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Research Article

Vegetable oils influence the persistence of entomopathogenic fungi isolates *Beauveria bassiana* and *Metarhizium anisopliae*

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ABSTRACT

The microbial biocontrol agents *Beauveria bassiana* (*B. bassiana*) and *Metarhizium anisopliae* (*M. anisopliae*) are highly effective in controlling various insect pests. However, their effectiveness often depends on biopesticide formulation, which could be affected by unfavourable environmental conditions. This study aimed to determine the compatibility of eight vegetable oils derived from raw palm, refined palm, sunflower, cotton, soybean, groundnut, neem, and canola in formulations of *B. bassiana* and *M. anisopliae*. This compatibility of oils with fungi was investigated by measuring fungal hyphal growth, conidial yield, fungal biomass, and conidial viability. The results showed a significant inhibition of *B. bassiana* growth in neem (2.08%) and raw palm oils (0.52%) and *M. anisopliae* in neem oil (0.28%). Conidia yield was high in the soybean (29.3 x 10^6 conidia/mL), groundnut (27.2 x 10^6 conidia/mL) and refined palm (23.83 x 10^6 conidia/mL) oils as well as vegetative growth for *B. bassiana*, while conidiogenesis of both fungal species was highest in soybean oil (29.3 x 10^6 conidia/mL and 44.4 x 10^6 conidia/mL for *B. bassiana* and *M. anisopliae* respectively). Fungal viability varied considerably, with the highest density of viable propagules obtained in Potato Dextrose Agar (PDA) culture media amended by sunflower oil for *B. bassiana* (43.2 x 10^4 viable propagules/ml) and in raw palm oil for *M. anisopliae* (97.4 x 10^4 viable propagules/ml). Taking these four parameters into account, it was concluded that the compatibility or toxicity of the oils to the two fungal species was mixed. The results of this study were relevant to the formulation of biopesticides based on effective strains of the two fungal species and constituted an essential component for this research program aimed at formulating biopesticides and testing their pathogenicity against insect pests under field conditions.

Keywords: Vegetable oil, Cameroonian indigenous isolates, entomopathogens, compatibility, biopesticide.

RÉSUMÉ

Les agents microbiens de biocontrôle *Beauveria bassiana* (*B. bassiana*) et *Metarhizium anisopliae* (*M. anisopliae*) se sont révélés très efficaces pour lutter contre divers insectes nuisibles. Cependant, leur efficacité dépend souvent de la formulation du biopesticide, qui peut être affectée par des conditions environnementales défavorables. Cette étude visait à déterminer la compatibilité de huit huiles végétales dérivées (huile de palme brute, de palme raffinée, du tournesol, du coton, du soja, de l'arachide, du neem et du canola) dans les formulations à base de *B. bassiana* et de *M. anisopliae*. Cette compatibilité des huiles avec les champignons a été étudiée en mesurant la croissance des hyphes fongiques, la production de conidies, la biomasse fongique et la viabilité des conidies. Les résultats ont montré une inhibition significative de la croissance de *B. bassiana* dans les huiles de neem (2,08%) et de palme brute (0,52%) et de *M. anisopliae* dans l'huile de neem (0,28%). La production de conidies était élevée dans les huiles de soja (27,2 x 10^6 conidies/mL), d'arachide (23,83 x 10^6 conidies/mL) et de palme raffinée, de même que la croissance végétative de *B. bassiana*, tandis que la conidiogenèse des deux espèces fongiques était plus élevée dans l'huile de soja (29,3 x

10^6 conidies/mL et 44,4 x 10^6 conidies/mL for *B. bassiana* and *M. anisopliae* respectivement). La viabilité fongique a considérablement varié, la densité la plus élevée de propagules viables étant obtenue dans le milieu de culture PDA (Pomme de terre Dextrose Agar) amendé par l'huile de tournesol pour *B. bassiana* (43,2 x 10^4 propagules viables /ml) et par l'huile de palme brute pour *M. anisopliae* (97,4 x 10^4 propagules viables /ml). En tenant compte de ces quatre paramètres, la compatibilité ou la toxicité des huiles à l'égard des deux espèces fongiques est restée mitigée. Les résultats de cette étude semblent pertinents pour la formulation de biopesticides à base de ces souches efficaces des deux espèces fongiques et constitueraient un élément essentiel pour les programmes de recherche visant à formuler des biopesticides et à tester leur pathogénicité vis-à-vis des insectes nuisibles dans des conditions de terrain.

Mots-clés: Huile végétale, isolats camerounais endogènes, entomopathogènes, compatibilité, biopesticides.

