

**EFFICACY OF LABORATORY PREPARED WETTABLE POWDER FORMULATION OF ENTOMOPATHOGENOUS FUNGI *BEAUVERIA BASSIANA*, *METARHIZIUM ANISOPLIAE* AND *PAECILOMYCES FUMOSOROSEUS* AGAINST THE *POLYPHAGOTARSONEMUS LATUS* (BANK) (ACARI: TARSONEMIDAE) (BROAD MITE) ON *CAPSICUM ANNUM* (CHILLI)**

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**Abstrak:** Efikasi formulasi serbuk basah (WP) *Beauveria bassiana* (BbGc), *Paecilomyces fumosoroseus* (PfPp) dan *Metarhizium anisopliae* (MaPs) sebagai rawatan tunggal atau campuran ke atas *Polyphagotarsonemus latus* (Bank) (Acari: Tarsonemidae) (hamama lebar) dan kerosakannya terhadap *Capsicum annum* (pokok cili) berumur satu bulan telah dibandingkan dengan racun hamama amitraz di lapangan. Kesemua formulasi WP itu telah disemur berselang empat kali ke atas pucuk cili pada kadar  $1 \times 10^{10}$  konidia  $\text{ml}^{-1}$ . Kedua-dua *B. bassiana* dan *P. fumosoroseus* didapati mengurangkan populasi hamama secara signifikan dan seterusnya menghasilkan peratus pemulihan pucuk yang tinggi selepas semburan keempat berbanding dengan rawatan kawalan. Rawatan campuran *M. anisopliae* dan *P. fumosoroseus*, dan *M. anisopliae* dan *B. bassiana* tidak dapat menindas populasi hamama dengan memuaskan, oleh itu secara bandingannya lebih banyak pucuk telah diserang. Pucuk baru yang dirawat dengan *B. bassiana* menghasilkan pemulihan yang sungguh baik (93.33% perlindungan pucuk) dengan perbezaan yang tidak signifikan berbanding amitraz (96.67%). Amitraz lebih pantas menindas populasi hamama, iaitu kepupusan populasi hamama dicapai selepas semburan kedua. Walau bagaimanapun, formulasi WP *B. bassiana* sebagai mitisid adalah sama-sama berkesan dengan amitraz ke atas hamama lebar.

**Abstract:** The efficacies of wettable powder (WP) formulations of *Beauveria bassiana* (BbGc), *Paecilomyces fumosoroseus* (PfPp) and *Metarhizium anisopliae* (MaPs) as a single or combined treatments against the *Polyphagotarsonemus latus* (Bank) (Acari: Tarsonemidae) (broad mite) and its damage on one month old *Capsicum annum* (chilli) plants were compared with the acaricide amitraz in the field. The WP formulations were applied at four spray intervals on the chilli shoots at the rate of  $1 \times 10^{10}$  conidia  $\text{ml}^{-1}$ . Both *B. bassiana* and *P. fumosoroseus* significantly reduced the mite population and subsequently resulted in a high percentage of shoot recovery by the fourth spray compared with the control. Combined treatments of *M. anisopliae* and *P. fumosoroseus*, and *M. anisopliae* and *B. bassiana* could not adequately suppress mite population hence comparatively more shoots were infested. New shoots treated with *B. bassiana* resulted in excellent recovery (93.33% shoot protection) which was not significantly different from that of amitraz (96.67%). Amitraz could suppress the mite population faster whereby zero level of mite population was achieved after the second spraying. Nevertheless, WP formulation of *B. bassiana* as a miticide was as effective as amitraz against the broad mites.

