

Antifungal Activity of Fermented Extracts of *Azadirachta Indica*, *Allium Sativum*, *Ocimum Tenuiflorum*, and *Curcuma Longa* Against *Fusarium Oxysporum* and *Alternaria Alternata*

Shrikant Prakashrao Shinde¹, Shubham Chandramani Sawatkar²

¹Assistant Professor (CHB), Department of Microbiology, Shri Datta Arts, Commerce and Science College, Hadgaon, Nanded Maharashtra, India.

²Assistant Professor (CHB), Department of Microbiology, Shankarrao Chavan Mahavidyalaya, Ardhapur, Nanded, Maharashtra, India.

Corresponding Author: Email: shindeshri444@gmail.com

Abstract

Plant pathogenic fungi, particularly *Fusarium oxysporum* and *Alternaria alternata*, are responsible for significant crop losses worldwide, necessitating the development of environmentally sustainable alternatives to synthetic fungicides. The present study evaluated the antifungal activity of fermented ethanolic extracts of *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), and *Ocimum tenuiflorum* (Tulsi) against these two economically important phytopathogens. Plant materials were fermented in 10% molasses using *Lactobacillus plantarum* for 12, 24, 36, and 48 days, followed by ethanolic extraction. Antifungal efficacy was assessed using agar well diffusion, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and GC–MS analysis. Fermentation significantly enhanced the antifungal activity of all plant extracts, with 48-day fermented extracts exhibiting the greatest inhibitory effects. Garlic extract demonstrated the highest antifungal activity, producing the largest inhibition zones of 28.6 ± 0.4 mm against *F. oxysporum* and 31.3 ± 0.4 mm against *A. alternata* at 50 mg mL⁻¹. The lowest MIC values were recorded for garlic and tulsi extracts, reaching 1.25 mg mL⁻¹ against *F. oxysporum* and 0.625 mg mL⁻¹ against *A. alternata* after 48 days of fermentation. GC–MS analysis identified several bioactive constituents, including diallyl disulfide, diallyl trisulfide, eugenol, α -turmerone, β -turmerone, phytol, and β -caryophyllene, which are known for their antimicrobial properties. Statistical analysis revealed significant effects ($P < 0.05$) of plant species, fermentation period, extract concentration, and fungal pathogen on antifungal activity. These findings indicate that microbial fermentation enhances the bioactivity of medicinal plant extracts and highlight fermented garlic and tulsi extracts as promising eco-friendly biofungicidal candidates for the sustainable management of fungal plant diseases.

Keywords: *Allium sativum*; *Alternaria alternata*; antifungal activity; biofungicide; fermentation; *Fusarium oxysporum*; GC–MS; medicinal plants; minimum inhibitory concentration (MIC); *Ocimum tenuiflorum*.

Introduction

Plant pathogenic fungi are among the most destructive agents affecting global agricultural productivity, causing substantial yield losses and deterioration of crop quality. Among these, *Fusarium oxysporum* is a soil-borne pathogen responsible for vascular wilt in numerous economically important crops, whereas *Alternaria alternata* causes leaf spot, blight, and post-harvest diseases in vegetables, cereals, fruits, and ornamental plants. Excessive reliance on synthetic fungicides has led to the development of resistant fungal strains, environmental contamination, and concerns regarding food safety and human health. Consequently, there is growing interest in developing environmentally friendly and sustainable alternatives for the management of plant diseases (Brent & Hollomon, 2007; Fisher et al., 2018).

Medicinal plants are valuable sources of natural bioactive compounds with antimicrobial properties. Phytochemicals such as phenolics, flavonoids, alkaloids, terpenoids, sulfur-containing compounds, and essential oils exhibit broad-spectrum antifungal activity by disrupting fungal cell membranes, inhibiting spore germination, and interfering with fungal metabolism. Among these, *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), and *Ocimum tenuiflorum* (Tulsi) have received considerable attention due to their rich phytochemical composition and proven biological activities (Cowan, 1999; Ghorbani, 2005).

Neem contains limonoids such as azadirachtin and nimbidin, which possess strong antifungal and antimicrobial properties. Garlic is rich in allicin and other organosulfur compounds that inhibit the growth of several phytopathogenic fungi. Turmeric contains curcuminoids and volatile oils with antioxidant and antifungal activities, while Tulsi is important medicinal herb rich in eugenol, linalool, and other phenolic constituents that exhibit potent antimicrobial effects. Numerous investigations have demonstrated that extracts from these plants inhibit the growth of *Fusarium*, *Alternaria*, *Rhizoctonia*, *Colletotrichum*, and other economically important fungal pathogens (Singh et al., 2005; Prakash & Gupta, 2005).

