


Efficacy of aqueous and oil formulations of a specific *Metarhizium anisopliae* isolate against *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae) under field conditions

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Abstract

Cowpea (*Vigna unguiculata*) production is constrained by biotic and abiotic factors, among which Cowpea aphid (*Aphis craccivora*) is ranked a key insect pest that severely limits its potential for provision of food and nutritional security to millions of people in sub-Saharan Africa. The use of entomopathogenic fungi for *A. craccivora* management has been recently demonstrated at laboratory and field levels as alternative to synthetic insecticides, but with low adoption in Africa. This study assessed the efficacy of aqueous and oil formulations of *Metarhizium anisopliae* ICIZE 62 against *A. craccivora* under field conditions. *Metarhizium anisopliae* formulations and a commonly used insecticide Duduthrin[®] were applied using knapsack sprayers with target output of 350 L/ha. Data on aphid infestation levels were collected weekly. ICIZE 62 efficacy in inducing mortality was also assessed 24 hr post-treatment coupled with mycosis test. Further, leaf and grain yields were determined. After six weeks post-treatment in the wet season, there was no significant reduction in aphid density in fungus-treated plots compared to control and Duduthrin[®]-treated plots. However, in the dry season six weeks after applying the treatments, oil formulation spray resulted low aphid density compared to control and Duduthrin[®]-treated plots. ICIZE 62 formulations did not negatively affect the natural enemies' population. Leaf yield from the various treatments did not differ significantly in the wet season, but the two fungal formulations recorded higher yields in dry season compared to other treatments. Grain yields in wet and dry seasons were lower in control and Duduthrin[®]-treated plots compared to both ICIZE 62 formulations. This study showed that both *M. anisopliae* ICIZE 62 formulations are effective in suppressing *A. craccivora* population under field conditions without adverse effects on its beneficial insects. The study also revealed that efficacy of fungal-based biopesticides is highly dependent on environmental conditions.

KEYWORDS

cowpea, entomopathogenic fungi, ICIZE 62, Lambda-cyhalothrin, management, population suppression

1 | INTRODUCTION

African leafy vegetables including cowpea form an important component of diet and provide income to smallholder farmers in rural areas as well as urban and peri-urban dwellers (Abukutsa-Onyango, 2010; Rusike et al., 2013). Cowpea is the most important African leafy vegetable in Africa and in Kenya in terms of production volume and hectareage, as well as income (Aliyu & Makinde, 2016; Horticultural Crops Directorate (HCD), 2016). Cowpea is well adapted in the tropics because it tolerates dry weather conditions and does well even in poor soils (Bisikwa et al., 2014; Ddamulira, Santos, Obuo, Alanyo, & Lwanga, 2015; Mucheru-Muna et al., 2010). Cowpea leaves are rich in proteins, carbohydrates, minerals and vitamins and have medicinal attributes (Hall, 2012; Trehan et al., 2015).

Despite the nutritional and income generation potential of the crop to millions of people worldwide, its production has mainly been hampered by arthropod pests and diseases (Dugje, Omoigui, Ekeleme, Kamara, & Ajeigbe, 2009; Jackai & Daoust, 1986). As a major pest of the crop, cowpea aphid (*Aphis craccivora* Koch, 1854; Hemiptera: Aphididae) is a sap sucking soft-bodied insect that attacks cowpea from seedling to podding stage, and also a vector of some viral diseases to the host plant. It voraciously feeds on soft and actively growing plant tissues and directly impacts the crop by removing vital plant sap and interfering with photosynthetic functions that negatively affect the productivity or yield of the crop (Blackman & Eastop, 2006; Souleymane, Aken'Ova Fatokun, & Alabi, 2013).

Chemical pesticides are extensively used to manage *A. craccivora* partly because of its susceptibility to different groups of pesticides, and also because it is not feasible to grow cowpea profitably without the use of pesticides (Egho & Enujoke, 2012; Waddington, Li, Dixon, Hyman, & Vicente, 2010). However, the use of these synthetic chemical pesticides raises concerns about environmental pollutions such as contamination of soil and ground water, adverse effect on non-target species and accumulation of residues along the food chain and in the final products. Furthermore, the use of synthetic pesticides in cowpea as a leaf vegetable leads to loss of yield and income due to long post-harvest intervals requirements since cowpea leaves are regularly harvested in a very short period of time (Mweke, Ulrichs, Maniania, & Ekesi, 2016).

Lambda-cyhalothrin is a soft contact synthetic pyrethroid-based insecticide that induces mortality on insects by disrupting the normal functions of central nervous systems and is used in the management of sucking pests including aphids. In Kenya, it is commonly used in management of aphids and comes under different trade names and formulations, for example Duduthrin® 1.75EC (Twiga Chemical Industries Ltd), KARATE® 2.5WG, KARATE® 5SC (Syngenta East Africa Ltd), and LAMBDEX 5 EC (Amiran Kenya Ltd) among others. This insecticide has a known persistence in soil and has been detected in produce even when post-harvest intervals have been adhered to (Hornsby, Wauchope, & Herner, 1995; Kithure, Murung, Tum, Wanjau, & Thoruwa, 2017). These detrimental effects of synthetic insecticides have therefore led to search for alternative

strategies for the management of the pest (Asante, Tamo, & Jackai, 2001; Nampala et al., 2002; Ofuya, 1997).

