



The potential of entomopathogenic fungus *Aspergillus oryzae* in controlling subterranean termites (Isoptera: Termitidae) in Tanzania

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ABSTRACT

Subterranean termites pose significant economic and food loss during production requiring intensive use of pesticides that affect crop quality and market hence necessitating effective and sustainable biocontrol strategies for management. This study evaluated the efficacy of the entomopathogenic fungus *Aspergillus oryzae* Isolate; VB – MG938648 in controlling subterranean termites on crops. A field trial was conducted from April to June 2024 in different zones of Tanzania. All treatments were applied once using a 20 L knapsack pump fitted with fan spray nozzle which is ideal narrow calibrated to distribute liquid in all parts including flat surface of plant and undersides of the leaf. Application of all treatments was done once a week from 1730 h m. The results showed significant termite mortality rate ranging from 71.2 % to 87.6 % across different treatments and locations. The efficacy of *Aspergillus oryzae* as a biopesticide against subterranean termites in cocoa, macadamia, and coffee trees was significant high compared to control. The mortality rate was dose dependent where the highest concentration achieved high mortality rate compared to low concentration and control. The study demonstrates the efficacy of *Aspergillus oryzae* in attracting beneficial insect especially pollinators including; *Andrena vicina*, *Apis mellifera*, *Cruciscispidia chandleri*, *Hypotrigena gribodoi*, *Osmia lignaria*, *Pseudapis usambarae* suggesting its potential as a termite biocontrol and increase yield through pollination in coffee plantations, a crop with high economic value in Tanzania. This study suggests the application of biopesticide for control of subterranean termites to reduce crop loss and the impact of chemical pesticides on biodiversity.

Introduction

Termites play a significant role in ecosystem and agroecological landscape such as soil nitrification to improve nutrient availability for high crop yield. However, subterranean termites are devastating pests of food crops including maize, sugar cane, sorghum, rice, cocoa and others leading to food insecurity if uncontrolled (Rouland-Lefèvre, 2010). Maize as the main food crop in Africa including Tanzania, is threatened by several pests including subterranean termites (Demissie et al., 2019). About 185 species of termites are reported as significant crop pests especially coffee, cocoa, rice and macadamia crops in tropical regions, causing substantial economic losses and food insecurity (Rana et al., 2021). The damage and loss occur during manipulation of organic matters whereas the termites have been reported to pose high crop damage and loss especially on maize where the loss is up to 30 % in East Africa (Otieno, 2018). In West Africa, it has been reported that termites affect cocoa production and cause massive loss especially in old cocoa orchard than new farms (Djuideu et al., 2021). These subterranean termites attack crops and interfere with agricultural practices that

harbor pathogens, exacerbating crop damage and significant impact on crop yields and quality, particularly in tropical regions. Directly by feeding on roots, stems, and leaves, or indirectly by creating pathways for pathogens and other pests (Rana et al., 2021). In conventional agricultural systems such as coffee cultivation, management of termites is crucial to avoid loss and maintain quality coffee. Management of subterranean termites is practically through use of conventional pesticide with ecological and economic effects (Ahmad et al., 2021; Paul et al., 2017). The current reliance on chemical pesticides, have environmental and health concerns thus many studies recommend the use of bio-based solutions such as microbes, plant extracts and natural enemies to control termites while maintaining their ecological role (Oksari et al., 2025; Wattimena and Latumahina, 2021), although none is commercially available in Tanzania. The use of safe and integrated termites' control would be ideal in agricultural interface for sustainable and economic viability (Mishra et al., 2021; Khan and Ahmad, 2018). The use of biological control approach especially microbial based biopesticides is recommended due to their availability in ecosystem and integration with other soil microbes and synergic ecological role with

