Website : www.asthafoundation.in
Progressive Research : An International Journal
Vol 17 (1) : 65-69 (January-2022), Print ISSN : 0973-6417, Online ISSN : 2454-6003
Society for Scientific Development in Agriculture and Technology, Meerut (U.P.) India

Pathogenicity and Bioefficacy of AS formulations of *Beaveria bassiana* against Mealy Bug

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Abstract

Laboratory studies with two promising AS formulations of *Beauveria bassiana* by spraying the dilution series (2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 g/l of water or at concentrations 0.014, 0.021, 0.028, 0.035, 0.042 and 0.056%, respectively) of each of the formulations and formulation without adjuvants having three replications in completely randomized design were carried out in the biological control laboratory, Dept. of Entomology, MPKV, Rahuri with an object to evaluate the pathogenicity and bio-efficacy of two promising AS formulationsof *B. bassiana* against mealy bug (*M. hirsutus*).The results revealed that theLC_{50} value for formulation A was 0.030% (11.9 x 10^6cfu ml^{-1}) when it was 0.023% (9.72 x 10^6cfu ml^{-1}) for formulation B against mealy bug. It indicated that the formulation B was more virulent than formulation A against the mealy bug. The data elucidated that the LT_{50} values of formulation A and formulation B were 10.47 days and 9.74 days, respectively. Thus it was evident from this results that formulation B was more virulent than formulation A as it taken slightly less time to kill 50 per cent test population of mealy bug. At 10 DAT, the cumulative mortality was highest (80.00 %) in the formulation B 0.056 % which was on par with formulation A 0.056 % (76.67 %), 0.042 % (73.00 %), 0.035 % (70.00 %) and 0.028 % (66.67 %) and formulation B 0.042 % (76.67 %), 0.035 % (73.33 %) and 0.028 % (70.00 %). The lowest (56.67 %) kill was noticed in control (*B.b.* alone). At 14 DAT, the highest (86.67 %) mortality of the pest was observed in formulation A 0.056 % and formulation B 0.042 and 0.056 %. However, these were at par with formulation A 0.028 % (80.00 %), 0.035% (83.33 %) and 0.042 % (83.33 %) and formulation B 0.028 % and 0.035 % (83.33%). Control (*B.b.* alone) caused the lowest (63.33 %) mortality.

Key words : *Beauveria bassiana, mealy bug, H. armigera, LT_{50} and LC_{50}*

Introduction

The entomopathogenic fungi causing diseases to the insects and are practically more significant as they are epizootic in nature. Also they have the advantage of ease of mass production and contact action which allow direct penetration of the host cuticle without ingestion. (1) was the first to demonstrate that entomopathogenic fungus, *B. bassiana* could cause an infectious disease in silkworm and suggested the concept that, an infectious micro-organism might be used to control insect pests. More than 750 species of entomopathogenic fungi, representing 100 genera are currently known (2). *Beauveria bassiana* (Balsamo) Vuillemin is cosmopolitan fungus useful for the control of various insect pests of different crops. (3) reported that *B. bassiana* causes mycosis in 175 host insects from order Lepidoptera, Coleoptera and Hemiptera. (4) reported the pathogenicity of *B. bassiana* to *H. armigera* larvae. This fungus also found useful for the control of various sucking pests of important field crops. Aphids, *Aphis craccivora* Koch, *A. gossypii* and *Rhapalosiphum maidis* were found to be attacked by *B. bassiana* causing 16-80 % mortality (5) also reported the pathogenicity of *B. bassiana* to aphids. (6) reported pathogenicity of *M. anisopliae* and *B. bassiana* to citrus mealy bug (*Phenococcuscitri*). (7) found that *B. bassiana* was pathogenic to mango mealy bug, *Drosichamangiferae*.

The use of entomopathogenic fungi has potential in future strategies in insect pest management due to heath awareness among peoples and ill effects of chemical pesticides (8). Hence, the present study was taken up to evaluate the pathogenicity and bio-efficacy of promising AS formulations of *B.bassiana* against mealy bug (*M.hirsutus*).

Materials and Methods

The present investigation was carried out at Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra State, India during year 2009-2011.

Fungus culture : The pure fungus culture of *B. bassiana* was available at Biocontrol Research Laboratory, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Received : September-2021; Revised : September-2021; Accepted : November-2021

Medium : The medium used for multiplication and growth of the fungus was potato dextrose broth.

