



Research article

Virulence and horizontal transmission of *Metarhizium anisopliae* by the adults of the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) and the efficacy of oil formulations against its nymphsVongai M. Paradza^{a,b}, Fathiya M. Khamis^a, Abdullahi A. Yusuf^{b,c}, Sevgan Subramanian^a, Komivi S. Akutse^{a,*}^a International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772-00100, Nairobi, Kenya^b Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa^c Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa

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ABSTRACT

The pathogenicity of dry conidia and fungal suspensions of 16 entomopathogenic fungal isolates (10 *Metarhizium anisopliae* and six *Beauveria bassiana*) was evaluated against adults and second instar nymphs of the greenhouse whitefly, *Trialeurodes vaporariorum* respectively. All the tested isolates were pathogenic to *T. vaporariorum* and caused mortality of 45–93% against the adults and 24–89% against the nymphs. However, *M. anisopliae* strains showed higher virulence to both developmental stages as compared to *B. bassiana* strains. The three most virulent isolates that caused high mortalities in adults were *M. anisopliae* ICIPE 18, ICIPE 62 and ICIPE 69, with cumulative mortalities of 82, 91 and 93%, and median lethal times (LT₅₀) of 5.20, 5.05 and 4.78 days, respectively. These isolates were further assessed for spore acquisition and retention by the adult insects at 0, 24, 48 and 72 h after exposure to dry conidia spores. There was no significant difference among isolates on their acquisition by the insects, although the effect of time on the number of spores retained by each insect was significant. For *M. anisopliae* ICIPE 62 and ICIPE 69, spore number was significantly higher immediately after exposure at 0 h than at 24, 48 and 72 h, whereas for *M. anisopliae* ICIPE 18, the spore number remained constant for all the days. The infected “donor” insects were able to horizontally transmit the acquired spores to uninfected “recipient” insects causing high mortality rates in both donor and recipient groups. *Metarhizium anisopliae* ICIPE 7, ICIPE 18 and ICIPE 62 were the most virulent isolates against the nymphs in aqueous formulation during the first screening with >80% mortality. However, in 2% (v/v) oil formulations at 1×10^8 conidia/ml, canola formulated ICIPE 62, ICIPE 18 and olive formulated ICIPE 18 were the most effective, resulting in 87.8, 88.1 and 99.4% nymphal mortalities respectively and with lower LT₅₀. Oil formulations significantly enhanced the efficacy and virulence of the isolates against the nymphs compared to aqueous formulations.

1. Introduction

Whiteflies (Hemiptera: Aleyrodidae) are notorious pests across different agronomic cropping systems around the world (Palumbo et al., 2001). The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) falls among the important pests of horticultural crops, causing extensive damage in both open field and protected crops (Wang et al., 2017). *Trialeurodes vaporariorum* is the predominant species across Kenya's different agroecological zones, with wide spread presence on tomato and French bean (Khamis et al., 2021).

Both the adult and nymphal stages feed directly from the phloem bundle through withdrawing sap which induces physiological challenges such as reduced plant vigour, premature loss of leaves, fruits and stunting (Jones, 2009; Navas-Castillo et al., 2011). The honeydew excreted by whiteflies during feeding leads to the growth of sooty mould which causes discolouration and reduces the quality of harvestable leaves and fruits in crops like tomato, *Solanum lycopersicum* L. and bean, *Phaseolus vulgaris* L. (Anderson et al., 2005). This feeding process by whiteflies provides an easy mode for the transmission of plant viruses such as criniviruses and torradoviruses in a

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range of host plants such as beet pseudo yellows virus, tomato chlorosis virus, strawberry palliodosis associated virus and tomato torrado virus (Wintermantel, 2004; Navas-Castillo et al., 2011; Gao et al., 2017; Perring et al., 2018).

The main basis for whitefly control is the use of pesticides that target the different developmental stages (Kim et al., 2014; Palumbo et al., 2001). However, the complexity of whitefly control with pesticides lies in their ecological and morphological characteristics that favor their abundance despite control measures. Intensive use of pesticides against whiteflies has had a huge impact on the insect's population dynamics (Byrne et al., 1992), combined with the insect's inherent ability to quickly develop resistance leading to pesticide resistant whitefly populations (Gilbertson et al., 2015; Wisler et al., 1998). Production of high value vegetable and ornamental crops in greenhouses is often characterised by low pest tolerance threshold levels and heavy spraying (Reid and McKenzie, 2016; Schreinemachers et al., 2018). These conditions are ideal for rapid selection of resistance genes, with evidence of correlation between diversity of vegetables grown, intensity of pesticide use and development of resistance in *T. vaporariorum* (Omer et al., 1992). The escape of insects that are resistant to pesticides from the greenhouse into the open field creates high populations of pesticide resistant strains that put a limitation to control strategies on a wider scale (Denholm et al., 1998).

The ecology of whiteflies in the field also brings additional challenges in their control by pesticides, with their waxy cuticle layer making it difficult for the active components to penetrate through the insect's cuticle (Patel et al., 2001; James et al., 2003). This is further compounded by their cryptic behavior (Wang et al., 2017), and their wide host range made up of more than 859 host plants (Perring et al., 2018). This diversity of alternative hosts provides a refuge for insects escaping treatment, some of which would have been exposed to sub-lethal doses. Ultimately, these insects that been exposed to sub-lethal doses, may be carrying resistance genes and viruses originating from different taxonomic types, leading to the development of new disease complexes (Gilbertson et al., 2015).

Resistance to common groups of pesticides such as pyrethroids, organophosphates and neonicotinoids has been shown already (De Bon et al., 2014), rendering them ineffective (Legg et al., 2014; Saad et al., 2013). This resistance to pesticides, coupled with genetic plasticity and adaptability of whiteflies (Denholm et al., 1998; Hemingway, 2000; Ellsworth and Martinez-Carrillo, 2001; Kady and Devine, 2003), continue to pose challenges to conventional chemical control of whitefly. Therefore, a sustainable ecological approach which can achieve pest control with minimum negative human and environmental consequences is needed.

