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ORIGINAL PAPER

Antifungal Activity of Essential Oils of *Ocimum gratissimum* and *Cymbopogon citratus* Leaves Against Post–harvest Fruit Rot of *Citrus sinensis*

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ABSTRACT

Citrus sinensis fruit is susceptible to post-harvest fungal rots. Eight fungal isolates were isolated from diseased *Citrus sinensis* fruits ,they were identified via Sanger sequencing with *Lasiodiplodia theobromae* and *Aspergillus flavus* showing high pathogenicity (≥75%), hence most virulent causing post-harvest rot in *Citrus sinensis*. This study investigated the effectiveness of essential oils from *Ocimum gratissimum* and *Cymbopogon citratus* leaves against *Lasiodiplodia theobromae* and *Aspergillus flavus*. In vitro assays showed a dose-dependent antifungal effect. *Cymbopogon citratus* at 1.00 µg/ml exhibited the highest inhibition zones against *Lasiodiplodia theobromae* (86.10±0.11mm) and *Aspergillus flavus* (62.11±0.04mm), while *Ocimum gratissimum* caused inhibition zones of 72.18±1.90mm and 24.35±0.56mm, respectively. Both oils outperformed mancozeb at 2.00 µg/ml (25.33±0.58mm). The antifungal activity was linked to key compounds such as eugenol (7.73%), linalool(12.34%), nerolidiol 2 (5.28%) and beta.-isabolene(5.11%), cyclohexane (4.99%), caryophyllene (3.31%) in Ocimum gratissimum, and geranial(39.05%), neral(28.20%), geraniol(6.33%), limonene(5.08%), geranylacetate(2.46%), isogeranial(1.49%), limonene(0.83%) in *Cymbopogon citratus*. Gas chromatography-mass spectrometry (GC-MS) analysis characterized the essential oils, revealing bioactive compounds. The findings suggest that these plant-derived essential oils can serve as effective bio-fungicides for controlling post-harvest rots in *Citrus sinensis*, promoting sustainable fruit production.

Key words: *Citrus sinensis*; Essential oil; Ecofriendly; Post-harvest; Rot fungi

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INTRODUCTION

Citrus sinensis (L.) Osbeck (sweet orange), belonging to Rutaceae family, is an important fruit crop grown in several regions of the world notably in Nigeria due to its nutritional value and socio-economic importance (Olife *et al*., 2015). It is beneficial to health and contributes to the prevention of degenerative processes, particularly lowering incidence of degenerative process, cardio and cerebrovascular diseases (Uwidia *et al*., 2020). These benefits have been attributed to the various antioxidant phytonutrients especially ascorbic and folic acids (Ezejiofor *et al*., 2011 and Obidi *et al*., 2013). Aside being a significant source of Vitamin C and other vital nutrients, Citrus

contributes to overall health and well-being, potentially reducing healthcare costs. It also provides jobs in cultivation, harvesting, processing, packaging, and transportation (Zhong and Nicolosi, 2020).

Mechanical damage during harvest, microbial pathogens infection, poor storage, and post-harvest handling are major causes of post-harvest losses that threaten fruit production. According to Singh and Sharma (2018), microbial pathogens responsible for post-harvest fruit spoilage are diverse and yield loss attributed to fungal infection reaches 40-50 % in developing and undeveloped countries. To date, the management of post-harvest rot has been effective through the use of conventional fungicides but, with several limitations which includes fungicide resistance in fungal pathogens, environmental and human health concerns related to chemical use, possible harm to non-target organisms, impact on crop physiology and fruit quality; increased risk of high-level toxic residues in food products. These issues prompted researchers to explore alternative approaches (Singh *et al*.,2021; Strano *et al*.,2022 and Rhouma *et al*., 2023). These concerns about environmental and human health impacts of chemical fungicides have led to increased interest in the development of natural alternatives.

In vitro *e*valuation of the efficacy of some essential oils in controlling common post-harvest fungi has been reported by Kontaxakis *et al*. (2020) and Remolif *et al*. (2024). Some tropical aromatic plants namely *Azadirachta indica* (neem), *Ocimum gratissimum* and *Cymbopogon citratus*, have shown exert high antimicrobial activities against *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus niger* and other molds and since they are natural products, mostly consumed by man, there is little or no fear of poisoning even at very high concentrations (Adegoke *et al*., 2010). The present study aimed at assessing the potency of the essential oil from *Ocimum gratissimum* and *Cymbopogon citratus* leaves on two post-harvest rot fungi isolates of *Citrus sinensis* fruit.

