

# Crop hosts and genotypic resistance influence the biological activity of *Bacillus thuringiensis* towards *Helicoverpa armigera*

I. Paramasiva<sup>a, b</sup>, P.V. Krishnayya<sup>b</sup>, A.R. War<sup>a</sup>, H.C. Sharma<sup>a, a</sup>

Received 19 July 2013, Revised 20 May 2014, Accepted 22 May 2014, Available online 1 July 2014

## **Crop Protection**

Volume 64, October 2014, Pages 38-46

DOI: http://dx.doi.org/10.1016/j.cropro.2014.05.010

This is author version post print archived in the official Institutional Repository of ICRISAT www.icrisat.org

Crop Protection

Crop hosts and genotypic resistance influences the biological activity of *Bacillus* thuringiensis towards *Helicoverpa armigera* 

Paramasiva I<sup>1, 2</sup>, Krishnayya P V<sup>2</sup>, War AR<sup>1</sup>, and Sharma H C<sup>1\*</sup>

1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India. 2. Acharya NG Ranga Agricultural University, College of Agriculture, Bapatla, Andhra Pradesh, India.

## \*Corresponding author

H. C. Sharma

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),

 <sup>&</sup>lt;sup>a</sup> International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324 Andhra Pradesh, India
 <sup>b</sup> Acharya NG Ranga Agricultural University, College of Agriculture, Bapatla, Andhra Pradesh, India

Email: h.sharma@cgiar.org

**ABSTRACT** 

Studies on the influence of genotypic resistance on biological activity of a commercial formulation of Bacillus thuriengiensis (Bt) and pure Bt toxin Cry1Ac were carried out to develop appropriate strategies for pod borer, Helicoverpa armigera management in chickpea, sorghum, pigeonpea and cotton. The interaction effect of host plant resistance and biological

activity of commercial Bt/Cry1Ac was studied by incorporating the lyophilized tissues of chickpea leaves, milk stage sorghum grain, pigeonpea pods and cotton squares into the

artificial diet with and without Bt formulation or Cry1Ac. The H. armigera larval weights were significantly lower in insects reared on diets with square powder of the insect – resistant Bt-cotton RCH 2 + Bt/Cry1Ac and pod powder of insect - resistant pigeonpea genotype, ICPL

332WR + Bt/Cry1Ac as compared to the larvae reared on diets with leaf powder of H. armigera susceptible chickpea genotype, ICCC 37 and the standard artificial diet. Pupation and adult emergence were significantly lower in insects reared on diets with tissues of pod

borer-resistant genotypes + Bt/Cry1Ac as compared to insects reared on diets with tissues of the insect susceptible genotypes + Bt/Cry1Ac. Insects reared on diets containing insectresistant and -susceptible genotypes of sorghum, pigeonpea and cotton and pod borer-resistant

genotype of chickpea (ICC 506EB) + Bt/Cry1Ac did not lay any eggs. However, eggs were laid by the insects reared on diets containing pod borer-susceptible genotype of chickpea, ICCC 37 and on the standard artificial diet + Bt/Cry1Ac. The insects reared on diets with sorghum genotype, ICSV 745, and Bt-cotton, RCH 2 without Bt/Cry1Ac also did not lay

eggs. The results suggested that Bt/Cry1Ac is more effective for management of H. armigera when deployed in combination with insect-resistant genotypes of cotton, chickpea, pigeonpea

and sorghum.

Keywords: Genotypic resistance, Bacillus thuringiensis, Helicoverpa armigera, Cry1Ac,

biological activity, Pest management

1. Introduction

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is one of the most

important constraints to crop production globally. It is widely distributed in Asia, Africa,

Australia, and the Mediterranean Europe (Sharma, 2005). It is a polyphagous pest, and attacks

more than 300 plant species including cotton, grain legumes, cereals, vegetables, fruits, and

forest trees (Manjunath et al., 1989; Fitt, 1991; Sharma, 2005). In India, it has been recorded

from over 20 crops and 180 wild hosts (Manjunath et al., 1989). Control of H. armigera is

largely based on synthetic insecticide. As a result of repeated insecticide application for

controlling this pest, it has developed high levels of resistance to commonly used insecticides

2

(Kranthi et al., 2002). There is a need to develop alternative methods for controlling this pest to minimize over dependence on synthetic insecticides. Insect-resistant cultivars, natural enemies, biopesticides, natural plant products and agronomic practices are some of the alternative methods of pest control to reduce pesticide use for pest management. Of these, host plant resistance is compatible with all the other methods of pest control. Host plant resistance to insect pests is economically feasible and the ecologically preferred alternative to other pest management strategies, particularly to synthetic pesticides (Sharma et al., 1999).

The effectiveness of different methods of pest control may vary across crops and genotypes with different levels of resistance/susceptibility to the target pests. Therefore, there is need to assess the effectiveness of different methods of pest control in combination with crop cultivars with resistance to insect pests. The efficacy of *Bt* varies across different host plants and insect species. The biological activity of *Bt* against *Lymantria dispar* L. is 2-5 times lower on *Quercus* spp. than on aspen (Appel and Schultz, 1994); while the LC<sub>50</sub> value of *Bt* towards forest tent caterpillar, *Malacosoma disstria* (Hubner) is 100-fold greater on quaking aspen (*Populus tremuloides* Michx.) than on sugar maple (*Acer saccharum* Marsh.) (Kouassi et al., 2001). The neonates emerging from eggs laid by the *H. armigera* larvae collected from pearl millet have been found to be two times more tolerant to *B. thuringiensis* sp. *kurstaki* than those collected from cotton and sunflower (Gujar et al., 2004). *H. armigera* larvae reared on diets containing cabbage, cauliflower, and pearl millet were more susceptible to *Bt* than those fed on diets with chickpea, green pea, and pigeonpea, suggesting that host plant has a major bearing on the biological activity of *Bt* towards *H. armigera*.

The interactions between host plant resistance and  $\delta$ -endotoxins of Bt might be one of the major factors influencing the biological activity of B. thuringiensis under field conditions (Luthy et al., 1985). The Bt toxin Cry1Ac is more effective on cotton cultivars with low tannin content as compared to the cultivars with high tannins (Olsen et al., 1998). The toxicity of Bt against lepidopteran larvae is greater when fed on maize cultivars with inherent resistance to lepidopteran insects (Mohan et al., 2008).

Expression of *Bt* toxin genes in crop plants to enhance host plant resistance to insects has shown considerable potential to achieve a more effective control of target insect pests for sustainable food production (Sharma et al., 2004; James, 2007), and efforts are underway to develop transgenic chickpea and pigeonpea plants with resistance to *H. armigera* (Sharma et al., 2005; Acharjee et al. 2010), and sorghum plants with resistance to spotted stem borer, *Chilo partellus* (Swin.) (Girijashankar et al., 2005). However, the effectiveness of the *Bt* toxin proteins may be influenced by the inherent genotypic susceptibility to insects (Sharma, 2009).

The efficacy of biopesticides and natural enemies is greatly enhanced if they are compatible with host plant resistance to insect pests. While *Bt* formulation is used as sprays in pest management, the toxin protein Cry1Ac has been deployed in several transgenic crops for pest management (Sharma et al., 2004). The present studies were undertaken to assess the biological activity of *Bt* and Cry1Ac on insect-resistant and –susceptible genotypes of different crops to gain an understanding of their compatibility with insect-resistant cultivars. Such information is important to develop appropriate strategies for integrated pest management in different crops, and to identify crop - genotype-*Bt* toxin protein combinations that will be most effective for developing transgenic plants for pest management and sustainable crop protection.

