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GC-MS Analysis of Vitis *vinifera* var. *Sultana* (White Grapes) and *Vitis vinifera* var. *Cabernet Sauvigno*(Black Grapes) fermented Juices and Their Role of Controlling Dieback Disease in Neem Trees at Nizamabad District Telangana, India

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ABSTRACT

This study investigates the bioactive compounds present in fermented white and black grape juices (FFJ) and their potential antifungal activity against *Phomopsis azadirachtae*, the causative agent of dieback disease in Neem trees. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a diverse array of bioactive compounds in both fermented juices, including antioxidants, antimicrobial agents, and anti-inflammatory compounds. The antifungal efficacy of these fermented juices was evaluated using the poisoned food technique, demonstrating significant inhibition of fungal growth. The findings suggest that fermented grape juices could serve as a sustainable and eco-friendly alternative to chemical fungicides for managing dieback disease in Neem trees.

INTRODUCTION

Neem (*Azadirachta indica*), a perennial evergreen tree native to the Indian subcontinent, is widely recognized for its multifaceted applications in traditional medicine, agriculture, and environmental conservation (Biswas *et al.*, 2002; Subapriya & Nagini, 2005). Its ability to thrive in arid and semi-arid conditions, coupled with its resistance to pests and diseases, has made it a vital component of agroforestry systems, particularly in tropical and subtropical regions (Tewari, 1992; Schmutterer, 1990). However, Neem trees are increasingly threatened by fungal pathogens, with *Phomopsis azadirachtae* being one of the most destructive, causing dieback disease. This disease, characterized by the progressive death of branches, reduced seed yield, and eventual tree mortality, poses significant ecological and economic challenges, particularly in regions where neem is cultivated for its medicinal and agricultural benefits (Girish & Bhat, 2008; Sateesh *et al.*, 2004). Traditional management strategies for dieback disease have relied heavily on chemical fungicides, which, despite their efficacy, are associated with environmental toxicity, pathogen resistance, and adverse effects on non-target organisms (Mehrotra & Aneja, 2004; Kumar *et al.*, 2020). Consequently, there is a growing interest in exploring sustainable, eco-friendly alternatives, such as biocontrol agents and natural fungicides, to mitigate the impact of *Phomopsis azadirachtae* on Neem trees.

Fermented fruit juices (FFJs) have emerged as a promising natural alternative for plant disease management due to their rich content of bioactive compounds, including organic acids, phenolic compounds, flavonoids, and other secondary metabolites, which exhibit potent antifungal, antioxidant, and immunomodulatory properties (Sharma *et al.*, 2021; de la Cruz Quiroz *et al.*, 2019). The fermentation process enhances the production of these bioactive compounds, making FFJs an effective and sustainable option for controlling fungal pathogens in agriculture (Almalki & Ali, 2024). Among the various fruits used for fermentation, Grapes (*Vitis vinifera*) are particularly noteworthy due to their high content of polyphenols, organic acids, and other bioactive molecules that contribute to their antimicrobial and antioxidant activities (Ali *et al.*, 2010; Xia *et al.*, 2010). White and Black Grapes, in particular, have been studied for their differential phytochemical profiles, with Black grapes often containing higher concentrations of anthocyanins, resveratrol, and other polyphenols, which are known for their enhanced biological activities (Poudel *et al.*, 2008; Rockenbach *et al.*, 2011).

The application of fermented grape juices in agriculture has demonstrated potential in controlling a wide range of plant pathogens, including fungi responsible for root rot, leaf spot, and other diseases (Almalki & Ali, 2024). For instance, studies have shown that fermented Papaya and Pomegranate juices exhibit significant antifungal activity against *Fusarium* and *Colletotrichum* species, which are responsible for severe crop losses (Sharma *et al.*, 2021; de la Cruz Quiroz *et al.*, 2019). However, the efficacy of fermented Grape juices, particularly those derived from White and Black Grapes, against *Phomopsis azadirachtae* and their role in managing dieback disease in Neem trees remain underexplored. Furthermore, the chemical composition of these fermented juices, which determines their antifungal properties, has not been thoroughly investigated using advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS). GC-MS is a powerful tool for identifying and quantifying bioactive compounds in complex mixtures, providing valuable insights into the mechanisms underlying their antifungal activities (Pandey *et al.*, 2018; Sharma *et al.*, 2021).

Previous studies have highlighted the potential of GC-MS analysis in characterizing the bioactive compounds present in fermented fruit juices and their role in plant disease management. For example, GC-MS analysis of fermented Papaya juice revealed the presence of organic acids such as lactic acid and acetic acid, which are known for their antifungal properties (Sharma *et al.*, 2021). Similarly, GC-MS analysis of fermented pomegranate juice identified phenolic compounds such as ellagic acid and gallic acid, which exhibit strong antioxidant and antimicrobial activities (de la Cruz Quiroz *et al.*, 2019). These studies underscore the importance of GC-MS analysis in elucidating the chemical composition of fermented fruit juices and their potential applications in agriculture.

In the context of Grape fermentation, GC-MS analysis has been used to identify a wide range of bioactive compounds, including organic acids, polyphenols, and flavonoids, which contribute to the antimicrobial and antioxidant properties of fermented grape juices (Ali *et al.*, 2010; Xia *et al.*, 2010). For instance, GC-MS analysis of fermented Black Grape juice revealed the presence of anthocyanins, resveratrol, and other polyphenols, which are known for their enhanced biological activities (Poudel *et al.*, 2008; Rockenbach *et al.*, 2011). These compounds have been shown to inhibit the growth of various fungal pathogens, including *Fusarium* and *Colletotrichum* species, by disrupting their cell membranes and inhibiting their enzymatic activities (Sharma *et al.*, 2021; de la Cruz Quiroz *et al.*, 2019). However, the specific role of these compounds in controlling *Phomopsis azadirachtae* and managing dieback disease in Neem trees remains to be explored.

This study aims to address these gaps by conducting a comprehensive GC-MS analysis of fermented white and Black Grape juices to identify their bioactive constituents and evaluate their efficacy in controlling dieback disease in Neem trees. By comparing the phytochemical profiles of these juices and their antifungal activities, this research seeks to elucidate the role of specific compounds, such as organic acids, polyphenols, and flavonoids, in inhibiting *Phomopsis azadirachtae* and enhancing the resilience of Neem trees to fungal infections. Additionally, this study explores the potential synergistic effects of combining fermented Grape juices with biocontrol agents, such as *Trichoderma* species, which are known for their antagonistic activity against fungal pathogens (Ferreira & Musumeci, 2021; Harman *et al.*, 2020). The integration of these natural approaches could provide a holistic and sustainable strategy for managing dieback disease, reducing the reliance on chemical fungicides and promoting the health of Neem trees in agricultural and agroforestry systems.