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## INTRODUCTION

The *Polyphagotarsonemus latus* (Bank) (Acari: Tarsonemidae) (broad mite) has been documented as a serious pest on *Capsicum annum* (chilli) (Jeppson 1975; Liu *et al.* 1991; Ibrahim & Low 1998) and other solanaceous plants in Malaysia. The diagnostic symptom of infestation on chilli is the downward curling of leaf margin and bronzing of new leaves and shoots. The shoots crinkled and become rosetted and then die back ensues (Ferron 1978). This mite alone has been reported to cause severe damages on chilli plants up to 100% in China (Liu *et al.* 1991). Attempts to control this mite have produced mixed results. A common cultural control practice is to allow the production area free of weeds or any host plants that can serve as hosts during the non-cropping period. Ensuring mite-free seedlings for new plantings is another approach to a successful crop. However, the latter approach is difficult to achieve since this mite survives on a large variety of plant species. Lowering infested plants into water held at 43°C–49°C for 15 minutes will destroy the broad mite without damaging the plants (Anonymous 2001). However, this method is not practical nor economical for large scale or field planting. Miticides have been used to control this species and some successful ones include those such as formamidines, dinitrophenols and synthetic pyrethroids. However, chemicals should be applied judiciously while care should be taken to minimise development of resistance and not to interfere with the natural enemies that offer biological control of other members of the pests' complex. Biological control agents, such as the predatory mites *Amblyseius* spp. and *Neoseiulus* spp., are the most commonly reported natural enemies in tropical Asia, either in the greenhouses or in the fields. Fan and Pettitt (1994) reported that releasing ten or more *Neoseiulus barkeri* per plant of *Capsicum cv.* Hungarian Wax effectively reduced populations of *P. latus* to zero within a week in the glasshouse. However, their perpetual presence in commercial groves is very low.

The entomopathogenous fungi as mycoinsecticides are being intensively studied in the laboratory and field trials in recent years. They are known to infect all life stages of insects and are commonly found in aquatic, terrestrial and subterranean habitats (Ferron 1978). The apparent interest in using fungal pathogens is due to the self-inflicted problems that have arisen with chemical controls, and since then several successful programmes have been developed (Burgess & Hussey 1971; Ferron 1978). In Malaysia, serious scientific research in crop protection using entomopathogenous fungi against agricultural pests only started in the early 1990s with studies against cabbage caterpillars and other vegetable insect pests. By the year 2003 many of the important vegetable pest species from Lepidoptera, Homoptera, Isoptera and Coleoptera have been tested to be susceptible to various fungal isolates (Ibrahim & Low 1993; Ibrahim & Yew 1994; Ibrahim & Lee 1996; Ibrahim & Hashim 1998; Ibrahim & Tan 1999; Ibrahim & Liu 2001; Priyatno & Ibrahim 2002, 2004; Ibrahim & Yeong 2002; Hashim *et al.*

2002; Hashim & Ibrahim 2003; Ihsan & Ibrahim 2004; Ibrahim & Ihsan 2005). Amongst these significant findings was the discovery that these living insecticides were also efficacious against the red spider mites. Pena *et al.* (1996) had noted the potential of fungi as biocontrol agent of the broad mite; unfortunately, there is a dearth of additional information on the use of entomopathogenous fungus against the broad mite in the tropics (Ihsan & Ibrahim 2004). The current investigation therefore was carried out to evaluate the field efficacy of three genera of indigenous isolates of entomopathogenous fungi *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* as biological insecticides against the broad mite *P. latus* in Malaysia.

## MATERIALS AND METHODS

### Conidia Production

Air-dried conidia of the entomopathogenous fungi *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* singly isolated from infected cadaver were produced by long grain rice fermentation technique. The rice (150 g) were autoclaved with 60 ml of sterile water in autoclavable polyethylene bags (10 × 33 cm) for 15 min at 121°C and a pressure of 1.05 kg/cm<sup>3</sup>. The bags were loosely sealed with a 3 cm polyvinyl chloride pipe (3 cm diameter) and plugged with cotton during autoclaving. The rice was then cooled for 24 h after which 1 ml of 1 × 10<sup>8</sup> conidia ml<sup>-1</sup> suspension in 0.05% aqueous Tween 80 was introduced to each bag and left to incubate in the dark at 26 ± 1°C for 2 weeks. The 2-week old fermented culture was then harvested and the colonised substrate was spread evenly to air-dry and sandwiched between paper towels for 4 days to induce full sporulation. The dried conidia were then sieved through a 125 µm particle size following the methods of Daoust *et al.* (1983) and Belloa *et al.* (2000).

