Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Direct and indirect infection effects of entomopathogenic fungi on two morphs of Frankliniella schulzei (Trybom) (Thysanoptera: Thripidae) through direct and indirect infections

**David K. Mfuti**

dkupesa@icipe.org

International Centre of Insect Physiology and Ecology

**A. O. Akinyemi**

Osun State University

**Takemore Chagomoka**

International Centre of Insect Physiology and Ecology

**W. J. Kimemia**

International Centre of Insect Physiology and Ecology

**K. S. Akutse**

International Centre of Insect Physiology and Ecology

**Research Article**

**Keywords:** Fungal suspension, contamination chamber, common blossom thrips, dark and pale forms, survival, management

**Posted Date:** May 20th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-9503951/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

**Additional Declarations:** No competing interests reported.

# Abstract

This study evaluated two infection techniques of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* isolates for controlling the dark and pale forms of common blossom thrips (*Frankliniella schultzei)*. The methods included indirect infection through fungal-contaminated food sources and direct infection using dry spores in a contamination chamber. Significant differences were observed in mortality and lethal time ($LT_{50}$) among the 17 fungal strains for both *F. schultzei* forms. The indirect infection of, *M. anisopliae* isolates ICIPE 7, ICIPE 18, ICIPE 20, and ICIPE 69 caused over 50% mortality on the dark form, with ICIPE 69 achieving 90.0 ± 3.33% mortality and the shortest $LT_{50}$ (4.20 days). Direct infection produced mortalities above 75% across isolates, with ICIPE 7 (100.0 ± 0.00%) and ICIPE 69 (93.33 ± 3.33%) performing best. For the pale form, indirect infection with ICIPE 20, ICIPE 18, and ICIPE 32 caused 69–72% mortality, while direct infection with ICIPE 62 achieved 94.55 ± 1.74% mortality and the shortest $LT_{50}$ (0.35 days). Overall, both infection techniques were effective, but indirect infection revealed higher pathogenicity of *M. anisopliae* isolates compared to *B. bassiana*. Isolates ICIPE 7, ICIPE 18, ICIPE 32, and ICIPE 69 were the most virulent against both forms of *F. schultzei*, with ICIPE 69 identified as the most potent candidate for further field validation in sustainable pest management.


# Introduction

The common blossom thrips, *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae) is an important crop pest infesting several ornamental and vegetable plants (Kakkar et al. 2012; Khan et al. 2020). Worldwide they feed on 83 species of ornamental and vegetable plants in 55 families (Palmer 1990; Milne et al. 1996; Dakshina et al. 2014; Kakkar et al. 2017; Odanga et al. 2017). The pest can cause direct damage to plants and also indirectly transmit tospoviruses. Direct damage is caused by feeding and oviposition activities on leaves, flowers and fruits resulting in necrotic spots on tender leaves and dark dotted blemishes in fruits (Kakkar et al. 2012). Oviposition causes brown lesions on fruits and flowers, and at high densities it may lead to premature abscission of flowers and fruits (Seal et al. 2014). Indirect damage by this thrips is caused by the transmission of viral diseases to the host plants. *Frankliniella schultzei* is a known as vector of several pathogenic viruses including tomato spotted wilt tospovirus (TSWV) (Sakimura 1969; Wijkamp et al. 1995; Wangai et al. 2001; Macharia et al. 2015), tomato chlorotic spot tospovirus (TCSV)(Khan et al. 2020), groundnut ring spot tospovirus (GRSV) (Wijkamp et al. 1995), tobacco streak ilarvirus (TSV) (Klose et al. 1996), stem necrosis virus and capsicum chlorosis virus (Jones and Sharman, 2005). *Frankliniella schultzei* can cause significant yield losses varying between crops. For instance, up to 80% yield losses has been estimated on tomatoes (*Solanum lycopersicum* L) in Kenya (Wangai et al. 2001) while in Brazil on melon (*Cucumis melo* L.), yield loss of 40% was recorded (Diamantino et al. 2021).

The pest exists in two distinct forms which are the dark and pale forms. The two forms reproduce sexually and parthenogenically (Gikonyo et al. 2017). The pale form exists as only virgin females and has solely female offsprings (thelytoky), while virgin dark females have male offspring but once mated, the dark females produce both male and female offsprings (arrhenotoky). Morphological and molecular

studies conducted on both forms showed that they belong to distinct clusters (Gikonyo et al. 2017). Among the two species, the dark forms can transmit five types of tospoviruses while the pale form can only transmit two types of tospoviruses (Gikonyo et al. 2017; Kakkar et al. 2017).

To control this pest, farmers often rely on chemical control (Abtew et al. 2016; Mouden et al. 2017). However, misuse and overuse of those chemical products lead to detrimental consequences to animals, humans environment and biodiversity. In addition, there have been records of this pest developing resistance to continuous use of pesticides for its control (Mouden et al. 2017). The development of nature positive and ecologically friendly management strategies could reduce synthetic insecticide use and thus make food safer, reduce the impacts on the environment and increase the yields. The use of entomopathogenic fungi for pest control is becoming an important safe alternative, because they are considered environmentally safe. The International Centre of Insect Physiology and Ecology (*icipe*) over the years, has identified a number of these beneficial biocontrol agents. In collaboration with Real IPM-Biobest a Kenya-based private company, the major biopesticides supplier in Africa, four strains (*Metarhizium anisopliae* strains ICIPE 69, ICIPE 78, ICIPE 62 and ICIPE 7) from *icipe*'s germplasm have been commercialized against different insect pest species and now available in the market (Akutse et al. 2020a, https://realipm.com/c/biopesticides/). Initial screening has shown that some of these entomopathogenic fungi are effective against several insect pests including the invasive fall armyworm (*M. anisopliae* strains ICIPE 78 – commercially known as Mazao Achieve and ICIPE 7 – Mazao Detain and Tickoff: Akutse et al. 2019; 2020a), and other thrips species such as *F. occidentalis* and *Megalurothrips sjostedsti*, fruit flies and mealybugs (*M. anisopliae* ICIPE 69 – Mazao Campaign: Ekesi et al. 1998; Niassy et al. 2012) and some have also been tested at the field on thrips (*M. anisopliae* ICIPE 69: Mfuti et al. 2016; 2017). However, these strains have not yet been tested against *F. schultzei* both in the laboratory and field despite the significant threat it poses to ornamentals and food crops.

Laboratory screening test for the effectiveness of entomopathogenic fungi strains before field trials and deployment is fundamental since it provide adequate information not only on the efficacy but also on the virulence as well as the appropriate concentration/dose to be used. Although external field conditions can influence the effectiveness of entomopathogenic fungi (Mantzoukas et al. 2022), most insects' pests get infected through direct contact with the fungi or when they feed on treated (fungal contaminated) food sources.

Therefore, the aim of this research study was to screen and select potent entomopathogenic fungal strains for the management of dark and pale forms of *F. schultzei*. To authenticate results, two inoculation techniques namely the pod infestation (indirect infection) and contamination chamber (direct infection) were used to assess the pathogenicity, virulence and effectiveness of these fungal strains under controlled laboratory conditions.

## Materials and methods

# Thrips rrearing

The study was conducted at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The colonies of the two-colour forms of *F. schultzei* were reared at *icipe*'s Animal Rearing and Containment unit. The population of dark and pale were originally collected from *Malvaviscus grandiflorus* Kunth at Kasarani, Nairobi County (GPS coordinates: −1.221, 36.897) and from a population on *Phaseolus vulgaris* (Linnaeus) in Kitui, Eastern County (GPS coordinates: −1.298, 37.944), respectively (Gikonyo et al. 2017). The two forms were reared for several generations to maintain the colony stability before being used for the experiments.

Both colour forms were reared in ventilated plastic jars (1 litre) with thrips-proof nets covering the top to prevent the insects from escaping. The two-colour forms were reared separately on surface-sterilized French bean (*P. vulgaris* (L.) var. *samantha*) pods as described earlier (Niassy et al. 2012 and Akinyemi et al.2021).

