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Evaluation of entomopathogenic fungi against *Raoiella indica* Hirst (Acari: Prostigmata: Tenuipalpidae)

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ABSTRACT: Entomopathogenic fungi *Metarrhizium anisopliae, Beauveria bassiana* and *Lecanicillium lecanii* tested against immature and adults of *Raoiella indica* under laboratory condition with five different concentrations of each sprayed on leaf discs containing larvae, nymphs, and adults, indicated that all life stages were susceptible. Larval and nymphal stages were generally less susceptible than adults. Based on probit analysis, *L. lecanii* was the most virulent with LC_{50} of 8.15×10^5 conidia ml^{-1} and 1.30×10^5 conidia ml^{-1} followed by *M. anisopliae* 18.05 x 10^5 conidia ml^{-1} and 2.70×10^5 conidia/ml and *B. bassiana* (27.13×10^5 conidia ml^{-1} and 4.80×10^5 conidia ml^{-1}) for immature and adults, respectively. However the efficacy of the fungal pathogens evaluated clearly differs from that of the controls. These entomopathogenic fungi could be considered as an environmentally friendly alternative for biocontrol of *R. indica.*

KEYWORDS: Red palm mite; *Metarrhizium anisopliae, Beauveria bassiana, Lecanicillium lecanii*; pathogenicity

INTRODUCTION

Arecanut is an important commercial crop and it is attacked by an array of insect and non-insect pests. The pests infest all parts of the palm *viz.,* stem, leaves, inflorescence, root and nuts. As many as 102 insect and non-insect pests have been reported to be associated with arecanut palm (Nair and Daniel, 1982). Among these, the red palm mite, *Raoiella indica* Hirst. (Acarina: Tenuipalpidae) is the most serious pest mainly in young areca plantations and active infestation of leaves occurs after the onset of hot weather. The mite feeds on the underside of palm fronds of various hosts in the orders Arecales and Zingiberales. The mite attained economic significance when it was first reported as an invasive species in the Caribbeans in 2004 (Flechtmann and Etienne, 2004). It was reported as a serious pest of economically important fruit-producing trees like the coconut, *Cocos nucifera* and banana, *Musa* spp (Nagesha-Chandra and Channabasavanna, 1984; Welbourn, 2006) and it formed the first mite species in which feeding was observed through the stomata of its host plants (Ochoa *et al.,* 2011). Through this specialized feeding habit, *R. indica* interferes with the photosynthesis and respiration processes of its host plants. Mite infested palms display stunted growth and withering of leaves. *R. indica* is primarily controlled by acaricides in India. Long-term reli-ance on chemical acaricides results in pest resistance and residue problems. It is necessary to

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search for alternatives such as biopesticides to control *R. indica*. Advantages of biopesticides are their low mammalian toxicity, short environmental persistence, safety to beneficial and non-target organisms, as well as minimum risk of resistance development. Biological control, including the use of entomopathogenic fungi as part of an inte-grated pest management (IPM) strategy, is expected to reduce the dependence on synthetic acaricides. Most reports on the subject deal with insects and only few reports are available on Acari. However, research reports on the use of *Metarrhizium anisolpliae* (Metsch)*, Beaveria bassiana* (Bal.) and *Lecanicillium lecanii* (Zimm.) against *R .indica* are limited. In this respect, this study focused on examining the effectiveness of these entomopathogens against *R. indica* populations under laboratory conditions. The objective was to evaluate their virulence against this important mite pest and facilitate progress in microbial control with fungal pathogens. The experiment was conducted under laboratory conditions at Organic Farming Research Centre (OFRC), Organic Farming Research Centre, University of agricultural and horticultural Sciences (UAHS), Shivamogga.

MATERIALS AND METHODS

Preparation of fungal pathogen suspension{ Entomopathogenic fungi (*Beauveria bassiana Metarhizium anisopliae* and *Lecanicillium lecanii*) were used for evaluation against *Raoiella indica*. All the three fungi were cultured in standard Potato Dextrose Agar medium. After ten days of incubation, the spores were harvested, spore suspension prepared with distilled water and filtered through a double layered muslin cloth to get a clear spore suspension. Tween 80 (0.02%) was used to disperse the conidia uniformly in the solution. One ml of the spore suspension was poured on to haemocytometer to count the fungal spores and adjusted to required level. Serial dilutions were made from the stock spore suspensionto obtain the required concentrations for bioassay studies.