Biological control which relies on the use of other organisms including microorganisms could offer a sustainable alternative to

synthetic pesticides (Kim et al., 2014). Within this group of microbes, entomopathogenic fungi are species of fungi that can parasitise and kill host insects by releasing toxins and depleting nutrients as they grow and proliferate inside the host's haemocoel (Shah and Pell, 2003; Pinnamaneni and Potineni, 2010). The most common fungal pathogens associated with arthropods are those in the order Hypocreales genera *Beauveria*, *Isaria*, *Lecanicillium* and *Metarhizium* (Inglis et al., 2001; Keyhani, 2018; Lee et al., 2015). These fungi propagate asexually and are able to colonise and kill several insects from different orders (Shin et al., 2017). They occur naturally and their role is to regulate arthropod pest populations by causing epizootic diseases among the pest population (Hall and Papierok, 1982; Lacey et al., 2015). All these attributes make them ideal candidates for selection as biological control agents. The aim of the study was, therefore, to screen for the most virulent fungal isolates against both the adult and nymphs of *T. vaporariorum*, evaluate spore retention and horizontal transmission of the acquired conidia by adult insects to their conspecifics, and assess how oil formulations enhance efficacy and virulence of these fungal-based biopesticides against the pest.

2. Materials and methods

2.1. Insects

Experiments were conducted in screenhouses and the Arthropod Pathology Unit laboratories at the International Centre of Insect Physiology and Ecology (*icipe*), Duguville Campus, Nairobi - Kenya. Whitefly populations were initially collected from eggplants, *Solanum melongena* L. at *icipe* campus (1°13'14.50" S; 36°53'43.82" E) and reared on French bean, *Phaseolus vulgaris* L., cv Goal in Plexiglas cages with fine muslin netting on the sides (40 cm × 60 cm × 80 cm) for more than three generations before use in experiments (Jaber et al., 2018; Kakimoto et al., 2007). The whiteflies were confirmed as *T. vaporariorum* using WF-F and WF-R primers (mitochondrial 16S rRNA gene) (Alhudaib et al., 2014; Frohlich et al., 1999). The colony was maintained inside a screenhouse with natural light conditions at 25 ± 2 °C, 65% relative humidity and a photoperiod of 12:12 h light/dark. Newly emerged whitefly adults ≤5 days old were used for all bioassays (Pakkianathan et al., 2015).

2.2. Fungal culture, viability and suspension preparation

The fungal isolates that were used were obtained from the *icipe*'s Arthropod Germplasm Centre for subculture (Table 1). Ten *Metarhizium anisopliae* (Metschnikoff) Sorokin and six *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) isolates were cultured and used in all the bioassays against adults and second instar nymphs of the

Table 1. List of fungal isolates and their origin and germination rates after 18 h on SDA/PDA plates at 25 ± 2 °C used in the study.

Fungal species	Isolate	Source	Locality	% Germination (±SE)
<i>Metarhizium anisopliae</i>	ICIPE 7	<i>Amblyoma variegatum</i>	Kenya	99.87 ± 0.12 c
	ICIPE 18	Soil	Kenya	99.25 ± 0.41 c
	ICIPE 20	Soil	Kenya	98.37 ± 0.7 bc
	ICIPE 30	<i>Busseola fusca</i>	Kenya	97.75 ± 0.77 bc
	ICIPE 62	Soil	DRC*	95.62 ± 1.03 ab
	ICIPE 68	Soil	DRC	97.75 ± 0.81 bc
	ICIPE 69	Soil	DRC	99.12 ± 0.51 c
	ICIPE 74	Soil	Kenya	99.37 ± 0.62 c
	ICIPE 78	<i>Temnoschoita nigroplagiata</i>	Kenya	97.23 ± 0.45 bc
	ICIPE 84	<i>Ornitacris turbida</i>	Senegal	93.87 ± 0.69 a
	<i>Beauveria bassiana</i>	ICIPE 273	Soil	Kenya
ICIPE 279		Coleopteran larva	Kenya	99.62 ± 0.37 c
ICIPE 281		Soil	Mauritius	100 abc
ICIPE 621		Soil	Kenya	100 abc
ICIPE 622		Soil	Kenya	100 abc
ICIPE 644		Soil	Mauritius	100 abc

* Democratic Republic of Congo.

greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). *Metarhizium anisopliae* isolates were sub-cultured on Sabouraud Dextrose Agar (SDA) and *B. bassiana* isolates on Potato Dextrose Agar (PDA) and incubated in complete darkness at 25 ± 2 °C for 14–21 days. Conidial viability was assessed before each bioassay under a microscope (Leica DM500) by inoculating 0.1 ml of 3×10^6 conidia/ml suspension onto four fresh plates of media for each isolate using a glass spreader. The Petri dishes were then incubated in complete darkness for 18 h at 25 ± 2 °C. Percentage germination was calculated by counting germinated conidia per hundred randomly selected conidia in a selected field covered by four cover slips under a microscope at 400× magnification. Conidia were scored as viable if the visible germ tubes were about twice the diameter of the conidium. Fungal suspensions for bioassays were prepared by scrapping the sporulated fungus off the agar surface into 10 ml sterile 0.05% Triton X-100 water in a universal bottle containing glass beads. The resulting suspension was vortexed and the spore concentration adjusted to 1×10^8 conidia/ml using a Neubauer haemocytometer prior to bioassays (Inglis et al., 2012).

2.3. Screening of fungal isolates against *Trialeurodes vaporariorum* adults

For each of the isolates, forty newly emerged adults of *T. vaporariorum* were exposed and infected with dry spores. The spores were harvested from well sporulated 14–day old culture plates. Exposure was carried out following a method by Dimbi et al. (2003) and Opisa et al. (2018). A cylindrical contamination device (3.5 cm length; 2.5 cm diameter) with an inner side lined with a velvet material was inoculated with 0.1 g of dry conidia spores. The insects were aspirated into the contamination device and left for three minutes to walk on the velvet material and acquire the fungal spores. Immediately after exposure, the insects were released onto a plant inside a ventilated Plexiglas cage (30 cm × 30 cm × 30 cm). For the control group, a fungus-free contamination device was used. The insects were monitored for mortality for seven days. All the dead whiteflies were surface sterilised with 1% sodium hypochlorite solution for 30 s and rinsed thrice with sterile distilled water. The surface sterilised insects were then placed on a moist filter paper in a Petri dish, sealed with Parafilm and kept at room temperature for mycosis examination on the cadavers under a dissecting microscope (×35, Leica EZ4 HD). The experiment consisted of seventeen treatments (16 isolates and the control) with four replicates per treatment organised in a completely randomised design and replicated over time.