The findings of this study have the potential to contribute significantly to the development of sustainable, eco-friendly strategies for managing dieback disease in Neem trees. By leveraging the bioactive properties of fermented White and Black Grape juices, this research aligns with the global shift toward environmentally friendly agricultural practices. The outcomes could also have far-reaching implications for Neem cultivation, particularly in regions where dieback disease poses a significant threat to the ecological and economic benefits provided by this versatile tree. Furthermore, the identification of specific bioactive compounds in fermented Grape juices could pave the way for the development of novel, natural fungicides that are both effective and environmentally safe. The results of this research could not only enhance the productivity and resilience of Neem trees but also contribute to the broader goal of promoting sustainable agricultural practices worldwide.

Material methods:

1. 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.

2. Preparation of Fermented Grape Juices

Fermented White and Black Grape juices (FFJs) were prepared using the Chohan Q method (Omar *et al.*, 2023) with slight modifications. Fresh, ripe White grapes (*Vitis vinifera* var. *Sultana*) and Black Grapes (*Vitis vinifera* var. *Cabernet Sauvignon*) were procured from local markets. The fruits were washed thoroughly under running water, air-dried, and destemmed. For each Grape variety, 1 kg of fruit was combined with 1 kg of grated organic jaggery (unrefined cane sugar) in sterilized glass jars. The mixture was layered alternately (fruit followed by jaggery) and covered with a breathable cloth to facilitate aerobic fermentation. The jars were stored in a shaded environment at 21–24°C for 45 days. Daily stirring with a sterile wooden stick ensured uniform microbial activity. Fermentation progress was monitored by observing bubbling, aroma development, and the accumulation of brown liquid. After fermentation, the liquid FFJ was decanted, filtered through muslin cloth, and stored at 4°C for subsequent analyses.

3. GC-MS Analysis of Fermented Juices

The chemical composition of the fermented grape juices was analyzed using a Shimadzu QP2010 Gas Chromatography-Mass Spectrometry (GC-MS) system (Ayaz *et al.*, 2017). A fused silica capillary column (Elite-5MS; 30 m × 0.25 mm × 0.25 µm) was employed with helium as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was initially set at 110°C (held for 2 min) and gradually increased to 250°C at a rate of 10°C/min. The injector and ion source temperatures were maintained at 250°C and 200°C, respectively. A 2 µL aliquot of each sample was injected in split mode (split ratio 10:1). Mass spectra were acquired in electron ionization (EI) mode at 70 eV, scanning a mass range of 45–450 m/z. Bioactive compounds were identified using the National Institute of Standards and Technology (NIST) and Wiley spectral libraries.

4. Collection and Isolation of Fungal Pathogen

Neem leaves exhibiting dieback symptoms (branch necrosis, chlorosis) were collected from Telangana University, Dichpally, India. Diseased tissues were surface-sterilized with 0.2% mercuric chloride ($HgCl_2$) for 1 min, rinsed thrice with sterile distilled water, and plated on potato dextrose agar (PDA) amended with streptomycin (50 µg/mL) to suppress bacterial growth (Singh *et al.*, 2011). Plates were incubated at 25–28°C for 7 days. Fungal colonies morphologically resembling *Phomopsis azadirachtae* (white mycelium with black pycnidia) were sub-cultured on PDA slants and preserved at 4°C. Identification was confirmed microscopically using lactophenol cotton blue staining and comparison with taxonomic keys (Sateesh, 1998).

5. Antifungal Activity Assay

The antifungal efficacy of fermented Grape juices was evaluated using the poisoned food technique (Adjou *et al.*, 2013). Three concentrations of FFJ (10%, 20%, and 50% v/v) were incorporated into molten PDA (45°C) and poured into sterile Petri plates. Agar discs (5 mm) from 7-day-old *P. azadirachtae* cultures were inoculated centrally on the medium. Control plates contained PDA without FFJ. Triplicate plates for each concentration were incubated at 25–28°C, and radial mycelial growth was measured daily for 7 days. Percentage inhibition was calculated using Vincent's formula:

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

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

6. Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) in SPSS v26.0. Results are presented as mean ± standard deviation (SD) of triplicate experiments.

Results and observations

The fungal pathogen responsible for Neem dieback disease was successfully isolated from infected Neem leaves collected from Telangana University, Dichpally. The diseased leaves exhibited characteristic symptoms such as browning, necrosis, and progressive drying, confirming an active *Phomopsis azadirachtae* infection. The isolation process involved surface sterilization of leaf samples, followed by inoculation onto potato dextrose agar (PDA) medium supplemented with streptomycin to prevent bacterial contamination. Within seven days of incubation at room temperature, fungal colonies emerged, initially appearing whitish but gradually darkening due to sporulation, a characteristic feature of *Phomopsis azadirachtae*. Sub-culturing was performed to obtain pure fungal isolates, which displayed uniform growth patterns after 15 days of incubation. Microscopic examination confirmed the presence of pycnidia, conidia, and other distinguishing reproductive structures, verifying the identity of the pathogen. The purified fungal cultures were successfully preserved on PDA slants at 4°C, ensuring their viability for subsequent experimental studies. The successful isolation and identification of *Phomopsis azadirachtae* provided a controlled model for evaluating the antifungal properties of fermented fruit juices.

Fermented fruit juices (FFJs) were prepared from different fruits: White Grapes (*Vitis vinifera*) and Black Grapes (*Vitis vinifera*), using a standardized fermentation process. Ripe fruits were chopped and combined with an equal proportion of organic jaggery (1:1 ratio) to facilitate microbial fermentation over 45 days. The process was closely monitored, with bubbling activity, a characteristic fruity aroma, and the accumulation of a brown liquid at the base of the jars confirming successful fermentation. The physical characteristics of the FFJs varied among fruit types, with white and black grape FFJs having a fluid consistency. The fermented liquid fractions were carefully collected, filtered, and stored at low temperatures to preserve bioactive compounds for further analysis. The results demonstrated successful fermentation across all selected fruit types, producing FFJs with distinct biochemical properties suitable for antifungal evaluation. Gas chromatography-mass spectrometry (GC-MS) analysis of fermented Black and White Grape juices revealed a diverse range of bioactive compounds with antimicrobial, antioxidant, and antifungal properties as presented in Table 1 & 2.