Maintenance of culture : Sabouarauds Dextrose Agar (SDAY) used by (9) and Potato Dextrose agar (PDA) suggested by (10) was utilized to maintain the culture.

Pathogenicity and bioefficacy of formulations of *B. bassiana* : The bioassay of the two promising 7% AS formulations*viz.,* Formulation A ($B_{40}G_{1/2}H_{1/1}$) and Formulation B ($B_{40}S_{1/1}C_{1/2}$) of *B. bassiana* was carried out by spraying the dilution series (2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 ml/l of water or at concentrations 0.014, 0.021, 0.028, 0.035, 0.042 and 0.056%, respectively) of each of the formulations and formulation without adjuvants. The two promising 7% AS formulations of *B. bassiana* were tested against mealy bug, *M. hirsutus*and calculated the LC_{50} and LT_{50} of respective AS formulations.

Bioassay method for determining LC_{50} values of mealy bug, *M. hirsutus* : The 250 ml sized plastic containers were filled with sandy soil up to 2/3 depth of the containers. The soil was saturated with 10 per cent sugar solution. The fresh sprouted potatoes were rinsed in water for 10 minutes and surface sterilized in 0.2 per cent sodium hypochlorite solution for 3 minutes, rinsed three times with distilled water and air dried in laminar flow cabinet. The 30 nymphs of grape mealy bug (*M. hirsutus*) were released per potato. Sprouted potato remained in satisfactory condition for at least 8 days in the presence of mealy bug.

The sprouted potatoes were kept in small bowl and sprayed individually with requisite test concentrations of promising A and B aqua suspension formulations of *B. bassiana*. The surrounding area around each potato was covered with tissue paper. The paper was soaked in glucose solution. The sugary tissue paper served as food for insect and barrier to protect dead insect from spoilage. Each of potato was covered with duly, finely and uniformly perforated polythene bags (50 perforations/bag). The perforation ensured appropriate R.H and temperature inside the bag. The numbers of dead insects were recorded daily for 7 days. Fungal infection was confirmed with help of microscope on 7^{th} day by ensuring mycosis. The data were subjected to probit analysis as suggested by (11) and LC_{50} and LT_{50} values, lower and upper fiducial limits and Chi-square values were calculated.

Bioefficacy of *B. bassiana* against Mealy bug, *M. hirsutus* : The laboratory experiment was laid out in completely randomized design (C.R.D.) with three replications. Three sprouted potatoes were used for each replication for each treatment. Thirty 1^{st} instar nymphs of mealy bug were released per sprouted potato with a camel hair brush. There were 13 treatments and an untreated control. These comprised of0.014, 0.021, 0.028, 0.035, 0.042 and 0.056 % concentration of each of formulation A and B, control (*B.b.* alone) and untreated control (water spray). Hand sprayer was used to treat the potato with respective concentrations.

Single spray was given. Initially untreated control was sprayed with water, followed by low and then their higher concentrations of the formulations to reduce error in spray concentrations. The sprayer was rinsed with water before switching to next treatment.

The live and dead insects were counted with the help of hand lens. The mortality of insect at 3, 7, 10 and 14 DAT was recorded. The per cent mortality was worked out on the basis of total number of live and dead insects. The data was converted to arcsin square root transformation (12) and further subjected to statistical analysis.

Results and Discussion

Pathogenicity of 2 promising 7% AS formulations of *B. bassiana* to mealy bug : The LC_{50} and LT_{50} values for mealy bug were determined through bioassay and probitanalysis. The results are presented in table-1 and 2.

LC_{50} values for mealy bug : The Table-1 elucidated that the LC_{50} value for formulation A was 0.030% (11.9 x 10^6cfu ml^{-1}) when it was 0.023% (9.72 x 10^6cfu ml^{-1}) for formulation B, using mealy bug, *M. hirsutus* as test insect. It indicated that the formulation B was more virulent than formulation A against the mealy bug.

LT_{50} values for mealy bug : The data in the Table-2 elucidated that the LT_{50} values of formulation A and formulation B were 10.47 days and 9.74 days, respectively.

Thus it is evident from this results that formulation B was more virulent than formulation A as it taken slightly less time to kill 50 per cent test population of mealy bug (*M. hirsutus*).

