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Short Communication

Beauveria bassiana: Entomopathogenic Fungi as Biopesticide reported from Cachar, Southern Assam

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***Key words*:** Entomopathogenic fungi, *Beauvaria bassiana,* Greater wax moth, Biopesticide, Organic farming

The concept of biological pest control system originated in early 19th century, when the Italian pathologist named Agostino Bassi spent years in studying white muscardine disease in silkworms, *Bombyx mori* [1]. Enotomopathogenic fungi are found in the division Eumycota. Currently, the two most widely studied and used species of entomopathogenic fungi are *Beauveria bassiana* and *Metarhizium anisopliae* [2]. The genus *B.* was described by Vuillemin Maclleod (1912). *Beauveria bassiana* have wide host range infect over 700 species belonging to order Lepidoptera, Coleoptera, Orthoptera, Hemiptera, Diptera and Hymenoptera [3]. Ectotoxins produced by these pathogenic fungi are beauvericin, isarolides, bassianolide and isarolides [4]. Disease caused by *Beauveria bassiana* known as white muscardine, its infection symptom is-mummification of cadavers, followed by white mycelial growth on the insect cuticle and formation of conidia [5].

Scientific classification

Kingdom: Fungi
Division: Ascomycota
Class: Sordariomycetes
Order: Hypocreales
Family: Cordycipitaceae
Genus: *Beauveria*
Species: *B. bassiana*

The mode of action of Enotomopathogenic fungi

Its infection strategy includes- attachment of the conidium to the insect cuticle, germination of the conidium on the insect outer surface, penetration into the cuticle, growth of the fungus in the haemocoel, production of toxins and lastly death of the host [6].

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Pathogenicity of Enotomopathogenic fungi

Several scientists tested the pathogenicity of *Beauveria bassiana* against different insect hosts. In an experiment Kpindou *et al*. [7] found that mortality rate in larval stages of *Helicoverpa armigera* increased with increased doses of *Beauveria bassiana*. Vijayavani *et al*. [8] tested the potency of two isolates of *Beauveria bassiana* on *Spodopteralitura* pupae under in vitro conditions. Both the strains found to be equally efficient causing 100% mortality and the mortality is directly proportional to the conidial concentration. Similarly, Gulati *et al*. [9] reported laboratory efficacy of *Beauveria bassiana* on *H. armigera* and *Spodoptera litura* depends on conidial concentrations. Godonou *et al*. [10] tested the efficacy of eight isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against diamond back moth (DBM), *Plutella xylostella* larvae. Result revealed *Beauveria bassiana* (Bba5653) to be most potent and caused 94% mortality. Thus, proving *Beauveria bassiana* as potent biocontrol agent.

The present study was conducted in Cachar, Southern Assam during February 2020 to May 2020. Experimental investigation was carried out in the Biotech Hub, department of Zoology, Cachar College, Silchar. Various soil samples were collected from different farm locations of Cachar district (Fig 1). Using metal shovel 15-20 cm pit dugout and 5 soil sample collected in sterile plastic bag from each site. Plastic bags were labelled with date & place of collection, and also soil related to which vegetation. In the laboratory soil were cleaned by removing debris like rocks, dead insects, wood bark pieces, leaves, etc to avoid further contamination with saprophytic microorganisms.

Insect bait method

For isolating indigenous strain of EPF from soil sample Greater wax moth (GWM), *Beauveria bassiana* larvae used as insect bait. GWM larvae were maintained in laboratory condition under artificial diet. For soil treatment to each soil sample of 250g in a container 10-12 medium size last instar GWM larvae were added. Containers were covered with a lid and turned upside down. Containers were maintained in the dark and at temperature 22-25°C. The soil was checked daily to observe dead bait larvae and also time at which larvae were died recorded.

Cultivation of Enotomopathogenic fungi

The collected cadavers from soil were cleaned with sterile water using point 1 painting brush twice to remove soil and dirt cling to its body setae or integuments. For surface sterilization cadaver dipped in 1% Na-hypochlorite for five seconds to prevent external growth of saprophytic fungi. Cadaver was placed in petri dish lined with a single layer of Whatman filter paper moisten with 3-5 drops of sterilized water for few days until mummified body i.e., muscardine disease were observed.

