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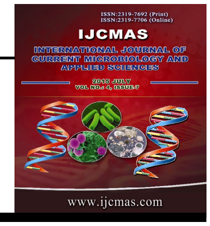

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Original Research Article

Combined effect of *Bacillus thuringiensis* and *Bacillus subtilis* against *Helicoverpa armigera*

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A B S T R A C T

Bacillus thuringiensis (*Bt*), *B. subtilis* (*Bs*) and cell suspensions of *Bt/Bs* mixture were added to an artificial diet of *Helicoverpa armigera* and the biological effects of the bacteria were evaluated by measuring feeding, growth, food utilization and behavioral response of *H. armigera* under laboratory conditions. Effects of *Bt* and *Bs* in the artificial diet were evaluated with the different spore concentration of 1×10^2, 1×10^4, 1×10^6, 1×10^8 and 1×10^{12} spores/ml. *Bt*/*Bs* food consumption, digestion, relative consumption and growth rate, efficiency of conversion of ingested and digested food values declined significantly, compared to the different concentration of *Bt* and *Bs* in the artificial diet, while at the same time a significant increase in approximate digestibility was also observed. The consumption dependent growth efficiency of larvae fed a bacteria-free diet was significantly higher than the growth efficiency of larvae fed amended diets. The LC_{50} values were estimated to calculate the lethal dose for mortality of *H. armigera*, the lethal concentration values were significantly lower level in the *Bt*/*Bs* mixture than *Bt* and *Bs* individually.

Keywords

Bacillus thuringiensis, Bacillus subtilis, Nutritional indices

Introduction

In the past decades, synthetic pesticides and chemical fertilizers in modern agriculture are being removed from the market for their hazardous encroachment to the natural environment. To overcome with these problems, biological control agents, which admit effective microorganisms and microbial products, and organic fertilizers have been attracting aid recently as alternatives to chemical agents (Shoda, 2000).

Bacillus thuringiensis (*Bt*) and *Bacillus subtilis* (*Bs*) are gram positive bacterium, ubiquitous, spore-forming soil bacterium. *Bt* produces crystalline inclusions containing entomocidal proteins, also referred as *Bt* toxins, or δ-endotoxins, during the sporulation process. Preparations containing spores and protein crystals of *Bt* have been used as microbial pesticides since the 1970s (Navon, 2000; Inatsu *et al.*, 2006). *Bt* strains produce a variety of crystal proteins each with its distinct host ranges (Kumar *et al.*, 1996). The inactive protoxins are proteolytically digested in the insect midgut to form active toxins. Their toxicity is achieved by binding to the midgut cells of insects and causing osmotic lysis through pore formation in the midgut (Gill *et al.*, 1992).

Gupta and Vyas, (1989) described *B. subtilis* cause mortality to *Anopheles culicifacies*, vector of malaria in India. However, efficient formulation consisting of *B. subtilis* lipopeptide biosurfactant used for control of *Drosophila melanogaster* (Assie *et al.*, 2002), *Culex quinquefasciatus* (Das and Mukherjee, 2006), *A. stephensi* (Geetha and Manonmani, 2008) and *Aedes aegypti* (Geetha *et al.*, 2010). Interestingly, *B. subtilis* has been revealed that biological control agent against *Spodoptera littoralis* (Abd El-Salam *et al.*, 2011; Ghribi *et al.*, 2012).

Bt resistance *Helicoverpa armigera* is widely spread occurring in Asia, Africa, Australia and Europe. It causes heavy economic losses in India. *H. armigera* developed resistance to many synthetic pesticides and chemical fertilizer (Kranthi *et al.*, 2000; Baskar *et al.*, 2009). It is prevalent pest of legume crops in India (Cowgill and Bhagwat, 1996). In India, economic losses in yield, 158 million US$ during 1996-1997 and 54% of the total insecticides was exhausted for the shelter of the cotton crop for the dangerous pests (Jalali *et al.*, 2004). In high pest consideration arises from the predilection of foraging larvae for plant structures rich in nitrogen such as flowers, pods and panicles (Fitt, 1989).

H. armigera has developed resistance to most established pesticides (Armes *et al.*, 1996) that is illustrated by its capability to formulate resistance against the *Bt* protein in laboratory condition (Kranthi *et al.*, 2000). Resistance development in insects against *Bt* proteins can be managed by various evasive actions (Tabashnik, 1994; Kumar, 1996; Babu *et al.*, 2002) of which gene roataion and pyramiding are more predicting as means to delay onset of resistance (Chakrabarti *et al.*, 1998; Gould, 1998)

Hence, the search of *Bt* protein mixed with *Bs*, they could combat resistant *H. armigera*. Our results indicate, that best alternatives insecticide used for transgenic plants and resistance insect pests.

Materials and Methods

Bacterial strains and culture conditions

Bacillus strains were isolated from soils of the Kadayam range, Triunelveli (District), Tamil-Nadu. Several isolates of *Bacillus* spp. were selected using screening procedures described previously (Cavaglieri *et al.*, 2004). Selected isolates were identified as *Bs* by testing biochemical characterization according to Bergey's manual.

