

## Role of Microorganisms in Sustainable Tea Cultivation in North East India: Recent Advances and Current Scenario

Azariah Babu\*

Department of Tea Research Association, North Bengal Regional R&D Centre, Nagrakata-735225, West Bengal, India

### ABSTRACT

Sustainable tea cultivation rely more on adopting alternative control strategies for effective tea pest management in an environment-friendly way which could replace some insecticides and thereby reduce the amount of pesticide residues in manufactured tea. Conservation of natural enemies and biodiversity of microorganisms in tea ecosystem is inevitable because, these natural enemies including the entomopathogens can play a vital role by providing effective control of target pests. With this background an attempt has been made to isolate *Beauveria* spp, and *Metarhizium* spp from Darjeeling and Dooars tea ecosystems of West Bengal, India and identified potential isolates like (BKN 1/14 and (MET 5/1) as *Beauveria bassiana* and *Metarhizium anisopliae* respectively. Stock suspension of BKN 1/14, MET 5/1 were prepared at concentration  $1 \times 10^8$  CFU/ml, further diluted to 0.25%, 0.5%, 0.75% and 1% using distilled water were sprayed on different life stages of tea mosquito (*Helopeltis theivora*), as well as red spider mite (*Oligonychus coffeae*) respectively. A Baculovirus, belonging to the group Nucleopolyhedrosis Virus (NPV) which infects the *Hyposidra talaca* larvae has also been isolated, identified, and characterized. Based on the findings of the laboratory studies, these beneficial microbial were developed in to a 5% AS formulations by the industry partner, following standard protocol. The developed 5% AS formulations were evaluated their field bio-efficacy under *in vivo* conditions following the Standard Operational Procedure (SOP) in three different locations against tea mosquito, red spider mite with encouraging results. The NPV was formulated in to an oil based suspension has also been tested at different concentration against tea lopper both under laboratory and field conditions with encouraging results in comparison to untreated control. All the three formulations were found to be non-phytotoxic to the tea plants, safer to the insect natural enemies, there is no tainting effect on made tea and were found to have a longer shelf life at room temperature without any change in their bio-efficacy. These strains could be commercialized after fulfilling the requirements for its registration and label claim on tea for the benefit of the tea industry.

**Keywords:** Microbial control; Entomopathogens; Tea pests; *Beauveria bassiana*; *Metarhizium anisopliae* Nucleopolyhedrosis Virus (NPV)

### INTRODUCTION

Tea (*Camellia* sp.) is an economically important, perennial plantation crop and requires warm humid climate for adequate growth and production. Such perennial nature, coupled favorable micro as well as macroclimate for flourishing of different insect pests and fungal diseases, which together cause huge crop loss in terms of quantity and quality. Synthetic

agrochemicals have been used since long back to get rid of these enemies.

Although, these synthetic chemicals provide better control of insect pests for a longer period; their recurrent and imprudent usage invite numerous additional problems associated with such as, environmental pollution, pesticides resistance, resurgence of secondary pests etc., [1,2]. The Biological Control Agents

**Correspondence to:** Azariah Babu, Department of Tea Research Association, North Bengal Regional R&D Centre, Nagrakata-735225, West Bengal, India, E-mail: azariah.babu@gmail.com

**Received:** June 17, 2020; **Accepted:** July 03, 2020; **Published:** July 10, 2020

**Citation:** Babu A (2020) Role of Microorganisms in Sustainable Tea Cultivation in North East India: Recent Advances and Current Scenario. Appli Microbiol Open Access 6:170. Doi: 10.35248/2471-9315.20.6.170

**Copyright:** © 2020 Babu A. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(BCAs) like species of *Beauveria*, *Metarhizium* and a Baculovirus, belonging to the group Nucleopolyhedrosis Virus (NPV) are safer and promising components of Integrated Pest Management programme (IPM), which have been adopted in various crops including tea for control of insect pests a (Papavizas, 1985) [3-7].

In Darjeeling and other tea growing area of West Bengal, different liquid and Wettable Powder (WP) formulations of such beneficial fungi are already in practice since last several decades, however they are outsourced ones. Although; in West Bengal, research work has been done in this direction [8-10] under lab conditions. A very little information on commercialization of such fungi is available. Our present study aimed at the isolation of *B. bassiana* and *M. anisopliae*, a Baculovirus (NPV) and development of suitable formulations for *in vitro* as well as *in vivo* evaluation against targeted insect pests.

