



Research Article

Susceptibility of white grub, *Brahmina coriacea* (Hope) infesting potato to local strains of *Beauveria brongniartii* (Saccardo) in Himachal Pradesh

SAURBH SONI*, P. K. MEHTA and R. S. CHANDEL

Department of Entomology, CSK Himachal Pradesh Krishi Vishvavidyalya Palampur - 176062, Himachal Pradesh, India

*Corresponding author E-mail: saurbhsoni@gmail.com

ABSTRACT: *Brahmina coriacea* (Hope) is the most widely distributed and destructive species of white grubs having potential to inflict 40-50 per cent yield losses to potato in Himachal Pradesh. The efficacy of two local isolates of *Beauveria brongniartii* (KH I and KH II) was evaluated against grubs of *B. coriacea* by dip treatment and oral feeding methods. Against first and second instar grubs, KH I in dip treatment proved to be highly effective, whereas for third instar grubs, KH I through oral feeding showed higher virulence. The LC_{50} values for first instar grubs for KH I and KH II were 2.55×10^5 conidia/ml and 2.80×10^5 conidia/ml in dip treatment, whereas in oral feeding method, the LC_{50} values were 7.27×10^5 conidia/ml and 9.69×10^5 conidia/ml, respectively. Against second instar grubs, LC_{50} values calculated were 2.91×10^5 conidia/ml and 3.98×10^5 conidia/ml for KH I and KH II in dip treatment, whereas corresponding values through oral feeding method were 5.36×10^5 conidia/ml and 8.82×10^5 conidia/ml. LC_{50} values for third instar grubs were 4.47×10^5 conidia/ml and 4.88×10^5 conidia/ml for KH I and KH II in dip treatment, whereas through oral feeding, LC_{50} values were 3.03×10^5 conidia/ml and 5.14×10^5 conidia/ml for KH I and KH II, respectively.

KEY WORDS: *Brahmina coriacea*, *Beauveria brongniartii*, Himachal Pradesh, potato, susceptibility

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INTRODUCTION

The larvae of family Scarabaeidae are recognized as pests of planted crops in many parts of the world and are almost universally known as 'white grubs'. In India, the white grubs have been categorized as a national pest and form a major component both in number of species and diversity of habits (Veeresh, 1988). The most widely distributed and destructive species in India are *Brahmina coriacea* (Hope), *Anomala dimidiata* Hope, *Holotrichia longipennis* Bl, *H. consanguinea* Bl, *H. serrata* (Fab.) and *Lepidiota mansueta* Burmeister. *Brahmina coriacea* has been recorded from most of the north western Indian hills occupying Himachal Pradesh, Utrakhhand and Jammu & Kashmir (Chandel *et al.*, 2015). In Himachal Pradesh, *B. coriacea* is the most predominant species constituting about 90 per cent of the total beetle population in higher hills (Chandel *et al.*, 2003).

In potato, the problem of white grubs is quite serious in hilly states where potatoes are grown during summer season as rain fed crop (Misra and Chandel, 2003). In higher hills of Uttar Pradesh, Himachal Pradesh, Jammu & Kash-

mir and north eastern states, white grubs are responsible for causing 40-90 per cent losses in potato yield (Misra, 2000). Besides potato, other cultivated crops such as maize, rice, vegetable, rajmah, ginger and fruit crops are also attacked by white grubs (Chandel *et al.*, 2015b). In majority of farming situations, control of these pests have become increasingly difficult because of the lack of control over the damages they cause. Application of chemical is practically uneconomical, difficult and associated with high cost, environmental pollution and other problems. Among alternative strategies for the control of white grubs, entomopathogenic fungi constitute a potential group of biocontrol agents in the integrated management of white grubs because the pest activity period provides ideal humid conditions for their proliferation (Srikanth *et al.*, 2010). Among them, *Beauveria* is one of the most studied fungi which infect many insect species in different parts of the world (Hajek and St. Leger, 1994). *Beauveria* exists saprophytically in the soil and often causes widespread epizootics wiping out insect populations on crops (Leathers *et al.*, 1993). Three species of *Beauveria*, viz., *B. bassiana* (Balsamo), *B. brongniartii* (Saccardo)

and *B. amorphpha* (von Hoehnel) are active against white grubs (Gupta, 2001). Entomopathogens of native region are of paramount importance in managing soil pests, as they sustain themselves in soil, after proper establishment. *B. brongniartii* is of great importance and can be effectively utilized as one of the components in the management of white grubs in summer planted potatoes. Susceptibility of insect developmental stages to entomopathogenic fungi is a key point in developing a control strategy. In the present study, we investigated the pathogenecity of two isolates of the *B. brongniartii* native to Himachal Pradesh against various larval instars of *B. coriacea*.