Isolation of *Bt* was conducted according to the method of (Ohba and Aizawa, 1986; Travers *et al.*, 1987). One gram of each sample was suspended in 10 ml sterile distilled water and pasteurized at 80°C for 30 min. *Bt* was selected by adding 1ml of each suspension to 10ml of Luria-Bertani (LB) broth buffered with 0.25M sodium acetate pH 6.8. The suspensions were incubated at 30°C for 4h and then heated to 80°C for 3min. Suspensions were diluted and plated on nutrient agar medium and incubated at 30°C for 24 h. Smears were examined under the light microscope to verify presences of the parasporal bodies found in *Bt*.

Molecular identification

The PCR amplification was performed using a PCR machine (Peltier). The 16S rRNA gene in *Bs* was amplified by PCR using the following universal primers forward primer 5’- CGGAACGCCGCACGATATGTA-3’ reverse primer 3’- GGCGGCTTCCACT AGTTTTCC- 5’ PCR program was set to denaturation at 94ºC for 1 min annealing at 55ºC for 1 min and extension at 72ºC for 1 min for a total of 35 cycles. Also, the Cry gene in *Bt* was amplified by PCR using the following Cry primers forward primer 5’-TGACCAGGTCCCTTGATTAC-3’ reverse primer 3’-GGTGCTTCCTATTCCTTTG GC- 5’ and the PCR program was set to denaturation at 94ºC for 1 min. annealing at 45ºC for 1 min. and extension at 72ºC for 1 min for a total of 25 cycles. The PCR product was determined by comparing them with 1kbp ladder marker (Medox).

Mixed culture

Previously isolated, *Bs* and *Bt* were subcultured on separate nutrient agar plates and incubated at 37°C for 24 h. Single colonies of these microorganisms were transferred separately into 100 ml of the nutrient broth medium (0.8%) and incubated for 24 h at 37ºC on an orbital shaker at 100 rpm. Aliquots (50 ml) of each culture medium were centrifuged (Eltek) at 9820g for 15 min. The cell pellets were washed and resuspended in autoclaved normal saline (0.89% NaCl in distilled water) and the optical density (OD) at 660nm (Spectro UV–vis RS Spectrophotometer, USA) was adjusted to 0.7 with normal saline. Five milliliters of each cell suspension was mixed in a sterilized screw-capped test tube. The resultant cell suspension was the mixed bacterial culture (Shabir *et al.,* 2008).

Insect culture

Nearly 250 larvae were collected from the cotton field in Alwarkurichi, Tirunelveli, Tamil Nadu to establish as a stock culture of *H. armigera*.

They were cultured on a semi-synthetic diet, which contains chickpea as its main component (Patel *et al.,* 1968). Larvae were inspected regularly to ensure that they remained pathogen-free, and were reared and maintained. The colony was maintained in a culture room with a mean temperature of 27C, 60% RH and with a photoperiod of 14:10 (L: D).

Bioassay

Bioassays were performed on first to fourth instars of *H. armigera* using concentrations of (1×10^2, 1×10^4, 1×10^6, 1×10^8 and 1×10^{12} spores/ml) of *Bs*, *Bt* and *Bs/Bt*. untreated served as a control. A minimum of 20 larvae per concentration was used for all the experiments and the experiments were replicated five times (total, n = 100). The lethal concentration (LC_{50}) was calculated using probit analysis (Finney, 1971).

For mortality studies, 20 larvae each of first, second, third, and fourth instars were introduced to a 250-ml glass beaker containing various concentrations of the *Bs*, *Bt* and *Bs/Bt*. The treatments were replicated five times, and each replicate set contained one control (total, n = 100). Percentage mortality in the treatments was corrected when necessary for mortality in the controls using Abbott’s (1925) formula (Senthil-Nathan *et al.,* 2008).

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Larval duration, food consumption and utilization

The evaluations of nutritional indices of *H. armigera* were done (Waldbauer, 1968; Henn and Solter, 2000; Senthil-Nathan *et al.*, 2007). Relative consumption rate RCR = dry weight of food eaten/duration of feeding (days) × mean dry weight of the larva during the feeding period, relative growth rate RGR = dry weight gain of larva during the period/duration of feeding (days) × mean dry weight of the larva during the feeding period, approximate digestibility AD = 100 × dry weight of food eaten - dry weight of feces produced)/dry weight of food eaten, Efficiency of conversion of ingested food ECI = 100 × dry weight gain of larva/dry weight of food eaten, and efficiency of conversion of digested food ECD = 100 × dry weight gain of larva/(dry weight of food eaten-dry weight of feces produced).

Larval growth and food utilization were calculated after 24 h. Differences in average weight of the larvae recorded at the beginning and at the end of the period gave the gain in body weight while the mean larval body weight was calculated using the formula: mean weight = initial weight - final weight (Waldbauer, 1968).

Histology

Small pieces of gut tissue from treated and control larvae were fixed overnight for fixation. The dehydrated specimens were embedded in an embedding medium at 60ºC. The blocks were cooled for 3 h and cut into 1.5 µm ribbons with a microtome. The ribbons were stained with haematoxylin and eosin, mounted after drying. The sections were observed in light microscope (Optika) (Senthil-Nathan *et al.*, 2008).