MATERIALS AND METHODS

This study was carried out in the Environmental Biology Laboratory, Department of Biological Sciences, Yaba College of Technology, Yaba, Lagos State, Nigeria. The institution is located on geographical coordinates of approximately 6^{o}37’1.9”N latitude and 3°19’12.0”E longitude

Collection of Plant materials

Samples of fresh, healthy and diseased *Citrus sinensis* fruits, *Ocimum gratissimum* and *Cymbopogon citratus* leaves were obtained from three locations in Ketu market, at Kosofe Local Government Area of Lagos State. The materials were properly authenticated at the Department of Botany herbarium, University of Lagos, and assigned voucher numbers- LUH 100392(Citrus sinensis fruits), LUH6854(*Ocimum gratissimum)* and LUH 100392(*Cymbopogon citratus*)

Isolation and Identification of Fungi

The modified method of Balogun *et al*. (2005) was used for isolation; periphery of rotted portions of Citrus fruits were cut under aseptic conditions into small bits(5mm) with the aid of sharp sterile surgical blade. The small bits were surface-sterilized in 70 % ethanol for one minute, rinsed in sterile water, and air-dried. They were plated aseptically in 9 mm diameter Petri dishes of Potato dextrose agar (PDA) (prepared according to manufacturer’s specification) and amended with streptomycin sulfate and incubated at room temperature (28± 2^{o}C) in the dark for 72 hours. The fungal colonies observed from the incubated plates were sub-cultured into fresh PDA plates until pure cultures were obtained. The identity of the pure cultures was confirmed through colony and microscopic characteristics observed in comparison with fungal identification atlas (Snowdon, 1990). Pure cultures of the fungi

were stored in agar slants in the fridge at 4°C for later use.

Determination of Percentage of Fungal Occurrence

Percentage frequency of occurrence of the fungal isolates from diseased citrus fruits was determined according to modified method of Bashir et al., (2020). This was calculated and expressed as shown below:

Percentage of occurrence = X/N x100/2

X = Total number of each organism in all the fruits

N =Total number of the entire organism in all the fruits screening.

Molecular Identification of the Fungal Isolates

The DNA extraction of genomic DNA from each of the eight fungal isolates was extracted from a one-week-old Potato dextrose agar (PDA) cultures using NIMR Biotech DNA extraction kit (NIMR, Nigeria). The purity and concentration of the extracted DNA were evaluated using a NANODROP (ND 1000) Spectrophotometer (Thermo Scientific, USA). All the samples showed a DNA yield between 5ng- 25ng. Polymerase chain reaction was carried out to amplify the ITS gene of the fungi using the primer pair Primers ITS1- (5' - TCCGTAGGTGAACCTGCGG- 3') and ITS4- (5'-TCCTCCGCTTATTGATATGC-3') (Synbio Technology, USA). Amplifications were carried out in a final volume of 25ul, with the PCR reaction mix containing 17ul of PCR grade water (Solis biodyne, Estonia), 4 ul of Master Mix (Solis biodyne, Estonia) comprising of 1X PCR Buffer, 1.5mM MgCl, 200µM of each deoxynucleoside triphosphates (dNTP), 2 unit of FIREPol DNA polymerase Taq polymeras, 25pMol of each primer (Synbio Technology, USA) and the 3 ul of extracted DNA. Thermal cycling was conducted in a Techne thermal cycler 3 prime series (Cole Pamer, UK) for an initial denaturation of 95°C for 5 minutes followed by 35 amplification cycles of denaturation at 95°C for 30 seconds; annealing at 58°C for 1 minute and extension at 72°C for 1 minute. This was followed by a final extension step of 10 minutes at 72°C. As a negative control, sterile water was included in the reaction. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining and then photographed under a UV transilluminator (Biobase, China). 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker

The amplified products were purified using the QIA quick PCR purification kit (Qiagen, Germany) and sent to Epoch Life Science (USA) for Sanger sequencing. The corresponding sequences were trimmed, edited, identified and compared with other related sequences using the online NCBI-BLAST search tool. The phylogenetic tree was constructed using MEGA 11 software. The ITS sequences were deposited in NCBI GenBank.