1. INTRODUCTION

Food safety in Africa is a critical and complex issue that affects public health, economic development, and food security across the continent. Crop protection is essential for safeguarding agricultural production against pests, diseases, and weeds that can significantly reduce yields. A market study was carried out to examine pesticide residues in selected food products in Africa, while at the same time, providing a protocol for the study of pesticide residues is strongly required (Jiang et al., 2023). The results call into question control methods, which remain largely chemical, suggesting the urgent need to propose alternatives. Biopesticides and organic pesticides, derived from natural sources like plants, microbes, and minerals, are increasingly recognized as a safer alternative to synthetic pesticides. They often have specific modes of action, lower environmental impact, and can be integrated into Integrated Pest Management (IPM) programs. As part of a strategy to reduce insect pest damage towards some targeted crops, such as cocoa, coffee, fruits, and vegetables, etc. formulated entomopathogenic fungi (EPF) have been used as organic food in a production system where pests are essentially managed by biological agents (Batta et al., 2011). It has been furthermore reported that the persistence of EPF propagules in the environment is an important factor in their success as biological control agents (BCA).

Entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) Sorokin, are often applied as an aqueous suspension of conidia directly to the target insects either by immersion or by spraying (Behle, 2006; Mahot et al., 2019; Membang et al., 2020; Negrete González et al., 2018). Their effectiveness has been proven under laboratory conditions against the larvae of *Sahlbergella singularis* Haglund (Mahot et al., 2019). However, the effectiveness of the aqueous formulation under field conditions has not been confirmed, as abiotic factors such as high temperature, low humidity, and exposure to ultraviolet (UV) radiation could be detrimental to the conidia. Aqueous conidial formulations of *B. bassiana* and *M. anisopliae* have often shown a loss of conidial viability within a short period from application under field conditions (Behle, 2006; Paula et al., 2019).

Recent attention has shifted to using oils for formulating EPFs due to the high protective action post-application (Boruah et al., 2015; Mola & Afkari, 2012). The use of oil as an adjuvant in BCA applications is known to protect agents from harmful environmental factors and enhance their activity at the specific site on/in the targeted insect pest (Hong et al., 2005). Several oils used in formulated biopesticides might affect the action of entomopathogenic fungi (Mahot et al., 2019). Studies have reported that oil formulations increase adhesion of the conidia and promote infectivity of insects, even under unfavourable environmental conditions, such as relatively low or high humidity and/or temperatures (Alves, 1998; Inglis et al., 2002; Umaru & Simarani, 2022). Furthermore, oil could protect the fungal conidia from the UV spectrum of sunlight (Umaru & Simarani, 2022). Entomopathogen propagules must be appropriately formulated before their recommendation as biocontrol agents against insect pests or other harmful arthropods.

The overall objective of this study was to determine the compatibility of oil mixture with *B. bassiana* and *M. anisopliae* isolates from Cameroon. Specifically, we determined and evaluated the effects of eight vegetable oils on vegetative growth, conidiation yield, biomass, and germination of *B. bassiana* and *M. anisopliae* Cameroonian indigenous isolates.

2. MATERIALS AND METHODS

2.1. Fungal isolates

Two isolates of *Beauveria bassiana* (BIITAC 6.2.2) and *Metarhizium anisopliae* (MIITAC 11.3.4) obtained from the microbial collection of the International Institute of Tropical Agriculture (IITA-Cameroon) were used in this

study. These entomopathogenic fungi (EPF) strains were originally isolated from banana farm soils using the insect bait method and morphologically identified (Membang, 2013; Membang et al., 2020; Moore & Prior, 1993). They were selected among other EPF strains for their high virulence against *S. singularis* and for their high horizontal transmission potential (Mahot et al., 2019). The fungi were maintained at 25 ± 1°C and 85 ± 5% relative humidity and 12L:12D photoperiod on Potato Dextrose Agar (PDA) supplemented with 0.1% streptomycin.

2.2. Inoculum preparation

Fifteen-day-old cultures were used to prepare conidial suspension following the method described by (Meyling, 2007). Cultures were removed with a flame-sterilized spoonbill and suspended in tubes containing 20 mL of Sterile Deionized Water (SDW) mixed with a 0.1% Tween 80 solution, followed by vortexing for 60 seconds. The conidial suspension was filtered with sterile Whatman No. 1 filter paper, and the concentration was determined and adjusted to 1 x 10^4 conidia/ml using a haemocytometer. An aliquot of 1 ml suspension was transferred onto PDA plates, spread with a sterile bent glass rod, and incubated in the laboratory for 4 days.