## MATERIALS AND METHODS

### Isolation and identification of Entomopathogens

The soil samples were collected from tea growing areas of Dooars and Darjeeling, India. Sample collection was carried out with auger, sealed in different sterilized zip bags and kept in a container with ice bag (Ogunmwoyiet al.). The samples taken to the laboratory were serially diluted and inoculated in sterilized plate with different media and accordingly CFU values were recorded after two days using the methodology of Morris and Rideout (2005). The suspected fungus were transferred to sterilized media (PDA) plate and incubated at  $28 \pm 2^\circ\text{C}$  under the laboratory conditions.

The mycelium of full grown fungus was taken in glass slide and observed under binocular microscope (Olympus CX21i with Mag Vision). The isolates were identified on the basis of morphological characteristics and microscopic observations. All isolates were sent to Indian Type Culture Centre (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi - 110 012 to re-confirm their identity. The isolate BKN1/14 has been identified as *B. bassiana* and MET 5/1 has been identified as *M. anisopliae*.

The mother slants of BKN 1/14 (*B. bassiana*) and MET 5/1 (*M. anisopliae*) were sub-cultured under the laboratory conditions and incubated at  $28 \pm 2^\circ\text{C}$  in the BOD incubator. For broth culture, fragment of BKN 1/14 and MET 5/1 were taken from the mother slants and inoculated in Potato Dextrose Broth (PDB) in aseptic environment under laminar air flow and incubated in BOD under suitable controlled condition ( $28 \pm 2^\circ\text{C}$ ).

Larvae showing typical symptoms of NPV infection were collected from different tea gardens and the samples taken to the laboratory, homogenized and centrifuged to remove the insect cuticle and body skin.

Virus infection was primarily ascertained by the typical symptoms observed in the cadavers and later by light microscopic studies of polyhedron particles. Collected dead larvae were kept individually in separate centrifuge tube tube size: 1.5 mL with 1 mL sterile double distilled water and homogenized the dead larvae in a 1.5 mL tube with a plastic pestle. Centrifuge the tube at 500 rpm (to remove the insect cuticle and body skin) for 15 mins.

The supernatant was transferred to a new tube again centrifuged at 8000 rpm to sediment the polyhedral particle. Sediments were washed two times in 1 mL sterile distilled water and finally dissolved the Polyhedral Inclusion Bodies (PIBs). 1 mL distilled water and used for the bioassays.

The solution was sprayed on the leaves and also on the surface of the loopers dead insects were collected from the bioassay were reconfirmed the presence of polyhedron. Centrifuged again as mentioned above to confirm the presence of polyhedron.

The NPV formulation was prepared by mixing refined sunflower oil (25 ml) PEG 400 (2.5 g), Glycerin (2.5 g), Tween 20 (50  $\mu\text{l}$ ) with PIB suspension of 10 ml (up to 50 larval equivalent). The formulation should have about  $1 \times 10^{10}$  virus particles per ml and can be diluted 10 times before spraying.

## RESULTS AND DISCUSSION

### In vivo testing/ evaluation/bioefficacy of fungal species

The liquid fermentation technique of Bhat et al. [11] was adopted with minor modifications for the mass production of *B. bassiana* using 25 L capacity fermenter. The potato dextrose broth (Hi-media) was used as basal medium. The biomass was harvested after 11 days and wettable powder formulations were prepared. *M. anisopliae* was mass multiplied on PDB medium.

After two weeks, the medium and biomass was homogenized and used for field application. It was sprayed on tea stem and also drenched uniformly in collar region.

Different concentrations of the developed *B. bassiana* 5% AS formulation ( $2 \times 10^8$  cfu/g) of isolates and commercial formulation were tested against tea mosquito bug in Randomized Block Design (RBD) with three replications.

However, under the field conditions *B. bassiana* could cause almost 58.46 to 76.09 mean per cent reduction of tea mosquito bug infestation after two rounds of application when compared to other treatments (Table 1).

It's efficacy was found to be slightly inferior on 14th and 21st day of 2nd spray when compared with plots treated with *B. bassiana* 5% AS at 1000 ml/ha and 1200 ml/ha. Similarly, *B. bassiana* 5% AS at 1000 ml/ha and 1200 ml/ha was found to be significantly superior when compared to *B. bassiana* 5% AS at 600 ml/ha, 800 ml/ha and market sample, *B. bassiana* 1.15% WP at 2500 g/ha. Bio-efficacy was found to be dose dependent and increased with concentration.

