



## Effect of crude essential oils of clove seed on some soil-borne fungal pathogens of *Telfairia occidentalis*

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### Abstract

This study examined the effect of crude essential oils (EOs) of clove seeds on some soil-borne fungal pathogens of *T. occidentalis*. Aqueous extraction method of 20g crude EOs from clove seed was adopted, and it was replicated to 100%, 50% and 25% concentration levels. The fungal isolates identified were *Rhizoctonia* spp, *Fusarium* spp, *Candida* spp and *Aspergillus* spp. The infected soil had more occurrence of soil-borne fungal pathogen than healthy soil. All the fungal isolates were inhibited mostly at higher EOs concentration. Visual assessment method of percentage disease incidence of *T. occidentalis* revealed that leaf spot (51.25%), rust (30.00%), damping off (8.75%), chlorosis (6.25%) and necrosis (3.75%) occurrences. Crude extracts of clove seed EOs exhibited antimicrobial effect on the soil-borne fungal pathogen of *T. occidentalis* and can be used as biofungicide to improve agro-practices to ensure sustainable production of vegetable.

**Keywords:** Clove seed, essential oils, *t. occidentalis*, fungal pathogens, aqueous extraction

### Introduction

*Telfairia occidentalis*, commonly referred to as fluted pumpkin, is a leafy vegetable widely cultivated in West Africa, particularly Nigeria for its nutritional, medicinal, and economic importance (Schippers, 2000; Adebooye *et al.*, 2006) [1, 21]. It is appreciated for its nutritional value, with high levels of iron, vitamins A and C, protein, and essential amino acids (Akoroda, 1990) [4]. It belongs to the family Cucurbitaceae and is valued for its edible leaves, seeds, and medicinal uses in traditional medicine. Despite its significance, the crop is highly susceptible to several soil-borne fungal pathogens, which adversely affect its growth, yield, and overall quality (Martin and Loper, 1999; Agrios, 2005; Amadi and Oso, 2006; Ogar and Nwankiti, 2014) [2, 6, 13, 14]

Soil-borne fungal pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium* spp., and *Scierotium rolfsii* are commonly associated with diseases like root rot, damping-off, and wilting in *T. occidentalis*. These pathogens are persistent in the soil and often spread through contaminated water, tools, and infected plant residues. Their impact can lead to significant yield losses, reduced market value, and increased cost of production due to the need for chemical control (Agrios, 2005; Eziashi *et al.*, 2006) [2, 10].

Clove (*Syzygium aromaticum*) is a medicinal plant known for its potent antifungal, antibacterial, antioxidant, and anti-inflammatory properties. Its essential oil is extracted primarily from clove buds and seeds and is rich in active compounds like eugenol, caryophyllene, and eugenyl acetate. Eugenol, the major constituent (up to 85–90%), has been extensively documented for its ability to disrupt fungal cell membranes, inhibit spore germination, and reduce mycelial growth (Chaieb *et al.*, 2007) [9].

Several studies have demonstrated the antifungal efficacy of clove oil against a range of soil-borne pathogens: Clove oil inhibited *Fusarium oxysporum* and *Rhizoctonia solani* in *in vitro* studies (Pramila *et al.*, 2012) [19]. Its application reduced disease severity and increased plant vigor in various vegetable crops (Pandey *et al.*, 2017) [17]. It acts through

mechanisms such as altering fungal membrane permeability and causing cytoplasmic leakage (Bakkali *et al.*, 2008) [8]. Given the pressing need for safer alternatives to chemical fungicides, and the increasing interest in organic farming, the application of clove seed essential oil offers a promising solution for the management of fungal pathogens affecting *T. occidentalis*. The oil's bioactivity, biodegradability, and low toxicity to humans make it suitable for use in sustainable agriculture (Tripathi *et al.*, 2008) [24].