2.3. Vegetative growth response with different oils

Eight vegetable oils (raw palm, sunflower, cotton, refined palm, soybean, groundnut, neem, and canola) were incorporated into PDA at a concentration of 0.5% (v/v) prior to solidification at approximately 50ºC under aseptic conditions (Visalakshy et al., 2006). EPF isolates cultured on PDA for 4 days were cut into 4 mm discs. Following the solidification of the amended PDA, the cut discs were inverted and placed in the centre of Petri dishes (ChaiChing et al., 2014; Senthamizhlselvan et al., 2010). The control consisted of PDA without oil amendment. Five replicates were used per treatment. The fungi were incubated in the IITA laboratory for 30 days, and colony diameters were recorded every 5 days. The Area Under Mycelial Growth Curve (AUMGC) was calculated by using colony diameter measurements and the time interval (days) between successive observations (Campbell & Madden, 1990). AUMGC was calculated according to the following mathematical formula:

$$AUMGC = \sum_{i=1}^{n} \left(\frac{X_{i+1} + X_i}{2}\right) (t_{i+1} - t_i)$$

where n is the total number of observations, X_i is the diameter measurement in mm at the i-th observation, X_{i+1} is the colony diameter measurement in mm at the i+1-th observation, and t_i is the time (in days) at the i-th observation, respectively.

Inhibition ratios caused by oil were also calculated relative to the control using the equation by (Hendricks et al., 2017):

$$Inhibition\ Ratio\ (\%) = \frac{Average\ Control\ Diameter - Average\ Treatment\ Diameter}{Average\ Control\ Diameter}$$

2.4. Conidia production assessment response in different oils

Conidia production was determined from 30-day-old cultures of the previous vegetative growth assessment. All Petri dishes used in vegetative growth were scraped with a flame-sterilized spoonbill. The fungi were then suspended in tubes containing 10 ml of sterile deionized water (SDW) mixed with a 0.1% solution of Tween 80. The conidial suspension from each treatment and replicate was vortexed for 60 seconds. The conidial concentrations of the suspensions were enumerated using a haemocytometer (Senthamizhlselvan et al., 2010).

2.5. Assessment of the oil's effect on Biomass Production

Fungal biomass production was evaluated in Potato Dextrose Broth (PDB). Eight test tubes with 20 ml of liquid medium of Potato Dextrose Broth (PDB) were prepared, and each was amended with 0.5% (v/v) of the respective oil. The conidial suspension was adjusted to 10^6 conidia/ml as previously described. Each test tube was inoculated with 1 mL of conidial suspension (Otgonjargal et al., 2015; Senthamizhlselvan et al., 2010). The control consisted of PDB medium without oil. The test tubes were incubated at 25 ± 1°C for 15 days. On day 15, the mycelium was filtered through pre-weighed Whatman No. 1 filter paper and oven-dried at 70°C 40ºC. Final weights were recorded using a high-precision jewellery balance (Smart Weigh, China).

2.6. Conidia viability

The determination of conidial viability through germination rate is typically challenging in the presence of oil for species with small round conidia (e.g., *B. bassiana*) due to the formation of tiny emulsion droplets on the medium surface, which complicates distinguishing germinated from ungerminated conidia (Inglis et al., 2012). To overcome this challenge, conidial viability was assessed through an indirect enumeration method described by (Inglis et al., 2012). For instance, a dilution plating method was adopted and developed. Original suspensions of *B. bassiana* and *M. anisopliae* were serially diluted tenfold. An aliquot of 100 µl from the 10^{-2} dilution was uniformly spread on the PDA medium surface amended with 0.5% (v/v) oil, and then the culture was incubated. The 10^{-2} dilution culture contained well-distributed spores, and colonies were readily counted. Control treatments were prepared by inoculation on PDA not amended with oil. The cultures were incubated for 7 days in a dark room at 25 ± 1°C, 70–80% (RH). The number of colonies was counted as Colony Forming Unit (CFU), and the density of viable propagules was calculated per unit volume following the formula (Inglis et al., 2012):

$$Number\ of\ viable\ propagules\ per\ mL\ (CFU/ml) = \frac{Number\ of\ colonies\ \mathrm{x}\ Tube\ dilution\ factor}{Plating\ dilution\ factor}$$

2.7. Data analysis

The data on AUMGC, conidia production, fungal biomass, and CFU of *B. bassiana* and *M. anisopliae* were summarized in a spreadsheet program such as Microsoft Excel. Due to the non-normal distribution of residuals (Shapiro-Wilk test, significance at 5%), AUMGC, Biomass, and CFU were used in Generalized Linear Models (PROC GLM), with oil as a fixed effect. The Gaussian model and log-link function were applied for AUMGC and biomass, and the Quasi-Poisson model with logit-link function for CFU values. Post-hoc comparisons of means were followed by the Tukey Honestly Significant Difference (HSD) test to separate the means at $P \leq 0.05$. All statistical analyses were performed in R 3.4.3.

3. RESULTS

3.1. Vegetative growth – oil response

The AUMGC of *B. bassiana* and *M. anisopliae* varied with vegetable oil and post-exposure duration. In general, AUMGC values obtained in both cases (Tables 1 and 2) with different oil treatments, including control, increased with the number of exposure days.