2.3.1. Assessment of potent candidate isolate spore retention by *Trialeurodes vaporariorum* adults

The three most virulent isolates, *M. anisopliae* ICIPE 18, ICIPE 62 and ICIPE 69 were selected from the above experiment (screening bioassay section), based on high mortality rates and corresponding lower median lethal times, (LT₅₀) to evaluate spore retention by the insects. Newly emerged insects were inoculated with dry conidia using inoculation/contamination device as described above in the screening bioassay (Dimbi et al., 2003; Opisa et al., 2018). A group of five insects were randomly picked at each evaluation time, 0, 24, 48 and 72 h after exposure, with 0 h representing the time immediately after exposure (Opisa et al., 2019). Insects were transferred individually into 1.5 ml Eppendorf tubes containing 0.1 ml of sterile 0.05% Triton X-100 water. The tube was vortexed briefly at low speed in order to dislodge conidia while also making sure the insect remained intact. The number of conidia retained by each insect was counted using the Neubauer haemocytometer (Inglis et al., 2012).

2.3.2. Horizontal transmission between male and female *Trialeurodes vaporariorum* adults

Horizontal transmission between sexes was assessed from male to female insects and vice versa using the most potent selected isolates. The sex of the insects were selected based on morphometric features where female whiteflies are larger than males (Shan et al., 2014). Three

replicates of groups of twenty newly emerged male adults of *T. vaporariorum* were inoculated with dry spores of *M. anisopliae* ICIPE 18, ICIPE 62 and ICIPE 69 as previously described and then released onto a plant in a ventilated Plexiglas cage (30 cm × 30 cm × 30 cm). An equal number of clean/healthy female “recipient” insects were introduced into the cage containing the infected male “donor” insects and kept together for 24 h. After the 24 h, the insects were separated by sex and held in separate cages for ten days. The experiment was repeated as described above, but this time using inoculated “donor” females and mixing them with clean/healthy “recipient” males. Mortality of both “donor” and “recipient” groups was recorded daily for ten days, including the controls according to procedures described in Akutse et al. (2020) and Dimbi et al. (2013). All the dead insects were examined for mycosis as described above in screening bioassay section.

2.4. Screening of fungal isolates against second instar nymphal stages of *Trialeurodes vaporariorum*

Ten *Metarhizium anisopliae* and six *Beauveria bassiana* isolates previously used in *T. vaporariorum* adult bioassay (Table 1) were also used here to assess their pathogenicity against second instar nymphs. The nymphs for the experiment were obtained by exposing French bean plants at three–five leaf stage to *T. vaporariorum* adults for 24–48 h for oviposition and then removed. The nymphs developed into second instar after 11–13 days post exposure (Mascarin et al., 2013) and used for the bioassay. A mark was made near each nymph using a fine tipped permanent marker to indicate the position of 40 s instar nymphs on the infested leaves. Three replicates of 40 s instar nymph infested leaves were excised and sprayed with 2 ml of 1×10^8 conidia/ml fungal suspension of each isolate using a 50 ml hand sprayer (Gökçe et al., 2005; Mascarin et al., 2013). The control groups were sprayed with sterile 0.05% Triton X-100 water and the leaves were left to air dry before placing on 3% (w/v) Technical Agar supplemented with 0.25 g/L chloramphenicol in Petri dishes. The Petri dishes were sealed with Parafilm for 24 h to allow for adequate humidity for the initiation of fungal infection. The seal was then removed after the 24 h in order to limit condensation (Jandrić et al., 2014). Mortality of nymphs was monitored using a dissecting microscope and recorded daily for seven days (Islam et al., 2018).

2.4.1. Oil formulation of the effective fungal isolates

Based on mortality results from the nymph screening experiment above, *M. anisopliae* ICIPE 7, ICIPE 18 and ICIPE 62 outperformed all the other isolates and were selected for different formulations. Three commercial vegetable oils, namely corn oil, olive oil (obtained from Bidco Africa Limited, Thika, Kenya) and canola oil (obtained from Agventure Limited, Nakuru, Kenya) were used for the formulation as follows. Dry conidia from the three fungal isolates were formulated with the oils by emulsifying 2% (v/v) of each oil in 0.05% Triton X-100 water containing 1×10^8 conidia/ml fungal suspension (Kirubakaran et al., 2014). Water suspension containing 0.05 % Triton X-100 water and 1×10^8 conidia/ml of the fungi was used as the aqueous formulated conidia. Further experiments followed similar procedures with those of the nymph screening experiment were conducted with four replicates, each with an average of 80 nymphs per treatment (Nauen et al., 2008). Second instar nymph infested leaves were excised and sprayed with formulations of each fungal isolate using a 50 ml hand sprayer (Gökçe et al., 2005; Mascarin et al., 2013). Mortality of the infected nymphs was monitored and recorded daily for seven days using a dissecting microscope (Islam et al., 2018).

2.5. Statistical analyses

Proportional data on conidial germination, mortality and mycosis were analysed using binomial regression of the Generalised Linear Model (GLM) and means for different treatments separated using Tukey's HSD test. Percentage mortality data were corrected for natural mortality

(Abbott, 1925) before being submitted to analysis. The median lethal time (LT₅₀) for mortality data were analysed using a GLM model assuming binomial distribution error and probit link using the *dose.p* function from the MASS package in R (Opisa et al., 2019). For spore uptake and retention data, the spore number count data were fitted to quasipoisson regression model (Bayissa et al., 2017). All data analyses were performed using R statistical package version 4.0.5 (R Core Team, 2021).

3. Results

3.1. Pathogenicity of fungal isolates against *Trialeurodes vaporariorum* adults

The results showed that all isolates were pathogenic to *T. vaporariorum*, although the level of pathogenicity at seven days post exposure ($\chi^2 = 726.52$, $df = 15$, $P < 0.0001$) varied significantly among isolates (Table 2). The highest mortality was observed on insects exposed to *M. anisopliae* isolates ICIPE 18 (81.9%), ICIPE 62 (91.5%) and ICIPE 69 (92.9%) with shorter median lethal times, (LT₅₀) of 5.2, 5.1 and 4.8 days, respectively compared to other isolates (Table 2). The percentage of mycosis of the cadavers ranged between 75.4–89.3% with no significant differences among the isolates.

