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Laboratory and Field Performance of *Metarhizium anisopliae* Isolate ICIPE 41 for Sustainable Control of the Invasive Fall Armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract: To overcome the negative impacts of invasive fall armyworm (FAW), this study assessed the laboratory and field efficacy of aqueous and oil-based formulations of *Metarhizium anisopliae* ICIPE 41 as an alternative source for FAW management. Three oil formulations, including canola, corn and olive oils and an aqueous formulation of ICIPE 41, were assessed against the second-instar larvae of FAW in the laboratory. Field experiments were also conducted at Mbita and Migori, Kenya, using the formulation that performed best in the lab, with four treatments: (a) A corn oil formulation of ICIPE 41; (b) Mazao Achieve® biopesticide, with the *M. anisopliae* ICIPE 78 strain as the active ingredient; (c) spinetoram-based synthetic pesticide; (d) a control (water + corn oil). FAW incidence, infestation, larval mortality and the effects on parasitoids and yield were evaluated. The laboratory results showed no significant differences in the efficacies among the oil-based formulations, with mortality rates of >70% and an LT_{50} of ~2 days compared to 15.15% in the controls with an LT_{50} of 8.11days. Under the field conditions, no significant differences in the FAW infestation were observed between ICIPE 41 and Mazao Achieve®, compared to the control treatments in both agro-ecological zones. From the field-collected samples, both fugal-based biopesticides exhibited high mortality and mycosis rates, with no negative impacts on *Cotesia icipe* compared to the spinetoram pesticide. There were significant differences in the total grain yields among the treatments in Mbita and Migori. Therefore, ICIPE 41 formulated with corn oil could be used for sustainable FAW management in maize cropping systems.

Keywords: entomopathogenic fungus; *Metarhizium anisopliae*; biopesticide; formulations; larvae; *Cotesia icipe*; fall armyworm

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1. Introduction

The majority of people in Sub-Saharan Africa, particularly East Africa, rely on maize as their major staple food crop, as it is cultivated on over 25 million hectares of land [1]. In Kenya, there has been an unstable trend in the country's maize production over the last two decades, which threatens household food security and income sources [2].

Sustainable maize production in Kenya is highly affected by various abiotic and biotic constraints that cause more than 50% losses [3]. One of the major biotic constraints is the insect pests that cause diverse damage at all the developmental stages of the crops [4]. Recently, the invasion of fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), has posed high risks towards sustainable maize production and productivity and consequently threatens the food security basket in many countries, including Kenya [5]. Studies have shown that a yield loss of between 32–47% per year is caused by FAW due to the lack of proper management strategies [6]. Maize growers have continuously depended on synthetic chemical pesticides to control this invasive polyphagous pest across the invaded areas [7]. However, the use of these pesticides has proven to be unsuitable due to their adverse toxic effects on the environment, human health, non-target species, and the accumulation of their residues along the food chain and possible resistance development [8].

It is, therefore, crucial to encourage the development of new sustainable management approaches for fall armyworm management as maize production is vital for the country's social and economic development [9]. In the last decades, several studies have mostly focused on the implementation of integrated pest management (IPM) strategies, which led to increased/improved maize production and its safe consumption [3,7,10]. The development and implementation of environmentally safe strategies, such as biological control—using parasitoids and formulated entomopathogenic fungi (EPF)—in combination with other IPM tools to counter FAW, has recently been identified as an alternative method that will positively impact maize productivity; since formulated fungal spores can withstand adverse environmental conditions, increase the adherence time to the surface of maize leaves as well as increase fungal infectivity [11–13]. For instance, Shylesha and colleagues reported the performance of egg, larval, and larval-pupal parasitoids and predators attacking different stages of FAW in maize cropping systems [14]. Further, a biocontrol-based IPM approach was formulated by combining EPF and FAW pheromone into traps (pheromone traps) for the sustainable management of the pest [1,15]. Furthermore, oil-based formulations have also received great attention due to their insecticidal, repellent or antifeedant activities. A study in Ghana reported that neem seed oil-based biopesticide products were as efficient as the synthetic ones containing emamectin benzoate in the FAW control [16,17]. In addition, biopesticides may offer an essential alternative to the indiscriminate use of synthetic pesticides as they are generally less toxic than conventional pesticides and, therefore, are considered safer for human health. Biopesticides often only affect the target pest and, therefore, do not negatively affect the environment and other non-target organisms, such as parasitoids and predators; furthermore, their use does not lead to a resistance build-up in target pests [18].

In the recent past, the International Centre of Insect Physiology and Ecology (*icipe*), in partnership with private sector partners, has developed and commercialized numerous entomopathogenic fungal-based biopesticides for the management of key crops and livestock pests in Africa [1,18,19]. Recently, the efficiency of the entomopathogenic fungus, *Metarhizium anisopliae* ICIPE 41 sensu lato (s.l.), against FAW eggs and neonate larvae demonstrated a 97.5% cumulated mortality of these immature stages under laboratory conditions [11]. We also demonstrated the effects of some formulations on FAW's associated parasitoids under laboratory conditions to establish the interactions between the EPF and parasitoids for the effective management of FAW (our unpublished data). Consequently, this study further assessed the laboratory and field efficacies of the aqueous and oil-based formulations of this potent fungus in the management of FAW and its effects on FAW's associated parasitoids, and compared its performance against a commonly used spinetoram-based synthetic pesticide and commercially available biopesticide, known as Achieve, with the *M. anisopliae* ICIPE 78 strain as the active ingredient.

