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Antifungal Activity of Extracts of Scent Leaf (*Ocimum gratissimum)* and Alligator Pepper *(Aframomum melegueta)* on the Post Harvest Decay of Carrot in Calabar, Nigeria

Arikpo Ikpi Okoi[1], Samuel Effiong Udo[1], Moses Eso Eka[2], Kingsley Hovana Enyi-Idoh[*1], Nsor O. Alobi[2] AND Magdalene Obi-Abang[2]
[1]Department of Biological Sciences, Cross River University of Technology, PMB 1123, Calabar, Nigeria.
[2]Department of Chemical Sciences, Cross River University of Technology, PMB 1123, Calabar, Nigeria.
[*] kingenyi@gmail.com

Abstract
The bioactive and antifungal effects of ethanol extracts of *Aframomum melegueta* and *Ocimum gratissimum* were investigated in vitro on causative agents of post harvest decay of carrots. The phytochemical screening of the two plants were obtained by analysis of their extracts using established procedures. Results showed moderate presence of saponins, tannins, flavonoids, alkaloids, glycosides and polyphenols, in *O. gratissimum* and high presence of saponnins, glycosides and polyphenols in *A. melegueta,* indicating high antifungal potency of the plants. The microbial pathogens isolated were *Rhizopus stolonifer, Fusarium culmorum, Sclerotinia sclerotiarum* and *Penicllium expansum.* The fungi were cultured with the two plant extracts using potato dextrose agar (PDA), and mycelia growth and inhibition zones of the agar was observed with a corresponding increase in concentration of the plant extracts. At 100% concentration, extract of *A melegueta* recorded the highest inhibition of mycelia growth in *P. expansum* (83.33±42%)while that of *O. gratissimum* was 73.78±3.69%. At 100% concentration, extract of *A. melegueta* is comparable with Dowicide in the in vitro inhibition of mycelia growth of the fungi. There is evidence of fungicidal attribute in *A. melegueta* and could be further developed pharmacologically for controlling post harvest rot of carrot caused by several pathogenic fungi.
Key words: Antifungal effect, post harvest, plant extract carrot.

1. Introduction

Carrot (*Daucus corota* Linn.) is a swollen underground vegetable root, popularly known and cherished for its nutritive value in vitamins A,B,C and pectin fibre found to contain cholesterol lowering properties (Krichevsky, 1999). Food science has shown that carrots are rich in vitamins A, a fat soluble vitamin that enhances eye sight, increased cell reproduction and prevents cancer (Harris et al, 1991). It also produces anti-oxidants which act as immune system enhancer (Kobaek-Larsen et al, 2005). Carrots can be eaten raw, cooked or used in flavouring soups, stews, couriee or salads (Wood, 1988).

In Nigeria, cultivation of carrots and other vegetable fruits is done in the northern part of the country where the climate is cool with a well drained and light loamy soil. After harvest and storage, transportation to market places within the country is often faced with some post harvest diseases leading to substantive crop losses (Finlayson *et al*, 1989). The most prevalent diseases of carrots during storage include carrot black rot (*Sclerotinia scloerotiarum)*, Botrytis rot (*Botrytis spp),* bacterial soft rot (*Erwinia carotovora)* and soar rot (*Geotrichum sp),* (*Pritchard et al, 1992).* Control of carrot diseases in storage is normally carried out by synthetic chemicals such as Bravo 500 and Dowicide (98g/l) which are not cost effective, and often require high foreign exchange to import them into Nigeria. There is a strong need, therefore, to source for plant extracts as alternative to synthetic chemicals in plant disease control that are biodegradable, readily available and environmentally friendly (Akueshi *et al*, 2002).

Ocimum gratissimum (L.) is a herbaceous, perennial shrub of the family Lamiaceae, often found growing around villages with its characteristic odour for seasoning dishes (Okujagu, 2008). It is a common spice with medicinal value, often known as 'scent leaf', among localities in Nigeria. Owalade et al, (2000) reported that leaf extracts of *O. gratissimum* and *Veronia amygdalina* effectively protected maize seeds from seed borne infection of *Fusarium moniliforme*. The fungitoxic effect of extracts of *O. gratissimum* completely inhibited conidial germination of *Mycosphaerella fijiensis* than sigatoka disease of banana (Okigbo and Emoghene, 2003). Also Okoi and Afuo, (2009) reported that crude extracts of *O. gratissimum* effectively exhibited antifungal activity on *Cercospora arachidicola*, the causal organism of leaf spot disease of groundnut.

