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Efficacy of different formulations of *Beauveria bassiana* (Bb 112) against *Bemisia tabaci* on tomato

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Abstract
Investigations were carried out to evaluate the efficacy of formulations of *Beauveria bassiana* (Bb 112) against whitefly, *Bemisia tabaci* on tomato under microplot condition. Among the different formulations tested *viz.,* crude, talc and oil formulations, *B. bassiana* (Bb 112) oil formulation was most effective against whitefly on tomato with 45.86 % reduction in population over control followed by talc (29.62 %) and crude formulations (21.63 %). Present study wide open the scope of using an oil formulation of *B. bassiana* (Bb 112) against other sucking pests also.

Keywords: *Bemisia tabaci, Beauveria bassiana,* tomato, efficacy, oil formulation

1. Introduction
Tomato, *Lycopersicum esculentum* L. is the world's largest cultivated vegetable crop occupying an outstanding place among the important vegetables of the world. It is commercially cultivated over an area of 0.88 million ha with an annual production of 18.23 million tonne and productivity of 20.70 tonne hectare^{-1} in India (Indian Horticulture Database-2013). The crop is infested by various insect pests *viz.,* whiteflies (*Bemisia tabaci* Gennadius), aphids (*Aphis gossypii* Glover), thrips (*Thrips tabaci* Lindeman) and fruit borer (*Helicoverpa armigera* Hubner).

Of which whiteflies are the most damaging pest as they act as a vector of tomato leaf curl virus (TLCV) and causes heavy loss of 47 to 95 % during the early stage of the crop (Saikia and Muniyappa, 1989) [16]. Farmers heavily rely on chemical pesticides for the management of this cosmopolitan pest in tomato fields. However, extensive use of pesticides has resulted in adverse effects on environment as well as an increase in cost of production. Injudicious application of pesticides also affected ecological imbalance resulting in serious problems such as insecticide resistance, pest resurgence and pesticide residues. This scenario has given impetus for the development of non-chemical control methods for ecofriendly pest management *viz*., biological control, botanicals and pheromone based pest management programmes.

Among the biological control options, entomopathogenic fungi (EPF) are the most potent microbial agents, due to their wide host range that often results in natural epizootics. EPF unlike entomopathogenic bacteria or viruses directly infect through insect cuticle and do not require ingestion for infection. Thereby EPF offer great potential for the management of sucking pests (Ramanujam, 2004) [15]. Improved mycoproducts, provide good control of whiteflies in both greenhouse and field crops. Keeping this in view, the present investigation was undertaken to evaluate the efficacy of formulations of promising fungal pathogen *Beauveria bassiana* (Bb 112) available at the Department of Agricultural Entomology, TNAU, Coimbatore against tomato whitefly, *B. tabaci* under microplot condition.

2. Materials and Methods
Formulations of *B. bassiana* (Bb 112)
Different formulations of *B. bassiana* (Bb 112) *viz*., crude, talc and oil based formulations were prepared as per the protocol given below.

2.1 Preparation of crude formulation of *B. bassiana* (Bb 112)

Two hundred and fifty ml of SMY broth was taken in a round bottom flask
(500 ml capacity) and autoclaved at 121 °C for 20 minutes. After cooling, 1.0 ml of spore suspension (10^6 spores ml^{-1}) of *B. bassiana* fungal isolate was inoculated into a flask and kept in an orbitrary shaker for three days and incubated at room temperature for 10 days. After sporulation, the fungus along with broth was ground in a mixer and filtered through double layered muslin cloth. The suspension was shaken thoroughly with 0.25 ml of Tween 80® in order to disperse the spores in solution. The conidial suspension was vortexed for 5 min to produce a homogenous conidial suspension and utilized for field evaluation immediately (Saranya *et al*., 2013) [18].

2.2 Preparation of talc based formulation

The fungus, *B. bassiana* (Bb 112) was multiplied in SMY broth as above and after multiplication, it was mixed with powdered talc at 1:2 ratio (500 ml: 1 kg). To the mixture, 5 g of CMC (Carboxy methyl cellulose) was added as sticker and dried in shade for 72 h, powdered and stored in polypropylene bags until further use (Jeyarajan *et al*., 1994) [8].