Determination of concentration range and pathogenicity: Before conducting bioassay in the laboratory, the procedure suggested by Daoust and Roome (1974) for bracketing of pathogens was followed. Accordingly**,** the serial dilutions were prepared to arrive at approximate range of concentrations inflicting mortality of mites between 10 and 90 per cent. Five different concentrations (10^4, 10^5, 10^6, 10^7, 10^8) were selected within each range and used for the determination of median lethal concentration (LC_{50}).

Two square centimeter leaf discs were cut from the areca palm, sterilized with 0.1 per cent sodium hypochlorite solution, later transferred to 5 ml distilled water blanks to remove excess solution**,** and dried (Gerson *et al.,* 1982). Such leaf discs were placed on water saturated cotton contained in petri dishes. In each leaf discs 50 immatures (larvae and nymphs)/ adults were transferred carefully using sterilized brush. Ten ml spore suspensions of the different entomopathogenic fungi were prepared by using 0.05 per cent Tween 80 solution to get uniform spray solution .The spore suspension of different concentrations were sprayed on mites contained on leaf discs with a hand atomizer spray. Such three replications and a control was maintained for each entomopathogenic fungi. The plates were incubated at 24±1^{0}C, 90 to 92 per cent RH in BOD incubators. Mortality of the mites was recorded 2, 4 and 6 days after spraying.

Lethal effects of entomopathogenic fungi were evaluated as per cent corrected mortality in the control variant according to Abbott's (1925) formula. For each concentration, mortality data from all the replicates were pooled and subjected to probit analysis. These data were analysed by IBM SPSS 23.

RESULTS AND DISCUSSION

Pathogenicity of Lecanicillum lecanii:

Maximum mortality of mites at two days after treatments (DAT) was recorded in $2x10^8$ conidia ml^{-1} (20.67 per cent ± 2.82 in immature and 27.33per cent ± 2.10 in adult stage) followed by $2x10^7$ conidia ml^{-1} which recorded 18.00 ±2.10 and 20.00 ± 1.66 per cent mortality in immature and adults respectively. At 4 DAT, the highest per cent mortality was observed at 2×10^8 conidia ml^{-1}

(64.00 ± 2.60 in immature and 72.00 ± 2.95 in adults), which was followed by 2×10^7 conidia ml^{-1} (58.67 ± 2.22 inimmature and 64.00 ± 1.02 in adult). The mortality rate of active stages of mite decreased gradually as the concentration of conidia decreased. At 6 DAT significantly higher mortality of both immature and adults was observed at 2×10^8 conidia ml^{-1} (92.00 ± 3.18 % in adults and 89.33 ± 2.60 % in immature), compared to rest of the concentrations (Table 1). The lethal concentration (LC_{50}) value at six days after treatment was (8.15×10^5 conidia ml^{-1} and 1.30×10^5 conidia ml^{-1})

Table 1. Bioefficacy of entomopathogenic fungi against immature and adult stages of *R. indica*

