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ORIGINAL ARTICLE

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Efficacy of *Metarhizium anisopliae* (Mechnikov) Sorokin ICIPE 69 against the melon fly *Zeugodacus cucurbitae* (Coquillett) infesting courgette (*Cucurbita pepo* L.) in field cages

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Abstract

Effective integrated pest management (IPM) of the melon fly *Zeugodacus cucurbitae* (Coquillett), a devastating pest threatening horticultural production in Africa, is urgently needed. In this study, a caged field experiment was implemented in Thika, Kenya to test the efficacy of *Metarhizium anisopliae* (Mechnikov) Sorokin ICIPE 69 for *Z. cucurbitae* control in courgette *Cucurbita pepo* L. Treatments included: (1) dry conidia of *M. anisopliae* ICIPE 69 applied in an autodissemination device (fungus); (2) dry conidia of *M. anisopliae* ICIPE 69 in an autodissemination device combined with cuelure, a male attractant (fungus + cuelure); (3) a commercial insecticide (profenofos + cypermethrin) (insecticide); and (4) an untreated control (control). Each treatment was replicated thrice and the experiment was conducted twice during two seasons. Flies (300/tunnel) and treatments were introduced at flowering. One day later, 25 flies/tunnel were randomly collected from each cage to assess conidial acquisition (for fungus and fungus + cuelure treatments only) and mortality during a 10-day interval in the laboratory. Flies in the fungus + cuelure treatment acquired more conidia (18.02 ± 0.48 conidia/fly) than those in the fungus treatment (11.93 ± 0.40 conidia/fly). Flies in the fungus + cuelure treatment experienced the highest mortality (95.31% ± 1.69%), while those in the insecticide treatment experienced the lowest (38.70% ± 4.32%). Dry conidia were collected from the autodissemination devices (in the fungus and fungus + cuelure treatment) daily for 7 days after flowering to check for compatibility through germination tests. Percentage germination reduced over time from 86.45% ± 1.77% and 87.72% ± 1.71% on day 1 to 16.39% ± 2.11% and 42.76% ± 1.74% on day 7 (seasons 1 and 2, respectively). The yield was significantly different among treatments and was, across seasons, highest in the fungus + cuelure (6961 ± 550 kg/ha) and insecticide (7267 ± 352 kg/ha) treatments and lowest in the control treatment (2089 ± 155 kg/ha).

KEYWORDS
autodissemination, cucurbit, entomopathogenic fungus, fruit fly, insecticide

1 | INTRODUCTION

In Africa, cucurbits are among the most important consumed and traded vegetables. They are a key source of income for smallholder farmers (HCDA, 2020) and are rich in vitamins and minerals (Patel & Rauf, 2017; Rolnik & Olas, 2020; Shayanowako et al., 2021). In Kenya, cucurbit production in 2019–2020 stood at 14,606 tonnes/year for butternut *Cucurbita moschata* (Duchesne), 3918 tonnes/year for courgette *Cucurbita pepo* L., 35,829 tonnes/year for pumpkin *Cucurbita maxima* L. and 55,325 tonnes/year for melon *Cucumis melo* L. (HCDA, 2020). However, cucurbit production in the country faces serious challenges from insect pests, especially Tephritid fruit flies (De Meyer et al., 2015; HCDA, 2020; Kambura et al., 2018; Leblanc et al., 2012). Direct losses caused by Tephritid fruit flies result from females ovipositing under the skin of the fruits, with emerging larvae feeding inside the fruit, leading to rotting that renders fruit unsuitable for human consumption, while indirect economic losses result from stringent quarantine measures enforced by importing countries (Badii et al., 2015; Shafiq Ansari et al., 2012).

