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IN-VITRO STUDIES ON THE MANAGEMENT OF WHITEFLY, *BEMISIA TABACI* WITH *BEAUVERIA BASSIANA*

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ABSTRACT

Bioassays were conducted with three strains of an entomopathogenic fungus *Beauveria bassiana* against third nymphal instar of *Bemisia tabaci* on soybean cultivar JS-335 under laboratory conditions at 25± 2^0 C, 70±10% RH, 13 photophase, at the Biocontrol Research and Production Centre, Department of Entomology, College of Agriculture, JNKVV, Jabalpur during 2016-2017. The study revealed that the *B. bassiana* strain-S3 wasthe most effective at 168 hours after treatment. The mortality at $1.72x10^9$ spores/ml with observed with very high lower and very low upper fiducial limits ($5.64x10^7$ and 5.24x 10, respectively) followed by Strain-2 ($4.32x10^8$ and $5.20x\ 10^{18}$) and Strain-1 ($1.40x10^9$ and $1.34x\ 10^{14}$). Among the doses $1x10^{12}$ spores/ml recorded maximum mortality (60.00%) followed by $1x10^{10}$ spores/ml (46.67%) and $1x10^8$spores/ml(38.89%).

Keywords: *Beauveria bassiana*, *B. tabaci*, bioassay,3rd instar nymphs, strains, spore concentration, mortality response

Soybean (*Glycine max* (L.) Merrill) is an important legume crop, and Madhya Pradesh is the leading state. There is a gradual reduction in the soybean yield because of various abiotic and biotic factors. Among the biotic factors,whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)is the most seriouscausing severe yield losses. It damages the leaf both directly and indirectly (Hoodle, 2013; Hilje and Morales,2008). Controlling insect pests in soybean by spraying of insecticides is widely adopted by the farmers (Song and Swinton,2009). Excessive application of insecticides resulting in the development of pest resurgence, pest resistance to pesticides and lethal effects on the non-target organisms(Carmo et al., 2010; Palumbo et al., 2001). Further, the use of insecticides in the soybean does not lead to higher productivity in the field when compared with biological control (Bueno et al., 2011). Among the biocontrol agents, entomopathogenic fungi are most versatile and environmentally safe. These act pathogenetically by contact action through penetration (Tank, 2015; Zimmermann, 2007). They are safe to the target organisms with no chance of development of resistance (Wright, 1992).*Beauveria bassiana* is considered to be the most promising EPF against whiteflies. The fungus also has the potentiality to infect a widerange of insect pests (Mohammad and Deghairi, 2008). Laboratory bioassay have revealed *B. bassiana* to be an effective pathogen against whiteflies when applied directly as concentrated conidial suspension (Wraight et al., 2000). This study evaluates three strains at different spore concentrations against *B. tabaci.*

MATERIALS AND METHODS

The pure culture of *Bemisia tabaci* was multiplied and maintained on the potted plants of soybean variety JS-335 under caged conditions. The plants were grown in screen house in disposable plastic pots having diameter and height of 10 and 20cm, respectively. The pots were filled with vermicompost, soil and sand in the ratio of 2:1:1. Watering was done manually once in every two days. Initially whitefly adults were collected from the soybean field by using an aspirator and were released on the soybean plants which were kept inside the screen house.

The *Beauveria bassiana* strain 1 (NAIMCC-F-00410), strain 2 (NAIMCC-F-02125) was obtained from National Bureau of Agriculturally Important Microorganisms(NBAIM)Mau, Uttar Pradesh and Strain 3 was isolated from silkworm infected larvae. Pure mother culture of fungus was maintained on Potato Dextrose Agar (PDA) at 4°C under refrigerated conditions till further use. Regular maintenance was done for further multiplication at 25±2°C and 70±10 % RH Aqueous conidial suspensions (10 ml) were made from conidia harvested from the slants which prepared in conical flasks (250 ml) after 14 days of inoculation. Tween-80 (0.02%) was used to disperse the conidia and

then filtered through a double layered muslin cloth. The number of conidia per ml was enumerated using plate count method (Reddy et al., 2016) Initially the highest required concentration (1×10^{12} conidia ml^{-1}) of the fungal suspension was prepared. This filtrate was the stock solution and further lower concentrations (upto 1×10^{8} conidia ml^{-1}) were prepared from it by serial dilution technique (Geroh et al., 2015).