### Formulation and Application Equipment

Wettable powder formulation was prepared by thoroughly mixing pure air-dried conidia of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* with a commercial diluent clay at a ratio of 1:4 (20% w/w a.i.) in a sterile room. For comparison, the acaricide (Amitraz, 21.7% a.i.) applied at the recommended rate (1 ml L<sup>-1</sup> of water) was used. All treatments were sprayed using a Sigma<sup>®</sup> hand atomizer which could deliver an even volume diameter droplet spray of 75 µm (VMD) at the rate of 1 ml of the treatment on the terminal shoot of each plant. Spraying was done at late evening in order to minimise the possible suppressive effect of the sun's rays on the conidial germination.

### Test Plants

Chilli seedlings (Langkap var.) were raised in two sets, one for the observation in the greenhouse and the other for the field experiment. For the greenhouse study the seedlings were grown in plastic cups measuring 6 cm diameter by 10 cm, while for the field experiment the seedlings were grown in 22.5 cm polybags under insect-proof muslin cloth enclosure in the greenhouse. The growth medium consisted of a mixture of red clay soil, river sand and peat soil in the ratio of 3:2:1

by weight. The plants were watered daily and sprayed with foliar compound-fertilizer "Welgrow Standard" (NPK, 15:30:15) at the rate of 2 g L<sup>-1</sup> over the canopy every three days. All auxiliary buds and lateral shoots were carefully nipped off leaving only the terminal shoot to be maintained for ease of assessment. One month old plants of approximately equal height were used in the experiments.

### Greenhouse Trial

Plants showing symptoms of natural infestations with the leaves curled downward were chosen for this study. There were seven treatments with four replications arranged in a completely randomised design. The treatments were: (1) *B. bassiana* (BbGc); (2) *P. fumosoroseus* (PfPp); (3) *M. anisopliae* (MaPs); (4) *M. anisopliae* + *P. fumosoroseus* (MaPs+PfPp = 1:1); (5) *M. anisopliae* + *B. bassiana* (MaPs+BbGc = 1:1); (6) Mitac<sup>®</sup> (Amitraz) (Mi) and (7) Control (0.05% Tween 80) (Ctrl). The respective wettable powder formulated entomopathogenous fungi were suspended in 0.05% aqueous Tween 80 + 0.3 % w/v (3 g L<sup>-1</sup>) gelatine. Ten plants for every replicate were sprayed individually on the terminal shoots outside the insect-proof enclosure in order to minimise spray drift with one ml of the conidial suspension (1 × 10<sup>10</sup> conidia ml<sup>-1</sup>) four times spaced five days apart. Amitraz was applied at the rate of 1 ml L<sup>-1</sup> of water which is the standard recommended field rate against mite. Only plants showing the typical symptom of infestation with the leaves curled downward were chosen for this experiment. Plants showing no infested terminal shoots 1 week after the final spray were considered as successful treatments.

### Field Experiment

The field experiment was conducted in the Research Park of Universiti Putra Malaysia during the dry months of July and August, 2001. Plants from the greenhouse free of pests were transferred to the open field spaced 100 cm between blocks and 60 cm within and between rows. Each plant was transplanted with the bottom of the polybag torn open to minimise transplanting shock. Standard cultural practices were employed until termination of the experiment; the plants were watered daily and fertilized weekly with a compound fertilizer (NPK 15:15:15) at a rate of 1 g per plant. All plants were infested with 20 young adult female broad mites on the top leaves three days after transplanting when the plants had fully recovered. The treatment protocols were similar to that applied in the greenhouse study except that MaPs as a single treatment was excluded and as such there were six treatments with four replications arranged in a randomised complete block design. Treatments were applied once the typical symptom of downward leaf curls was manifested by most of the shoots (10 days after infestation). Thirty-five plants were used per replicate and each plant was treated separately within a portable cylindrical plastic barrier (50 cm diameter, 70 cm high) to minimise spray drift. Plants showing uninfested terminal shoots one week after the final spray were considered as successful treatments. Destructive sampling of shoots was taken randomly for mite and egg count four days after each spray. Four open leaves from two plants, i.e., two per shoot from each plant, were sampled from each treatment replicate and taken in plastic bag to the

laboratory for the count. Fifteen plants at the centre of each treatment plot (replicate) were assessed for uninfested shoots one week after the last spray.