## 2. Materials and Methods

The influence of host plant resistance on biological activity of commercial Bt formulation and the pure Bt toxin Cry1Ac was studied by using diet incorporation assay (Sharma et al., 2008). The compatibility of commercial Bt with insect-resistant and susceptible genotypes of chickpea, pigeonpea, sorghum and cotton was studied to develop appropriate strategies for pest management in these crops using biopesticides. The biological activity of pure Bt toxin Cry1Ac was evaluated in combination with genotypes to determine the genotype – Cry1Ac combinations that would be most effective for deployment of Bt toxins in transgenic plants for pest management.

The interaction between host plant resistance and biological activity of a commercial Bt formulation and the pure Bt toxin Cry1Ac was studied on resistance and susceptible

genotypes of chickpea (ICCC 37- susceptible and ICC 506EB - resistant to H. armigera) (Narayanamma et al., 2007), sorghum (IS 18698 - with high tannin content and resistant, and ICSV 745 - with no/low tannins and susceptible to H. armigera) (Sharma et al., 1993), pigeonpea (ICPL 87 -susceptible and ICPL 332WR - resistant to H. armigera) (Sharma et al., 2009), and cotton (RCH 2 – susceptible, and Bt RCH 2 – resistant to H. armigera) (Dhillon and Sharma, 2012). The interaction of commercial Bt formulation and pure Bt toxin Cry1Ac with insect-resistant and -susceptible genotypes on survival and development of H. armigera was studied by incorporating the lyophilized chickpea leaves, pigeonpea pods, sorghum grains, and cotton squares (the plant parts upon which H. armigera feeds on these crops under natural conditions) into the artificial diets (Sharma, 2009; Dhillon and Sharma, 2010; Narayanamma et al., 2013). Biolep®, a commercial formulation of Btk (strain Z-52, serotype H-3a, 3b), obtained from Biotech International Ltd; New Delhi, India, and the Bt Cry1Ac toxin (obtained from Dr. Marianne P. Carey, Case Western Reserve University, Department of Biochemistry, Cleveland, Ohio, USA) were used for studying host genotype - Bt interactions at the ED<sub>50</sub> (effective dose to reduce the larval weight by 50%) concentrations of 0.0125% and 5 ng ml<sup>-1</sup> of diet, respectively (Sharma et al., 2008).

#### 2.1. Insect culture

Larvae of H. armigera used in the bioassays were obtained from a laboratory culture maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Larvae were reared on chickpea based artificial diet (Armes et al., 1992) at  $27 \pm 2$  °C. The laboratory culture was supplemented with field-collected populations 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 H. armigera neonates (200 to 250) were reared in 200 ml plastic cups (having 2 to 3 mm layer of artificial diet on the bottom and sides) for five days. After five days, the larvae were transferred individually to six cell-well plates (each cell well 3.5 cm in diameter, 2 cm in depth) to avoid cannibalism. Each cell-well had sufficient amount of diet (7 ml) to support larval development until pupation. The pupae

were removed from cell-wells, sterilized with 2% 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. Nappy liners, which have a rough surface, were hung inside the cage as an oviposition substrate. They were removed daily and the eggs sterilized in 2% sodium hypochlorite solution. They were then dried under a table fan, and then placed inside the plastic cups with artificial diet. After egg hatching, the larvae moved to the artificial diet, and the liners were removed after 4 days. Newly emerged larvae of *H. armigera* were used for bioassays by using diet impregnation assay (Sharma et al., 2005).

2.2. Effectiveness of commercial Bt formulation and pure Bt toxin Cry1Ac against H. armigera on insect-resistant and susceptible genotypes of chickpea, sorghum, pigeonpea, and cotton

Freeze-dried plant parts upon which the *H. armigera* feeds under natural conditions [terminal branches of chickpea (ICCC 37 and ICC 506EB), dough-stage grain of sorghum (IS 18698 and ICSV 745), tender green pods of pigeonpea (ICPL 87 and ICPL 332WR), and squares of cotton (RCH 2 and Bt RCH 2)], were added to the artificial diet with and without Bt or Cry1Ac. The plant material was collected from the plants grown under field conditions at the appropriate stage. The samples were collected in an ice-box and lyophilized in a freeze drier for three days. The freeze-dried material was powdered in a Willey mill to obtain a fine powder (<80 µm), and stored in desiccators until used. Freeze-dried and powdered chickpea leaves, sorghum grain, pigeonpea pods (20 g), and cotton squares (7.5 g) (Table 1), used as a replacement for chickpea flour in the artificial diet, were mixed with the artificial diet ingredients sufficient for 300 ml diet. Artificial diet (7 ml) was poured into small plastic cups (25 ml capacity), and the neonates were released individually into the cups to assess the bioefficacy of Bt/Cry1Ac on H. armigera - resistant and -susceptible genotypes of chickpea, pigeonpea, sorghum and cotton. The experiment was conducted in a completely randomized design (CRD) with three replications. There were 20 insects in each replication. Data were recorded on larval weights at 5 and 10 days after initiating the experiment (DAI). The larvae

were removed from the rearing cups, cleaned, weighed and then placed back in the cups. Pupae from each replication were placed in a 1 L plastic jar containing moist Vermiculite. The pupal weights were recorded one day after pupation. Percentage pupation and adult emergence were computed in relation to number of neonate larvae released in each replication. Data were also recorded on larval and pupal periods and adult longevity. Five pairs of adults emerging from each treatment were released inside an oviposition cage and provided with 10% sucrose solution on cotton swab. Numbers of eggs laid per female were recorded from five pairs of adults in each treatment.

#### 2.3. Statistical analysis

Data were subjected to analysis of variance by using GENSTAT version 10.1 by factorial analysis. The significance of differences between the treatments, genotypes, and the interaction effects was judged by the F-test ( $P \le 0.05$ ), while significance of the differences between the treatments and genotypes was judged by Duncan's Multiple Range Test (DMRT) ( $P \le 0.05$ ).

## 3. Results

3.1. Biological activity of Bt against H. armigera in artificial diets containing insect -resistant and -susceptible genotypes of chickpea, sorghum, pigeonpea, and cotton

#### 3.1.1. Larval and pupal weights

There were significant differences in larval weights at 5 DAI between the treatments  $(F_{(1,16)} = 154.23, P \le 0.001)$ , genotypes  $(F_{(8,16)} = 52.23, P \le 0.001)$ , and the interaction effects  $(F_{(8,16)} = 7.81, P \le 0.01)$ . The larval weights were significantly lower in diets with leaf, grain or square powder of different crops + Bt than the corresponding diets without Bt, except in diets with square powder of cotton genotype, RCH 2 (Fig. 1A). The H. armigera larval weights were significantly reduced in diets with square powder of cotton Bt RCH 2 + Bt (1.02 mg) and with pod powder of pod borer-tolerant pigeonpea genotype, ICPL 332WR + Bt (1.49 mg) and ICPL 87 + Bt (1.88 mg) as compared to the larvae reared on diets with leaf powder of chickpea genotype, ICCC 37 and the standard artificial diet + Bt. At 10 DAI, the larval

weights differed significantly between the treatments ( $F_{(1,16)} = 183.57$ ,  $P \le 0.001$ ), the genotypes ( $F_{(8,16)} = 124.07$ ,  $P \le 0.001$ ), and the interaction effects ( $F_{(8,16)} = 14.16$ ,  $P \le 0.001$ ). The larval weights were significantly lower in diets impregnated with leaf, grain or square powder of different crops + Bt than the corresponding diets without Bt, except in diets with grain powder of sorghum genotype ICSV 745, pigeonpea genotypes, ICPL 87 and ICPL 332WR, and Bt cotton RCH 2 (Fig. 1B). In diets without Bt, the larval weights were significantly reduced in insects reared on diets with square powder of cotton Bt RCH 2 (12.40 mg) and pod powder of pigeonpea genotype, ICPL 332WR (16.80 mg) than on the standard artificial diet. The larval weights were significantly lower in insects reared on diets with Bt RCH 2 + Bt (2.70 mg), ICPL 332WR + Bt (4.70 mg) and ICPL 87 + Bt (5.80 mg) as compared to the larvae reared on the standard artificial diet + Bt.