3.1.1. Spore acquisition and retention by *Trialeurodes vaporariorum* adults

The number of spores acquired by the insects at 0 h did not differ significantly ($\chi^2 = 1.53$, $df = 2$, $P = 0.15$) among the three potent isolates, *M. anisopliae* ICIPE 18, ICIPE 62 and ICIPE 69 (Figure 1). However, the number of spores retained by the insects decreased significantly with time ($\chi^2 = 5.18$, $df = 3$, $P < 0.0001$) (Figure 1). A higher spore number was observed immediately (0 h) after exposure than at 24, 48 and 72 h. For example, *M. anisopliae* ICIPE 62 and ICIPE 69 recorded the highest number of spores at 0 h (immediately after exposure), followed by 60.5 and 43.3% loss of the acquired spores after 24 h respectively, with no significant variation for the remaining days. However, for *M. anisopliae* ICIPE 18, the number of spores acquired and retained by the insects remained constant ($\chi^2 = 0.36$, $df = 3$, $P = 0.26$) for the entire evaluation period (Figure 1).

3.1.2. Horizontal transmission of spores by *Trialeurodes vaporariorum* adults

Both the infected males and females, referred to as “donors”, were able to horizontally transmit the spores to their conspecifics. There were

Table 2. Cumulative mortality and median lethal time (LT₅₀) seven days post treatment of *Trialeurodes vaporariorum* adults with dry conidia spores of different isolates of entomopathogenic fungi.

Fungal species	Isolate	% Mortality (\pm SE)	LT ₅₀ (days) (95% FL)
<i>Metarhizium anisopliae</i>	ICIPE 7	60.93 \pm 17.13 c	6.43 (6.43–6.52)
	ICIPE 18	81.87 \pm 12.39 e	5.20 (5.15–5.25)
	ICIPE 20	48.15 \pm 11.96 ab	-
	ICIPE 30	58.80 \pm 12.62 bc	6.39 (6.28–6.50)
	ICIPE 62	91.47 \pm 6.76 f	5.05 (5.00–5.10)
	ICIPE 68	75.8 \pm 14.69 e	5.08 (5.02–5.14)
	ICIPE 69	92.89 \pm 7.10 f	4.78 (4.74–4.82)
	ICIPE 74	62.35 \pm 7.10 cd	6.28 (6.19–6.37)
	ICIPE 78	50.28 \pm 14.73 abc	6.83 (6.17–6.96)
	ICIPE 84	72.30 \pm 16.49 de	5.50 (5.44–5.56)
<i>Beauveria bassiana</i>	ICIPE 273	45.00 \pm 5.86 a	-
	ICIPE 279	57.50 \pm 7.70 bc	6.91 (6.81–7.10)
	ICIPE 281	53.12 \pm 8.18 abc	6.63 (6.50–6.76)
	ICIPE 621	60.62 \pm 18.85 c	6.27 (6.19–6.35)
	ICIPE 622	44.37 \pm 6.79 a	-
	ICIPE 644	79.40 \pm 12.56 e	5.24 (5.18–5.30)

Means within the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's HSD). ^a LT₅₀ values not estimated for <50% mortality. FL represents 95% fiducial limits.

significant differences between mortality rates of “donor” and “recipient” insects when either males ($\chi^2 = 350.58$, $df = 5$, $P < 0.0001$) or females ($\chi^2 = 191.62$, $df = 5$, $P < 0.0001$) were used as donors. The total cumulative mortality rates for “donor” insects were generally higher than for “recipient” insects regardless of the sex, except for *M. anisopliae* ICIPE 18 when the females were used as “donors” (Figure 2).

The median times to death for “donor” insect groups were shorter than those of “recipient” insects, except for *M. anisopliae* ICIPE 69 where females were used as “donors” (Table 3). In treatments where males were used as the “donors”, LT₅₀ values ranged from 4.54 to 5.16 days, while for the “recipients” females, the median time to death was between 6.33–8.84 days. Similarly, when females were used as “donors”, shorter median time to death of 4.63–5.21 days were recorded in comparison to male “recipients” that had LT₅₀ values ranging between 3.77–7.88 days (Table 3). There were significant differences in mycosis levels when males were used as “donors” ($\chi^2 = 100.5$, $df = 5$, $P < 0.0001$) as they exhibited higher mycosis levels than the “recipient” females for all the isolates. However, when the female whiteflies were used as “donors”, mycosis levels for the “donor” and “recipient” insects did not vary significantly ($\chi^2 = 10.9$, $df = 5$, $P = 0.1$) (Table 3).

3.2. Pathogenicity of fungal isolates against second-instar nymphs of *Trialeurodes vaporariorum*

Infection was visible three days post treatment for most isolates, and the progression of the nymphal infection is shown in Figure 3. In the early infection stage, fungal mycelia could be seen on the cuticle surface, and the nymphs changed from a normal translucent greenish colour to opaque white in appearance. As the infection progressed, fungal mycelia could be seen extensively covering the cuticle and the nymphs became flattened, with some nymphs showing a brown discolouration, desiccation and followed by fungal sporulation on the cadaver cuticle (Figure 3).

The pathogenicity and virulence of the various fungal isolates against the nymphs are presented in Table 4. The isolates showed significantly varied degrees of infective potential towards *T. vaporariorum* nymphs ($\chi^2 = 959.4$, $df = 15$, $P < 0.0001$), with mortality ranging from 24.0–89.3%. The three most virulent isolates, *M. anisopliae* ICIPE 7, ICIPE 18 and ICIPE 62 caused the highest mortality rates of 81.0, 83.3 and 89.3% with LT₅₀ values ranged of 5.9, 6.2 and 6.4 days, respectively (Table 4). The efficacy and virulence of *M. anisopliae* ICIPE 7, ICIPE 18 and ICIPE 62 outperformed all the other isolates in infecting *T. vaporariorum* nymphs were therefore assessed using different oil formulations (see the section 3.2.1).