Aphids are susceptible to infection by micro-organisms including entomopathogenic fungi (EPF) that are responsible for regulation of their populations under natural environment (Feng, Johnson, & Kish, 1990; Hajek & St. Leger, 1994; Selvaraj, Gurati, & Sharma, 2010; Shah & Pell, 2003; St. Leger & Wang, 2009). Commercial biopesticides derived from entomopathogenic fungi in Zygomycetes and Hyphomycetes groups, mainly *Lecanicillium lecanii* (Zimm.) Viegas, *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria bassiana* (Bals.) Vuill. and *Isaria* spp., have been successfully used in the management of several insect pests including different aphid species. These EPF are cheap and easy to mass-produce on organic substrates like rice, maize and sorghum and produce stable infective spores (Milner, 1997; Jaronski, 2013). In addition, these biopesticides have advantages of wide host range infections and are compatible with integrated management interventions (Lacey, Frutos, Kaya, & Vail, 2001; Lacey et al., 2015; Milner, 1997). However, adoption and application of EPF-based biopesticides is hampered by their short shelf life, inconsistent performance and low persistence under field conditions.

Different fungal-based biopesticide formulations have been used to improve these undesirable characteristics and improve market share and performance of these bioproducts under different environmental conditions (Chandler et al., 2011; Gasic & Tanovic, 2013; Guinossi, Moscardi, Oliveira, & Sosa-Gómez, 2012; Inglis, Jaronski, Wraight, Beattie, & Watson, 2002). The EPF *M. anisopliae* sensu lato (s.l.) isolate ICIPE 62 was previously demonstrated to be highly pathogenic to *A. craccivora* under laboratory conditions (Mweke et al., 2018). Formulation of EPF and application methods significantly influence their efficacy in pest management under field conditions (Inglis et al., 2002). Additionally, distribution of infective spores has been shown to be influenced by formulation; for example, conidia formulated in oil are usually evenly distributed on the insect cuticle and leaf surface while aqueous spore formulations remain as drops on the surface after application and thereby enhancing the efficacy of EPF-based biopesticides (Inglis, Ivie, Duke, & Goettel, 2000; Inyang et al., 2000; Wraight, Filotas, & Sanderson, 2016). Previous studies have reported pathogenicity of *M. anisopliae* isolates against *A. craccivora* under laboratory studies (Ekesi, Akpa, Onu, & Ogunlana, 2000; Mweke et al., 2018; Sahayaraj & Borgio, 2010; Saranya, Ushakumari, Jacob, & Philip, 2010). However, these isolates were not evaluated under field conditions neither of them was commercialized for control of *A. craccivora*. Though there is a commercial biopesticide based on ICIPE 62 for control of other aphid species (<http://www.realipm.com/>), there are no previous studies on efficacy of ICIPE 62 against *A. craccivora* under field conditions and there is no biopesticide specifically registered for use against this aphid species. Therefore, exploring efficacy of ICIPE 62 under field conditions would provide useful data for validation and use as biological control agent against the pest.

This study was therefore undertaken to evaluate field efficacy of *M. anisopliae* ICIPE 62 propagules formulated as aqueous and oil in the management of *A. craccivora* and compare its performance against commonly used synthetic pyrethroid-Duduthrin® 1.75EC

under field conditions. The effect of ICIPE 62 on non-target beneficial organisms, Cowpea leaf and grain yields was also evaluated.

2 | MATERIALS AND METHODS

2.1 | Fungal culture and inoculum preparation

Fungal isolate ICIPE 62 (*Metarhizium anisopliae* s.l.) used in this study was obtained from *icipe's* Arthropod Germplasm Centre and was recently demonstrated to be pathogenic against *A. craccivora* under laboratory conditions (Mweke et al., 2018).

ICIPE 62 conidia were mass produced on sterilized whole long grain rice substrate in Milner bags (60 × 35 cm). The substrate was first autoclaved for 1 hr at 120°C, then transferred to plastic buckets 35 (Ø) × 25 (width) × 15 cm (depth) and allowed to cool at room temperature, after which it was inoculated with a 3-day-old culture of blastopores (50 ml), and covered with sterile polyethylene bag. The culture was then incubated for 21 days at ambient conditions (20–26°C, 40%–70% RH) (Maniania, 1993). After 21 days, the bag was removed and dried for 5 days at room temperature. Conidia were harvested by sifting the substrate through a sieve (295-µm mesh size) and stored in a refrigerator (4–6°C, 40%–50% RH) for <2 weeks before being used in field experiments. Viability of the fungus was determined prior to treatment application by spread plating 100 µl of conidial at a concentration of 3×10^6 conidia/ml in Sabouraud dextrose agar (SDA) plates. Afterwards, the plates were incubated at $25 \pm 2^\circ\text{C}$ in darkness and examined after 18 hr. The percentage of germination was determined by counting randomly 100 selected conidia in a surface area per spot covered by cover slip under a light microscope (400×) (Goettl & Inglis, 1997). The conidia were scored as viable or germinated when the germ tubes were at least as long as twice the diameter of the conidium (Schumacher & Poehling, 2012). Conidial germination of >90% after 18 hr on SDA was obtained and was considered adequate for use in the field trials. Conidia concentration per gram was calculated by dissolving 0.1 g of conidia in 10 ml of Triton water that had been autoclaved. The concentration was adjusted to 1×10^{12} conidia/ml through serial dilution prior to application.

