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Evaluation of the Effectiveness of Entomopathogenic Fungus (*Metarhizium anisopliae* var. major) on Cabbage Semilooper (*Chrysodeixis eriosoma* Doubl.)

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ABSTRACT. *Laboratory bioassays and field evaluations were carried out to determine the effectiveness of an entomopathogenic fungus isolated from field infected larvae of cabbage semilooper, Chrysodeixis eriosoma Doubl. Fungus was identified and confirmed as Metarhizium anisopliae var. major (Johnson). In laboratory bioassays a fungal spore concentration of $40x10^8$ spores/ml caused 92% and 90% mortality of 3–5 days old and 5–8 days old cabbage semilooper larvae respectively, within 7 days after treatment. Susceptibility of 10–12 days old semilooper larvae was very low as the mortality caused by the spore concentration of $40x10^8$ spores/ml was only 8%. A spore concentration of $86x10^7$ spores/ml was found to be the optimum spore concentration under laboratory conditions. In the field experiments where aqueous suspensions of dried powder of dead infected semilooper larvae were used, spore concentrations of $9.3x10^9$, $8.4x10^{11}$ and $8.8x10^{13}$ spores/ml caused a cumulative mortality of 65% of 3–5 days old semilooper larvae.*

The considerably high mortality of cabbage semilooper larvae under field conditions indicates the suitability of Metarhizium anisopliae var. major as an effective biocontrol agent to be incorporated in cabbage semilooper management systems.

INTRODUCTION

Cabbage semilooper (*Chrysodeixis eriosoma*) is a major pest that affects cabbage cultivations in different parts of the world (Prasad, 1963). It is one of the most harmful and widely spread pests that thrive on cabbage and other Brassica crops grown in Sri Lanka (Ketipearachchi *et al.*, 1992). The cabbage semilooper is very often found in up country than elsewhere. Early larval stages, especially, first and second instars feed on lower epidermis of cabbage leaves, whilst later instars eat young and moderately matured leaves forming semi circular patches on the foliage. At severe infestation level entire leaf lamina may be destroyed by the pest.

The damage caused by semiloopers reduces the yield of the marketable crop by 64% to 78% (Prasad, 1963). These losses limit the attempts to promote the cabbage cultivation. To reduce the loss of yield and to improve the quantity and quality of produce

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the pest control on cabbage is very important. In Sri Lanka, farmers depend mainly on chemical insecticides to control cabbage caterpillars. Very often farmers do not adhere to the recommended insecticides and application rates. They use highly toxic insecticides in high doses. Some farmers do not follow the pre-harvest safety intervals after the application of pesticides. This indiscriminate use of insecticides has lead to many adverse effects such as environmental pollution, health hazards, residual effects, development of secondary pest outbreaks, development of resistance, biomagnification *etc.* In addition to above adverse effects, the farmer has to face ever increasing cost of production due to high pesticide prices.

Therefore, paying more attention to the safer and less expensive insect specific insecticides is very important. Use of botanical pesticides is one alternate safe method for vegetable pest management and pesticides based on Neem products are now been used (Schmutterer, 1990). Pathogenic microbes such as bacteria, fungi, viruses have potential in pest control. Out of these pathogenic microbes entomogenous fungus *Metarhizium* sp. is reported to be effective in controlling numerous agriculturally important insect pests (Fargues *et al.*, 1997; Milner, 1997; Milner *et al.*, 1996; Quintela and McCoy, 1998; Rath and Tidbury, 1996; Thomas *et al.*, 1996). In this experiment, an attempt is made to determine the efficiency of *Metarhizium anisopliae* var. major for control of cabbage semilooper under laboratory and field conditions.

MATERIALS AND METHODS

Laboratory and field trials were carried out with *Metarhizium anisopliae* var. major isolated from field infected larvae of *Chrysodeixis eriosoma* collected from cabbage cultivations from the village Pananwala in Kandy district. Fungus was cultured on Potato Dextrose Agar (PDA) media and pure *Metarhizium* cultures were obtained by subculturing and the identification of the fungus was confirmed by CABI Bioscience, United Kingdom.

