eggs laid on flowers/bracts and on bolls were non significant and leaves were preferred more than flowers/bracts and bolls. But differences were not indicating any preference of hybrids on genetic basis. These observations are in accordance with the earlier findings of Vennila *et al*. (2004) and Basavaraja *et al*. (2012) on ovipositional preference of *H. armigera*.

CHAPTER-V

SUMMARY AND CONCLUSION

The present studies on "Evaluation of *Bt* cotton hybrids against *Earias vittella* (Fabricius) under laboratory conditions" were carried out in the laboratory and at Research Farm, Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar during kharif season 2011-12. Total six hybrids of cotton *viz.,* BIO 6488 *Bt*, BIO 6488 BGII, BIO 6488 non *Bt*, RCH 134 *Bt*, RCH 134 BGII and RCH 134 non *Bt*, were used for the study and were grown by adopting recommended in Package and Practice of Kharif crops (Anonymous, 2011).

The survival of first instar larvae of *E. vittella* was observed on squares and bolls for five days at an interval of 60, 90, 105 and 125 days of crop age. Observations at 60 and 90 DAS showed a significant adverse effect of *Bt* (Cry 1 Ac) and BGII (Cry 1Ac + Cry 2Ab) on survival of *E. vittella* larvae on both squares and bolls. The larval survival was very low at 60 days after sowing on both *Bt* and BGII hybrids.

At 60 days after sowing of crop the larval survival ranged from 0.00 to 1.33 per cent on *Bt* and BGII hybrids and 93.33 to 96.66 per cent on non *Bt* hybrids. Among the hybrids none of the larvae survived on RCH 134 BGII and BIO 6488 BGII. On *Bt* hybrids the larval survival was significantly less than non-*Bt* hybrids i.e. RCH 134 (0.66%) followed by BIO 6488 (1.33%). BGII hybrids BIO 6488 and RCH 134 recorded minimum larval survival; however the differences between *Bt* and BGII hybrids were at par with each other. At 90 DAS of crop age similar trends followed where lowest per cent larval survival was recorded (1.33%) on BIO 6488 BGII hybrid followed by RCH 134 BGII (1.66%). At 105 DAS the lowest larval survival (6.66%) was recorded on both BGII hybrids of RCH 134 and BIO 6488 and were statistically at par with each other, while highest larval survival (83.33%) was recorded on BIO 6488 non *Bt*. At 125 DAS larval survival ranged from 66.66 to 73.33 per cent on non *Bt*, 33.33 to 46.66 per cent on *Bt* hybrids, 26.66 to 30.00 per cent on BGII hybrids. There was no significant difference between *Bt* and BGII hybrids.

The various biological parameters of *E. vittella* were studied on third instar larvae onwards on squares and bolls at various intervals (60, 90, 105 and 125 days after sowing). Significant effects of *Bt* and BGII hybrids were observed at 60, 90, 105 and 125 DAS on squares. The maximum effects of *Bt* and BGII hybrids on biological parameters were observed at 60 days of crop age on squares where the larval period ranged from 12.50 to 14.16 days on *Bt* and BGII hybrids and 8.50 to 9.16 days on non *Bt* hybrids. Larval weight ranged from 29.32 to 35.41 mg on *Bt* and BGII hybrids and 62.32 to 63.12 mg on non *Bt* hybrids. Lowest larval survival (39.68%) was recorded on RCH 134 BGII followed by BIO 6488 BGII (40.01%), while highest (76.68%) on BIO 6488 non *Bt* followed by (73.32%) on RCH 134 non *Bt* hybrids. Pupal period ranged from 11.33 to 13.33 days on *Bt* and BGII hybrids and 7.66 to 8.33 days on non *Bt* hybrids. Among the hybrids the minimum pupal weight (23.53 mg) was recorded on BIO 6488 *Bt*, while maximum (54.31 mg) on RCH 134 non *Bt* hybrid. The lowest adult emergence (23.33%) was recorded on BIO 6488 *Bt*, while highest (63.32%) on BIO 6488 non *Bt* hybrid. Similar trend was observed on bolls where the larval period ranged from 11.5 to 14.33 days on *Bt* and BGII hybrids and 7.66 to 8.16 days on non *Bt* hybrids. Larval weight ranged from 31.66 to 38.67 mg on *Bt* and BGII hybrids and 67.12 to 67.66 mg on non *Bt* hybrids. Lowest larval survival (36.67 %) was recorded on BIO 6488 BGII followed by RCH 134 BGII (36.68 %), while highest (76.68 %) on RCH 134 non *Bt* followed by (73.34%) on BIO 6488 non *Bt* hybrids. Pupal period ranged from 10.33 to 12.83 days on *Bt* hybrids and 6.83 to 8.00 days on non *Bt* hybrids. Among the hybrids the minimum pupal weight (26.21 mg) was recorded in BIO 6488 *Bt*, while maximum (58.40 mg) on RCH 134 non *Bt* hybrid. The lowest adult emergence (23.34%) was recorded on BIO 6488 *Bt*, while highest (60.01%) on RCH 134 non *Bt* hybrid. At 90, 105 and 125 DAS similar trend was observed on both squares and bolls.

