Bio-efficacy of BPA/B7 formulations against *Helopeltis theivora* Waterhouse

Preety Ekka*[1], Lakhi Ram Saikia[1] and Azariah Babu[2]

ABSTRACT

Tea garden soil harbors several microorganisms that help in maintaining the crop agro-ecosystem. Some soil microorganisms are beneficial and some may cause harm. The beneficial entomopathogenic fungus (BPA/B7) isolated from Tinsukia (Assam) was found to be more effective against *Helopeltis theivora* Waterhouse compared to other strains of *Beauveria bassiana*. This pathogenic fungus has been mass produced and formulated into powder and liquid formulation and its different properties have been screened under laboratory and field conditions and both the formulations were found to be effective against *H. theivora*.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, *Helopeltis theivora*, Formulations

MS History: 30.03.2023 (Received)- 20.06.2023(Revised)- 28.06.2023 (Accepted).

Citation: Ekka, P., Saikia, L.R. and Babu, A. 2023. Bio-efficacy of BPA/B7 formulations against *Helopeltis theivora* Waterhouse. *Journal of Biopesticides,* **16**(1):63-67. **DOI:10.57182/jbiopestic.16.1.63-67**

INTRODUCTION

Tea is a consumable product and pesticide-residue free organic tea are in demand in the world tea market. Tea gardens of North-East India is known as world's largest tea producing belts. This large agriculture land also provides suitable micro-climate for the occurrence of vast pest diversity and chemical measures used to manage this pest population often causes different ailments of agro-ecosystem. The soil of the tea garden harbours a number of micro-organisms like fungal and bacterial species that helps in maintenance of the crop agro-ecosystem (Madhab *et al*. 2009, Sharma *et al.* 2012; Bhattacharjee *et al*. 2013). Some micro-organisms may be harmful or beneficial or some also contribute to the enhancement of the plant productivity (Kumar and Bezbaruah 1997 and Phukan *et al.* 2012). Beneficial fungi fall under four groups, like Oomycetes, Zygomycetes, Chytridiomycetes and Deuteromycetes, where the first three groups have a narrow host range while the last one has a wide host range and can be formulated as bio-pesticides which eco-friendly, bio-degradable and have less or no residue (Wahab 2004). The tea gardens soil of Tinsukia and Dibrugarh districts of Assam have been screened and a fungal strain BPA/B7 (*Beauveria bassiana*) was isolated from Tinsukia that caused high mortality against *Helopeltis theivora* Waterhouse compared to other strains of *B. bassiana* (Ekka *et al.* 2019). This pathogenic fungus has been mass-produced and formulated into powder and liquid formulations in collaboration with Varsha Bio-Science and Technology India Pvt. Ltd.

The virulence rate of bio-formulation must be tested in laboratory conditions against both target and non-target pests and should have longer shelf life and can be easily stored in both room and control conditions are some of the criteria for its registration as bio-pesticides (Soper and Ward 1981, Babu A. 2009; Strasser *et al.* 2010). Lower production cost of bio-pesticides than that of chemical pesticides and its inclusion in integrated pest management could reduce the heavy dependence on chemical pesticides for the reduction of pest infestation. The market for bio-pesticides is gradually growing, so an attempt has been made to screen the different properties of the bio-formulations of BPA/B7 for its suitable application in field conditions against the major hemipteran pest, *Helopeltis theivora* Waterhouse.

MATERIAL AND METHODS

Formulation of BPA/B7 into powder and liquid bio-formulations were prepared in collaboration with Varsha Bio-Science and Technology India Pvt. Ltd. (ISO 9001:2015 certified company).

Determination of spore viability: 1 gram of dried culture of fungus was mixed with 10 ml of distilled water inoculated with a small volume of 0.01% tritonX 100. The suspension was filtered and spore was adjusted to spore concentration of 1 X 10^7 spore/ml.

Determination of spore concentration: 1 gram of the dried culture was taken into 10ml of sterilized distilled water containing 0.1% triton X 100 solutions. The flask was shaken for 10 minutes and the suspension was filtered and mixed in 10 ml of sterilized distilled water. The spore suspension was serially diluted and each dilution was used for counting of spore using haemocytometer per gram of fungal biomass.

Evaluation of the formulations

In-vitro assessment: The liquid and powder formulation BPA/B7 was prepared in collaboration with Varsha Bio-Science and Technology India Pvt. Ltd. (ISO 9001:2015 certified company). This commercially formulated isolate was evaluated against *Helopeltis theivora* to determination of LC_{50} value of BPA/B7.

