



## Efficacy of *Aspergillus oryzae* strains in reducing *Thaumatotibia leucotreta* damage and improving postharvest quality of avocado fruits

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### ABSTRACT

Avocado productivity is strongly constrained by insect-mediated fruit damage, because feeding injury reduces marketable yield and can also compromise postharvest quality. Field trial was purposively conducted in 3 districts growing Hass avocado namely Siha, Moshi and Hai. The minimum farm size was 2800 m<sup>2</sup> which was enough to provide a minimum of 30 plants to cater for one set for the treatment which was replicated thrice. A randomized complete block design was used with six treatments namely; *Aspergillus oryzae*- JF20, JF21, JF22, JF23, JF24 at concentration of  $4.0 \times 10^8$  conidia/mL in 4 mls/Litre and 6-negative control, whereby only water was applied without addition of any form of insecticide. In the present study, the unsprayed control consistently showed the greatest fruit damage across all three sites, reaching  $2.95 \pm 0.069$  in Siha,  $3.88 \pm 0.067$  in Moshi, and  $3.35 \pm 0.186$  in Hai, which establishes a clear baseline of vulnerability under untreated conditions. This pattern indicates that, avocado orchards without protection remained highly exposed to damage associated with False Codling Moth, *Thaumatotibia leucotreta*. The magnitude of control damage also underscores the agronomic importance of interventions that can suppress pest injury while maintaining orchard productivity. Fruit damage is not only a direct loss of harvestable produce but also a pathway to reduced economic value through lower grades and shortened shelf-life. This framing is important for interpreting the treatment effects in a production system where pest suppression, yield formation, and quality preservation are functionally linked increasing export value for avocado.

### Introduction

Agricultural pests pose a significant threat to global food security, and their management is a major concern for farmers and policymakers worldwide (Adom et al., 2021). Production of avocado in East Africa is threatened by invasion of *Thaumatotibia leucotreta*, False codling moth (FCM) causing massive damage and loss to smallholder farmers (Odanga et al., 2020). In Tanzania, FCM causes significant yield losses, impacting food security and livelihoods of smallholder farmers (Shikoshi et al., 2025; Onamu et al., 2024). The effect of *Thaumatotibia leucotreta* on avocado cause substantial economic losses (Shikoshi et al., 2025). Studies report that an increase in climate change related events has increased the spread and impact of the pest especially in Northern Tanzania and southern Kenya (Mkiga et al., 2021; Odanga et al., 2020). The increasing spread of FCM has reported to cause fruit drop, low quality leading to market loss in Tanzania (Yangaza, Kiwonde, 2026). Current pest management strategies rely heavily on chemical pesticides, which have environmental and health risks, and are often ineffective due to pesticide resistance (Mutymbai et al., 2020; Grové, 2025). The

use of pheromone traps is also reported with little success as attract only adult leaving out larvae, the destructive stage (Kemigisha, 2022). The use of chemical pesticides is reported to pose many challenges including pest resistance which increases production cost to smallholder farmers. Hence biological control strategies including plant extracts, microbial based products such as entomopathogenic fungi (EPF) have emerged as a promising source for biological pest control (Bara & Laing, 2020). *Beauveria bassiana* and *Metarhizium anisopliae* have been widely used for controlling various insect pests in different crops with many economic and ecological benefits compare to chemical pesticides (McGuire & Northfield, 2020; Oliveira et al., 2014). In Tanzania, fungal isolates such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Aspergillus oryzae* have been reported for controlling several pests such as tomato moth, *Tuta absoluta*, (Boni et al., 2021; Zekeya et al., 2019). These fungal based biopesticides have emerged as a promising alternative for biological pest control, producing secondary metabolites with insecticidal properties in tomato fields in Tanzania (Zekeya et al., 2022). Studies on entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae*, and *Aspergillus oryzae* in Tanzania field have been successful in increasing

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yield after controlling various insect pests that reduces vigor (Zekeya et al., 2022)). *Aspergillus oryzae*, a fungus commonly used in food fermentation, has been reported to have entomopathogenic properties, producing secondary metabolites such as kojic acid and aspergilline, which have insecticidal activities (Wang, Zhang, 2024). Secondary metabolites produced by *A. oryzae*, have been shown to have insecticidal activities against a wide range of agricultural pests (Mahmoud et al., 2023). These metabolites can be used as biopesticides, providing a sustainable and environmentally friendly alternative to chemical pesticides (Ibrahim et al., 2024). Despite of efforts in commercializing biocontrol for pest management, none have been registered for control of FCM in East Africa making false codling moth, a major pest of avocado, causes significant damage to fruit, leading to losses of up to 20% in some regions (Odanga et al., 2020).

