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Assessing the bioefficacy of entomopathogenic fungi bio formulations against whitefly (Genadius) on cotton under screen house conditions

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Abstract : Polyphagous whiteflies are the most invasive economically important pest that cause serious damage in various ornamental and vegetable crops globally. The damage potential of this pest may owe to its higher fecundity, polyphagous nature, resistance to chemical insecticides. Some alternative measures are required to combat this devastating pest. Biopesticides serves as environment friendly, biodegradable, target specific alternative to insecticides. There is an urgent need for the identification of virulent strains of entomopathogenic fungi (EPF) which could be used as an important component of an IPM program for the management of *B. tabaci*.

In this study the efficacy of different commercial, native and procured entomopathogenic fungi along with 1% Diafenthouron has been evaluated against whitefly under screen house conditions. The result revealed that bioformulation of L. *lecanii* MTCC 956 at dose 12g/ltr and 10g/ltr recorded maximum percent reduction of whitefly population over control. According to the outcomes it is suggested that formulation of *L. lecanii* is most virulent against *B. tabaci* and could be employed as a significant component of integrated pest management program.

Key words: Biopesticides, EPF, *L. lecanii*, polyphagus, whiteflies

Biological control has been emerged as a environmental friendly and sustainable method for the control of arthropod pests that poses major economic loss to agricultural crops. Among the arthropod pests *B.tabaci* has emerged as most devastating pests in recent years causing major yield losses to cotton crop. The whitefly is highly polyphagous capable of infesting more than 900 cultivated crops across the globe and it also serve as vector of plant viruses (Navas-Castillo *et al.*, 2011). In 2015-2016, this pest cause severe outbreak northern cotton-growing zone which caused 50–60 per cent yield loss in cotton (Singh *et al.*, 2016). It causes indirect damage to crop by transmitting cotton leaf curl disease (CLCuD). The control of this notorious pest rely on chemical insecticides but unfortunately it developed tremendous resistance to insecticides (Naveen *et al.*, 2017). Till date, almost 40 active ingredients of insecticides have been shown to be ineffective for control of whiteflies. Indeed, the repetitive usage of same compounds and application of insecticides at higher doses within a given cropping season are the main contributing factors for pest resurgence and insecticide resistance development in case of whiteflies (Kranthi *et al.*, 2001). Due to insecticide resistance, biological control with entomopathogenic fungi is gaining impetus in insect pest management (Shahid *et al.*, 2012). Sustainability of cotton production can be maintained by exploring some novel approaches to combat this invasive pest.

Entomopathogenic fungi (EPF) have been recognized as the natural enemies of the insect population. Entomogenous fungi infect insects and lead to epidemic disease in proper conditions. Many of fungal entomopathogens have been developed as mycoinsecticide. The fungal agents with advantages of environmental friendly and lack of insect resistance along with higher persistence are considered as an alternative means for the control of sucking pests

thus attracting more public and scientific interests. Species of EPF from several genera have been demonstrated to cause natural mortality of the *B. tabaci* population, with more than 20 species identified to be effective against this insect. Species such as *Beauveria bassiana*, Metarhizium anisopliae, Isaria fumosoroseus, *Ashersonia* spp., and *Verticillium lecanii* are the most common EPF with potentials as biocontrol agents for *B. tabaci* (Chen *et al.*, 2015; Lacey *et al.*, 2015; Sani *et al.*, 2020). These mycoinsectides control whiteflies by nutritional deficiency, release of toxins and secreting cuticle degrading enzymes (Butt *et al.*, 2016; Gebramariam *et al.*, 2022; Pedrini 2018). The infection process of EPF are mediated by direct invasion of insect cuticle mediated by two types adhesion proteins hydrophobins and adhesion factors (MAD1 and MAD2) which allow close adhesion of fungal spore with insect body using hydrophobic and electrostatic forces (Skinner *et al.*, 2013). Spore germination led to emergence of appresoria and production of lytic enzymes (lipases, chitinase and proteinase enzyme that led to mechanical disruption of insect cuticle (Pedrini *et al.*, 2007; Pedrini 2018, Silva *et al.*, 2009, Santi *et al.*, 2010). Complete invasion of host tissue is mediated by production of secondary metabolites that causes paralysis and disruption of the host's immune response which led to mechanical damage and nutrition depletion (Donzelli *et al.*, 2016; Fan *et al.*,2017). Progression of infection led to stiffness in insect's body due to fluid absorption by the fungus and ultimately cause killing of the insect. Success of biological control of insect pests will largely dependent upon the screening of fungal strains is (virulence and persistence). As the number of target pests shown to be susceptible to EPFs has continued to increase and their employment in pest management strategies require the selection of fungal strains with high virulence against target pests. Hence, present study is being conducted for evaluating the potential EPF against whiteflies in order to successfully adopt them in field conditions and to further validate them as significant component of IPM technology for combating deadly pest *B. tabaci.*

