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OIL BASED FORMULATION OF *BEAUVERIA BASSIANA* (BB 112) AGAINST ONION THRIPS *THRIPS TABACI* LINDEMAN

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ABSTRACT

Successful usage of entomopathogenic fungi for pest control under field conditions includes delivery techniques, infectivity and persistence of their inoculum in the environment. Pathogenicity tests performed with oil-based formulation of *Beauveria bassiana* (Bals.) Vuill (Bb 112) against onion thrips *Thrips tabaci* Lindeman revealed maximum virulence with the least LC_{50} and LT_{50} values of 1.25 x 10^5 spores/ ml and 76.11 hr, respectively. Microplot trials showed oil-based formulation of Bb 112 was effective against *T. tabaci* when sprayed with Controlled Droplet Applicator (CDA) sprayer giving maximum cumulative reduction of 54.04%. Field experiments revealed oil-based formulation of Bb 112 @ 10^8 spores ml/ l applied with CDA sprayer led to maximum cumulative reduction in thrips incidence (44.69 and 41.01%, respectively), at Kumarapalayam village, Coimbatore and Ambilikkai village, Dindigul districts. In addition, maximum onion yield of 14.66 and 16.23 t/ ha was observed at Kumarapalayam and Ambilikkai, respectively. Mycoinsecticides applied with the CDA sprayer can be incorporated in management of *T. tabaci.*

Key words: Onion, *Thrips tabaci*, *Beauveria bassiana*, oil-based formulation, Bb 112, pathogenicity, delivery techniques, infectivity, persistence, CDA sprayer, LC_{50}, $LT_{50,}$ bulb yield

Onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a polyphagous pest of onion (*Allium cepa* L.) causing extensive economic losses in greenhouse and open-field (Diaz-Montano et al., 2011; Reitz et al., 2011). Damage to onion is caused by adults and nymphs in green plant tissues (Trdan et al., 2005), with damaged areas become desiccated causing a silvery flecked appearance and bulbs become undersized (Diaz-Montano et al., 2011). In addition to direct quantitative and qualitative damage caused by its feeding *T. tabaci* acts as a vector of Iris yellow spot virus (Family: Bunyaviridae; Genus: Tospoviruses, IYSV), which can reduce bulb size (Gent et al., 2004). Failure to control this pest by timely and effective means causes yield loss up to 50%. Use of insecticides to manage thrips is difficult owing to the cryptic nature of the insect. Effective alternatives could include economical and ecofriendly microbial agents. Biological control with entomopathogenic fungi (EPF) has potential for thrips management in developed countries (Arthurs et al., 2013). The EPF *Beauveria bassiana* (Bals.) Vuill. is a biopesticide for use in IPM because of its host specificity with proven safety (Bateman et al., 1993). Successful use of EPF as microbial control agents of thrips depends on strain virulence, an appropriate delivery system and timing of application. Keeping this in view, in this study the pathogenicity of oil-based formulation of *B. bassiana* (Bb 112) against *T. tabaci* was evaluated with identifying the best equipment to effectively deliver the EPF.

MATERIALS AND METHODS

The basal inoculum of *T. tabaci* was collected from onion fields and maintained on 25 to 30 days old healthy onion plants (var. Co 1) at the Insectary Unit of the Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore. Thrips from base cultures were used to carry out experiments. Pure culture of the fungal isolate, *B. bassiana* (Bb 112) was obtained from the Department of Agricultural Entomology and was maintained as follows: The isolate was cultured in petridishes (9 cm dia) containing Sabouraud's Maltose Agar enriched with 1% yeast extract (SMAY) solid medium and incubated at 25± 2ºC for 10 to 14 days. After complete sporulation, spores were scraped from the surface of SMAY plates and suspended in 20 ml sterile distilled water containing 0.05% Tween 80®. The conidial suspension was vortexed for 5 min to produce a homogenous suspension, then prepared and used to produce spores by diphasic liquid-solid fermentation method for the preparation of formulation. 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 (Lomer et al., 1997).