(6) recorded LC_{50} values of *M. anisopliae* against second instar citrus mealy bug (*Phenococuscitri*) in both water and oil suspensions were 6.4 x 10^5 and 3.4 x 10^4 conidia ml^{-1}, respectively. Lesser LC_{50} in oil suspension than water ones supporting present findings. (13) found that the LC_{50} values of *B. bassiana* against *Myzuspersicae* eight days after inoculation were ranged from 1.20 to 1.55 x 10^6 conidia ml^{-1} and also reported that the LT_{50} values for the 6 isolates ranged from 3.7 to 8.9 days with the dose of 10^6 conidia ml^{-1}. (10) determined that the LC_{50} and LT_{50} values of Bb5a for three day old nymph of *A. gossypii* was 6.57 x 10^5 spores ml^{-1} and 9.67 days respectively for the lowest dose of 10^6 spores ml^{-1}. The highest dose 10^9 spores ml^{-1} recorded lowest LT_{50} value of 1.76 days.

Table-1 : LC_{50} values of final stage 7% AS formulations of *B. bassiana* against mealy bug (*M. hirsutus*).

Sr. No.	Formulations (B.b.)*	Host tested	Chi-square	Regression equation	LC50 based on		Fiducial limit	
					BAI (%)	cfu ml_{-1} x 10^6	Lower	Upper
1.	Form. A	Mealy bug (*M.hirsutus*)	0.11	Y=3.8953+0. 7411X	0.030	11.9	0.017	0.055
2.	Form. B	Mealy bug (*M.hirsutus*)	0.45	Y=3.0631+1. 4048X	0.023	9.72	0.017	0.033

Table-2 : LT_{50} values of final stage 7 % AS formulations of *B. bassiana* against mealy bug (*M. hirsutus*).

Sr. No.	Formulations (B.b.)*	Host tested	Chi-square	Regression equation	LT_{50} (Days)	Fiducial limit	
						Lower	Upper
1.	Form. A	Mealy bug *(M. hirsutus)*	13.13	Y= 1.473 + 3.457 X	10.47	8.82	13.12
2.	Form. B	Mealy bug *(M. hirsutus)*	0.79	Y= 2.222 + 2.809 X	9.74	7.97	12.59

Table-3 : Bioefficacy of two promising 7% AS formulations of *B. bassiana* against mealy bug (*M. hirsutus*).

Tr. No.	Treatments**	Dose based on		Nymphal mortality (%) on			
		BAI conc. (%)	Product (%)	3 DAT	7 DAT	10 DAT	14 DAT
T_1	Formulation A	0.014	0.2	6.67 (12.29)	40.00 (39.14)	60.00 (50.85)	70.00 (56.78)
T_2		0.021	0.3	10.00 (15.00)	43.33 (41.15)	63.33 (52.85)	73.33 (59.00)
T_3		0.028	0.4	16.67 (23.85)	50.00 (45.00)	66.67 (54.78)	80.00 (63.43)
T_4		0.035	0.5	26.67 (30.75)	53.33 (46.92)	70.00 (56.99)	83.33 (66.14)
T_5		0.042	0.6	30.00 (33.00)	56.67 (48.84)	73.33 (59.00)	83.33 (66.14)
T_6		0.056	0.8	40.00 (39.14)	63.33 (52.85)	76.67 (61.22)	86.67 (68.85)
T_7	Formulation B	0.014	0.2	10.00 (15.00)	40.00 (39.14)	60.00 (50.76)	70.00 (56.78)
T_8		0.021	0.3	16.67 (23.85)	43.33 (41.07)	63.33 (52.85)	73.33 (59.00)
T_9		0.028	0.4	30.00 (33.00)	53.33 (46.92)	70.00 (56.99)	83.33 (66.14)
T_{10}		0.035	0.5	36.67 (37.14)	56.67 (48.84)	73.33 (59.00)	83.33 (66.14)
T_{11}		0.042	0.6	40.00 (39.14)	60.00 (50.85)	76.67 (61.22)	86.67 (68.85)
T_{12}		0.056	0.8	46.67 (43.07)	66.67 (54.78)	80.00 (63.43)	86.67 (68.85)
T_{13}	B.b. alone	0.028	0.4	13.33 (21.14)	36.67 (37.14)	56.67 (48.84)	63.33 (52.85)
T_{14}	Control (Water spray)	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E. ±				4.65	3.00	3.11	2.93
C.D. at 5%				13.53	8.73	9.05	8.54

*Figures in the parentheses indicate arcsin transformed values, ***B.b.*= *Beauveriabassiana*,
Formulation A = ($B_{40}G_{2/1}H_{1/1}$), Formulation B = ($B_{40}S_{1/1}C_{1/2}$), DAT = Days after treatment.