Studies have shown that clove oil exhibits strong antifungal activity against various plant pathogens, including species of *Fusarium*, *Aspergillus*, and *Pythium* (Singh *et al.*, 2011; Mahmoud, 2013) [22]. For instance, Okigbo and Ogbonnaya (2006) [15] reported significant inhibition of *Fusarium* spp. using clove oil in *in-vitro* conditions. However, research on the direct application of clove essential oil to manage soil-borne fungal pathogens in *T. occidentalis* is limited, despite the crop's susceptibility and the potential benefits of this natural product.

Fungal diseases are primarily managed with synthetic fungicides. While effective in the short term, these chemicals present challenges, including the development of resistant fungal strains, environmental pollution, health hazards to humans, and negative effects on non-target organisms (Tripathi and Dubey, 2004) [23]. As a result, there were and is a growing demand for environmentally sustainable safer alternatives and cost effective control agent for the management of soil borne fungal infection/diseases of plant and especially *T. occidentalis* for supplementary income and reduced food costs (Adebooye *et al.*, 2006; Akinyele and Akinyosoye, 2005) [1, 3].

Chaieb *et al.* (2007) [9], reported that eugenol, which makes up 70–90% of CSEO, disrupts fungal cell membranes, leading to leakage of cellular contents and inhibition of growth. Pandey *et al.* (2017) [17] showed that clove oil reduced the severity of anthracnose and powdery mildew on treated plants, especially when applied as nanoemulsions. Amadi *et al.* (2012) [7] reported that clove oil showed a clear zone of inhibition and significantly reduced spore

germination and radial growth of *F. oxysporum* in the poisoned food assay. Oyewole and Bolarinwa (2012) [16] found that *F. oxysporum* was highly susceptible to clove extract, achieving complete inhibition (100%) at 2% concentration. Amadi *et al.* (2012) [7] concluded that clove oil was equally effective as carbendazim and safer for non-target organisms.

Ali-Shtayeh *et al.* (2003) [5] reported that clove oil's fungitoxicity is comparable to mancozeb, especially when integrated with organic farming practices. Okigbo and Ogbonnaya (2006) [15] found that clove extract suppressed *Sclerotium* more consistently than captan, especially when used preventively.

Given the increasing need for sustainable agriculture and the known bioactivity of clove seed oil, this study seeks to assess the effectiveness of crude clove essential oil in controlling key soil-borne fungal pathogens affecting *T. occidentalis*. The work was aimed at examining the effect of crude essential oils from clove seeds on some soil-borne fungal pathogens of *T. occidentalis* while the findings may provide a basis for developing safer, plant-based disease management strategies suitable for organic farming.

## Materials And Methods

### Study Area

These experiments were carried out at the Department of Plant Science and Biotechnology, Rivers State, Rivers State, Nigeria. The area lies at approximately latitude 4.797°N and longitude 6.979°E.

### Materials Used for the Experiment

Materials used for this research work were; sample collection polythene bags, hand gloves, test tubes, 90ml disposable sterile Petri dish, inoculating needle, spirit lamp, autoclave, test tube, aluminum foil, paper tape, ethanol, conical flask, Beaker, Sabouraud Dextrose Agar (SDA), micro pipette, tetracycline (antibiotics), pipette tip, Cork borer, cotton wool, weigh balance, measuring cylinder, conical flask, healthy and infected *T. occidentalis* soil and clove seeds.

### Sample Collection

Diseased and healthy soil from *T. occidentalis* was collected from Department of Geology opposite Faculty of Law, Rivers State University. The sample was then taken to the Department of Plant Science and Biotechnology for onward research.

### Preparation of Media

Sabouraud Dextrose Agar (SDA, 36g) was measured into a 1000ml of sterile conical flask. Thereafter 900ml of distilled water was added and shaken for 10 minutes. This was autoclaved for 15 minutes at 121°C, it was allowed to cool for 5 minutes before taken out of the autoclave and allowed to cool to about 45°C before adding 250g of tetracycline for the inhibition of fungal growth. The SDA was then poured into disposable sterile Petri dish and swirled gently and was allowed to solidify before inoculation of the *T. occidentalis* soil. The same process was repeated for fungal isolate.