In addition to the economic losses, FCM also have significant impacts on the environment and human health due to over and misuse of synthetic pesticides (Kadoić Balaško et al. 2020). Some of these of chemical pesticides to control the pest have been linked to the decline of pollinator populations, such as bees, and the contamination of water sources (Ara & Haque, 2021). Therefore, there is a need for sustainable and environmentally friendly pest management strategies that can effectively control these pests without harming the environment and human health for improving avocado yield and market across borders without impacts. This study aimed to identify fungal isolates with potential entomopathogenic activity for controlling agricultural pests especially FCM contributing to sustainable pest management strategies for improving food production in Tanzania.

## Material and methods

### Identification of isolates

The new isolates were isolated and identified from unmanaged local avocado variety at Kibosho Mashariki, Moshi, District, Kilimanjaro in March 2024. Isolates were purified through several subculturing using Sabouraud Dextrose agar as growth media. In order to clear uncertainty of isolates, molecular characterization of all isolates was conducted. DNA from each fungal isolates was extracted according to Zhang et al. (2015) with modifications. To generate the genome sequence of *Aspergillus oryzae*, fungal cultures were grown on appropriate media under controlled conditions. Genomic DNA was extracted using a standardized fungal DNA extraction protocol of the Wizard® Genomic DNA Purification Kit, which provides a solution-based method for isolating DNA from various sources, including fungi. Thereafter DNA quality and concentration were assessed using a NanoDrop spectrophotometer and Qubit fluorometer. DNA integrity was confirmed by agarose gel electrophoresis. Sequence analysis of fungi isolates was done with the BLAST program available at the National Centre for Biological Information (NCBI). Then the isolates showed 100% similarity with *Aspergillus oryzae* fungal sequences of the present invention have been deposited on the NCBI Gene Bank.

### Library Preparation

The high-quality DNA was fragmented using a Covaris sonicator where fragmented DNA was subjected to end-repair, A-tailing, and adapter ligation. Thereafter library size selection was performed using SPRI beads or gel electrophoresis where prepared libraries were quantified using qPCR or Bioanalyzer. Sequencing was done by the whole-genome sequencing was performed using the Oxford Nanopore Sequencing (ONT) whereby long-read sequencing for improved assembly quality and resolution of complex genomic regions was achieved. The sequencing depth was optimized to achieve at least  $100 \times$  coverage for accurate assembly. Raw reads were processed using Fast QC quality control tool to remove low-quality sequences and adapters. The De novo genome assembly was performed using long-read assemblers for Oxford

Nanopore data. Hybrid assembly approaches were used when both short- and long-read data were available. The assembled genome was scaffolded and gap-filled using PBJelly. Gene prediction and annotation were carried out using the structural annotation; Augustus, GeneMark-ES, or SNAP for gene prediction and functional annotation: BLASTp, InterProScan, and EggNOG for protein function assignment and secondary metabolite gene cluster identification was done by antiSMASH for detecting biosynthetic gene clusters.

### Secondary metabolite biosynthetic gene cluster identification

The identification of biosynthetic gene clusters (BGCs) was performed using antiSMASH (Antibiotics & Secondary Metabolite Analysis Shell), a bioinformatics tool designed to predict secondary metabolite gene clusters in fungal and bacterial genomes. This involved uploading the assembled genome sequence to the Fungal antiSMASH web server and enabling detection parameters to identify known and novel BGCs thereafter running the analysis and retrieving results, including predicted BGC locations, cluster types, and similarities to known secondary metabolites. Each identified BGC was classified based on its biosynthetic type such as Non-Ribosomal Peptide Synthetases (NRPS) & NRPS-like Clusters. Each identified BGC was compared against existing secondary metabolite databases, MIBiG (Minimum Information about a Biosynthetic Gene cluster), to determine similarities with known clusters. AntiSMASH provided information on the similarity Scores (% Match) that indicate the degree of conservation with known secondary metabolite clusters and putative Metabolites based on homology to characterized compounds (e.g., FR901512, Clavric acid, PR-toxin). For BGCs with no significant similarity to known clusters, additional analyses were conducted. Gene Ontology (GO) and KEGG Pathway Analysis for functional characterization of genes within each cluster Whereas mMotif and Domain Analysis was done using Pfam and InterPro tools to predict protein functions within unidentified BGCs followed by comparative Genomics to examine conservation of these clusters across other *Aspergillus* species.

