

Efficacy of Two Serotypes of *Bacillus thuringiensis* on the African Bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), on Snap Bean (*Phaseolus vulgaris* L.) in the Central Rift Valley of Ethiopia

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Abstract

African bollworm (ABW), *Helicoverpa armigera* Hübner, is an important insect pest inflicting damage on snap bean (*Phaseolus vulgaris* L.) produced as vegetable in the Central Rift Valley (CRV) of Ethiopia. The efficacy of two serotypes of *Bacillus thuringiensis* (Bt) namely *kurstaki* (Delfin[®]) and *aizawai* (Probit[®]), were evaluated against African bollworm at Ethio-Vegfru farm, Koka, in the Central Rift Valley. The experiment was laid out in a factorial randomized complete block design with three replications. The two serotypes of Bt were applied at three rates (0.25, 0.5 and 0.75 kg ha⁻¹) and at two different application frequencies (4 and 7 day intervals) from flower initiation until harvest. Treatments were applied five and three times for the 4 and 7 days application intervals, respectively. Deltamethrin (Decis[®] 25 EC) applied at 0.3 L ha⁻¹ weekly and untreated control were included as checks. Data on number of ABW larvae, pod damage both by number and weight and marketable yield were recorded. Damage level was reduced with increased rate and frequency of application. The high rate of Delfin applied both at 4 and 7 days interval and the high rate of Probit applied at 4 days interval significantly reduced pod damage compared with the untreated control. Delfin at the high rate performed significantly better than Probit and its low rate treatments in reducing infestation level and minimizing yield loss. In conclusion, application of high rate of Delfin weekly from flower initiation up to harvest for three times was the most effective in reducing the pod damage and associated yield loss, and can be used in place of pyrethroid insecticides such as deltamethrin that are currently in use in the central rift valley.

Introduction

Snap bean (*Phaseolus vulgaris* L.) is one of the most important fresh vegetables and more than 90% of snap bean produced in Eastern Africa is exported to the regional and international markets (CIAT, 2006). In Ethiopia, snap bean covers about 13,200 hectare of land with annual production of 5,779 tons (FAOSTAT, 2008). According to Smelt and de Jager (2002) cited by Negasi (2008) commercial snap bean production is mainly practiced on certain farms in central Ethiopia where infrastructure and irrigation facilities are available. The area allotted to the production of green beans is expanding in the central rift valley of Ethiopia. However, productivity of the crop is constrained by a number of biotic and abiotic factors. A number of insect pests including mites, thrips, whiteflies and African bollworm are inflicting damage on snap bean. Of these, African bollworm is the most destructive one (Tsedek et al., 1985). Mohammed Dawd et al. (2004) reviewed studies on ABW management in Ethiopia on various crops including cotton, vegetables and different legume species. The main control method has been the use of chemical insecticides mainly synthetic pyrethroids including cypermethrin, cyhalothrin and deltamethrin (Gashawbeza and Lemma 2004; Gebre et al., 2004; Tebekew and Seid, 2004). Decline in efficacy of these products on ABW has been reported and are attributed to the development of pesticide resistant populations (Geremew and Ermias, 2005). Keeping safety period of applied pesticides before taking the first harvest is affecting their produces and is forcing vegetable farms in Central Rift Valley region to look for easily degradable and IPM compatible pest control such as bio-pesticide like *Bacillus thuringiensis*, which has been found effective mainly against lepidopterous larvae (Lacey et al., 2001). *Bacillus thuringiensis* is one of the most important spore forming bacterial pathogens of insects. In the process of sporulation, it produces a crystal of toxic protein (delta indotoxin). When spores and crystals of *Bt* are eaten by susceptible host insect, a general paralysis usually develops and the insects die within a few hours to 4-5 days

depending on the *Bt* serotype and the susceptibility of the insect (Luckmann, 1982).

Various serotypes of *Bt* have been developed and used for the control *H. armigera*. This is because chemicals had failed to control *H. armigera* and because of their additional advantage of conserving natural enemies of *H. armigera*. No records for *Bt* resistance in insects had been reported for 30 years since its introduction for control of insect pest in the world, except in Lepidoptera pests of stored grain products *Plodia interpunctella* (Hama, 1990). Previous studies on the efficacy of *Bacillus thuringiensis* against diamondback moth, *Plutella xylostella*, on cabbage in the Central Rift Valley of Ethiopia indicated the effectiveness of the product at a rate of 0.5 kg per ha (Gashawbeza 2006; Lidet et al. 2009). In order to increase the volume of export of snap beans without facing restrictions by importing countries, it was deemed necessary that alternatives to the use of pesticides, which require longer safety period, be found. Hence, the efficacy of two commercially available *Bt* serotypes in terms of their effective dosages and optimum application frequencies were evaluated against ABW on snap beans under field condition in the central rift valley.