Data analysis

Data from mortality and nutritional indices were expressed as the mean of three replications and normalized by arcsine-square root transformation of percentages. The transformed percentages were subjected to analysis of variance (ANOVA) and were fitted with linear regression using Minitab®16 software package. Differences between the three treatments were determined using the Tukeys family error rate ($P \leq 0.05$) via the Minitab®16 software package. The lethal concentrations LC_{50} were calculated using probit analysis (SPSS).

Results and Discussion

Effects of insecticidal activity against *H. armigera*

Higher larval mortality of *H. armigera* was observed at 48 h following treatment for larvae exposed to identified (Fig. 1) *Bs, Bt*, and the *Bs/Bt* combination than for control larvae not exposed to bacterial suspensions. The different spore concentrations applied (1×10^2, 1×10^4, 1×10^6, 1×10^8 and 1×10^{12} spores/ml) differed in their effects on larval mortality when larvae were treated with *Bs* (F = 26.11, df = 4, P = 0.001), with *Bt* (F = 27.84, df = 4, P = 0.001), and with the *Bs/Bt mixture* (F = 49.43, df = 4, P = 0.001) for first instar of *H. armigera* and *Bs* (F =42.60, df = 4, P = 0.001), with *Bt* (F = 37.19, df = 4, P = 0.001), and with the *Bs/Bt mixture* (F = 32.33, df = 4, P = 0.001) for second instar of *H. armigera*.

Exposure of *H. armigera* larvae to either *Bs* or *Bt* alone caused only 80% and 70% mortality respectively compared to the mortality levels found with the *Bs/Bt* mixture (Fig. 2, 3 and 4).

The larval mortality third instar larvae *Bs* (F = 16.75, df = 4, P = 0.001), with *Bt* (F = 13.85, df = 4, P = 0.001), and with the *Bs/Bt mixture* (F = 34.07, df = 4, P = 0.001) and *Bs* (F = 11.23, df = 4, P = 0.001), with *Bt* (F = 14.79, df = 4, P = 0.001), and with the *Bs/Bt mixture* (F = 44.57, df = 4, P = 0.001) for fourth instar of *H. armigera*. The LC_{50} values were determined with different concentrations (Table 1). The mortality rate caused by the *Bs/Bt* mixture was almost 100% compared to control for the untreated larvae. *Bt* crystal toxins and *Bs* were affected *H. armigera* only at higher doses but the *Bs/Bt* mixture gave higher mortality with almost 100% of the individual larvae affected.

Larval duration versus larval weight

H. armigera were fed with bacterial cultures containing spore concentrations of 1×10^{12}spores/ml as mentioned and effects on larval survival are showed (Fig. 5). The weight of larvae changed day by day in growing insects. The larval period was longest in larvae exposed to the *Bs/Bt* mixture and shortest in the control larvae, and differences were significant (F = 2.61, df = 39, P = 0.0001). The survival of larval fed on the control diet was 95 % after 48 h compared to larval survivals of 52.50%, 57.80%, and 38.50% for larvae fed on diets containing *Bs*, *Bt*, and the *Bs/Bt* mixture, respectively.

Nutritional indices of *H. armigera* after treatment with bacterial culture

The *Bs, Bt*, and *Bs/Bt* mixture all reduced the nutritional indices RCR, RGR, ECI, ECD and AD of the third instar larvae (Table 2). The reduction depended on the doses included in the diet. A significant reduction (P < 0.001) in all nutritional indices was observed at concentrations of (1×10^2, 1×10^4, 1×10^6, 1×10^8 and 1×10^{12} spores/ml). Only the ECI and RGR were reduced by the dose of lowest concentration. Plotting the RCR against the RGR revealed that the extract had substantial toxic effects as shown by the relatively low growth rates of larvae fed on treated diets. The regression coefficients of the RCR–RGR relationships for control and treated larvae were significantly different (F = 47.32, df = 3, P = 0.001), indicating that the reduction in growth of larvae fed on bacterial culture-containing diets was not entirely a result of lower food intake (Fig. 6).

Histology of *H. armigera* after treatment

Midgut sections of *Bs*, *Bt* and *Bs/Bt* treated larvae and control larvae of *H. armigera*. *Bs/Bt* treated larvae epithelial cells were fully disrupted and complete disappearance of cellular components. High concentrations *Bs* and *Bt* treated larvae were damaged in columnar cells of *H. armigera*. In control larvae of *H. armigera* appears clearly columnar cells globet cells, brush border membrane, basement membrane, peritrophic membrane and epithelium of midgut (Fig. 7). Biocontrol, over the years, has launched a leading role in issuing the most exciting work in all aspects of biological control of invertebrates, and plant diseases (Eric-Wajnberg, 2009). In this study, we compared *Bt*, *Bs*, and a *Bs*/*Bt* mixture for insecticide activity. In those comparisons, bacterial amendments included in their food affected third instar larvae of *H. armigera*by extending larval mortality. In other studies, larval performance and mortality were influenced by variation in the nutritional food quality (Bauce *et al.*, 2002). Food ingestion, digestion, gain in body weight and efficiencies of utilization significantly decline as a result of *Bt* infection. *Btk* seems to carry particular promising uses in Brazil (Giustolin *et al.*, 2001, Desneux *et al.*, 2010).