2.3 Preparation of oil formulation

2.3.1 Spore production by diphasic liquid-solid fermentation technique

The aerial conidia of *B. bassiana*, which is best suited to be formulated in oil was produced by diphasic liquid – solid fermentation technique developed by LUBILOSA (*Lutte Biologique contre les Locustes et Sauteriaux*, www.lubilosa.org) project to produce *Metarhizium flavoviride* (Lomer *et al*., 1997) [11].

2.3.2 Preparation for vegetative phase

The first stage in diphasic technique is to encourage the production of hyphal bodies and mycelium of an isolate that can then be used for inoculation into the second (solid) stage of production. The SMY broth was used as substrate for vegetative phase of fungal growth.
Seventy five ml of SMY broth was taken in two hundred and fifty ml conical flasks. The flasks were plugged with non-absorbent cotton wool, covered with aluminium foil and kept for sterilization at 121 °C (15 psi) for 20 minutes. After cooling, the broth was inoculated with a spore suspension containing approximately 6 x 10^6 spores ml^{-1} and kept in a shaker at approximately 150 rpm for three days at room temperature (~25 to 30°C).

2.3.3 Inoculation of solid media (rice) for solid state fermentation

Broken white rice is often the preferred substrate for a second phase, since individual rice particles provide a large surface area and optimum aeration which could favour the formation of conidia. Five hundred gram of broken rice sprinkled with 100 ml sterile distilled water was filled in polythene bags (45x35cm; 300 gauge). The rice bags were then autoclaved at 121 °C (15 psi) for 30 minutes. One flask of liquid inoculum (75 ml) for each 500 g bag of rice was utilized. After cooling, each rice bags were inoculated with 75 ml of liquid inoculum of the fungal isolate. The bag was massaged gently from outside to evenly distribute the inoculum over the rice grains. The top of the bag was folded loosely to aerate the substrate as the fungus grows. The bags were incubated at room temperature (~25 to 30°C) for about 10 to 15 days for sporulation.
After sporulation, the bag was opened and the rice was allowed to dry by spreading the grains in sterile stainless steel trays of uniform size (20 x 15 x 4 cm), covered by sterile muslin cloth under aseptic condition for seven to ten days depending on the temperature and humidity in the environment. Once dried, the spores were extracted from rice by manual sieving with 36 micron mesh sieve. After sieving, the strength of the spore powder was assessed using Neubauer haemocytometer. Then the spores were kept in sterile vials and stored in refrigerator for further experimental purposes.
Spores produced were used for the preparation of oil formulation of Bb 112 by adopting the protocol standardized by Sangamithra (2015) [17] and stored under room temperature until further use.

2.4 Evaluation of formulations under microplot

Studies were conducted in microplots at the insectary of the Department of Agricultural Entomology, TNAU, Coimbatore in 2016 to evaluate the efficacy of different formulations of *B. bassiana* (Bb 112) at 10^8 spores ml^{-1} against whitefly (*B. tabaci*) on tomato in comparison with talc formulation of *B. bassiana* (B2) available in the Department of Plant Pathology, TNAU, Coimbatore and standard insecticide checks (imidacloprid 17.8 SL and dimethoate 30 EC). Tomato seedlings (cv. PKM 1) were raised in protrays. After 15 days of sowing, tomato seedlings were transferred to the microplots of size 12 m^2 (4x3 m^2). The experiment included seven treatments with four replications.
Two rounds of treatments were imposed at fortnightly interval with the help of a hand sprayer. The pre and post treatment observation on live whitefly populations were assessed at 0, 3, 5, 7, 10 and 14 days after application. Three leaves one each from top, middle and bottom canopies of each plant were assessed for the live whiteflies (both nymphs and adults) on the undersurface of the leaves. The observations were made randomly on the place where the maximum population was noticed. The treatment details are as follows.