Aframomum melegueta (K. Schum) of the family Zingiberacea, is a perennial herb with short stems, highly branched with lanceolate leaves and adventitious roots (Okujagu, 2008). In tropical Africa, and indeed Nigeria, the plant is cultivated mainly for its use in ethnomedicine than as a spice (Norton, 2004). The phytochemical screening of plant extracts as antimicrobial agents of plant diseases showed that *A. melegueta* contain high doses of tannins saponins, glycosides and polyphenols than many other weed plants (Odebiyi and

Sofowora, 1978). Also, the fruit and seeds contain a volatile oil with a pungent, peppery taste due to the presence of aromatic ketones called (6) – paradole (Awua, 1989; Sofowora, 1984). This compound has potent antifungal and antibiotic property in the plant, thus, used in the treatment of human diseases like measles, leprosy, excessive lactation and post partum haemorage (John, 1984); and also, sickle cell anaemia (http//:www.afrmomum.net. Apart from its use in the traditional medication of human diseases in Africa and Nigeria, no information on the antimicrobial or inhibitory effect associated with the use of *A. melegueta* extracts in the control of crop diseases has been documented. However, plant extracts with similar biofungicidal efficacies have been reported on various fungal diseases of vegetable crops. Ajayi and Olufolaji (2008) reported that extracts of *Zingiber officinale* and *Gmelina aborea* significantly inhibited the mycelial growth and sporulation of *Colletotrichum* capcici (in vitro), the causal organism of brown blotch of cowpea. Also, the application of neem leaves and bark were reported to have successfully controlled damping off of soyabean caused by *Rhizoctonia solani* (Adeyeye and Olufolaji, 2004); and anthracnose disease of soybean caused by *Colletotrichum truncatum* (Ojo and Olufolaji, 2005). Also, Wokocha and Okereke, (2005) reported that extracts of neem seeds, bitter kola, pawpaw roots and rhizomes of ginger effectively inhibited the sclerotial germination of *Sclerotium rolfsii,* in vitro, the causal organism of the basal rot disease of tomato.

This study, therefore, investigates the antifungal activity of ethanolic extracts of *O. gratissimum* and *A. melegueta* on the post harvest decay of carrot in Calabar, Nigeria.

2. Materials and Methods

2.1. Plant materials

The three plant samples: leaves of *Ocimum gratissimum,* seeds of Aframomum melegueta and roots of *Daucus carota* used for this work were purchased from Watt Market in Calabar Metropolis, Cross River State. The plants were identified at the Herbarium unit of the Department of Biological Sciences, Cross River University of Technology, Calabar.

2.2 Isolation of test fungi from carrot

Fresh roots of carrots (200g) were washed in sterile distilled water in the laboratory. They were cut into smaller pieces (2x2cm) and put into sterile Petri dishes and left on the laboratory benches at room temperature (28 ± 2^0c) for 4 days. Each Petri dish contained 4 pieces (2x2cm) of carrots. The partially decayed carrots were soaked in a 500ml beaker containing sterile distilled water for 10 minutes. The content of the beaker was stirred with a sterile glass rod to dislodge more microbial agents into the water. The carrots were later removed from the beaker and the contents filtered through sterile Whatman No 1 filter paper. Extactly 1.0ml aliquot of the filtrate was aseptically inoculated into each of the 4 Petri dishes containing sterile potato dextrose agar (PDA) medium. The media plates were incubated at 28^0c for 4 days and then observed for microbial growth. Pure cultures were obtained by sub-culturing onto fresh potato dextrose agar in Petri dishes and used as fungal inoculum for the plants. Fungal colonies observed were identified on the basis of spore characteristics and nature of mycelia using a compound microscope and identification guides.

2.3 Preparation of the plant extracts

Fresh leaves of *O. gratissimum* and seeds of *A. melegueta* were thoroughly washed with sterile distilled water, and air dried at 28^0c for 2 hours. Both plant samples were dried in an oven at 45^oc for 30 minutes and then pulverized separately with a household blender (Model 830L, Hongkong). The crude extracts of the two plant samples were prepared using standard procedures (Fatope *et al* 1999; Muktur and Huda, 2005). This involved soaking 50g of the powdered extract in 95% ethanol for 48 hrs at room temperature to allow for maximum extraction of the components. This was followed by evaporation of the filtrate to eliminate the solvent using a rotary evaporator (STUARC SCIENTIFIC, England). The residue was used as crude extract for each of the test plants and stored in reagent bottles in the refrigerator at 12^0c until they were used.

