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Original Research Article

INVESTIGATING THE ANTIFUNGAL POTENTIAL OF ESSENTIAL OILS FROM DIFFERENT PLANTS AGAINST BREAD MOLD CONTAMINANTS: A FAISALABAD, PAKISTAN PERSPECTIVE

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Abstract *Bread spoilage caused by molds poses a significant challenge to the bakery industry, resulting in financial losses for both producers and consumers due to microbial deterioration and waste. Traditional methods rely on chemical preservatives like propionic acid to extend bread shelf life, but there's growing consumer demand for natural alternatives. Essential oils (EOs) have garnered interest for their potential as natural preservatives, given their wide-ranging applications in food preservation and beyond. This study assessed the antifungal properties of EOs derived from Rosemary, Lemongrass, Clove, Cinnamon, Cardamom, and Garlic using agar well diffusion and micro-dilution methods. Samples of stale bread contaminated with various fungal species were tested, revealing that rosemary, clove, and cinnamon exhibited significant antifungal activity against common molds such as Mucor piriformis, Penicillium, Aspergillus, Alternaria, and Cladosporium possessing zones of inhibition ranging from 16±7 to 40±5. The mean MIC of rosemary, lemongrass, clove, and cinnamon against Mucor piriformis was 0.054, 0.032, 0.002, and 0.01µg respectively. 0.035, 0.033, 0.002, and 0.033µg were observed for A. fumigatus. Regarding A. flavus, the mean MIC values were 0.035, 0.003, 0.0023, and 0.009µg, while 0.037µg, 0.0023µg, 0.002µg, 0.022µg and 0.037µg, 0.032µg, 0.002µg, and 0.023µg were observed in P. chrysogenum and A. niger respectively. The MFC of the rosemary, lemongrass, clove, and cinnamon were: (M. piriformis: 0.016, 0.009, 0.002, and 0.004µg. A. fumigatus: 0.016, 0.0106, 0.0036, and 0.0043µg. A. flavus: 0.016, 0.014, 0.0043, and 0.0053µg. P. chrysogemum: 0.017, 0.014, 0.005, and 0.004µg. A. niger: 0.016, 0.014, 0.005, and 0.0043µg). While the tested EOs showed promising results in inhibiting fungal growth, further research is needed to fully understand their mechanisms of action and explore additional EO sources for potential application in the bakery industry.*

Keywords: *Essential Oils; Antifungal; Bread; Mold; Spoilage*

Introduction

Cereal-based products like bread play a vital role in human nutrition, with wheat (*Triticum aestivum* L) as the primary (Torri and Piochi, 2016). According to current guidelines, bread should be included in a healthy person's daily food intake (Torri and Piochi, 2016). The baking industry's scientific advancement focuses on enhancing bread and bakery goods to meet dietary balance needs, emphasizing high-protein bread production (Shatnyuk *et al*., 2012). However, bread's susceptibility to microbiological spoilage, particularly mold, poses challenges to its safety and quality, crucial for its widespread consumption (Chaplygina *et al.*, 2020).

Variations in bread composition arise from regional flora, climate, and seasonal changes. Elite bread, predominantly wheat or barley-based, undergoes leavening in ovens, with production methods significantly impacting final product safety and reliability (Chaplygina et al., 2020). Microbial spoilage, especially mold and fungus decay, significantly affects bread quality and economic viability (Garcia et al., 2019). Common molds such as Penicillium and Aspergillus contribute to spoilage, posing health risks and economic burdens (Melini & Melini, 2018). Preservative-free bread has a limited shelf life, typically 3-7 days, with mold contamination posing health risks and economic losses (Vasafi *et al*., 2019). Fungal infections during post-baking stages, such as cooling and slicing, further contribute to bread spoilage (Valková et al., 2021).

As consumers demand natural food products, essential oils (EOs) emerge as potential antimicrobial agents for food preservation, offering advantages over synthetic preservatives (Elshafie *et al*. 2022)The term "essential oils" (EOs) refers to the fragrant, liquid, flammable, colorless, volatile byproducts of secondary metabolites in plants primarily from spices and herbs that have applications in practically every aspect of life, notably traditional medicine, food flavoring, and preservation (Satyavani *et al*., 2015).

EOs' diverse biological activities, including antimicrobial properties, make them attractive for food industry applications (Mendonca *et al*., 2018). The study aims to identify and isolate fungal genera in stale bread, assess the antifungal properties of various plant EOs against these

[Citation: Ijaz, A., Naz, G., Majeed, K., Mughal, V.A., Amjad, K., Sarwar, A., Mahmood, S. (2024). Investigating the antifungal potential of essential oils from different plants against bread mold contaminants: a Faisalabad, Pakistan perspective. *Biol. Clin. Sci. Res. J.*, **2024***:900*. doi: https://doi.org/10.54112/bcsrj.v2024i1.900]

fungi, and determine the Minimum Fungicidal Concentration (MFC) and Minimum Inhibitory Concentration (MIC) of the EOs.

Materials and methods

Sample Collection

A total of 60 samples of stale bread were gathered. After that, the samples were brought to the postgraduate lab of the Department of Microbiology at Government College University Faisalabad.

Isolation of Fungi

Each sample was separately grown on Sabouraud dextrose agar (SDA). The sample was cut into small segments with a sterilized blade the small segment was then placed in the center of a petri plate containing Sabouraud dextrose agar (SDA) and then incubated for 2-3 days at 28ºC. Pure culture was obtained by sub-culturing different colonies that appear on SDA plates (Mahmoudi et al., 2018).

Identification of Fungi

The isolated fungi were further identified using morphological and cultural traits such as pigmentation, colony growth pattern, and colony features (colour, shape, size, and hyphae). Utilizing cotton blue lacto phenol stain, identification was accomplished (Bonneville et al., 2020).