Entomopathogenic fungi	Concentration (Conidia/ml)	Mortality± SE (%)					
		2 DAT		4 DAT		6 DAT	
		Immature stage	Adult	Immature stage	Adult	Immature stage	Adult
Lecanicilliumlecanii	$2x10^8$	20.67± 2.82^c	27.33± 2.10^c	64.00± 2.60^d	72.00± 2.95^d	89.33± 2.60^{cd}	92.00± 3.18^d
	$2x10^7$	18.00± 2.10^{bc}	20.00± 1.66b^c	58.67± 2.22^c	64.00± 1.02^{bc}	68.00± 1.98^c	80.67± 2.66^c
	$2x10^6$	16.00± 1.62^b	14.00± 1.24^b	40.00± 1.58^{bc}	58.67± 1.41^c	47.33± 1.66^b	65.33± 1.90^c
	$2x10^5$	11.33± 1.89^b	10.00± 1.45a	26.00± 1.83^b	36.00± 1.62^b	36.00± 1.91^b	43.33± 2.24^b
	$2x10^4$	6.67± 1.10^a	8.67± 0.90^a	12.67± 1.42^a	24.00± 1.35^a	16.00± 1.04^a	32.67± 1.22^a
Metarhiziumanisopliae	$1.4x10^8$	20.00± 2.05^c	26.00± 1.90^c	60.00± 2.11^d	68.00± 1.60^d	82.67± 3.33^d	90.00± 3.10^d
	$1.4x10^7$	17.33± 1.30^c	22.00± 1.22^c	52.67± 1.95^d	59.33± 1.54^c	64.00± 2.90^d	77.33± 2.42^c
	$1.4x10^6$	13.33± 1.20^{bc}	13.33± 0.90^{ab}	36.00± 1.41^c	48.00± 1.22^{bc}	40.67± 1.66^{bc}	58.00± 1.60^b
	$1.4x10^5$	9.30± 1.48^b	10.00± 1.23^a	24.00± 1.62^b	32.67± 1.60^b	28.00± 1.84^b	39.33± 1.85^{ab}
	$1.4x10^4$	6.00± 0.69^a	8.00± 0.65^a	9.33± 1.10^a	22.67± 0.98^a	14.00± 1.20^a	28.00± 1.24^a
Beauveriabassiana	$2.6x10^8$	20.00± 1.92^d	24.00± 1.66^c	58.00± 1.39^d	66.67± 1.54^d	76.67± 2.22^d	86.67± 2.10^d
	$2.6x10^7$	16.00± 1.83^c	20.00± 1.90^b	49.33± 1.20^{cd}	54.67± 0.93^d	63.33± 1.90^c	73.33± 1.60^d
	$2.6x10^6$	17.33± 1.20^b	12.67± 1.27^{ab}	32.67± 0.88^c	43.33± 0.66^c	36.00± 0.90^{bc}	54.00± 0.98^c
	$2.6x10^5$	8.00± 1.68^a	9.33± 1.57^a	20.00± 1.10^b	28.67± 1.24^b	26.67± 1.22^b	32.00± 0.14^b
	$2.6x10^4$	7.33± 0.59^a	6.67± 0.44^a	9.33± 0.49^a	18.00± 0.40^a	14.67± 0.38^a	26.67± 0.24^a
Untreated Control	0.00	0.00	0.00	5.00± 0.82^e	3.87± 1.23^e	8.24± 0.44^e	5.33± 0.89^e

Table 2.Probit analysis of concentration-mortality responses of immaturesof *R. indica* to entomopathogenic fungi (6 DAT)

Entomopathogenic fungi	Regression Equation (Y= a + bx)	LC_{50} (Conidia/ml) (x 10^5)	LC_{90} (Conidia/ml) (x 10^5)	Fiducial limit (Conidia/ml) (x 10^5) at 95 % CI	Chi^2(÷2)
Lecanicilliumlecanii	Y= - 3.12+0.53 x	8.15	2421.22	5.23-12.66	4.59
Metarhiziumanisopliae	Y= - 3.11+0.50 x	18.05	6727.66	11.46-28.91	1.74
Beauveriabassiana	Y= - 2.91+0.39 x	27.13	17579.00	16.50-46.00	3.27

Table 3. Probit analysis of concentration-mortality responses of adults of *R. indica* to entomopathogenic fungi (6 DAT)

Entomopathogenic fungi	Regression Equation (Y= a + bx)	LC_{50} (Conidia/ml) (x 10^5)	LC_{90} (Conidia/ml) (x 10^5)	Fiducial limit (Conidia/ml) (x 10^5) at 95 % CI	Chi^2(÷2)
Lecancilliumlecanii	Y = - 1.96 + 0.33 x	1.30	751	0.70-2.30	1.83
Metarhiziumanisopliae	Y = - 1.96 + 0.33 x	2.70	1436	1.50-4.30	1.81
Beauveriabassiana	Y = - 2.59 + 0.46 x	4.80	3225	2.92-7.98	4.30

against immature and adults of *R. indica* respectively (Table 2 and 3).

