Potential of new strain of *Beauveria bassiana* isolated from Tinsukia (Assam) against tea mosquito bug *Helopeltis theivora* Waterhouse (Heteroptera: Miridae)

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ABSTRACT

Tea mosquito bug, *Helopeltis theivora* Waterhouse, is the major sucking pest of tea plant. It mainly attacks the young shoots essential for tea production. In lacunae of proper management, the pest remains active throughout the year resulting in almost 100% crop loss. To manage the population of this pest below Economic Threshold Level (ETL) of 5%, several rounds of chemical pesticides are applied during the season. Tea, being a consumable product; the unwanted pesticide residue cause a major concern for the tea industry. Thus, incorporation of alternate strategies like Biological Control Agents (BCA) in pest management programme is important to overcome the problems besides prevent the pest from developing resistance. The BCAs like entomopathogenic bacteria, fungi and virus are effective in an eco-friendly management of the pest population. The entomopathogenic fungi, *Beauveria bassiana* has been found to be effective pest control agent in several agro ecosystems including tea. Commercial *B. bassiana* formulations are available but the local strains are reported to be more efficient in managing the pest population. The present study was aimed to analyze the potential of a new strain of *B. bassiana* named as BPA/B7 (I.D. No. 10,928.8) isolated from tea soils of Tinsukia (Assam) against *H. theivora*. The efficacy of six concentrations (5 mlL^{-1}, 10 mlL^{-1}, 15 mlL^{-1}, 20 mlL^{-1} and 25 mlL^{-1}) of powder formulation of BPA/B7 was compared with a commercial formulation to estimate the LC_{50} of the same. The BPA/B7 (*B. bassiana*) with a spore density of $1.68X10^6$ spores/ml was found to kill 50% of *H. theivora* at a concentration of 21.87 mlL^{-1} within 96hrs. Further studies on the standardization of both liquid/powder formulations, shelf life studies, followed by their field evlauation, will ensure the possibility of utilizing this strain as a potential componemt of intergrated management of *H. theivora*.

Keywords: Tea mosquito bug, soil fungus, *Beauveria bassiana*, biocontrol

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INTRODUCTION

Helopeltis theivora Waterhouse (Miridae: Heteroptera) is the key sucking pest of tea, (*Camellia sinensis*) responsible for enormous crop loss to the tea industry. They primarily attack the harvestable young shoot and remain active from March to November (Sarmah and Phukan, 2004). Both adult and nymphal stages of tea mosquito bug pierce the plant tissue and may lead to complete death of shoots thus causing about 10-50% crop loss (Hazarika *et al*., 2001). Severe infestation of *H. theivora* may also encourage the growth of secondary pathogen like *Fusarium solani*, which causes die back disease resulting in further loss to the tea plantations. The pest since remains active throughout the year and unchecked infestation may lead to 100% crop loss if appropriate management strategies are not adopted. Timely execution of suitable control measures can minimize the losses of the tea planters. In general, application of chemical insecticides like Thiamethoxam, Deltamethrin, Clothianidin, Thiacloprid and Quinalphos are preferred to keep this pest below the Economic Threshold Level (ETL) of 5%. However, continuous and haphazard use of these chemical insecticides may also lead to certain undesirable issues like, water pollution, degradation of valuable soil microbes, decline in biological control agents, resurgence, development of resistance in pest, and pesticide residues in manufactured tea (Bora and Gurusubramanian, 2007; Roy *et al*., 2008). Being a consumable product, residue traces is a matter of concern for tea industry. The Maximum Residual Limit for most synthetic pesticides in European Commission has become a concern to the tea exporting countries like India. Residues of synthetic pesticides in tea has encouraged the planters to look for an eco-friendly approaches to deal with the pests which includes selective use of pesticides and incorporation of Biological Control Agents (BCAs) like predators and parasitoids and micro-organisms occasionally.

Among the BCAs, entomopathogenic fungi (EPF) effectively reduce the crop infestation by Hemipterans pest mainly because of its breaching property (Bordoloi, 2012; Keswani *et al*., 2013). Application of such beneficial fungi is worldwide popular and are commercially developed for the management of pest (Ferron, 1978; Ferron, 1985; Keswani *et al.*, 2013).These efficient hyphomycetes are economical for mass production and are easy to store in powder or liquid formulation. The *Beauveria bassiana*, commonly called as "white muscardine fungus" is one among the potential hypomycetes that has a high success rate in controlling wide range of pests in different agro-ecosystems (Ferron, 1981; Wagner, 1997).

Therefore, planned implementation of different means of pest control strategies may reduce the load of pesticides on the tea ecosystem and environment, ensuring the maintenance of quality of manufactured tea. Several microbial formulations are used now-a-days on tea and local strains are found to be more efficient in pest management than the commercial ones isolated and developed from other ecosystems (Babu and Kumhar, 2014). The present study was aimed to analyze the potential of different concentrations of the new fungal strain isolated (named as BPA/B7) and identified as *B. bassiana* from tea soils of Tinsukia (Assam) against the tea mosquito bug *H. theivora*.

