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Efficacy of the entomopathogenic fungus *Metarhizium anisopliae* (Clavicipitaceae: Hypocreales) against the black bean aphid (*Aphis fabae* Scopoli, 1763) under laboratory conditions

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ABSTRACT
Black bean aphid (BBA) is a serious insect pest that severely significantly impacts French bean production. Over-reliance on synthetic chemicals to reduce economic losses caused by aphids poses serious environmental and human health threats. Biopesticides containing entomopathogenic fungi (EPF) have potential as viable alternatives that can be incorporated into integrated to manage such insect pests. The efficacy of *Metarhizium anisopliae* against BBA was assessed in the laboratory under a complete randomized design (CRD) design with five replicates. Data was analysed using SAS 9.4 (2021). In part one, *Metarhizium anisopliae* products caused bean aphid mortality etween 60.1% and 95.5%. Mazao supreme (4×10^8 spores/ml) and Metarril (4×10^8 spores/ml) caused the highest mortality (95.5%) and the lowest concentration of irrespective product caused the lowest mortality (60.1%) after treatment application. In part two, alpha-cypermethrin caused the highest aphid mortality (99.3%), followed by Metarril (84.2%), Mazao (80.7%) and biomagic had the lowest (68.4%) mortality after treatment application. Alpha-cypermethrin had the lowest lethatl time (LT_{50}), causing 50% aphid mortality, followed by Metarril, Mazao supreme, and biomagic. Differents treatments had varying effects on nymph numbers. Alpha-cypermethrin caused 50% aphid mortality, followed by Metarril, Mazao supreme, and biomagic, with varying effects on nymph numbers. The study recommends incorporating *Metarhizium anisopliae*-based biopesticides, particularly Metarril by small holder farmers to offer a cost-effective integrated pest management approach for managing BBA (adults and nymphs) in French bean production while reducing reliance on synthetic chemicals.

Keywords: Black bean aphid, biopesticides, French beans

RÉSUMÉ

Efficacité du champignon entomopathogène Metarhizium anisopliae (Clavicipitaceae : Hypocreales) contre le puceron noir du haricot (Aphis fabae Scopoli, 1763) en conditions de laboratoire

Le puceron noir du haricot (BBA) est un insecte nuisible sérieux qui a un impact considérable sur la production de haricot vert. Une dépendance excessive à l'égard de produits chimiques synthétiques pour réduire les pertes économiques causées par les pucerons pose de graves menaces pour l'environnement et la santé humaine. Les biopesticides contenant des champignons entomopathogènes (EPF) constituent des alternatives viables qui peuvent être intégrées pour lutter contre ces insectes nuisibles. L'efficacité de *Metarhizium anisopliae* contre le BBA a été évaluée en laboratoire dans le cadre d'un plan randomisé complet (CRD) avec cinq répétitions. Les données ont été analysées à l'aide de SAS 9.4 (2021). Dans la première partie, les produits *Metarhizium anisopliae* ont provoqué une mortalité des pucerons du haricot comprise entre 60,1 % et 95,5 %. Mazao suprême (4 × 108 spores/ml) et Metarril (4 × 108 spores/ml) ont provoqué la mortalité la plus élevée (95,5 %) et la concentration la plus faible du produit, quel que soit le produit, a provoqué la mortalité la plus faible (60,1 %) après l'application du traitement. Dans la deuxième partie, l'alpha-cyperméthrine a causé la mortalité de pucerons la plus élevée (99,3 %), suivie par Metarril (84,2 %), Mazao (80,7 %) et biomagic a eu la mortalité la plus faible (68,4 %) après application du traitement. L'alpha-cyperméthrine avait le temps de mortalité le plus bas (LT50), provoquant une mortalité des pucerons de 50 %, suivie par le Metarril, le Mazao suprême et le biomagic. Différents traitements ont eu des effets variables sur le nombre de nymphes. L'alpha-cyperméthrine a causé 50 % de mortalité des pucerons, suivie par le Metarril, le Mazao suprême et le biomagic, avec des effets variables sur le nombre de nymphes. L'étude recommande d'incorporer des biopesticides à base de *Metarhizium anisopliae*, en particulier Metarril, par les petits agriculteurs afin d'offrir une approche de lutte antiparasitaire intégrée rentable pour gérer le BBA (adultes et nymphes) dans la production de haricots verts tout en réduisant la dépendance aux produits chimiques synthétiques.

Mots-clés : Puceron noir du haricot, biopesticides, haricot vert

INTRODUCTION

Vegetable crops account for 12% (1.1 billion tonnes) of the total world crop production [9.3 billion tonnes] (FAO 2022). According to the Food and Agriculture Organization (FAO), Kenya, among developing countries in Africa, accounted for 4.2% of total vegetable production (thousand tonnes) in 2022 (FAO 2022). French bean (*Phaseolus vulgaris* L.) is a leading exotic vegetable crop grown primarily for export in Kenya (Fulano *et al*. 2021). According to Food and Agriculture Authority (AFA), this makes Kenya the second-largest exporter of French beans in Africa after Morocco, contributing about 52% and 61% in value and volume, respectively, of total exports which translates to about $24.01 million in 2021 (AFA 2022; FAO 2022). French bean crop also accounts for 4.32% in value produced in 2021compared to spinach (5.25%), garden peas (13.94%), kales (15.27%), cabbage (17.52%) and tomatoes (32.5%) out of the total vegetables produced (3.1 million tonnes) in 2021 thus valued at $558.8 million (AFA 2022). Despite French bean's economic and nutritional importance, its productivity is still below the estimated yield potential in Kenya. This low yield is linked to a number of biotic and abiotic constrains such as: insect pests, diseases, drought and soil fertility, that affect the productivity and profitability of the crop (Shaban 2021; Yang *et al*. 2023). The most common pests of French bean are the sap-sucking insect pests, among them being aphids, thrips, leafhoppers and spider mites that cause significant losses and quality reduction in the vegetable.