Materials and Methods

The study was conducted at Koka Ethio-Vegfru Farm in the Central Rift Valley of Ethiopia for two experimental periods from August - October 2010 and December - February 2011.

The experiment was laid out in a factorial randomized complete block design (RCBD) with three replications. The unit plot size was 2.4 m x 3.2 m with row spacing of 40 cm and plant spacing of 8 cm. Spacing between plots and blocks were 1 m and 1.5 m, respectively. The treatments were applied using a knapsack sprayer with a water volume of 400 to 600 L per ha using a flat fan nozzle. Two seeds per hill were seeded and thinned to one plant per hill 15 days after seeding to maintain a population of 240 plants per plot. Four

different fertilizers were applied during the trial periods. These include monoammonium phosphate (MAP), Diamonium Phosphate (DAP), Potassium nitrate and urea. DAP was broadcasted once before planting at 150 kg/ha. The other three fertilizers were applied using fertigation system daily for twelve days after planting until harvest, at different rates, depending on the time from planting. Fields were drip irrigated daily from sowing to a day before harvest for 15 to 45 minutes depending on level of soil moisture. Management practices like weeding and cultivation were carried out as needed.

Two serotypes of *Bt* (*kurstaki* and *aizawai*), hereafter referred by their trade names Delfin® and Probit®, respectively, were evaluated along with the standard insecticide deltamethrin (Decis® 25 EC) and untreated control from flower initiation up to about a week before harvest, which are the susceptible stages of the crop to ABW attack. The *Bt* formulations were sprayed at three different rates i.e., 0.25, 0.5 and 0.75 kg ha⁻¹ on 4 and 7 days interval. Application of Delfin® and Probit® was made for a total of five and three times for the 4 and 7 days application intervals, respectively. Decis® 25 EC was applied weekly during the same period at 0.3 L per ha.

Data on the number of larvae present was recorded from central rows of each plot prior to treatment application from ten randomly selected plants. Leaves and pods of each plant were examined for ABW larvae and counts were recorded. At harvest, pods from the central rows were harvested and sorted into damaged and undamaged based on visual damage symptoms, clear circular holes. Percent damaged pods by number and weights were calculated as a proportion of total pod number and weight, respectively. Marketable yield was recorded by weighing undamaged pods from each plot.

Data analysis

Analyses of variance (ANOVA) were carried out using SAS statistical version 9.2 software (SAS, 1999). Data from four days application intervals were averaged to generate mean

values for a week. Treatment evaluation dates coincided with 41, 48 and 56 days after planting (DAP) of the crop. The data set was analyzed in one way ANOVA as this allowed direct comparison of mean differences between different treatment combinations with the untreated and standard checks. To assess the interaction effect between *Bt* formulations and their application rates and frequencies, the data was reanalyzed in three way ANOVA by excluding the standard and untreated control treatments. Student Newman Keuls (SNK) test (SAS, 1999) was used to separate the means.

Results

Main and interaction effects

Interaction effects between *Bt* formulations, rate and frequency were insignificant ($P > 0.05$) for all the parameters measured with the exception of pod damage by weight ($F = 12.17$; $df = 2, 59$; $P < 0.0001$) and larvae number ($F = 3.48$; $df = 2, 59$; $P = 0.037$) at 56 days after planting. Pod damage both by number ($F = 43.86$; $df = 1, 59$; $P < 0.0001$) and weight ($F = 43.78$; $df = 1, 59$; $P < 0.0001$) was significantly lower in the first than in the second season. Pod damage both by number and weight and larvae number at 48 and 56 days after planting were significantly lower and yield was higher in Delfin® than in Probit® treated plots. Larvae number was significantly lower in the high rate treatments both at 48 ($F = 6.69$; $df = 2, 59$; $P = 0.0024$) and 56 ($F = 28.14$; $df = 2, 59$; $P < 0.0001$) days after planting. Similarly pod damage both by number and weight was significantly lower and yield was higher in the high rate treatment.

