

In vitro efficacy of *Beauveria bassiana* (Balsamo) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin and *Lecanicillium lecanii* (Zimmerman) against *Maconellicoccus hirsutus* (Green) and *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae)

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ABSTRACT

Maconellicoccus hirsutus (Green) and *Ferrisia virgata* (Cockerell) were mass cultured in insectary using hibiscus plants, potato sprouts and the different stages were maintained to conduct bioassay. Entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* was sub-cultured on SDAY. Spore suspensions of concentration (1×10^8 conidia/ml) of seven isolates (BbBp1, BbGW1, BbBM1, MaBp1, MaBm1, LiBm1, LiMo1) were prepared from the 15 day old culture of the fungi. A preliminary study on *B. bassiana* against *Maconellicoccus hirsutus* (Green) and *Ferrisia virgata* (Cockerell) female adults was done. untreated (sterile water) were used as controls. Mortality of pink mealy bug (*Maconellicoccus hirsutus* (Green)) adults under laboratory condition was recorded at different intervals where entomopathogens (% mortality after 9th day) like LiMo1 (93.33%), BbGW1 (80.00%) and MaBm1 (63.33%). The mortality of guava mealy bug [*Ferrisia virgata* (Cockerell)] adults under laboratory condition was recorded at different intervals where entomopathogens (% mortality after 9th day) like LiMo1 (96.55%), BbBp1 (86.21%) and MaBp1 (65.52%).

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INTRODUCTION

Mealy bugs (Hemiptera: Pseudococcidae) are important insect pests worldwide (Williams, 1985; Williams and Granara de Willink, 1992; Miller *et al.*,

2005; Abbas *et al.*, 2009 and Khuhro *et al.*, 2012). The *Maconellicoccus hirsutus*, infests hibiscus, citrus, coffee, guava, mango, mora, pigeonpea, peanut, grapevine, maize, asparagus, chrysanthemum, beans, cotton, soybean, cocoa and many other plants. The

mature female lays eggs in an egg sack of white wax, usually in clusters on the twigs, branches and bark of the host plant and also on the plant's leaves and terminal ends. The stripped mealy bug, *Ferrisia virgata* (Cockerell) was found to infest the leaves of different ornamental plants. It is a polyphagous pest damaging coffee, sweet potato, jute, groundnut, tomato, citrus, cotton, guava etc. causing enormous damage to them. The female lays about 300-400 eggs which hatch in few hours. The life cycle is completed in about 40 days. The small and big black ants tend *F. virgata* and they keep other insects including hymenopteran parasites away from the mealy bug. Insecticides of synthetic origin have been used to manage insect pests for more than 50 years (Charnley and Collins, 2007). Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include low cost, high efficiency, safety for beneficial organisms, reduction of residues in environment, and increased biodiversity in human managed ecosystems (Lacey *et al.*, 2001). In contrast to bacteria and viruses, they do not need to be ingested and can invade their host directly through the cuticle. That is why entomopathogenic fungi are capable of infecting non feeding mesh like eggs (Ujian and Shahzad, 2007 and Anand and Tiwary, 2009) and pupae of insects (Nguyen *et al.*, 2007 and Anand *et al.*, 2008). Fungal pathogen particularly, *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests (Lingappa *et al.*, 2005). The objective of present study was to critically evaluate the potential of entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae* (Metsch.) Sorokin and *Lecanicillium lecanii* (Zimmerman) (= *Verticillium lecanii*) for biocontrol of Mealy bugs.

MATERIAL AND METHODS

Rearing of pink mealy bug and guava mealy bug:

Pink mealy bug and Guava mealy bug were collected from the field and reared on hibiscus plant, potato sprouts in the insect rearing room of the department of Entomology, Tamil Nadu Agricultural University. New plants were added roughly every 4 weeks, and old plants were removed after the mealy bug had settled on the new plants. Population of the mealy bugs were maintained until completion of the research work.