Pupal weights differed significantly between the treatments ( $F_{(1,16)} = 6.58$ ,  $P \le 0.01$ ) and genotypes ( $F_{(8,16)} = 6.58$ ,  $P \le 0.01$ ), however, the interaction effects were non-significant. Significant differences were observed in pupal weights between the insects reared on Bt treated and the control diets having leaf powder of chickpea genotype ICC 506EB, square powder of Bt cotton RCH 2 and the standard artificial diet (Fig. 2). The pupal weights were lowest in insects reared on diets with grain flour of the sorghum genotype, ICSV 745 (281.5 mg) and IS 18698 (292.5 mg), and square powder of Bt cotton, RCH 2 (296.6 mg). Among the Bt treated diets, lowest pupal weights were observed in insects reared on diets with Bt RCH 2, RCH 2 and ICSV 745 (273.3, 280.1, and 286.6 mg, respectively). Heaviest pupae were recovered in insects reared on diets with leaf powder of chickpea genotype, ICCC 37 (335.20 mg), and with the pod powder of pigeonpea genotype, ICPL 332WR (335.40 mg).

#### 3.1.2. Larval and pupal periods

There were significant differences in the duration of larval period across treatments  $(F_{(1,16)} = 38.41, P \le 0.001)$  and the genotypes  $(F_{(8,16)} = 36.36, P \le 0.001)$ . The interaction effects were also significant  $(F_{(8,16)} = 3.77, P \le 0.05)$ . Significant differences were observed in larval periods between the larvae reared on diets with and without Bt within a genotype, except those reared on the diet containing leaf powder of the chickpea genotype ICCC 37

(Table 2). In general, larval period was shorter in insects reared on diets without Bt (19.15 days) as compared to those reared on diets with Bt (21.8 days). Larval period was prolonged in insects reared on diets with pod powder of pigeonpea [ICPL 332WR + Bt (30.0 days), and ICPL 87 + Bt (28.3 days)] as compared to the insects reared on diets with leaf powder of chickpea (15.6 days on ICCC 37 and 15.8 days on ICC 506EB).

The pupal period showed significant differences between treatments ( $F_{(1,16)} = 144.95$ ,  $P \le 0.001$ ), genotypes ( $F_{(8,16)} = 47.33$ ,  $P \le 0.001$ ), and the interaction effects ( $F_{(8,16)} = 38.77$ ,  $P \le 0.001$ ). Pupal period of the insects reared on diets with and without Bt showed significant difference, except those reared on diets containing pod powder of the pigeonpea genotype, ICPL 332WR and on the standard artificial diet (Table 2). Longest pupal period was observed in insects reared on diets containing leaf powder of chickpea genotype, ICCC 37 (13.8 days), and the shortest on diets containing grain powder of the sorghum genotype, IS 18698 (9.5 days). In diets containing Bt, the longest pupal period was recorded in insects reared on diet containing leaf powder of ICC 506EB (13.3 days) and the shortest on diets containing square powder of RCH 2 (8.7 days). No adults emerged in insects reared on diets containing sorghum grain powder + Bt (ICSV 745 and IS 18698) and the pod powder of pigeonpea genotypes, ICPL 87.

## 3.1.3. Pupation and adult emergence

There were significant differences in percentage pupation between the treatments  $(F_{(1,16)} = 94.41, P \le 0.001)$ , genotypes  $(F_{(8,16)} = 12.21, P \le 0.001)$ , and the interaction effects  $(F_{(8,16)} = 4.61, P \le 0.01)$ . Percentage pupation was significantly lower in insects reared on diets with leaf, grain, pod or square powder of different crops with Bt than in corresponding diets without Bt (Fig. 3A). Lowest pupation was recorded in the larvae fed on diets with pod powder of pigeonpea genotype, ICPL 87 + Bt (10%), followed by the insects reared on diets with ICPL 332WR + Bt (13.3%), and square powder of Bt RCH 2 + Bt (26.7%). Maximum pupation (90%) was recorded in insects reared on the standard artificial diet without Bt, while 70% pupation was recorded in insects fed on diets with leaf powder of chickpea genotype, ICCC 37 without Bt.

Adult emergence differed significantly across treatments ( $F_{(1,16)} = 198.10$ ,  $P \le 0.001$ ), the genotypes ( $F_{(8,16)} = 10.00$ ,  $P \le 0.001$ ), and the interaction effects ( $F_{(8,16)} = 3.21$ ,  $P \le 0.05$ ). Least adult emergence was recorded in insects reared on diets containing leaf powder of ICC 506EB + Bt (13.3%), followed by 18.9% adult emergence on the standard artificial diet + Bt (Fig. 3B). Among the diets without Bt, adult emergence was greater (73.2%) in insects reared on diets with pod powder of pigeonpea (ICPL 87), leaf powder of chickpea (ICCC 37, 71.3%), and the standard artificial diet (70.4%).

#### 3.1.4. Adult longevity and fecundity

The female longevity differed significantly across treatments ( $F_{(1,16)} = 145.54$ ,  $P \le 0.001$ ) and the genotypes ( $F_{(8,16)} = 7.35$ ,  $P \le 0.001$ ), but the interaction effects were non-significant. There were significant differences in female longevity of the insects reared on diets with and without Bt within the genotypes (Table 3). Female longevity was shortest in insects reared on diets with square powder of Bt cotton RCH 2 (3 days). Females survived for a longer period of time when reared on standard artificial diet without Bt (10.2 days), and on diets with leaf powder of chickpea genotype, ICCC 37 (9.8 days). In diets containing Bt, the females lived longer (5.7 days) when reared on diets with leaf powder of chickpea genotype, ICCC 37, followed by those reared on the standard artificial diet (4.3 days).

Significant differences were observed in male longevity across treatments ( $F_{(1,16)} = 57.92$ ,  $P \le 0.001$ ), the genotypes ( $F_{(8,16)} = 6.69$ ,  $P \le 0.001$ ), and the interaction effects were also significant ( $F_{(8,16)} = 2.47$ ,  $P \le 0.05$ ). Male longevity of the insects reared on the diets with Bt was significantly shorter than those reared on diets without Bt across genotypes (Table 3). The males lived longer when the insects reared on diets containing leaf powder of the chickpea genotypes, ICCC 37 (9.1 days) and ICC 506EB (8.2 days). The shortest male longevity was shown by insects reared on diet containing square powder of Bt cotton RCH 2 (4.0 days). In diets containing Bt, maximum longevity was shown by insects reared on diets containing square powder of cotton RCH 2 (8.1 days), followed by those reared on diets containing leaf powder of chickpea genotype, ICCC 37 (7.3 days). The lowest longevity was observed in insects reared on artificial diet containing Bt (1.3).

Insect fecundity showed significant differences between treatments ( $F_{(1,16)} = 110.91$ , P  $\leq 0.001$ ), the genotypes ( $F_{(8,16)} = 22.26$ , P  $\leq 0.001$ ), and the interaction effects ( $F_{(8,16)} = 7.98$ , P  $\leq 0.001$ ). Significant differences were observed in egg laying between the insects reared on the diets with and without Bt within a genotype (Table 3). Maximum egg laying was recorded in insects reared on diets with leaf powder of the chickpea genotype, ICCC 37 (350 eggs female<sup>-1</sup>) without Bt. No eggs were laid by the insects reared on the diets with Bt, except on those reared on diets with ICCC 37 leaf powder + Bt (153.3 eggs female<sup>-1</sup>) and the standard artificial diet + Bt (58.3 eggs female<sup>-1</sup>). The insects reared on diets with grain flour of sorghum genotype ICSV 745, and square powder of cotton genotype Bt - RCH 2 did not lay any eggs.