Field assessments

Dosage of synthetic chemicals per hectare and the spray interval were listed in the table I (a). The control plot was used for comparison and the total duration of experiment has been one month (based on pest incidence). The experiments were conducted in the TRA plot and in the small tea garden (Assam). Randomized block design has been followed. Spraying has been carried out using a motorized mist blower. Spray volume employed was 400 liters per ha. Two rounds of spraying have been carried for seven days. During every harvest (once in 7 days), sampling has been taken for the assessment of insect infestation. The data on insect infestation assessment were subjected to statistical analysis of variance. Pre-treatment and Post treatment sampling are done based on the harvesting interval (10-12 days) and data is subjected to appropriate statistical analysis to calculate the percentage bio-efficacy by using Henderson and Tilton (1955) formula.

Henderson and Tilton formula

$$\%\ \textit{bio-efficacy} = \left(1 - \frac{Ta \times Cb}{Tb \times Ca}\right) \times 100$$

Ta=N in T after treatment Tb=N in T before treatment (T=Treated)

Ca= N in Co after treatment Cb=N in Co before treatment (Co=Control)

Table 1: List of combinations used for the treatment against *Helopeltis theivora* Waterhouse

Treatments	Combination	Dosage/ concentration
T1	*B.bassiana* powder formulation	750g / ha
T2	*B. bassiana* powder formulation	1000g / ha
T3	*B. bassiana* powder formulation	1250g / ha
T4	*B. bassiana* liquid formulation	250 mL / ha
T5	*B. bassiana* liquid formulation	400 mL / ha
T6	*B. bassiana* liquid formulation	500 mL / ha
T7	Thiamethoxam	1:4000
T8	Quinalphos	1:400
T9	Commercial formulation of *B. bassiana*	1250g / ha
T10	Crude extract of *B. bassiana*	10^8conidia/ml
T11	Control	

RESULTS

Quality assessment: The spore loads were higher in powder formulations of both the isolates Table1(b). The mean CFU was highest on powder formulation of BPA/B7 (174.91 X 10^8 CFU) followed by liquid formulation of BPA/B7(154.04 X 10^8 CFU) and minimum was recorded on powder formulation of BKN-20 (75.50 X 10^8 CFU) followed by liquid formulation of BKN-20(98.72 X 10^8 CFU). The highest spore load were recorded from powder and liquid formulations of

Table 2. Spore load of liquid (LF) and powder formulations (PF) of BPA/B7* and BKN-20

Days	TF*	LF*	TF	LF	Mean
0	245.22[a]	212.51[b]	236.21[a]	200.11[b]	223.51
15	236.28[a]	200.10[b]	152.11[d]	175.45[c]	190.99
30	201.00[b]	190.31[c]	118.15[f]	124.87[e]	158.58
45	194.12[c]	169.23[c]	95.22[f]	114.03[f]	143.15
60	181.23[c]	155.25[d]	73.12[f]	100.01[f]	127.40
75	176.45[c]	152.00[d]	55.21[f]	98.78[f]	120.61
90	162.11[d]	145.18[d]	30.96[f]	74.66[f]	103.23
105	158.13[d]	134.33[e]	26.11[g]	64.82[f]	95.85
120	152.10[d]	126.09[e]	25.09[g]	51.23[f]	88.63
135	145.12[d]	115.11[e]	13.22[g]	49.75[f]	80.80
150	72.22[f]	94.28[f]	5.11[g]	32.22[g]	50.96
Mean	174.91	154.04	75.50	98.72	
SEM	14.21	11.05	21.35	15.92	

* Mean sharing the same letter are not significantly different at p=0.05 by LSD
CD (P = 0.05)
Between days: 0.12 Between Formulations : 0.08

In-vitro assessment of bio-formulations

Table 3. Effect of the treatments on the of *Helopeltis theivora* Waterhouse. DAT: Day after treatment