MATERIALS AND METHODS

Isolation and maintenance of *Beauveria brongniartii*

Beauveria brongniartii was isolated from the diseased grubs of *B. coriacea* from the potato fields. The diseased grubs showed white mycelial growth on their body and such grubs were collected in screw cap vials and brought to laboratory for isolation of the fungus. The fungus infected grubs were surface sterilized by immersing them into 5% sodium hypochloride solution for 2 minutes and then rinsed with sterile distilled water thrice under aseptic conditions. The sterilized specimen was cut open in a sterile Petri plate and a small portion of infected tissue was streaked on Potato Dextrose Agar (PDA) slants. The slants were kept at $26\pm 1^\circ\text{C}$. The fungus was identified by Dr YS Paul, Senior Mycologist, Department of Plant Pathology, CSKHPKV Palampur.

Maintenance of insect culture in laboratory

Adult beetles of *Brahmina coriacea* were collected from apple orchards during late evening hours around 8:00 PM onwards near the host trees in Shimla hills during mid-June. Beetles were reared in glass jars on fresh pear twigs provided with about 15 cm soil layer at bottom for oviposition. Eggs were separated from jars and transferred to Petri dishes containing moist soil. Newly emerged grubs were transferred to small paper cups containing 4-5 days old maize seedlings. Second and third instar grubs were reared on potato tubers.

Inoculation and treatment of *Brahmina coriacea* grubs for bioassay studies

A laboratory experiment was conducted to determine the virulence of *B. brongniartii* on first, second and third instar grubs of *B. coriacea*. Fungal isolates collected from Kheradhar and coded as KH I and KH II were tested by dip treatment and by oral feeding methods. There were 7 treatments (6 conidial suspension and a check) each replicated thrice with 15 larvae/replicate. The conidia were harvested

by adding 10 ml of sterile distilled water in PDA slants containing 20 days old well sporulated culture. The conidial count was taken through haemocytometer and different conidial suspensions ranging from 9.5 to 0.5×10^5 conidia/ml were prepared in sterilized distilled water. The concentration of stock solution was 9.5×10^5 conidia/ml, and further concentrations were obtained through serial dilution method. For obtaining concentration of 7.5×10^5 conidia/ml, 79 ml of stock solution was taken and final volume of 100 ml was prepared by adding sterilized distilled water. To make lower concentrations of 5.5, 3.5, 1.5 and 0.5×10^5 conidia/ml, 73 ml, 64 ml, 43 ml and 33 ml suspension was taken from each serial dilution, and final volume was prepared to 100 ml using distilled water, respectively.

Dip treatment

The first and second instar grubs were dipped for about 10 seconds in the conidial suspension @ 1ml/grub in Petri plate, whereas for dip treatment of third instar grubs, the volume was standardized to 5ml/grub. Treated grubs were immediately placed in paper cups containing moist sterilized soil. First instar grubs were released in cups containing 4-5 days old maize seedlings. However, for second and third instar grubs, small potato tubers were put in soil.

Oral feeding method

For first instar grubs, maize roots were dipped in conidial suspension, whereas for second and third instar grubs, small potato tubers were treated. After treatment, maize seedlings were given to first instar grubs and potato tubers were given to second and third instar grubs for feeding. Single grub was released in each cup and three replications were maintained having 15 grubs per replication.

Observations on grub mortality were recorded at weekly interval up to eight weeks as per method standardized under AINP on soil arthropod pests (Anonymous, 2009). The dead larvae were counted, transferred to new Petri dishes containing moistened filter paper and examined for another seven days for growth of fungal hyphae to confirm mycosis. Eight week mortality data were subjected to probit analysis and LC_{50} values were calculated as outlined by Finney (1971) and using computer based probit software. Corrected per cent mortality was calculated as per Abbott (1925) formula.