## Entomopathogenic fungi

Thirteen isolates of *M. anisopliae* and four isolates of *B. bassiana* used in this study were obtained from the *icipe*'s Arthropod Germplasm Centre (Table 1). Most of the isolates were randomly selected because of their virulence to other arthropod pests in previous studies (Niassy et al. 2012; Akutse et al. 2020a). *Metarhizium anisopliae* and *Beauveria bassiana* isolates were cultured respectively on Sabouraud dextrose agar (SDA) and on Potato dextrose agar (PDA) in Petri dishes at 25 ± 2°C under complete darkness. Conidia were harvested by scraping the surface of 3-week-old sporulated cultures of these fungi grown on SDA. Conidia were suspended in 20 ml sterile distilled water containing 0.05% Triton X-100. The conidial suspension was vortexed for 5 min at about 700 rpm to produce a homogenous conidial suspension. The viability of conidia was then determined by spread-plating 0.1 ml of the suspension (titrated to 3.0 x $10^6$ conidia $ml^{-1}$) on SDA plates. Plates were incubated at 25 ± 2°C and examined after 18–24 h. Four sterile microscope coverslips were placed on the surface of each plate. Percentage germination was determined by counting randomly 100 germinated conidia beneath each coverslip under a light microscope; for each plate the number of germinated conidia out of the total number of conidia determined. Conidia were considered to have germinated when the length of the germ tube was at least twice the diameter of the conidium (Inglis et al. 2012). Four replicates were used for each isolate. Prior to starting the experiment, the initial viability for all the fungal strains used was above 90%.

Table 1
Fungal isolates tested against adult of both forms of *Frankliniella schultzei*

| Fungal species | Isolates | Source | Country |
|---|---|---|---|
| *Metarhizium anisopliae* | ICIPE 7 | *Amblyoma variegatum* | Kenya |
| | ICIPE 18 | Soil | Kenya |
| | ICIPE 20 | Soil | Kenya |
| | ICIPE 30 | *Buseola fusca* | Kenya |
| | ICIPE 32 | Not known | Australia |
| | ICIPE 40 | Soil | Kenya |
| | ICIPE 41 | Soil | DRC |
| | ICIPE 62 | Soil | DRC |
| | ICIPE 69 | Soil | DRC |
| | ICIPE 78 | *Temnoschoita nigroplagiata* | Kenya |
| | ICIPE 50 | *Rhipicephalus appendiculatus* | Kenya |
| | ICIPE 655 | Soil | Kenya |
| | ICIPE 656 | Soil | Kenya |
| *Beauveria bassiana* | ICIPE 603 | Hymenoptera | Kenya |
| | ICIPE 706 | Maize | Kenya |
| | ICIPE 275 | Soil | Kenya |
| | ICIPE 279 | Coleopteran larvae | Kenya |

Screening Techniques of Entomopathogenic Fungal Isolates Against Adult of Both Forms of *Frankliniella schultzei*

## Fungal suspension preparation and indirect infection

A working concentration of 1 x $10^8$ conidia $ml^{-1}$ was prepared from the stock suspension of each fungal isolate. French bean pods were surface sterilized by dipping in 10% Sodium hypochlorite for 5 minutes, then the suspension was discarded following by three times rinsing in distilled water. The pods were sliced into small pieces of 3 cm and soaked in a conidial suspension of 1 x $10^8$ conidia $ml^{-1}$ of the fungal isolates for 2 min followed by 5 min air-drying. Dried pods were placed singly into sterile Eppendorf tubes using sterile pair of forceps. Insects for the control were exposed to surface sterilized fungal-free pods. For each separate form of *F. schultzei*, ten 2-day-old adult thrips, irrespective of sex,

were used per replicate and introduced into individual Eppendorf tubes containing the treated pods. The experimental treatments were arranged in completely randomized design (CRD) where each treatment was replicated three times. The mortality of the exposed insects was monitored at 24 h intervals for 7 days. For all mortality observed, mycosis test was conducted to confirm whether death was due to infection by the fungus used in the bioassays. The insect cadavers were surface sterilized with 70% ethanol, rinsed three times in distilled water, and then kept separately in 9 cm Petri dishes lined with sterile moistened filter paper to allow for fungal outgrowth and verify if mortality could be attributed to the respective fungal isolates they were treated with. Mortality due to fungal infection was confirmed by the presence of hyphae and conidia on the surface of the cadavers. These procedures were conducted for both forms of *F. schultzei* as described earlier by Niassy et al. (2012).

## Contamination chamber technique or direct infection

For pathogenicity and virulence bioassays, screening was conducted against both forms of *F. schultzei* using direct infection. Conidia of all fungal isolates were harvested from three-week-old sporulated culture by scraping the surface of the plate using a spatula. Approximately, 1–2 g of dry conidia was spread evenly on the velvet cloth of a contamination chamber. Ten live adult insects (2-day old) were introduced individually in the contamination chamber for 20 seconds. This is to enable contamination of test insects with the dry spores of the fungus that were spread on the velvet of the contamination chamber. The technique has also been previously used to infect several arthropod pests such as *Spoladea recurvalis* (Lepidoptera: Crambidae) (Opisa et al. 2019), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Akutse et al. 2020b), *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) (Akutse et al. 2020c). The exposed insects were then removed and placed into sterile Eppendorf tubes containing a clean French bean pod as food source. The same procedure was conducted for all the 17 fungal strains. For the control, insects were also exposed to fungal-free contamination chamber and introduced into sterile Eppendorf tubes containing a clean French bean pod without being previously infected. The mortality of the exposed insects was assessed at 24 h intervals for 7 days. The experiment was arranged in completely randomized design (CRD) where each treatment was replicated three times, and mycosis test was also conducted with the dead insects as described above.

## Dose response bioassay

A dose–response bioassay was performed for the best four and five fungal isolates from the indirect and direct infections respectively. The best fungal isolates were selected based on cumulative mean mortality and lethal time to 50% mortality ($LT_{50}$) values from the initial screening bioassays. The isolates were tested at four different conidial concentrations as follows: $1 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$ and $1 \times 10^8$ conidia/ml. French bean pods used in this experiment were prepared as described above in the fungal suspension technique to infect the insects. Only the indirect procedure was used for the dose response bioassay. Each selected potent fungal isolate and defined dosage/concentration were replicated three times under a completely randomized design. The same experimental procedure was conducted for both

*F. schultzei* forms. Insects for the control were exposed to fungal-free pods. The survival of the exposed insects was monitored daily. The adult thrips that were monitored were fed with treated pods; they produced a progeny which emerged from egg to adult. The monitoring for survival was conducted from the progeny fed with treated pods up to the new adult passing through all different stages.

# Statistical analysis

Data on percentage mortalities were corrected for natural control mortality using Abbott's formula (Abbott 1925). Data on percentage conidial germination and percentage thrips mortality were subjected to a generalized linear model (GLM), assuming a binomial distribution and logit link. Whenever there are significant differences, means were separated using Student Newman Keuls (SNK) test and $P = 0.05$ was considered as significance level. Lethal time to 50% mortality ($LT_{50}$), lethal dose to 50% mortality ($LD_{50}$) values were estimated using the probit regression model implemented in the *ecotox* package (Hlina, 2020). The survival data were subjected to the Cox regression model and summarized using Kaplan-Meier survival distribution curves. The normality of the conidial germination data was performed using Shapiro-Wilk test before subjecting them to generalized linear mixed model. The analyses were performed in R software version 4.2.2 (R Core Team 2022).

# Results

### Efficacy of entomopathogenic fungi isolates against adult of both forms of Frankliniella schultzei

### Efficacy of entomopathogenic fungi against adult of Frankliniella schultzei dark form using fungal suspension in indirect infection

In the indirect infection bioassay, a significant difference was observed between the different fungal strains on mortality of dark form of *F. schultzei* ($\chi^2 = 12.32$; df = 32; $p < 0.001$). Fungal strains of *M. anisopliae* ICIPE 7, ICIPE18, ICIPE 20 and ICIPE 69 recorded mortalities of over 50% (Table 2). However, *M. anisopliae* ICIPE 69 recorded the highest mortality rate of 90.00 ± 3.33% and the shortest $LT_{50}$ of 4.20 (3.53–5.01) days, while *B. bassiana* ICIPE 706 recorded the lowest mortality rate (13.33 ± 6.67%) (Table 2).