Treatment	Code	Percent mortality after				
		3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
BPA/B7@ 750 g/h	T1	0.00 [a]	0.00 [a]	3.33 [a]	3.33 [a]	10.00 [b]
BPA/B7@ 1000 g/h	T2	0.00 [a]	13.33 [b e]	26.67 [c]	36.67 [e d]	40.00[d]
BPA/B7@ 1250 g/h	T3	10.00 [b]	16.67 [b e]	36.67 [e d]	43.33[d]	56.67[e]
BPA/B7@ 400 ml/h	T4	0.00 [a]	6.67 [a b]	23.33 [c]	26.67[c d]	30.00[d]
BPA/B7@ 600 ml/h	T5	10.00 [b]	26.67 [c d]	36.67 [c d]	46.67[d e]	53.33[e]
BPA/B7@ 800 ml/h	T6	16.67 [b c]	40.00 [d]	53.33 [e]	70.00[g]	76.67[g h]
Thiamethoxam	T7	83.30[h]	96.70[i j]	100.00 [j]	100.00 [j]	100.00 [j]
Quinalphos	T8	76.70 [g h]	90.00[i]	93.30[i]	100.00 [j]	100.00 [j]
Commercial *B.bassiana* @ 800 ml/h	T9	0.00 [a]	13.33 [b e]	23.33 [c]	23.33[c]	23.33[c]
Control	T10	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	3.33 [a]
SEm (+)		1.58	2.91	2.67	2.58	4.28
CD		7.617	10.772	8.795	9.329	14.913
CV %		22.361	20.850	13.018	12.172	17.749

* Mean sharing the same letter are not significantly different at p=0.05 by LSD

BPA/B7.The decline in spore load with time is slower in liquid formulations compared to that of powder formulations.

The synthetic chemicals (Thiamethoxam and Quinalphos) showed maximum efficacy of killing (100%) the pest within a period of 5-6 days. The commercial formulation of *Beauveria bassiana* @ 800 ml/ hectare gave a maximum mortality of 23.33% even after 7 day of treatment. The higher doses of powder formulation killed 40% and 56.67% of the pest population respectively. The liquid formulation at a concentration of 800ml/h was noted with maximum mortality of 76.67% followed by liquid formulation at a concentration of 600 ml/h and 400 ml/h with mortality of 53.33% and 30.00% respectively. The controlled environment of laboratory trial showed a higher percent mortality of pest was caused by liquid formulation than powder formulation of BPA/B7.

Field assessments

At TRA plot after the first round treatment the maximum % bio-efficacy was recorded by Thiamethoxam followed by quinalphos and liquid formulation (800ml/hectre) with 84.85%, 70.91% and 60.79% respectively and minimum was recorded by commercial *B. bassiana*(39.59%). At small tea garden (STG), maximum % was recorded from Thiamethoxam followed by quinalphos and liquid formulation of BPA/B7 (800ml/hectre) with 85.46%, 73.41% and 61.97% respectively and minimum % bio-efficacy after first round treatment was recorded by commercial *B.bassiana*.

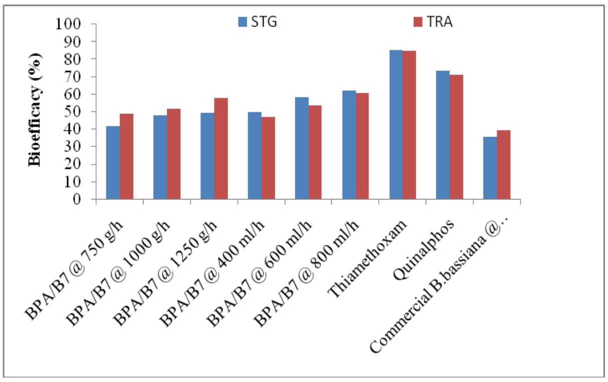

Fig 1. Percent Bio-efficacy trial of different treatments after first round of application at two trial plots

The second round application showed that both synthetic chemicals have higher % bio-efficacy than the formulation. At TRA plot Thiamethoxam was recorded with 84.92% bio-efficacy followed by Quinalphos and liquid formulation of BPA/B7, 800ml/hectre and 600ml / hectre with 37.83% and 31.39% respectively. At STG plot the second-round treatment showed similar trend with maximum % bio-efficacy of synthetic chemicals (Thiamethoxam followed by quinalphos) than the formulation with 79.59% and 52.24% respectively followed by BPA/B7 (powder formulation, 1250gm/hectre) and BPA/B7 (liquid formulation, 800ml/hectre) with 43.41% and 41.13% respectively.

