

## Full Length Research Paper

## Screening for attractants compatible with entomopathogenic fungus *Metarhizium anisopliae* for use in thrips management

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Several thrips attractants were screened for compatibility with *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and a subset of these for attraction to *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). Conidial germination and germ tube length of *M. anisopliae* were used as indicators of its compatibility with thrips attractant. Conidial germination and germ tube length differed significantly according to volatiles of different attractants. The highest conidial germination (76.5±3.5%) and longest germ tube length (130.3±13.4 µm) were recorded in the control, followed by methyl anthranilate (63.8±3.8%; 103.8±8.4 µm), cis-jasmone (61.8±5.9%; 93.8±14.4 µm) and trans-caryophyllene (57.7±6.5%; 96.3±15.5 µm) which were found compatible with *M. anisopliae*. A Pearson correlation test indicated a significant positive correlation between conidial germination and germ tube length ( $r = 0.6$ ;  $P < 0.0001$ ). The attraction of *M. sjostedti* to selected thrips attractant also varied significantly among the attractants. Under field conditions, methyl anthranilate was equally attractive to *M. sjostedti* as Lurem-TR and could be recommended as a thrips attractant that can be combined with *M. anisopliae* in autoinoculation devices for potential control of *M. sjostedti*.

**Key words:** Semiochemicals, conidial germination, germ tube length, *Megalurothrips sjostedti*, attraction, persistence, field.

### INTRODUCTION

In many flower dwelling thrips, host finding is linked to visual, odour and morphological (shape) cues (Rieske and Raffa, 2003; Mainali and Lim, 2011). Subsequently,

semiochemical-based products such as Lurem-TR and Thripline have been developed for use in thrips monitoring and management (Sampson and Kirk, 2013;

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Teulon et al., 2014; Broughton et al., 2015). These semiochemicals can be integrated with other control strategies to improve thrips management in horticulture (Suckling et al., 2012; Sampson and Kirk, 2013).

Entomopathogenic fungi (EPF) are among the alternatives to synthetic chemical pesticides being considered for the management of thrips in horticulture (Ekesi and Maniania, 2007). EPF are generally applied through inundative spray, which requires high amount of inocula, thereby enhancing its cost (Jaronski, 2010). Further, the persistence of conidia applied on foliage is challenged by several environmental parameters such as UV light, rain, temperature (Inglis et al., 2000; Jaronski, 2010). The use of "lure and kill" strategy using autoinoculation device or spot spray application could reduce the amount of inoculum, the cost and sustain fungal persistence in the field (Dimbi et al., 2003; Nana et al., 2014; Mfuti et al., 2016). However, the success of this technology depends on the use of powerful attractants and their compatibility with the entomopathogens. For example, the tick attraction-aggregation-attachment pheromone (AAAP) could attract adult ticks from a distance of 6 m (Nchu et al., 2009) but could not be used in combination with conidia of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) because of inhibition of fungal conidia by the pheromone (Nana et al., 2012). Niassy et al. (2012a) and Mfuti et al. (2016) have also reported inhibitory effects of conidia of *M. anisopliae* by the semiochemical Lurem-TR in autoinoculation device in a greenhouse and field experiments.

Considering the growing interest in integrating attractants with EPF in thrips management (Niassy et al., 2012a; Mfuti et al., 2016), there is a need to identify compounds that are both attractive to thrips and compatible with EPF. The objective of the present study was therefore to identify thrips attractants that are compatible with *M. anisopliae* in terms of conidial germination and germ tube length since the latter plays a crucial role in fungal infection (Ortiz-Ribbing and Williams, 2006).

## MATERIALS AND METHODS

### Thrips attractants

Seven compounds used for thrips attraction or with potential attraction for thrips were tested for their compatibility with *M. anisopliae* isolate ICIPE 69. They were selected on the basis of structural analogies to known attractant such as methyl isonicotinate (Lurem-TR) (Teulon et al., 2007, 2010) but also based on previous studies of Koschier et al. (2000). Information on their chemical characteristics and manufacturers is presented in Table 1. The commercial attractant, Lurem-TR which was earlier reported to be toxic with the entomopathogen (Niassy et al., 2012a) was included in the study as a reference. It is a commercial product which quantity and release rate is standardized; therefore, could not be diluted. In preliminary bioassays, no significant effect of different concentrations (0.1, 10 and 100%) of attractants was observed on

conidial germination and subsequently only the recommended concentration of 10% of the pure product was used in the screening bioassays. The pure concentration of all attractants was diluted in paraffin oil.