2.2 | Lambda-cyhalothrin–(Duduthrin® I.75EC)

Duduthrin® I.75EC with active ingredient Lambda-cyhalothrin which is commonly used to control aphids in Kenya was used as standard in this field experiment. This pesticide was bought from Twiga Chemical Industries Ltd after confirmation of its authenticity and validity by expiry date. The pesticide was prepared by mixing 65 ml of the pesticide with 20 L of clean water in a knapsack sprayer and adding 0.05% (1 ml) Integra (sticker, Greenlife Crop Protection Africa Ltd.) and shaking thoroughly to produce a homogenous mixture before application.

2.3 | Experimental sites

The experiment was carried out at *icipe's* Mbita point campus (00.4305S, 034.2057 E, 1150 metres above sea level (m.s.l)),

Homabay County, Western Kenya, for two seasons. The first season was wet, and experiment was carried out between January and April 2016, while the second season was dry, and trials were undertaken from May to August 2016. The mean annual rainfall in season one was 120.4 mm, and its minimum and maximum temperatures were 23°C and 29.2°C, respectively, with relative humidity ranging between 60% and 70%. In the second season, the mean rainfall was 53.67 mm and minimum and maximum temperatures were 20.7 and 28.5°C, respectively, with relative humidity between 60% and 65% (ICIPE, 2016; Kenya Meteorological department, 2017). There were 16 experimental plots per season (four treatments replicated four times) arranged in randomized complete block design.

2.4 | Crop

The land was first ploughed and harrowed in preparation for planting. Cowpea landrace Ex-Luanda known for its high susceptibility to *A. craccivora* was obtained from *icipe's* germplasm collection and used in the experiment. Four cowpea seeds per hole were sown in 10 × 10 m plots at a spacing of 20 cm intra-row by 75 cm inter-row. Two weeks after germination, weeding and thinning were done to keep only two plants per hole, giving a total plant density of 570 plants in each experimental plot. Overhead irrigation (sprinkler) was used for the first 3 weeks to support the crop as it was relatively dry in January during planting and irrigation discontinued after the onset of the rains. Weeding was done twice a month before the crop established and smothered the weeds, and thereafter, the frequency of weeding was reduced to once per month. All the necessary and recommended agronomic practices were undertaken, and the crops were then left for natural aphid infestation. The infestation level was monitored weekly by sampling randomly five plants in each of the inner rows in all the experimental plots and inspecting them for presence of aphids prior to treatments applications.

2.5 | Treatments, layout and design

The experiment was conducted in two seasons as described above, and for each season, experiment involved four different treatments: (a) an aqueous and (b) oil formulations of *M. anisopliae* ICIPE 62, (c) Lambda-cyhalothrin (Duduthrin® I.75EC) and (d) the control. In the aqueous formulation, spores were suspended in water containing 0.05% Integra (sticker, Greenlife Crop Protection Africa Ltd) with 0.1% nutrient agar, 0.1% glycerine and 0.5% molasses added as protectants and attractant, respectively (Maniania, 1993), whereas in oil formulation, spores were suspended in vegetable oil-elianto (Elianto, Bidco Africa Ltd) with similar proportions of the sticker, nutrient agar, glycerine and molasses as described above in aqueous formulation. The formulations were prepared at the time of treatment application. The insecticide Duduthrin® I.75EC was applied at the rate of 1.75 g (Active Ingredient [AI]) ha⁻¹ with 0.1% Integra. Control treatment was sprayed with water containing 0.05% Integra, 0.1% nutrient agar, 0.05% molasses and 0.1%

TABLE 1 Mean aphid population density per plant 6 weeks after treatment in wet and dry seasons

Treatment	Aphid density per plant	
	Wet season	Dry season
<i>M. anisopliae</i> oil formulation	2.1 ± 1.4aA	1.0 ± 0.8bB
<i>M. anisopliae</i> aqueous formulation	3.9 ± 2.1aA	1.4 ± 1.4bB
Duduthrin [®]	4.6 ± 2.4aB	6.9 ± 5.7aA
Control	2.2 ± 1.2aB	4.0 ± 1.4aA
χ^2	4.2	8.2
<i>p</i> value	.36	.04
<i>df</i>	3, 48	3, 48

Note: Means followed by the same lower (upper) case letters within a column (row) are not significantly different, respectively, by Tukey HSD at $p < .05$.

glycerine without any fungal conidia and any insecticide solution. In both aqueous and oil formulations, conidia were applied at the rate of 1×10^{12} conidia/ml. The spray applications started on day 56 after planting due to late aphids' infestation and thereafter done on a weekly basis for a period of 6 weeks. The fungus formulations and the insecticide were applied with different knapsack sprayers with target output of 350 L/ha, and spraying was done late in the evening between 17:00 and 18:00 hr. The experimental design was randomized complete block with four replications and was carried out for two seasons (January–April and May–August 2016).