3.2. Biological activity of Bt toxin Cry1Ac against H. armigera in artificial diets containing insect -resistant and -susceptible genotypes of chickpea, sorghum, pigeonpea, and cotton

#### 3.2.1. Larval and pupal weights

The larval weight at 5 DAI showed significant differences between the treatments  $(F_{(1.16)} = 162.66, P \le 0.001)$ , genotypes  $(F_{(18.16)} = 58.71, P \le 0.001)$ , and the interaction effects  $(F_{(8.16)} = 8.28, P \le 0.001)$ . The larval weights were significantly lower in insects reared on diets with leaf, grain or square powder of different crops + Cry1Ac than in the insects reared on diets without Cry1Ac, except in diets with square powder of the cotton genotypes RCH 2 and Bt RCH 2, and grain powder of sorghum genotype, ICSV 745 (Fig. 4A). Lowest larval weights were recorded in insects reared on diets (without Cry1Ac) with square powder of Bt RCH 2 (1.9 mg) and highest in insects reared on diets with leaf powder of chickpea genotype, ICCC 37 (14.8 mg). In diets containing Cry1Ac, lowest larval weights were observed in insects reared on diets with pod powder of the pigeonpea genotype, ICPL 332WR (0.9 mg), followed by ICPL 87 (1.0 mg). Maximum larval weights were observed in insects reared on diets with leaf powder of ICCC 37 (6.3 mg). At 10 DAI, there were significant differences in larval weights across treatments ( $F_{(1.16)} = 369.86$ ,  $P \le 0.001$ ), genotypes ( $F_{(8.16)} = 134.60$ ,  $P \le 0.001$ ). The interaction effects were also significant ( $F_{(8.16)} = 28.40$ ,  $P \le 0.001$ ). The larval weights were significantly lower in insects reared on diets with Cry1Ac compared to those

reared on diets without Cry1Ac, except in insects reared on diets with pod powder of pigeonpea genotype, ICPL 332WR and square powder of the cotton genotype, *Bt* RCH 2 (Fig. 4B). The larval weights were lower in insects reared on diets with square powder of *Bt* RCH 2 (18.2 mg), followed by insects reared on diets with pod powder of ICPL 332WR (26.8 mg). Maximum larval weights were shown by insects reared on diets containing leaf powder of ICCC 37 (308.4 mg) and the standard artificial diet (294.1 mg). In diets containing Cry1Ac, lowest larval weights were recorded in insects reared on diets with square powder of *Bt* RCH 2 (4.1 mg), and the pod powder of pigeonpea genotype, ICPL 332WR (12.0 mg). Highest larval weights were recorded in insects reared on diets with leaf powder of chickpea genotype, ICCC 37 (132.3 mg), followed by those reared on the standard artificial diet (104.4 mg).

The pupal weights differed significantly across treatments ( $F_{(1,16)} = 39.25$ ,  $P \le 0.01$ ) and the genotypes ( $F_{(18,16)} = 3.25$ ,  $P \le 0.001$ ). The interaction effects were non-significant. The pupal weights were significantly lower in diets impregnated with leaf, grain or square powder of different crops + Cry1Ac than on diets without Cry1Ac, except in diets with square powder of the cotton genotype, RCH 2 (Fig. 5). Pupal weights were lowest in insects reared on diets with grain flour ICSV 745 (259.8 mg), followed by those fed on diets with pod powder of ICPL 332WR (264.5 mg), and square powder of *Bt* cotton RCH 2 (273.1 mg). Pupal weight was highest in insects fed on diets containing pod powder of ICPL 87 (303.0 mg), followed by those reared on diets with leaf powder of ICCC 37 (300.9 mg) and square powder of RCH 2 (300.6 mg).

## 3.2.2. Larval and pupal periods

Larval periods showed significant differences between the treatments ( $F_{(1,16)} = 17.52$ ,  $P \le 0.001$ ), and the genotypes ( $F_{(8,16)} = 30.12$ ,  $P \le 0.001$ ). The interaction effects were non-significant. Larval periods differed significantly between the insects reared on the diets containing leaf, grain or square powder of different crops + Cry1Ac than those reared on diets without Cry1Ac, except in diets with leaf powder of the chickpea genotype, ICCC 37 (Table 4). Larval period was prolonged (26.57 days) in insects reared on diets with pod powder of

the pigeonpea genotype, ICPL 332WR + Cry1Ac than those reared on diet without Cry1Ac. In diets containing Cry1Ac, larval period was shortest in insects reared on diets with leaf powder of ICCC 37 (16.6 days), followed by those reared on diets with leaf powder ICC 506EB (17.5 days) and the standard artificial diet (17.8 days). The longest larval period was observed in insects fed on diets containing pod powder of ICPL 332WR, with and without Cry1Ac (29. 7 and 23.5 days, respectively).

Pupal period also differed significantly across treatments ( $F_{(1.16)} = 348.39$ ,  $P \le 0.001$ ), genotypes ( $F_{(8.16)} = 85.84$ ,  $P \le 0.001$ ), and the interaction effects ( $F_{(8.16)} = 97.98$ ,  $P \le 0.001$ ). Pupal period of the insects reared on the diets with Cry1Ac + leaf powder of the chickpea genotypes, ICCC 37 and ICC 506EB, and those reared on the standard artificial diet were significantly different than those reared on the corresponding diets without Cry1Ac (Table 4). Pupal period was longer in insects reared on diets containing pod powder of ICPL 332WR (13.67 days) and leaf powder of ICCC 37 (12.7 days) than in insects reared on diets with square powder of Bt RCH 2 (10.4 days) and RCH 2 (10.5 days), and grain powder of IS 18698 (10.5 days). In diets containing Cry1Ac, pupal period was prolonged in insects fed on diets containing leaf powder of ICC 506EB (13.3 days) than those reared on diets containing square powder of Bt RCH 2 (10.2 days) and RCH 2 (10.6 days). No adults emerged from insects reared on Bt diets containing grain powder of the sorghum genotypes, ICSV 745 and IS 18698, and the pod powder of the pigeonpea genotype, ICPL 332WR.

#### 3.2.3. Pupation and adult emergence

There were significant differences in percentage pupation between the treatments  $(F_{(1,16)} = 187.23, P \le 0.001)$  and the genotypes  $(F_{(8,16)} = 52.20, P \le 0.001)$ . The interaction effects were also significant  $(F_{(8,16)} = 5.69, P \le 0.001)$ . Percentage pupation was significantly lower in diets impregnated with leaf, grain or square powder of different crops + Cry1Ac than on diets without Cry1Ac (Fig. 6A). Lowest pupation was recorded in insects reared on diets with square powder of Bt cotton RCH 2 (33.3%), followed by the insects reared on diets containing pod powder of ICPL 332WR (40.0%). In diets containing Cry1Ac, lower pupation was recorded in insects reared on diets with square powder of Bt cotton RCH 2 (13.3%) and

the pod powder of the pigeonpea genotype, ICPL 332WR (23.3%) as compared to the insects reared on the standard artificial diet, and the diets containing leaf powder of ICCC 37 (63.3% each).

The percentage adult emergence differed significantly between the treatments ( $F_{(1.16)}$  = 291.15,  $P \le 0.001$ ) and the genotypes ( $F_{(8.16)}$  = 72.02,  $P \le 0.001$ ). Percentage adult emergence was significantly greater in insects reared on diets without Cry1Ac than the corresponding diets with Cry1Ac (Fig. 6B). Lowest adult emergence was observed in insects reared on diets containing square powder of Bt RCH 2 (23.3%), followed by those reared on diets containing pod powder of ICPL 332WR (26.2%). In diets containing Cry1Ac, adult emergence was lower in insects reared on diets containing square powder of Bt RCH 2 (10.0%) and leaf powder of ICC 506EB (20.0%) than in insects reared on diets containing leaf powder of ICCC 37 (52.4%). There was no adult emergence in insects reared on diets containing Cry1Ac with grain powder of sorghum (ICSV 745 and IS 18698), and pod powder of the pigeonpea genotype, ICPL 332WR.