Lacto phenol cotton blue staining

A drop of LPCB stain was combined with a tiny amount of a fungal colony on a glass microscope slide to create LPCB wet mounts. the mixture on a coverslip, and then examined under a microscope.

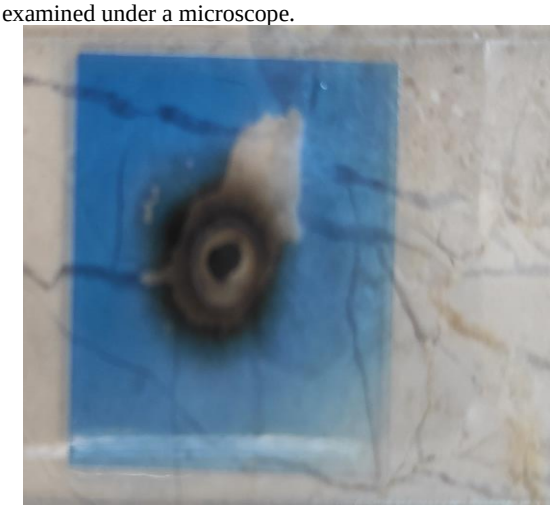

Figure 1: scotch tape method for fungal identification

Slide culture technique

Sterile microscope slide was taken. A small drop of molten SDA media was put then a drop of microbiological culture (fungal spore) was taken on molten media. A cover slip was placed on it with the help of forceps. The slide was set up in airtight chamber and placed two moist cotton balls in both the zone petri dishes and incubated for 2 to 3 days (Prakash & Bhargava, 2016).

Fungal spore suspension

Fungal spore inoculum was prepared for every fungal isolate in normal saline by following the instructions in Annexure V. (Queener and Capone1974). Screw-capped glass test tubes (10ml each) were filled with normal saline (0.9%) and sterilized in an autoclave. With the aid of an inoculation needle, fungal spores were extracted from each pure culture of a different fungus genus and placed in test tubes containing normal saline before being appropriately mixed by turning the tubes upside down. Using an upgraded Neubauer chamber, spore counts were performed (sterilized by ethanol and air-dried). A sterile micropipette was used to apply spore suspension (10µl) to the coverslip that had been placed on the chamber's engraved region. A standard spore inoculum (approx.106 spores /ml) was made for pure culture after fungal spores were counted at 40X.

Extraction of oils

The method for extracting the essential oils was as stated by Utegenova *et al* (2018). To extract the oils, air-dried plant components such as leaves, roots, tubers, fruiting bodies, and bars were used (Ahangari et al., 2021). For the extraction of their oils, several plants including Rosemary, Lemongrass, Clove, Cinnamon, Cardamom, and Garlic were used. A Clevenger-style apparatus and the standard hydro distillation method were used to extract the oils. Plant material was first completely submerged in water before being boiled. Water was utilized in the distillation process because it enveloped the plant material and prevented it from malfunctioning at temperatures below 100°C. The final step involved condensing steam and essential oil vapors into an aqueous fraction. Within one hour, the entire hydrodistillation process was completed.

Dimethyl sulfoxide (DMSO) was used for dilution to calculate their antifungal activity. One milliliter of DMSO and one milliliter of each essential oil were taken to prepare different oil solutions in test tubes (1:1 ratio). Oil stock solutions were kept for later processing.

Table 1: Essential oils used for antifungal activity against fungal species

Common name	Scientific name	Parts of plants used for oil extraction
Clove	*Syzygium aromaticum*	Dried flower buds
Cinnamon	*Cinnamomum verum*	Bark or leaves
Rosemary	*Salvia rosmarinus*	branches and leaves
Lemongrass	*Cymbopogon*	Leaves and stalks
Cardamom	*Elettaria cardamomum*	Seeds
Garlic	*Allium sativum*	Steams

Figure 2: Stock solution of EOs

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Agar Well diffusion method to assess plant Eos's antifungal activity

Using the good diffusion assay method published by Zamanian *et al*., (2021), the antifungal effectiveness of plant EOs against *Mucor piriformis, A. fumigatus, A. flavus, Penicillium chrysogenum,* and *A. niger* were evaluated. The tests were performed using SDA plates. Three to four wells per petri dish were made, each holding 100 L of each essential oil, on the surface of SDA agar plates, prepared for fungal cultures that were visually matched to a 0.5 McFarland standard were subcultured and disseminated using cotton swabs in Stock solution which was prepared in DMSO (Sharma *et al.* 2012). The incubation was done at 28 °C for 24-48 hours. The antifungal activity of essential oils was determined by measuring the diameter of zones of inhibition represented in mm of inhibition against tested strains.

Evaluation of Minimum Inhibitory Concentrations (MIC)

The approach was used to measure the minimal inhibitory concentration to determine the effectiveness of plant essential oils. The MIC procedure used the micro-dilution method (Afonso et al., 2021). In each of the 12 wells of a micro-titration plate, 50µl of Sabourad dextrose broth (SDB) was introduced. To preserve an equal proportion in each well, 50µl of the solution from the tenth well of the micro-titration plate was removed after each essential oil had been serially diluted two-fold up to the first swell. Up until the eleventh well, plates were injected with the fungal suspension. Due to the presence of bacteria and broth, the 11th well serves as a positive control, and the 12th well contains simply SDB as a negative control. The plates were properly sealed with their lids before being incubated at 37ºC for at least 24 hours. Results were noticed visually by observing the presence of spores.

Determination of minimal fungicidal concentration (MFC)

The minimal amount of the substance needed to stop microbial growth was discovered by MFC. For MFC, 50l of solution were taken from all microplate wells that displayed MIC as well as those that had no obvious fungal growth, and they were subcultured on sterile petri plates with SDA with the aid of a glass spreader (El Boumlasy *et al*., 2021). Agar plates were incubated for 28 hours for 2-3 days at 28°C. MFC status was assigned to plates that had no discernible growth.