3.2.1. Effect of oil formulations on pathogenicity against second instar *Trialeurodes vaporariorum* nymphs

All the three vegetable oils tested were compatible with the three most potent isolates based on the viability assay where >95% conidia germinated. Significant variation in mortality was observed among the various formulations of the different isolates ($\chi^2 = 333.93$, $df = 11$, $P < 0.0001$) (Table 5). When compared to their aqueous formulations, the most potent formulation was that of *M. anisopliae* ICIPE 18 formulated in olive which increased mortality by 19.3%, together with formulations of canola with *M. anisopliae* ICIPE 7 and *M. anisopliae* ICIPE 18 which enhanced pathogenicity by 5.30 and 5.76% respectively. However, some formulations (ICIPE 62 formulated in olive oil and ICIPE 18 in corn oil) significantly reduced pathogenicity of the fungus, resulting in lower nymph mortality rates than their aqueous formulations (Table 5). A relatively shorter median time to death of 4.72–5.14 days was observed for the three potent formulations highlighted above in comparison to 6.04–6.39 days registered for their aqueous formulations (Table 5).

4. Discussion

Metarhizium anisopliae and *B. bassiana* make up the majority of entomopathogens with high pathogenicity against a broad range of insect species (Kirubakaran et al., 2014; Zimmermann, 2007a, 2007b). The

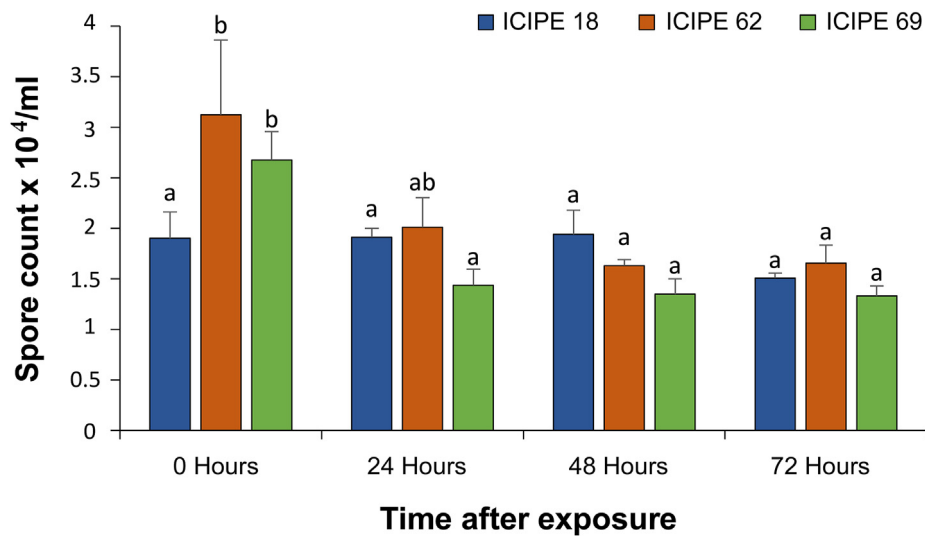


Figure 1. Number of conidia acquired and retained per whitefly after exposure to dry spores of *Metarhizium anisopliae* isolates ICIPE 18, ICIPE 62 and ICIPE 69. Bars denote means \pm standard errors. Means followed by the same letter across days for each isolate are not significantly different by Tukey’ HSD test at $P < 0.05$.

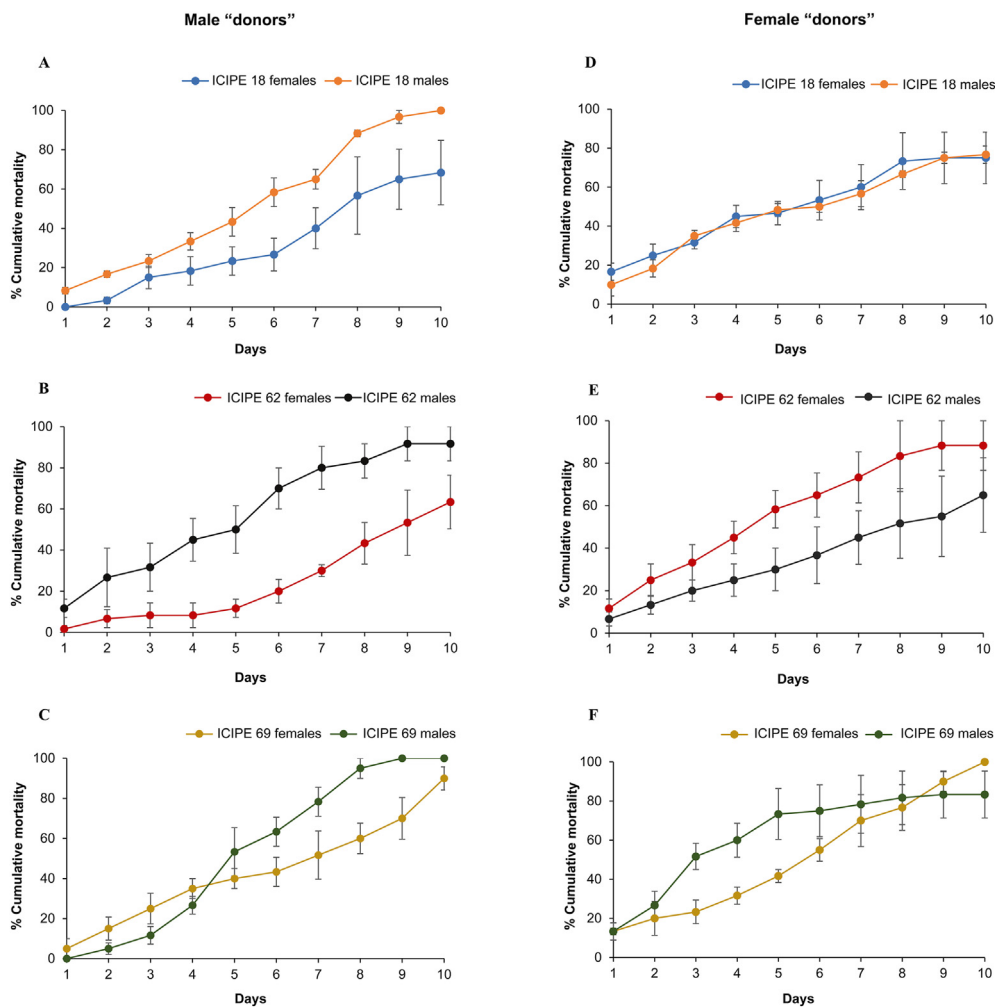


Figure 2. Corrected cumulative mortality rates (\pm SE) of *Trialeurodes vaporariorum* “donor” and “recipient” insects as per sexes of the insects. “Donor” insects are infected with dry spores of the fungi and “recipient” insects (healthy insects) acquire spores after exposure to infected insects. (A), (B) and (C) males as donors; (D), (E), and (F), females as donors as per the various isolates, *Metarhizium anisopliae* ICIPE 18, ICIPE 62 and ICIPE 69.