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termites (Sindhu et al., 2011). The use of biopesticide for management of termites is promising as some studies revealed the effectiveness of plant extract on woody and buildings termites (Oksari et al., 2025; Sindhu et al., 2011). On other hand, studies report on application of plant based biopesticide for controlling crop damaging termites (Coulbaly et al., 2024; Lopes et al., 2017). However, the use of fungal based biopesticide would be ideal for controlling termites due to compatibility with the pest and applicability especially in tropical regions. Studies report the efficacy and suitability of entomopathogenic fungi as a biological control agent of wide range of pests including termites. Entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Trichodema harzianum* are reported to possess entomopathogenic activity against different species promising the commercialization of microbial biocontrol (Subekti et al., 2024). Despite the effectiveness of most entomopathogenic fungi, a study by Yanagawa et al. (Yanagawa et al., 2008) shows that some termites species exhibit resistance against non-spore forming fungi. Hence the spore forming fungi such as *Aspergillus oryzae* exhibit high activity and virulence through cuticle penetration (Jessica et al., 2019). *Aspergillus oryzae* is one of the common a fungus used in food fermentation, particularly in Asian revealing its safety on food and environment (Daba et al., 2021). In Tanzania, *Aspergillus oryzae* has been reported to control devastating pest such as *Tuta absoluta*, ticks with proven safety on food crops and environment as compared to synthetic pesticides that are reported to pose many health-related issues (Msangi et al., 2022; Zekeya et al., 2019). It also produces various enzymes and secondary metabolites which effectively control insect pests (Hassan et al., 2024). The present study aimed to determine the of potential of *Aspergillus oryzae* as biological control for management of subterranean termites in agricultural fields for improving marketable yield of crops especially coffee with high export market in Tanzania.

Material and methods

Isolation of fungus as biopesticide

Fungal isolate; *Aspergillus oryzae* isolate VB – MG938648 was isolated from waste water from Moshi Leather Industry, Moshi-Tanzania. In order to clear uncertainty of isolates, molecular characterization of all isolates was conducted. DNA from each fungal isolates was extracted according to the methods described by Zekeya et al. 2019. About 200 mg of fresh fungal mycelia were transferred to a sterilized 1.5 mL Eppendorf (micro centrifuge) containing 300 μ L of TES, extraction buffer (0.2 M Tris-HCl [pH 8], 10 mM EDTA [pH 8], 0.5 M NaCl, 1 % SDS) and 800 mg of acid washed sterilized sea sand. Micro-centrifuge tube 1.5 mL containing mycelium were fitted into homogenizer (Retsch) and macerated for 4 min. Samples were then vortexed for 30 s followed by addition of 200 μ L of TES extraction buffer containing proteinase K. The mixture was vortexed to thoroughly mix and tubes were placed on water bath at 65°C for 30 min. 250 μ L of 7.5 M ammonium acetate was added, mixed thoroughly and incubated on ice –5 °C for 10 min. The mixture was centrifuged for 15 min at 12,000 g to pellet DNA and the supernatant was transferred to a new tube and 500 μ L of ice-cold isopropanol was added. Tubes were then incubated –20 °C for overnight. The mixture was centrifuged for 10 min at 12,000 g to pellet DNA. The supernatant was decanted and DNA pellet was washed with 800 μ L of cold 70 % ethanol. Tubes were upside-down on clean sterile paper towel for 10 min to air dry. DNA was eluted from the pellet with twice repeated extraction with 100 μ L of 1x TE buffer (10 Mm Tris-HCl [pH 8], 1Mm EDTA) each time centrifuged for 2 min to avoid collecting polysaccharides. DNA solution was transferred to micro-centrifuge tube, followed by addition of 5 μ L of RNase (20mg/ mL) and incubated at 37° C for 60 min. Finally, 1 drop (1 μ L) DNA solution was dropped into Nano drop to determine concentration and purity of DNA.