The melon fly *Zeugodacus cucurbitae* Coquillett is among the most destructive and widespread fruit fly species in the tropics (Mir et al., 2014). The pest is highly polypgahous, with damage reported from over 81 plant species (Dhillon et al., 2005). In Africa, De Meyer et al. (2015) listed 45 plant species as hosts, of which 29 were from the Cucurbitaceae family. *Zeugodacus cucurbitae* invaded Africa around 1936 from the Indian sub-continent (De Meyer et al., 2015) and has become a pest of significant economic importance, including through quarantine restrictions, in more than 25 African countries (De Meyer et al., 2015). In Kenya, *Z. cucurbitae* has been reported to be the dominant species on the most cucurbit crops (Kambura et al., 2018).

Synthetic chemical insecticides are routinely used to control *Z. cucurbitae*, yet they are neither effective nor affordable for most resource-poor cucurbit growers. Their mis- and overuse negatively impact on animal, human and environmental health, including the elimination of associated natural enemies of the pest, and may lead to the development of insecticide resistance (Ryckewaert et al., 2010; Vontas et al., 2011). Therefore, integrated pest management (IPM) approaches are being sought for the sustainable management of *Z. cucurbitae*. The sterile insect technique (SIT) is effectively used against the pest, mainly as part of area-wide IPM programs (Dyck et al., 2014; Ito et al., 2003). Egg, larval and pupal parasitoids of *Z. cucurbitae* are being used in its classical and augmentative biological control (Garcia et al., 2020; Vargas et al., 2012; Zhao et al., 2013). For instance, *Fopius arisanus* (Sonan) parasitize eggs; *Psyttalia fletcheri* (Silvestri), *Tetrastichus dacicida* Silvestri, and *Tetrastichus giffardianus* Silvestri parasitize larvae; while *Dirhinus giffardii* Silvestri and *Spalangia endius* Walker parasitize pupae (Bautista et al., 2004; Ramadan & Messing, 2003; Ullah et al., 2021; Vargas et al., 2004). Other management methods include the use of protein baits (Piñero et al., 2020; Ramasamy, 2020) and augmentoria (Deguine et al., 2015).

Entomopathogenic fungi are being researched as effective biological control agents against *Z. cucurbitae* and are the focus of this study. Previous studies have identified *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Paecilomyces fumosoroseus* (Wize) Brown and Smith for control of fruit flies (Castillo et al., 2000; Cossentine et al., 2010). Recently, Onsongo et al. (2022) tested the effectiveness of various isolates of *M. anisopliae* and *B. bassiana* against *Z. cucurbitae* in the laboratory. The authors identified isolate *M. anisopliae* ICIPE 69 as the most potent isolate against the pest as it caused the highest mortality (96.2%) and lowest LT_{50} (2.61 days) while producing the highest number of conidia (90.5×10^7/mL). The isolate has been previously commercialized as Campaign (RealIPM) and is currently applied on 80,420 ha, targeting mealybugs, thrips, leafminers and fruit flies (Akutse et al., 2020). Entomopathogenic fungi are usually introduced as cover sprays in farmer fields to control fruit fly populations (Daniel & Wysse, 2010; Ortu et al., 2009) but have also been used through a 'lure-and-infect' approach (Ekesi et al., 2007; Flores et al., 2013; Liedo et al., 2007). The approach works by attracting an insect into an autodissemination device with pheromones or other attractants, where it becomes contaminated with conidia and subsequently disseminates the pathogen to other conspecifics in the population (Toledo et al., 2017), increasing efficiency and reducing the amount of inoculum needed compared to inundative applications. The male sex pheromone cuelure [4-(*p*-acetoxyphenyl)-2-butanone] attracts male adults of *Z. cucurbitae* and can therefore be used in a lure-and-infect approach (Onsongo et al., 2022). The use of autodissemination devices have been evaluated for several fruit fly species in the field, but not yet for *Z. cucurbitae*. For example, Dimbi et al. (2003) designed an autodissemination device that proved efficient in the laboratory and field cages for contaminating fruit flies. In the study, autodissemination devices laced with *M. anisopliae* ICIPE 20 were evaluated against *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa* var. *fasciventris* Karsch and caused 70%–93% mortality of adults of both species in 12 days after capture and rearing in the laboratory. Studies carried out in the laboratory by Onsongo et al. (2022) reported successful autodissemination of *M. anisopliae* ICIPE 69 to suppress *Z. cucurbitae*. However, the entomopathogen has not yet been tested under field conditions against *Z. cucurbitae*. Therefore, the aim of the current study was to evaluate the efficacy of *M. anisopliae* ICIPE 69 to control *Z. cucurbitae* in the field and test the performance of a lure-and-infect approach through the use of autodissemination devices.