Recently, fermentation has emerged as an effective approach for enhancing the biological activity of plant extracts. During microbial fermentation, beneficial microorganisms produce hydrolytic enzymes capable of degrading complex plant polymers into simpler bioactive molecules with improved solubility and bioavailability. Fermentation also generates organic acids, peptides, and antimicrobial metabolites that may act synergistically with plant-derived phytochemicals, resulting in enhanced antifungal efficacy. Fermented plant extracts have therefore gained attention as sustainable biofungicides suitable for integrated plant disease management and organic agriculture (Hur et al., 2014; Swain et al., 2014).

Despite increasing reports on the antimicrobial properties of medicinal plants, comparative information regarding the antifungal activity of fermented extracts of Neem, Garlic, Turmeric, and Tulsi against important phytopathogens remains limited. Therefore, the present study was designed to evaluate the antifungal activity of fermented extracts prepared from these four medicinal plants against *Fusarium oxysporum* and *Alternaria alternata*. The study further aimed to compare antifungal efficacy following different fermentation periods using minimum inhibitory concentration (MIC) and related antifungal assays to identify promising plant-based biofungicidal formulations.

Certainly. Below is a journal-style introduction suitable for a manuscript entitled "**Antifungal Activity of Fermented Plant Extracts Against *Fusarium oxysporum* and *Alternaria alternata***". The references included are established sources appropriate for the statements made.

Introduction

Plant pathogenic fungi are among the most destructive agents affecting global agricultural productivity, causing substantial yield losses and deterioration of crop quality. Among these, *Fusarium oxysporum* is a soil-borne pathogen responsible for vascular wilt in numerous economically important crops, whereas *Alternaria alternata* causes leaf spot, blight, and post-harvest diseases in vegetables, cereals, fruits, and ornamental plants. Excessive reliance on synthetic fungicides has led to the development of resistant fungal strains, environmental contamination, and concerns regarding food safety and human health. Consequently, there is growing interest in developing environmentally friendly and sustainable alternatives for the management of plant diseases (Brent & Hollomon, 2007; Fisher et al., 2018).

Medicinal plants are valuable sources of natural bioactive compounds with antimicrobial properties. Phytochemicals such as phenolics, flavonoids, alkaloids, terpenoids, sulfur-containing compounds, and essential oils exhibit broad-spectrum antifungal activity by disrupting fungal cell membranes, inhibiting spore germination, and interfering with fungal metabolism. Among these, *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), and *Ocimum tenuiflorum* (Tulsi) have received considerable attention due to their rich phytochemical composition and proven biological activities (Cowan, 1999; Ghorbani, 2005).

Neem contains limonoids such as azadirachtin and nimbidin, which possess strong antifungal and antimicrobial properties. Garlic is rich in allicin and other organosulfur compounds that inhibit the growth of several phytopathogenic fungi. Turmeric contains curcuminoids and volatile oils with antioxidant and antifungal activities, while Tulsi is an important medicinal herb rich in eugenol, linalool, and other phenolic constituents that exhibit potent antimicrobial effects. Numerous investigations have demonstrated that extracts from these plants inhibit the growth of *Fusarium*, *Alternaria*, *Rhizoctonia*, *Colletotrichum*, and other economically important fungal pathogens (Singh et al., 2005; Prakash & Gupta, 2005).

Recently, fermentation has emerged as an effective approach for enhancing the biological activity of plant extracts. During microbial fermentation, beneficial microorganisms produce hydrolytic enzymes capable of degrading complex plant polymers into simpler bioactive molecules with improved solubility and bioavailability. Fermentation also generates organic acids, peptides, and antimicrobial metabolites that may act synergistically with plant-derived phytochemicals, resulting in enhanced antifungal efficacy. Fermented plant extracts have therefore gained attention as sustainable biofungicides suitable for integrated plant disease management and organic agriculture (Hur et al., 2014; Swain et al., 2014).

Despite increasing reports on the antimicrobial properties of medicinal plants, comparative information regarding the antifungal activity of fermented extracts of Neem, Garlic, Turmeric, and Tulsi against important phytopathogens remains limited. Therefore, the present study was designed to evaluate the antifungal activity of fermented extracts prepared from these four medicinal plants against *Fusarium oxysporum* and *Alternaria alternata*. The study further aimed to compare antifungal efficacy following different fermentation periods using minimum inhibitory concentration (MIC) and related antifungal assays to identify promising plant-based biofungicidal formulations.

2. Materials and Methods

2.1 Chemicals and Reagents

Analytical-grade ethanol (99.9%), methanol, dimethyl sulfoxide (DMSO), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Carbendazim (analytical standard), and other analytical-grade chemicals were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Sterile distilled water was used throughout the study.

