

Effects of Fungal Biodeterioration on Food Values (Qualities) of Carrot and Fungicidal Effects of Plant Oils of *Cymbopogon citratus*, *Cananga odorata* and *Hyptis suaveolens* on Rot Pathogens of Carrots

Ijato JY^{1*}, Ajisafe TJ¹ and Ojo BO²

¹Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, P.M.B 5363, Ado-Ekiti, Ekiti State, Nigeria.

²The Polytechnic, Ibadan, Department of Biology, P.M.B. 22, U.I. Post Office, Ibadan, Oyo State, Nigeria.

*Correspondence:

Ijato JY, Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, P.M.B 5363, Ado-Ekiti, Ekiti State, Nigeria.

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ABSTRACT

This study was carried out to determine the effects of fungal infection on nutritional qualities of carrot and also to evaluate the fungicidal effects of some essential oils extracts on fungal rot pathogens of carrot. Carrot samples were gotten from Ado-Ekiti market and the test plant samples namely; *Cymbopogon citratus* (Tea leaf), *Cananga odorata* (Ylang Ylang) and *Hyptis suaveolens* (Pignut) as sources of test plant oil were gotten from a garden in Ado-Ekiti, the fungal rot pathogens were isolated from rotten carrot and pathogenicity of the fungal isolates established. The nutrient compositions of both the healthy and fungal pathogens infected carrots were assayed. The infected carrots showed a reduction in the nutrient composition compared with the fresh and healthy carrot. The test plant samples were thoroughly washed in running tap water, washed once in sterile water, and shade dried in aseptic condition. The samples were dried for 15 days under room temperature (28 °C). The dried samples were then separately pulverized using an electric blender (Model M 20 IKA Universal Mill, IKA Group Japan). Five hundred grams (500g) of each ground sample was weighed into a filter paper folded together and these were tied with a thread to avoid being volatile. This was separately packed into the extractor chamber of Soxhlet extractor setup. The results of proximate analyses of infected carrot with various fungal rot pathogens showed that *Sclerotinia sclerotiorum* (83.10%) and *Aspergillus niger* (82.60%) infected carrot exhibited highest percentage moisture content while *Sclerotinia sclerotiorum* (13.82%) and *Mucor sp* (13.35%) infected carrot had highest crude fiber, ash of infected carrot was mostly recorded by *Sclerotinia sclerotiorum* (4.80%) and *Rhizopus sp* (4.70%), fat content was highest by *Rhizopus sp* (1.23%) infected carrot, highest crude protein of infected carrot was mostly exhibited by *Penicillium species* (13.43%) and *Rhizopus sp* (13.01%) infection, *Penicillium species* (17.87%) and *Aspergillus niger* (17.07%) infected carrot recorded highest value of carbohydrate. Carrot infected with *Sclerotinia sclerotiorum* had the highest moisture content (83.10%), crude fiber (4.80%) and Ash (4.80%). All the oil extracts had considerable fungicidal effects on all the fungal pathogens, *Cymbopogon citratus* oil extract possessed high fungicidal activity on the isolate. Oil extract of *Cymbopogon citratus* at 100.0/mL mostly inhibited *Rhizopus spp* (47.05%) and *Aspergillus niger* (46.15%), oil extract of *Cananga odorata* at 100.0/mL mostly inhibited *Aspergillus niger* (45.15%) and *Rhizopus spp* (45.00%) while oil extract of *Hyptis suaveolens* at 100.0/mL mostly inhibited *Rhizopus sp* (43.20%) and *Aspergillus niger* (43.10%).

Keywords

Biodeterioration, Food values (qualities), Fungicidal effects, Plant oils, Carrots pathogens rot.