2 | MATERIALS AND METHODS

2.1 | Insect source and rearing conditions

A *Z. cucurbitae* colony was initiated from infested fruits of four cucurbit hosts (watermelon *Citrullus lanatus* (Thunb.), cucumber *Cucumis sativus* L., *C. pepo*, and *C. moschata*) collected from the Coastal Region in Kenya in November 2020. Infested fruits were

incubated in the laboratory at the International Centre for Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, until adult emergence. Emerged adults were transferred to clean Perspex cages (30 cm × 30 cm × 30 cm), maintained on an artificial diet containing sugar and yeast hydrolysate-based artificial diet and supplied with a source of water in a falcon tube lid filled with pumice granules according to Sookar et al. (2014). Adults were kept in a rearing room at 12 h: 12 h light: dark, 27 ± 2°C and 45% relative humidity (RH). Adults were provided with *C. maxima* fruits for oviposition. After 24 h of exposure, the fruits were removed and placed in a plastic container (35 cm × 20 cm × 12 cm) containing sterilized sand up to a depth of 5 cm and a wire mesh placed at 15 cm above, which was used to hold the infested fruits and allow the mature larvae to drop into the sand to pupate. Pupae were collected in 90 mm diameter plastic Petri dishes and placed in Perspex cages (30 cm × 30 cm × 30 cm) for adults to emerge. Emerged adults were maintained in the same way and under the same conditions as described above for 10 days. Prior to release, flies were separated by sex based on the presence of an ovipositor and transported to the field in the Perspex cages.

2.2 | Fungus

The isolate *M. anisopliae* ICIPE 69 was obtained from *icipe*'s germplasm repository. The fungus was subcultured on Sabouraud dextrose agar (SDA) (Oxoid) at 26 ± 2°C in complete darkness for 21 days. Conidia of *M. anisopliae* ICIPE 69 were mass-produced on whole rice *Oryza sativa* L. substrate in Milner bags (60 cm long × 35 cm wide) as described by Tumuhaise et al. (2018). Prior to inoculation, the rice was autoclaved for 1 h at 121°C and inoculated with a 3-day-old culture of blastopores (Jenkins et al., 1998). The rice containing fungal spores was then allowed to dry for 5 days at 26 ± 2°C. Conidia were harvested by sifting the substrate through a 295 μm mesh sieve and stored at 4°C in total darkness until used. Conidial viability was determined by suspending the inoculum in 10 mL of sterile 0.01% Triton in a 30 mL universal bottle containing glass beads measuring 3 mm in diameter. The conidial suspension was vortexed for 3 min at 700 rpm to attain homogeneity, from which a final concentration of 3×10^6 conidia/mL was prepared using an improved Neubauer haemocytometer (Bulldog Bio) under a light microscope (LEICA DM 2000, Leica Microsystems) at 40 × magnification. A volume of 0.1 mL of conidial suspension was spread onto sterilized SDA in 90 mm Petri dishes. The plates were incubated at 26 ± 2°C for 16–18 h, followed by fixing with lactophenol cotton blue (Millipore Corporation) to terminate fungal growth. Sterile slide cover slips (2 cm × 2 cm) were placed on the top of each Petri dish and viability was determined by counting a total number of 100 conidia per coverslip using a compound microscope (LEICA DM 500). Conidia were considered to have germinated when the length of the germ tube was at least twice the diameter of the conidium, according to Inglis et al. (2012).