Fragments containing the region encoding ITS-1,5.8S rDNA and ITS-2 were amplified using universal primer ITS-1 (F 5'-CTTGGTCATTTA

GAGGAAGTAA-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC- 3') as described by (Zhang et al., 2015). PCR was conducted with Taq™ mix polymerase in a thermocycler amplification of total of 20 μ L DNA mixture. The initial denaturation temperature was 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and incubation at 72 °C for 10 min. Electrophoresis of all PCR products were performed on the 1.5 % agarose gel and was run against the 1 kb ladder (Promega), DNA was stained using Gel red stain for easy visualization and the Buffer used was TAE. Products were visualized through gel documentation system. The 5.8 s gene of fungal isolates were aligned with the sequences in the gene bank using MEGA 5 software methods. Sequence analysis of fungi isolates were analyzed with the BLAST program available at the National Centre for Biological Information (NCBI). The most effective isolate against ticks was deposited on NCBI with accession number; MG938648.

Preparation of *A. oryzae* concentration

Working concentration were prepared and selected based on Zekeya et al. 2019 methods. Fungal isolate was sub-cultured on Potato Dextrose Agar (PDA) in Petri dishes to confirm its viability. After 5 days of full maturation, spores were gently scrapped from the media by suspending into 10 mL sterile distilled water with 59 %,40 %sunflower, 1 % lavender extract as emulsifier to make a stock suspension. After mixing suspension in a flask, the concentration of stock solution was accessed by a haemocetometer neubauer (Manfield, German) that was 1.0×10^9 conidial/mL which was further diluted to get working concentrations of 2.0×10^6 , 3.0×10^6 and 5.0×10^6 conidia/mL and 1.0×10^7 conidia/mL by the addition of sterile distilled water.

Field trial

This study was conducted from April to June 2024in three regions namely; Mbeya and Songwe (Southern highlands), Kagera (Western zone), Singida (Central and Kilimanjaro (Northern zone) representing main agroecological zones for coffee, cocoa and macadamia. The selection was due to climatic variation that affect crop cultivation especially coffee (Mmbwambo et al., 2022=). In each region, districts were purposively selected based on history and agronomic features of target crops. Field trial on efficacy was conducted using VB-Liquid (*Aspergillus oryzae*) supplied by Plant Biodefenders Ltd at 3 different concentration reference biopesticide (*Metarhizium anisopliae*) supplied by Real IPM and water as negative control. The biopesticide was stored at room temperature around 30 °C before use. The original VB-Liquid containing 1×10^9 conidial/mL as stock concentration was diluted in 3 different concentrations; (2 ml, 3 ml, 5ml/L) with the concentration of 2.0×10^6 , 3.0×10^6 and 5.0×10^6 and 0 conidial/ml respectively of the VB-Liquid, and 5ml/L of positive control (*Metarhizium anisopliae*) and 0 conidia in negative control.). The experiments were arranged in randomized complete block design (RCBD) with five treatments in each crop and three block per treatment were selected. The distance between blocks was 20 m and between treatments was 60 m to avoid treatment contamination. The selection of experiment blocks was based on high rate of infection in macadamia and cocoa and early infestation with active and growing plant of plants for coffee for monitoring plant growth. The treatments were replicated four times and the pre assessment of the test and reference products to assess the presence of termites on soil around the cocoa, coffee and macadamia trees was done a day before application of biopesticides. All treatments were applied once by spraying around infested crops on stem/trunk in cocoa and macadamia while a whole plant was sprayed in coffee using a 20 L knapsack pump fitted with fan spray nozzle which is ideal narrow calibrated to distribute liquid in all parts including flat surface of plant and undersides of the leaf. Application of all treatments was done once a week from 1730 h since is the best time for microbial based pesticides to allow maximum time overnight for best fungal conditions such as penetration to soil,

plant materials and termites to avoid dry during the day time with high temperature which affect fungal activity level. Data on mortality of termites, destruction of structure, beneficial insects' retention and crops recovery were collected only 10 selected plants in each block based on early infested plants after 7 days for eight weeks consecutively. Data termites' mortality of termites in cocoa, macadamia and coffee whereas plant recovery, beneficial insect retention and structure destruction was only assessed on coffee plants due to long crop cycle, tree architecture (tall and dense canopy) on cocoa and macadamia which would have not been achieved within the timeframe. Data across treatments were analyzed and compared by Tukey's test at $\alpha < 0.05$ using Statistical Analysis Software (SAS Institute Inc., USA) version 9.4.