### Preparation of Normal Saline solution and Serial Dilution

A stock solution of 500ml of water and 4.25g of NaCl was prepared. Micropipette was used to measure 1 of saline solution into 20 test tubes and was corked with cotton wool. The test tubes alongside micropipettes were sterilized for 15

minutes at 121°C. The sterilized test tube was allowed to cool down at 40°C. Test tubes were arranged in the test tube racks and were labeled ( $10^{-1}$ ,  $10^{-2}$  &  $10^{-3}$ ). A spatula was used to pick the soil sample into the first test tube ( $10^{-1}$ ) and was shaken vigorously to mix with the sample in it. 1ml of saline solution was pipetted from the first test tube ( $10^{-1}$ ) into the second test tube ( $10^{-2}$ ) and properly mixed. 1ml was also measured from the ( $10^{-2}$ ) into the third test tube ( $10^{-3}$ ) and was properly mixed with the pipette making it a threefold dilution. 0.1ml of each saline solution was inoculated into the plate (three replicates for each concentration).

### Extraction of Crude Essential Oils from Clove Seeds

The work surface in the laboratory was sterilized with 95% ethanol. All working equipment were washed and sterilized with 10% hydrochloric water. Polypropylene filter cloth was soaked in a solution of 2.5 Sodium metabisulphate salt, 50ml of ethanol and 150ml of water. Clove seed was grinded using grinding machine and 20g was weighed into a beaker and 20ml of distilled water was added to help the extracting process and it was allowed to stand for about 20 minutes before filtering using polypropylene filter cloth through a funnel into a sterile sample bottle and was stored in the fridge for 24hrs. Thereafter all equipment was washed and sterilized with 10% hypochloric water.

### Isolation and Identification of Fungal Isolate

The plates were incubated at room temperature for 72 hours (3 days) for the fungal to grow. The fungal was later sub-cultured into Petri dish containing SDA in order to obtain pure culture. The fungal isolated was identified at the pathology laboratory, Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria.

### Inoculation of Fungal Isolate

Exactly 1ml, 2ml, 3ml of the crude extract from clove seed were respectively introduced into some disposable Petri dishes with the aid of a sterile syringe, after which 15ml of SDA (this time without tetracycline because the extract was presumed to inhibit fungal growth) was poured into the petri dish, swirled gently and was allowed to cool and gel. The isolates were inoculated into the surface of the medium using 5mm Cork borer. The treatment of various quantities was in triplicate, including their control experiment and incubated at room temperature. Examination and measurement of the radial mycelia growth was taken using a meter rule for each of the quantities and their concentration.

### Inhibitory Potential of Crude EOs from Clove Seed

The fungal isolate was inoculated in the center of 9cm (90ml) sterile plastic Petri dish with (SDA). This was done by flaming dissecting needle, allowing to cool and the aerial mycelium of the fungal isolate was picked and aseptically placed in the center of the dish. A marker pen was used to indicate the point where the mycelium was placed. This was incubated at room temperature 25°C for 3 days. The growth rate was determined by the use of ruler to measure the vertical radial growth from the transverse of the plate.

### Determination of Number of Colony Forming Unit (NCFU) and Microbial Load of Microflora

Number of colony forming units (per unit volume) is a measure of viable micro-toxic fungal cells in a sample. It is

also used to calculate the number of CFU per unit volume (CFU/ml) of the original culture;

$$NCFU = \frac{\text{Number of Colomes}}{\text{Volume of Weight of Sample}} \times \text{Dilution Factors} \quad 3.1$$

Where  
 Number of Colonies = Number of Colonies Counted on the Plate  
 Dilution Factor = Dilution Factor Used to Prepare the Plate Culture (e.g. 10<sup>-3</sup>, 10<sup>1</sup>)  
 Volume of Culture Plate = Volume of Culture Actually Plated (0.1g, 1g)

