Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 2 (15-16): 1079-1082, 2003
ISSN 1682-3974

The Use of CAMB Biopesticides to Control Pests of Rice (*Oryza sativa*)

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Abstract: The biopesticides based on fungus *Metarhizium anisopliae* and bacteria *Bacillus thringiensis* (Bt) were used on stem borer and leaf folder of rice, which have reduced the population of these pests effectively both in laboratory and in the field. In laboratory bioassays CAMB biopesticides were very useful on insect larvae of *Helicoverpa armigera*. By using the combination of fungal and bacterial formulation the increase in mortality was studied and it was 100% after 96 h. In the field trials a significant effect on stem borer and leaf folder was observed. On the basis of these results, biopesticides can safely be recommended for the control of rice pests with no harmful effect on its predators as in case with chemical pesticides.

Key words: *Metarhizium anisopliae*, *Bacillus thringiensis* (Bt), biopesticide, rice, *Helicoverpa armigera, Cnaphalocrocis medinalis* and *Scirpophaga incertulas*

INTRODUCTION

Rice (*Oryza sativa*) is the most important food crop of the world. In Pakistan, the yield of rice is very low as compared to other rice producing countries. Several factors contribute for such low yield and among them most important are loses due to insect attack. The leaf folder (*Cnaphalocrocis medinalis)* and yellow stem borer (*Scirpophaga incertulas*) are very serious insect pest of rice. Losses due to these insects usually occur 5-10% and sometime reaches to 60% (Pathak and Khan 1994). For the control of these pests, chemical spray is most common practice. Concern about environment pollution, resistance to pesticides, residues in food and biodiversity make new and novel strategies for the control of pests like rice leaf folder and yellow stem borer. Critically important is to secure food for a rapidly growing population. In view of these considerations, biopesticides offers a technically feasible and environmentally acceptable strategy for controlling agronomically important insects. The use of biopesticides in many countries is limited for a variety of reasons most notable among them is the poor efficacy of imported products under local conditions(Prior, 1989).

Previous workers (Karim *et al.,* 1999) have made extensive studies on efficacy, resistance buildup and economics of CAMB biopesticide. The studies carried out by Zafar *et al.* (2000) represented shelf life and field evaluation of commercial product against *Helicoverpa armigera* on tomato. They reported that the CAMB Bt formulation based on indigenous strain of bacteria successfully controlled insect population in small scale tomato field after a storage period of 1-2 years. In America Badilla *et al.* (2000) reported *M. anisopliae* for the control of sugarcane pests. Therefore, microbial control preparation may be considered as an alternative to the chemical control. The present studies were carried out to see the effect of CAMB biopesticides for the control of rice leaf folder and yellow stem borer.

MATERIALS AND METHODS

Insects: For laboratory bioassay the insects were reared on the diet based on chickpea flour in the insectory of the centre. The insect colony was constantly supplied with moths from the field populations to prevent inbreeding and subsequent loss of vigor. It was maintained under conditions of ambient temperature, humidity and light.

CAMB Biopesticides: For large-scale production of formulation, local strain of bacteria *Bacillus thuringiensis* (Bt) was propagated in 14 liter "Microfern Fermenter" New Brunswick, USA model MF-114 (Zafar *et al.*, 2001 and Zafar *et al.*, 2003). The fungus biopesticide was prepared by propagating an entomopathogenic fungi *Metarhizium anisopliae.*

Bioassay procedure: The bioassays were carried out on 2nd instar of *H. armigera* larvae for pathogenic studies. Spores of *M. anisopliae* were harvested from 15-20 days culture and suspended in 0.03% solution of Tween 80. The spores were counted under microscope by using haemocytometer and diluted to desired concentrations. The bioassays were made using a dose of $1X10^7$ spore/ml. The insects were inoculated by fungal spore suspension or Bt formulation as described by Hall (1976). After inoculation, the larvae of the insects were transferred to small glass jar by providing moisture on filter paper and cotton leaves as diet. The control insects were treated similarly with 0.03% tween 80 or water. The incubation temperature was 22±1°C in 16:8 (light: dark) photoperiod and humid conditions were maintained for the first 24 h by placing the boxes between paper towels.

Each assay consisted of three replicates, 10 larvae per replicate and assay was repeated three times. Insects were examined daily and dead individuals were transferred to petri dishes lined with moist filter paper to encourage external conditions for the germination of fungal spores. The data of mortality rate was recorded after 48, 72 and 96 h.

Rice field: The field trial experiment was conducted in premises of National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore. Total plant area was divided into four treatments and four replicates according to randomized complete block design (RCBD). The field was sown with supper basmati cultivar of rice. Infestation of rice leaf folder and stem borer started after 35-40 days of rice plantation. The biopesticides were sprayed three times by looking the severity of infestation.

Treatments: Timing of biopesticide application and larval behaviors are very important for the effective control of target pests (Ghidiu and Zehnder, 1993). For best results, it was recommended to apply biopesticide treatments in the evening hours (Karim *et al*., 1999). For each treatment separate backpack hand sprayers were used and treatments were applied one hour before sunset.