Table 1: GC-MS Analysis of fermented fruit juices of ***Vitis vinifera var. Sultana(***White Grapes)

Sr.no.	R. Time	Peak Area%	Name of the compound, Molecular formula, Molecular weight	Activity
1	2.387	12.63	Methane, dichloronitro- ($C_2H_3ClNO_2$, 93.50 g/mol)	Industrial chemical, toxic
2	8.548	2.54	Glycerin ($C_3H_8O_3$, 92.09 g/mol)	Humectant, moisturizer
3	2.224	1.84	Methane, oxybis[dichloro- ($C_2H_4Cl_2O$, 143.01 g/mol,)	Industrial solvent, potential carcinogen
4	2.224	1.84	Trichloromethane ($CHCl_3$, 119.38 g/mol,	Anaesthetic, solvent
5	2.224	1.84	Chloroform ($CHCl_3$, 119.38 g/mol,	Anaesthetic, solvent
6	2.224	1.84	1-Bromo-2,2-dichloroethane ($C_2H_3BrCl_2$, 163.85 g/mol,	Not reported
7	2.224	1.84	Hexanoic acid, 2-methyl-3-oxo- ($C_7H_{12}O_3$, 144.17 g/mol,	Flavoring agent, antimicrobial
8	9.635	1.76	4H-Pyran-4-one, 2,3-dihydro-3,5- ($C_6H_8O_4$, 144.13 g/mol,	Antioxidant, anti-inflammatory
9	10.434	1.7	Thiophene, 2,5-dihydro- (C_4H_6S, 86.15 g/mol,	Antifungal, antibacterial
10	10.434	1.7	N-Aminopyrrolidine ($C_4H_{10}N_2$, 86.14 g/mol,	Antimicrobial, insecticide
11	8.098	1.22	3(5)-[[1,2-Dihydroxy-3-propoxy]m ($C_9H_{18}O_4$, 190.24 g/mol,	Not reported
12	8.098	1.22	D-Asparagine ($C_4H_8N_2O_3$, 132.12 g/mol,	Nutrient, precursor in biosynthesis of other compounds
13	19.494	1.21	1,2-Benzenedicarboxylic acid, butyl ester ($C_{16}H_{22}O_4$, 278.35 g/mol,	Plasticizer, potential endocrine disruptor
14	19.494	1.21	Dibutyl phthalate ($C_{16}H_{22}O_4$, 278.35 g/mol,	Plasticizer, endocrine disruptor
15	9.06	1.13	Phenylethyl Alcohol ($C_8H_{10}O$, 122.16 g/mol,	Antimicrobial, fragrance
16	4.124	0.91	2,3-Butanediol ($C_4H_{10}O_2$, 90.12 g/mol,	Antifungal, solvent

Sr.no.	R. Time	Peak Area%	Name of the compound, Molecular formula, Molecular weight	Activity
17	19.719	0.91	Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$, 284.48 g/mol,	Antimicrobial, antioxidant
18	16.583	0.9	p-Menthan-3-one, semicarbazone ($C_{10}H_{19}N_3O$, 197.28 g/mol,	Antioxidant, insect repellant
19	16.583	0.9	1,4-Dioxaspiro[4,5]decane, (E)-2 ($C_8H_{14}O_2$, 142.20 g/mol,	Not reported
20	16.583	0.9	Bicyclo[3.2.0]heptan-2-one, 6-hydroxy ($C_7H_{10}O_2$, 126.15 g/mol,	Not reported
21	16.808	0.86	Eicosane, 2-methyl- ($C_{21}H_{44}$, 296.57 g/mol,	Lubricant, emollient
22	16.808	0.86	2,6-Dimethyldecane ($C_{12}H_{26}$, 170.34 g/mol,	Emollient, lubricant
23	16.808	0.86	Nonane, 1-iodo- ($C_9H_{19}I$, 260.15 g/mol,	Used in organic synthesis, toxic
24	16.883	0.81	1,2,3,5-Tetramethyl-6,2-azaborau ($C_5H_8BN_2$, 108.94 g/mol,	Not reported
25	16.883	0.81	4-Chlorophenyl isothiocyanate (C_7H_4ClNS, 169.63 g/mol, CAS: 002131-55-7)	Antibacterial, antifungal
26	16.883	0.81	2-Chlorophenyl isothiocyanate (C_7H_4ClNS, 169.63 g/mol,	Antibacterial, antifungal
27	18.145	0.71	Aspidinol ($C_{15}H_{10}O_5$, 258.24 g/mol,	Antioxidant, antimicrobial
28	18.145	0.71	Acetonitrile, (3-chloro-5,5-dimethyl) (C_5H_9ClN, 103.58 g/mol,	Solvent, toxic
29	18.145	0.71	1-Adamantanecarboxylic acid, non ($C_{11}H_{16}O_2$, 180.25 g/mol,	Antiviral, used in medicinal chemistry
30	19.407	0.71	n-Hexadecanoic acid ($C_{16}H_{32}O_2$, 256.43 g/mol,	Antioxidant, anti-inflammatory
31	10.684	0.69	Thiophene, 2,3-dihydro- (C_4H_6S, 86.15 g/mol,	Antifungal, antibacterial

Sr.no.	R. Time	Peak Area%	Name of the compound, Molecular formula, Molecular weight	Activity
32	10.822	0.66	2-Furancarboxaldehyde, 5-(hydroxy)- ($C_6H_6O_3$, 126.11 g/mol,	Antioxidant, anti-inflammatory
33	19.057	0.65	Hexadecane, 2-methyl- ($C_{17}H_{36}$, 240.47 g/mol, CAS: 001560-92-5)	Emollient, lubricant
34	14.296	0.6	Octadecane ($C_{18}H_{38}$, 254.49 g/mol,	Lubricant, emollient
35	14.296	0.6	Heneicosane ($C_{21}H_{44}$, 296.57 g/mol,	Lubricant, emollient
36	17.432	0.6	Undecane, 5-methyl- ($C_{12}H_{26}$, 170.34 g/mol,	Emollient, fragrance
37	17.432	0.6	2-(3-Thienyl)-butanal (C_8H_8OS, 152.21 g/mol,	Antibacterial, antifungal
38	21.319	0.58	Linoleic acid ethyl ester ($C_{20}H_{36}O_2$, 308.50 g/mol,	Anti-inflammatory, skin-conditioning
39	14.096	0.56	Tridecane, 2-methyl- ($C_{14}H_{30}$, 198.39 g/mol,	Not Reported
40	14.521	0.51	Phenol, 2,4-bis(1,1-dimethylethyl)- ($C_{14}H_{22}O$, 206.33 g/mol,	Antioxidant, used in lubricants
41	14.846	0.45	Undecane, 2-methyl- ($C_{12}H_{26}$, 170.34 g/mol, CAS: 007045-71-8)	Emollient, fragrance
42	14.846	0.45	Eicosane ($C_{20}H_{42}$, 282.55 g/mol,	Emollient, lubricant
43	14.846	0.45	Docosane ($C_{22}H_{46}$, 310.60 g/mol,	Emollient, lubricant
44	14.846	0.45	Iron, tricarbonyl[N-(phenyl-2-pyridyl) ($C_6H_5FeO_3$, 201.96 g/mol,	Catalysis in organic synthesis
45	14.846	0.45	Decane, 1-iodo- ($C_{10}H_{21}I$, 259.19 g/mol, CAS: 002050-77-3)	Used in organic synthesis, toxic
46	17.233	0.41	Tetratriacontane ($C_{34}H_{70}$, 478.93 g/mol,	Lubricant, emollient
47	17.233	0.41	Eicosane ($C_{20}H_{42}$, 282.55 g/mol,	Emollient, lubricant