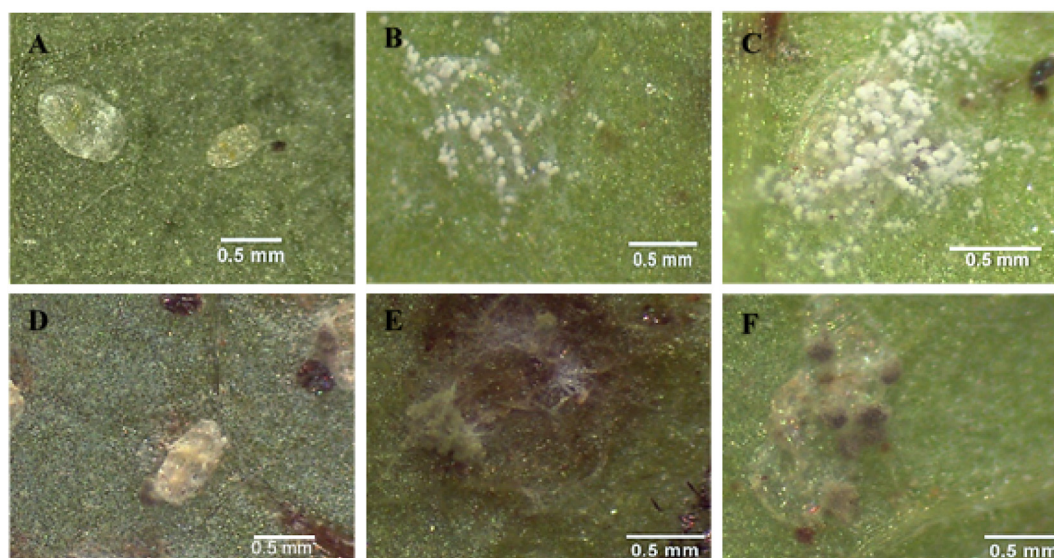
results from the current study showed that all *M. anisopliae* and *B. bassiana* species were pathogenic to *T. vaporariorum* adults and nymphs. However, the level of virulence based on mortality and median

lethal time values showed inter and intra species differences among the isolates. Greater mortality was observed with *M. anisopliae* isolates than *B. bassiana*, and this trend was consistent with both the adult and

Table 3. Percent mortality, mycosis rates and median lethal time (LT₅₀) in days of “donor” and “recipient” *Trialeurodes vaporariorum* ten days after horizontal transmission treatment with *Metarhizium anisopliae* isolates ICIPE 69, ICIPE 62 and ICIPE 18.

Isolates	Male “donors”			Female “donors”		
	% Mortality (±SE)	LT ₅₀ ± SE (days)	% Mycosis (±SE)	% Mortality (±SE)	LT ₅₀ ± SE (days)	% Mycosis (±SE)
ICIPE 18 *M	100 ± 0 c	5.10 ± 0.14	68.33 ± 6.01 c	76.6 ± 4.04 b	5.82 ± 0.20	71.8 ± 8.32 a
ICIPE 18 **F	68.3 ± 16.41 a	7.78 ± 0.41	45.0 ± 8.66 a	75.0 ± 13.22 ab	5.48 ± 0.41	71.9 ± 3.93 a
ICIPE 62 M	91.6 ± 8.33 b	4.54 ± 0.34	80.0 ± 11.6 d	65.0 ± 7.55 a	7.88 ± 0.61	55.3 ± 20.7 a
ICIPE 62 F	63.3 ± 13.01 a	8.84 ± 0.43	68.62 ± 16.1 c	88.3 ± 11.66 c	4.63 ± 0.39	72.2 ± 8.28 a
ICIPE 69 M	100 ± 0 c	5.16 ± 0.13	61.6 ± 8.33 bc	83.3 ± 12.01 bc	3.77 ± 0.57	54.5 ± 16.0 a
ICIPE 69 F	90 ± 5.77 b	6.33 ± 0.29	53.3 ± 13.0 ab	100 ± 0 d	5.21 ± 0.19	71.5 ± 2.20 a

Means followed by the same letter within a column do not differ significantly at P < 0.05 (Tukey's HSD). *M denotes male insects; **F denotes female insects in the various treatment groups.

**Figure 3.** The progression of *Trialeurodes vaporariorum* nymphal infection by entomopathogenic fungi: (A) healthy nymphs, (B) early infection showing the onset of fungal growth, (C) proliferation of mycelial growth, (D) nymph colour changing from a translucent green to opaque white as the fungus grows inside the nymph, (E) flattened nymphs exhibiting brown discoloration, (F) sporulation on a desiccated cadaver.

nymphal stages. Whilst the infection process for all entomopathogenic fungi is known to be generally the same, variability in pathogenicity among isolates might be attributed to differences in the factors that

Table 4. Mortality and median lethal time (LT₅₀) of second instar nymphs of *Trialeurodes vaporariorum* sprayed with fungal aqueous suspension at 1×10^8 conidial/ml seven days post treatment.