2.6 | Evaluation of treatments

Two leaflets, each from the base and the top from 20 randomly selected cowpea plants in the middle rows were sampled from each plot for aphid infestation assessment. The aphids were dislodged from the host plant with a fine hair brush into a vial containing 70% alcohol, labelled and thereafter counted in the laboratory. Sampling was done on weekly basis from day seven after planting until the cowpea leaves begun to dry out. Aphid infestation was assessed using the following scale: 1–5 rating scale (1 = a few individual aphids, 2 = few small individual colonies, 3 = several small colonies, 4 = large individual colonies and 5 = large continuous colonies) as described by Souleymane et al. (2013).

2.7 | Assessment of cowpea leaf damage and aphid mortality induced by *Metarhizium anisopliae* ICIPE 62

Leaf damage (leaf quality and fitness for human consumption) was visually assessed weekly using the following scale: 0 = visual damage on leaves and flower buds <10%, 1 = visual damage on leaves and flower buds 10%–25%, 2 = visual damage on leaves and flower buds 26%–50%, 3 = visual damage on leaves and flower buds 51%–75%, and 4 = visual damage on leaves and flower buds 76%–100% (Benchasri, 2009).

Aphid mortality due to the fungus was also assessed weekly 24 hr after treatment application by actively picking 30 aphids from each plot and placing them in plastic dishes (11.3 cm (Ø) × 4 cm (depth)) lined with moist filter paper containing sterilized cowpea leaves serving as food source. The lids of the plastic dishes were covered by muslin cloth with apertures (300 × 300 µm) to allow for free circulation of air. The dishes were kept at room temperature and mortality recorded daily for seven days. The leaves in the dishes were changed daily with fresh ones. Dead aphids were collected and placed in Petri dishes with sterilized moistened filter papers and kept at room temperature for assessing mycosis. Mortality due to the fungus was confirmed by observing mycosis on dead aphid under a dissecting microscope, and slides were prepared for confirmation with the applied fungus.

2.8 | Leaf vegetable and grain yield

Leafy vegetable yield data were collected at seven days interval starting from day 21 after planting (Saidi, Itulya, Aguyoh, Mshenga, & Owour, 2010). The total leaf vegetable weight for each treatment was calculated by cumulatively adding the fresh leaf weights obtained per treatment at the different leaf harvesting dates and expressed in kilograms per hectare (kg/ha). At plant maturity, the pods were harvested, sun dried, threshed and the grain weight recorded using electronic balance (PM 15, Mettler Instruments Ltd) and yield computed.

2.9 | Natural enemies' assessment

Ladybird beetles, spiders, lacewing and parasitoids were the beneficial arthropods assessed in the study. Predators were assessed weekly by counting their numbers on five randomly selected plants in inner rows of each plot. Parasitoids were assessed by collecting 20 mummies per plot, transferred into perforated Petri dishes, and incubated under laboratory conditions, and the number of emerged parasitoids species was identified and recorded.

2.10 | Statistical analysis

The aphid density, natural enemies and count data as well as leaf and grain yield were first log transformed before analysis using generalized linear mixed model and means separated using Tukey HSD and presented in chi-square values. Per cent aphid damage data were analysed using ANOVA. To evaluate aphid mortality induced by *M. anisopliae* ICIPE 62, data on mortality were corrected for natural mortality (Abbott, 1925), then tested for normality (Shapiro & Wilk, 1965), arcsine transformed then subjected to ANOVA and means separated using Tukey HSD. Data on mycosis were tested for normality (Shapiro & Wilk, 1965), arcsine transformed and analysed using ANOVA and means separated using Tukey HSD. Two-sample *t* test was used to compare performance of the aqueous and oil formulations. Data were analysed using R software (R Development Core Team, 2013).

3 | RESULTS

3.1 | Effect of treatment application on aphid infestation

During wet season, there was no significant difference between the treatments and the control ($\chi^2_{3,48} = 4.2, p = .36$) with regard to aphid population density per plant after 6 weeks of treatment application (Table 1). However, in dry season, the mean aphid infestation per plant 6 weeks post-treatment was lower than in the wet season for the two ICIPE 62 formulations, but higher in control and Duduthrin[®] treatments and differed significantly between the treatments ($\chi^2_{3,48} = 8.2, p = .04$). The aphid infestation varied significantly across the seasons ($\chi^2_{3,96} = 8.11, p = .004$; Table 1).

