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Article

Compatibility of the aqueous leaf extract of *Mimosa pudica* and the entom-acaropathogenic fungus *Beauveria bassiana* in controlling the broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae)

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ABSTRACT

The broad mite *Polyphagotarsonemus latus* (Banks) is a polyphagous pest with a cosmopolitan distribution. The combined application of botanical pesticides and entom-acaropathogenic fungi may serve as an eco-friendly approach in controlling the mite. In this study, the *Mimosa pudica* (Fabales: Fabaceae) leaf aqueous extract (*MpAE*) was initially tested to determine its effects on the mycelial growth, sporulation, and conidia viability of the entom-acaropathogenic fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae). The compatibility was then determined based on the biological index (BI) value. The bioefficacy of *MpAE* and *B. bassiana* at various concentrations was evaluated against *P. latus*, both alone and in combinations. Each treatment was sprayed into an arena containing a citrus leaf disc and gravid females of *P. latus*. The mortality and oviposition rate of the females were observed until 120 h after application. The results showed no reduction in *B. bassiana* mycelial growth, sporulation, and conidia viability when *MpAE* was incorporated into the culture medium. The BI value ranged from 100.01 to 136.30, indicating that *MpAE* was highly compatible with *B. bassiana*. The individual and combined applications of *MpAE* and *B. bassiana* significantly increased the mite mortality and simultaneously inhibited the mite oviposition rate. The mite mortality varied from 90 to 100% at 120 h after application in the treatment groups. The mite oviposition rate was significantly decreased, ranging from 11.50 to 29.00 eggs in the treatment groups, while it was 57.25 eggs in control. Therefore, the application of *MpAE* and *B. bassiana* propose a valuable alternative to integrate into pest management programs.

KEY WORDS: Bioefficacy; botanical pesticides; eco-friendly approach; mortality; oviposition rate; pest management programs.

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INTRODUCTION

The broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) is an extremely polyphagous pest with a cosmopolitan distribution. More than 60 plant families are registered as its hosts (Jeppson *et al.* 1975; Gerson 1992). The high biotic potential of *P. latus* permits the mite population to achieve an economic injury level rapidly (Peña and Bullock 1994). Severe losses

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caused by the mite have been observed worldwide, especially on solanaceous and cucurbitaceous crops (Grinberg *et al.* 2005; Kousik *et al.* 2007; Onzo *et al.* 2012; Nasrin *et al.* 2020). However, significant damage caused by the mite has also been reported in some ornamental, fruit, and industrial crops (Peña *et al.* 1996; Luypaert *et al.* 2014; Rosado *et al.* 2015; Akyazi *et al.* 2019), for instance, in Indonesia, the mite has caused a great deal of injury on chili, eggplant, citrus, tea, and physic nut (Puspitarini *et al.* 2021a). On the infested plants, the mite mostly feeds in apical parts of the plants (Rosado *et al.* 2014) and ruptures the plant cell wall with its chelicerae (Jeppson *et al.* 1975), which results in the leakage of intracellular content (Martins *et al.* 2016). The plant's morpho-physiological alterations induced by the mite feeding would lead to growth retardation of the infested plants, flower and fruit drop, also a rejection by consumers because of the cosmetic injuries in the like of leaf distortion, flower discoloration, and fruit blistering (Gerson 1992; Grinberg *et al.* 2005; Weintraub 2007; Sarmiento *et al.* 2011).

The broad mite dispersal occurs through several mechanisms. One of them is being carried by insect pests (Alagarmalai *et al.* 2009). A myriad of studies evidenced that the mite has a phoretic association with whiteflies (Hemiptera: Aleyrodidae) (Parker and Gerson 1994; Fan and Pettitt 1998; Palevsky *et al.* 2001; Soroker *et al.* 2003). This association facilitates the colonization of crops by the mite and makes its management more difficult. The difficulties in combating broad mite infestation have made farmers depend entirely on chemical pesticides and often apply the pesticides frequently (Herron *et al.* 1996; Onzo *et al.* 2012; Akyazi *et al.* 2019). Considering the indirect hazardous effects of chemical pesticides on the environment and human health, there is a necessity to develop eco-friendly approaches to controlling this noxious pest.

Botanical pesticides are viewed as a suitable method to control phytophagous mites compared to chemical pesticides (Azaizeh *et al.* 2007; Mohammadi *et al.* 2016; Ziaee *et al.* 2017; Venzon *et al.* 2020). Among them, the effectiveness of neem seed extract against the broad mite was evaluated by Venzon *et al.* (2008). Akyazi *et al.* (2019) studied the toxicity of plant extracts derived from garlic bulb and tobacco leaf on this mite. It is generally accepted that botanical pesticides are less toxic to non-target organisms (Tsolakis and Ragusa 2008; Nexticapan-Garcéz *et al.* 2021; Tavares *et al.* 2021). Hence, they could be co-applied with biocontrol agents. Among several biocontrol agents, the use of entomo-acaropathogenic fungi (EAF) is considered suitable upon application with botanical pesticides (Hernández *et al.* 2012; Nana *et al.* 2012; Ali *et al.* 2018; Sohrabi *et al.* 2019). The combined application of EAF with botanical pesticides may provide a more powerful tool for mite management, that is, when compatibility is achieved (Hernández *et al.* 2012; Halder *et al.* 2021). Neves *et al.* (2001) and Usha *et al.* (2014) stated that the compatibility between EAF and botanical pesticides may ease the selection of appropriate products, enhance the control efficacy by reducing the required dose rate, environmental pollution threats, and build-up of pest resistance. However, the utilization of EAF in suppressing populations of the broad mite has been demonstrated by only a few researchers (Peña *et al.* 1996; Maketon *et al.* 2008; Martins *et al.* 2016). Moreover, the bioefficacy of EAF is known to be strongly species- and strain-specific (Liu *et al.* 2014; Wu *et al.* 2016; Puspitarini *et al.* 2021a).

Based on our previous work, the BbHPT-1 isolate of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) showed high compatibility with four different botanical pesticides, including leaf extract of neem, chinaberry, Mexican sunflower, and lantana (Afandhi *et al.* 2020). Nevertheless, for broadening its effectiveness, evaluation of the compatibility of this fungus isolate with extract of other plant species is needed. *Mimosa pudica* L. (Fabales: Fabaceae) is an underused invasive plant that possesses numerous antimicrobial chemicals (Muhammad *et al.* 2016). However, little is known regarding the potential effect of *M. pudica* extract against phytophagous mites. The study conducted by Zhang *et al.* (2011) suggested that the leaf extract of *M. pudica* contains a large number of phenolic compounds, especially flavonoids. Flavonoids are known to have pesticidal features against insects (Boulogne *et al.* 2012; Mathesius 2018). Therefore, this study aimed to

determine the compatibility of *B. bassiana* with the aqueous leaf extract of *M. pudica*. Additionally, their individual and combined application were evaluated against the broad mite, *P. latus*, one of the most damaging phytophagous mites in Indonesia.

MATERIALS AND METHODS

Preparation of the aqueous leaf extract of M. pudica

Leaves of *M. pudica* were collected from the municipality of Malang (7° 57' 19.67" S; 112° 39' 41.42" E), East Java, Indonesia. For the preparation of the aqueous extract, the method described by Ribeiro *et al.* (2012) was followed with a slight modification. The collected *M. pudica* leaves were dried in an oven for 48 h at 40 °C. Thereafter, the dried leaves were ground in an electric blender. The obtained powder was stored in a glass flask and covered with an aluminum foil and stored at 4 °C until used. The *M. pudica* aqueous extract (*MpAE*) was made by the addition of 100 g *M. pudica* powder to 100 ml distilled water as the solvent. This stock solution (100%) was kept in an air-tight Erlenmeyer flask and subsequently were agitated using an orbital shaker (Protech®, 722 type) overnight at 220 rpm for the extraction of hydrosoluble compounds. After 24 h, the solution was filtered through filter papers with a pore size of 6 µm. The filtered solution was stored in a glass flask, covered with aluminum foil, and placed at 4 °C or readily employed for experiments.

Preparation of B. bassiana concentration

The BbHPT-1 isolate of *B. bassiana* was obtained from the Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya, Indonesia. The fungus was grown on potato dextrose agar (PDA) amended with 1% chloramphenicol in the dark for two weeks. The fungus conidia were harvested from the culture plates by surface scraping (Sohrabi *et al.* 2019). The fungal conidia were suspended with 10 ml of sterilized distilled water containing 0.02% Tween 80 in a falcon tube. The tube was centrifuged at 3,000 rpm for 5 minutes to separate the conidia from the mycelium. The obtained conidia pellet was mixed with 5 ml of distilled water and the conidial concentration was estimated using a hemocytometer. The conidial suspension was then diluted to obtain the desired concentrations. The concentrations used in this study were 10⁴, 10⁶, and 10⁸ conidia ml⁻¹. These concentrations are often used when testing the effectiveness of EAF against insect and mite pests (Nugroho and Ibrahim 2004; Fernández-Grandon *et al.* 2020; Puspitarini *et al.* 2021a).