Black bean aphid (BBA), *Aphis fabae* Scopoli, 1763 (Hemiptera: Aphididae), is the most destructive phytophagous insect pest of French beans, with more than 200 host species globally (Saruhan 2018). BBA causes severe damage and loss of economically important crops, including French beans, through direct feeding and/or as efficient vectors of numerous plant viruses (Ullah *et al*., 2023). French beans are usually grown primarily for their edible immature pods, the damage to the plant is to all plant parts, such as leaves, stems, pods and flowers, through sap-sucking, resulting in stunted growth and reduced yields besides playing an essential role as a vector for viral diseases (Srinivasan *et al*. 2019). In addition, the feeding habit of black bean aphids also produces honeydew. This sticky substance attracts sooty mold and other fungi, further reducing the pods' crop productivity and aesthetic value. The severe destructive nature of BBA is associated with their rapid reproduction and parthenogenesis (Zhao *et al*. 2020). Therefore, managing insect pests is a pre-requisite for enhanced horticultural crop productivity and subsequent impact because of their significant damage and loss to the crop (Srinivasan *et al*. 2019). Currently, management of BBA is limited and relies heavily on organophosphate, pyrethroid, neonicotinoids, chordotonal organ modulators and carbamate-based synthetic insecticides (Zaller 2020; Ullah *et al*. 2023). While attempting to suppress BBA, farmers regularly use a cocktail of synthetic chemicals, increasing the farmers' production cost. Continued use of these synthetic chemicals, which target the insect central nervous system, renders them ineffective as BBA rapidly develops resistance to the commonly used insecticides (Mirza *et al*. 2020). Synthetic pesticides are usually non-selective and most often disrupt the activity of natural enemies that would otherwise contribute to keeping the BBA under control. Furthermore, due to their non-selective nature (Ochieng *et al*., 2022), and linkage to multiple health and environmental hazards, there has been an increase in the global ban on most of the synthetic pesticides (WHO 2020). This results in indiscriminative and frequent use of insecticide, which negatively impacts the environment and may lead to the development of resistance by aphids. In addition, several studies (Zaller 2020; Alfaro-Tapia et al. 2021; Ochieng et al. 2022) have agreed that many synthetic insecticides used to manage aphids are not easily degradable. Besides, they persist in soil, leach to groundwater and contaminate the environment. These synthetic chemicals have been documented to pose a serious threat to natural enemies of insect pests and pollinators such as ladybirds beetles, parasitoids, lacewing and hoverflies that can significantly impact food security (Ochieng *et al*., 2022). Therefore, an urgent need to develop alternative approaches and more sustainable means of aphid control to synthetic chemical use in managing insect pests is essential to reduce environmental impact and enhance crop productivity.

Biological control is considered one of the most effective direct green management approaches for many sucking insect pests of French beans with no or limited harm to humans and the environment (Mweke et al. 2018, 2019; Srinivasan et al. 2019; Akutse et al. 2020; Kumar *et al*. 2021). This is considered the first line of protection in an integrated pest management (IPM) program (Srinivasan *et al*. 2019). In the latter case, recent studies have reported that using biopesticide products such as entomopathogenic fungi (EPF) containing *Metarhizium anisopliae* is comparatively safe for natural enemies and the environment with less residue levels. Fungal biopesticides are also biodegradable, more host-specific, considered safer, more sustainable and documented with varying abilities to kill insects thus as a promising alternative to synthetic insecticides (Warra and Prasad 2020; Kumar *et al*. 2021). Effective use of *M. anisopliae* against *Aphis craccivora* (Mweke et al. 2020; Mohamed-Ali et al. 2021), *Aphis gossypii* Glover (Mweke et al. 2018; Erol et al. 2020; Nawaz et al. 2022) *Brevicoryne brassicae* and *Sitobion avenae* Fab (Gebreyohans et al. 2022), *Megoura japonica* (Trinh *et al*. 2020), *Shizaphis graminum* (Sulahudin *et al*. 2020), *Pemphigus bursarius* (Chandler 2010) has been reported. However, there is still need for empirical research to ascertain the effectiveness and efficacy of *M. anisopliae* products against BBA, thus a process that leads to more understanding of the product and its effectiveness as used by farmers. Moreover, there is limited information about the overall efficacy and sustainability of microbial biopesticides containing *M.* species as a management strategy for black bean aphids in sub-Saharan Africa. Because of this, there is a need to evaluate and test microbial biopesticides containing *M. anisopliae* as a potential alternative approach that could minimize residues in produce compared to synthetic

chemicals (Gwynn and Maniania 2017). Therefore, the general objective of the study was to contribute to improved French bean productivity through the use of entomopathogenic fungus containing *Metarhizium anisopliae* to control BBA. With the specific objective to determine the efficacy of *Metarhizium anisopliae*-based concentration against the BBA in French beans, focusing on establishing the optimal dose. The hypothesis was that *Metarhizium anisopliae* entomopathogenic fungi had no significant bioactivity against BBA in French beans.