MATERIALS AND METHODS

The experiment was laid out on the Regional Research Station PAU Faridkot. The cotton *G.hirsutum* cotton cultivar RST9 was sown on having plot size of 4.5 x 4.5 m with 90 x 90 cm spacing in randomized block design with six treatments replicated four times.

Strains of the entomopathogenic fungi and their sources (Table 1).

Two native and two standard isolates procured from different sources along with three commercial formulation isolate were evaluated for the pathogencity against adults of *B. tabaci. Bioformulations* of two procured *Lecanicillium lecanii* isolates *viz.*, *L. lecanii* MTCC 956 and *L. lecanii* NIPHM, two native *B. bassianai* solates B1 and B5, three commercial formulation *B. bassiana, M. anisopliaeand L. lecanii* along with chemical control Diafenthiuron 50WP and untreated control were evaluated against adults *B. tabaciin* cotton under screen house conditions at Entomological Research Farm, PAU, Ludhiana during the year 2018. Viability of spores and bioassay was tested at different concentration *i.e.* 8g/ltr, 10g/ltr and 12g/ltr. Native and procured isolates were grown and maintained on potato dextrose agar (PDA) comprising 2 per cent dextrose, 20 per cent potatoes infusion form, 5 per cent agar and 0.5 per cent chloramphenicol and stored at refrigeration temperature till further use.

Green house house evaluation of entomopathgenic fungi

The experiment was carried out in net house condition. The earthen pots each with *Bt-cotton* plant approximately 50-60 days old carrying 90-100 adult white flies were used for the biopesticides to be tested were sprayed with a

hand sprayer covering both sides of leaves. The experiment was laid out in randomized block design (RBD) with each concentration of birational as one treatment and three replications were kept for all the treatments. Apart from these biorational treatments, one untreated check was also maintained to compare the efficacy of treatments against whitefly adults. Two sprays were done at ten days interval. The observations were recorded after 3, 7, 10 days of application. The pre population record from three leaves (top, middle and bottom) was also being taken before every spray. The whitefly populations were recorded from the randomly selected 3 leaves from top, middle, and bottom canopy of randomly selected plant before treatment and after 3, 7, and 10 days of spray, respectively. To compare the efficacy of different EPF, percent reduction in the population of the whitefly over control was calculated, using (Henderson and Tilton's 1955) formula

$$\text{Corrected mortality (\%)} = 100\left\{1 - \frac{Ta \times Cb}{Tb \times Ca}\right\}$$

Here *Tb* is the number of whiteflies per plant before treatment; Ta is whiteflies/plant after treatment; *Cb* is the number of whiteflies/plant from the control before treatment, and *Ca* is whiteflies/plant from the control after-treatment.

Statistical analysis: The data were subjected to arc sine transformation and analyzed statistically for comparing treatments following analysis of variance (ANOVA) and mean values were compared by Duncan multiple range test (DMRT) and results were interpreted at 5 per cent level of significance (SPSS 2015).

RESULTS AND DISCUSSION

The data was recorded after whitefly infestation on cotton plant under glasshouse conditions which showed significant decrease of whitefly population in all treatments when compared to untreated control where the population remained high. Dose dependent mortality was observed after evaluating different EPF at different doses. *Bioformulation* of *L. lecanii* MTCC 956 at 12ml/ltr recorded minimum whitefly population (47.0 adults/plant) and was *at par* with chemical control (43.67adults /plant) after ten days of first spray. This was followed by *L. lecanii* MTCC 956 at 10 and 8ml/ltr and *L. lecanii* commercial formulation at 12ml/ltr which recorded 50.33, 53.00 and 51.00 whitefly adults/plants, respectively, and were *at par* with each other and with *L. lecanii* MTCC 956 at 12ml/liter (Table 2). Whitefly adult population in treatment *L. lecanii* MTCC 956 at 12ml/ltr recorded 59.67, 54.0 and 47.0 adults/plant with