### Crop

Cowpea, *Vigna unguiculata* L. Walp variety Ken-Kunde1, was planted in 80 m<sup>2</sup> plots with an inter- and intra- row spacing of 10 and 45 cm, respectively, in Mbita Thomas Odhiambo Campus (ITOC) (0° 26' 06.19" S, 34° 12' 53.13" E; 1,137 above sea level) earlier during rainy season (March 2014). The size of the cowpea farm was about 94 x 22 m. The field experiment of selected attractants was conducted during flowering stage of the crop (45 days after planting). No fertilizers, organic matter or synthetic chemical insecticides were applied during the experiment.

### Fungal culture

*M. anisopliae* isolate ICIPE 69 was obtained from the Arthropod Germplasm Centre of *icipe*. It is currently commercialized as Campaign® by the Real IPM Ltd, Kenya, for the control of thrips, papaw mealy bug and fruit flies (<http://www.realipm.com>). It was cultured on Sabouraud Dextrose Agar (SDA) in 9 cm Petri dishes and incubated at 25 ± 2°C in complete darkness. Conidia were harvested from three week-old culture by scraping the surface using a spatula. Conidia were suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads. Conidial suspensions were vortexed for 5 min to produce a homogeneous suspension. Spore concentrations were determined using a haemocytometer.

### Effect of thrips attractants on conidial viability and germ tube length of *M. anisopliae*

The conidial suspension was prepared as described earlier and titrated to 1 × 10<sup>7</sup> conidia ml<sup>-1</sup>. The spores were retained on a nitrocellulose filter membrane (diameter 47 mm, pore size 0.45 µm, Sigma Chemicals) by pouring 10 ml suspension through a filter holder unit (MFS) under aspirator vacuum (Maniania, 1994). The nitrocellulose filter membranes were dried for 30 min under a laminar flow cabinet and transferred to glass desiccators (2.5 L) for exposure to the attractant volatile. Cotton wicks were soaked in 0.5 ml suspensions of each attractant diluted in paraffin oil and placed in desiccators to allow volatile diffusion. Cotton wicks were used as dispenser (Sidahmed et al., 2014). Fungus-treated nitrocellulose membranes were exposed to different thrips attractants and sampled for viability observation at different time intervals of 1, 2, 3, 6 and 8 days. An untreated control without thrips attractant was included. The commercial thrips attractant, Lurem-TR was included as a check. Treatments were randomized and the experiment repeated three times over time.

To determine conidial germination, nitrocellulose filter membranes containing conidia were removed from the desiccators and transferred into 10 ml sterile distilled water containing 0.05% Triton X-100 and vortexed for 3 min to dislodge conidia. Suspension (0.1 ml) titrated to 3 × 10<sup>6</sup> conidia ml<sup>-1</sup> was spread-plated on SDA plates. Plates were incubated at 26 ± 2°C, L12: D12 photoperiod and examined after 18 to 24 h for conidial germination and germ tube length thereafter. Samples that could not be processed the same day were fixed by pouring a drop of lactophenol cotton blue onto the plate to stop further growth. Percentage germination was determined by counting approx. 100 spores per plate under a microscope Leica DMLB at 40 X magnification. The length of germ tubes was measured using a