3.2 | Aphid mortality and infection by *Metarhizium anisopliae* ICIPE 62

In the wet season, mean mortality of aphids induced by *M. anisopliae* ICIPE 62 formulated in oil was $74.3 \pm 4.1\%$ while in aqueous formulation the mortality was $66.58 \pm 3.5\%$; however, there was no significant difference between the two formulations in the wet season ($t = 1.43, df = 34, p = .16$) (Table 2). Oil and aqueous formulations of *M. anisopliae* s.l. isolate ICIPE 62 caused $71.9 \pm 3.2\%$ and $64.8 \pm 3.5\%$ infection (mycosis rates), respectively, but did not differ significantly ($t = 1.85, df = 34, p = .07$) (Table 2). In dry season, the mean mortality induced by oil formulation and aqueous formulations did not differ significantly ($t = 1.66, df = 34, p = .10$). In the same season, the mycosis due to the two ICIPE 62 formulations was not significantly different ($t = 0.55, df = 34, p = .58$) (Table 2).

3.3 | Aphid damage on cowpea crop

In the wet season, percentage aphid damage on cowpea plants was higher in Duduthrin[®] ($40.4 \pm 1.8\%$) and untreated

control ($30.3 \pm 2.3\%$) plots compared to the two fungal formulations ($25.6 \pm 5.1\%$ and $21.2 \pm 1.1\%$ for aqueous and oil formulations, respectively) with significant differences between the treatments ($F = 3.43, df = 3, p = .02$; Table 3). Similarly, in the dry season the percentage leaf damage differed significantly among the treatments ($F = 5.31, df = 3, p = .001$) with less damage obtained in fungal treatments as compared to Duduthrin[®] ($47.10 \pm 4.2\%$) and the control (52.1 ± 8.1) (Table 3).

3.4 | Natural enemies associated with *Aphis craccivora* in the various treatment plots

During the field trial surveys, predators (Ladybird beetles), spiders (*Leucocage decorata* Blackwall, 1864; Arachnida: Tetragnathidae), lacewing and parasitoids (*Aphidius colemani* Viereck, 1912; Hymenoptera: Aphidiidae) were the key natural enemies identified to be associated with *A. craccivora* in the experimental sites. In the wet season, among the natural enemies, the spiders (*L. decorata*) were more abundant across all the treatments but were significantly lower in plots treated with Duduthrin[®] compared to control and fungal-treated plots ($\chi^2_{3,48} = 11.8, p = .008$). The number of ladybird beetles differed significantly among the treatments ($\chi^2_{3,48} = 28.4, p = .001$) and was higher in the two fungal formulations treated plots than in the plots treated with Duduthrin[®] and untreated control (Table 4). However, the number of lacewings did not differ significantly across the treatments ($\chi^2_{3,48} = 1.8, p = .61$), while the number of parasitoids was the lowest compared to other recorded natural enemies of *A. craccivora*. Parasitoid numbers were higher in untreated control plots and in ICIPE 62 aqueous and oil formulations plots than in Duduthrin[®], with significant differences between the treatments ($\chi^2_{3,48} = 12.7, p = .005$; Table 4).

In the dry season, the mean numbers of ladybird beetles varied significantly among the treatments ($\chi^2_{3,48} = 28.2, p = .001$) and with the lowest values obtained in Duduthrin[®]-treated plots (Table 3), which was also similar to the wet season trend. In addition, the

TABLE 2 Mean mortality and mycosis obtained from aphids collected from the two fungal formulations 24 hr post-treatment and reared at the laboratory in wet and dry seasons

Fungal formulations	Mortality (%)	Mycosis (%)
Wet season		
<i>M. anisopliae</i> oil formulation	$74.33 \pm 4.1a$	$71.9 \pm 3.2a$
<i>M. anisopliae</i> aqueous formulation	$66.58 \pm 3.5a$	$64.8 \pm 3.5a$
Control	$0.0 \pm 0.0b$	$0.0 \pm 0.0b$
Dry season		
<i>M. anisopliae</i> oil formulation	$80.0 \pm 3.7a$	78.7 ± 3.1
<i>M. anisopliae</i> aqueous formulation	$77.9 \pm 3.8a$	75.5 ± 3.4
Control	$0.0 \pm 0.0b$	$0.0 \pm 0.0b$

Note: Means followed by the same letter within a column are not significantly different for each season at $p < .05$.

TABLE 3 Cowpea crop infestation or damage as influenced by different treatments during the two seasons 6 weeks post-treatment

Treatment	Cowpea crop damage (%)	
	Wet season	Dry season
<i>M. anisopliae</i> oil formulation	$25.65 \pm 5.1bA$	$25.6 \pm 2.4bA$
<i>M. anisopliae</i> aqueous formulation	$22.0 \pm 1.1bA$	$21.1 \pm 0.04bA$
Duduthrin [®]	$40.44 \pm 1.8aA$	$47.10 \pm 4.2aA$
Control	$30.24 \pm 2.3abB$	$52.1 \pm 8.1aA$
F-value	3.0	5.31
p-Value	.02	.001
df	3	3

Note: Means followed by the same lower case (upper) letter within a column (row) are not significantly different, respectively, by Tukey HSD at $p < .05$.

