Susceptibility of Cotton Mealy Bugs, *Phenacoccus solenopsis* and *Paracoccus marginatus* at Different Developmental Stages to Entomopathogenic Fungi

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Cotton mealy bugs, *Phenacoccus solenopsis* and *Paracoccus marginatus* have been reported from 15 genera of family Malvaceae, including cotton and many other plants of economic importance (Ben-Dov 1994). *P. solenopsis* appeared during the year 2005 and attained the status of serious pest in the cotton growing areas of India. Out break of mealy bugs on cotton and other hosts has threatened the economic production of many crops including cotton (Arif *et al.* 2009). The large populations reached by this insect are attributed, in large measure, to its polyphagous nature, short developmental cycles and high fecundity. Although mainly chemical control has been used against mealy bug, it is not always successful, possibly due to the waxy nature of the cuticle and nature of hiding (Blumberg and Van Driesche 2001; Ujjan and Shahzad 2007). The reduced efficiency of insecticidal control and ecological awareness have revived the interest in biological control. With the increasing importance of mealy bug control worldwide, there is more interest in the use of fungi for sustainable management of these important pests (Latge and Papierok 1988; Hajek and St Leger 1994). For the successful introduction of a fungal agent, information is not only needed on the biology of control agent but also on the most susceptible stage of pest species (Cubbertson *et al.* 2003). Hence, this study was conducted to determine the pathogenicity of *Metarrhizium anisopliae, Beauveria bassiana* and *Verticillium lecanii* on various developmental stages of cotton mealy bugs *viz.*, *P. solenopsis* and *P. marginatus.*

The present study was conducted at Central Institute for Cotton Research (CICR), Regional station, Coimbatore, Tamil Nadu, India. The experiment was laid out in completely randomized design (CRD). Homogeneous cultures of *P. solenopsis* and *P. marginatus* maintained at glass house were utilised for the experiment. The fungal isolates utilised in these experiments were collected form National Bureau for Agriculturally Important Insects (NBAII), Bangalore. Multiple dose assays were carried out with different conidial concentrations containing $1x10^5$ to $1x10^8$ viable conidia ml^{-1} in 0.02 per cent Tween 80® (Negasi *et al*., 1998). A sample of hundred mealy bugs (respective stages) was surface sterilized with 0.1% sodium hypochlorite solution and inoculated by immersing them in 10 ml each of conidial suspension of *M. anisopliae, B. bassiana* and *V. lecanii* for 10 seconds. For untreated control, insects were immersed in the 0.02 per cent Tween 80®. The treated insects were carefully transferred to petridishes with cotton leaves moistened with filter paper to maintain the turgidity. To determine the LC_{50} and LT_{50} of the fungal isolates, the insect's mortality count was recorded at 24 hours intervals until thirteenth day of treatment. From the tenth day, percentage of mortality due to observable mycosis was calculated for assay. The cadavers were incubated for 48 hours in a moist chamber and were monitored for hyphal emergence; mycelia from six randomly selected cadavers per isolate were sampled and cultured on SDAY (Saboured Dextrose Agar Yeast Medium) plates for confirming the identity. Each treatment was replicated thrice. Corrected percent mortality was worked out using Abbott's formula (Abbott 1925). The LC_{50} and LT_{50} were worked out using Finney (1967) method.

The LC_{50} of *M. anisopleae* on 1st instar of *P. solenopsis* was $8.7x10^5$ spores ml^{-1} whereas, it was $1.3x10^6$ and $5.4x10^6$ for second instar and adults, respectively (Table 1). The LC_{50} for *P. marginatus* was $5.0x10^5$ spores ml^{-1} for first instar grubs, $9.8x10^5$ for second instar and $1.3x10^6$ for adults. LC_{50} of *B. bassiana* on crawlers of *P. solenopsis* was $9x10^5$ spores ml^{-1} whereas, it was $3.9x10^6$ and $5.3x10^7$ for second instar and adults, respectively. The LC_{50} for *P. marginatus* was $8.2x10^5$ spores ml^{-1} for first instar grubs, $2.5x10^6$ for second instar and $1.4x10^7$ for adults (Table 2). *V. lecanii* tested against *P. solenopsis*, at $1x10^8$ spores ml^{-1}, recorded an LC_{50} value of $1.5x10^6$, $3.2x10^6$ and $1.3x10^7$ on first instar, second instar and adults respectively. *In case of P. marginatus* adults recorded an LC_{50} of $1.2x10^7$, $1.7x10^6$ and $5.9x10^5$ were recorded for adults, second and first instar respectively.