Effect of MpAE on the mycelial growth, sporulation and conidia viability of B. bassiana

Different concentrations of *MpAE* were incorporated into the PDA. Autoclaved PDA, cooled to 45 °C, was thoroughly mixed with the *MpAE* at the desired concentrations, and poured into Petri dishes (9 cm diameter). The *B. bassiana* was inoculated to the culture plate by using a paper-dip method. A 5 mm diameter filter paper was dipped into *B. bassiana* suspension and was placed in the middle of the culture plate. There were 12 treatments in this experiment that consist of the combinations of four levels of *MpAE* concentration (0, 2, 3, and 4%) and three levels of *B. bassiana* concentration (10⁴, 10⁶, and 10⁸ conidia ml⁻¹). Treatments with *MpAE* at 0% were considered as controls. Four replicates were made for each of the treatments. The culture plates were incubated in the dark at 27 °C. The radial mycelial growth, sporulation (conidia production), and conidia germination (viability) were calculated at 6 days after the beginning of the incubation as described by Depieri *et al.* (2005). The concentrations of *MpAE* were selected from the preliminary toxicity bioassay of *MpAE* against *P. latus*. Based on the preliminary trials, the 4% concentration of *MpAE* was enough to cause 100% mortality of the tested mites at 120 h after application and therefore was chosen as the highest concentration applied.

Compatibility calculations

The compatibility of *B. bassiana* tested with *MpAE* was calculated through the formula of the biological index. Classification of compatibility is based on the toxicity of chemical products to EAF *in vitro* by the calculation of the BI value (Rossi-Zalaf *et al.* 2008). The formula was

$$BI = [47(VG) + 43(SP) + 10(GER)]/100$$

where VG is the percent mycelial or vegetative growth, SP is the percent sporulation, and GER is the percent germination compared to the control. The obtained BI value from 0 to 41 indicates that the *MpAE* is considered toxic to *B. bassiana*, from 42 to 66 is moderately toxic, and above 66 represents compatibility (Rossi-Zalaf *et al.* 2008). This formula has been used in several studies (Ribeiro *et al.* 2012; Diniz *et al.* 2020).

Mite colony

The stock population of the broad mite was collected from infested mandarin orange [*Citrus reticulata* Blanco (Sapindales: Rutaceae)] orchards in Malang located in the Indonesian Citrus and Subtropical Fruits Research Institute. The mites were brought to the Laboratory of Plant Pests, Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya, Indonesia. The mites were reared on the leaves of *C. reticulata* and held in an arena (Puspitarini *et al.* 2021a). To make an arena, a water-soaked sponge (5 × 5 × 1.5 cm) was placed inside a Petri dish (9 cm diameter). The sponge was covered with a moistened cotton, and after that, a *C. reticulata* leaf disc (3 cm diameter) was placed above the cotton with the abaxial surface facing up. Water was added regularly to keep the leaf in turgid condition and to prevent the desiccation of the mites (Puspitarini *et al.* 2021b). The arenas were put in a plastic tray, covered with muslin, and placed into a cabinet. The rearing was performed under laboratory conditions at 24 ± 1 °C, 66 ± 2% RH, and a photoperiod of 12:12 L:D h.

Effects of *MpAE* and *B. bassiana* on *P. latus*

This bioassay was conducted to evaluate the effect of *MpAE* and *B. bassiana* on mortality and oviposition rate of *P. latus*. Ten mated females of *P. latus* were placed into an arena (Martins *et al.* 2016; Thakur and Sood 2019). The females used in the bioassay were 1–2 days old. Thereafter, males were introduced into the arena and allowed to mate with the females. After copulation, the males were removed from the arena. There were 16 treatments which were set as follows: control (water); three levels of *MpAE* concentration (2, 3, and 4%); three levels of *B. bassiana* concentration (10⁴, 10⁶, and 10⁸ conidia ml⁻¹); and nine combinations of *MpAE* and *B. bassiana*. Each treatment was applied using a glass atomizer (Roy and Mukhopadhyay 2012) with a volume of 1 ml per arena (Martins *et al.* 2016), which was sufficient to cover the surface area of the leaf as well as the mites. The applied volume of *MpAE* and *B. bassiana* was halved in combination treatments as 1:1 (Ali *et al.* 2018). Each treatment was replicated four times. The tested mites were observed at 72, 96, and 120 h after application to record mortality. Mites were assumed dead if there was no movement when touched with a camel hair brush. The daily mortality was shown in a cumulative percent from the previous observation(s). Eggs laid by the females were also recorded daily and immediately removed to avoid double counting. After the last observation, the oviposition rate was shown in terms of the difference in the number of eggs deposited by the females in the control and treatment groups. The discrimination quotient (DQ) was calculated using the following formula:

$$DQ = (C - T) / (C + T)$$

where C is the number of eggs on the control and T is the number of eggs on treatment groups

(Roobakkumar *et al.* 2010).

Statistical analysis

All data obtained in this study were initially subjected to the Shapiro-Wilk test to determine if the assumption of normality was met. Based on the analysis, only the data on mortality did not show a normal distribution, therefore the mortality data were transformed using the arcsine square root formula (Lü and Zhang 2016). In addition, the mortality data were not corrected by Abbot's formula as the mean mortality in the control was less than 5% (WHO 2016). All data were then submitted to a two-way analysis of variance (ANOVA) (Ali *et al.* 2018; Sohrabi *et al.* 2019). The means were compared by applying Tukey's test at $P < 0.05$. Statistical analyses were carried out with R statistics (R Core Team 2020).

RESULTS

Mycelial growth, sporulation and conidia viability of *B. bassiana*

A significant interaction was observed between different concentrations of *MpAE* and *B. bassiana* when assessing the mycelial growth ($F_{6, 36} = 5.05$; $P = 0.0008$), conidia production ($F_{6, 36} = 12.01$; $P < 0.0001$), and conidia viability ($F_{6, 36} = 3.07$; $P = 0.0156$) of the fungus. The tested *MpAE*, regardless of the concentrations used, did not show adverse effects towards *B. bassiana*. There was no reduction in mycelial growth, conidia production, and conidia viability when *MpAE* at different concentrations was incorporated into the PDA. For instance, the mycelial growth, conidia production, and conidia viability of "*B. bassiana* 10^4 conidia ml^{-1} " grown in *MpAE*-enriched PDA were higher (although not always significant) than the control without the incorporation of *MpAE* (Table 1).

Table 1. Radial mycelial growth, conidia production and conidia viability (mean \pm SD) of *B. bassiana* at 6 days after inoculation in a PDA containing different concentrations of *MpAE*.