Treatments	Dosage
B. bassiana (Bb 112) (oil formulation)	10^8 Spores ml^{-1}
B. bassiana (Bb 112) (talc formulation)	10^8 Spores ml^{-1}
B. bassiana (Bb 112) (crude formulation)	10^8 Spores ml^{-1}
B. bassiana (B 2) 5g lit^{-1} (Commercial talc formulation)	10^8 Spores ml^{-1}
Dimethoate 30 EC	0.7 ml lit^{-1}
Imidacloprid 17.8 SL	0.5 ml lit^{-1}
Control (water spray)	-

3. Results and Discussion

Formulation is mandatory in order to enhance field application and efficacy.
The type of formulation selected depends upon the biological and physical properties of the pathogens, location and habitats of the target pest (Daoust *et al*., 1983) [2]. Good formulation will increase the efficacy of microbial biopesticides by improving their application efficiency, coverage, activity and persistence on the leaf surface (Jones and Burges, 1998) [9].
The present study revealed that there were significant differences with respect to whitefly control among different formulations. The results of the experiments on pre and post treatment population of whiteflies are presented in Table 1.
Before spraying, the mean number of whiteflies in tomato was

not significant which ranged from 23.53 to 25.01 leaf^{-1}. Oil based formulation of *B. bassiana* (Bb 112)was found significantly superior to other *B. bassiana* formulations with the lowest whitefly population of 17.64, 15.41, 12.91, 14.84 and 16.72 numbers leaf^{-1} at 3, 5, 7, 10 and 14 days after treatment, respectively with the highest population reduction of 37.80 % after first round of spraying. This was followed by talc and crude formulations of *B. bassiana* (Bb 112) with a reduction in whitefly population of 22.77 and 15.76 %, respectively (Table 1). In case of standard insecticide checks, imidacloprid 17.8 SL (0.5ml lit^{-1}) and dimethoate 30 EC (0.7 ml lit^{-1}) were found to be significantly superior to all other treatments and recorded the highest population reduction of 70.16 and 68.49 %, respectively.

Table 1: Efficacy of *B. bassiana* (Bb 112) formulations against *Bemisia tabaci* on tomato (cv. PKM 1) – Microplot

Treatments	PTC (No. of whiteflies/ leaf)	Days after first spraying (No. of whiteflies / leaf)					Mean no. of whiteflies / leaf	% reduction over control
		3	5	7	10	14		
B. bassiana (Bb 112) - 10^8 spores ml^{-1} (oil formulation)	24.40	17.64 (4.20)^b	15.41 (3.93)^b	12.91 (3.59)^b	14.84 (3.85)^b	16.72 (4.09)^c	15.50	37.80
B. bassiana (Bb 112) - 10^8 spores ml^{-1} (talc formulation)	23.80	20.47 (4.52)^d	19.07 (4.37)^c	16.74 (4.09)^d	18.91 (4.35)^d	21.07 (4.59)^e	19.25	22.77
B. bassiana (Bb 112) - 10^8 spores ml^{-1} (crude formulation)	24.96	21.03 (4.59)^{de}	20.29 (4.50)^d	19.76 (4.45)^e	21.29 (4.61)^e	22.63 (4.76)^f	21.00	15.76
B. bassiana (B 2) - 10^8 spores ml^{-1} (talc formulation)	25.01	19.75 (4.44)^c	16.53 (4.07)^c	14.26 (3.78)^c	16.04 (4.00)^c	18.52 (4.30)^d	17.02	31.72
Dimethoate 30 EC 0.7 ml l^{-1}	23.53	13.41 (3.66)^a	10.04 (3.17)^a	5.39 (2.32)^a	3.27 (1.81)^a	7.16 (2.68)^b	7.85	68.49
Imidacloprid 17.8 SL 0.5 ml l^{-1}	24.82	12.94 (3.60)^a	9.73 (3.12)^a	4.98 (2.23)^a	2.86 (1.69)^a	6.68 (2.58)^a	7.44	70.16
Control (water spray)	23.88	22.32 (4.72)^e	23.49 (4.85)^e	25.81 (5.08)^f	26.25 (5.12)^f	26.77 (5.17)^g	24.93	-
SE (d)	NS	0.24	0.18	0.22	0.21	0.16	-	-
C.D (P=0.05)	-	0.49	0.37	0.45	0.42	0.34	-	-

