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In Vitro Potency of Scent Leaf (*Ocimum gratissimum*) on fungi causing post harvest rot of Lemon fruit

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Abstract
In vitro potency of Scent leaf (Ocimum gratissimum) on fungi causing post harvest rot of lemon fruit was conducted. Two fungi namely, Aspergillus niger and Yeast were isolated from the lemon samples. Pathogenicity tests showed that the fungal isolates induced rot in healthy lemon fruits. Rot severity ranged from 4 – 5 which indicate 61 – 100% of fruit tissue damage. Analysis of Variance revealed significant differences at P = 0.05 in the disease causing potential of the fungal isolates on the lemon fruits. Water extract of O. gratissimum at 25g/ml, 50g/ml and 75g/ml was tested against the fungal isolates in vitro. The mean inhibitory effect of the plant extract at 25g/ml, 50g/ml and 75g/ml on Aspergillus niger ranged from 0.73 – 0.81. Analysis of Variance showed that there was significant difference P > 0.05 in the inhibitory effect of the extract on the test organism with respect to the control. The mean inhibitory effect of Ocimum gratissimum at 25g/ml, 50g/ml and 75g/ml on Yeast ranged from 0.76 – 0.80. Analysis of Variance showed that there was significant difference P > 0.05 in the potency of the extract on Yeast with respect to the control. The antifungal characteristics of O. gratissimum can be improved pharmacologically to improve its fungicidal attribute against pathogenic fungi of crop diseases.

Keywords: *In vitro, lemon fruit, post harvest, potency, Scent leaf.*

I. Introduction

Lemon (*Citrus limon*) belongs to the citrus family "Rutaceae". It is indigenous to northern India and widely cultivated in the Mediterranean countries. The current annual world production of citrus fruits is approximately 110 million tons [1]. In Nigeria, about 930,000 tons of citrus fruits are produced annually from an estimate of three million hectares [2]. Lemon have essential oils which are complex mixtures of chemical compounds like limonene, citral, Y-terpinene, linalool, and β-caryophyllene among others, which can be represented by three main classes, namely terpenes, oxygenates and sesquiterpenes [3]. Lemon juice contains sugar, gum and a very little potash and it is the best of all antiscorbutics. It is valuable as a cooling drink in fevers and for allaying thirst. Locally, it is a good astringent, whether as a gargle in sore throat, in prurities of the scrotum, in uterine haemorrhage after delivery or as lotion in sunburn. Despite the importance of the lemon fruit, about 30-50% gets spoilt. Post harvest diseases due to fungal infections cause significant economic losses for the citrus industry during storage, transport and marketing. Diseases that destroy the citrus fruits are through the impairment of biochemical processes resulting in the reduction of nutritional and market value of the fruits in a given environment [4].*Ocimum gratissimum* is a herbaceous perennial shrub which belong to the family "Lamiaceae". Among localities in Nigeria, it is a common spice with medicinal value, often called "scent leaf" or "clove basil". It possesses some anti microbial properties and is found in many tropical countries. Some of its vernacular names in Nigeria include "Ncho-anwu" or "Ahuji" in Igbo; "Efinrin" in Yoruba, "Aranogbo" in Edo and "Daidoyo" in Hausa. Scent leaf has numerous medical uses. The oil extracted from the leaf has been found to be active against several species of bacteria and fungi. Experiments were therefore undertaken to evaluate the in vitro potency of scent leaf on fungi causing post harvest rot of lemon fruit in Makurdi.

II. Materials and Methods

2.1 Collection of Samples: Lemon fruits with symptoms of rot were collected in polythene envelops from a store house in Lobi quarters, Makurdi and taken to the botany laboratory in the Benue State University for isolation of fungal pathogens.

2.2 Media Preparation: The medium used for isolation of fungi was Potato Dextrose Agar (PDA) which was prepared according to the manufacturer's instruction.

2.3 Isolation of Fungi: Small sizes were cut from lemon fruits infected with rot and surface sterilized by dipping in 1% sodium hypochlorite (NaOCl) solution for one minute. They were removed and rinsed in several changes of sterile distilled water then placed on sterile paper towels to dry. They were then placed on solidified Potato Dextrose Agar medium. Three replicates were made for each sample. The inoculated plates were incubated at 25 - 30^{o}C and observations were made for microbial growth. After 6 - 7 days of growth, sub culturing was done to obtain pure cultures of the isolates.