Materials and methods

Metarhizium anisopliae Formulation

This study used two *Metarhizium anisopliae* based commercial products, Mazao supreme® and Metarril® WP E9, and one synthetic pesticide, Alpha 10 EC (Table 1). Alpha 10 EC is registered in Kenya under the pest control products board (PCPB) for the control of several sucking crop pests including aphids. Mazao supreme®, supplied by Real Ipm Co.(Kenya) Ltd, is registered for the management of aphids while Metarril® WP E9 is not registered in Kenya (PCPB 2022). All test products were sourced from the manufacturers/ distributors; the mycoinsecticides were stored at 4°C, while the synthetic pesticide was kept at room temperature until required for use in the bioassays. The details for the different treatment combinations are shown in Table 1.

Table 1. Description of selected products used in the study

Common name	Active Ingredient	Mode of action	Chemical subgroup	Formulation	Concentration of active ingredient	Manufacturer/distributor in Kenya
Mazao supreme ®	*M. anisopliae*	Contact	EPF	Oil	2×10^8 spores/ml	Real IPM Co. (K) Ltd.
Metarril WP E9	*M. anisopliae*	Contact	EPF	Powder	2×10^8spores/g	Koppert Biological Systems
Biomagic 1.5LF	*M. anisopliae*	Contact	EPF	Liquid	2×10^8 spores/ml	Osho Chemical Industries Ltd.
Alpha 10 EC	Alpha-cypermethrin	Systemic	Pyrethroid	Liquid	8 ml/20l	Osho Chemical Industries Ltd.

Note: WP = Wettable Powder, LF= liquid formulation, EC= Emulsifiable concentrate, EPF= Entomopathogenic fungi, ml= milliliters: Source: Products' label 2022.

Capture, identification and establishment of black bean aphid *invitro* colony

Adult aphids were captured from infested common bean plants grown within the Horticulture Research and Demonstration Field at Egerton University, regardless of sex. The adult aphids were captured by plucking the aphid infested leaves from a bean plant and placed inside a plastic container (7.5 × 5.5 × 9.8 cm) with a screened lid. The aphids were transferred to the Microbiology and Ecology Laboratory, Egerton University, and identified under a stereo microscope using the polyphagous aphid keys (Blackman and Eastop 2000). Only aphids that were identified as the BBA were individually picked out and used to establish an *in-vitro* colony. To establish the *in-vitro* BBA colony, individual French bean leaves were placed on top of moist sterile cotton wool contained in sterile 90 mm plastic Petri dishes. Twelve (12) adult BBA were placed on top of the French bean leaf in the Petri dish. The plates were sealed and incubated on a laboratory bench at 25 ± 2 °C and the BBA were left to multiply over a 21-day period (Boni *et al.*, 2020).

Mass rearing of black bean aphids

After the invitro BBA colony was established, the insects were gently transferred from the Petri dishes with a camel hair brush onto the leaves of French bean plants (*cv.* Enclave)following the method described by Nyaanga *et al.* (2012). The insects were maintained on 3- to 5-weeks old potted plants in cages (2.5 × 2 × 1.5 m, L × W × H) covered with insect-proof net (mesh size: 0.4 mm, AgroZ® Insect Net Nairobi, Kenya). Fresh, aphid-free potted plants were introduced into the cage twice a week.

Metarhizium anisopliae products

Prior to use in the bioassay, the spore concentration for the test products was determined. Mazao Supreme ® (0.1 ml) and Metarril WP E9 (0.1 g) were individually suspended in 100 ml of sterile 0.05% (v/v) Triton X-100 and the fungal spores were quantified using a Neubauer hemocytometer under a light microscope at 400× magnification. Furthermore, spore viability for the two mycoinsecticides was determined by individually spreading 100 µl of spore suspension (3.0×10^6 spores /ml) on Sabouraud dextrose agar (SDA) media in 90 mm sterile Petri dishes. Four sterile microscope coverslips were placed randomly per Petri dish, the Petri dishes were sealed with parafilm and left in an incubator (25 ± 2 °C) for 18 hours. After 18 hours, spore germination was determined by randomly selecting approximately 100 spores under the coverslip and both the germinated and non-germinated spores were counted using a light microscope (400× magnification). The spores were considered to have germinated when the germ tube length was at least twice the diameter of the conidia (Mweke *et al.* 2018). Four replicate Petri dishes were used for each mycoinsecticide.

Using a twenty millilitre (20 ml) syringe procured from a registered pharmaceutical shop in Nakuru town, 10 ml of triton water was measured and put inside a sterile clean universal bottle to reduce the possibility of contamination from pathogens. A stock solution was prepared by measuring 10 ml (Mazao, Biomagic) using a micropipette or 10g (Metarril) using an analytical balance LINB-A10 and dissolving it in a litre of triton water. In addition, 10ml of cypermethrin was also measured from the stock solution, where 0.4ml had been dissolved in one litre of

triton water, which is the recommended dose by the manufacturer. For all *M. anisopliae* products, serial dilution of the stock solution (1×10^{-6} to 1×10^{-1} spores/ml) was done. Before use, each concentration was vortexed for 5 minutes to achieve suspension homogeneity, according to Sayed *et al.* (2019). The serial dilutions were then used to determine the concentration [colony forming units, spores at dilution factor (d.f) of 10^{-6} and 10^{-2} dilution factor hemocytometer determination], the spore viability percentage test was also carried out.