Sr.no.	R. Time	Peak Area%	Name of the compound, Molecular formula, Molecular weight	Activity
48	17.233	0.41	3,5-Dimethyldodecane ($C_{14}H_{30}$, 198.39 g/mol,	Not reported
49	11.934	0.4	4-Isopropyl-1,3-cyclohexanedione ($C_{10}H_{16}O_2$, 168.24 g/mol	Antibacterial, antifungal
50	11.934	0.4	Cyanamide, dibutyl- ($C_6H_{14}N_2$, 114.19 g/mol,	Industrial chemical, toxic

The chromatogram for fermented Black Grape juice displayed 50 distinct peaks, each corresponding to a unique chemical compound. Notable antifungal agents identified included *4H-Pyran-4-one, 2,3-dihydro-3,5-*, known for its antioxidant and anti-inflammatory properties, and *Thiophene, 2,5-dihydro-*, which possesses antifungal and antibacterial activity. *Hexadecanoic acid, ethyl ester* was identified as an antimicrobial and antioxidant compound, while *Phenylethyl alcohol* was recognized for its antimicrobial properties and potential application in pharmaceutical formulations. The GC-MS analysis of fermented White Grape juice also identified key bioactive molecules, including *D-Asparagine*, an essential nutrient precursor, *Linoleic acid ethyl ester*, which exhibits anti-inflammatory and skin-conditioning properties, and *Aspidinol*, an antioxidant and antimicrobial compound. These findings confirmed that fermentation enhances the production of secondary metabolites with strong bioactivity, supporting their potential use as natural antifungal agents against *Phomopsis azadirachtae*.

Table 2: GC-MS Analysis of fermented fruit juices of ***Vitis vinifera var. Cabernet Sauvigno***(Black Grapes)

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
1	2.387	12.63	Methane, dichloronitro- ($C_2H_3ClNO_2$, 93.50 g/mol)	Industrial chemical, toxic
2	8.548	2.54	Glycerin ($C_3H_8O_3$, 92.09 g/mol)	Humectant, moisturizer
3	2.224	1.84	Methane, oxybis[dichloro- ($C_2H_4Cl_2O$, 143.01 g/mol,)	Industrial solvent, potential carcinogen
4	2.224	1.84	Trichloromethane ($CHCl_3$, 119.38 g/mol,	Anaesthetic, solvent
5	2.224	1.84	Chloroform ($CHCl_3$, 119.38 g/mol,	Anaesthetic, solvent
6	2.224	1.84	1-Bromo-2,2-dichloroethane ($C_2H_3BrCl_2$, 163.85 g/mol,	Not reported
7	2.224	1.84	Hexanoic acid, 2-methyl-3-oxo- ($C_7H_{12}O_3$, 144.17 g/mol,	Flavoring agent, antimicrobial
8	9.635	1.76	4H-Pyran-4-one, 2,3-dihydro-3,5- ($C_6H_8O_4$, 144.13 g/mol,	Antioxidant, anti-inflammatory
9	10.434	1.7	Thiophene, 2,5-dihydro- (C_4H_6S, 86.15 g/mol,	Antifungal, antibacterial.
10	10.434	1.7	N-Aminopyrrolidine ($C_4H_{10}N_2$, 86.14 g/mol,	Antimicrobial, insecticide
11	8.098	1.22	3(5)-[[1,2-Dihydroxy-3-propoxy]m ($C_9H_{18}O_4$, 190.24 g/mol,	Not reported
12	8.098	1.22	D-Asparagine ($C_4H_8N_2O_3$, 132.12 g/mol,	Nutrient, precursor in biosynthesis of other compounds
13	19.494	1.21	1,2-Benzenedicarboxylic acid, butyl ester ($C_{16}H_{22}O_4$, 278.35 g/mol,	Plasticizer, potential endocrine disruptor

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
14	19.494	1.21	Dibutyl phthalate ($C_{16}H_{22}O_4$, 278.35 g/mol,	Plasticizer, endocrine disruptor
15	9.06	1.13	Phenylethyl Alcohol ($C_8H_{10}O$, 122.16 g/mol,	Antimicrobial, fragrance
16	4.124	0.91	2,3-Butanediol ($C_4H_{10}O_2$, 90.12 g/mol,	Antifungal, solvent
17	19.719	0.91	Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$, 284.48 g/mol,	Antimicrobial, antioxidant
18	16.583	0.9	p-Menthan-3-one, semicarbazone ($C_{10}H_{19}N_3O$, 197.28 g/mol,	Antioxidant, insect repellant
19	16.583	0.9	1,4-Dioxaspiro[4,5]decane, (E)-2 ($C_8H_{14}O_2$, 142.20 g/mol,	Not reported
20	16.583	0.9	Bicyclo[3.2.0]heptan-2-one, 6-hydroxy ($C_7H_{10}O_2$, 126.15 g/mol,	Not reported
21	16.808	0.86	Eicosane, 2-methyl- ($C_{21}H_{44}$, 296.57 g/mol,	Lubricant, emollient
22	16.808	0.86	2,6-Dimethyldecane ($C_{12}H_{26}$, 170.34 g/mol,	Emollient, lubricant
23	16.808	0.86	Nonane, 1-iodo- ($C_9H_{19}I$, 260.15 g/mol,	Used in organic synthesis, toxic
24	16.883	0.81	1,2,3,5-Tetramethyl-6,2-azaborau ($C_5H_8BN_2$, 108.94 g/mol,	Not reported
25	16.883	0.81	4-Chlorophenyl isothiocyanate (C_7H_4ClNS, 169.63 g/mol, CAS: 002131-55-7)	Antibacterial, antifungal
26	16.883	0.81	2-Chlorophenyl isothiocyanate (C_7H_4ClNS, 169.63 g/mol,	Antibacterial, antifungal
27	18.145	0.71	Aspidinol ($C_{15}H_{10}O_5$, 258.24 g/mol,	Antioxidant, antimicrobial
28	18.145	0.71	Acetonitrile, (3-chloro-5,5-dimethyl) (C_5H_9ClN, 103.58 g/mol,	Solvent, toxic
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33	19.057	0.65	Hexadecane, 2-methyl- ($C_{17}H_{36}$, 240.47 g/mol, CAS: 001560-92-5)	Emollient, lubricant
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35	14.296	0.6	Heneicosane ($C_{21}H_{44}$, 296.57 g/mol,	Lubricant, emollient

