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## *In vitro* studies on the management of whitefly with entomopathogenic fungi

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**Abstract**

Three entomopathogenic fungi (EPF) viz., *Beauveria bassiana*, *Lecanicillium* (= *Verticillium*) *lecanii*, and *Metarhizium anisopilae* at three spore concentration ( $10^8$ ,  $10^{10}$  and  $10^{12}$  spores/ml) were evaluated for their efficacy against third instar nymphal stage of *Bemisia tabaci* on soybean cultivar JS 335. The highest nymphal mortality (70.00%) was recorded in EPF *L. lecanii*, followed by *B. bassiana* (53.33%) and *M. anisopilae* (43.33%) at 168 hours after spraying with  $1 \times 10^{12}$  spores/ml. However, the two lower doses i.e.  $1 \times 10^{10}$  and  $1 \times 10^8$  spores/ml were not as effective as the highest doses even up to 168 spores/ml against *B. tabaci* third instar nymphs.

**Keywords:** *Bemisia tabaci*, *Beauveria bassiana*, *Metarhizium anisopilae*, *Verticillium lecanii*, Soybean

**Introduction**

Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most serious, cosmopolitan sucking pest that causes severe yield losses in soybean. It can lead to damage either directly or indirectly (Hoddle 2013) [1]. Direct damage occurs when the stylet of the whitefly pierces the leaves and sucks the liquid that causes chlorosis in plants. While, the indirect damage occurs due to the accumulation of honeydew that catalyzes the growth of sooty mold on the entire surface of the leaf and disrupts the process of photosynthesis (Hilje and Morales, 2008) [2].

Chemical control using synthetic sprays remains the most commonly used method for the management of whitefly populations in soybean (Vieira *et al.*, 2011) [3]. The prophylactic use of insecticides in the soybean does not lead to higher productivity in the field when compared with the technique of integrated pest management (IPM) and biological control (Bueno *et al.*, 2011) [4]. Excessive insecticide applications also have a negative impact on the environment; one of them is impairing the efficiency of all existing biological control agents for soybean (Carmo *et al.*, 2010) [5].

There is a need for an effective alternative and an environmentally safe pest management strategy. The use of entomopathogenic fungi is an environmental –friendly, alternative to plant protection chemicals (Balazy 2004) [6]. The success of fungal entomopathogens as a biological control agent depends not only on its high efficacy against insect pests, but also on its low virulence against non- target insects (Thungrabeab and Tongma 2007) [7]. Keeping this in view, the present investigation was undertaken to evaluate the efficacy of promising fungal pathogen *B. bassiana*, *L. lecanii* and *M. anisopilae* on soybean cultivar JS 335 under laboratory conditions.

**2. Materials and Methods**

The *in-vitro* studies were carried at Biocontrol Research and Production Centre, Department of Entomology, JNKVV, Jabalpur (M.P.) during the year 2017-2018 with three fungus at three spore concentration with Completely Randomized Design (CRD).

**2.1 Media preparation**

Potato dextrose agar (PDA) is the most commonly used media for the growth of entomopathogenic fungi. For this purpose, 250 g of potato was washed, the skin peeled off and sliced into small pieces. To the sliced potato 500 ml water and 20g agar was added and boiled for 30 minutes in an open vessel.

Collected the potato extract by filtering through a muslin cloth. Added 20g dextrose to the potato extract and mixed thoroughly and made the volume to 1 litre with distilled water. Poured it in 250 ml conical flask, plugged with non-absorbent cotton wool, covered it with a paper sheet, and tied tightly with a rubber band. Sterilized them in an autoclave at 15 lbs pressure at 121°C for 15 minutes [agritech.tnau.ac.in](http://agritech.tnau.ac.in) [18].

## 2.2 Maintenance of insect culture

The culture of *B. tabaci* was multiplied and maintained on the potted plants of soybean variety JS 335. Initially whitefly adults were collected from the field using an aspirator and were released on the soybean plants which were kept inside the screen house. The whiteflies were allowed to develop and multiply on those plants. The second generation of the non-virulent *B. tabaci* adults were used for the study.