Table 1. AUMGC of *Beauveria bassiana* with different oil treatments

Oil	Area Under Mycelial Growth Curve (AUMGC) ± SD across observation (days)					
	5	**10**	**15**	**20**	**25**	**30**
Refined palm	30.8 ± 4.9[a]	64.5 ± 6.2[a]	86 ± 13.8[a]	106 ± 13.8[a]	121.5 ± 21.3[a]	141.7 ± 30.9[a]
Soybean	29.0 ± 2.5[a]	60.5 ± 7.5[a]	85 ± 16.4[a]	104.5 ± 14.3[a]	120.7 ± 16.5[a]	138.5 ± 20.6[a]
Groundnut	28.2 ± 3,3[a]	59.7 ± 9.7[a]	83.7 ± 14.6[a]	102.5 ± 19.4[a]	116.7 ± 14.5[a]	133.7 ± 20.3[a]
Cotton	28.2 ± 3.3[a]	54.7 ± 8.4[4]	71.5 ± 6.6[a]	91.7 ± 1.7[a]	105.2 ± 21.5[a]	126.2 ± 27.3[a]
Sunflower	26.6 ± 4.1 [a]	53.5 ± 4.4[a]	71.2 ± 4.4[a]	90 ± 3.4[a]	105.7 ± 3.6[a]	123.2 ± 3.1[a]
Canola	25.0 ± 8.4[a]	52.7 ± 13.6[a]	70.2 ± 19.6[a]	86.5 ± 14.1[a]	105.7 ± 6.0[a]	121.7 ± 2.3[a]
Control	25.6 ± 3.1[a]	52.0 ± 5.5[a]	69.5 ± 3.2[a]	88.2 ± 6.1[a]	104.7 ± 9.7[a]	121.0 ± 17.1[a]
Raw palm	25.0 ± 6.0[a]	51.5 ± 5.2[a]	69.2 ± 14.0[a]	87.2 ± 19.0[a]	103.0 ± 14.3[a]	118.2 ± 10.0[a]
Neem	22.8 ± 2.2[a]	49.2 ± 4.7[a]	65.7 ± 7.1[a]	82.5 ± 6.8[a]	99.5 ± 7.5[a]	116.0 ± 10.2[a]
Statistics	**P = 0.28** $F_{(8,36)} = 1.28$	**P = 0.08** $F_{(8,36)} = 1.95$	**P = 0.07** $F_{(8,36)} = 2.01$	**P = 0.05** $F_{(8,36)} = 2.37$	**P = 0.14** $F_{(8,36)} = 1.66$	**P = 0.30** $F_{(8,36)} = 1.23$

*Means followed with the same letter in a column are not significantly different at (P=0.05), according to HSD Tukey.

For *B. bassiana*, AUMGC ranged from 22.8 ± 1.0 on day 5 for neem oil to 141.7 ± 13.8 on day 30 for refined palm oil. There was no significant difference in AUMGC between the tested substance and the control across observation periods. No significant difference in AUMGC was observed between oils, regardless of the day of observation. The highest values of AUMGC were obtained with refined palm oil (30.8 ± 2.2) and soybean oil (30.8 ± 1.5) on day 5, and refined palm oil (141.7 ± 13.8) on day 30, respectively. The lowest AUMGC values were recorded with neem oil and raw palm oil, which ranged from 22.8 ± 1.0 and 25.0 ± 3.8 on day 5 to 116.0

± 4.5 and 118.2 ± 4.5 on day 30, respectively. AUMGC values for cotton, sunflower, canola, and control were intermediates (Table 1).

There were significant differences among the AUMGC values of *M. anisopliae* exposed to oils at each day post-exposure, except on day 5 (Table 2). Throughout the exposure period, *M. anisopliae* consistently showed higher AUMGC values in the presence of refined palm oil with 33.2 ± 1.2 and 183.5 ± 1.7 on days 5 and 30, respectively. In contrast, the lowest AUMGC values for this fungus were observed with neem oil, with the values of 28.2 ± 0.8 and 171.2 ± 4.2 on days 5 and 30, respectively.

Table 2. AUMGC of *Metarhizium anisopliae* with different treatments with oils.

