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RESEARCH ARTICLE

Black soldier fly frass and its derivatives as biofungicide to control Fusarium wilt in bananas

Jia Xin Ong[1], Nur Baiti Abd Murad[1], Siti Rasila Ainaa Mohd Rasli[2], Mohd Rezuwan Shah Zakaria[2], Shanmugaprakasham Selvamani[2], Hesham Ali El-Enshasy[3], Martin Jose Zorrilla[4], and Noor Baity Saidi[1*]

[1]Universiti Putra Malaysia, Faculty of Biotechnology and Biomolecular Sciences, 43400, Serdang, Selangor, Malaysia.
[2]Nutrition Technologies, R&D Biotechnology Division, 79150 Iskandar Puteri, Johor, Malaysia.
[3]Universiti Teknologi Malaysia, Institute of Bioproduct Development, 81310 UTM, Johor Darul Ta'zim, Malaysia.
[4]Nutrition Technologies, 048423, Singapore, Singapore.
[*]Corresponding author (norbaity@upm.edu.my).
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ABSTRACT

Fusarium wilt disease has greatly threatened banana (*Musa acuminata* Colla) plantations worldwide. Sustainable approaches utilizing bio-based agricultural products are a promising alternative to managing the disease. In this study, the potential of composted black soldier fly (BSF; *Hermetia illucens* [Linnaeus], 1758) frass and its fermented liquid derivative as biofungicides against the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4), were investigated in vitro and in vivo. The minimum inhibitory concentration (MIC) of sterile (filter-sterilized) and non-sterile filtrates prepared from composted BSF that inhibit *Foc* TR4 spores was recorded at 40% and 25% (v/v), respectively. However, only the non-sterile filtrate (50%, v/v) achieved the fungicidal effect against *Foc* TR4 spores and mycelium in the in vitro inhibition assay. The in vivo pot experiment showed that preventive treatment of soil with the composted BSF frass in combination with the fermented liquid derivative reduced the Fusarium wilt disease index to 47.66%. Analysis of the microbial community in the composted BSF frass revealed a high abundance of *Firmicutes* (64%) and *Actinobacteriota* (23%) phyla, whereas *Proteobacteria* (44%), *Planctomycetota* (19%), and *Bacteroidota* (18%) dominated the fermented liquid derivative, which likely explains the observed disease-suppressing effects. This study pioneers the exploration of BSF frass as a biofungicide against Fusarium wilt in bananas, revealing that composted BSF frass and its fermented liquid derivative could serve as effective, sustainable alternatives to conventional fungicides in managing the disease.

Key words: Biological control, BSF, *Foc* TR4, *Fusarium oxysporum* f. sp. *cubense*, *Hermetia illucens*, *Musa acuminata*, suppression.

INTRODUCTION

The soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is the cause of major losses to banana (*Musa acuminata* Colla) plantations globally (Staver et al., 2020). *Foc* can survive in the soil for decades by producing chlamydospores that can withstand desiccation and unfavorable environments, thus presenting a great challenge in controlling the disease once it is established (Pegg et al., 2019). Among the *Foc* races, tropical race 4 (TR4) is considered the most virulent.

Disease control approaches, including physical, chemical, and biological, have been practiced singly or in combination with other disease management strategies to prevent and manage Fusarium wilt in bananas (Jamil et al., 2019). Among them, the application of chemical fungicide is by far the most adopted strategy despite being marginally effective (Cannon et al., 2022). However, the continuous use of chemical fungicides can lead to fungal resistance and is harmful to human health in the long term. On the other hand, biological control offers a promising disease management approach due to its safety and less environmental impact (Bubici et al.,

2019). Organic soil amendment, which is a form of biological control methods holds great potential in plant disease control due to its ability to improve the soil's physicochemical and biological properties. Several studies reported that organic amendments can increase soil suppressiveness against numerous plant pathogens, including fungi (Liu et al., 2018; van der Sloot et al., 2024). Exploiting these positive effects of organic materials can also address rising environmental concerns about the use of inorganic fertilizers and chemical fungicides.

Insect frass, a waste generated from insect farming, is one of the organically derived soil amendments currently undergoing substantial research in terms of the effects on soil microbiome, plant performance, and disease suppression (Arabzadeh et al., 2024; van de Zande et al., 2024). It is composed of insect feces, exuviae, fragments of the exoskeleton, diet material, and microorganisms (Barragán-Fonseca et al., 2022). The insect frass can also be repurposed as an organic fertilizer with a positive impact on plant yields, equivalent to those obtained with synthetic N fertilizers and more traditional organic fertilizers (Beesigamukama et al., 2020). As insect frass represent commodities derived from waste products, they provide excellent opportunities for practically implementing circular economy models (van Huis et al., 2021).