Result and discussion

The efficacy of *Aspergillus oryzae* (VB-Liquid) as a biopesticide against subterranean termites in cocoa, macadamia, and coffee trees was significant ($P = 0.05$) high compared to *Metarhizium anisopliae* and control. The mortality rate was dose dependent where the highest concentration achieved high mortality rate compared to low concentration and control. The results showed the highest termite mortality rate at 5.0×10^6 conidial/ml compared to low concentrations and the control, with mean mortality rates ranging from 71.2 % to 87.6 % across different treatments and locations (Tables 2–4). The biopesticide, *Aspergillus oryzae* (VB-Liquid) showed high termite mortality rate in cocoa trees at Kyela district where at 5ml/L achieved 85.4 % mortality with an LT50 of 2.0 days, comparable to *Metarhizium anisopliae* (84.1 % mortality, LT50 2.45 days) and control with 00.00 % mortality (Table 1). On Macadamia plants, *Aspergillus oryzae* showed high efficacy on subterranean termite by causing mortality of 86.4 and 85.2 % in Kyela than *Metarhizium anisopliae* with 81.1 % and 83.2 % mortality in Kyela and Ikungi districts respectively whereas control 00.0 % mortality in both districts (Table 2). On coffee plantations, the effect of VB-Liquid on termite was similarly high in Siha and Biharamulo districts with 85.0 % termite mortality at 5 ml dose compared to *Metarhizium anisopliae* with 85.0 % and 83.0 % termite mortality whereas no mortality was observed in control in Siha and Biharamulo districts respectively (Table 3) Similarly, VB-Liquid showed high efficiency on subterranean termites in coffee plantations with the highest mortality rate of 86.8 % and 87.6 % Mbozi and Moshi district respectively compared to *Metarhizium anisopliae* with 84.0 % and 82.2 % termite mortality whereas no termite mortality was observed in control (Table 4). The lethal time (LT50) to indicate time for death ranged from 2.4 to 3.7 days, showing that high dose of *A. oryzae* especially 5ml/L has quick effect on termite. On other hand *Aspergillus oryzae* showed high recovery rate on affected coffee plants by recovering after treatment compared to *Metarhizium anisopliae* and control. The effect of biopesticide was dose dependent where the high dose of 5mls of *A. oryzae* improved plant recovery within short time compared to low dose of 2mls. In *A. oryzae* an average of 92 % coffee plants recovered compared to 85 % and 4 % of coffee plants in *M. anisopliae* and control plots after 8 weeks of observation (Fig. 1). Additionally, *A. oryzae* treated blocks attracted beneficial insects such as different species of

Table 1
Effect of VB-Liquid- (*Aspergillus oryzae*) against subterranean termite on cocoa.

Treatments	Kyela district (Southern)		Ikungi district (Central)	
	Mean mortality (%)	LT50 (days)	Mean mortality (%)	LT50 (days)
VB – Liquid@2ml/L	71.2ab	3.9	74.0ab	3.8
VB – Liquid @3ml/L	80.1a	2.48	81.2a	2.45
VB – Liquid @5ml/L	85.4a	2.00	83.4a	2.00
<i>Metarhizium anisopliae</i> @5ml/L	84.1a	2.45	81.2a	2.45
Negative control	0.00c	—	0.00c	—

Mean in columns with the same letter are not significantly different at $\alpha < 0.05$, Tukey's Test.

Table 2
Effect of VB-Liquid (*Aspergillus oryzae*) against subterranean termite on macadamia.

Treatments	Kyela district (Southern)		Ikungi district (Central)	
	Mean mortality (%)	LT50 (days)	Mean mortality (%)	LT50 (days)
VB – Liquid @2ml/L	74.4ab	3.5	72.1ab	3.5
VB-Liquid @3ml/L	81.2a	2.46	82.4a	2.46
VB – Liquid @5ml/L	86.40a	2.00	85.2a	2.00
<i>Metarhizium anisopliae</i> @5ml/L	81.1a	2.45	83.2a	2.45
Negative control	0.00c	—	0.00c	—

Mean in columns with the same letter are not significantly different at $\alpha < 0.05$, Tukey's Test.