Experiment I

Investigation of effectiveness of *Metarhizium anisopliae* against different stages (3–5, 5–8 and 10–12 days old larvae) of cabbage semilooper (*C. eriosoma*)

Young cabbage leaves were removed from plants and ends of leaf stalks were plugged with moistened cotton wool. Each leaf was placed in a beaker lined with moistened filter paper to prevent withering of the leaves. Ten healthy similar size larvae (3–5 days old) were introduced in to each beaker. Two concentrations $40x10^8$ and $30x10^8$ spores/ml of *Metarhizium* water suspensions were applied on to each larva. Distilled water was used as control. One ml of each suspension was applied on to the dorsal region of each larva using a micro syringe and allowed them to feed on fresh leaves. Beakers were covered with net cloth in order to permit ventilation and to prevent the escape of larvae. Control was kept separately, however under the same environmental conditions to avoid possible contamination by *Metarhizium* spores. Experiment was conducted under Randomized Complete Block design with four replicates.

This experiment was repeated using 5–8 days old larvae and 10–12 days old larvae in order to evaluate the effectiveness of *Metarhizium* on different growth stages of larva. During each experiment, each beaker was observed at 24 h intervals and number of dead larvae was recorded. Fresh leaves were introduced daily until the death or pupation of all larvae. Mortality data in the treatments were corrected using Abbott's Formula. Data were analysed by ANOVA after arcsin transformation.

Experiment II

Determination of the optimum spore concentration range required to control *C. eriosoma* under laboratory conditions

Young cabbage leaves were removed from the plants and leaf stalks were plugged with moistened cotton wool to prevent withering of leaves. Each leaf was placed in a beaker. Concentrations of $430x10^7$, $86x10^7$, $17.2x10^7$, $3.44x10^7$ and $0.688x10^7$ spores/ml, *Metarhizium* water suspension were used as treatments. Ten of three to six days old semilooper larvae were introduced in to each beaker and treated with 1 ml of spore concentrations on to the dorsal region of each larva using a micro syringe. Distilled water was sprayed on larvae used in control.

Experiment was laid out in Randomized Complete Block design with four replicates. Mortality of larvae was recorded at 24 h intervals. Fresh leaves were provided daily until the death or pupation of all the larvae. Mortality data in treatments were corrected using Abbott's Formula.

Experiment III

Investigation of effectiveness of *Metarhizium anisopliae* var. major against larvae of cabbage semilooper under field conditions

As attempts to mass culture *Metarhizium anisopliae* isolated from Cabbage semilooper on rice and fish meal media were not successful, dry powder of dead infected larvae was used for field testing. Cabbage semilooper larvae were infected by applying the spores of *Metarhizium* fungus maintained on Potato Dextrose Agar (PDA) media. Also naturally infected larvae were collected from the fields and were used after confirmation of the fungus species using spore characters. These infected larvae were dried and crushed well and converted to a powdery form.

In order to ascertain the effectiveness of the field application, 12 field plots were prepared at Pananwala in Kandy district. Each plot was 3 m × 3 m in size and were separated by a 30 cm wide drain. Nurseries were established and sixteen days old cabbage seedlings were planted in experimental plots at a spacing of 50 cm and other recommended agronomic practices were carried out. Treatments were replicated three times.

The fields and plots were observed daily to ascertain the infestation of plants by semiloopers. All the cabbage semiloopers appeared on the plants of each plot were counted

and recorded, one month after establishment of the plots. Concentrations of 9.3×10^{9}, 8.4×10^{11} and 8.8×10^{13} spores/ml, *Metarhizium* water suspensions were prepared using ground powder of infected larvae. Even though, 86×10^{7} spores/ml was found to be the optimum spore concentration under laboratory conditions where the fungal spore suspensions were applied directly on to the larvae, higher spore concentrations were used in the field application as the fungal suspensions were sprayed on the cabbage plants. Each spore concentration was applied to the plots in the afternoon at a rate of 350 ml of spore suspension per plot using a hand sprayer until run off occurred on its foliage. Treatments were arranged in a Randomized Complete Block design. For control plots pure water was applied using a separate hand sprayer.