Table.1 The LC_{50} values for different concentrations of *Bt, Bs, Bt/Bs*

S.No	Treatment	LC_{50} value	Chi square test	95% confidence level	
				Upper limit	Lower limit
1.	*Bt*	4.19	5.115	4.78	3.78
2.	*Bs*	4.81	6.023	5.65	4.31
3.	*Bt/Bs*	3.19	6.096	3.55	2.84

Table.2 Nutritional indices of *H. armigera* after treatment with *Bt*. Mean standard error (±) followed by the same letter within columns indicate no significant difference (p≤0.05) in a Turkey test

S.No	Treatments	RCR	RGR	ECI	ECD	AD
1	1×10^{2}	3.54 ± 0.005^{b}	0.65 ± 0.005^{b}	18.55 ± 0.050^{b}	26.50 ± 0.292^{b}	70.01 ± 0.009^{e}
2	1×10^{4}	3.52 ± 0.006^{bc}	0.63 ± 0.010^{bc}	18.14 ± 0.010^{b}	25.22 ± 0.010^{c}	72.28 ± 0.010^{d}
3	1×10^{6}	3.49 ± 0.005^{bc}	0.62 ± 0.006^{c}	17.96 ± 0.006^{bc}	24.56 ± 0.10^{d}	73.14 ± 0.020^{c}
4	1×10^{8}	3.45 ± 0.001^{cd}	0.60 ± 0.005^{d}	17.51 ± 0.010^{c}	23.28 ± 0.009^{e}	75.24 ± 0.011^{b}
5	1×10^{12}	3.40 ± 0.011^{d}	0.57 ± 0.010^{e}	16.94 ± 0.540^{d}	22.14 ± 0.084^{f}	76.52 ± 0.303^{a}
6	Control	4.13 ± 0.072^{a}	0.74 ± 0.020^{a}	19.98 ± 0.010^{a}	28.02 ± 0.015^{a}	64.67 ± 0.570^{f}

± standard deviation, RCR- Relative consumption rate, RGR- Relative growth rate, ECI- Efficiency of conversion of ingested food, ECD- Efficiency of conversion of digested food, AD- Apporoximate digestibility.

Table.3 Nutritional indices of *H. armigera* after treatment with *Bs*. Mean standard error (±) followed by the same letter within columns indicate no significant difference (p≤0.05) in a Turkey test

S.No	Treatments	RCR	RGR	ECI	ECD	AD
1	1×10^{2}	3.98 ± 0.010^{ab}	0.84 ± 0.005^{a}	21.23 ± 0.011^{b}	28.50 ± 0.010^{b}	74.52 ± 0.990^{c}
2	1×10^{4}	3.96 ± 0.020^{b}	0.83 ± 0.005^{ab}	21.08 ± 0.010^{b}	28.49 ± 0.001^{b}	74.54 ± 0.010^{bc}
3	1×10^{6}	3.92 ± 0.010^{b}	0.82 ± 0.010^{ab}	21.73 ± 0.020^{a}	27.96 ± 0.010^{c}	76.58 ± 0.010^{ab}
4	1×10^{8}	3.89 ± 0.049^{b}	0.79 ± 0.011^{b}	20.00 ± 0.280^{c}	26.95 ± 0.006^{d}	76.95 ± 0.020^{a}
5	1×10^{12}	3.50 ± 0.010^{c}	0.69 ± 0.050^{c}	19.98 ± 0.020^{c}	25.64 ± 0.370^{e}	77.95 ± 0.021^{a}
6	Control	4.13 ± 0.070^{a}	0.88 ± 0.02^{d}	22.02 ± 0.021^{a}	29.01 ± 0.010^{a}	64.05 ± 0.049^{d}

± standard deviation, RCR- Relative consumption rate, RGR- Relative growth rate, ECI- Efficiency of conversion of ingested food, ECD- Efficiency of conversion of digested food, AD- Apporoximate digestibility.

Table.4 Nutritional indices of *H. armigera* after treatment with *Bt/Bs*. Mean standard error (±) followed by the same letter within columns indicate no significant difference (p≤0.05) in a Turkey test.

S.No	Treatments	RCR	RGR	ECI	ECD	AD
1	1×10^{2}	3.60 ± 0.010^{b}	0.64 ± 0.026^{b}	18.01 ± 0.015^{ab}	24.30 ± 1.100^{b}	75.07 ± 1.010^{c}
2	1×10^{4}	3.54 ± 0.400^{b}	0.59 ± 0.010^{b}	16.94 ± 0.061^{b}	22.31 ± 1.10^{b}	77.62 ± 0.640^{bc}
3	1×10^{6}	3.52 ± 0.035^{bc}	0.49 ± 0.010^{c}	14.40 ± 0.015^{c}	18.01 ± 0.435^{c}	78.01 ± 0.075^{a}
4	1×10^{8}	3.40 ± 0.020^{cd}	0.48 ± 0.043^{c}	14.28 ± 0.064^{c}	16.19 ± 0.131^{cd}	78.91 ± 0.260^{a}
5	1×10^{12}	3.37 ± 0.015^{d}	0.37 ± 0.043^{d}	11.90 ± 0.021^{d}	15.93 ± 0.309^{d}	79.38 ± 0.26^{a}
6	Control	4.13 ± 0.078^{a}	0.74 ± 0.035^{d}	17.92 ± 1.700^{a}	28.02 ± 1.75^{a}	64.01 ± 2.470^{d}

± standard deviation, RCR- Relative consumption rate, RGR- Relative growth rate, ECI- Efficiency of conversion of ingested food, ECD- Efficiency of conversion of digested food, AD- Apporoximate digestibility.