Dose-depended mortality of Black bean aphids

To establish the most effective spore concentration for Mazao supreme® and Metarril WP E9 against the BBA, twelve (12) adult insects were placed on a French bean leaf on top of moist sterile cotton wool in a sterile 90 mm Petri dish. The BBAs were gently transferred using a moistened camel hairbrush. The BBA were sprayed with 10 ml of either Mazao supreme® suspension at 1×10^8, 2×10^8 and 4×10^8 spore/ml or Metarril WP E9 suspension at 1×10^8, 2×10^8 and 4×10^8 spore/ml. For each mycoinsecticide, the three test spore concentrations used in the study were guided by the manufacture's recommended application rate, and these were determined to be: (1) the manufacture's recommended rate, (2) half the recommended rate and (3) double the recommended rate. Control BBA was sprayed with 10 ml of sterile 0.05% Triton X-100. Spraying was performed using Alison's hand sprayer held at 0.5 m from the treatment Petri dishes to ensure uniform delivery of the fungal spores (Erdos *et al*. 2020). Each treatment was replicated five times. Petri dishes were sealed with parafilm and maintained in a sterilized chamber at 26 ± 2 °C, photoperiod of 12:12h (light: dark) and relative humidity of 70 - 75%. Treatments were arranged in a completely randomized design and the experiment was repeated once in time. Observation of dead BBA was conducted daily over a seven-day period. A BBA would be considered dead when no movement was observed after probing with the tip of a camel hairbrush (Macuphe *et al*. 2021). Dead BBA was counted and disinfested with 1% sodium hypochlorite and 70% ethanol, then rinsed three times in sterile distilled water. The disinfected dead BBA were transferred to sterile 90 mm Petri dishes lined with a moist sterile filter paper. The Petri dishes were sealed with parafilm and incubated (25 ± 2 ◦C) for 7 days to monitor for mycosis (Mweke *et al*. 2020).

Comparison with synthetic insecticide

In this study, mortality of adult BBA due to exposure to Metarril WP 90 and Mazao supreme was compared with that of a commercial insecticide alpha-cypermethrin and *M. anisopliae* based biocontrol agent Biomagic 1.5 LF. In Kenya, Biomagic is a registered biological control agent against aphids (PCPB 2022). The experiment set up in this bioassay was designed as described above; however, in this bioassay, a single spore concentration for each test product was used. The spore concentrations that caused the highest BBA mortality for Mazao supreme (2×10^8 spores/ml) and Metarril WP (2×10^8spores/ml) were compared to Biomagic 1.5 LF (2×10^8 spores/ml) and alpha-cypermethrin (0.4ml /litre). Both Biomagic and alpha-cypermethrin were prepared according to manufacturer's guidelines. Ten (10) ml of the test product were sprayed per Petri dish containing 12 adult BBA. Control BBA was sprayed with 10 ml of sterile 0.05% Triton X-100. Treatments were replicated five times and arranged in a Completely Randomized Design (CRD). Plates were sealed with parafilm and maintained in a sterilized chamber at 26 ± 2 °C. Assessments for mortality and mycosis were performed as described before.

Data analysis

Mortality data were first corrected for natural mortality (Abbott 1925) and for the fulfilment of normality (Shapiro & Wilk, 1965) and homogeneity of variances (O'Neill & Mathews, 2002) prior to analysis. The statistical software SAS version 9.4 (SAS Institute, Cary, NC, 2022) was used for data analysis. Corrected percent BBA mortality data were subjected to analysis of variance (ANOVA) using a generalized linear model assuming a binomial distribution. Probit regression analysis was conducted to determine the median lethal time (LT_{50}) for treatments that caused > 50% mortality of aphids over time post-treatment application. Tukey's honestly significant difference test was used to separate treatment means significance at 5%.

Results

Spore percentage viability

The spore viability percentage tests results showed that *M. anisopliae* products did not differ significantly, where Mazao supreme had 95.6%, Metarril wettable powder had 96.9% and biomagic 1.5 liquid formulations treatment had 94.5% (Table 2).

Table 2: Spore viability (Mean ± standard error) of different products of M. anisopliae. Means in a column followed by same letter are not significantly different using Tukey's test at $p < 0.05$

***Metarhizium anisopliae* products**	**Spore viability (%)**
Mazao supreme	95.6± 0.63[a*]
Metarril E9	96.9± 1.60[a]
Biomagic	94.5± 1.00[a]

*Means followed by the same letter within a column are not significantly different according to Tukey's Honestly Significant Difference at ($p \leq 0.05$).

Mycosis test

The results showed that *M. anisopliae* biopesticide caused 96 % of the aphid death for treatment application. Green muscardine was observed on the cadavers of aphids

treated with *Metarhizium anisopliae* as confirmation for fungal growth compared to water and alpha-cypermethrin treatments which showed no sign of green muscardine (Fig.1).

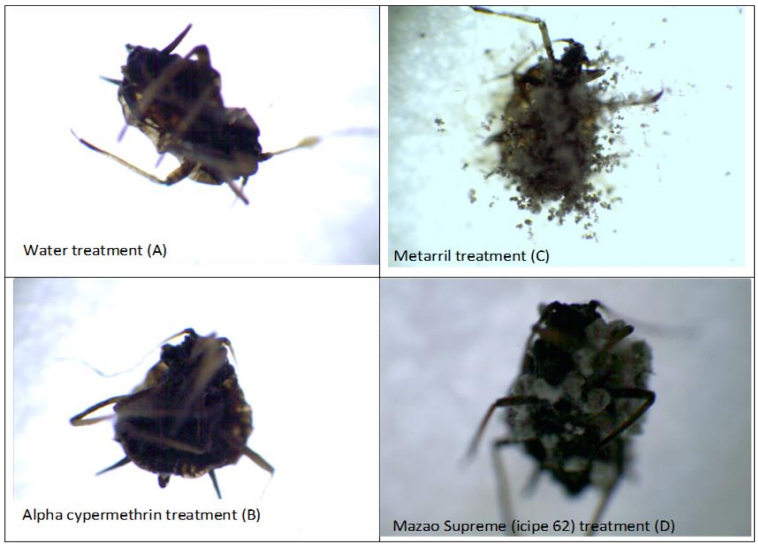

Figure 1. Microscopic mycosis test in the laboratory efficacy, where water treatment (a) and alpha-cypermethrin (b) all without green muscardine growth, while Metarril (c) and Mazao supreme treatment (d) showed green muscardine growth on the cadaver of black