**Table 1.** General information of the tested thrips attractant compounds.

Label Name	Chemical formula	CAS number	Chemical group	Company	Purity (%)	Dilution range for thrips attraction and species attracted
4-anisaldehyde	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	19486-71-6	Aldehyde	Sigma-Aldrich Chemicals GmbH, Germany	98	0.1-10% (applied in 1 microliter paraffin oil) (Koschier et al., 2000) <i>Frankliniella occidentalis</i>
Ethyl benzoate	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	93-89-0	Ester of benzoic acid and ethanol	Sigma-Aldrich Chemicals GmbH, Germany	99	<i>Thrips obscuratus</i> ; <i>Thrips tabaci</i> (Koschier et al., 2000)
<i>Cis</i> -jasmone	C <sub>11</sub> H <sub>16</sub> O	488-10-8	Jasmonate (organic compound)	Sigma-Aldrich Chemicals GmbH, Germany	≥ 99	10mg/200 microliters hexane <i>T. obscuratus</i> , <i>T. tabaci</i> (El-Sayed et al., 2009)
Linalool	C <sub>10</sub> H <sub>18</sub> O	78-70-6	monoterpene	Sigma-Aldrich Chemicals GmbH, Germany	97	1-10% (in 1 microliter paraffin oil) <i>F. occidentalis</i> , <i>T. tabaci</i> (Koschier et al., 2000)
Methyl anthranilate	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>	134-20-3	Ester of anthranilic	Sigma-Aldrich Chemicals GmbH, Germany	98	<i>T. coloratus</i> , <i>T. hawaiiensis</i> (Murai et al., 2000; Imai et al., 2001)
<i>trans</i> caryophyllene	C <sub>15</sub> H <sub>24</sub>	87-44-5	Sesquiterpene	Sigma-Aldrich Chemicals GmbH, Germany	≥98.5	1-10% (in 1 microliter paraffin oil) (Koschier et al., 2000)
Phenylethanol	C <sub>8</sub> H <sub>10</sub> O	60-12-8	Alcohol	Sigma-Aldrich Chemicals GmbH, Germany	≥ 99	<i>T. tabaci</i> (Teulon et al., 2007)
Methyl-isonicotinate	C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>	2459-09-8	Pyridine	Pherobank Wageningen, The Netherlands.	-	Several thrips species such as <i>F. occidentalis</i> , <i>T. tabaci</i> (Davidson et al., 2007) <i>F. schultzei</i> , <i>Hydatothrips adolffriderici</i> and <i>Megalurothrips sjostedti</i> (Muvea et al., 2014)

Leica Application Suite (LAS EZ V1.5.0). Average germ tube lengths were obtained from 5 spores taken at random in each cover slip (22 x 22 mm) and replicated three times.

#### Effect of selected thrips attractants on the attraction of *M. sjostedti*

Attractants that were found compatible with *M. anisopliae* from the screening experiment were selected for field experiment to evaluate the attraction of Bean Flower Thrips (BFT), *M. sjostedti* (Trybom) (Thysanoptera: Thripidae) on cowpea. Lurem-TR was included as reference. Attractants were diluted in Paraffin oil as indicated above. Each attractant suspension was poured in 5 ml Eppendorf tube and suspended in the middle surface of the blue sticky card (10 x 25 cm) (Plate 1). The two items were placed at 30 cm above ground level. Blue sticky cards were

separated 10 m from one another to avoid interference. An untreated blue sticky card with no attractant was used as a control. The experiment was conducted during flowering and podding stages of cowpea when BFT populations are high (Ezueh, 1981; Nyasani et al., 2013). Cards were replaced every three days. Numbers of adult BFT were recorded on each card and the experiment was replicated four times over time.

#### Statistical analysis

Data on conidial germination of *M. anisopliae* were normalized by arcsine transformation before subjecting them to linear mixed model. Data on *M. anisopliae* conidial germ tube length and *M. sjostedti* catches were also analyzed using linear mixed model. Means were separated using Student–Newman–Keuls (SNK) test. A Pearson

correlation analysis was carried out to relate conidial viability with the germ tube length. All data analyses were performed using R (R Development Core Team, 2014). The level of significance was maintained at 95%.

## RESULTS

### Effect of thrips attractants on conidial viability and germ tube length of *M. anisopliae*

Overall, the effects of thrips attractants on germination of conidia of *M. anisopliae* varied significantly between the attractants ( $F_{9,268} = 22.1$ ;  $P < 0.0001$ ) (Table 2). The interaction day x attractant was statistically significant ( $F_{9,268} = 3.8$ ;



**Plate 1.** *M. sjostedti* attracted in the surface of the blue sticky card baited with attractant suspension poured in 5 ml Eppendorf tube.