Fungus	Strain code	Host source	Collection site
<i>Beauveria</i>	BbBp1	BPH	PBS, TNAU
<i>bassiana</i>	BbGW1	Grasshopper	Wetland, TNAU
	BbBM1	BPH	Mandya, Karnataka
<i>Metarhizium</i>	MaBp1	BPH	PBS, TNAU
<i>anisopliae</i>	MaBm1	BPH	Mandya, Karnataka
<i>Lecanicillium</i>	LiBm1	BPH	Mandya, Karnataka
<i>lecanii</i>	LiMo1	Mealybug	Orchard, TNAU

Laboratory bioassay:

The laboratory bioassay was carried out against female adults of mealy bug using the spray solutions prepared for field conditions by leaf dipping method. Hibiscus leaves and guava leaves were collected from plants and surface sterilized with alcohol before treatment. Treated leaves were kept on sterile moist filter paper in Petri plates and the stalk end of the leaf was put in eppendorf tube containing sucrose solution to maintain its turgidity. The eppendorf tube was plugged with cotton and cello tape. Female adults were released on each treated leaf in a Petri plate. To avoid any error in counting due to escape or other settling problem, the adults were first allowed to settle on treated leaf for 12 h, the leaf was then taken out of the Petri plate and transferred to another fresh and sterilized Petri plate and the settled adults of mealy bug were counted carefully. The Petri plate containing treated leaf with settled mealy bug adults was sealed with paraffin film to further reduce the chances of escape. Petri plates were stored at 25±2°C in BOD incubator. The data on mortality was recorded at different intervals after inoculation. To verify mortality or survival a fine hair brush was used to touch the body of insect to induce or detect movement. Individual were considered dead based on the absence of leg movement after stimulus or colour change. Treated insects cadavers were surface sterilized in absolute alcohol and placed on SDYA (Sabourou Dextrose Yeast Agar) medium for growth of fungi.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Laboratory evaluation:

In the bioassay, mortality of pink mealy bug adults

under laboratory condition was recorded at different intervals where entomopathogens (% Mortality after 9th day) like *V. lecanii* (93.33%), *B. bassiana* (76.67%) and *M. anisopliae* (63.33%). The mortality of guava

mealy bug adults under laboratory condition was recorded at different intervals where entomopathogens (% mortality after 9th day) like *V. lecanii* (90.00%), *B. bassiana* (80.00%) and *M. anisopliae* (66.67%).

Table 1 : *In vitro* efficacy of different isolates of entomopathogenic fungi on per cent net mortality of pink mealy bug at different hours of interval

Treatments	Dosage	Corrected cumulative mortality of pink mealy bug				
		1 DAT	3 DAT	5 DAT	7 DAT	9 DAT
T ₁	1x 10 ⁸ cfu /ml	13.33 (21.41) c	26.67 (31.09)c	46.67 (43.09)c	60.00 (50.77)d	76.67 (61.11)cd
T ₂	1x 10 ⁸ cfu /ml	6.67 (14.96)e	23.33 (28.89)d	40.00 (39.23)d	63.33 (52.73)b	80.00 (63.43)c
T ₃	1x 10 ⁸ cfu/ml	13.33 (21.42) c	26.67 (31.09)c	50.00 (45.00)b	60.00 (50.77)d	73.33 (58.92)d
T ₄	1x 10 ⁸ cfu/ml	3.33 (10.52) f	13.33 (21.41)f	26.67 (31.09)f	46.67 (43.08)e	63.33 (52.73)e
T ₅	1x 10 ⁸ cfu/ml	10.00 (18.44) d	20.00 (26.57)e	30.00 (33.21)e	43.33 (41.17)f	60.00 (50.77)e
T ₆	1x 10 ⁸ cfu/ml	16.67 (24.09) b	33.33 (35.27)b	56.67 (48.83)a	80.00 (63.46)a	90.00 (75.15)b
T ₇	1x 10 ⁸ cfu/ml	20.00 (26.57)a	36.67 (37.27)a	56.67 (48.83)a	76.67 (61.11)b	93.33 (84.67)a
Untreated control	--	0.00 (0.58)g	0.00 (0.58)g	0.00 (0.58)g	0.00 (0.58)g	0.00 (0.58)f
Mean	--	17.25	26.52	36.23	45.46	54.31
S.E.±	--	0.230	0.268	0.381	0.769	1.4291
C.D. (P=0.01)	--	0.672	0.784	1.115	2.248	4.1743
CV	--	1.63	1.24	1.29	2.07	3.22

Table 2 : *In vitro* efficacy of different isolates of entomopathogenic fungi on per cent net mortality of guava mealy bug at different hours of interval