The LT_{50} ranges for different concentrations of *M. anisoplea* were 3.56 to 4.50 days against 1st instar, 4.87 to 5.97 days against 2nd instar and 5.66 to 6.27 days against adults in the

Table 1. Dose mortality response (LC_{50}) of different developmental stages of *P. solenopsis* and *P. marginatus to* entomopathogenic fungi

Stage of the insect	χ^2	Regression equation	LC_{50}(Days)	Fiducial limits Lower	Fiducial limits Upper
i. M. anisoplae					
P. solenopsis					
1st instar	0.1507	0.4603x + 7.7345	8.7 x 10^5	3.0 x 10^5	2.5 x 10^6
2nd instar	1.3487	0.4241x + 6.3245	1.3 x 10^6	4.3 x 10^5	4.0 x 10^6
Adult	0.2209	0.3002x + 7.0215	5.4 x 10^6	1.2 x 10^6	2.4 x 10^7
P. marginatus					
1st instar	0.2480	0.3297x + 6.8811	5.0 x 10^5	1.1 x 10^5	2.2 x 10^6
2nd instar	0.0342	0.4707x + 7.8191	9.8 x 10^5	3.4 x 10^5	2.7 x 10^6
Adult	1.0338	0.4216x + 6.321	1.3 x 10^6	4.5 x 10^5	4.1 x 10^6
ii.B. bassiana					
P. solenopsis					
1st instar	0.487	0.424x + 7.505	9.0 x 10 5	2.9 x 10 5	2.8 x 10 6
2nd instar	0.588	0.378x + 7.494	3.9 x 10 6	1.1 x 10 6	1.3 x 10 7
Adult	0.445	0.369x + 7.856	5.3x 10 7	1.3x 10 7	2.3x 10 8
P. marginatus					
1st instar	0.370	0.426x + 7.519	8.2 x 105	2.6 x 10 5	2.5 x 10 6
2nd instar	1.190	0.401x + 7.568	2.5 x 10 6	7.9 x 10 5	8.0 x 10 6
Adult	0.739	0.338x + 7.407	1.4 x 10 7	3.3 x 10 6	5.4 x 10 7
iii.V. lecanii					
P. solenopsis					
1st instar	0.602	0.478x + 2.044	1.5 x 10^6	5.6 x 10^5	4.2 x 10^6
2nd instar	0.214	0.407x + 2.355	3.2 x 10^6	9.8 x 10^5	1.0 x 10^7
Adult	0.458	0.475x + 1.620	1.3 x 10^7	4.7 x 10^6	3.7 x 10^7
P. marginatus					
1st instar	0.715	0.440x + 2.439	5.9 x 10^5	1.7 x 10^5	1.9 x 10^6
2nd instar	0.104	0.394x + 2.495	1.7 x 10^6	4.0 x 10^5	7.3 x 10^6
Adult	1.248	0.413x + 2.070	1.2 x 10^7	3.5 x 10^6	4.3 x 10^7