Table 2
Mortality and lethal time of *Frankliniella schultzei* dark form fed on French bean pod infested with fungal suspension.

| Fungal strains | Mortality ± SE (%) | $LT_{50}$ ± SE (day) |
|---|---|---|
| *Metarhizium anisopliae* ICIPE 69 | 90.00 ± 3.33 a | 4.20 (3.53–5.01) |
| *Metarhizium anisopliae* ICIPE 18 | 73.33 ± 6.67 ab | 6.34(5.16-11.0) |
| *Beauveria bassiana* ICIPE 279 | 66.67 ± 3.33 bc | 5.85 (5.56–6.21) |
| *Metarhizium anisopliae* ICIPE 20 | 66.67 ± 14.52 bc | 6.45 (6.04-7.00) |
| *Metarhizium anisopliae* ICIPE 7 | 60.00 ± 5.77 bcd | 6.53 (6.07–7.20) |
| *Metarhizium anisopliae* ICIPE 32 | 50.00 ± 5.77 bcde | 7.50 (6.82–8.18) |
| *Metarhizium anisopliae* ICIPE 40 | 46.67 ± 3.33 cde | - |
| *Beauveria bassiana* ICIPE 275 | 46.67 ± 3.33 cde | - |
| *Metarhizium anisopliae* ICIPE 30 | 46.67 ± 3.33 cdef | - |
| *Metarhizium anisopliae* ICIPE 41 | 40.00 ± 5.77 cdef | - |
| *Beauveria bassiana* ICIPE 603 | 36.67 ± 3.33 def | - |
| *Metarhizium anisopliae* ICIPE 78 | 36.67 ± 3.33 def | - |
| *Metarhizium anisopliae* ICIPE 62 | 30.00 ± 5.77 ef | - |
| *Metarhizium anisopliae* ICIPE 655 | 26.67 ± 3.33 ef | - |
| *Beauveria bassiana* ICIPE 656 | 23.33 ± 6.67 ef | - |
| *Metarhizium anisopliae* ICIPE 50 | 23.33 ± 6.67 ef | - |
| *Beauveria bassiana* ICIPE 706 | 13.33 ± 6.67 f | - |
| | $\chi^2$ = 12.32; df = 38; P < 0.001 | |

Different lowercase letters within the same column indicate a significant difference according to Student Newman Keuls (SNK) test at $p < 0.05$.

**Efficacy of entomopathogenic fungi against adult of Frankliniella schultzei dark directly infected with the dry spores using contamination chamber**

All the fungal strains used in this experiment were pathogenic to *F. schultzei* dark form when they were infected directly with the dry conidia in the contamination chamber. Mortalities obtained were all over 75% and varied significantly among fungal strains ($\chi^2 = 50.59$; df = 32; $p < 0.001$). The highest mortality was recorded with *M. anisopliae* ICIPE 7 (100.00 ± 0.00%) while the lowest mortality was obtained with *B. bassiana* ICIPE 706 (76.67 ± 3.33%) (Table 3). Furthermore, the shortest and longest lethal times were

obtained with *M. anisopliae* ICIPE 7 and *Metarhizium anisopliae* ICIPE 32 with 3.54 (3.25–3.82) days and 6.72 (5.99–7.98) days, respectively (Table 3).

Table 3
Mortality and lethal time of *Frankliniella schultzei* dark form directly exposed to dry conidia of the different fungal strains in a contamination chamber.

| **Fungal strains** | **Mortality** | **$LT_{50}$ ± SE (day)** |
|---|---|---|
| *Metarhizium anisopliae* ICIPE 7 | 100.00 ± 0.00 a | 3.54(3.25–3.82) |
| *Metarhizium anisopliae* ICIPE 40 | 96.67 ± 3.33 ab | 3.93(3.53–4.31) |
| *Metarhizium anisopliae* ICIPE 18 | 96.67 ± 3.33 ab | 4.11 (3.87–4.35) |
| *Metarhizium anisopliae* ICIPE 20 | 96.67 ± 3.33 ab | 3.81(3.57–4.04) |
| *Metarhizium anisopliae* ICIPE 41 | 96.67 ± 3.33 ab | 4.23(3.91–4.56) |
| *Metarhizium anisopliae* ICIPE 30 | 93.33 ± 3.33 ab | 4.02(3.72–4.31) |
| *Metarhizium anisopliae* ICIPE 69 | 93.33 ± 3.33 ab | 3.60 (3.28–3.91) |
| *Beauveria bassiana* ICIPE 279 | 90.00 ± 5.77 b | 4.71(4.30–5.20) |
| *Metarhizium anisopliae* ICIPE 655 | 90.00 ± 5.77 b | 4.04(3.46–4.69) |
| *Metarhizium anisopliae* ICIPE 603 | 86.67 ± 3.33 bc | 4.11(3.68–4.55) |
| *Beauveria bassiana* ICIPE 656 | 83.33 ± 3.33 c | 4.65(4.19–5.22) |
| *Metarhizium anisopliae* ICIPE 50 | 80.00 ± 5.77 c | 4.79(4.48–5.13) |
| *Beauveria bassiana* ICIPE 275 | 80.00 ± 5.77 c | 5.32(4.98–5.74) |
| *Metarhizium anisopliae* ICIPE 78 | 80.00 ± 5.77 c | 4.57 (4.33–4.84) |
| *Metarhizium anisopliae* ICIPE 32 | 76.67 ± 3.33 d | 6.72 (5.99–7.98) |
| *Metarhizium anisopliae* ICIPE 62 | 76.67 ± 3.33 d | 5.16(4.87–5.50) |
| *Beauveria bassiana* ICIPE 706 | 76.67 ± 3.33 d | 4.34(4.00-4.71) |
| | $\chi 2 = 109.49$; df = 38; $P < 0.001$ | |

Different lowercase letters within the same column indicate a significant difference according to Student Newman Keuls (SNK) test at $p < 0.05$

**Lethal dose of selected potent fungal isolates to control Frankliniella schulzei dark form**

From the results of the above bioassays, the fungal strains of *M. anisopliae* ICIPE 7, ICIPE 18, ICIPE 20 and ICIPE 69, were selected due to their pathogenicity and virulence against *F. schulzei* dark form fed with the French bean pods infected with fungal suspension and through the direct infection with dry

conidia in a contamination chamber. The $LD_{50}$ values obtained varied among the selected fungal isolates. *Metarhizium anisopliae* ICIPE 7 and ICIPE 69 recorded the lowest doses as compared to *M. anisopliae* ICIPE 18 and ICIPE 20. Furthermore, the mortality of the dark form that has occurred was similar among the selected fungal isolates ($\chi 2 = 13.88$, df = 10, p = 0.18) (Table 4).

Table 4
Lethal dose of selected potent fungal isolates to control *Frankliniella schulzei* dark form.

| **Fungal strains** | **$LD_{50}$ (± 95% CL× $10^6$)** | **Mortality ± SE (%)** |
|---|---|---|
| *M. anisopliae* ICIPE 7 | $5.48 \times 10^{-25}$($4.58\ 10^8$, $5.46\ 10^4$) | 35.24 ± 0.35 a |
| *M. anisopliae* ICIPE 18 | $2.91 \times 10^2$($1.87\ 10^1$, $1.88\ 10^{16}$) | 21.59 ± 3.34 a |
| *M. anisopliae* ICIPE 20 | $5.01 \times 10^2$ ($2.23\ 10^1$, $3.00\ 10^{32}$) | 21.74 ± 0.32 a |
| *M. anisopliae* ICIPE 69 | $2.83 \times 10^{-6}$ ($4.71\ 10^1$, $9.1\ 10^5$) | 41.12 ± 0.36 a |
| | | $\chi 2 = 13.88$, df = 10, P = 0.18 |

**Survival of Frankliniella schultzei dark form fed on infested French bean pods**

The survival of *F. schultzei* dark form from infected French bean pods was monitored for a period of two weeks, using the four best selected fungal isolates to evaluate their effect on *F. schultzei* dark from during their developmental stage. The survival of the infected *F. schultzei* dark form was monitored on the progeny from the larval stage up to the possible emergence of adult. The survival of *F. schultzei* dark form was significantly different between fungal isolates (p = 0.018). *Metarhizium anisopliae* ICIPE 7 significantly reduced *F. schultzei* dark form survival as compared with the control and other isolates (*M. anisopliae* isolates ICIPE 18, ICIPE 20, and ICIPE 69) (Fig. 1). At five- and fourteen-days post exposure, the survival rates in *M. anisopliae* ICIPE 7 were 42.8 ± 5.76% and 0.00 ± 0.00% respectively as compared to 64.4 ± 5.04% and 8.8 ± 1.77% for the control (Fig. 1).