TABLE 4 Beneficial arthropods density recorded in the various treatments in wet and dry seasons

Treatments	Lady bird beetles	Spiders (<i>Leucauge decorata</i>)	Green Lacewing	Parasitoid (<i>Aphidius colemani</i>)
Wet season				
<i>M. anisopliae</i> oil formulation	2.5 ± 1.0a	8.3 ± 2.1a	1.9 ± 0.8a	0.66 ± 0.5a
<i>M. anisopliae</i> aqueous formulation	2.5 ± 1.4a	7.75 ± 1.9a	1.9 ± 0.7a	0.75 ± 0.4a
Duduthrin®	1.0 ± 0.5b	6.33 ± 2.2b	1.7 ± 0.8a	0.33 ± 0.2b
Control	1.9 ± 1.1b	9.03 ± 1.9a	1.7 ± 0.6a	0.54 ± 0.4a
Dry season				
<i>M. anisopliae</i> oil formulation	7.6 ± 3.4a	8.6 ± 2.8a	4.6 ± 1.9a	1.1 ± 0.7a
<i>M. anisopliae</i> aqueous formulation	6.9 ± 3.4a	4.0 ± 2.6b	4.7 ± 1.9a	1.2 ± 0.5a
Duduthrin®	2.3 ± 2.0b	1.3 ± 0.4c	0.8 ± 0.5b	0.37 ± 0.1b
Control	7.2 ± 3.6a	10.6 ± 2.2a	4.7 ± 1.9a	1.0 ± 0.5a

Note: Means followed by the same letter within a column are not significantly different for each season by Tukey HSD at $p < .05$.

mean numbers of spiders (*L. decorata*) were significantly different among the treatments ($\chi^2_{3,48} = 24.4$, $p = .001$) and were lower in Duduthrin®-treated plots compared to both ICIPE 62 formulations treated and untreated control plots (Table 4). Similarly, the number of lacewings was significantly different between the treatments ($\chi^2_{3,48} = 34.4$, $p < .001$) and was high in control and ICIPE 62-treated plots compared to Duduthrin®-treated plots. There was significant difference in parasitoid numbers (*A. colemani*) among the treatments ($\chi^2_{3,48} = 13.9$, $p = .001$) where the lowest number was obtained in plots treated with Duduthrin® compared to other treatments, and their numbers were generally low compared to other recorded natural enemies, predators (Table 4).

3.5 | Leaf and grain yield

Cumulative leaf yield obtained in the wet season did not differ significantly ($\chi^2_{3,48} = 0.22$, $p = .97$) among the treatments (Table 5). However, in the dry season cumulative leaf yield was significantly different ($\chi^2_{3,48} = 8.0$, $p = .04$) among treatments and was higher in the two fungal formulations (24.5 and 23.2 kg/ha for oil and aqueous respectively) compared to control (10.5 kg/ha) and Duduthrin® (10.9 kg/ha) treatments (Table 5). Season-wise comparison of cowpea leaf yield also showed that the season type affected the yield significantly ($\chi^2_{3,48} = 42.5$, $p < .001$), and the wet season recorded higher yield than the dry season (Table 5).

The grain yield was significantly different among treatments in the first season that experienced heavy and frequent rainfall ($\chi^2_{3,48} = 15.5$, $p = .02$) where the two ICIPE 62 fungal formulations produced more yield (560 and 553 kg/ha for oil and aqueous, respectively) compared to control (343 kg/ha) and Duduthrin® (325 kg/ha) treatments (Table 5). Similarly, in the dry season the grain yield was lower than the wet season ($\chi^2_{3,48} = 9.8$, $p = .02$) and differed significantly among the treatments ($\chi^2_{3,48} = 11.1$, $p = .01$) (Table 5). The yield obtained also varied significantly between the seasons ($\chi^2_{3,48} = 155.6$,

$p = .001$). In the same season, the oil and aqueous formulations of ICIPE 62 produced higher grain yields (345 and 322.5 kg/ha, respectively) compared to Duduthrin® (281.4 kg/ha) and control (277.8 kg/ha) treatments.

4 | DISCUSSION

The results of this study have confirmed the potential of *M. anisopliae* s.l. isolate ICIPE 62 in the management of *A. craccivora* under field conditions. The efficacy of this isolate in inducing high mortality on *A. craccivora* and other aphid species has been also demonstrated under laboratory conditions and greenhouse in previous studies (Bayissa et al., 2016a; Mweke et al., 2018). Application of both *M. anisopliae* ICIPE 62 formulations did not result in significant decline in aphid densities in wet season compared to untreated control. This could be attributed to the fact that the wet season was characterized by heavy and frequent rains that could have not only reduced infective amount of the conidia by washing them off the cowpea leaf surfaces, but also could have washed aphid colonies off plant surfaces thus reducing the levels of infection and subsequent mortality, as well as fungal persistence after field application. Uniform distribution of infective spores on leaf surface is an important factor in the infection process, and the droplet distribution of the spores can be affected by the treatment application method (foliar spray) used, as well as by the type of formulation (Bateman & Alves, 2000; Bateman, Carey, Moore, & Prior, 1993; Santi et al., 2011; Wraight et al., 2016; Wraight & Ramos, 2002).