Table 3
Effect of VB-liquid (*Aspergillus oryzae*) against subterranean termite on coffee.

Treatments	Siha district (Southern)		Biharamulo district (Western)	
	Mean mortality (%)	LT50 (days)	Mean mortality (%)	LT50 (days)
VB – MG938648 @2ml/L	76.0ab	3.7	74.0ab	3.9
VB – MG938648 @3ml/L	84.0a	2.68	83.0a	2.75
VB – MG938648 @5ml/L	87.0a	2.40	85.0a	2.50
<i>Metarhizium anisopliae</i> @5ml/L	85.0a	2.45	83.0a	2.65
Negative control	0.00c	—	0.00c	—

Mean in columns with the same letter are not significantly different at $\alpha < 0.05$, Tukey's Test.

Table 4
Effect of VB-Liquid- (*Aspergillus oryzae*) against subterranean termite on coffee.

Treatments	Mbozi district (Southern)		Moshi district (Northern)	
	Mean mortality (%)	LT50 (days)	Mean mortality (%)	LT50 (days)
VB – Liquid @2ml/L	77.2ab	3.7	75.2ab	3.65
VB– Liquid @3ml/L	82.4a	2.55	84.2a	2.56
VB– Liquid @5ml/L	86.8a	2.40	87.6a	2.50
<i>Metarhizium anisopliae</i> @5ml/L	84.0a	2.55	82.2a	2.45
Negative control	0.00c	—	0.00c	—

Mean in columns with the same letter are not significantly different at $\alpha < 0.05$, Tukey's Test.

bees where 13 bee species including *Apis mellifera* were observed compared to *M. anisopliae* which attracted only 8 bee species with low diversity whereas control attracted only 2 species were observed in control plots over eight weeks (Table 5). The study also revealed health and vigor leaves and berry were observed in coffee plantations without pathogenesis from fungus- VB Liquid and beneficial insects such as bees were recorded in coffee compared to other treatments which might influence increase in yield suggesting that VB-Liquid is safe for use on crops and attract market making it a viable option for integrated pest management in these crops.

The effect of *A. oryzae* on destructing mound structure on coffee plantations was high where the highest dose of 5mls of *A. oryzae* destructed the structure within 7 days compared to control with no effect on structure). The effected could be accelerated by mortality rate of termites which affected their activity of manipulating organic matter from crops for constructing and maintaining the structure in coffee plantations. This study revealed the effectiveness of VB-Liquid in northern compared to Southern zone farms which could be affected by



Fig. 1. Effect of *Aspergillus oryzae* on recovery of coffee plants after 8 weeks of treatment.

Table 5

Showing diversity and abundance of different species of bees in coffee plantations cross treatments for 8 weeks.

Species Name	<i>A. oryzae</i> - 2mls - Mls 2	<i>A.oryzae</i> -3mls- Mls 3	<i>A. oryzae</i> - 5mls -Mls 5	<i>M. anisopliae</i>	Control
<i>Andrena vicina</i>	0.25	0.25	0.75	0.25	0
<i>Apis mellifera</i>	1	1.125	2.125	0.75	0.25
<i>cruciscispidia</i>	0.25	0.5	0.125	0	0
<i>chandleri</i>					
<i>Hypotrigona</i>	0.25	0.125	0.5	0	0
<i>gribodoi</i>					
<i>Lithurgus</i>	0.125	0	0.25	0.25	0
<i>species</i>					
<i>Osmia lignaria</i>	0.125	0.25	0.625	0.125	0
<i>Pseudapis</i>	0.25	0.125	0.25	0.125	0.125
<i>usambarae</i>					
<i>seladonia</i>	0.125	0	0.375	0.5	0
<i>africana</i>					
<i>Seladonia</i>	0.25	0.375	0.125	0.375	0
<i>lucidepenis</i>					
<i>Xylocopa</i>	0.25	0.5	0.125	0.125	0
<i>caffra</i>					
<i>Xylocopa</i>	0.125	0.125	0.75	0.375	0
<i>flavorufa</i>					
<i>Xylocopa</i>	0.25	0.375	0.25	0	0
<i>nigrita</i>					
<i>Xylocopa</i>	0.375	0.125	0.375	0.125	0
<i>scionensis</i>					