Effect of *B. bassiana* AS formulations on behavior of mealy bug (*M. hirsutus*) : When the 1st instar nymphs of mealy bug (*M. hirsutus*) were contaminated with the various concentrations of *B. bassiana* 7 % AS formulation, most of the nymphs stop feeding within 48 hrs and left the food. The nymphal mortality observed from one day after spraying. The visible mycosis on the nymphs was observed from 10 to 14 days after treatment (DAT). However, it was confused with the wax. The wax is glittering, microgranular type and sprayed erratically; while mycosis is smooth, fibrous and uniformly covered on the body. In early instar mouth parts and other

appendages were blackened and hidden under white mass. In contrary, some of the nymphs survived after contamination but they stop feeding and died after two weeks.

(7) reported that mortality of 1st instar nymphs of the mango mealy bug, *Drosichamangiferae* by *B. bassiana* was noticed after 3 days, and cumulative mortality was found to increase with time for all concentrations.

Bioefficacy of two promising 7% AS formulations of *B. bassiana* against mealy bug (*M. hirsutus*) : The data on bioefficacy of 7% AS formulations*viz*., formulation A ($B_{40}G_{2/1}H_{1/1}$) and formulation B ($B_{40}S_{1/1}C_{1/2}$) along with control (*B.b.* alone) against mealy bug recorded at 3, 7, 10 and 14 DAT. Results are presented in Table-3.

At 3 DAT the nymphal mortality in various treatments was 6.67 to 46.67 per cent. The highest (46.67%) kill was observed in 0.056 % concentration of formulation B. However, it was at par with formulation A 0.035 % (26.67 %), 0.042 % (30.00 %) and 0.056 % (40.00 %) and formulation B 0.028 % (30.00 %), 0.035 % (36.67 %) and 0.042 % (40.00 %). The lowest (6.67%) nymphal mortality was recorded in 0.014 % concentration of formulation A. At 7 DAT the nymphal mortality ranged from 36.67 to 66.67 per cent. The highest (66.67%) nymphal mortality was recorded in formulation B 0.056 %. It was at par with formulation B 0.042 % (60.00 %), 0.035 % (56.67 %), 0.028 % (53.33 %) and formulation A 0.056 % (63.33 %), 0.042 % (56.67 %) and 0.035 % (53.33 %). However, *B. b. alone* registered the lowest (36.67 %) mortality.

At 10 DAT, the cumulative mortality was highest (80.00 %) in the formulation B 0.056 % which was on par with formulation A 0.056 % (76.67 %), 0.042 % (73.00 %), 0.035 % (70.00 %) and 0.028 % (66.67 %) and formulation B 0.042 % (76.67 %), 0.035 % (73.33 %) and 0.028 % (70.00 %). The lowest (56.67 %) kill was noticed in control (*B.b.* alone).

At 14 DAT, the highest (86.67 %) mortality of the pest was observed in formulation A 0.056 % and formulation B 0.042 and 0.056 %. However, these were at par with formulation A 0.028 % (80.00 %), 0.035% (83.33 %) and 0.042 % (83.33 %) and formulation B 0.028 % and 0.035 % (83.33%). Control (*B.b.* alone) caused the lowest (63.33 %) mortality.

(7) observed that mortality of 1st instar nymphs of the mango mealy bug, *Drosichamangiferae* was noticed after 3 days, and cumulative mortality was found to increase with time for all concentrations. They also reported 100 % mortality of 1st instar nymphs of the pest at 14 days after treatment (DAT) with the highest concentration (6 X 10^9 conidia ml^{-1}). (14) reported that at 72 h after treatment, groundnut oil formulation recorded 100 per cent mortality of the adults (*Bemicia tabaci*) followed by coconut (97.8%), sunflower (85.6%) and castor oil (64.4%). These studies clearly suggest that, *B. bassiana* is a potent bioagent against *B. tabaci*, when it was formulated with 1% coconut oil followed by groundnut, sunflower and castor oils. (15) reported the potential of oil emulsion formulations of *B. bassiana* against silverleaf whitefly in greenhouse. (16) reported that the liquid formulations A (VGTA 50512) and B (VGTA 502105) of *V. lecanii* comprising various adjuvants glycerol, Tween 80 and arachnid oil were significantly superior to untreated control recording 74.22 to 92.22 per cent mortality of grape mealy bug (*M. hirsutus*) at 14 DAT. The increase in bioefficacy of *B. bassiana* against mealy bug by addition of oils, glycerol, Tween 80 as adjuvants is in agreement with present findings.

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