Treatment	Mycelial growth (cm)	Conidia amount (... $\times 10^9 \text{ ml}^{-1}$)	Conidia viability (%)	
<i>MpAE</i> 0%	<i>B. bassiana</i> 10^4 conidia ml^{-1}	3.61 \pm 0.71 d	8.02 \pm 0.58 c	54.76 \pm 2.18 b
	<i>B. bassiana</i> 10^6 conidia ml^{-1}	4.65 \pm 0.46 cd	8.17 \pm 0.96 bc	55.88 \pm 1.45 b
	<i>B. bassiana</i> 10^8 conidia ml^{-1}	6.65 \pm 0.66 ab	8.75 \pm 0.82 a	61.39 \pm 1.73 ab
<i>MpAE</i> 2%	<i>B. bassiana</i> 10^4 conidia ml^{-1}	6.25 \pm 0.34 abc	8.18 \pm 1.71 bc	60.83 \pm 2.55 ab
	<i>B. bassiana</i> 10^6 conidia ml^{-1}	6.80 \pm 0.06 ab	8.67 \pm 1.73 a	59.80 \pm 0.61 b
	<i>B. bassiana</i> 10^8 conidia ml^{-1}	6.66 \pm 0.14 ab	8.78 \pm 1.26 a	61.97 \pm 1.25 ab
<i>MpAE</i> 3%	<i>B. bassiana</i> 10^4 conidia ml^{-1}	6.81 \pm 0.09 ab	8.13 \pm 0.96 bc	61.04 \pm 2.17 ab
	<i>B. bassiana</i> 10^6 conidia ml^{-1}	7.69 \pm 0.09 a	8.66 \pm 1.71 a	69.05 \pm 1.09 a
	<i>B. bassiana</i> 10^8 conidia ml^{-1}	6.80 \pm 0.18 ab	8.70 \pm 3.56 a	62.02 \pm 1.41 ab
<i>MpAE</i> 4%	<i>B. bassiana</i> 10^4 conidia ml^{-1}	5.83 \pm 0.05 bc	8.22 \pm 1.29 b	57.11 \pm 1.86 b
	<i>B. bassiana</i> 10^6 conidia ml^{-1}	6.33 \pm 0.08 ab	8.80 \pm 1.41 a	60.35 \pm 1.62 b
	<i>B. bassiana</i> 10^8 conidia ml^{-1}	6.63 \pm 0.04 ab	8.77 \pm 0.58 a	61.76 \pm 0.99 ab
Statistics				
<i>MpAE</i> ($df = 3, 36$)	$F = 21.83$; $P <$	$F = 28.49$; $P < 0.0001$	$F = 8.36$; $P = 0.0002$	
<i>B. bassiana</i> ($df = 2, 36$)	$F = 10.49$; $P =$	$F = 253.40$; $P < 0.0001$	$F = 4.70$; $P = 0.0153$	
<i>MpAE</i> \times <i>B. bassiana</i> ($df = 6, 36$)	$F = 5.05$; $P = 0.0008$	$F = 12.01$; $P < 0.0001$	$F = 3.07$; $P = 0.0156$	

Means followed by the same letters within each column are not significantly different at $P < 0.05$ according to Tukey's test.

Potential synergy between MpAE and B. bassiana

Depending on the calculated BI values, the MpAE at all tested concentrations showed high compatibility with *B. bassiana*. The BI value ranges from 100.01 to 136.30 (Table 2).

Table 2. MpAE compatibility classification with *B. bassiana* based from the calculation of the biological index (BI) values.

Treatment		BI value	Classification
MpAE 2%	<i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	136.30	Compatible
	<i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	124.99	Compatible
	<i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	100.37	Compatible
MpAE 3%	<i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	143.38	Compatible
	<i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	135.55	Compatible
	<i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	100.92	Compatible
MpAE 4%	<i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	130.29	Compatible
	<i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	120.95	Compatible
	<i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	100.01	Compatible

Table 3. Cumulative percent mortality (means ± SD) in *P. latius* after the treatment with MpAE and *B. bassiana* alone and in combinations.

Treatment	Time		
	72 h	96 h	120 h
Control (Water)	2.50 ± 5.00 a	2.50 ± 5.00 b	2.50 ± 5.00 c
MpAE 2%	20.00 ± 14.14 a	40.00 ± 14.14 ab	92.50 ± 9.57 ab
MpAE 3%	25.00 ± 12.91 a	45.00 ± 19.15 ab	100.00 ± 0.00 a
MpAE 4%	27.50 ± 12.58 a	47.50 ± 12.58 ab	100.00 ± 0.00 a
<i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	25.00 ± 17.32 a	42.50 ± 12.58 ab	90.00 ± 8.16 b
<i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	22.50 ± 9.57 a	42.50 ± 9.57 ab	95.00 ± 5.77 ab
<i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	22.50 ± 5.00 a	47.50 ± 9.57 ab	100.00 ± 0.00 a
MpAE 2% + <i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	22.50 ± 15.00 a	52.50 ± 32.02 ab	100.00 ± 0.00 a
MpAE 2% + <i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	30.00 ± 8.16 a	77.50 ± 22.17 a	100.00 ± 0.00 a
MpAE 2% + <i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	27.50 ± 17.08 a	72.50 ± 9.57 a	100.00 ± 0.00 a
MpAE 3% + <i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	25.00 ± 12.91 a	77.50 ± 26.30 a	100.00 ± 0.00 a
MpAE 3% + <i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	30.00 ± 8.16 a	80.00 ± 18.26 a	100.00 ± 0.00 a
MpAE 3% + <i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	22.50 ± 9.57 a	72.50 ± 34.03 a	100.00 ± 0.00 a
MpAE 4% + <i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	30.00 ± 0.00 a	65.00 ± 30.00 a	100.00 ± 0.00 a
MpAE 4% + <i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	30.00 ± 11.55 a	60.00 ± 9.12 a	100.00 ± 0.00 a
MpAE 4% + <i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	27.50 ± 15.00 a	72.50 ± 8.53 a	100.00 ± 0.00 a
Statistics			
MpAE (df = 3, 48)	F = 2.29; P = 0.0893	F = 9.40; P < 0.0001	F = 224.30; P < 0.0001
<i>B. bassiana</i> (df = 3, 48)	F = 1.82; P = 0.1559	F = 9.22; P < 0.0001	F = 185.50; P < 0.0001
MpAE x <i>B. bassiana</i> (df = 9, 48)	F = 0.75; P = 0.6563	F = 0.64; P = 0.7556	F = 151.70; P < 0.0001

Mortality data were arcsine square-root transformed before analysis, actual mean values are presented based on the interpretation from the transformed data. Means followed by the same letters within each column are not significantly different at $P < 0.05$ according to Tukey's test.

Efficacy of individual and combined application of MpAE and *B. bassiana* against *P. latus*

The mortality of *P. latus* was detected at 72 h after the application, but the mite mortality was not statistically different between the control and the treatment groups. At 96 h after the application, the mite mortality on the combination treatments (except “MpAE 2% + *B. bassiana* 10⁴ conidia ml⁻¹”) was higher than the control. Nevertheless, on the last day of observation, all treatments significantly caused higher mortality of the tested mites than the control. However, there were no differences in the mite mortality between the individual application of MpAE and *B. bassiana* (except “*B. bassiana* 10⁴ conidia ml⁻¹”) and the combination treatments. Additionally, at 120 h after the application, the mite mortality was seen as significantly affected by the interaction between different concentrations of MpAE and *B. bassiana* ($F_{9, 48} = 151.70$; $P < 0.0001$) (Table 3).

Effect of MpAE and *B. bassiana* on oviposition rate of *P. latus*

The oviposition rate of *P. latus* was significantly affected by the different concentrations of MpAE ($F_{3, 48} = 5.88$; $P = 0.0017$) and *B. bassiana* ($F_{3, 48} = 4.07$; $P = 0.0118$), but not by their interaction ($F_{9, 48} = 1.83$; $P = 0.0854$). The number of eggs laid on the control group was 57.25, and the numbers of eggs laid were significantly lower on all of the treatment groups, ranging from 11.50 to 29.00. The DQ value ranges from 0.40 to 0.66. Higher DQ values indicate a stronger ovipositional deterrence of the treatments on *P. latus* (Table 4).

Table 4. Oviposition rate (mean \pm SD) of *P. latus* and discrimination quotient (DQ) values after the treatment with MpAE and *B. bassiana* alone and in combinations.

Treatment	Number of eggs laid at 120 h	DQ value
Control (Water)	57.25 \pm 18.14 a	-
MpAE 2%	23.25 \pm 8.84 b	0.42
MpAE 3%	24.00 \pm 9.48 b	0.40
MpAE 4%	22.75 \pm 13.35 b	0.43
<i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	29.00 \pm 13.83 b	0.32
<i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	22.75 \pm 9.17 b	0.43
<i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	24.00 \pm 11.78 b	0.40
MpAE 2% + <i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	19.50 \pm 20.07 b	0.48
MpAE 2% + <i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	19.75 \pm 4.50 b	0.48
MpAE 2% + <i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	23.00 \pm 13.54 b	0.42
MpAE 3% + <i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	11.50 \pm 8.85 b	0.66
MpAE 3% + <i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	13.75 \pm 2.50 b	0.61
MpAE 3% + <i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	20.25 \pm 11.18 b	0.47
MpAE 4% + <i>B. bassiana</i> 10 ⁴ conidia ml ⁻¹	18.75 \pm 9.17 b	0.50
MpAE 4% + <i>B. bassiana</i> 10 ⁶ conidia ml ⁻¹	24.50 \pm 6.55 b	0.40
MpAE 4% + <i>B. bassiana</i> 10 ⁸ conidia ml ⁻¹	17.75 \pm 7.36 b	0.52
Statistics		
MpAE ($df = 3, 48$)	$F = 5.88$; $P = 0.0017$	
<i>B. bassiana</i> ($df = 3, 48$)	$F = 4.07$; $P = 0.0118$	
MpAE x <i>B. bassiana</i> ($df = 9, 48$)	$F = 1.83$; $P = 0.0854$	

Means followed by the same letters within each column are not significantly different at $P < 0.05$ according to Tukey's test.

