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Evaluation of Essential Oils for the in Vivo Management of Fusarium Tuber Rot Disease of White Yam (*Dioscorea rotundata* Poir)

Obiora Albert Onwuta [1, 2] ***and Victor Ohileobo Dania***[1*]

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria
[2]Department of Agricultural Education, Federal College of Education 9Technical), Akoka, Lagos State, Nigeria

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ABSTRACT

Yam is an important economic crop that is widely cultivated in Africa and other sub-tropical continents. Its cultivation is, however, constrained by huge postharvest yield losses in tubers and setts meant for next season planting due to microbial-induced rot. The essential oils (EOs) of three botanicals, *Cymbopogon nardus*, *Ocimum gratissimum* and *Citrus sinensis* were evaluated for their phytochemical composition and efficacy in the management of *Fusarium* soft rot disease in white yam (*Dioscorea rotundata* Poir). Chemical constituents were analyzed and quantified using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detectors (GC-FID). The experimental design for the in *vivo* trial was a 15×3×3 factorial in completely randomized design with three replications, which comprised fifteen isolates of *Fusarium oxysporum*, three EOs and three concentrations. Geraniol (51.73%), thymol (50.52%) and limonene (45.84%) were the major compounds found in *C. nardus*, *O. gratissimum* and *C. sinensis* EOs, respectively. Rot development in inoculated but treated tubers was significantly ($p<0.05$) reduced by 9.3 -16.4%, 10.1-17.6% and 10.2-18.3%, respectively at 0.8 µl/ml EO concentration. The efficacy of the EOs in this study indicates their potential as biofungicides in the management of *Fusarium* soft rot disease in white yam.

Keywords: *Cymbopogon nardus*, *Fusarium*, Geraniol, *In vivo*, Soft rot disease

INTRODUCTION

White yam (*Dioscorea rotundata* Poir) is the most important and widely cultivated yam species in Nigeria. The starchy tuber is the economically important part of the crop and rich carbohydrate source in the diet of rural and urban dwellers. It is a critical crop in the food value chain which is geared toward ensuring food security, particularly in developing countries (Kouakou et al. 2023). It is considered a famine crop because of its long shelf-life and availability during the off-season periods, especially at times of food scarcity. Yam occupies a high premium in the customs and traditions of several communities in Nigeria, where an annual festival is celebrated to mark the arrival of new yams (Anuagasi et al. 2024). It is the second most important tuber crop after cassava and contributes about 10% of the total root and tubers production worldwide.

Yam production is often constrained by foliar and resident soil-borne pathogens, leading to concomitant reduction in tuber yield. It is susceptible to several plant pathogens, including bacteria, fungi, viruses, and

* Corresponding author. E-mail: victorohileobo@gmail.com

nematodes. However, tuber soft rot, which is mainly caused by fungi, is the most devastating disease of yams. Li et al. (2024) reported that about 30 and 40% of postharvest yams are lost to yam rot pathogens. *Fusarium oxysporum* is an important soil-borne fungal pathogen causing soft rot disease in yams both in the field and storage, with a consequential decrease in output and market value of harvested tubers. Under optimal conditions and storage in hygienic conditions, yam tubers have a longer shelf life of 5 to 6 months. Yams particularly store better when unbruised, healthy tubers are brought to the store for storage (Dania et al. 2016). Yam tuber rot is often facilitated when infected, cut, or injured tubers are brought from the field to storage. Thus, the injuries provide easy access to the tuber by the pathogens, which increases the incidence and severity of rot in ware yams.

Due to the biodeterioration and significant yield losses associated with yam tuber rot, effective strategies are needed to manage the disease. The application of synthetic fungicides is undesirable because of their threat and danger to biodiversity. Botanicals are biopesticides of plant origin that are readily available, biodegradable, and harmless to man and the environment. Essential oils (EOs) have been reported to possess several volatile organic metabolites that are against in the management of plant diseases. (Lee et al. 2020; Hiwandika et al. 2021; Sawadogo et al. 2023).

Citrus sinensis L.is an important commercial fruit crop that is cultivated worldwide with high economic and medicinal value. The fruit peels have medicinal properties and have been reported to reduce cough, sore throat, hyperlipidemia and other ailments (Sharma et al. 2018; Yang et al. 2023). Also, Citrus EO from various species has shown antioxidant, antibacterial, and antifungal activity against plant pathogens (Zhang et al. 2019; Luro et al. 2020).

Citronella grass (*Cymbopogon nardus* (L) Rendle is a member of the Poaceae family, with several antimicrobial properties acting synergistically against various bacterial and fungal pathogens of many crop varieties (Wildeska et al. 2018; Martinazzo et al. 2019; Valková et al. 2022). *Occimum gratissimum* L. leaves have been reported as an effective antidote against several bacterial infections with antipyretic, anticarcinogenic properties (Kumar et al. 2017). It has also been used as food preservative against *Colletotrichum musae*, causing anthracnose disease in banana (Madjouko et al. 2019). These botanicals are ecologically safe and are a promising alternative to the use of harmful chemical fungicides. This study aimed to determine the chemical composition of *O. gratissimum*, *C. nardus*, and *C. sinensis* EOs and their efficacy in the management of *Fusarium* tuber rot disease in yam.

Materials and methods

Collection of samples

The fifteen pathogenic isolates of *Fusarium oxysporum* used in this study were obtained from the yam fungal culture collection in the Pathology Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. The cultures were preserved on acidified potato dextrose agar and stored at 4°C until needed. Healthy leaves of *Cymbopogon nardus* and *Ocimum gratissimum* were obtained from the premises of the University of Ibadan, Nigeria, while green peels of *Citrus sinensis* were obtained from fruits purchased from the open market. The botanicals were identified at the Taxonomy Unit in the Department of Forest Production and Products, University of Ibadan, Nigeria. Voucher specimen numbers FPP 11.107, FPP 11.108, and FPP 11.109 have been deposited in the Herbarium repository. Tubers of a susceptible variety confirmed in the previous experiment were obtained from yam barns in cultivated farms.

