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Comparative Study of *Bacillus thuringiensis* Biopesticides Against Cotton Bollworms

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Abstract: Three *Bacillus thuringiensis* biopesticides were sprayed at their recommended rate against cotton bollworms, which have controlled these pests effectively both in lab and field trials. In the lab CAMB-Bt was found the best biopesticide giving 100% mortality with in 48hrs against spotted bollworms. Similarly Lepinox-Bt gave 100% mortality against spotted bollworm. However it was least effective against American bollworm with less than 60 % killing. Comparatively Larvo-Bt gave $\leq 20\%$ mortality against both the insects. There were made 3 sprays of Bt on cotton crop. In the field four days after each spray CAMB-Bt rank 1st by giving (65, 34 and 32%) mortality. While second was Larvo-Bt giving (62, 28 and 30%). Lepinox-Bt ranked 3rd (53, 2 and 26%). After three sprays the residual efficacy of CAMB-Bt was (55, 26 and 15%) followed by Larvo-Bt having (26, 29 and 13%) mortality. While Lepinox-Bt has (49, 3 and 1%) mortality as compared to control (11, 14 and 7.5%). The results suggests that CAMB-Bt is more effective than imported Bt formulations.

Key words: *Bacillus thuringiensis*, biopesticides, efficacy, spotted bollworms, cotton

Introduction

Cotton (*Gossypium hirsutum* L.) is a major cash crop of Pakistan. It is also a source of foreign exchange earning. Unluckily it is attacked by a number of sucking and chewing pests. Spotted bollworm *Earis vitella* is one of the serious chewing pest for cotton buds, bolls, flowers and shoots. Before flowering, larvae bore into terminal shoots, which wither and die. Squares, flowers and young bolls drop down after the attack of spotted larvae. Due to boring into main shoots followed by drying, side branches emerge from plant stem. There fore the energy which should have to be used for boll production, is consumed up by vegetative growth of plant (Ahmed, 1996).

To control this pest chemical control is most common. But in an integrated pest management system, it is the last tool. As it pollutes the environment and demands high cost and sometime it becomes the cause of insect resistance. Due to discovery of new potential bacterial strains and also their development, the trend for using microbial insecticide is increasing (Karim and Riazuddin, 1997).

CAMB *Bacillus thuringiensis* microbial insecticide controlled successfully *H. armigera* and *E. vitella* larvae in Okra field (Karim *et al.*, 2000 and Zafar *et al.*, 2001). CAMB Bt formulation was also found effective to control *H. armigera* in Tomato crop (Zafar *et al.*, 2000). Present work reports findings of comparative studies for CAMB-Bt biopesticides against spotted bollworm on cotton crop.

Materials and Methods

Insects: For lab bioassay insects were reared in the insectary for 14 h/10 h light and dark period at 30 °C on synthetic diet. Second instar larvae of *Helicoverpa armigera* and *Earis vitella* were used in lab bioassay.

Leaf bioassay: Infectivity test was conducted to check the fat of three Bt formulations in laboratory against *Helicoverpa armigera* and *Earis vitella*. There were four treatments, containing 25 replicates in each treatment. Three biopesticides Larvo-Bt, CAMB-Bt and Lepinox-Bt solution was prepared according to field spray. Cotton leaves of same size were sprayed with these solutions. While control leaves were sprayed with sterilized distill water. Second instar larvae were allowed to feed on these leaves. Data of mortality rate after 48 and 72 hrs was recorded.

Bt biopesticides: For large scale production of Bt biopesticide, CAMB-Bt was propagated in 14 L "Microferm Fermenter" New Brunswick, USA model MF-114 (Zafar *et al.*, 2002). Commercial biopesticides Larvo-Bt (Zagro) and Lepinox-Bt (FMC) were used for comparative studies.

Cotton field: The test was conducted in premises of National Centre of Excellence in Molecular Biology, University of the Punjab Lahore during June-Sept. 2000. Total plot area was divided into four treatments and four replicates according to randomized complete block design. The field was sown with Karishma cultivar of cotton in 2nd fortnight of June-2000. Injurious infestation of spotted bollworm started in 3rd week of July \approx 30 days of cotton plantation. In fourth week it increased fastly. There were made three sprays after 10 days interval in the month of August.

Pest scouting: Criteria for evaluation of *Bacillus thuringiensis* was based on pre and post spray pest scouting. Then % mortality data was calculated by % infestation (Amer *et al.*, 1999).