Determination of microbial load

$$NCFU = \frac{\text{Dilution Factor}}{\text{Volume of Weight of Sample}} \times 10 \quad 3.2$$

Where  
 ML = Microbial Load  
 NCFU = Number of Colony Forming Units  
 Dilution factor = dilution Factor used to Prepare Plate Culture (e.g. 10<sup>-5</sup>)  
 Volume of sample = Volume (e.g. ml) of Original Sample

This formula takes into account of the original sample to calculate the microbial load. It also provides a more accurate calculation of microbial load considering the dilution factor and the sample volume.

**Determination of Plant Disease Symptom and Incidence**

Visual assessment method was used to describe and estimate the quantitative damage that manifested in plant leave. The percentage disease incidence was derived.

Thus

$$DI = \frac{X}{Y} \times \frac{100}{1}$$

Where

DI = Disease Incidence  
 X = Total Number of each Organism  
 Y = Total Number of all Identified Organism

**Results**

This shows the results from effects of crude essential oils of clove seeds on some soil-borne fungal pathogens of *T. occidentalis*. The result is presented as thus, fungal isolate from *T. occidentalis* soil, inhibitory potential of clove seed EOs and physical disease manifestation on *T. occidentalis*. However, fig.1 shows crude extract from clove seed.



Fig 1: Clove Seed Essential Oils

The following (*Rhizoctonia spp*, *Candida spp*, *Fusarium spp*, *Aspergillus spp*) were the soil- borne fungal isolates

Table 1: Morphology and Identification of Fungal

Isolate Code	Morphological Characteristics	Microscopic Characteristics	Fungal isolate
H <sub>2</sub>	Orange color mucerid growth	Oval shaped cells	Rhizoctonia
H <sub>3</sub>	Milk color mucerid growth	Oval shaped cells	Candida spp
H <sub>4</sub>	Milk color mucerid growth	Oval shaped cells	Candida spp
H <sub>5</sub>	Brown dusty growth with black reverse	Separate branchy hyphae columnar, no spores, no head	Aspergillus spp
H <sub>6</sub>	White laury growth with brown reverse	Separate branchy hyphae with spores	Fusarium spp

Table 2 below shows the frequency of occurrence of fungal isolate in healthy and infected *T. occidentalis* soil. The

result revealed that the fungal isolate occur more in the infected soil/ *T. occidentalis* than healthy soil.

**Table 2:** Fungal Isolate in Healthy and Infected *T. occidentalis* Soil

Isolate	Healthy Soil	Infected Soil
Rhizoctonia spp	+	++
Candida spp	+	++
Fusarium spp	+	++
Aspergillus spp	+	++

Note: moderate (+), severe (++)

Table 3 represents soil higher frequency of fungal isolates (Rhizoctonia spp, Candida spp, Fusarium spp, Aspergillus spp) than healthy soil.

All four fungi occurred severely (++) in infected soil, while only moderately (+) in healthy soil, indicating a strong correlation between fungal presence and plant health.

**Table 3:** Frequency of Soil-Borne Fungal on *T. Occidentalis*

Day	Dilution Factor (ml)	Healthy	Infected
1.	10 <sup>-1</sup>	1.5	4.0
	10 <sup>-2</sup>	2.3	3.3
	10 <sup>-3</sup>	2.5	4.0
2	10 <sup>-1</sup>	2.0	3.1
	10 <sup>-2</sup>	4.7	8.0
	10 <sup>-3</sup>	5.0	7.5

Table 4 shows microbial load or colony forming unit of infected soil of *T. occidentalis* at the various concentration (100%, 50% and 20%).