Introduction

Carrot, *Daucus carota* L, is an edible taproot, belongs to the family Apiaceae (Umbelliferae) which other vegetables such as celery;

celeriac, parsley and other herbs are members. Carrot consumption is popular due to the pleasant flavour and health benefits accruing from the presence of carotenes, vitamins, minerals and fibre. The most abundant pro-vitamin A precursor is β -carotene [1,2], often exceeding 50% of the vitamin A content. Epidemiological studies have indicated that human consumption of foods rich in carotenoids and antioxidants lead to diminished risk against cardiovascular diseases and some forms of cancer [3]. Despite the nutritional importance of carrots, production levels in smallholdings are stagnant or declining. Some of the major problems in carrot production are sporadic and delayed seedling emergence leading to low population and resultant root yield [4]. Over the past two decades, many researchers from a number of developed countries have been screening wild plants for their potential antimicrobial, pesticidal, herbicidal and biostimulatory properties [5]. The use of crude extracts from wild plants for carrot plant wellness have exhibited either plant growth stimulatory, pesticidal or herbicidal activity [6-9]. Other advantages of plant extracts include their low toxicity to fauna and short life span in the environment. Plant secondary metabolites are synthesized by higher plants; these compounds do not seem to have a recognized role in primary plant processes but are important in the interaction of the plant with the environment [10]. Secondary metabolites have been extensively studied over the past two decades and have been ascribed many functions including their allelopathic effect on other plants [11], their herbicidal [9], insecticidal [7,8], antimicrobial [12,13] and bio-stimulatory properties [6,14].

Materials and Methods

Samples Collection

Carrots that showed symptoms of rot were randomly selected from different market places at Oba Market, Ado Ekiti. The samples were collected and kept separately in sterile polythene bags and brought to the laboratory of Microbiology Department, Ekiti State University, Ado-Ekiti. The plants fruits were identified and authenticated at the herbarium unit of Ekiti State University, Ado-Ekiti.

Plant Materials

Fresh samples of *Cymbopogon citratus*, *Cananga odorata* and *Hyptis suaveolens* were collected from a garden at Ado Ekiti, Ekiti State. The samples were identified and authenticated at the herbarium unit of Ekiti State University, AdoEkiti.

Preparation of Plant Extracts

Fresh samples of three test plants namely: *Cymbopogon citratus*, *Cananga odorata* and *Hyptis suaveolens* were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried without any contamination. The samples were air dried for 15 days under room temperature (28°C). The dried samples were then separately ground into fine powder using an electric blender (Model M 20 IKA Universal Mill, IKA Group Japan).

Extraction of Plant Materials

Five hundred grams of each ground samples of test plants were weighed into a filter paper folded together and tied with a thread.

It was separately packed into the extractor chamber of Soxhlet extractor setup, which was set to temperature 50°C. The samples were separately extracted with ethanol which is a solvent, this solvent evaporate and moves up to the condenser where it was converted back to liquid and move slowly into the extraction chamber through the loaded sample and back to the boiling flask.

The solvent was heated to reflux and the solvent vapour move up to the distillation arm and floods into the chamber housing the sample. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm. After about four rounds, the boiling flask containing the solvent and extracted metabolites was removed, placed in the rotatory evaporator where there was separation of the oil and the solvent (ethanol) in which the metabolites was collected into a cleaned bottle.

Preparation of the Media

Thirty-nine grams of potato dextrose agar (PDA) was weighed and dissolved into 1000 mL of distilled water. This was boiled to dissolve completely and then sterilized in an autoclave at 121°C at a pressure of 1.05 kg/cm² for 15 minutes. It was allowed to cool for about 45°C and then poured into sterile Petri dishes to solidify.

Isolation of Associated Fungi

The diseased samples of carrots were first surface sterilized by washing under running tap water to remove dirt such as sand. A flamed blade was used to cut partly diseased and partly healthy portion of the sample, the cut portions were then surface sterilized using 70% alcohol after which they were rinsed in successive changes of sterile distilled water. They were then inoculated on potato dextrose agar (PDA). And the plates were incubated at 28°C. Fungal growth was observed daily. After five days of incubation, a small portion of mycelium from each fungal colony was transferred aseptically unto fresh plates containing the medium used. The fungi were purified by repeated sub-culturing.

