

Bioactive Compounds in Fermented Papaya (*Carica Papaya*) And Banana (*Musa Paradisiaca*) Fruit Juices on Dieback Disease (*Phomopsis Azadirachtae*) in Neem Using GC-MS and Their Efficacy t Nizamabad District, Telangana, India

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Abstract The current study explores the antifungal potential of fermented fruit juices (FFJs) derived from *Carica papaya* (Papaya) and *Musa paradisiaca* (Banana) against *Phomopsis azadirachtae*, the causative agent of Neem dieback disease. Utilizing Gas Chromatography–Mass Spectrometry (GC-MS), a detailed chemical profiling of the FFJs was conducted, which revealed a rich diversity of bioactive metabolites, including ethyl esters, phenylethyl alcohol, linoleic acid derivatives, and thiophene compounds. These metabolites are known for their antimicrobial, antioxidant, and anti-inflammatory activities. Antifungal assays demonstrated that both FFJs exerted significant inhibitory effects on fungal growth in a concentration-dependent manner, with the 50% concentration of each juice achieving complete inhibition by Day 3 and maintaining this effect through Day 7. The GC-MS analysis substantiated the presence of compounds capable of disrupting fungal membranes, inhibiting enzymatic activity, and inducing oxidative stress. These findings suggest that Papaya and Banana FFJs can serve as eco-friendly alternatives to synthetic fungicides in controlling Neem dieback disease, providing a cost-effective and sustainable approach suitable for organic and small-scale agricultural practices. The study also underscores the role of natural fermentation in enhancing the bioactivity of fruit-derived phytochemicals, presenting new opportunities for integrated plant disease management.

Keywords *Neem dieback (Phomopsis azadirachtae), fermented Papaya (Carica papaya) and juice, fermented Banana (Musa paradisiaca) juice, GC-MS profiling, natural antifungal agent, bioactive compounds, eco-friendly fungicide.*

INTRODUCTION

Neem (*Azadirachta indica*), a drought-tolerant species native to the Indian subcontinent, is a cornerstone of arid and semi-arid ecosystems due to its multifaceted ecological and socio-economic contributions. It flourishes in poor soils and extreme climates, stabilizing degraded landscapes through its deep-rooted structure and enhancing microbial soil health via nutrient-rich leaf litter (Sustainable Oasis, n.d.). Its ecological benefits extend globally, with over 18 million hectares under Neem cultivation, particularly in agroforestry systems across Sub-Saharan Africa and South America. These systems leverage Neem's carbon sequestration capacity—estimated at 50–60 kg of carbon per tree per year—and its ability to improve soil organic content (Britannica, n.d.; Miller *et al.*, 2022). In regions such as Burkina Faso, Neem-based land restoration efforts have successfully reversed up to 40% of land degradation, significantly bolstering agroecological resilience (Miller *et al.*, 2022). Economically, Neem supports a global biopesticide industry projected at USD 6.5 billion, primarily fueled by azadirachtin, a highly effective insect growth regulator known for its minimal environmental toxicity (Britannica, n.d.). Despite these benefits, Neem populations face an escalating threat from dieback disease, a vascular and foliar pathology caused primarily by the fungal pathogens *Phomopsis azadirachtae* and *Cytospora azadirachtae*. These pathogens secrete a battery of hydrolytic enzymes such as cellulases and pectinases, and phytotoxins like oxalic acid, which damage xylem tissues and

induce necrotic lesions (Al-Khayri *et al.*, 2023). Molecular studies show that *P. azadirachtae* produces extracellular polysaccharides that block water transport, leading to systemic wilting and decline (Al-Khayri *et al.*, 2023). Originally reported in India in the early 1990s, dieback has since spread to Neem populations in Nigeria's Sahel and Brazil's semi-arid zones, causing tree mortality rates exceeding 40% and reducing azadirachtin yield by up to 30% (FAO, 2023). This expansion poses a serious threat to the sustainability of Neem-based livelihoods and agroforestry systems. Recognizing the severity of the issue, the FAO (2023) has flagged dieback as a critical phytosanitary risk for regions where Neem is central to both food security and environmental restoration. Currently, dieback is primarily managed using copper and sulfur-based chemical fungicides. Although effective in the short term, these compounds present long-term ecological drawbacks. For instance, copper residues persist in soil and disrupt microbial communities, particularly nitrogen-fixing *Pseudomonas* spp., resulting in a 25% decline in biological nitrogen fixation (Britannica, n.d.). Additionally, chemical residues adversely affect beneficial pollinators such as *Apis mellifera*, with studies reporting a 50% increase in mortality following fungicide exposure, thereby threatening Neem pollination and associated ecosystem services (Zhao *et al.*, 2020). In light of these impacts, policy frameworks like the European Union's Farm to Fork Strategy (2020) mandate a 50% reduction in synthetic pesticide usage by 2030 (European Commission, 2020), emphasizing the urgent need for safer, sustainable, and scalable disease control alternatives. In this context, fermented fruit juices (FFJs) have emerged as promising biocontrol agents with antifungal and plant-strengthening capabilities. Produced through lactic acid bacteria (LAB)-mediated fermentation of substrates such as Papaya, Banana, Pineapple, and citrus, FFJs are enriched with antimicrobial compounds including organic acids (e.g., lactic and acetic acids), phenolics, and volatile organic compounds (VOCs). These bioactives exhibit broad-spectrum efficacy against fungal pathogens like *Fusarium oxysporum* and *Botrytis cinerea* by lowering pH, producing bacteriocins, and disrupting fungal membranes (Gajbhiye & Kapadnis, 2016; Szutowaska, 2020; Garcia *et al.*, 2020). For instance, fermented pineapple juice inhibits *Fusarium* by delivering proteolytic enzymes like bromelain, while LAB-fermented apple juice suppresses *Botrytis* via synergistic acidification and ROS scavenging (Barrios-Roblero *et al.*, 2019; Tang *et al.*, 2023). Among potential FFJ substrates, Papaya (*Carica papaya*) and Banana (*Musa* spp.) are particularly valuable due to their distinct bioactive profiles and widespread availability. Papaya FFJs are rich in ascorbic acid (~120 mg/100 g) and papain, both of which exhibit antifungal activity through oxidative stress modulation and proteolytic action. Fermentation boosts their phenolic content especially ferulic acid by over 200%, enhancing antifungal potency (Dube *et al.*, 2022). Banana FFJs, in contrast, contain dopamine (8.5 µg/g) and gallic catechin, compounds known to compromise fungal membrane integrity and inhibit spore germination by up to 70%. Additionally, LAB fermentation produces acetic acid, leading to acidic environments (pH < 3.5) that are hostile to fungal growth (Kim *et al.*, 2021). Beyond disease suppression, FFJs also promote plant resilience by activating systemic acquired resistance (SAR), enhancing phytoalexin synthesis, and improving soil microbial diversity (El-Baky & Amara, 2021; Ranjith *et al.*, 2021). However, practical implementation of FFJs faces several challenges, including variability in fermentation conditions, inconsistent metabolite profiles, and potential phytotoxicity at high concentrations. Regulatory hurdles further complicate their commercial adoption (Avirvarei *et al.*, 2023; Yuan *et al.*, 2024). Standardizing microbial consortia, fermentation protocols, and delivery methods is essential to ensure consistency and efficacy (Plessas, 2021). Addressing these gaps requires interdisciplinary research integrating metabolomics, plant pathology, and field agronomy alongside policy support that fosters circular economy models and educates smallholder farmers on low-input biocontrol strategies (Doriya *et al.*, 2022; Wang *et al.*, 2024). Recent advancements in analytical chemistry, particularly gas chromatography–mass spectrometry (GC-MS), offer tools to characterize the bioactive landscape of FFJs with high precision. When paired with solid-phase microextraction (SPME), GC-MS allows for sensitive detection of fermentation-derived volatiles. Zheng *et al.* (2022), for example, used a 65 µm PDMS/DVB fiber and 30-minute extraction to identify 52 VOCs in fermented *Citrus medica*, including