#### 3.2.4. Adult longevity and fecundity

The female longevity showed significant differences between the treatments ( $F_{(1.16)}$  = 48.54,  $P \le 0.001$ ), genotypes ( $F_{(8.16)}$  = 13.45,  $P \le 0.001$ ), and in interaction effects ( $F_{(8.16)}$  = 2.93,  $P \le 0.05$ ). Female longevity was significantly shorter in Cry1Ac treated diets containing leaf powders of ICCC 37 and ICC 506 EB, pod powder of ICPL 87, square powder of RCH 2 and the standard artificial diet than the corresponding treatments without Cry1Ac (Table 5). Female longevity was shorter in insects reared on diets containing pod powder of ICPL 332WR (3.0 days) as compared to those reared on the standard artificial diet (11.0 days). In diets containing Cry1Ac, the female longevity was shortest in insects reared on diets with square powder of RCH 2 (3.0 days), followed by those reared on diets with pod powder of ICPL 87 (5.7 days). Significant differences were observed in male longevity between the insects reared on the diets with and without Cry1Ac containing leaf powder of ICC 506EB and pod powder of ICPL 87 (Table 5). Longevity of males was shortest in insects reared on diets with pod powder of ICPL 332WR (2.3 days), and longest in insects reared on diets

containing leaf powder of ICCC 37 and ICC 506EB (9.7 and 9.0 days, respectively). Among the Cry1Ac containing diets, shortest longevity of males was observed in insects reared on diets containing pod powder of ICPL 87 (4.3 days) and the highest in those reared on diets containing leaf powder of ICCC 37 (9.0 days). Fecundity of the insects reared on diets with Cry1Ac + leaf powder of ICCC 37, pod powder of ICPL 87 and the standard artificial diet was significantly reduced than the insects reared on the corresponding diets without Cry1Ac (Table 5). There was no egg laying by the insects reared on diets with leaf powder of ICC 506EB + Cry1Ac, grain flour of IS 18698 + Cry1Ac, and ICSV 745 with and without Cry1Ac, pod powder of ICPL 332WR with and without Cry1Ac, square powder of RCH 2 + Cry1Ac, and Bt RCH 2 with and without Cry1Ac. Highest numbers of eggs were laid by the insects reared on the standard artificial diet (450 eggs female<sup>-1</sup>), followed by those reared on diets with leaf powder of the chickpea genotype, ICCC 37 (416.7 eggs female<sup>-1</sup>). The lowest numbers of eggs were laid by the insects reared on diets containing square powder of RCH 2 and grain powder of IS 18698 (200 eggs, each).

#### 4. Discussion

Biological control in combination with host plant resistance will form an important component of insect pest management. However, the compatibility between different components of pest management is highly important. Host plant resistance may improve or reduce the effectiveness of insect pathogens, depending on the nature of plant resistance. Allelochemicals, one of the important components of resistance to insects, at times antagonize the biological activity of biopesticides because of their antifeedant activity. In some cases, the biological activity of the microbial pesticides increases as a result of synergistic activity. Weights of *H. armigera* larvae were significantly lower on diets containing leaf, grain, pod or square powder in combination with *Bt* or Cry1Ac than the corresponding diets without *Bt* or Cry1Ac. However, significantly lower larval weights were recorded in insects fed on *Bt* treated diet containing *Bt* RCH 2 and the pigeonpea and sorghum genotypes at 5 DAI; and in diets with *Bt* RCH 2 and the pigeonpea genotypes at 10 DAI. These differences may be because of the differences in nutritional quality of the artificial diets because of the secondary

metabolites in these crops on the biological activity of Bt. Square powder of Bt cotton RCH 2 along with Bt/Cry1Ac resulted in lowest weights of H. armigera larvae because of the possible synergistic effect of secondary plant metabolites such as gossypol, tannins, and anthocyanins present in cotton (Sharma and Agarwal, 1982) on the biological activity of Bt. Maximum growth of H. armigera larvae has been reported earlier on chickpea leaves and flowers, followed by pigeonpea (Bilapate, 1988). The Helicoverpa zea Boddie and H. armigera larvae prefer to feed on reproductive parts, which contain more nitrogen than the foliage (Fitt, 1991). Larval period was prolonged in insects reared on diets with pod powder of pod borer resistant pigeonpea genotype, ICPL 332WR +Bt as compared to the insects reared on diets with leaf powder of chickpea genotype, ICCC 37. Low larval weights and prolonged larval periods of the larvae reared on diets with pod powder of pigeonpea may be because of the antibiotic effects of secondary metabolites in pigeonpea (Green et al., 2002).

The ability of host crops to influence the toxicity of microbial insecticides has been well documented (Gore et al., 2003). Gypsy moth larvae fed on alder are less susceptible to *B. thuringiensis* than the larvae fed on Douglas fir (Moldenke et al., 1994). Meade and Hare (1993) also observed high efficacy of *B. thuringiensis* sp. *kurstaki* on insect-resistant genotype (P 1357333) of *Apium graveolens* var. *rapaceum* (L.) as compared to the susceptible one (P 1223333). No eggs were laid by the insects reared on the diets with *Bt*, except the insects reared on diets with ICCC 37 leaf powder + *Bt* (153.3 eggs female<sup>-1</sup>), and the standard artificial diet + *Bt* (58.3 days), suggesting that *Bt* in combination with insect-resistant genotypes not only have adverse effects on the survival and development, but also influences the fecundity of the surviving insects adversely. There is a significant reduction in susceptibility of *H. armigera* and *Helicoverpa punctigera* (Wallen.) to *B. thuringiensis* formulations MVP II and Dipel<sup>®</sup> when fed on sweet corn than on cotton (Holloway and Dang, 2000).

The influence of host crop on insect susceptibility to biopesticides could be due to differential rates of growth on various host plants, because of the differences in their nutritional quality, and the amounts of secondary metabolites. The proteinaceous diets – such

as those of chickpea and pigeonpea, were more nutritious for insect growth and development than the non-proteinaceous diets such as those of sorghum and cotton. Plant resistance may improve or reduce the effectiveness of insect pathogens depending on the nature of host plant resistance to insects (Barbosa, 1988). In the present study, survival, development, and fecundity of H. armigera were poor on diets containing lyophilized tissue of insect-resistant genotypes +Bt as compared to that on the insect susceptible genotypes +Bt. The present studies demonstrated that biological activity of Bt is not only influenced by the host plant, but also by the level of genotypic resistance to insect pests. Therefore, it is important to take into consideration genotypic resistance to the target insect species for developing transgenic plants expressing Bt toxin proteins for pest management.

Development of improved crop varieties with resistance or tolerance to H. armigera is highly desirable, particularly for the subsistence farming systems in the developing countries. Plant resistance improves the effectiveness of B. thuringiensis. In some cases, the biological activity of the microbial pesticides increases as a result of synergistic activity. Insect pathogens can be more effective in a pest management programs if antibiosis factors of host resistance are compatible with the biopesticides. Therefore, Bt genes deployed in transgenic plants will be more effective in pigeonpea and cotton, and that it will be more useful to deploy the Bt genes in genotypes with same degree of resistance to target insect pests.

#### Acknowledgements

The authors are thankful to staff of entomology, ICRISAT, for their help in these studies, and to the Department of Agriculture and Cooperation, Govt of India, for the financial support.

#### References

Acharjee, S., Sarmah, B.K., Kumar, P.A., Olsen, K., Mahon, R., Moar, W.J., Moore, A., Higgins, T.J.V., 2010. Expression of a sequence-modified cry2Aa gene for resistance to *Helicoverpa armigera* in chickpea (*Cicer arietinum* L.). Plant Sci. 178, 333-339.