Extraction of essential oils from botanicals

Healthy leaves of *C. citratus* and *O. gratissimum* and green peels of *C. sinensis* fruits were used for essential oil (EO) extraction. The botanicals were air-dried in the laboratory at room temperature (28±2°C) until they became crisp at 10-14 days. They were ground to a fine powder using a Warring 4-Litre CB25ST Commercial Blender, Warring Company, New York City, USA. A 500-gramme weight of each of the three test botanicals

was passed through the process of hydrodistillation using a Clevenger-type apparatus. The samples were distilled at 105°C till all the oil was completely extracted. The EOs were collected, dried under anhydrous sodium sulphate to eliminate any source of moisture, and preserved in properly sealed vials to avoid evaporation and stored in the dark at 4 °C till required for use.

Gas Chromatography–Mass Spectrometry analysis

A gas chromatography-mass spectroscopy experiment was conducted at Hydrochrom Resource Laboratory, Lagos, Nigeria, according to the method of Yang et al. (2023). Analysis of the chemical constituents of the EOs was carried out using a quadrupole TQ8045NX gas chromatographer fitted with a flame ionization detector (GC-FID) and a 5-MS capillary column of 60 m×0.25 mm×0.25 mm dimension. The temperature of the injector capillary was set at 250±2°C, while the flow rate of the helium carrier gas was 1.0 mL/min. The temperature of the oven was set at 60°C for 5 min, which was later elevated to 70°C with a flow rate of 20°C/min. The temperature was further stepped up to 200°C for 15 min at 10°C/min and later increased to 320°C for 5 min at 10°C/min. The mass spectrometry parameters consisted of the following operating conditions: The electron ionization mode and ion source temperature were set at 70 eV and 260°C, respectively, while the mass spectra range was 55–500 amu. The various EO volatile compounds were identified through comparison of their respective mass spectra to those reported in the National Institute of Standards and Technology (NIST) library. Identification of individual constituents was done based on their retention times, retention indices relative to C5–C18 n-alkanes, and matching spectral peaks with available published data (Adams, 2007).

Effect of essential oils on rot development in inoculated yam tubers

Healthy, clean tubers of a susceptible variety measuring 30 cm in length and 10 cm in width were used in the *in vivo* trial. The experimental design was a 15×3×3 factorial in a completely randomized design with three replications. The treatments comprised 15 *Fusarium* isolates, three essential oils (*C. nardus, O. gratissimum* leaves, and *C. sinensis* peels), and three concentrations (0.2, 0.4, and 0.8 µl). The tubers were surface sterilized with 0.5% v/v sodium hypochlorite and rinsed using distilled water. A 0.5-cm width and 1-cm length hole was made in the tubers using a sterile cork borer at the distal, middle, and proximal regions of each tuber. The bored section of the tuber was inoculated with 0.5 cm of mycelium of each test fungus from pure cultures. Inoculated tubers were treated with the three EO concentrations as described above and sealed with sterile mastic tape to prevent secondary contaminants. Yam tubers that were inoculated with the pathogen, but without extract application, served as a control for each of the treatments. All the treatments and controls were incubated in sterile, clean perforated trays in a humid environment for 14 days. At the end of the period, the tubers were cut through to expose the internal tissue of the transverse section. Rot development was recorded by measuring the length and width of the lesion on each tuber according to the modified method of Dania et al. (2016):

$$\text{Rot inhibition} = \frac{\text{Area of healthy tissue} \times 100}{\text{Total surface area}}$$

where area of healthy tissue = $1/3 \times \pi \times r \times l$ (r = radius of healthy tissues, l = length of tuber)

Total surface area of tuber = $1/3 \times \pi \times d \times l$ (d = diameter of tuber, l = length of tuber)

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and means of treatment differences compared with Duncan Multiple Range Test (DMRT) at 5% probability level using Statistical Analysis System (SAS) Institute Cary, NC, and USA, Version 9.1 (SAS 2002).

Results

Gas chromatography-mass spectrometry of essential oil constituents

Gas Chromatography-Mass Spectrometry (GC-MS) showed that the essential oil (EO) of *C. nardus* consisted

of 28 constituent compounds, and these accounted for 97.79% of its composition (Table 1). The EO composition varied from 0.23 to 51.73%. The sesquiterpenoid geraniol was the most prevalent compound, which accounted for 51.73% of its composition. The GC-MS analysis showed that the EO was of high quality, varying between 80 and 97%. The retention time of the constituent compounds ranged between 3.62 and 14.60%. The chromatogram (Figure 1) shows the pattern of release of the constituent compounds during the GC-MS analysis and their corresponding peaks of elution. The EO composition of *O. gratissimum* consisted of 31 constituent compounds, and these accounted for 98.57% of its composition. Metabolite composition of the EO ranged between 0.31 and 50.52% (Table 1). The sesquiterpenoid thymol was the most prevalent, with 52.52% of the EO composition. This was followed by the limonene with 10.61%. The retention time of the volatile compounds varied between 2.71 and 16.36 minutes, depending on their molecular weights. The quality of the EO constituents was relatively high, and this varied from 83 to 99%. The pattern of elution of the constituent compounds and their respective peaks are shown in Figure 2. The EO of *C. sinensis* consisted of 36 volatile compounds which accounted for 94.90% of its composition (Table 1). Monoterpenoid limonene was the most predominant compound comprising 21.81% of the EO constituents. This was followed by linalool and α - Terpineol with 10.79 and 10.08%, respectively. The quality of the EO was relatively high and varied from 90 to 99%. The chromatogram (Figure 3) shows the pattern of elution of the volatile compounds during the GC-MS analysis. The retention time of the metabolites ranged between 3.80 to 11.36 minutes depending on the molecular weight of the various compounds.