Conidia formulated in oil are usually evenly distributed on the insect cuticle and leaf surface while aqueous spore formulations remain as drops on the surface after application and thereby enhancing the efficacy of EPF-based biopesticides (Inglis et al., 2000; Inyang et al., 2000; Wraight et al., 2016). However, the results of this study showed similar performance of oil and aqueous formulations.

TABLE 5 Cowpea leaf and grain yield of the two seasons as influenced by the different treatments

Treatment	Cowpea leaf yield (kg/ha)	
	Wet season	Dry season
M. anisopliae oil formulation	28.8 ± 0.57aA	24.5 ± 4.8aB
M. anisopliae aqueous formulation	28.1 ± 0.57aA	23.2 ± 1.8aB
Duduthrin®	30.2 ± 0.57aA	10.9 ± 3.5bC
Control	28.2 ± 0.57aA	10.5 ± 3.9bC
χ ² -value	0.22	8.0
p-Value	.97	.04
df	3, 48	3,48

Treatment	Cowpea grain yield (kg/ha)	
	Wet season	Dry season
M. anisopliae oil formulation	560 ± 60.0aA	345.0 ± 29.8aB
M. anisopliae aqueous formulation	553 ± 60.0aA	322.5 ± 29.8aB
Duduthrin®	325 ± 60.0bA	270.0 ± 29.8bA
Control	343 ± 60.0bA	222.5 ± 29.8bB
χ ² -value	15.5	11.1
p-Value	.02	.01
df	3,48	3,48

Note: Means followed by the same lower case (upper) letter within a column (row) are not significantly different, respectively, by Tukey HSD at $p < .05$.

This could be explained by the fact that efficacy of EPF-based biopesticides might not solely be influenced by formulation but also by prevailing environmental conditions including rainfall, temperature and relative humidity (Hajek & St. Leger, 1994; Inglis et al., 2002; Nussenbaum, Lewylle, & Lecuona, 2013). Rainfall has also been shown to negatively influence the efficacy of EPF in pest management under field conditions (Inglis et al., 2000; Wraight & Ramos, 2002). Similarly, Inyang et al. (2000) have previously proved that susceptibility of pests to EPF is not only influenced or reduced by the formulation type, but also by rainfall.

Furthermore, leaf growth leading to expansion of the leaf surface has also been shown to dilute infective spores' efficacy (Inyang et al., 1998). Though oil formulations are known to prolong conidial infectivity and survival, and reduce sensitivity to UV radiation compared to aqueous formulations (Alves, Bateman, Prior, & Leather, 1998; Inglis, Johnson, & Goettel, 1995; Moore, Bridge, Higgins, Bateman, & Prior, 1993), their performance has not been consistent under varying environmental conditions. Some studies have also reported similar findings where aqueous formulations of *M. anisopliae* and *M. flavoviride* performed the same way as oil formulations (Ouedraogo, Fargues, Goettel, & Lomer, 1997). Performance of different species of EPF has also been shown to vary depending on formulation types. For example, Guinossi et al. (2012) reported better dispersal of *B. bassiana* conidia formulated in oil after field application but did not equally favour spatial distribution of *M. anisopliae* conidia. Inconsistent efficacy

of EPF-based biopesticides under different environmental conditions could therefore be addressed through improved formulations. For example, Ritu et al. (2012) demonstrated that incorporating bentonite in oil-based formulation of *B. bassiana* improved its efficacy against *Helicoverpa armigera* (Hübner; Lepidoptera: Noctuidae).

Application of Duduthrin® did not result in decline in aphid population density as expected with many synthetic pesticides. This could partly be attributed to the low number of natural enemies recorded in the plots treated with Duduthrin®. We also hypothesize that there could be possible aspects of resistance which has not been previously reported, since aphid species are known to develop resistance against insecticides after years of exposure (Abdallah, Abou-Yousef, Fouad, & Kandil, 2016; Van Emden & Harrington, 2007). Similar observations on the population explosion of aphid species after use of Karate, a synthetic insecticide with same active ingredient (Lambda-cyhalothrin) as Duduthrin®, were earlier reported (Bayissa, 2016c).

In this study, application of treatments in both seasons did not prevent cowpea aphid damage compared to control. Additionally, Duduthrin® performed poorly compared to untreated control in the wet season, where spiders and parasitoids populations were higher in the control compared to Duduthrin® plots. The nature of aphid reproduction where eggs are retained inside the body and young nymphs are borne and mature within 2–3 days increases the chance of fast population build-up under favourable environmental conditions, and therefore, there would be inevitable crop damage (Omoigui et al., 2017; Schreiner, 2000). However, the damage was less in the wet season because infestation occurred late and plants were old enough and hence less susceptible to aphid damage compared to dry season where infestation occurred at early stages when plants were more susceptible to damage. Therefore, the stage of crop infestation by aphids and the level of natural enemies' populations might influence the level of damage that occurred. Evaluation of mycosis caused in oil and aqueous formulations in both seasons showed >95% of the aphid cadavers died as a result of fungal infection. This demonstrates the ability of *M. anisopliae* ICIPE 62 to effectively cause mortality to *A. craccivora* under field conditions. Production of spores on cadavers could trigger secondary infection especially for insects that have aggregatory behaviour, and this could further reduce the population especially under favourable environmental conditions (Pettersson, Karunaratne, Ahmed, & Kumar, 1998; Purandare & Tenhumberg, 2012).