limonene, α -terpineol, and cymene compounds that increased significantly over fermentation duration. GC-MS profiling of *Rhazya stricta* similarly revealed antifungal fatty acids like hexadecanoic acid (Al-Khayri *et al.*, 2023). Additionally, chemometric tools such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) now facilitate correlation between metabolite profiles and antifungal efficacy, streamlining formulation development (Zheng *et al.*, 2022).

Despite growing interest in FFJs for crop protection, their application in managing woody perennial diseases such as Neem dieback remains underexplored. Most existing research has focused on Neem leaf or bark extracts, often neglecting the enhanced bioactivity derived from microbial fermentation or failing to correlate phytochemical profiles with antifungal efficacy. This study aims to bridge that gap by systematically profiling bioactive compounds in LAB-fermented Papaya and Banana juices using GC-MS, evaluating their efficacy against *P. azadirachtae* through spore and hyphal inhibition assays, and conducting in vivo field trials on Neem saplings. The central hypothesis posits that fermentation enhances synergistic interactions among phenolics, organic acids, and VOCs, leading to effective suppression of dieback symptoms and potentially contributing to azadirachtin recovery. This work aligns with FAO's broader objectives for climate-resilient, low-cost biocontrol interventions in support of smallholder farming systems (FAO, 2023).

MATERIALS & METHODOLOGY

Collection and Authentication of Plant Material

The leaves of *Azadirachta indica* were collected from Arts and science college, Telangana University, Dichpally Village is located in Nizamabad District, of Telangana State, India during february/ March in the year 2021. The plant was authenticated by My Supervisor Dr.M.Mamatha , Professor in forest Botany,Basic and Social sciences department, Forest college and Research institute, Hyderabad at Mulugu.

Preparation of Fermented Fruit Juices

Fermentation of papaya and banana was conducted using a modified natural fermentation method inspired by Chohan Q's protocol (Omar *et al.*, 2023). After thorough washing and peeling, 1 kg each of chopped Papaya and sliced Banana were mixed separately with 1 kg of grated organic jaggery in sterile glass jars. The fruits and jaggery were added in alternating layers, and the jars were loosely covered with sterile muslin cloth to facilitate aerobic fermentation. Fermentation was carried out for 45 days at ambient room temperatures (21–24°C) in a shaded location. The mixtures were stirred once daily using sterile wooden spatulas to promote microbial activity. Indicators of active fermentation included bubbling, fruity aroma development, and brown liquid formation. After 45 days, the fermented liquids were carefully decanted, filtered through double-layered muslin cloth, and stored at 4°C until analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To identify and characterize the bioactive compounds present in the fermented Papaya and Banana juices, GC-MS analysis was performed using a Shimadzu QP2010 GC-MS system equipped with an Elite-5MS capillary column (30 m \times 0.25 mm \times 0.25 μ m). Helium served as the carrier gas at a flow rate of 1 mL/min. The injection volume was 2 μ L (split ratio 10:1), and the injector temperature was set at 250°C. The oven temperature was programmed as follows: initial temperature of 110°C (held for 2 minutes), ramped to 250°C at 10°C/min. The ion source temperature was maintained at 200°C, and electron ionization (EI) was conducted at 70 eV, with a mass scan range of 45–450 m/z. Compounds were identified by comparing their mass spectra with those in the NIST and Wiley libraries. Only peaks with a similarity index above 90% were considered for further analysis.

Isolation and Identification of Dieback Pathogen

Neem leaves showing clear symptoms of dieback, such as chlorosis, necrosis, and branch die-off, were collected from affected Neem trees at Telangana University, Dichpally. Small tissue sections from the symptomatic

regions were surface-sterilized using 0.2% mercuric chloride for one minute, rinsed three times with sterile distilled water, and plated on potato dextrose agar (PDA) supplemented with streptomycin (50 µg/mL) to suppress bacterial growth. Plates were incubated at $26 \pm 2^\circ\text{C}$ for 7 days. Emerging fungal colonies showing morphological traits of *Phomopsis azadirachtae*—notably white mycelium and dark pycnidia—were sub-cultured onto fresh PDA and maintained at 4°C . Fungal identity was confirmed through microscopic examination using lactophenol cotton blue staining and validated using taxonomic descriptions (Sateesh, 1998).

Evaluation of Antifungal Activity

The antifungal potential of the fermented fruit juices was assessed using the poisoned food technique. Each fermented juice (papaya and banana) was tested at three concentrations: 10%, 20%, and 50% (v/v), mixed with sterilized PDA medium maintained at 45°C . The media were poured into Petri dishes under aseptic conditions. After solidification, a 5 mm diameter agar plug from a 7-day-old culture of *P. azadirachtae* was placed at the center of each plate. Control plates were prepared without FFJs. All experiments were carried out in triplicate. Plates were incubated at $26 \pm 2^\circ\text{C}$ for 7 days, and radial mycelial growth was measured daily. The percentage inhibition of fungal growth was calculated using the formula by Vincent (1927):

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where C = radial growth in control (mm) and T = radial growth in treatment (mm).