Efficacy of *Metarhizium anisopliae* fungi against black bean aphid

Bioassay one result showed that two biopesticide products of Mazao supreme and Metarril E9, at different concentrations, significantly (X^2= 2.202, df=1, p < 0.00007) influenced the mortality of black bean aphids. There was a significant difference (X^2=2.070, df=1, p < 0.05) in black bean aphid mortality between Metarril E9 applied at 1×10^8 spores /g and Mazao supreme at 1×10^8 spores /ml only at day 5- and 6-days exposure time, with Metarril E9 causing more black bean aphid mortality compared to Mazao supreme post-treatment application (Fig. 2). Also, Metarril E9 applied at 2×10^8 spores /g caused significantly more black bean mortality compared to Mazao supreme 2×10^8 spores /ml at 3-, 4- and 5-day exposure time (Fig. 2). There was, however, no significant difference (X^2=0.023, df=1, p > 0.05) in black bean mortality for Metarril WP E9 applied at 4×10^8 spores /g and Mazao supreme applied at 4×10^8 spores/ml post-treatment application. Among all the treatments, Mazao supreme (4×10^8 spores /ml) and Metarril E9 (4×10^8 spores /g) caused highest average mortality (95%) of black bean aphid, followed by 2×10^8 spores /ml of Mazao supreme and 2×10^8 spores /g of Metarril E9 (86%) and lowest mortality (67%) was obtained at 1×10^8 spores /ml of Mazao supreme and 1×10^8 spores /g of Metarril E9.

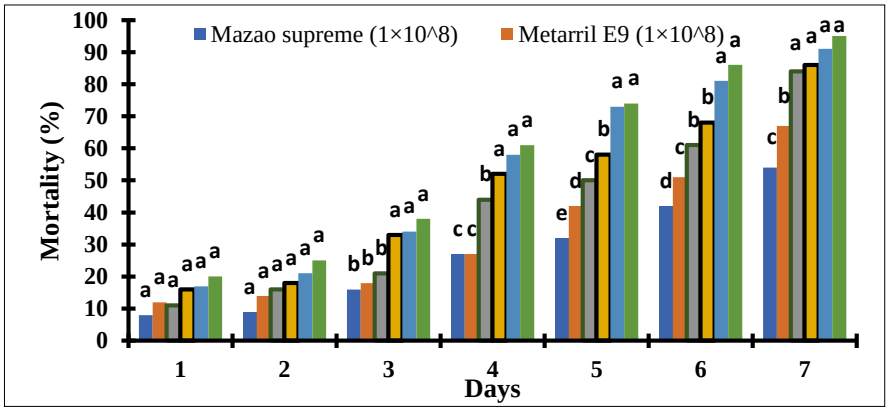

Figure 2. Mortality of black bean aphids exposed to the Metarhizium anisopliae products at different concentrations, comparison between 1×10^8 conidia/ml of Mazao supreme and 1×10^8 spores/g of Metarril E9-, 2×108 spores /ml of Mazao supreme and 2×10^8 spores /g of Metarril E9- concentration as a control, lastly 4×108 spores /ml of Mazao supreme and 4×108 spores /g of Metarril E9 concentration, respectively over time.

Median lethal time (LT50) results in a comparison between Mazao supreme and Metarril isolated (time each concentration took to cause 50% of aphid mortality) were significantly different (F=10.06, df=1, p < 0.00004) at different concentrations. The 1×108 spores /ml concentrations of Mazao supreme and 1×108 spores /g of Metarril took the longest time to kill 50% of the bean aphid population, whereas 4×108 spores /ml of Mazao supreme and 4×108 spores /g of Metarril concentration took the shortest time of four (4) days this is as shown in Table.3.

Table 1. Median Lethal time (LT_{50}) for Mazao supreme and Metarril at different concentrations as the time it took to cause 50% aphid mortality after treatment application

***Metarhizium anisopliae* products**	**Concentration**	**LT_{50}***
Mazao supreme	1×10^8 spores/ml	7.0±0.9a
	2×10^8 spores/ml	5.0±0.4b
	4×10^8 spores/ml	4.8+0.3b
Metarril E9	1×10^8 spores/g	7.0±0.9a
	2×10^8 spores/g	5.0±0.4b
	4×10^8 spores/g	4.0±0.3b

Means followed by the same letter within a column are not significantly different according to Tukey test ($p < 0.05$). Bioassay one, probit analysis results showed that concentration and exposure time-dependent increase in the efficacy of Mazao supreme and Metarril WP against black bean aphids (Fig 3 and 4). The black bean aphid mortality increased with increased biopesticide concentrations for both Mazao supreme and Metarril WP; however, this depended on the exposure time. The seven-day exposure time showed higher bean aphid mortality at all concentrations tested for Mazao supreme (Fig 3) and Metarril E9 (Figure 4). Shorter exposure times of 2, 6 and 7 days for Mazao supreme applied at 1×10^8 spores/ml (a) and at 2×10^8 spores /ml (b) at 3,6 and 7 days were significant, while the same product applied at 4×10^8 spores /ml (c) was significant at all day's post-treatment exposure. Metarril E9 applied at 1×10^8 spores /g at 5, 6 and 7 days (a) applied at 2×10^8 spores /g at 1, 6 and 7 and applied at 4×10^8 spores /g at 4 and 7, were significant, respectively (Fig 4).