Oils	AUMGC ± SD across observation (days)					
	5	**10**	**15**	**20**	**25**	**30**
Refined palm	33.2 ± 2.8^{a}	76.7 ± 3.1^{a}	116.0 ± 5.1^{a}	152.2 ± 6,2^{a}	171.2 ± 5.0^{a}	183.5 ± 3.8^{a}
Soybean	32.0 ± 2.9^{a}	74.7 ± 4.9^{ab}	110.2 ± 8.6^{ab}	142.5 ± 9.3^{ab}	167.5 ± 5.4^{ab}	180.5 ± 4.5^{ab}
Groundnut	32.2 ± 2.4^{a}	74.2 ± 6.5^{ab}	109.2 ± 5.2^{ab}	141.0 ± 4.6^{ab}	165.2 ± 4.4^{ab}	178.0 ± 3.0^{ab}
Cotton	31.8 ± 3.8^{a}	70.7 ± 6.0^{ab}	108.5 ± 7.4^{ab}	139.5 ± 7.1^{ab}	164.2 ± 4.2^{ab}	177.0 ± 4.0^{ab}
Sunflower	31.8 ± 1.9^{a}	70.5 ± 7,1^{ab}	105.2 ± 4.9^{ab}	136.7 ± 4.8^{b}	164.0 ± 2.8^{ab}	176.0 ± 3.8^{ab}
Canola	31.0 ± 2.9^{a}	70.5 ± 2.7^{ab}	104.0 ± 2.2^{b}	135.5 ± 8.8^{b}	161.0 ± 3.9^{ab}	175.7 ± 7.6^{ab}
Control	30.8 ± 1.9^{a}	67.0 ± 4.0^{ab}	105.5 ± 5.5^{ab}	134.0 ± 5.2^{b}	160.2 ± 8.2^{ab}	175.0 ± 2.0^{ab}
Raw palm	30.6 ± 3.87^{a}	68.5 ± 3.2^{ab}	105.0 ± 1.5^{ab}	133.5 ± 6.4^{b}	158.2 ± 8.6^{b}	171.7 ± 2.1^{b}
Neem	28.2 ± 1.8^{a}	65.0 ± 4.1b	102.2 ± 5.5^{b}	131.5 ± 3.2^{b}	156.7 ± 2.9^{b}	171.2 ± 9.4^{b}
Statistics	P = 0.28 $F_{(8,36)}$ = 1.29	P = 0.001 $F_{(8,36)}$ = 3.05	P = 0.015 $F_{(8,36)}$ = 2.84	P < 0.001 $F_{(8,36)}$ = 4.76	P = 0.004 $F_{(8,36)}$ = 3.56	P = 0.011 $F_{(8,36)}$ = 2.96

*Means followed with the same letter in a column are not significantly different at (P=0.05), according to HSD Tukey.

Table 3. Mean percentage inhibition of radial growth of colonies of *Beauveria bassiana* and *Metarhizium anisopliae* on PDA after day 30, treated with different vegetable oils.

Oil	*B. bassiana*		*M. anisopliae*	
	Diameter (mm)	% inhibition	Diameter (mm)	% inhibition
Refined palm	56.7	-16.93	73.4	-6.85
Soybean	55.4	-15.8	72.2	-5.11
Groundnut	53.5	-10.8	71.2	-3.64
Cotton	50.5	-3.82	70.8	-3.07
Sunflower	49.3	-3.56	70.3	-2.50
Canola	48.7	-2.03	70.4	-2.30
Raw palm	47.3	0.52	70	-1.90
Neem	46.4	2.08	68.5	0.28

The inhibitory effects of vegetable oils on the growth of *B. bassiana* and *M. anisopliae* on day 30 were revealed. The growth of *B. bassiana* on PDA media was inhibited by neem oil (2.08%) and raw palm oil (0.52%). Except for the above oils, the radial growth of *B. bassiana* was enhanced in the PDA media amended by oils from 2.03% to 16.93%. Refined palm and soybean oils had the most positive effects on radial growth, with inhibition percentages of -16.93% and -15.80%, respectively. The sensitivity of the mycelial growth of *M. anisopliae* showed that almost all the oils were favourable. Their effect on *M. anisopliae* growth was enhanced from 1.90 to 6.85% (Table 3). Only neem oil inhibited M. anisopliae growth (0.28%).

3.2. Fungal conidia yield

Fungal conidia yield varied between the tested vegetable oils. Neem oil and raw palm had the most substantial negative effects on *B. bassiana* and *M. anisopliae* conidia yield, respectively. Conidial production ranged from 9.1 x 10^6 conidia/mL in neem oil to 29.3 x 10^6 conidia/mL in soybean oil, respectively. For *M. anisopliae* it ranged from 10.8 x 10^6 conidia/mL in raw palm to 44.4 x 10^6 conidia/ml soybean oil (Table 4).

Table 4. Fungal yield on PDA treated with different vegetable oils on day 30.

Oil carrier	*B. bassiana* concentration (x10^6 conidia/Ml)	*M. anisopliae* concentration (x10^6 conidia/Ml)
Soybean	29.3	44.4
Groundnut	27.2	17.3
Refined palm	23.8	32.5
Cotton	23.2	15.6
Raw palm	21.4	10.4
Sunflower	16.5	17.2
Control	14.9	19.2
Canola	13.3	32.0
Neem	9.1	11.2

3.3. Fungal Biomass - oil response

The fungi tested in PDB revealed the production of *B. bassiana* and *M. anisopliae* biomass at the end of the incubation period, as observed on the surface of the liquid culture media (Figure 1).