Identification of Isolated Fungi

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with $\times 10$ and $\times 40$ objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Adebayo-Tayo et al., [15].

Pathogenicity of the Isolated Fungi

Fresh and healthy was washed from the carrot with tap water and the sterilized superficially with 0.1% sodium hypochlorite solution 2-3 Minutes. Then, the carrots were washed with water until the odor disappears, and the cylindrical cores of the tubers

are removed in diameter 5mm by Cork borer. Different fungal isolates were inserted into puncture and closed with paraffin wax to prevent spores from spreading out. Control was used without fungal isolates. Treatment tubers were placed separately in sterile polythene bags and incubated at $27 \pm 2^\circ\text{C}$ for 10 days. Samples are examined from time to time and the appearance of rot is observed until the end of the incubation time [16].

Antifungal Activities of Test Plant Oils

Antifungal activities of oil extracts from each of *Cymbopogon citratus*, *Carnagal odorata* and *Hyptis suaveolens* were determined by agar well diffusion modified method. The fungal isolates used in this study were cultivated on potato dextrose agar at 26°C for 48 to 72 hr. Aliquot of culture (100 μl) was evenly spread on the surface of the solidified agar. Wells of 7mm were bored in the agar with sterile Cork borers. The three extracts were separately dissolved in 10% dimethyl sulfoxide (DMSO) to the concentration of 25-100 mg/mL and filtered through 0.22 μm membrane filter. A 100 μl volume of the extracts from each concentration were placed in each well. The plates were incubated at 26°C for 48 to 72 hr. Nystatin was used as a standard antifungal agent. The diameters of the inhibition zones were measured in millimeters. Inhibition zones were measured in triplicates (three plates per indicator organism). Agar well in which 10% DMSO was added served as negative control. The inhibitory action of negative control was not visible.

Proximate Analysis

The proximate composition was determined according to AOAC,

i. Moisture content determination

Two grams of each of the sample(s) were placed in an oven maintained at 100 - 103°C for 16 hours with the weight of the wet sample and the weight after drying noted. The drying was repeated until a constant weight was obtained. The moisture content was expressed in terms of loss in weight of the wet sample.

$$\% \text{ moisture content} = \frac{\text{weight of moisture}}{\text{weight of sample}} \times 100$$

ii. Ash content determination

Two grams of each of the oven-dried samples in powder form were accurately weighed and placed in crucible of known weight. These were ignited in a muffle furnace and ashed for 8 hr at 550°C . The crucible containing the ash was then removed, cooled in a desiccator and weighed and the ash content expressed in term of the oven-dried weight of the sample.

$$\% \text{ Ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

iii. Protein content determination

The protein nitrogen in 1g of the dried samples each was converted to ammonium sulphate by digestion with concentrated H_2SO_4 and in the presence of CuSO_4 and Na_2SO_4 . These were heated and the ammonia evolved was steam distilled into boric acid solution. The nitrogen from ammonia was deduced from the titration of the

trapped ammonia with 0.1M HCl with Tashirus indicator (double indicator) until a purplish pink color was obtained. Crude protein was calculated by multiplying the value of the deduced nitrogen by the factor 6.25mg.

iv. Crude fibre content determination

Two grams of each sample was weighed into separate beakers, the samples were then extracted with petroleum ether by stirring, settling and decanting 3 times. The samples were then air dried and transferred into a dried 100mL conical flask. 200cm³ of 0.127M sulphuric acid solution was added at room temperature to the samples. The first 40cm³ of the acid was used to disperse the sample. This was heated gently to boiling point and boiled for 30 minutes. The contents were filtered to remove insoluble materials, which was then washed with distilled water, then with 1% HCl, next with twice ethanol and finally with diethyl ether. Finally the oven-dried residue was ignited in a furnace at 55°C . The fibre contents were measured by the weight left after ignition and were expressed in term of the weight of the sample before ignition.

v. Fat content determination

The lipid content was determined by extracting the fat from 10g of the samples using petroleum ether in a Soxhlet apparatus. The weight of the lipid obtained after evaporating off the petroleum ether from the extract gave the weight of the crude fat in the sample.

vi. Carbohydrate content determination

The carbohydrate content of the samples were determined as the difference obtained after subtracting the values of protein, lipid, ash and fibre from the total dry matter.