% infestation = $\frac{\text{post spray infestation}}{\text{pre spray infestation}} \times 100$

% mortality = $100 - \% \text{infestation}$

Infestation rate was calculated as:

Infestation rate = $\frac{\text{No of larvae}}{\text{No of plants}} \times 100$

Data was recorded after 4-days and 7-days of spray

Results

In leaf bioassay against American bollworm CAMB-Bt gave 100% mortality after 48 hrs as compared to Larvo-Bt and Lepinox-Bt having 20 and 50% mortality respectively. The same bioassay against spotted bollworm resulted in 100 % mortality in CAMB-Bt and Lepinox-Bt after 48hrs. While Larvo-Bt gave 10% mortality only (Fig. 1).

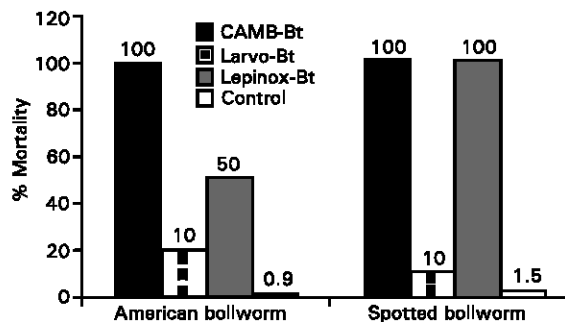


Fig. 1: Leaf bioassay against American bollworm and spotted bollworm

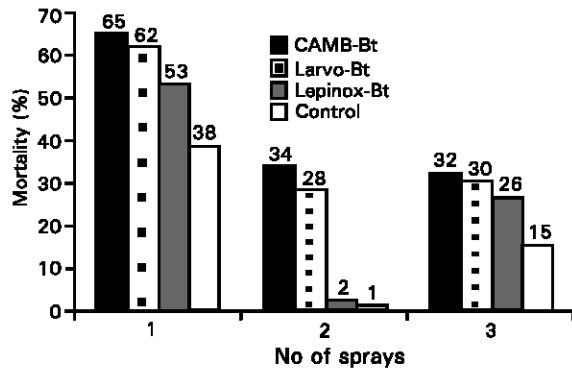


Fig. 2: Mortality in spotted bollworm 4-days after application of Bt biopesticides

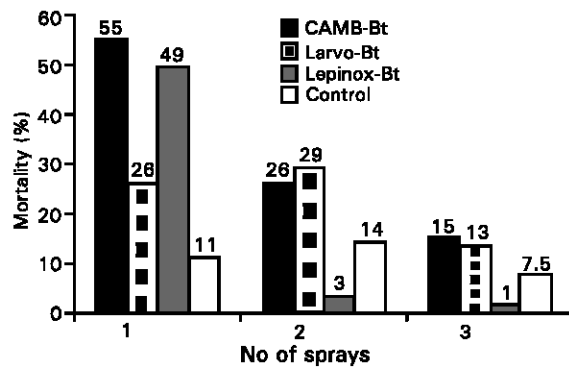


Fig. 3: Mortality in spotted bollworm 7-days after application of Bt biopesticides

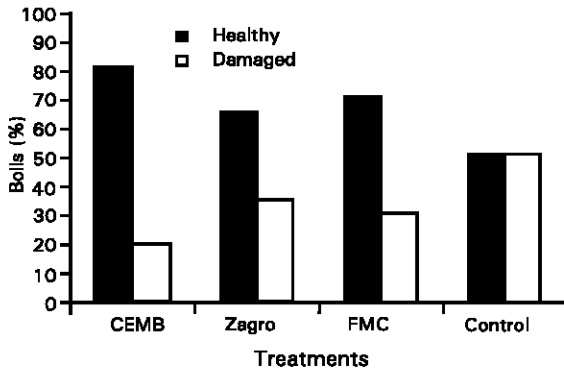


Fig. 4: Healthy and damaged bolls yield (%) after *Bacillus thuringiensis* biopesticides treatments

In fields injurious infestation of spotted bollworm began in 3rd week of July, i.e. 30 days after cotton sowing. Plots sprayed with CAMB-Bt gave the best control. On the basis of decrease in pest infestation, mortality of spotted bollworm (*Earias vitella* F.) was recorded 4 days after 1st spray as: 65 % in the plot treated with CAMB-Bt, 62 % in Larvo-Bt, 53 % in Lepinox-Bt plot & control with 38 % (Fig. 2).