In addition to providing critical nutrients for plant growth, insect frass is also rich in microbial diversity, which has potential roles in eliminating pathogens, toxins, and pollutants from the environment (Mannaa et al., 2024). Another significant component of insect frass is chitin, a high-molecular-weight amino-sugar polysaccharide that is also present in fungal cell walls and the exoskeleton of many crustaceans. Chitin soil amendments have also been demonstrated to promote plant growth and stimulate naturally occurring beneficial microbes in the soil (Fan et al., 2022).

The most common insect utilized in insect agriculture is the black soldier fly (BSF; *Hermetia illucens* [Linnaeus], 1758), a non-pest detritivore able to consume a wide range of organic waste. Recent studies have reported enhanced growth, yield, and nutrient quality in plants grown using composted BSF frass (Beesigamukama et al., 2020; Anyega et al., 2021). In addition to its application as an organic fertilizer, BSF frass has been shown to positively modulate the soil microbial community and suppress various crop diseases (Barragán-Fonseca et al., 2022; Arabzadeh et al., 2022). For example, BSF frass reared on a specific larval-rearing diet greatly suppressed root colonization by the tomato pathogen, *Fusarium oxysporum* f. sp. *lycopersici* and reduced the severity of tomato Fusarium wilt (Arabzadeh et al., 2024). Meanwhile, Anedo et al. (2024) presented evidence of the potential of BSF frass fortified with chitin as a multipurpose organic fertilizer amendment for enhancing potato yield and suppressing potato cyst nematodes. In addition, the value of BSF frass can be further increased through bioengineering, which includes fermentation using beneficial microbes (Tepper et al., 2024). The use of microorganism-rich biofertilizers in liquid form not only improves microbial diversity and nutrient availability but can also last for longer periods and require less application (Allouzi et al., 2022; Han et al., 2024).

We hypothesized that composted BSF frass, including the fermented BSF frass in liquid form, can potentially control Fusarium wilt in bananas due to their high chitin content, richness of microbiota, and ability to promote soil microbial diversity and functions. Therefore, this work aimed (1) to evaluate the inhibitory effect of composted BSF frass filtrates against *Foc* TR4 in vitro and determine its minimum inhibitory concentration (MIC), (2) to determine the bio-fungicidal effects of single and combined applications of composted BSF frass and its fermented liquid derivative as soil amendment against Fusarium wilt in vivo and (3) to discover the mechanism behind the disease-suppressing effects of BSF frass in bananas.

MATERIALS AND METHODS

Experimental site

The in vitro experiment was conducted at the Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai, Johor, while the in vivo bioassay was conducted at the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, from August to November 2023.

Composted BSF frass filtrate preparation

The composted black soldier fly (BSF) (*Hermetia illucens* [Linnaeus], 1758) frass containing *Bacillus halotolerans* used in this study was provided by Nutrition Technologies Sdn Bhd., Iskandar Puteri, Johor, Malaysia. The BSF frass underwent a windrow composting process for a period of 90-120 d. A filtrate was prepared by first

incubating 50% (w/v) composted BSF frass in sterile 0.85% NaCl at 27 °C with shaking at 300 rpm for 1 h, following the method by Vishan et al. (2017) with modifications. The liquid mixture was then centrifuged for 10 min at 4000 rpm through a sterile gauze cotton cloth to collect supernatant. The filtrate was tested in two conditions: Sterile and non-sterile. The supernatant was filtered using a 0.44 µm syringe filter to produce a non-sterile filtrate. To produce a sterile filtrate, the first-round filtrate was passed through a 0.22 µm syringe filter, which removed bacterial cells. The filtrates were then prepared at different concentrations for the in vitro antifungal assays (1%, 5%, 7%, 10%, 25%, 40% and 50% v/v).