2. Materials and Methods

2.1. Study Site

The laboratory experiments were conducted at the Arthropod Pathology Unit, *icipe*, Duduvile Campus in Nairobi, Kenya (1.221 S, 36.896 E; 1616 m a.s.l.). The field experiments were carried out between January and April 2021 in Kenya, specifically at Mbita (00.4305 S, 034.2057 E, 1150 m a.s.l.), Homabay county, and at Awendo (00.86 S, 34.57 E, 1411 m), Migori county. The mean annual rainfalls in Mbita and Migori were 120.4 and 118 mm, respectively, and their minimum and maximum temperatures were 23, 29 °C and 20, 26 °C, respectively, in the same order.

2.2. Insects

A colony of *Spodoptera frugiperda* was established using larvae collected on maize from the Siaya and Homabay counties (0.61401 S, 34.09095 E; 1215 m a.s.l.), Kenya. The field-collected larvae were reared on a semi-synthetic diet until pupation [20]. The pupae were held in sleeved Perspex cages (40 × 40 × 45 cm) until adult emergence. Moths were fed with a 10% honey solution soaked in balls of cotton wool. Potted maize plants were placed in the cages for oviposition. After oviposition, the plants were removed after 24 h and transferred to separate, clean, ventilated cages (50 × 50 × 60 cm) to facilitate the egg hatch. Leaves with larvae were removed from these plants 3 days after the eggs hatched and were placed into clean cages (50 × 50 × 60 cm) lined with paper towels (to absorb the excess moisture) and a fine netting-infused lid for ventilation [11]. The larvae were supplied daily with fresh maize leaves as a food source until they pupated. The pupae were collected from the cages using a fine camel hairbrush and placed inside sleeved Perspex cages (40 × 40 × 45 cm) for adult eclosion. The newly eclosed adults were released into sleeved cages (40 × 40 × 45 cm). In these cages, butter papers were used as an oviposition substrate instead of maize plants, and the papers were replaced every 24 h to ensure the eggs were of homogenous age for the bioassays [11]. The colony was maintained in a rearing room at 26 ± 2 °C, 50–70% RH and a 12:12 L:D photoperiod at the Animal Rearing and Quarantine Unit (ARQU) of *icipe*.

2.3. Fungal Isolate Culture, Viability and Suspension Preparation

For the laboratory experiments, *M. anisopliae* ICIPE 41 (s.l), obtained from *icipe*'s repository collections in the Arthropod Germplasm Centre, was cultured on Sabouraud dextrose agar (SDA) and maintained at 26 ± 2 °C in complete darkness. *Metarhizium anisopliae* ICIPE 41 fungal conidia were harvested from a 2- to 3-week-old sporulated culture and suspended in 10 mL of distilled water with 0.05% Triton X-100 in universal bottles containing glass beads (5–10 beads of 3 mm in diameter per bottle). The suspension was vortexed for 5 min at about 700 rpm to break the conidial clumps and ensure a homogeneous suspension. The conidial concentration was quantified using a haemocytometer under a light microscope. Prior to the bioassays, the conidial suspensions were adjusted to a concentration of 1×10^8 conidia/mL through dilution [11,21]. Prior to the experiments, viability tests were conducted, where a concentration of 3×10^6 conidia/mL was prepared, 0.1 mL of the suspension was evenly spread on SDA media, and three sterile microscope coverslips were placed randomly on the surface of each inoculated plate. Each plate represented a replicate, and four replicates were done for the fungal isolate, *M. anisopliae* ICIPE 41. The plates were sealed with Parafilm and incubated under complete darkness at 26 ± 2 °C. Conidia germination was assessed after 18 h by counting 100 randomly selected conidia beneath each coverslip under a light microscope. Conidia were considered germinated when the length of the germ tube was at least twice the diameter of the conidium [22].

2.4. Formulation of the Fungal Isolate Metarhizium anisopliae ICIPE 41

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 formulations. Dry conidia (0.3 g) of the fungal isolate 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 of the fungal suspension [23]. Water suspension, containing 0.05% Triton X-100 water and 1×10^8 conidia/mL of the fungus, was used as the aqueous-formulated conidia.

2.5. Pathogenicity of the Formulations of Metarhizium anisopliae ICIPE 41 against Second Instar of FAW in the Laboratory

All four fungal formulations were screened against the second instar of *S. frugiperda* obtained from the colony, as described above. Fresh maize leaves of similar size and age, approximately 2–3 weeks old, were cleaned, cut, and placed individually in Petri dishes

(90 mm in diameter). First, a group of twenty second-instars representing a replicate were sprayed with each of the formulations (10 mL of 1×10^8 conidia/mL) using a Potter Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth, UK). The treated larvae were then transferred into the Petri dish containing the cut leaf using a camel hairbrush. This was replicated four times per treatment in a completely randomized design. The insects were supplied with fresh maize leaves daily as food. The larval mortality was recorded daily for seven days at 25 ± 2 °C, 50–70% RH and a 12:12 L:D photoperiod [19,24]. Mycosis was assessed to confirm mortality due to infection by the fungus treated, where the dead cadavers were surface-sterilized with 70% alcohol for 1-2 min and then rinsed thrice in distilled water. The surface-sterilized cadavers were kept separately in Petri dishes lined with sterile moistened filter paper to record the fungal outgrowth and verify if their mortality could be attributed to the respective fungal formulations they were treated with. Mortality due to fungal infection was confirmed by the presence of hyphae and conidia on the surface of the cadaver. The same process was repeated for all the formulations/treatments, but the control groups were treated with sterile distilled water containing 0.05% Triton X-100.

2.6. Field Experiments

2.6.1. Mazao Achieve® Biopesticide

Mazao Achieve® is a contact fungal-based biopesticide with the entomopathogenic fungus, *M. anisopliae* ICIPE 78 (s.l.), as the active ingredient. This bioproduct was initially registered and commercialized by RealIPM-Biobest against red spider mites. Recently, the label of this biopesticide was extended for FAW management in Kenya. The biopesticide is very effective against egg and neonate larvae of fall armyworm and prevents the build-up of the pest population [11]. In addition, our other laboratory bioassays on the effects of these formulations on FAW-associated parasitoids showed insignificant effects on the parasitoids, where a canola oil formulation induced a high mortality rate (52%) compared to the corn oil formulation (12.5%) and hence, the corn oil formulation was selected for the field experiment (our unpublished data). We, therefore, assessed its effects on the major FAW-associated parasitoids during the field experiments, as described below in Section 2.7.3.