Fig.1 Molecular identification of *Bt* by using Cry gene amplification and *Bs* by using 16s rRNA.

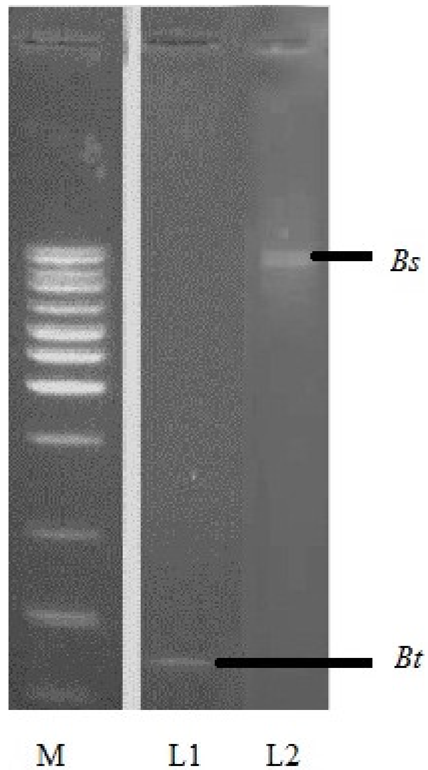


Fig.2 Mortality of *Bt* against first, second, third and fourth instar larvae of *H. armigera*. Mean (±SEM) followed by the bars indicate no significant difference (P<0.05) in a Tukey's test.

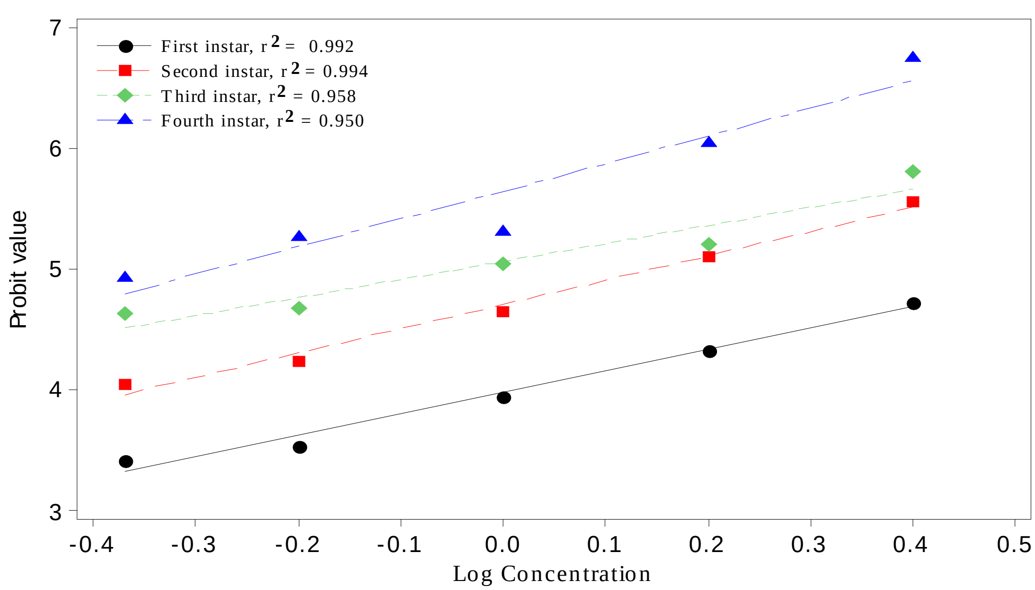


Fig.3 Mortality of *Bs* against first, second, third and fourth instar larvae of *H. armigera*. Mean (±SEM) followed by the bars indicate no significant difference (P<0.05) in a Tukey's test.