2.3 | Site, *Cucurbita pepo* and field cages

Twelve field cages measuring 3 m × 10 m × 1.5 m were constructed using a white netting material of mesh size 0.7 mm × 0.9 mm and mounted along a north–south orientation at 2 m apart at the Kenya Agricultural and Livestock Research Organization (KALRO), Thika, Kenya (Figure 1). Thika is characterized by an average annual temperature of 19.0°C and a total annual precipitation of 1014 mm, with the highest rainfall during March and November. Two experiments were conducted from February to April 2020 (season 1) and September to November 2020 (season 2). Thirty *C. pepo* seeds (Hybrid Squash, Starke Ayres) were directly planted in each of the cages at the

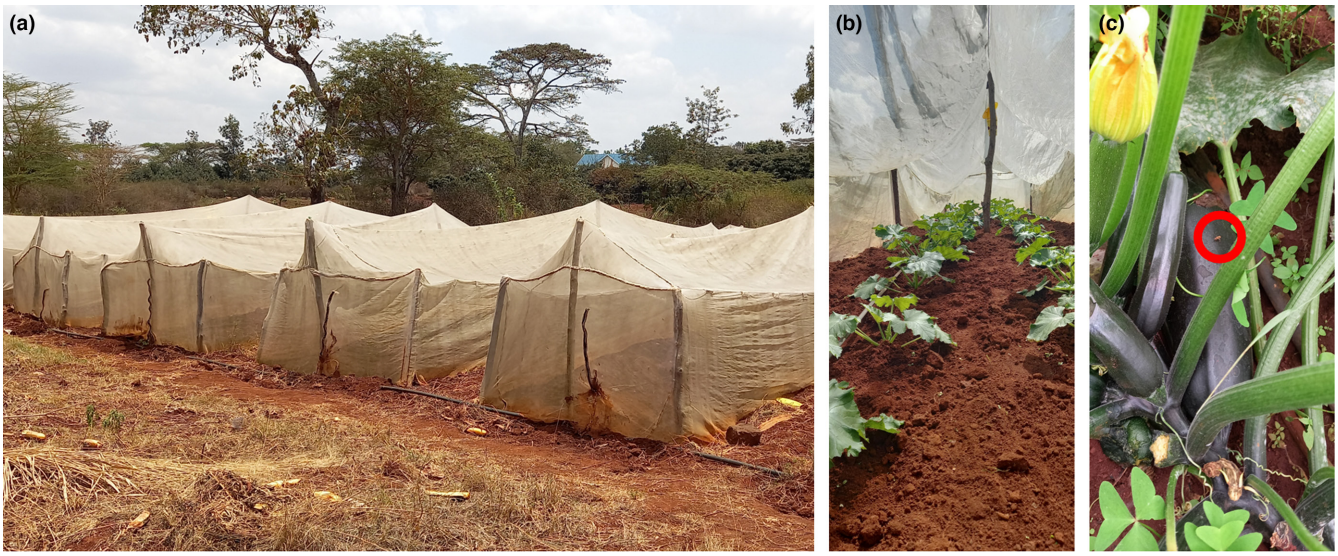

FIGURE 1 Experimental setup of cages for testing the effect of *Metarhizium anisopliae* ICIPE 69 on the melon fly *Zeugodacus cucurbitae* on courgette (*Cucurbita pepo*). (a) Exterior view of the cages, (b) interior view of the cages with growing courgette plants, (c) adult *Z. cucurbitae* on courgette plant.