In vitro antifungal assay on *Foc* TR4 spores

To prepare *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4) spore suspension for the in vitro assay, the fungus was grown on potato dextrose agar (PDA) at room temperature (around 25-27 °C) on the lab bench for 7 d. The fungal colonies were flooded with approximately 1 mL sterile saline containing 1% Tween 80, and the spores were harvested by probing the colonies with the tip of a Pasteur pipette. The mixture was filtered through a sterile Miracloth (Millipore, Burlington, Massachusetts, USA) gauze to separate the spores and the mycelia and the collected spore was diluted to 1×10^6 conidia mL^{-1} using sterile double distilled water. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined according to Wei et al. (2020) with modifications. A total of 20 µL each filtrate (sterile and non-sterile) at different concentrations was added into the wells of the 96-well plate in 6 replicates, each containing 100 µL *Foc* TR4 spore suspension and 80 µL potato dextrose broth (PDB). For negative control, spore suspensions were only mixed with PDB without filtrate. The spore suspension was mixed with PDB and an antifungal solution (nystatin, 30 mg L^{-1}) for the positive control. The plates were then incubated at 27 °C for 5 d. Subsequently, 100 µL each of the mixtures was spread onto plates containing PDA and antibiotic streptomycin (100 µg mL^{-1}) to prevent the growth of bacteria. The plates were then incubated for 3 d at 27 °C. The number of fungal colonies was counted, and the percentage of inhibition was calculated using the formula A-B × 100, where A is the number of fungal colonies in the negative control plates, and B is the number of fungal colonies in the treatment plates. The minimum filtrate concentration, which produces 60% inhibitory activity against *Foc* TR4 spores, was selected as the MIC. Meanwhile, the minimum concentration of filtrate that could kill 99% of the fungal spores was selected as the MFC.

In vitro antifungal assay on *Foc* TR4 mycelium plug

The antifungal characteristics of composted BSF frass filtrate against *Foc* TR4 fungal mycelium plug were determined according to Castaldi et al. (2021) with modifications. A total of 500 µL each filtrate (sterile and non-sterile) at different concentrations was added into the wells of the 26-well plate in four replicates, containing 500 µL 2× PDB. A fungal mycelium plug (4 × 4 mm) was added to each well. For negative control wells, the mycelium plugs were only mixed with PDB without filtrate. The mycelium plugs were mixed with PDB and an antifungal solution (nystatin, 50 mg L^{-1}) for the positive control. The plates were then incubated for 5 d at 27 °C. The mycelium was collected and transferred to a filter paper in a petri dish and then dried at 45 °C for one night. The dried mycelium was weighed, and the inhibition percentage was calculated using formula (A-B)/A × 100, where A is the weight of fungal mycelium from the negative control plate, and B is the weight of fungal mycelium from the treatment plates.

Chemical analysis of potting mix and BSF products

The potting mix was sieved through a 2 mm sieve and sent for pH and CNS analysis at the Centre of Tropical Soil Studies, Faculty of Agriculture, Universiti Putra Malaysia. The BSF products were sent for NPK analysis at SGS Malaysia Sdn. Bhd., Shah Alam, Selangor.

Pot experiment

Two-month-old *Musa acuminata* var. Cavendish plantlets (20 cm height) from Simple Farm Sdn Bhd., Kluang, Malaysia, were used in this study. The pot experiment was conducted using composted BSF frass and a fermented liquid derivative of the BSF frass. The liquid was fermented for 3-5 d with an additional *B. halotolerans* inoculant (added before the fermentation process) and provided by Nutrition Technologies Sdn Bhd. The bioassay was conducted in 15 replicates for each treatment in completely randomized design. The

experimental design is shown in Table 1. The plantlets were transferred into 1 L pots with a potting mix comprising cocopeat, sawdust, and burnt soil (1:2:2), weighing 1 kg after watering. The total N in each pot was standardized to approximately 1 g throughout the experiment, which was supplied through BSF frass based on its ammoniacal N content and a commercial NPK (10:20:20) fertilizer. The first 7 d following plantlet transfer were regarded as week 1. For the preventive treatment, the banana plantlets were transferred to pots containing composted BSF (35 g) only or composted BSF mixed with 1% (v/v) fermented liquid derivative (50 mL) (mixed earlier during the preparation of the potting mix) and grown for 14 d before being inoculated with *Foc* TR4. Composted BSF frass and its fermented liquid derivative were added to the potting mix 14 d after inoculation with *Foc* TR4 for the curative treatment. To even up the N contents between treatments, the commercial NPK fertilizer was added as a top dressing (T1 and T2, 2.355 g; T3 and T4, 2.058 g; T5, T6, and T7, 5.600 g) immediately after the plantlets being transferred to the individual pot. The BSF frass-treated plantlets (curative and preventive) were supplemented with 10 g composted BSF frass as a top dressing and 25 mL 1% (v/v) fermented liquid derivative as a foliar spray and the balance was poured onto the soil (soil drench application) every 3 wk starting from week 6 of the experiment. The plantlets were allowed to grow for a total of 12 wk. The height and leaf number of plantlets were recorded every 3 wk to assess their growth.