Statistical Analysis

All experimental data were expressed as mean \pm standard deviation (SD) from three independent replicates. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Analyses were performed using IBM SPSS Statistics software version 26.0.

RESULTS

The fungal organism implicated in Neem dieback was successfully isolated from visibly infected Neem foliage collected from the campus of Telangana University, Dichpally. The symptomatic leaves exhibited classical signs of disease progression, including chlorotic patches, necrotic lesions, and extensive leaf drying strongly indicative of *Phomopsis azadirachtae* infection. Isolation of the pathogen involved surface disinfection of leaf tissue using a mild mercuric chloride solution, followed by aseptic placement onto potato dextrose agar (PDA) medium containing streptomycin to inhibit bacterial interference. After incubation at ambient room temperature ($26 \pm 2^\circ\text{C}$) for seven days, fungal growth was observed. Initial colonies presented as white, fluffy mycelium, which gradually developed into darker, spore-forming structures—a distinguishing trait of *Phomopsis azadirachtae*. Subsequent sub-culturing produced uniform and stable fungal growth within 15 days. Microscopic analysis revealed the formation of characteristic pycnidia and conidia, aiding in conclusive identification of the isolate. These purified cultures were preserved on PDA slants at 4°C to maintain viability for downstream antifungal testing. The successful isolation and authentication of *P. azadirachtae* established a reliable bioassay model for screening the antifungal efficacy of naturally derived formulations. In parallel, fermented fruit juices (FFJs) were prepared from fully ripe Papaya and Banana using a modified natural fermentation protocol. The process involved chopping the fruits and layering them with organic jaggery in a 1:1 ratio to provide fermentable sugars and enhance microbial activity. The mixtures were stored in clean, breathable containers and allowed to ferment at room temperature for 45 days under semi-aerobic conditions. Daily stirring with sterile wooden spatulas ensured homogeneity and prevented surface contamination. Observable signs of active fermentation included gas bubble formation, a distinct fruity odor, and the

accumulation of dark-colored liquid at the bottom of the fermentation jars. At the end of the fermentation period, the liquid portions were carefully separated from solid residues, filtered through muslin cloth, and stored at refrigeration temperature (4°C) to retain their bioactive content. The Papaya and Banana FFJs displayed consistent fermentation behavior and were visually characterized by fluid consistency and distinct aroma, suggesting the presence of secondary metabolites with potential antifungal properties. These fermented extracts were subsequently utilized in bioassays to determine their inhibitory activity against the isolated fungal pathogen. Gas chromatography-mass spectrometry (GC-MS) analysis of fermented Banana and Papaya juices revealed a diverse range of bioactive compounds with antimicrobial, antioxidant, and antifungal properties as presented in Table 1 & 2. The GC-MS analysis of fermented Banana juice revealed a rich and diverse spectrum of bioactive compounds with established antimicrobial, antioxidant, and therapeutic functions. Ethyl oleate emerged as the predominant constituent, accounting for 15.08% of the total peak area. This compound is recognized for its dual role as an antimicrobial agent and a transdermal penetration enhancer, making it highly valuable in pharmaceutical and dermatological applications. Hexadecanoic acid ethyl ester was the second most abundant compound (12.55%), known for its antioxidative and antimicrobial efficacy, contributing to the FFJ's ability to neutralize oxidative stress and inhibit microbial proliferation. Other major compounds included linoleic acid ethyl ester and 9,12-octadecadienoic acid ethyl ester (both 7.70%), which are associated with anti-inflammatory properties and skin-conditioning effects, indicating potential use in therapeutic and cosmetic product development. Additionally, phenylethyl alcohol (2.95%) was detected, a compound with documented antimicrobial action and frequent usage in fragrance and personal care formulations. Minor compounds such as methyl β -D-galactopyranoside and β -D-glucopyranoside, methyl, though present in smaller proportions, are relevant as enzymatic substrates with applications in industrial biotechnology. Notably, 2,3-butanediol (1.65%) was identified as an antifungal metabolite, reinforcing FBJ's potential role in agricultural disease suppression, especially against *Phomopsis azadirachtae*.

Table 1: GC-MS Analysis of fermented fruit juices of Banana (*Musa paradisiaca*)

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
1.	21.444	15.08	Ethyl Oleate (C ₂₀ H ₃₈ O ₂ , 310.53 g/mol)	Transdermal penetration enhancer, antimicrobial
2.	19.782	12.55	Hexadecanoic acid, ethyl ester (C ₁₈ H ₃₆ O ₂ , 284.48 g/mol)	Antioxidant, antimicrobial
3.	21.369	7.7	Linoleic acid ethyl ester (C ₂₀ H ₃₆ O ₂ , 308.50 g/mol)	Anti-inflammatory, skin-conditioning
4.	21.369	7.7	9,12-Octadecadienoic acid, ethyl ester (C ₂₀ H ₃₆ O ₂ , 308.50 g/mol)	Anti-inflammatory, anticancer, antioxidant
5.	21.606	3.7	Octadecanoic acid, ethyl ester (C ₂₀ H ₄₀ O ₂ , 312.53 g/mol)	Emollient, antimicrobial
6.	2.537	3.16	Methane, oxybis[dichloro- (C ₂ H ₄ Cl ₂ O, 167.86 g/mol)	Industrial solvent, potential carcinogen
7.	9.073	2.95	Phenylethyl Alcohol (C ₈ H ₁₀ O, 122.16 g/mol)	Antimicrobial, fragrance