- Appel, H.M., Schultz, J.C., 1994. Oak tannins reduce effectiveness of Thuricide (*Bacillus thuringiensis*) in the gypsy moth (Lepidoptera: Lymantridae). J. Econ. Entomol. 87, 1736-1742.
- Armes, N.J., Bond, G.S., Cooker, R.J., 1992. The laboratory culture and development of *Helicoverpa armigera*. Natural Resources Institute Bulletin No. 57, Natural Resources Institute, Chatham, UK, pp. 20-21
- Barbosa, P., 1988. Natural enemies and herbivore-plant interactions: influence of plant allelochemicals and host specificity. In: Barbosa P, Letourneau DK (eds.), Novel Aspects of Insect-plant-interactions. John Wiley and Sons, New York, USA. pp. 201-229.
- Dhillon, M.K., Sharma, H.C., 2010. Influence of seed treatment and abiotic factors on damage to *Bt* and non-*Bt* cotton genotypes by the serpentine leaf miner *Liriomyza trifoli* (Diptera: Agromyzidae). Int. J. Trop. Insect Sci. 30, 127-131.
- Dhillon, M.K., Sharma, H.C., 2012. Comparative studies on the effects of Bt-transgenic and nontransgenic cotton on arthropod diversity, seed cotton yield and bollworms control.J. Environ. Biol. 34, 67-73
- Fitt, G.P., 1991. Host plant selection in *Heliothinae*. In: Bailey WJ, Ridsdill-Smith TJ (Eds.), Reproductive Behavior in Insects-Individuals and Populations. Chapman and Hall, London, United Kingdom, pp. 172-201.
- Girijashankar, V., Sharma, H.C., Sharma, K.K., Swathisree, V., Prasad, L.S., Bhat, B.V., Royer, M., Secundo, B.S., Narasu, M.L., Altosaar, I., Seetharama, N., 2005. Development of transgenic sorghum for insect resistance against the spotted stem borer (*Chilo partellus*). Plant Cell Rep. 24, 513-22.
- Gore, J., Leonard, B.R., Jones, R.H., 2003. Influence of agronomic hosts on the susceptibility of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) to genetically engineered and non-engineered cottons. Environ. Entomol. 32, 103-110.

- Green, P.W.C., Stevenson, P.C., Simmonds, M.S.J., Sharma, H.C., 2002. Can larvae of the pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), select between wild and cultivated pigeonpea [(*Cajanus* sp. (Fabaceae)]. Bull. Entomol. Res. 92, 45-51.
- Gujar, G.T., Mittal, A., Kumari, A., Kalia, V., 2004. Host crop influence on the susceptibility of the American bollworm, *Helicoverpa armigera*, to *Bacillus thuringiensis* ssp. *kurstaki* HD-73. Entomol. Exp. Applic. 113, 165-172.
- Holloway, J.W., Dang., H., 2000. Monitoring susceptibility of *Bt* toxins in Australian *Helicoverpa* species. Proceedings of the 10<sup>th</sup> Australian Cotton Conference, Australian Cotton Co-operative Research Centre, Brisbane, Australia, pp. 189-194.
- James, C., 2009. Global status of commercialized biotech/GM crops. International Service for Acquisition on Agri-Biotech Applications (ISAAA), Ithaca, New York, USA.
- Kouassi, K.C., Lorenzetti, F., Guertin, C., Cabana, J., Mauffette Y., 2001. Variation in the susceptibility of the forest tent caterpillar (Lepidoptera: Lasiocampidae) to *Bacillus thuringiensis* variety *kurstaki* HD-1: Effect of the host plant. J. Econ. Entomol. 94, 1135-1141.
- Kranthi, K.R., Jadhav, D.R., Kranthi. S., Wanjari, R.R., Ali S.S., Russel, D.A., 2002. Insecticide resistance in five major pests of cotton in India. Crop Prot. 21, 449-460.
- Luthy, P., Hoffmann, C., Jaquet, F., 1985. Inactivation of delta-endotoxin of *Bacillus thuringiensis* by tannin. FEMS Microbiol. Lett. 28, 31-33.
- Manjunath, T.M., Bhatnagar, V.S., Pawar, C.S., Sitanatham, S., 1989. Economic Importance of *Heliothis* spp. in India and an assessment of their natural enemies and host plants.
  In: King EG, Jackson RD (Eds.), Proceedings of the Workshop on Biological Control of *Heliothis* Increasing the Effectiveness of Natural Enemies. US Department of Agriculture, New Delhi, India, pp. 196-278.

- Meade, T., Hare, J.D., 1993. Effects of differential host plant consumption by *Spodoptera* exigua (Lepidoptera: Noctuidae) on *Bacillus thuringiensis* efficacy. Environ. Entomol. 22, 432-437.
- Mohan, S., Ma, P.W.K., Williams, W.P., Luthe, D., 2008. A naturally occurring plant cysteine protease possesses remarkable toxicity against insect pests and synergizes *Bacillus thuringiensis* toxin. PLoS ONE 3, 1-7.
- Moldenke, A.F., Berry, R.E., Miller, J.C., Wernz, J.G., Li, X.H., 1994. Toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to gypsy moth, *Lymantria dispar*, fed with alder or Douglas-fir. J. Invert. Pathol. 64, 143-145.
- Narayanamma, V.L., Sharma, H.C., Gowda, C.L.L., Sriramulu, M., 2007. Incorporation of lyophilized leaves and pods into artificial diets to assess the antibiosis component of resistance to pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. Int. J. Trop. Insect Sci. 27, 191-198.
- Narayanamma, V.L., Gowda C.L.L., Sriramulu M., Ghaffar, M.A., Sharma, H.C., 2013. Nature of gene action and maternal effects for pod borer, *Helicoverpa armigera* resistance and grain yield in chickpea, *Cicer arietinum*. Am. J. Pl. Sci. 4, 26-37.
- Olsen, K.M., Daly, J.C., Tanner, G.J., 1998. The effect of cotton condensed tannin on the efficacy of the Cry1Ac δ-endotoxin of *Bacillus thuringiensis*. Proceedings of the 9<sup>th</sup> Australian Cotton Conference 337-342. Australian Cotton Growers Research Association, Wee Waa, New South Wales, Australia.
- Sharma, H.C. (Ed.), 2005. *Heliothis/Helicoverpa* management: emerging trends and strategies for future research. Oxford and IBH Publishers,, New Delhi, India, 469 pp.
- Sharma, H.C. (Ed.), 2009. Applications of biotechnology in pest management and ecological sustainability. CRC Press, Taylor and Francis, Boca Raton, Florida, USA, 526 pp.

- Sharma, H.C., Agarwal, R.A., 1982. Effect of some antibiotic compounds in *Gossypium* on the post-embryonic development of spotted bollworm (*Earias vittella* F.). Entomol. Exp. Appl. 31: 225-228.
- Sharma, H.C., Dhillon, M.K., Arora, R., 2008. Effects of *Bacillus thuringiensis* δ-endotoixn-fed *Helicoverpa armigera* on the survival and development of the parasitoid *Campoletis chlorideae*. Entomol. Exp. Applic. 126, 1-8.
- Sharma, H.C., Pampapathy, G., Lanka, S.K., Ridsdill Smith, T.J., 2005. Antibiosis mechanism of resistance to pod borer, *Helicoverpa armigera* in wild relatives of chickpea. Euphytica 142, 107-117.
- Sharma, H. C., Sharma, K.K., Crouch, J.H., 2004. Genetic transformation of crops for insect resistance: potential and limitations. CRC Crit. Rev. Plant Sci. 23, 47-72.
- Sharma, H.C., Singh, B.U., Hariprasad, K.V., Bramel-Cox, P.J., 1999. Host plant resistance to insects in integrated pest management for a safer environment. Proc. Acad. Env. Biol. 8, 113-136.
- Sharma, H.C., Sujana, G., Manohar Rao, D., 2009. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. Arth. Plant Inter. 3, 151-161.
- Sharma, H.C., Vidyasagar, P., Subramanian, V., 1993. Antibiosis component of resistance in sorghum to sorghum midge, *Contarinia sorghicola*. Ann. Appl. Biol. 123, 469-483.