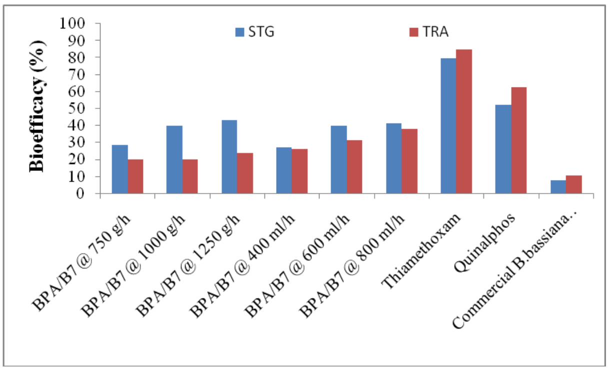

Fig 2. Percent Bio-efficacy trial of different treatments after second round of application at two trial plots

The infestation % of control at TRA plot increased from pre-treatment infestation of 46.20 % to 72.01%, 87.65% and 91.11% by 21th day of the experiment. In the treated plot a decrease in pest infestation was observed with a maximum reduction of 70% was recorded in plot treated with Thiamethoxam followed by quinalphos, and BPA/B7 (liquid formulation, 800ml per hectre) with infestation reduction of 64% and 40% respectively. In STG plot the infestation % of control increased from pre-treatment infestation of 50.60 % to 69.80%, 82.60% and 92.01% by 21th day of the experiment. In the treated plot a decrease in pest infestation was observed and maximum reduction of 78% was recorded in plot treated with Thiamethoxam followed by quinalphos and BPA/B7 (liquid formulation, 800ml per hectre) with infestation reduction% of 63.71% and 40.31% respectively.

The BPA/B7 formulations are effective against *H. theivora*. The synthetic chemicals have higher % bio-efficacy followed by liquid formulation and powder formulation of BPA/B7 suggesting that bio-formulations could be effectively used under field conditions. This bio-formulation should be applied at the preliminary stage of the pest infestation which would suppress the pest population at an early stage reducing the application of chemical pesticides.

ACKNOWLEDGEMENTS: The authors are thankful Dr. John Peter, Chairman, M/S Varsha Bioscience and Technology India Pvt Ltd, Hyderabad-500059, Telangana, for his help in manufacturing and supply of powder and liquid formulation of the BPA/B7.

REFERENCE

Babu, A. 2009. Eco- friendly insect pest management in tea in south India. *Pest management and Environmental Safety*. **4**(2):49-56.

Battacharjee, M.K., Mazumdar, P.B and Sharma, G.D. 2013. Isolation and identification of bacteria of genus *Pseudomonas* from tea rhizosphere of South Assam, India. *Journal of Biological Sciences*. **2** (1) : 5-7.

Ekka P., Babu A. and Saikia L.R. 2019. Potential of new strain of *Beauveria bassiana* isolated from Tinsukia (Assam) against tea mosquito bug *Helopeltis theivora* Waterhouse (Heteroptera: Miridae). *Journal of Biopesticides.* **12**(1): 104-108.

Henderson C. F and Tilton E. W. 1955. Test with acaricides against the brown wheat mite. *Journal of Economic Entomology.* **48**(2): 157-161.

Kumar, B. D and Bezbaruah, B. 1997. Plant growth promotion and fungal pest control through an antibiotic and Siderophore producing fluorescent *Pseudomonas* strain from tea (*Camellia sinensis* L.) O. Kuntze plantation. *Indian Journal of Experimental Biology*. **35**:289-292.

Madhab, M., Saikia, L. R and Barthakur, B. K. 2009. Microbial diversity in tea soils of Brahmaputra Valley. *Two Bud*, **56:**48-51.

Phukan, I., Madhab, M., Bordoloi, M., Sharma, S. R., Dutta, P., Begum, R., Tanti, A.,Bora, S., Debnath, S and Barthakur, B.K. 2012. Exploitation of PGP microbes of tea for improvement of plant growth and pest suppression: A novel approach. *Two Bud*, **59:**69-74.

Sharma, B. C., Subba, R and Saha, A. 2012. *Kurthia sp.* a novel member of phosphate solubilizing bacteria from rhizopheric tea soil of Darjeeling Hills, India. *Journal of Pharmacy and Biological Sciences.* **2**(3) : 36-39.

Strasser H., Vey A and Butt T.M. 2010. Are there any risks in using entomopathogenic fungi for pest control with particular reference to bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technology*. **10**(6): 717-735.

Soper R. S and Ward M. G. 1981. Production, formulation and application of fungi for insect control. 161-18. *In: Biological Control in Crop Production* (G.C. Papavizas, ed.). Allanheld Osmun Publisher, London.

Wahab S. 2004. Tea pests and their management with bio-pesticides. https://repository.up.ac.za/bitstream/handle/2263/8414/Wahab_2004.

Preety Ekka*[1], Lakhi Ram Saikia[1] and Azariah Babu[2]

[1]Department of Life Sciences, Dibrugarh University, Assam-786004, India

[2]Tocklai Tea Research Institute, Jorhat, Assam-785 008, India

***Corresponding author**

E-mail: preetyekka42@gmail.com