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
8.	9.073	2.95	Benzeneethanol (CAS) \$ Phenethyl Alcohol (C ₈ H ₁₀ O, 122.16 g/mol)	Antimicrobial, fragrance
9.	19.545	2.54	E-11-Hexadecenoic acid, ethyl ester (C ₁₈ H ₃₄ O ₂ , 282.46 g/mol)	Antimicrobial, antioxidant
10.	19.545	2.54	Ethyl 9-hexadecenoate (C ₁₈ H ₃₄ O ₂ , 282.46 g/mol)	Antioxidant, antimicrobial
11.	16.145	1.76	Methyl .beta.-D- galactopyranoside (C ₇ H ₁₄ O ₆ , 194.18 g/mol)	Enzyme substrate
12.	16.145	1.76	.beta.-D-Glucopyranoside, methyl (C ₇ H ₁₄ O ₆ , 194.18 g/mol)	Enzyme substrate
13.	4.161	1.65	2,3-Butanediol (C ₄ H ₁₀ O ₂ , 90.12 g/mol)	Not Reported
14.	4.161	1.65	2,3-Butanediol (C ₄ H ₁₀ O ₂ , 90.12 g/mol)	Bio-based chemical, antifungal
15.	26.942	1.46	2,6,10,14,18,22- Tetracosahexaene (C ₂₄ H ₃₈ , 326.57 g/mol)	Anticancer, anti-inflammatory
16.	26.942	1.46	Squalene (C ₃₀ H ₅₀ , 410.72 g/mol)	Antioxidant, anti- inflammatory, skin- conditioning
17.	10.485	0.99	Thiophene, 2,5-dihydro- (C ₄ H ₆ S, 86.15 g/mol)	Antifungal, antibacterial
18.	8.11	0.88	Diglycerol (C ₆ H ₁₄ O ₅ , 166.17 g/mol)	Humectant, moisturizer
19.	8.11	0.88	Propanoic acid, 2-mercapto-, ethyl ester (C ₅ H ₁₀ O ₂ S, 134.19 g/mol)	Antioxidant, antimicrobial
20.	8.11	0.88	Erythritol (C ₄ H ₁₀ O ₄ , 122.12 g/mol)	Antioxidant, sugar substitute
21.	21.157	0.76	Hexadecanoic acid, 2- methylpropyl ester (C ₂₀ H ₄₀ O ₂ , 312.53 g/mol)	Antimicrobial, antioxidant
22.	21.157	0.76	Hexadecanoic acid, butyl ester (C ₂₀ H ₄₀ O ₂ , 312.53 g/mol)	Antimicrobial, antioxidant
23.	23.281	0.75	Eicosanoic acid, ethyl ester (C ₂₂ H ₄₄ O ₂ , 340.58 g/mol)	Emollient, antimicrobial
24.	10.735	0.67	N-Aminopyrrolidine (C ₄ H ₁₀ N ₂ , 86.14 g/mol)	Antimicrobial, insecticide

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
25.	10.735	0.67	Thiophene, 2,3-dihydro- (C ₄ H ₆ S, 86.15 g/mol)	Antifungal, antibacterial
26.	7.885	0.6	D-Arabinitol (C ₅ H ₁₂ O ₅ , 152.15 g/mol)	Antioxidant, sugar substitute
27.	7.885	0.6	Xylitol (C ₅ H ₁₂ O ₅ , 152.15 g/mol)	Antimicrobial, dental health
28.	17.695	0.58	Tetradecanoic acid, ethyl ester (C ₁₆ H ₃₂ O ₂ , 256.43 g/mol)	Antimicrobial, skin- conditioning
29.	9.635	0.55	4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl- (C ₆ H ₈ O ₄ , 144.13 g/mol)	Antioxidant, anti- inflammatory
30.	23.506	0.55	Phytol (C ₂₀ H ₄₀ O, 296.53 g/mol)	Antioxidant, anti- inflammatory
31.	23.506	0.55	Decane, 2,7,7-trimethyl- (CAS) (C ₁₃ H ₂₈ , 184.37 g/mol)	Emollient, fragrance component
32.	23.506	0.55	9-Octadecenoic acid (Z)-, pentyl ester (C ₂₃ H ₄₄ O ₂ , 352.59 g/mol)	Antioxidant, emollient
33.	16.895	0.52	Acetic acid, 4-(3,5,12- trioxatricyclo... (C ₁₀ H ₁₆ O ₅ , 216.23 g/mol)	Solvent, antimicrobial
34.	16.895	0.52	Acetate, 4-(3,5,12- trioxatricyclo... (C ₁₀ H ₁₆ O ₅ , 216.23 g/mol)	Solvent, antimicrobial
35.	1.024	0.5	Trichloromethane (CHCl ₃ , 119.38 g/mol)	Anesthetic, solvent
36.	1.024	0.5	Chloroform (CHCl ₃ , 119.38 g/mol)	Anesthetic, solvent
37.	22.031	0.45	Pentadecanoic acid, 3- methylbutyl ester (C ₂₀ H ₄₀ O ₂ , 312.53 g/mol)	Antioxidant, antimicrobial
38.	22.031	0.45	3-Methylbutyl Decanoate (C ₁₅ H ₃₀ O ₂ , 242.40 g/mol)	Antioxidant, antimicrobial
39.	22.031	0.45	3-Methylbutyl hexadecanoate (C ₂₁ H ₄₂ O ₂ , 326.57 g/mol)	Antibacterial, fragrance component
40.	16.595	0.44	p-Menthan-3-one, semicarbazone (C ₁₀ H ₁₉ N ₃ O, 197.28 g/mol)	Antioxidant, insect repellent
41.	10.835	0.38	2-Furancarboxaldehyde, 5- (hydroxymethyl)- (C ₆ H ₆ O ₃ , 126.11 g/mol)	Antioxidant, anti- inflammatory

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
42.	10.835	0.38	5-Hydroxymethylfurfural (C ₆ H ₆ O ₃ , 126.11 g/mol)	Antioxidant, anti-inflammatory
43.	22.681	0.37	Oleic Acid (C ₁₈ H ₃₄ O ₂ , 282.46 g/mol)	Anti-inflammatory, cardiovascular benefits
44.	22.681	0.37	9-Octadecenoic acid (Z)- (C ₁₈ H ₃₄ O ₂ , 282.46 g/mol)	Emollient, anti-inflammatory
45.	22.681	0.37	9-Octadecenoic acid (E)- (C ₁₈ H ₃₄ O ₂ , 282.46 g/mol)	Moisturizing, skin-conditioning
46.	8.348	0.35	Glycerin (C ₃ H ₈ O ₃ , 92.09 g/mol)	Humectant, moisturizer
47.	16.808	0.3	Undecane, 2-methyl- (C ₁₂ H ₂₆ , 170.34 g/mol)	Emollient, fragrance
48.	16.808	0.3	Octadecane, 2-methyl- (C ₁₉ H ₄₀ , 268.52 g/mol)	Lubricant, emollient
49.	16.808	0.3	Heneicosane (C ₂₁ H ₄₄ , 296.57 g/mol)	Lubricant, emollient
50.	15.471	0.26	Dodecanoic acid, ethyl ester (C ₁₄ H ₂₈ O ₂ , 228.37 g/mol)	Antibacterial, used in fragrance formulations