Data Analysis

The data were analyzed using the PROC ANOVA procedure of GENSTAT version 15 and significant differences among the means and compared using Fisher's protected LCD at 5% probability level. Linear regression analysis was performed to establish any correlations among different concentrations of the essential oil and their antifungal activity.

Results

The results of proximate analysis of infected carrot with various fungal rot pathogens showed that carrot infected with *Sclerotinia sclerotiorum* had the highest Ash (4.80%), moisture content (83.10%) and crude fiber (4.80%). Both *Sclerotinia sclerotiorum* (83.10%) and *Aspergillus niger* (82.60%) exhibited highest percentage moisture content on infected carrot while highest crude fiber was noticed with *Sclerotinia sclerotiorum* (13.82%) and *Mucor* sp (13.35%) infected carrot, carrot infected with *Sclerotinia sclerotiorum* (4.80%) and *Rhizopus* sp (4.70%) had the highest ash, carrot infected with *Rhizopus* sp (1.23%) had highest fat content, crude protein of infected carrot was mostly noticed by *Penicillium* species (13.43%) and *Rhizopus* sp (13.01%) infection, *Penicillium* species (17.87%) and *Aspergillus niger* (17.07%) infected carrot exhibited highest value of carbohydrate.

Effects of different concentrations of oil extract from *Cymbopogon citratus* on mycelial growth inhibition of isolated fungi from spoiled carrot indicates that the oil extract possess fungicidal activity on the isolated fungi (Table 2). All the oil extracts had considerable fungicidal effects on all the fungal pathogens, *Cymbopogon citratus* oil extract possess high fungicidal activity on the isolate. Oil extract of *Cymbopogon citratus* at 100.0/mL mostly inhibited *Rhizopus* spp (47.05%) and *Aspergillus niger* (46.15%).

Effect of different concentrations of oil extract from *Cananga odorata* on isolated fungi from spoiled carrot is shown (Table 3), fungicidal effect of oil extract of *Cananga odorata* at 100.0/

mL was mostly expressed against *Aspergillus niger* (45.15%) and *Rhizopus* spp (45.00%).

Effect of different concentrations of oil extract from *Hyptis suaveolens* on isolated fungi from spoiled carrot is shown (Table 4).

Discussion

Al-Hindi et al., [7] considered toxigenic or pathogenic fungi isolated from spoiling fruits and vegetables as causes of food spoilage. Efiuvwevwe et al., [18] reported prevalence of fungi as the rot organisms of fruits and vegetables which are due to a wide range of factors that are encountered at each stage of handling from

Table 1: Result of proximate analysis of infected carrots.

Parameters (%)	Control	Sample code				
		A	B	C	D	E
Moisture content	80.76a	81.23a	83.10bc	82.60d	81.51c	80.35a
Crude fiber	12.81a	12.90c	13.82d	12.06b	12.20b	13.35bc
Ash	4.54b	4.56bc	4.80c	4.40a	4.70a	4.66c
Fat content	0.16b	0.28a	1.06b	0.21c	1.23c	1.08a
Crude protein	12.81bc	13.43ab	12.27c	12.87d	13.01c	12.61d
Carbohydrate	16.06a	17.87c	16.03b	17.07b	16.74c	16.65bc

Key: A = *Penicillium* species; B = *Sclerotinia sclerotiorum*; C = *Aspergillus niger*; D = *Rhizopus* sp; E = *Mucor* sp.

Table 2: Effect of different Concentrations of oil extract from *Cymbopogon citratus* on Isolated Fungi from carrot Test organisms.