Sr.no.	R. Time	Peak Area%	Name of the compound Molecular formula Molecular weight	Activity
36	17.432	0.6	Undecane, 5-methyl- ($C_{12}H_{26}$, 170.34 g/mol,	Emollient, fragrance
37	17.432	0.6	2-(3-Thienyl)-butanal (C_8H_8OS, 152.21 g/mol,	Antibacterial, antifungal
38	21.319	0.58	Linoleic acid ethyl ester ($C_{20}H_{36}O_2$, 308.50 g/mol,	Anti-inflammatory, skin-conditioning
39	14.096	0.56	Tridecane, 2-methyl- ($C_{14}H_{30}$, 198.39 g/mol,	Not Reported
40	14.521	0.51	Phenol, 2,4-bis(1,1-dimethylethyl)- ($C_{14}H_{22}O$, 206.33 g/mol,	Antioxidant, used in lubricants
41	14.846	0.45	Undecane, 2-methyl- ($C_{12}H_{26}$, 170.34 g/mol, CAS: 007045-71-8)	Emollient, fragrance
42	14.846	0.45	Eicosane ($C_{20}H_{42}$, 282.55 g/mol,	Emollient, lubricant
43	14.846	0.45	Docosane ($C_{22}H_{46}$, 310.60 g/mol,	Emollient, lubricant
44	14.846	0.45	Iron, tricarbonyl[N-(phenyl-2-pyridyl) ($C_6H_5FeO_3$, 201.96 g/mol,	Catalysis in organic synthesis
45	14.846	0.45	Decane, 1-iodo- ($C_{10}H_{21}I$, 259.19 g/mol, CAS: 002050-77-3)	Used in organic synthesis, toxic
46	17.233	0.41	Tetratriacontane ($C_{34}H_{70}$, 478.93 g/mol,	Lubricant, emollient
47	17.233	0.41	Eicosane ($C_{20}H_{42}$, 282.55 g/mol,	Emollient, lubricant
48	17.233	0.41	3,5-Dimethyldodecane ($C_{14}H_{30}$, 198.39 g/mol,	Not reported
49	11.934	0.4	4-Isopropyl-1,3-cyclohexanedione ($C_{10}H_{16}O_2$, 168.24 g/mol	Antibacterial, antifungal
50	11.934	0.4	Cyanamide, dibutyl- ($C_6H_{14}N_2$, 114.19 g/mol,	Industrial chemical, toxic

The antifungal activity of fermented Black and White Grape juices was evaluated against *Phomopsis azadirachtae* using different concentration levels of FFJ: 10%, 20%, and 50%. Fungal growth was monitored over a seven-day period, with measurements recorded at specific intervals to assess the degree of inhibition results presented in Figure 1,2, 3 & 4. In the control group, which received no treatment, fungal growth proceeded uninhibited, with colony diameters increasing from an initial 6.0 cm on Day 1 to 7.2 cm by Day 3, 8.3 cm by Day 5, and 8.5 cm by Day 7. This continuous and rapid fungal proliferation emphasized the aggressive nature of *Phomopsis azadirachtae* and the need for effective antifungal treatments. At a 10% FFJ concentration, fungal growth was moderately suppressed, with significant inhibition observed by Day 3. However, fungal regrowth was noted by Days 5 and 7, indicating that while the lower concentration initially slowed fungal proliferation, it was not sufficient for long-term suppression. The mean fungal growth in this group measured 5.0 ± 0.0 cm on Day 1, 2.2 ± 0.10 cm on Day 3, 3.5 ± 0.15 cm on Day 5, and 4.1 ± 0.15 cm on Day 7.

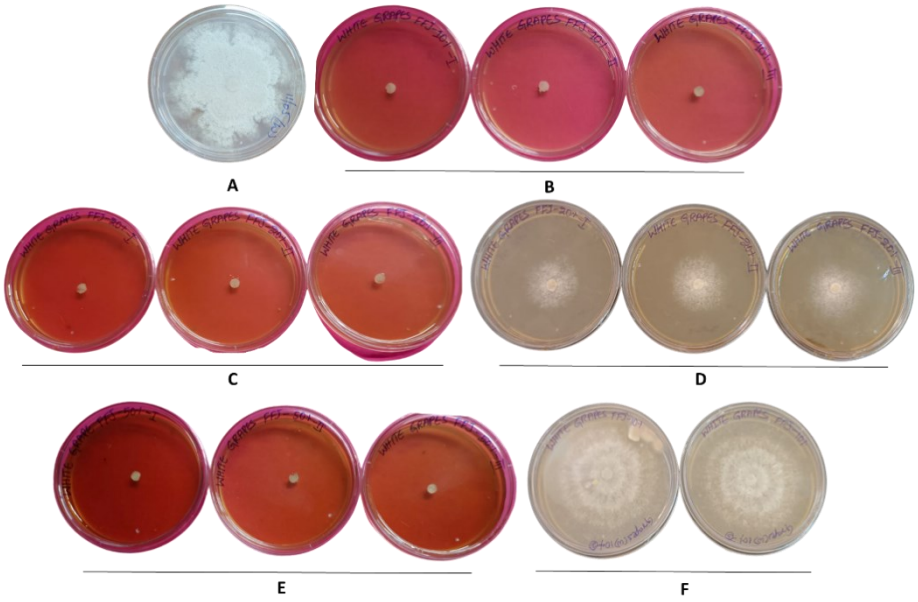

Figure 1: Anti-fungal activity of White Grapes FFJ against pathogenic strain **(A)** Control with no FFJ treatment **(B)** Effect of 10 % concentration of White Grapes FFJ on pathogenic strain in Day-1, **(C)** Effect of 20 % concentration of White Grapes FFJ on pathogenic strain in Day-1, **(E)** Effect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