Fungal species	Isolate	% Mortality ±SE	LT ₅₀ (days) (95% FL)
<i>Metarhizium anisopliae</i>	ICIPE 7	81.00 ± 8.02 def	6.04 (5.98–6.0)
	ICIPE 18	83.33 ± 6.66 ef	6.39 (6.35–6.43)
	ICIPE 20	75.66 ± 7.44 cde	6.21 (6.15–6.27)
	ICIPE 30	65.66 ± 13.86 bc	6.61 (6.53–6.69)
	ICIPE 62	89.33 ± 0.66 f	5.56 (5.52–5.60)
	ICIPE 68	80.66 ± 9.33 def	6.29 (6.23–6.35)
	ICIPE 69	59.33 ± 5.81 b	6.55 (6.48–6.62)
	ICIPE 74	41.33 ± 4.70 a	–
	ICIPE 78	69.00 ± 11.37 bcd	6.52 (6.46–6.58)
	ICIPE 84	26.33 ± 11.56 a	–
<i>Beauveria bassiana</i>	ICIPE 273	24.00 ± 6.00 a	–
	ICIPE 279	69.00 ± 4.00 bcd	6.71 (6.66–6.76)
	ICIPE 281	27.33 ± 10.74 a	–
	ICIPE 621	75.66 ± 14.33 cde	6.55 (6.51–6.59)
	ICIPE 622	66.25 ± 6.16 bc	6.62 (6.58–6.66)
	ICIPE 644	70.66 ± 8.03 bcd	6.62 (6.59–6.65)

Means within the same column followed by the same letter do not differ significantly at P < 0.05 (Tukey's HSD). ^aLT₅₀ values not estimated (<50% mortality). FL represents 95% fiducial limits.

mediate virulence, such as proteinases, chitinases and lipases which are responsible for cuticle degradation and penetration into the insect (Ortiz-Urquiza and Keyhani, 2013). Since fungal species or isolates express these proteins differently, this might therefore lead to differences observed among different isolates, and may explain the variability in pathogenicity and “speed of kill” observed among the isolates in the study (Mondal et al., 2016; Ortiz-Urquiza and Keyhani, 2013; Shah and Pell, 2003). Previous studies have also reported similar trends with entomopathogenic fungi belonging to different species or isolates of the same genera causing variable mortality rates on insects such as pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) (Migiro et al., 2010), silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Norhelina et al., 2013), western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Niassy et al., 2012) and two spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (Shin et al., 2017).

Our results also showed that the adults of *T. vaporariorum* were more susceptible to the fungal isolates than the second instar nymph, where the isolates generally took longer time to kill nymphs compared to the adults. *Metarhizium anisopliae* ICIPE 69 was the most virulent isolate against adults causing a mortality of 93% with an LT₅₀ value of 4.78 days, compared to the nymphs where *M. anisopliae* ICIPE 62 induced the highest mortality of 89% with a longer LT₅₀ value of 5.43 days. In a similar study with three different aphid species, *Myzus persicae* (Sulzer), *Aphis gossypii* (Glover) and *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae), it was reported that the mortality rates obtained after

Table 5. Mortality and median lethal time (LT₅₀) of second instar nymphs of *Trialeurodes vaporariorum* seven days post treatment with aqueous and 2% (v/v) oil formulations of *Metarhizium anisopliae* ICIPE 7, ICIPE 18 and ICIPE 62 at 1×10^8 conidia/ml.

Isolate	Aqueous		Canola		Corn		Olive	
	%Mortality	LT ₅₀ (days)	%Mortality	LT ₅₀ (days)	%Mortality	LT ₅₀ (days)	%Mortality	LT ₅₀ (days)
ICIPE 7	81.0 ± 8.0 aAB	6.04 (5.98–6.0)	85.3 ± 6.70 aB	5.00 (4.96–5.04)	80.6 ± 7.63 bAB	5.70 (5.65–5.75)	78.2 ± 8.04 aA	5.96 (5.91–6.01)
ICIPE 18	83.3 ± 6.66 abB	6.39 (6.35–6.43)	88.1 ± 5.0 aB	5.14 (5.10–5.18)	62.4 ± 21.2 aA	6.02 (5.96–6.08)	99.4 ± 0.63 bC	4.72 (4.68–4.76)
ICIPE 62	89.3 ± 0.66 bC	5.56 (5.52–5.60)	87.8 ± 5.4 aBC	4.99 (4.94–5.04)	82.1 ± 4.14 bAB	5.34 (5.30–5.38)	76.9 ± 11.2 aA	5.60 (5.55–5.65)

Within column, means (±SE) followed by the same lower case letters and within rows, means (±SE) followed by the same upper case letters are not significantly different at $P < 0.05$ (Tukey's HSD).

exposure to entomopathogenic fungi were significantly higher in adult than nymph groups (Jandricic et al., 2014). The difference in the speed of kill by entomopathogenic fungi in relation to the life stage of an insect is associated with insect morphology and behaviour (Ortiz-Urquiza and Keyhani, 2013). In the current study, the spore retention bioassay confirmed the ability of adult whiteflies to retain high level of spores after acquisition for up to 72 h. Likewise, spore retention by adults of *B. tabaci* showed that high densities of *M. anisopliae* and *B. bassiana* conidia become attached to the legs, wings and the thorax (Soliman et al., 2019). During movement, the appendages and cavities such as those between the head capsule and the thorax present an easy route for fungal entry for the attached spores and subsequent infection (Butt and Goettel, 2000). By comparison, whitefly nymphs are immobile with limited vulnerable entry points, in addition to lipids that form a waxy layer on the cuticle and inhibit conidial germination (James et al., 2003). The significance of the cuticle structure was further supported by results from a study by Panyasiri et al. (2007). The comparative pathogenicity of entomopathogenic fungal suspensions against whitefly, *B. tabaci*; thrips, *Ceratohripoides claratris* (Shumsher) (Thysanoptera: Thripidae) and mealybug, *Pseudococcus cryptus* (Hempel) (Hemiptera: Pseudococcidae) nymphs showed that whitefly and mealybug nymphs were less susceptible than thrips, and this was attributed to the presence of waxy cuticular lipids that cover their cuticles (Panyasiri et al., 2007). Since the mode of action of entomopathogenic fungi is by direct penetration through the insect's cuticle, it could be postulated that the differences on morphology and behaviour highlighted earlier accounted for shorter lethal time in *T. vaporariorum* adults than their nymphs. However, in some instances, contrasting results have been reported; for example, the oak lace bug, *Corythucha arcuate* (Say) (Hemiptera: Tingidae) nymphs were more susceptible to entomopathogenic fungal infection than the adults (Sönmez et al., 2016). All these observations seem to support the theory that susceptibility of an insect to entomopathogenic fungi depends on its developmental stage (Inglis et al., 2001), although no generalisation can be made on which stage is deemed more susceptible for various insect species.

