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RESEARCH ARTICLE

BEHAVIORAL RESPONSE OF *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE) TOWARDS *METARHIZIUM ANISOPLIAE* VOLATILES

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ABSTRACT

The aim of this study was to investigate the behavioral response of *Bemisia tabaci* towards *Metarhizium anisopliae* (isolates PR₁ and GT₃) volatiles. Behavioral response of *B. tabaci* was investigated based on adult feeding and oviposition preference in eggplant *Solanum melongena* L. The highest mean number of adult (87) and egg (418) of *B. tabaci* were observed in the control plant, while the lowest mean number of adult (26) and egg (107) of *B. tabaci* were deposited in the PR₁-treated plant. There were 8 and 5 compounds identified from the isolates PR₁ and GT₃, respectively. The highest amount of compounds of 1-Hydroxy-2-aminopropane (61.96%) and 1, 4-Dioxane-2-ol (54.18%) were released by PR₁ and GT₃ respectively. The results obtained so far revealed that whitefly avoided the eggplants provided with cultures of the *M. anisopliae* isolates emanating the volatile organic compounds and suitability largely depended upon the volatile profile.

KEYWORDS

Entomopathogenic fungus, Whitefly, Adult feeding, Oviposition preference, IPM

1. INTRODUCTION

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is considered as a significant pest of global agriculture which is one of the most destructive pests of many vegetable, horticultural and ornamental crops at the tropical and subtropical countries including Bangladesh (Khatun et al., 2018) in the world (Oliveria et al., 2001; Simmons et al., 2008). This insect damages plants through direct feeding on phloem (Islam and Ren, 2009). It is an extremely problematic to agricultural production, because it has a high propensity to develop resistance to insect growth regulators (Horowitz et al., 2003) and neonicotinoid insecticides (Elbert and Nauen, 2000; Horowitz et al., 2004).

Chemical control is an essential component of whitefly management programs worldwide (Ellsworth and Martinez-Carrillo, 2001). This insect has developed resistance to chemical insecticides; therefore, alternative pest control strategies including biological control agent *viz.* bacteria, viruses, fungi, nematodes and protozoa are needed to introduce for their ongoing spreading (Huang et al., 2010; Cuthbertson et al., 2011). The entomopathogenic fungus *Metarhizium anisopliae* as an alternative approach to whitefly control has already developed in worldwide (Flores et al., 2012; Islam et al., 2014, 2016; Norhelina et al., 2013); because, there are approximately 200 species of insects, in a variety of different crops including greenhouse brown vegetables and ornamentals are infected by this entomopathogen (de Faria and Wraight, 2007). However, the entomopathogenic fungi can produce a wide assortment of metabolites, some of which are important to the host-specialization of this group of fungi.

Volatile organic compounds (VOCs) are a large group of carbon-based chemicals with low molecular weights and high vapor pressure produced by living organisms as part of their metabolic process (Bennett and Inamdar, 2015). Fungal metabolites are indicators of food and feed spoilage, flavor (Schnürer et al., 1999), as well as indoor building contamination (Sunesson et al., 1995). The information concerning the volatile organic compounds in relation to response the attraction or repellency of insect are available (Saini and Hassanali, 2007; Sullivan and Mori, 2009; Sun et al., 2010). The information concerning a strong positive correlation between virulence and repellency of entomopathogen towards the termite has been reported by Mburu et al. (2009). However, very few entomopathogenic fungi have been examined in detail for their metabolites and in even fewer cases is there information on the rule of these metabolites in disease process.

A growing body of research indicates that insects respond to fungal volatile organic compounds associated with their sensory environment, but few fungal volatiles have been tested to date for activity in laboratory or natural settings (Davis et al., 2013). Electrophysiological and behavioral responses of *Xylosandrus germanus* volatiles associated with its fungal symbiont *Ambrosiella grosmanii* was investigated (Ranger et al., 2021). Many fungal species produce low molecular weight volatile organic compounds (VOCs) with insect behavior modifying properties (Butt et al., 2016). Some compounds clearly possess pesticidal or repellent properties (Herrera et al., 2015; Holighaus and Rohlf, 2016, 2019). Most notable are 1-octen-3-ol, 3-octanone and 1-octene, which are produced by a wide range of fungi including saprophytic molds, mushrooms and entomopathogenic fungi including species of *Beauveria* and *Metarhizium*

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(Bojke et al., 2018; Combet et al., 2006; Yanagawa et al., 2011, 2015). Exactly why entomopathogenic fungi produce these compounds is unclear but the fact that so many insects exhibit avoidance behavior suggest that they are responding to VOCs (Butt et al., 2016; Mburu et al., 2013).