Oil based formulation of *B. bassiana* (Bb 112) was prepared as per the protocol developed by Sangamithra (2015). Oil based formulation was prepared by dissolving 1 g of pure conidia (10^{10} conidia g^{-1}) of Bb 112 in 100 ml of light paraffin oil, along with adjuvants to enhance the efficacy of the formulation. The prepared formulation was stored under ambient temperature (28± 2°C). The pathogenicity assay of the oil based formulation was carried out once in fortnight using 15 days old onion seedlings (var. Co 1) raised in small plastic cups (9 x 8 cm) @ one seedling per cup. The seedlings were artificially inoculated with twenty numbers of thrips in each plant. Fortnight after inoculation, observations on number of thrips per plant was recorded before imposing treatment. Five spore concentrations ($1x10^8$ to $1x10^4$ spores ml^{-1}) of oil formulation of *B. bassiana* (Bb 112) was prepared and ten ml of respective concentrations were sprayed on the onion seedlings infested with thrips using glass atomizer. Plants sprayed with 0.05% Tween 80® served as control. After spraying, the post treatment counts were taken at 24 hours interval up to 7 days. The percent mortality of the larvae was calculated using Abbott's formula (Abbott's 1925). Statistical analysis for the concentration and time mortality responses of oil formulation of *B. bassiana* (Bb 112) against *T. tabaci* were subjected to probit analysis (Finney, 1971).

The microplot trial was conducted at Insectary in a red loamy soil. Ridges and furrows were made at 45 cm spacing. Onion bulbs, var. Co.1 were directly planted to the field on both sides of the ridges 10 cm apart at Kumarapalayam, Coimbatore, in a sandy loam soil and var. Co (on) 5 was planted in a field at Ambilikkai, Dindigul, in a red loamy soil with a spacing of 20 × 12 cm. (Anonymous, 2013).The oil based formulation of Bb 112 at 10^8 spores ml^{-1} was delivered through : T1, ASPEE Maruyama engine sprayer (capacity: 20 l, nozzle: hollow cone, droplet size: 225.66 µm); T2, Avenger ULV sprayer (capacity: 4 l, nozzle: spinning disc, droplet size: 111.15 µm); T3, ASPEE battery sprayer (capacity:16 l, nozzle: flood jet, droplet size: 203.70 µm); T4, ASPEE knapsack hand sprayer (capacity: 16 l, nozzle: hollow cone, droplet size: 287.49 µm); T5, ASPEE Hitech hand sprayer (capacity: 16 l, nozzle: hollow cone, droplet size: 283.80 µm) (ASPEE sprayers from Bhuvana Enterprises, Coimbatore, Tamil Nadu) and T6, Controlled droplet applicator (CDA) sprayer (capacity: 0.5 l, nozzle: spinning disc, droplet size: 108.40 µm) (Bhuvana Enterprises, Coimbatore) and compared to T7, a talc based formulation of *B. bassiana* (B2) at 10^8 spores ml^{-1} (Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore), the insecticide checks, T8, imidacloprid 17.8 SL at 0.5 ml/ l and T9, dimethoate 30 EC at 0.7 ml/ l; or T10, water only control were applied with a knapsack hand sprayer. The experiments were carried out from January to March, 2016 in microplots at insectary and field experiments at Kumarapalayam and Ambilikkai were conducted from March to May, 2016 in a randomized block design with a plot size of 2.5x 2.5 m for microplots and 4x 5 m for field trials. Treatments were replicated thrice. The first treatment was imposed when thrips were first observed. Two rounds of treatments were imposed at two weeks interval. Pre- and post-treatment counts on thrips incidence (nymphs and adults) were made on 0 (day of treatment), 3, 7, 10 and 14 days after application from 5 plants selected at random in each plot for number of thrips/ plant. The thrips were counted using a 10x hand lens. Yield data was recorded for onion in field trials, with thrips incidence and % reduction in over the control computed from microplot and field experiments subjected to square root (x+0.5) and arc sine transformation. ANOVA and means separated by least significant difference were carried out in Statistical Package for Social Sciences (ver.16, SPSS, Inc., Chicago, IL). Data on total yield were analysed using AGRES (SPSS, Inc.).

RESULTS AND DISCUSSION

The effect of fungal biocontrol agents against target pests are usually estimated by median lethal concentrations (LC_{50}) and time (LT_{50}). Such an effect, however is part of the full potential of a fungal candidate and could be more implicative of the persistency in the field (Negasi et al., 1998; Shi and Feng, 2009). The pathogenicity assays carried out at 15 days interval revealed significant differences in the LC_{50} values with 1.20 to 10.60 x 10^5 spores ml^{-1} from the first fortnight. At the concentration of 10^8 spores ml^{-1}, the LT_{50} value was 76.11 hr on first and it gradually increased over time and reached the maximum of 138.49 hr on 180 DAS (12^{th} fortnight) (Table 1). Investigation on the thrips cadavers confirmed mycosis as the cause of death. Thrips infected by fungi were mummified and brittle. Mycelial growth developed after 24 to 48 hr of death. Initially, growth of the fungus was inconspicuous

through the intersegmental membrane of abdomen and legs and finally the entire cadaver was fully covered with fungal growth.