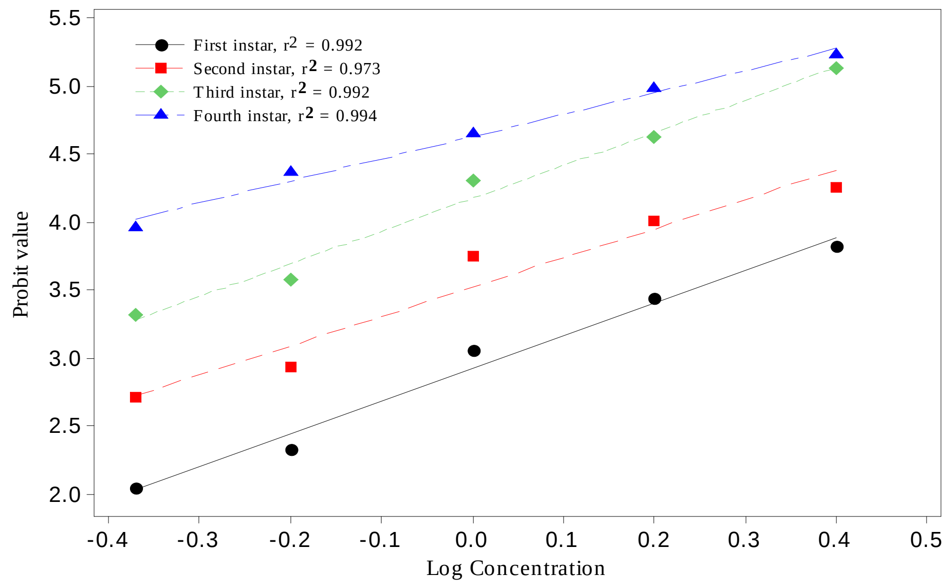


Fig.4 Mortality of *Bt/Bs* against first, second, third and fourth instar larvae of *H. armigera*. Mean (±SEM) followed by the bars indicate no significant difference (P<0.05) in a Tukey's test.

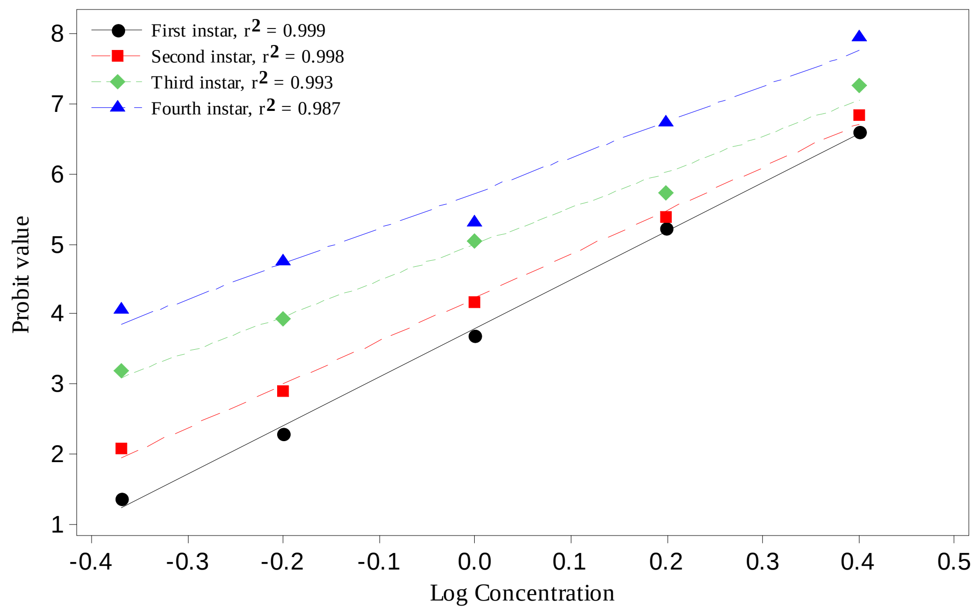


Fig.5 Survival rate of third instar larvae of *H. armigera* after treatment with *Bt*, *Bs* alone and *Bt* /*Bs* (24 h treated larvae).

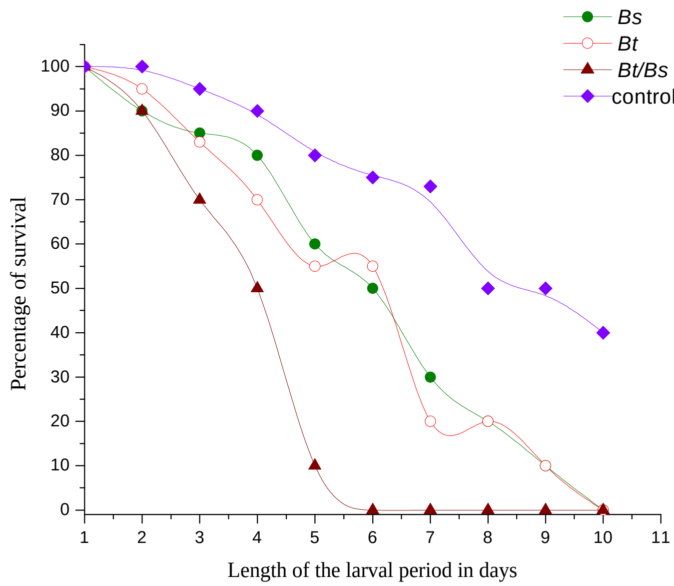


Fig.6 Correlation between RGR and RCR of *H. armigera* fed on different amount of control diet and different concentrations of bacterial cultures. Two regressions co-efficient lines are significantly different. Regression equations are displayed.