### Evaluation of potential bioactivity and toxicity

Based on identified biosynthetic pathways, clusters were further assessed for their potential to produce bioactive or toxic metabolites. Presence of Toxicity-Related Genes: Clusters encoding enzymes linked to toxin production (NRPS-derived alkaloids, PR-toxin synthesis genes) were flagged for further study. Literature review for cross-referencing known secondary metabolites associated with *A. flavus*, including aflatoxins and other mycotoxins was conducted. Graphical outputs from antiSMASH were analyzed using Genome Browser Tools: Artemis and IGV for visualizing gene cluster architecture whereas the Phylogenetic Analysis was done by constructing evolutionary relationships among biosynthetic genes using MEGA software. Thereafter, metabolic Network Reconstruction was done by predicting potential biosynthetic pathways using Pathway Tools.

### Formulation of biopesticide

A novel biopesticide was formulated by using spores as active ingredient. A new biocide is composed of new formulation from 9.5% fermented broken rice mixed with mineral 0.1% mineral (potassium hydroxide) adjuvant 0.4%, incubated at  $35^{\circ}\text{C}$  in pH 8.5–9.0 for 7 days in incubator. Thereafter, fungal mats were gently grinded to get homogeneous mixture followed by addition of water 30%, 50% triple refined sunflower oil, 18% molasis and 2% lavender oil. Then conidia from stock suspension was injected into haemocytometer neubauer chamber (Manfield, German) that was viewed under stereo microscope and known to have  $10 \times 10^9$  conidia/mL which was then diluted to measure concentrations of  $4.0 \times 10^7$  conidia/mL for efficacy trail. The resulting suspension were used for bioassays to confirm activity of fungal isolates

against False codling moth on avocado in field.

### Field trial

Field trial was purposively conducted in 3 districts growing Hass avocado namely Siha, Moshi and Hai from February to April 2026 during flowering and fruiting stage where FCM infest mostly. Prior to trials set up, brief pest assessments were made using traps in the respective farms. The avocado orchard with Hass avocado plants aged between 6 and 8 years were selected for the experiment in each district. The mandatory minimum farm size was more than 2800 m<sup>2</sup> which was enough to provide a minimum of 30 plants to cater for one set (replication) for the treatment. Each treatment set was replicated thrice and inter-row space was at least a row of 10 plants. A randomized complete block design was used with six treatments namely; *Aspergillus oryzae*-JF20, JF21, JF22, JF23, JF24 at concentration of  $4.0 \times 10^8$  conidia/mL in 4 mls/Litre and 6-negative control, whereby only water was applied without addition of any form of insecticide. All *Aspergillus oryzae* isolate were separately measured using a cylinder to provide recommended dosage of 4 mL/L. A 100 mL measuring cylinder was used to measure biopesticide volume measured was either 80mls for a 20litre knapsack sprayer 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 fruit/leaf. Thorough mixing was done and the mixture applied to the avocado plant covering the whole canopy from bottom up. Motorized sprayers were used on tall trees to ensure good coverage to the tip leaves. All *Aspergillus oryzae* isolate JF20–24 spraying was made in the evening of the sunny day starting at 1700hrs through 1900hrs as 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. The application was repeated every week providing a seven days interval for eight weeks consecutively. The experiment was conducted during fruiting season from March to May 2026.

### Data collection

The data on FCM incidence, FCM damage severity and abundance of beneficial insect (bees) was collected on a weekly basis. The FCM damage severity score was based on the observable damage signs estimated in percentage using the scale; 0, 1, 2, 3, 4 & 5 whereby, 0 = healthy fruits (not damaged), 1 = up to 5% damaged, 2 = 6–12% damage, 3 = 13–25% damage, 4 = 26–75% damage, 5 > 75% damage. Yield data was recorded at harvesting in form of number and weights of FCM infected and healthy fruits obtained from each treatment while data on effect of *A. oryzae* on avocado postharvest quality was obtained after selecting 10 fruits from each treatment exposing in room temperature for 10 days.