**Efficacy of entomopathogenic fungi against adult of Frankliniella schultzei pale form using fungal suspension technique in indirect infection**

The results of the indirect infection bioassays showed that there was a significant difference between different fungal strains on *F. schultzei* pale form mortality ($\chi^2 = 17.36$; df = 32; $P < 0.001$). Fungal suspension of *M. anisopliae* ICIPE 7, ICIPE 18, ICIPE 20 and ICIPE 32 strains recorded higher mortalities when compared to others. However, *M. anisopliae* ICIPE 20 and ICIPE 18 recorded the highest mortality rates of 72.38 ± 6.83% and 71.43 ± 7.66%, respectively. In addition, the shortest $LT_{50}$ of 1.16 (1.71–2.57) days was recorded with *M. anisopliae* ICIPE 18 (Table 5).

Table 5
Mortality and lethal time of *Frankliniella schultzei* pale fed on French bean pod infected with fungal suspension.

| **Fungal strains** | **Mortality ± SE (%)** | **$LT_{50}$ ± SE (day)** |
|---|---|---|
| *Metarhizium anisopliae* ICIPE 20 | 72.38 ± 6.83a | 3.15 (2.78–3.53) |
| *Metarhizium anisopliae* ICIPE 18 | 71.43 ± 7.66a | 1.16 (1.71–2.57) |
| *Metarhizium anisopliae* ICIPE 32 | 69.52 ± 8.60a | 3.76(2.61–5.27) |
| *Metarhizium anisopliae* ICIPE 7 | 68.57 ± 7.05a | 2.29 (1.75–2.78) |
| *Metarhizium anisopliae* ICIPE 30 | 66.19 ± 7.88ab | 3.93(3.22–4.86) |
| *Metarhizium anisopliae* ICIPE 62 | 62.94 ± 7.79ab | 1.70 (0.51–2.59) |
| *Beauveria bassiana* ICIPE 706 | 56.02 ± 8.48b | 2.37(1.29–3.31) |
| *Beauveria bassiana* ICIPE 275 | 55.18 ± 8.32b | 2.71(2.28–3.13) |
| *Metarhizium anisopliae* ICIPE 603 | 54.74 ± 8.18b | 2.86 (1.96–3.78) |
| *Metarhizium anisopliae* ICIPE 69 | 52.42 ± 8.79b | 3.31 (2.96–3.69) |
| *Metarhizium anisopliae* ICIPE 655 | 50.98 ± 7,66b | 3.27 (2.08–4.68) |
| *Metarhizium anisopliae* ICIPE 41 | 48.02 ± 8.62bc | - |
| *Beauveria bassiana* ICIPE 279 | 47.06 ± 8.01c | |
| *Metarhizium anisopliae* ICIPE 50 | 47.04 ± 8.92c | - |
| *Metarhizium anisopliae* ICIPE 78 | 46.11 ± 8.62c | - |
| *Metarhizium anisopliae* ICIPE 40 | 44.58 ± 8.85c | - |
| *Beauveria bassiana* ICIPE 656 | 41.32 ± 8.13c | - |
| | $\chi^2$ = 17.36; df = 32; P < 0.001 | |

Different lowercase letters within the same column indicate a significant difference according to Student Newman Keuls (SNK) test at p < 0.05

**Efficacy of entomopathogenic fungi against adult of Frankliniella schutlzei pale directly infected with the dry spores using contamination chamber technique**

Direct exposure of *F. schutlzei* pale form to the dry conidia of the different fungal strains using a contamination chamber resulted in a significant difference ($\chi^2$ = 27.45; df = 32; P < 0.001) in the insect mortality. All the fungal strains were pathogenic and virulent to the exposed insect pest at over 70% level of mortality (Table 6). The fungal strains which recorded the highest mortality rates and the shortest $LT_{50}$

values included *M. anisopliae* isolates ICIPE 62, ICIPE 69, ICIPE 32, ICIPE 40, ICIPE 41, ICIPE 7, ICIPE30 and *Beauveria bassiana* ICIPE 656 (Table 6).

Table 6
Mortality and lethal time of *Frankliniella schultzei* pale form directly exposed to dry spores of the different fungal strains using a contamination chamber technique.

| **Fungal strains** | **Mortality** | **$LT_{50}$ ± SE (day)** |
|---|---|---|
| *Metarhizium anisopliae* ICIPE 62 | 94.55 ± 1.74 a | 0.35 (0.10–0.62) |
| *Beauveria bassiana* ICIPE 656 | 94.29 ± 3.21 a | 1.02 (0.75–1.24) |
| *Metarhizium anisopliae* ICIPE 69 | 92.99 ± 3.17 a | 0.59 (0.22–0.95) |
| *Metarhizium anisopliae* ICIPE 40 | 91.69 ± 3.10 a | 0.76 (0.41–1.04) |
| *Metarhizium anisopliae* ICIPE 32 | 91.21 ± 3.30 a | 0.92 (0.54–1.22) |
| *Metarhizium anisopliae* ICIPE 41 | 90.26 ± 3.59 a | 0.69 (0.22–1.10) |
| *Metarhizium anisopliae* ICIPE 7 | 90.74 ± 2.97 a | 0.45 (0.10–0.81) |
| *Metarhizium anisopliae* ICIPE 30 | 90.26 ± 3.84 a | 0.92 (0.54–1.22) |
| *Metarhizium anisopliae* ICIPE 603 | 85.06 ± 6.13 a | 1.33 (0.62–1.86) |
| *Beauveria bassiana* ICIPE 706 | 84.76 ± 6.25 a | 1.52 (1.25–1.78) |
| *Beauveria bassiana* ICIPE 725 | 78.57 ± 6.25 ab | 1.60 (0.53–2.40) |
| *Metarhizium anisopliae* ICIPE 655 | 77.75 ± 6.52 ab | 1.74 (1.48–1.98) |
| *Metarhizium anisopliae* ICIPE 50 | 75.50 ± 6.92 ab | 1.14 (0.50–1.65) |
| *Metarhizium anisopliae* ICIPE 18 | 74.56 ± 3.52 ab | 1.02 (0.99–1.79) |
| *Beauveria bassiana* ICIPE 279 | 74.50 ± 6.45 ab | 1.88(1.77–1.98) |
| *Metarhizium anisopliae* ICIPE 20 | 73.12 ± 5.61 ab | 1.49(0.84–2.01) |
| *Metarhizium anisopliae* ICIPE 78 | 60.73 ± 4.95 b | 1.93(1.03–2.82) |
| | $\chi^2 = 27.45$; df = 32; $P < 0.001$ | |

Different lowercase letters within the same column indicate a significant difference according to Student Newman Keuls (SNK) test at $p < 0.05$

**Lethal doses of the selected potent stains against Frankliniella schulzei pale form**

The fungal strains *M. anisopliae* ICIPE 20, ICIPE 40, ICIPE 62, ICIPE 656 and ICIPE 69 were selected due to their pathogenicity and virulence on *F. schulzei* pale form fed with the French bean pods infected with fungal suspension and directly infected through contamination chamber. Similarly to the *F. schulzei* dark

form, the $LD_{50}$ values obtained here also varied among all the selected isolates. However, *M. anisopliae* ICIPE 62 and ICIPE 69 recorded the lowest lethal doses as compared to the other isolates (Table 7). Furthermore, the mortality obtained on the pale form thrips was similar among the selected fungal isolates ($\chi 2 = 7.29$; df = 10; p = 0.70).

Table 7
Lethal dose of selected potent fungal isolates to control *Frankliniella schulzei* pale form.

| **Fungal strains** | **$LD_{50}$ (± 95% CL× $10^6$)** | **Mortality ± SE (%)** |
|---|---|---|
| *M. anisopliae* ICIPE 20 | $4.18\ 10^{-7}$ ($1.22\ 10^{12}$, $3.11\ 10^{4}$) | 55.71 ± 0.55 a |
| *M. anisopliae* ICIPE 32 | $3.59\ 10^{-19}$($6.9910^{9}$, $1.25\ 10^{15}$) | 52.5 ± 0.57 a |
| *M. anisopliae* ICIPE 62 | $1.15\ 10^{-75}$ (1.67 109, $9.59\ 10^{3}$) | 56.07 ± 0.58 a |
| *M. anisopliae* ICIPE 656 | $7.04\ 10^{7}$ ($6.41\ 10^{8}$, $4.06\ 10^{-1}$) | 45.00 ± 0.55 a |
| *M. anisopliae* ICIPE 69 | $4.40\ 10^{-27}$ ($5.00\ 10^{10}$ ,$2.28\ 10^{3}$) | 55.71 ± 0.54 a |
| | | $\chi 2 = 7.29$; df = 10; P = 0.70 |

**Survival of Frankliniella schultzei pale form fed on infected French bean pod**

The survival of *F. schultzei* pale form fed on infected French bean pods using the various suspensions at the different concentrations of the selected potent fungal strains was monitored for a period two weeks. The survival rates of *F. schultzei* pale form were monitored on the progenies development up to the emergence of the newly adult stage. The survival of the progenies observed was significantly different among the different fungal isolates (P = 0.0026). However, *M. anisopliae* ICIPE 32 and ICIPE 69 significantly affected *F. schultzei* pale form survival as compared to the other isolates (*M. anisopliae* ICIPE 20, ICIPE 62 and ICIPE 656) and control (Fig. 2). At five- and fourteen-days post exposure, the survival rates were 42.8 ± 10.8% and 0.0 ± 0.0% in *M. anisopliae* ICIPE 32 and 38.5 ± 9.5% and 0.0 ± 0.00% in *M. anisopliae* ICIPE 69 treatments, respectively as compared to 63.6 ± 5.04% and 9.1 ± 4.3% for the control (Fig. 2).