Table 1. Experimental design for in vivo bioassay.

Group	Treatment
T1	Preventive-composted black soldier fly (BSF) frass + *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4)
T2	Curative-*Foc* TR4 + Composted BSF frass
T3	Preventive-composted BSF frass + Fermented liquid derivative + *Foc* TR4
T4	Curative-*Foc* TR4 + Composted BSF frass + Fermented liquid derivative
T5	Positive control-Benomyl + *Foc* TR4
T6	Inoculated control-*Foc* TR4
T7	Negative control-Water

The spore suspension for *Foc* TR4 inoculation was prepared as described previously. The spore suspension (200 mL) was slowly poured on the potting medium of the potted plantlets (García-Bastidas et al., 2019). Inoculation of *Foc* TR4 was done on day 3 following the plantlets transfer for the curative treatment (before the BSF treatment) and on day 14 for the preventive treatment (after the BSF treatment). At the end of week 12, the external symptoms of Fusarium wilt were recorded by observing the wilting and yellowing symptoms. The plantlets were then up-rooted and cut longitudinally at the rhizome to record the internal symptoms. The leaf symptom index (LSI) (%), rhizome discoloration index (RDI) (%), disease index (DI), and the efficiency of the treatments in reducing the DI were calculated according to Dita et al. (2021) and Widodo et al. (2020), respectively.

DNA Filtration and sample preparation for metagenomics analysis

Total DNA was filtrated using at least 250 mg composted BSF frass and 250 µL fermented liquid fertilizer using the ZymoBIOMICS DNA Kit (Zymo Research, Irvine, California, USA) according to the manufacturer's instructions. Triplicate samples pooled equimolarly were sent for sequencing at Patriot Biotech Sdn Bhd., Selangor, Malaysia. An amplicon library was constructed for Illumina paired-end sequencing using bacterial primers for the V3 hypervariable region of the bacterial 16S rRNA. The PCR was performed using SolarBio PCR MasterMix (Solarbio Science & Technology, Beijing, China). The PCR products were purified using a 2.0% agarose gel and were used as the template for eight cycles of index PCR to incorporate the complete Illumina adapter and Illumina-compatible dual-index barcodes. The constructed libraries were subsequently size-selected using 0.8 X vol of SPRI bead and pooled into a single tube. Sequencing of the pooled libraries was performed on a NovaSEQ6000 (Illumina, San Diego, California, USA) using the 2 x 150 bp paired-end sequencing configuration. The demultiplexing and primer trimming of the raw paired-end reads were performed with cutadapt v1.18 (Martin, 2011). The trimmed reads were subsequently merged using fastp v0.21 (Chen et al.,

2018). The processed reads were then imported into QIIME2 v.2022.8 (Bolyen et al., 2019) and denoised into Amplicon Sequence Variant (ASV) with dada2 (Callahan et al., 2016). Taxonomic assignment of the ASV used q2-feature-classifier that has been trained on the latest GreenGenes2 database (2022.10) (McDonald et al., 2024).

Statistical analysis

The data set for the in vitro bioassay was analyzed using one-way ANOVA, and the difference in means was determined using Tukey's HSD post hoc test at a 5% significance level. The data set for in vivo bioassay was analyzed using one-way ANOVA on ranks (Kruskal-Wallis's test).

RESULTS

In vitro antifungal assay

The inhibitory effects of sterile and non-sterile composted BSF frass filtrate in different concentrations are shown in Table 2. The results indicated that both the sterile and non-sterile filtrates have inhibitory effects on spores and mycelium of *Foc* TR4. The MIC of the sterile filtrate is 40% (v/v), while the MIC of the non-sterile filtrate is lower at 25% (v/v). At 50% (v/v), the non-sterile filtrate achieved a fungicidal effect, which kills at least 99% of the spores and mycelium. However, the sterile filtrate could not achieve the fungicidal effect against the *Foc* TR4.

Table 2. The inhibitory effects of sterile and non-sterile composted black soldier fly (BSF) frass filtrate on *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) colony growth and mycelium dry weight.