Table 1. Chemical constituents and percent concentration of the essential oils

Peak area concentration (%)						
S/N	**Composition**	**Klovat index**	***C. nardus***	***O. gratissimum***	***C. sinensis***	**Quality (%)**
1	Pinene	926	0.65	0.94	3.18	93.3
2	Camphene	950	0.50	0.38	-	90.7
4	β-Pinene	970	0.23	-	1.54	87.2
5	Sabinene	975	0.89	1.47	10.79	97.3
6	Citronellol	985	-	-	-	90.5
7	Mrycene	990	0.94	0.63	1.66	85.3
8	Octanal	1008	-	-	0.56	96.3
9	Squalene	1015	-	0.35	-	91.4
10	α-Terpineol	1024	-	4.73	0.89	90.7
11	Carvacrol	1028	0.71	0.81	3.21	86.7
12	β-Ocimene	10.42	0.61	-	-	98.1
13	γ-Terpinene	1070	-	0.69	1.8	88.8
14	Limonene	10.88	1.77	10.61	45.84	96.1
15	Pirillene	1098	-	0.31	0.78	93.3
16	Azulene	1088	-	0.38	-	84.7
17	Linalool	1088	0.67	-	1.81	86.1
18	Nonanal	1102	0.77	-	0.59	90.6
19	Thujanol	1113	-	0.68	1.26	87.3

20	Nonanal	1122	0.75	1.12	0.59	94.7
21	Terpinen-1-ol	1139	-	0.46		96.4
22	trans-Verbenol	1143	-	1.68	2.56	90.2
23	Citronellal	1154	13.14	4.58	1.42	98.7
24	Borneol	1163	0.50	-	-	90.4
26	Farnesol	11.77	0.24	-	1.2	95.8
27	α- Terpineol	1191	0.66	0.38	2.64	83.3
28	Tridecanal	1204	-	0.34	-	87.5
29	Decanal	1209	1.03	2.13	0.83	93.8
30	Nerol	1228	-	-	-	96.6
31	trans-Sabinene	1220	-	7.33	2.04	91.5
32	Neral	1242	0.89	0.37	1.48	88.3
33	Citral	1243	1.46	-	0.62	82.7
34	Geraniol	1244	51.73	0.35	1.76	90.3
35	Caryophyllene	12.45	-	-	-	98.4
36	Vamillin	12.47	0.60	0.41	0.64	99.3
37	Carvone	1251	-	-	2.67	80.2
40	Geranial	1276	0.87	1.38	1.09	86.3
41	Thymol	1289	-	50.52	-	97.6
42	Carvacrol	1294	-	-	-	98.4
43	Azathymine	13.01		-	0.47	87.2
44	α - Capaene	1371	0.95	-	1.89	90.7
45	ß-cubebene	1387	-	0.69	0.81	94.6
46	Selinene	1484	0.89	-	0.55	93.3
47	β-Copaene	1430	-	-	1.17	93.5
48	Eugenol	1359	0.60	0.36	1.51	98.3
49	ß-caryophyllene	1418	-	0.87	-	81.2
50	α- Farnesene	1457	0.90	0.52	-	87.3
51	Elemol	1481	-	0.34	-	84.5
52	β--Elemene	1372	1.45	-	-	90.5
53	Silane	14.02	-	-	0.51	94.1
54	Humulene	1467	0.68	2.80	-	85.5
58	Cubenol	1581	1.03	-	-	96.3
59	Indol	1636	.	-	-	90.1
	Total		97.79	98.57	99.40	