2.6.2. Field Site

The land used for the efficacy trials at each site (Migori and Mbita), measuring 45×45 m, was first ploughed and harrowed in preparation for planting. Experimental plots of 7×7 m were made with a plot interspacing of 2 m. Maize seeds (variety, Duma 43), known for their high susceptibility to FAW and used in our field experiments, were obtained from Seed Company Limited, Kenya. Two maize seeds per hole were sown in the 7×7 m experimental unit at a spacing of 35 cm intra-row by 70 cm inter-row. Two weeks after germination, weeding and thinning were completed to keep only one plant per hole, giving a total plant density of 231 plants in each experimental unit. Surface irrigation was used to support the crop, as it was relatively dry during the season; however, irrigation was discontinued after the onset of the rains. Weeding was done thrice a month until the maize crop was fully established smothering the weeds; and thereafter, the frequency of weeding was reduced to twice per month. All the necessary and recommended agronomic practices, such as di-ammonium phosphate (DAP) and calcium ammonium nitrate (CAN) fertilizers, were applied. DAP was applied twice at three-week intervals at a rate of 10 g per maize at the vegetative stage, while CAN was applied once at the same rate at the tasseling and silking stage, and the crops were then left for natural FAW infestation. The infestation level was monitored weekly by randomly sampling ten plants from each experimental unit and inspecting for the incidence of FAW prior to the treatment applications. A single well-designed delta trap with FAW pheromone (obtained from Kenya Biologics Limited, Makuyu, Kenya) was placed in the field path adjacent to every plot to monitor the FAW population dynamics during the pre- and post-treatment applications.

2.6.3. Treatments and Experimental Layout

The experiment was conducted in two agro-ecological zones (Mbita and Migori) as described above, and for each zone, the experiment consisted of four different treatments: (i) a corn oil formulation of *M. anisopliae* ICIPE 41; (ii) Mazao Achieve® (with *M. anisopliae* ICIPE 78 as the active ingredient and considered as standard); (iii) spinetoram synthetic pesticide, registered in Kenya for FAW control; (iv) the control (water + corn oil). All the field treatments were arranged in a randomized complete block design. One gram of dry conidia of *M. anisopliae* ICIPE 41 was formulated with the corn oil by emulsifying 2% (v/v) of the oil in 0.05% Triton X-100 water containing 1×10^8 conidia/mL of the fungal suspension and 0.1% of Integra sticker (Greenlife Crop Protection Africa Ltd.) was added to the formulation. The formulation was prepared during the treatment application. The pesticide was applied at the rate of 10 mL in 20 L of water, while the Mazao Achieve® biopesticide was applied at the rate of 40 mL in 20 L of water. The control was treated with water mixed with corn oil. The treatments were applied one week after germination and at the third leaf collar, with a two-weeks interval afterwards between each application. Prior to treatment application, the FAW infestation rates and adult abundance were assessed as described below. In addition, the FAW damage associated with larval incidence/infestation, the effect of the treatment on parasitoid abundance and the treatment's effect on the grain yield were also evaluated, as described in the below sections.

2.7. Assessment of FAW Adults before Treatment Application

Before the application of the treatments, the FAW populations at the experimental sites were monitored from one week after germination. One delta trap per experimental plot, which was fitted with sticky paper and charged with Falltrack® lure, was used for monitoring. The sticky papers were removed from the trap every week and replaced with new ones. The number of adult FAW on each sticky paper was counted and recorded. After the two weeks of monitoring, the number of moth/trap/week was computed. The delta trap remained there for FAW-population monitoring for two months.

2.7.1. Assessment of FAW Damage and Larval Mortality Induced by Corn Oil Formulation of *Metarhizium anisopliae* ICIPE 41

Maize damage (leaf, tassel, ear and corn damage) was evaluated weekly by assessing 10 randomly selected plants in each experimental plot using a scale, as described previously [20]. The scale ranged from 0 to 100%, where 0–10% (no visual damage on maize plant), 10–20% (visual damage on leaves), 20–30% (visual damage on maize leaves and tassel), 30–50% (visual damage on maize leaves, tassel and internode), 50–60% (visual damage on leaves, tassels and ears), 60–70% (visual damage on leaves, tassels, ears, internode and corns) and 70–100% (extreme visual damage on tassels, leaves and maize corns). The larval mortality, due to the fungus, was also assessed weekly 24 h after the treatment application by actively picking 20 larvae from each experimental plot and placing them in plastic dishes (11.3 cm $\times$ 4 cm (depth)) lined with moist filter paper containing sterilized maize leaves, which served as a food source. The lids of the plastic dishes were covered by Parafilm with apertures (300 $\times$ 300 µm) to allow for the free circulation of air. The dishes were kept at room temperature, and the mortality was recorded daily for seven days. The leaves in the dishes were changed daily with fresh ones. Dead larvae were collected to assess mycosis, as described above in Section 2.5, and the slides were prepared for confirmation with the applied fungus in comparison with the mother plates.