case of P. *solenopsis* (Table 2), while, the respective LT_{50} ranges were 3.88 to 4.71 days, 5.19 to 6.00 days, and 6.52 to 7.02 days, for *P. marginatus*. However, the shortest LT_{50} was recorded on at $1x10^7$ spores ml^{-1} on 1st instar (3.56 days) followed by second instar (4.87 days) and adults (5.66 days). For *B. bassiana*, the LT_{50} range was 4.09 to 4.95 days against 1st instar, 5.60 to 6.10 days against 2nd instar and 6.71 to 7.17 days against adults *P. solenopsis* while in case of *P. marginatus,* the respective LT50 ranges varied between 3.88 and 4.71 days against 1st instar, 5.19 to 6.00 days against 2nd instar and 6.52 and 7.02 days against adults. For *V. lecanii*, the LT50 ranges were 4.79 to 5.72 days against 1st instar, 5.65 to 6.47 days against 2nd instar and 7.05 to 7.22 days against adults of *P. solenopsis*. The LT 50 ranges in the case of *P. marginatus* were 4.55 to 5.46 days, 5.22 to 6.21 days and 6.93 to 6.96 days, respectively.

In the present study, the LC_{50} and LT_{50} values indicated that the reduction in virulence with the advancement of the developmental stages. The grub stages were more

Table 2. Time-mortality response (LT_{50}) of entomopathogenic fungi on different developmental stages of *P. solenopsis* and *P. marginatus*

Stage of the insect	χ^2	Regression equation	LT_{50}(Days)	Fiducial limits Lower	Upper
M. anisoplae* at 1x10^5 spores ml^{-1} on *P. solenopsis					
1st instar	2.9738	3.0528x + 3.0041	4.50	3.95	5.13
2nd instar	0.7694	3.1215x + 2.5773	5.97	5.30	6.73
Adult	0.8282	3.558x + 2.1623	6.27	5.63	6.98
M. anisoplae* at 1x10^6 spores ml^{-1} on *P. solenopsis					
1st instar	3.4183	3.3123x + 2.8459	4.47	3.95	5.05
2nd instar	5.3468	3.6702x + 2.2316	5.67	5.11	6.30
Adult	4.2066	3.8581x + 1.9328	6.24	5.64	6.89
M. anisoplae* at 1x10^7 spores ml^{-1} on *P. solenopsis					
1st instar	2.9231	3.6346x + 2.8375	3.94	3.47	4.45
2nd instar	2.5504	4.5032x + 1.6823	5.45	4.99	5.96
Adult	3.5926	4.3274x + 1.5647	6.22	5.68	6.81
M. anisoplae* at 1x10^5 spores ml^{-1} *P. marginatus					
1st instar	2.922	2.9207x + 3.1797	4.20	3.64	4.83
2nd instar	1.9051	3.4269x + 2.5942	5.03	4.50	5.64
Adult	7.0747	2.9714x + 2.6867	6.00	5.30	6.80
M. anisoplea* at 1x10^6 spores ml^{-1} *P. marginatus					
1st instar	4.2254	2.7041x + 3.3741	3.99	3.41	4.67
2nd instar	4.2868	3.4098x + 2.6481	4.89	4.36	5.49
Adult	6.6314	3.6847x + 2.1941	5.77	5.20	6.40
M. anisoplae* at 1x10^7 spores ml^{-1} *P. marginatus					
1st instar	1.2981	3.3692x + 3.1424	3.56	3.09	4.11
2nd instar	0.7751	4.107x + 2.1756	4.87	4.41	5.38
Adult	2.3158	4.3111x + 1.754	5.66	5.16	6.20
B. bassiana* at 1x10^5 spores ml^{-1} on *P. solenopsis					
1st instar	3.681	3.24x + 2.75	4.95	4.39	5.57
2nd instar	2.254	3.48x + 2.27	6.10	5.47	6.80
Adult	6.688	3.12x + 2.33	7.17	6.29	8.16
B. bassiana* at 1x10^6 spores ml^{-1} on *P. solenopsis					
1st instar	3.18	2.84x + 3.12	4.60	4.01	5.28
2nd instar	4.24	4.29x + 1.69	5.89	5.38	6.46
Adult	2.13	3.70x + 1.91	6.83	6.13	7.60
B. bassiana* at 1x10^7 spores ml^{-1} on *P. solenopsis					
1st instar	3.02	3.19x + 3.04	4.09	3.58	4.67
2nd instar	3.53	3.93x + 2.06	5.60	5.09	6.18
Adult	5.58	4.08x + 1.62	6.71	6.10	7.40