## 2.3 Sources of entomopathogenic fungi (EPF)

*B. bassiana* - Isolated from *Bombyx mori* larvae (JNKVV, Jabalpur, Madhya Pradesh)

*M. anisopliae* - Isolated from *Spodoptera litura* larvae (JNKVV, Jabalpur, Madhya Pradesh)

*L. lecanii* - Isolated from *Bemisia tabaci* (JNKVV, Jabalpur, Madhya Pradesh)

(JNKVV: Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur)

## 2.4 Culturing of Entomopathogenic fungi

Pure mother culture of all the three fungus was maintained on PDA slants at 4°C under refrigerated conditions until further use. Regular maintenance was done for further multiplication at 25±2°C and 70±10 % RH.

## 2.5 Materials used

The following materials were used to conduct the efficacy studies: stereo zoom binocular microscope, camel hair brush, needle, pipette, filter paper, marking tags, Petri dishes, soybean cultivar JS 335 (susceptible), atomizer, Tween-80 (0.02%), 20 megapixel camera and walk-in BOD chamber, whitefly third instar nymphs and three entomopathogenic fungi.

## 2.6 Preparation of fungal suspension

Aqueous conidial suspensions (10 ml) were made from conidia harvested from the slants prepared in conical flasks (250 ml) after 14 days of inoculation. Tween-80 (0.02%) was used to disperse the conidia; it was then filtered through a double-layered muslin cloth. The number of conidia per ml was enumerated using the Plate count method (Reddy *et al.*, 2016) [9]. Initially highest required concentration (1×10<sup>12</sup> spore ml<sup>-1</sup>) of the fungal suspension was prepared. This filtrate was the stock solution and further lower concentrations (up to 1×10<sup>8</sup> spore ml<sup>-1</sup>) were prepared from it by serial dilution technique (Geroh *et al.*, 2015) [10].

## 2.7 Bioassay against whitefly third nymphal stage

The virulence test was conducted against third instar nymph

of whitefly as per the methodology proposed by (Wraight *et al.*, 1998) [11]. For this purpose 3<sup>rd</sup> instar nymph was obtained from soybean cultivar (JS 335) grown in plastic pots. Three different entomopathogenic fungi were tested for their efficacy against whitefly nymphs along with a control (untreated check). In control, the nymphs were treated with distilled water + Tween-80 @ 0.02%. Each treatment was replicated thrice. A filter paper was wetted with distilled water and inserted in Petri dishes and infested soybean leaves having at least 10 third instar nymphs of about the same age were placed on it. Soybean petiole was wrapped with cotton swap containing water to keep the leaves fresh. The conidial suspension was sprayed with atomizer on the leaf surface @1ml of the diluted spore suspension of different spore concentrations (1×10<sup>12</sup>, 1×10<sup>10</sup>, 1×10<sup>8</sup> spores ml<sup>-1</sup>). Petri dishes were placed at 25 ± 2°C, 70± 10% RH, and 13h light exposure in the walk-in BOD chamber. Observations on mortality of the 3<sup>rd</sup> instar *B. tabaci* nymphs were recorded at every 24 hours interval and was continued up to 168 hours *i.e.* up to the adult emergence stage. The data was statistically analyzed by using Factorial CRD and the corrected mortality was calculated by using Abbott's formula. (Prasad, 2014) [12]. Also, mortality data were arcsine square-root transformed to meet the assumptions of the ANOVA.

$$T - C / 100 - C \times 100$$

Where

T = % mortality in the treatment,

C = % mortality in the control

## 3. Results

The third instar nymphal stage of *B. tabaci* was highly susceptible to infection by entomopathogenic fungi (EPF). Out of the three EPF viz., *B. bassiana*, *Lecanicillium (=Verticillium) lecanii* and *M. anisopliae* along with three spore concentrations (10<sup>8</sup>, 10<sup>10</sup> and 10<sup>12</sup> spores /ml), *L. lecanii* was found to be most virulent at highest spore concentration (10<sup>12</sup> spores /ml). At 24 hours after spraying with 1×10<sup>12</sup> spores/ ml, no mortality was recorded in all the three EPF. At 48 hours after spraying with 1×10<sup>12</sup> spores/ ml among the EPF, *L. lecanii* recorded the highest mortality (16.67%) this was followed by *B. bassiana* (6.67%). while no mortality was recorded in *M. anisopliae* including control. At 72 hours after treatment with 1×10<sup>12</sup> spores/ ml among EPF, *L. lecanii* was found to be most effective as it recorded the highest nymphal mortality (23.33%), followed by *B. bassiana* (20.00%). The least effective EPF was *M. anisopliae* (6.67%) and was significantly superior to control. A similar trend was recorded at 96, 120, 144 and 168 hours after spray. However, the lower doses *i.e.* 1×10<sup>10</sup> spores/ml and 1×10<sup>8</sup> spores/ml were not so effective as was evident by the nymphal mortality even after 168 hrs of spray (Table:1). Thus *L. lecanii* was found to be most virulent at a higher dose of 1×10<sup>12</sup> spores /ml and recorded more than 70% nymphal mortality at 168 hours after treatment, followed by *B. bassiana*. (Table:2)