Fermented papaya juice exhibited a chemically diverse profile dominated by compounds with strong antifungal and antioxidant activities. Key antimicrobial constituents included 2-propanone, 2,4-dithiapentane, and various methyl esters of fatty acids, all of which have been previously reported to interfere with fungal cell metabolism and membrane integrity. These compounds are likely contributors to the inhibitory effects observed against *Phomopsis azadirachtae* in *In vitro* assays. The detection of thiazole and furfural derivatives further enhanced the antimicrobial and antioxidant potential of FPJ. These heterocyclic compounds are known to disrupt microbial enzyme systems, enhancing the overall biocidal effect of the juice. Linoleic acid ethyl ester and structurally related unsaturated fatty acid esters were also present, offering additional benefits such as anti-inflammatory activity and skin-rejuvenating properties. The multifunctionality of FPJ is evident in its broad-spectrum antifungal capacity, oxidative stress mitigation potential, and its relevance in health-oriented formulations. The combination of these properties makes fermented papaya juice a compelling candidate for use in integrated disease management (IDM) systems in agriculture and in the development of eco-friendly therapeutic and cosmetic products.

Table 2: GC-MS Analysis of fermented fruit juices of Papaya(*Carica papaya*)

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
1.	2.318	1.747%	2-Propanone, 1-hydroxy CH ₃ COCH ₂ OH 74.08	Not reported
2.	2.882	3.03	2,4-Dithiapentane	Not reported

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
			C ₃ H ₈ S ₂ 108.226	
3.	2.882	3.03	Methyl 2-(methylthio)acetate CH ₃ SCH ₂ COOCH ₃ 120.17	Not reported
4.	2.882	3.03	N-(2-Sulfanylethyl)-2-oxopropana C ₅ H ₉ NO ₂ S 147.20	Not reported
5.	2.882	3.03	Hydroperoxide, 1-methylhexyl C ₃ H ₈ O ₂ 76.09	Antimicrobial, antioxidant
6.	3.147	1.87	Isobutyl acrylate CH ₂ =CHCOOCH ₂ CH(CH ₃) ₂ 128.17	Not reported
7.	3.147	1.87	Propargyl alcohol HC≡CCH ₂ OH 56.06	Antimicrobial
8.	3.147	1.87	2-Propenoic acid, methyl ester C ₈ H ₁₀ O ₅ 186.16	Antimicrobial, Antihyperglycemic, Cytotoxic activity, Antioxidant, Insecticidal activity
9.	3.362	2.44	Pentanoic acid, 3-hydroxy-, methyl ester C ₁₂ H ₁₈ O ₃ S, 242.34	Not reported
10.	3.362	2.44	Methyl acetoacetate CH ₃ COCH ₂ COOCH ₃ , 116.12	Not reported
11.	3.362	2.44	Butanoic acid, 3-oxo-, methyl ester C ₅ H ₈ O, 116.12	Not reported
12.	3.362	2.44	Acetic acid, hydrazide C ₉ H ₉ N ₂ NaO ₄ S, 264.237	Anti-inflammatory, Analgesic, & Anti-microbial
13.	3.526	0.55	2,3-Butanediol CH ₃ COCOCH ₃ , 86.09	Not reported
14.	3.614	2.97	Propanoic acid, 2-oxo-, methyl ester C ₄ H ₆ O ₃ , 102.08	Not reported
15.	4.157	0.19	d-Glycero-d-galacto-heptose C ₇ H ₁₄ O ₇ , 210.18	Antioxidant

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
16.	4.157	0.19	Allyl mercaptan $\text{CH}_2=\text{CHCH}_2\text{SH}$, 74.14	Anti-cancer (Hep G2)
17.	4.157	0.19	1,2,3-Propanetriol $\text{HOCH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$ 92.09	Kinase activity
18.	4.157	0.19	(R)-(-)-Methyl 3-hydroxybutyrate $\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{CO}_2\text{CH}_3$ 118.13	Not reported
19.	4.245	0.10	Benzimidazole, 1-[2-(1-piperidyl) $\text{C}_{12}\text{H}_{15}\text{N}_3$ 201.27	Antidiabetic Anti-Alzheimer Antioxidant. Antituberculosis
20	4.245	0.10	4-(chloro-difluoro-methoxy)- phenylamine $\text{C}_7\text{H}_6\text{ClF}_2\text{NO}$ 193.58	Not reported
21	4.245	0.10	Thiazole, 5-(2-chloroethyl)- $\text{C}_6\text{H}_8\text{ClNS} \cdot \text{HCl}$ 198.11	Not reported
22	4.245	0.10	1-Piperidinepropanol, $\text{C}_8\text{H}_{17}\text{NO}$ 143.23	Not reported
23	4.296	0.26	Furfural $\text{C}_5\text{H}_4\text{O}_2$ 96.08	Anti-microbial
24.	4.637	1.17	2-Furanmethanol $\text{C}_5\text{H}_6\text{O}_2$ 98.09	Antiviral
25.	4.864	0.40	3-Amino-2-oxazolidinone $\text{C}_3\text{H}_6\text{N}_2\text{O}_2$ 102.09	Not reported

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
26.	4.864	0.40	Urea NH_2CONH_2 60.06	Anticancer, antiviral, antibacterial, antihypertensive antibacterial, antifungal and antitubercular
27.	4.864	0.40	Di-n-propyl ether $(\text{CH}_3\text{CH}_2\text{CH}_2)_2\text{O}$ 102.17	Not reported
28	5.066	3.60	4-Aminoisoxazolidin-3-one $\text{C}_3\text{H}_6\text{N}_2\text{O}_2$ 102.09	Not reported
29	5.066	3.60	N,N-BIS(2-methoxyethyl)-methylamine $(\text{CH}_3\text{OCH}_2\text{CH}_2)_2\text{NH}$ 133.19	Not reported
30	5.066	3.60	2,2-Dimethyl-3-oxobutyric acid, methyl ester $\text{C}_{11}\text{H}_{22}\text{O}_3\text{Si}$ 230.38	Not reported
31.	5.634	2.22	Dihydroxyacetone $\text{C}_3\text{H}_6\text{O}_3$ 90.08	Not reported
32	5.798	3.89	2-Cyclopenten-1-one, 2-hydroxy- $\text{C}_5\text{H}_6\text{O}_2$ 98.0999	Not reported
33.	5.798	3.89	alpha.-Pyrrolidone, N-methyl-5- $\text{C}_5\text{H}_9\text{NO}$ 99.13	Antimicrobial
34.	6.139	3.68	Carbonic acid, dimethyl ester $(\text{CH}_3\text{O})_2\text{CO}$ 90.08	Not reported
35.	6.278	3.26	dl-Glyceraldehyde dimer $\text{C}_3\text{H}_6\text{O}_3$ 90.08	Not reported