**Table 2.** Conidial germination and germ tube length of *Metarhizium anisopliae* after exposition to thrips attractants (temperature:  $26 \pm 2^\circ\text{C}$ ; photoperiod: L12: D12; time of incubation: 18-24 h)

Treatments	Mean % germination $\pm$ SE (transformed)	Mean length $\pm$ SE
Control	62.4 $\pm$ 3.5 <sup>a</sup>	130.5 $\pm$ 10.0 <sup>a</sup>
Methyl anthranilate	53.7 $\pm$ 3.5 <sup>ab</sup>	103.8 $\pm$ 10.0 <sup>ab</sup>
Trans-Caryophyllene	51.2 $\pm$ 3.5 <sup>ab</sup>	96.3 $\pm$ 10.0 <sup>ab</sup>
Cis-jasmone	50.9 $\pm$ 3.5 <sup>ab</sup>	93.8 $\pm$ 10.0 <sup>ab</sup>
Solvent (paraffin oil)	48.4 $\pm$ 3.5 <sup>b</sup>	90.9 $\pm$ 10.0 <sup>b</sup>
Linalool	33.3 $\pm$ 3.5 <sup>bc</sup>	67.1 $\pm$ 10.0 <sup>c</sup>
Phenylethanol	32.4 $\pm$ 3.5 <sup>c</sup>	50.9 $\pm$ 10.0 <sup>c</sup>
4-Anisaldehyde	30.8 $\pm$ 3.5 <sup>c</sup>	45.5 $\pm$ 10.0 <sup>c</sup>
Lurem-TR	24.4 $\pm$ 3.5 <sup>c</sup>	37.1 $\pm$ 11.7 <sup>c</sup>
Ethyl benzoate	20.1 $\pm$ 4.0 <sup>c</sup>	36.1 $\pm$ 10.0 <sup>c</sup>

Means bearing the same letters are not significantly different by the Student–Newman–Keuls test (SNK).

$P < 0.0001$ ). The time of exposure had significant effects on conidial germination, except at 1 day post-exposure when no significant effect was observed ( $F_{9,45} = 1.5$ ;  $P = 0.2$ ) (Table 3). Significant reduction in conidial germination was observed in all the treatments from day 2 ( $F_{9,45} = 6.1$ ;  $P < 0.0001$ ), day 3 ( $F_{9,45} = 6.8$ ;  $P < 0.0001$ ), Day 6 ( $F_{9,45} = 8.3$ ;  $P < 0.0001$ ) and day 8 post-exposure ( $F_{9,45} = 8.7$ ;  $P < 0.0001$ ) (Table 3). The conidial germination was significantly higher in the control ( $62.5 \pm 10.0\%$ ), followed by cis-jasmone ( $44.8 \pm 16.6\%$ ), Solvent (paraffin oil) ( $42.8 \pm 11.0\%$ ), methyl anthranilate ( $36.6 \pm$

$8.0\%$ ) and trans-caryophyllene ( $31.3 \pm 16.8\%$ ) treatments after 8 days of exposure and was significantly different (Table 3). No conidial germination was observed in Lurem-TR treatment at day 8 post-exposure (Table 3).

The effect of thrips attractants on germ tube length followed the same trend as with conidial germination where treatments differed significantly ( $F_{9,268} = 12.6$ ;  $P < 0.0001$ ) (Table 2). The interaction day  $\times$  attractant was not statistically significant ( $F_{9,268} = 1.0$ ;  $P = 0.5$ ). Exposure time had significant effects on length of the germ tube of *M. anisopliae* at day 1 ( $F_{9,45} = 4.3$ ;  $P = 0.003$ ), Day 2