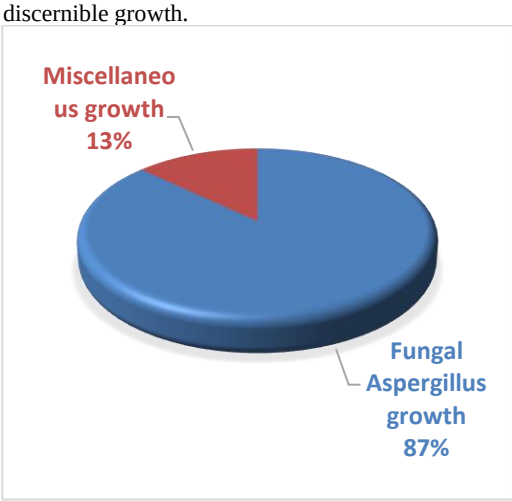

Figure 3: Occurrences of fungal species in bread

Results and discussion

Occurrence of Fungal Species in Moldy Bread

Out of 60 moldy bread samples collected from different bakeries, 52 were shown for fungal growth including *Mucor piriformis, A. fumigatus, A. flavus, A. niger, Penicillum chrysogenum,* and a few species of *Alternaria*. While the other 8 samples showed *miscellaneous* growth.

Morphology characteristics of fungal species

A. fumigatus colonies are often blue-green with suede-like surfaces, while Mucor piriformis colonies are floccose, pale greyish-brown. A. flavus colonies are powdery masses with reddish gold on the lower surface and yellowish-green spores on the upper surface. The surface of *Penicillum chrysogenum* is smooth, while the conidiophores and spores of *A. niger* are smooth and colourless.