**Table 1:** Impact of application of *B. bassiana* 5% AS on shoot damage caused by tea mosquito bug on tea.

Treatment details	Dose/ha	Pre-spray shoot damage incidence (%)	Shoot damage reduction over control (%)				Mean
			Day 7 after 1st spray	Day 7 after 2nd spray	Day 14 after 2nd spray	Day 21 after 2nd spray	
<i>B. bassiana</i> 5% AS	600 ml	25.7(30.42)	34.79 <sup>d</sup> (36.08)	53.24 <sup>c</sup> (46.86)	60.19 <sup>d</sup> (50.88)	67.66 <sup>c</sup> (55.33)	53.97 <sup>b</sup> (46.91)
<i>B. bassiana</i> 5% AS	800 ml	23.0 (28.49)	43.53 <sup>c</sup> (41.26)	54.55 <sup>c</sup> (47.59)	66.39 <sup>b</sup> (54.55)	70.15 <sup>b</sup> (56.88)	58.66 <sup>b</sup> (50.07)
<i>B. bassiana</i> 5% AS	1000 ml	22.3 (28.05)	49.02 <sup>bc</sup> (44.42)	70.84 <sup>b</sup> (57.3)	75.83 <sup>a</sup> (60.54)	81.82 <sup>a</sup> (64.73)	69.38 <sup>a</sup> (56.74)
<i>B. bassiana</i> 5% AS	1200 ml	21.3 (27.46)	51.87 <sup>b</sup> (46.06)	72.66 <sup>ab</sup> ( )	79.26 <sup>a</sup> (62.92)	83.47 <sup>a</sup> (66.07)	71.82 <sup>a</sup> (58.34)
<i>B. bassiana</i> 1% WP*	2500g	25 (29.99)	33.59 <sup>d</sup> (35.4)	52.35 <sup>c</sup> (46.34)	58.70 <sup>c</sup> (50)	62.90 <sup>b</sup> (52.46)	51.89 <sup>b</sup> (46.05)
Thiamethoxam 25% WG	120 g	20.3 (26.75)	61.77 <sup>a</sup> (51.8)	77.21 <sup>a</sup> (61.52)	73.06 <sup>b</sup> (58.78)	64.29 <sup>c</sup> (53.30)	69.08 <sup>a</sup> (56.31)
Untreated control	-	21.7 (27.69)	0.00 <sup>e</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>e</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>c</sup> (0.00)
CD	-	N/A	4.48	3.69	3.5	2.59	5.98

Mean of three replications, Figures in parenthesis are angular transformed values; \*Market sample

Under the laboratory conditions, the developed *M. anisoplae* 5% AS formulation against red spider mite recorded up to 68.2% efficacy over control. However, under the field conditions *M. anisoplae* could cause almost 48.4 to 70.8 mean per cent reduction of red spider mite population after two rounds of application when compared to other treatments (Table 2).

Mortality was found to be concentration dependent. Roobak Kumar et al [12] observed that *Pseudomonas putida* was capable of controlling of red spider mites under laboratory conditions.

The mites (nymphs and adults) when exposed to this bacterium, it resulted in to reduced mobility and cessation of feeding and ultimately died.

Addisu et al [13] assessed in vitro bio-efficacy of four isolates of *M. anisoplae* and *B. bassiana* against termite by spraying of  $1 \times 10^5$  to  $1 \times 10^9$  conidia per ml concentrations and they found that *M. anisoplae* and *B. bassiana* could cause 60-100 and 25-95 per cent mortality, respectively at different doses.

Selvasundaram and Muraleedharan [14] established that *B. bassiana* in combination with Triton AE and Teepol enhanced

the mortality of shot hole borer beetles (*Euwallacea fornicatus*) of tea plants under field conditions.

Annamalai et al. reported that *B. bassiana* and *L. lecanii* were effective for the control of *T. tabaci*. In the case of Nucleopolyhedrosis Virus (NPV), a RBD field trial using the NPV formulation against the tea looper (late 2nd and early 3rd instar) was initiated with 50 bushes per treatment.

Pre-treatment assessment was done on the population of tea loopers in the plot. The NPV formulation @400, 800 ml in 400 L was prepared and sprayed in the plot to evaluate the efficacy [15,16].

Observations were taken on the mortality of loopers at 24h interval and the results showed 85.5% reduction of looper after 7th day of spraying in comparison to the standard treatments which showed reduction 88.6% to 90.1%. Percent reduction of looper after application of NPV formulation under field conditions is given in Table 3.