# Discussion

Thrips (Thysanoptera) are among the most destructive pests of agricultural and horticultural crops, causing substantial economic losses through direct feeding damage and indirect effects such as virus transmission (Reitz et al. 2019; Liang-De Tang et al. 2023). Previous studies, particularly those conducted at *icipe*, have extensively investigated key genera including *Frankliniella*, *Thrips*, and *Megalurothrips* (Ekesi et al. 1998; Nyasani et al. 2012; Niassy et al. 2012, 2019; Muvea et al. 2014; Mfuti et al. 2016, 2021; Diabate et al. 2019). Building on this foundation, the present study evaluated the efficacy of entomopathogenic fungi (EPF) against the dark and pale forms of *Frankliniella schultzei* as a

sustainable alternative to synthetic insecticides. Two techniques namely the conidial suspension and contamination chamber were used to assess the pathogenicity, virulence and effectiveness of different fungal strains against both forms of *F. schultzei*. The indirect infection through pods contamination with fungal suspension and the direct infection using contamination chamber showed high susceptibility of the two thrips forms to the entomopathogenic fungi.

The results of our bioassays using fungal suspension (indirect infection) revealed high pathogenicity rates of *Metarhizium anisopliae* isolates as compared to *B. bassiana* on dark and pale forms. The isolates of *M. anisopliae* that performed well included *M. anisopliae* ICIPE 69, ICIPE 62, ICIPE 32, ICIPE 18, ICIPE 20 and ICIPE 7 with the isolates *M. anisopliae* ICIPE 7, ICIPE 18 and ICIPE 20 being highly pathogenic and virulent to both pale and dark forms of *F. schultzei*. The isolates *M. anisopliae*, ICIPE 69 has been reported previously to be pathogenic to thrips (Ekesi et al. 1998; Niassy et al. 2012). Previous studies demonstrated the efficacy of *M. anisopliae* ICIPE 69 against some other thrips species including *Megalurothrips sjostedti* (Trybom) (Ekesi et al. 1998), *Frankliniella occidentalis* (Niassy et al. 2012). The isolate *M. anisopliae* ICIPE 69 is already being commercialized in partnership with Real IPM biobest as Mazao Campaign against thrips, mealybugs and fruit flies (Akutse et al. 2020a, https://realipm.com/c/biopesticides/). This finding is showing the efficacy of the same isolate against two other forms of *F. schultzei* thrips species, which would lead to label extension of this biopesticide. In addition, *M. anisopliae* ICIPE 69 was also found to be effective against the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Mkiga et al. 2019), the greenhouse whiteflies *Trialeurodes vaporariorum* (Westwood) (Paradaza et al. 2021), and leafminers *Liriomyza huidobrensis* (Blanchard) (Migiro et al. 2010). However, for the isolates *M. anisopliae* ICIPE 7, ICIPE 18 and ICIPE 20 apart from being more virulent and pathogenic to both forms of *F. schultzei* during our experiment using indirect contamination, they have been reported to be pathogenic to several insect pests and vectors. For instance, the isolate *M. anisopliae* ICIPE 18 has been found to be highly pathogenic to several tropical insect pests including the African tephritid fruit flies, tomato leafminer, whiteflies (Dimbi et al. 2003; Onsongo et al. 2019; Akutse et al. 2020a; Mama Sambo et al. 2022; Paradaza et al. 2021; Agbessenou et al. 2021) and bean pod borer *Maruca vitrata*. (Tumahaise et al. 2018). Moreover, *M. anisopliae* ICIPE 7 has been found to be virulent against *T. urticae* (Bugeme et al. 2015), and *F. occidentalis* (Niassy et al. 2012), Ticks (Oundo et al. 2024), Mosquitoes (Ong'wen et al. 2024), fall armyworm (Akutse et al. 2019), while *M. anisopliae* ICIPE 20 has been reported very effective against *Tuta absoluta* (Akutse et al. 2020c, Agbessenou et al. 2021). In addition, *M. anisopliae* ICIPE 32 was reported effective in the control of fruit fly species (Dimbi et al. 2003).

The method of inoculation significantly influenced fungal efficacy. Direct exposure in contamination chambers resulted in higher mortality compared to indirect exposure through treated plant material, likely due to increased conidial contact and infection efficiency. Nevertheless, isolates that performed well under indirect exposure maintained consistent virulence patterns, indicating their potential effectiveness under field conditions where horizontal transmission plays a critical role. These results underscore the importance of considering exposure pathways when screening EPF and highlight the need to align laboratory bioassays with field-relevant conditions.

Differences in susceptibility between the dark and pale forms of *F. schultzei* were observed, with certain isolates (e.g., ICIPE 32 and ICIPE 62) showing greater efficacy against the pale form. Such variation may be attributed to genetic differences between morphs, which can influence host-pathogen interactions (Valero-Jiménez et al. 2016; Wang et al. 2021). In addition, variability in virulence among fungal isolates is consistent with previous reports of inter- and intra-specific variation in EPF performance across arthropod hosts such as three adult fruit flies' species (Dimbi et al. 2003), two-spotted spider mite *Tetranychus urticae* (Bugeme et al. 2015; Shin et al. 2017), *Maruca vitrata* (Tumahaise et al. 2015), greenhouse whitefly *Trialeurodes vaporariorum* (Paradaza et al. 2021).

As expected, pathogenicity and the virulence of the tested EPF isolates were higher when contamination of both *F. schultzei* thrips species was done directly using contamination chamber. However, the isolates that were pathogenic to both forms of *F. schultzei* through the suspension technique confirmed their trend and maintain their virulence. This was more consistent with the trend observed on *F. schultzei* dark form. Our findings showed that the two screening techniques have been complementary and have allowed to validate the direct and indirect efficacy of the potent isolates against both forms of *F. schultzei*. This current study demonstrated that the fungal suspension technique (indirect infection) played a critical role in authenticating the potent entomopathogenic fungi through horizontal contamination of the inoculum.

Survival analyses further confirmed the impact of EPF on thrips populations. Isolate ICIPE 7 significantly reduced survival of the dark form, whereas ICIPE 32 and ICIPE 69 were more effective against the pale form. These results are consistent with the observed dose–response relationships where isolates with lower lethal doses correspondingly reduced insect survival. The best performance of *M. anisopliae* ICIPE 7 in controlling *F. schultzei* dark form has been elucidated with its least lethal dose obtained when tested using both techniques and could be recommended for the management of this insect pest species. Interestingly, *M. anisopliae* ICIPE 7 has been earlier reported to control of red spider mites *T. urticae* (Bugeme et al. 2015), ticks (Oundo et al. 2024) and recently for the control of the fall armyworm (Akutse et al. 2019) and it is currently been commercialized as Mazao Tickoff (against ticks) and Mazao Detain (against fall armyworm) by the Real IPM-Biobest. The least lethal dose to control *F. schulzei* pale form was obtained with *M. anisopliae* ICIPE 62 and ICIPE 69. Previous studies have demonstrated that *M. anisopliae* ICIPE 62 has significant potential in controlling aphid species (Bayissa et al. 2015; Mweke et al. 2018) and currently registered as Mazao Supreme by Real IPM-Biobest company. The performance of *M. anisopliae* ICIPE 69 in controlling the pale form of *F. schultzei* corroborates with its performance of reducing the survival of the *F. schulzei* pale form when fed with infested French bean pod. The fungal isolate *M. anisopliae* ICIPE 69 has been extensively reported to control thrips (Ekesi et al. 1998; Niassy et al. 2012; Mfuti et al. 2017).