**Table 1:** Efficacy of Entomopathogenic fungi (EPF) (1×10<sup>12</sup>, 1×10<sup>10</sup>, 1×10<sup>8</sup> spores ml<sup>-1</sup>) on *Bemisia tabaci* (III<sup>rd</sup> instar nymphs) at different intervals after treatment

Hr	1×10 <sup>12</sup> spores ml <sup>-1</sup>						1×10 <sup>10</sup> spores ml <sup>-1</sup>						1×10 <sup>8</sup> spores ml <sup>-1</sup>					
	EPF						EPF						EPF					
	<i>Bb</i>	<i>Ma</i>	<i>LI</i>	Control	SEm±	CD@5%	<i>Bb</i>	<i>Ma</i>	<i>LI</i>	Control	SEm±	CD@5%	<i>Bb</i>	<i>Ma</i>	<i>LI</i>	Control	SEm±	CD@5%
24	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-	-	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-	-	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-	-

48	6.67 (13.96)	0.00 (4.05)	16.67 (24.25)	0.00 (4.05)	2.81	9.32	3.33 (9.01)	0.00 (4.05)	13.33 (21.58)	0.00 (4.05)	2.82	9.32	3.33 (9.01)	0.00 (4.05)	6.67 (13.96)	0.00 (4.05)	3.50	NS
72	20.00 (26.92)	6.67 (13.96)	23.33 (29.12)	3.33 (9.01)	3.67	12.15	13.33 (21.58)	3.33 (9.01)	20.00 (26.92)	3.33 (9.01)	3.75	12.41	10.00 (16.63)	3.33 (9.01)	10.00 (16.63)	3.33 (9.01)	4.85	NS
96	23.33 (29.12)	13.33 (21.58)	40.00 (39.44)	3.33 (9.01)	3.46	11.47	16.67 (24.25)	10.00 (16.63)	26.67 (31.32)	3.33 (9.01)	4.51	14.94	10.00 (16.63)	6.67 (13.96)	20.00 (26.92)	3.33 (9.01)	4.85	NS
120	40.00 (39.44)	23.33 (29.12)	46.67 (43.37)	3.33 (9.01)	3.34	11.06	33.33 (35.52)	16.67 (24.25)	36.67 (37.52)	3.33 (9.01)	3.15	10.43	23.33 (29.12)	16.67 (24.25)	26.67 (31.32)	3.33 (9.01)	3.22	10.65
144	50.00 (45.29)	36.67 (37.52)	50.00 (45.29)	6.67 (13.96)	3.56	11.79	36.67 (37.52)	26.67 (31.32)	43.33 (41.37)	6.67 (13.96)	3.86	12.81	30.00 (33.32)	26.67 (31.12)	30.00 (33.32)	6.67 (13.96)	4.14	13.72
168	53.33 (46.92)	43.33 (41.07)	70.00 (57.00)	10.00 (18.43)	2.85	9.44	46.67 (43.08)	33.33 (35.22)	56.67 (48.93)	10.00 (18.43)	2.93	9.69	33.33 (35.22)	30.00 (33.00)	46.67 (43.67)	10.00 (18.43)	2.84	9.39

Note: Figures in parentheses are (x+0.5) arcsin transformed values and values outside parentheses are per-cent mortality values

*Bb* = *Beauveria bassiana*

*Ma* = *Metarhizium anisopila*

*Ll* = *Lecanicillium (= Verticillium) lecanii*

M= Mortality

HAT= Hours after treatment

NS = Non significant

**Table 2:** Effect of Entomopathogenic fungi (EPF) and spore concentration (Sc) on *Bemisia tabaci* (III<sup>rd</sup> instar nymphs) at different hours after treatment