### Pest scouting and identification

Pheromone trap and Pan-trap were used to collect and identify insect groups. Five sampling plots were selected randomly for sampling, each consisted of at least 20 plants. Pheromone traps were set on avocado trees to identify and tap FCM whereas pan traps were placed in quadrant shape, in a space of 1 m between one trap and the other to collect adults or other pests. Data on traps were collected after 24 h of setting traps.

### Pathogenicity test and confirmation of fungal isolates

After pest death of all larvae of from all treatments including control were collected and stored separately to verify whether the death of individual has been infected or non-infected with conidia of *A. oryzae*. The dead larvae were separately placed in moisty petri dishes lined with sterile paper towel and incubated at  $28 \pm 2$  °C for 7 days after which

they were observed for mycelial growth and checked for isolate similarities using Stereo microscope, 400x magnification (Carl Zeiss, Germany). Data were collected by taking pictures of each fungal stage up to 7th day of incubation to observe and change in color and mycelia growth without contamination.

### Data analysis

Data on pest incidences were calculated based on the formula: Pest incidence (%) = (Number of affected plants / Total number of plants) x 100. 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.

### Results

This study revealed that all the five isolates belong to the genus *Aspergillus*. Some isolates are identified as *Aspergillus oryzae* while others as *Aspergillus flavus*. The phylogenetic analysis revealed that isolates are clonally related, that is overall more closely related to each other than to the nearest references. The isolates are overall genetically more similar to *A. oryzae* than to *A. flavus*. *A. oryzae* is generally identified as non-toxicogenic used for centuries in fermented food production in Japan, whereas some *A. flavus* produce aflatoxin B (AFB1, AFB2) and other toxins. Although *A. oryzae* and *A. flavus* are genetically very closely related, *A. oryzae* is presumed to be a domesticated descendant and the ~0.3% ANI difference may not be significant. Also, this is only based on average nucleotide identity (ANI) across the genome. The results revealed that all isolates are genetically related to non-toxicogenic strain SU-16, which is classified as *A. flavus* but which sits with the *A. oryzae* genotypes and is widely used in China for fermentation; inversely KY132 is classified *A. oryzae* but sits with the *A. flavus* in the typings (Fig. 1).

Fruit damage differed markedly among treatments and sites, with the unsprayed control showing the highest damage in every location. In Siha, control damage was  $2.95 \pm 0.069$ , compared with  $0.06 \pm 0.02$  for JF24, which was the lowest observed value and was assigned a distinct significance letter from the control. In Moshi, the control reached  $3.88 \pm 0.067$ , whereas JF24 reduced damage to  $0.08 \pm 0.11$ . In Hai, control damage was  $3.35 \pm 0.186$ , while JF24 again had the lowest value at  $0.31 \pm 0.08$ . These differences indicate that JF24 provided the most consistent damage suppression across all sites. Other isolates also reduced damage relative to the control, but their effectiveness was more variable. JF20 produced low damage in Siha and Moshi, with  $0.11 \pm 0.016$  and  $0.48 \pm 0.114$ , respectively, but damage was higher in Hai at  $2.11 \pm 0.087$ . JF21 showed intermediate suppression in Siha and Hai, yet Moshi retained relatively elevated damage at  $2.03 \pm 0.038$ . JF22 performed better than the control but still left notable damage in Hai, where it recorded  $1.51 \pm 0.172$ . JF23 was generally stronger than JF20 to JF22, especially in Hai where damage was  $1.21 \pm 0.162$ , but it still did not match JF24. The lettering patterns reinforce these differences and show that the treatments separated into statistically distinguishable groups within columns (Table 1).