In summary, *M. anisopliae* isolates, particularly ICIPE 7, ICIPE 18, ICIPE 32, and ICIPE 69, demonstrated high pathogenicity against both forms of *F. schultzei*. Although direct exposure resulted in higher mortality, indirect infection confirmed the potential for horizontal transmission under more realistic conditions. Among the tested isolates, ICIPE 69 showed the most consistent performance across both

infection methods and thrips forms. These findings highlight its strong potential for integration into sustainable pest management programmes. However, further validation under field conditions, including formulation and application strategies, is required prior to large-scale deployment.

## Declarations

# Declaration, author contributions and data availability

### Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Ethical approval

The authors approve the ethical guidelines of the journal.

### Consent for publication

All authors gave consent to the publication of the study.

# Author Contribution

Conceptualization: D.K .M., K.S.A.; Methodology: K.S. A., W.J. K.; Formal analysis: D.K. M., A.O.A.; Writing-original draft preparation: D. K. M.; Writing-review and editing: D. K. M., A.O. A., T. C., W.J. K., and K.S.A. All authors have read and agreed to the final version of the manuscript.

# Acknowledgement

We are thankful to Gladys Karira Wairumu, Mary Achieng, Andrew Agrey Abiya and Nelson Mwando for their technical assistance.

# Data availability

The data that support the findings of this study are available at https://dmmg.icipe.org/dataportal/dataset/efficacy-of-entomopathogenic-fungi.

## References

1. Abbott WS (1925) A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

2. Abtew A, Niassy S, Affognon H, Subramanian S, Kreiter S, Garzia GT, Martin T (2016) Farmers' knowledge and perception of grain legume pests and their management in the eastern province of Kenya. J. Crop Prot. 87: 90–97
3. Agbessenou A, Akutse KS, Yusuf AA, Wekesa SW, Khamis F (2021) Temperature-dependent modelling and spatial prediction reveal suitable geographical areas for deployment of two *Metarhizium anisopliae* isolates for *Tuta absoluta* management. Sci. Rep. 11: 23346. https://doi.org/10.1038/s41598-021-02718-w
4. Akinyemi AO, Subramanian S, Mfuti DK, Pope TW, Tamiru A, Kirk WD (2021) Mating behaviour, mate choice and female resistance in the bean flower thrips (Megalurothrips sjostedti). Sci. Rep.11: 1–10. https://doi.org/10.1038/s41598-021-93891-5
5. Akutse KS, Kimemia JW, Ekesi S, Khamis FM, Ombura OL, Subramanian S (2019) Ovicidal effects of entomopathogenic fungal isolates on the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Appl. Entomol. 143 : 626–634. https://onlinelibrary.wiley.com/doi/epdf/10.1111/jen.12634. DOI: 10.1111/jen.12634.
6. Akutse KS, Subramanian S, Maniania NK, Dubois T, Ekesi S (2020a) Biopesticide Research and Product Development in Africa for Sustainable Agriculture and Food Security. Experiences from the International Centre of Insect Physiology and Ecology (*icipe*). Front. sustain. food syst. 4: 563016. doi: 10.3389/fsufs.2020.563016
7. Akutse KS, Khamis FM, Ambele FC, Kimemia JW, Ekesi S, Subramanian S (2020b) Combining insect pathogenic fungi and a pheromone trap for sustainable management of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 177: 107477. https://doi.org/10.1016/j.jip.2020.107477
8. Akutse KS, Subramanian S, Khamis FM, Ekesi S, Mohamed SA (2020c) Entomopathogenic fungus isolates for adult *Tuta absoluta* (Lepidoptera: Gelechiidae) management and their compatibility with Tuta pheromone. J. Appl. Entomol. 144: 777–787.
9. Bayissa W, Ekesi S, Mohamed SA, Kaaya GP, Wagacha JM, Hanna R, Maniania NK (2016) Selection of fungal isolates for virulence against three aphid pest species of crucifers and okra. J. Pest Sci. 90: 355–368, 10.1007/s10340-016-0781-4.
10. Bugeme DM, Knapp M, Ekesi S, Chabi-Olaye A, Boga HI, Maniania NK (2015) Efficacy of *Metarhizium anisopliae* in controlling the two-spotted spider mite *Tetranychus urticae* on common bean in screenhouse and field experiments. J. insect sci. 22:121–128. doi: 10.1111/1744-7917.12111.
11. Dakshina RS, Vivek K, Garima K (2014) Common Blossom Thrips, *Frankliniella schultzei* (Thysanoptera: Thripidae) Management and Groundnut Ring Spot Virus Prevention on Tomato and Pepper in Southern Florida, Fla. Entomol. 97: 374–383. https://doi.org/10.1653/024.097.0206.
12. Diabate S, Deletre E, Murungi L, Fiaboe K, Subramanian S, Wesonga J, Martin T (2019) Behavioural responses of bean flower thrips (Megalurothrips sjostedti) to vegetative and floral volatiles from different cowpea cultivars. Chemoecology 29: 73–88. https://doi.org/10.1007/s00049-019-00278-0.

13. Diamantino ML, Ramos RS, Sarmento RA, Pereira PS, Picanço MC (2021) Decision-making system for the management of *Frankliniella schultzei* thrips in commercial melon fields. J. Crop Prot. 139: 105346. doi: 10.1016/j.cropro.2020.105346
14. Dimbi S, Maniania NK, Lux SA, Ekesi S, Mueke JM (2003) Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin to three adult fruit by species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). Mycopathologia 156: 375–382.
15. Ekesi S, Maniania NK, Ampong-Nyarko K, Onu I (1998) Potential of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin for control of the legume flower thrips, *Megalurothrips sjostedti* (Trybom) on cowpea in Kenya. J. Crop Prot. 17: 661–668.
16. Gikonyo MW, Niassy S, Moritz GB, Khamis FM, Magiri E, Subramanian S (2017) Resolving the taxonomic status of *Frankliniella schultzei* (Thysanoptera: Thripidae) colour forms in Kenya. A morphological-, biological-, molecular- and ecological-based approach. Int. J.Trop. Insect Sci. 37: 57–70. https://doi.org/10.1017/S1742758416000126.
17. Hlina BL (2020) Ecotox: A package for analysis of ecotoxicology. R Package Version 1.4.2, 2020. Available online: https://cran.rproject.org/package=ecotox (accessed on 25 February 2022).
18. Inglis GD, Enkerli JUERG, Goettel MS (2012) Laboratory techniques used for entomopathogenic fungi: Hypocreales. Manual of techniques in invertebrate pathology. vol. 2.
19. Jones RAC, Sharman M (2005) Capsicum chlorosis infecting *Capsicum annum* in the East Kimberley region of Western Australia. Australas. Plant Pathol.34 : 297–299.
20. 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: e103350. https://doi.org/10.1371/journal.pone.0103350
21. Kakkar G, Seal DR, Kumar V (2012) Assessing abundance and distribution of an invasive thrips *Frankliniella schultzei* (Thysanoptera: Thripidae) in south Florida. Bull. Entomol. Res.102: 249 – 59. doi: 10.1017/S0007485311000599.
22. Kakkar G, Seal DR, Kumar V (2017) Common blossom thrips, *Frankliniella schultzei* Trybom (Insecta: Thysanoptera: Thripidae). Tropical Research and Education Center, Entomology and Nematology Department, UF/IFAS Extension, Gainesville, FL 32611.
23. Klose MJ, Sdoodee R, Teakle DS, Milne JR, Greber RS, Walter GH (1996) Transmission of three strains of tobacco streak ilarvirus by different thrips species using virus-infected pollen. J Phytopathol. 144: 281–284.
24. Khan RA, Seal DR, Zhang S, Liburd OE, Srinivasan R, Evans E (2020) Distribution Pattern of Thrips (Thysanoptera: Thripidae) and Tomato Chlorotic Spot Virus in South Florida Tomato Fields. Environ. Entomol. 49: 73–87. doi: 10.1093/ee/nvz153. PMID: 31922551.
25. Liang-De T, Ling-Hang G, Jian-Hui W, Lian-Sheng Z (2023) Thrips in genus *Megalurothrips* (Thysanoptera: Thripidae): biodiversity, bioecology, and IPM, J. Integr. Pest Manag. 14: 8. https://doi.org/10.1093/jipm/pmad006