**Table 3.** Effect of thrips attractants on conidial germination of *M. anisopliae* over time.

Treatment (Thrips attractants)	Day after exposure					
	1	2	3	6	8	ANOVA
Control	91.3±3.0 <sup>aA</sup>	85.4±3.7 <sup>aAB</sup>	78.3±4.0 <sup>aB</sup>	65.5±10.0 <sup>aC</sup>	62.5±10.0 <sup>aC</sup>	F <sub>4,22</sub> =14.65; P<0.0001
4-anisaldehyde	85.7±2.0 <sup>aA</sup>	48.7±14.3 <sup>bcdB</sup>	9.2±7.9 <sup>dC</sup>	0.1±0.1 <sup>cC</sup>	0.1±0.1 <sup>cC</sup>	F <sub>4,22</sub> =38.2; P<0.0001
Ethyl Benzoate	79.8±6.8 <sup>aA</sup>	44.1±14.1 <sup>abcB</sup>	17.5±6.1 <sup>cdBC</sup>	11.9±4.4 <sup>bcBC</sup>	7.5±3.4 <sup>bcC</sup>	F <sub>4,22</sub> =35.8; P<0.0001
Jasmone	83.8±4.0 <sup>aA</sup>	74.6±7.5 <sup>abcA</sup>	61.1±12.9 <sup>abAB</sup>	44.6±15.5 <sup>abB</sup>	44.8±16.6 <sup>abB</sup>	F <sub>4,22</sub> =6.1; P=0.001
Linalool	75.5±4.3 <sup>aA</sup>	63±3 <sup>abcB</sup>	50.6±6.2 <sup>abcC</sup>	2.5±1.4 <sup>cD</sup>	0.5±0.5 <sup>cD</sup>	F <sub>4,22</sub> =195.5; P<0.0001
Methyl anthranilate	85.8±3.6 <sup>aA</sup>	78.8±2.7 <sup>abA</sup>	60.7±2.3 <sup>abBC</sup>	56.8±4.6 <sup>abB</sup>	36.6±8.0 <sup>aC</sup>	F <sub>4,22</sub> =28.2; P<0.0001
Phenylethanol	76.1±8.5 <sup>aA</sup>	50.1±14.6 <sup>bcdB</sup>	36.5±15.3 <sup>bcdB</sup>	18.2±11.5 <sup>bcC</sup>	5.5±4.1 <sup>bcC</sup>	F <sub>4,22</sub> =21.5; P<0.0001
Trans caryophyllene	80.0±5.0 <sup>aA</sup>	74.5±7.0 <sup>abA</sup>	53.8±16.6 <sup>abcAB</sup>	49.3±16.4 <sup>abAB</sup>	31.3±16.8 <sup>abB</sup>	F <sub>4,22</sub> =6.2; P=0.001
Lurem-TR	75.7±4.2 <sup>aA</sup>	20.6±3.7 <sup>dB</sup>	13.6±3.1 <sup>cdC</sup>	0.03±0.0 <sup>cD</sup>	0.0±0.0 <sup>D</sup>	F <sub>4,13</sub> =199.8; P<0.0001
Solvent (paraffin oil)	82.0±3.0 <sup>aA</sup>	72.7±5.2 <sup>abcAB</sup>	58.6±7.1 <sup>abB</sup>	43.9±11.0 <sup>abC</sup>	42.8±11.0 <sup>aC</sup>	F <sub>4,22</sub> =14.7; P<0.0001
ANOVA	F <sub>9,45</sub> =1.5; P=0.2	F <sub>9,45</sub> =6.1; P<0.0001	F <sub>9,45</sub> =6.8; P<0.0001	F <sub>9,46</sub> =8.3; P<0.0001	F <sub>9,46</sub> =8.7; P<0.0001	

Within column, means (±SE) followed by the same small letters are not significantly different Student–Newman–Keuls test (SNK), Within rows, means (±SE) followed by the same capital letters are not significantly different Student–Newman–Keuls test (SNK).