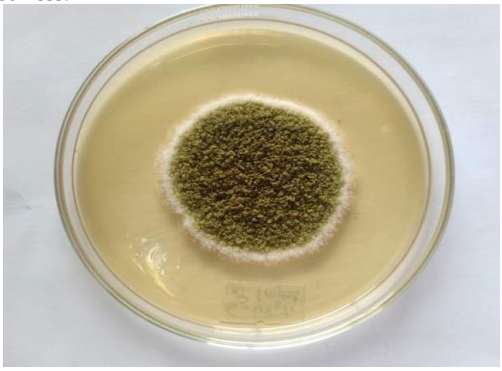

Figure 4: Colony morphology of *Aspergillus fumigatus*

Figure 5: Colony morphology of *Aspergillus flavus*

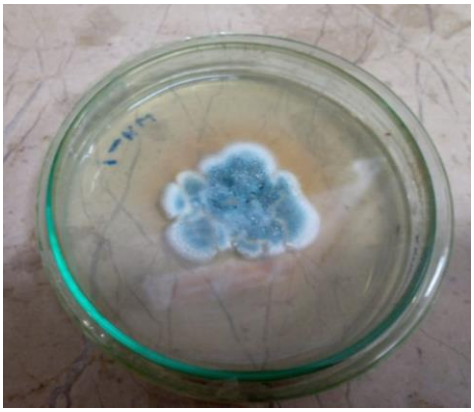

Figure 6: Morphology characterization of *Penicillium chyrosegenum*

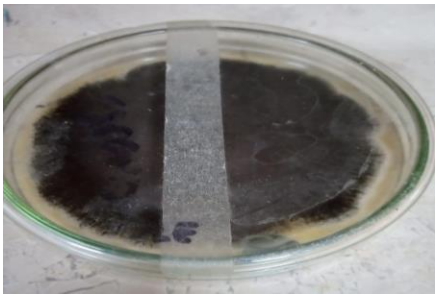

Figure 7: Morphology characterization of *Aspergillus niger*

Figure 8: Morphological characterizations of *Mucor piriformis*

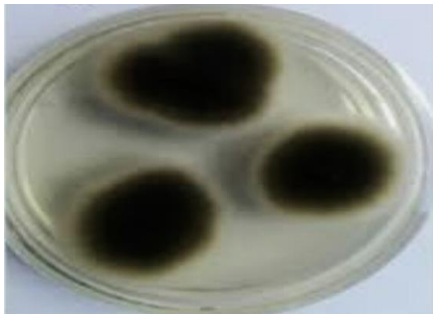

Figure 9: Morphology observation of *Alterinaria*

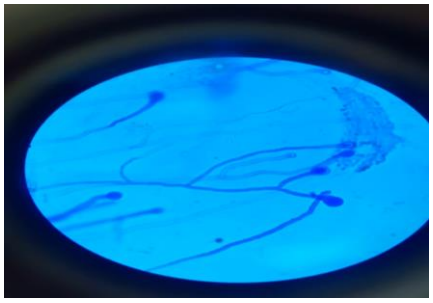

Figure 10: Microscopic examinations of *Mucor piriformis*

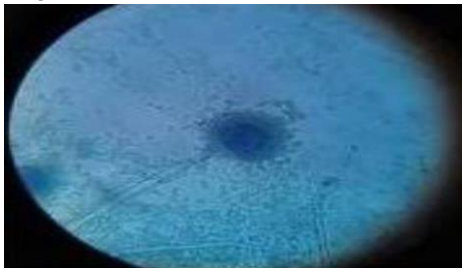

Figure 11: Microscopic examinations of *Aspergillus flavus*

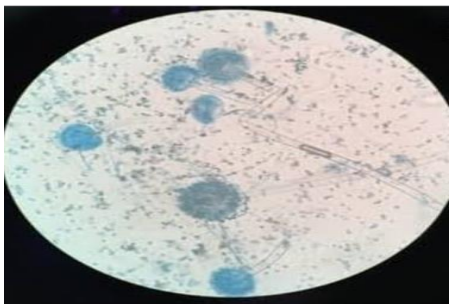

Figure 12: Microscopic examination of *Aspergillus niger*

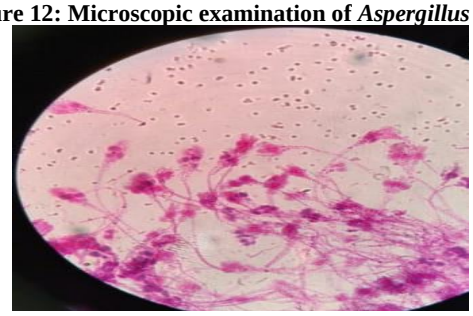

Figure 13: Microscopic examination of *Penicillium chrysogenum*

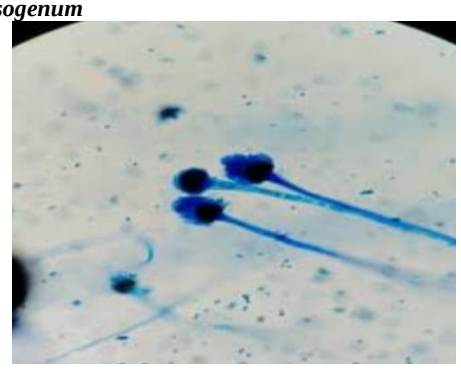

Figure 14: Microscopic examinations of *Aspergillus fumigatus*

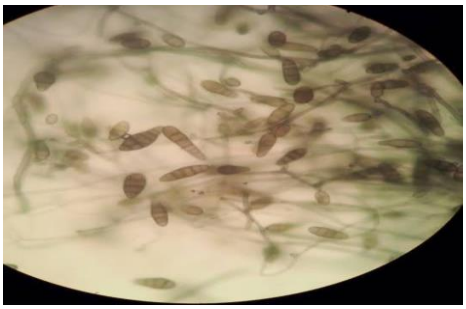

Figure 15: Microscopic examinations of *Alterniaria*

Microscopic examination of fungal species

After performing scotch tape method and slide culture techniques microscopic examination were performed at 10x and 40x. Five fungal species were identified including *Mucor piriformis, A. fumigatus, A. flavus, Penicillium chrysogenum,* and *A. niger*.

Prevalence of fungal species

The prevalence of Mucor, Fumigatus, Flavus, Penicillium, and Niger are shown in the following table. Out of 52, 19(37%) were positive for Mucor piriformis, 8(15%) were positive for A. fumigatus, 9(17%) were confirmed for A. flavus, 7(13%) were positive for Penicillium chrysogenum

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6(12%) were positive for A. niger while 3(6%) were positive for Alternaria.

Table 2: Positive prevalence of different fungal species from mold bread

Total no positive isolates	*Mucor piriformis*	*A. niger*	*A. fumigatus*	*Penicillium chrysogenum*	*A. flavus*	*Alternaria*
52	19(37%)	6(12%)	8(15%)	7(13%)	9(17%)	3(6%)

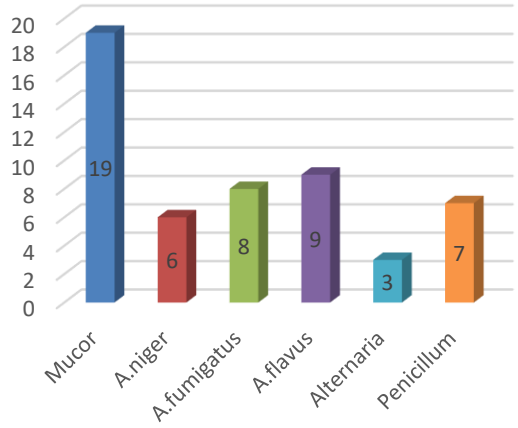

Figure 16: Prevalence of fungal species

Investigation of antimicrobial potential of EOs by Agar well diffusion method

By using an agar well diffusion assay, the antimicrobial potential of 6 different essential oils against fungal species including *Mucor prirformis*, *A. fumigatus*, *Penicillium chrysogenum*, *A. flavus*, and *A. niger* were assessed. Out of 6 oils, only four oils showed their effectiveness against 5 fungal species. Clove, cinnamon, lemongrass, and rosemary essential oils showed zones of inhibition while garlic and cardamom oils had no zone of inhibition. The diameter of zones of inhibition about each well containing essential oil has been presented in Table. The zone of inhibition against fungal species had been shown.

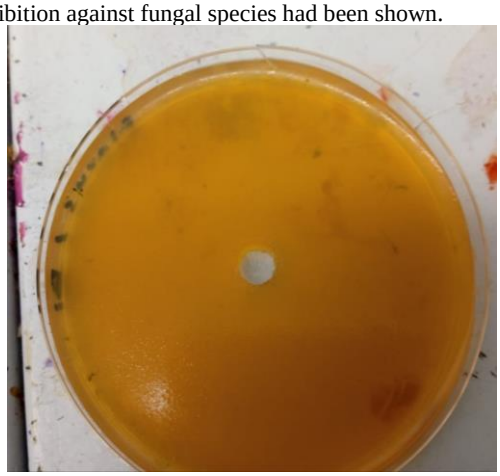

Figure 17: Antifungal potential of EOs by agar well diffusion method

Antifungal Activity of the EOs Against *M. piriformis*

Table 3 below shows the antifungal activity of the Eos against *M. piriformis*. Essential Oil of rosemary showed the highest zone of inhibition which was 35±5 while cinnamon EO showed the lowest zone of inhibition, 20±5. Garlic and Cardamon showed no zone of inhibition.

Table 3: **Antifungal Activity of the Eos Against *M. piriformis***

Fungal specie	*Essential oils*	*Zone of inhibition*	*Mean ZOI± S.D*
Mucor piriformis	Rosemary	40	35±5
		35	
		30	
	Lemongrass	25	23.33±2.88
		20	
		25	
	Clove	30	30±5
		25	
		35	
	Cinnamon	20	20±5
		15	
		25	
	Cardamon	NZ	Nill
	Garlic	NZ	Nill

Antifungal Activity of the EOs Against *A. Fumigatus*

Table 4 below shows the antifungal activity of the Eos against *A. fumigatus*. Essential Oil of rosemary showed the highest zone of inhibition which was 35±5 while lemongrass EO showed the lowest zone of inhibition, 20±5. Garlic and Cardamon showed no zone of inhibition.