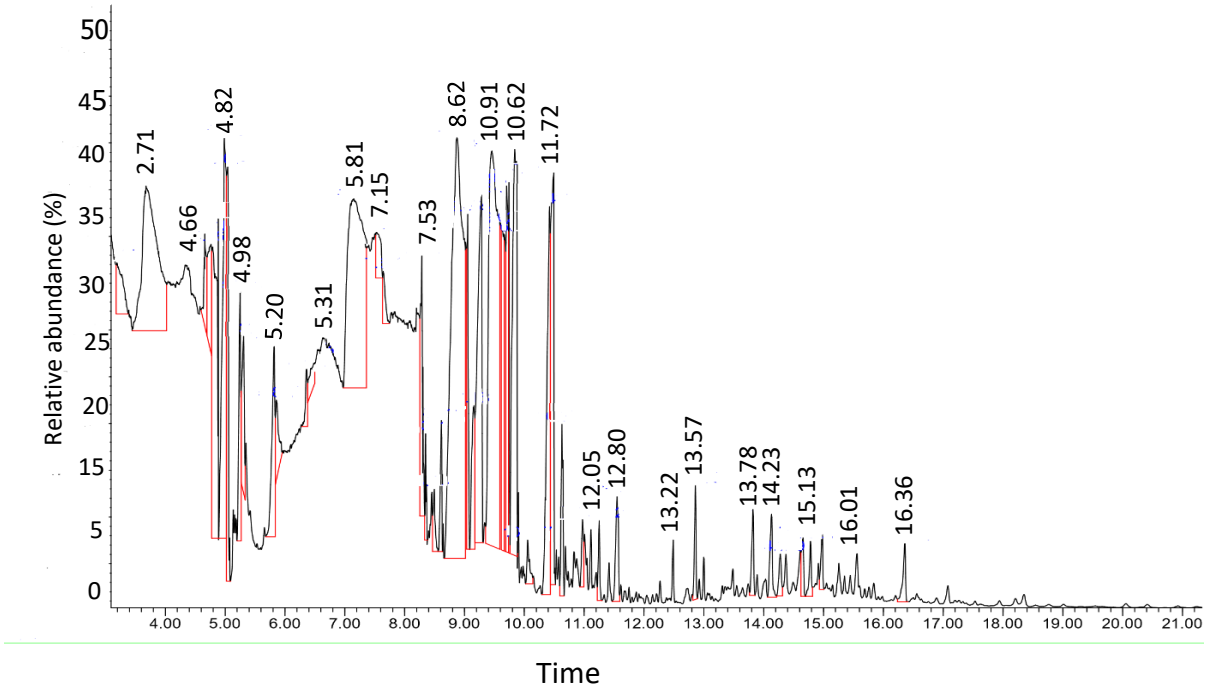

Figure 1. The GC-MS chromatogram of *Cymbopogon nardus* essential oils

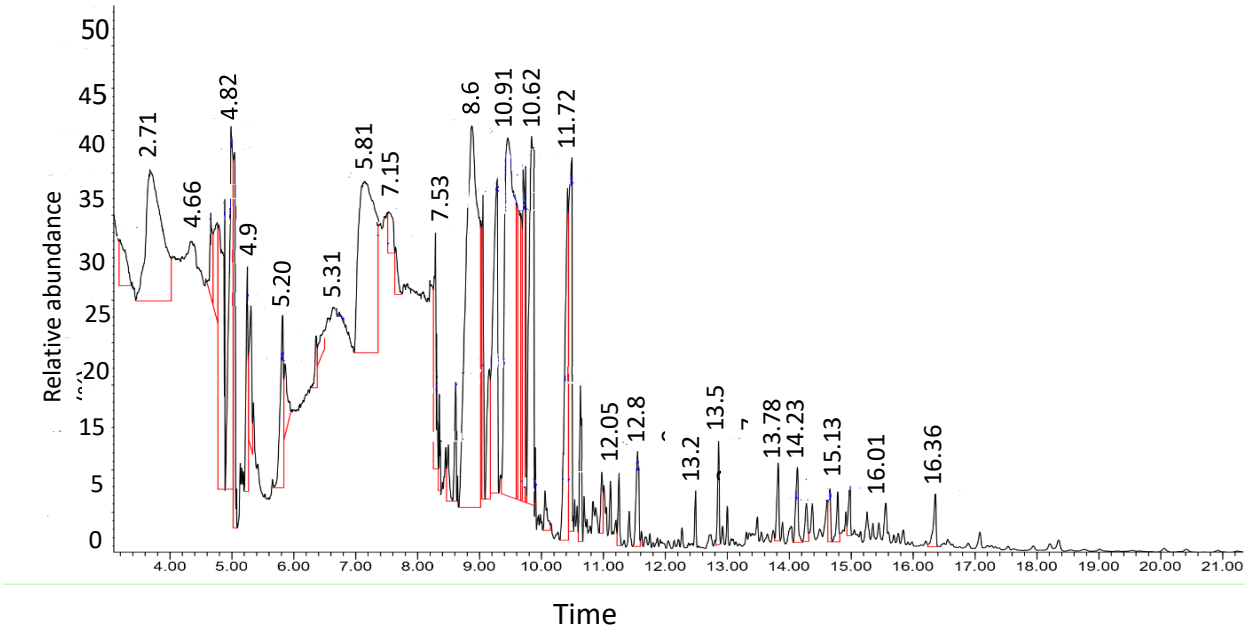

Figure 2. The GC-MS chromatogram of *Ocimum gratissimum* essential oils

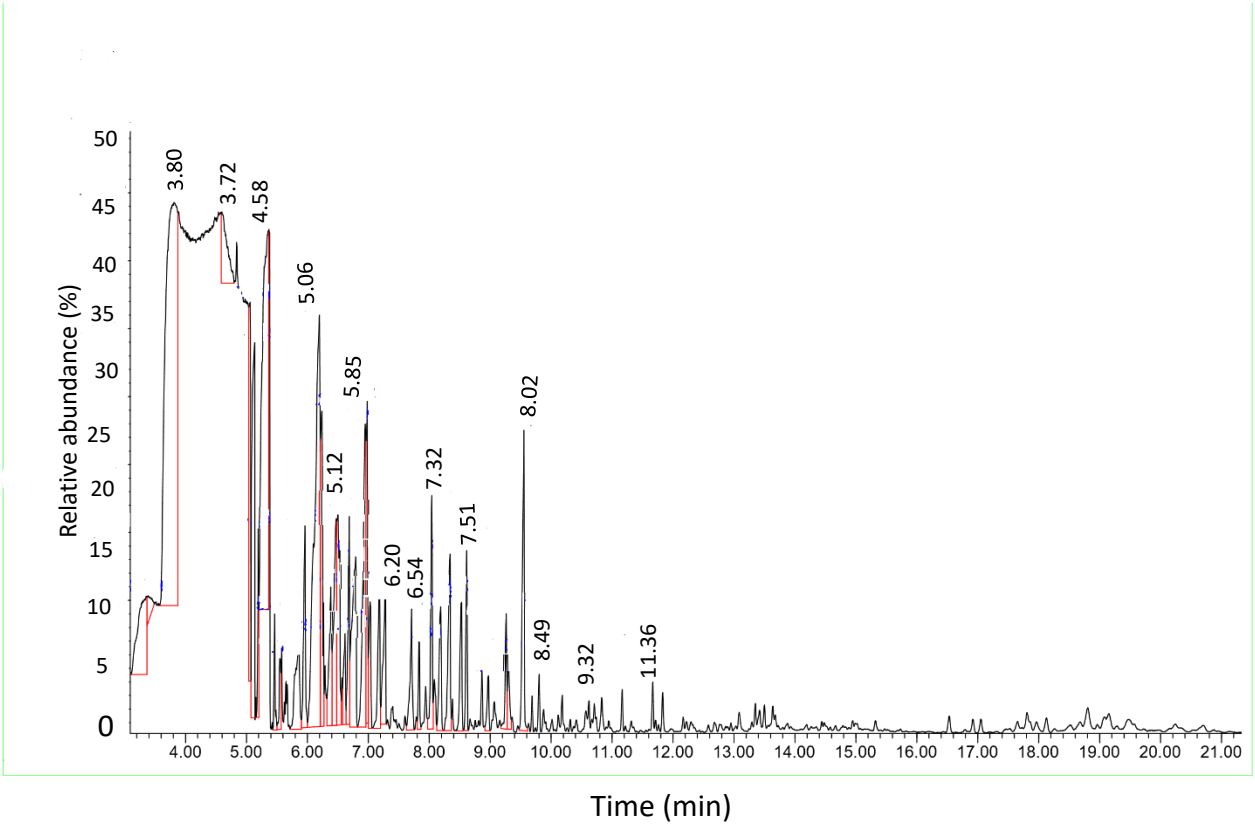

Figure 3. The GC-MS chromatogram of *Citrus sinensis* essential oils

***In vivo* inhibitory effect of essential oils on rot development**

All three botanicals that were evaluated for their inhibitory effect on yam tuber rot induced by *Fusarium oxysporum* in inoculated tubers exhibited varying efficacy at the test concentrations. Although they were less effective at lower concentrations, their efficacy peaked at the highest concentration of 0.8µl/ml. The efficacy of *O. gratissimum* varied from 18.1 to 29.3% at 0.2 µl/ml. Rot development was further reduced to between 15.1 and 25.1% when the EO concentration was doubled to 0.4 µl/ml and this was significantly ($p<0.05$) higher than the control with 25.5 to 41.6% rot range. The best inhibitory action was recorded at the highest EO concentration of 0.8 µl/ml. Rot development was significantly ($p<0.05$) reduced to between 10.1-17.6% compared to the control, with 22.8 and 40.1% rot range at this concentration. The application of *C. nardus* EO reduced rot initiation in inoculated but treated yam tubers to between 17.3 and 27.1% compared to values obtained in control tubers (24.3 to 40.1% at 0.2 µl/ml concentration (Table 2). Bioactivity of the EO increased considerably at 0.4 µl/ml concentration with rot development ranging from 13.4 to 20.4% which was significantly ($p<0.05$) lower than the rot values obtained in the control tubers (19.3 to 41.3. Tuber decay was also further inhibited when the EO concentration was increased to 0.8 µl/ml with rot varying between 9.3 and 16.4% and this was significantly