RESULTS AND DISCUSSION

The fungus infected grubs of *Brahmina coriacea* were collected from potato fields at Kheradhar (1950 m amsl) in Sirmaur district of Himachal Pradesh. The cadavers of grubs were completely engulfed in white hyphae.

Chandel *et al.* (2015) collected a total of 321 grubs of *B. coriacea* and out of which 20.2 per cent were found to be infected with fungus. In infections of adult beetles, the fungus often emerged from intersegmental joints. The fungus was isolated on PDA from field infected white grubs and

after purification, was identified as *B. brongniartii*. The *B. brongniartii* was identified by its shorter conidiogenous rachis and ellipsoid conidia which measured $2-3 \times 1.5-2.5 \mu\text{m}$ in size. Colonies on PDA grew quickly, and based on morphological features the fungus was categorized into two

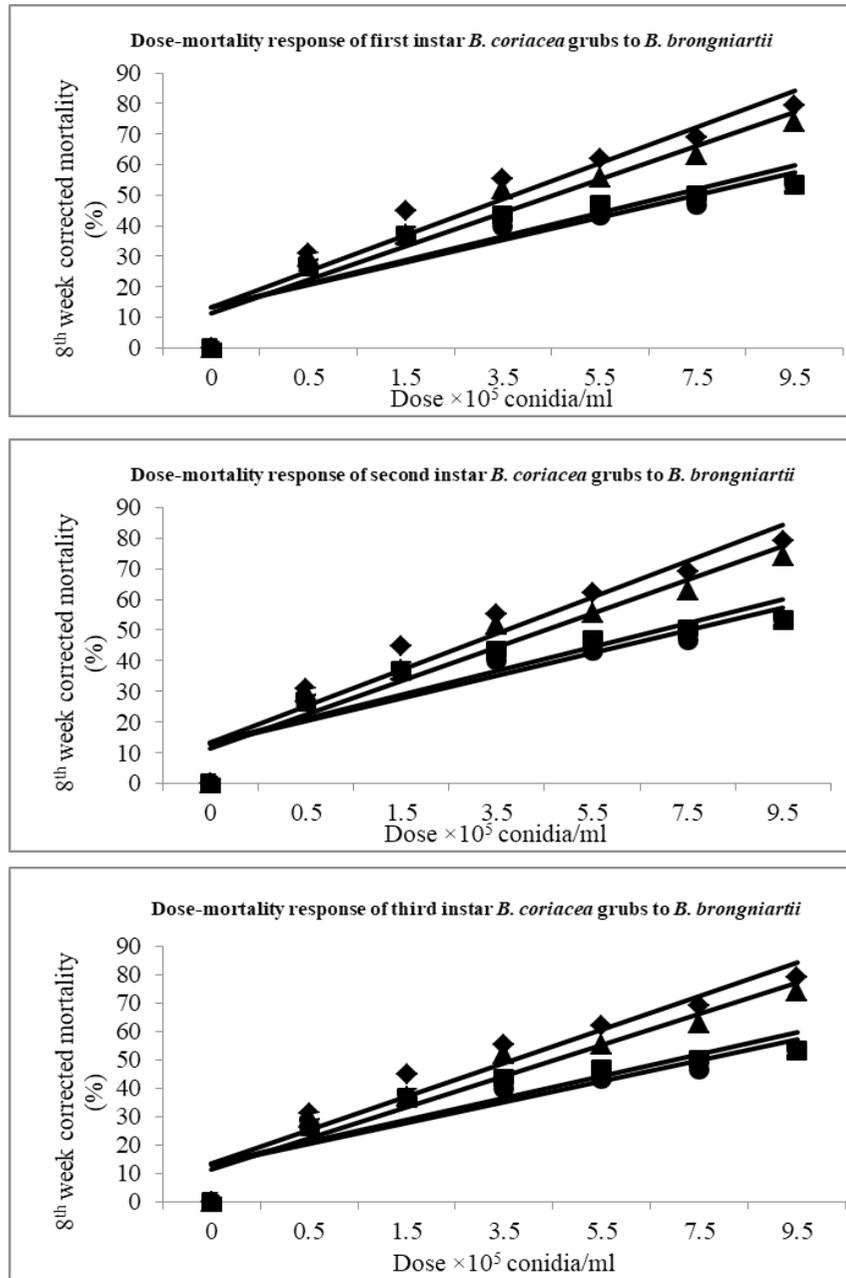


Fig. 1. Dose-mortality response of *Brahmina coriacea* grubs to *Beauveria brongniartii*.

