Pathogenicity of entomopathogenic fungi on *Paracoccus marginatus* Williams and Granara de Willink

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ABSTRACT

Studies were conducted to determine the pathogenicity of *Lecanicillium lecanii, Metarhizium anisopliae* and *Beauveria bassiana* against papaya mealybug (*Paracoccus marginatus*) II instar nymphs under laboratory conditions. The nymphs of *P. marginatus* were susceptible to all three entomopathogenic fungi at varying levels. In general, insect mortality was increased with an increase in the exposure dose and period. When *L. lecanii*, *M. anisopliae* and *B. bassiana* were tested against *P. marginatus* nymphs, *L. lecanii* recorded the highest mortality of 73.33 % at 144 hrs after treatment (HAT). *M. anisopliae* and *B. bassiana* recorded 63.33 and 56.66% mortality, respectively, at 144 HAT. The LC_{50} values of *L. lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* for II instar nymphs of *P. marginatus* were 1.7 x 10^6 CFU/ml, 1.3 x 10^7 CFU/ml and 7.0 X 10^7 CFU/ml, respectively.

Keywords: Papaya Mealybug, *Lecanicillium lecanii, Metarhizium anisopliae* and *Beauveria bassiana*.

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INTRODUCTION

The papaya plant (*Carica papaya* Linnaeus) originated from Southern Mexico. India is the largest producer of papaya, contributing 25 per cent of total world production. In India around 1, 33, 000 ha of area is under cultivation with 56, 39, 000 MT fruit production (Anonymous, 2014). The fruit is known for its nutritional, digestive and medicinal properties. In addition, the immature papaya fruit contains a milky latex containing papain. It has several uses in industry *viz*., food processing, tanning and textile. Papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), natives of Mexico and Central America were introduced in the Caribbean and had become a pest in the early 1990's; since then it invaded most of the Caribbean archipelago in 1994 and spread to South America in 1999, the Pacific Island in 2002 and South Asia in 2008. Papaya mealybug is an exotic pest that recently invaded India (Muniappan *et al*., 2008). Its extensive spread to neighbouring countries is also reported. Most recently papaya mealybug has expanded to Bangladesh, Combodia, Phillippines and Thailand reaching the Reunion Island by 2010 (Muniappan *et al.,* 2011).

The infestation of mealybug appears as clusters of cotton like mass on the above ground portion of plants with long waxy filaments. Immature and adult stages of *P. marginatus* suck the sap of the plant and weaken it. The leaves become crinkled, yellowish and wither. The honeydew excreted by the bug and the associated black sooty mould formation impairs photosynthetic efficiency of the affected plant. In India, the insect assumed the status of major pest in 2009 when it caused severe damage to economically important crops.

Biopesticides are gaining importance in crop protection; hence to tackle the pest menace, a number of chemical insecticides are liberally sprayed on fruit crop which leads to several problems like toxic residues, elimination of natural enemies, environmental disharmony

and development of resistance. The demand is ever increasing for organically produced agricultural commodities all round the world and biological control agents have a vital role to reduce the pest damage. Entomopathogenic fungi (EPF) have a unique ability to attack insect by penetrating/ invading the insects cuticle and disabling them for control of sucking pests. Pathogenic fungi are easy for mass production using low-input technology, cause minimal effects on non-target arthropods, have a low mammalian toxicity and therefore ideal for IPM strategies. Though more than 900 species of entomopathogenic fungi representing 100 genera are currently known, only a few are commercially exploited. Interest on entomopathogenic fungi has been renewed in last quarter of 20^{th} century to better understand the role played by them in the natural regulation of insect populations. Only ten genera of EPF belonging to deuteromycetes are exploited for insect control, many species belonging to *Beauveria, Metarhizium, Lecanicillium, Nomuraea, Hirsutella* and *Paecilomyces* etc. have great potential for insect pest management.

MATERIAL AND METHOD

A laboratory experiment was conducted at All India Coordinated Research Project on Biological Control of Crop Pest and Weed, Research Laboratory, College of Agriculture, Pune during 2014 -2015 on hibiscus leaves as a factitious host at 27 ± 2^0C and RH 65 ± 5 %.

The working concentrations were prepared from the available EPF culture collected from the Biocontrol Laboratory, MPKV, Rahuri. A series of six concentration *viz.*, 2 X 10^5, 2 X 10^6, 2 X 10^7, 2 X 10^8, 2 X 10^9 and 2 X 10^{10} for entomopathogenic fungi compared with standard check *i.e.* buprofezin 25 SC (0.16, 0.32, 0.62, 1.25, 2.5 and 5.0 ml/L) were prepared for determining LC_{50} value of EPF under laboratory conditions.