Values are means of four replications
PTC- Pretreatment count
Figures in the parentheses are $\sqrt{x+0.5}$ transformed values
Means followed by the common letter (s) are not significant at P=0.05 level by LSD

A similar trend was also observed after second round of treatment (Table 2). Oil based formulation of *B. bassiana* (Bb 112) was found to be highly effective with a cumulative mean population reduction of 45.86 % after two rounds of spraying which was followed by talc and crude formulations of *B. bassiana* (Bb 112),with cumulative mean reduction of 29.62 and 21.93 % over control, respectively. The standard insecticide checks, imidacloprid 17.8 SL (0.5ml lit^{-1}) and dimethoate 30 EC (0.7 ml lit^{-1}) recorded the highest cumulative mean reduction of 78.13 and 76.60 %, (Fig. 1) respectively.

Table 2: Efficacy of B. bassiana (Bb 112) formulations against *Bemisia tabaci* on tomato (cv. PKM 1) – Microplot

Treatments	Days after second spraying (No. of whiteflies / leaf)					Mean no. of whiteflies / leaf	% reduction over control	Pooled mean (No/leaf)	Cumulative % reduction over control
	3	5	7	10	14				
B. bassiana (Bb 112) - 10^8 spores ml^{-1} (oil formulation)	14.89 (3.86)^b	12.06 (3.47)^b	10.78 (3.28)^b	13.47 (3.67)^b	15.63 (3.95)^c	13.37	53.92	14.44	45.86
B. bassiana (Bb 112) - 10^8 spores ml^{-1} (talc formulation)	20.35 (4.51)^d	18.67 (4.32)^d	15.22 (3.90)^d	17.68 (4.20)^d	20.21 (4.50)^e	18.43	36.48	18.84	29.62
B. bassiana (Bb 112) - 10^8 spores ml^{-1} (crude formulation)	21.54 (4.64)^e	20.04 (4.48)^e	19.36 (4.40)^e	21.32 (4.62)^e	22.02 (4.69)^f	20.86	28.10	20.93	21.93
B. bassiana (B 2) - 10^8 spores ml^{-1} (talc formulation)	16.71 (4.09)^c	14.98 (3.87)^c	12.56 (3.54)^c	14.53 (3.81)^c	17.98 (4.24)^d	15.35	47.07	16.19	39.40
Dimethoate 30 EC 0.7 ml l^{-1}	5.36 (2.32)^a	4.12 (2.03)^a	3.28 (1.81)^a	2.59 (1.61)^a	6.83 (2.61)^b	4.44	84.71	6.15	76.60
Imidacloprid 17.8 SL 0.5 ml l^{-1}	4.92 (2.22)^a	3.83 (1.96)^a	2.92 (1.71)^a	2.48 (1.57)^a	6.02 (2.45)^a	4.03	86.09	5.74	78.13
Control (water spray)	28.05 (5.30)^f	27.64 (5.26)^f	28.43 (5.33)^f	30.12 (5.49)^f	30.79 (5.55)^g	29.01	-	26.97	-
SE (d)	0.23	0.17	0.21	0.13	0.19	-	-	-	-
C.D (P=0.05)	0.47	0.35	0.43	0.27	0.38	-	-	-	-

Values are means of four replications
Figures in the parentheses are $\sqrt{x+0.5}$ transformed values
Means followed by the common letter (s) are not significant at P=0.05 level by LSD

The results from the present study indicates that the formulations of *B. bassiana* (Bb 112) was effective against whitefly infesting tomato and among the formulations, oil formulation of Bb 112 was highly effective. Manjula *et al.* (2003) [12] reported that *B. bassiana* formulated with coconut oil was effective against *B. tabaci,* which in turn limit the