Figure 1. Cultures of *Beauveria bassiana* in Potato Dextrose Broth (PDB) liquid culture media

Biomass yield in PDB ranged from 0.59 mg to 0.69 mg for *B. bassiana* (Figure 2A). The biomass of *B. bassiana* recorded in PDB amended by oils was significantly different among treatments ($p < 0.0001$, $F_{(8,36)} = 19.5$). There was a consistent and significant difference in the production of *B. bassiana* biomass between refined palm and neem oil. Except for neem oil, there was no significant difference in biomass production between refined palm and the rest of the used oil. Numerically, the highest biomass of *Beauveria bassiana* was produced in PDB amended by refined palm oil, while the lowest was observed in neem oil.

Metarhizium anisopliae produced fungal biomass in the presence of all oils tested. The fungal mycelial weight of *M. anisopliae* ranged from 0.60 mg to 0.64 mg (Figure 2B). Biomass production by *M. anisopliae* was significantly different among treatments ($p < 0.001$, $F_{(8,36)} = 9.7$). Although there was no significant difference between the response of refined palm oil and that of soybean oil, the maximum mycelial biomass was still obtained with refined palm oil. In contrast, the lowest was observed with neem oil and the control.

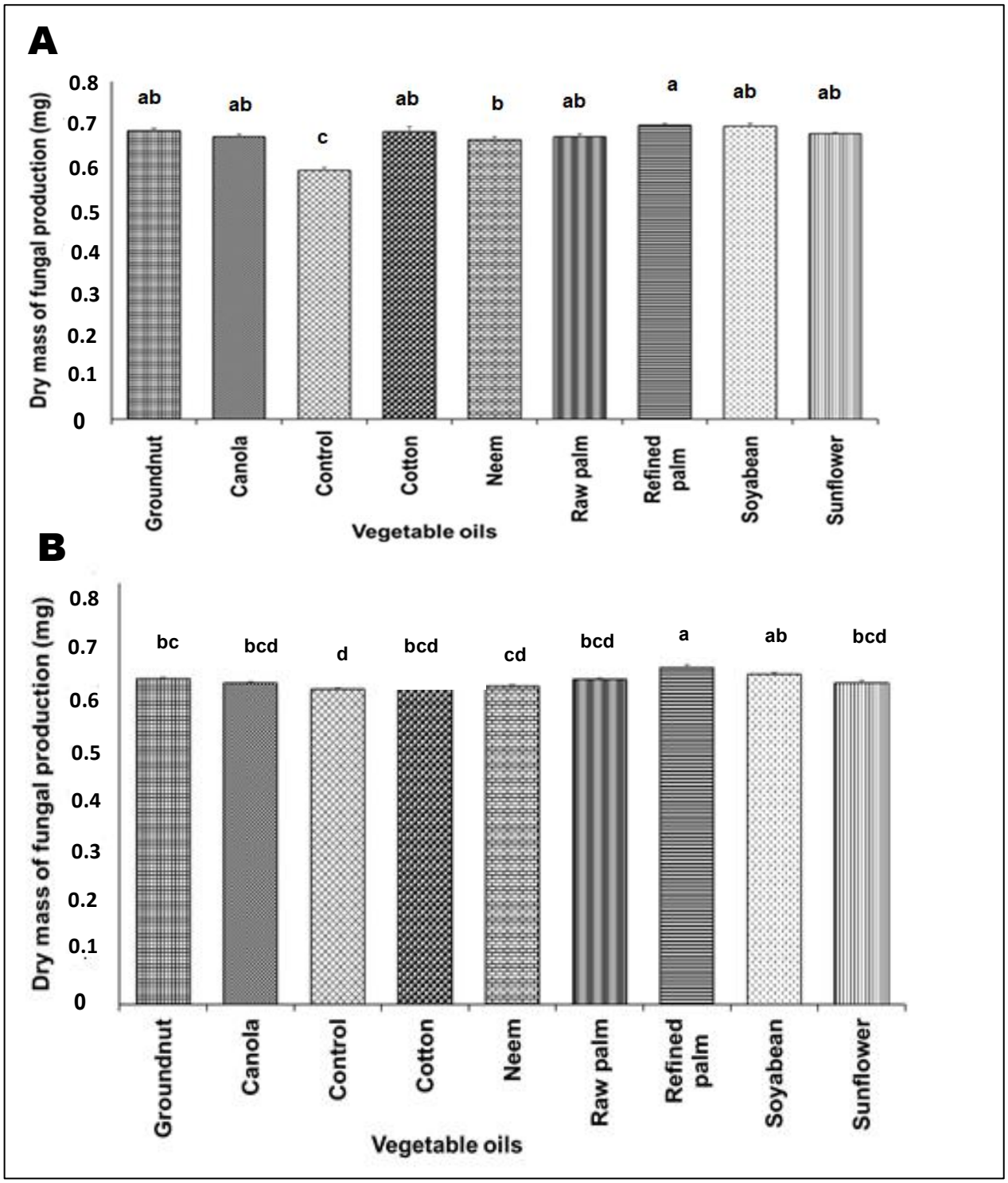

Figure 2. Fungal Biomass (mg) produced (A) *Beauveria bassiana* and (B) *Metarhizium anisopliae* in different vegetable oils 10 days post-exposure.

3.4. Effect of oil on fungal conidial viability

B. bassiana and *M. anisopliae* were incubated in oil-amended PDA for 7 days in the dark room; all treatments showed viable propagules, as assessed by the Colony Forming Unit (CFU). However, the result indicated variability in the viability of fungal spores. CFU ranged from 15.9 ± 1.3 x 10^4/ml with raw palm oil to 43.2 ± 2.6 x 10^4/ml with sunflower oil for *B. bassiana*, from 16.6 ± 1.1 x 10^4/ml with cotton oil to 97.4 ± 0.8 x 10^4/ml with raw palm oil for *M. anisopliae* (Table 5).

The number of viable propagules, as indicated by the Colony Forming Unit (CFU), differed significantly among tested vegetable oils for each fungal strain and between tested oils and control ($p < 0.001$). The density of

viable *B. bassiana* propagules was highest in PDA amended by sunflower oil (43.2 ± 2.6 x 10^4/ml), while raw palm oil resulted in the highest CFU count for *M. anisopliae* (97.4 ± 0.8 x 10^4/ml).