Fig 1 Soil collected from various farm land of Cachar district villages- (A) Borkhola (B) Dwarband

Culture medium preparation

For the adequate growth fungi optimal nutrient medium is necessary. In the present study, potato dextrose agar (PDA) medium selected. PDA is prepared by boiling mixture of infused potatoes (200 g peeled potatoes scrubbed and boiled in 1000 ml water for at least 30mins to 1 hour, then extract is filtered using fine trainer to obtain smooth paste) 1000ml, glucose 20.0 g and agar 15.0 g followed by sterilization of medium in autoclave at 121°C for 15 mins. Then the mixture is aseptically transferred into sterile petri dishes. As the medium is both light and temperature sensitive, so, plates were stored in dark at 2-8°C and are used within a week of preparation [11].

pH of culture medium

To study the effect of pH on biomass growth and radial expansion of colonies of EPF, five pH levels ranging from 3.5 to 6.8 (i.e., 3.5, 4.5, 5.5, 6.5 and 6.8) were tested. For each pH three petri dishes prepared to take mean of three replicates. The pH of culture medium was adjusted by adding 1 N HCl and 1 N NaOH with the help of indicator paper prior to autoclaving. Fungal growth was assessed in the form of radial expansion.

Cultivation of fungal spore

The fungal spore was cultivated in total fifteen petri dishes (3 petri dishes for each pH to take mean of three replicates) measuring 5cm × 1 cm containing potato dextrose agar (PDA) medium & kept in BOD incubator at temperature 25°C, 90±5% relative humidity and pH 3.5-6.8. After appearance of mycelial margin and sporulation, the fungus was taken for further identification.

Based on Humber [12] manual following observations were made and species is confirmed. On the onset of mortality, the cadaver body was covered with black dot (Fig 2) suggesting infestation by *B. bassiana*. After 5-7 days post mortality mummified bodies look woolly, floccose and appeared powdery due to abundant conidia formation (Fig 3) indicating occurrence of muscardine disease by *Beauveria* spp. Isolates of *B. bassiana* were cultured in five different pH levels (3.5-6.8) for 13 days in PDA medium. After 14 days on PDA, colonies look velvet to powdery, rarely forming synnemata. At the beginning colonies had whitish mycelial growth, which is the symptom of infection by *B. bassiana* (Fig 4), which became pale yellow to creamish spores with time. The underneath surface or reverse side of the colonies is observed to be colourless to yellowish. Conidia are globose (ovoid) to sub-globose in shape in globose clusters with smooth walled of 2-3 microns in diameter. Conidiophore were smooth and colourless, conidial chains were long and conidial heads diffused. Also, the spp. reproduces by production of dry spores on the conidiophore that grew sympodially, thus, confirming the local fungal isolate of this study to be *Beauveria bassiana*.