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
36.	6.682	0.84	1,3-Dioxane, 4-methyl- $C_5H_{10}O_2$ 102.13	Not reported
37.	6.682	0.84	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- $C_6H_8O_4$ 144.12	Antioxidant
38.	6.682	0.84	cis-2,3-Dimethylthiophane $C_6H_{10}(CH_3)_2$ 112.21	Anti-cancer
39.	6.972	0.47	Formyl glutamine $H_2NCOCH_2CH_2CH(NH_2)CO_2H$ 146.14	Not reported
40.	6.972	0.47	1H-Tetrazole, 1-methyl $C_2H_4N_4$ 84.08	antibacterial, antifungal, anticancer, analgesic, anti-inflammatory, antidiabetic, anti-hyperlipidemic and antitubercular activities
41.	6.972	0.42	N-[3-Methylaminopropyl]aziridine $C_6H_{14}N_2$ 114.19	Anti-microbial
42.	7.667	0.34	L-Mannose $C_6H_{12}O_6$ 180.16	Anti-cancer
43.	7.667	0.34	Formic acid $HCOOH$ 46.03	Antimicrobial
44.	7.730	0.22	Isosorbide Dinitrate $C_6H_8N_2O_8$ 236.14	Not reported
45.	7.730	0.22	Methylsilyl Formate HCO_2CH_3 60.05	Not reported

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
46.	7.831	1.40	2(R),3(S)-1,2,3,4-Butanetetrol $C_{18}H_{18}O_6$ 330.3	Not reported
47.	7.831	1.40	Piperazine, 1,4-dimethyl- $C_6H_{14}N_2$ 114.19	Not reported
48.	8.096	2.44	N-Methoxy-N-methylacetamide $CH_3CON(OCH_3)CH_3$ 103.12	Not reported
49.	8.096	2.44	1,3-Diethoxy-2-propanol $C_7H_{16}O_3$ 148.20	Not reported
50.	9.548	2.40	1,3-Benzenediol, 5-chloro- $C_6H_5ClO_2$ 306.45	Antiproliferative
51	9.889	0.88	2(3H)-Furanone, dihydro-4-hydroxy $C_8H_{14}O_2$ 142.20	Not reported
52.	9.889	0.88	Imidodicarbonic diamide, N-formyl- $C_3H_5N_3O_3$ 131.09	Not reported
53.	9.889	0.88	Propanenitrile, 3-amino-2,3-di $C_4H_8N_2$ 84.12	Anti-bacterial
54	9.977	0.36	Hydrazine, N,N-dimethyl-N'-dieth... $(CH_3)_2NNH_2$ 60.10	Insecticidal activities
55.	9.977	0.36	Imidazole-4-acetic acid sodium salt $C_5H_5N_2NaO_2$ 148.10	Antitumor

The antifungal efficacy of fermented papaya and banana fruit juices (FFJ) against *Phomopsis azadirachtae* was evaluated over a seven-day period at concentrations of 10%, 20%, and 50%. Fungal growth was measured in triplicates, with mean values and standard deviations calculated to ensure statistical reliability.

Antifungal Activity of Papaya FFJ

In the untreated control group, fungal growth increased steadily from 6.0 cm on Day 1 to 8.5 cm by Day 7, confirming uninhibited proliferation (Table 6). At 10% papaya FFJ, fungal growth was reduced to 5.0 cm on Day 1, followed by a mean suppression to 1.7 ± 0.24 cm on Day 3. However, partial regrowth occurred by Day 5 (2.3 ± 0.12 cm) and Day 7 (3.6 ± 0.21 cm), indicating moderate, transient inhibition. The 20% concentration exhibited stronger effects, reducing growth to 1.1 ± 0.09 cm on Day 3 and maintaining minimal growth through Day 5 (2.0 ± 0.05 cm), though slight regrowth (2.3 ± 0.22 cm) was observed by Day 7. The 50% papaya FFJ demonstrated complete inhibition, with fungal growth suppressed to 0.0 ± 0.0 cm from Day 3 onward, showing no resurgence through Day 7 (Figure 1 & 2) & Table 3.

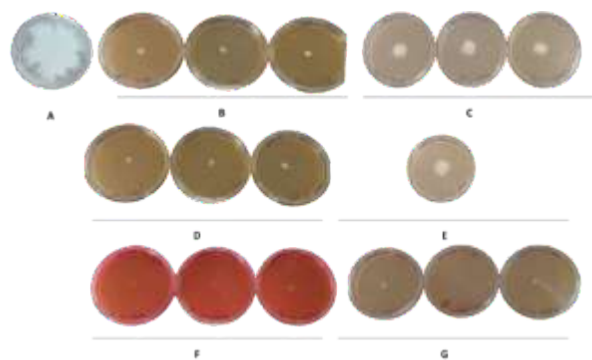


Figure 1: Anti-fungal activity of Papaya FFJ against pathogenic strain (A) Control with no FFJ treatment (B) Effect of 10 % concentration of Papaya FFJ on pathogenic strain in Day-1, (C) Effect of 20 % concentration of Papaya FFJ on pathogenic strain in Day-1, (E) Effect of 50 % concentration of Papaya FFJ on pathogenic strain in Day-1, (D) Effect of 10 % concentration of Papaya FFJ on pathogenic strain in Day-3, (F) Effect of 20 % concentration of Papaya FFJ on pathogenic strain in Day-3