Limited information is available on the pathogenicity of *B. bassiana* against *T. tabaci*. Ekesi et al. (1998) reported a LC_{50} value of 7.9 x 10^6 spores ml^{-1} for *B. bassiana* strain against legume flower thrips, *Megalurothrips sjostedti* (Trybom) which is in close agreement with the present results. Ugine et al. (2005) also reported that the *B. bassiana* (strain GHA) was highly effective to western flower thrips *Frankliniella occidentalis* (Pergande) at very low doses (LD_{50} of 33 to 66 conidia/ insect). Similarly, Sabbour and Abbas (2007) also reported the lowest LC_{50} value of 110 spores ml^{-1} for *B. bassiana* against *T. tabaci*. Annamalai (2010) reported the LC_{50} and LT_{50} values as 1.23 x 10^7 spores $ml^{-1}$and 140.08 hr at 1x 10^8 spores ml^{-1} of *B. bassiana*, respectively against *T. tabaci*. Hemalatha et al. (2014) reported that Bb 111 isolate of *B. bassiana* was highly virulent against *T. tabaci* with an LC_{50} of 1.6x 10^5 spores ml^{-1} and LT_{50} of 104.91 hr on tomato. Sangamithra (2015) reported the potential of *B. bassiana* (Bb 101) against *T. tabaci* with a LC_{50} value of 1.01x 10^6 spores ml^{-1} and LT_{50} of 94.43 hr. Present investigation revealed the efficacy of oil-based formulation of *B. bassiana* (Bb 112) against onion thrips which makes it as a promising candidate for thrips management. For IPM, selection of right plant protection methods coupled with right appliance is important to tackle the target pest in an effective manner (Gowda et al., 2005). In this context, it is important to carry out field trials to evaluate the efficacy of the treatments that were best performing under laboratory (controlled) conditions.

Table 1. Dose and time mortality response of oil-based formulation of *B. bassiana* (Bb 112) against *Thrips tabaci*

Bioassay interval (in days)	Heterogeneity ($\chi 2$)*	Regression equation	LC_{50} (x 10^5 spores ml^{-1})	95% Fiducial limits ($\times 10^5 ml^{-1}$)
		Dose mortality		
0	5.36	Y=0.620x+1.839	1.20	0.78-1.83
15	6.23	Y=0.690x+1.358	1.99	1.36-2.92
30	3.72	Y=0.604x+1.778	2.17	1.43-3.30
45	3.90	Y=0.634x+1.603	2.36	1.56-3.57
60	4.16	Y=0.586x+1.825	2.67	1.72-4.14
75	3.33	Y=0.464x+2.443	3.05	1.83-5.07
90	3.96	Y=0.411x+2.688	3.93	2.26-6.81
105	3.39	Y=0.628x+1.466	4.42	3.01-6.48
120	3.75	Y=0.395x+2.747	4.64	2.63-8.20
135	3.68	Y=0.415x+2.603	5.56	3.23-9.56
150	4.29	Y=0.392x+2.717	6.25	3.53-11.09
165	5.95	Y=0.374x+2.799	7.47	4.10-13.60
180	7.66	Y=0.296x+3.204	10.60	5.08-22.08
		Time mortality		
Bioassay interval (in days)	Heterogeneity ($\chi 2$)*	Regression equation	LT_{50} # (h)	95% Fiducial limits (h)
0	3.30	Y=3.390x-1.393	76.11	67.70-85.57
15	2.31	Y=3.653x-1.931	79.10	71.60-87.39
30	3.80	Y=3.887x-2.438	81.80	73.84-90.61
45	3.31	Y=3.859x-2.398	82.42	74.31-91.41
60	2.88	Y=3.661x-2.084	86.03	76.72-96.46
75	2.53	Y=3.811x-2.461	90.89	82.23-100.46
90	2.84	Y=2.734x-0.394	93.15	80.99-107.13
105	3.54	Y=2.247x+0.542	94.87	79.77-112.83
120	3.29	Y=1.913x+1.153	101.63	81.40-126.90
135	3.31	Y=3.060x-1.233	111.71	93.16-133.94
150	3.56	Y=2.734x-0.394	127.19	91.49-176.81
165	5.23	Y=1.695x+1.392	129.33	95.01-176.04
180	2.54	Y=1.572x+1.617	138.49	95.87-200.04

*All lines significantly good fit @ $p \leq 0.05$; # LT_{50} at maximum of 10^8 spores ml^{-1}