2.4 Phytochemical screening

The phytochemical screening (qualitative determination) of the bioactive ingredients of the plants were determined using the methods of Culier (1982), Sofowora (1984) and Gundiza (1985).

2.5 Preparation of extract concentrations

The extracts were reconstituted in sterile distilled water to obtain various concentrations of the extracts as follows: 100ml of the extract was reconstituted in 100ml of sterile distilled water to obtain 100 percent concentration of the extract. Also, 80ml of the 100 percent concentration was diluted with 20ml of sterile distilled water to obtain 80 percent concentration. The serial dilution procedure was continued to obtain lower concentrations of the extract as follows; 100, 80, 60, 40, and 20 percent.

2.6 Effect of plant extract on fungal growth

Five milliliter (5ml) from each of the concentration of the extract (20,40,60,80 and 100%) was dispensed into 9cm diameter Petri dish and agitated thoroughly with 20ml of melted PDA forming potato dextrose leaf extract agar (PDLA). Dowicide solution (98g/l), obtained by mixing 98g in 1000ml of water was

used as standard fungicide. Exactly 5ml from each of these extract concentrations, including the Dowicide solution was added to each Petri dish containing PDA medium before the medium solidified to form potato dextrose Dowicide agar (PDDA) (Emoghene *et al*, 1998). The agar-extract mixture or Dowicide-agar was allowed to solidify and then inoculated centrally with a 6mm diameter mycelia disc obtained from the colony of 4-day old cultures of each of the test fungi using a sterile inoculating needle. PDA plates inoculated with the test fungus but without the extract or Dowicide served as the control. All the plates were incubated at 27^0c, after which the zones of inhibition on colony diameter was measured for five days. Percentage inhibition was determined according to Whipps (1987).

Percentage inhibition $= \frac{R_1 - R_2}{R_1} x \frac{100}{1}$

Where, R_1 = Furthest radial distance of fungus in control plates (PDA only)

R_2 = Furthest radial distance of fungus in treatment plates.

The experiment was repeated thrice in a randomized complete block design with 6 treatments and 3 replicates.

2.7 Statistical analysis

Results were expressed as mean ± S.D. Comparison of mean of 3 determinations; and results adjudged significant at P<0.05

3. Results

Results of the phytochemical screening of the test plants are shown in Table 1. A *melegueta* shows high levels of saponins, glycosides and polyphenols with moderate presence of tannins and steroids. Also, there were low content of flavonoids and alkaloids while anthraquinones was absent. On the other hand, moderate levels of saponins, flavonoids, alkaloids glycosides and polyphenals were recorded in O. *gratissimum* with low levels of tannins and steroids while anthraquinones was absent.

Table 1 Phytochemicals present in ethanolic extract of plants used

Component	*Aframomum melegueta*	*Ocimum gratissimum*
Saponins	+++	++
Tannins	++	+
Flavonoids	+	++
Alkaloids	+	++
Glycosides	+++	++
Polyphenols	+++	++
Anthraquinones	-	-
Steroids	++	+

Key

\+ = Low presence
++ = Moderate presence
+++ = High presence
\- = Not present

The pathogens isolated from post harvest decay of carrots were: *Rhizopus stolonifer, Fusarium culmorum, Sclerotiania scleratiarum* and *Penicillium expansum*. The fungi and their prevalence of occurrence are shown in Table 2. The results showed that *Sclerotinia sclerotiarum* was most prevalent (50%) followed by *Rhizopus stolonifer* (30%) while the least was *Fusarium culmorum* (10%)

Table 2. Percentage occurrence of Pathogens isolated from post harvest decay of carrot.