Continued

Table 2. *continued*

Stage of the insect	χ^2	Regression equation	LT_{50}(Days)	Fiducial limits	
				Lower	Upper
B. bassiana* at 1x10^5 spores ml^{-1} on *P. marginatus					
1st instar	3.435	2.98x + 3.00	4.68	4.11	5.34
2nd instar	2.319	3.35x + 2.40	6.00	5.36	6.71
Adult	6.244	4.02x + 1.59	7.02	6.34	7.77
B. bassiana* at 1x10^6 spores ml^{-1} on *P. marginatus					
1st instar	2.437	3.39x + 2.72	4.71	4.19	5.29
2nd instar	7.279	3.27x + 2.62	5.37	4.78	6.02
Adult	1.840	3.72x + 1.89	6.80	6.11	7.57
B. bassiana* at 1x10^7 spores ml^{-1} on *P. marginatus					
1st instar	1.207	3.40x + 2.99	3.88	3.41	4.43
2nd instar	2.188	4.07x + 2.09	5.19	4.71	5.72
Adult	1.900	3.66x + 2.02	6.52	5.86	7.25
V. lecanii* at 1x10^5 spores ml^{-1} on *P. solenopsis					
1st instar	7.548	3.20x + 2.57	5.72	5.09	6.43
2nd instar	5.199	3.46x + 2.19	6.47	5.79	7.23
Adult	2.921	3.51x + 1.98	7.22	6.42	8.11
V. lecanii* at 1x10^6 spores ml^{-1} on *P. solenopsis					
1st instar	3.187	3.17x + 2.69	5.35	4.76	6.02
2nd instar	5.281	3.12x + 2.57	5.99	5.32	6.75
Adult	1.774	3.67x + 1.89	7.07	6.33	7.89
V. lecanii* at 1x10^7 spores ml^{-1} on *P. solenopsis					
1st instar	6.149	3.15x + 2.86	4.79	4.23	5.42
2nd instar	2.364	3.51x + 2.36	5.65	5.07	6.30
Adult	1.758	3.60x + 1.94	7.05	6.31	7.89
V. lecanii* at 1x10^5 spores ml^{-1} on *P. marginatus					
1st instar	7.14	2.99x + 2.79	5.46	4.82	6.19
2nd instar	2.997	3.40x + 2.30	6.21	5.55	6.94
Adult	1.866	3.78x + 1.82	6.93	6.23	7.70
V. lecanii* at 1x10^6 spores ml^{-1} on *P. marginatus					
1st instar	3.686	3.28x + 2.76	4.82	4.28	5.43
2nd instar	3.161	3.36x + 2.49	5.56	4.67	6.22
Adult	2.683	3.69x + 1.88	6.98	6.25	7.78
V. lecanii* at 1x10^7 spores ml^{-1} on *P. marginatus					
1st instar	5.721	2.85x + 3.13	4.55	3.96	5.22
2nd instar	5.674	3.57x + 2.44	5.22	4.69	5.82
Adult	2.759	3.21x + 2.30	6.96	6.15	7.87

susceptible to infection by entomopathogenic fungi than adult stages. Earlier, Gindin et al. (2000) found that pathogenicity of *V. lecanii* to silverleaf whitefly, *Aschersonia aleyrodis* decreased with developmental stage and the older instars were less susceptible and adults were seldom infected (Fransen et al. 1987), whereas in the case of *Helicoverpa* spp, early stages were found less susceptible to *Nomuraea rileyi* (Mohamed et al. 1977). The susceptibility of different stages of the mealy bug to the pathogen and its ability to transmit infection among the various developmental stages and generations, lend support to the potential of entomopathogens for biological control of mealy bug.

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