## Figure captions

- **Fig. 1** Weights of *H. armigera* larvae at 5 (A) and 10 (B) days after inoculation on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without *Bt*.
- **Fig. 2** Weights of *H. armigera* pupae on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EBEB), pod powder of pigeonpea (ICPL 87 and ICPL 332WRWR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without *Bt*.
- **Fig. 3** Percentage pupation (A) and adult emergence (B) of *H. armigera* on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without *Bt*.
- **Fig. 4** Weights of *H. armigera* larvae at 5 (A) and 10 (B) days after inoculation on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without Cry1Ac.
- **Fig. 5** Weights of *H. armigera* pupae on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without Cry1Ac.
- **Fig. 6** Percentage pupation (A) and adult emergence (B) of *H. armigera* on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without Cry1Ac.

**Table 1** Composition of the artificial diet used to assess effectiveness of Bt on different host plants.

Ingredients	Quantity			
Chickpea flour	55 g			
Lyophilized leaf/ pod powder	20 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	112.5 mL			
Agar-agar solution				
Agar-agar	4.325 g			
Water	200 mL			

Table 2 Larval and pupal periods of *H. armigera* reared on artificial diet with lyophilized chickpea leaf, sorghum grain, pigeonpea pod, and cotton square powders and *Bt* (0.0125%).

Genotypes _		Larva	l period (days)	s) Pupal period (days)
		Without I	Bt With Bt	Without Bt With Bt
Chickpea	ICCC 37	15.56 <sup>a</sup>	15.89 <sup>ab</sup>	13.77 <sup>fg</sup> 12.00 <sup>efg*</sup>
_	ICC 506EB	15.75 <sup>ab</sup>	17.87 <sup>abc*</sup>	$10.72^{\text{cde}}$ $13.33^{\text{fg*}}$
Sorghum	IS 18698	19.33 <sup>cde</sup>		9.53 <sup>bc</sup> -
	ICSV 745	19.37 <sup>cde</sup> 20.53		
Pigeonpea	ICPL 87	$21.54^{\rm efg}$		11.90 <sup>defg</sup> -
	ICPL 332WR	23.31 <sup>gh</sup>	$30.00^{i^*}$	$12.17^{\rm efg}$ $11.67^{\rm def^*}$
Cotton	RCH 2	18.50 <sup>bcd</sup>	19.40 <sup>cde*</sup>	
	Bt RCH 2	$22.60^{fg}$	25.43 <sup>h*</sup>	$11.20^{\text{cde}}$ $12.01^{\text{efg}*}$
	Standard artificial diet	16.44 <sup>ab</sup>	18.76 <sup>bcd*</sup>	11.39 <sup>cde</sup> 11.67 <sup>def</sup>
	Mean	19.15	21.79	7.71 11.40
	For comparing	DF	Fp SE± I	LSD DF Fp SE± LSD
	Bt treatments	1,16 <0	001 0.42 0	0.86** 1,16 <0.001 0.30 0.62**
	Genotypes	8,16 <0	.001 0.90 1	1.83** 8,16 <0.001 0.65 1.32**
	Bt X Genotypes	8,16 0.0	02 1.27 2	2.59* 8,16 <0.001 0.92 1.87**

Figures followed by the same letter within a column are not significantly different at  $P \le 0.05$ . Figures with an '\*' within a row indicates significant differences between the diets with and without Bt within a genotype. DF = Degrees of freedom. LSD = Least significant difference. SEM = standard error of mean.

Table 3 Longevity and fecundity of H. armigera adults reared on artificial diet with lyophilized chickpea leaf, sorghum grain, pigeonpea pod, and cotton square powders and Bt (0.0125%).

		Female longevity (days)		Male longevity (days)		Fecundity (eggs laid female <sup>-1</sup> )	
Genotypes		Without Bt	With Bt	Without Bt	With Bt	Without Bt	With Bt
Chickpea	ICCC 37	9.83	5.67*	9.14 <sup>e</sup>	7.33 <sup>cde*</sup>	350.0 <sup>e</sup>	153.3 <sup>bc*</sup>
	ICC 506EB	8.67	-	8.17 <sup>de</sup>	4.67 <sup>bcd*</sup>	$300.0^{de}$	-
Sorghum	IS 18698	6.00	-	$7.50^{\text{cde}}$	-	83.3 <sup>ab</sup>	-
	ICSV 745	6.67	-	$4.00^{\mathrm{bc}}$	-	-	-
	ICPL 87	7.89	-	7.44 <sup>cde</sup>	-	296.7 <sup>de</sup>	-
Pigeonpea	ICPL 332WR	9.00	2.67*	6.83 <sup>cde</sup>	-	106.7 <sup>b</sup>	-
Cotton	RCH 2	6.67	-	$7.40^{\rm cde}$	8.12 <sup>de</sup>	216.7 <sup>cd</sup>	-
	Bt RCH 2	3.00	-	$3.99^{bc}$	-	-	-
	Standard artificial diet	10.17	4.33*	7.89 <sup>de</sup>	1.33 <sup>ab*</sup>	316.7 <sup>e</sup>	58.3 <sup>ab*</sup>
	Mean	7.54	1.41	6.93	2.38	242.2	105.8
	For comparing	DF Fp	SE± LSD	DF Fp S	SE± LSD	DF Fp	SE± LSD
	Bt treatments	1,16 < 0.001	0.50 1.03**	1,16 < 0.001 (	0.59 1.21**	1,16 < 0.001	15.64 31.8**
	Genotypes	8,16 < 0.001	1.07 2.19**	8,16 < 0.001	1.26 2.56**	8,16 < 0.001	33.18 67.4**
	Bt X Genotypes	8,16 0.285	1.52 NS	8.16 0.032	1.79 3.64*	8,16 < 0.001	46.92 95.4**

Figures followed by the same letter within a column are not significantly different at  $P \le 0.05$ . Figures with an '\*' within a row indicates significant differences between the diets with and without Bt within a genotype. DF = Degrees of freedom. LSD = Least significant difference. SEM = standard error of mean.

Table 4. Larval and pupal periods of H. armigera reared on artificial diet with lyophilized chickpea leaf, sorghum grain, pigeonpea pod, and cotton square powders and Bt toxin protein Cry1Ac (5 ng ml<sup>-1</sup>).