T1 = CAMB Bt. (Biopesticide)	@ 250g/100lit. / Acre
T2 = CAMB Fungus (Biopesticide)	@ 250g/100lit. / Acre
T3 = CAMB Bt + CAMB Fungus (Biopesticide)	@ 250g +250g/100lit. / Acre
T4 = Control	

Pest scouting: The criteria of fungus and Bt biopesticide evaluation were based on pre and post spray pest scouting. The mortality percentage data was calculated by looking the severity of infestation (Amer *et al*., 1999). Data was recorded after spray on 1, 2, 7 and 9 days.

RESULTS

Laboratory bioassay: Some experiments were completed in the laboratory to check the effect of fungal and Bt formulation on the larvae before applying the biopesticides as a spray in the field. The data was collected upto 96 h. The mortality percentage was

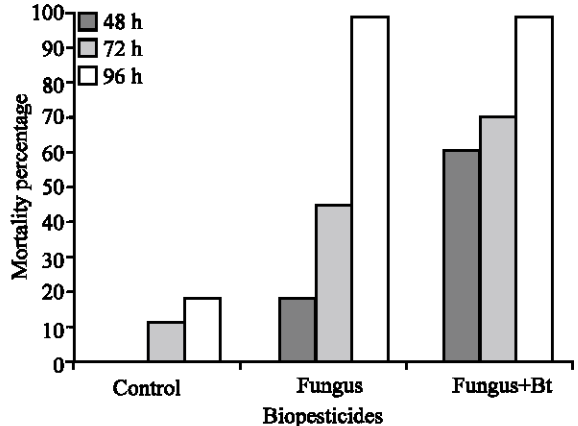

Fig. 1: Laboratory bioassay on larvae of *Helicoverpa armigera*, showing the mortality percentage due to biopesticides

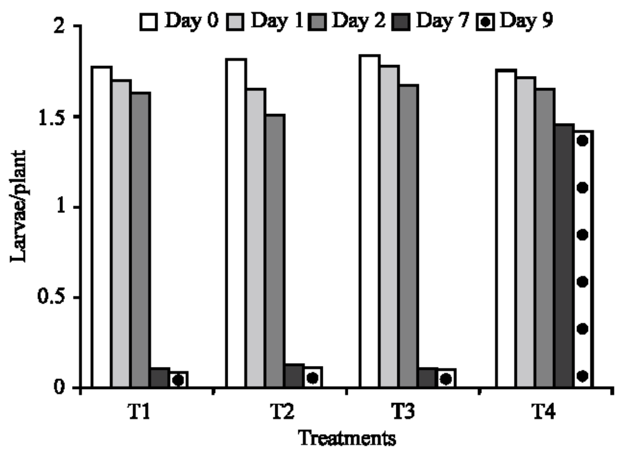

Fig. 2: Effect of biopesticides on rice leaf folder, pre and post-spray population of larvae/plant was calculated in all treatments

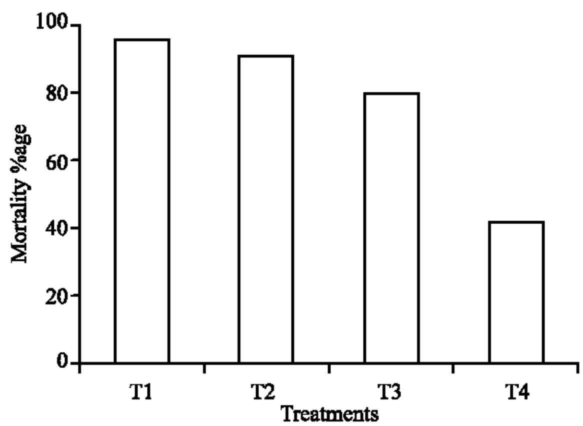

Fig. 3: Mortality percentage of rice leaf folder due to effect of biopesticides

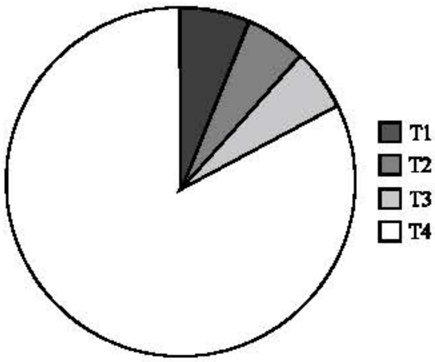

Fig. 4: Effect of biopesticides on whiteheads of rice

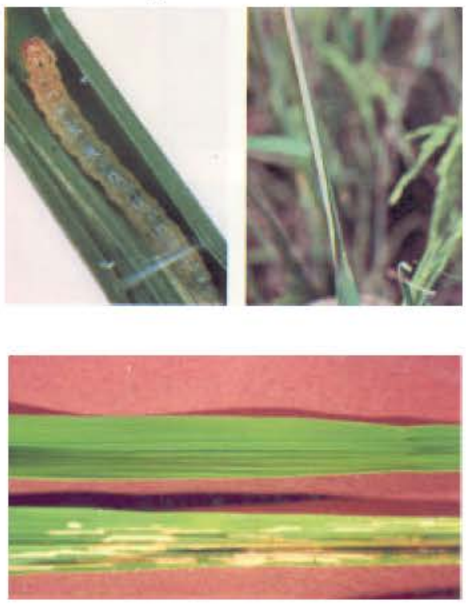

Fig. 5: Rice leaf folder and damage on leaves of rice. Larvae feed on leaf and fold the leaf to form a tube. Severely damaged plants appear burnt

calculated after 48, 72 and 96 h and it was observed 20% after 48 h and 49% after 72 h. By using the combination of fungal and Bt formulation the increase in mortality was observed. It was 65 and 75% after 48 and 72 h. The mortality was 100% after 96 h (Fig. 1).