Fungi isolates	**% occurrence**
Rhizopus stolonifer	30
Fusarium culmorum	10
Sclerotinia sclerotiarum	50
Penicillium expansum	20

The fungicidal activity of the plant extracts on the test fungi is presented in Table 3. The extracts inhibited the growth of all the fungi tested at various concentrations. Increased antifungal activity was observed with a corresponding increase in the concentration of the two plant extracts studied. The highest percentage inhibition of mycelia growth of the fungi was recorded at 100% extract concentration of *A. melegueta,* with *R. stolonifer* (52.90 ± 2.64%), *F. culmorum* (68.44 ± 3.42%), *S. sclerotiarum* (76.66 ± 3.83%) and *P. expansum* (83.33 ± 4.17%) (Table 3). At 20% concentration, the least antifungal activity was recorded in *O. gratissimum* extract with *F. culmorum* (5.78 ± 0.29%), *R. stolonifer* (7.56 ± 0.38%), *S. sclerotiarum* (8.67 ± 0.43%) and *P. expansum* 10.67 ± 0.53%). Extract of *A. melegueta* significantly (p>0.05) showed higher antifungal activity than *O. gratissimum* at all the concentrations. Also *P. expansum (73.78* ± 3.69%*)* significantly, (p>0.05) showed highest

inhibition with *O. gratissimum* at 100% extract concentration than *R. stolonifer* (51.56 ± 2.58%), *F. culmorum* (60.89 ± 3.04%) and *S. sclerotiarum (*65.56 ± 3.28%*).* The control plates, without the incorporation of the extracts grew very well with no inhibition of the fungus.

Dowicide (98g/l) which was applied as standard chemical often used for control of carrot diseases showed 100% inhibition of mycelia growth in *P. expansum,* closely followed by *F. culmorum* (97.78 ± 4.89%), and *S. sclerotiarum (*80.00 ± 4.00%*)* while *R. stolonifer (*77.78 ± 3.89%*)* was least.

Table 3: In vitro antifungal efficacy of ethanolic extracts of *A. melegueta and O. gratissimum* (incubated at 27^0c for 5 days after inoculation

Extract conc (%)	***O. gratissimum (percentage inhibition)***				***A. melegueta (percentage inhibition***			
	R. stolonifer	*F. culmorum*	*S. sclerotiarum*	*P. expansum*	*R. stolonifer*	*F. culmorium*	*S. sclerotiarum*	*P. expansum*
Control (0) untreated	0	0	0	0	0	0	0	0
20	*7.56±0.38	5.78±0.29	8.67±0.43	10.67±0.53	23.33±1.17	23.33±1.17	30.22±1.51	32.66±1.63
40	12.22±0.61	13.56±0.68	15.78±0.79	17.56±0.88	28.00±1.40	36.44±1.82	44.00±2.20	48.44±2.42
60	27.11±1.36	27.56±1.38	28.67±1.43	30.22±1.51	31.33±1.57	54.44±2.72	53.78±2.69	55.11±2.76
80	36.66±1.83	45.33±2.27	46.67±2.33	48.44±2.42	41.11±2.06	62.90±3.14	64.66±3.23	68.44±3.42
100%	51.56±2.58	60.89±3.04	65.56±3.28	73.78±3.69	52.90±2.64	68.44±3.42	76.66±3.83	83.33±4.17
98g/l *Dowicide					77.78±3.89	97.78±4.89	80.00±4.00	100±5.00

- Values are mean ± standard deviation from three replication

4. Discussion

The pathogens associated with the post harvest decay of carrots in this study were. *R. stolonifer F. culmorum, S. sclerotiarum* and *P. expansum. S. sclerotiarum* showed the highest occurrence while *F. culmorum* was the least. The fungal organisms causing decay in the carrots must have been present right from the field, and after harvest transferred to storage houses and market places.

This study has shown that *R. stolonifer, F. culmorum, S. Sclerotiarum* and *P. expanum* associated with the post harvest decay of carrots were inhibited by the ethanolic extracts of *O. gratissimum* and *A. melegueta* at all the concentrations in vitro. These results also agreed with earlier works on the inhibitory action of plant products employed on the mycelia and spore germination of other pathogenic fungi (Wokocha and Okereke, 2005; Adeyeye and Olufolaji, 2004; Ajayi and Olufolaji, 2008). This study corroborates the report of other workers (Owolade *et al,* 2000; Okigbo and Emoghene, 2003; Okoi and Afuo, 2009), that *O. gratissimum* is among important plants whose extracts are capable of checking the spread of many fungal diseases of food crops.

Extract from *A. melegueta* appeared to possess a stronger antifungal property against the mycelia growth of all the test fungi over *O. gratissimum* at same concentrations. The high fungitoxic effect of *A. melegueta* observed in this study could be due to the presence of a volatile oil of the seed which was reported to contain (6) – paradole, an aromatic ketone which contains a pungent, peppery hot taste with a high antifungi property (Norton, 2004; Awua, 1989 and Sofowora, 1984). Extracts from *A. melegueta* at 100% concentration compared favourably well with Dowicide (98g/l) in this study, which is one of the standard fungicides for control of carrot rot in storage.