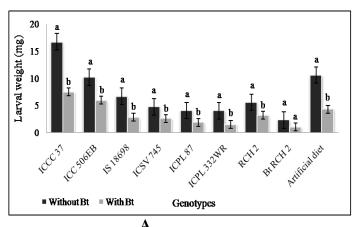
Genotypes			al period lays)	Pupal period (days)		
		Without Cry1Ac	With Cry1Ac	Without Cry1Ac	With Cry1Ac	
Chickpea	ICCC 37	16.04	16.58	12.71 <sup>de</sup>	11.67 <sup>cd*</sup>	
	ICC 506EB	16.48	17.4*	11.67 <sup>cd</sup>	13.33 <sup>e*</sup>	
Sorghum	IS 18698	19.79	20.78*	10.50 <sup>bc</sup>	-	
	ICSV 745	21.89	21.83	12.50 <sup>de</sup>	-	
Pigeonpea	ICPL 87	22.03	24.59*	12.03 <sup>d</sup>	12.17 <sup>d</sup>	
	ICPL 332WR	23.47	29.67*	13.67 <sup>e</sup>	-	
Cotton	RCH 2	17.51	19.00*	10.53 <sup>bc</sup>	10.56 <sup>bc</sup>	
	Bt RCH 2	22.28	24.48*	10.42 <sup>bc</sup>	10.24 <sup>b</sup>	
	Standard artificial diet	16.23	17.75*	12.42 <sup>de</sup>	12.89 <sup>de*</sup>	
	Mean	19.52	21.34	11.83	7.87	
	For comparing	DF Fp	SE± LSD	DF F	p SE± LSD	
	Cry1Ac treatments	1,16 < 0.00	1 0.43 0.88**	1,16 <0.0	001 0.21 0.43**	
	Genotypes	8,16 < 0.00	1 0.92 1.87**	8,16 < 0.0	001 0.45 0.91**	
	Bt X Genotypes	8,16 0.082	1.30 NS	8,16 <0.0	001 0.63 1.29**	

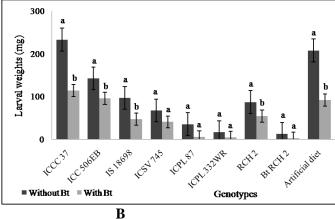
Figures followed by the same letter within a comparison are not significantly different at  $P \le 0.05$ . Figures with an '\*' within a row indicates significant differences between the diets with and without Cry1Ac within a genotype. DF = Degrees of freedom. LSD = Least significant difference. SEM = standard error of mean.

Table 5. Longevity and fecundity of H. armigera adults reared on artificial diet with lyophilized chickpea leaf, sorghum grain, pigeonpea pod, and cotton square powder and Bt toxin protein Cry1Ac (5 ng ml<sup>-1</sup>).

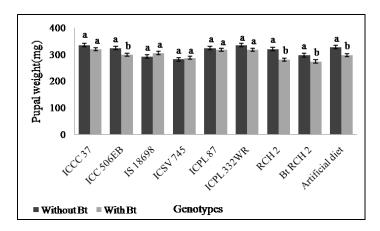
Genotypes		Female longevity (days)		Male longevity (days)		Fecundity (eggs laid female <sup>-1</sup> )	
		Without Cry1Ac	With Cry1Ac	Without Cry1Ac	With Cry1Ac	Without Cry1Ac	With Cry1Ac
Chickpea	ICCC 37	10.33 <sup>cd</sup>	8.33 <sup>cd*</sup>	9.67 <sup>d</sup>	9.00 <sup>d</sup>	416.70 <sup>f</sup>	100.00 <sup>bc*</sup>
	ICC 506EB	9.33 <sup>cd</sup>	7.33 <sup>cd*</sup>	$9.00^{d}$	5.67 <sup>c*</sup>	266.70 <sup>e</sup>	-
Sorghum	IS 18698	$8.00^{cd}$	-	8.33 <sup>cd</sup>	-	$200.00^{de}$	-
	ICSV 745	9.67 <sup>d</sup>	-	8.67 <sup>d</sup>	-	-	-
Pigeonpea	ICPL 87	9.00 <sup>cd</sup>	5.67 <sup>bc*</sup>	8.33 <sup>cd</sup>	4.33 <sup>b*</sup>	233.30 <sup>de</sup>	50.00 <sup>ab*</sup>
	ICPL 332WR	3.00 <sup>ab</sup>		2.33 <sup>ab</sup>	-	-	-
Cotton	RCH 2	8.67 <sup>bcd</sup>	$3.00^{ab^*}$	8.33 <sup>cd</sup>	-	$200.00^{\mathrm{ed}}$	-
	Bt RCH 2	-	-	$0.00^{a}$	-	-	-
	Standard artificial diet	11.00 <sup>e</sup>	$7.00^{\mathrm{bc}*}$	8.33 <sup>cd</sup>	8.33 <sup>cd</sup>	$450.00^{\rm f}$	150.00 <sup>cd*</sup>
	Mean	7.67	3.48	7.00	2.85	196.30	33.30
	For comparing	DF Fp	SE± LSD	DF Fp	SE± LSD	DF Fp	SE± LSD
	Cry1Ac treatments	1,16 < 0.001	0.60 1.22**	1,16 <0.00	01 0.47 0.96**	1,16 <0.0	01 15.47 31.45**
	Genotypes	8,16 < 0.001	1.24 2.59**	8,16 < 0.00	01 1.00 2.03**	8,16 < 0.0	01 32.83 66.71**
	Bt x Genotypes	8,16 0.013	1.80 3.66*	8,16 < 0.00	01 1.41 2.88**	8,16 < 0.0	01 46.42 94.34**

Figures followed by the same letter within a comparison are not significantly different at  $P \le 0.05$ . Figures with an '\*' within a row indicates significant differences between the diets with and without Cry1Ac within a genotype. DF = Degrees of freedom. LSD = Least significant difference. SEM = standard error of mean.

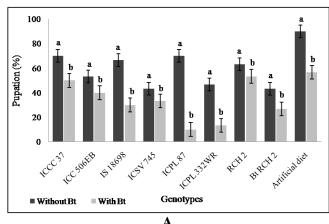


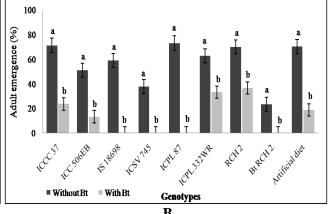


**Fig. 1** Weights of *H. armigera* larvae at 5 (A) and 10 (B) days after inoculation on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without *Bt*.

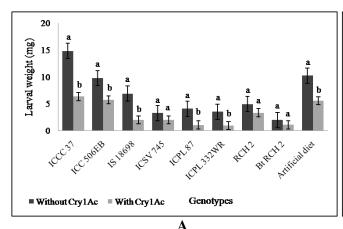


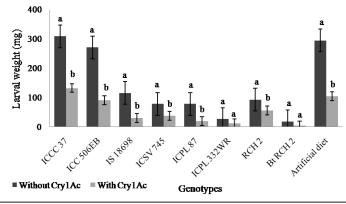
**Fig. 2** Weights of *H. armigera* pupae on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EBEB), pod powder of pigeonpea (ICPL 87 and ICPL 332WRWR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2)and non *Bt* cotton (RCH 2) with and without *Bt*.



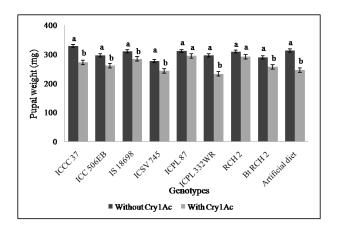


**Fig. 3** Percentage pupation (A) and adult emergence (B) of *H. armigera* on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without *Bt*.

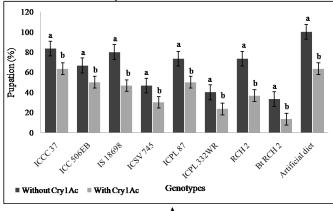


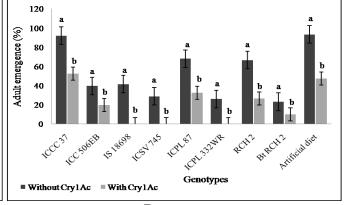


**Fig. 4** Weights of *H. armigera* larvae at 5 (A) and 10 (B) days after inoculation on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without Cry1Ac.



**Fig. 5** Weights of *H. armigera* pupae on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without Cry1Ac.





**Fig. 6** Percentage pupation (A) and adult emergence (B) of *H. armigera* on artificial diets with leaf powder of chickpea (ICCC 37 and ICC 506EB), pod powder of pigeonpea (ICPL 87 and ICPL 332WR), grain powder of sorghum (ICSV 745 and IS 18698), and *Bt* (*Bt* RCH 2) and non *Bt* cotton (RCH 2) with and without Cry1Ac.