### Statistical Assessment

Data from the greenhouse observation were analysed using one-way ANOVA while those from the field study were analysed using two-way ANOVA. All ANOVA were performed on  $\arcsin \sqrt{x}$  transformed values to stabilised the variance prior to analyses and the means were separated using Least Significant Difference (LSD) test at  $\alpha = 0.05$ . Statistical analyses were carried out using SAS version 8.12 (SAS System 2001).

## RESULTS AND DISCUSSION

### Effect of Treatments on Shoot Recovery

Results from the protected glasshouse trial were consistent with that from the field experiment. The highest percentage recoveries of chilli shoots from the glasshouse and the field was obtained from amitraz (100% and 96.67%) followed by BbGc (93.33%), BbGc+MaPs (80.0% and 76.67%), PfPp+MaPs (80.0% and 66.67%), PfPp (73.33% and 83.33%) and MaPs (46.67%) while essentially no protection was obtained from the control (0% and 8.33%) (Table 1).

These successful treatments as indicated by the high percentage recovery of shoot also recorded a low (highest being 8.75 mites) or zero mite infestation in the field seven days after the final spray application. Recovered shoots with absolutely no mite infestation occurred in amitraz and *B. bassiana* treatments. *Beauveria bassiana* was significantly the best amongst the fungal treatments, not only in terms of shoot recovery but also by zero mite infestation after the final spray application (Fig. 1). Shoots in the controls were severely stunted and heavily infested with the mites. The percentage of shoot recovery was shown to be negatively correlated with mite infestation (Fig. 2).

**Table 1:** Mean percent recovery of chilli shoots seven days after the final spray.

Treatments	Glasshouse <sup>a</sup>	Field <sup>b</sup>
BbGc	93.33 a	93.33 a
PfPp	73.33 bc	83.33 b
MPs	46.67 c	–
MPs+PfPp	80.00 ab	66.67 b
MPs+BbGc	80.00 ab	76.67 b
Mitac <sup>®</sup>	100.00 a	96.67 a
Control	0.00 d	8.33 c

Means within columns followed by the same letter are not significantly different at  $P = 0.05$  according to LSD.

<sup>a</sup> Ten chilli shoots per replicate.

<sup>b</sup> Fifteen chilli shoots per replicate.

This relationship was clearly demonstrated in the control with the lowest shoot recovery and the highest mite infestation. Amitraz and BbGc gave zero mite infestation and the highest rate of recovery of chilli shoot. Figure 1 shows a decrease in mite population in all the treatments after the first spray. It seemed that this was assisted by the high rainfall of 49.2 mm (July 28, 2002) (Fig. 3) that occurred one day before the second spray that had contributed to the decrease in the population. Thereafter, the population increased exponentially in the control while the population continued to decrease in the treated plots.