recommended spacing of 60 cm × 60 cm. Plants were watered thrice a week and plots were weeded every 2–3 weeks. Farmyard manure was applied 1 week before planting, followed by 10 g/plant of diammonium phosphate (Yaramila Complex Fertilizer) during planting and 10 g/plant of calcium ammonium nitrate (YaraBela CAN) as a top dressing 3 weeks after planting. One week after flowering, hand pollination was carried out by gently rubbing the male flowers on the female flowers. After 5 weeks, when the plants had started to flower, 300 adult flies (1: 2 male: female) were introduced in each cage. Treatments included: (1) dry conidia of *M. anisopliae* ICIPE 69 applied in an autodissemination device (fungus); (2) dry conidia of *M. anisopliae* ICIPE 69 in an autodissemination device combined with cuelure (fungus + cuelure); (3) a commercial insecticide (400 g/L profenofos + 40 g/L cypermethrin) (Polytin C, Sygenta) (insecticide); and (4) an untreated control (control). The autodissemination device consisted of a 500 mL yellow polyethylene terephthalate (PET) container measuring 14.0 cm × 8.5 cm, with two diametrically opposed holes of 3 cm diameter in the upper part of the container which allowed pests inside. For the fungus + cuelure treatment, the inside of the autodissemination device was covered with brown velvet material, with 3 g of dry *M. ansiopliae* ICIPE 69 conidia applied to the external surface of this cloth using a spatula. Cuelure (20 g, Cue-Lure Plug, Farmtrack) was unwrapped on-site and placed at the bottom of the autodissemination device. One autodissemination device was placed at the center of each cage. For the insecticide treatment, 30 mL of the product was mixed with 15 L water and sprayed as cover sprays. During each season, the treatments were organized in randomized complete block design, with three replicates for each treatment.

2.4 | Conidial acquisition and mortality assessment

Data on conidial acquisition by flies in the fungus and fungus + cuelure treatments were collected by randomly aspirating five flies from the autodissemination device in each cage 1 day after fly release. Flies were individually transferred into 10 mL of water containing 1 mL 0.05% Triton X-100 (Merck) in 30 mL universal bottles containing glass beads measuring 3 mm in diameter. The bottles were vortexed for 5 min at 700 rpm to dislodge conidia from the insect body, after which 1 mL of the solution was examined in an improved Neubauer haemocytometer covered with sterile cover slips. Simultaneously, 20 flies from each cage were randomly aspirated across all treatments, placed in Plexiglas cages and maintained at room conditions for 10 days as described above. Mortality was monitored daily and dead flies were removed, surface-sterilized with 3% sodium hypochlorite and 70% alcohol and rinsed thrice with sterile distilled water. To assess mycosis, cadavers were placed on sterile wet filter paper in 90 mm diameter sterile plastic Petri dishes sealed with parafilm. Petri dishes were kept for 4 days in an incubator (12 h: 12 h light: dark, 27 ± 2°C and 45% RH) to assess for development of mycosis on the cadavers. Mortality due to *M. anisopliae* ICIPE 69 was confirmed by the presence of green mycelium on the surface of the cadavers after 2–5 days, which was compared with mother cultures. If in doubt, slides were prepared from mycelial outgrowth and conidia to confirm fungal identity.

2.5 | Conidial germination over time

Three samples of conidia were collected daily from each autodissemination device for seven consecutive days using a moist cotton bud. The end of the cotton bud containing conidia was cut, suspended in 10 mL 0.05% Triton X-100 and vortexed for 3 min at 700 rpm. The conidial suspension was quantified and adjusted to 3×10^6 conidia/mL using an improved Neubauer haemocytometer as described above. A sample of 100 µL was spread-plated on four SDA plates and incubated for 16–18 h at 25 ± 2°C, after which each plate was covered with four cover slips. Germination of conidia was determined by counting 100 germinated spores under each cover slip, according to Inglis et al. (2012).

2.6 | Yield data collection

Two weeks after flowering, the first fruits attained physiological maturity and were harvested. Harvesting continued thereafter weekly for 3 weeks. Only marketable fruits (fruit surface without any sign of infestation) were harvested and considered for analysis. Fruit weight was measured on a weighing balance and extrapolated to kg/ha.