One week after treatment the infected larvae were collected and recorded from each plot. Number of healthy semilooper larvae in each plot too were recorded. Second round of infected larvae were collected and recorded 12 days after treatment and this was repeated until pupation of all the larvae in experimental plots. Mortality percentages were calculated and were analysed using ANOVA after arcsine transformation.

RESULTS AND DISCUSSION

In the lab experiment disease symptoms appeared from 2–3 days after treatment. Greenish body colour of the larvae changed into pale whitish colour at first and ultimately the whole larval body was covered with white mycelium. Both spore concentrations showed significant effect on semilooper larvae. For 3–5 day old larvae spore concentration of 40×10^{8} spores/ml gave 92% mortality and spore concentration of 30×10^{8} spores/ml gave 75% mortality within six days after treatment. In the case of 5–8 day old larvae, spore concentration of 40×10^{8} spores/ml gave 90% mortality and 30×10^{8} spores/ml gave 71% mortality within six days after treatments (Fig. 1 and Fig. 2). However, there was no significant treatment difference between these two spore concentrations.

The susceptibility of 10–12 days old mature cabbage semilooper larvae to *Metarhizium anisopliae* var. major was significantly low compared to the susceptibility of 3–5 and 5–8 days old larvae. Fungal spore concentration of 40×10^{8} and 30×10^{8} spore/ml showed only about 8% mortality and 92% of larvae pupated and emerged as normal adults (Fig. 1 and Fig. 2). Very few pupae showed signs of fungal infection.

This reduced susceptibility of mature *C. eriosoma* larvae to *Metarhizium anisopliae* fungus may be due to difficulties in penetration of the fungus into the insect body or may be due to increased defence mechanisms of mature larvae. Thus to obtain, effective control of cabbage semilooper population the fungal species should be applied when the pest larvae are in an early stage of development. Early control of early larval stages will be of value as the crop can be saved before feeding by later developmental stages of larvae.

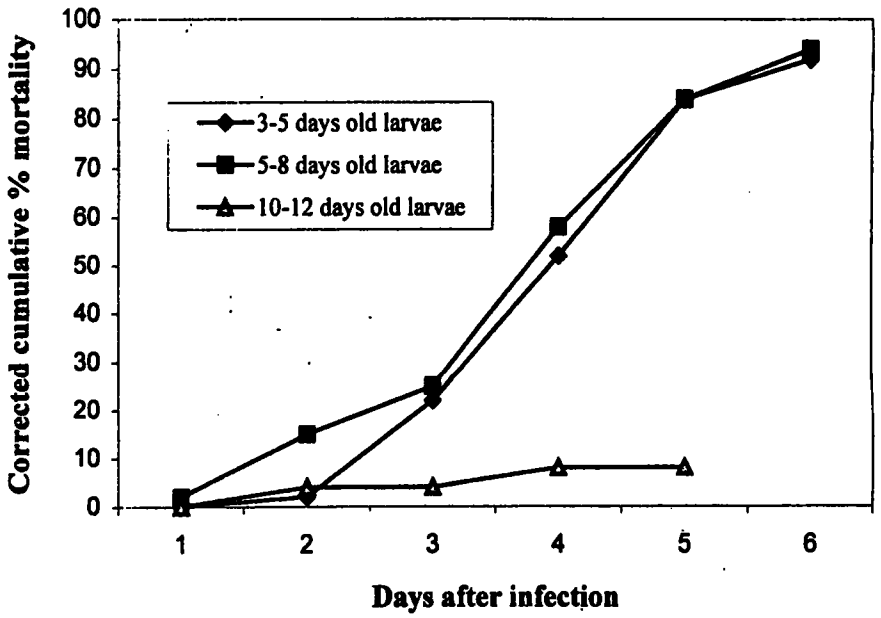

Fig. 1. Effectiveness of *Metarhizium anisopliae* ($40x10^8$ spores/ml) against different stages of cabbage semilooper, *C. eriosoma* larvae.