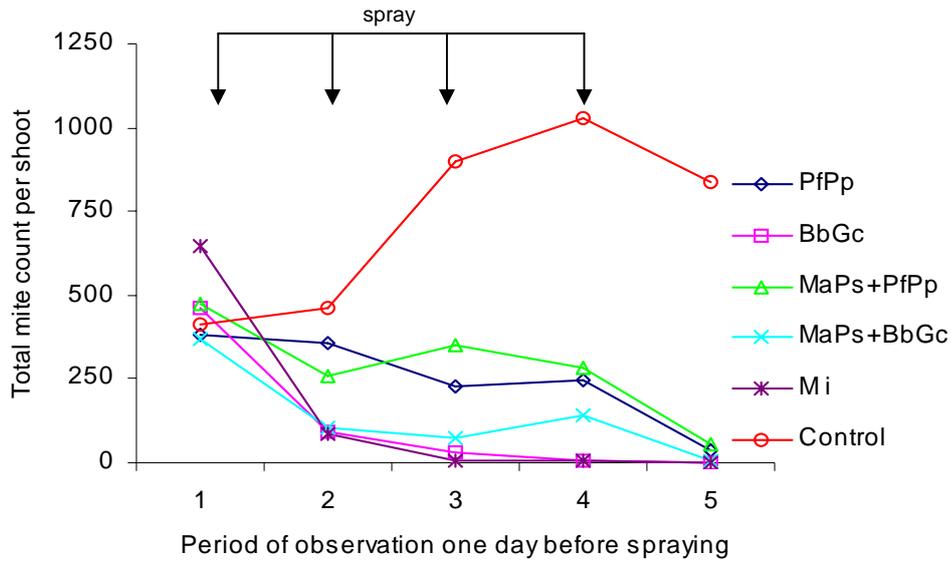


Figure 1: Broad mites sampled prior to each spraying.

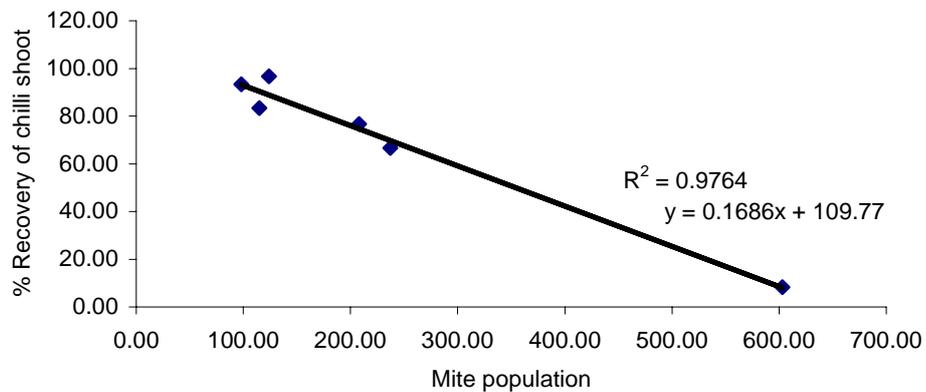


Figure 2: Relationship between mite population and percent recovery of shoot.

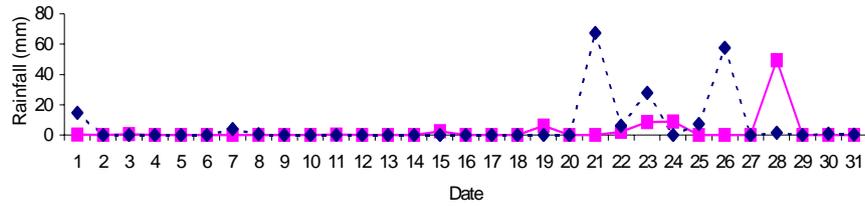


Figure 3: Total daily rainfall during field experiment, — July, ---- August 2002.

**Effect of Treatments on Mite Count**

In general, all the treatments successfully suppressed the mite population by the fourth and final spray compared to the control. However, compare to amitraz the entomopathogenous fungi killed fewer mites due to the typically slower action during the early periods of treatments. *Beauveria bassiana* successfully suppressed the broad mite population to zero by the fourth spray, thus repeated application was necessary. However, the suppressive effect of amitraz was faster whereby zero population level was achieved after the second spray application (Fig. 1). A similar trend was shown with the broad mite's egg count.