Microplot experiments against *T. tabaci* revealed that the oil based formulation of *B. bassiana* (Bb 112) @ 10^8 spores ml^{-1} sprayed with controlled droplet applicator (CDA) was significantly superior with a cumulative mean reduction of 54.04% in *T. tabaci*. Two field experiments one each at Kumarapalayam and Ambilikkai villages were conducted. In both the experiments, oil-based formulation of *B. bassiana* (Bb 112) @ 10^8 spores ml^{-1} sprayed with controlled droplet applicator (CDA) was significantly superior (44.69 and 41.01% reduction, respectively) after two rounds of spraying (Table 2). These observations agree with those

Table 2. Efficacy of oil-based formulation of *B. bassiana* (Bb 112) against *T. tabaci* on onion (var. Co 1) with different delivery equipment- microplot and field

Treatment	Delivery system	Application number	Microplot		Trial I -Kumarapalayam		Trial II - Ambilikkai	
			No. thrips/ leaf[a]	% Reduction[b]	No.thrips/ plant[a]	% Reduction[b]	No. of thrips/ leaf[a]	% Reduction[b]
Oil formulation of *B. bassiana* (Bb 112; 10^8 spores mL^{-1}) @ 4 ml/ l	Aspee Maruyama engine sprayer	1	5.79 (2.40)c[a]	35.16	8.63 (2.93)[de]	33.36	10.48 (3.23)[c]	31.39
		2	5.31 (2.30)[d]	48.29	8.02 (2.83)[cd]	43.12	10.70 (3.27)[de]	40.90
	Avenger ULV sprayer	1	5.72 (2.39)[c]	35.94	8.11 (2.84)[d]	37.37	9.84 (3.13)[bc]	35.60
		2	5.49 (2.34)[d]	46.54	7.03 (2.65)[c]	47.44	10.35 (3.21)[cd]	42.49
	Aspee battery sprayer	1	7.41 (2.72)[e]	17.02	8.57 (2.92)[de]	24.62	10.78 (3.28)[e]	29.45
		2	6.77 (2.60)[f]	34.07	8.26 (2.87)[d]	41.27	11.23 (3.35)[e]	37.60
	Aspee Knapsack hand sprayer	1	6.42 (2.53)[d]	28.10	8.45 (2.90)[de]	25.68	10.62 (3.25)[d]	30.53
		2	6.14 (2.47)[e]	40.21	7.94 (2.81)[cd]	43.47	10.96 (3.31)[e]	39.10
	Aspee hitech hand sprayer	1	7.06 (2.65)[e]	20.94	8.50 (2.91)[de]	25.24	10.71 (3.27)[e]	29.92
		2	7.05 (2.65)[f]	31.35	8.02 (2.83)[cd]	42.83	11.00 (3.31)[e]	38.86
	CDA sprayer	1	4.88 (2.20)[b]	45.63	7.41 (2.72)[c]	42.78	9.52 (3.08)[b]	37.72
		2	4.59 (2.14)[c]	55.28	6.75 (2.59)[c]	52.12	10.10 (3.17)[bc]	43.83
Talc based formulation of *B. bassiana* (B2) @ 5g/ l	Knapsack hand sprayer	1	7.49 (2.73)[e]	16.12	8.83 (2.97)[e]	22.34	11.64 (3.41)[f]	23.84
		2	7.41 (2.71)[g]	27.84	8.75 (2.95)[d]	38.53	11.98 (3.60)[f]	27.83
Imidacloprid 17.8 S @ 0.5 ml/ l	Knapsack hand sprayer	1	3.44 (1.85)[a]	65.14	3.67 (1.91)[a]	67.66	5.28 (2.29)[a]	65.42
		2	2.59 (1.61)[a]	75.90	2.57 (1.60)[a]	81.90	3.32 (1.82)[a]	81.55
Dimethoate 20 EC @ 0.7 ml/ l	Knapsack hand sprayer	1	3.89 (1.97)[b]	60.61	5.14 (2.26)[b]	60.25	5.97 (2.44)[a]	60.89
		2	2.75 (1.65)[b]	74.74	3.53 (1.87)[b]	72.83	3.91 (1.97)[a]	78.23
Control (water)	Knapsack hand sprayer	1	8.93 (2.98)[f]	-	11.37 (3.37)[f]	-	15.28 (3.90)[g]	-
		2	10.27 (3.20)[h]	-	14.24 (3.77)[e]	-	17.99 (4.24)[g]	-