Effects of *Bt* serotype on larval population

Larvae number ranged between 0.07 and 0.4 per plant in the first season and between 0.03 and 0.5 per plant in the second season (Table 1). Differences between treatments were not significant in the first two sampling dates in the first season. Differences appeared to be significant with delay in sampling. The high

rate of Delfin® applied at 4 days interval resulted in significantly lower number of larvae than its low rate and the medium rate of Probit® applied both at four and seven days interval at 56 DAP in the first experiment. Similarly in the second season, the high rate of Delfin® applied at 4 days interval resulted in significantly lower number of larvae than in plots treated with the low rate of Delfin® applied both at four and seven days interval, medium rate of Delfin® applied weekly, the low rate of Probit® applied both at four days interval and weekly, medium rate of Probit® applied weekly and the untreated plot at 56 DAP.

Effects of *Bt* serotype on pod damage

Percent pod damage ranged between 2.7 and 13.3 in the first season and between 3.4 and 20.1 in the second season (Table 2). Differences between treatments were significant in both experimental periods. The minimum and maximum damage levels in the first season were recorded from the standard insecticide (Decis®) and untreated control, respectively. Among *Bt* treatments, the high

rate of both Delfin® and Probit® resulted in lower damage than the low rates in the first season. Both the medium and high rate of the two *Bt* formulations resulted in significantly lower damage than the untreated check in the first season regardless of the application frequencies. Similar trends were observed in the performance of the treatments in the second season. The high rate of both *Bt* formulations resulted in lower damage than the corresponding low rates (Table 2). The medium and high rate of Delfin® applied at 4 days interval, the high rate of Delfin® applied weekly and the high rate of Probit® applied at 4 days interval resulted in significantly lower pod damage than the rest of the treatments including the standard insecticide (Table 2). Mean of the two experimental seasons showed that the high rate of Delfin® at both frequencies and the high rate of Probit® applied at 4 days interval resulted in significantly ($P < 0.05$) lower pod damage than the standard insecticide treatment, Decis®. Differences between frequencies of application appeared to be significant with the low rates of application (Table 2).

Table 1. Mean number of ABW larvae per plant on snap bean treated with *Bt* formulations during 2010 and 2011

Treatments	During 2010				During 2011			
	41 DAP	48 DAP	56 DAP	Mean	41 DAP	48 DAP	56 DAP	Mean
Delfin® 0.25 Kg/ha at 4 days	0.33a	0.4a	0.36a	0.37a	0.3a	0.26ab	0.26ab	0.3abc
Delfin® 0.5 Kg/ha at 4 days	0.17a	0.23a	0.13bc	0.2cd	0.33a	0.17b	0.1bc	0.2cd
Delfin® 0.75 Kg /ha at 4 days	0.2a	0.23a	0.07c	0.2cd	0.23a	0.17b	0.03c	0.14d
Delfin® 0.25 Kg/ha at 7 days	0.37a	0.37a	0.23abc	0.32ab	0.3a	0.3ab	0.3a	0.3abc
Delfin® 0.5 Kg /ha at 7 days	0.26a	0.23a	0.2abc	0.23abcd	0.37a	0.26ab	0.26ab	0.3abc
Delfin® 0.75 Kg/ha at 7 days	0.26a	0.2a	0.13bc	0.2bcd	0.26a	0.23ab	0.06c	0.2cd
Probit® 0.25 Kg /ha at 4 days	0.3a	0.3a	0.23abc	0.28abcd	0.3a	0.33ab	0.3a	0.31abc
Probit® 0.5 Kg/ha at 4 days	0.23a	0.33a	0.3ab	0.29ab	0.37a	0.5a	0.2abc	0.4a
Probit® 0.75 Kg/ha at 4 days	0.3a	0.26a	0.23abc	0.3abcd	0.23a	0.26ab	0.17abc	0.2bcd
Probit® 0.25 Kg/ha at 7 days	0.33a	0.33a	0.4a	0.34a	0.33a	0.4ab	0.33a	0.4a
Probit® 0.5 Kg /ha at 7 days	0.26a	0.33a	0.26ab	0.28abcd	0.43a	0.3ab	0.33a	0.4a
Probit® 0.75 Kg/ha at 7 days	0.36a	0.2a	0.23abc	0.3abcd	0.23a	0.33ab	0.2abc	0.23abc
Untreated	0.33a	0.4a	0.3ab	0.34a	0.37a	0.4ab	0.33a	0.3ab
Decis® 25 EC @0.3 L ha	0.2a	0.176a	0.06c	0.14d	0.4a	0.17b	0.1bc	0.2bcd
CV (%)	31.6	25.96	31.47	16.97	24.43	28.92	32.42	15.15

Means in a column followed by the same letter are not significantly different from each other at 5% significance level. Means were separated by Student-Newman-Keuls (NSK) test. Data were converted to log (n +1) prior to analysis but untransformed values are given in the table.