(p<0.05) lower than the control (13.3 to 41.5%). The rot recorded in control tubers was significantly (p<0.05) higher than the recorded values in the control.

Rot initiation in inoculated yam tubers that were treated with *C. sinensis* EO was reduced to between 15.8 and 26.5% and this was significantly lower than the control tubers at 0.2 µl/ml (Table 2). The EO efficacy increased at 0.4 µl/ml concentration with rot values ranging from 14.1 to 22.1% relative to the decay in control tubers without EO application (23.1 to 36.1%). The inhibitory effect at this concentration was significantly (p<0.05) higher than the control. The best inhibitory effect was obtained when the EO concentration was increased to 0.8 µl/ml with rot development reduced to between 10.2 and 18.3%. This was significantly (p<0.05) higher than rot values obtained in the control, which varied from 24.4 to 33.3%. Generally, the efficacy of *C. sinensis* was significantly (p<0.05) higher in treated tubers than in the control.

Comparatively, the EO of *C. sinensis* peels was most effective with 15.8 to 26.5% rot inhibition at 0.2 µl/ml relative to *C. nardus* and *O. gratissimum* with 17.3 to 27.2% and 18.1 to 29.3% inhibition, respectively. *Cymbopogon nardus* EO had the highest rot inhibitory effect of 13.4 to 20.4% when the concentration was increased to 04 µl/ml than the other botanicals. Overall, the best rot reduction of 9.3 to 16.4% was recorded at the highest concentration of 0.8 µl/ml obtained in inoculated tubers that were treated with *C. nardus* EO. Generally, all EO treatments at various concentrations were effective against yam rot inhibition and were significantly (p<0.05) higher than the control (Fig. 4).