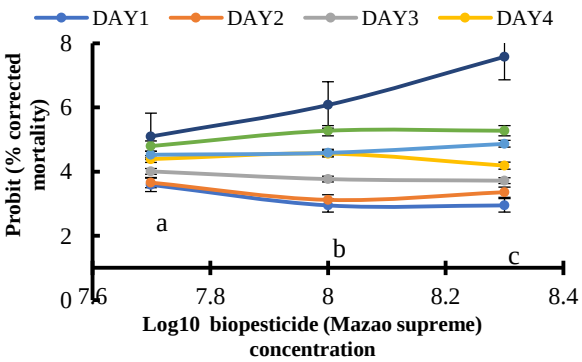

Figure 3. Concentration-response curves for Mazao supreme over time (days), effects Mazao supreme applied at 1×10^8 spores /ml (a), 2×10^8 spores /ml (b) and 4×10^8 spores /ml concentration against aphid mortality days post-treatment application. Error bars connected within the different days within each concentration show no significant difference in aphid mortality caused.

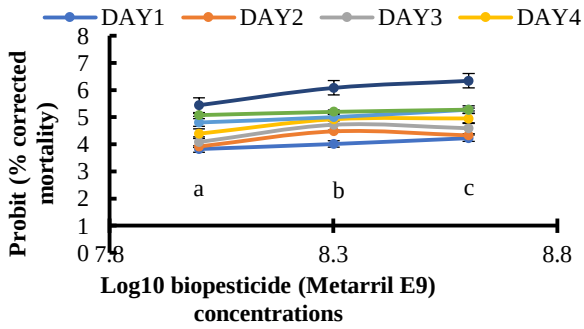

Figure 4: Concentration-response curves for Metarril E9 over time (days), effects Metarril E9 applied at 1×10^8 spores /g (a), 2×10^8 spores /g (b) and 4×10^8 spores /g concentration against aphid mortality days post-treatment application. Error bars connected within the different days within each concentration show no significant difference in aphid mortality caused.

Results for probit regression analysis for median lethal concentrations (LC_{50}- concentration which caused >50% of black bean aphid mortality) values showed that exposure time indicating >50% mortality was $0.40\text{x}10^8$ at 7 days for Mazao supreme Metarril WP, and $0.43\text{x}10^8$ at day 7 as shown in Table. 4 below.

Table 2. LC_{50} values for Mazao supreme and Metarril WP for exposure time

Product	**7 days**
Mazao supreme	$0.40\text{x}10^8$
Metarril WP	$0.43\text{x}10^8$

Efficacy of *Metarhizium anisopliae* fungi against black bean aphids

Bioassay two (2) experiment, where Mazao supreme (2×10^8 spores /ml) and Metarril E9 (2×10^8 spores/g) were further compared to biomagic (2×10^8 spores /ml), a registered biopesticide containing *Metarhizium anisopliae* and alpha-cypermethrin a synthetic commercial insecticide against black bean aphid for efficacy. The results showed that Mazao supreme and Metarril WP E9 *Metarhizium anisopliae* biopesticide applied significantly affected black bean aphid mortality (X^2=2.070, df=1, $p < 0.0005$). Alpha-cypermethrin caused the highest corrected percentage mortality (99.3%a) of aphids, followed by Metarril (84.2% ab), Mazao supreme (80.7% ab) and biomagic recorded the lowest (68.4% b) aphid percentage mortality (Fig.5). However, there were no significant differences between Metarril E9 (84.2% ab) and Mazao supreme (80.7% ab) in percentage mortality caused to black bean aphids. In general, among the biopesticides, Metarril E9 causes the highest mortality, followed by Mazao supreme and the lowest mortality was recorded for the treatment of biomagic. Metarril E9 caused higher mortality percentage compared to Mazao supreme post-treatment application. Comparison between Mazao supreme and Metarril E9 to biomagic and alpha-cypermethrin for the median lethal time, LT_{50}, against black bean aphids over time. The results showed a significant difference in the median lethal (LT_{50}) (F=13.22, df=3, $p < 0.0005$) between the different treatments against black bean aphid post-treatment application. Alpha-cypermethrin had the lowest median

lethal time (LT_{50}), followed by Metarril E9, Mazao supreme and biomagic had the longest time to cause 50% aphid mortality, respectively (Fig.5). Among the biopesticides, there was no significant difference in the median lethal time (LT_{50}); however, Metarril had the lowest LT_{50}, followed by Mazao supreme and the highest for the treatment of biomagic (Fig.5)

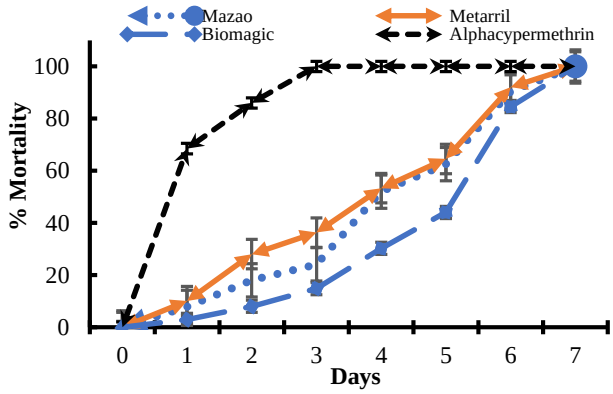

Figure 5. Percent mortality (mean ± S.E.) of black bean aphid treated with biopesticide products containing *M. anisopliae* as Metarril (2×10^8 spores /g), Mazao supreme (2×10^8 spores /ml), biomagic (2×10^8spores /ml) and synthetic pesticide alpha-cypermethrin response over time (days).