- ◆ (KH I Dip treatment)
- (KH I Oral feeding)
- ▲ (KH II Dip treatment)
- (KH II Oral feeding)

isolates named as KH I and KH II. There was sparse growth of fungus in KH I and in KH II isolate the growth was very dense. The colony colour in KH II was pure white, whereas in KH I the colony colour was off white. Kalia (2013) revealed that wide variation exists among the isolates of *B. brongniartii* in Himachal Pradesh and has characterized 7 isolates which are clustered in separate subclade.

Susceptibility of *Brahmina coriacea* grubs to *Beauveria brongniartii*

B. coriacea completes its life cycle in one year and undergoes three larval stages in duration of 230-267 days from June-April. The first, second and third instars occupy 19-24 days, 20-26 and 211-217 days, respectively (Pathania and Chandel, 2017). The susceptibility of first, second and third instar grubs of *B. coriacea* to KH I and KH II isolates was tested using dip treatment and oral feeding methods.

Dip treatment method

Dip treatment of first instar grubs with KH I and KH II isolates of *B. brongniartii* produced a mortality of 46.66 per cent at highest dose (9.5×10^5 conidia/ml) after one week of treatment. However, after 8 weeks of treatment, the mortality varied from 33.33-80.00 per cent in KH I and 36.66-76.66 per cent in KH II isolate, respectively. Kalia (2013) also observed 66.67 and 76.67 per cent mortality of first instar grubs treated with 95×10^4 and 75×10^4 conidia/ml (KH I isolate of *B. brongniartii*) and 66.66 and 76.67 per cent mortality with KH II isolate at same dosage, respectively, which is in concordance with our results. Jayaramaiah and Veeresh (1983) reported that *B. bassiana* @ 10^9 spores/ml produced 92.0 per cent mortality of first instar grubs of *H. serrata*. The LC_{50} value of KH I was 2.55×10^5 conidia/ml, and in KH II, the LC_{50} value was 2.80×10^5 conidia/ml. Khagta (2006) reported LC_{50} of 10^7 conidia/ml of *B. bassiana* for first instar grubs of *B. coriacea*.

Against second instar grubs, dip treatment with KH I and KH II isolates caused 20.00 per cent and 16.67 per cent mortality, respectively, at lowest tested concentration (0.5×10^5 conidia/ml) after one week of treatment. The mortality data at different dosages ranged between 23.33-60.00 per cent for both the isolates, after four weeks of treatment. After 8 weeks of treatment, the maximum mortality was 76.66 per cent in KH I, whereas in KH II, the mortality was recorded to be 73.33 per cent (Figure 1). Jayaramaiah and Veeresh (1983) reported that *B. brongniartii* @ 10^9 spores/ml caused 86.00 per cent mortality of second instar grubs of *H. serrata*. Sharma *et al.* (1999) inoculated second instar grubs of *H. consanguinea* by dipping in spore suspension of $2 - 4.5 \times 10^6$ conidia/ml and observed 100 per cent mortality with *B. bassiana* and *B. brongniartii* after 4-6 weeks of

treatment. Kalia (2013) reported 46.67 per cent mortality with KH I (29.5×10^4 conidia/ml) and 43.33 per cent mortality with KH II (29.5×10^4 conidia/ml). The LC_{50} value for KH I was 2.91×10^5 conidia/ml and 3.98×10^5 conidia/ml for KH II (Table 1). Khagta (2006) calculated higher LC_{50} value of 9.886×10^5 conidia/ml of *B. bassiana* against second instar grubs of *B. coriacea*.

When third instar grubs were dip treated with varying dosages of *B. brongniartii*, the mortality data ranged from 16.66- 40.00 per cent (KH I) and 16.66- 46.66 per cent (KH II) at different dosages after one week of the treatment. After eight weeks of treatment, 63.33 per cent mortality with KH I and 60.00 per cent mortality with KH II was observed @ 7.5×10^5 conidia/ml. Jayaramaiah and Veeresh (1983) reported that *B. brongniartii* at a dose of 10^9 spores/ml resulted in 80.0 per cent mortality in third instar grubs of *H. serrata*. The LC_{50} value for KH I was 4.47×10^5 conidia/ml and 4.88×10^5 conidia/ml for KH II (Table 1). These LC_{50} values are almost similar with the values (4.4×10^4 conidia/ml for KH I and 6.23×10^5 conidia/ml) reported earlier by Kalia (2013). Khagta (2006) computed LC_{50} of 4.645×10^7 conidia/ml for third instar grubs of *B. coriacea*.