incidence and spread of tomato leaf curl virus. Several workers also documented the efficacy of *B. bassiana* against *B. tabaci* (Negasi *et al*., 1998) [14], (Herrera *et al*., 1999) [6], (Vicentini *et al*., 2001) [19], (Moraga *et al*., 2006) [13] and *Trialeurodes vaporariorum* (Fargues *et al*., 1992) [4], (Benuzzi and Santopolo, 2001) [1]. Sangamithra (2015) [17] investigated the potential of nine different isolates of entomopathogenic fungi as effective alternative to synthetic pesticides against onion thrips, *T. tabaci*. Among the nine isolates tested, *B. bassiana* (Bb 101) was found to cause the fifty percent mortality at low concentration of 1×10^6 spores ml^{-1} and also within a short time (94.43 h) than the other isolates. Hemalatha *et al.* (2015) [5] reported that application of *B. bassiana* (Bb 112) oil formulation at 10^8 spores ml^{-1} had the highest cumulative mean thrips population reduction of 59.73% and 53.88% in pot culture experiments.

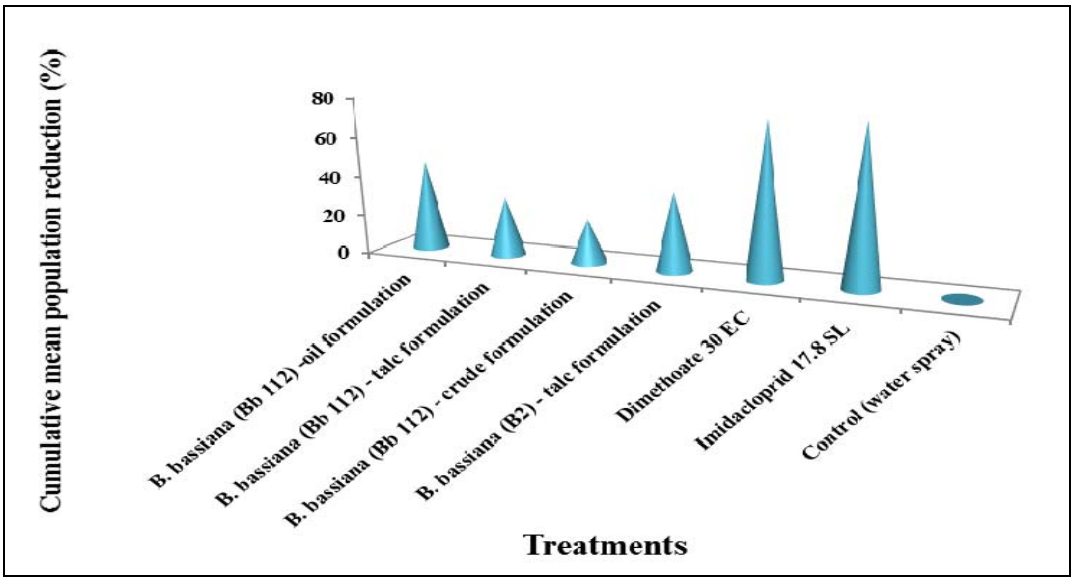

Fig 1: Efficacy of different formulations of *B. bassiana* (Bb 112) against *B. tabaci* on tomato

4. Conclusion

Environmental safety and ecosystem stability considerations lead to the conclusion that the use of native isolates in a microbial control program is more convenient (Lockwood 1993) [10]. Also, mycoinsecticides may be most effective in pest managements programmes integrating beneficial arthropods, or in greenhouse crops where favourable environmental conditions (high humidity and low UV exposure) can be manipulated (Jacobson *et al.,* 2001) [7], (Down *et al.,* 2009) [3]. The oil based formulation used in the present study made from the indigenous fungal strain Bb 112 maintained at the Department of Agricultural Entomology, was proved very effective against tomato whitefly and hence, it can fit very well with the integrated pest management programmes. However, to make it as a holistic component in IPM programme on vegetables, additional research to optimize its use against other sucking pests needs to be carried out.