(F<sub>9,45</sub> = 6.7; P<0.0001), day 3 (F<sub>9,45</sub> = 6.9; P<0.0001), day 6 (F<sub>9,45</sub> = 6.5; P<0.0001) and day 8 (F<sub>9,45</sub> = 5.6; P<0.0001) post-exposure (Table 4). The highest germ tube length was observed in the control treatment (89.1±32.4 µm) followed by methyl anthranilate (69.6±12.9 µm), solvent (paraffin oil) (54.7 ± 16.4 µm), trans-caryophyllene (42.1 ± 13.9 µm) and cis-jasmone (41.9 ± 16.7 µm) and was not significantly different at day 8 post-exposure (Table 4). No germ tube developed in Lurem-TR treatment at day 6 and 8 post-exposure (Table 4). A significant correlation was found between conidial germination and germ tube length of *M. anisopliae* (r = 0.6; P<0.0001) (Figure 1).

#### Effect of selected thrips attractants on the attraction of *M. sjostedti*

The number of adult BFT caught on the baited sticky cards varied significantly (F<sub>3,28</sub> = 7.9; P<

0.0001) between the treatments. More adult BFT were caught on sticky cards treated with Lurem-TR and methyl anthranilate than cards treated with cis-jasmone (Figure 2). No significant difference in thrips catches was found between Lurem-TR and methyl anthranilate. Similarly, there was no significant difference between cis-jasmone and control treatments (Figure 2).

#### DISCUSSION

Most studies on compatibility of *M. anisopliae* have focused on agrochemicals and botanicals (Nana et al., 2012; Niassy et al., 2012b) overlooking the potential of semiochemical attractants in insect pest management (IPM). However, a study on compatibility between attractants and EPF is required before their integration in an IPM strategy.

Thrips attractants tested in the present study affected conidial germination and germ tube

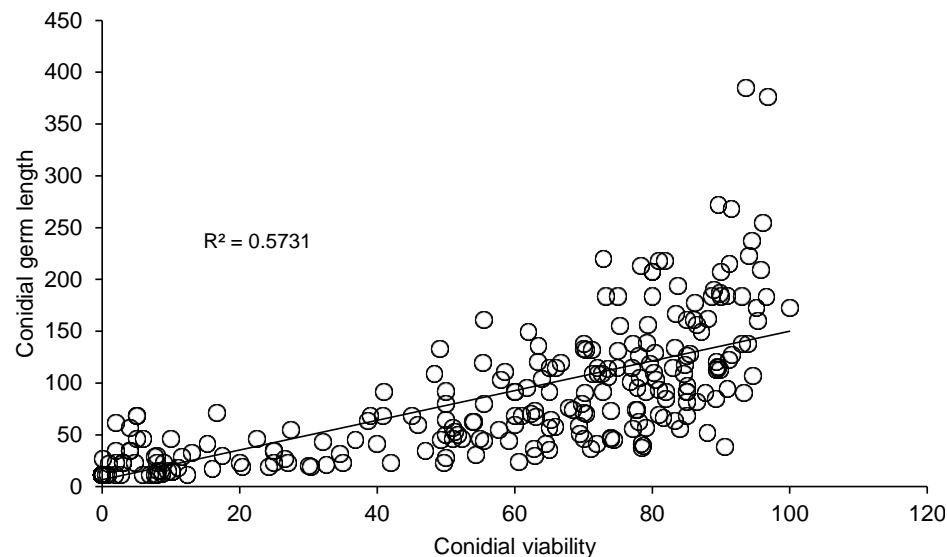
length differently, with time of exposure being the determining factor. Three out of eight attractants tested namely methyl anthranilate, cis-jasmone and trans-caryophyllene, did not have deleterious effects on conidial germination and germ tube length of *M. anisopliae* at day 8 post-exposure (Tables 3 and 4). Interestingly, these three attractants have been reported elsewhere to have effects on fungal pathogens. For instance, methyl anthranilate has been reported to significantly reduce the growth of strawberry pathogens such as *Botrytis cinerea* (Helotiales: Sclerotiniaceae), *Colletotrichum gloeosporioides* and *C. acutatum* (Glomerellale: Glomerellaceae). In addition, medium supplemented with methyl anthranilate resulted in complete cessation of growth in those pathogens (Chambers et al., 2013).