Comparison between Mazao supreme and Metarril E9 to biomagic and alpha-cypermethrin for the median lethal time, LT_{50}, against black bean aphids over time. The results showed a significant difference in the median lethal (LT_{50}) ($F=12.54$, $df=3$, $p < 0.0005$) between the different treatments against black bean aphid post-treatment application. Alpha-cypermethrin had the lowest median lethal time (LT_{50}), followed by Metarril E9, Mazao supreme and biomagic had the longest time to cause 50% aphid mortality, respectively (Fig. 6). Among the biopesticides, there was no significant difference in the median lethal time (LT_{50}); however, Metarril had slightly the lowest LT_{50}, followed by Mazao supreme and the highest for the treatment of biomagic (Fig. 6)

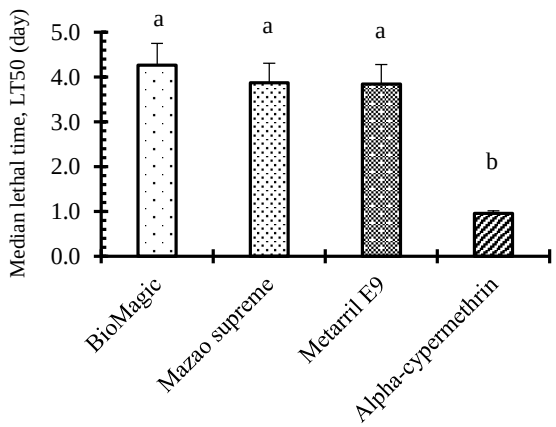

Figure 6. Median lethal time (LT50) against black bean aphids, a comparison between Mazao supreme (2×10^8 spores /ml), Metarril E9 (2×10^8 spores /g), biomagic (2×10^8 spores /ml) and alpha-cypermethrin over time. Different letters above bars (Mean ± SD) within the same day indicate significant differences according to Tukey test ($p < 0.05$).

Effects of different products on the cumulative number of live aphid nymphs.

At the 7-day post-treatment exposure, the results indicate that the number of nymphs significantly varied between products ($X^2=0.0002$, $df=8$, $p<0.00002$). The average means after seven days of the nymphs varied significantly among products. Distilled water had the highest mean (13.5 ±0.56, number of aphid nymphs and standard error) and alpha-cypermethrin with the least mean number of aphid nymphs (0.2±0.56). Treatments of biomagic (12.7±0.7) and distilled water (13.7±0.9) were not significantly different ($X^2=0.512$, $df=1$, $p > 0.05$) in the number of aphid nymphs post treatments application. Mazao at $1\text{x}10^8$ spores / ml (9.1±0.5) and Metarril at $1\text{x}10^8$ spores /g (3.0±0.1) differed significantly on the number of aphid nymphs post treatment application as shown in Fig.7.

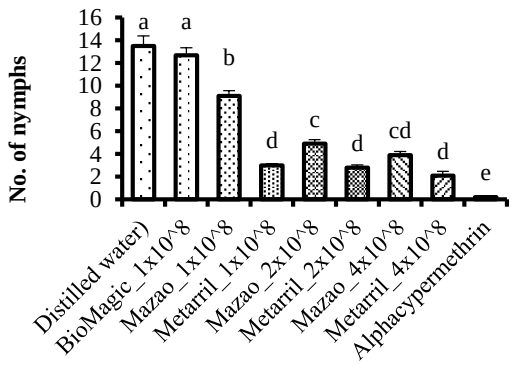

Figure 7. Effects of the different doses of products on the cumulative number of live aphid nymphs under laboratory conditions.

Similar trend was observed between Mazao at $2\text{x}10^8$ spores / ml and Metarril at $2\text{x}10^8$ spores /g, Mazao at $4\text{x}10^8$ spores /ml and Metarril at $4\text{x}10^8$ spores /g in regard with the number of live nymph's post treatment application. The number of live nymphs on Metarril treated plates were fewer than those of Mazao supreme treatments plates. Among the *Metarhizium anisopliae* treatments, Metarril had the lowest average number of live nymphs, followed by Mazo and then biomagic treatment.

Discussion

The use of biopesticides has the potential to reduce the economic losses caused by black bean aphids. Several research studies evidenced the promising ability of some biopesticides containing particularly *Metarhizium anisopliae* (Mweke et al. 2018, 2019; Akutse et al. 2020; Boni et al. 2020). Therefore, biopesticide products containing *Metarhizium anisopliae* use have been recommended as an important add-up to the integrated pest management programs. Based on this study, the efficacy results demonstrated the effect of *Metarhizium anisopliae*, such as Mazao supreme (ICIPE62) and Metarril E9 were effective against black bean aphids. This study showed that all different concentrations caused mortality to the aphid. This study's results also showed

that the efficacy of black bean aphid mortality varied between 60.1%, the lowest and 95.5%, the highest. A similar study by Trinh *et al.*. (2020) reported maximal mortality (85.3%) and lowerest (60.0%) at the highest concentration of *Metarhizium anisopliae* at eight days post-treatment. Mweke *et al.*. (2018) conducted a study on different isolates of *Metarhizium anisopliae* and reported ICIPE62 (1×10^8 spores/ml) mortality on *Aphis craccivora* (90% and 45%) and LT_{50} (3.3 to 6.3 days) post-treatment. The latter findings by Mweke *et al.*. (2018) were associated with the difference in fungal strains as some strains take longer for the pathogen to contact and cause harm to the target insect pest. Srinivasan *et al.* (2019) reported a mortality rate (83.23%) caused by *Metarhizium anisopliae* against aphids and also attributed the effects to pathogen strain isolated and its ability to cause secondary infection to target pests or hosts within a given period.