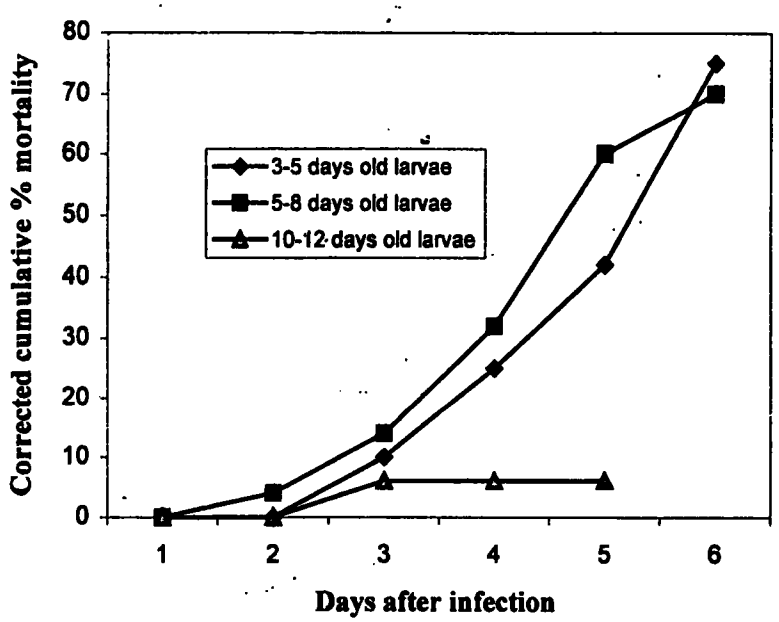

Fig. 2. Effectiveness of *Metarhizium anisopliae* ($30x10^8$ spores/ml) against different stages of cabbage semilooper, *C. eriosoma* larvae.

Experiment II

Determination of the optimum spore concentration range of *Metarhizium anisopliae* var. major to control *C. eriosoma* larvae

When 3–5 days old larvae of *C. eriosoma* were treated with five different spore concentrations $430x10^7$, $86x10^7$, $17.2x10^7$, $3.44x10^7$ and $0.688x10^7$ spores/ml of *Metarhizium* spore suspension, all the spore concentrations cause significant mortality of cabbage semilooper larvae. Spore concentration of $430x10^7$ and $86x10^7$ spores/ml gave the highest mortality of 85 and 90% mortality rate respectively, within six days after infection (Fig. 3). There was no significant difference between the two spore concentrations. Thus, a spore concentration of $86x10^7$ spores/ml can be considered as the optimum spore concentration under laboratory conditions.

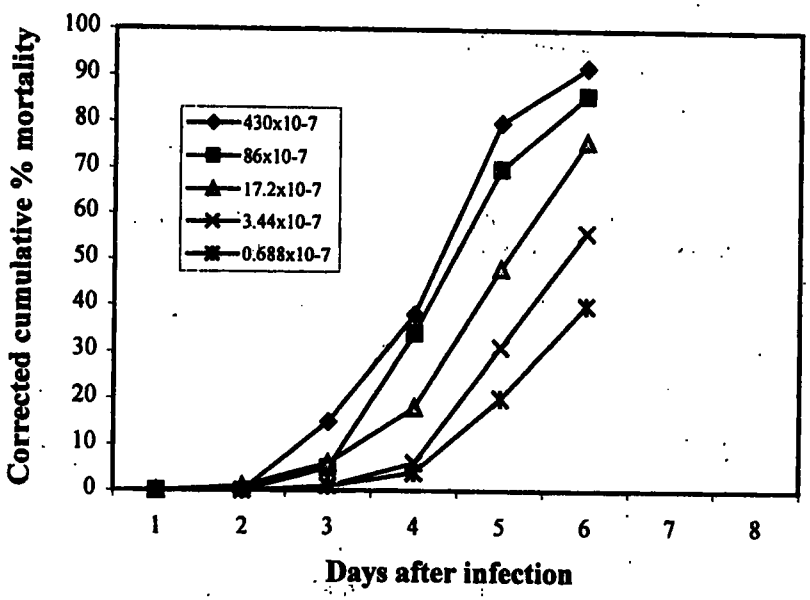

Fig. 3. Effect of different concentrations of *Metarhizium anisopliae* var. major spores on 3-6 days old cabbage semilooper, *C. eriosoma* larvae.