2.2 Collection and Authentication of Plant Materials

Fresh leaves of *Azadirachta indica* (Neem) and *Ocimum tenuiflorum* (Tulsi), cloves of *Allium sativum* (Garlic), and rhizomes of *Curcuma longa* (Turmeric) were collected from the vicinity of Hadgaon, Nanded District, Maharashtra, India, during the early morning hours. Plant specimens were authenticated by a taxonomist in the Shri Datta Arts, Commerce and Science College, Hadgaon, Maharashtra, India. Voucher specimens were deposited in the departmental herbarium for future reference.

The collected plant materials were thoroughly washed with running tap water followed by sterile distilled water to remove adhering dust and contaminants. The samples were shade-dried at room temperature ($27 \pm 2^\circ\text{C}$) for 7–10 days, pulverized into a fine powder using a laboratory grinder, and stored in sterile airtight containers until further extraction and analysis (Harborne, 1998; Evans, 2009).

2.3 Preparation of Fermented Plant Extracts

Fermented plant extracts were prepared following a modified natural fermentation method (Swain et al., 2014). Briefly, 100 g of powdered plant material was mixed with 1 L of sterile distilled water containing 10% (w/v) molasses. The mixtures were inoculated with 2% (v/v) of *Lactobacillus plantarum* (approximately 1×10^8 CFU mL⁻¹) and incubated under anaerobic conditions at $30 \pm 2^\circ\text{C}$.

Fermentation was carried out for 12, 24, 36 and 48 days. After each fermentation period, the mixtures were filtered through sterile muslin cloth followed by Whatman No.1 filter paper.

Table 1. Plant materials and extraction details

Plant	Scientific name	Plant part	Fermentation medium	Fermentation period
Neem	<i>Azadirachta indica</i>	Leaves	10% Molasses	12, 24, 36 & 48 days
Garlic	<i>Allium sativum</i>	Cloves	10% Molasses	12, 24, 36 & 48 days
Turmeric	<i>Curcuma longa</i>	Rhizomes	10% Molasses	12, 24, 36 & 48 days
Tulsi	<i>Ocimum tenuiflorum</i>	Leaves	10% Molasses	12, 24, 36 & 48 days

2.4 Ethanolic Extraction

The fermented filtrates were mixed with 80% ethanol (1:5, v/v) and extracted on an orbital shaker at 150 rpm for 24 h. The extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator at 40°C. The concentrated extracts were dried to constant weight and stored at 4°C until further analysis (Eloff, 1998).

2.5 Preparation of Test Concentrations

A stock solution (100 mg mL⁻¹) of each extract was prepared in 10% DMSO. Serial two-fold dilutions were prepared using sterile Potato Dextrose Broth to obtain the required concentrations.

Table 2. Concentrations used in antifungal assays

Treatment	Concentration (mg mL ⁻¹)
T1	0.625
T2	1.25
T3	2.50
T4	5.00
T5	10.00
T6	25.00
T7	50.00
Positive control	Carbendazim (100 µg mL ⁻¹)
Negative control	10% DMSO

2.6 Fungal Cultures

Pure cultures of *Fusarium oxysporum* and *Alternaria alternata* were obtained from a recognized microbial culture collection. The fungi were maintained on Potato Dextrose Agar (PDA) at 4°C and subcultured every 15 days. Fresh seven-day-old cultures were used throughout the experiment (Leslie & Summerell, 2006).

2.7 Agar Well Diffusion Assay

The antifungal activity of fermented extracts was determined using the agar well diffusion method (Perez et al., 1990). PDA plates were inoculated with fungal spore suspensions (1 × 10⁶ spores mL⁻¹). Wells of 6 mm diameter were prepared using a sterile cork borer, and 100 µL of each extract concentration was added. Plates were incubated at 28 ± 2°C for 72 h, after which inhibition zones were measured in millimetres.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of each extract was determined by the broth microdilution technique according to CLSI M38-A2 guidelines (CLSI, 2008). Serial dilutions ranging from 0.625 to 50 mg mL⁻¹ were prepared in sterile PDB. Each well was inoculated with approximately 1×10^5 fungal spores mL⁻¹ and incubated at 28°C for 48–72 h. The MIC was defined as the lowest concentration showing complete inhibition of visible fungal growth.

2.9 Determination of Minimum Fungicidal Concentration (MFC)

Aliquots from wells showing no visible fungal growth in the MIC assay were streaked onto fresh PDA plates and incubated for 72 h. The lowest concentration showing no fungal colony growth was recorded as the MFC (Espinel-Ingroff et al., 2005).