Table 1. List of biopesticides tested against *B. tabaci*

Sr. No.	Treatment	Dose (ml/liter)	Trade Name	Source
1.	*Beaveria bassiana* (1.0%) ($1X10^8$ cfu/ml)	8,10 and 12ml/liter	Daman	International Panaacea limited, New Delhi
2.	*Metarhizium anisopliae* (1.0%) ($1X10^8$ cfu/ml)	8,10 and 12ml/ltr	Kalichakra	International Panaacea limited, New Delhi
3.	*Lecanicillium lecanii* (1.50%) ($1X10^8$ cfu/ml)	8,10 and 12/ltr	Biocatch	T. stanes and company limited, Tamilnadu
4.	*Lecanicillium lecanii* MTCC956 ($1X10^8$ cfu/ml)	8,10 and 12ml/ltr	-	Institute of Microbial technology (IMTECH), Chandigarh
5.	*Lecanicillium lecanii* N1PHM ($1X10^8$ cfu/ml)	8,10 and 12ml/ltr	-	National Institute of Plant Health Management (NIHPM), Hydrabad
6.	Native *Beauveria bassiana* B1($1X10^8$ cfu/ml)	8,10 and 12ml/ltr	-	Biocontrol Lab
7.	Native Beauveria bassiana B5 ($1X10^8$ cfu/ml)	8,10 and 12ml/ltr	-	Biocontrol Lab
8.	Diafenthouron @50 WP	8,10 and 12ml/ltr	Polo	Syngenta India limited

Table 2. Number adults of *Bemisiatabacion* cotton under screen house conditions after

Treatments	Number of adult whiteflies /3leaves /plant*							
	Dose (g or ml/ltr)	Pretreatment	I Spray			II Spray		
			3DAS	7DAS	10DAS	3DAS	7DAS	10DAS
B. *bassiana*	8	80.30	72.33^{efgh}	67.67^{fghi}	62.67^{ghij}	59.00^{ghij}	52.00^{ghij}	48.00^{ghi}
Commercial	10	80.00	70.33^{defg}	66.33^{efg}	59.67^{fghi}	55.33^{efgh}	49.67^{fghi}	43.00^{efg}
formulation	12	78.00	70.00^{cdefg}	64.00^{efg}	55.67^{cdef}	52.00^{cdef}	44.33^{cdef}	40.00^{cdef}
($1x10^8$ cfu/ml)								
M. anisopliae	8	82.00	79.33^{hi}	73.67^{i}	67.33^{j}	66.33^{ij}	59.00^{kl}	55.00^{jk}
Commercial	10	82.30	$77.33g^{hi}$	71.33^{hi}	65.00^{ij}	63.67^{ij}	57.00^{ij}	52.00^{ijk}
formulation	12	80.00	$78.00g^{hi}$	68.33^{ghi}	63.00^{ghij}	62.00^{ijk}	53.67^{hijk}	49.67^{hij}
($1x10^8$ cfu/ml)								
L. lecanii	8	80.70	72.67^{efgh}	62.00^{cdef}	57.00^{defg}	55.33^{efgh}	51.00^{ghij}	45.33^{fgh}
Commercial	10	83.30	70.67^{defg}	59.33^{bcde}	54.00^{cdef}	50.33^{cdef}	47.33^{defg}	41.67^{defg}
formulation	12	80.30	67.67^{bcdef}	56.67^{abc}	51.00^{bcd}	46.67^{bc}	41.00^{bc}	35.33^{bc}
($1x10^8$ cfu/ml)								
B. bassiana -B1	8	80.30	73.33^{efgh}	71.33^{hi}	67.67^{j}	63.67^{ij}	62.33^{l}	57.33^{k}
($1x10^8$ cfu/ml)	10	79.70	72.33^{efgh}	$68.33g^{hi}$	65.33^{ij}	61.67^{hijk}	58.67^{jkl}	53.33^{ijk}
	12	80.70	72.00^{efgh}	67.00^{efg}	63.67^{ghij}	59.00^{ghij}	56.00^{hij}	51.00^{ij}
B. bassiana -B5	8	79.30	74.00^{fgh}	69.67^{ghi}	67.00^{j}	63.33^{ijk}	59.00^{kl}	54.67^{jk}
$1x10^8$ cfu/ml)	10	78.70	73.33^{efgh}	67.67^{fghi}	64.00^{hij}	60.67^{hijk}	57.00^{ijk}	52.33^{ijk}
	12	80.70	72.33^{efgh}	66.67^{efg}	62.67^{ghij}	60.00^{hijk}	55.67^{ij}	50.33^{hij}
L. Lecanii -	8	79.70	68.00^{cdef}	63.67^{defg}	60.00^{fghi}	56.33^{fghi}	48.00^{efgh}	43.00^{efg}
N1PHM	10	81.30	67.67^{bcdef}	60.33^{cde}	57.67^{efgh}	53.00^{defg}	46.67^{cdefg}	41.00^{def}
($1x10^8$ cfu/ml)	12	80.00	65.33^{bcde}	57.33^{abc}	55.00^{cdef}	50.00^{cde}	42.67^{bcde}	38.33^{bcde}
L. Lecanii -	8	80.70	63.67^{bcd}	58.00^{bcd}	53.00^{bcde}	49.00^{cd}	46.33^{cdefg}	42.67^{efg}
MTCC956	10	79.70	62.00^{abc}	56.67^{abc}	50.33^{bc}	46.00^{bc}	41.33^{abc}	36.33^{bcd}
($1x10^8$ cfu/ml)	12	81.00	59.67^{ab}	54.00^{ab}	47.00^{ab}	42.67^{ab}	38.00^{ab}	33.00^{ab}
Diafenthouron 50WP (Chemical control)	1%	81.30	55.67^{a}	51.67^{a}	43.67^{a}	39.00^{a}	34.33^{a}	28.33^{a}
Untreated Control		82.10	83.33^{i}	81.00^{j}	80.67^{k}	80.33^{l}	80.67^{m}	80.00^{l}