Treatments	Dosage	Corrected cumulative mortality of guava mealy bug				
		1 DAT	3 DAT	5 DAT	7 DAT	9 DAT
T ₁	1x 10 ⁸ cfu /ml	13.33 (21.41) e	33.33 (35.26)b	50.00 (45.00) d	70.00 (56.79)b	86.21 (71.34)c
T ₂	1x 10 ⁸ cfu /ml	23.33 (28.88)c	33.33 (35.27) b	53.33 (46.91) c	70.00 (56.79) b	82.76 (65.47)c
T ₃	1x 10 ⁸ cfu/ml	23.33 (28.88) c	33.33 (35.26) b	46.67 (43.09)e	63.33 (52.74)c	79.31 (62.98) d
T ₄	1x 10 ⁸ cfu/ml	6.67 (14.96) f	20.00 (26.57) c	30.00 (33.23) f	46.67 (43.09) e	65.52 (54.04)e
T ₅	1x 10 ⁸ cfu/ml	16.67 (24.09)d	20.00 (26.57)c	30.00 (33.21) f	50.00 (45.00) d	65.52 (54.05) e
T ₆	1x 10 ⁸ cfu/ml	33.33 (35.26) a	46.67 (43.09) a	66.67 (54.74) a	80.00 (63.44) a	93.10 (75.00) b
T ₇	1x 10 ⁸ cfu/ml	26.67 (31.08)b	46.67 (43.09)a	63.33 (52.74)b	80.00 (63.44)a	96.55 (80.16) a
Untreated control	--	0.00 (0.58)g	0.00 (0.58)d	0.00 (0.58)g	0.00 (0.58)f	3.33 (10.52)f
Mean	--	23.14	30.71	38.68	47.73	57.53
S.E.±	--	0.401	0.407	0.521	0.561	1.889
C.D. (P=0.01)	--	1.173	1.189	1.522	1.638	5.517
CV	--	2.13	1.62	1.65	1.44	4.02

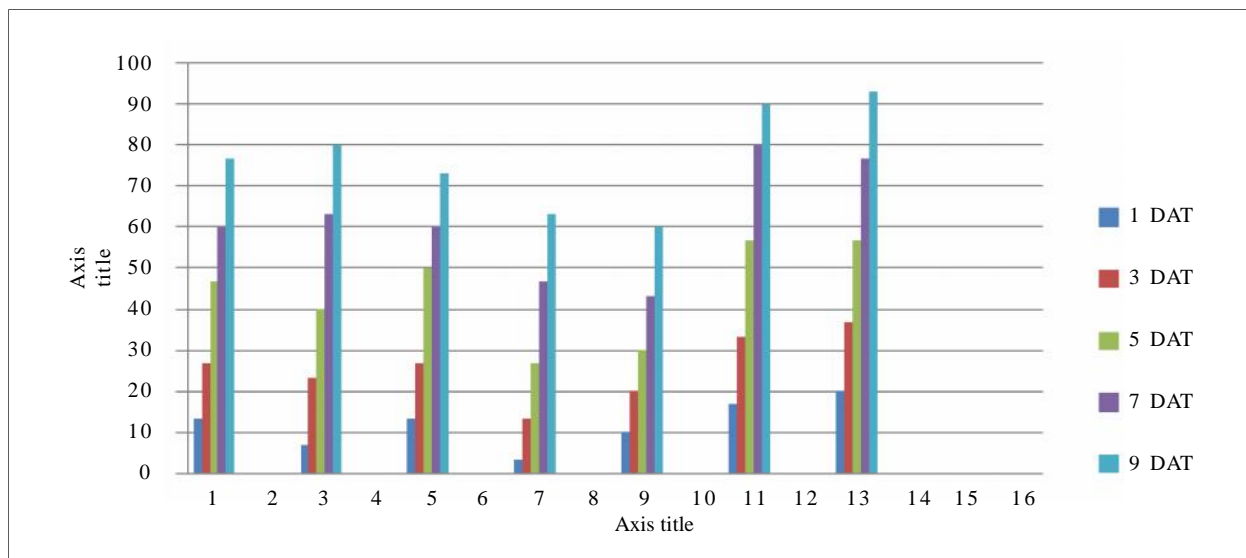


Fig. 1 : Different treatments on per cent net mortality of pink mealy bug at different hours of interval