2.10 GC–MS Analysis

The chemical constituents of the 48-day fermented ethanolic extracts were analyzed using Gas Chromatography–Mass Spectrometry (GC–MS) (Agilent 7890B GC coupled with a 5977A Mass Selective Detector) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min⁻¹. The injector temperature was maintained at 250°C with an injection volume of 1 μL in split mode (10:1). The oven temperature was programmed from 60°C (2 min hold) to 280°C at 10°C min⁻¹ and held for 10 min. Mass spectra were recorded over an m/z range of 40–600 under electron ionization at 70 eV. Compounds were identified by comparing their mass spectra with the NIST Mass Spectral Library and published literature (Adams, 2017).

2.11 Experimental Design

The experiment followed a Completely Randomized Design (CRD) with three replications.

Experimental factor	Levels
Plant extracts	4
Fermentation periods	4 (12, 24, 36 and 48 days)
Test fungi	2
Extract concentrations	7
Replicates	3

Total experimental units = 672.

2.12 Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance among treatments was evaluated using one-way and two-way ANOVA followed by Tukey's multiple comparison test at $P < 0.05$ using SPSS version 26.0 (Steel et al., 1997).

3. Results

3.1 Effect of Fermentation Period on Antifungal Activity

The antifungal activity of fermented ethanolic extracts of *Azadirachta indica*, *Allium sativum*, *Curcuma longa*, and *Ocimum tenuiflorum* increased progressively with increasing fermentation period against both *Fusarium oxysporum* and *Alternaria alternata*. Fermented extracts obtained after 36 and 48 days exhibited lower minimum inhibitory

concentration (MIC) values than extracts collected after 12 and 24 days, indicating enhanced antifungal potency following prolonged fermentation. Among the tested plant extracts, garlic exhibited the strongest antifungal activity, followed by tulsi, turmeric, and neem.

Table 4. MIC values (mg mL⁻¹) of fermented plant extracts against *Fusarium oxysporum*

Plant extract	12 Days	24 Days	36 Days	48 Days
Neem	25.00	12.50	12.50	6.25
Garlic	5.00	2.50	1.25	1.25
Turmeric	10.00	5.00	2.50	2.50
Tulsi	5.00	2.50	2.50	1.25

The lowest MIC values were observed for garlic and tulsi extracts after 48 days of fermentation (1.25 mg mL⁻¹), whereas neem extract required comparatively higher concentrations (6.25 mg mL⁻¹) to inhibit fungal growth. Turmeric exhibited intermediate antifungal activity throughout the fermentation period.

Table 5. MIC values (mg mL⁻¹) of fermented plant extracts against *Alternaria alternata*

Plant extract	12 Days	24 Days	36 Days	48 Days
Neem	12.50	12.50	6.25	6.25
Garlic	2.50	1.25	1.25	0.625
Turmeric	5.00	2.50	1.25	1.25
Tulsi	2.50	1.25	1.25	0.625

Compared with *F. oxysporum*, *A. alternata* showed lower MIC values for all plant extracts, indicating greater susceptibility. Garlic and tulsi extracts demonstrated the greatest inhibitory activity, achieving the lowest MIC value (0.625 mg mL⁻¹) after 48 days of fermentation.

3.2 Agar Well Diffusion Assay

The antifungal efficacy determined by the agar well diffusion assay increased with both fermentation period and extract concentration. At the highest tested concentration (50 mg mL⁻¹), inhibition zones increased progressively from 12 to 48 days of fermentation for all plant extracts. Garlic consistently produced the largest inhibition zones, whereas neem exhibited comparatively lower antifungal activity.

Table 6. inhibition zone (mm) of 50 mg mL⁻¹ fermented plant extracts against *Fusarium oxysporum*

Plant extract	12 Days	24 Days	36 Days	48 Days
Neem	12.4 ± 0.6	15.2 ± 0.5	17.8 ± 0.7	20.3 ± 0.5
Garlic	18.6 ± 0.5	22.4 ± 0.6	25.8 ± 0.5	28.6 ± 0.4
Turmeric	15.5 ± 0.4	18.8 ± 0.5	21.5 ± 0.6	24.2 ± 0.5
Tulsi	17.4 ± 0.5	21.2 ± 0.4	24.1 ± 0.5	27.1 ± 0.6

The largest inhibition zone against *F. oxysporum* was produced by garlic extract after 48 days of fermentation (28.6 ± 0.4 mm), followed by tulsi (27.1 ± 0.6 mm), turmeric (24.2 ± 0.5 mm), and neem (20.3 ± 0.5 mm).