Figure 4 shows that there was a steady decline in egg counts in all the treatments and this coincided with the decreasing mite count after each application period. The performance of amitraz and *B. bassiana* (BbGc) were consistent with the zero egg count by the third and fourth spray respectively. Studies by Wright and Chandler (1992) also reported that *B. bassiana*, could suppress the *Anthonomus grandis* (boll weevil) in the field but comparatively

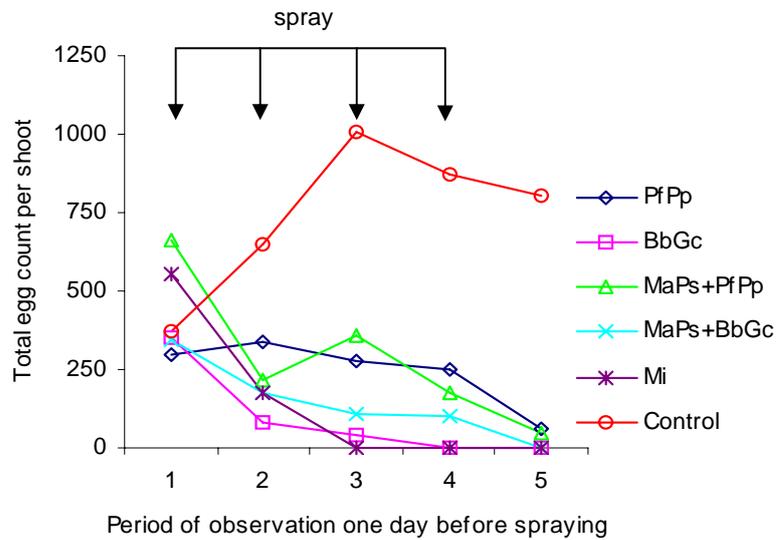


Figure 4: Total number of eggs recorded prior to each spraying.

slower than the insecticide dicotophos. Combined treatments of *M. anisopliae* and *B. bassiana*, and *M. anisopliae* and *P. fumosoroseus* did not adequately suppress mite population hence overall shoot re-growth was comparatively poor.

Microclimatic factors such as temperature and relative humidity are important in improving the efficacy of fungal treatment in the field. Total rainfall (July–August 2002) of 323.9 mm with daily average of 5.22 mm and 92.6% RH, and temperatures between 23.05°C–33.28°C were conducive for fungal growth. This is especially true with *B. bassiana* which has a thermal threshold of 35°C–37°C (Fargues *et al.* 1997). A similar situation was recorded by Hallsworth and Magan (1999) who reported that *B. bassiana* grew faster than *M. anisopliae* or *P. fumosoroseus* when moisture was freely available in the atmosphere. This study suggests that *B. bassiana* was the most effective in the field spray compared to *M. anisopliae* or *P. fumosoroseus*, and consistent with the lower EC<sub>50</sub> value for *B. bassiana* obtained in a pathogenicity test conducted earlier (Ihsan & Ibrahim 2004). *Beauveria bassiana* was also noted to be significantly effective when sprayed against the *Plutella xylostella* (diamondback moth) on crucifers (Vandenberg *et al.* 1998), *Hypothenemus hampei* (De La Rosa *et al.* 2000) and the *Eureuma loftini* (Mexican rice borer) (Legaspi *et al.* 2000).

Combinations of *M. anisopliae* with *B. bassiana* or *P. fumosoroseus* (1:1) did not give added advantage since they could not adequately suppress the mite population rapidly in the field, hence effective control was not achieved compared to *B. bassiana*. This could be due to the fact that *M. anisopliae* was the least effective and the combination contained lesser density of *B. bassiana* or *P. fumosoroseus* conidia. Thus, the infection was slow as the speed of kill is related to the number of conidia received by the individual pest (Bateman *et al.* 1993). Three repeated sprays spaced five days apart were needed before *B. bassiana* could completely suppress the broad mite population, an indication that its virulence was maintained throughout the duration of the experiment. In a permanent or a continuous cropping system *B. bassiana* could give long-term or permanent suppression of the mite if it could spread from the release site in subsequent seasons as had been demonstrated by Milner *et al.* (1982) with the *Zoophthora radicans* on the spotted *Therioaphis trifoliumaculata* (alfalfa aphid) in Israel.

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