Biological Control of *Myllocerus subasciatus* Guerin Infesting Brinjal (*Solanum melongena* L.) Using *Bacillus thuringiensis* ssp. *tenebrionis*

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Keywords: Weevil, Coleopteral pest, insecticide, crystal protein, lethal concentration, soil drenching, root dip

Abstract

In Karnataka and other neighbouring states of South India, Coleopteran pest *Myllocerus subfaciatus* infesting brinjal (*Solanum melongena* L.) has assumed a major pest status often resulting in 100% crop loss. Chemical control methods are not ecofriendly and have several problems of resistance development and residual toxicity. Therefore, biological control methods were tried to control the insect. To start with methods were standardized to rear the insect in the laboratory. Insecticidal crystal protein was extracted from *Bacillus thuringiensis ssp. tenebrionis* grown on the sporulation medium. The specific activity of the insecticidal protein in 50 ml 48 h old culture containing 10^{-9} cells was 2.5 μg mL^{-1}. Bioassay was standardized in replicated sets to test the insecticidal property of the toxic protein on *Myllocerus* larvae by soil drenching and root dip methods. In the soil drenching procedure, the freshly emerged larvae from the egg were transferred to the pots containing the brinjal seedlings, which was treated with 10 mL of 10^{2} to 10^{-4} ng/ mL concentrations of Bt toxins. In the root dip method, the root tissue of known size treated with the same concentrations of Bt toxins and dried in air were fed to the larvae. Suitable controls were compared. Mortality was recorded after 24 hours. Lethal concentration of Bt was determined by Probit analysis. The LC_{50} values were 2.69 ng mL^{-1} and Fiducial limit was 1.4-511 ng mL^{-1}. In the root dip method LC_{50} value was 44.936 ng mL^{-1} and Fiducial limit was 20.4-110.5 ng mL^{-1} . Bt toxins (25 ng/mL concentrations) was also tested in *Myllocerus* infested field of brinjal 'Purple Long'plants in 5m x 5m replicated plots by soil drenching. The toxin from *Bacillus thuringiensis ssp. tenebrionis* was effective against *Myllocerus subfasciatus* infesting brinjal crop.

INTRODUCTION

Myllocerus subfasciatus Guerin, which was once a minor pest of brinjal (Ayyar, 1920) and other solanaceous crops has now assumed a major pest status in Southern India especially Karnataka State often resulting in 100 per cent crop loss (Siddappaji, 1976; Tewari and Krishna Kumar, 1983). *Myllocerus* is a plant feeder, both adults and larvae are potentially injurious to the crops. The adult feeds on the leaves and the larvae are subterranean and are exclusively root feeders through out the year. Their breeding is restricted to March to November when damage to the roots by larvae can be expected. The plant starts dying at the time of flowering. A single adult lays more than 100 eggs of which 80% hatch. This pest can be effectively eliminated by employing chemical insecticides. But the indiscriminate use of chemical pesticides has led to several environmental problems including development of resistance in insects to insecticides, resurgence of minor pests, pesticide residue in food, fodder, soil and feed and destruction of beneficial insects. Further, besides the impairment of ecosystem, the modern chemical input based agriculture technology has led to poor economic returns.

Biological control agents such as insect pathogens, predators and parasites can be effective and ecofriendly. Hence the present work was taken up to control *Myllocerus subfaciatus* using the cry 3 gene toxin from *Bacillus thuringiensis* s.sp. *tenebrionis* (Btt) as a controlling agent (Hofte and Whiteley, 1998). It was of interest to test if Btt brought

Proc. XXVI IHC – Sustainability of Horticultural Systems
Eds. L. Bertschinger and J.D. Anderson
Acta Hort. 638, ISHS 2004
Publication supported by Can. Int. Dev. Agency (CIDA)

about effective control of *Myllocerus* a coleopteran pest. Bt insecticides are highly specific, biodegradable and non-accumulative. Their applications offer little risk of long-term impact on the ecosystem, contamination of the environment and insects may not develop resistance to microorganisms as easily as the chemical insecticides.