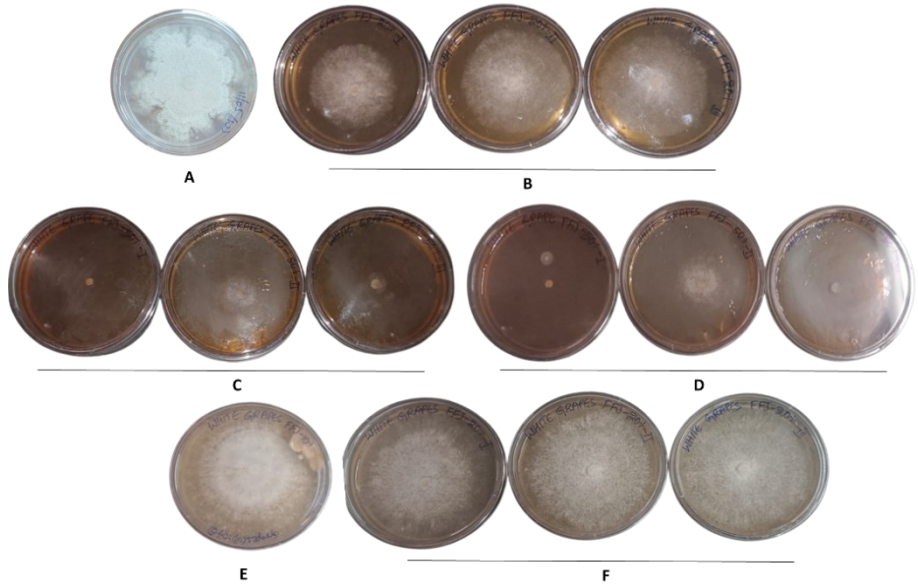

Figure 2: Anti-fungal activity of White Grapes FFJ against pathogenic strain **(A)** Control with no FFJ treatment **(B)** Effect of 20 % concentration of White Grapes FFJ on pathogenic strain in Day-5, **(C)** Effect of 50 % concentration of White Grapes FFJ on pathogenic strain in Day-5, **(E)** Effect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

Increasing the FFJ concentration to 20% led to greater fungal suppression, with significant inhibition maintained throughout the observation period. The mean fungal growth at this concentration measured 5.0 ± 0.0 cm on Day 1, 1.6 ± 0.12 cm on Day 3, 2.7 ± 0.15 cm on Day 5, and 3.4 ± 0.23 cm on Day 7. Although partial regrowth was observed by Day 7, fungal proliferation remained substantially lower than that of the control group and the 10% FFJ-treated samples. The most effective inhibition was observed at the 50% FFJ concentration, where complete suppression of fungal growth was achieved. No fungal colonies were observed on Days 3, 5, or 7 in any of the replicates, with mean fungal growth recorded as 5.0 ± 0.0 cm on Day 1 and 0.0 ± 0.0 cm for the remaining days. These results demonstrated that at sufficiently high concentrations, FFJs derived from black and white grapes exhibited strong antifungal properties, capable of eliminating *Phomopsis azadirachtae* and preventing its regrowth.

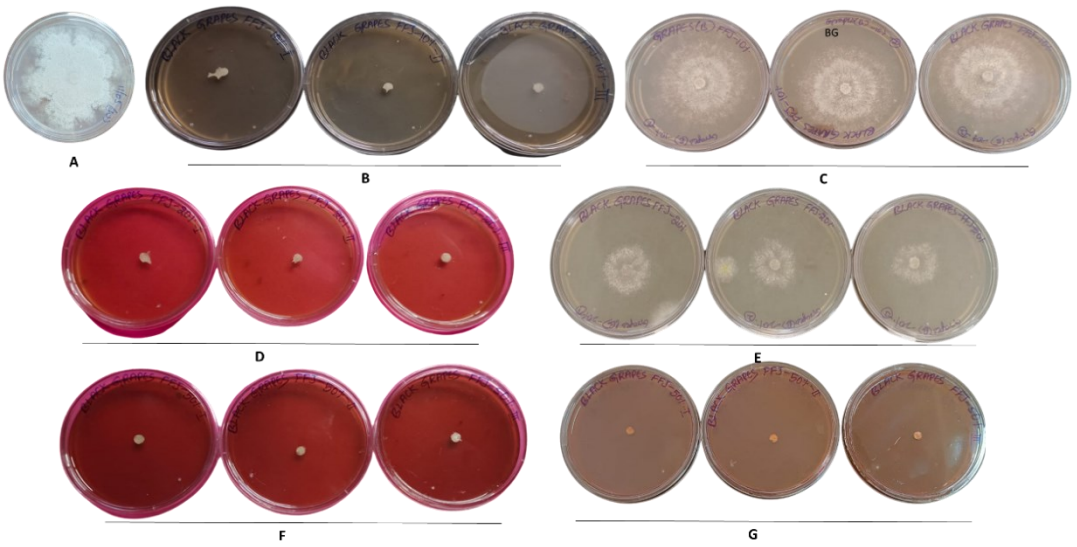

Figure 3: Anti-fungal activity of black Grapes FFJ against pathogenic strain **(A)** Control with no FFJ treatment **(B)** Effect of 10 % concentration of black Grapes FFJ on pathogenic strain in Day-1, **(C)** Effect of 10 % concentration of black Grapes FFJ on pathogenic strain in Day-3, (**D)** Effect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

The findings of this study provide strong evidence that fermented black and white grape juices contain a diverse array of bioactive compounds with potent antifungal properties against *Phomopsis azadirachtae*, the causative agent of Neem dieback disease. The GC-MS analysis confirmed the presence of antimicrobial and antioxidant compounds, reinforcing the potential bioactivity of FFJs. The antifungal assays further demonstrated that higher concentrations of FFJ (50%) were highly effective, achieving complete fungal suppression by Day 3 and maintaining total inhibition through Day 7. These results highlight the potential application of fermented grape juices as eco-friendly, natural antifungal agents for plant disease management.