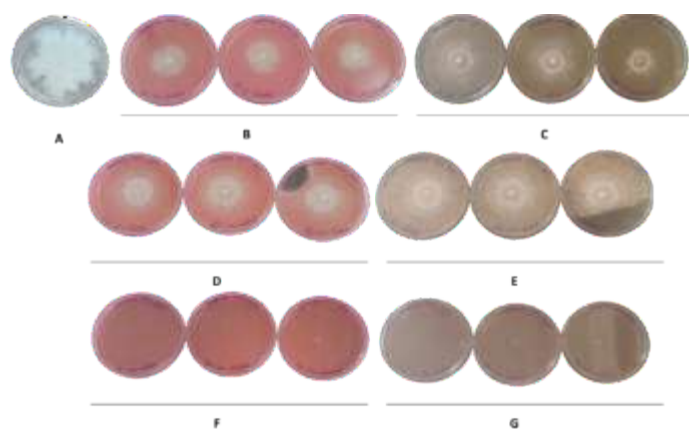


Figure 2: Anti-fungal activity of Papaya FFJ against pathogenic strain (A) Control with no FFJ treatment (B) Effect of 20 % concentration of Papaya FFJ on pathogenic strain in Day-5, (C) Effect of 50 % concentration of Papaya FFJ on pathogenic strain in Day-5, (E) Effect of 50 % concentration of Papaya FFJ on pathogenic strain in Day-7, (D) Effect of 50 % concentration of Papaya FFJ on pathogenic strain in Day-7, (F) Effect of 20 % concentration of Papaya FFJ on pathogenic strain in Day-7

Antifungal Activity of Banana FFJ

Similar trends were observed for Banana FFJ. The control group exhibited progressive growth from 6.0 cm (Day 1) to 8.5 cm (Day 7) (Table 7). At 10% Banana FFJ, mean fungal growth declined to 1.3 ± 0.29 cm by Day 3 and 0.9 ± 0.31 cm by Day 5, but regrowth to 2.3 ± 0.06 cm occurred by Day 7. The 20% concentration showed enhanced suppression, reducing growth to 0.3 ± 0.06 cm on Day 3 and 0.8 ± 0.26 cm on Day 5, with limited regrowth (2.1 ± 0.17 cm) by Day 7. The 50% Banana FFJ achieved complete inhibition (0.0 ± 0.0 cm) from Day 3 onward, with no fungal recovery observed during the study period (Figure 3 & 4) & Table 3.

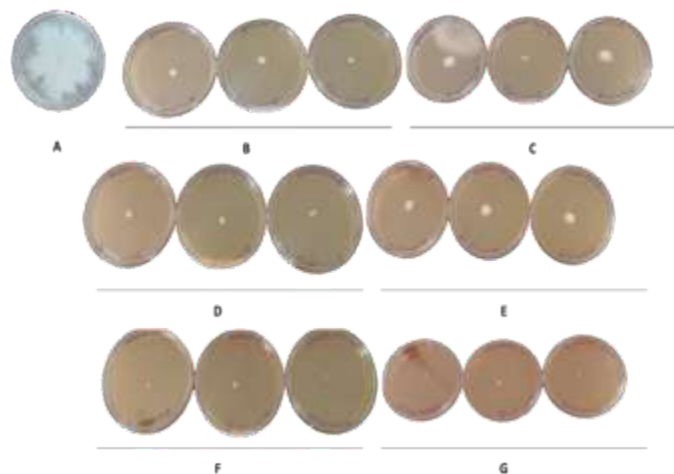


Figure 3: Anti-fungal activity of Banana FFJ against pathogenic strain (A) Control with no FFJ treatment (B) Effect of 10 % concentration of Banana FFJ on pathogenic strain in Day-1, (C) Effect of 10 % concentration of Banana FFJ on pathogenic strain in Day-3, (D) Effect of 20 % concentration of Banana FFJ on pathogenic strain in Day-1 (E) Effect of 20 % concentration of Banana FFJ on pathogenic strain in Day-3, (F) Effect of 50 % concentration of Banana FFJ on pathogenic strain in Day-1, (G) Effect of 50 % concentration of Banana FFJ on pathogenic strain in Day-3

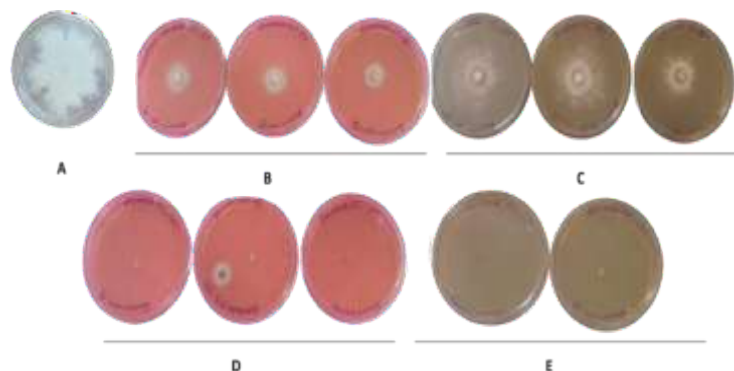


Figure 4: Anti-fungal activity of Banana FFJ against pathogenic strain (A) Control with no FFJ treatment (B) Effect of 10 % concentration of Banana FFJ on pathogenic strain in Day-5, (C) Effect of 10 % concentration of Banana FFJ on pathogenic strain in Day-7, (D) Effect of 20 % concentration of Banana FFJ on pathogenic strain in Day-5 (E) Effect of 20 % concentration of Banana FFJ on pathogenic strain in Day-7, (F) Effect of 50 % concentration of Banana FFJ on pathogenic strain in Day-5, (G) Effect of 50 % concentration of Banana FFJ on pathogenic strain in Day-7

Table 3: Anti-fungal activity of various FFJ against *Phomopsis azadirachtae* in comparison

FFJ Type	Fruit Juice source	Day-1 (mm)	Day-3 (cm)	Day-5 (cm)	Day-7 (cm)
Control	Banana	5	7.2	8.3	8.5
Control	Papaya	5	7.2	8.3	8.5
10% FFJ	Banana	5.0±0.0	1.3±0.29	0.9±0.31	2.3±0.06
10% FFJ	Papaya	5.0±0.0	1.7±0.24	2.3±0.12	3.6±0.21
20% FFJ	Banana	5.0±0.0	0.3±0.06	0.8±0.26	2.1±0.17
20% FFJ	Papaya	5.0±0.0	1.1±0.09	2.0±0.05	2.3±0.22
50% FFJ	Banana	5.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
50% FFJ	Papaya	5.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Both Papaya and Banana FFJ exhibited concentration-dependent antifungal activity. Lower concentrations (10% and 20%) provided partial inhibition but allowed regrowth over time, whereas 50% concentrations of both juices completely suppressed fungal growth after Day 3, sustaining total inhibition through Day 7. These results underscore the superior efficacy of higher FFJ concentrations in managing *Phomopsis azadirachtae*, positioning fermented fruit juices as potent natural antifungal agents.