MATERIALS AND METHODS

To Develop Standard Insect Rearing Techniques in the Laboratory

Adult weevils of *M. subfaciatus* collected on brinjal crop were reared in the laboratory on potted plants enclosed in wire mesh cage. Freshly emerged adults were sexed and individual pairs were enclosed in petri plates with filter paper. The filter paper was kept moist using distilled water to facilitate egg laying. The eggs laid on the filter paper or between the filter paper and the wall of the plate were retained for hatching and the incubation period and percentage of hatching were recorded. Larval development was studied by enclosing individual larvae immediately after emerging, in plastic pots in which brinjal seedlings were grown. This allowed the larvae to feed on established roots. The larvae were removed from the pot regularly and instars measured. In other experiments, larvae were changed to the pots which contained 3 to 4 day- old finger millet (*Eleusine coracana* L.) seedling. This was done to develop an alternate source of food for the larvae.

Mass Culture of Btt and Extraction of Toxin

One L of sterile T3 medium (tryptone 3g, tryptose 1g, yeast extract 1g and sodium phosphate 0.05g, MgCl 0.005 per litre water, pH 6.8) was inoculated with Btt grown on nutrient agar plates and incubated at 30 C for 72 hours. Cells were harvested by centrifuging at 8000 rpm at 4 ^{o}C for 5 minutes and the pellet was washed with I N NaCl and 0.01% Triton x-100 . The suspension was centrifuged once more and the pellet was washed twice with distilled water, followed by dissolving in 2N NaOH and incubating for 1 hour and centrifuging at 8000 rpm, for 5 minutes. The supernatant was used in bioassay as stock solution.

Standardisation of Bioassay for Control of *M. subfaciatus* Using Btt Soil Drenching

Seven different workable concentration from 10^{2} to 10^{-4} ng/mL of the Btt toxins suspended in distilled water was used for the bioassay. Surface sterilised brinjal seed were sown in paper cups containing 100 g sterile soil. The pots were covered with mesh. When the seedlings were 10 day old 25 first instar larvae were introduced to the pots. After 24 hours when the larvae established on the roots of the host plant 20 ml each of Btt endotoxin of concentration mentioned in Fig 1 were sprayed on to each pot. The experiment was carried out in five replications at 27 ^{o}C. Mortality rate of the larvae was recorded after 36 hours. In control pots distilled water was sprayed. Any mortality in control was used for correcting the mortality in treatment using the following formula

$$\text{Corrected mortality \%} = \frac{(\text{\% mortality in treatment - \% mortality in control})}{(100 - \text{\% mortality in control})} \times 100$$

The lethal concentrations of LC_{50} for each concentrations was determined by Probit analysis. Two trials were carried out to confirm the results.

Root Dip Method

Seven different workable concentrations (10^{2} to 10^{-4} ng/mL) of Btt were selected. Root tissue, which measured three to four centimetres and weighed about 2 grams were dipped in the various toxin concentrations and air dried. Twenty five larvae were allowed to feed on these treated roots. The larvae were starved for twelve hours before allowing them to feed on these treated roots. The larvae were allowed to feed on the treated root for

five hours and then normal diet was given. Experiments was replicated five times. Mortality was recorded 24 hours to 72 hours after starting treatment. Moribund larvae were considered as dead. Twenty five larvae were used for each concentration. A control batch of twenty five larvae were fed roots treated with sterile distilled water. Mortality of the larvae was recorded after 36 hours. The LC_{50} for each of concentration of the Btt toxin was determined by Probit analysis. Two trials were carried out to confirm the results.

Field Study

Six plots 6m x 6m were sown with brinjal ‘ purple long’ at a distance of 60 x 30 cm in a field having loamy soil , pH 6.5. Twenty plants were maintained in each plot. When the plants were 30 day old the larvae were introduce near the root zone of each plant at the rate of 25 larvae per plant. The larvae were allowed to establish on the plant root by feeding on it. Each root zone was sprayed with 25 ng mL^{-1} concentration of Btt endotoxin. Control plants were sprayed with distilled water. The mortality of the plants were recorded over a period of 15 days. Plants not treated with the weevils were also maintained as second controls.