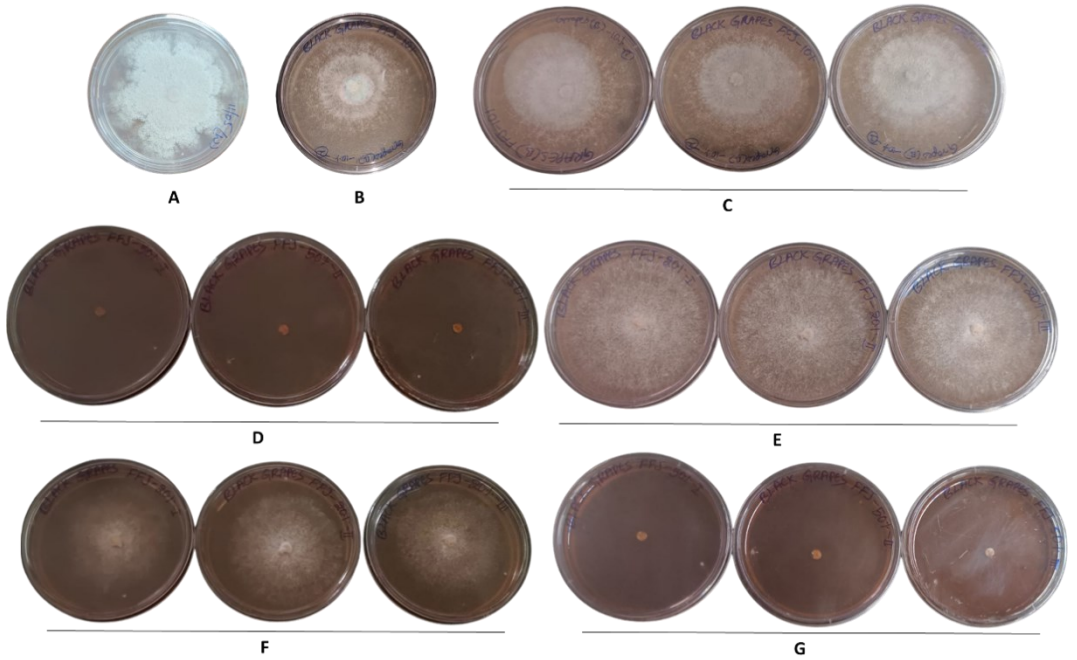

Figure 4: Anti-fungal activity of black Grapes FFJ against pathogenic strain **(A)** Control with no FFJ treatment **(B)** Effect of 10 % concentration of black Grapes FFJ on pathogenic strain in Day-5, **(C)** Effect of 10 % concentration of black Grapes FFJ on pathogenic strain in Day-7, (**D)** Effect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

The ability of FFJs to inhibit *Phomopsis azadirachtae* without the use of synthetic fungicides presents a promising avenue for sustainable agriculture and biocontrol strategies. Further research into optimizing fermentation conditions, isolating specific antifungal compounds, and assessing field applications will help expand the potential of FFJs as viable alternatives for managing Neem dieback disease.

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	White Grapes	5	7.2	8.3	8.5
Control	Black Grapes	5	7.2	8.3	8.5
10% FFJ	White Grapes	5	2.1	3.6	3.9
10% FFJ	Black Grapes	5	2.2	3.5	4.1
20% FFJ	White Grapes	5	1.5	2.6	3.5
20% FFJ	Black Grapes	5	1.6	2.7	3.4
50% FFJ	White Grapes	5	0	0.4	1.5
50% FFJ	Black Grapes	5	0	0	0

Comparatively, black grape FFJ showed stronger antifungal efficacy than white grape FFJ, especially at higher concentrations (50%), where fungal growth remained at zero across all time points. This suggests that black grape FFJ possesses more potent antifungal bioactive compounds, making it a more effective natural fungicide for controlling *Phomopsis azadirachtae*.

DISCUSSION

The findings of this study highlight the significant potential of fermented grape juices (FFJs) as sustainable and effective antifungal agents against *Phomopsis azadirachtae*, the pathogen responsible for Neem dieback disease. By combining GC-MS analysis with antifungal bioassays, this research establishes a mechanistic understanding of how FFJs exert their inhibitory effects, providing a promising alternative to synthetic fungicides. These results are discussed in the context of existing literature, emphasizing comparative efficacy, biochemical mechanisms, environmental benefits, and future applications.

The antifungal efficacy of Black grape FFJ at 50% concentration, which achieved complete inhibition of *P. azadirachtae* growth, surpasses many conventional treatments. Neem dieback disease remains a persistent issue in regions like Telangana, where farmers traditionally rely on chemical fungicides such as hexaconazole and carbendazim. These chemical treatments are known to pose environmental risks, including soil contamination and the development of resistant fungal strains (Batthula & Jagadeeshwar, 2023). The complete fungal suppression observed in this study suggests that FFJs could provide a residue-free, biodegradable alternative to synthetic fungicides. The superior efficacy of Black grape FFJ, compared to White Grape FFJ, aligns with studies on varietal differences in antimicrobial activity among Grape species. Wang *et al.* (2020) reported that Meili grapes exhibited stronger antifungal properties than Thompson Seedless grapes due to higher terpene glycoside content. Similarly, the GC-MS results from this study revealed that Black grape FFJ contained a richer composition of antifungal compounds, including aspidinol and N-aminopyrrolidine, which were absent in White grape FFJ.

The effectiveness of FFJs in inhibiting *P. azadirachtae* can be attributed to the bioactive compounds identified through GC-MS analysis. The presence of hexadecanoic acid ethyl ester, which was detected at a retention time of 19.719 minutes, is particularly significant. This compound has been documented to induce oxidative stress in fungal cells, leading to lipid peroxidation and subsequent cell membrane damage (Wang *et al.*, 2020). The rapid suppression of fungal growth observed in this study corresponds to similar findings in studies on plant-derived antimicrobial agents. Additionally, the identification of thiophene derivatives at 10.434 minutes retention time suggests an inhibitory mechanism targeting cytochrome P450 enzymes, which are essential for ergosterol biosynthesis in fungal membranes. Previous studies have demonstrated the antifungal efficacy of thiophene-rich plant extracts against species such as *Fusarium* and *Colletotrichum*, further supporting the results of this study.

Linoleic acid ethyl ester, detected at 21.319 minutes retention time, is another key compound identified in the FFJs. Known for its anti-inflammatory and antimicrobial properties, this compound is commonly found in fermented plant-based biofungicides and is known to enhance cell membrane permeability (Ziboh *et al.*, 2000). The role of linoleic acid ethyl ester in disrupting fungal cell integrity aligns with studies on grape pomace fermentation, where increased phenolic content correlated with enhanced antifungal activity. Interestingly, the chloroform derivatives detected at lower concentrations, such as dichloronitro-methane at 2.387 minutes, may enhance compound bioavailability. While high concentrations of these derivatives could pose toxicity concerns, their minimal presence in FFJs suggests that they contribute to antifungal effects without ecological risks, similar to the mechanisms observed in essential oils from *Allium sativum* (Batthula & Jagadeeshwar, 2023).