	Negative control (distilled water)	Positive control (nystatin 30 mg L^{-1})	Sterile filtrate (% v/v)							Non-sterile filtrate (% v/v)						
			1	5	7	10	25	40	50	1	5	7	10	25	40	50
Average Colony Count, cfu mL^{-1}	1.20×10^{6}	0.00	1.18×10^{6}	1.16×10^{6}	1.11×10^{6}	1.10×10^{6}	8.90×10^{5}	4.30×10^{5}	1.60×10^{5}	1.15×10^{6}	1.09×10^{6}	1.05×10^{6}	9.70×10^{5}	4.10×10^{5}	8.00×10^{4}	1.00×10^{3}
Inhibition percentage	0.00^{a}	100.00	1.67^{a}	3.33^{a}	7.50^{ab}	8.30^{ab}	25.80^{b}	64.17^{c*}	86.67^{d}	4.17^{a}	9.17^{ab}	12.50^{ab}	19.17^{b}	65.83^{c*}	93.33^{d}	99.94^{d**}
Mycelium dry weight, g	0.0048	0.000	0.047	0.042	0.040	0.036	0.029	0.028	0.020	0.041	0.039	0.033	0.029	0.009	0.003	0.000
Inhibition percentage	0.00	100.00	2.08	12.5	16.67	25.00	39.58	41.67	58.33	14.58	18.75	31.25	39.58	81.25^{*}	93.75	100.00^{**}

Potting mix and BSF frass analysis

The pH value, content of C, N, and S of the potting mix, and the content of N, P, and K of BSF frass are shown in Table 3. The potting mix is rich in C but contains a low level of N and S. The two forms of BSF frass differ greatly in their N content, with a higher amount in composted BSF frass (4.04%) compared to the fermented liquid derivative (0.35%). The total ammoniacal N present in the composted BSF frass was 1.18%, which was used as a guide to calculate the amount of available N per pot in the subsequent pot experiment.

Table 3. The chemical profiles of black soldier fly (BSF) frass and the potting mix.

Sample	pH	C (%)	S (%)	N (%)	P (%)	K (%)
Potting mix	6.33	30.50	0.07	0.16	-	-
Composted BSF frass	-	-	-	4.04	1.68	1.30
Fermented liquid derivative	-	-	-	0.35	0.10	0.21

In vivo bioassay

The effects of the treatments on the LSI and RDI of the banana plantlets are presented in Figure 1. The results revealed that preventive treatment with both composted BSF frass and its fermented liquid derivative (T3) recorded the lowest RDI among the BSF treatments, followed by preventive treatment with composted BSF frass only (T1), curative treatment with composted BSF frass and its fermented liquid derivative (T4) and curative treatment with composted BSF frass only (T2). Similar observations were recorded for LSI except for T1, which had the lowest LSI. Preventive treatment with BSF frass, specifically a combination of both composted BSF frass and its fermented liquid derivative (T3), decreased the DI to as low as 39.74% and showed the highest efficiency in reducing the severity of Fusarium wilt disease in banana plantlets (47.66%) (Table 4).