MATERIAL AND METHODS

Isolation and identification

The soil samples were collected from 5 different tea gardens of Tinsukia district of Assam. Sample collection was done with auger and sealed in the sterilized zip bag and kept in container with ice bag (Ogunmwonyi *et al.*, 2008). The soil samples taken to the laboratory were serially diluted and inoculated in sterilized plate with different media and accordingly CFU values were noted down after two days using the methodology of Morris and Rideout (2005). The suspected fungus were transferred to sterilized media (PDA) plate and incubated at 28±2° C. The mycelium of full grown fungus was taken in glass slide and observed under binocular microscope (Olympus CX21i with Mag Vision). After identification, the pure cultures were maintained on PDA slants and used for subsequent studies. The strains were coded and sent to ITCC, IARI, New Delhi for identification, and BPA/B7 has been identified as *B. bassiana* (I.D. No. 10,928.18).

Fungal pathogens

The mother slant of BPA/B7 (*B. bassiana*) was sub-cultured and incubated at 28±2 ºC in the BOD incubator. For broth culture, a fragment of BPA/B7 was taken from the mother slant and inoculated in PDB in aseptic environment under laminar air flow and incubated in BOD under suitable controlled condition (28±2 ºC).

Preparation of fungal solution

The fungal strain BPA/B7 (*B. bassiana*) was grown in PDB for 10-14 days in conical flasks. The broth culture was grinded for 5 minutes to generate homogeneous conidial suspension and was double filtered by means of fine muslin cloth. The aqueous spore suspensions of different dilutions were prepared using sterilized distilled water. Concentration of the conidia was calculated in a Neubauer chamber following the methodology of Annamalai *et al.*, 2015; and spore density was counted accordingly using the formula:

Number of spores= X x 400 x 10 x 1000 x D where; X is the Average number of spores per Small Square; 400 is the Number of small squares; 10 is the Depth factor; 1000 is the Conversion factor for mm^3 to cm^3 and D is the dilution factor. The fungal strain BPA/B7 (*B. bassiana*) was formulated in to a powder formulation for its bioefficacy studies.

Bioassay procedure

The experiment was conducted by a completely randomized design (CRD) with seven treatments including a control, following the methodology Akmal et al., 2013 with slight modifications. Each treatment was replicated three times. Ten tea mosquito adults of same age group were released inside a glass chimney (7cm in diameter at top and 9cm bottom respectively with a height of 20 cm) with filter paper at the base and the mouth of chimney was covered with the muslin cloth. Different concentrations of powder formulation of *B. bassiana* BPA/B7 were applied on the tea shoots along with the test insects using an atomizer sprayer. Mortality of the insect was counted every day at 24 hours interval for 7-days after treatment. After 48 h, the old tea shoots were replaced with fresh ones. The cumulative percent mortality was recorded for seven days. Insect cadavers collected were surface sterilized and transferred to individual sterilized sample containers (with moist filter paper) for fungal growth and sporulation. The fungus was then observed under binocular microscope (Olympus CX21i with Mag Vision) to reconfirm the insects' death by fungal infection. The mean temperature in the laboratory during this period was 28±2˚C and RH 70-74%. The LC_{50} value was analyzed in SPSS-16 by Finney Probit Analysis Method (Finney, 1973) and ANOVA was tested at α= 0.05.

RESULTS

CFU value of five soil samples from different Tea estates of Tinsukia (Assam) showeds that; T5 sample (Sri Krishna T.E.) was found to be rich with maximum number of fungal colonies followed by T3 sample, T4 sample, T1 sample and T2 sample has minimum fungal colony. The fungus, BPA/B7 was isolated from T5 sample (pH= 4.33±0.33) and was identified as *B. bassiana*. In almost all the soil samples analyzed, number of fungal colonies formed on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) are similar while lesser colony formation was observed on Rose Bengal Chloramphenicol Agar (RBC) (Fig. 1).

Fig 1. Enumeration of culturable fungi from different Tea estates of Tinsukia (Assam) grown on different media.* Range of CFU 10^4-10^6
T1: Powai T.E., T2: Namdang T.E., T3: Borbil T.E., T4: Dehing T.E. and T5: Sri Krishna T.E.

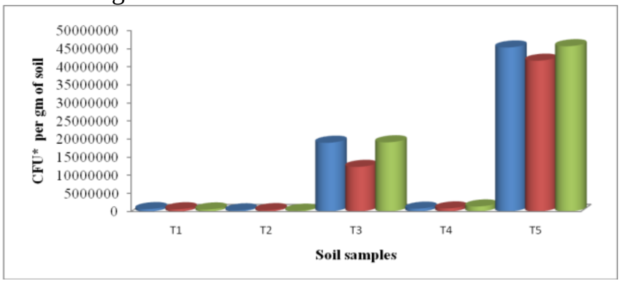
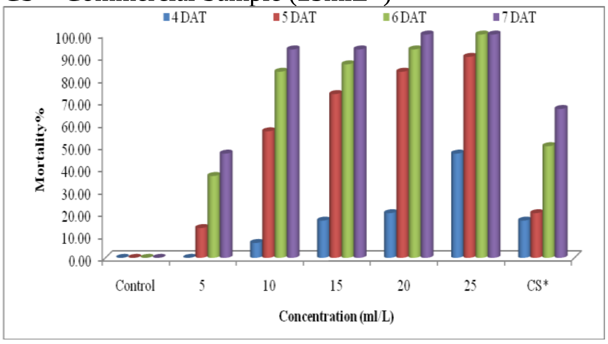