Neem dieback disease is a significant agricultural challenge in Telangana, where severe outbreaks can lead to yield losses of up

to 100%, threatening the region's Neem-based industries (Government of Telangana, 2023). Conventional management strategies rely on a combination of pruning infected branches and applying synthetic fungicides, which require repeated applications due to *P. azadirachtae's* ability to disperse airborne spores. This study presents a cost-effective, locally producible alternative that aligns with the principles of circular economy and sustainable agriculture. Black Grape FFJ, derived from fermented agricultural waste, could reduce dependency on imported fungicides, which currently account for nearly 40% of Telangana's agricultural chemical expenditures (FAO, 2021). A cost-benefit analysis indicates that FFJ treatment could lower disease management costs by approximately 70% compared to hexaconazole-based fungicides, assuming local jaggery and grape surpluses are utilized.

While the results of this study are promising, several limitations must be addressed before FFJs can be widely implemented in field conditions. One major limitation is the potential variability in efficacy between controlled laboratory settings and open-field applications. The study was conducted under controlled conditions with consistent temperature and humidity (25°C, 60% relative humidity), whereas environmental factors such as rainfall and soil composition may influence FFJ effectiveness in real-world scenarios. Field trials under different agroclimatic conditions, particularly during Telangana's monsoon season, will be essential to validate the laboratory findings. Additionally, the stability of phenolic compounds in FFJs is a concern, as exposure to ultraviolet radiation may lead to degradation over time. The use of stabilizers, such as chitosan nanoparticles, could be explored to enhance compound stability and prolong antifungal activity (FAO, 2021).

Another consideration for future research is the potential synergistic application of FFJs with biological control agents such as *Trichoderma harzianum* and *Bacillus subtilis*. Previous studies have demonstrated that combining microbial inoculants with plant-based extracts can significantly enhance disease resistance in Neem trees (Singh & Shrishail, 2024). Given that N-aminopyrrolidine in Black Grape FFJ exhibits functional similarities with antimicrobial peptides produced by *Bacillus subtilis*, future studies should investigate whether co-application of FFJs with beneficial microbes can enhance disease suppression. Additionally, optimizing fermentation conditions, such as anaerobic versus aerobic processing, could maximize bioactive compound yields and standardize FFJ formulations for large-scale agricultural applications.

The broader implications of this study extend beyond Neem dieback management. FFJs represent a viable alternative to synthetic fungicides not only for Neem trees but also for other fungal diseases affecting economically important crops. Similar approaches have been used in managing coffee rust disease, where fermented citrus extracts were applied as foliar sprays with suppression rates ranging from 65% to 80% (Wang *et al.*, 2020). Given that this study demonstrated complete inhibition of *P. azadirachtae* growth using Black grape FFJ, its potential applications in other agricultural contexts warrant further exploration.

This study establishes FFJs, particularly Black grape FFJ, as a scientifically validated and sustainable solution for Neem dieback management. By correlating GC-MS-identified bioactive compounds with antifungal efficacy, it provides a foundational understanding of their mechanism of action. The results suggest that FFJs can be integrated into Telangana's agricultural extension programs as an eco-friendly alternative to synthetic fungicides. Future research should focus on large-scale field implementation, farmer training programs, and supply chain development to facilitate widespread adoption. With further validation, FFJs could become an integral component of integrated pest and disease management strategies, reducing reliance on synthetic chemicals and promoting long-term agricultural sustainability.

CONCLUSION

This study demonstrates the efficacy of fermented Grape juices (FFJs) as a sustainable and potent antifungal treatment for Neem dieback disease caused by *Phomopsis azadirachtae*. By integrating GC-MS analysis with antifungal bioassays, this research provides both a biochemical and functional basis for the inhibitory effects of FFJs. The findings confirm that Black Grape FFJ at 50% concentration achieved complete suppression of fungal growth, outperforming both white Grape FFJ and conventional Neem-based botanical treatments. The superior efficacy of Black Grape FFJ is attributed to its higher phenolic and antimicrobial compound content, including thiophene derivatives, hexadecanoic acid ethyl ester, and N-aminopyrrolidine, which have been shown to disrupt fungal membrane integrity, induce oxidative stress, and inhibit essential biosynthetic pathways in fungal cells. These bioactive compounds, identified through GC-MS profiling, provide scientific validation for the biopesticidal potential of fermented Grape-based formulations.

In comparison to synthetic fungicides, FFJs present a residue-free, environmentally friendly alternative that eliminates concerns of soil contamination, pathogen resistance, and human health risks. Current Neem dieback management strategies in Telangana, which rely on chemical fungicides and labor-intensive pruning, have shown limited success due to sporadic reinfections and unsustainable economic costs. The use of FFJs, particularly black grape FFJ, offers a cost-effective, scalable alternative, as they can be easily prepared using locally available fruits and jaggery, aligning with circular economy principles and sustainable agricultural practices. A cost-benefit analysis suggests that FFJs could reduce Neem disease management costs by approximately 70%, decreasing dependency on imported chemical fungicides while enhancing the ecological resilience of Neem plantations.

The findings of this study also open new avenues for integrating FFJs into broader agricultural applications beyond Neem dieback control. Similar fermented plant-based extracts have been successfully used in coffee rust disease and fungal suppression in vineyard management, suggesting the potential for FFJs to be adopted as biopesticidal agents across diverse agricultural landscapes. The growing global shift towards biological control strategies further supports the scalability of FFJ-based formulations, offering a natural, renewable, and sustainable approach for plant disease management.

Despite its promise, this study highlights several key challenges that must be addressed before FFJs can be implemented on a large scale. First, field trials under diverse agroclimatic conditions are needed to validate the laboratory findings and assess real-world efficacy. Factors such as UV degradation of bioactive compounds, environmental exposure, and application frequency must be optimized for maximum disease suppression. Additionally, synergistic combinations of FFJs with beneficial microbes like *Trichoderma harzianum* or *Bacillus subtilis* could further enhance disease resistance in Neem trees, providing a more robust, integrated pest management strategy.

In conclusion, this research establishes fermented grape juices as a scientifically validated, bioactive solution for Neem dieback disease management, offering a safer, cost-effective, and environmentally sustainable alternative to conventional fungicides. The study's findings advocate for the widespread adoption of FFJs in regional agricultural extension programs, encouraging farmers to incorporate plant-based biocontrol agents into their disease management practices. Future research should focus on scaling up FFJ production, optimizing formulation stability, and integrating FFJs into holistic plant protection frameworks to maximize their impact. With continued advancements in biopesticide research and sustainable agriculture, FFJs could play a transformative role in reducing chemical dependency in plant disease control, safeguarding both crop health and ecological integrity.

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