2.4 Identification of Fungi: Identification was done microscopically and macroscopically. Colony characteristics such as appearance, change in medium colour and growth rate were observed. Shape of the conidia and conidiophores were taken note of. These features were matched with standards in [5].

2.5 Pathogenicity Test: Mycelia plugs of the fungal isolates from 5 day old cultures were used to inoculate four lemon fruits per pathogen. On appearance of symptoms, the tissues at the margin of the healthy and diseased parts were excised, sterilised and placed onto Potato Dextrose Agar (PDA) and incubated at 25 - 30^{o}C for 5 - 7 days. At the end of this period, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates. One lemon fruit each was used for each fungal isolate replicated four times and arranged in completely randomized design. Controls were lemon fruits inoculated with sterile PDA only. After 8-14 days of post inoculation, rot severity index was assessed on a scale of 0 -5 where 0- no disease manifestation, 1 – 1 - 20%rot, 2 – 21 - 40% rot, 3 – 41 - 60% rot, 4 – 61 - 80%rot, 5 - 81-100% rot.

2.6 Collection of Plant Material: Fresh leaves of scent leaf (*Ocimum gratissimum) were* collected in polythene bags and transported to the botany laboratory of the Benue state University for preparation of the extract.

2.7 Preparation of Extracts: Water extracts of *Ocimum gratissimum* was prepared. The leaves were washed under running tap water and soaked in 1% of sodium hypochloride chloride for 30 seconds. They were rinsed in sterile distilled water and air dried at room temperature. 35g of the fresh leaves of *Ocimum gratissimum* was weighed for water extraction. The leaves were ground using mortar and pestle after which the macerate was transferred into a beaker containing 100ml of sterile distilled water.

2.8 Concentration of Crude Extracts: Serial dilutions of the crude extract were prepared to give different concentrations of 25g/ml, 50g/ml and 75g/ml.

2.9 Susceptibility Test: 2mls of the extract concentration was dispensed in Petri dishes after which 15 - 20mls of molten PDA was added. The Agar-extract mixture was swirled gently on the work bench to ensure even dispersion of the extracts and allowed to solidify. 4mm diameter of mycelia obtained from the edge of a five day old culture of each test fungi was inoculated centrally into the medium. Four replicates were used for each fungal isolate. Controls were Petri plates with the organism with no botanical extract. The Petri dishes were arranged in completely randomized design & incubated at 27- 30^{o}C for 5 - 7 days. Inhibition for fungal growth was calculated using the formula:

$$\frac{K1 - K2}{K1} \times 100$$

Where K_1-growth of the pathogen in control

K_2-growth of the pathogen with treatment

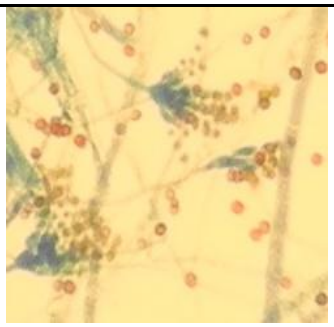
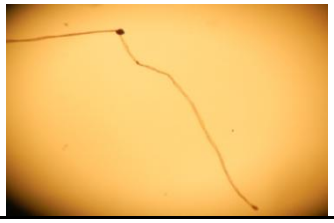
2.10 Data Analysis: Data obtained was analylized using Analysis of Variance (ANOVA) and the Fishers Least Significant Difference was used to separate the means at 5% level of significance.

III. Results

3.1 Fungi Isolated From Lemon Samples

A total of two fungi where isolated from the samples. They are *Aspergillus niger* and Yeast as shown in table 1.

Table 1: Characterization of fungi isolates from lemon samples

Macro/Microscopic appearance	Appearance on PDA	Photomicrograph	Probable organism
Colony grows fast consisting of a compact white to yellow mycelium, which bears abundant erect conidia structure. Two series of conidia bearing cells are produced.			*Aspergillus niger*
Colony is fast growing in shades of green with white margin. A germ tube protrusion growing out of the yeast cell is seen			Yeast

3.2 Pathogenicity Test: The Pathogenicity test showed that the fungal isolates induced rot in healthy lemon fruits after 8 - 14 days of inoculation. Rot severity ranged from 4 – 5 which indicated 61 – 100% of fruit rot as shown in table 2. Analysis of Variance revealed a significant difference in the disease causing potential of the fungi isolates on healthy lemon fruits with respect to their controls as shown in table 3.