DISCUSSION

In this study, we demonstrated that MpAE did not possess deleterious effects on *B. bassiana*. The effects of MpAE on *B. bassiana* mycelial growth, conidia production, and conidia viability were

positive and depended on the concentrations of both the botanical pesticide and fungus. Contradictorily, inhibitory effects caused by various botanical pesticides to EAF were reported by several studies (Hirose *et al.* 2001; Mohan *et al.* 2007; Saharayaj *et al.* 2011; Ribeiro *et al.* 2014; Jamali *et al.* 2021). For instance, Ribeiro *et al.* (2012) reported that the incorporation of aqueous extract of tobacco powder at 2.5 to 10% into the culture medium significantly inhibited vegetative growth, sporulation, and conidia germination of *B. bassiana*. Similarly, Puspitarini *et al.* (2021a) reported the toxicity of aqueous leaf extract of soursop to *B. bassiana*, even at the lowest concentration used (0.5%). However, other studies indicated that the positive or negative effects of botanical pesticides on EAF varied according to the concentrations or formulation used (Depieri *et al.* 2005; Sohrabi *et al.* 2019; Diniz *et al.* 2020). The variations in the effects of botanical pesticides on EAF could depend on several factors. Ribeiro *et al.* (2012) stated that the quantities and the components of the botanical pesticide formulation, and their influence on the medium pH, may affect mycelial growth and conidia production of EAF. Some phytochemicals have been reported to serve as fungitoxic compounds (Ma *et al.* 2000; Curir *et al.* 2003). Additionally, Forim *et al.* (2010) mentioned that the quality of the raw material used and the phenological variations of the source plants may alter the qualitative and quantitative composition of bioactive compounds contained in the products. The genetic variability of different EAF species and isolates also needs to be considered because each species or isolate may have different degrees of susceptibility upon exposure to botanical pesticides (Mohan *et al.* 2007; Diniz *et al.* 2020).

Since the effects of botanical pesticides on EAF are influenced by the concentration used, a preliminary *in vitro* assessment is needed to determine the concentration threshold of botanical pesticides when one wants to use the products simultaneously with EAF. Our results indicated that the mycelial growth and conidia viability of *B. bassiana* showed a tendency to decrease with the higher concentration of *MpAE*. In general, although insignificant, the mycelial growth and conidia viability of the fungus was relatively higher on “*MpAE* at 3%” treatments rather than at 4%. Hence, we hypothesize that the addition of *MpAE* higher than 4% may produce antagonistic effects toward EAF. This evaluation of the effects of botanical pesticides on conidia viability or germination of EAF is important. One of the determining factors that influence the success of EAF infection on arthropods is the conidia germination (Neves *et al.* 2001; Nana *et al.* 2012). Hence, the use of excessive concentrations of botanical pesticides may inhibit the development and reproduction of EAF, resulting in low efficiency of pest control.

Based on the BI value, *MpAE* at all concentrations was compatible with *B. bassiana* which could envisage the positive synergism between the botanical pesticide and EAF for the control of insect and mite pests. Vice versa, the combined application of botanical pesticides and EAF must be avoided when contradictory interactions are detected. Our result showed that at 96 h after application, the combined application of *MpAE* and *B. bassiana* produced higher mortality of *P. latus* compared to the control. These results are in line with some other studies that showed that combined application of botanical pesticides and EAF yielded higher mortality of the tested pests (Islam *et al.* 2010; Hernández *et al.* 2012; Ali *et al.* 2018; Sohrabi *et al.* 2019; Diniz *et al.* 2020; Rehman *et al.* 2020; Puspitarini *et al.* 2021c). The enhanced bioefficacy of the combined application of botanical pesticides and EAF is due to the action of botanical pesticides as physiological stressors, affecting the behavior of insects and mites, which become more prone to EAF infection (Paula *et al.* 2011). The use of botanical pesticides may also facilitate the penetration of EAF into the insect and mite cuticle (Diniz *et al.* 2020).

Other than this study, the toxicity of *M. pudica* extract has also been documented against coleopteran pests (Ahad *et al.* 2015, 2016). The toxicity may be attributed to the high concentration of flavonoids found in the *M. pudica* extract (Zhang *et al.* 2011). Flavonoids in botanical pesticides have been known to possess varying levels of toxicity against numerous insect pests (Panche *et al.* 2016; Hikal *et al.* 2017). Flavonoids also alter the behavior (mobility and feeding activity) and

metabolism (growth and development) of insects (Simmonds 2003), hence confirming that botanical pesticides may also serve as a synergist for EAF application.

Regarding *B. bassiana*, its virulence against insects and mites has been reported by a plethora of studies (Alves *et al.* 2005; Rossi-Zalaf and Alves 2006; Zimmermann 2007; McKinnon *et al.* 2016; Wu *et al.* 2016), including *P. latus* (Nugroho and Ibrahim 2004; Martins *et al.* 2016). During mycosis, *B. bassiana* secretes hydrolytic enzymes such as carboxypeptidase, chitinase, lipase, and proteinase that digest the host's cuticle (Wang and Wang 2017; Harith-Fadzilah *et al.* 2021). The fungus will then produce defensive molecules and enzymes to tackle the host's immune response and establish itself inside the host hemocoel (Butt *et al.* 2016; Chen *et al.* 2018). The fungus also produces various secondary metabolites that are cytotoxic, leading to the death of the host. However, the present study showed that the *P. latus* mortality was lower when *B. bassiana* at 10^4 conidia ml^{-1} was applied singly. It indicates that the conidia concentration is one of the determining factors that influences the effectiveness of EAF. The lower initial number of inoculums may decrease the number of adhered conidia in the host's cuticle (Puspitarini *et al.* 2021a).

Lower oviposition rate was observed when *P. latus* was exposed to MpAE and *B. bassiana* at all concentrations, both singly and in combinations. Similarly, Roobakkumar *et al.* (2010), as well as Roy and Mukhopadhyay (2012), demonstrated that some plant extracts showed strong ovipositional deterrence against the red spider mite *Oligonychus coffeae* (Nietner) (Acari: Tetranychidae) as indicated by the lower numbers of eggs laid and the high DQ values in the treatment groups. Furthermore, it is proposed that the ingestion of phytochemicals in plant extracts such as flavonoids by insects could reduce their fecundity (Boué and Raina 2003; Goławska *et al.* 2014). The application of *B. bassiana* has also been known to inhibit the oviposition rate of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) (Chandler *et al.* 2005; Duso *et al.* 2008). In addition, Ali *et al.* (2018) reported that combined application of botanical pesticides and EAF significantly lowered the fecundity of *Sitobion avenae* (Fab.) (Hemiptera: Aphididae). Similarly, Puspitarini *et al.* (2021c) demonstrated that the combined application of *Jatropha curcas* L. (Malpighiales: Euphorbiaceae) seed extract and *Akanthomyces lecanii* (Zimm.) (Hypocreales: Cordycipitaceae) dramatically reduced the oviposition rate of the citrus red mite *Panonychus citri* (McGregor) (Acari: Tetranychidae).

In conclusion, MpAE was highly compatible with *B. bassiana*, and their application showed promising results against the broad mite *P. latus* when applied alone or in combinations. The application of MpAE and *B. bassiana* may present valuable eco-friendly alternatives to integrate into management programs of *P. latus*. Studies have reported the safety of botanical pesticides and EAF application on beneficial arthropods, including predatory mites (Bernardi *et al.* 2013; Castillo-Ramírez *et al.* 2020; Savi *et al.* 2021). In addition, the simple preparation of MpAE could be adopted by farmers since *M. pudica* is ubiquitous and abundant, while water is an inexpensive extraction solvent. Therefore, our findings highlight that the application of MpAE and *B. bassiana* is ecologically and economically feasible.