Fungal isolates from rotten carrots	Concentration (%) of <i>Cymbopogon Citratus</i> Oil Extract				
	Control (DMSO)	12.5/mL	25.0/mL	50.0/mL	100.0/mL
<i>Penicillium</i> species	0.00±0.00a	13.3±3.43b	15.15±1.22b	28.15±3.21bc	39.0±2.02c
<i>Sclerotinia sclerotiorum</i> .	0.00±0.00a	25.0±0.00b	34.25±2.05c	39.15±0.00bc	40.3±0.00d
<i>Aspergillus niger</i>	0.00±0.00a	15.1±2.21b	38.25±0.04c	41.35±3.15d	46.15±0.55e
<i>Rhizopus</i> sp	0.00±0.00a	15.4±3.25b	28.30±0.00c	42.20±0.81d	47.05±0.00e
<i>Mucor</i> sp.	0.00±0.00a	2.30±0.00b	15.35±3.62c	26.25±0.00c	32.25±2.12d

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at $P < 0.05$.

Table 3: Effects of different Concentrations of oil extract from *Cananga odorata* on Isolated Fungi from carrot Test.

Fungal isolates from rotten carrots	Concentration (%) of <i>Cananga odorata</i> oil extract				
	Control (DMSO)	12.5/mL	25.0/mL	50.0/mL	100.0/mL
<i>Penicillium</i> species	0.00±0.00a	13.25±3.43b	15.15±1.22b	25.15±3.21bc	40.00±2.02c
<i>Sclerotinia sclerotiorum</i> .	0.00±0.00a	25.10±0.00b	37.25±2.05c	40.15±0.00bc	42.25±0.00d
<i>Aspergillus niger</i>	0.00±0.00a	15.15±2.21b	32.15±0.04c	42.30±3.15d	45.15±0.55e
<i>Rhizopus</i> sp	0.00±0.00a	15.10±3.25b	28.30±0.00c	43.25±0.81d	45.00±0.00e
<i>Mucor</i> sp.	0.00±0.00a	2.25±0.00b	25.30±3.62c	28.3±0.00c	31.25±2.12d

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at $P < 0.05$.

Table 4: Effects of different concentrations of oil extract from *Hyptis suaveolens*. Fungitoxicity of oil extract of *Hyptis suaveolens* at 100.0/mL mostly potent against *Rhizopus* sp (43.20%) and *Aspergillus niger* (43.10%).

Fungal isolates from rotten carrots	Concentration (%) of <i>Hyptis suaveolens</i> oil extract				
	Control (DMSO)	12.5/mL	25.0/mL	50.0/mL	100.0/mL
<i>Penicillium</i> species	0.00±0.00ab	12.10±3.43b	15.10±1.22c	25.15±3.21b	38.20±2.00c
<i>Sclerotinia sclerotiorum</i> .	0.00±0.00ab	22.25±0.0a	33.20±2.05c	38.20±0.00b	40.20±0.00
<i>Aspergillus niger</i>	0.00±0.00ab	17.00±2.21a	30.25±0.04c	40.25±3.15d	43.10±0.55e
<i>Rhizopus</i> sp	0.00±0.00ab	12.20±3.25a	30.00±0.00c	39.20±0.81d	43.20±0.00e
<i>Mucor</i> sp.	0.00±0.00ab	18.10±0.00a	23.20±3.62c	30.10±0.00c	35.10±2.12

Values are expressed as means ± SEM (Standard error of means). Means having different superscripted alphabets along columns are significantly different at $P < 0.05$.

pre-harvest to consumption and are related to the physiological and physical condition of the produce as well as the extrinsic parameters to which they are exposed. High moisture and relative humidity led to greater fungal growth in farm produce which tends to lower the storability of fruits and vegetables Efiuvewwere et al., [18].