Experiment III

Investigation of effectiveness of *Metarhizium anisopliae* var. major against larvae of cabbage semilooper *C. eriosoma* under field conditions

In the field experiments, the three spore concentrations, $9.3x10^9$, $8.4x10^{11}$ and $8.8x10^{13}$ spores/ml gave 63%, 67% and 63% mortality of cabbage semilooper larvae 7 days after treatment with the fungal spore suspensions. The mortality percentages shown by the

three spore concentrations were not significantly different. The untreated control plots too showed 41% mortality of the pest larvae. This indicates a possible drift of applied spores from treated plots to the control plots with the wind. Some cabbage semilooper larvae, which were healthy when sampling was done at 7 days after treatment were found to be dead when mortality counts were taken 12 days after treatment. All the three fungal spore concentrations showed a cumulative mortality of 60–65% and the control plots showed a cumulative mortality of 50% when mortality data were recorded 12 days after treatment (Table 1). This type of cumulative mortality was reported in field application of *Metarhizium flavoviridae* against *Zonocerus variegatus* (Langewald *et al.*, 1997). The authors have suggested an incubation delay of some fungal spores on susceptible larvae.

Table 1. The percentage mortality of cabbage semilooper (*C. eriosoma*) larvae treated with different spore concentrations of *Metarhizium anisopliae* var. major under field conditions.

Days after treatment	Spore Concentration, No. of spores/ml			Control
	9.3×10^{9}	8.4×10^{11}	8.8×10^{13}	
7	62.5	66.6	62.5	41.0
12	62.5	64.5	50.0	53.4
Cumulative mortality	65%	66%	60%	50%

Observed significantly high percentage mortality in untreated plots showed the natural dispersal ability of the fungal spores in the field. As sophisticated mass culturing methods were not utilized as the fungus was successfully cultured on insects themselves this method of spore preparation and application will be a feasible method to be popularized among farmers at village levels.

This considerably high amount of mortality (60–65%) of cabbage semilooper larvae under field conditions shows the feasibility of incorporation of this crude microbial pesticide in IPM programmes for cabbage semilooper management systems and will help the farmers to reduce dependence on synthetic insecticides.

It was observed during field experiments that the Diamond back moth (*Plutella xylostella*) larvae found on treated cabbage plots were not affected by the applied fungal species.

CONCLUSIONS

The fungus species isolated from field collected diseased cabbage semilooper, *C. eriosoma* larvae was confirmed as *Metarhizium anisopliae* var. major. In laboratory bioassays 3–5 days old cabbage semilooper larvae treated with spore concentration of

$40x10^7$ spores/ml showed symptoms of disease development and a mortality of 92% was observed 7 days after treatment. Same concentration of spores gave 90% mortality of 5–8 days old larvae. A spore concentration of $86x10^7$ spores/ml was found to be the optimum spore concentration under laboratory conditions against cabbage semilooper larvae.

In the field application, where the fungus spores were obtained by grinding dead diseased larvae 60–65% mortality was observed when 3–5 days old semilooper larvae in the field were treated with spore concentrations of $9.3x10^9$, $8.4x10^{11}$ and $8.8x10^{13}$ spore/ml. Significantly high mortality of 50% was observed in larvae in untreated plots indicating the dispersal capacity of spores under field conditions. Because of the high mortality observed under field conditions, this microbial agent can be considered as an effective biocontrol agent to be incorporated in cabbage IPM programmes in the areas where cabbage semilooper occurs as a major pest species. As no sophisticated mass culture methods of the fungus are involved, this will be a feasible agent to be popularized among farmers at village level.

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