Pathogenicity Test

To establish Koch's postulates, all the organisms isolated from the fruits with rot symptoms were tested for their pathogenicity using modified method described by Bashir *et al*., (2020). Healthy Citrus fruits were surface-sterilized with 75% ethanol for one minute and dried under sterile conditions. Using a sterilized 2 mm cork borer, cylindrical plugs of tissue were removed from the fruits. Agar discs containing one-week-old cultures of the fungi isolated from spoiled fruits were aseptically placed into the holes of the fresh, healthy fruits. The inoculated sites were then covered and sealed with petroleum jelly. Both the inoculated samples and the control were placed in sterile polythene bags and incubated at 28 ± 3°C for 14 days. After incubation, the severity of the disease caused by the fungi was assessed by measuring and recording the diameter of the rotten areas on the fruits. The fungi were subsequently re-isolated from the inoculated fruits and compared with the original isolates to confirm their role in spoilage. Fungi was considered pathogenic

on the fruit when new mycelia emerged and extended radially and upwards from the originally inoculated disc, and became visible outside the original wound hole on the fruit surface thereby causing fruit rot. On this basis, growth and pathogenicity were rated as follows; Low (rot covered less than 25% of the fruit surface); Medium (covered 25- 50% of the fruit surface); High (51- 75% covered) and very high (covered 75% and above).

Extraction and GC-MS analysis of essential oil from *Ocimum gratissimum* and *Cymbopogon citratus* leaves

About 250 g of fresh leaves of *Ocimum gratissimum* and *Cymbopogon citratus* were subjected to steam distillation method (Ngujen *et al*.2022). The components of the essential oils were identified based on chromatographic retention indices and by comparison of the recorded spectra with computed data libraries (Anonymous, 1998).

Antifungal activity of the two essential oils

In this study, the antifungal efficacy of essential oils from *Ocimum gratissimum* and *Cymbopogon citratus* leaves was assessed against the pathogenic fungi *Aspergillus flavus* and *Lasiodiplodia theobromae* using the modified disc diffusion method (Allizond *et al*.,2023). Sterilized Potato Dextrose Agar (PDA) was poured into 9 mm Petri dishes and allowed to solidify. Each plate was then inoculated with a microbial suspension (10^6 CFU/mL) of the target fungi. Sterile paper discs 6mm were immersed in varying concentrations (0.25 µg/mL, 0.5 µg/mL, and 1.00 µg/mL) of the essential oils and allowed to dry under ultraviolet light. A standard fungicide, Mancozeb, at 2.00 µg/mL served as a control. The treated discs were placed onto the inoculated PDA medium, which were then incubated at 28 ± 2 °C for 1–5 days. Post-incubation, inhibition zones were measured to evaluate antifungal activity. This procedure was replicated three times to ensure accuracy.

Statistical analysis

Results obtained from experiments were performed in triplicates and expressed as mean values, and *p*-values lower than 0.05 were considered significant. Standard error means were determined using one Duncan test ($P \leq 0.05$). Data were analysed with statistical software (SPSS version 20) (Bryman and Gramer (2012).

RESULTS AND DISCUSSION

Isolation and Identification of the associated Fungi

Eight fungal isolates were identified and purified according to their colony and microscopic characteristics (Snowdon, 1990). They were three strains of *Aspergillus niger* van Tieghem *(A,B,C), and Aspergillus flavus* Link, *Wikerhamomyces anomalus* E.C Hansem, *Trichoderma asperellum* Samuel Lieckf, *Penicillium citrinum* Thom & Niren, *Lasiodiplodia theobromae* (Pat.) Griff. & Manbl. *Lasiodiplodia theobromae* had the highest percentage abundance of all fungal pathogens in the three sampled locations with 25.00 % frequency followed by *Aspergillus flavus* with 22.44 % and *Aspergillus niger* strain A with 12.24 %, *and Wikerhamomyces anomalus* and *Penicillium citrinum* with moderate abundances of 10.20 %. *Aspergillus niger* strain B, *Aspergillus niger* strain C and *Trichoderma asperellum* had low percentage abundances of 8.16, 8.16 and 4.08, respectively (Table 1).