The virulence test was conducted against 3rd instar nymphs as per the methodology proposed by Wraight et al., (1998). For this purpose only 3rd instar nymphs were obtained based on morphological observation, which is oval and flat remains attached to the leaves initially pale in yellow and later turns to dark yellow with clear appearance of mycetomes (Kedar and Saini., 2014), which were maintained on soybean cultivar (JS-335) grown in plastic pots, remaining stages i.e., first instar crawlers, second instar and pupal stages were removed with brush. Three strains of *B.bassiana* were tested for their efficacy against 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 petridishes and infested soybean leaves having at least 10 third instar nymphs of about same age were placed in it. Soybean petiole was wrapped with cotton swap containing water in order to keep the leaves fresh. Conidial suspension was sprayed with atomizer on the leaf surface@1ml of the spore suspension of different spore concentration ($1x10^{12}$, $1x10^{10}$, $1x10^{8}$ spores ml^{-1}). Petridishes were placed at 25 ± 2°C, 70± 10% RH and 13hr light exposure in walk-in BOD chamber under caged conditions. Observations on mortality of the 3rd instar *B. tabaci* nymphs were recorded at 24 hr interval and was continued upto their mortality of adult emergence stage whichever was earlier. Abbott's formula was used to correct the mortality and the statistical analysis adopted was factorial CRD to know the degree of variation among all the treatments, For testing the toxicity probit analysis was used and LC_{50} values were computed.

RESULTS AND DISCUSSION

The results revealed that the third instar *B.tabaci*nymphs were highly susceptible to infection by *Beauveria bassiana*. These results confirm the findings of Vincentini et al.(2001), James et al. (2003) and AI-Deghairi (2008). They also reported that nymphs of *B.tabaci* and *B. argentifolii* were highly vulnerable to *B. bassiana* infection. Fourth instar cuticular lipids had the toxic or inhibitory effect on the conidia of *B.bassiana* (James et al., 2003).The effects of the fungal infection included impaired fertility, production of malformation or external variations and reduced survival of later generations (Torrado-leon et al.,2006).

Observations given in Table 1 reveal that strain S-3 is the most virulent at highest spore concentration (10^{12} spores/ml). At 24 hours after treatment (HAT) at highest spore concentration ($1x10^{12}$ spores/ml), differences in the nymphal mortality among different *B. bassiana* strains were not significant. Among the strains, strain S-3 recorded highest nymphal mortality (6.67%), followed by strain S-1(3.33%), while no mortality was recorded in strain S-2 and control. At 48 HAT, the differences in the nymphal mortality among different strains were significant, but the trend was same as in 24 HAT.At 72 HAT, the strain S-3@$1x10^{12}$ spores/ml was found to be most effective as it recorded highest nymphal mortality (23.33%) followed by strain S-1(13.33%), but both were at par with each other. The least effective strain was S-2(10.00%) but was significantly superior than control. However, Ramazeame (2012) reported that at 72 HAT the mortality ranged from 72 to 98.33%. The differences in the mortality in the present studies might be due to the variation in the virulency of the tested *B. bassiana* strains and spore concentration.

At 96 HAT at $1x10^{12}$spores/ml, the differences in the mean nymphal mortality among different strains were significant. Among the strains, S-3 was found to be most effective as it recorded highest nymphal mortality (36.67%), followed by strain S-1(26.67%), but both were statistically at par with each other. The least effective strain was S-2(16.67%), but significantly superior than control (3.33%).Similar trend was observed at 120, 144 and 168 HAT at $1x10^{12}$ spores/ml but with increased nymphal mortality *i.e* highest (76.67%) recorded in strain S-3, followed by S-1 (56.67%) and S-2 (46.67%).

At 24 HAT at $1X10^{10}$spores/ml, differences in the nymphal mortality among different *B. bassiana* strains were not significant. Among the strains, strain S-3 recorded highest nymphal mortality (3.33%), while no nymphal mortality was recorded in the strains S-1, S-2 and untreated control. At 168 HAT there was an increase in the nymphal mortality with highest mortality registered by S-3 (53.33%), followed by S-1(46.66%) and S-2(40.00%). At 48 HAT at $1x10^{8}$ spores/ml, the differences in the mortality were non-

Table1. Efficacy of *B.bassiana* ($1x10^{12}$, $1x10^{10}$, $1x10^{8}$ spores ml^{-1}) on *B. tabaci* (3^{rd} instar nymphs)