Table 4: Antifungal Activity of the EOs Against *A. Fumigatus*

Fungal specie	*Essential oil*	*Zone of inhibition*	*Mean ZOI ± S. D*
Aspergillus fumigatus	Rosemary	30	35±5
		35	
		40	
	Lemongrass	20	20±5
		25	
		15	
	Clove	35	26.66±7.64
		20	
		25	
	Cinnamon	15	23.33±7.64
		30	
		25	
	Cardamon	NZ	Nill
	Garlic	NZ	Nill

Antifungal Activity of the EOs Against *A. flavus*

Table 5 below shows the antifungal activity of the Eos against *A. flavus*. Essential Oil of clove showed the highest zone of inhibition which was 35±5 while cinnamon, and lemomgrass EOs showed the lowest zone of inhibition, 20±5. Garlic and Cardamon showed no zone of inhibition.

Table 5: Antifungal Activity of the EOs Against *A. flavus*

Fungal specie	*Essential oils*	*Zone of inhibition*	*Mean ZOI± S.D*
Aspergillus flavus	Rosemary	20	30±13.22
		25	
		45	
	Lemongrass	15	20±5
		20	
		25	
	Clove	30	35±5
		35	
		40	
	Cinnamon	15	20±5
		20	
		25	
	Cardamom	NZ	Nill
	Garlic	NZ	Nill

Antifungal Activity of the EOs Against *Penicillium chrysogenum*

Table 6 below shows the antifungal activity of the Eos against *P. chrysogenum*. Essential Oil of rosemary showed the highest zone of inhibition which was 40±5 while cinnamon EO showed the lowest zone of inhibition, 16.66±7.64. Garlic and Cardamon showed no zone of inhibition.

Table 6: Antifungal Activity of the EOs Against *Penicillium chrysogenum*

Fungal specie	*Essential oils*	*Zone of inhibition*	*Mean ZOI ± S.D*
Penicillium chrysogenum	Rosemary	40	40±5
		45	
		35	
	Lemongrass	15	21.66±5.77
		25	
		25	
	Clove	30	25±5
		25	
		20	
	Cinnamon	15	16.66±7.64
		10	
		25	
	Cardamon	NZ	Nill
	Garlic	NZ	Nill

Antifungal Activity of the EOs Against *A. niger*

Table 7 below shows the antifungal activity of the Eos against *A. niger*. Essential Oil of rosemary showed the highest zone of inhibition which was 38.33±7.64 while lemongrass EO showed the lowest zone of inhibition, 16.66±7.64. Garlic and Cardamon showed no zone of inhibition.

Evaluation of Minimum Inhibitory Concentration of Different Plant EOs

By using broth micro-dilution method MIC of plant EOs was performed. Only those oils were used in MIC which showed effectiveness in the form of zone of inhibition against 5 fungal isolates. Clove, cinnamon, lemongrass, and rosemary showed a zone of inhibition so their MIC was performed. MICs were illustrated by broth micro-dilution method by use of 96 well microtitration plates as shown in (Figure 19). Initially as well as after 24 hours of incubation plates were observed for absorbance measurement at an optical density (OD) of 620 nm under microplate reader. Inhibition of fungal growth was observed in starting wells in the form of less absorbance, which increased gradually in the last wells due to fungal growth. Maximum MIC value of MIC was demonstrated as showed in Table 4. Graphically MICs of *Mucor pirformisr*, *A. fumigatus*, *A. flavus*, *Penicillium chrysogenum*, and *A. niger* isolates showed in figure 20

Table 7: Antifungal Activity of the EOs Against *A. niger*

Fungal specie	*Essential oils*	*Zone of inhibition*	*Mean ZOI ± S.D*
Aspergillus niger	Rosemary	45	38.33±7.64
		40	
		30	
	Lemongrass	10	16.66±7.64
		15	
		25	
	Clove	25	23.33±2.89
		20	
		25	
	Cinnamon	10	18.33±10.40
		15	
		30	
	Cardamon	NZ	Nill
	Garlic	NZ	Nill

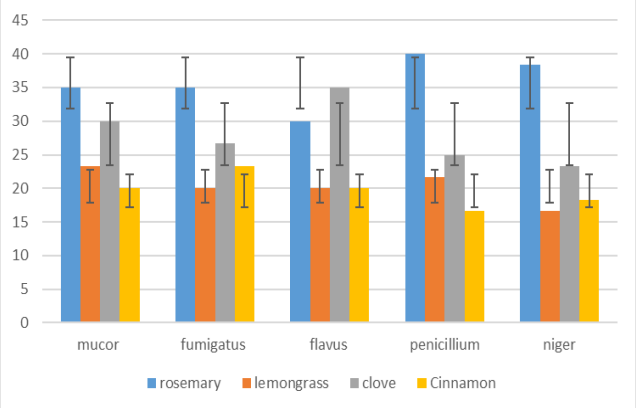

Figure 18: graphically representation of zone of inhibition (mm)