**Table 2:** Field bioefficacy of *Metarhizium anisopliae* 5%AS against red spider mite in tea during season

Treatment details	Dose/ha	Pre-spray	% Reduction of mite population over control				Mean
			7th day after 1st spray	7th day after 2nd spray	14h day after 2nd spray	21st day after 2nd spray	
<i>Metarhizium anisopliae</i> 5% AS	600 ml	25.47 (30.3)	43.32 <sup>d</sup> (41.14)	50.95 <sup>d</sup> (45.53)	58.63 <sup>d</sup> (9.95)	62.31 <sup>c</sup> (52.11)	53.80 (47.18) <sup>c</sup>
<i>Metarhizium anisopliae</i> 5% AS	800 ml	25.67 (30.42)	51.16 <sup>c</sup> (45.64)	61.59 <sup>c</sup> (51.68)	68.45 <sup>c</sup> (55.84)	71.19 <sup>b</sup> (57.52)	63.10 (52.66) <sup>b</sup>
<i>Metarhizium anisopliae</i> 5% AS	1000 ml	26.21 (30.78)	57.43 <sup>b</sup> (49.26)	69.17 <sup>b</sup> (56.26)	73.74 <sup>ab</sup> (59.15)	79.79 <sup>a</sup> (63.27)	70.03 (56.98) <sup>a</sup>
<i>Metarhizium anisopliae</i> 5% AS	1200 ml	25.18 (30.95)	59.13 <sup>b</sup> (50.25)	73.71 <sup>ab</sup> (59.14)	76.30 <sup>a</sup> (60.88)	81.50 <sup>a</sup> (64.5)	72.66 (58.68) <sup>a</sup>
<i>Metarhizium anisopliae</i> 1.15% WP*	2500 g	26.47 (31.3)	46.85 <sup>d</sup> (43.18)	52.64 <sup>d</sup> (46.49)	59.70 <sup>d</sup> (50.57)	63.77 <sup>cd</sup> (52.98)	55.74 (48.3) <sup>c</sup>
Fenpyroximate 5% EC	250 ml	25.71 (30.46)	63.83 <sup>a</sup> (53.01)	76.52 <sup>a</sup> (61.01)	72.38 <sup>b</sup> (58.27)	65.90 <sup>c</sup> (54.25)	69.66 (56.63) <sup>ab</sup>
Control	-	26.52 (30.98)	0.00 <sup>e</sup> (0.00)	0.00 <sup>e</sup> (0.00)	0.00 <sup>e</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)
CD	-	N/A	2.08	1.9	2.14	1.66	4.29

Mean of three replications, Figures in parenthesis are angular transformed values, \* Indicates Market sample

**Table 3:** Effect of NPV formulation against tea looper *Hyposidra talaca* under field conditions

Treatments	Pre-treatment looper/bush	(Mean % of reduction of looper population*		% of yield increase
		After 24h	After 7 days	
NPV @400 ml/ha	6.4 ± 0.9 <sup>a</sup>	1.1 ± 0.0 <sup>a</sup>	81.2 ± 5.6 <sup>b</sup>	26.1 ± 4.5 <sup>bc</sup>
NPV @800 ml/ha	6.4 ± 0.7 <sup>a</sup>	3.4 ± 1.8 <sup>a</sup>	85.5 ± 1.0 <sup>b</sup>	19.4 ± 3.2 <sup>b</sup>
Flubendiamide 20 WG 80 g/ha	6.7 ± 1.0 <sup>a</sup>	70.5 ± 1.1 <sup>bc</sup>	90.1 ± 4.2 <sup>bc</sup>	29.7 ± 1.3 <sup>c</sup>
Emamectin benzoate 5% SG 40 g/ha	7.9 ± 0.5 <sup>ab</sup>	67.7 ± 2.8 <sup>b</sup>	88.6 ± 3.7 <sup>cd</sup>	23.8 ± 4.4 <sup>bc</sup>
Control	5.9 ± 0.9 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>

\*Values are represented as mean ± SE of five replications followed by the same letter in a row do not differ significantly

## CONCLUSION

The isolates, BKN 1/14 and MET 5/1 were identified as *Beauveria bassiana* and *Metarhizium anisopliae* respectively through DNA finger printing. Isolates have been registered with West Bengal Bio-Diversity Board, India. *Beauveria bassiana* (BKN 1/14) and *Metarhizium anisopliae* (MET 5/1) were shortlisted and formulated for conducting efficacy studies against tea mosquito bug red spider mite respectively.