*Mean of three replicates; DAS -day after spray

Means followed by the same letter (a, b, c) in vertical column are not significantly different at 0.05% level of probability using Duncan LSD *post hoc* test

26.40, 33.61 and 42.21 per cent reduction in population over control after 3,7 and 10 DAS, respectively which was *at par* with *L. lecanii* MTCC 956 at 10ml/ltr and recorded 62.0, 56.67 and 50.33 adults/plant with per cent reduction in population over control 23.77, 30.33 and 38.11 after 3,7 and 10 days respectively after the first spray. Commercial *L. lecanii* at 12ml/ltr recorded 67.67, 56.67 and 51.0 adults/plant with 16.8, 30.33 and 37.41 per cent reduction in population over. Whitefly population in the treatments of commercial *L. lecanii* and procured *L. lecanii* - NIPHM, commercial *B. bassiana*, *commercial M. anisopliae* at 8ml/ltr and 10ml/ltr and native *B. bassiana* B1 and B5 at 12ml/ltr were found to be *at par* with each other (Table 2). The maximum per cent reduction over control was obtained in chemical control (Diafenthiuron 50 WP) 31.56, 36.48 and 46.31 (Table2) after 3,7 and 10 days of first spray. After the second spray, bioformulation of *L. lecanii* MTCC956 at 12ml/liter recorded whitefly population (42.67,38.0 and 33.0 adults/plant) and Diafenthiuron 50 WP recorded (39.0, 34.33 and 28.33 adults /plant) after 3,7 and 10 days of second spray and were *at par* with each other (Table 3).

Table 3. Percent reduction of *Bemisiata bacion* cotton under screen house conditions after