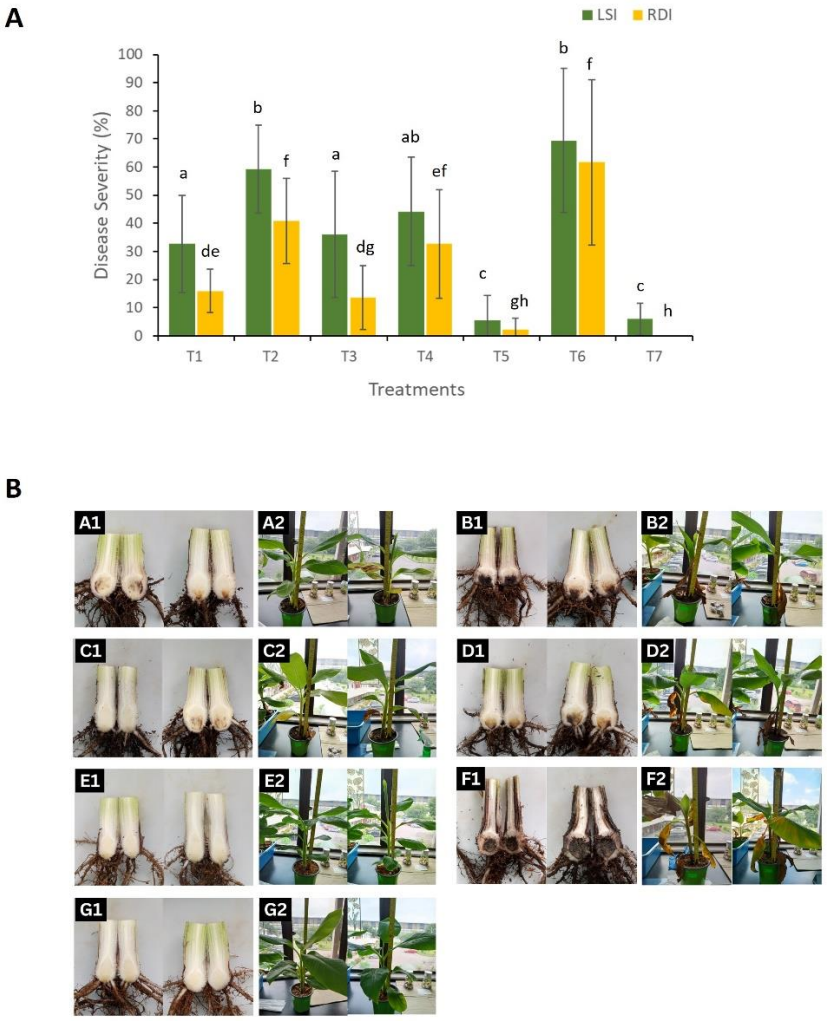

Figure 1. Effects of black soldier fly (BSF) frass treatments on the Fusarium wilt disease severity in bananas. A. Percentage of disease severity based on leaf symptom index (LSI) and rhizome discoloration index (RDI) across treatments. Different letters show significant values at $p > 0.05$ using one-way ANOVA on ranks (Kruskal-Wallis's test). B. External and internal symptoms of Fusarium wilt in banana plantlets recorded on week 12. The alphabet represents different treatment groups, while the numbers 1 and 2 represent internal and external symptoms for each treatment group, respectively. A = T1; B = T2; C = T3; D = T4; E = T5; F = T6; G = T7.

Table 4. Effects of black soldier fly (BSF) frass treatment on the Fusarium wilt disease in banana plantlets. Bold values – BSF frass treatment with the highest efficiency.

Group	Disease index (DI) (%)	Treatment efficiency (%)
T1	45.83	39.64
T2	66.67	12.20
T3	**39.74**	**47.66**
T4	60.26	20.64
T5	15.15	80.05
T6	75.93	-

Effects of BSF frass on banana growth

Plant height and leaf number were recorded every 3 wk from the start of the experiment to determine the effects of BSF frass treatment on the growth of the banana plantlets. While both plant height and leaf number increased in the first 3 wk in samples treated with BSF frass (T1-T4), the differences were nonsignificant. Additionally, no differences were observed between treatments after week six and therefore, the data was not included.

Diversity of bacterial population in the composted BSF frass and its fermented liquid derivative

Figure 2 shows the relative abundance of bacterial diversity in the composted BSF frass and its fermented liquid derivative. The composted BSF frass is dominated by members from *Firmicutes* (64%) and *Actinobacteriota* (23%) phyla. The former is mainly represented by members from the genera *Novibacillus*, *Planifilum*, *Ammoniibacillus*, and *Bacillus*. Meanwhile, the *Actinobacteriota* population is dominated by genera *Actinomyces*, *Saccharomonospora*, *Thermobifida*, and *Mycobacterium*. The fermented liquid derivative, having gone through a fermentation process, is enriched with different bacterial taxa. Members from the phyla of *Proteobacteria* (44%), *Planctomycetota* (19%), and *Bacteroidota* (18%) dominate the bacterial population. Notably, the population of *Actinobacteriota* decreased to 1.6% in the fermented liquid derivative as compared to the composted BSF frass. In contrast, the population of plant growth-promoting bacteria, represented by *Bacteroidota*, had increased. Additionally, the population of the genus *Burkholderia*, which is widely distributed in terrestrial environments, is more abundant in the fermented liquid derivative than the composted BSF frass.

