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Laboratory Bioassay of Some Entomopathogenic Fungi Against Broad Mite (*Polyphagotarsonemus latus* Bank)

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ABSTRACT

Laboratory bioassay of three entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metch.) Sorokin, *Paecilomyces fumosoroseus* (Wise) Brown & Smith against broad mite (*Polyphagotarsonemus latus* Bank) was evaluated. Effect of these fungi on the broad mite egg was also investigated. Dose mortality bioassay revealed that *B. bassiana* (*Glenia celia* isolate, BbGc) caused mortality up to 80.88% at a dose 1 x 108 conidia mL^{-1} while *M. anisopliae* (*Phylotreta striolata* isolate, MaPs) and *P. fumosoroseus* (*Pteroma pendula* isolate, PfPp) caused 60 and 90% mortality, respectively. Significant relationship (P=0.05) was obtained between log concentration and probit mortality value for all the three isolates. The effective concentration of *B. bassiana* to kill 50% mite treated (EC_{50}) was the lowest (2.74 x 106 conidia mL^{-1}) followed by that of *P. fumosoroseus* (3.23 x 106 conidia mL^{-1}) and *M. anisopliae* (2.77 x 107 conidia mL^{-1}). The LT50 at comparable dosage (1 x 108 conidia mL^{-1}) of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* were 3.4 (1.4 - 5.1), 4.3 (3.3 – 5.8) and 2.8 (1.9 – 3.9) days, respectively. Therefore *B. bassiana* proved to be the most effective followed by *P. fumosoroseus* and *M. anisopliae*. However *P. fumosoroseus* caused mortality more quickly than others. There was poor infection on the mite eggs (10%) caused by *M. anisopliae* while no infection was recorded by *B. bassiana* and *P. fumosoroseus*.

Key Words: Bioassay; Broad mite (*Polyphagotarsonemus latus*); *Beauveria bassiana; Metarhizium anisopliae*

INTRODUCTION

The broad mite was recorded for the first time by Bank (1904) and described as *Tarsonemus latus* from the terminal buds of mango in a greenhouse. In Malaysia, broad mite better known as the yellow tea mite, is a constant problem in areas where chilli (*Capsicum annum)* is cultivated (Ibrahim & Low, 1998). It is distributed throughout the tropics and in greenhouses in the temperate regions. A wide variety (about 50) of agriculture crops, ornamentals, and wild plants have been recorded as host in the temperate (Jeppson *et al*., 1975). In Philippines, this mite is a pest of young plants in greenhouses (tomato, potato, tobacco) and ornamental plants. *Polyphagotarsonemus latus* has an extensive host range. Ibrahim and Low (1998) reported that five annual broad leaf weed species were harbouring these mites in the absence of chilli plant in the glasshouse, while Yang and Chen (1982) reported 21 species of weeds to be suitable as shelters for this mite.

Symptoms of infestation on chilli include downward curling of leaf margin and bronzing of new leaves and shoots. Severely attacked chilli leaves turn pale golden yellow with the underside of the leaf becoming purplish followed by abscission. The plants develop rosette and than die back (Ferron, 1978; Ibrahim & Low, 1998). This mite feeds by piercing plant cells and sucking up the sap that oozes from the wound (Waterhouse & Norris, 1987). It feeds on almost entirely lower leaf surface causing the leaves to become rigid and rolled down at the edges. Feeding injury is confined to young foliage or flower parts. As the leaves age they may split or crack open, producing a ragged appearance of various shapes. The lower leaf surface becomes bronzed, and injured flowers have parts or all the rays distorted or discolored. Severely attacked plants stop growing and die (Smith, 1939; Jeppson *et al.*, 1975). It has been reported to destroy 50% of the bean crop in New Guinea and lemon crop in part of South Africa. Liu *et al*. (1991) reported the damage by this species up to 100% on sweet peppers (*Capsicum* spp.) grown in greenhouses in Taiwan. It is difficult to control *P. latus* on certain plants, presumably because the mites are protected in curly leaves. Moreover, the mites are polyphagous, have short life cycle and quickly multiply in the dry season. As such effective management and control of this mite needs to be developed.