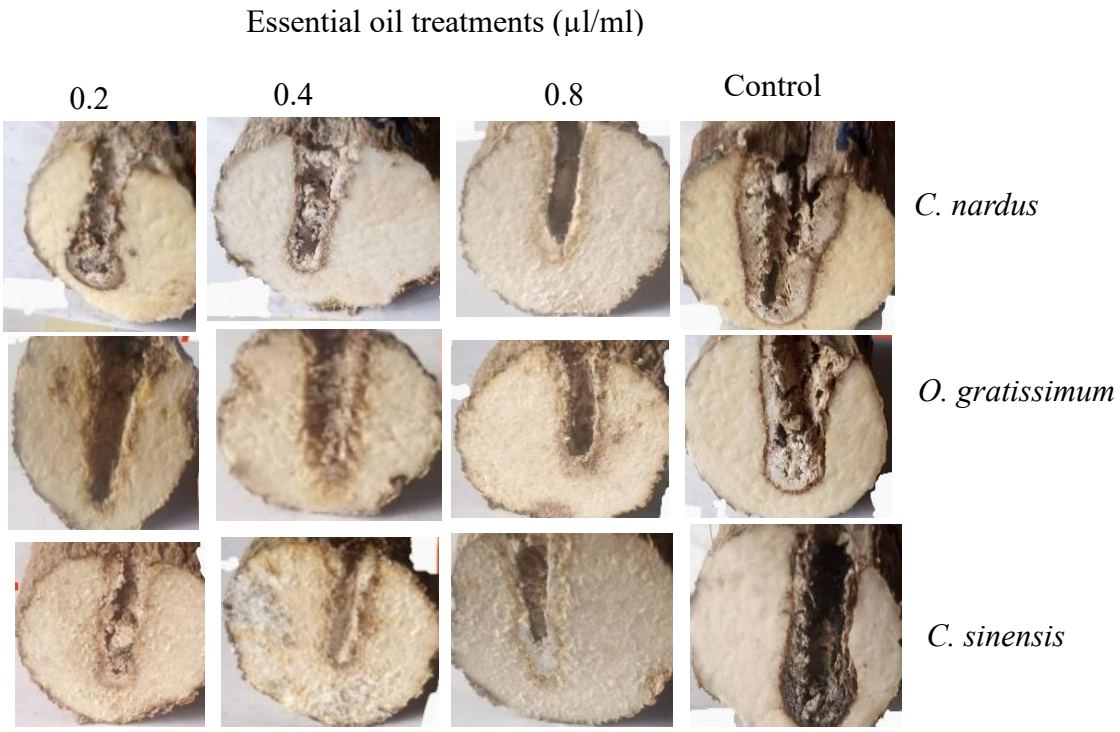

Figure 4. Rot inhibition in yam tubers inoculated with a virulent isolate of *Fusarium oxysporum* and treated with essential oils

Table 2. Effect of concentration of the essential oils on percent *in vivo* yam rot development

	Essential oil concentration (µ/ml)								
	Cymbopogon nardus			*Ocimum gratissimum*			*Citrus sinensis*		
Fusarium spp.	0.2	0.4	0.8	0.2	0.4	0.8	0.2	0.4	0.8
F. oxysporum 012	23.4e	16.2f	10.4d	20.1d	19.3e	14.3f	26.5a	21.5d	17.3c
Control	30.7c	29.1c	35.7b	26.4c	40.3a	35.7b	31.3a	27.3c	31.2a
F. oxysporum 015	24.7e	19.0e	14.8d	24.2c	21.2e	16.8f	23.3b	16.1e	11.6d
Control	40.1a	40.3a	27.8c	24.7c	27.1d	29.1c	32.1a	26.8c	27.1b
F. oxysporum 017	27.2d	18.7e	13.3d	24.1c	16.8f	17.5f	17.1d	15.6e	10.8d
Control	36.4b	31.8c	41.3a	21.2d	26.8d	24.3d	27.3b	23.1d	18.5c
F. oxysporum 021	23.4e	20.1e	12.6d	27.3c	21.7e	16.3f	23.6b	17.1e	13.1d
Control	30.7c	35.2b	28.5c	30.4b	26.1d	34.4b	26.4b	36.3a	29.4a
F. oxysporum 023	24.3e	20.4e	16.1d	20.7d	17.5f	15.8f	25.4a	21.3d	19.1c
Control	27.1d	30.9c	39.3a	29.6b	26.7d	30.3c	31.7a	26.1c	33.3a
F. oxysporum 027	24.4e	19.8e	10.1e	26.8c	22.1e	16.1f	24.4c	16.5e	13.6d
Control	24.3e	19.3e	13.3d	30.3b	31.6c	25.1d	30.2a	27.3c	25.1b
F. oxysporum 031	17.3f	13.4f	15.5d	21.1d	17.3f	15.3f	20.1c	17.3e	12.7d
Control	27.3d	21.1e	27.2c	25.2c	41.6a	26.1d	30.3a	35.8a	24.4b
F. oxysporum 033	26.5d	18.2e	16.4d	19.2d	15.1f	13.1f	20.7c	14.1e	10.2d
Control	37.4b	31.2c	27.6c	33.1a	37.1b	27.1d	26.3b	27.5c	30.5a
F. oxysporum 035	22.6e	19.3e	15.1d	24.3c	20.8e	17.6f	21.3c	15.5e	11.1d
Control	29.9c	30.4c	40.3a	25.1c	27.9d	30.1c	31.9a	36.1a	26.2b
F. oxysporum 040	17.4f	15.2f	10.7e	18.4d	16.7f	14.4f	15.8d	14.3e	13.9d
Control	26.3d	20.1e	14.3d	29.8b	25.7d	40.1a	25.1b	32.8b	27.1b
F. oxysporum 042	25.6d	20.4e	15.7d	26.4c	22.7e	17.4f	20.3c	17.4e	15.8c
Control	27.1d	35.7b	39.7a	30.9b	32.1c	25.6d	32.4a	30.7b	29.4a
F. oxysporum 043	23.9e	16.9f	13.3d	21.7d	16.2f	10.1g	25.3a	22.1b	18.3c
Control	36.6b	41.3a	28.1c	29.4b	27.7d	30.6c	26.1b	32.1b	30.3a
F. oxysporum 046	24.7e	20.1e	16.4d	18.1d	15.4f	11.3g	21.4c	16.4e	11.4d
Control	30.1c	26.3d	28.7c	27.6c	30.7c	22.8e	33.1a	27.3c	25.4b
F. oxysporum 051	23.1e	17.7f	13.1d	24.6c	21.4e	16.3f	26.2a	20.1d	15.3c
Control	31.4c	29.3c	27.3c	34.7a	30.1c	25.5d	29.4a	30.1b	25.7b
F. oxysporum 055	21.1e	17.3f	16.4d	29.3b	25.1d	16.7f	21.8c	16.1e	12.6d
Control	27.3d	31.1c	9.3e	25.3c	27.1d	21.1e	31.4a	26.3c	30.1a

Values are means of three replicates. Means with the same letter along the column are not significantly different. ($p<0.05$), using Duncan Multiple Range Test (DMRT).