In this study, alpha-cypermethrin treatments had the highest aphid mortality (99.3%), followed by Metarril (84.2%) and Mazao supreme (80.7%) compared to the control biomagic (68.4%). However, Mazao supreme (2×10^8 spores /ml) and Metarril WP (2×10^8 spores /g) did not differ in mortality rate caused, respectively. Significant effects at different concentration levels of Mazao and Metarril on aphid mortality were also recorded. Although there were variations in efficacy rate among the different concentrations of Metarhizium anisopliae products regarding mortality, all concentrations caused mortality to aphids, as recorded in this study. A study by Latiff *et al*. (2022) reported and confirmed that aphids are susceptible to *Metarhizium* species fungal treatment irrespective of the host plant. The strain, characteristic and concentrated levels are associated with the virulence rate of the pathogen isolate of fungi. Conversely, the concentration of each isolate, as in the number of conidial per gram or ml, is associated with enhancing the ability of the isolate to cause mortality to the target pest (Iqbal *et al*. 2021; Qubbaj and Samara 2022). Mweke *et al.* (2018) found that *Metarhizium anisopliae* isolates ICIPE 62 produced more conidia on aphid cadavers and with a high ability and enhanced chances for secondary infection to the host. The ability of the pathogen to cause secondary infection to the new host increases its efficacy rate and the mortality percentage of the target insect pest species. As a result, secondary infection could enhance the ability to manage the population target pest and lower survival rates of target pest post-treatment application. Latiff *et al.*. (2022) findings reported a difference in mortality and virulence rate of five *Metarhizium anisopliae* against *Aphis gossypii* reared on *Capsicum annum* (chilli) and *Solanum melongena* (brinjal). The finding was associated with the initial isolate concentration that the insect received and the fungal strain. The difference in efficacy could also be associated with the ability of the isolate to produce an immense extent of toxins associated with higher mortality or focus more energy on vegetative growth, thus lowering the mortality rate on the target insect pest. A study by Erol *et al.*. (2020) suggested that the difference in the fungal ability to cause mortality depends on the secondary bioactive metabolites produced by pathogen associated with the initial formulation of the isolate affecting the isolate infection rate. Similar result by Mweke *et al*. (2019) reported enhanced performance of *Metarhizium anisopliae* oil formulation than aqueous due to increased contact with the host insect pest. Kisaakye *et al*. (2021) study reported *Beauveria bassiana* isolates, ICIPE 648, ICIPE 660 and ICIPE 273 caused over 80% mortality to adult banana weevil associated to with highest spores per cadaver.

The study results also showed that application dose significantly differed in the number of live nymphs at 7 days post-treatment. Metarril double concentration and Mazao supreme recommended concentration had average live nymphs of 2.1 and 2.8, respectively, compared to untreated control (13.5 nymphs). This is could be attributed to fungal strain formulation. Mweke *et al*. (2019) reported performance on oil than aqueous formulations. In addition, Mweke *et al*. (2018) also found that *M. anisopliae* isolate ICIPE 62 produced more conidia on aphid cadavers, increasing their virulence against post-treatment. This could be attributed to the lower survival average live nymphs 7 days post-treatment application. Based on the effect of dose on the average nymphs' number 7 days post-treatment application. This study found that Metarril double and Mazao supreme recommended doses lowered the average number of live nymphs 7 days post exposure and aphid mortality rate but with no significant difference. This study indicates that the *Metarhizium anisopliae* Mazao supreme (ICIPE62) and Metarril are potential candidates for the environmentally sustainable management of black bean aphids. Thus, further evaluation is necessary to confirm the efficacy potential under field conditions.

Conclusion

This study revealed that *M. anisopliae* product concentrations caused mortality to black bean aphids after treatment application. Alpha-cypermethrin, a synthetic insecticide, was very effective against black bean aphids, causing up to 99.33% mortality rate and a lower median lethal time (LT_{50}) to cause 50% mortality to black bean aphids in one day compared to other treatments. Among biopesticides, Metarril E9 (2×10^8 spores/g) concentration comparative to alpha-cypermethrin caused the highest mortality rate (84.2%) and with a median lethal time of 3.8 days, while Mazao supreme also caused aphid mortality (80.7%) and took 3.9 days to cause mortality to 50% of aphids. *Metarhizium anisopliae* treatment caused mortality to aphid nymphs. Based on the concentration and cost of the product comparison, 2×10^8 spores /g of Metarril with the highest mortality and reduced cost compared to Mazao supreme was recommended for further trials under field conditions to evaluate their effectiveness with border cropping. Also, to explore the possibilities of incorporating *Metarhizium anisopliae* as part of the integrated pest management of black bean aphids in the open field condition. Small holder farmers could use microbial biopesticides like Metarril E9 on their increased accessibility, affordability and ease in application methods for safer insect pest management with no pre- and post-harvest interval requirement,

adhering to food safety risks and avoiding environmental pollution and non-target beneficial arthropod killing. Different aphid species may vary in their susceptibility to biopesticides, *Metarhizium anisopliae* showed promising potential against black bean aphids but further research is needed for other species, ecological implications and different crop systems.

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