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Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. on 5th Instar Larvae of *Pieris brassicae* (L.) in Laboratory Condition

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ABSTRACT

Beauveria bassiana, is an entomopathogenic fungus is often relied on as important components of integrated pest management in agriculture, either as biocontrol agent or as naturally occurring microbe exploited against the major economic crop pests. Cabbage butterfly, *Pieris brassicae* is one of the major insect pest of cabbage to cause serious damage to the crop. It is an emerging pest of the Brassicaceae family. An experiment was conducted in the insect physiology laboratory, department of entomology, Assam Agricultural University, Jorhat-13. In the present study, the fungal isolate of *B. bassiana* viz., BBJ-S-1 (1×10^7 conidia/ml) showed mortality rate in 5th instar larvae of *P. brassicae*. The mortality rate of *P. brassicae* was found to be significantly different between treated and control insects. Pathogenicity test showed the mortality ranged as 16.00, 52.00, 84.00% at three, five, seven days after treatment (DAT) respectively, in response to *B. bassiana* infection. The mortality rate depend on the species of fungi are used and also dose of the concentration. This investigation shows that *B. bassiana* could be considered as a promising biocontrol agent to control the *P. brassicae* in the future.

Keywords Pathogenicity, *Pieris brassicae, Beauveria bassiana, Treated,* Control.

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata)* is one of the most popular winter season vegetable grown in India. Cabbage butterfly, *Pieris brassicae* (L.) also known as large white butterfly. It is a strong flier, cosmopolitan in distribution and one of the major insect pest of cabbage to cause serious damage to the crop. It is polyphagous in nature, infesting the radish, turnip, cauliflower, toria and other cruciferous vegetables. To overcome the losses different types of insecticides were being used by the farmers from time to time. The residual toxicity of pesticides in cabbage was another big threat to our vegetable exports in the foreign markets. Therefore we need to manage this pest in an ecofriendly manner so that non-chemical approaches such as application of bio-pesticides, botanicals and biological control agents by the introduction of *Apanteles glomeratus*.

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Among the entomopathogenic organisms, *Beauveria bassiana* (Bals.) Vuill., a naturally occurring entomopathogenic fungus was occupied an important place in the pest management. It was one of the most versatile parasite, capable of attacking and penetrating in various developmental stages of the host body. The spores of *B. bassiana* (Bals.) Vuill. adhere to the body surface, germinate and enter into the integument whereby it eats away the content of hemolymph and ramify ultimately resulting in the death of the host tissue causing white muscardine disease (Hazarika and Puzari 1995). *B. bassiana* as a major disease-causing pathogen of silkworm, *Bombyx mori* (L.) caused 100% mortality when applied singly (Chandrashekaran and Nataraju 2008). The pathogenicity test of *B. bassiana* to *Periplaneta americana* (L.) was tested in three different ways, where 100 per cent mortality was observed by direct contact with spore mass, 67-100% by a spore-wheat flour mixture and 17-75% in anaqueous spore suspension (Mohan *et al*.1999). Several crop pests have been effectively controlled by this entomopathogenic fungi viz. Whitefly, *Bemisia tabaci* (Genn.), Cotton leaf worm, *Spodoptera littoralis* (Boisd.) Cabbage butterfly, *Pieris rapae* (L).

MATERIALS AND METHODS

Collection and laboratory rearing of *Pieris brassicae* (L.)

The adults of *P. brassicae* (L.) were collected, reared and maintained in wooden rearing cages (90 x 60cm) on cabbage leaves in laboratory 26°C, 85% relative humidity (RH) and 14:10 L:D. Cabbage leaves inside the cage on which eggs were deposited. The egg period was approximately 5 days, after which larvae hatched. Larvae ate non-contaminated cabbage leaves on which they will born until the fifth day after egg collection.

(a) Stock solution of *B. bassiana*

(b) Radial growth of *B. bassiana*

(c) Pure culture of *B. bassiana*

(d) Serial dilution of *B. bassiana*

Plate 1. Culture of *Beauveria bassiana.*

Source of *Beauveria bassiana* (Bals.) Vuill. strain

Beauveria bassiana strain namely, BBJ-S-1 (collected from the Department of Plant Pathology, Assam Agricultural University) was maintained in insect physiology laboratory, department of Entomology, Assam Agricultural University, Jorhat. (Plates1a, 1b).

Culturing of fungal strain and inoculum preparation

Fungal strain was used in this work and obtained from *B. bassiana* culture. This strain was grown on petri dishes and incubated in darkness for 10-14 days at 22°C and 70% relative humidity (RH) (Plate 1).

Preparation of potato dextrose agar (PDA) medium

To prepare the PDA medium, 39.9 gm of PDA powder (Himedia) was mixed with 1 liter of distilled water. The medium was poured into culture tubes and conical flasks, plugged with non- absorbent cotton wool and then sterilized in an autoclave at 121°C (15 lb pressure per square inch) for 1 hr.

Isolation procedure

Each cadaver was cut into many pieces, each measuring 0.5 to 1mm in length and surface- sterilized in 1% sodium hypo chlorite solution ($NaOCl_2$) for 1 min. Then they were placed on damp filter paper within a sterile petri plates containing PDA (Potato Dextrose Agar) medium and incubated at a 26± 2^0C for 14 days for complete sporulation.

Pure culture

The pure culture was prepared in PDA medium (Potato Dextrose Agar) with streptomycin sulfate in culture tubes and incubated them at a temperature of 26±1°C for complete growth and stored in a refrigerator at 4°C (Plate 1c).