Discussion

The use of natural products from botanicals such as essential oils (EOs) and their inherent secondary metabolites has stimulated significant focus in recent times for the management of plant diseases as an alternative to the use of chemical pesticides with minimal adverse effects. Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that the essential oil (EO) of *C. nardus* consisted of 28 constituent compounds. Geraniol and citronellal were the most prevalent organic compounds, and these constituted 51.73 and 13.14% of its composition, respectively. Other bioactive constituents included limonene, linalool, and camphene. These findings are consistent with Sawadogo et al. (2022) and Dangol et al. (2023), who reported sesquiterpenoid geraniol and aldehyde citronellal or its oxidized product, citronellol, as the primary constituents of *C. nardus* EO. In related experiments, the occurrence of limonene, linalool, and camphene has also been obtained in the EO analysis of this botanical by other authors (Chandra et al. 2016; Pontes et al. 2019). Although the major constituents associated with *C. nardus* EO have been corroborated with other studies, however, some considerable differences were observed between the percentage composition of constituent compounds obtained in this study and previous reports. The variability in EO yield recovery and inherent volatile compounds could be influenced by the time of harvest, age of the plant, drying and extraction methods, geographical area, part of the plant used, and environmental stress factors (De Tolebo et al. 2016; Tang et al. 2018; Dania and Olaleye, 2022). Sharma et al. (2018) and Dangol et al. (2023) reported that EO yields from storage organs such as roots, corms, fruits, and seeds produced significantly higher yields compared to the leaves or flowers.

The EO composition of *Ocimum gratissimum* consisted of 31 constituent compounds. Thymol and limonene were the most predominant with 50.52% and 10.61% composition, respectively. Madjouko et al. (2019) reported thymol as the major constituent of *O. gratissimum* EO with 42.5% recovery. This result, however, contradicts Huong et al. (2020), who reported eugenol as the most predominant compound found in the EO of the botanical. This contradiction may be attributed to differences in seasons, geographical location, and prevailing environmental conditions at the time of leaf sample collection (Nakasugi et al. 2021). Pandey et al. (2014) reported limonene as an active principle in *O. gratissimum* EO, though to a higher degree compared to the results obtained in this study. This disparity may be because while the authors extracted EO from the seeds, which are expectedly to have higher yield potential, leaves were used in this study. Seeds have been reported to have higher EO concentrations than leaves (Mutlu-Ingok et al. 2020; Hu et al. 2021). GC-MS analysis showed that the EO constituent of *C. sinensis* comprised 33 volatile compounds, which accounted for 99.40% of its composition. Limonene and sabinene were the most predominant constituents with 45.84 and 10.79% occurrence, respectively. Limonene has been reported as the major constituent of EO extracted from green peels of *C. sinensis* (Farouk et al, 2022; Anwar et al., 2023; Yang et al., 2023).

All three botanicals that were evaluated for their inhibitory effect on yam tuber rot induced by *F. oxysporum* in inoculated tubers exhibited varying efficacy at the test concentrations. The application of *C. nardus* EO in the treatment of tubers inoculated with the pathogen significantly reduced rot development to between 9.3 and 16.4% at 0.8 µl/ml concentration, and this was significantly ($p<0.05$) lower than the control. Dangol et al. (2023) reported the effectiveness of *C. nardus* against *Aspergillus niger* and attributed its efficacy to the existence of a likely interaction among the different constituent compounds contained in the leaves. In a related research, Sawadogo et al. (2022) reported the efficacy of *C. nardus* EO in the reduction of the aflatoxin-producing fungi, *A. flavus* and *A. parasiticus*, contamination in stored maize grains. Although citral was a minor constituent, consisting of 1.46% of the EO composition of the botanical obtained in this study, it has, however, been reported by Wang et al. (2019) to exert an

inhibitory effect on the growth, mycotoxin biosynthesis, and transcriptomic profile of *Alternaria alternata* causing brown leaf spot disease in potato. This gives credence to earlier reports by Dania and Olaleye (2022) that each of the compounds contained in the EO can influence its bioactivity and the existence of a possible synergy among these compounds in enhancing the bioactivity against pathogens causing plant diseases.

Significant inhibitory action was recorded at the highest EO concentration of 0.8 µl/ml when *O. gratissimum* was used in the treatment of inoculated tubers with rot development reduction varying between 10.1 and 17.6% compared to the control with 22.8 and 40.1% rot range. Madjouko et al. (2019) reported the effectiveness of *O. gratissimum* EO against *Colletotrichum musae*, causing anthracnose disease in bananas. Similarly, Prakash et al. (2015) had used the EO of this botanical as a preservative in the management of mould, mycotoxin contamination, and oxidative reaction in harvested apple fruits. The bioactivity of thymol, which accounted for 50.52% of the EO composition of *O. gratissimum* in this study, had been reported against fumonisin production by *F. verticillioides* in the preservation of postharvest maize grains (Dambonelena et al. 2010). However, it must be emphasized that there is usually an interplay between major and trace compounds contained in EO for successful suppression of plant disease incidence.