Pathogenicity of Metarhizium anisopliae:

At 2 DAT *M. anisopliae* at 1.4 × 10^8 conidia/ ml caused highest mortality in immatures (20.00% ± 2.05) and adults (26.00 % ± 1.90). The concentrations 1.4 × 10^8 and 1.4 × 10^7conidia/ ml performed on par with each other and significantly superior to other concentrations of *M. anisopliae* (Table 1). *M. anisopliae* at 1.4 × 10^8 conidia/ml caused 60.00 ± 2.11 per cent mortality of immatures and 68.00 ± 1.60 per cent of adult mortality at 4 DAT followed by 2 × 10^7 conidia/ ml which recorded 52.67±1.95 and 59.33 ± 1.54 per cent mortality in immatures and adult mites respectively. The mean mortality of mite increased steadily as conidial concentration increased. At 6 DAT among the different concentration of *M. anisopliae*maximum per cent mortality was recorded at 2 × 10^8 conidia ml^{-1} both in immatures (82.67 ± 3.33) and adult (90.00 ± 3.10) mites, followed by 2 × 10^7 conidia/ ml (64.00 ± 2.90 in immatures and 77.33 ± 2.42 in adults) and were on par each other (Table 1). At 6 DAT and the lowest calculated LC_{50} value was 18.05 x 10^5 conidia/ml with fiducial limit ranging from 11.46 × 10^5 to 28.91 × 10^5 conidia/ml against immature and 2.70 × 10^5 conidia/ml with fiducial limit ranging from 1.50 × 10^5 to 4.50 × 10^5 conidia ml^{-1} against adults of *R. indica* (Table 2 and 3).

Pathogenicity of Beauveria bassiana:

At 2 days after treatment maximum mortality per cent of 20.00 ± 1.92 was recorded in immature and 24.00 ± 1.66 per cent in adults at 2x10^8 conidia/ ml, followed by 2 × 10^7 conidia ml^{-1} , where mortality rate of 16.00 ±1.83 per cent was recorded in immature and 20.00 ± 1.90 per cent in adult mites (Table 1). Different conidial concentration of *B. bassiana* was evaluated against active stages of mites and at four days after treatment, the highest mortality percentage was observed at 2x10^8 conidia/ ml concentration with 58.00 ± 1.39 in immature stages and 66.67 ± 1.54 per cent in adults, followed by 2 × 10^7 conidia/ ml which recorded 49.33 ± 1.20 and 54.67 ± 0.93 per cent mortality of adult and

immatures respectively. However, the mortality decreased gradually with the decrease in concentration. At six days after treatment *B. bassiana*at 2×10^8 conidia/ ml recorded significantly higher per cent mortality in adult stage (86.67 ± 2.10) and 76.67 ± 2.22 in immature stages compared to rest of the concentrations (Table 1). At 6 DAT the calculated lowest LC_{50} values for *B. bassiana* was 27.13×10^5 conidia ml^{-1} and 4.80×10^5 conidia/ml against immatures and adults of *R. indica* respectively (Table 2 and 3).

Overall, the efficacy of entomopathogenic fungi indicated that higher conidial concentration was more effective compared to rest of the treatments. All the five dosages of fungi proved their supremacy to uninoculated treatments. The least mortality of mites was observed at lower conidial concentration, this may be due to hyphal and conidial characters vary between the concentrations. These results indicate variability among the concentration which needs to be realized when being used for the development of effective bioinoculant and mass production. In the present study, it was noticed that at four and six days after infestation maximum mortality was observed in all the three entomopathogenic fungi *viz.,* of *L. lecanii, M. anisopliae* and *B. bassiana.* The different motile stages of *R. indica* varied in their susceptibility to these entomopathogenic fungi. It was observed that immature stages were generally less susceptible to fungal infection than adults. This might be due to integument being penetrated by the fungus and ecdysis. Moulting has been reported to be an important factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals (Sewify and Mabrouk, 1991).obtained are close to those reported by Sewify and Mabrouk (1991) who found that adult stages of the citrus brown mite were susceptible to the entomopathogenic fungus, *V. lecanii* Similarly, El Hady (2004) reported that adult stage *Eutetranychus orientalis*, was highly susceptible to *V. lecanii* compared to other motile stages.

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