Fresh and tender hibiscus leaves were collected from untreated hibiscus plant and washed with fresh water and dried under shade to evaporate the moisture. The petiole of each leaf was wrapped with wet cotton wool to keep the leaves fresh for a longer time and better movement of mealybug and placed individually in a petri dish. All the treatments were replicated thrice. The leaves were dipped in respective entomopathogenic fungi concentration and dried under shade and placed individually in petri dish. Ten II instar nymphs were released per replication in each petri dish on the treated leaf (Mandal *et al*., 2013). Mortality of II instar mealybugs were recorded at 24, 48, 72, 96,120 and 144 HAT. That the insect was dead confirmed by non movement when touch with the help of a hair brush. The data was statistically analysed by using probit analysis. The dose mortality regression lines were plotted by following the method of Finney (1971). LC_{50} values were expressed in terms of CFU/ml and ml/L.

Table 1. Efficacy of differential spore concentration of *L. lecanii* against II instar nymph of papaya mealybug under laboratory conditions

Spore conc. (CFU/ml)	Per cent mortality (HAT)						
	24	**48**	**72**	**96**	**120**	**144**	**Total**
1 x 10^5	0.00	0.00	0.00	3.33	10.00	16.67	30.00
1 x 10^6	0.00	0.00	3.33	6.67	13.33	16.67	40.00
1 x 10^7	0.00	0.00	3.33	10.00	16.67	20.00	50.00
1 x 10^8	0.00	0.00	6.67	10.00	16.67	23.33	56.67
1 x 10^9	0.00	3.33	6.67	13.33	16.67	23.33	63.33
1 x 10^{10}	0.00	3.33	10.00	13.33	20.00	26.67	73.33
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

RESULT **68**

Fungal species that were tested on *P. marginatus* and used for the pathogenicity test were *L. lecanii*, *M. anisopliae* and *B. bassiana*. The results of the bioassay of entomopathogenic fungi and buprofezin with six dosages at different exposure period against II instar nymph of papaya mealybug, *P. marginatus* are presented in Tables 1 -4. The LC_{50} value for 2nd instar nymph of *P. marginatus* was 1.7 x 10^6 CFU/ml with 1.3 X 10^5 and 1.4 X 10^7 CFU/ml as lower and upper fiducial limits, respectively (Probit equation = Y = 0.219 + 3.417 X; $\chi^2 = 0.058$).

Data on efficacy of *L. lecanii* 1.15 % WP showed that cumulative mean mortality ranged from 30.00 to 73.33 % (Table 2). From the results, it was revealed that the highest cumulative mean mortality of 73.33 % was observed with the highest *L. lecanii* dose *i.e.* 1 x 10^{10} CFU/ml. However, the lowest cumulative mean mortality of 30.00 % was recorded with the lowest dose i.e. 1 x 10^5 CFU/ml. The data on pathogenicity of *M. anisopliae* to *P. marginatus* the LC_{50} value for second instar nymph of *P. marginatus* was 1.3 x 10^7 CFU/ml with 1.7 X 10^6 CFU/ml as lower fiducial limit and 2.5 X10^8 CFU/ml as upper fiducial limits (Probit equation = Y = 0.218 + 3.231 X; Chi-square =0.106). Data indicated in Table 2 revealed that efficacy of *M. anisopliae* 1.15 % WP showed the cumulative mean mortality ranged from 23.33 to 63.33 %. From the results, it was revealed that the highest cumulative mean mortality of 63.33 % was observed with the highest *M. anisopliae* dose *i.e.* 1 x 10^{10} CFU/ml. However, the lowest cumulative mean mortality was recorded with the lowest dose *i.e.* 1 x 10^5 CFU/ml. Perusal of data from the Table 5 revealed that the LC_{50} value for II instar nymph of *P. marginatus* was 7.0 X 10^7 CFU/ml with 7.6 X 10^6 and 9.3 X 10^9 (CFU/ml) as lower and upper fiducial limits (Y = 0.199 + 3.235 X; $\chi^2 = 0.151$).