Rot initiation was considerably reduced at 0.8 µl/ml concentration in tubers that were inoculated with *F. oxysporum* and treated with *C. sinensis* EO, and this varied between 10.2 and 18.3%, which was significantly ($p<0.05$) higher than rot values obtained in the control. This result is consistent with Anwar et al (2023), who reported the efficiency of EO extracted from peels of *C. sinensis* against three fungal pathogens, *A. flavus, A. niger,* and *A. alternata,* causing different plant diseases. Farouk et al. (2022) assessed the impact of nanoencapsulation on volatile compounds in *C. sinensis* and reported their effectiveness against *Penicillium* and *Fusarium* species. Bioactivity of limonene, which was found as the major constituent of *C. sinensis* EO in this study, has been reported against postharvest pathogens causing biodeterioration of tomato fruits (Magalhães et al. 2020).

It was observed in this study that the efficacy of the EOs increased at higher concentrations, suggesting that an optimal dosage needs to be established before embarking on their application. Also, it was noted that terpenoids and aromatic groups, particularly phenolic compounds, were most prevalent among the EO constituents of the botanicals. Antonioli et al. (2020) reported that the bioactivity of botanicals against plant pathogens may be directly related to the structural composition of the volatile compounds. It has also been hypothesized that phenolic compounds have a wide spectrum of action against plant pathogenic fungi (Obeidat 2018; Kamel et al. 2022). The antimicrobial potential of EOs may also be attributed to the alteration that takes place in many of the enzymatic processes that are associated with the production of energy and synthesis of cellular constituents (Bellik et al. 2019). It has been reported that the movement of phenolic compounds containing oils and their subsequent interaction with the cell membrane may lead to cellular enzymes and proteins initiating a reverse transport of protons. This action by the secondary metabolites drastically reduces cellular metabolic activity, leading to lysis and likely death of the pathogen. (Madjouko et al. 2019; Poudel et al. 2021). Also, it has been suggested that the bioactivity of EOs could be enhanced by the occurrence of aromatic nucleus and a phenolic hydroxyl anion that usually react and produce hydrogen bonds in the active sites of target enzymes. This process eventually culminates in the immobilization and inactivation of fungal enzymes, causing biodegradation of infected plant tissue with a consequential reduction in disease incidence (Cofelice et al. 2021; Muala et al. 2021). Citrus EO has been reported to possess the capacity to produce reactive oxygen species which can eliminate pathogens by causing cell wall porosity with alteration of the structural components thereby leading to a burst of the protective outer membrane and out flow of the cell constituents (Anwar et al. 2023).

Conclusion

Findings from this study showed the efficacy of EOs obtained from *C. nardus*, *O. gratissimum*, and *S. sinensis*, which had a significant reduction of *Fusarium* soft rot in inoculated but treated tubers and their prospects as biofungicides in the management of the disease in yams. However, further research needs to be conducted on the formulation, stabilization, and evaluation of the possible existence of undesirable effects on the flavour or odour of treated yam tubers.

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Conflict of interest

Conflict of interest: The Authors declare that they have no conflict of interest in the conduct of this research.

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تقييم الزيوت العطرية في المكافحة الحية لمرض عفن درنات الفيوزاريوم في نبات اليام الأبيض (Dioscorea rotundata Poir)

Obiora Albert Onwuta [1, 2] and Victor Ohileobo Dania[1*]

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria
[2]Department of Agricultural Education, Federal College of Education 9Technical), Akoka, Lagos State, Nigeria

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ملخص

ليام هو محصول اقتصادي مهم يزرع على نطاق واسع في أفريقيا والقارات شبه الاستوائية الأخرى. ومع ذلك، فإن زراعته مقيدة بخسائر كبيرة في محصول ما بعد الحصاد في الدرنات والبذور المخصصة للزراعة في الموسم التالي بسبب العفن الناجم عن الميكروبات. تم تقييم الزيوت العطرية (EOs) لثلاثة نباتات، Cymbopogon nardusو Ocimum gratissimumو Citrus sinensis من حيث تركيبها الكيميائي النباتي وفعاليتها في إدارة مرض العفن اللين Fusariumفي اليام الأبيض .(Dioscorea rotundata Poir) تم تحليل المكونات الكيميائية وتحديد كميتها باستخدام كروماتوغرافيا الغاز–مطياف الكتلة (GC–MS) وكروماتوغرافيا الغاز–كاشف التأين اللهبي .(GC–FID) كان التصميم التجريبي للتجربة الحية عبارة عن عامل 15 × 3 × 3 في تصميم عشوائي تمامًا مع ثلاث مكررات، والتي تضمنت خمسة عشر عزلة من Fusarium oxysporum وثلاثة زيوت عطرية وثلاثة تركيزات. كانت المركبات الرئيسية الموجودة في الزيوت العطرية لـ C. nardus و O. gratissimumو C. sinensisهي جيرانيول (51.73٪) والثيمول (50.52٪) والليمونين (45.84٪) على التوالي. انخفض تطور العفن في الدرنات الملقحة والمعالجة بشكل ملحوظ ($p < 0.05$) بنسبة 9.3 –16.4٪ و10.1–17.6٪ و10.2–18.3٪ على التوالي عند تركيز 0.8 مل / مل من الزيوت العطرية. تشير فعالية الزيوت العطرية في هذه الدراسة إلى إمكاناتها كمبيدات حيوية للفطريات في إدارة مرض العفن اللين فيوزاريوم في البطاطا البيضاء.

الكلمات الدالة: سيمبوبوغون ناردوس، فيوزاريوم، جيرانيول، في الجسم الحي، مرض العفن الطري.

* الباحث المعتمد للمراسلة: victorohileobo@gmail.com