Losses caused by post-harvest diseases are greater than generally realized because the value of fresh fruits decreases several-folds while passing from the field to the consumer. Good storage limits losses of good products over relatively long period of time (Ademoh *et al*., 2017). At Ketu market, the traders store the fruits in bags and

baskets and the fruits probably get contaminated during transportation and packaging in bags and baskets that are unsterilized. *Lasiodiplodia theobromae and Aspergillus flavus* had the highest percentage frequency of occurrence in the Citrus fruits. This is in consonance with the reports of Bashir *et al*. (2020) who found that these organisms were associated with spoilage of *Citrus sinensis* fruits. The amount of sugar in these fruits, high moisture content and high temperature (state the average temperature) in Lagos metropolis can facilitate the growth and development of these fungi on citrus fruits and cause the spoilage of these fruits. The highest frequency of *Lasiodiplodia theobromae* and *Aspergillus flavus,* and other species of *Aspergillus* may be attributed to the facts that these pathogens are fast growing organisms and thus can grow faster than most of the fungi isolated in the study. The association of strains of *Aspergillus niger, Aspergillus flavus, Wikerhamomyces anomalus, Trichoderma asperellum, Penicillium citrinum, Lasiodiplodia theobromae* with Citrus fruit rot have been previously reported by Bashir *et al*., (2020) and Zakaria, (2024).

Table 1. Occurrence and frequency of fungi associated with diseased *Citrus sinensis* fruits.

	Number of fungi isolates				
Fungi isolates	**1st sample**	**2nd sample**	**3rd sample**	**Total**	**% Frequency**
Aspergillus niger strain A	2	3	1	6	12.24
Aspergillus niger strain B	1	2	1	4	8.16
Aspergillus niger strain C	1	2	1	4	8.16
Aspergillus flavus	3	4	4	11	22.44
Wikerhamomyces anomalus	1	3	1	5	10.20
Trichoderma asperellum	1	0	1	2	4.08
Penicillium citrinum	2	3	0	5	10.20
Lasiodiplodia theobromae	4	3	5	12	25.00
Total	15	20	14	49	24.49

Pathogenicity

In a pathogenicity assessment of the eight fungal isolates, Las*iodiplodia theobromae* and *Aspergillus flavus* exhibited high virulence, causing between 74 and/over 75% significant rot on the inoculated fruits. Conversely, *Aspergillus niger* and *Penicillium citrinum* were moderately pathogenic, affecting less than 50% of the fruits. *Trichoderma asperellum* and *W. anomalus* were deemed non-pathogenic, as they did not induce any rot symptoms (Table 2). These findings underscore the primary role of *L. theobromae* and *A. flavus* in post-harvest decay of sweet oranges, highlighting the necessity for targeted management strategies against these pathogens. The mechanisms which enabled the pathogens to establish infections on the fruit could be possible host tissue adhesion, production of enzymes that damage host cells, and evasion of host immune defenses. The differences in the pathogenicity of the fungi isolates from the Citrus fruits might be due to the fungi ability to overcome the natural defense mechanism of the Citrus fruits or their ability to induce resistance in the fruits when infected. Mojo *et al*., (2024) reported that citrus fruit peels contain essential oils rich in monoterpenes group; limonene, sabinene, β-mycene and their antimicrobial property differs between individual organisms. These compounds induced chemical defense mechanism against a broad range of fungal pathogens as reported by Divekar *et al*. (2022). Physiologically, spoilage fungi are considered toxigenic or pathogenic (Al-Hindi *et al*., 2011). These toxigenic fungi

produce secondary metabolites potentially harmful to humans. Thus, extra care should be taken during handling of these fruits. The two fungal isolates, *W. anomalus* and *Trichoderma asperellum*, which are nonpathogenic, could be regarded as opportunistic organisms. According to Kurtzman (2011) and Sehim *et al*. (2023), these fungi have been reported to act as preventive agents against undesirable fungi. Although, *W. anomalus* had been implicated by Ioannou *et al.*, (2024) as an emerging human pathogen.

Molecular characterization of the fungal isolates

Blast analysis for the rDNA sequence of the eight isolates at the NCBI database showed sequence identity up to 100% with that in the database (Table 3).

Table 2. Pathogenicity of eight fungal isolates on healthy Citrus fruits.

S/N	Fungi	%Virulence	Description
1	*Aspergillus niger* (PQ796069)	≤50%	Mycelium growth/fruit rot on surface
2	*Aspergillus niger* (PQ796070)	≤50%	Mycelium growth/fruit rot on surface
3	*Aspergillus niger* (PQ796072)	≤50%	Moderate mycelium growth/fruit rot on surface
4	*Aspergillus flavus* (PP862691.1)	≤74%	High mycelium growth/fruit rot on surface
5	*Wikerhamomyces anomalus* (PQ796075)	0%	No sign of rot symptom
6	*Trichoderma asperellum* (PQ796076)	0%	No sign of rot symptom
7	*Penicillium citrinum* (PQ796077)	≤50%	Mycelium growth/fruit rot on surface
8	*Lasiodiplodia theobromae* (PP862890.1)	≥75%	Very high mycelium growth covering more than half of fruit surface