Recent evidence suggests that jasmonic acid is involved in the induction of genes that act primarily in defense against plant pathogens rather than insects (Halim et al., 2006). Jasmonic acid is part of the plant's alarm system and

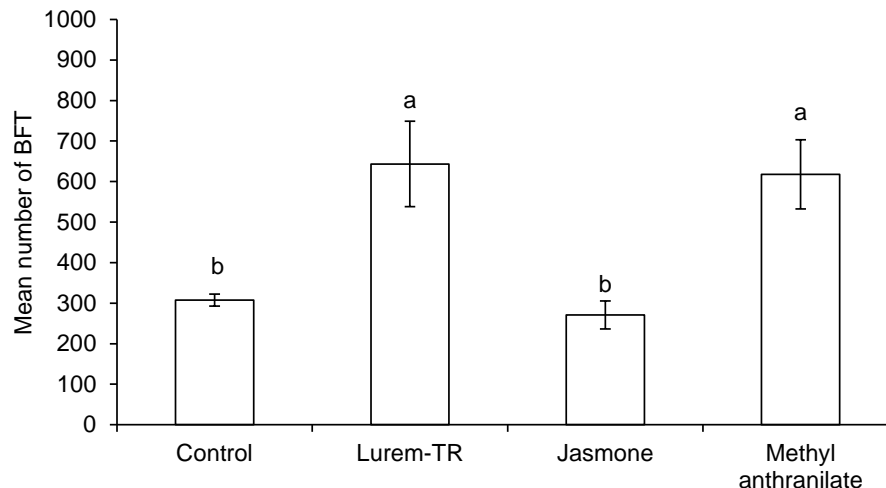
**Table 4.** Effect of thrips attractants on conidial germ tube length (µm) of *M. anisopliae* over time.

Treatment (Thrips attractants)	Day after exposure					ANOVA
	1	2	3	6	8	
Control	190.7±2.0 <sup>aA</sup>	146.9±25.4 <sup>aB</sup>	123.2±28.2 <sup>aBC</sup>	102.4±28.9 <sup>aBC</sup>	89.1±32.4 <sup>aC</sup>	F <sub>4,22</sub> =8.3; P=0.003
4-anisaldehyde	102.5±19.0 <sup>bcA</sup>	74.6±12.4 <sup>bcA</sup>	27.7±16.2 <sup>cb</sup>	11.5±0.0 <sup>cb</sup>	11.5±0.0 <sup>cb</sup>	F <sub>4,22</sub> =12.6; P<0.0001
Ethyl benzoate	77.8±20.4 <sup>ca</sup>	40.9±13.4 <sup>cb</sup>	26.8±5.4 <sup>cb</sup>	20.5±4.6 <sup>cb</sup>	14.4±2.0 <sup>cb</sup>	F <sub>4,22</sub> =7.2; P=0.0007
Jasmone	185.4±42.6 <sup>aA</sup>	103.8±22.1 <sup>abB</sup>	90.6±21.9 <sup>abBC</sup>	47.3±16.3 <sup>bcC</sup>	41.9±16.7 <sup>bcC</sup>	F <sub>4,22</sub> =13.5; P<0.0001
Linalool	122±26.4 <sup>abcA</sup>	94.3±15.5 <sup>abA</sup>	83.9±12.8 <sup>abA</sup>	22.2±5.7 <sup>cb</sup>	13.4±1.9 <sup>cb</sup>	F <sub>4,22</sub> =17.58; P<0.0001
Methyl anthranilate	134.4±22.6 <sup>abcA</sup>	120.5±20.4 <sup>abA</sup>	108.6±17 <sup>aAB</sup>	86.1±11.4 <sup>abBC</sup>	69.6±12.9 <sup>abC</sup>	F <sub>4,22</sub> =8.4; P=0.0002
Phenylethanol	102.9±25.7 <sup>bcA</sup>	71.9±21.6 <sup>bcAB</sup>	45.9±18.2 <sup>bcBC</sup>	20.4±5.9 <sup>cc</sup>	13.4±1.9 <sup>cc</sup>	F <sub>4,22</sub> =7.1; P=0.0007
Transcaryophyllene	179.3±49.1 <sup>abA</sup>	112.9±34.1 <sup>abB</sup>	81.0±21.6 <sup>abBC</sup>	66.2±21.8 <sup>abcBC</sup>	42.1±13.9 <sup>bcC</sup>	F <sub>4,22</sub> =9.9; P<0.0001
Lurem-TR	75.7±22.6 <sup>bcA</sup>	20.6±6.1 <sup>cb</sup>	13.6±3.3 <sup>cb</sup>	15.4±7.9 <sup>cb</sup>	11.5±0.0 <sup>cb</sup>	F <sub>4,13</sub> =14.3; P=0.0001
Solvent (paraffin oil)	139.3±19.9 <sup>abcA</sup>	107.9±21.4 <sup>abB</sup>	87.7±19.0 <sup>abBC</sup>	64.7±18.0 <sup>abcBC</sup>	54.7±16.4 <sup>abC</sup>	F <sub>4,22</sub> =8.3; P=0.0003
ANOVA	F <sub>9,45</sub> =4.3; P=0.003	F <sub>9,45</sub> =6.7; P<0.0001	F <sub>9,45</sub> =6.9; P<0.0001	F <sub>9,45</sub> =6.5; P<0.0001	F <sub>9,45</sub> =5.6; P<0.0001	