Yield responses were closely related to the damage results, with JF24 producing the highest yields in all three sites. In Siha, JF24 yielded 8937.28 kg/ha, clearly exceeding all other treatments and the control, which produced only 2816.80 kg/ha. In Moshi, JF24 again ranked first at 7597.62 kg/ha, and in Hai it reached 8806.24 kg/ha. The control remained the lowest-yielding treatment in every site, with 2764.39 kg/ha in Moshi and 2951.42 kg/ha in Hai, indicating a broad production penalty under unsprayed conditions. The remaining isolates produced intermediate yields that were consistently higher than the control but lower than JF24. JF21 gave relatively strong yields, particularly in Siha and Moshi, where it exceeded 7600 kg/ha in both sites. JF20 and JF23 also improved yield over the control, with JF23 showing especially favorable performance in Hai at 6806.42 kg/ha. JF22 was the weakest of the treated groups, with yields of 5425.33 kg/ha in Siha, 5532.00 kg/

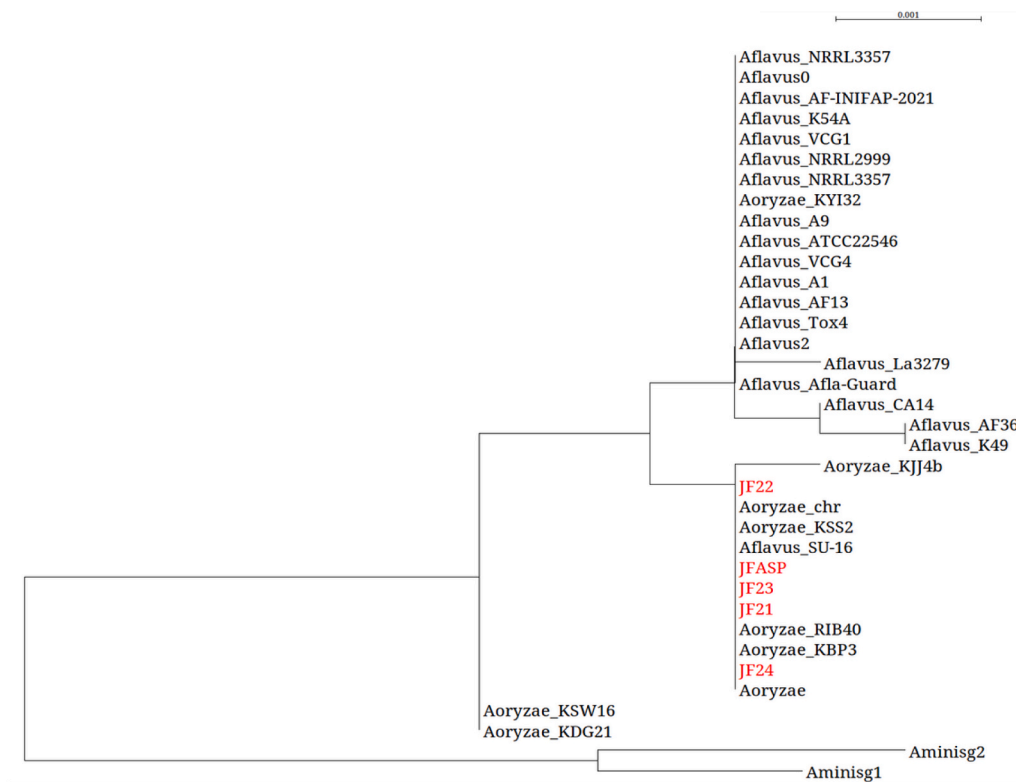


Fig. 1. A phylogenetic tree placing the five fungal isolates (Colored red) in the reference genomes. The tree was constructed based on the conserved regions namely: -beta-tubulin (benA/tub1), calmodulin (CaM) and rRNA with ITS1 and ITS2.

**Table 1**  
Efficacy of *Aspergillus oryzae*-JF24 in preventing avocado fruits from *Thaumastobita leucotreta* damage.

| Treatment                       | Siha          | Moshi          | Hai           |
|---------------------------------|---------------|----------------|---------------|
| <i>Aspergillus oryzae</i> -JF20 | 0.11 ± 0.016d | 0.48 ± 0.114d  | 2.11 ± 0.087b |
| <i>Aspergillus oryzae</i> -JF21 | 1.38 ± 0.021c | 02.03 ± 0.038d | 2.87 ± 0.108b |
| <i>Aspergillus oryzae</i> -JF22 | 0.55 ± 0.040d | 062 ± 0.036d   | 1.51 ± 0.172b |
| <i>Aspergillus oryzae</i> -JF23 | 0.13 ± 0.003d | 0.51 ± 0.011d  | 1.21 ± 0.162c |
| <i>Aspergillus oryzae</i> -JF24 | 0.06 ± 0.02d  | 0.08 ± 0.11d   | 0.31 ± 0.08d  |
| Unsprayed (Control)             | 2.95 ± 0.069a | 3.88 ± 0.067a  | 3.35 ± 0.186a |

Means within columns followed by the same letters are not significantly different (P > 0.05)

ha in Moshi, and 5359.32 kg/ha in Hai. The lettering patterns show clear separation between the top-performing JF24 and the lower-yielding treatments (Table 2).