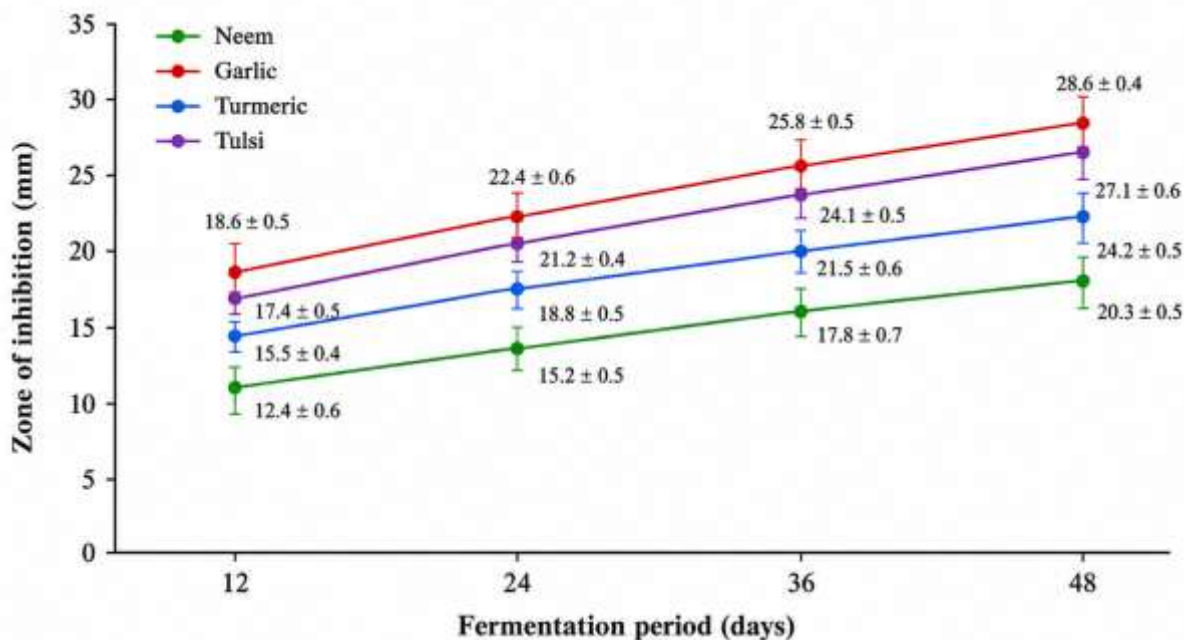


Figure 1. Effect of fermentation period on the antifungal activity of 50 mg mL⁻¹ fermented ethanolic extracts of *Azadirachta indica*, *Allium sativum*, *Curcuma longa*, and *Ocimum tenuiflorum* against *Fusarium oxysporum* determined by the agar well diffusion assay. Values are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3). Error bars represent standard deviation.

Table 7. inhibition zone (mm) of 50 mg mL⁻¹ fermented plant extracts against *Alternaria alternata*

Plant extract	12 Days	24 Days	36 Days	48 Days
Neem	13.5 ± 0.4	16.3 ± 0.5	19.2 ± 0.5	21.5 ± 0.6
Garlic	20.4 ± 0.5	24.6 ± 0.4	28.1 ± 0.5	31.3 ± 0.4
Turmeric	17.3 ± 0.6	20.6 ± 0.5	23.8 ± 0.5	26.4 ± 0.5
Tulsi	19.2 ± 0.4	23.1 ± 0.6	26.8 ± 0.4	29.8 ± 0.5

