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Pot culture studies of entomopathogenic fungi against, selected species of mealybugs present in different crops

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Abstract
In Pot culture studies, LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *Phenococcus solenopsis* population were 278.61, 266.69, 250.79, 334.09, 301.71, 217.65 and 190.30 hours, respectively. It shows that LlMO2 is most effective against *P. solenopsis.* LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. marginatus* population were 270.11, 278.42, 260.89, 357.14, 312.02, 226.47 and 197.82 hours, respectively. It clearly indicates that is most effective against *P. maraginatus.* Pot culture studies on *M. hirsutus,* revealed that LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations were 256.12, 263.38, 247.92, 305.98, 293.99, 222.28 and 194.77 hours, respectively. Results clearly shows that that LlMO2 is most effective against *M. hirsutus.* The LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *F. virgata* population were 249.96, 258.44, 241.68, 320.06, 293.98, 211.03 and 187.67 hours, respectively. Results clearly indicates that that LlMO2 is most effective against *F. virgata.*

Keywords: Pot culture, *Lecanicillium lecanii,* mealy bugs, LT_{50}

1. Introduction
Entomopathgenic fungi nearly 700 species are existing but currently known, only 10 species have been, or are presently being, developed for control (Robert and Hajek, 1992; Hajek & Leger, 1994) [6, 7]. These entomopathogenic hypomycetes fungus have great ability as biological control agents against insect pests and in an important component within integrated pest management systems. In world-wide they are being developed for the control of many pests of agricultural importance (Ferron, 1985) [4]. It has emerged as the most promising and extensively researched biocontrol agent that can suppress a variety of economically important insect pests (Kaur and Padmaja, 2008) [8]. *Lecanicillium lecanii* (= *Verticillium lecanii*) (Zimm.) Zare & W. Gams is having the ability for control of whiteflies, aphids and other insect pests.
Brown (1971) [3] explained that some 130 species of arthropods of agricultural and veterinary importance and importance to human health (102 species) have been found to be resistant to chemical insecticides. A large number of pesticides being used are poisoning in nature to men and other warm blooded animals and also leave residues. Residues of pesticides is due to inherent physio-chemical properties and depends on a number of namely (1) crop and their varieties with particularly leaf, stem, fruits etc., (2) climate conditions such as temperature, rainfall (3) pH of soil type. (4) Texture of soil etc.
By understanding the present scenario, the ill effects of a chemical pesticides on human health and the environment, development of resistance in pests to pesticides and higher level of pesticides residue in food items. Now, it is a time to develop suitable alternatives to chemical pesticides for use in pest control. In the search for new avenues in biological control, the importance of entomopathogens has been highlighted as an environmentally friendly pest control method. Therefore, it is imperative to evolve an effective and ecofriendly method for the management of four species of mealybugs (*Phen*a coccus solenopsis, *Paracoccus marginatus, Maconellicoccus hirsutus, Ferrisia virgata*) infesting different crops under pot culture conditions

2. Material and Methods
2.1 Exploration of entomopathogenic fungi *Beauveria bassiana, Metarhizium anispoliae* and *Lecanicillium lecanii* present in different agro climatic regions
2.1.1 Fungal collection and isolation
Entomopathogenic fungi were collected from different areas. Collection sites are listed in Table 1. Infected insect specimens were generally collected in 20 ml plastic containers lined

with dry tissue paper that were sterilized by autoclaving. In most cases specimens were stored at 4 °C for up to a week before isolation of cultures. Specimens were examined under a dissection microscope to confirm fungal infection. Each field-collected cadavar was cut into many pieces, and these pieces were then surface sterilized by 1% sodium hypo chloride solution ($NaOCl_2$) for 30 sec. The sterilized pieces were then transferred to both Potato Dextrose Agar (PDA) and Sabouraud dextrose agar yeast (SDAY) with streptomycin sulphate and incubated at a temperature of 25 ^{0}c for 15-20 days for complete growth.

For isolations, a flamed inoculating needle was used to cut a small (approximately 1mm^3) cube of agar which was gently wiped over conidiophores to pick up conidia. Conidia were inoculated at four equidistant points on each of two or three plates and incubated at 20 °C. Cultures were examined daily to confirm germination and to check contamination of fungi. For preliminary identification of fungal species, conidiogenous structures were mounted in lactic acid and examined at 100x magnification.