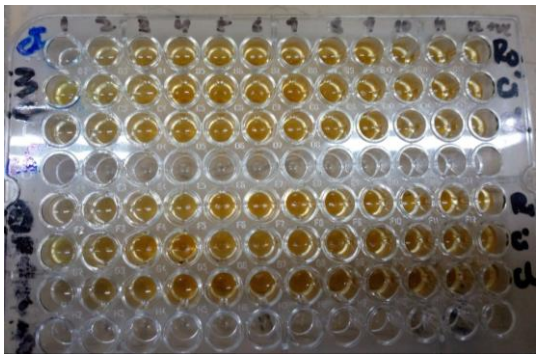

Figure 19: MIC observation of different EOs

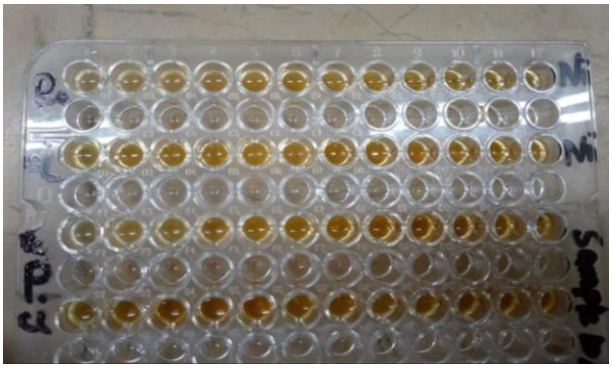

Figure 20: Observation of different EOs

MIC (µg/ml) of plant EOs for the isolate of *Mucor piriformis*

Table 8 below shows the minimum inhibitory concentration of the Eos against *M. piriformis.* Essential Oil of rosemary showed the highest MIC which was 0.054±0.019 while cinnamon EO showed the lowest MIC, 0.010±0.0046

Table 8: MIC (µg/ml) of plant EOs for the isolate of *Mucor piriformis*

Fungal specie	*Essential oils*	*MIC (µg/ml)*	*Mean + S.D*
Mucor piriformis	Rosemary	0.032	0.054±0.019
		0.065	
		0.065	
	Lemongrass	0.031	0.032±0.0315
		0.064	
		0.001	
	Clove	0.001	0.0023±0.0015
		0.002	
		0.004	
	Cinnamon	0.008	0.010±0.0046
		0.016	
		0.008	

MIC (µg/ml) of plant EOs Against *A. fumigatus*

Table 9 below shows the minimum inhibitory concentration of the Eos against *A. fumigatus.* Essential Oil of rosemary showed the highest MIC which was 0.035±0.0286 while clove EO showed the lowest MIC, 0.0023±0.00152.

Table 9: MIC (µg/ml) of plant EOs for the isolate of *A. fumigatus*

Fungal specie	*Essential oils*	*MIC (µg/ml)*	*Mean ± S.D*
Aspergillus fumigatus	Rosemary	0.008	0.035±0.0286
		0.032	
		0.065	
	Lemongrass	0.001	0.033±0.0325
		0.066	
		0.032	
	Clove	0.001	0.0023±0.00152
		0.004	
		0.002	
	Cinnamon	0.002	0.033±0.0315
		0.065	
		0.032	

MIC (µg/ml) of plant EOs Against *A. flavus*

Table 10 below shows the minimum inhibitory concentration of the Eos against *A. flavus.* Essential Oil of rosemary showed the highest MIC which was 0.035±0.0286 while clove EO showed the lowest MIC, 0.0023±0.00152.

Table 10: MIC (µg/ml) of plant EOs Against *A. flavus*

Fungal specie	*Essential oils*	*MIC (µg/ml)*	*Mean + S.D*
Aspergillus flavus	Rosemary	0.032	0.035±0.0286
		0.008	
		0.065	
	Lemongrass	0.006	0.0033±0.0025
		0.003	
		0.001	
	Clove	0.001	0.0023±0.00152
		0.004	
		0.002	
	Cinnamon	0.016	0.0093±0.0061
		0.008	
		0.004	

MIC (µg/ml) of plant EOs Against *Penicillium chrysogenum*

Table 11 below shows the minimum inhibitory concentration of the Eos against *P. chrysogenum.* Essential Oil of rosemary showed the highest MIC which was 0.037±0.0249 while clove EO showed the lowest MIC, 0.002±0.00173.

Table 11: MIC (µg/ml) of plant EOs Against *Penicillium chrysogenum*

Fungal specie	*Essential oils*	*MIC (µg/ml)*	*Mean + S.D*
Penicillium chrysogenum	Rosemary	0.065	0.037±0.0249
		0.032	
		0.016	
	Lemongrass	0.004	0.0023±0.0015
		0.001	
		0.002	
	Clove	0.001	0.002±0.00173
		0.004	
		0.001	
	Cinnamon	0.002	0.022±0.0366
		0.001	
		0.065	

MIC (µg/ml) of plant EOs Against *A. niger*

Table 12 below shows the minimum inhibitory concentration of the Eos against *A. niger*. Essential Oil of rosemary showed the highest MIC which was 0.037±0.024 while clove EO showed the lowest MIC, 0.0026±0.0020

Table 12: MIC (µg/ml) of plant EOs Against *A. niger*

Fungal specie	*Essential oils*	*MIC(µg/ml)*	*Mean± S.D*
	Rosemary	0.064	0.037±0.024
		0.031	
		0.017	
	Lemongrass	0.032	0.0323±0.0305
		0.063	
		0.002	
	Clove	0.001	0.0026±0.0020
		0.005	
		0.002	

Aspergilllus niger	Cinnamon	0.001	0.023±0.0375
		0.003	
		0.067	

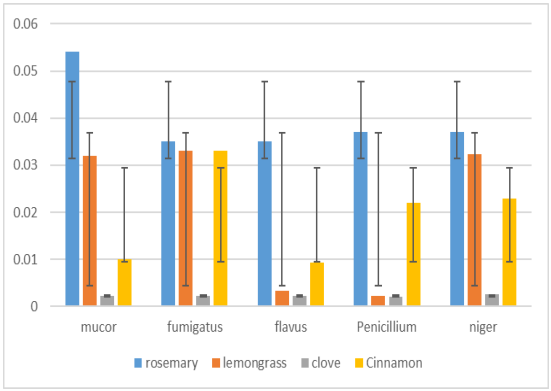

Figure 21: Graphical representations of MICs of plant EOs

Evaluation of minimal fungicidal concentration (MFC) of the different plant EOs.