Treatments	Dose (g or ml/ltr)	Per cent reduction over control *					
		I Spray			II Spray		
		3DAS	7DAS	10DAS	3DAS	7DAS	10DAS
Commercial formulation	8	11.07	16.80	22.95	27.46	36.07	40.98
B.bassiana	10	13.52	18.44	26.64	31.97	38.93	47.13
$1x10^8$ cfu/ml)	12	13.93	21.31	31.56	36.07	45.49	48.41
Commercial formulation	8	2.46	9.43	17.21	18.44	27.46	32.38
M.anisopliae	10	4.92	12.30	20.08	21.72	29.92	36.07
($1x10^8$ cfu/ml)	12	4.10	15.98	22.54	23.77	34.02	38.93
Commercial formulation	8	11.48	23.77	29.92	31.97	37.30	44.26
L.lecanii	10	13.11	27.05	33.61	38.11	41.80	48.77
($1x10^8$ cfu/ml)	12	16.80	30.33	37.30	42.62	49.59	56.56
Native *B.bassiana*	8	9.84	12.30	16.80	21.72	23.36	29.51
B1 ($1x10^8$ cfu/ml)	10	11.07	15.98	19.67	24.18	27.87	34.43
	12	11.48	17.62	21.72	27.46	31.15	37.30
Native *B.bassiana*	8	9.02	14.34	17.62	22.13	27.46	32.79
B5 ($1x10^8$ cfu/ml)	10	9.84	16.80	21.31	25.41	29.92	35.66
	12	11.07	18.03	22.99	26.23	31.56	38.11
Procured *L.Lecanii*	8	16.39	21.72	26.23	30.74	40.98	47.13
N1APHM ($1x10^8$ cfu/ml)	10	16.80	25.82	29.10	34.84	42.62	49.59
	12	19.67	29.51	32.38	38.52	47.54	52.87
Procured *L. Lecanii* MTCC956	8	21.72	28.69	34.84	39.75	43.03	47.54
($1x10^8$ cfu/ml)	10	23.77	30.33	38.11	43.44	49.18	55.33
	12	26.64	33.61	42.21	47.54	53.28	59.43
Chemical control (Diafenthouron)	1%	31.56	36.48	46.31	52.05	57.79	65.16

*Percent reduction calculated using Abbott's Formula; DAS -day after spray

Bioformulation of Procured L. lecaniiMTCC956 at 12ml/ltr recorded 46.41, 52.21and 58.19 per cent population reduction over control after 3,7 and 10 days of second spray (Table) Adult whitefly population in commercial *L. lecanii* at 12ml/ltr (46.67,41.0 and 35.33 adults/plant), *L. lecanii* MTCC956 at 10ml/ltr (46.0,41.33 and 36.33 adults/plant) and *L. lecanii* NIPHM at 12ml/ltr (50.0, 42.67 and 38.33 adults/plants) were recorded after 3,7 and 10 days of second spray and were at par with each other. Maximum whitefly population was recorded in commercial *M. anisoplia* at 8 and 10 ml/ltr followed by *B. bassiana* B1 and B5 at 8 and10ml/ltr. In the present studies, it was observed that Diafenthouron 1 per cent and *L. lecanii* bioformulation at 12 ml/ltr were significantly better than all other fungal formulations in management of the whiteflies. Although all other biorationals employed in the study were better than untreated control (80 adults/plant) in reducing whitefly population. In recent years, the biocontrol agents used for whiteflies control are mainly *Beauveria bassiana*, *Metarhizium anisopliaes*.l., and *Lecanicillium lecanii* strains (Wu *et al.*, 2018). In the present study, *Lecanicillium lecanii* was found to be most effective for the control of whiteflies. *Lecanicillium* spp are known to be effective biological control agents for several diseases of plants, arthropod pests, and plant-parasitic nematodes (Goettel *et al.*, 2008). The high virulence against whiteflies may be due to its ability to adapt under various climatic conditions. Several previous studies had reported high efficacy of *L. lecanii* against *B. tabaci* in the field/green house conditions. *L. lecanii* MTCC956 bioformulation at doses 10 and 12 g/ltr was found to be highly virulent against *Myzus*, *persicae* and *B. tabacion* capsicum under

polyhouse conditions (Singh and Joshi 2020). The commercial formulation *L. muscarium* (Mycotal, Koppert Biological Systems Ltd., UK) was found to be most effective against *B. tabaci* under controlled laboratory and glasshouse conditions (Cuthbertson and Walters, 2005).

Similarly, potentialof six strains of *L. lecanii* was evaluated against sweet potato whitefly *B. tabaci* and found three strains of *L. lecanii* V16063, *L. lecanii* V3450 and *L. lecanii* Vp28 to be highly virulent. In addition, toxins (V3450 and Vp28) extracted from *L. lecanii* at a concentration of 400 mg/ ltr led to significant reduction in egg hatching and survival rate of the nymphs and the adults of *B. tabaci* (Wang *et al.*, 2007).

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