2.7.2. Conidial Persistence Assessment under Field Conditions

The persistence of *M. anisopliae* ICIPE 41 conidia in the formulated suspensions was assessed over one month in both seasons and at the two locations. This was performed weekly by randomly cutting maize leaves in small sizes (about 5 cm) from 10 randomly selected plants in each experimental unit (as described above in Section 2.7.1) from all replicates sprayed with the fungal formulation. The leaves were then suspended in 10 mL of 0.05% (v/v) Triton X-100 in a universal bottle containing glass beads (5–10) and vortexed for two minutes to dislodge the conidia from the surface of the leaves. A sample of 0.1 mL was aliquoted from the homogenous mixture, spread-plated on SDA media mixed with 0.25 g of streptomycin antibiotic and incubated for 18 h at 26 ± 2 °C and an L12:D12 photoperiod with each replicate having two plates and being replicated four times. The percentage germination was calculated by counting the germinated conidia per hundred randomly selected conidia in a selected field, covered by four coverslips under a microscope at 400× magnification. Conidia were scored as viable if the visible germ tubes were about twice the diameter of the conidium. The same process was repeated for four weeks on days 7, 14, 21 and 28 after treatment.

2.7.3. Effect of Different Treatments against FAW-Associated Parasitoids in Field

Key FAW-associated parasitoid populations were assessed weekly, as described above in Section 2.7.1, and recorded [25,26]. For larval parasitoids (*Cotesia icipe*) and egg parasitoids (*Telenomous remus* and *Trichogramma* spp.), parasitoid performance was assessed by actively collecting larvae and eggs clusters, respectively, from all plots and transferred into perforated Petri dishes, labeled according to the treatment and incubated under laboratory conditions. The number of emerged parasitoid species was identified and recorded daily for 14 days.

2.8. Maize Grain Yield

Maize yield data were collected at maturity at the end of the experiment by cutting down maize, harvesting the cobs in each treatment plot and collecting maize corn—sun-dried and threshed. The grain weight was recorded using an electronic balance (PM 15, Mettler Instruments Ltd., Tokyo, Japan), and the yield was computed. The total maize grain weight for each treatment was calculated by cumulatively adding the weights obtained per treatment and estimating in kilograms per hectare (kg/ha), following the method described by Mweke et al. [8].

2.9. Data Analysis

The FAW incidence and infestation/damage data were checked for normality using the Shapiro–Wilks test before being subjected to repeated-measures ANOVA analysis. The percentage of FAW infestation and damage data were analysed using ANOVA. To evaluate the FAW mortality induced by *M. anisopliae* ICIPE 41, data on mortality were corrected for natural mortality [27], then tested for normality [28], arcsine transformed, then subjected to ANOVA and means were separated using Tukey's HSD. Data on the percentage conidia germination of *M. anisopliae* ICIPE 41 were subjected to a logistic linear mixed model with an intercept slope using the 'glmer' function from the lme4, and the percentage FAW infestations were arcsine transformed and analysed using one-way ANOVA, followed by Tukey's post hoc test for mean separation. The estimated maize yield in kg/ha and the percentage yield increase, as a result of the treatments applied, were also analysed using one-way ANOVA after testing the normality of the by Shapiro-Wilks test. All data analyses were performed using the R (version 3.2.5) statistical software packages [29].

3. Results

3.1. Pathogenicity of the various Metarhizium Anisopliae ICIPE 41 Formulations against Second-Instar FAW in the Laboratory

For all fungal formulations, the viability exceeded 98% after 18-h incubation at 26 ± 2 °C. The second-instar FAWs were found to be susceptible to all the tested fungal formulations. There was a significant difference between the larval mortality in oil-based and aqueous formulations ($F_{3,12} = 50.3$; $p < 0.01$). The canola oil-formulated *M. anisopliae* ICIPE 41 performed better by causing total mortality of 76.07 ± 6.43%, compared to the corn oil and olive oil-formulated *M. anisopliae* ICIPE 41, with 72.5 ± 5.58% and 70.36 ± 6.66%, respectively, at 7 days post-treatment (Table 1). There was no significant difference between the three oil-based formulations. The results further showed that there was a significant difference ($F_{3,12} = 50.2$; $p < 0.01$) in the lethal time of 50% (LT_{50}) between the oil-based formulations and the aqueous formulation (Table 1). The canola oil formulation recorded the shortest LT_{50} of 2.06 days versus 8.11 days in the aqueous formulation, while the corn and olive oil formulations recorded an LT_{50} of 2.26 and 2.52 days, respectively (Table 1). There was no significant difference in the LT_{50} values between the three oil-based formulations (Table 1). Further, the corn, olive and canola oil-based formulations and aqueous formulations recorded mycosis rates of 78.45, 65.85, 58.25 and 25.75%, respectively, of the dead incubated insects.

Table 1. Cumulated larval mortality induced by fungal formulations and their respective lethal time 50% (LT_{50}) 7 days post-treatment.

Fungal Formulation	Larval Cumulated Mortality (%)	Lethal Time 50% (LT_{50}) (Days) ± SE
Canola oil-formulated *M. anisopliae* ICIPE 41	76.0 ± 6.4 b	2.0 (1.9–2.2) a
Corn oil-formulated *M. anisopliae* ICIPE 41	72.5 ± 5.5 b	2.2 (2.0–2.5) a
Olive oil-formulated *M. anisopliae* ICIPE 41	70.4 ± 6.6 b	2.5 (2.0–2.9) a
Aqueous-formulated *M. anisopliae* ICIPE 41	15.2 ± 2.8 a	8.1 (6.5–0.6) a

Note: The values represent mean ± SE. Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls (SNK) test at $p < 0.05$.

3.2. Assessment of the Effect of FAW Sex Pheromone Traps on the Population of FAW Moths

The presence of the FAW sex pheromone had a significant effect on moth attraction between the treatment and control plots (t = 15.1, df = 3.15, $p < 0.001$) two weeks after the treatments. Moths were less attracted to the delta trap containing the sex pheromone in the treatment plots compared to the control plots that recorded the highest number of moths captured (Table 2). However, at the beginning of the experiment and prior to the treatment applications, there was no significant difference between the number of moths captured in the treatments compared to the control plots.