EPF	48 hours									
	Sc <sub>1</sub>	Sc <sub>2</sub>	Sc <sub>3</sub>	Mean	Sem			CD		
					EPF	Sc	EPFXSc	EPF	Sc	EPFXSc
<i>Bb</i>	6.67(13.96)	3.33(9.01)	3.33(9.01)	4.44(10.66)	0.68	0.68	2.04	2.02	NS	NS
<i>Ma</i>	0.00(4.05)	0.00(4.05)	0.00(4.05)	0.00(4.05)						
<i>Ll</i>	16.67(24.25)	13.33(21.58)	6.67(13.96)	12.22(19.93)						
Mean	7.78(14.09)	5.56(11.55)	3.33(9.01)	-						
72 hours										
<i>Bb</i>	20.00(26.92)	13.33(21.58)	10.00(16.63)	14.44(21.71)	0.73	0.73	2.20	2.18	2.18	NS
<i>Ma</i>	6.67(13.96)	3.33(9.01)	3.33(9.01)	4.44(10.66)						
<i>Ll</i>	23.33(29.12)	20.00(26.92)	10.00(18.91)	17.78(24.98)						
Mean	16.67(23.33)	12.22(19.17)	7.78(14.85)	-						
96 hours										
<i>Bb</i>	23.33(29.12)	16.67(24.25)	10.00(16.63)	16.67(23.33)	0.79	0.79	2.36	2.33	2.33	NS
<i>Ma</i>	13.33(21.58)	10.00(16.63)	6.67(13.96)	10.00(17.39)						
<i>Ll</i>	40.00(39.44)	26.67(31.32)	20.00(26.92)	28.89(32.56)						
Mean	25.56(30.05)	17.78(24.07)	12.22(19.17)	-						
120 hours										
<i>Bb</i>	40.00(39.15)	33.33(35.22)	23.33(28.78)	32.22(34.38)	0.47	0.47	1.40	1.38	1.38	NS
<i>Ma</i>	23.33(28.78)	16.67(23.86)	16.67(23.86)	18.89(25.50)						
<i>Ll</i>	46.67(43.08)	36.67(37.22)	26.67(31.00)	36.67(37.10)						
Mean	36.67(37.00)	28.89(32.10)	22.22(27.88)	-						
144 hours										
<i>Bb</i>	50.00(45.00)	36.67(37.22)	30.00(33.00)	38.89(38.41)	0.66	0.66	1.98	1.96	1.96	NS
<i>Ma</i>	36.67(37.22)	26.67(31.00)	26.67(30.79)	30.00(33.00)						
<i>Ll</i>	50.00(45.00)	43.33(41.07)	30.00(33.00)	41.11(39.69)						
Mean	45.56(42.41)	35.56(36.43)	28.89(32.26)	-						
168 hours										
<i>Bb</i>	53.33(46.92)	46.67(43.08)	33.33(35.22)	44.44(41.74)	0.64	0.64	1.91	1.90	1.90	NS
<i>Ma</i>	43.33(41.07)	33.33(35.22)	30.00(33.00)	35.56(36.43)						
<i>Ll</i>	70.00(57.00)	56.67(48.93)	46.67(43.08)	57.78(49.67)						
Mean	55.56(48.33)	45.56(42.41)	36.67(37.10)	-						

Note: ( ) = Figures in parentheses are x+0.5 arcsin transformed values

Sc<sub>1</sub> = 1x10<sup>12</sup> spores/ml, Sc<sub>2</sub>=1x10<sup>10</sup> spores/ml, Sc<sub>3</sub>= 1x10<sup>8</sup> spores/ml, NS=Nonsignificant

*Bb*=*Beauveria bassiana*, *Ma*=*Metarhizium anisopila*, *Ll*=*Lecanicillium (=Verticillium) lecanii*