Under field conditions, the efficacy of *Beauveria bassiana* against tea mosquito bug recorded as 53.97% to 71.82% efficacy over control. Similarly, in the case of *M. anisopliae* against red spider mite, the average efficacy ranged between 53.80 to 72.66 at different concentrations over control and the efficacy of these formulations were found to be concentration dependent.

The NPV of *Hyposidratacalaca* was isolated and characterized by genome sequencing. Viral particles isolated from the lab infected insect were used for preparation of a formulation for

field application. Laboratory, micro plot and large scale field trials on the NPV formulation's effectiveness against *Hyposidratacalaca*. Further studies are required for registration of the product including the toxicology have to be completed. Commercialization and utilization of these microbial formulations may be helpful in solving the problems associated with tea cultivation including the pesticide residues in made teas to a great extent.

## REFERENCES

1. Babu A. Pest management in tea: the south Indian scenario. Bull UPASI Tea Res Found. 2010; 55:23-30.
2. Roy S, Gurusubramanian G. Bioefficacy of azadirachtin content of neem formulation against three major sucking pests of tea in sub Himalayan tea plantation of North Bengal, India. Agric Trop Subtrop. 2011;44 3:134-142.
3. Roberts DW, St Leger RJ. *Metarhizium* spp., cosmopolitan insect pathogenic fungi: mycological aspects. Adv Appl Microbiol. 2004; 54: 1-70.
4. Steve R, Ellen B. A *Beauveria* phylogeny inferred from nuclear ITS and EF1- sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia. 2005;
5. Hall RA, Papierok B. Fungi as Biological control agents of Arthropods of Agricultural and Medical Importance. Parasitology. 1982; 84: 205-240.
6. Mukhopadhyay A, Khewra S, De D. Characteristics and virulence of nucleopolyhedrovirus isolated from *Hyposidratacalaca* (Lepidoptera: Geometridae), a pest of tea in Darjeeling Terai, India. Int J Trop Insect Sci. 2011; 31 1-2:13-19.
7. Nguyen TT, Suryamohan K, Kuriakose B, Janakiraman V, Reichelt M, Chaudhuri S, et al. Comprehensive analysis of single molecule sequencing-derived complete genome and whole transcriptome of *Hyposidratacalaca* nuclear polyhedrosis virus. Scientific Reports. 2018; 8:8924.
8. Debnath S. Studies on pathogenicity of *Paecilomyces tenuipes* (Peck) Samson to *Lesperysialeucostoma*, a flushworm of tea. Two and A Bud. 1997; 44 2: 19-22.
9. Babu A, Kumhar KC. New record of two fungal pathogens of red slug caterpillar. Tocklai News, Tea Research Association, Tocklai Experimental Station, Jorhat. 2013;19: 2-3.
10. Babu A, Kumhar KC. Pathogenicity of indigenous *Beauveria bassiana* (BKN 20) against tea mosquito and its compatibility with a few insecticides. Two and a Bud. 2014; 61 1- 2: 64-67.
11. Bhat K, Anwar A, Lone G, Hussain K. Shelf life of Liquid Fermented product of *Trichoderma harzianum* in Talc. Mycology and Plant Pathol. 2009; 39.
12. Roobak kumar A, Babu A, VasanthaKumar D, Rahman VKJ, Sarkar S. *Pseudomonas fluorescens* as an efficient entomopathogen against *Oligonychus coffeae* Nietner (Acari: Tetranychidae) infesting tea. J Entomol Nematol. 2011; 3 5:73-7.
13. Addisu S, Waktole S, Mohamed D. Laboratory evaluation of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against termite, *Macrotermes* (Isoptera: Termitidae). Asian J Plant Sci. 2013; 12: 1- 10.
14. Selvasundaram R, Muraleedharan N. Occurrence of the entomogenous fungus *Beauveria bassiana* on the shot hole borer of tea. J Plantation Crops 2000; 28 3: 229-230.
15. Yin Ye G, Xiao Q, Chen M, Chen XX, Yuan ZJ, Stanley DW, et al. Tea: Biological control of insect and mite pests in China. Biological Control. 2014; 68:73-91.
16. Rehner SA. Phylogenetics of the insect pathogenic genus *Beauveria*. In: Vega FE, Blackwell M (ed) Insect-Fungal Associations: Ecology and Evolution. Oxford University Press. 2005; 3-27.