Fig 2. Cumulative mortality graph of *Helopeltis theivora* at different Day After Treatment (DAT) CS*=Commercial Sample (25mL^{-1})



Virulence of BPA/B7 ***Beauveria bassiana***

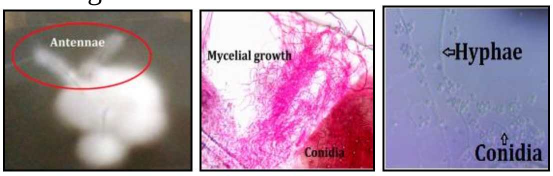
Mortality in the first three days was not significantly different from the commercial formulation and control hence, data from fourth day onwards was considered. With the advancement of days after treatment (DAT), BPA/B7 showed increased mortality with LC_{50} of 12.23 mlL^{-1} (P<0.001) after five days. The spore density recorded was 1.68X10^6 spores/ml. After 3-4 days, fungal growth started appearing on the surface of the cadaver that were collected after death during the experiment and stored in the sterilized container (with moist filter paper) under laboratory conditions. The cadavers were then surface sterilized and transferred to the PDA plates. On 4th-7th day, the entire body of *H. theivora* was almost masked with the white-colored mycelia.

Table 1. LC_{50} of BPA/B7 (*B. bassiana*) on different days against *Helopeltis theivora*

BPA/B7	Regression equation	Chi square	LC_{50}	FD Limit
4th Day	Y =0.0933*X+-2333	1.189	21.87	(18.825-33.204)
5th Day	Y=0.15*X+-2.25	3.739	12.229	(8.808-15.416)
6th Day	Y=0.133*X+-1.1667	5.078	7.643	(4.238-10.380)
7th Day	Y =0.116*X+-0.4667	4.243	6.200	(3.362-8.561)

Microscopic observation of white mycelia showed that hyphae bear conidiogenous cell that have denticle with conidia at the end. The spores were globose with smooth surface and had a size of 3.63 ± 0.68 µm in diameter. Observation of generation time of single inoculums on a PDA plate (9cm in diameter) showed that by 15 days it grows up to a diameter of 5.26±0.48 cm at 28 ± 2˚C.

Fig 3. (a) White cottony growth of *Beauveria bassiana* on *H. theivora*, **(b)** Transverse section of cadaver showing hyphal growth inside the body and on cuticle, **(c)** Observation of spore of 10 days old fungus re-isolated from the cadaver.



a **b** c

DISCUSSION

In general soil condition is one of the most important ecological factors that determine the distribution of the beneficial fungi. In this current investigation**,** after the fungal isolation from soil samples of five different tea gardens, we have recorded *Beauveria bassiana* from T5 sample only. The strains of *B. bassiana*, as a biological control agent is more effective in managing the population when it is collected from the same ecosystem compared to the commercial formulations (Babu and Kumhar, 2014). Efficacy of a strain depends on efficient endo-cutinase activity to penetrate the cuticle which is the primary barrier of insect against fungal infection (Pekrul and Grula, 1979; Wagner, 1997). The results of present study showed BPA/B7 notably accounts for the 50% mortality of the insect by fifth day at 12.23 mlL^{-1} and within sixth day after spraying, it significantly killed 90% of the insect population at a minimal concentration of $10mlL^{-1}$ whereas, commercial formulation ($25mlL^{-1}$) of *B. bassiana* showed a mortality of only 20% during the same period of observation. Throughout the experiment, BPA/B7 showed the highest mortality than the commercial formulation of *B. bassiana* and significantly reduced the adults' population as compared to control on the 7th day after treatment under laboratory conditions. The mycelial growth appeared on the cuticle of the cadaver after 3-4 days of death of the insect. The cadaver on being sectioned transversely (Fig. 3) showed the development of mycelia inside the body and microscopic study of hyphae and spores confirmed the development of *B. bassiana* inside the insect body, thus confirming the death of insect by fungal infection.

Results of this study clearly indicated that, BPA/B7 strain of *B. bassiana* was found to be an effective biocontrol agent that could be used against *H. theivora* which could be developed into a formulation for evaluation under field conditions. Further studies on mortality at different conidial concentration ($1X10^5$ to $1X\ 10^{9)}$ and standardization of both liquid/powder formulations, shelf life studies, followed by their field evlauation, will ensure the possibility of utilizing this strain as a potential componemt of intergrated management of tea mosquito in tea plantations.

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