Table 2. Percent damage of Snap bean pods treated with Bt formulations against ABW at Ethio-Vegfru Farm during 2010 and 2011 experimental periods.

Treatments	During 2010	During 2011	Mean
Delfin® 0.25 Kg/ha at 4 days	7.1bc	15.5b	11.3c
Delfin® 0.5 Kg/ha at 4 days	5.1cde	3.5e	4.3f
Delfin® 0.75 Kg /ha at 4 days	3.1e	3.4e	3.2f
Delfin® 0.25 Kg/ha at 7 days	12.1a	20.1a	16.1a
Delfin® 0.5 Kg /ha at 7 days	8.0b	11.4c	9.7d
Delfin® 0.75 Kg/ha at 7 days	3.6e	4.2e	3.9f
Probit® 0.25 Kg/ha at 4 days	7.88b	17.3b	12.5c
Probit® 0.5 Kg/ha at 4 days	4.3de	17.9b	11.1c
Probit® 0.75 Kg/ha at 4 days	3.5e	4.6e	4.1f
Probit® 0.25 Kg/ha at 7 days	11.4a	16.9b	14.1b
Probit® 0.5 Kg /ha at 7 days	6.2bcd	17.6b	11.9c
Probit® 0.75 Kg/ha at 7 days	3.6e	9.6cd	6.6e
Untreated	13.3a	17.6b	15.5a
Decis® 25 EC @0.3 L ha at 7 days	2.7e	9.1d	5.9e
CV (%)	12.74	11.65	8.84

Means in a column followed by the same letter are not significantly different from each other at 5% significance level.

Percent damaged pod by weight ranged between 5.5 and 13.0 in the first season and between 5.0 and 20.2 in the second season. Damage level was reduced with increased concentration and frequency of application (Table 3). In the first year the minimum and maximum damage levels were recorded from Decis® and untreated plots, respectively. Damage level from the high rate of application of both Delfin and Probit was at par with Decis®, regardless of frequency of application.

In the second season, pod damage was significantly higher in Decis® than the high rate of Delfin® applied at 4 and 7 days interval and the high rate of Probit® applied at 4 days interval. When mean of the two seasons are considered, the high rate of Delfin® at both application frequencies and the high rate of Probit® applied at 4 days interval resulted in significantly lower damage than Decis®. The low rate of Probit® applied weekly did not differ significantly from the untreated check.

Table 3. Percent weight damage of snap bean pods treated with Bt formulations against ABW during 2010-11.

Treatments	During 2010	During 2011	Mean
Delfin® 0.25 Kg/ha at 4 days	8.5b	12.8bc	10.6de
Delfin® 0.5 Kg/ha at 4 days	7.2b	7.7cd	7.4fg
Delfin® 0.75 Kg /ha at 4 days	5.6b	5.0d	5.3g
Delfin® 0.25 Kg/ha at 7 days	13.0a	20.2a	16.6a
Delfin® 0.5 Kg /ha at 7 days	7.4b	16.7ab	12.1c
Delfin® 0.75 Kg/ha at 7 days	5.6b	4.81d	5.2g
Probit® 0.25 Kg /ha at 4 days	8.3b	19.1a	13.7abcd
Probit® 0.5 Kg/ha at 4 days	6.7b	19.0a	12.8bcd
Probit® 0.75 Kg/ha at 4 days	5.7b	6.0d	5.9fg
Probit® 0.25 Kg/ha at 7 days	12.1a	19.7a	15.9ab
Probit® 0.5 Kg /ha at 7 days	8.3b	18.6a	13.4abcd
Probit® 0.75 Kg/ha at 7 days	6.2b	10.5cd	8.3ef
Untreated	12.5a	17.8a	15.2abc
Decis® 25 EC @0.3 L ha	5.5b	11.8bc	8.6ef
CV (%)	12.74	11.65	8.84

Means in a column followed by the same letter are not significantly different from each other at 5% significance level.