Table 2. Response of FAW moths to the attraction device containing the sex pheromone two weeks post-treatment.

Treatments	Percentage of FAW Moths	
	Migori	Mbita
Spinetoram pesticide	0.00 d	0.00 d
Corn oil formulation of *M. anisopliae* ICIPE 41	79.25 ± 11.85 b	92.76 ± 7.83 b
Mazao Achieve®	32.10 ± 8.49 c	37.50 ± 9.15 c
Control	88.00 ± 12.21 a	95.50 ± 5.45 a

Means within the same column followed by the same letters are not significantly different by Tukey's HSD test at $p < 0.05$.

3.3. Fall Armyworm Larval Incidence and Damage in the Field

There were no significant effects observed between the control and the treatment plots regarding the incidence of FAW prior to the treatment applications and one week after treatment. However, there was a significant difference in FAW damage in Migori ($F_{3,16} = 77.4$; $p < 0.0001$) and Mbita ($F_{3,16} = 55.7$; $p < 0.0001$) between the treatments and control plots one week after the treatment evaluation (Table 3). The highest damage was recorded in the control plots at both Migori and Mbita sites, with 75.1 ± 8.3% and 71.7 ± 7.1%, respectively, while the spinetoram pesticide plots recorded the least damage in Migori and Mbita. Furthermore, significant differences in FAW damage were observed between the ICIPE 41 corn oil-based formulation and the commercial biopesticide, Achieve, in both study sites (χ^2 = 19.32; p< 0.0001) (Table 3).

Table 3. Mean FAW population damage per plant one week after treatment in Migori and Mbita.

Treatments	FAW Damage per Plant (%)	
	Migori	**Mbita**
Spinetoram pesticide	2.2 ± 0.7 a	2.4 ± 0.6 a
Corn oil formulation of *M. anisopliae* ICIPE 41	13.3 ± 2.0 b	12.0 ± 2.8 b
Mazao Achieve®	36.1 ± 5.5 c	35.1 ± 5.0 c
Control	75.7 ± 8.3 d	71.7 ± 7.3 d

Means within the same column followed by the same letters are not significantly different by Tukey's HSD test at $p < 0.05$.

3.4. Cumulated Mortality of Collected Second-Instar FAW in the various Field Treatments after Applications

From the collected and incubated larvae, a significant difference was observed in the larval mortality between the treatments seven days post-treatment in both Migori (F = 21.37, df = 3, $p < 0.0001$) and Mbita (F = 29.83, df = 3, p< 0.0001). The spinetoram pesticide exhibited the highest mortality rates, followed by the ICIPE 41 corn oil-based formulation and Mazao Achieve® biopesticide in both experimental areas (Table 4). In addition, the results showed 70.0% and 66.3% mycosis rates of the cadavers from the Migori and Mbita field sites, respectively. Furthermore, a significant difference in lethal time 50% (LT_{50}) was noted among the treatments in Migori and Mbita (F = 11.12, df = 3, $p < 0.0001$). The spinetoram pesticide recorded the shortest LT_{50} of 3.8 and 3.8 days in both Migori and Mbita, respectively, while the Mazao Achieve® biopesticide and corn oil-formulated *M. anisopliae* ICIPE 41 LT_{50} values ranged between 4.6–5.2 days (Table 4).

Table 4. Cumulated larval mortality induced by different treatments and their lethal time 50% (LT_{50}).

Treatments	% FAW Larvae Mortality	% Mycosis	Lethal Time 50% ± SE
		Migori	
Spinetoram-based pesticide	96.1 ± 2.5 a	0.0 ± 0.0 c	3.8 (3.80–3.90) b
Mazao Achieve®	73.0 ± 1.3 c	30.0 ± 0.7 b	5.1 (5.12–5.26) a
Corn oil formulation of *M. anisopliae* ICIPE 41	81.3 ± 2.6 b	70.0 ± 0.5 a	5.2 (5.22–5.32) a
Control	-	0.0 ± 0.0 c	-
		Mbita	
Spinetoram-based pesticide	98.7 ± 1.3 a	0.0 ± 0.0 c	3.8 (3.80–3.88) b
Mazao Achive®	74.3 ± 2.5 c	25.0 ± 0.4 b	4.7 (4.70–4.82) a
Corn oil formulation of *M. anisopliae* ICIPE 41	83.6 ± 1.5 b	66.3 ± 0.7 a	4.6 (4.56–4.68) a
Control	-	0.0 ± 0.0 c	-

Means within the same column followed by the same letters are not significantly different via Tukey's HSD for each agro-ecological zone test at $p < 0.05$.

3.5. Conidial Persistence Assessment after Application

Conidial persistence in the experimental plots that were treated with corn oil-formulated *M. anisopliae* ICIPE 41 was conducted to assess the field persistence/viability of the spores in the field. The results showed that conidial persistence remained high (>98%) within the first seven days post-treatment in both zones (Figure 1). There was a significant difference between conidial persistence with time in both Migori ($F_{4,20}$ = 3.95, $p < 0.0001$) and Mbita ($F_{4,20}$ = 3.95, $p < 0.0001$). In addition, the findings also showed a significant decline in conidial persistence from the 14th, 21st and 28th days in Migori (87%, 74% and 61%, respectively) and Mbita (88%, 72% and 61%, respectively) (Figure 1).