For its evaluation, the samples were taken from the MIC well and above wells. MFC values were evaluated by the absence of growth of fungus on agar plates. Plates that showed no visible growth after 24 hours were considered MFC as shown in Figure 20, 4.21, and 22 respectively. MFC values of rosemary were found 0.016±0.002, clove showed 0.002±0.001, Cinnamon showed 0.004±0.001 as shown in Table 12 to 16 Graphically MFCs of fungal species.

Figure 22: Evaluation of rosemary by MFC

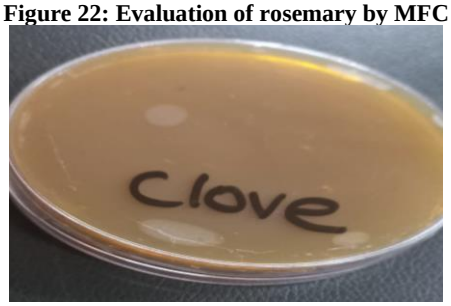

Figure 23: Evaluation of Clove by MFC

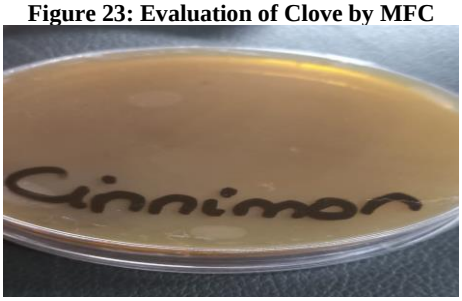

Figure 24: Evaluation of Cinnamon by MFC

MFC (µg/ml) of different plant EOs Against *Mucor piriformis*

Table 13 below shows the minimum fungicidal concentration of the EOs against *M. piriformis.* Essential Oil of rosemary showed the highest MFC which was 0.016±0.002 while clove EO showed the lowest MFC, 0.002±0.001

Table 13: MFC (µg/ml) of different plant Eos Against *Mucor piriformis*

Fungal specie	*Essential oils*	*MFC(µg/ml)*	*Mean ± SD*
Mucor piriformis	Rosemary	0.016	0.016±0.002
		0.018	
		0.014	
	Lemongrass	0.013	0.0096±0.0049
		0.004	
		0.012	
	Clove	0.002	0.002±0.001
		0.001	
		0.003	
	Cinnamon	0.004	0.004±0.001
		0.005	
		0.003	

MFC (µg/ml) of Different Plant EOs Against *A. fumigatus*

Table 14 below shows the minimum fungicidal concentration of the EOs against *A. fumigatus.* Essential Oil of rosemary showed the highest MFC which was 0.016±0.001 while clove EO showed the lowest MFC, 0.0036±0.002.

Table 14: MFC (µg/ml) of different plant EOs for the isolate of *A. fumigatus*

Fungal specie	*Essential oils*	*MFC(µg/ml)*	*Mean ± SD*
Aspergillus fumigatus	Rosemary	0.015	0.016±0.001
		0.017	
		0.016	
	Lemongrass	0.013	0.0106±0.0049
		0.005	
		0.014	
	Clove	0.002	0.0036±0.002
		0.003	
		0.006	
	Cinnamon	0.006	0.0043±0.002
		0.005	
		0.002	

MFC (µg/ml) of Different Plant EOs Against *A. flavus*

Table 15 below shows the minimum fungicidal concentration of the EOs against *A. flavus.* Essential Oil of rosemary showed the highest MFC which was 0.016±0.001 while clove EO showed the lowest MFC, 0.0043±0.002.

Table 15: MFC (µg/ml) of different plant Eos Against *A. flavus*

Fungal specie	*Essential oils*	*MFC(µg/ml)*	*Mean± SD*
Aspergillus flavus	Rosemary	0.016	0.016±0.001
		0.015	
		0.017	
	Lemongrass	0.015	0.014±0.001
		0.014	
		0.013	
	Clove	0.002	0.0043±0.002
		0.005	
		0.006	
	Cinnamon	0.005	0.0053±0.0015
		0.007	
		0.004	

MFC (µg/ml) of Different plant EOs Against *P. chrysogenum*

Table 16 below shows the minimum fungicidal concentration of the EOs against *P. chrysogenum.* Essential Oil of rosemary showed the highest MFC which was 0.017±0.001 while cinnamon EO showed the lowest MFC, 0.004±0.002.

Table 16: **MFC (µg/ml) of different plant Eos Against *P. chrysogenum***

Fungal specie	*Essential oils*	*MFC(µg/ml)*	*Mean ± SD*
Penicillium chrysogenum	Rosemary	0.016	0.017±0.001
		0.018	
		0.017	
	Lemongrass	0.016	0.0146±0.0015
		0.015	
		0.013	
	Clove	0.004	0.005±0.001
		0.005	
		0.006	
	Cinnamon	0.004	0.004±0.002
		0.002	
		0.006	

MFC (µg/ml) of Different Plant EOs Against *A. niger*

Table 17 below shows the minimum fungicidal concentration of the EOs against *A. niger*. Essential Oil of rosemary showed the highest MFC which was 0.016±0.001 while clove EO showed the lowest MFC, 0.005±0.002.