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REFERENCES

- Afandhi, A., Pratiwi, V.R., Hadi, M.S., Setiawan, Y. & Puspitarini, R.D. (2020) Suitable combination between *Beauveria bassiana* (Balsamo) Vuillemin and four plant leaf extracts to control *Spodoptera litura* (Fabricius). *Agrivita*, 42(2): 341–349. DOI: [10.17503/agrivita.v42i2.2678](https://doi.org/10.17503/agrivita.v42i2.2678)

- Ahad, M.A., Bhuyain, M.M.H., Muzammel, M. & Hoque, M.F. (2016) Eco-friendly management of lesser grain borer, *Rhizopertha dominica* F. (Bostrichidae: Coleoptera) on wheat in storage. *American Journal of Life Science Researches*, 4(4): 145–151. DOI: [10.21859/ajlsr-040407](https://doi.org/10.21859/ajlsr-040407)
- Ahad, M.A., Nahar, M.K., Amin, M.R., Suh, S.J. & Kwon, Y.J. (2015) Biological activities of five weed extracts against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *Korean Journal of Applied Entomology*, 54(2): 83–89. DOI: [10.5656/KSAE.2015.04.0.003](https://doi.org/10.5656/KSAE.2015.04.0.003)
- Akyazi, R., Sekban, R., Soysal, M., Akyol, D., Colee, J. & Bostan, S.Z. (2019) Ecofriendly control approaches for *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on tea (*Camellia sinensis* L.). *International Journal of Acarology*, 45(1–2): 79–89. DOI: [10.1080/01647954.2018.1544989](https://doi.org/10.1080/01647954.2018.1544989)
- Alagarmalai, J., Grinberg, M., Perl-Treves, R. & Soroker, V. (2009) Host selection by the herbivorous mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Journal of Insect Behavior*, 22: 375–387. DOI: [10.1007/s10905-009-9179-y](https://doi.org/10.1007/s10905-009-9179-y)
- Ali, S., Farooqi, M.A., Sajjad, A., Ullah, M.I., Qureshi, A.K., Siddique, B., Waheed, W., Safraz, M. & Asghar, A. (2018) Compatibility of entomopathogenic fungi and botanical extracts against the wheat aphid, *Sitobion avenae* (Fab.) (Hemiptera: Aphididae). *Egyptian Journal of Biological Pest Control*, 28: 97. DOI: [10.1186/s41938-018-0101-9](https://doi.org/10.1186/s41938-018-0101-9)
- Alves, S.B., Tamai, M.A., Rossi, L. & Castiglioni, E. (2005) *Beauveria bassiana* pathogenicity to the citrus rust mite *Phyllocoptruta oleivora*. *Experimental and Applied Acarology*, 37(1–2): 117–122. DOI: [10.1007/s10493-005-0314-y](https://doi.org/10.1007/s10493-005-0314-y)
- Azaizeh, H., Kobaisy, M., Dakwar, S., Saad, B., Shaqir, I. & Said, O. (2007) Botanical pesticides as a source of safe bio-acaricides for the control of *Tetranychus cinnabarinus* mites. *Acta Phytopathologica et Entomologica Hungarica*, 42(1): 143–152.
- Bernardi, D., Botton, M., da Cunha, U.S., Bernardi, O., Malausa, T., Garcia, M.S. & Nava, D.E. (2013) Effects of azadirachtin on *Tetranychus urticae* (Acari: Tetranychidae) and its compatibility with predatory mites (Acari: Phytoseiidae) on strawberry. *Pest Management Science*, 69(1): 75–80. DOI: [10.1002/ps.3364](https://doi.org/10.1002/ps.3364)
- Boué, S.M. & Raina, A.K. (2003) Effects of plant flavonoids on fecundity, survival, and feeding of the Formosan subterranean termite. *Journal of Chemical Ecology*, 29: 2575–2584. DOI: [10.1023/A:1026318203775](https://doi.org/10.1023/A:1026318203775)
- Boulogne, I., Peiti, P., Ozier-Lafontaine, H., Desfontaines, L. & Loranger-Merciris, G. (2012) Insecticidal and antifungal chemicals produced by plants: a review. *Environmental Chemistry Letters*, 10: 325–347. DOI: [10.1007/s10311-012-0359-1](https://doi.org/10.1007/s10311-012-0359-1)
- Butt, T.M., Coates, C.J., Dubovskiy, I.M. & Ratcliffe, N.A. (2016) Entomopathogenic fungi: New insights into host-pathogen interactions. *Advances in Genetics*, 94: 307–364. DOI: [10.1016/bs.adgen.2016.01.006](https://doi.org/10.1016/bs.adgen.2016.01.006)
- Castillo-Ramírez, O., Guzmán-Franco, A.W., Santillán-Galicia, Ma.T. & Tamayo-Mejía, F. (2020) Interaction between predatory mites (Acari: Phytoseiidae) and entomopathogenic fungi in *Tetranychus urticae* populations. *BioControl*, 65: 433–445. DOI: [10.1007/s10526-020-10004-3](https://doi.org/10.1007/s10526-020-10004-3)
- Chandler, D., Davidson, G. & Jacobson, R.J. (2005) Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology*, 15(1): 37–54. DOI: [10.1080/09583150410001720617](https://doi.org/10.1080/09583150410001720617)

- Chen, A., Wang, Y., Shao, Y., Zhou, Q., Chen, S., Wu, Y., Chen, H. & Liu, E. (2018) Genes involved in *Beauveria bassiana* infection to *Galleria mellonella*. *Archives of Microbiology*, 200: 541–552. DOI: [10.1007/s00203-017-1456-0](https://doi.org/10.1007/s00203-017-1456-0)
- Curir, P., Dolci, M., Dolci, P., Lanzotti, V. & De Cooman, L. (2003) Fungitoxic phenols from carnation (*Dianthus caryophyllus*) effective against *Fusarium oxysporum* f. sp. *dianthi*. *Phytochemical Analysis*, 14(1): 8–12. DOI: [10.1002/pca.672](https://doi.org/10.1002/pca.672)
- Depieri, R.A., Martinez, R.R. & Menezes Jr., A.O. (2005) Compatibility of the fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. *Neotropical Entomology*, 34(4): 601–606. DOI: [10.1590/S1519-566X200500040010](https://doi.org/10.1590/S1519-566X200500040010)
- Diniz, A.G., Barbosa, L.F.S., Santos, A.C.S., de Oliveira, N.T., da Costa, A.F., Carneiro-Leão, M.P. & Tiago, P.V. (2020) Bio-insecticide effect of isolates of *Fusarium caatingaense* (Sordariomycetes: Hypocreales) combined to botanical extracts against *Dactylopius opuntiae* (Hemiptera: Dactylopiidae). *Biocontrol Science and Technology*, 30(4): 384–395. DOI: [10.1080/09583157.2020.1720601](https://doi.org/10.1080/09583157.2020.1720601)
- Duso, C., Malagnini, V., Pozzebon, A., Castagnoli, M., Liguori, M. & Simoni, S. (2008) Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). *Biological Control*, 47: 16–21. DOI: [10.1016/j.biocontrol.2008.06.011](https://doi.org/10.1016/j.biocontrol.2008.06.011)
- Fan, Y. & Pettitt, F.L. (1998) Dispersal of the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Experimental and Applied Acarology*, 22: 411–415. DOI: [10.1023/A:1006045911286](https://doi.org/10.1023/A:1006045911286)
- Fernández-Grandon, G.M., Harte, S.J., Ewany, J., Bray, D. & Stevenson, P.C. (2020) Additive effect of botanical insecticide and entomopathogenic fungi on pest mortality and the behavioral response of its natural enemy. *Plants*, 9: 173. DOI: [10.3390/plants9020173](https://doi.org/10.3390/plants9020173)
- Forim, M.R., da Silva, M.F.G.F., Cass, Q.B., Fernandes, J.B. & Vieira, P.C. (2010) Simultaneous quantification of azadirachtin and 3-tigloylazadirachtol in Brazilian seeds and oil of *Azadirachta indica*: application to quality control and marketing. *Analytical Methods*, 2(7): 860–869. DOI: [10.1039/C0ay00008f](https://doi.org/10.1039/C0ay00008f)
- Gerson, U. (1992) Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Experimental and Applied Acarology*, 13: 163–178. DOI: [10.1007/BF01194934](https://doi.org/10.1007/BF01194934)
- Goławska, S., Sprawka, I., Łukasik, I. & Gołowski, A. (2014) Are naringenin and quercetin useful chemicals in pest-management strategies? *Journal of Pest Science*, 87(1): 173–180. DOI: [10.1007/s10340-013-0535-5](https://doi.org/10.1007/s10340-013-0535-5)
- Grinberg, M., Perl-Treves, R., Palevsky, E., Shomer, I. & Soroker, V. (2005) Interaction between cucumber plants and the broad mite, *Polyphagotarsonemus latus*: from damage to defense gene expression. *Entomologia Experimentalis et Applicata*, 115(1): 135–144. DOI: [10.1111/j.1570-7458.2005.00275.x](https://doi.org/10.1111/j.1570-7458.2005.00275.x)
- Halder, J., Divekar, P.A. & Rani, A.T. (2021) Compatibility of entomopathogenic fungi and botanical against sucking pests of okra: an ecofriendly approach. *Egyptian Journal of Biological Pest Control*, 31(1): 30. DOI: [10.1186/s41938-021-00378-6](https://doi.org/10.1186/s41938-021-00378-6)
- Harith-Fadzilah, N., Ghani, I.A. & Hassan, M. (2021) Omics-based approach in characterizing mechanism of entomopathogenic fungi pathogenicity: A case example of *Beauveria bassiana*. *Journal of King Saud University*, 33: 101332. DOI: [10.1016/j.jksus.2020.101332](https://doi.org/10.1016/j.jksus.2020.101332)