RESULTS

To Develop Standard Insect Rearing Techniques in the Laboratory

Myllocerus subfaciatus were reared successfully in the laboratory. The adult female laid more than 100 eggs which averaged 92% hatched. The newly hatched eggs were transferred to the pots with 10 days old brinjal seedlings. The larvae were fed on finger millet seedlings as an alternative. Immediately after hatching the grub looked creamy, crinkled and cylindrical. Not many structural differences between different larval instars were noticed. Total larval period ended after 40 days. Availability of roots, optimum moisture in the soil and soil temperature were the important factors favouring development of larvae. The first second, third and fourth instar larvae measured 2.9 mm by 1.36 mm, 5.09 mm by 1.91mm, 7.19 mm by 2.61 mm, and 7.63 by 3.28 mm in length and breadth, respectively for each instar.

Standardisation of Bioassay

The lethal concentration (LC_{50}) of Btt to larvae of *Myllocerus* was calculated from the mortalities shown in Figure 1. LC_{50} is the value of endotoxin that is able to kill 50% of the treated larvae. The observed LC_{50} values was obtained directly from the figure plotted between empirical Probit and the toxin concentration of the *Bacillius thruringiensis.* All the data were further subjected to probit analysis to get an appropriate value of LC_{50} and its percent confidence interval (i.e Fiducial limit), the values of the observed and estimated LC_{50} of both soil drenching and root dip method are given in Table 1.

Soil Drenching Method

The first instar larvae were the most sensitive to Bt toxins. As the concentration increases mortality also increases. Over 70% of the larve were killed at the highest concentration (2500 ng/mL) used. The time taken by the toxin to kill increased as the concentration of the toxins decreases. Concentration verses mortality graph is shown in Figure 1. The LC_{50} value estimated for Bt toxin by this method is 2.690ng/mL. The Fiducial limit is 1.4 ng/mL. This methods was more efficient than root dip method.

Root Dip Method

The mortality verses concentration of btt endotoxin is represented in Figure 2. The LC_{50} value is 44.963 ng/mL and the fudicial limit is 20.4 ng/mL. As the concentration of the Bt toxins increases the mortality percentage also increases. The percentage of mortality was less compared to the soil drenching. Mortality of the larvae depended on the maintenance of humidity and temperature.

Field Study

Myllocerus was partially controlled on Brinjal plants when treated with 25 ng/mL of btt endotoxin. Three out of 20 infested plants treated with btt toxin died, while 14 out of 20 control infested plants died. . In controls were weevils were not used, all the 20 plants were healthy and the yield was the highest, Table 2.

DISCUSSION

Intensive cultivation practises with enhanced crop protection strategies have led to many newer aggravated pest problems. The present study using Btt endotoxin clearly demonstrated the possibilities of controlling the insect *M. subfasciatus* by biological means. *B. thuringiensis ssp. kurstaki* controlling ball worm of cotton is very well documented. But biological control of *M. subfaciatus* has not been studied so far. This study is the first to demonstrate successful the control of brinjal weevil with btt endotoxin. As *M. subfaciatus* is a coleopteran insect, btt should be able to be safely used to control this pest in large-scale field trials. In the present study, a standard method is developed for rearing the insect in laboratory conditions. The results obtained from soil drenching were better than the root dip method. Purified toxins used in this study were found to be more effective than applying the active cells as found in our earlier study (Saravanan, 1999). The advantage of toxin over live bacterial application is that, these bacteria may multiply in the soil and may affect the other beneficial organism in the future. The mechanism of action of the endotoxin is similar to other bt strains (Bernad, 1986). In the field trials many factors influence the effectiveness of the Bt toxins to control the insect. There is a need to study the different soil factors which effect the concentration of the endotoxin and their residual effects. Few companies have already placed Btt products on the market. *Bacillus thuringiensis ssp. tenebrionis* can very well be used to control *M. subfaciatus.*

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Tables

Table 1. LC 50 and fudicial limit value of the bioassay conducted with Bt toxin on *M. subfaciatus* first instar larvae.