Bee abundance was substantially higher in the treated orchards than in the unsprayed control, which suggests that the treatments were not ecologically suppressive. JF24 recorded the highest bee abundance in all three sites, with 5.12 ± 0.03 in Siha, 8.12 ± 0.17 in Moshi, and 6.19 ± 0.53 in Hai. JF23 also fell in the high-abundance group, with values of

**Table 2**  
Effect of *Aspergillus oryzae* on avocado yield (kg/ha).

| Treatment                       | Siha     | Moshi    | Hai       |
|---------------------------------|----------|----------|-----------|
| <i>Aspergillus oryzae</i> -JF20 | 6722.33c | 6773.25c | 6822.71c  |
| <i>Aspergillus oryzae</i> -JF21 | 7654.67b | 7628.31b | 7622.18b  |
| <i>Aspergillus oryzae</i> -JF22 | 5425.33d | 5532.00d | 5359.32d  |
| <i>Aspergillus oryzae</i> -JF23 | 6437.24c | 6597.64c | 6806.42c  |
| <i>Aspergillus oryzae</i> -JF24 | 8937.28a | 7597.62a | 8806.24a  |
| Unsprayed (Control)             | 2816.80e | 2764.39e | 2951.42 e |

Means within columns followed by the same letters are not significantly different (P > 0.05)

4.92 ± 0.03, 5.82 ± 0.17, and 5.19 ± 0.53 in Siha, Moshi, and Hai, respectively. By contrast, the control had the lowest bee abundance across all sites, at 0.15 ± 0.19 in Siha, 0.18 ± 0.17 in Moshi, and 0.94 ± 0.27 in Hai. The intermediate isolates, JF20, JF21, and JF22, all supported moderate bee presence, with values generally clustered between about 2 and 4 across the sites. The shared lettering shows that these treatments formed a lower category than JF23 and JF24 but a higher category than the control. This pattern is important because it suggests that the best-performing isolates did not do so at the expense of beneficial insect activity. Instead, the highest-yielding treatments were also associated with the strongest bee presence, which is consistent with ecological compatibility in orchard management (Table 3).

The quality of avocado was also influenced by the preharvest effectiveness of the *A. oryzae* JF24 which revealed high quality of avocado compared to all other isolates and control after harvest. Postharvest quality after 10 days further strengthens the case for JF24 as the leading treatment. JF24 recorded the highest quality scores in every site, with 94.20 ± 0.62 in Siha, 92.66 ± 0.88 in Moshi, and 86.33 ± 0.295 in Hai. The control had the poorest quality in all locations, at 69.33 ± 0.62 in Siha, 69.33 ± 0.62 in Moshi, and 58.333 ± 2.051 in Hai. These differences indicate that treatment effects extended beyond the field into the storage period, where quality retention is essential for market value

**Table 3**  
Effect of *Aspergillus oryzae* on beneficial insects (bees) in avocado orchards.

| Treatment                       | Siha         | Moshi        | Hai          |
|---------------------------------|--------------|--------------|--------------|
| <i>Aspergillus oryzae</i> -JF20 | 2.14 ± 0.05b | 2.25 ± 0.33b | 3.87 ± 0.21b |
| <i>Aspergillus oryzae</i> -JF21 | 2.16 ± 0.04b | 2.13 ± 0.17b | 2.37 ± 0.46b |
| <i>Aspergillus oryzae</i> -JF22 | 2.18 ± 0.06b | 2.28 ± 0.21b | 3.81 ± 0.45b |
| <i>Aspergillus oryzae</i> -JF23 | 4.92 ± 0.03a | 5.82 ± 0.17a | 5.19 ± 0.53a |
| <i>Aspergillus oryzae</i> -JF24 | 5.12 ± 0.03a | 8.12 ± 0.17a | 6.19 ± 0.53a |
| Unsprayed (Control)             | 0.15 ± 0.19c | 0.18 ± 0.17c | 0.94 ± 0.27c |