Table 5. Density of viable propagules per unit volume through enumeration by the Most Probable Number (MPN) method with different treatments.

Oil treatment	Number of viable propagules ± SE **(x10^4 conidia/Ml)**	
	B. bassiana	*M. anisopliae*
Groundnut	27.1 ± 1.9^{d}	17.7 ± 3.5^{h}
Canola	33.7 ± 2.9^{b}	30.2 ± 3.0^{b}
Cotton	25.3 ± 1.8^{g}	16.6 ± 1.1^{i}
Neem	28.0 ± 1.7^{c}	21.8 ± 3.3^{g}
Raw palm	15.9 ± 1.3^{i}	97.4 ± 0.8^{a}
Soybean	25.5 ± 1.2^{f}	25.3 ± 1.3^{c}
Sunflower	43.2 ± 2.6^{a}	23.6 ± 2.9^{e}
Refined palm	26.9 ± 2.9^{e}	24.7 ± 2.1^{d}
Control (no oil)	22.8 ± 0.5^{h}	22.0 ± 1.6^{f}
Statistics	**P < 0.001; Df = 8**	**P < 0.001; Df = 8**

*Means followed by a common letter in a column are not significantly different at (P=0.05) by HSD Tukey.

4. DISCUSSION

The compatibility of BCAs with natural product-based additives is crucial for their effective application in disease and pest management programs (Liu, 2012). This study demonstrates that the compatibility or toxicity of oils to *B. bassiana* and *M. anisopliae* varies widely. Furthermore, our research reveals that Oil-amended PDA media affected the Area Under Mycelial Growth Curve (AUMGC) of both fungi, with some oils causing growth inhibition. The growth of *B. bassiana* was notably inhibited by neem oil and raw palm oil, while only neem oil inhibited *M. anisopliae*. The AUMGC was inversely proportional to the percentage of inhibition, indicating the antifungal potential of neem oil and raw palm oil on mycelial growth. This potential may be attributed to the presence of active compounds such as organic acids in these oils, as suggested for other fungal species than *B. bassiana* and *M. anisopliae*. So previous studies reported antifungal activities of essential oils on mycelial growth of *Fusarium verticillioides*, *Aspergillus niger*, *A. oryzae*, *Mucor pusillus,* and *Fusarium oxysporum* (Ferdes et al., 2017). The AUMGC results for *M. anisopliae* showed a difference between the oils throughout the period, except on day 5. The exception of day 5 result may indicate that *M. anisopliae* isolate has not yet produced the enzymes needed to degrade the metabolites of different oils. This is because the types of metabolites in the different oils certainly influence fungi growth. Results also showed weak or no inhibition of vegetative growth by the oils used, suggesting their pronounced fungistatic action on entomopathogenic fungi and enabling a choice to be made in favor of these oils.