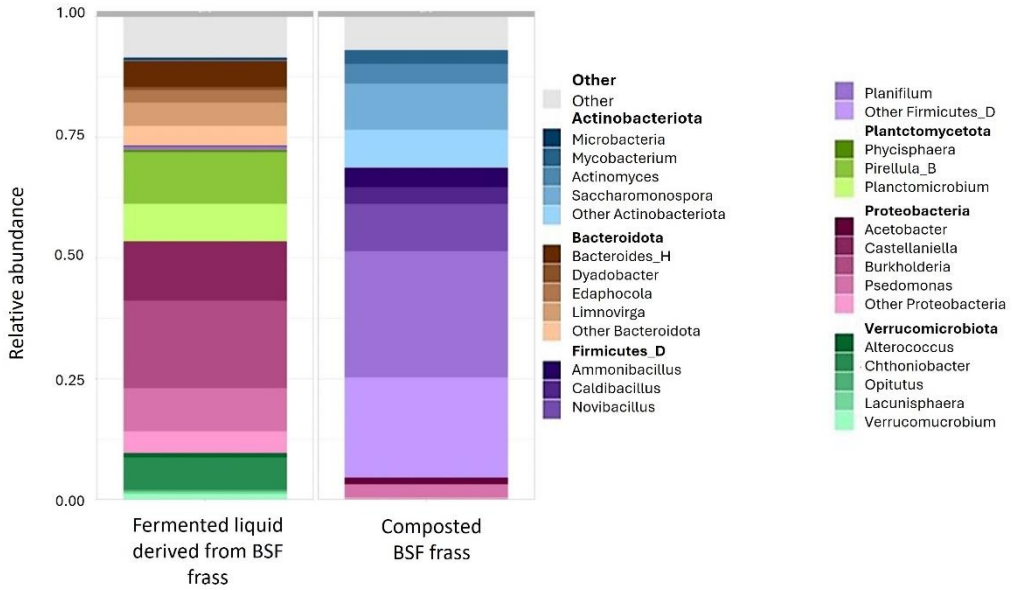

Figure 2. Relative abundance of bacterial taxa in the fermented black soldier fly (BSF) frass and its fermented liquid derivative at the genus level.

DISCUSSION

Our data shows that both sterile and non-sterile filtrates of the composted black soldier fly (BSF) frass inhibited the spores and mycelium of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4) through the in vitro assay. Interestingly, the non-sterile filtrates exhibited stronger inhibition (minimum inhibitory concentration [MIC] at 25%, minimum fungicidal concentration [MFC] at 50%) compared to the sterile filtrate (MIC at 40%). These findings indicate that the antifungal properties of the composted BSF frass are primarily attributed to the presence of beneficial microbes, which could potentially include the bacterial inoculant, *Bacillus halotolerans*. The bacterium was shown to have antagonistic effects against plant pathogens, including *Fusarium* sp. (Slama et al., 2019). Other studies also suggest that the effectiveness of compost or vermicompost against plant fungal pathogens is associated with living microorganisms (reviewed in Yatoo et al., 2021). Hence, it is not surprising that sterilization reduces the disease-suppressive capacity of composts. Mannai et al. (2018) showed that the sterile organic composted filtrates failed to inhibit the mycelial growth of *F. oxysporum* and *F. solani.* Arabzadeh et al. (2022) also reported that both non-sterile and sterile BSF frass filtrates exhibited inhibitory effects on plant pathogens, including *F. oxysporum*. Consistent with our findings, the sterile filtrate only displayed a low level of inhibitory activity. The inhibitory effect of the sterile filtrate, albeit less effective, is most likely due to the antifungal metabolites, such as hydrolytic enzymes and siderophores, secreted from the bacteria present in the filtrate.

The composted BSF frass and its fermented liquid derivative in this study are enriched with bacterial genera that exhibit plant growth-promoting and antagonistic properties. *Firmicutes* was identified as the most abundant phylum in the composted BSF frass, likely originating from the BSF larvae gut and feed residues (Gold et al., 2020). Actinomycetes and thermo-tolerant *Bacillus* sp. found in the composted BSF frass have also been detected in other composted organic fertilizers (Chang et al., 2021). Additionally, the insect chitin residues in BSF frass may selectively reduce specific microbial populations while promoting the enrichment of beneficial microbes in the final composted product. Nurfikari et al. (2024) found that exuviae and frass residues are enriched chitinolytic microbial inhabitants, including *Bacilli* and *Actinobacteria*, which played a significant role in suppressing phytopathogenic fungal communities. On the other hand, the fermentation process promoted the development of a different set of bacterial populations dominated by *Bacteroidota* and *Proteobacteria*. Members of *Bacteroidota* are known to be plant-borne and present in the human microbiota, reflecting the organic nature of the BSF feed sources. Members of *Bacteroidota* are also reported to possess pathogen-suppressing and phosphate-solubilizing potentials (Lidbury et al., 2021). Similarly, the *Burkholderia* genus present in the fermented liquid derivative has been reported to promote the growth of crops in immature soil (Li et al., 2021).