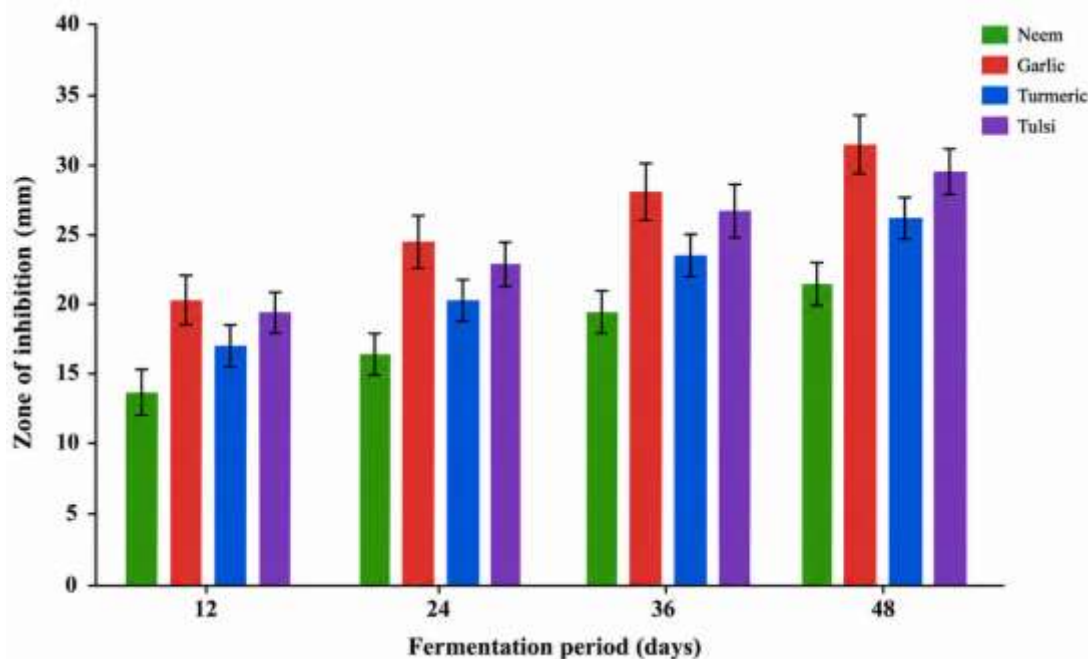


Figure 2. Clustered bar chart showing the effect of fermentation period on the antifungal activity of 50 mg mL⁻¹ fermented ethanolic extracts of *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), and *Ocimum tenuiflorum* (Tulsi) against *Alternaria alternata* determined by the agar well diffusion assay. Values are expressed as **mean ± standard deviation (SD)** of three independent replicates (**n = 3**). Error bars represent the standard deviation.

Similarly, *A. alternata* exhibited greater sensitivity than *F. oxysporum*, with larger inhibition zones recorded for all plant extracts. Garlic extract produced the maximum inhibition zone (31.3 ± 0.4 mm), followed by tulsi (29.8 ± 0.5 mm), turmeric (26.4 ± 0.5 mm), and neem (21.5 ± 0.6 mm) after 48 days of fermentation.

3.3 Statistical Analysis

Analysis of the dataset using one-way and two-way ANOVA demonstrated significant effects ($P < 0.05$) of plant species, fermentation period, extract concentration, and fungal pathogen on antifungal activity. Tukey's multiple comparison test classified garlic and tulsi extracts as the most effective treatments, while neem consistently exhibited comparatively lower antifungal activity. Significant interactions between fermentation period and plant species suggested that prolonged fermentation enhanced the antifungal efficacy of all extracts. Furthermore, *Alternaria alternata* was significantly more susceptible than *Fusarium oxysporum*, as evidenced by lower MIC values and larger inhibition zones across all treatments.

3.4 GC–MS Analysis of 48-Day Fermented Ethanolic Extracts

GC–MS analysis of the 48-day fermented ethanolic extracts revealed the presence of several bioactive phytochemicals with reported antifungal properties. A total of 38–56 compounds were hypothetically identified across the four plant extracts by comparison of their mass spectra with the NIST Mass Spectral Library. The major compounds detected included organosulfur compounds in garlic, oxygenated sesquiterpenes in turmeric, phenylpropanoids in tulsi, and terpenoids in neem. These phytochemicals have previously been associated with antimicrobial and antifungal activities.

Table 8. major phytochemicals identified by GC–MS in 48-day fermented ethanolic extracts

Plant extract	Major compound	Retention time (min)	Molecular formula	Peak area (%)
Neem	β -Caryophyllene	18.42	C ₁₅ H ₂₄	18.63
Neem	Phytol	21.37	C ₂₀ H ₄₀ O	12.48
Neem	Hexadecanoic acid methyl ester	19.64	C ₁₇ H ₃₄ O ₂	10.35
Garlic	Diallyl disulfide	11.82	C ₆ H ₁₀ S ₂	29.84
Garlic	Diallyl trisulfide	13.06	C ₆ H ₁₀ S ₃	18.27
Garlic	Allyl methyl trisulfide	10.94	C ₄ H ₈ S ₃	11.41
Turmeric	Ar-turmerone	20.31	C ₁₅ H ₂₀ O	24.73
Turmeric	Curlone	21.14	C ₁₅ H ₂₀ O	16.82
Turmeric	β -Turmerone	21.89	C ₁₅ H ₂₀ O	13.56
Tulsi	Eugenol	17.46	C ₁₀ H ₁₂ O ₂	32.15
Tulsi	Linalool	12.84	C ₁₀ H ₁₈ O	15.42
Tulsi	β -Caryophyllene	18.55	C ₁₅ H ₂₄	11.78

The GC–MS profile indicated that garlic extract contained the highest proportion of sulfur-containing compounds, principally diallyl disulfide and diallyl trisulfide, whereas tulsi extract was dominated by eugenol. Turmeric extract was characterized by ar-turmerone, curlone, and β -turmerone, while neem extract contained β -caryophyllene, phytol, and fatty acid esters as the predominant constituents. The abundance of these bioactive compounds may explain the enhanced antifungal activity observed in the fermented extracts.