Fungal biomass was significantly affected by the oils used in PDB. *B. bassiana* and *M. anisopliae* produced the highest biomass in refined palm oil. Refined palm oil seems to enhance more fungal mycelium mass, probably due to the presence of some biocompatible metabolites. The conidia yield was higher with soybean oil for both fungal strains. Soybean oil may contain a substance that promotes fungal growth and productivity. Hofer et al. (2018) emphasized that soybean oil is often used as a media supplement in fungal bioprocesses, likely due to lecithin, a key component of soybean oil with beneficial effects on fungal growth. This finding suggests that soybean oil, or more specifically lecithin, could be used as an adjuvant in biopesticide preparation. In contrast, neem oil resulted in reduced fungal biomass production. Also, conidia yield was lower with neem and raw palm oils for *B. bassiana* and *M. anisopliae*, respectively. The reduction of fungal conidiogenesis as well as fungal growth and biomass with neem oil is probably due to the presence of azadirachtin which may have toxic effect on EPF. In addition, Depieri et al. (2005) has shown that the neem oil main compound azadirachtin, can inhibit *B. bassiana* vegetative growth, decreasing conidial production and viability. Hirose et al. (2001) even advised against using neem in mixtures with *B. bassiana* and *M. anisopliae*. Our result indicates a potential correlation between conidial production and fungal growth, both negatively affected by neem and raw palm oils. The lower response of *M. anisopliae* conidia yield in raw palm oil could have been due to the presence of organic acids

(palmitic, palmitoleic, linoleic, and oleic), as Alves et al. (1998) revealed the toxicity of organic acids to *M. Anisopliae*. These results suggest the importance and necessity of chemical analysis of used oils.

The germination of fungal conidia on oil-supplemented media was not a novel finding. Rocha et al. (2024) reported that conidia of entomopathogenic fungi could germinate in oils; and Mathulwe et al. (2023) revealed variability in conidial viability, suggesting that conidia germination depends on oil type. The highest density of viable propagules was observed in PDA amended by sunflower and raw palm oil for *B. bassiana* and *M. anisopliae,* respectively. These findings suggested a compatibility effect with nearly all of the oils used, particularly with sunflower oil and raw palm oil for *B. bassiana* and *M. anisopliae*, respectively. Growth, conidia yield, and germination were essential for the entomopathogenic infection process (Harith-Fadzilah et al., 2021): (1) Host arthropod adhesion, (2) penetration of arthropod cuticle, and (3) arthropod haemocoel colonization. Thus, a correlation between these parameters in oil treatments could be expected. This correlation is particularly significant, as Gebremariam et al. (2021) found these parameters sometimes positively correlated with fungal virulence. In certain cases, compatible products may enhance the efficiency of propagules (Quintela & McCoy, 1998) with the benefits of protection against their physical damage due to adverse environmental factors. Conversely, some oils may hinder to the propagules effective. More, the use of incompatible products may inhibit the development and reproduction of entomopathogens, adversely affecting pest control (Rivera-Malo, 1993). Conidia are crucial for the survival, spread, and persistence of entomopathogens in nature. And at the onset of the epizootic, the conidia were responsible for the first disease foci. Therefore, if the germination is inhibited, pathogen control efficiency would likely be compromised. Thus, these trials need replications to avoid premature conclusions.

Studies on the compatibility of entomopathogenic fungi with several oils such as neem's found a significant reduction of *Metarhizium rileyi* radial growth (Bharti et al., 2023). Islam et al. (2010) also noted that emulsifiable neem oil negatively impacted *B. bassiana* growth (including germination, colony formation, and sporulation). Additionally, Hirose et al. (2001), observed that oils did not always maximize effects on BCAs in field conditions and Alves et al. (1998) further showed that high *in vitro* toxicity did not always translate to field outcomes in the field.

5. CONCLUSION AND APPLICATION OF RESULTS

The importance of our work lies in the results relating to the compatibility of fungi with oils that can be used in the formulation of mycoinsecticides. The selection of mycoinsecticidal oils based on entomopathogenic fungi through fungal growth, conidial production, and germination highlights the crucial importance of developing a robust oil-pathogen synergy for effective and efficient management of agricultural pests. Without any pretension of drawing hasty conclusions, our results allow us to say that refined palm oil, soybean oil, sunflower oil, and raw palm oil are compatible with the EPFs tested. On the other hand, a large grey area remains to be elucidated on the contradiction observed with raw palm oil, our findings show that neem oil should be avoided in formulation processes against *B. bassiana* and *M. anisopliae*. These studies offer exceptional advantages, as the results will serve as a basis for future research programmes into the testing of entomopathogenic fungi formulated as potential bio-pesticides for the biological control of economically important crop pests.

However, to complete this work, it is necessary to test a range of concentrations, to validate viability by direct observation of germination, and to carry out further analysis of the chemical composition of the oils. But you should mention these at the end of your discussion or conclusion, as limitations or prospects of this research work.

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