2.1.2 Preparation of media

2.1.2.1 Potato dextrose agar (PDA) medium

To prepare the PDA medium, 200g peeled potatoes were cut into small chips and boiled in 500 ml distilled water. Fully boiled potato extract was separated by using doubled layer muslin cloth, and 20 g of dextrose was added to the extract. In another flask, remaining 500 ml distilled water was taken and allowed agar to melt by boiling. The molten agar was stained through double layer muslin cloth and mixed with potato extract solution. The volume was made up to 1000 ml by adding distilled water and pH of the medium was maintained at 6.5. The medium was poured into culture tubes and conical flasks, plugged by non-absorbent cotton wool and then sterilized in an autoclave at 121 ^{0}C (15 lb pressure per square inch) for 30 min.

2.1.2.2 Sabouraud dextrose agar yeast extract (SDAY) medium

To prepare the SDAY medium, 20g of dextrose, 10g of peptone and 5g of yeast extract were added in one flask containing 500ml distilled water. In another flask, remaining 500 ml distilled water was taken and allowed 20g of agar to melt by boiling. The volume was made up to 1000 ml by adding both and pH of the medium was maintained at 6.5. The medium was poured into culture tubes and conical flasks, plugged by non-absorbent cotton wool and then sterilized in an autoclave at 121 ^{0}C (15 lb pressure per square inch) for 30 min.

2.1.3 Mass production of entomopathogenic fungi

Broken rice of 50g was taken into the bottle and added 50 ml of 1% yeast extract, Soak it for overnight and sterilize in autoclave at 15 PSI for 30 minutes. After cooling, inoculate with 3 ml spore suspension (10^8 conidia per ml) in laminar air flow chamber, incubate at room temperature of (26^0 C) and relatively humidity of 80 percent condition for 20 days. Harvest and air dry the digested material and grind the digested material and dry once again to bring down the moisture below 9 percent. Assess spore concentration per g using haemocytometer and then dilute with commercial talc to bring down the conidial load to required strength. Pack in bag and store under refrigerator (4 ^{0}C) (Nagaraja, 2005).

2.1.4 Mass production of pure cultures of *M. anisopliae, B. bassiana* and *L. lecanii*

Sabourad's Dextrose Agar media enriched with yeast extract (SDAY) was used for production of *M. anisopliae, B. Bassiana* and *L. lecanii.* The media composed of Dextrose 40 g, peptone 10 g, Agar 15 g, yeast extract 10 g in 1000 ml distilled water (Bell, 1981). The inoculated plates were incubated at room temperature (26 ± 1 ^{0}C) and observed daily for the development of colonies. From such colony, a small quantity of inoculum was taken and transferred to SDAY slants and maintained as pure culture.

2.2 Pot culture experiment on *Phenacoccus solenopsis, Paracoccus marginatus, Maconellicoccus hirsutus* and *Ferrisia virgata*

Earthen pots (30 cm height and 15 cm dia.) were filled with sun dried loamy soil and cow dung manure (3:1 w/w) and the pots were irrigated two days before sowing the seed. The cotton variety (Surabhi) was selected based on their known susceptibility to test insect. The cotton seeds were soaked in water for 12 h before the sowing. Three young seedlings were kept intact and remaining plants were thinned after a foot height growths, remaining two were also thinned and one plant was maintained per pot. All the recommended fertilizers and irrigations were followed and female insects were released in to each plant, 15 days earlier to spray regimes. The numbers of harvested spores per gram of substrate are counted by using haemocytometer and the required concentration made over. Each treatment of entomopathogenic fungal spores (1.0 x 10^8 spores per ml) was sprayed with hand atomizer. The control plants were sprayed with the spores and bio pesticide free Tween-80 (0.02% v/v) solution. Population of insects on plant was counted before the spray and the changes in populations noted at different time intervals (3, 5, 7, 10 and 15 days) after the spray. The adult mealybug population were counted on 5 cm length on twig. At the end of season, plants were harvested and their lint + seeds from dehiscent capsule (cotton) were collected and weighed (Plate 8). The differences between control and among treatments were analyzed. The experiment was completely randomized designed and the mortality percentage was corrected using Henderson and Tilton (1955) formula. The median lethal time (LT_{50}) was calculated from the cumulative mortality data using probit analysis (Finney, 1971) [5].