Means within columns followed by the same letters are not significantly different (P > 0.05)

**Table 4**  
Postharvest Effect of *Aspergillus oryzae* on Maintaining Avocado quality 10 days after harvesting.

| Treatment                       | Siha           | Moshi         | Hai             |
|---------------------------------|----------------|---------------|-----------------|
| <i>Aspergillus oryzae</i> -JF20 | 88.67 ± 0.87a  | 86.00 ± 1.32a | 80.56 ± 0.28a   |
| <i>Aspergillus oryzae</i> -JF21 | 84.33 ± 1.17ab | 83.67 ± 1.03a | 80.46 ± 3.41a   |
| <i>Aspergillus oryzae</i> -JF22 | 81.67 ± 0.98b  | 85.00 ± 1.29a | 75.83 ± 1.07a   |
| <i>Aspergillus oryzae</i> -JF23 | 88.66 ± 0.68a  | 87.61 ± 0.88a | 82.73 ± 0.95a   |
| <i>Aspergillus oryzae</i> -JF24 | 94.20 ± 0.62a  | 92.66 ± 0.88a | 86.33 ± 0.295a  |
| Unsprayed (Control)             | 69.33 ± 0.62c  | 69.33 ± 0.62b | 58.333 ± 2.051b |

Means within columns followed by the same letters are not significantly different (P > 0.05)

(Table 4).

The results showed that *A. oryzae* Isolate JF24 was the most effective and pathogenic to larvae with high propagation rate on dead larvae revealing that all it was responsible for FCM larval mortality through pathogenesis while there was slow growth in isolate JF20–23 and no fungal growth was observed in dead larval from control plots (Fig. 2).

**Discussion**

The findings revealed that JF24 outperformed the other isolates because it combined the strongest suppression of fruit damage with the greatest yield gains across all three sites. The treatment that minimized pest injury also produced the highest orchard productivity. In Siha, for instance, JF24 reduced damage to near-zero levels relative to the control and increased yield to 8937.28 kg/ha. A similar pattern appeared in Moshi and Hai, where JF24 remained the top treatment for both damage suppression and yield. The data suggest that the relationship between damage reduction and yield improvement was especially tight for JF24. Treatments with moderate damage suppression, such as JF20, JF21, and JF22, produced intermediate yields rather than matching the output of JF24. This indicates that partial pest suppression may not be sufficient to maximize productivity if fruit injury remains substantial in at least some site conditions (Shikoshi et al., 2025; Nengovhela, 2020). JF24 therefore appears to have crossed a functional threshold in which pest pressure was reduced enough to preserve a larger proportion of productive fruit. The significance letters reinforce this interpretation by separating JF24 from the lower-performing groups in most data. The combined pattern of lower damage, higher yield, and greater bee abundance suggests a plausible positive linkage among these outcomes. Because JF24 and

JF23 produced the highest bee counts and also ranked among the strongest treatments for yield, the data imply that beneficial insect presence may be associated with orchard productivity. This does not prove causality, but it does indicate that the most productive treatments were also those most compatible with bee activity. The unsprayed control, in contrast, showed both the highest damage and the lowest bee abundance, which aligns with its poor yield. This study revealed that a better pest suppression may reduce stress on fruiting trees and help maintain conditions that support orchard function more broadly. The observed bee abundance could reflect a favorable orchard environment under treated conditions but in this study was attributed by the formulation of biopesticide using lavender and molasis which attract the bees as revealed by other studies (Deshpande & Naik, 2016). Nonetheless, the treatment pattern suggests that biocontrol interventions can be designed to preserve beneficial insects while improving production outcomes. This is particularly relevant for avocado systems where pollinator activity is part of the broader production ecology (Jailiang, 2019). JF24 was consistently superior, the magnitude of responses differed by site, showing that agroecological context and history of isolation influenced treatment performance. Moshi often showed the highest damage in the control and also the highest bee abundance under JF24, while Hai sometimes showed more moderate treatment separation. Siha displayed especially low damage under several treatments, suggesting that the baseline pest environment or orchard response may differ from the other two sites. These site differences indicate that treatment effects were not uniform across landscapes, even when the ranking of treatments remained similar. The variation among sites is also visible in the intermediate isolates. JF21 and JF22 performed reasonably well in some locations but less effectively in others, especially in Hai where damage remained comparatively elevated for several treatments. This suggests that isolate performance may be modulated by local field conditions, orchard history and environmental context, where isolate J4 is local to Moshi environment which might influence the performance. The consistency of JF24 across all three sites is therefore especially notable because it suggests broader robustness to site variation than the other isolates because was isolated from local variety avocado which were resistant to FCM. The broader agronomic value of the treatments lies in this linkage between preharvest protection and post-harvest preservation. JF24 not only reduced damage and improved yield but also maintained fruit quality more effectively than the other isolates. JF23 also performed well in postharvest quality, but it remained consistently below JF24 in the top-performing sites. This suggests that