Previous study indicates that the isolates PR₁ and GT₃ of *M. anisopliae* are virulent to *B. tabaci* (Islam et al., 2014, 2016), but which factors are involved for host preference and suitability of *B. tabaci* was unknown. Therefore, the present study was conducted to investigate the behavioral response based on adult feeding and oviposition preference of *B. tabaci* towards volatile organic compound (VOC) of the mentioned above two isolates of *M. anisopliae*.

2. MATERIALS AND METHODS

2.1 Study site

The experiment was conducted in the Laboratory of Toxicology, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia. The present study was carried out under laboratory conditions at 25 ± 1 °C, 70 ± 10% relative humidity (r.h.), and 12 h light:12 h dark photoperiod.

2.2 Insect and host plant

The sweetpotato whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) biotype Q was used in this experiment (Islam et al., 2014). The population of *B. tabaci* was maintained on eggplants *Solanum melongena* L. for at least two successive generations before being used in experiment. Seeds of eggplant (cv. Malaysian dark red) were obtained from Baker Creek Heirloom Seed Co Ltd., Malaysia. They were grown individually in 15 cm-diameter plastic pots containing 1 kg autoclaved fertile soil, and at the 4-5 leaf stage, they were used in the experiment. These pots were placed into cages (60 cm × 60 cm × 60 cm). Mixed fertilizer (N:P:K = 13:7:15) were used 7 days after transplanting at the rate of 1 gm per pot.

2.3 Entomopathogenic fungi

The two strains- PR₁ (accession number- JX041509) and GT₃ (accession number- JX041511) of *M. anisopliae* originally isolated from *Coptotermes gestroi* (Rhinotermitidae: Isoptera) (Islam et al., 2014). The isolates were cultured on Sabouraud dextrose agar (SDA: 4% dextrose, 1% peptone, 2% agar) medium. Conidia of the isolates were collected from 15-day-old cultures (maintained at 25 ± 1°C, 70 ± 10% RH, and L12:D12 photoperiod) and were suspended in water containing 0.02% Tween 80 (Bio Basic Inc., Canada). The conidia were quantified using a hemocytometer and under a light microscope. The required fungal suspension (conidia/ml) was adjusted with regard to conidial viability (Islam et al., 2010a, 2014).

2.4 Host preference and suitability of *B. tabaci* to *M. anisopliae* isolates

When eggplants were 6 weeks old (4-5 true leaves), the adaxial and abaxial leaf surfaces were sprayed with the isolates of *M. anisopliae* (10⁸ conidia/ml) until runoff. The plants were then arranged for a choice test with 3 plants (1 control + 1 PR₁ + 1 GT₃) per rearing cage; and after 3 h, approximately 200 adult whiteflies (2-d-old) were released onto each cage (Islam et al., 2010a). Tween 80, 0.02% without fungal suspension served as control. There were 10 cages for each treatment. After 48 h, which was sufficient time for the adult whiteflies to choose a plant and oviposit, the adult whiteflies that had settled on each plant were counted. The adults were then removed from the plants and the number of egg per plant was counted using a dissecting microscope at 40× magnifications.

2.5 Analysis of VOC's released by *M. anisopliae* isolates

One hundred fifty ml Erlenmeyer flasks containing 50 ml of medium were sterilized at 121 °C for 20 min. After cooling, 5 ml of a fungal suspension (10⁸conidia/ml) were inoculated into each flask and shaking at 180 rpm with 30 °C for 5 d. There were 10 flasks for each treatment. After incubation period, the content of each flask was filtered through 0.2 µm mess filter paper (Gema Medical S.L.). The test tubes containing 1 ml of the filtrate culture were centrifuged at 12,000 g for 20 min (4 °C) and the