- Hernández, M.M., Martínez-Villar, E., Peace, C., Pérez-Moreno, I. & Marco, V. (2012) Compatibility of the entomopathogenic fungus *Beauveria bassiana* with flufenoxuron and azadirachtin against *Tetranychus urticae*. *Experimental and Applied Acarology*, 58: 395–405. DOI: [10.1007/s10493-012-9594-1](https://doi.org/10.1007/s10493-012-9594-1)
- Herron, G., Jiang, L. & Spooner-Hart, R. (1996) A laboratory-based method to measure relative pesticide and spray oil efficacy against the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Experimental and Applied Acarology*, 20: 495–502. DOI: [10.1007/BF00048282](https://doi.org/10.1007/BF00048282)
- Hikal, W.M., Baeshen, R.S. & Said-Al Ahl, A.H. (2017) Botanical insecticide as simple extractives for pest control. *Cogent Biology*, 3: 1404274. DOI: [10.1080/23312025.2017.1404274](https://doi.org/10.1080/23312025.2017.1404274)
- Hirose, E., Neves, P.M.O.J., Zequi, J.A.C., Peralta, C.H. & Moino Jr., A. (2001) Effect of biofertilizers and neem oil on the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. *Brazilian Archives of Biology and Technology*, 44(4): 419–423. DOI: [10.1590/S1516-89132001000400013](https://doi.org/10.1590/S1516-89132001000400013)
- Islam, M.T., Castle, S.J. & Ren, S. (2010) Compatibility of the insect pathogenic fungus *Beauveria bassiana* with neem against sweet potato whitefly, *Bemisia tabaci*, on eggplant. *Entomologia Experimentalis et Applicata*, 134: 28–34. DOI: [10.1111/j.1570-7458.2009.00933.x](https://doi.org/10.1111/j.1570-7458.2009.00933.x)
- Jamali, F., Sohrabi, F. & Kohanmoo, M.A. (2021) Entomopathogenic fungi and plant essential oils are not compatible in controlling *Tribolium castaneum* (Herbst). *Journal of Plant Diseases and Protection*, 128: 799–808. DOI: [10.1007/s41348-021-00430-5](https://doi.org/10.1007/s41348-021-00430-5)
- Jeppson, L., Keiffer, H. & Baker, E. (1975) *Mites injurious to economic plants*. University of California Press, Los Angeles, USA, 614 pp.
- Kousik, C.S., Shepard, B.M., Hassell, R., Levi, A. & Simmons, A.M. (2007) Potential sources of resistance to broad mites (*Polyphagotarsonemus latus*) in watermelon germplasm. *HortScience*, 42(7): 1539–1544. DOI: [10.21273/HORTSCI.42.7.1539](https://doi.org/10.21273/HORTSCI.42.7.1539)
- Luypaert, G., Witters, J., van Huylenbroeck, J., Maes, M., de Riek, J. & de Clerq, P. (2014) Temperature-dependent development of the broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on *Rhododendron simsii*. *Experimental and Applied Acarology*, 63: 389–400. DOI: [10.1007/s10493-014-9777-z](https://doi.org/10.1007/s10493-014-9777-z)
- Liu, W., Xie, Y., Dong, J., Xue, J., Zhang, Y., Lu, Y. & Wu, J. (2014). Pathogenicity of three entomopathogenic fungi to *Matsucoccus matsumurae*. *PLoS ONE*, 9(7): e103350. DOI: [10.1371/journal.pone.0103350](https://doi.org/10.1371/journal.pone.0103350)
- Lü, J. & Zhang, H. (2016) The effect of acclimation to sublethal temperature on subsequent susceptibility of *Sitophilus zeamais* Mostchulsky (Coleoptera: Curculionidae) to high temperatures. *PLoS ONE*, 11(7): e0159400. DOI: [10.1371/journal.pone.0159400](https://doi.org/10.1371/journal.pone.0159400)
- Ma, W.G., Fukushi, Y., Tahara, S. & Osawa, T. (2000) Fungitoxic alkaloids from Hokkaido Papaveraceae. *Fitoterapia*, 71(5): 527–534. DOI: [10.1016/S0367-326X\(00\)00193-3](https://doi.org/10.1016/S0367-326X(00)00193-3)
- Maketon, M., Orosz-Coghlan, P. & Sinprasert, J. (2008) Evaluation of *Metarhizium anisopliae* (Deuteromycota: Hyphomycetes) for control of broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) in mulberry. *Experimental and Applied Acarology*, 46: 157–167. DOI: [10.1007/s10493-008-9153-y](https://doi.org/10.1007/s10493-008-9153-y)
- Martins, C.C., Alves, L.F.A., Mamprim, A.P. & Souza, L.P.A. (2016) Selection and characterization of *Beauveria* spp. isolates to control the broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae). *Brazilian Journal of Biology*, 76(3): 629–637. DOI: [10.1590/1519-6984.22614](https://doi.org/10.1590/1519-6984.22614)

- Mathesius, U. (2018) Flavonoid functions in plants and their interactions with other organisms. *Plants*, 7(2): 30. DOI: [10.3390/plants7020030](https://doi.org/10.3390/plants7020030)
- McKinnon, A.C., Saari, S., Moran-Diez, M.E., Meyling, N.V., Raad, M. & Glare, T.R. (2016) *Beauveria bassiana* as an endophyte: a critical review on associated methodology and biocontrol potential. *BioControl*, 62: 1–17. DOI: [10.1007/s10526-016-9769-5](https://doi.org/10.1007/s10526-016-9769-5)
- Mohammadi, S., Ziaee, M. & Seraj, A.A. (2016) Sublethal effects of Biomite® on the population growth and life table parameters of *Tetranychus turkestanii* Ugarov and Nikolskii on three cucumber cultivars. *Systematic and Applied Acarology*, 21(2): 218–226. DOI: [10.11158/saa.21.2.6](https://doi.org/10.11158/saa.21.2.6)
- Mohan, M.C., Reddy, N.P., Devi, U.K., Kongara, R. & Sharma, H.C. (2007) Growth and insect assay of *Beauveria bassiana* with neem to test their compatibility and synergism. *Biocontrol Science and Technology*, 17(10): 1059–1069. DOI: [10.1080/09583150701714551](https://doi.org/10.1080/09583150701714551)
- Muhammad, G., Hussain, M.A., Jantan, I. & Bukhari, S.N.A. (2016) *Mimosa pudica* L., a high-value medicinal plant as a source of bioactives for pharmaceuticals. *Comprehensive Reviews in Food Science and Food Safety*, 15: 303–315. DOI: [10.1111/1541-4337.12184](https://doi.org/10.1111/1541-4337.12184)
- Nana, P., Maniania, N.K., Maranga, R.O., Boga, H.I., Kutima, H.L. & Eloff, J.N. (2012) Compatibility between *Calpurnia aurea* leaf extract, attraction aggregation, and attachment pheromone and entomopathogenic fungus *Metarhizium anisopliae* on viability, growth, and virulence of the pathogen. *Journal of Pest Science*, 85: 109–115. DOI: [10.1007/s10340-011-0399-5](https://doi.org/10.1007/s10340-011-0399-5)
- Nasrin, M., Amin, A.R., Miah, M.R.U., Akanda, A.M., Miah, M.G., Kwon, O. & Suh, S.J. (2020) Occurrence and severity of mite *Polyphagotarsonemus latus* (Tarsonemidae) on chili plants: Analysis of pest-weather and host plant characteristics. *Entomological Research*, 51 (6): 273–281. DOI: [10.1111/1748-5967.12491](https://doi.org/10.1111/1748-5967.12491)
- Neves, P.O.M.J., Hirose, E., Tchujo, P.T. & Moino Jr., A. (2001) Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotropical Entomology*, 30(2): 263–268. DOI: [10.1590/S1519-566X2001000200009](https://doi.org/10.1590/S1519-566X2001000200009)
- Nexticapan-Garcéz, Á., Cua-Basulto, M., Martín-Mex, R., Pérez-Brito, D., Larqué-Saavedra, A., Villanueva-Couoh, E., Pérez-Gutiérrez, A. & Ruiz-Sánchez, E. (2021) Effects of botanical acaricides on *Raoiella indica* and *Oligonychus* sp. and their toxicity on two species of phytoseiid predatory mites. *Archive of Phytopathology and Plant Protection*. DOI: [10.1080/03235408.2021.1925515](https://doi.org/10.1080/03235408.2021.1925515)
- Nugroho, I. & Ibrahim, Y.B. (2004) Laboratory bioassay of some entomopathogenic fungi against broad mite (*Polyphagotarsonemus latus* Banks). *International Journal of Agriculture & Biology*, 6(2): 223–225.
- Onzo, A., Houedokoho, A.F. & Hanna, R. (2012) Potential of the predatory mite, *Amblyseius swirskii* to suppress the broad mite, *Polyphagotarsonemus latus* on the gboma eggplant, *Solanum macrocarpon*. *Journal of Insect Science*, 12(1): 7. DOI: [10.1673/031.012.0701](https://doi.org/10.1673/031.012.0701)
- Palevsky, E., Soroker, V., Weintraub, P., Mansour, F., Abo-Moch, F. & Gerson, U. (2001) How species-specific is the phoretic relationship between the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae), and its insect hosts? *Experimental and Applied Acarology*, 25: 217–224. DOI: [10.1023/A:1010645315630](https://doi.org/10.1023/A:1010645315630)
- Panche, A.N., Diwan, A.D. & Chandra, S.R. (2016) Flavonoids: an overview. *Journal of Nutritional Science*, 5: e47. DOI: [10.1017/jns.2016.41](https://doi.org/10.1017/jns.2016.41)
- Parker, R. & Gerson, U. (1994) Dispersal of the broad mite, *Polyphagotarsonemus latus* (Banks) (Heterostigmata: Tarsonemidae), by the greenhouse whitefly, *Trialeurodes vaporariorum*