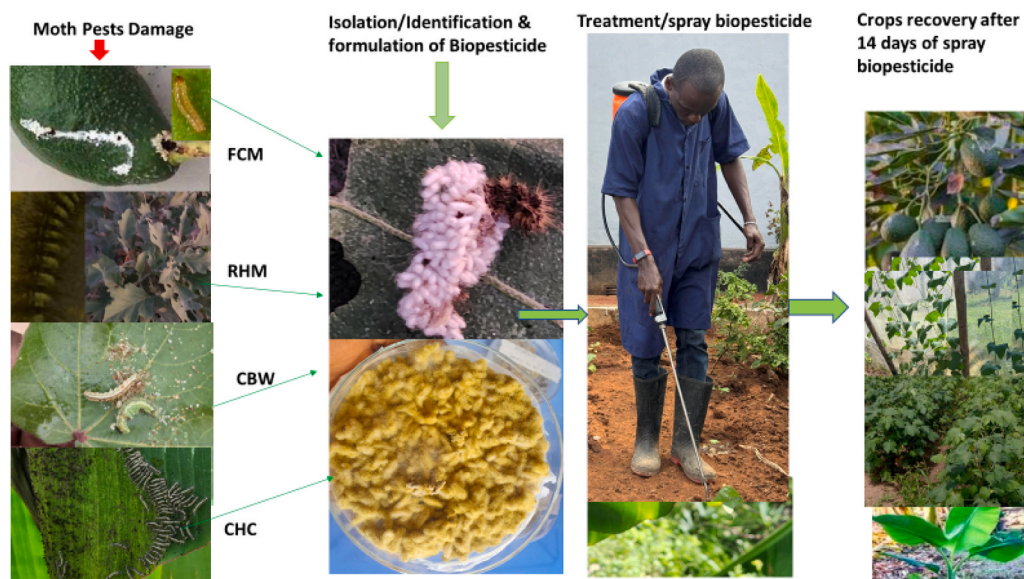


Fig. 2. Showing mode of action of *A. oryzae* JF–24 and pathogenesis on moth pests.

the most effective treatment was the one that integrated multiple desirable outcomes across the production chain. In practical terms, such a treatment has greater value especially for export market of avocado than an intervention that improves only one endpoint. The strongest overall evidence points to JF24 as the leading isolate because it dominated all four outcome domains assessed here. It produced the lowest fruit damage, the highest yield, the highest or near-highest bee abundance, and the best postharvest quality across Siha, Moshi, and Hai. The control treatment showed the opposite profile, with the highest damage, the lowest yield, the lowest bee abundance, and the poorest quality. This inverse relationship between JF24 and the control provides a coherent pattern of treatment response.

## Conclusion

Generally, findings revealed that JF24 delivered the most favorable overall performance among the tested *Aspergillus oryzae* isolates. It consistently produced the lowest fruit damage, the highest yield, the strongest bee presence among the top treatments, and the best postharvest quality in Siha, Moshi, and Hai. By contrast, the unsprayed control performed worst on every major outcome and therefore represents a clear benchmark of vulnerability. The treatment hierarchy was also supported by the significance letters, which separated JF24 from the control and most of the other isolates across treatments. This integrated pattern suggests that JF24 was not simply effective in one dimension but beneficial across the production sequence. Damage suppression likely contributed to improved yield, while the preservation of quality extended the advantage into the postharvest phase. The bee data further indicate that the treatment was compatible with orchard ecological function. Taken together, these outcomes make JF24 the strongest candidate for pest control in avocado.

## CRedit authorship contribution statement

**Never Zekeya:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition.

## Declaration of Competing Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Never Zekeya reports financial support was provided by Plant Biodefenders. There are no other authors, hence 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 will be made available on request.

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