Sowing of cotton in pot culture

Experimental setup for bioassay in pot culture

Fig 1: Pot culture studies on different species of mealybugs

3. Results and Discussion

3.1 Results

3.1.1 Pot culture studies of entomopathogenic fungi against, selected species of mealybugs present in different crops

3.1.1.1 Pot culture studies of entomopathogenic fungi against *Phenacoccus solenopsis*

The LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. solenopsis* population were 278.61, 266.69, 250.79, 334.09, 301.71, 217.65 and 190.30 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. solenopsis* population were 704.70, 680.70, 629.26, 879.74, 764.25, 520.44 and 433.68 hours, respectively (Table 1).

Table 1: Effect of entomopathogenic fungi against *Phenacoccus solenopsis* in pot culture (Time-mortality response) (Location: Insectary, TNAU)

Treatment	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
BbBP1	y = 2.57x - 1.26	5.4798	278.61	244.96	316.88	704.70	536.39	925.82
BbGW2	y = 2.46x - 0.93	6.0637	266.69	235.35	302.20	680.70	519.89	891.25
BbBM3	y = 2.50x - 0.92	4.2518	250.79	223.22	281.77	629.26	489.50	808.91
MaBP1	y = 2.78x - 2.07	3.3920	334.09	285.03	391.58	879.74	635.40	1218.05
MaBM2	y = 2.67x - 1.64	4.0196	301.71	262.64	346.59	764.25	573.28	1018.82
LlBM1	y = 2.84x - 1.43	3.5947	217.65	196.96	240.51	520.44	422.78	640.67
LlMO2	y = 3.42x - 2.45	6.2616	190.30	173.96	208.18	433.68	365.89	514.02

All lines are significantly a good fit at 1% (P = 0.05)

3.1.1.2 Pot culture studies of entomopathogenic fungi against *Paracocus marginatus*

The LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. marginatus* population were 270.11, 278.42, 260.89, 357.14, 312.02, 226.47 and 197.82 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. marginatus* population were 700.54, 718.55, 688.07, 1051.91, 845.93, 570.58 and 483.21 hours, respectively (Table 2).

Table 2: Effect of entomopathogenic fungi against *Paracoccus marginatus* in pot culture (Time- mortality response) (Location: Insectary, TNAU)

Treatment	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
BbBP1	y = 2.29x - 0.52	0.4143	270.11	237.52	307.17	700.54	529.87	926.17
BbGW2	y = 2.32x - 0.66	0.1798	278.42	244.13	317.53	718.55	541.83	952.92
BbBM3	y = 2.23x - 0.33	0.4007	260.89	229.79	296.19	688.07	519.58	911.21
MaBP1	y = 1.96x - 0.16	1.8266	357.14	295.85	431.14	1051.91	705.10	1569.31
MaBM2	y = 2.15x - 0.43	0.2150	312.02	267.61	363.81	845.93	609.55	1173.98
LlBM1	y = 2.57x - 0.86	0.2072	226.47	203.19	252.41	570.58	450.79	722.23
LlMO2	y = 3.03x - 1.64	0.7174	197.82	179.31	218.23	483.21	395.18	590.86

All lines are significantly a good fit at 1% (P = 0.05)

3.1.1.3 Pot culture studies of entomopathogenic fungi against *Maconellicoccus hirsutus*

The LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *M. hirsutus* population were 256.12, 263.38, 247.92, 305.98, 293.99, 222.28 and 194.77 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *M. hirsutus* population were 611.88, 626.65, 600.63, 751.16, 741.89, 528.58 and 467.72 hours, respectively (Table 3).

Table 3: Effect of entomopathogenic fungi against *Maconellicoccus hirsutus* in pot culture (Time-mortality response) (Location: Insectary, TNAU)

Treatment	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
BbBP1	y = 2.75x - 1.55	3.0178	256.12	228.94	286.53	611.88	484.57	772.62
BbGW2	y = 2.84x - 1.81	2.7235	263.38	234.92	295.30	626.65	494.53	793.43
BbBM3	y = 2.66x - 1.26	2.8802	247.92	221.87	277.02	600.63	475.32	758.97
MaBP1	y = 3.11x - 2.73	4.1051	305.98	267.14	350.49	751.16	570.63	988.80
MaBM2	y = 2.71x - 1.68	4.3960	293.99	256.90	336.43	741.89	560.06	982.77
LlBM1	y = 2.84x - 1.46	2.6321	222.28	201.03	245.77	528.58	428.92	651.40
LlMO2	y = 3.05x - 1.69	3.2347	194.77	176.95	214.38	467.72	385.91	566.87

All lines are significantly a good fit at 1% (P = 0.05)

3.1.1.4 Pot culture studies of entomopathogenic fungi against *Ferrisia virgata*

The LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *F. virgata* population were 249.96, 258.44, 241.68, 320.06, 293.98, 211.03 and 187.67 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *F. virgata* population were 621.29, 632.90, 594.95, 840.34, 754.30, 500.18 and 440.70 hours, respectively (Table 4).