2.7 | Data analysis

Control mortality equaled 8.33% and 15.00% during seasons 1 and 2, respectively. Daily percentage mortality was corrected by adjusting the treatment mortality with control mortality using Abbott's correction (Abbott, 1925). All data were pooled across seasons and analysed separately in case of significant differences. Conidial acquisition and mortality were $\log_{10}$-transformed prior to analysis of variance (ANOVA). Conidial germination was subjected to logistic regression. Yield was analysed using a generalized linear mixed effect model implemented in the *lme4* package (Bates et al., 2014), with week as random variable and season and treatment as fixed variables. Means for factors showing significant effects were separated using the *lsmeans* package (Lenth, 2015) with the *p*-value adjusted using Tukey's honestly significant difference. Data analyses were performed using R software (R Core Team, 2020).

3 | RESULTS

3.1 | Conidial acquisition and mortality

There was no significant difference in conidial acquisition between seasons ($\chi^2 = 6.67$; df = 1; $p = 0.29$). There was, however, a significant difference among treatments ($\chi^2 = 555.10$; df = 3; $p < 0.0001$), with

flies from the fungus + cuelure treatment acquiring more conidia (18.02 ± 0.48 conidia/fly) than those from the fungus treatment (11.93 ± 0.40 conidia/fly). There was no significant difference in mortality between seasons ($\chi^2 = 2.31$; df = 1; $p = 0.13$) but there was significant difference in mortality among treatments ($\chi^2 = 501.09$; df = 2; $p < 0.0001$; Table 1). Flies from the fungus + cuelure treatment experienced the highest mortality, while those from the insecticide treatment experienced the lowest mortality.

3.2 | Conidial germination over time

Conidial germination differed significantly between seasons ($\chi^2 = 15.86$; df = 1; $p < 0.0001$), among days ($\chi^2 = 1935.94$; df = 6; $p < 0.0001$) and among treatments ($\chi^2 = 183.65$; df = 1; $p < 0.0001$; Table 2). There was also an interaction between treatment and day ($\chi^2 = 64.23$; df = 6; $p < 0.0001$). Across both seasons, conidial germination reduced over time (from day 1 to day 7). Across both seasons, whereas conidial germination was not significantly different among treatments on day 1, it was significantly lower on day 7 in the fungus + cuelure treatment than in the fungus treatment.

3.3 | Yield

Cucurbita pepo yield did not differ significantly between seasons ($\chi^2 = 1.74$; df = 1; $p = 0.19$), but there was a significant difference among treatments ($\chi^2 = 139.83$; df = 3; $p < 0.0001$; Table 1). Across seasons, yield was highest in the fungus + cuelure and insecticide treatments and lowest in the control treatment.

4 | DISCUSSION

Our findings showed that *Z. cucurbitae* acquired high conidial doses of *M. anisopliae* ICIPE 69 from the autodissemination device, either with or without the cuelure, but acquisition was significantly higher in the autodissemination devices with cuelure. Cuelure is the main attractant for *Z. cucurbitae* and likely increased visits by flies to the autodissemination devices, resulting in higher conidial acquisition (Inskeep et al., 2018). However, the attractiveness of cuelure is less pronounced over longer distances (Shelly et al., 2010) and the effectiveness of autodissemination devices laced with cuelure to attract *Z. cucurbitae* and infest them with *M. anisopliae* ICIPE 69 conidia may need to be tested in open fields. Nevertheless, flies also visited autodissemination devices without cuelure present, as illustrated by their conidial acquisition. Presumably, the bright yellow colour of the autodissemination devices may have attracted male and female *Z. cucurbitae* adults without the presence of cuelure. Yellow-coloured traps have indeed been found to catch more olive fruit fly *Bactrocera oleae* (Rossi) males than traps made from other colours (Katsoyannos & Kouloussis, 2001). This indicates that the flies are also attracted to the traps using visual cues in addition to odour cues, increase the attractiveness to the traps.