Data analysed with least square means and means separated with least significant difference, $p<0.05$; [a] values in column followed by the same letter not significantly different; values in parentheses square root (x + 0.5) transformed; [b] % Reduction = % reduction calculated over control using (No. of thrips in control- No. thrips in treatment)/ No. thrips in control x 100; "-" Indicates there will be no value for control.

of Singh et al. (2011) with *B. bassiana* and Vishalakshy and Krishnamoorthy (2012) with *M. anisopliae* against *T. tabaci*. The potential entomopathogenic fungi promote plant growth and improves yield (Dara, 2013). In onion, oil-based formulation of *B. bassiana* (Bb 112) @ 10^8 spores ml^{-1} sprayed with CDA sprayer against *T. tabaci* recorded the highest bulb yield of (var. co 1) 14.66 and (var. co (on) 5) 16.23 t ha^1 in Kumarapalayam and Ambilikkai villages with a yield increase of 22.68 and 23.29%, respectively (Table 3). This observation is in accordance with that of Singh et al. (2011). Visalakshy and Krishnamoorthy (2012) also reported the superior performance of oil-based formulation of *M. anisopliae* @ $1x10^9$ spores ml^{-1}. The present findings are also in accordance with Shiberu et al. (2013) who reported that *B. bassiana* (PPRC-6) and *M. anisopliae* sprayed against *T. tabaci*.

Higher efficacy of oil- based formulations of *B. bassiana* (Bb 112) in the present study might be due to oil that could coat the dry, dusty type of conidia allowing them to suspend easily in oil and spread rapidly over the surface of leaves which helps better contact of conidia with insect cuticle. The variation in virulence might be attributed to the number of conidia received by the individual pest (Bateman et al., 1993). Being non-evaporative, oil formulation of mycoinsecticides is readily compatible with ultra-low volume (ULV) application techniques for spraying at low relative humidity (Bateman, 1997). According to Bateman and Alves (2000), CDA represents a very specialised delivery system for oil formulations which can only be used with specialised application equipment (often rotary atomisers). In laboratory conditions, thrips acts as good host for fungal pathogens, since these are generally soft bodied and inhabit environments with humid microclimates which favours infection and disease transmission (Hajek and Ledger, 1994). Whereas the field performance of fungal candidates was dependent on both virulence to target pests and adaptation to field condition (Luz and Fargues, 1997). Multiple applications of microbial pesticides may improve the ability of infectious inoculum, thereby providing efficient control of target pests. Nevertheless, in a single spray all the population may not be encountered by *B. bassiana* and is dependent on persistence of conidia

Table 3. Effect of oil-based formulation of *B. bassiana* (Bb 112) applied with different delivery equipment on onion bulb yield

Treatments	Delivery equipments	Trial I (Location: Kumarapalyam) – (var. Co 1)		Trial II (Location: Ambilikkai) – (var. Co (on)5)	
		Bulb yield t ha^{-1}	% increase over control	Bulb yield t ha^{-1}	% increase over control
Oil formulation of *B. bassiana* (Bb 112) @ 10^8 spores ml^{-1}	Aspee Maruyama engine sprayer	13.82^{bc}	15.65	14.21^{de}	12.38
	Avenger ULV sprayer	13.90^{b}	16.32	14.44^{cd}	13.78
	Aspee battery sprayer	12.89^{de}	7.87	13.66^{f}	8..85
	Aspee Knapsack hand sprayer	13.23^{cd}	10.71	14.02^{efg}	11.19
	Aspee hitech hand sprayer	13.21^{cd}	10.54	13.98^{efg}	10.94
	CDA sprayer	14.66^{b}	22.68	16.23^{c}	23.29
Talc based formulation of *B. bassiana* (B2) @ 5g/ lit (10^8 spores ml^{-1})	Knapsack hand sprayer	12.78^{de}	6.95	13.01^{g}	4.30
Imidacloprid 17.8 SL @ 0.5ml/ lit	Knapsack hand sprayer	15.32^{a}	28.20	18.01^{a}	30.87
Dimethoate 30 EC @ 0.7 ml lit^{-1}	Knapsack hand sprayer	15.21^{a}	27.28	16.24^{b}	23.33
Control (water spray)	-	11.95^{e}	-	12.45^{h}	-

In a column means followed by a common letter (s) not significantly different (p = 0.05, LSD)

on foliage, probably because of the limited ability of individual thrips to acquire secondary conidia from the treated surface (Gatarayiha et al., 2011). Thus, the effective control by fungus is largely attributed to the favorable environmental condition. Hence, the repeated applications of *B. bassiana* with the right formulation could directly target new emerging adults, thereby providing a better control.

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