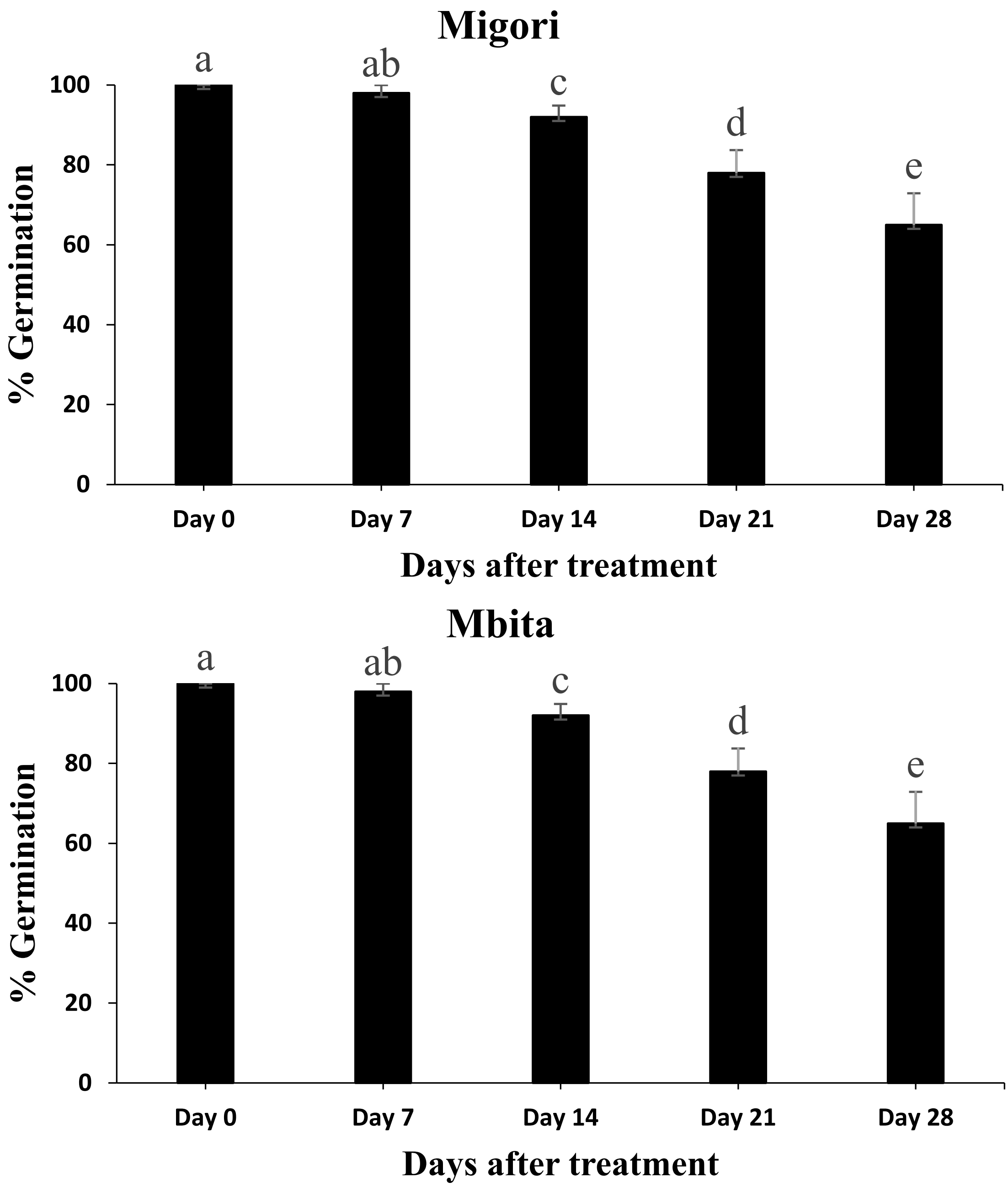

Figure 1. Persistence of corn oil-formulated *Metarhizium anisopliae* ICIPE 41 treatment over time in the field. Bars denote means ± standard errors, and means followed by the same letters are not significantly different by GLM, Tukey's HSD test, at $p < 0.05$.

3.6. Effect of Entomopathogenic Fungi on Parasitoids Emergence

A significant difference was observed in the parasitoid population between all the treatments at Migori ($F = 1.71$; $df = 3$; $p < 0.0001$) and Mbita ($F = 3.13$; $df = 3$; $p < 0.0001$). From the incubated eggs and larvae collected from the various treatments after applications, only the larval parasitoid *Cotesia icipe* was obtained. The highest number of *C. icipe* parasitoids was observed in the control and ICIPE 41 corn oil-based formulation treatments in both study areas (Table 5). Furthermore, no parasitoids were recorded from the incubated FAW eggs/larvae in spinetoram pesticide plots in the two trial sites.

Table 5. Beneficial parasitoids (*Cotesia icipe*) performance recorded from immature stages of FAW in the various treatments in Migori and Mbita.

Treatments	Percentage of *Cotesia icipe* Emerged	
	Migori	**Mbita**
Spinetoram pesticide	0.00	0.00
Corn oil formulation of *M. anisopliae* ICIPE 41	9.25 ± 0.85 b	11.00 ± 1.83 b
Mazao Achive®	2.00 ± 0.49 c	2.50 ± 0.15 c
Control	10.00 ± 0.71 a	14.75 ± 1.25 a
χ^2	1.71	3.13
Df	3	3
p-value	<0.0001	<0.0001

Means within the same column followed by the same letters are not significantly different via Tukey's HSD test at $p < 0.05$ for each experimental site.

3.7. Maize Grain Yield

There was a significant difference in the total maize yield between the two agro-ecological zones (t = 3.30, df = 3, $p = 0.0029$), where the maize grain yields from each experimental plot in the Mbita trials site were relatively higher than those of Migori (Figure 2). In addition, a significant difference in maize grain yields among all the treatments in both the Mbita (F = 4.4, df = 3, $p < 0.001$) and Migori (F = 3.8, df = 3, $p < 0.001$) agro-ecological zones was observed (F = 3.8, df = 3, $p < 0.001$), respectively. The experimental plots treated with the spinetoram pesticide and ICIPE 41 corn oil-based formulation from both the Migori and Mbita sites produced the highest grain yields.