Various miticides have been used to control this species. However, control is more difficult in winter than in the summer due to lower greenhouse temperature. Several chemicals have been found effective such as dinitrophenol and synthetic pyretroids (Vaissayre, 1982). Foliar sprays directed at flushes or new growths have been the best approach thus far. In Hawaii fenbutatin (Vendex) and diazinon provided satisfactory control while endosulfan provided superior control while dicofol required two applications a week in severe attack (Hill, 1983). However, chemical control should be carefully applied to minimise pests resistance and interference with natural enemies that

offer biological control of other members of the pest complex. Fungal pathogens as biocontrol agents now are accepted and have become important as one of the biological control components in the IPM programmes. Therefore, this study was designed to investigate the potential of three entomopathogenic fungi *Beauveria, Metarhizium* and *Paecilomyces* against broad mite (*P. latus*).

MATERIALS AND METHODS

Fungal culture. Three fungi of entomopathogenic Hyphomycetes were used in this study; *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces fumosoroseus.* All the isolates were maintained on Potato Dextrose Agar (PDA) supplemented with 0.5% yeast extract (Difco) which had been sterilized for 20 min at 121°C at 1.05 kg cm^3. To prepare fungal inocula , conidia from 2 – 3 week old cultures were scraped from the surface of the plates with a sterile scalpel and suspended in 0.05% aqueous Tween 80. A Neubeur Haemocytometer was used to estimate the conidial concentration and subsequent appropriate dilutions were made thereof. A single spore re-isolation from mite cadaver was done before.

Mite culture. Chilli seedlings were grown in cups measuring 6 cm in diameter to be used to culture mite in the glasshouse. The mite was maintained and changed with fresh chilli seedling periodically. To prepare the same age of mite (homogenous), adult broad mites were transferred in the fresh chilli seedling periodically and observed daily.

Laboratory bioassay. One month chilli seedlings infested with mites were used in this experiment. The whole chilli leave infested with mites was placed underside up in a Petri dish (9 cm diameter) on a water-soaked 2-layer filter papers to ensure high relative humidity. Twenty young adult female mites were selected on chilli leaves described earlier with a fine soft brush. Conidia from 14-21days old culture suspended in 0.05% aqueous Tween 80 were inoculated against the eggs and adults of broad mites using a Sigma® hand atomizer. Bioassays were conducted using conidial concentration ranging from 1×10^2 to 1×10^8 conidial mL^{-1} in 0.05% aqueous Tween 80. All treatments were replicated six times. Percentage of eggs and adult infected were recorded up to seven days after treatment. Only cadavers with fungal growth were considered as a successful infection. Estimates of EC_{50} and LT_{50} and regression relationship for the dosage mortality line with 95% fiducial limit were obtained using a Probit programme (S103, Statistical Research Service, Canada Department of Agriculture) based on the procedure by Finney (1971).

RESULTS AND DISCUSSION

The pathogenicity of the three entomopathogenic fungi against the broad mite differed among each others. *Metarhizium anisopliae* (MaPs), *B. bassiana* (BbGc), and *P. fumosoroseus* (PfPp) could infect the broad mite and sporulated outside on the body of mite. The broad mite entered the state of moribund two days after treatment and fungal sporulation was observed over the surface of mite's cadaver 4-5 days after treatment. *Beauveria ba*ssiana sporulation over the mite's cadaver took a whitish colour. Wright *et al*. (1997) similarly reported that the silverleaf whitefly *Bemisia argentifolii* infected by *B. bassiana* displayed the colour that rapidly faded to white. Like *B. bassiana*, *P. fumosoroseus* and *M. anisopliae* demonstrated rapid external hyphal development and sporulation under moist condition. Initially, hyphal strands emerged from the anal region of the broad mite cadaver and then quickly covered the cadaver with profused hyphal growth followed by sporulation. Low infection on the eggs of the broad mite (10%) was observed at a dosage of 1×10^8 conidia mL^{-1} by *M. anisopliae* while no sign of infection displayed by *B. bassiana* and *P. fumosoroseus. Metarhizium anisopliae* appeared greenish colour when sporulation occurred while *P. fumosoroseus* appeared pinkish upon sporulation on the mite's cadaver.