The observed antifungal potential of the composted BSF frass filtrates in vitro was extended to the greenhouse, where the preventive treatments with BSF frass (T1 and T3) significantly reduced the severity of Fusarium wilt, both externally and internally. The combined application of the composted BSF frass and its fermented liquid derivative (T3) significantly suppressed the internal symptoms and was the most efficient in reducing the disease index (DI). Other studies also suggest that combining foliar spray with soil application is more effective in reducing plant disease incidence (Harish, 2022). The application of the fermented liquid derivative, in addition to the composted BSF frass, is likely to increase the abundance of beneficial microbes and metabolites accessible to the banana plantlets, thus improving the suppression of the pathogen (Allouzi et al., 2022). The disease-preventive effect of BSF frass in this study is consistent with a previous study of BSF frass treatment on sugar beet against *Rhizoctonia solani* (Elissen et al., 2023).

However, compared to the sole treatment with composted BSF frass (T1), the leaf symptom index (LSI) for T3 is slightly higher, though this difference is nonsignificant. In this case, the application dosage of the fermented liquid derivative must be further optimized to avoid excessive application of soluble nutrients to the plants. On the other hand, applying the BSF frass after the banana plantlets had been inoculated with *Foc* TR4 and the disease had already taken hold (T2 and T4) did not effectively reduce the Fusarium wilt symptoms compared to the antifungal agent benomyl that was used as positive control in this study.

In addition to the potential direct antagonistic role of microbes, the high chitin content in BSF frass could also induce a defense response in banana plants. Chitin and chitosan filtrated from BSF pupal exuviae have been shown to reduce wilting symptoms caused by the bacteria *Ralstonia solanacearum* in tomato plants

(Kemboi et al., 2022). Since the preventive treatment with BSF frass proved to be more effective than the curative treatment in this study, it is tempting to speculate that the chitin in BSF frass might play an important role through the activation of plant defense mechanisms or the formation of mechanical barriers to prevent pathogen invasion. Although curative treatments using BSF frass did not significantly suppress Fusarium wilt in this study, field trials are still warranted to explore the preventive and curative potential of BSF frass, as well as its long-term effects on the growth of the banana plants. The interactions between the soil microbiome, the beneficial microbes present in the BSF frass, and various abiotic factors will likely have a significant impact on the treatment's effectiveness.

CONCLUSIONS

Through the in vitro antifungal assays, this study shows that sterile composted black soldier fly (BSF) filtrates significantly inhibited the mycelium growth and spore germination of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4). Both the composted BSF frass and its fermented liquid derivative are enriched with bacterial genera that exhibit plant growth-promoting and antagonistic properties. The findings from the in vivo bioassay indicated that the combination of composted BSF frass and its fermented liquid derivative applied as preventive treatment was the most effective in reducing the severity of Fusarium wilt disease in banana plantlets. However, the growth-promoting effects of the BSF treatments on the banana plantlets in this study were nonsignificant. Nevertheless, this study shows the promising potential of BSF frass-based biofertilizer to substitute chemical treatments for managing Fusarium wilt in bananas.

Author contribution

Conceptualization: N.B.S. Validation: S.R.A.M.R., S.S., M.J.Z. Formal analysis: J.X.O., N.B.A.M. Investigation: Resources: S.R.A.M.R., S.S., M.J.Z., N.B.S. Data curation: Writing-original draft: J.X.O. Writing-review & editing: H.A.E.E., N.B.S. Supervision: N.B.S. Project administration: M.R.S.Z., M.J.Z., N.B.S. Funding acquisition: N.B.S. All co-authors reviewed the final version and approved the manuscript before submission.

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Conflict of interest

This project was partially funded by Nutrition Technologies Sdn Bhd. Co-authors of Nutrition Technologies Sdn. Bhd. contributed resources and support for the project. The authors affirm that this involvement did not compromise the integrity or objectivity of the research. The funders had no role in the design, collection, analysis, or interpretation of data, nor in the decision to publish or write the manuscript.

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