Radial growth of Beauveria bassiana

Inoculum of *B. bassiana* was produced by growing the fungus on PDA plates for seven days in a BOD

(a) **Treated with *B. bassiana* against *P. brassicae***

(b) **Control**

(c) Topical application of *B. bassiana* on *P. brassicae*

Plate 2. Pathogenicity of *Beauveria bassiana* against *Pieris brassicae.*

(a) Control

(b) Treated with *B. bassiana*

Plate 3. Effect of *Beauveria bassiana* (1×10^7conidia/ml) on mortality of 5th instar larvae of *Pieris brassicae.*

incubator. With the help of a 0.8 cm diameter cork borer, a piece was cut out from the actively growing region of a 7- day old culture, and the same was placed aseptically in the center of a fresh petri plate (9 cm diameter) containing PDA medium. Plates were prepared, sealed with cellophane tapes and incubated in BOD incubator at a temperature of 26±1°C. Strain was taken for measuring the radial growth. After 3 days of inoculation five orthogonal diameters were measured at an interval of 24 hrs up to 7 days (Plate 1b).

Statistical analysis

The data on mortality were analyzed statistically with Paired t-test (t-test). Before analysis the mortality data were transformed to angular values.

RESULTS AND DISCUSSION

Pathogenicity of *Beauveria bassiana* (1×10^7conidia/ml) on mortality of 5th instar larvae of *Pieris brassicae*

The average radial growth of *B. bassiana* was recorded 30.46±0.54 mm (Plate1b). Radial growth of the fungus was recorded on the daily basis upto 7 days after inoculation. The mortality of *P. brassicae* was found to be significantly different between treated and control insects. Results of the pathogenicity test showed that mortality of 5th instar larvae of *P. brassicae* ranged from 16.00 to 84.00% at seven days after treatment (DAT) in response to *B. bassiana,* BBJ-S-1 (1×10^7conidia/ml) infection (Plate.). The maximum cumulative mortality was recorded 84.00% at seven

Table 1. Pathogenicity of *Beauveria bassiana* (1×10^7conidia/ml) on mortality of 5th instar larvae of *Pieris brassicae.*

Strain	5th instar (Mean ± SD)		
	3 DAT	5 DAT	7 DAT
Treated (BBJ-S-1)	16.00± 4.45 (23.30)	52.00±2.57 (46.15)	84.00±4.45 (66.68)
Control	0.00±0.00	0.00±0.00	0.00±0.00
t- value ($p<0.05$)	11.70	40.06	33.48
Significant (2-tailed)	0.0001 (S)	0.0001 (S)	0.0001 (S)

Data presented are the means of 5 replications (10 insects / replication)
Data in parenthesis are angular transformed value.

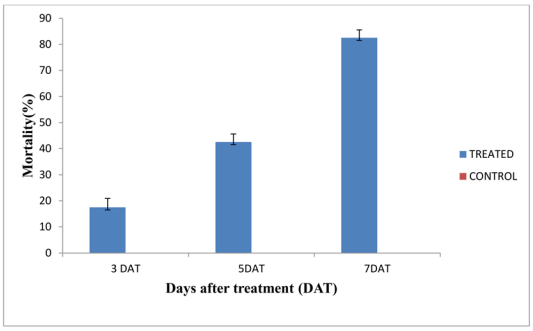

Fig. 1. Pathogenicity of *Beauveria bassiana* (1×10^7 conidia/ml) on mortality of 5th instar larvae of *Pieris brassicae.*

and minimum 16.00% at three days after treatment in response to *B. bassiana*. The mortality rate depend on the species of fungi used, dose of the concentration and also genetic makeup of the strain which produces the toxin for inducing the immunity response of the insect. Treated larvae transformed into pupa but the pupa was died after 2 days without transforming into adult whereas in control the larvae transformed into pupa and further to adult stage without any deformities was observed. No death was recorded in the control. The mortality of *P. brassicae* might be due to the fungal toxin which completely destroy the fat bodies, immune cells, depletion of the nutrients or it might be due to the innate ability of the fungus (Table 1, Fig.1 and Plate 3).

Radial growth of the *B. bassiana* (BBJ-S-1) was recorded 30.46±0.54 mm. Similarly, Nussenbaum (2013) and Das *et al.* (2012) reported that temperature and growth medium plays a significant role in radial growth of the fungus. Similar results were agreement with the findings of Alali *et al.* (2019) where they reported the biological parameters of *B. bassiana* and found that temperature influences the superior growth rate of the fungus. Mudoi *et al.* (2018) recorded the strain wise variation in related to the radial growth of the *B. bassiana.* Hussain *et al*. (2009) reported the 5th instar larvae of *Ocinara varians* were transformed into pupa but died within one week without result in the adult emergence. Faraji *et al*. (2013) reported that mortality of 3rd instar larvae of mediterranean flour moth, Ephestia kuehniella (Zeller) ranged from 17.00 to 88.00% in response to *B. bassiana*. Similarly, Puzari and Hazarika (1992) recorded the mortality of *Dicladispa armigera* (Olivier) showed more than 90, 50 and 7% in response to *B. bassiana, Aspergillus flavus* and *Fusarium heterosporum,* respectively. Chandrashekaran and Nataraju (2008) reported the 100% mortality of silkworm, *Bombyx mori* (L.) in response to *B. bassiana.*

CONCLUSION

The present study demonstrates that native strain of Beauveria bassiana can be a promising biocontrol agent to manage the Pieris brassicae. This study suggested that B. bassiana could be incorporated in an overall IPM program.

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