compounds were identified using GC-MS (QP5050A, Shimadzu, Japan), equipped with Zebron ZB-FFAP column (30 m × 0.25 mm ID., 0.25 µm film thickness). Helium (> 99% purity) was used as the carrier gas with a constant flow of 1.0 ml/min. The oven was held at 50 °C for 2 min. The temperature was then raised to 80 °C at a rate of 5 °C/min, followed by a gradient to 150 °C at a rate of 20 °C/min, held for 5 min. Finally, the temperature was raised to 250 °C at a rate of 30 °C /min, and held at 250 °C for 5 min. Mass spectra were repetitively scanned from 35–395 amu. Ionization was used in the electron impact mode (EI) at 70 eV. Compounds were identified by mass spectral matches to Wiley275.1 and NIST98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) libraries. Three replicates for each fungal isolate were analyzed. The peaks observed in the control (medium) were deleted from the samples.

2.6 Data analysis

Data concerning number of adult and egg of *B. tabaci* were subjected to one-way ANOVA by using SAS software (SAS Institute, 2001). The means were separated using the Tukey's honestly significant difference (HSD) test at 5% level of significance. The volatile organic compounds (VOCs) of the two isolates PR₁ and GT₃ of *M. anisopliae* were identified by GC-MS.

3. RESULTS AND DISCUSSION

Behavioral response of *B. tabaci* was investigated based on adult feeding and oviposition preference in eggplant. Extensive phenotypic variability was observed between the two isolates PR₁ and GT₃ of *M. anisopliae*. One-way ANOVA results showed significant differences according to the HSD test between the strains of *M. anisopliae* on two variables, including adult feeding ($F = 437.38$; $df = 2, 89$; $P < 0.0001$) and oviposition preference ($F = 4487.99$; $df = 2, 89$; $P < 0.0001$) of *B. tabaci* when compared with their respective controls (Figure 1). The highest mean number of adult (87) and egg (418) of *B. tabaci* were observed in the control plant, while the lowest mean number of adult (26) and egg (107) of *B. tabaci* were deposited in the PR₁-treated plant (Figure 1). This study clearly demonstrates that the adult of *B. tabaci* will preferentially feed on non-treated plants when surrounding plants have been treated with the isolates of *M. anisopliae*. Several studies concerning adult feeding and oviposition preference of *B. tabaci* were investigated earlier (Nardo et al., 1997; Hammad et al., 2000, 2001; Islam et al., 2010a, 2010b).

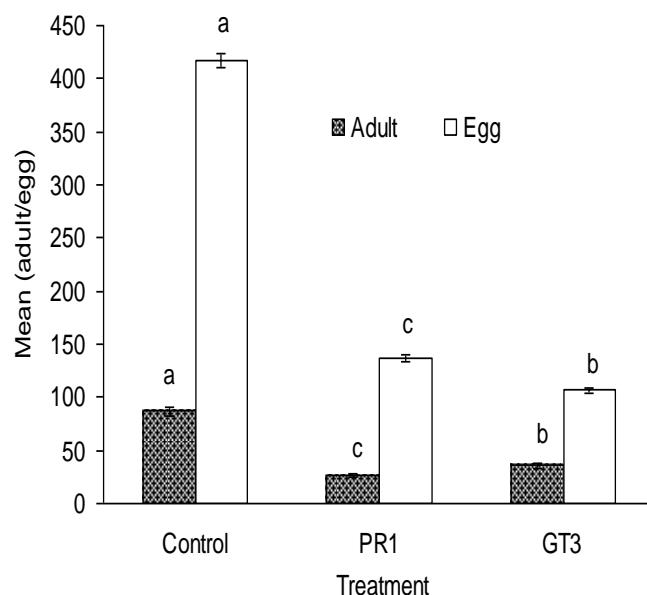


Figure 1: Mean (± standard error [SE]) number of adult and egg of *Bemisia tabaci* on eggplant treated with the two isolates of *Metarhizium anisopliae* at 10⁸ conidia/ml compared with control. Means with the same letter within a column are not significantly different (HSD-test, $P < 0.05$).