Table 3. Identified organisms with their accession numbers and similarities

S/N	NCBI BLAST relative	Accession number
1	*Aspergillus niger*	PQ796069
2	*Aspergillus niger*	PQ796070
3	*Aspergillus niger*	PQ796072
4	*Aspergillus flavus*	PP862691.1
5	*Wikerhamomyces anomalus*	PQ796075
6	*Trichoderma asperellum*	PQ796076
7	*Penicillium citrinum*	PQ796077
8	*Lasiodiplodia theobromae*	PP862690.1

The molecular identification was carried out by DNA barcoding using the ITS region sequencing. The ITS rDNA sequences were compared to those in the databases using NCBI-BLAST. Eight fungal isolates were identified using DNA barcoding with 100 % identity. It is also proposed that ITS rDNA region sequence is one of the most important tools for the identification of the filamentous fungi (Kwiatkowski *et al*., 2012; Raja *et al*., 2017). ITS rRNA genes are excellent candidates for the phylogenetic analysis because they are universally distributed, functionally constant, sufficiently conserved, and of adequate length to provide a deep view of

evolutionary relationships (Madigan *et al*., 2012).

From the dendrogram, (Figure 1) the tree grouped the isolates into 4 major clusters. *Pneumocystis jiroveci* isolate recovered from the NCBI database (accession number MK300664/1) was used to root the tree. The cluster at the top of the tree contained the *Aspergillus niger* isolates (PQ796069, PQ796070 and PQ796072) clustered together with other *Aspergillus niger* isolates (MF422165.1, MT550027.1) recovered from the GenBank database. *Aspergillus flavus* PP862691.1 exhibited a clustering relationship with *Aspergillus flavus* ON351498.1, which was isolated from animal feed and raw bovine milk as reported in Mexico (Álvarez-Días *et al*.,2022), in addition to *Aspergillus flavus* PQ036835.1, reported in Pakistan. Furthermore, *Lasiodiplodia theobromae* PP862690.1 was observed to cluster with *Lasiodiplodia theobromae* ON368091.1, which was isolated from guava in Ghana (Zee *et al*.2021), as well as *Lasiodiplodia theobromae* KR260793.1, isolated from mango gummosis in China (Li *et al*., 2013), and *Lasiodiplodia theobromae* OR18815.1, reported in Brazil (Marques *et al*., 2013). *Penicillium citrinium* isolate (PQ796077) clustered with other *Penicillium citrinium* isolates recovered from the GenBank database. Isolates clustered together in the same clade with a reasonable confidence level (bootstrap score more than 50%) indicate a significant level of similarity within the isolates.