Likely because of higher conidial doses acquired in the autodissemination devices with cuelure than those without, we found that *Z. cucurbitae* adults from the fungus + cuelure treatment experienced the highest mortality, followed by those from the fungus treatment. Using a similar study testing the effect of *M. anisopliae* and *B. bassiana* in autodissemination devices in field cages in Kenya, Migiro et al. (2010) also reported a positive correlation between conidial density acquired by the pea leafminer *Liriomyza huidobrensis* (Blanchard) and its mortality. The use of autodissemination devices baited with sex pheromones has previously been demonstrated to be effective in the field by Vargas et al. (2008) in Hawaii, USA on various species of fruit flies, with *Z. cucurbitae* population densities reduced to near zero. The performance of *M. anisopliae* ICIPE 69 against *C. capitata* was also demonstrated in the field in Spain by Navarro-Llopis et al. (2015), who reported mortality of up to 60% after flies had been exposed to an autodissemination device containing an attractant and the fungus. Mkiga et al. (2020) found that, under field conditions in Kenya, *M. anisopliae* ICIPE 69 in combination with pheromone traps, was most effective in controlling the false codling moth *Thaumatotibia leucotreta* (Meyrick).

Presumably, *M. anisopliae* ICIPE 69 conidia acquired by flies in the autodissemination devices allowed for horizontal transmission and increased the efficacy of *M. anisopliae* ICIPE 69. Previously, in laboratory studies, Onsongo et al. (2022) demonstrated successful horizontal transmission of *M. anisopliae* ICIPE 69 among male and female *Z. cucurbitae*, resulting in 59%–67% mortality. Indeed, horizontal transmission of a similar entomopathogenic fungus, *M. anisopliae* ICIPE 62, was equally demonstrated by Dimbi et al. (2013) in a laboratory experiment, with uninfected adults of *C. capitata*, the mango fruit fly *Ceratitis cosyra* (Walker) and the Natal fruit fly *Ceratitis fasciventris* (Bezzi) acquiring conidia from infected adults, resulting in high mortality of 83%–100%, 72%–85% and 71%–93%, respectively. Furthermore, *M. anisopliae* ICIPE 69 negatively affected *Z. cucurbitae* oviposition in laboratory studies (Onsongo et al., 2022) and it would be interesting to assess this suppression effect of the entomopathogen during consecutive crop cycles with multiple *Z. cucurbitae* generations.

TABLE 1 Effect of different treatments testing the entomopathogen *Metarhizium anisopliae* ICIPE 69 and the pheromone cuelure on percentage mortality of the melon fly *Zeugodacus cucurbitae* and courgette *Cucurbita pepo* yield.

Treatment	Mortality (%)	Yield (kg/ha)
Fungus + cuelure	95.31 ± 1.69a	6961 ± 550a
Fungus	72.78 ± 4.19b	4411 ± 200b
Insecticide	38.70 ± 4.32c	7267 ± 352a
Control	–	2089 ± 155c

Note: Data is pooled across two seasons. Mortality was assessed on a total of 480 flies (60 flies/treatment/season). Means with the same letter within a column are not significantly different according to Tukey test at $\alpha = 0.05$.