In the present study, the population of aphid-associated natural enemies remained high in plots treated with *M. anisopliae* ICIPE 62 as compared to Duduthrin® in both seasons. This indicates that the application of the aqueous and oil formulations of ICIPE 62 did not negatively affect the population of aphid-associated natural enemies. For example, the mean numbers of ladybird beetles obtained in oil and aqueous formulations were 3 times higher than Duduthrin®-treated plots in dry season. The aphid predatory spider (*L. decorata*) population was similar in control and both fungal formulations, while least numbers were obtained in Duduthrin®-treated plots. The lacewing population was 6 times lower in Duduthrin® treatment during dry season compared to *M. anisopliae*-treated plots. In previous studies, *M. anisopliae* has been shown to have minimal negative impacts on natural enemies

(Zimmermann, 2007). Bayissa et al. (2016b) also demonstrated the low toxicity of ICIPE 62 to the predatory coccinellid *Cheilomenes lunata* Fabricius, 1775; Coleoptera: Coccinellidae) under laboratory conditions, while Mweke, Ulrichs, Fiaboe, Maniania, and Ekese (2017) previously reported the non-target effect of ICIPE 62 to natural enemies of aphids under field conditions. This non-target effect presents an advantage of ICIPE 62 as a potential candidate for biopesticide development and commercialization against *A. craccivora*, since it is considered as one of the key criteria used in registration of biopesticide (Goettel, 1994; Goettel & Hajek, 2000; Montesinos, 2003). Entomopathogenic fungi play a key role in supplementing the activities of natural enemies in suppressing arthropod pests, and therefore, positive synergism between them could provide a sustainable option of pest management (Ferguson & Stiling, 1996; Roy & Pell, 2000).

Duduthrin® appeared to have negative effects on the natural enemies, and this could probably have contributed to higher population of aphids observed in plots treated with Duduthrin®. Previous studies have shown that Lambda-cyhalothrin (active ingredient of Duduthrin®) has detrimental effects on (*Trichogramma evanescens* Westwood, 1833; Hymenoptera: Trichogrammatidae) (Shoeb, 2010) while other synthetic pyrethroids have been reported to be toxic to other natural enemies (Tillman & Mulrooney, 2000).

In the wet season, leaf yield data collection commenced four weeks post-germination. Treatment application therefore did not influence significantly leaf yield level because, by the time aphid population build-up to warrant treatment application, leaf yield data collection was advanced. In addition, the frequent rainfall observed during this season reduced aphid infestation and consequently without affecting leaf yield. However, the grain yield was influenced by treatment applications where it was lower in Duduthrin®-treated plots compared to fungal treatments. This could be attributed to the fact that Duduthrin® recorded high numbers of aphids per plant and consequently could have directly affected the grain yield since treatment applications stopped at leaf senescence while the pods were still developing. In addition, since *A. craccivora* is known to attack the pods which remain green and succulent as other plant parts dried, the concentration of aphid colonies on the pods might negatively affect their development and subsequently reduce grain yield.

The dry season was characterized by reduced rainfall, moderate temperatures, with early and heavy infestation of *A. craccivora* on cowpea. As a result, ICIPE 62 was able to suppress aphid population and enabled the crop to recover from aphid damage and produce more leaf yields compared to synthetic insecticide application. The two fungal formulations produced more yields (23.2 and 24.4 kg/ha for aqueous and oil formulations respectively) compared to untreated control (10.5 kg/ha) and Duduthrin® (10.9 kg/ha). Aphid infestation affects quantitatively and qualitatively leaf yield because the aphid colonies not only reduced the photosynthetic ability of the plant, but also honeydew deposited on leaf surfaces renders the leaves unfit for human consumption and also leads to growth of moulds that further reduce the harvestable leaves.

In conclusion, *M. anisopliae* ICIPE 62 has the potential to control *A. craccivora* under field conditions and could therefore be

incorporated into integrated pest management strategies as it does not negatively affect key beneficial non-target arthropods. However, there is need for further research to develop and optimize formulation types by incorporating suitable carriers that could prolong storage period and improve EPF application techniques while maintaining their efficacy under different environmental conditions. The application of *M. anisopliae* ICIPE 62-based biopesticide could thus be recommended as a sustainable management strategy against cowpea aphid. In addition, since *M. anisopliae* ICIPE 62 is already commercialized in partnership with *icipe* private sector Real IPM against other aphid species, label extension might therefore be required to increase the portfolio of this biopesticide against *A. craccivora*.

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CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTIONS

All authors conceived and designed the research. AM conducted experiments and analysed the data. NKM, KKM, KSA and SE provided the fungal isolates, reagents materials, analytical tools and the facilities. AM, CU, KSA, NKM, KKM and SE wrote the manuscript. KKM and SE secured funding. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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