- (Westwood) (Homoptera: Aleyrodidae). *Experimental and Applied Acarology*, 18: 581–585. DOI: [10.1007/BF00051720](https://doi.org/10.1007/BF00051720)
- Paula, A.R., Carolino, A.T., Paula, C.O. & Samuels, R.I. (2011) The combination of the entomopathogenic fungus *Metarhizium anisopliae* with the insecticide imidacloprid increases virulence against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Parasites & Vectors*, 4: 8. DOI: [10.1186/1756-3305-4-8](https://doi.org/10.1186/1756-3305-4-8)
- Peña, J.E. & Bullock, R. (1994) Effects of broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) feeding on vegetative plant growth. *The Florida Entomologist*, 77(1): 180–184. DOI: [10.2307/3495885](https://doi.org/10.2307/3495885)
- Peña, J.E., Osborne, L.S. & Duncan, R.E. (1996) Potential of fungi as biocontrol agents of *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Entomophaga*, 41(1): 27–36. DOI: [10.1007/BF02893289](https://doi.org/10.1007/BF02893289)
- Puspitarini, R.D., Afandhi, A. & Fernando, I. (2021a) Evaluation of indigenous fungal entomopathogens and aqueous leaf extract of *Annona muricata* against *Polyphagotarsonemus latus* infesting *Jatropha curcas* in Indonesia. *Biodiversitas*, 22(7): 2648–2655. DOI: [10.13057/biodiv/d220713](https://doi.org/10.13057/biodiv/d220713)
- Puspitarini, R.D., Fernando, I., Rachmawati, R., Hadi, M.S. & Rizali, A. (2021b) Host plant variability affects the development and reproduction of *Tetranychus urticae*. *International Journal of Acarology*, 47(5): 381–386. DOI: [10.1080/01647954.2021.1915377](https://doi.org/10.1080/01647954.2021.1915377)
- Puspitarini, R.D., Fernando, I., Sianturi, Y.P.P.A. & Rachmawati, R. (2021c) Compatibility of *Jatropha curcas* seed extract and entomopathogenic fungus *Akanthomyces lecanii* against the citrus red mite *Panonychus citri*. *Biocontrol Science and Technology*. DOI: [10.1080/09583157.2021.1993134](https://doi.org/10.1080/09583157.2021.1993134)
- Rehman, Hu., Rasul, A., Farooqi, M.A., Aslam, H.M.U., Majeed, B., Sagheer, M. & Ali, Q. (2020) Compatibility of some botanicals and the entomopathogenic fungus, *Beauveria bassiana* (Bals.), against the red flour beetle, *Tribolium castanenum* (Herbst) (Coleoptera: Tenebrionidae). *Egyptian Journal of Biological Pest Control*, 30: 131. DOI: [10.1186/s41938-020-00329-7](https://doi.org/10.1186/s41938-020-00329-7)
- Ribeiro, L.P., Blume, E., Bogorni, P.C., Dequech, S.T.B., Brand, S.C. & Junges, E. (2012) Compatibility of *Beauveria bassiana* commercial isolate with botanical insecticides utilized in organic crops in southern Brazil. *Biological Agriculture & Horticulture*, 28(4): 223–240. DOI: [10.1080/01448765.2012.735088](https://doi.org/10.1080/01448765.2012.735088)
- Ribeiro, L.P., Mota, L.H.C., D'alessandro C.P., Vendramin, J.D. & Delalibera Júnior, I. (2014) *In vitro* compatibility of an acetogenin-based bioinsecticide with three species of entomopathogenic fungi. *Florida Entomologist*, 97(4): 1395–1403. DOI: [10.1653/024.097.0414](https://doi.org/10.1653/024.097.0414)
- Roobakkumar, A., Subramaniam, M.S.R., Babu, A. & Muraleedharan, N. (2010) Bioefficacy of certain plant extracts against the red spider mite, *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae) infesting tea in Tamil Nadu, India. *International Journal of Acarology*, 36(3): 255–258. DOI: [10.1080/01647951003652592](https://doi.org/10.1080/01647951003652592)
- Rosado, J.F., Picanço, M.C., Sarmiento, R.A., da Silva, R.S., Pedro-Neto, M., Carvalho, M.A., Erasmo, E.A.L. & Silva, L.C.R. (2015) Seasonal variation in the populations of *Polyphagotarsonemus latus* and *Tetranychus bastosi* in physic nut (*Jatropha curcas*) plantations. *Experimental and Applied Acarology*, 66: 415–426. DOI: [10.1007/s10493-015-9911-6](https://doi.org/10.1007/s10493-015-9911-6)
- Rosado, J.F., Sarmiento, R.A., Pedro-Neto, M., Galdino, T.V.S., Marques, R.V., Erasmo, E.A.L. & Picanço, M.C. (2014) Sampling plans for pest mites on physic nut. *Experimental and Applied*