26. Macharia I, Backhouse D, Skilton R, Ateka E, Wu S-B, Njahira M, Maina S, Harvey J (2015) Diversity of thrips species and vectors of tomato spotted wilt virus in tomato production systems in Kenya. J. Econ. Entomol. 108: 20–28; DOI: 10.1093/jee/tou010.
27. Mama SS, Akutse KS, du Plessis H, Aigbedion-Atalor PO, Mohamed SA, Ndlela S (2022) Interactions between the Entomopathogenic Fungus *Metarhizium anisopliae* ICIPE 20 and the Endoparasitoid *Dolichogenidea gelechiidivoris*, and Implications for Combined Biocontrol of *Tuta absoluta*. Biology (Basel)11:1323. doi: 10.3390/biology11091323.
28. Mantzoukas S, Kitsiou F, Natsiopoulos D, Eliopoulos PA (2022) Entomopathogenic Fungi: Interactions and applications. Encyclopedia 2: 646–656. https://doi.org/10.3390/encyclopedia 2020044.
29. Mfuti DK, Tamiru A, Kirk WDJ, Akinyemi AO, Campbell H, O'Brien M, Drijfhout FP, Pope TW, Niassy S, Subramanian S (2021) Exploiting Thrips Aggregation Pheromones to Develop a Lure-and-Kill Strategy for the Management of the Bean Flower Thrips. Agronomy 11 : 1269. https://doi.org/10.3390/agronomy11071269
30. Mfuti DK, Niassy S, Subramanian S, du Plessis H, Ekesi S, Maniania NK (2017) Lure and infect strategy for application of entomopathogenic fungus for the control of bean flower thrips in cowpea. Biol. Control 107: 70–76.
31. Mfuti DK, Subramanian S, van Tol RWHM, Wiegers GL, De Kogel WJ, Niassy S, Du Plessis H, Ekesi S, Maniania NK (2016) Spatial separation of semiochemical Lurem-TR and entomopathogenic fungi to enhance their compatibility and infectivity in an autoinoculation system for thrips management. Pest Manag. Sci. 72: 131–139.
32. Migiro LN, Maniania NK, Chabi-Olaye A, Van den Berg J (2010) Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) isolates to the adult pea leafminer (Diptera: Agromyzidae) and prospects of an autoinoculation device for infection in the field. Environ. Entomol. 39: 468–475.
33. Milne JR, Jhumlekhasing M, Walter GH (1996). Understanding host plant relationships of polyphagous flower thrips, a case study of *Frankliniella schultzei* (Trybom). In: Goodwin, S., Gillespie, P. (eds), Proceedings of the 1995 Australia and New Zealand Thrips Workshop: Methods, Biology, Ecology and Management, NSW Agriculture, Gosford. 8–14.
34. Mkiga AM, Mohamed SA, du Plessis H, Khamis FM, Ekesi S (2019). Field and laboratory performance of false codling moth, *Thaumatotibia Leucotreta* (Lepidoptera: Troticidae) on orange and selected vegetables. Insects 10: 63. https://doi.org/10.3390/insects1003006.
35. Mouden S, Sarmiento KF, Klinkhamer PGL, Leiss KA (2017) Integrated pest management in western flower thrips: past, present and future. Pest Manag. Sci. 75, 813–822. doi: 10.1002/ps.4531.
36. Muvea AM, Waiganjo MM, Kutima HL, Osiemo Z, Nyasani JO, Subramanian S (2014) Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. Int. J.Trop. Insect Sci. 34: 197–206.

37. Mweke A, Ulrichs C, Nana P, Akutse KS, Fiaboe KKM, Maniania NK, Ekesi S (2018) Evaluation of the entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria* sp. for the management of *Aphis craccivora* (Hemiptera: Aphididdae). J. Econ. Entomol.111: 1587–1594,10.1093/jee/toy135,
38. Nana P, Maniania NK, Maranga RO, Boga HI, Kutima HL, Eloff JN (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. J. Pest Sci. 85: 109–115.
39. Niassy S, Maniania NK, Subramanian S, Gitonga LM, Ekesi S (2012) Performance of a semiochemical-baited autoinoculation device treated with *Metarhizium anisopliae* for control of *Frankliniella occidentalis* on French bean in field cages. Entomol. Exp. Appl.142: 97–103.
40. Niassy S, Tamiru A, Hamilton JGC, Kirk WDJ, Mumm R, Sims C, de Kogel WJ, Ekesi S, Maniania NK, Bandi K, et al. (2019) Characterization of male-produced aggregation pheromone of the bean flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). J. Chem. Ecol. 45: 348–355.doi: 10.1007/s10886-019-01054-8.
41. Nyasani J O, Meyhöfer R, Subramanian S, Poehling H-M (2012) Effect of intercrops on thrips species composition and population abundance on French beans in Kenya. Entomol. Exp. Appl.142: 236–246.
42. Palmer JM (1990) Identification of the common thrips of Tropical Africa (Thysanoptera: Insecta). Trop. Pest. Manage. 36 : 27–49. DOI: 10.1080/09670879009371431.
43. Paradza VM, Khamis FM, Yusuf AA, Subramanian S, Akutse KS (2021) 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 nymphs. Heliyon 7: e08277. doi: 10.1016/j.heliyon. 2021.e08277.
44. Odanga JJ, Mohamed S, Olubayo F, Nyankanga R, Otieno IA, Mwalusepo S, Mwachala G, Johansson TP (2017) Spatial characterization of common blossom thrips (Frankliniella schultzei) in smallholder avocado orchards along slopes of Taita Hills and Mount Kilimanjaro, IJEAB.2: 2675–2678. https://doi.org/10.22161/ijeab/2.5.50.
45. Onsongo SK, Gichimu BM, Akutse KS, Dubois T, Mohamed SA (2019) Performance of three isolates of *Metarhizium anisopliae* and their virulence against *Zeugodacus cucurbitae* under different temperature regimes, with global extrapolation of their efficiency. Insects 10: 270. https://doi.org/10.3390/insects10090270
46. Ong'wen F, Njoroge M M, Fillinger U, Lutermann H, Bukhari T (2024) Efficacy of *Metarhizium anisopliae*, Isolate ICIPE 7, against *Anopheles arabiensis*, *Glossina fuscipes*, and *Rhipicephalus* spp. Insects 15: 449. https://doi.org/10.3390/insects15060449.
47. Opisa S, Du Plessis H, Akutse KS, Fiaboe KKM, Ekesi S (2019) Horizontal transmission of *Metarhizium anisopliae* between *Spoladea recurvalis* (Lepidoptera: Crambidae) adults and compatibility of the fungus with the attractant phenylacetaldehyde. Microb. Pathog. 131: 197–204.

48. Oundo JW, Kalayou S, Gort G, Bron GM, Koenraadt CJM, Bosch QT, Masiga AD (2024) Randomized controlled trial of Tickoff® (*Metarhizium anisopliae* ICIPE 7) for control of tick infestations and transmission of tick-borne infections in extensively grazed zebu cattle in coastal Kenya. Parasite Epidemiol Control. 27 : e00384,https://doi.org/10.1016/j.parepi.2024.e00384.
49. Reitz SR, Gao Y, Kirk WDJ, Hoddle MS, Leiss KA, Funderburk JE (2020) Invasion biology, ecology, and management of western flower thrips. Annu. Rev. Entomol. 65: 17–37. https://doi.org/10.1146/annurev-ento-011019-024947.
50. R Development Core Team (2022) A language and environment for statistical computin. Vienna, Austria: R Foundation for statistical computing, Vienna, Austria.
51. Tumuhaise V, Ekesi S, Mohamed SA, Ndegwa PN, Irungu LW, Srinivasan R, Maniania NK (2015) Pathogenicity and performance of two candidate isolates of *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) in four liquid culture media for the management of the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae). Int. J.Trop. Insect Sci. 35: 34–47.
52. Sakimura K (1969) A comment on the color forms of *Frankliniella schultzei* (Thysanoptera:Thripidae) in relation to transmission of the tomato-spotted wilt virus. Pac. Insects. 11: 761–762.
53. Seal DR, Kumar V, Kakkar G (2014) Common blossom thrips, *Frankliniella schultzei* (Thysanoptera: Thripidae) management and Groundnut ring spot virus prevention on tomato and pepper in Southern Florida. Fla. Entomol. 1: 374–383.
54. Shin TY, Bae SM, Kim DJ, Yun HG, Woo SD (2017) Evaluation of virulence, tolerance to environmental factors and antimicrobial activities of entomopathogenic fungi against two-spotted spider mite, *Tetranychus urticae*. Mycoscience 58: 204–212. 10.1016/j.myc.2017.02.002.
55. Valero-Jiménez CA, Wiegers H, Zwaan BJ, Koenraadt CJ, van Kan JA (2016) Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*. J. Invertebr. Pathol. 133: 41 – 9. doi: 10.1016/j.jip.2015.11.011.
56. Wangai AW, Mandal B, Pappu HR, Kilonzo S (2001) Outbreak of tomato spotted wilt virus in tomato in Kenya. Plant Dis.85: 1123. doi: 10.1094/PDIS.2001.85.10.1123B.
57. Wijkamp I, Almarza N, Goldbach R, Peters D (1995) Distinct levels of specificity in thrips transmission of tospoviruses. Phytopathology 85: 1069–1074.
58. Wang Y, Cui C, Wang G, Li Y, Wang S (2021) Insects defend against fungal infection by employing microRNAs to silence virulence-related genes. Proc. Natl. Acad. Sci. U.S.A. 118(19): e2023802118. doi: 10.1073/pnas.2023802118.