All the isolated fungi species in this study have been found to cause spoilage of the carrots; this is congruent with findings of [19,20]. It can be deduced from this study that *Aspergillus niger*, *Sclerotinia sclerotium*, *Penicillium species*, *Mucor sp.*, and *Rhizopus sp* were associated with spoilage of carrots sold in Ado Ekiti. These results obtained from this study largely corroborated the results of, Ewekeye et al., [21] in Nigeria, Khatoon et al., [16] in India Kurt et al., [22] in Turkey and Elsayed et al., [23] in Saudi Arabia that isolated these spoilage fungi from carrot. Similarly, Baiyewu et al., [19]; Chukwuka et al., [20] reportedly isolated these fungal species from pawpaw fruits in Nigeria. The isolation of *Aspergillus niger*, *Penicillium species* from carrot in this study is also congruent with findings of Efiuvewwere, et al., [18] who reported that *Aspergillus niger* has been responsible for the pineapple fruit rot. The isolation of *Rhizopus spp.* and *Mucor spp.* from carrot in this study agreed with work of [18].

The fungi isolated in this study have been reported to produce secondary metabolites in plants tissues. These secondary metabolites are potentially harmful to humans and animals [19]. A good example is Aflatoxin which has been associated in cancer of the liver (hepatoma), aflatoxicosis and also with acute hepatitis in humans, especially in the developing countries. Pathogenic fungi, on the other hand, could cause infections or allergies [24]. *Aspergillus niger* are known to produce several toxic metabolites, such as malformins, naphthopyrones [25] and they can produce Ochratoxins (OTA), a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health [26] thus extra care should be taken during personnel handling of these fruits; such as harvesting, cleaning, sorting, packaging, transport and storage.

Results have shown the antifungal activities of the botanical oil extracts of *Cymbopogon citratus*, *Cananga odorata* and *Hyptis suaveolens* on the common isolated fungi from carrot. The results revealed that the oil extracts was dose independent. This result is supported by the past researchers on the antimicrobial effects of certain plant based extracts [27].

Cymbopogon citratus oil extracts significantly inhibited most of the fungi at higher concentration (100 mg/mL) with highest effects on *Aspergillus species*. Shafique et al. [28] reported total inhibition of some fungi a *C. citratus* essential oil, with an IC50 of 279.13 µL/L, as also reported by [29]. The antifungal activity of *C. citratus* essential oil has also been reported against other fungi, including *Aspergillus niger*. These activities can be ascribed to the presence of various components such as citral, geraniol, and β- myrcene [30]. According to some studies, citral and geranol can indeed inhibit the mycelium growth of *Fusarium oxysporum*,

Colletotrichum gloeosporioides, *Bipolaris sp.* and *A. alternata* [31].

Also, the results also showed that oil extracts from *H. suaveolens* can be used effectively to inhibit the growth of fungi associated with post-harvest bio-deterioration of carrots. Thus, *H. suaveolens* possesses antifungal properties as reported by Okonogi et al., [32] and Sharma et al., [33]. Antifungal activities observed in the present study corroborates the works of Parichad and Krittaporn et al., [34] who found that oil extracts of *H. suaveolens* possess antimicrobial properties. *Mucor sp* and *Aspergillus niger* was more sensitive to the inhibitory effect of the oil extracts of *H. suaveolens*. Sharma et al., [33] had made a similar observation, where it was suggested that various extracts from *H. suaveolens* showed better antifungal activity against *A. niger* when compared with other fungi. The inhibitory effect exerted by 50 and 100mg/mL extract concentrations on mycelia growth of the tested fungi were higher than that caused by other lesser concentrations (12.5 and 25 mg/mL). This agreed with the report of Babu et al., [34] who observed higher inhibition of fungal growth at higher concentrations of plant extracts.

Also, the results indicated that the tested oil extract of *Cananga odorata*, like other test plant oils, caused a significant reduction in the radial growth of the pathogens. The radial growth and spore of germination of *Fusarium oxysporum* was reported to be inhibited by extracts of *Cananga odorata* [36].

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