- Acarology*, 63: 521–534. DOI: [10.1007/s10493-014-9804-0](https://doi.org/10.1007/s10493-014-9804-0)
- Rossi-Zalaf, L.S. & Alves, S.B. (2006) Susceptibility of *Brevipalpus phoenicis* to entomopathogenic fungi. *Experimental and Applied Acarology*, 40(1): 37–47. DOI: [10.1007/s10493-006-9024-3](https://doi.org/10.1007/s10493-006-9024-3)
- Rossi-Zalaf, L.S., Alves, S.B., Lopes, R.B., Silveira, N.S. & Tanzini, M.R. (2008) Interação de microrganismos com outros agentes de controle de pragas e doenças. In: Alves, S.B. & Lopes, R.B. (Eds.), *Controle microbiano de pragas na América Latina*. Fundação de Estudos Agrários Luiz de Queiroz, Piracicaba, pp. 279–302.
- Roy, S. & Mukhopadhyay, A. (2012) Bioefficacy assessment of *Melia azedarach* (L.) seed extract on tea red spider mite, *Oligonychus coffeae* (Nietner) (Acari: Tetranychidae). *International Journal of Acarology*, 38(1): 79–86. DOI: [10.1080/01647954.2011.594811](https://doi.org/10.1080/01647954.2011.594811)
- Saharayaj, K., Namasivayam, S.K.R. & Rathi, M.J. (2011) Compatibility of entomopathogenic fungi with extracts of plants and commercial botanicals. *African Journal of Biotechnology*, 10(6): 933–938.
- Sarmiento, R.A., Rodrigues, D.M., Faraji, F., Erasmo, E.A.L., Lemos, F., Teodoro, A.V., Kikuchi, W.T., dos Santos, G.R. & Pallini, A. (2011) Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curca* plants in Brazil. *Experimental and Applied Acarology*, 53: 203–214. DOI: [10.1007/s10493-010-9396-2](https://doi.org/10.1007/s10493-010-9396-2)
- Savi, P.J., Martins, M.B., de Moraes, G.J., Hountondji, F.C.C. & Andrade, D.J. (2021) Bioactivity of oxymatrine and azadirachtin against *Tetranychus urticae* (Acari: Tetranychidae) and their compatibility with the predator *Phytoseiulus longipes* (Acari: Phytoseiidae) on tomato. *Systematic and Applied Acarology*, 26(7). DOI: [10.11158/saa.26.7.7](https://doi.org/10.11158/saa.26.7.7)
- Simmonds, M.S.J. (2003) Flavonoid-insect interactions: recent advances in our knowledge. *Phytochemistry*, 64: 21–30. DOI: [0.1016/S0031-9422\(03\)00293-0](https://doi.org/0.1016/S0031-9422(03)00293-0)
- Sohrabi, F., Jamali, F., Morammazi, S., Saber, M. & Kamita, S.G. (2019) Evaluation of the compatibility of entomopathogenic fungi and two botanical insecticides tondexir and palizin for controlling *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Crop Protection*, 117: 20–25. DOI: [10.1016/j.cropro.2018.11.012](https://doi.org/10.1016/j.cropro.2018.11.012)
- Soroker, V., Nelson, D.R., Bahar, O., Reneh, S., Yablonski, S. & Palevsky, E. (2003) Whitefly wax as a cue for phoresy in the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Chemoecology*, 13: 163–168. DOI: [10.1007/s00049-003-0243-3](https://doi.org/10.1007/s00049-003-0243-3)
- Tavares, W.R., do Carmo Barreto, M. & Seca, A.M.L. (2021) Aqueous and ethanolic plant extracts as bio-insecticides – establishing a bridge between raw scientific data and practical reality. *Plants*, 10: 920. DOI: [10.3390/plants10050920](https://doi.org/10.3390/plants10050920)
- Thakur, S. & Sood, A.K. (2019) Lethal and inhibitory activities of natural products and biopesticide formulations against *Tetranychus urticae* Koch (Acarina: Tetranychidae). *International Journal of Acarology*, 45(6–7): 381–390. DOI: [10.1080/01647954.2019.1666920](https://doi.org/10.1080/01647954.2019.1666920)
- Tsolakis, H. & Ragusa, S. (2008) Effects of a mixture of vegetable and essential oils and fatty acid potassium salts on *Tetranychus urticae* and *Phytoseiulus persimilis*. *Ecotoxicology and Environmental Safety*, 70(2): 276–282. DOI: [10.1016/j.ecoenv.2007.10.001](https://doi.org/10.1016/j.ecoenv.2007.10.001)
- Usha, J., Babu, M.N. & Padmaja, V. (2014) Detection of compatibility of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. with pesticides, fungicides and botanicals. *International Journal of Plant, Animal and Environmental Sciences*, 4(2): 613–624.

- Venzon, M., Rosado, M.C., Molina-Rugama, A.J.M., Duarte, V.S., Dias, R. & Pallini, A. (2008) Acaricidal efficacy of neem against *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Crop Protection*, 27(3–5): 869–872. DOI: [10.1016/j.cropro.2007.10.001](https://doi.org/10.1016/j.cropro.2007.10.001)
- Venzon, M., Togni, P.H.B., Perez, A.L. & Oliveira, J.M. (2020) Control of two-spotted spider mites with neem-based products on a leafy vegetable. *Crop Protection*, 128: 105006. DOI: [10.1016/j.cropro.2019.105006](https://doi.org/10.1016/j.cropro.2019.105006)
- Wang, C. & Wang, S. (2017) Insect pathogenic fungi: Genomics, molecular interactions, and genetic improvements. *Annual Review of Entomology*, 62: 73–90. DOI: [10.1146/annurev-ento-031616-035509](https://doi.org/10.1146/annurev-ento-031616-035509)
- Weintraub, P.G. (2007) Integrated control of pests in tropical and subtropical sweet pepper production. *Pest Management Science*, 63(8): 753–760. DOI: [10.1002/ps.1366](https://doi.org/10.1002/ps.1366)
- WHO (2016) *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. 2nd ed. World Health Organization, Geneva, 48 pp.
- Wu, S., Xie, H., Li, M., Xu, X. & Lei, Z. (2016) Highly virulent *Beauveria bassiana* strains against the two-spotted spider mite, *Tetranychus urticae*, show no pathogenicity against five phytoseiid mite species. *Experimental and Applied Acarology*, 70(4): 421–435. DOI: [10.1007/s10493-016-0090-x](https://doi.org/10.1007/s10493-016-0090-x)
- Zhang, J., Yuan, K., Zhou, W.L., Zhou, J. & Yang, P. (2011) Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn. from southern China. *Pharmacognosy Magazine*, 7(25): 35–39. DOI: [10.4103/0973-1296.75899](https://doi.org/10.4103/0973-1296.75899)
- Ziaee, M., Nikpay, A., Koohzad-Mohammadi, P. & Behman-Oskuyee, S. (2017) The toxicity of Biomite®, GC-mite®, Oberon® and Envidor® acaricides against sugarcane yellow mite, *Oligonychus sacchari* (Acari: Tetranychidae). *Persian Journal of Acarology*, 6(2): 137–141. DOI: [10.22073/pja.v6i2.28024](https://doi.org/10.22073/pja.v6i2.28024)
- Zimmermann, G. (2007) Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology*, 17(6): 553–596. DOI: [10.1080/09583150701309006](https://doi.org/10.1080/09583150701309006)

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سازگاری عصاره آبی برگ *Mimosa pudica* و قارچ انتومو-آکاروپاتوزن *Beauveria bassiana* در مهار کتفه پهن *Polyphagotarsonemus latus* (Acari: Tarsonemidae)

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چکیده

کتفه پهن *Polyphagotarsonemus latus* (Banks) آفتی چندخوار با پراکنش جهانی است. کاربرد ترکیبی آفت‌کش‌های گیاهی و قارچ انتومو-آکاروپاتوزن ممکن است به عنوان رویکردی سازگار با محیط زیست در مهار کتفه عمل کند. در این مطالعه، عصاره آبی برگ *Mimosa pudica* (Fabales: Fabaceae) (MpAE) در ابتدا برای تعیین اثرهای آن بر رشد میسلیم، اسپورزایی و زنده‌مانی قارچ انتومو-آکاروپاتوزن *Beauveria bassiana* (Hypocreales: Cordycipitaceae) مورد بررسی قرار گرفت. سپس سازگاری بر اساس محاسبه شاخص زیستی (BI) تعیین شد. کارایی زیستی MpAE و *B. bassiana* در غلظت‌های مختلف در برابر *P. latus*، هم به تنهایی و هم در ترکیب مورد بررسی قرار گرفت. هر تیمار در بستری حاوی دیسک برگی از مرکبات و ماده‌های جفتگیری کرده *P. latus* اسپری شد. میزان مرگ و میر و تخمگذاری ماده‌ها تا ۱۲۰ ساعت پس از مصرف مشاهده شد. نتایج نشان داد که زمانی که MpAE در محیط پرورش اضافه شد هیچ کاهش در رشد میسلیم *B. bassiana*، اسپورزایی، و زنده‌مانی کتفه‌ها دیده نشد. گستره شاخص زیستی از ۱۰۰/۰۱ تا ۱۳۶/۳۰ بود که نشان می‌دهد MpAE با *B. bassiana* بسیار سازگار است. کاربرد انفرادی و ترکیبی MpAE و *B. bassiana* به میزان زیادی موجب افزایش مرگ و میر کتفه و به طور هم‌زمان میزان تخمگذاری کتفه را محدود می‌کند. مرگ و میر کتفه از ۹۰ تا ۱۰۰ درصد در ۱۲۰ ساعت پس از استفاده در گروه‌های تیمارها متفاوت بود. میزان تخمگذاری کتفه به طور معنی‌داری کاهش یافت و از ۱۱/۵۰ تا ۲۹/۰۰ تخم در گروه‌های تیمارها متغیر بود، در حالی که در شاهد ۵۷/۲۵ تخم بود. بنابراین، پیشنهاد کاربرد MpAE و *B. bassiana* جایگزین ارزشمندی برای برنامه‌های مدیریت تلفیقی آفات خواهد بود.

واژگان کلیدی: کارایی زیستی؛ آفت‌کش‌های گیاهی؛ رویکرد سازگار با محیط زیست؛ مرگ و میر؛ میزان تخمگذاری؛ برنامه‌های مدیریت آفات.

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