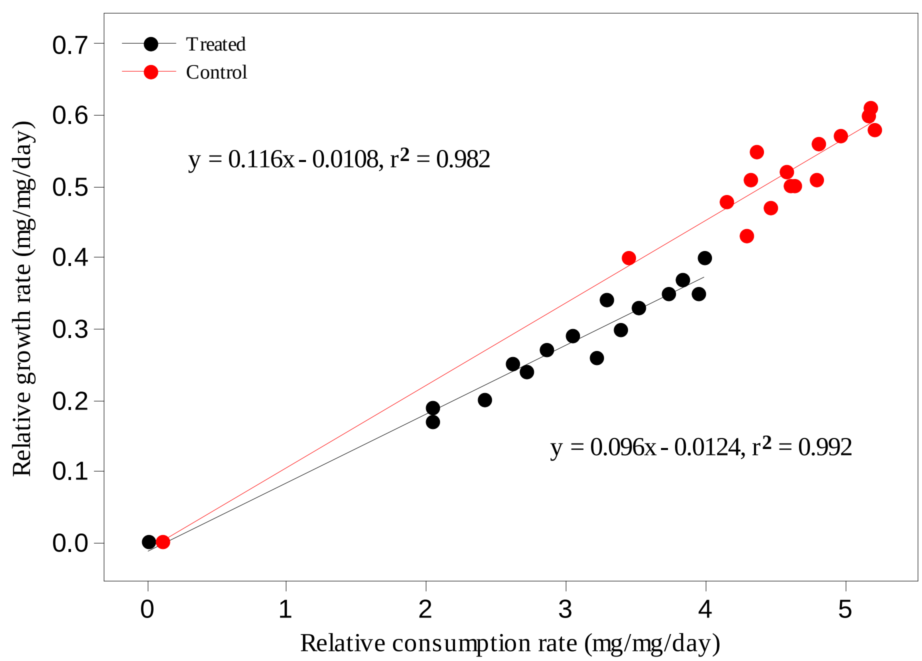
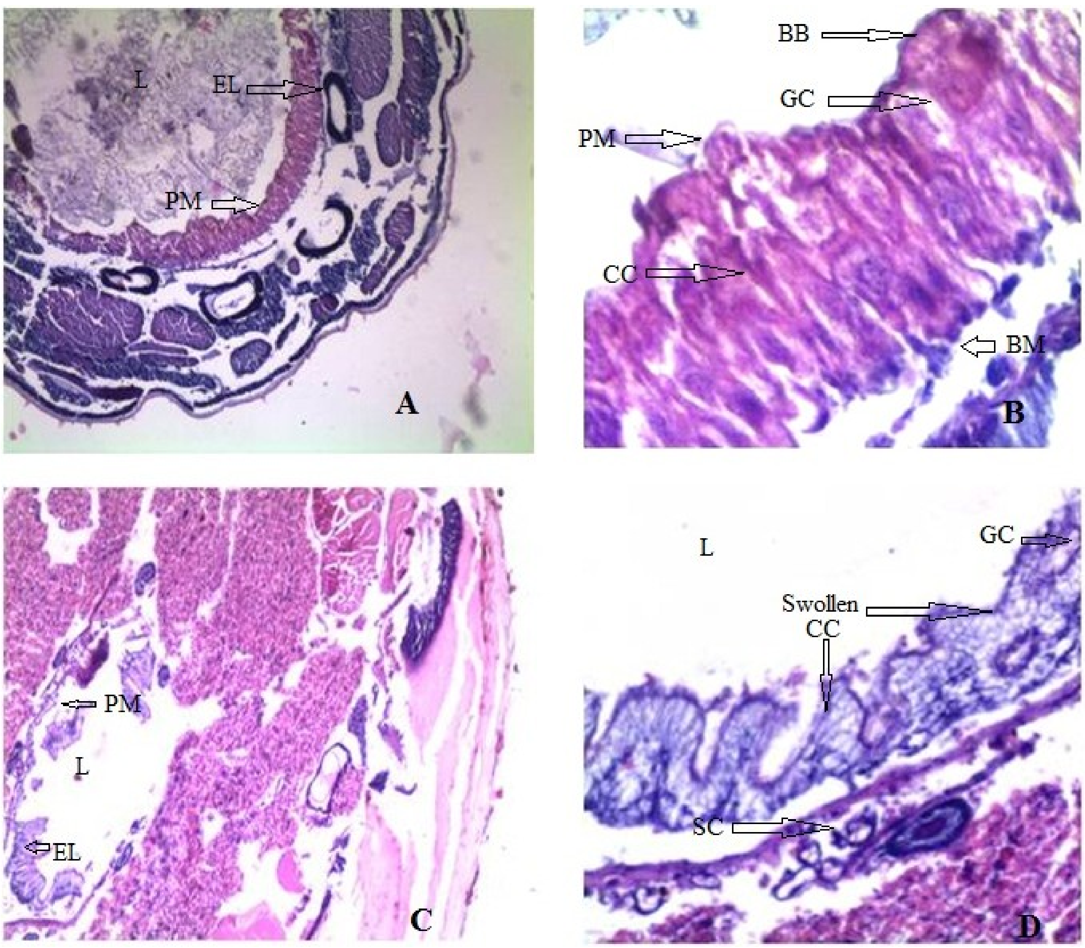


Fig.7 Light microscopy of the midgut third-instar larvae fed for 20 h on *Bt/Bs* and control. Lumen (L), columnar cells (CC), goblet cells (GC), brush border (BB) of the columnar cells, stem cells (SC) peritrophic membrane (PM), and epithelial layer (EL) are as labeled. A and B are the midgut epithelium of third-instar larva after ingestion of control diet. C and D is the treated larva. The diameter of the gut is smaller than the gut in control larvae. The width of the cell layer was greater than the control larvae. CCs were lengthened and swollen and some had burst exhausting granular material into the lumen. The departure of brush border at the apex of columnar cells is showed by arrows. The peritrophic membrane was not visible.