# Figures

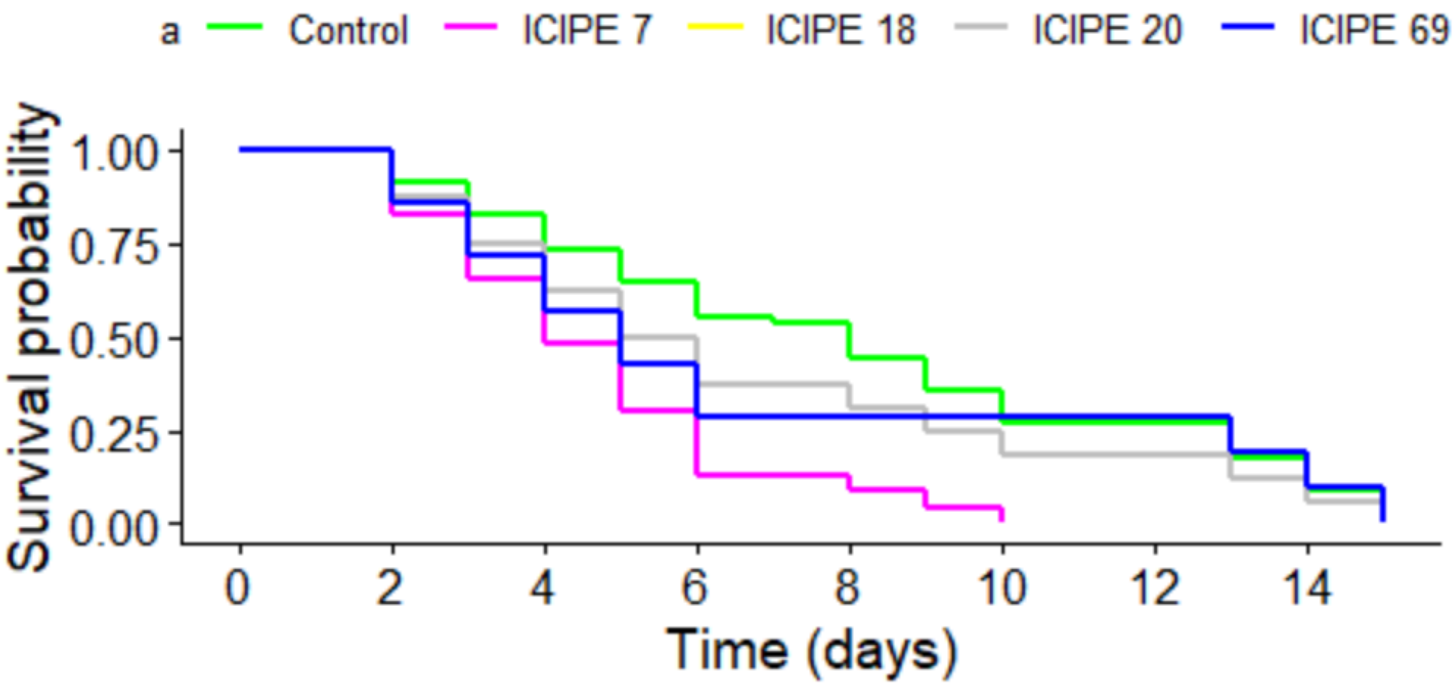



**Figure 1**

Survival of *Frankliniella schultzei* dark form fed on French bean pods infected with selected potent fungal strains

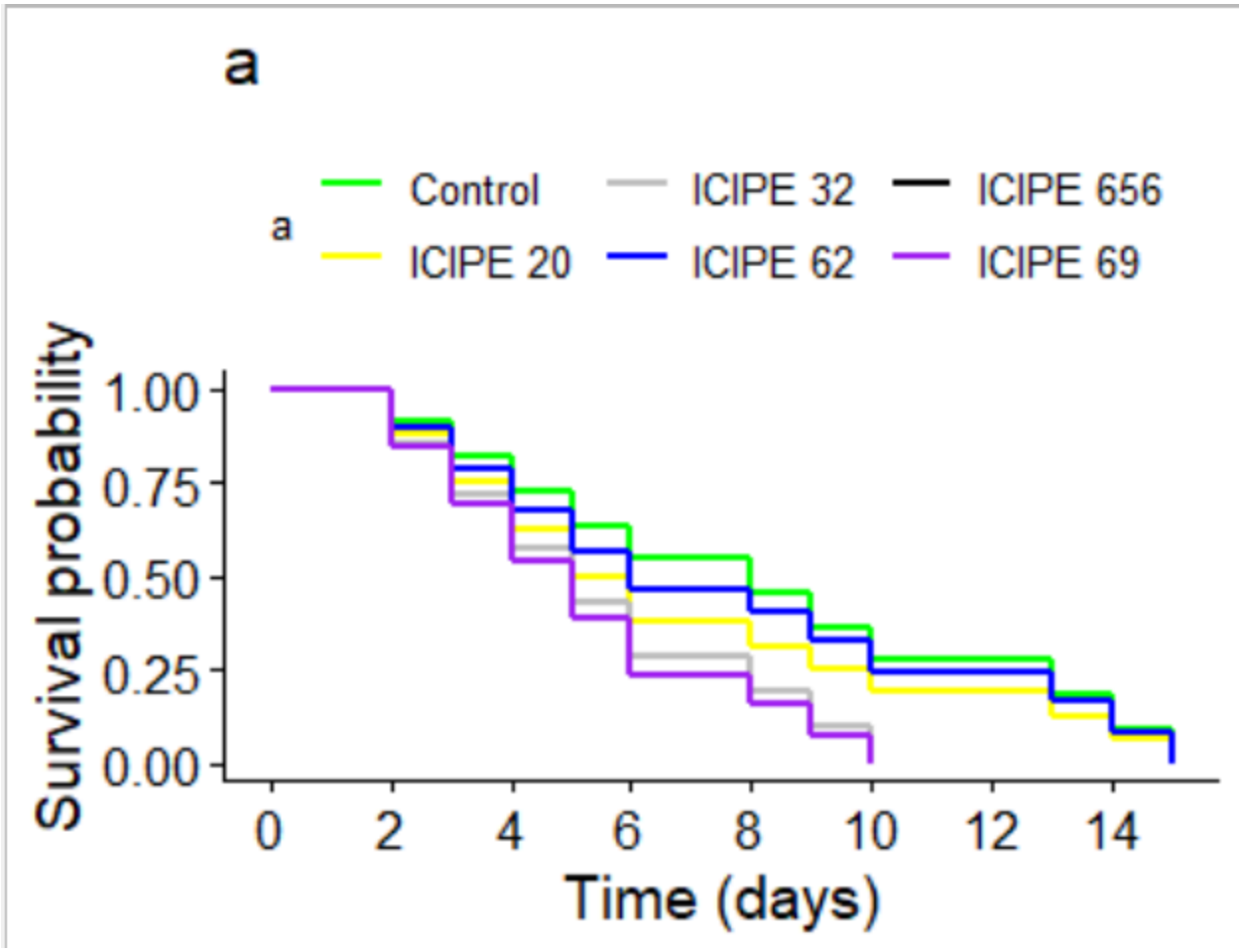



**Figure 2**

Survival of *Frankliniella schultzei* pale form fed on French bean pods infected with the selected potent *Metarhizium anisopliae* fungal strains