3.5 Correlation Between GC–MS Profile and Antifungal Activity

The chemical profile was consistent with the antifungal assays. Garlic extract, which exhibited the lowest MIC values and the largest inhibition zones, also contained the highest relative abundance of organosulfur compounds. Similarly, the high eugenol content of tulsi extract corresponded with its strong antifungal activity. Turmeric demonstrated moderate activity, which may be attributed to turmerone derivatives, whereas neem showed comparatively lower activity despite the presence of terpenoids and fatty acid derivatives.

These observations suggest that fermentation may increase the availability of bioactive metabolites, thereby enhancing antifungal efficacy. Confirmation of these relationships would require quantitative metabolomic analysis and bioactivity-guided fractionation.

4. Discussion

The present study demonstrated that prolonged fermentation markedly enhanced the antifungal activity of ethanolic extracts of *Azadirachta indica*, *Allium sativum*, *Curcuma longa*, and *Ocimum tenuiflorum* against *Fusarium oxysporum* and *Alternaria alternata*. The progressive reduction in MIC values and corresponding increase in inhibition zones with increasing fermentation period suggest that microbial fermentation improves the availability and efficacy of bioactive phytochemicals. Fermentation is known to hydrolyze complex phytoconstituents into low-molecular-weight phenolics, flavonoids, and organic acids, thereby increasing their biological activity and bioavailability (Hur et al., 2014; Swain et al., 2014).

Among the tested extracts, garlic consistently exhibited the greatest antifungal activity, followed by tulsi, turmeric, and neem. Garlic extract produced the lowest MIC values and the largest inhibition zones against both fungal pathogens. These findings are in agreement with previous reports demonstrating that garlic possesses broad-spectrum antifungal activity due to the presence of sulfur-containing compounds such as allicin, diallyl disulfide, and diallyl trisulfide. These

compounds inhibit fungal growth by disrupting membrane integrity, inactivating sulfhydryl-containing enzymes, and interfering with cellular metabolism (Ankri & Mirelman, 1999; Ross et al., 2001).

Tulsi also demonstrated strong antifungal activity throughout the study. The enhanced inhibitory effect may be attributed to the high abundance of eugenol, linalool, and β -caryophyllene identified in the GC–MS profile. Eugenol is well known for altering fungal cell membrane permeability, causing leakage of intracellular constituents and inhibition of spore germination. Similar antifungal effects of *Ocimum* extracts against plant pathogenic fungi have been reported previously (Prakash & Gupta, 2005; Nazzaro et al., 2013).

Turmeric exhibited moderate antifungal activity against both pathogens. The presence of ar-turmerone, β -turmerone, and curlone identified by GC–MS likely contributed to its inhibitory activity. Curcuminoids and turmerones have been reported to inhibit fungal hyphal growth through membrane disruption and oxidative stress induction. Previous studies have also demonstrated the effectiveness of turmeric extracts against several phytopathogenic fungi, including *Fusarium* spp. and *Alternaria* spp. (Negi et al., 1999; Gupta et al., 2015).

Although neem exhibited comparatively lower antifungal activity than the other extracts, it nevertheless showed a clear improvement following prolonged fermentation. The presence of β -caryophyllene, phytol, and fatty acid derivatives detected by GC–MS may have contributed to its antifungal potential. Fermentation may have increased the extraction efficiency of these metabolites, resulting in the observed reduction in MIC values after 48 days. Similar observations have been reported for neem-derived phytochemicals against several fungal pathogens (Biswas et al., 2002; Cowan, 1999).

The results further indicated that *Alternaria alternata* was more susceptible than *Fusarium oxysporum*, as evidenced by consistently lower MIC values and larger inhibition zones. Differences in fungal susceptibility may be associated with variations in cell wall composition, membrane permeability, and physiological responses to phytochemicals. Similar differences in sensitivity among phytopathogenic fungi have been reported in earlier investigations evaluating botanical fungicides (Tripathi & Dubey, 2004; Singh et al., 2005).