Table 4: Effect of entomopathogenic fungi against *Ferrisia virgata* in pot culture (Time-mortality response) (Location: Insectary, TNAU)

Treatment	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
BbBP1	y = 2.54x - 1.01	2.9967	249.96	222.82	280.42	621.29	485.59	794.90
BbGW2	y = 2.64x - 1.29	3.1511	258.44	230.07	290.31	632.90	495.14	809.00
BbBM3	y = 2.61x - 1.11	3.3243	241.68	216.34	269.98	594.95	469.57	753.79
MaBP1	y = 2.51x - 1.34	2.2293	320.06	274.88	372.66	840.34	612.68	1152.59
MaBM2	y = 2.47x - 1.11	1.8850	293.98	256.27	337.24	754.30	564.89	1007.22
LlBM1	y = 2.93x - 1.59	4.1398	211.03	191.47	232.60	500.18	409.68	610.68
LlMO2	y = 3.27x - 2.11	5.2373	187.67	171.02	205.93	440.70	368.60	526.83

All lines are significantly a good fit at 1% (P = 0.05)

3.2 Discussion

3.2.1 Pot culture studies of entomopathogenic fungi against *Phenacoccus solenopsis*

The LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. solenopsis* population were 278.61, 266.69, 250.79, 334.09, 301.71, 217.65 and 190.30 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. solenopsis* population were 704.70, 680.70, 629.26, 879.74, 764.25, 520.44 and 433.68 hours, respectively. Aziz ahmed (2013) reported that *P. solenopsis* mortality was caused by four strains *B. bassiana* PDRL1187 (LT50 321.6 hrs), *M. anisopliae* PDRL526 (LT50 331.2 hrs) and *I. fumosorosea* PDRL891 (LT50 417.6 hrs) under screen house conditions. Vestergaard *et al*. (1995) and Brownbridge *et al*. (1994) showed that high mortality of *Frankliniella occidentalis* could be obtained with *B. bassiana*, *M. anisopliae* and *L. lecanii* at concentration between 10^7–10^8 conidia per ml.

3.2.2 Pot culture studies of entomopathogenic fungi against *Paracocus marginatus*

In the present study, LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. marginatus* population were 270.11, 278.42, 260.89, 357.14, 312.02, 226.47 and 197.82 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *P. marginatus* population were 700.54, 718.55, 688.07, 1051.91, 845.93, 570.58 and 483.21, respectively. This result corroborate with the findings of Espin *et al*. (1989) who observed probit analysis of the time mortality response (LT50) was 12.59 days shorter for *L. lecanii* than that of *B. bassiana* (16.06 days) and *M. anisopilae* (26.07 days).

3.2.3 Pot culture studies of entomopathogenic fungi against *Maconellicoccus hirsutus*

In the present experiment, LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *M. hirsutus* population were 256.12, 263.38, 247.92, 305.98, 293.99, 222.28 and 194.77 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *M. hirsutus* population were 611.88, 626.65, 600.63, 751.16, 741.89, 528.58 and 467.72 hours, respectively. The result in respect of effectiveness of *B. bassiana* was in conformity with Suresh *et al*. (2010) who reported that *B. bassiana* was moderately effective against mealy bugs.

3.2.4 Pot culture studies of entomopathogenic fungi against *Ferrisia virgata*

In the present study, LT_{50} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *F. virgata* population were 249.96, 258.44, 241.68, 320.06, 293.98, 211.03 and 187.67 hours, respectively. The LT_{95} of BbBP1, BbGW2, BbBM3, MaBP1, MaBM2, LlBM1 and LlMO2 formulations assessed against *F. virgata* population were 621.29, 632.90, 594.95, 840.34, 754.30, 500.18 and 440.70 hours, respectively.

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