The high pathogenicity exhibited by the fungal isolates used in the study has also been reported for other insect orders in several studies. For instance, *M. anisopliae* ICIPE 69 was reported to be virulent against *F. occidentalis* (Niassy et al., 2012), false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Mkiga et al., 2020) and bean pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) (Tumuhaise et al., 2015). In addition, *M. anisopliae* ICIPE 18 and ICIPE 62 were both reported highly virulent against sweet potato weevil, *Cylas puncticollis* (Bohemen) (Coleoptera: Curculionidae) (Ondiaka et al., 2008), fruitflies, *Ceratitits capitata* (Weidmann) and *C. rosa* var. *fasciventris* (Karsch) (Diptera: Tephritidae) (Dimbi et al., 2003), and legume flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) (Ekesi et al., 1998). *Metarhizium anisopliae* ICIPE 18 was also shown to cause mortality of up to 95 % in tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Akutse et al., 2020) and stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Maniania, 1992). Furthermore, *M. anisopliae* ICIPE 7 was proved virulent against *T. urticae* (Bugeme et al., 2008), and *F. occidentalis* (Niassy et al., 2012). A broad

host range ability demonstrated by these isolates in the laboratory bioassays presents an advantage in crop protection because of their potential to control more than one pest. However, further experiments are needed in order to evaluate their efficacy against *T. vaporariorum* in the natural environment to validate these results.

In the present study, *M. anisopliae* ICIPE 18, ICIPE 62 and ICIPE 69 were assessed for spore acquisition and retention by *T. vaporariorum*, and the horizontal transmission of acquired conidia to conspecifics established. Horizontal transmission occurs when uninfected individuals get into contact with infected individuals or surfaces (Baverstock et al., 2010). The results showed that the insects were able to retain the spores for up to 72 h, which gives ample time for horizontal transmission to their conspecifics during their association. Selection of a candidate entomopathogen based on its environmental fitness by evaluating additional attributes other than just the virulence improves their success as a biocontrol agent in the environment. Horizontal transmission of a pathogen amongst insects is an important indicator of how efficiently it can establish and spread in the environment, and consequently create more epizootics in target host populations. Our findings also showed that both sexes were able to horizontally transmit the spores to the opposite sex as confirmed by the high mortality rates and mycosis observed on the cadavers from the two “donor” and “recipient” groups. However, median time to death for the “recipient” insects was generally longer than for the “donor” insects for most of the treatments. Because the speed of kill depends on the number of conidia on the insect's cuticle (Butt and Goettel, 2000), the “recipient” insects in this case may have received less spores compared to the “donor” insects which primarily acquired spores from the inoculated contamination device, and this may explain the trend observed. The cadavers from both groups of insects were able to mycose, and this is important because mycosed cadavers become sources of inoculum and this serves as a self-propagative route for the entomopathogen within the environment (Mascarin and Jaronski, 2016; Shah and Pell, 2003). Evaluation of spore retention and horizontal transmission by insects are important aspects when employing the autodissemination technique as a control strategy (Dimbi et al., 2003).

When conidia were formulated in the different oil types, efficacy increased by 5–19%, resulting in higher mortality in insects treated with oil formulations than those exposed to aqueous formulations. However, variations in mortality were evident for the different oil formulations, with olive oil showing the highest efficacy when formulated with *M. anisopliae* ICIPE 18, followed by *M. anisopliae* ICIPE 18 and ICIPE 62 formulated in canola oil. Despite the inherent compatibility of these vegetable oils with *M. anisopliae* (Polar et al., 2005), quantitative and qualitative differences in the composition of fatty acids for different vegetable oils still exists (Luz and Batagin, 2005), possibly resulting in differential efficacy among the oil formulations. With regards to the role of oils in enhancing pathogenicity and virulence of the isolates, this could be attributed to the ability of the hydrophilic groups in oils to readily bind to the insect's hydrophilic cuticle (Luz and Batagin, 2005; Polar et al., 2005; Wraight and Ramos, 2017). Furthermore, the hydrophobic conidia can also readily suspend in oils which then easily spreads into the segmented parts of the cuticle

unlike the aqueous form (Butt and Goettel, 2000). For entomopathogenic fungi whose mode of action is contact, higher spore adhesion to the nymph cuticle facilitates more spore germination leading to an enhanced infection process observed with oil formulations than aqueous formulations (Ummidi and Padmaja, 2014; Wraight and Ramos, 2017). For insects with a cryptic behavior in the field like whiteflies, greater spore adhesion of those propagules that successfully make contact with the insect cuticle is advantageous since achieving high conidial contact in such insects is already difficult. Higher virulence with oil formulations has also been demonstrated with the larvae of the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (Hicks, 2016), *Triatoma infestans* nymphs (Klug) (Hemiptera: Reduviidae) (Luz and Batagin, 2005), ticks, *Boophilus microplus* (Canestrini) (Acari: Ixodidae) (Polar et al., 2005) and desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) (Bateman et al., 1993).

5. Conclusion

The results of the study suggest that *M. anisopliae* isolates ICIPE 18, ICIPE 62 and ICIPE 69 were highly pathogenic to adults of *T. vaporariorum*, with ICIPE 69 showing more potential to be developed as fungal-based biopesticide against the pest. The adult whiteflies managed to retain spores for up to 72 h and were able to horizontally transmit these spores to their conspecifics causing high mortality rates in both the “donor” and “recipient” groups. These findings provide a basis for the assessment of an improved approach to control *T. vaporariorum* in the field where the use of dry spores targeting the adult insects could be improved with an autodissemination device. However, additional studies are warranted to assess the compatibility of these potent fungal isolates with some semiochemicals that could be used in “attract and kill” approach. Further studies on the performance of *M. anisopliae* ICIPE 18 in olive oil formulation against the nymphs in the field are needed to evaluate and validate their efficacy under natural environmental conditions.

Declarations

Author contribution statement

Vongai M. Paradza: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sevgan Subramanian: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fathiya M. Khamis, Komivi S. Akutse: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdullahi A. Yusuf: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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