Table 2. Efficacy of differential spore concentration of *M. anisopliae* against II instar nymph of papaya mealybug under laboratory conditions

Spore conc. (CFU/ml)	Per cent mortality (HAT)						
	24	**48**	**72**	**96**	**120**	**144**	**Total**
1 x 10^5	0.00	0.00	0.00	3.33	3.33	16.67	23.33
1 x 10^6	0.00	0.00	3.33	6.67	6.67	16.67	33.33
1 x 10^7	0.00	0.00	3.33	6.67	10.00	20.00	40.00
1 x 10^8	0.00	0.00	6.67	10.00	13.33	20.00	50.00
1 x 10^9	0.00	3.33	6.67	10.00	16.67	23.33	60.00
1 x 10^{10}	0.00	3.33	6.67	10.00	20.00	23.33	63.33
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3. Efficacy of differential spore concentration of *B. bassiana* against II instar nymph of papaya mealybug under laboratory conditions

Spore conc. (CFU/ml)	Per cent mortality (HAT)						
	24	**48**	**72**	**96**	**120**	**144**	**Total**
1 x 10^5	0.00	0.00	0.00	0.00	6.67	13.33	20.00
1 x 10^6	0.00	0.00	0.00	3.33	10.00	16.67	30.00
1 x 10^7	0.00	0.00	3.33	3.33	10.00	20.00	36.67
1 x 10^8	0.00	0.00	3.33	10.00	10.00	20.00	43.33
1 x 10^9	0.00	0.00	6.67	10.00	13.33	23.33	53.33
1 x 10^{10}	0.00	3.33	6.67	10.00	13.33	23.33	56.67
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data on efficacy of *B. bassiana* 1.15 % WP showed that cumulative mean mortality ranged from 20.00 to 56.67 % (Table 4). From the results, it was revealed that the highest cumulative mean mortality of 56.67 % was observed with the highest *B. bassiana* dose *i.e.* 1 x 10^{10} CFU/ml. However, the lowest cumulative mean mortality of 20.00 % was recorded with the lowest dose *i.e.* 1 x 10^5 CFU/ml. In case of buprofezin bioassay study the LC_{50} value for II instar nymph of *P. marginatus* was 0.474 (ml/L) with 0.252 as lower fiducial limit and 0.742 (ml/L) as upper fiducial limits (Y = 1.002 + 5.324 X; Hetero-genicity = 0.093).

Table 4. Efficacy of buprofezin 25 SC against II instar nymph of papaya mealybug under laboratory conditions

Dose ml/l	Per cent mortality (HAT)						
	24	48	72	96	120	144	Total
0.16	0.00	0.00	3.33	3.33	10.00	16.67	33.33
0.31	0.00	0.00	3.33	6.67	13.33	20.00	43.33
0.63	0.00	0.00	3.33	10.00	16.67	23.33	53.33
1.25	0.00	3.33	6.67	13.33	16.67	23.33	63.33
2.50	0.00	3.33	10.00	16.67	20.00	26.67	76.67
5.00	0.00	6.67	13.33	16.67	20.00	30.00	86.67
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data indicated in Table 4 revealed that efficacy of buprofezin 25 SC the cumulative mean mortality ranged from 33.33 to 86.67 %. From the results, it was revealed that the highest cumulative mean mortality of 63.33 % was observed with the highest buprofezin 25 SC dose *i.e.* 5.0 ml/L. However, the lowest cumulative mean mortality was recorded with the lowest dose *i.e.* 0.16 ml/L. The pathogenicity of entomopathogenic fungi *viz*., *L. lecanii* 1.15 % WP, *M. anisopliae* 1.15 % WP and *B. bassiana* 1.15 % WP was carried out against II instar nymph of *P. marginatus* on hibiscus leaves which was easily available. The lowest LC_{50} value of 1.7 x 10^6 CFU/ml recorded in *V. lecanii* 1.15 % WP. However, the LC_{50} values for *M. anisopliae* 1.15 % WP and *B. bassiana* 1.15 % WP were recorded as 1.3 x 10^7 and 7.0 X 10^7 CFU/ml, respectively. These findings are in conformity with those of Jayachakravarthy (2002) who reported LC_{50} values of *L. lecanii* 5.98 x 10^6 CFU/ml against grape mealybug. Arthurs (2013) reported relatively less effective control of *S. dorsalis* treated with *B. bassiana*. Similarly, Benserradj and Mihoubi (2014) who reported the highest concentration of EPF cause higher mortality in mosquito larvae. Similar results were recorded by Indira *et al.* (2014) who recorded the lowest LC_{50} values of 1.03 x 10^7 and 3.47 x 10^7 spores/ml, respectively in *B. bassiana* against papaya mealybug. In case of buprofezin bioassay study, the LC_{50} value for II instar nymph of *P. marginatus* was 0.474 ml/L and it was confirmed with the results reported by Irulandi *et al*. (2006) who observed the effect of buprofezin (Applaud) 25 SC against mealybug, *P. lilacinus* under the laboratory conditions.

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