agricultural practices in southern highlands which involve intercropping attracting more pest and diseases (Otieno, 2018). The findings suggest that *Aspergillus oryzae* is a promising biopesticide for controlling subterranean termites, as compared to *Metarhizium anisopliae* and control with potential for integrated pest management especially in agricultural fields. The efficacy of *Aspergillus oryzae* VB-MG938648 could be attributed to its ability to infect and kill termites, disrupting termite's structure through cuticle penetration and causing pathogenesis (Zekeya et al., 2019; Zulfiana et al., 2020). Similar mode of action was reported in Study by which showed how conidia forming entomopathogenic fungi particularly *Metarhizium anisopliae* effectively controlled termites than other microbes (Syazwan et al., 2021) and revealed to be more effective

when combined with other substances (EE. et al., 2016) [. This is supported by studies on other fungal biopesticides, which have shown similar effects of causing pathogenesis on termite colonies (Ambele et al., 2020). The effectiveness of biopesticide was dose-dependent across experiments which is supported with previous research on fungal biopesticides, where higher conidia concentrations resulted in faster termite mortality (Sileshi et al., 2013). This highlights the importance of optimizing application rates for effective termite control within short time to prevent high loss especially during dry season where in southern highlands of Tanzania the impact has been revealed by a study done by Mbwambo (Mbwambo et al., 2022). *Aspergillus oryzae* showed the effectiveness in controlling termite on coffee all zones of Tanzania where the quality Arabica coffee is mostly cultivated (Otieno, 2019). *A. oryzae* was most effective coffee plantations whereby mound building termites which were observed and reported to affect yield due to space occupancy and destruction of coffee trees. The effect on bees' attraction was dose dependent where the highest dose of *A. oryzae* attracted a diversity of bees compared to low doses and Control. The high attraction rate of bees in *A. oryzae* treated plots could be attributed by lavender aroma which is used as adjuvant in VB-Liquid which is reported to attract bees as reported by Sari & Sarvia (Sari and Sarvia, 2025). The formulation of *A. oryzae* with lavender have influence on high abundance and diversity of bees in *A. oryzae* treated coffee plantations which would have contributed the high pollination rate enhancing the quality and yield to coffee as reported in previous studies (Roba Jilo, 2025; Pereira Machado et al., 2024), increasing economic and ecological value. The use of entomopathogenic fungi including *A. oryzae* have been revealed to increase yield and conserve biodiversity in contrast to chemical pesticide which affect soil and agriculture ecosystem (Wang and Zhang, 2024; Shi et al., 2025). This study proves the effectiveness of *A. oryzae* as a biocontrol for improving crop production while reducing impacts associated with chemical pesticides in Tanzania.

Conclusion

In conclusion, the study demonstrates the effectiveness of *Aspergillus oryzae* (VB-MG938648) as a biopesticide against subterranean termites in cocoa, macadamia, and coffee trees. VB-Liquid showed high activity

on coffee by improving plant recovery hence preventing loss, attracted pollinators such as bees and showed no plant pathogenesis hence promise for integrated pest management especially on coffee which is intensively cultivated in Tanzania. Further research is required to optimize application rates and evaluate long-term effect in macadamia and cocoa which take long time cultivation with complex tree architecture affecting effective application of biopesticide on canopy.

CRedit authorship contribution statement

Never Zekeya: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Never Zekeya reports financial support was provided by PLANT BIODEFENDERS. Reports a relationship with that includes: Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data included in manuscript

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