#### 4. Discussion

The present studies revealed that the third instar nymphal stage of *B. tabaci* was highly susceptible to infection by entomopathogenic fungi. The present findings confirm the findings of Vincentini *et al.*, (2001) [13] James *et al.*, (2003) [14]; AI-Deghairi (2008) [15] and Malekan *et al.*, (2015) [16]. In the present study, out of the three spore concentration of three entomopathogenic fungi (EPF), *L. lecanii* was found to be most virulent at the highest spore concentration (10<sup>12</sup> spores/ml) against third instar nymphal stage of *B. tabaci*. It confirms the findings of Karthikeyan and Selvanarayanan (2011) [17]. They also found a linear relationship between mortality and dose concentration. At 48 hours after spraying the differences in the nymphal mortality among different EPF

were significant. *L. lecanii* recorded highest nymphal mortality (16.67%), followed by *B. bassiana* (6.67%). The present findings conform with those of Mascarin *et al.*, (2013) [18], as they also stated that the mortality began after 2 days of exposure with conidia. The fungus *M. anisopila*, including control did not cause any mortality at this stage. At 72 hours after spray the EPF *L. lecanii* at spore concentration of 1x10<sup>12</sup> spores/ml was found to be most effective as it recorded the highest nymphal mortality (23.33%). The present findings corroborate the findings of Bouhous and Larous (2012) [19] and Cuthbertson *et al.*, (2005) [20]. They also reported that *L. lecanii* is particularly infectious for nymphal stages. The differences in the mortality in the present studies might be due to the variation in the virulence of the tested

entomopathogenic fungi (EPF) strain and also spore concentration. At 96 hours after spray at a spore concentration of  $1 \times 10^{12}$  spores/ml, the differences in the nymphal mortality among different strains were significant. Among the EPF, *L. lecanii* was found to be most effective as it recorded highest nymphal mortality (40.00%), followed by *B. bassiana* (23.33%), but both were statistically at par with each other. The least effective EPF was *M. anisopila* (13.33%), but significantly superior to control (3.33%). A similar trend was observed at 120, 144 and 168 hrs after spray at  $1 \times 10^{12}$  spores/ml but with increased nymphal mortality i.e. highest (70.00%) recorded in EPF *L. lecanii*, followed by *B. bassiana* (53.33%) and lowest in *M. anisopila* (43.33%), respectively. Mortality recorded in control was 10%. The present findings confirm the findings of Reyad (2017) [21]. He also reported *L. lecanii* to be highly toxic at the highest concentration among the three fungi under laboratory conditions.

Further, Al- Alawi *et al.*, (2014) [22] and Zafar *et al.*, (2016) [23] reported *B. bassiana* as highly effective in the management of whitefly nymphs. This is in accordance with the present findings, where *B. bassiana* has proved to be an effective fungus next to *L. lecanii*. At 168 hrs after spray at  $1 \times 10^8$  spores/ml, there was a slight increase in the nymphal mortality, the highest mortality (46.67%) was recorded in isolate *L. lecanii*, followed by *B. bassiana* (33.33%) and lowest in *M. anisopila* (30%). However mortality recorded in control was 10%. The present findings confirm the findings of Abdel-Raheem and AL- Keridis (2017) [24]. They also reported *L. lecanii* to be the most effective fungus that registered up to 100% mortality, compared to *M. anisopila* and *B. bassiana*. Ramos *et al.*, (2000) [25], Kuang (2005) [26] and Islam (2009) [27] reported that after 7 days of spray of *B. bassiana* @  $1 \times 10^8$  conidia/ml, nymphal mortality ranged from 62 to 71%, 84.88 to 86.81%, and 38.78 to 72.9%, respectively. However, mortalities in the present research contradict their findings, where *L. lecanii* had shown the highest nymphal mortality of about 70% at the highest spore concentration of  $1 \times 10^{12}$  spore/ml. The differences in the mortality in the present studies might be due to the variations in the virulency of the EPF and spore concentration Quesada *et al.*, (2006) [28]. The findings also indicate that there is a linear relationship between mortality and dose concentration and are in conformity with the findings of Karthikeyan and Selvanarayanan (2011) [17].

#### 4. Conclusion

The study revealed that *Lecanicillium* (= *Verticillium*) *lecanii* is the most potent fungus at a higher dose of  $1 \times 10^{12}$  spores /ml and recorded more than 70% nymphal mortality at 168 hours after treatment, followed by *B. bassiana* and *M. anisopila*. The overall conclusion represents that the three entomopathogenic fungi (EPF) were significantly superior over control. However, EPF *Lecanicillium* (= *Verticillium*) *lecanii* was the most virulent against *B. tabaci* third instar nymphs and recorded the highest mortality @  $1 \times 10^{12}$  spores /ml dose.

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