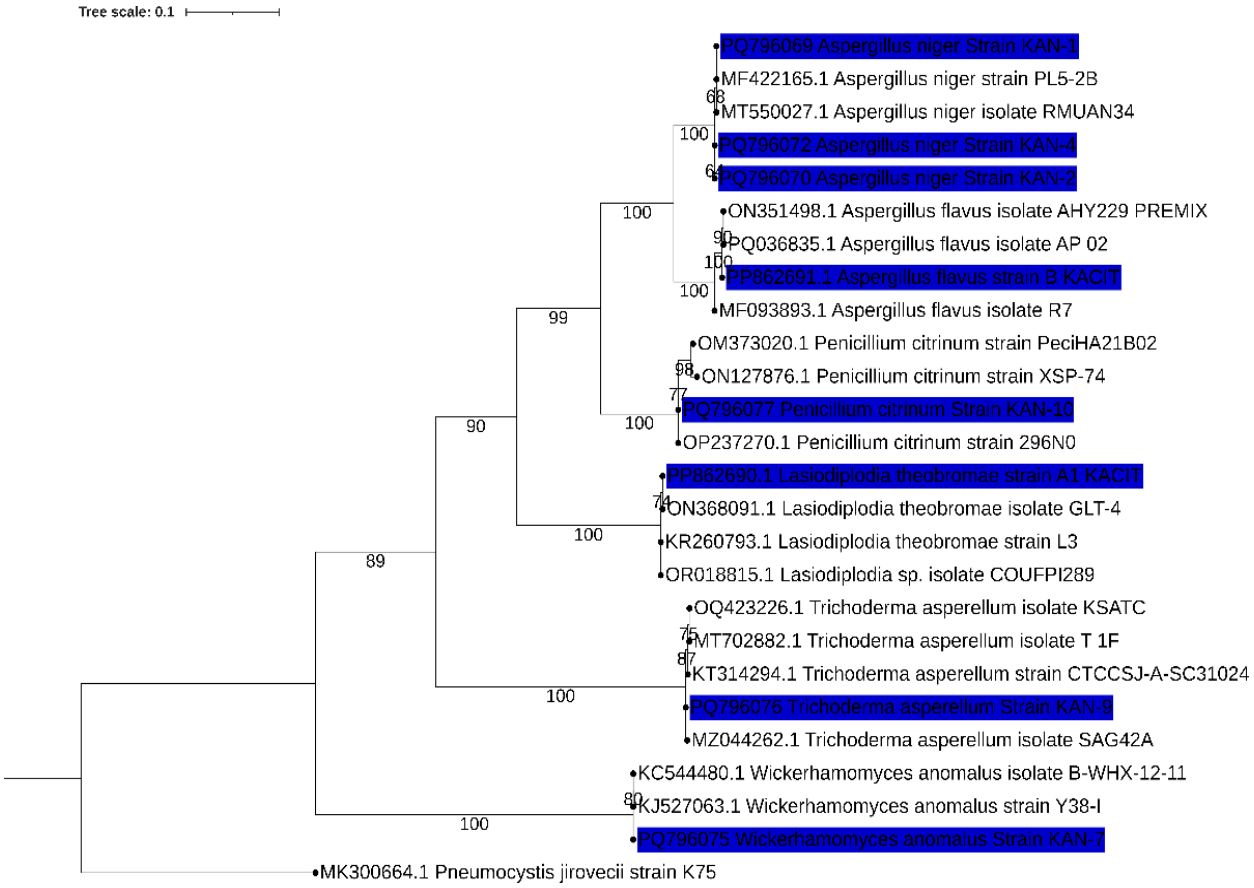

Fig 1. Dendrogram of the eight fungal isolates constructed using UPGMA method with 1000 bootstrap replicates value. Evolutionary distances were computed using the maximum composite likelihood method. *Pneumocystis jorvecii* was included as an outgroup. The phylogenetic analysis was performed using MEGA11 software

Chemical component of essential oils extracted from *Ocimum gratissimum* and *Cymbopogon citratus* leaves

The GC-MS analysis of the two essential oils revealed the presence of some compounds such as eugenol (7.73%), linalool (12.34%), nerolidiol 2 (5.28%) and beta.-isabolene (5.11%), cyclohexane (4.99%), caryophyllene (3.31%), phytol (2.83%) in *Ocimum gratissimum*, and geranial (39.05%), neral (28.20%), geraniol (6.33%), limonene (5.08%), geranylacetate(2.46%), isogeranial (1.49%) in *Cymbopogon citratus*. These results are in agreement with the reports of Mankandan *et al*., (2021), Mukarram *et al*., (2022), Rhinu *et al*., (2022).

Antifungal activity of the essential oils against the most pathogenic rot fungi

The results in Tables (4-5) show that in general, the essential oils from *Ocimum gratissimum and Cymbopogon citratus* leaves exhibited inhibitory effects on the two fungal pathogens; *Lasiodiplodia theobromae* and *Aspergillus flavus* at varying concentrations.

Table 4. Zone of inhibition incited by *Ocimum gratissimum* leaves oil in the case of *Lasiodiplodia theobromae* and *Aspergillus flavus* (n=3).

Concentrations	***Lasiodiplodia theobromae***				
	Day1	**Day2**	**Day3**	**Day4**	**Day5**
0.25µg/ml	19.98^{B}±0.0	26.77^{B}±0.21	33.62^{A}±0.03	40.45^{B}±0.51	52.43^{B}±0.03
0.50µg/ml	32.04^{C}±0.0	42.23^{C}±0.25	43.66^{B}±0.35	51.38^{C}±1.20	61.42^{C}±1.06
1.00µg/ml	43.74^{D}±0.6	50.94^{D}±0.70	52.59^{C}±0.90	61.86^{D}±0.78	72.18^{D}±1.90
2.00 µg/ml (C)	12.05^{A}±0.0	11.45^{A}±0.58	14.50^{D}±0.46	20.15^{A}±0.13	25.33^{A}±0.58
	Aspergillus flavus				
0.25µg/ml	14.01^{B}±0.0	13.33^{B}±0.01	10.66^{B}±0.01	9.65^{B}±0.01	6.66^{B}±0.01
0.50µg/ml	23.00^{C}±0.0	21.06^{C}±0.78	19.01^{C}±0.57	15.68^{C}±0.59	13.33^{C}±0.58
1.00µg/ml	38.66^{D}±0.0	33.89^{D}±1.01	31.68^{D}±0.57	28.46^{D}±0.53	24.35^{D}±0.56
2.00 µg/ml (C)	9.34^{A}±4.67	8.00^{A}±3.49	7.31^{A}±3.37	4.66^{A}±2.06	4.41^{A}±1.92