**Table 4:** Effect of EOs from Clove Seed on Fungal Zone of Inhibition

Conc. (%) / Fungal Isolate	<i>Rhizoctonia spp</i>	<i>Candida Spp</i>	<i>Aspergillus Spp</i>	<i>Fusarium spp</i>
100	25	38	17	25
50	22	28		20
25	25	25		

Table 5 shows the percentage disease incidence of *T. occidentalis* at the Department of Geology, Rivers State University. The percentage of disease symptoms on *T. occidentalis*, based on visual observation of 96 leaves. Out of these, 80 leaves showed varying symptoms of infection, while only 16 were healthy.

Leaf spot had the highest occurrence, affecting 41 leaves, which accounted for 51.25% disease incidence. This suggests that leaf spot is the most widespread and dominant symptom in the surveyed field. Its high prevalence could be due to favorable environmental conditions for fungal growth, such as humidity and poor air circulation around the leaves. Followed by rust which had 30.0%, damping-off was observed in 8.75%, Chlorosis (6.25%) and necrosis (3.75%).

**Table 5:** Survey of Disease Manifestation on *T. Occidentalis* at Department of Geology, Rivers State University

Symptom	No. of Leaf	No. of Healthy Leaf	No. of Infected Leaf	% Incidence
Chlorosis	96	16	5	6.25
Necrosis	96	16	3	3.75
Damping off	96	16	7	8.75
Leaf spot	96	16	41	51.25
Rust	96	16	24	30.00

## Discussion

The results confirmed the pathogenic significance of soil-borne fungi in *T. occidentalis* soil, disease development, and the efficacy of essential oils of clove seed as antifungal agent.

The result revealed that Rhizoctonia spp., Fusarium spp., Aspergillus spp., and Candida spp. were isolated from both healthy and infected *T. occidentalis* soils, with higher severity noted in infected soils (Table 2). This corroborates with the findings of Ogar and Nwankiti (2014) [14] who reported similar fungal profiles in diseased fluted pumpkin fields across Nigeria.

The identification of Rhizoctonia and Fusarium known agents of damping-off and root rot; is consistent with the pathogenic roles described and greater fungal diversity and higher intensity in infected soils may indicate a breakdown in the plant's resistance or soil microbial balance have been reported in relation to organic farming (Agrios 2005; Reganold *et al.* 2016) [2, 20].

The CFU counts showed higher microbial loads in infected soils across all dilution factors and days. For example, at dilution 10<sup>-3</sup> on Day 2, the infected soil had 7.5 CFU/mL compared to 5.0 CFU/mL in healthy soil. This aligns with Agrios (2005) [2] who noted that infected rhizospheres often harbor more pathogens due to decaying organic matter and suppressed beneficial microbes.

These results reflect the trend described by Van-West *et al.* (2003) [25] regarding microbial imbalances in diseased soil environments, where pathogenic fungi proliferate rapidly due to weakened host defenses or nutrient leakage from

infected roots. The elevated microbial population serves as a disease pressure indicator and supports early intervention.

Table 4 demonstrated that clove essential oil positively inhibited fungal growth, especially Aspergillus spp. (38 mm) and Candida spp. (25 mm) at 100% concentration. This validates the findings of Chaieb *et al.* (2007) [9] and Pinto *et al.* (2009) [18] that linked the antifungal activity to eugenol as the major component of the essential oils (EOs) which disrupts fungal cell membranes, leading to leakage of cellular contents and inhibition of growth. The clear zone of inhibition observed against the fungal isolates suggests that clove essential oils possess high fungicidal potentials. Pandey *et al.* (2017) [17] showed that clove oil reduced the severity of anthracnose and powdery mildew on treated plants, especially when applied as nanoemulsions. Similarly, Amadi *et al.* (2012) [7] reported that clove oil showed a clear zone of inhibition and significantly reduced spore germination and radial growth of *F. oxysporum* in the poisoned food assay and concluded that clove oil was equally effective as carbendazim and safer for non-target organisms.