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6. References

1. Benuzzi M, Santopolo F. Naturalis: a bioinsecticide based on *Beauveria bassiana*. Informatore Fitopatologico (Italy). 2001; 51(4):61-64.
2. Daoust RA, Ward MG, Roberts DW. Effect of formulation on the viability of *Metarhizium anisopliae* conidia. Journal of Invertebrate Pathology. 1983; 41:151-160.
3. Down RE, Cuthbertson AGS, Mathers JJ, Walters KFA. Dissemination of the entomopathogenic fungi, *Lecanicillium longisporum* and *L. muscarium*, by the predatory bug, *Orius laevigatus* to provide concurrent control of *Myzus persicae*, *Frankliniella occidentalis* and *Bemisia tabaci*. Biological Control. 2009; 50:172-178.
4. Fargues J, Maniania NK, Delmas JC, Smits N. Influence de la temperature sur la croissance *in vitro* dhyphomycetes entomopathogenes. Agronomie. 1992; 12:557-564.
5. Hemalatha S. Management of thrips infesting chilli and tomato with entomopathogenic fungi and validation of delivery equipments. Ph. D. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India. 2015, 196.
6. Herrera F, Carballo M, Shannon P. Efficacy of native entomopathogenic fungal strains against *Bemisia tabaci* in the laboratory. Integrated Pest Management. 1999; 54:37-43.
7. Jacobson RJ, Chandler D, Fenlon J, Russell KM. Compatibility of *Beauveria bassiana* Balsamo Vuillemin with *Amblyseius cucumeris* Oudemans Acarina: Phytoseiidae to control *Frankliniella occidentalis* Pergande Thysanoptera: Thripidae on cucumber plants. Biocontrol Science and Technology. 2001; 11:391-400.
8. Jeyarajan R, Ramakrishnan G, Dinakaran D, Sridar R. Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot diseases. In Biotechnology in India Ed Dwivedi B.K Bioved Research society, Allahabad. 1994, 25-36.
9. Jones K, Burges HD. Technology of formulation and application. In: Formulation of microbial biopesticides: Beneficial microorganisms, nematodes and seed treatments. Ed. HD Burges, Kluwer Academic Publishers, Dordrecht, Netherlands. 1998, 412.
10. Lockwood JA. Environmental issues involved in biological control of rangeland grasshopper with exotic agents. Environmental Entomology. 1993; 22:5503-5518.

11. Lomer CJ, Prior C, Kooyman C. Development of *Metarhizium* sp. for the control of grasshoppers and locusts. In: Microbial control of Grasshoppers and Locusts. (Eds.,) MS. Goettel, DL. Johnson, Memoirs of the Entomological society of Canada. 1997, 265-286.
12. Manjula C, Nagaraju, Muniyappa V. Evaluation of different oil formulations of bioagent, *Beauveria bassiana* against different life stage of *Bemisia tabaci*, the vector of tomato leaf curl virus. Plant Disease Research. 2003; 18(1):25-28.
13. Moraga EQ, Maranhao EAA, Garcia PV, Alvarez SC. Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity. Biological Control. 2006; 36:274-287.
14. Negasi A, Parker BL, Brownbridge M. Screening and bioassay of entomopathogenic fungi for the control of silver leaf whitefly, *Bemisia argentifolii*. Insect Science and its Applications. 1998; 18(1):37-44.
15. Ramanujam B. Microbial control of crop pests using entomopathogenic fungi.
    In: NS. Rao, RJ Rabindra (ed.), Training programme on emerging trends in biological control. Project Directorate of Biological Control, Bangalore. 2004, 320.
16. Saikia AK, Muniyappa V. Epidemology and control of tomato leaf curl virus in Southern India. Tropical Agriculture. 1989; 66:350-354.
17. Sangamithra S. Investigations on the entomopathogenic fungal formulations for the management of onion thrips, *Thrips tabaci* Lindemann Thripidae: Thysanoptera. Ph.D. Ag. Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 2015.
18. Saranya S, Ramaraju K, Jeyarani S. Pathogenicity of entomopathogenic fungi to two spotted spider mite, *Tetranychus urticae* Koch Acari: Tetranychidae. Biopesticides International. 2013; 9(2):127-131.
19. Vicentini S, Faria M, Oliveira MRV. Screening of *Beauveria bassiana* Deuteromycotina: Hyphomycetes isolates against nymphs of *Bemisia tabaci* biotype B Hemiptera: Aleyrodidae with description of a new bioassay method. Neotropical Entomology. 2001; 30:97-103.