DISCUSSION

The antifungal efficacy of fermented fruit juices (FFJs) from Papaya (*Carica papaya*) and Banana (*Musa* spp.) against *Phomopsis azadirachtae*, the pathogen responsible for Neem dieback disease, is strongly supported by this study's findings. By combining chemical profiling (GC-MS) with biological assays, this research highlights FFJs as sustainable alternatives to synthetic fungicides. Below, the results are contextualized within broader scientific frameworks, emphasizing their novelty and implications. GC-MS profiling of Banana FFJ identified ethyl oleate (15.08%) and hexadecanoic acid ethyl ester (12.55%) as predominant constituents (Table 1). These compounds are known to compromise fungal membrane integrity by integrating into lipid bilayers, leading to cellular leakage (Sudha *et al.*, 2013; Ziboh *et al.*, 2000). Additionally, linoleic acid ethyl ester (7.7%) and phenylethyl alcohol (2.95%) disrupt mitochondrial function and hyphal elongation (Sung & Lee, 2010). Banana FFJ also contained squalene (1.46%), which inhibits ergosterol synthesis—a vital

component of fungal cell walls (Khan *et al.*, 2019)—and 2,3-butanediol (1.65%), known to induce oxidative stress (Yang *et al.*, 2011). In Papaya FFJ, 2,4-dithiapentane (3.03%) and methyl β -D-galactopyranoside (1.76%) were prominent (Table 2), both linked to enzymatic interference and membrane destabilization (Shapla *et al.*, 2018). Notably, furfural (0.26%) and thiazole derivatives (0.10%) disrupt fungal metabolism through thiol interactions (Al-Khayri *et al.*, 2023), while dihydroxyacetone (2.22%) and 4H-pyran-4-one (0.84%) mitigate oxidative damage (Sharma *et al.*, 2021). Fermentation amplified bioactive compound concentrations, such as a 200% increase in phenolics in Papaya FFJ via lactic acid bacteria (LAB) activity (Dube *et al.*, 2022). Banana FFJ's acetic acid content reduced pH to <3.5 , inhibiting fungal colonization (Kim *et al.*, 2021). *In vitro* assays revealed a dose-response relationship in suppressing *P. azadirachtae* (Table 3). At 50% concentration, both FFJs achieved complete inhibition (0.0 cm radial growth) from Day 3 onward, with no resurgence by Day 7 (Figures 1–4). This aligns with studies requiring high concentrations (40–60%) of plant extracts to overcome fungal defenses (de la Cruz Quiroz *et al.*, 2019). Lower concentrations (10–20%) showed transient effects. Papaya FFJ (10%) reduced growth to 1.7 ± 0.24 cm by Day 3 but allowed regrowth to 3.6 ± 0.21 cm by Day 7. Similarly, Banana FFJ (20%) suppressed growth to 0.3 ± 0.06 cm on Day 3 but exhibited partial recovery (2.1 ± 0.17 cm) by Day 7. Such patterns suggest sublethal stress at lower doses, permitting fungal adaptation (Almalki & Ali, 2024). Fermentation enhanced antifungal activity through synergistic pathways. LAB metabolism not only elevated organic acids (e.g., lactic acid) but also bioconverted precursors like linoleic acid into bioactive ethyl esters, improving solubility and membrane penetration (Ziboh *et al.*, 2000). Phenylethyl alcohol in Papaya FFJ impaired mitochondrial ATP production (Sung & Lee, 2010), while Banana FFJ's thiophene derivatives alkylated thiol groups in fungal spores (Shapla *et al.*, 2018). Volatile organic compounds (VOCs) like limonene and α -terpineol, identified via GC-MS, destabilized fungal membranes (Zheng *et al.*, 2022). Combined with acidic pH, these multi-modal mechanisms reduce resistance risks compared to single-target fungicides (Girish & Fathima, 2019). These findings validate FFJs as eco-friendly antifungal agents suitable for integrated disease management (IDM). Their biodegradability, cost-effectiveness, and local availability make them ideal for resource-limited regions. For instance, farmers in Nizamabad District, India, could repurpose fruit waste into FFJs, aligning with circular economy principles (Avîrvarei *et al.*, 2023). Prior studies on plant-derived antimicrobials, such as Neem essential oils (Girish & Fathima, 2019) and fermented Guava extracts (Singh & Shrishail, 2023), corroborate their efficacy against *P. azadirachtae*. This study extends these findings by introducing FFJs as fermentation-enhanced solutions with dual antifungal and soil-enriching properties.

Replacing copper-based fungicides with FFJs could mitigate ecological harm to soil microbiota and pollinators (Zhao *et al.*, 2020), supporting the EU's Farm to Fork Strategy (European Commission, 2020). However, standardizing fermentation protocols (e.g., microbial consortia, pH) is critical for consistent metabolite profiles (Wang *et al.*, 2024).

CONCLUSION:

This study provides robust evidence supporting the antifungal efficacy of fermented Papaya and Banana juices against *Phomopsis azadirachtae*, a major pathogen causing dieback disease in Neem trees. The integration of GC-MS analysis with *In vitro* antifungal assays allowed for the identification of key bioactive compounds responsible for the inhibitory effects, including ethyl oleate, linoleic acid ethyl ester, phenylethyl alcohol, and thiophene derivatives. These metabolites exhibit mechanisms such as fungal membrane disruption, oxidative stress induction, and enzymatic inhibition, contributing to the observed concentration-dependent suppression of fungal growth. Notably, both Papaya and Banana FFJs at 50% concentration demonstrated complete inhibition of the fungal pathogen by Day 3, with no signs of regrowth through Day 7, indicating a fungicidal rather than merely fungistatic effect. The findings highlight the potential of these FFJs as cost-effective, residue-free, and locally producible alternatives to synthetic fungicides. Such natural interventions

align with the principles of sustainable agriculture and are especially relevant for low-resource settings and organic farming systems. Moreover, the successful demonstration of fermentation as a value-adding step emphasizes the importance of bio-transformation in enhancing phytochemical efficacy.

Looking forward, further validation through field trials is necessary to establish real-world applicability. Factors such as stability under environmental stress, formulation for shelf life, and compatibility with biological control agents like *Trichoderma* spp. should be explored. Additionally, scaling up the production of FFJs using agro-waste and integrating them into broader integrated pest management (IPM) frameworks could amplify their impact. In conclusion, fermented Papaya and Banana juices offer a promising, environmentally sustainable tool for the control of Neem dieback and potentially other fungal phytopathogens, paving the way for future biopesticide innovation and green crop protection strategies.

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