When different instar grubs of *B. coriacea* were compared for their sensitivity to KH I and KH II isolates of *B. brongniartii*, there was gradual decrease in response with increasing age. Overall, first instar grubs were found to be 1.14-1.42 times more sensitive than second instar grubs to KH I and KH II in dip treatment. Similarly, second instar grubs were 1.23-1.54 times more sensitive as compared to third instar grubs. When LC_{50} values of first instar grubs were compared to the third instar grubs, there was 1.74-1.87 fold increase in LC_{50} values indicating decrease in sensitivity with age of grubs. The increase in LC_{50} in relation to change of instars may be due to increase in size of larvae. The full fed first, second and third instar grubs of *B. coriacea* are 11.6, 20.7 and 30.9 mm in length (Chandel *et al.*, 1995), and there is a growth increment of about 10 mm in each instar. Therefore, it seems possible that there is a proportional increase in LC_{50} values in relation to age and size of grubs. Busvine (1971) also reported that larger, heavier animals require a large dose than smaller animals and a means of comparison is via body weight.

Oral feeding method

In case of oral feeding method, when first instar grubs were treated with Kheradhar strains of *B. brongniartii*, the mortality varied from 13.33-36.66 per cent in case of KH I, whereas KH II gave a mortality of 20.00-36.66 per cent at different doses after one week of treatment. The maximum mortality obtained in KH I and KH II after 8 weeks

Table 1. Dose-mortality response of *Brahmina coriacea* grubs to *Beauveria brongniartii*

| Treatment methods/ isolates | LC ₅₀ (conidia/ml) | 95 % Fiducial limits (conidia/ml) | Slope (b) | Regression equation (Y) |
|-----------------------------|-------------------------------|-----------------------------------|-----------|-------------------------|
| First instar | | | | |
| KH I (Dip Treatment) | 2.55×10 ⁵ | 1.26-4.17×10 ⁵ | 1.007 | 3.5827+1.0074x |
| KH II (Dip Treatment) | 2.80×10 ⁵ | 1.25-5.11×10 ⁵ | 0.8599 | 3.7558+0.8599x |
| KH I (Oral feeding) | 7.27×10 ⁵ | 3.18-516.9×10 ⁵ | 0.5192 | 4.0355+0.5192x |
| KH II (Oral feeding) | 9.69×10 ⁵ | 3.87-67.67×10 ⁵ | 0.4731 | 4.0603+0.4731x |
| Second instar | | | | |
| KH I (Dip Treatment) | 2.91×10 ⁵ | 1.66-4.73×10 ⁵ | 0.9830 | 3.5604+0.9830x |
| KH II (Dip Treatment) | 3.98×10 ⁵ | 2.27-7.37×10 ⁵ | 0.9926 | 3.4117+0.9926x |
| KH I (Oral feeding) | 5.36×10 ⁵ | 2.48-37.55×10 ⁵ | 0.6009 | 3.9612+0.6009x |
| KH II (Oral feeding) | 8.82×10 ⁵ | 3.95-881.87×10 ⁵ | 0.5945 | 3.8435+0.5945x |
| Third instar | | | | |
| KH I (Dip Treatment) | 4.47×10 ⁵ | 2.70-8.52×10 ⁵ | 0.9937 | 3.3605+0.9937x |
| KH II (Dip Treatment) | 4.88×10 ⁵ | 2.84-10.61×10 ⁵ | 0.9553 | 3.3873+0.9553x |
| KH I (Oral feeding) | 3.03×10 ⁵ | 1.09-6.93×10 ⁵ | 0.6948 | 3.9703+0.6948x |
| KH II (Oral feeding) | 5.14×10 ⁵ | 2.53-22.14×10 ⁵ | 0.6585 | 3.8734+0.6585x |

of treatment was 53.33 per cent at highest concentration of 9.5×10^5 conidia/ml. The LC₅₀ value for KH I was calculated to be 7.27×10^5 conidia/ml and 9.69×10^5 conidia/ml for KH II. In second instar grubs, KH I and KH II isolates caused 16.67 per cent mortality at lowest concentration (0.5×10^5 conidia/ml) after one week of treatment. The mortality data at different dosages ranged between 30.00-60.00 per cent for KH I and 30.00-56.66 per cent for KH II, after 8 weeks of treatment. The LC₅₀ value for KH I was computed to be 5.36×10^5 conidia/ml and 8.82×10^5 conidia/ml for KH II.