Table 17: MFC (µg/ml) of different plant EOs Against *A. niger*

Fungal specie	*Essential oils*	*MFC(µg/ml)*	*Mean ± SD*
Aspergillus niger	Rosemary	0.015	0.016±0.001
		0.017	
		0.016	
	Lemongrass	0.017	0.014±0.003
		0.014	
		0.011	
	Clove	0.003	0.005±0.002
		0.006	
		0.007	
	Cinnamon	0.004	0.0043±0.0015
		0.003	
		0.006	

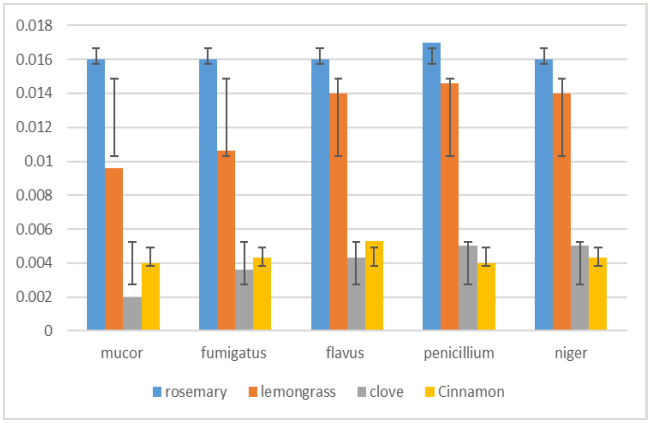

Figure 25: Graphical representations of MFCs of plant EOs for different fungal species

Discussion

The use of such preservatives is necessary to provide such things with the right shelf life under environmental processing settings since bread fungal deterioration is a recurrent problem in the food sector (Darughe *et al*., 2012). In bakery processes, mold formation is the most common type of microbial decomposition and commonly affects shelf-life. Many plant extracts and oils have been used as topical antiseptics or have been touted as having antibacterial properties in the past. (Darughe *et al*., 2012). The use of some synthetic food additives is restricted by the food industry and regulatory organizations, which has rekindled interest in finding natural antibacterial chemicals, particularly those originating from plants, as alternatives (Hu et al., 2019). EOs have gained a lot of notoriety recently for their all-natural, risk-free healing abilities. Recent years have seen a lot of work put into developing new effective coating preparation methods on pectin that can be used to protect fresh foods, enhance their nutritional and organoleptic qualities, and extend their shelf lives (Hu *et al*., 2019).

In the present findings, Out of 60 moldy samples collected, 52(87%) showed fungal growth. Similar results were observed by Amirthalingam *et al*., (2022), who reported 82% confirmed growth of fungus from bread in different samples. Regarding the prevalence of the fungal occurrence obtained, similar findings showed the 30% frequency of *Aspergillus niger* while *Fusarium avenaceum* (26%), *Aspergillus fumigatus* (13%), *Mucor piriformis* (10%), *Fusarium solani* (8%), *Aspergillus flavus* (5%), *Penicillium digitatum* (4%) and *Rhizopus stolonifer* (4%) by Ogbonna David & Udo, (2015). Another study by Sadeghi Dehkordi *et al*., (2017) also reported similar frequency of the identified species as follows: *Penicillium* spp. (28%), *Aspergillus flavus* (16%), *Aspergillus fumigatus* (16%), *Cladosporium* spp. (12%), *Rhizopus* spp. (12%), *Mucor* spp. (8%) and *Ulocladium* spp. (8%). In

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both studies, Aspergillus species possessed the highest frequency which is contrary to the findings of Sudawa *et al*., (2022).

Furthermore, the antibacterial activity of different EOs of plants (like rosemary, clove, oregano, lemongrass, cinnamon plant, ginger, and from the plant cardamon) was evaluated in this study. These plant essential oils were prepared by a Clevenger apparatus following a series of steps. Similar to this method, Mollaei *et al*., (2019) extracted different plant oils. In another study by Wang *et al*., (2018), Essential oils from the white and black papers were obtained by hydro distillation method. Our extraction method was also similar to the method of Fagbemi *et al*., (2021) and Wang& Zhang, (2020), they extracted the essential oils by collecting leaves of *Luodian Blumea balsamifera* from different harvest times in China.

The response of rosemary, lemongrass, clove, cardamom, garlic, and cinnamon essential oils observed in this study are in consistent with that of Nyamath & Karthikeyan, (2018), who evaluated the antifungal activity by using different concentrations of lemongrass and its leaves extract by using agar well diffusion assay.

The mean the zone of inhibition of rosemary oil (35mm), lemongrass (23.33mm) clove (30mm), and cinnamon (20mm), were slightly lower than the findings of Fontenelle *et al*., (2008) and slightly higher than that of Walia *et al*., (2020).

Similarly, the MICs of the EOs observed in the present study conform with those of De Toledo *et al* (2016) who studied the MIC of eucalyptus plant extracts against fungal growth. In a study that contradicts the current investigation, Hossain *et al*. (2016) found that eucalyptus and mandarin showed the least efficacy because they were unable to stop any fungal development. However, according to Wang *et al*. (2018), four EOs from *Cinnamomum cassia Presl*, *Litsea cubeba*, *Cymbopogon martini*, and *Thymus mongolicus Ronn* all exhibited higher inhibitory effects and lower minimal inhibitory concentrations (MICs) against the three different types of fungus.

Moreover, the MFCs obtained in the current study are in contrast to the findings of Saxena *et al*., (2012), who observed the MFC pf 1.55 µl /ml against yeast. The EOs of *Zataria multiflora* (165.4 and 88.9 g/ml), *Cuminum cyminum* (159 and 185.3 g/ml), *Foeniculum vulgare* (496.4 and 532.9 g/ml), Pinaceae (869.7 and 852.43 g/ml), and *Heracleum pers* (869.7 and 852.43 g/ml) completely block all of the non-toxigenic and poisonous (2010).

Conclusion

Overall study concluded that extract of plant EOs of rosemary; lemongrass, clove, and cinnamon have wonderful potential for the five different fungal species. The use of plants in a way of plant extract is a grateful and effective pathway as these provide good antifungal potential in an environmentally friendly and cost-effective manner. It is significant to utilize EOs as a viable option antifungal agent and can be used as preservatives in the bakery industry to increase bread shelf life. Plant essential oils of rosemary, lemongrass, clove, and cinnamon can be extracted easily and might be used as safer medicinal options for human beings for treating fungal infections The potential components of its antifungal activity require more investigation.

RECOMMENDATIONS

It is recommended that further research is required to determine the antibacterial activity of the essential oils used in this research to explore their potential in preventing some bacterial contaminants of food and beverages.

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Authors Contribution

All authors contributed equally.