Method of application	LC_{50} ng/ml	Fuducial limit
Soil drenching	2.69	1.4
Root dip method	42.527	17.8-113.9

Table 2. Field testing of Btt endotoxin on *M. subfaciatus* infesting brinjal.

Treatment	No. of plants wilted after 45 days	% of mortality
Treated with Bt	3	80
Untreated control	14	20
Untreated control without weevils	0	Nil
CD 5%	0.467	5.25

Figures

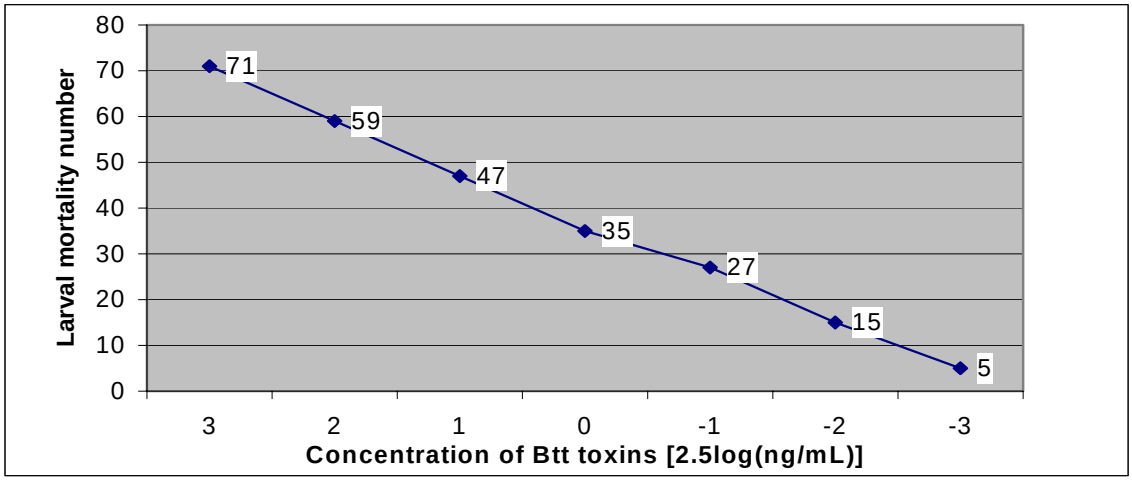

Fig. 1. Mortality of first instar larvae of *M. subfaciatus* treated with different concentrations of btt endotoxin by soil drenching method.

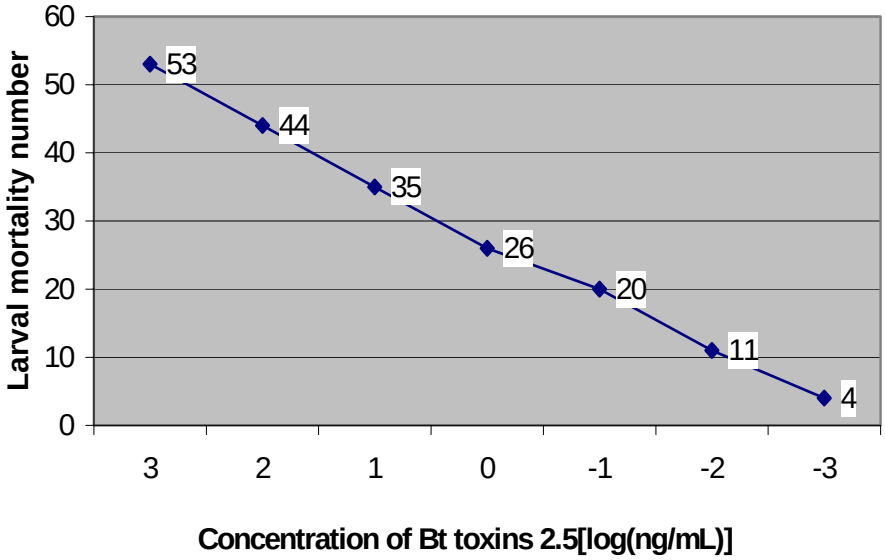

Fig. 2. Mortality of first instar larvae of *M. subfaciatus* treated with different concentrations of btt endotoxin by root dip method.