Table 2: Pathogenicity of fungal isolates on healthy lemon fruits

Fungal Isolates/ Replicates	C	R_1	R_2	R_3	R_4
Aspergillus niger	0	5	5	5	5
Yeast	0	5	4	5	4

Key
C – Control,
R_1 _ R_4 – Replicate 1 - 4

Severity Scale
0 – No infection
1 – 1 – 20% rot
2 – 21 – 40% rot
3 – 41 – 60% rot
4 – 61 – 80% rot
5 – 81 – 100% rot

Table 3: Analysis of Variance in the Pathogenicity of fungal isolates on lemon fruit

Replicates/Fungal Isolates	*Aspergillus niger*	Yeast
R_1	5.00 ± 0.00^{b}	5.00 ± 0.00^{b}
R_2	4.50 ± 0.50^{b}	4.50 ± 0.50^{b}
R_3	5.00 ± 0.00^{b}	5.00 ± 0.00^{b}
R_4	4.50 ± 0.00^{b}	4.50 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
LSD (0.05)	**1.14**	**1.14**

Footnote: Means tagged with different alphabets are significant at P=0.05

3.3 Mean Inhibitory Effect of *O. gratissimum* on *Aspergillus niger* on Day Four: The highest mean inhibitory effect was observed at 75g/ml (0.73cm) and the least significant inhibitory effect was observed at 50g/ml (0.81cm) while 25g/ml had a mean inhibitory effect of 0.76cm with respect to their control as shown in table 4.

Table 4: Mean inhibitory effect of *O. gratissimum* on *Aspergillus niger* on Day four.

Concentration (g/ml) /Test Organism	*Aspergillus niger*
25g/ml	0.76 ± 0.10^{a}
50g/ml	0.81 ± 0.00^{a}
75g/ml	0.73 ± 0.02^{a}
Control	5.27 ± 1.10^{b}
LSD (0.05)	**1.70**

Footnote: Means tagged with different alphabets are significant at P=0.05

3.4 Mean Inhibitory Effect of *O. gratissimum* on Yeast on Day Four: The highest mean inhibitory effect was observed at 75g/ml (0.76cm) and the least significant inhibitory effect was observed at 50g/ml (0.80cm) while 25g/ml had a significant inhibitory effect of (0.79cm) with respect to their controls as shown in table 5.

Table 5: Mean inhibitory effect of Scent leaf on Yeast at day 4.

Concentration g/ml/ Test organism	Yeast
25g/ml	0.79 ± 0.30^{a}
50g/ml	0.80 ± 0.00^{a}
75g/ml	0.76 ± 0.90^{a}
Control	4.67 ± 0.93^{b}
LSD (0.05)	**1.26**

Footnote: Means tagged with different alphabets are significant at P=0.05

IV. Discussion

This study revealed that two fungi namely *Aspergillus niger* and Yeast are associated with the post harvest rot of lemon fruits during storage. *Aspergillus niger* is a wound invading pathogen that causes decay on stored citrus fruits damaged by insects, animals, splits and mechanical harvesting. The occurrence of these organisms may be attributed to their ability to produce resistant spores from the field as reported by [6]. *Aspergillus* species have also been implicated in the spoilage of fruits and Vegetables in Nigeria [7]. Yeast grows typically on moist environments where there is plenty supply of soluble nutrients such as sugars and amino acids. For this reason they are common on leaf and fruit surfaces and in various types of food. This study has shown that *Aspergillus niger* and Yeast associated with post harvest decay of lemon fruit were inhibited by the water extracts of *O. gratissimum* at all concentrations. This agrees with the work of [8] who reported the inhibitory action of plant products on the mycelia and spore germination of other fungi. [9] reported that leaf extracts of *O. gratissimum* effectively protected maize seeds from infection against *Fusarium moniliforme.* Also, [10] reported the fungitoxic effects of extracts of *O. gratissimum* to completely inhibit the conidia germination of *Mycosphaerella fijiensis* and sigatoka disease of banana. Crude extracts of *O. gratissimum* effectively exhibited antifungal activity on *Cercospora arachidicola*, which causes leaf spot on groundnut [11].The antifungal potency shown by *O. gratissimum* on the fungal isolates could be due to the presence of active bioactive compounds such as tannins, polyphenols and saponins present in the extract.

V. Conclusion

The antifungal characteristics of *O. gratissimum* can be pharmacologically improved upon to boost its fungicidal attribute for the control of post harvest rot caused by pathogenic fungi of crop diseases.

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