Fourth instar larvae of *E. vittella* were used for studying consumption utilization indices at 70, 100 and 120 DAS on squares and bolls. There was significant reduction in CI, GR, ECI, AD/AE and ECD of larvae on *Bt* and BGII hybrids at 70,100 and 120 days of crop age on squares. At 70 days of crop age the CI ranged from 0.13 to 0.35 on *Bt* hybrids and 0.81 to 0.83 on non *Bt* hybrids. Growth rate of larvae ranged from 0.11 to 0.18 mg/day on *Bt* and BGII hybrids and 0.22 to 0.25 mg/day on non *Bt* hybrids. Among the hybrids the lowest growth rate (0.11 mg/day) was recorded on RCH 134 BGII, while highest (0.25 mg/day) on RCH 134 non *Bt*. The GR was highest on non *Bt* hybrids of RCH 134 (0.25 mg/day) and BIO 6488 (0.22 mg/day) which were found statistically at par with each other. Efficiency of conversion of ingested food (ECI) ranged from 11.93 to 18.86 per cent on *Bt* and BGII hybrids and 30.36 to 32.13 per cent on non *Bt* hybrids. Further, Approximate digestibility or Assimilation efficiency (AD/AE) ranged from 22.43 to 39.41 per cent on *Bt* and BGII hybrids and 58.66 to 59.13 per cent on non *Bt* hybrids. Efficiency of conversion of digested food (ECD) ranged from 13.76 to 28.43 per cent on *Bt* and BGII hybrids and 55.36 to 55.72 per cent on non *Bt* hybrids. Among the hybrids the lowest ECD (13.76%) was recorded on RCH 134 BGII, while highest (55.72%) on BIO 6488 non *Bt*. Similar trend followed on squares at 100 and 120 days of crop age. The observations were followed almost similar in bolls at an interval of 70, 100 and 120 days of crop age.

Twigs of different hybrids bearing leaves, squares, flowers and bolls were brought to laboratory at 80 days after sowing to observe ovipositional preference of *E. vittella* between *Bt*, BGII and non *Bt* hybrids on a choice test condition. No Significant differences were observed between *Bt*, BGII and non *Bt* hybrids. The total number of eggs laid on *Bt*, BGII and non *Bt* ranged from 405 to 531 eggs/1 twig/4 females.

It was concluded from the study that *Bt* (Cry 1 Ac) and BGII (Cry 1 Ac + Cry 2 Ab) cotton can be used as a potential control measure against *E. vittella*. Survival of first instar larvae was recorded very low at 60 and 90 days after sowing. The data after 105 and 125 days showed increased levels of larval survival which might be due to low production of Cry 1 Ac and Cry 2Ab toxins after 100 days of crop age.

Studies on some biological parameters revealed that *Bt* and BGII hybrids were more effective against *E. vittella* than non *Bt* hybrids. The observations on *Bt* and BGII at 60 and 90 DAS was highly significant which showed that it is effective against third instar also. At 105 and 125 DAS the effect was slightly less but control of the pest was good.

The data on consumption utilization indices of larvae showed that *Bt* and BGII had significant adverse effects on consumption and utilization indices *viz*., consumption index, growth rate, efficiency of conversion of ingested food, approximate digestibility/assimilation efficiency and efficiency of conversion of digested food.

E. vittella adults did not follow any pattern in egg laying under choice conditions when twigs of hybrids of *Bt*, BGII and non *Bt* were provided.

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ABSTRACT

Title of thesis	:	"Evaluation of *Bt* cotton hybrids against *Earias vittella* (Fabricius) under laboratory conditions"
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Major subject	:	Entomology
Total number of pages in thesis	:	40+iv
Number of words in the abstract	:	280
Key words	:	*Earias vittella,* Biological parameters, consumption- utilization indices, oviposition, *Bt* cotton

Experiments were conducted for evaluation of *Bt* cotton hybrids against *Earias vittella* (Fabricius) under laboratory conditions during the year 2011-12 in CCS Haryana Agricultural University, Hisar. Six hybrids namely BIO 6488 *Bt*, BIO 6488 BGII, BIO 6488 non *Bt,* RCH 134 *Bt,* RCH 134 BGII and RCH 134 non *Bt* were used for studying the biological parameters of *E. vittella viz.,* larval survival, larval weight, larval period ,pupal period, pupal weight and adult emergence at 60, 90, 105 and 125 days after sowing (DAS) of crop. At 60 and 90 DAS, *Bt* and BGII hybrids showed significant adverse effects on various biological parameters. However, larval survival increased at 05 and 125 DAS. Larval weight was recorded minimum on both *Bt* and BGII hybrids at 60, 90 and 105 DAS but an increased larval weight was observed after 125 DAS. Similar trends were observed in pupal weight and adult emergence on *Bt* and BGII hybrids. Larval period increased on both *Bt* and BGII hybrids at 60, 90 and 105 DAS but reduced at 125 DAS. Similar trends were observed in pupal period on both *Bt* and BGII hybrids. Consumption utilization studies were also conducted at 70, 100 and 120 days after sowing of crop. At 70 and 100 DAS, growth rate, efficiency of conversion of ingested food, approximate digestibility/assimilation efficiency and efficiency of conversion of digested food were observed minimum on *Bt* and BGII hybrids. After 120 DAS, effects were marginal. In case of ovipositional preference at 80 DAS under choice conditions, females of *E. vittella* showed no difference between *Bt*, BGII and non *Bt* hybrids under investigation. Leaves were preferred more over squares, flowers and bolls for egg laying by *E. vittella* females.

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