Clark *et al.* (2002) described mixed insecticides are becoming progressively popular in agricultural use because of their high efficiency, convenience and rapid actions and also well known that generally mixed insecticides cause substantial synergistic toxic effects on both target species (Ahmad *et al.,* 2009). Single-insecticide tries out fail to reflect field experiments where multiple insecticides or insecticide mixtures are used (Zhou *et al.,* 2011). Comparatively our present study demonstrates that mixed bio-insecticides are very effective and rapid action against *H. armigera*.

Bt sprays have been found to be effective for *H. armigera* control on chickpea (Balasubramanian *et al.,* 2002; Mandal *et al.,* 2003; Bhojne *et al.,* 2004; Singh and Ali, 2005). There were significant differences in the survival and development of *H. armigera* larvae on *Bt*-sprayed and unsprayed chickpeas (Devi *et al.*, 2011). Since our study to enhance toxicity of *Bt* by mixing *Bs* to *Bt* resistance *H. armigera*.

Bt and *Bs* containing spores that consist of the polypeptide, crystals and low levels of spores, were moderately potent. Mixtures of spores and crystals were highly potent. So, *H. armigera*, pathogenicity depends on the presence of both the infectivity of the spore and the toxicity of the crystal (Li *et al.*, 1987). In our present study also investigated mixture of *Bs/Bt* increases pathogenicity against insect.

Food consumption, RGR, RCR, ECI, ECD and AD was obviously extended on infected larvae. *Bt*-induced delays in development times were greater when spruce bud worm SBW larvae were exposed as fourth-instar larvae, but effects on pupal weight and fecundity were more pronounced when larvae were exposed as later instars (Pedersen *et al.*, 1997). The reduced digestive efficiency in insects exposed to *Bt* means more food must be ingested by the host to assure optimum growth for pupation and to collect sufficient nutrients for adult stages via extended larval periods. The bacterial parasites caused extended development periods for many lepidopteron species (Slansky and Scribe 1985; Rath *et al.*, 2000).

The reduction in dietary utilization suggests that reduction in growth may result from both behavioral and physiological (post-ingestives) effects (Senthil-Nathan *et al.*, 2008). SBW decreases in food quality leads to a decrease in pupal weights, an increase in development times and mortality rates of larvae (Harvey, 1974; Bidon, 1993; Bauce *et al.*, 2002). The resistant gene pool of a population was already shown in the laboratory. *Bt* formulations can be selected in several insect species and for several different toxins (Tabashnik, 1994; Ferre, 1995), used a vector express a *cry* gene from *Bt* in *Bs*. They placed the *cry* (c) gene from strain HD73 into the *Bs* chromosome, and after amplification of the inserted sequences. Low assimilation efficiencies and low net growth efficiencies cause the relative growth rate were lower than the relative consumption rate (Slansky and Scribe, 1985).

In this low absorption, efficiency evaluates are characteristic of organisms accommodated to feed (Mattson, 1980), and it may occur an accommodation to feeding on a food of poor nutritional capacity (Larsson and Tenow, 1979) or an effect of feeding on a food with a high content of non digestible fiber (Fogal, 1974). Slansky and Scribe (1985) indicated that the rates and efficiencies of food consumption and utilization have adaptive significance in that insects have an ideal growth rate and should, through selection, be able to change food consumption and utilization efficiencies in order to accomplish this ideal growth rate. The ECI decreases are related with energy-consuming physiological activities and also associated with recent molts and the approaching maturity (Carne, 1966). ECI measures of an insect ability to utilize the food that it ingests for growth (Senthil-Nathan *et al.*, 2008).

For the recreation of absorbed food to energy metabolism to ensue in a reduction in growth and that the growth must be energy-limited, that the growth of insect is more probably to be modified by the accessibility of nitrogen or water than by the availability of energy (Mattson, 1980; Slansky and Scribe, 1985; Schroeder, 1986). In this study, we mixed *Bt/Bs* for evaluations of insecticidal activity. Delaying insect resistance to *Bt* toxins has been improved by introducing more than two insect resistance genes with different insect resistance mechanisms into plants (Zhao and Shi, 1998).

Bt has no damaging effect to natural enemies and the environment (their low perseveration and high specialness), and are viewed to be compatible with integrated pest management (IPM) practices (de-Maagd *et al.*, 1999). These results cannot be promptly generalized to transgenic *Bt*-plants, since conflicts in the duration and insect's exposure to the toxin. Nevertheless, few of them studied on the effects of *Bt*-plants on beneficial insects have been issued to date (Hilbeck, 1998).

Since our study was carried infected larvae growth was a failure, it may be due to the lack of proteins and sugars or other nutrients, which are measurable by means of the utilization values. Further investigations are needed for the exact resource was utilized by the larvae, and also the isolated *Bacillus* spp were potentially used for controlling diseases in crops. We concluded that combined cell suspension of *Bs*/*Bt* having the action of insecticidal activity was high compared with individual cell suspension. In that further our mixed culture will apply for green house trials.

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