Inhibition zones are presented as mean ± SD. A column with a different superscript implies a significant difference ($p \leq 0.05$). (C): Mancozeb fungicide as control

Also, both essential oils were more potent than mancozeb (standard fungicide) even at the lower concentrations of 0.25 µg/ml to 1.00 µg/ml compared to the highest concentration of 2.00 µg/ml of mancozeb. Table (4) shows the effect of varying concentrations of *Ocimum gratissimum* leave oil on the growth of La*siodiplodia theobromae* incubated at 28 ^{o}C for five days. There was significant increase in zones of inhibitions (19.98±0.01 to 72.18±1.90 mm) with increasing concentrations of *Ocimum gratissimum* leave oil from 0.25 µg/ml to 1.00 µg/ml. In comparison with the standard fungicide Mancozeb (2 µg/ml), which caused inhibition zone of 25.33±0.58 mm even day five of incubation, the oil was more potent because even at lower concentrations of 0.05-1.00 µg/ml, inhibition of the growth of *Lasiodiplodia theobromae* ranged from 4±0.06 to 61.68±0.78 mm between day 1 to day 4 incubation period. These findings suggested fungicidal property of the oil.

Table 5. Zone of inhibition incited by *Cymbopogon citratus* leaves oil in the case of *Lasiodiplodia theobromae* and *Aspergillus flavus* (n=3).

Concentration	Day1	Day2	Day3	Day4	Day5
	Lasiodiplodia theobromae				
0.25ua/ml	20.07^{B}±0.11	28.24^{B}±0.2	41.37^{B}±0.5	51.73^{B}±0.6	64.71^{B}±0.6
0.50µg/ml	34.72^{C}±0.49	44.10^{C}±0.1	55.35^{C}±1.1	56.00^{C}±0.0	67.47^{C}±0.6
1.00µg/ml	45.78^{D}±0.6	56.12^{D}±0.1	65.34^{D}±0.1	72.33^{D}±1.1	86.10^{D}±0.1
2.00 µg /ml (C)	12.05^{A}±0.0	11.45^{A}±0.5	14.50^{A}±0.4	20.15^{A}±0.1	25.33^{A}±0.5
	Aspergillus flavus				
0.25µg/ml	21.78^{B}±0.65	26.12^{B}±0.0	35.13^{B}±0.1	42.15^{B}±0.1	55.34^{B}±0.0
0.50µg/ml	32.14^{C}±0.12	40.56^{C}±0.6	47.48^{C}±0.5	52.46^{C}±0.0	62.25^{D}±0.0
1.00µg/ml	39.01^{D}±0.5	49.34^{D}±0.0	53.28^{D}±0.0	55.14^{C}±0.0	62.11^{C}±0.0
2.00 µg /ml (C)	9.34^{A}±0.02	8.00^{A}±0.00	7.31^{A}±0.06	4.66^{A}±0.00	4.41^{A}±0.06

Inhibition zones are presented as mean ± SD. A column with a different superscript implies a significant difference ($p \leq 0.05$). (C): Mancozeb fungicide as control.

Table (4) shows the effect of varying concentrations of *Ocimum gratissimum* leave oil on the growth of A*spergillus flavus* incubated at 28 °C for five days. There was increase in inhibition zones ranged from 14.01±0.01 to 38.66±0.01 mm with increasing concentration gradient of 0.25 to 1.00 µg/ml of the oil when compared with the higher concentration of mancozeb (2.00 µg/ml) which caused minimal inhibition of 4.41±1.92 mm even at day 5 of incubation. Also, the *O. gratissimum* leaves oil showed fungistatic effect on *Aspergillus flavus* though the average decreases in zones of inhibition as the days of incubation increased from 1 to 5.

