ROLE OF MICROORGANISMS IN SUSTAINABLE TEA CULTIVATION IN NORTH EAST INDIA: RECENT ADVANCES AND CURRENT SCENARIO

Azariah Babu[1]
[1]Tea Research Association, North Bengal Regional R&D Centre, Nagrakata-735225, West Bengal, India

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 x 108 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 (Oligonychuscoffeae) 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.

Key words: Microbial Control, Entomopathogens, Tea Pests, Beauveria bassiana, Metarhizium anisoplae 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., (Babu and Muraleedharan, 2010, Roy et al, 2011). The biological control agents (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, Roberts & St. Leger, 2004; Rehner & Buckley, 2005, Hall & Papierok,1982). 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; inWest Bengal, research work has been done in this direction (Debnath, 1986, Debnath, 1996, Babu and Kumhar, 2013, 2014) 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.

METHODOLOGY

Isolation and identification of Entomopathogens

(i)Beauveria bassiana (ii) Metarhizium anisoplae:

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 (Ogunmwonyi et al., 2008). 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±2° 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. anisoplae) were sub-cultured under the laboratory conditions and incubated at 28±2 □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±2 □C).

Nucleopolyhedrosis Virus (NPV)

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 1mL sterile double distilled water and homogenized the dead larvae in a 1.5mL 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 1mL sterile distilled water and finally dissolved the polyhedral inclusion bodies (PIBs). 1mL 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 (25ml) PEG 400 (2.5g), Glycerin (2.5g), Tween20 (50µl) with PIB suspension of 10ml (up to 50 larval equivalent) The formulation should have about 1x1010 virus particles per ml and can be diluted 10 times before spraying.

FINDINGS

In vivo testing / evaluation / bioefficacy of fungal species

The liquid fermentation technique of Bhat et al. (2009) 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 (2x108cfu / 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 & 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.

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. anisopliae 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 (2011) 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 (2013) assessed in vitro bio-efficacy of four isolates of M. anisoplae and B. bassiana against termite by spraying of 1 x 105 to 1 x 109 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 (2000) 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. (2016) 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, 800ml in 400 L was prepared and sprayed in the plot to evaluate the efficacy. 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.

CONCLUSION

The isolates, BKN 1/14 and MET 5/1 were identified as Beauveria bassiana and Metarhizium anisoplae respectively through DNA finger printing. Isolates have been registered with West Bengal Bio-Diversity Board, India. Beauveria bassiana (BKN 1/14) and Metarhizium anisoplae (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. anisoplae 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 Hyposidra talaca 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 Hyposidra talaca.

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

Note: This work is partly presented at 3rd international conference on 7th Global Conference on Applied Microbiology and Biotechnology (November 18-19, 2019 Rome, Italy)