	Season 1		Season 2	
Day	Fungus + cuelure	Fungus	Fungus + cuelure	Fungus
1	86.45 ± 1.77aA	87.72 ± 1.71aA	90.93 ± 1.36aA	92.92 ± 1.45aA
2	75.21 ± 2.92bA	76.93 ± 1.56cA	79.52 ± 2.36bA	86.24 ± 2.32bB
3	56.86 ± 4.83cA	65.72 ± 3.81cB	61.88 ± 5cA	71.86 ± 5.26cB
4	50.6 ± 1.51cA	62.24 ± 3.25cB	51.92 ± 1.89cdA	59.56 ± 2.91 dB
5	37.52 ± 1.82dA	56.48 ± 2.79cdB	42.95 ± 3.29dA	56.46 ± 2.83 dB
6	25.27 ± 2.46eA	49.24 ± 2.95deB	31.51 ± 1.92eA	50.7 ± 0.88deB
7	16.39 ± 2.11fA	42.76 ± 1.74fB	20.16 ± 1.08fA	44.84 ± 2.26eB

Note: Means with the same lowercase letter within a column are not significantly different while those with the same uppercase letter within a row are not significantly different according to Tukey test at $\alpha = 0.05$.

TABLE 2 Percentage conidial germination of *Metarhizium anisopliae* ICIPE 69 in autodissemination devices with and without the pheromone cuelure over time.

Under field conditions, biotic factors such as sunlight, humidity and temperature in the field are responsible for reduced conidial germination of *M. anisopliae* (Jaronski, 2010) and also in our study viability of *M. anisopliae* ICIPE 69 reduced significantly over time. A decrease in conidial viability of *M. anisopliae* ICIPE 69 in traps over 8 days has also reported by Mfuti et al. (2016) when testing the same entomopathogen against the bean flower thrips *Megalurothrips sjostedti* (Trybom). Differences in conidial viability reduction between the two seasons may be explained by variability in weather conditions during the two seasons. Although total monthly rainfall is similar, mean monthly temperature is slightly higher in February–April (season 1) compared to September–November (season 2). *Metarhizium anisopliae* ICIPE 69 germinates best at temperatures of >25°C (Onsongo et al., 2019), which may have increased persistence of its viability during season 1. However, across seasons, viability of *M. anisopliae* ICIPE 69 reduced more sharply in autodissemination devices with cuelure than those without, highlighting some negative effects of cuelure on *M. anisopliae* viability under field conditions. Incompatibility of cuelure with *M. anisopliae* ICIPE 69 in the laboratory has previously been reported (Onsongo et al., 2022). Nevertheless, the positive effects of cuelure on increasing conidial loads and mortality of *Z. cucurbitae* outweighed its negative effects on *M. anisopliae* ICIPE 69 viability. Further studies are, however, warranted for spatial separation of the pheromone with conidia to improve their combined use and efficacy in the field and consequently reduce the frequency of *M. anisopliae* ICIPE 69 replacement in the traps.

Ultimately, yield was highest in cages treated with *M. anisopliae* ICIPE 69 and cuelure, further confirming that the positive effects of cuelure outweighed its negative effects. Yield in cages treated with *M. anisopliae* ICIPE 69 was lower than that in cages treated with *M. anisopliae* ICIPE 69 + cuelure, but still higher than yield in untreated cages. Importantly, yield in cages treated with *M. anisopliae* ICIPE 69 + cuelure was not different from that in cages treated with insecticides, illustrating that the use of autodissemination devices laced with cuelure and the entomopathogen constitutes a viable IPM option to sustainably control *Z. cucurbitae*. We, however, recommend further studies testing the use of *M. anisopliae* ICIPE 69 with cuelure in open field trials and over several cropping cycles. In addition, further modifications may be required to the autodissemination device to enhance the compatibility between *M. anisopliae* ICIPE 69 and cuelure.

AUTHOR CONTRIBUTIONS

Thomas Dubois: Conceptualization; methodology; validation; formal analysis; data curation; writing – original draft; project administration; funding acquisition. **Susan K. Onsongo:** Conceptualization; methodology; formal analysis; writing – original draft; data curation. **Joseph A. Odhiambo:** Methodology; data curation. **Evanson R. Omuse:** Validation; formal analysis; writing – original draft; data curation. **Komivi S. Akutse:** Writing – original draft; project administration; funding acquisition. **Samira A. Mohamed:** Writing – original draft; project administration; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.8104163.

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