Estimates of the median effective concentration (EC_{50}) with 95% fiducial limit computed for *B. bassiana* was 2.740×10^6 (4.73×10^5 - 3.67×10^7), *M. anisopliae* was 2.770×10^7 (1.46×10^7 – 6.03×10^7) and *P. fumosoroseus* was 3.230×10^6 (2.27×10^6 – 4.65×10^6) (Table I). These results showed that *B. bassiana* was the most pathogenic followed by *M. anisopliae* and lastly *P. fumosoroseus*.

Results of the probit analysis indicate significant relationships ($P < 0.05$) between log dosage and probit mortality. The LT_{50} values of BbGc, MaPs and PfPp isolate at comparable dosage of 1×10^8 conidia mL^{-1} was 3.349; 4.280 and 2.783 days, respectively (Table II).

The LT_{50} value at 1×10^7 conidia mL^{-1} for BbGc and PfPp increased to 5.495 and 3.693 days respectively while for MaPs isolate it was not computable because mortality was less than 50%. This means that a 10 fold increase in dosage significantly decreased the LT_{50} value of BbGc by

Table I. Result of probit analysis for isolates of *B. bassiana*. *M. anisopliae* and *P. fumosoroseus* against adult of the broad mite *P. latus* Bank

Isolate	A (intercept)	B ± SE (slope)	$\chi 2$	EC_{50}[c]	95 % FL[c]	EC_{95}[c]	95 % FL[c]
BbGc	1.871	0.486± 0.081	25.44	0.274E+07	0.473E+06- 0.367E+08	0.665E+10	0.247E+09- 0.285E+14
MaPs	1.459	0.476±0.038	5.928	0.277E+08	0.446E+08- 0.172E+11	0.603E+08	0.794E+11- 0.625E+12
Pf Pp	0.337	0.819±0.054	3.044	0.323E+07	0.227E+07- 0.465E+07	0.328E+09	0.169E+09- 0.754E+09

Probit analysis with 20 adult mite per replicate, 6 replicates per dose, 7 doses plus control per assay (N=960); control = zero mortality; [c] conidia mL^{-1}

Table II. Mortality infected and mean lethal time for varying dosages of *B. bassiana* (BbGc), *M. anisopliae* (MaPs) and *P. fumosoroseus* (PfPp) on broad mite (*P. latus* Bank)

Isolates	Dose[c]	Mean Infected (%)	a (intercept)	B ± SE (slope)	LT_{50} (days)	95 % FL
Bb	1×10^8	80.88	2.7662	4.2556 ± 1.2073	3.349	1.444 - 5.141
	1×10^7	52.50	2.6789	3.1367 ± 7588	5.495	4.092 - 10.82
	1×10^6	40.83	Na			
	1×10^5	6.67	Na			
	1×10^4	4.17	Na			
	1×10^3	0.00	Na			
	1×10^2	0.00	Na			
MPs	1×10^8	60.00	3.4207	2.5011 ± 0.4489	4.280	3.322 - 5.842
	1×10^7	35.83	Na			
	1×10^6	32.50	Na			
	1×10^5	10.83	Na			
	1×10^4	7.50	Na			
	1×10^3	0.00	Na			
	1×10^2	0.00	Na			
Pf	1×10^8	90.00	3.5145	3.3429 ± 0.5870	2.783	1.960 - 3.560
	1×10^7	65.83	3.6035		3.693	2.707 - 5.040
	1×10^6	28.88	Na			
	1×10^5	15.00	Na			
	1×10^4	1.67	Na			
	1×10^3	0.00	Na			
	1×10^2	0.00	Na			

Mortality infected with 20 adult mite per replicate, 6 replicates per dose, 7 dosages plus control per assay. Data from the control were not included since no death occurred. c: conidia mL^{-1} Na: LT_{50} is not available since mean % mortality infected was below 50 %; infection were recorded at seven days after inoculation

two days and PfPp by a day. The LT_{50} value recorded for *P. latus* was dependent on the dosage of the entomopathogenic fungi. As the dosages increased for all the three isolates tested, the LT_{50} value decreased. Based on their EC_{50} values, BbGc was the best isolate (lowest EC_{50}) followed by PfPp and MaPs isolates. However, the faster infectivity or shorter time to the broad mite mortality was caused by PfPp with 2.783 days to give 50% mortality, while *B. bassiana* took 3.349 days and *M. anisopliae* took 4.280 days to cause 50% mortality.

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