When third instar grubs were subjected to oral feeding of *B. brongniartii*, the mortality varied from 16.66-40.00 per cent at different doses after one week of treatment. At highest concentration of 9.5×10^5 conidia/ml, maximum mortality obtained for KH I was 66.66 per cent and 63.33 per cent for KH II after 8 weeks of treatment (Figure 1). The LC₅₀ value for KH I was calculated to be 3.03×10^5 conidia/ml and 5.14×10^5 conidia/ml for KH II (Table 1). Contrary to dip treatment, there was gradual increase in susceptibility with increase in age of the grubs. First instar grubs were 1.098-1.36 times less sensitive to treatment as compared to second instar grubs. A

comparison of first instar grubs with third instar grubs revealed 1.89-2.39 fold lesser sensitivity of first instar grubs. The second instar grubs, were found to be 1.72-1.77 times less sensitive to third instar grubs on the basis of their calculated LC₅₀ values in oral feeding tests. The higher sensitivity of third instar grubs in oral feeding is attributed to higher feeding potential of third instar grubs. The third instar grubs are most voracious feeders leading to more ingestion of conidia from treated tubers.

Comparison of dip treatment and oral feeding method

Against first and second instar, dip treatment of grubs in conidial suspension of *B. brongniartii* was found to be statistically at par with oral feeding method based on 95% fiducial limits which showed distinct overlap (Table 1). However, LC₅₀ value was calculated to be maximum (9.69×10^5 conidia/ml) for KH II in oral feeding method against first instar grubs. Against second instar grubs, KH II (Dip treatment) was found to be 2.22 times effective. The LC₅₀ value was calculated minimum in case of KH I in dip treatment and it was found to be 3.80 times more virulent as compared to KH II (oral feeding). KH I (dip treatment)

showed maximum virulence. Irrespective of isolates, dip treatment was 2.85-3.46 times more effective as compared to oral feeding method. Irrespective of method of treatment, isolate KH I was 1.10-1.33 times more virulent as compared to KH II. Against second instar grubs, KH II (dip treatment) was found to be 2.22 times more effective as compared to KH II (oral feeding). On the basis of LC_{50} value, it was 3.03 times more toxic as compared to KH II (oral feeding). Irrespective of isolates, dip treatment was 1.84 and 2.22 times more effective as compared to oral feeding method. Irrespective of method of treatment, isolate KH I was 1.37 and 1.65 times more virulent as compared to KH II. As opposed to younger instars of *B. coriacea*, the LC_{50} values were calculated to be on lower side in oral feeding method, although data were found to be statistically non-significant. The efficacy of KH I in oral feeding was 1.70 times higher as compared to KH II (oral feeding) which was least effective.

From the results, it can be concluded that method of treatment did not affect susceptibility of *B. brongniartii* in different instars of *B. coriacea*, but the susceptibility of younger instars was marginally higher than older instars. The susceptibility of insects to fungal infection is influenced by a number of factors, including pathogen, host properties and environmental conditions (Mullens, 1985; Benz, 1987; Fuxa and Tanada, 1987; Inglis *et al.*, 2001). Developmental stage is an important factor that has been reported to affect host susceptibility to entomopathogenic fungi (Ferron, 1985; Dimbi *et al.*, 2003). Yokoyama *et al.* (1998) also observed that younger instars of *Anomala corpulenta* Motschulsky are more sensitive than older instars to entomopathogenic fungi. The reason for this varying susceptibility is that the cuticle is the primary barrier to entomopathogenic infection of insects. It is likely that the structural components of the white grub cuticle change with increasing physiological age, resulting in changing susceptibility to infection (Nong *et al.*, 2011). This suggests that bio control using entomopathogenic fungi will be more effective when applied at earlier stages of target pest.

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