Effect of *Bt* serotypes on marketable yield of snap beans

Yield (t/ha) ranged between 16.5 and 20.8 in the first season and between 16.2 and 22.9 in the second season (Table 4). In the first season, yield was high in plots treated with the high rate of Delfin® (at both frequencies) and Decis® treatment followed by the high and medium rate of Probit® applied at 4 days interval. In the second season, treatments that gave higher yield than Decis® include the medium and high rate of Delfin® applied at 4

days interval and the high rate of Delfin® applied weekly.

The mean yield of the two seasons showed that the high rate of both Delfin® and Probit® (at both frequencies) gave 1.1 to 4.1 t/ha higher yields than the untreated check, which correspond with yield losses of 5.6 to 19.1%. Decis® gave 2.46 t/ha higher yield than the untreated check which corresponds to a yield loss of 12.4%. In general, in both seasons the performance of the high rate of Delfin® was at par with the standard insecticide and better than the rest of the treatments.

Table 4. Marketable yield (tons per ha) of snap bean sprayed with *Bt* formulations against ABW during 2010/11

Treatments	During 2010	During 2011	Mean
Delfin® 0.25 Kg/ha at 4 days	16.97c	16.72cd	16.84d
Delfin® 0.5 Kg/ha at 4 days	17.17c	22.92a	20.04ab
Delfin® 0.75 Kg /ha at 4 days	20.80ab	19.65bc	20.23ab
Delfin® 0.25 Kg/ha at 7 days	16.52c	16.04d	16.28d
Delfin® 0.5 Kg /ha at 7 days	17.90bc	18.46bcd	18.18bcd
Delfin® 0.75 Kg/ha at 7 days	21.82a	21.01ab	21.42a
Probit® 0.25 Kg /ha at 4 days	17.11c	16.65cd	16.88d
Probit® 0.5 Kg/ha at 4 days	19.84abc	16.62cd	18.23bcd
Probit® 0.75 Kg/ha at 4 days	19.87abc	18.96bcd	19.42abc
Probit® 0.25 Kg/ha at 7 days	17.03c	16.59cd	16.81d
Probit® 0.5 Kg /ha at 7 days	18.46bc	16.26d	17.36cd
Probit® 0.75 Kg/ha at 7 days	19.76abc	19.08bcd	19.42abc
Untreated	17.12c	17.52cd	17.32cd
Decis® 25 EC @0.3 L ha	20.02abc	19.53bc	19.78ab
CV (%)	6.58	6.32	5.15

Means in a column followed by the same letter are not significantly different from each other at 5% significance level.

Discussion and Conclusion

Both serotypes of *Bt* were found effective in reducing ABW the infestation levels and associated yield loss. The performance of the high rate of Delfin® was better than most of the rest of the treatments. Study conducted by Ravi *et al.* (2008) on the performance of *Bt* against ABW on tomato agree with results of the present experiment. *Bt* treatments were effective in reducing the larval population and fruit damage on tomatoes. Similar performance of *Bt* against ABW on chickpea has been reported (Khalique *et al.* 1994). The high rate of Delfin® at both frequencies and the high

rate of Probit® applied at 4 days interval resulted in significantly lower pod damage than the standard insecticide treatment, Decis®. The good performance of Delfin® observed in this study agrees with reports of Kesavan *et al.* (2003). Among five serotypes of *Bt* they compared against the sugarcane early shoot borer, *Chilo infuscatellus*, Delfin® was the best performing and better than the recommended insecticide, Sevidol. A study conducted on chickpea against ABW in southern India by Cherry *et al.* (1999) showed effective control of the pest in plots treated with endosulfan and Delfin. The reduction of damage level with increased application rate of Delfin® observed in this study agrees with reports of Sidde and Elshetty (2003).

Yield of the two seasons showed that the high rate of *Bt* treatments resulted in higher yield as compared to untreated check. Similar results were reported by Khalique *et al.* (1994) that the application *Bt* treatments increased yields compared to the control and compared favorably with synthetic chemicals insecticides in terms of reduced crop damage and increased yields. Gupta (2007) also reported that *Bt* were highly effective and comparable with the chemical pesticides Quinolphs (0.05%) and Cypermethrin (0.01%) in reducing ABW larval population and pod damage and consequently minimizing yield loss of chickpea.

In conclusion, application of high rate of Delfin weekly from flower initiation up to harvest for three times was the most effective in reducing the pod damage and associated yield loss. Therefore, Delfin could be used in place of pyrethroid insecticides such as deltamethrin that are currently in use in the central rift valley to control ABW in snap beans.

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