Within column, means (±SE) followed by the same small letters are not significantly different by the Student–Newman–Keuls test (SNK); Within rows, means (±SE) followed by the same capital letters are not significantly different by the Student–Newman–Keuls test (SNK).



**Figure 1.** Scattergram showing correlation between *Metarhizium anisopliae* conidial germination and germ tube length using the Pearson method.



**Figure 2.** Mean ( $\pm$  SE) number of *M. sjostedti* attracted to blue sticky card baited with Methyl anthranilate, Cis-jasmone, Lurem-TR and control. Means bearing the same small letters are not significantly different by the Student–Newman–Keuls test (SNK).

defense mechanism. It is a volatile (gas phase of Cis-jasmone) which is released during insect attack and controls the response to damage (Menzel et al., 2014).

Essential oil from *Perovskia atriplicifolia* Benth (Lamiales: Lamiaceae) containing 9.30% of trans-caryophyllene are reported to have antimicrobial activity against fungal strains (Erdemgil et al., 2007). The difference between these results and our results could be explained by the fact that attractants were used as volatiles in our study while they were used as oil supplements in culture media.

This study also confirmed previous findings on the antifungal effect of Lurem-TR on conidial germination (Niassy et al., 2012a). More recently, it was demonstrated that direct exposure of fungus without separation from Lurem-TR recorded the lowest conidial germination as compared with the other treatments where separation was made. However, fungal persistence increased with distance of separation of Lurem-TR (Mfuti et al., 2016).

The strong correlation observed between conidial germination and germ tube length suggests that fungal inoculum would still cause infection in the insects. The role of germ tube formation in the pathogenesis is well established (Ortiz-Ribbing and Williams, 2006). For instance, comparing four different growth stages of *Isaria fumosorosea* (*Paecilomyces fumosoroseus*) (Eurotiales: Trichocomaceae) (conidia, germinated conidia with either one or two germ tubes and hyphal bodies), Fargues et al. (1994) found that germinated conidia and hyphal bodies were more aggressive than ungerminated conidia against first-instar larvae of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae).

The catches of BFT were significantly higher on blue sticky cards baited with methyl anthranilate and Lurem-TR than the control and cis-jasmone baited cards. The

increased attraction of BFT to Lurem-TR and blue sticky traps was reported by (Muvea et al., 2014). No difference in BFT attraction was found between the two compounds. Methyl anthranilate has been reported to be attractive to four species of flower thrips, *Thrips hawaiiensis*, *Thrips coloratus*, *Thrips flavus*, and *Megalurothrips distalis*, irrespective of sex (Murai et al., 2000; Imai et al., 2001). However, this study is the first report on BFT response to methyl anthranilate. This study has identified methyl anthranilate as an attractant effective for BFT and also compatible with conidia of *M. anisopliae* and hence can be considered for a “lure and kill” management strategy for BFT. The “lure and kill” strategy could be adopted either as an autoinoculation device or spot spray. Further studies need to be carried out to validate this proof of concept.

### Conflict of Interests

The authors have not declared any conflict of interests.

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