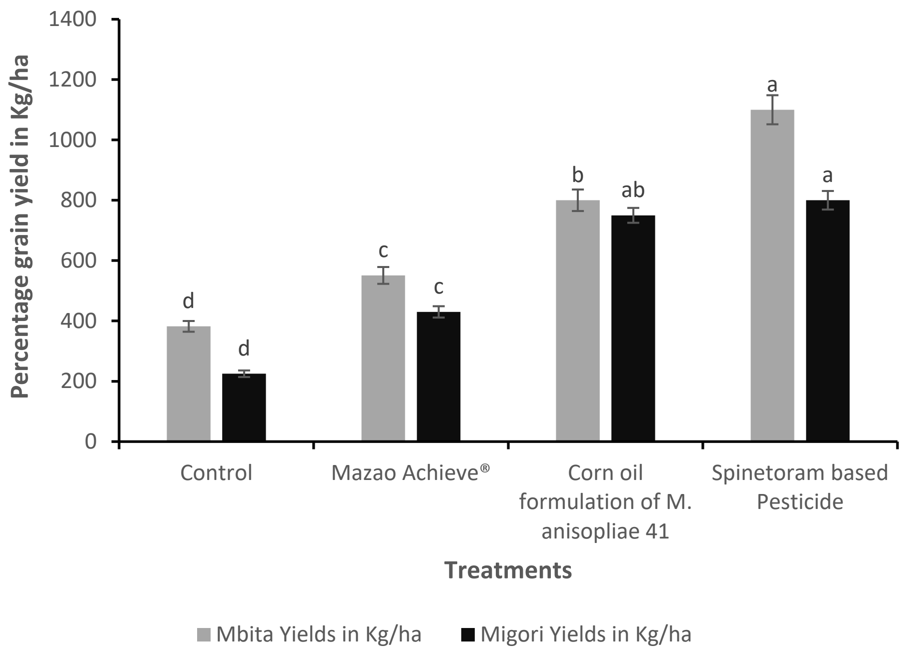

Figure 2. Maize grain yield in Migori and Mbita in Kg/ha. Bars represent means ± SE; means followed by the same letters are not significantly different via Tukey's HSD for each experimental site at $p < 0.05$.

4. Discussion

This study was designed to formulate new environmentally friendly and affordable biopesticides for the management and control of invasive FAW in maize production systems. Overall, the results of this study showed that the corn oil formulation of *M. anisopliae* ICIPE 41 could be a potential starting point towards the development of effective biopesticide against FAW. The preliminary screening of various oil formulations against second instars of FAWs showed a significant difference in the mortality rates between the oil-based and aqueous formulations. All the oil-based fungal formulations were pathogenic to the larvae, with a mortality rate of $\geq$ 70% compared to the aqueous formulation. From a chemistry point of view, these could be explained by the fact that all these vegetable oils are comprised of non-polar saturated and unsaturated fatty acids, among others [30]. The results could further be explained by the fact that the oils could be harmful to the insects, causing suffocation by blockage of the spiracles [31]. Further, the oils being non-polar might have a potentiation effect on the fungal isolate, hence, increasing/enhancing its infectivity rate and performance. Studies by Alavo, 2006 reported that integrating bentonite in oil-based fungal formulations of *Beauveria bassiana* improved its efficacy against *Helicoverpa armigera* (Hübner; Lepidoptera: Noctuidae) [32]. This finding correlated with the efficacy of *M. anisopliae* to induce high mortality rates and a short lethal time (LT_{50}), as was reported in other major arthropod pests [19,33].

In addition, under field conditions, the present study showed a significant difference in FAWs' attraction to the sex pheromone traps in both the Migori and Mbita trial sites. The positive correlation observed with FAWs' attraction could be explained by the frequent visits of the male moths in the device due to the presence of the lure and could, therefore, significantly contribute to monitoring FAW adults' dynamics prior to biopesticide applications.

In the current study, the application of treatments in both fields' efficacy trial sites reduced damage due to FAW compared to the control plots. Additionally, the spinetoram pesticide performed better than the untreated control. The reduced damage rates in the pesticide and fungal-based treated plots could be associated with the toxicity of the treatments to the second-instar FAWs, unlike in the control treatment that offered relatively favorable conditions for the larvae to feed and hence, increased the damages recorded. However, the overall damage level recorded was less at the Mbita site because low and late infestation occurred on the plants, and hence, the plants were less susceptible to FAW damage compared to the Migori site, where high infestation occurred at the early stages when the plants were more susceptible to damage.

Application of the spinetoram-based pesticide resulted in a sharp decline in the FAW larval population density in the respective treatment plots in both experimental sites, as was also observed in many synthetic pesticides, including those that were Lambda-cyhalothrin-based [8], chlorantraniliprole-based [34], cyantraniliprole-based [35] and flubendiamide-based [36]. The decline could be attributed to an unreported non-resistance of FAW against the spinetoram-based synthetic pesticides. Synthetic pesticides, such as spinetoram, are known to be phytotoxic to the pest as well as natural enemies, hence, making them non-target-specific pesticides with a broad spectrum of activity. However, the values obtained in both areas of our study were not significantly different in the presence of biopesticides. This could be attributed to the slow action characteristics of all biopesticides and the fact that the efficacy of EPF-based biopesticides is not solely influenced by the formulation but also the prevailing environmental conditions, including rainfall, temperature and relative humidity [24,37,38]. Our results demonstrated that the spinetoram-based pesticide recorded the highest FAW mortality rates, followed by *M. anisopliae* ICIPE 41 and Mazao Achieve® biopesticide. However, no mortality was recorded in the controls in both experimental zones (Table 4). In the experimental plots treated with pesticide, the percentage of the collected FAW larvae mortality was 96%. This performance was due to its strong/high toxicity to the larvae. Similar results in field trials against FAW mortality and infestation in grain sorghum indicated that spinetoram highly reduced the number of infested and damaged whorls [39]. Despite its good performance, this synthetic pesticide has to be considered with caution for pest management because being toxic, increases environmental pollution and pest resistance development, is non-target specific to natural enemies and costly [40]. Interestingly, the plots treated with ICIPE 41 caused a significantly higher mortality rate than the commercial biopesticide Mazao