Effect of biopesticide on rice: When the rice field was naturally infested with leaf folder. The biopesticides were sprayed to the field. The number of larvae was counted in each replicate. The data was collected on day 0 (before spray), on day 1, 2, 7 and 9. The number of larvae per plant was calculated and it is clear from the figure that all the treatments presented different response. In the first treatment (T1), on day 1 high number of larvae was observed and with the passage of time the insect population was reduced due to effect of biopesticides and very few insects were recorded on day 9 (Fig. 2). Similar results were observed in all other treatments. The damage caused by rice leaf folder is shown in Fig. 5.

Mortality percentage: The mortality percentage due to the effect of biopesticides was calculated. In all the treatments (T1, T2 and T3) high mortality was observed as compare to control (T4). In Bt and fungal formulation, almost equal mortality was observed showing the effect of biopesticides on insect population (Fig. 3).

Effect on whiteheads: Whitehead is the damage caused by stem borer to the rice crop. It is usually observed after flowering. Stem borer larvae migrate to between the leaf sheaths. It causes the entire panicle to dry. Average number of whiteheads was counted in each replicate. A significant reduction of whitehead was observed in all treatments due to effect of spray as compare to control. In control (T4) the number of whitehead was very high. The Fig. 4 demonstrates, the effect of biopesticides on stem borer, which ultimately resulted in reduction of whiteheads.

DISCUSSION

Public concern about chemical residues on fruits, vegetables and other crops has led to a progressive increase of interest in alternative strategies for the control of diseases and pests. The application of biological control is increasing largely because of greater environment awareness and food safety concerns plus the failure of conventional chemicals due to an increasing number of insecticide resistant species (Dent, 1993). The present study describes the use of CAMB biopesticides for the control of rice leaf folder and stem borer. Preliminary laboratory bioassay gave excellent results on insect larvae. The experiment was continued up to 96 h. Mortality due to fungal biopesticide was 20 and 49% after 48 and 72 hours. Increase in mortality was observed by using the combination of fungus and Bt formulation. It was 100% after 96 h (Fig. 1). CAMB Bt biopesticide was found very effective against lepidopteran insects *Helicoverpa armigera* and *Erias vitella* on tomato and okra crops (Karim *et al*., 2000 and Zafar *et al*., 2000).

In field trials on rice crop, spray data indicates that under natural infestation pest population in the field can never remain same. Plots sprayed with CAMB biopesticides represented decrease in percent infestation from 1st to 9th day (Fig. 2). These results suggest that biopesticides were effective not in laboratory bioassay but also in the field against rice pests. Yield of rice crop in same conditions of attack was also studied. In treated plots the crop looks healthier and gave better yield than non-treated, which recommends CAMB biopesticide as a best biological agent in crop protection.

In present studies the fungal biopesticide based on *M. anisopliae* was used along with Bt biopesticide. The fungus generally enters in the insect body through spiracles and pores. Hyphal growth continues until the insect is filled with fungal mycelia. The fungus can also produce secondary metabolites, such as destruxin, which have insecticidal properties on moth and larvae (Cloyd, 2002). The effect of biopesticide on rice stem borer was also studied which resulted in reduction of whiteheads in all the replicates as compare to control (Fig. 4). Bt biopesticide based on bacterial cry toxin, which binds to a specific receptor on the brush border membrane of insect midgut epithelial cells, Bt toxin insert rapidly and irreversibly in to plasma membrane of gut cells (Malik *et al.*, 2001) and either form an ion channel or act on some membrane components to open a pore, which resulted in cell lysis and eventually death of insect larvae (Knowles and Dow, 1993).

Our results are similar to previous reports of Godwin and David (1995), that entomopathogenic fungi can be use for the control of *Glossiana spp.* The application of biopesticides on rice crop indicated that CAMB biopesticides successfully controlled rice stem borer and leaf folder in small-scale rice field. This microbial control strategy may be considered as an alternative to the chemical pesticides for the control of pest population of field crops. Therefore, CAMB biopesticides can be use in integrated pest management strategies of rice without disturbing the agro-ecosystem and the quality of the environment.

ACKNOWLEDGMENTS

Authors are grateful to Mr. Khalid Masood and Mr. Shaheryar Iqbal for their cooperation in preparation of this manuscript. We also acknowledge the support of Insectory group, especially Raza Ali Zaidi and Muhammad Munir.

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