In case of 2nd spray, on the basis of decrease in pest infestation, four days after spray mortality was 34% in CAMB-Bt treated plot and 28% in Larvo-Bt plot. However in Lepinox-Bt plot mortality was only 2% and in control it was only 1%. Similarly on the basis of decrease in pest infestation after 3rd spray, mortality in Larvo-Bt treated plot was 30 % and in Lepinox-Bt treated plot it was 26%.

Maximum mortality was in the CAMB-Bt treated plot which was 32% and in the control only 15% mortality occurred (Fig. 2).

After 7 days residual efficacy of CAMB-Bt treated plots was significantly better as compared to other treatments and mortality was 55% in first spray while in 2nd & 3rd spray it was 26 and 15% respectively. Larvo-Bt gave 26, 29 and 3% mortality 7 days after spray while Lepinox-Bt treated plots gave 49, 3 and 1% as compared to control where the mortality was 11, 14 and 7.5% (Fig. 3). There was significant difference between healthy bolls of CAMB Bt and control. In CAMB-Bt treated plot, there was 80% healthy bolls and in control only 50%. Larvo-Bt & Lepinox-Bt had protected the bolls up to 65 and 70% respectively (Fig. 4).

Discussion

The importance of Bt based crop protection systems, increasing largely as a result of greater environmental awareness and food safety concerns plus the failure of conventional chemicals due to an increasing number of insecticide resistant species has provided a major niche for the development of Bt (Dent, 1993). The present study describes the efficacy of CAMB-Bt, in comparison with the imported biopesticides of Zagro and FMC.

Preliminary comparative test by Leaf bioassay in laboratory indicated that Zagro-Bt has less efficacy to control spotted bollworm than Lepinox-Bt and CAMB-Bt, while against American bollworm it is very less potent. Lepinox-Bt has a greater potency than Larvo-Bt. However CAMB-Bt gave excellent results against both American bollworm and spotted bollworm within 48 hours. CAMB biopesticide was found very effective against lepidopteran insects *Helicoverpa armigera*, H. and *Earias vitella*, F. on tomato & okra crops (Karim, 2000; Zafar *et al.*, 2000).

In field trials on cotton crop, spray data indicates that under natural infestation pest population in a field can never remain same in all plots. Plots sprayed with CAMB-Bt showed 65, 34, and 32% mortality after 4 days and in control 38, 1, & 15% mortality representing decreasing % infestation from 1st to 3rd spray. Field studies of CAMB-Bt showed (65 and 55%), (34 and 26%), (32 and 15%) i.e. (4d and 7d) after spray %mortality suggests that CAMB-Bt was effective not only in lab but also in the field against the Lepinox-Bt and Larvo-Bt (Fig. 2 and 3).

Yield of cotton crop in same condition of attack of non lepidopteran and lepidopteran insects damaging buds, bolls & flowers, CAMB-Bt treated plot gave more healthy bolls as compared to control, which recommends CAMB-Bt formulation as a best biological control agent in crop protection.

The results of the field trials shows that for the best control of spotted bollworm (*Earias vitella* F.), Bt should be sprayed at 1st larval instar. For this reason pest scouting technique is very important to check the larval instars present in the field. In 3rd & 4th instars spotted bollworm larvae bore into the top tender shoots and tunnel down the stem and molting into next instars takes place inside the tunnel. After 5th instar larva comes out for pupation. So spotted bollworm instars developing inside stem cannot be controlled.

Cotton is lush green crop having big leaves and is sown during hot months of year, therefore Bt biopesticides does not give ideal results as compared to chemical control because *Bacillus thuringiensis* protein degrades due to sunlight and temperature above 50 °C. It is suggested that Bt spray should be applied in afternoon for longer exposure to larvae at night. However in vegetables and other spring season crops, best control of Bt formulation against target pests have been found.

This data also suggests that all the three *Bacillus thuringiensis* formulations have potential to control the spotted bollworm. By spraying these biopesticides with other chemicals, farmer can reduce the load of chemicals on environment plus cost of input because it is cheaper than many chemicals. Thus the data of present studies show that CAMB *Bacillus thuringiensis* biopesticide is most effective than imported Bt formulations. The *Bacillus thuringiensis* strains of foreign countries may be stabilized and somewhat effective in their climate. But in Pakistan, the strains

that were locally isolated are proved to be the best control agents in our country.

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