Achieve®. To the best of our knowledge, the efficacity of Mazao Achieve® biopesticide against FAW has been reported here for the first time under field conditions, although its efficacity has been documented against red spider mites and other major arthropod pests. Furthermore, the higher performance of *M. anisopliae* ICIPE 41 as compared to Mazao Achieve® could also be due to the differences in the type of oil substrate used in formulating them, as spores' viability could usually reduce with time, depending on the formulation and environmental conditions [41]. Similar results were also reported for the decreased viability of the *M. anisopliae* ICIPE 18, 20, 32, 40, 41, 62 and 69 isolates upon their exposure to environmental conditions [19,38,42]. The current study also showed that 66% and 70% of the dead cadavers sprayed with ICIPE 41 in Mbita and Migori, respectively, had developed mycosis with hyphae and conidia, similar to the applied fungus. Such results were also obtained on dead neonate larvae where the hyphae and conidia of the inoculated fungus were observed on the surface of the incubated cadaver [11]. In addition, the current study reported few insect cadavers that developed hyphae and conidia from the plots treated with Mazao Achieve® biopesticide as compared to the ICIPE 41-based biopesticide from each experimental zone.

Data obtained from both agro-ecological zones showed a significant difference in conidial persistence with time in the plots treated with the formulated fungal-based biopesticide, with conidial germination being> 90% after the first two weeks and followed by a subsequent decline in the third- and fourth-weeks post-application. The oil-based formulations significantly enhanced the tolerance of conidia to UV radiation and other unfavorable environmental conditions with advanced germination of conidia, in comparison with conidia suspended in the water-based formulations [43]. The presence of sticker in the formulation increased the adherence of the spores onto the surface of maize leaves and prolonged their infectivity action to visiting larvae. This finding agrees with a similar research report on *M. acridum* IMI 330189, which showed declined percentage germinations of 67.4%, 39.4%, 45.4% and 28.6% after 4-, 8-, 16- and 24-h exposure, respectively [44]. Similar conidial persistence times of more than two weeks, and viability rates, were also reported for Mazao Achieve®, which has *M. anisopliae* ICIPE 78 as an active ingredient (https://realipm.com/products/, accessed on 5 August 2022). Additionally, the high persistence/viability of the conidia after two weeks showed that the frequency of the application of the corn oil-formulated ICIPE 41 could even go beyond 14 days. The corn oil formulation of the *M. anisopliae* ICIPE 41 strain presents an advantage as a potential lead for biopesticide development and commercialization for the sustainable control of FAWs.

The results also demonstrated that only *Cotesia icipe* parasitoids emerged from the field-collected larvae, even from the plots treated with the *M. anisopliae* ICIPE 41 corn oil-based formulation. No single egg parasitoids emerged from the dead eggs treated with ICIPE 41, spinetoram pesticide and Mazao Achieve®. This finding could be attributed to the possible toxicity nature of the biopesticide against these naturally occurring natural enemies. This finding concurred with similar studies conducted by Mweke et al. [8] on the effect of corn oil formulations on natural enemies of aphids. However, the mechanism of action of this isolate against FAWs' natural enemies could be further investigated since it is considered one of the key criteria used in the registration of a biopesticide [45]

Our findings showed a significant difference in the total plot grain yields among the treatments from both experimental sites. Generally, the grain yields produced in Mbita were much higher than in Migori. This could have been attributed to the low infestation rates at Mbita when the crops were still young as compared to Migori. The application of Radiant, a spinetoram-based pesticide, highly reduced pest damage and hence, impacted the overall grain yields produced. Similar results on the effect of pest reduction on maize yields were reported earlier [46]. It was also recorded that *M. anisopliae* ICIPE 41 treated plots recorded high yields in both trial sites. This finding on the performance of ICIPE 41 could be explained by the formulated biopesticide that was highly entomopathogenic/effective and hence, reduced the FAW damage in maize, which consequently impacted the increased yield obtained. Mazao Achieve® biopesticide treated plots had, however, a relatively lower yield from both sites as compared to ICIPE 41, but was higher than the one obtained in the control plots where high FAW infestation and damage were recorded. This may be a result of its low efficacy against FAW larvae compared to the eggs [11,19].

5. Conclusions

Overall, the present study reported a high susceptibility of second-instar larvae of *S. frugiperda* to oil-based *M. anisopliae* ICIPE 41 under laboratory and field conditions. Oil-formulated conidia of *M. anisopliae* ICIPE 41 are effective against FAW and could be used sustainably to manage the invasive FAW in maize cropping systems. However, further studies are warranted to establish the effects of the ICIPE 41 corn oil-based formulation on other FAW-associated natural enemies in addition to the field observations.

Author Contributions: All authors conceived and designed the research. Conceptualization, J.M., L.K.O., S.S., D.K.M., E.M.N. and K.S.A.; data curation, J.M., V.-A.N.-N. and K.S.A.; writing—original draft preparation, J.M., L.K.O., D.K.M., V.-A.N.-N. and K.S.A.; writing—review and editing, L.K.O., S.S., E.M.N. and K.S.A.; project administration, L.K.O., S.S. and K.S.A.; funding acquisition, L.K.O., S.S. and K.S.A. All authors have read and agreed to the published version of the manuscript.

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