B.bassiana Strains	Mean mortality of nymphs at different HAT (in %)																				
	24			48			72			96			120			144			168		
	10^{12}	10^{10}	10^{8}	10^{12}	10^{10}	10^{8}	10^{12}	10^{10}	10^{8}	10^{12}	10^{10}	10^{8}	10^{12}	10^{10}	10^{8}	10^{12}	10^{10}	10^{8}	10^{12}	10^{10}	10^{8}
Strain 1	3.33 (9.00)	0.00 (4.05)	0.00 (4.05)	6.67 (13.96)	3.33 (9.00)	6.67 (13.96)	13.33 (21.38)	10.00 (18.91)	10.00 (18.91)	26.67 (31.32)	16.67 (24.25)	10.00 (18.91)	43.33 (41.44)	33.33 (35.52)	23.33 (29.12)	53.33 (46.92)	43.33 (41.15)	26.67 (3.99)	56.67 (48.85)	46.66 (43.07)	36.67 (37.22)
Strain 2	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	10.00 (18.91)	6.67 (13.95)	6.67 (13.96)	16.67 (24.25)	10.00 (18.91)	10.00 (18.91)	26.67 (31.32)	20.00 (26.92)	13.33 (21.58)	46.67 (43.08)	30.00 (33.21	16.67 (23.85)	46.67 (43.08)	40.00 (39.23)	30.00 (33.21)
Strain 3	6.67 (13.96)	3.33 (9.00)	0.00 (4.05)	20.00 (26.45)	13.33 (21.58)	10.00 (18.91)	23.33 (29.12)	16.67 (24.25)	13.33 (21.58)	36.67 (37.52)	26.67 (31.32)	20.00 (26.45)	50.00 (45.29)	33.33 (35.52)	30.00 (33.32)	53.33 (46.92)	50.00 (45.00)	40.00 (39.23)	76.67 (61.22)	53.33 (46.92)	50.00 (45.00)
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	3.33 (9.00)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)
SEm±	3.50	2.47	0.00	4.09	3.74	3.5	3.02	3.74	3.74	3.18	3.02	3.25	3.32	2.85	3.52	1.66	0.96	1.74	1.75	1.36	1.00
CD@5%	NS	NS	NS	13.33	NS	NS	9.85	NS	NS	10.37	9.85	10.61	10.83	9.30	11.50	5.43	3.13	5.70	5.72	4.43	3.27

Figures in parentheses arcsin transformed values (X+0.5): NS: Non significant ; HAT: Hours after treatment

significant. Among the strains, S-3 recorded highest mortality (10.00%), followed by strain S-1(6.67%) and S-2(3.33%). At 168 HAT there was a slight increase in the mortality, highest (50%) was recorded in strain S-3, followed by S-1 (36.67%) and S-2 (30%) (Table 1).

On the contrary, Sinary EI (2002) reported 100% nymphal mortality after 96 HAT of *B.bassiana* @1x10^8 conidia/ml. Similarly, Ramos et al. (2000), Kuang (2005) and Islam (2009) reported nymphal mortality pf 62-71%, 84.88% to 86.81%, and 38.78% to 72.9% after 7 days of spray of *B.bassiana* @1x10^8 conidia/ml. The differences in the mortality in the present study might be due to the variations in the virulency of the *B. bassiana* strains and doses (Quesada et al., 2006).

Data in Table 2 revealed that at 168 HAT, the differences in mortality among strains and spore concentration were significant. Highest mortality was recorded with spore concentration 1x10^{12} spores/ml(60.00%) followed by spore concentration 1x10^{10} spores/ml (46.67%) and 10^8spores/ml (38.89%) and they differed significantly from each other. The results indicated that the nymphal mortality was spore concentration dependent and its interaction with strain exhibited significant impact on the nymphal mortality.

Toxicity of three strains of *B.bassiana* was determined against *B. tabaci* using probit analysis and the LC_{50} values were computed. The LC_{50} value at 24 HAT for strain S-3 was 8.67x10^{17}spores/ml with very high lower and very low upper fiducial limits (3.57x10^6 and 2.11 x 10^{29}, respectively). The present findings are in accordance with the findings of AlAlawi et al. (2014) and Zafar et al. (2016). They also reported LC_{50} values of 3.16x10^6, 1.17x10^6, 9.33x10^5, 5.62x10^5 and 7.76x10^5 against the test strains of *B. bassiana.* The LC_{50} value at 168 HAT for strain S-3 revealed the lower and upper fiducial limits of 5.64 x10^7 and 5.24x10, respectively. Thus, the *B. bassiana* strain S-3 was the most virulent against *B. tabaci* third instar nymphs with maximum mortality with 1x10^{12} spores /ml spore concentration.

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Table 2. Effect of *B. bassiana* and spore concentration, and mortality response relationship on *B. tabaci*

B.bassiana Strains	Mortality of *B.tabaci* (3rd instar nymphs) at 168 HAT(%)							
	D_1	D_2	D_3	Mean	Regression equation	LC_{50} (Spores	Fiducial limits Lower	Upper
Strain 1	56.66 (48.85)	46.66 (43.08)	36.66 (37.22)	46.67 (43.05)	Y=0.145x+3.305	4.33x10^{11}	1.40x10^9	1.34x10^{14}
Strain 2	46.66 (43.08)	40.00 (39.23)	30.00 (33.21)	38.89 (38.51)	Y=0.132x+3.197	4.74x10^{13}	4.32x10^8	5.20x10^{18}
Strain 3	76.66 (61.22)	53.33 (46.92)	50.00 (45.00)	60.00 (51.05)	Y=0.196x+3.189	1.72x10^9	5.64x10^7	5.24x10
	SEm±		CD (P=0.05)					
Strain	0.94		2.78					
Spore concentration	0.94		2.78					
Strain x Spore concentration	1.62		4.82					

D_1: 1x10^{12} spores/ml, D_2: 1x10^{10} spores/ml, D_3:1x10^8 spores/ml; HAT: Hours after treatment

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