The effectiveness of clove oil supports its use as a natural alternative to synthetic fungicides, particularly relevant considering the growing hazardous concerns of fungicide to humans, animals, environment and resistance of pathogens (Hahn, 2014) [12]. Additionally, studies by Ali-Shtayeh *et al.* (2003) [5] reported similar results, suggesting essential oils can disrupt fungal cell membranes and inhibit ergosterol biosynthesis.

The inhibition exhibited by clove essential oil at lower concentrations for some isolates may indicate that clove oil is effective even at low concentration. This agrees with (Oyewole, and Bolarinwa, 2012; Ghosh *et al.* 2013) <sup>[11, 16]</sup> who reported that *F. oxysporum* was highly susceptible to clove extract, achieving complete inhibition (100%) at 2% concentration and recommended critical formulation for essential oil application in agricultural system or fields. The fungal radial growth inhibition exhibited by clove essential oil at relation low concentrations on *Rhizoctonia* spp. and *Candida* spp. also indicates higher toxicity of the clove EOs to soil fungal pathogens of *T. occidentalis*. This agrees with (Ali-Shtayeh *et al.* (2003; Okigbo and Ogbonnaya 2006) <sup>[5, 15]</sup> who submitted that clove oil's fungitoxicity is comparable to mancozeb, especially when integrated with organic farming practices and found that clove extract suppressed *Sclerotium* more consistently than captan, especially when used preventively.

The field examination revealed that leaf spot had the highest occurrence, affecting 41 leaves, which accounted for 51.25% disease incidence. This suggests that leaf spot is the most widespread and dominant symptom in the surveyed field. Its high prevalence could be due to favorable environmental conditions for fungal growth, such as humidity and poor air circulation around the leaves. Next to leaf spot was rust which had 30.0% which typically affected the leaves surfaces and may reduce photosynthetic efficiency, thereby weakening the plant over time.

Damping-off was observed in 8.75% of the leaves, a condition often affecting younger plants. Though less prevalent, it is critical because it can cause sudden seedling collapse, reducing plant stand and yield potential. Chlorosis (6.25%) and necrosis (3.75%) were the least common symptoms. Chlorosis, the yellowing of leaves, may indicate early infection or nutrient deficiency, while necrosis reflects more advanced tissue damage, usually resulting in dead patches on the leaves. This trend suggests that foliar diseases are the most aggressive in *T. occidentalis*. These symptoms strongly correspond to fungal infections such as leaf spot and rust reported by Ogar and Nwankiti (2014) <sup>[14]</sup> and Amadi and Oso (2006) <sup>[6]</sup> in *T. occidentalis* that were attributed to *Fusarium* and *Aspergillus* species, of which were isolated in this study. The high incidence of leaf spot may suggest its relevance as a dominant disease symptom *T. occidentalis* under field conditions.

The visible manifestations of physiological stress, yellowing, localized tissue death and damping-off observed in *T. occidentalis* was typically associated with *Rhizoctonia* spp. and *Pythium* spp. This corresponds to (Agrios, 2005) <sup>[2]</sup> who earlier reported that chlorosis, necrosis, physiological stress and localized tissue death may be consistent with systemic fungal infections caused by *Rhizoctonia* spp. and *Pythium* spp.

## Appendix





### Conclusion

The threats posed by soil-borne fungal pathogens as have been widely reported do not only reduce yield but also undermine food security. However, this study confirmed the presence of soil-borne fungi such as *Rhizoctonia* spp., *Fusarium* spp., *Candida* spp., and *Aspergillus* spp. in *T. occidentalis* soil. The microbial load was higher in infected soils, reflecting the aggressive nature of these pathogens. Disease incidences such as leaf spot (42.7%), rust (25.0%), and damping-off (7.3%) further validated the field impact of these fungi. But clove essential oil demonstrated strong antifungal activity, especially at 100% concentration, indicating its potential as a bio-fungicide. The findings support the integration of eco-friendly disease management strategies to reduce fungal pressure and improve yield of *T. occidentalis* and encourages cultivation for large and small-scale farmers lacking access to expensive to expensive chemicals thereby increasing output.

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