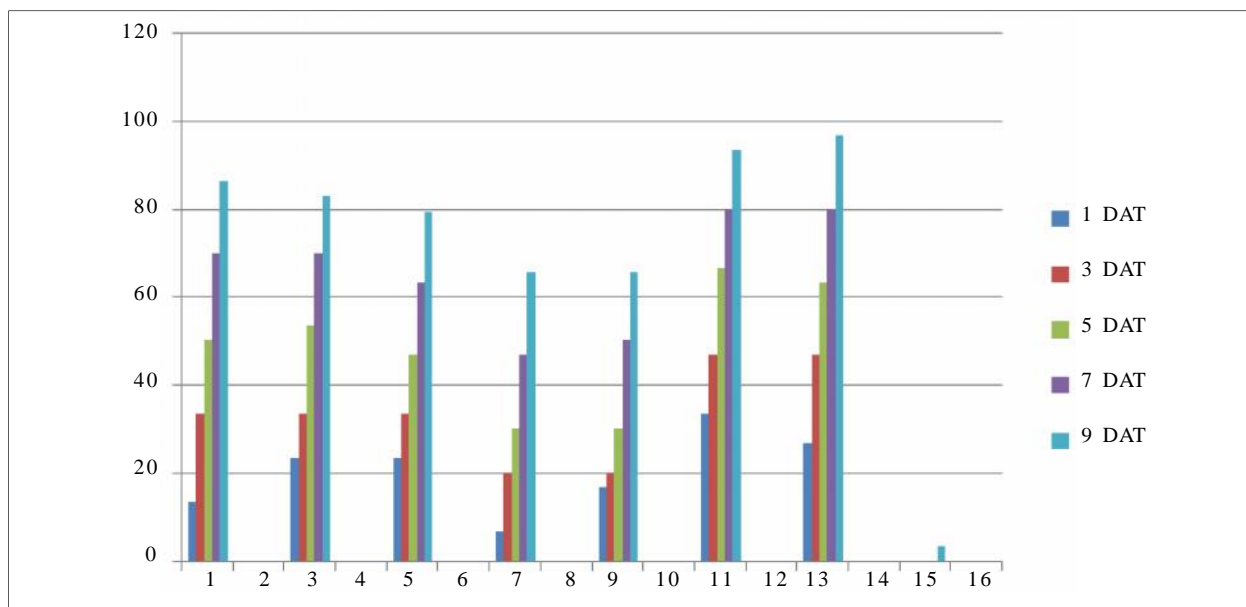


Fig. 2 : Different treatments on per cent net mortality of guava mealy bug at different hours of interval

Fungi forms one of the important biotic agents in biological control of insect pests. The fungus *Verticilium lecanii* (Zirm.) Veiges has been reported to infect mango leafhoppers, *A. atkinsoni* (Leth) (Nirwan and Upadhyaya, 1976), *I. clypeali* (Kumar *et al.*, 1983) and *I. nitidulus* walker (*I. niveosprosus*) and *I. nagpurensis* (Viraktamath *et al.*, 1994). Another entomopathogen *Beauveria bassiana* (Buls).Vuill has also been reported to infect mango leafhopper *I. clypealis* (Srivastava and Tandon, 1986). Entomopathogenic fungi, particularly the

deuteromycetes *Metarhizium* and *Beauveria* spp were alternative biopesticides for use in integrated pest management because of they have combine host specificity with proven safety (McCoy *et al.*, 1988), *B. bassiana* showed significant potential when applied as a mycoinsecticide against *Bemisia* spp in laboratory, green house and field test (Ben –Zeev *et al.*, 1994). Evaluation of n different isolates of *B. bassiana*, *M. anisopliae* and *V. Lecanii* against cotton mealy bug and papaya mealy bug under laboratory condition.

Pink mealy bug:

Entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* was sub-cultured on SDAY. Spore suspensions of concentration (1×10^8 conidia/ml) of seven isolates (BbBp1, BbGW1, BbBM1, MaBp1, MaBm1, LiBm1, LiMo1) were prepared from the 15 day old culture of the fungi. A preliminary study on *B. bassiana* against *Maconellicoccus hirsutus* female adults was done. untreated (sterile water) were used as controls. Mortality of pink mealy bug adults under laboratory condition was recorded at different intervals where entomopathogens (% Mortality after 9th day) like LiMo1 (93.33%), BbGW1 (80.00%) and MaBm1 (63.33%). However, the present findings are in comparison with Mote *et al.* (2003), reported that higher mortality of Gerbera aphid was observed in formulation of *V. lecanii* at 0.3 per cent (93.44%).

Guava mealy bug:

Spore suspensions of concentration (1×10^8 conidia/ml) of seven isolates (BbBp1, BbGW1, BbBM1, MaBp1, MaBm1, LiBm1, LiMo1) were prepared from the 15 day old culture of the fungi. A preliminary study on *B. bassiana* against *Ferrisia virgata* female adults was done. untreated (sterile water) were used as controls. Mortality of guava mealy bug adults under laboratory condition was recorded at different intervals where entomopathogens (% Mortality after 9th day) like LiMo1 (96.55%), BbBp1 (86.21%) and MaBp1 (65.52%). However, the present findings are in comparison with Mote *et al.* (2003), reported that higher mortality of Gerbera aphid was observed in formulation of *V. lecanii* at 0.3 per cent (93.44%).

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