Table 1: Volatile organic compounds identified by GC-MS from the isolates of *Metarhizium anisopliae* at 10⁸ conidia/ml grown on SDA medium

Isolate	Compounds	Molecular formula	Retention time (min)	Total area (%)
PR ₁	1-Hydroxy-2-aminopropane	C ₃ H ₉ NO	6.13	61.96
	N, N-dimethyl- Formamide	HCON(CH ₃) ₂	9.78	0.12
	2-Hydroxypropanoic acid	C ₃ H ₆ O ₃	13.07	0.09
	Propylene Glycol	C ₃ H ₈ O ₂	13.17	0.09
	3-methyl-1, 2-Cyclopentanedione	C ₆ H ₈ O ₂	15.05	0.25
	3-Methylcyclopentanone	C ₆ H ₁₀ O	15.89	0.19
	Cyclohexanone	C ₆ H ₁₀ O	15.94	0.19
	2-methyl-Octane	C ₉ H ₂₀	19.60	0.10
GT ₃	2-methylpropanoic acid	C ₄ H ₈ O ₂	12.58	0.18
	1, 4-Dioxane-2-ol	C ₄ H ₈ O ₂	12.88	54.18
	Benzeneacetaldehyde	C ₈ H ₈ O	13.68	0.40
	Phenylethyl alcohol	C ₈ H ₁₀ O	17.88	0.07
	6-butyl-3-methoxy-2-Cyclohexen-1-one	C ₁₁ H ₁₈ O ₂	20.73	0.17

The volatile organic compound production among entomopathogenic fungi may reflect the difference in their biological activities. There were 8 and 5 compounds identified from the isolates PR₁ and GT₃, respectively (Table 1). The highest amount of compounds of 1-Hydroxy-2-aminopropane (61.96%) and 1, 4-Dioxane-2-ol (54.18%) released by the isolates PR₁ and GT₃, respectively (Table 1). These compounds are caused to reduce *B. tabaci* population. There were 13 compounds identified from *M. anisopliae* (isolate- EBCL 02049), including one alkene (1.25%), nine alkanes (73.38%), two ketones (23.30%) and one alcohol (2.07). The volatiles of *M. anisopliae* (isolate- EBCL 02049), which are mainly comprised of paraffins such as n-tetradecane (56.81%), were strong enough to cause a majority of live termites to increase their distance from the fungal source and aggregated in the container farthest from the fungal source (Hussain *et al.*, 2010). For the different entomopathogenic fungi, the *Beauveria bassiana* strains tested, DB-2, caused changes in mole cricket behavior, including significantly less new surface tunneling, fewer vertical tunnels descending into the soil, less tunneling along the perimeter of the containers, and significantly more occurrences of the crickets remaining in an area that reduced exposure to the conidia (Thompson and Brandenburg, 2005).

The differential repellency, as depicted by RD₅₀ (repellency dose for 50%) value of the fungi suggested qualitative and/or quantitative variations in the composition of volatile blends emitted by different strains (Mburu *et al.*, 2009) that is similar with our study. Our data also agree with the study of Thompson and Brandenburg (2005) that the presence of environmentally friendly control agents, such as entomopathogenic fungi, may affect pest behavior and avoidance by the target insect. Previous laboratory concerning behavioral studies have shown that repellency is directly related to the isolates, and different isolates of entomopathogenic fungi may elicit different levels of repellent response in termites (Staples and Milner, 2000). Several behavioral studies of different insects towards VOC's of entomopathogenic fungi have also been conducted earlier (Gikonyo *et al.*, 2003; Hountondji *et al.*, 2005; Kepler and Bruck, 2006; De Bruyne and Baker, 2008; Crespo *et al.*, 2008; Myrick *et al.*, 2009; Mburu *et al.*, 2011).

4. CONCLUSIONS

Both the isolates PR₁ and GT₃ of *M. anisopliae* are susceptible to *B. tabaci*. The study results also showed that the isolate PR₁ is the most suitable for *B. tabaci* as measured using adult feeding and oviposition preference; as well as VOC's of *M. anisopliae*. The results obtained so far revealed that whitefly avoided the eggplants provided with cultures of the *M. anisopliae* isolates emanating the volatile organic compounds and suitability largely depended upon the volatile profile. Further research is needed to investigate the compatibility of *M. anisopliae* with botanicals under field condition.

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