Fig 2 Appearance of black dot in GWM larvae onset of mortality

Fig 3 Development of muscardine disease after 5-7 days post mortality

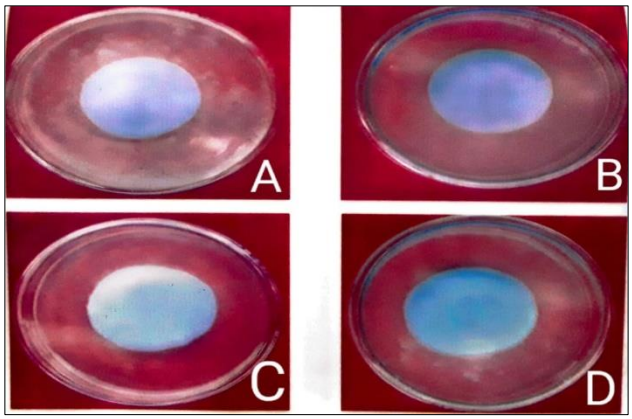


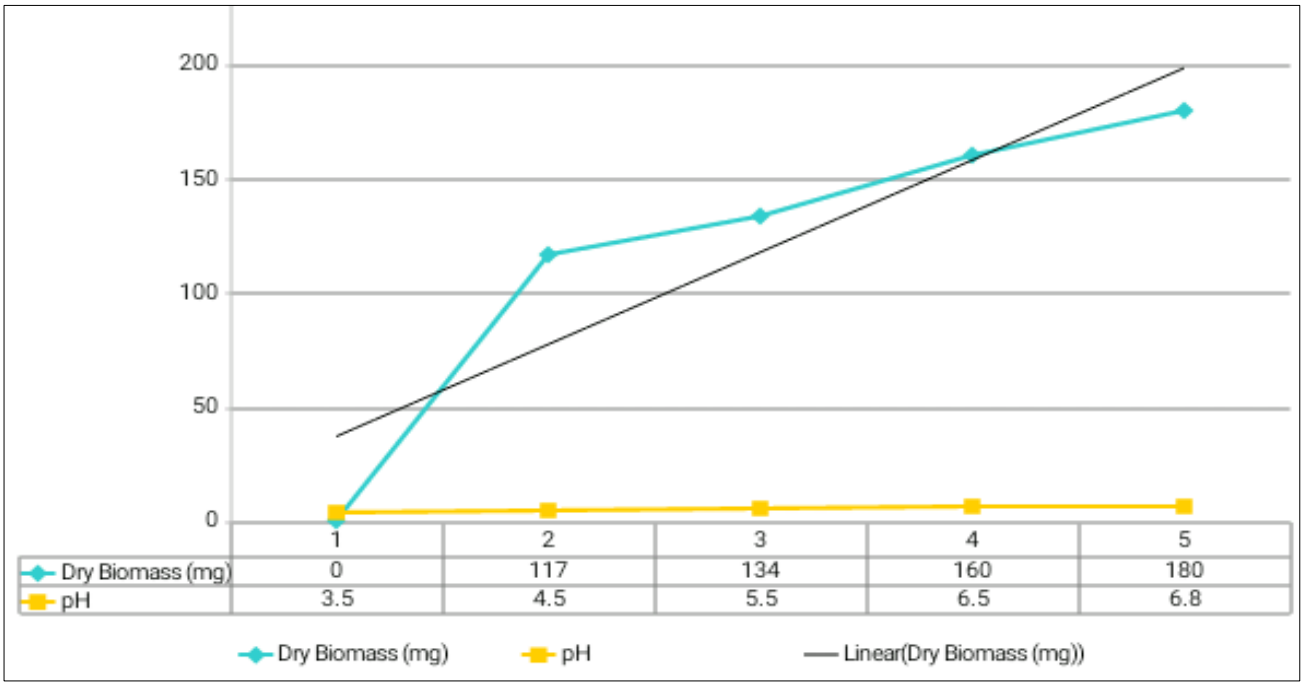
Fig 4 Sporulation growth of *Beauveria bassiana* in PDA medium at different pH levels- (A) pH 4.5 (B) pH 5.5 (C) pH 6.5 (D) pH 6.8

The variation in the pH level of the culture medium has been observed and noted that pH played significant role on the biomass production (Fig 4). With the increase in pH the growth of these organisms increased considerably. The pH 6.5-6.8 has been found to be favourable level for their vigorous growth. At pH 3.5 no growth recorded whereas from pH 6.5-6.8 subsequent growth visible and at pH 6.8 maximum biomass production of 180mg in *B. bassiana* observed (Fig 5). The change in radial expansion (growth) within different pH level upto 13th day has been observed. Result showed that maximum expansion visible in 6.8 pH petri dishes from day 1 onwards. Maximum diameter of the colony noted to be 4.57 mm on 13th day at pH 6.8 (Fig 6).

SUMMARY

Several attempts yielded those soils of Cachar district are rich in entomopathogens viz., entomopathogenic nematodes (EPN), entomopathogenic bacteria (EPB) and entomopathogenic fungi (EPF), therefore adequate knowledge of pathogen ecology- its spatial distribution, population dynamics and its efficacy are essential for improvement of sustainable, eco-friendly pest management practice in the district. Among all the entomopathogens, EPF are the most potential natural mortality agents because it has wide host range, host specific, viability in soil and infectivity on slight contact with the host. The present study was conducted to isolate and identify local strain of EPF from soil using Greater wax moth, *Galleria mellonela* as baiting insect. Based on

detailed morphological study through microscopic observation after lactophenol cotton blue staining, the species identified as *Beauvaria bassiana*. In potato dextrose agar (PDA) culture medium EPF was cultured at various pH level (3.5-6.8) for 13 days. The identified EPF, *B. bassiana* grew as white mould, it produces distinct spore balls made of many dry, powdery conidia. Each spore ball is made of group of numerous conidiogenous cells. The conidiogenous cells are short, ovoid and with elongated rachis. The optimum temperature, relative humidity and pH for abundant sporulation growth of EPF, *B. bassiana* in PDA culture medium was 25°C, 90±5% & 6.8 respectively. Sporulation growth directly proportional to pH level, as with the increase in pH the biomass of *B. bassiana* increased considerably. Maximum dry biomass growth recorded 180mg and radial expansion 4.57mm diameter at pH 6.8. PDA media also noted to be ideal for mass production of *B. bassiana*. Future scope of this research can be concluded that this experiment has already highlighted some aspects of efficacy of EPF, *B. bassiana* in effective pest management strategy. Extensive exploration with molecular characterization of indigenous isolates required for better adaptability of predator-prey association in different agroclimatic zones of Assam. Host specific virulent strains should be identified and maintained by mass production in appropriate nutrient supplement. Also, formulation storage technique for prolonged shelf life should be developed.



	1	2	3	4	5
Dry Biomass (mg)	0	117	134	160	180
pH	3.5	4.5	5.5	6.5	6.8

*Mean of three replicates

Fig 5 Effect of pH on the biomass of *Beauveria bassiana* in vitro

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