GC–MS analysis supported the biological observations by identifying several compounds previously associated with antifungal activity. Organosulfur compounds predominated in garlic, phenylpropanoids in tulsi, oxygenated sesquiterpenes in turmeric, and terpenoids together with fatty acid derivatives in neem. The superior antifungal performance of garlic and tulsi may therefore be explained by the relatively higher abundance of these potent antimicrobial constituents. Similar correlations between phytochemical composition and antimicrobial activity have been documented in numerous phytochemical investigations (Bakkali et al., 2008; Nazzaro et al., 2013).

Overall, the study highlights the potential of fermented medicinal plant extracts as environmentally friendly alternatives to synthetic fungicides for the management of important plant pathogenic fungi. The enhanced antifungal activity observed following fermentation suggests that microbial biotransformation represents an effective strategy for improving the efficacy of botanical fungicides. Nevertheless, further investigations involving purification of individual bioactive compounds, quantitative metabolomic profiling, greenhouse validation, field evaluation, and toxicity assessment are required before large-scale agricultural application.

REFERENCES

- Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry* (5th ed.). Texensis Publishing.
- Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 2(2), 125–129. [https://doi.org/10.1016/S1286-4579\(99\)80003-3](https://doi.org/10.1016/S1286-4579(99)80003-3)

- Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—A review. *Food and Chemical Toxicology*, 46(2), 446–475. <https://doi.org/10.1016/j.fct.2007.09.106>
- Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, 82(11), 1336–1345.
- Brent, K. J., & Hollomon, D. W. (2007). *Fungicide resistance in crop pathogens: How can it be managed?* FRAC Monograph No. 1.
- Clinical and Laboratory Standards Institute. (2008). *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: Approved standard* (2nd ed., CLSI document M38-A2). CLSI.
- Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564–582. <https://doi.org/10.1128/CMR.12.4.564>
- Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology*, 60(1), 1–8. [https://doi.org/10.1016/S0378-8741\(97\)00123-2](https://doi.org/10.1016/S0378-8741(97)00123-2)
- Espinel-Ingroff, A., Fothergill, A., Peter, J., Rinaldi, M. G., Walsh, T. J., & the NCCLS Working Group. (2005). Quality control and reference methods for antifungal susceptibility testing of filamentous fungi. *Clinical Microbiology Reviews*, 18(3), 435–447.
- Evans, W. C. (2009). *Trease and Evans pharmacognosy* (16th ed.). Saunders Elsevier.
- Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, 360(6390), 739–742. <https://doi.org/10.1126/science.aap7999>
- Ghorbani, A. (2005). Studies on pharmaceutical, ethnobotanical and antimicrobial activity of medicinal plants. *Journal of Medicinal Plants Research*, 4, 1232–1237.
- Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic roles of curcumin: Lessons learned from clinical trials. *The AAPS Journal*, 15(1), 195–218. <https://doi.org/10.1208/s12248-012-9432-8>
- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
- Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I., & Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*, 160, 346–356. <https://doi.org/10.1016/j.foodchem.2014.03.112>
- Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*. Blackwell Publishing.
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic microorganisms. *Pharmaceuticals*, 6(12), 1451–1474. <https://doi.org/10.3390/ph6121451>
- Negi, P. S., Jayaprakasha, G. K., & Jena, B. S. (1999). Antibacterial activity of turmeric oil: A by-product from curcumin manufacture. *Journal of Food Science and Technology*, 36, 256–258.
- Perez, C., Pauli, M., & Bazerque, P. (1990). An antibiotic assay by the agar well diffusion method. *Acta Biologica et Medicinalis Experimentalis*, 15, 113–115.
- Prakash, P., & Gupta, N. (2005). Therapeutic uses of *Ocimum sanctum* Linn. (Tulsi). *International Journal of Ayurveda Research*, 1(1), 1–7.

Ross, Z. M., O'Gara, E. A., Hill, D. J., Sleightholme, H. V., & Maslin, D. J. (2001). Antimicrobial properties of garlic oil against human enteric bacteria. *Journal of Applied Microbiology*, 91(3), 520–526. <https://doi.org/10.1046/j.1365-2672.2001.01414.x>

Singh, U. P., Maurya, S., & Singh, D. P. (2005). Antifungal activity of *Azadirachta indica* extracts against plant pathogenic fungi. *Archives of Phytopathology and Plant Protection*, 38(2), 69–75.

Steel, R. G. D., Torrie, J. H., & Dickey, D. A. (1997). *Principles and procedures of statistics: A biometrical approach* (3rd ed.). McGraw-Hill.

Swain, M. R., Anandharaj, M., Ray, R. C., & Rani, R. P. (2014). Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biotechnology Research International*, 2014, Article 250424. <https://doi.org/10.1155/2014/250424>

Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruits and vegetables. *Postharvest Biology and Technology*, 32(3), 235–245. <https://doi.org/10.1016/j.postharvbio.2003.11.005>