Effect of *Cymbopogon citratus* leave oil at 0.25 µg/ml to 1.00 µg/ml concentrations showed similar trend on the two fungal pathogens as observed in *Ocimum gratissimum* oil effect but is considered more potent against *Lasiodiplodia theobromae* and *Aspergillus flavus* since higher inhibition zones of 86.10±0.11 mm and 62.11±0.04 mm at highest concentration of 1.00 µg/ml were recorded at day 5 incubation period than *Ocimum gratissimum* oil which its effect caused 72.18±1.90 mm and 24.35±0.56 mm inhibition zones at day 5 incubation period (Tables 5). Fungicidal effect of *Cymbopogon citratus* oil was also observed to increase zones of inhibition as the number of days of incubation was increased from 1 to 5.

The essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* had fungitoxicity effect against the two rot fungi; *Lasiodiplodia theobromae* and *Aspergillus flavus*. These findings were also reported by Nyamath and Karthikeyan, (2018); Nakada-Freetas *et al*. (2022); Oribhabor and Iyekekpolor, (2023). Yeo *et al*. (2023) reported complete inhibition of the mycelial growth of *L. theobromae* by *C. citratus* and *O. gratissimum* oils at 700µg/ml and 1000µg/ml, respectively. The decrease in inhibitory effect of *O. gratissimum* on *A. flavus* with increasing time could be due to volatilization of the active compounds.

The inhibitory effect of the essential oils could be by targeting multiple fungal virulence mechanisms which include prevention of adhesion (biofilm disruption), neutralizing of tissue-damaging enzymes, and enhancement of immune recognition (membrane disruption, melanin inhibition) (Hernández-Ruiz *et al*., 2023). The high fungi toxicity exhibited by the essential oils could be due to the presence of strong bioactive compounds with antifungal properties such as neophytadiene, nerolidiol 2, β-bisabolene, humulene, phytol (*Ocimum gratissimum*) and high percentage of geranial (39.05%) and neral

(28.20%) amongst other components in *Cymbopogon citratus.* Akpo *et al*. (2023) reported strong antifungal activity of *O. gratissimum* oil against rot fungi of mango and tomato and attributed its potency to the phytochemical compounds present in the oil. The dose-dependent antifungal effect of the oil was a function of time

Geraniol increases the outward leakage rate of potassium ions, while citral damages the microtubules and exhibits cytotoxicity in fungi (Ekpenyong *et al*. 2015), Linalool, a monoterpene alcohol, comprises numerous fungicidal properties and It retards the overall development and propagation of different fungi through the respiratory restriction of their aerial mycelia. Additionally, other aldehydes of LEO can confer antimycotic activity through cross-linkage reaction within the fungal membrane (Jayasena and Jo, 2013; Boukhatem *et al*., 2014). Furthermore, the components of the two oils comprises of group of terpenes (monoterpenes, diterpenes, sesquiterpenes, phenylpropenes) and they exerted their antifungal effect through **m**embrane disruption, mitochondrial damage, cell wall weakening, oxidative stress and biofilm suppression **(**Hernández-Ruiz *et al*.,2023). The use of essential oils as alternatives lessons the drawbacks of synthetic fungicides such as resistance and environmental contamination. They are non-hazardous to environment, effective, and biodegradable in this regard. Their low toxicity to non-target organisms, degradability, relatively straightforward, inexpensive production processes, and reduced health risks during application because of low residue toxicity makes them to be preferred by agriculturists and plant biologist. The range of molecules that are available in natural oils enables them to diversify the biochemical and molecular targets that are directed at fungi pathogens and thus limit or delay the resistance phenomenon (Deresa and Diriba, 2023).

CONCLUSION

The findings of this study indicate that the essential oils of *Cymbopogon citratus* and *Ocimum gratissimum* have significant antifungal activity as compared with the standard fungicide; mancozeb. Since the two oils are natural, non-toxic, ecofriendly, they could be promising potential natural fungicides for effective management of post-harvest fungal disease of *Citrus sinensis* fruits.

FUTURE RESEARCH DIRECTION

The biosafety of the essential oils (*Ocimum gratissimum* and *Cymbopogon citratus*) will be evaluated by determining the oral LD50 through acute oral toxicity with mice and sub-acute toxicity studies will be performed using spraque-dawley rats. The animals will be observed for behavioural changes, and histopathology studies carried out on several organs to ascertain the safety of the oil usage in long term. A comprehensive In vivo testing of the oils will be carried out on several replicates of healthy and artificially infected sweet oranges and the proximate contents determined. The most effective concentrations of the essential oils will be tested on replicates of ripe and unripe sweet oranges on different storage systems.

AUTHOR CONTRIBUTIONS

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study, as it involved only plant and microbial samples, which are exempt from human or animal ethical review regulations.

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