The high antimicrobial property exhibited by *A. melegueta* over *O. gratissimum* on the test fungi in this study could be attributed to the higher concentration and potency of saponins, tannins, glycosides and polyphenols present in the extract of *A. melegueta* (Odebiyi and Sofowora, 1978) than that of *O. gratissimum.* The fruit and seeds of *A. melegueta* were also reported to contain antifungal and antibiotic activities. This gives credence to its traditional use for the treatment of human diseases like measles, leprosy and post partum haemorrages (John, 1984). Extract of the plant was also reported to play a significant role in the treatment of sickle cell anaemia as it could reverse the sickle shaped (S) blood in the vessels upto 75 -90% for the blood to have free passage into the vessels (http://ww.aframomum.net).

The fungicidal attribute evidenced in this study can further be developed pharmacologically for the control of post harvest rot of carrot caused by pathogenic fungi and other microbial agents of other crop diseases.

References

Adedeye, O. O. and Olufolaji, D. B. (2004). Control of damping off of soybean caused by *Rhizoctonia solani* using neem extract, in Proc. Nigerian Society for plant protection P16.

Ajayi, A. M. and Olufolaji, D. B. (2008). The biofungicidal attributes of some plant extracts on *Colletotrichum capsicum,* the fungal pathogen of brown blotch disease of cow pea. *Nigeria Journal Mycol. 1(1): 59-65.*

Akueshi, C. O. Kadir, C. O., Akueshi E. U., Agina, S. E. and Ngurukwen, C. (2002). Antimicrobial Potential of

Hyptis Sauveolen poit *(*Lamiaceae). *Nigeria Journal of Botany.* 15:37-41.

Awua, R. T. (1989). Fungitoxic effects of extracts from West African plants. *Ann. Applied Biology.* 115:451-452.

Culier, L. (1982). Methodology for analysis of vegetable Drugs. Practical Manuals on the Industrial Utilization of Medicinal and Aromatic Plants Centre Bidv. Romania 40:67-71.

Emoghene, A. O., Ikediugwu, F. E. O. and Okigbo, R. N. (1998). A rapid leaf segment method of testing pathogenicity of *Mycosphaerella fijiensis* Morolet. on banana and plantain *Nig. Journal of Microbiology*

Fatope, M. O., Ibrahim, H. and Takeda, Y. (1999) Screening of higher plants reputed as pesticides using brine shrimp lethality assay, Int. J. Pharmacol. 3(1): 250-260.

Finlayson, J. E., S. R. Rimmer, and Pritchard, M. K. (1989). Infection of Carrots by *Sclerotinia sclerotiarum. Canadian Journal of Plant pathology* 11:242.

Gundiaza, M. (1985). Phytochemical screening of some Zimbabwean Medicinal Plants. *The Central Africa Journal of Medicine 31:238-242*

Harris, R. A., Key, T. J. and Silcoks, P. B. (1991). A case control study of dietary carotene in men with lung cancer and in men with other epithelial cancers. *Nutrition and cancer* 15:63-68.

http://www.aframomum.net

John, L. (1984). The herb book Bantam Books, New York, USA *Journal of Agric and Food Chem.* 48: 1315-1321.

Kobaek-Larsen, M., Christensen, L. P; Vach, W. Ritskes-Hoitinga, J. Brandt, K. (2005). Inhibitory effects of feeding with carrots of Falcarinol on Development of Azoxymethane-induced preneoplastic lesions in the Rat colon. *J. Agric food Chem.* 53 (5): 1823-1827.

Kritchevsky, S. B. (1999). Beta-Carotene, Carotenoids and the Prevention of Caronary Heart Disease J.. *Nutr.* Jan; 129(1): 5-8.

Norton, M. (2004). *Cupboard Love. A dictionary of culinary curiosities.* Insomnia Press Toronto Canada.

Odebiyi, O. O. and Sofowora, E. A. (1978). Phytochemical screening of Nigerian Medicinal Plants part II, IIoydia pp 1-25.

Ojo, B. A. and Olufolaji D. B., (2005). Evaluation of the efficacy of neem crude back extract in enhancing germination and seedling establishment of anthracnose diseased soybean seeds. *Nig Jour. Plt. Protect 22. (*Special edition) 132-139.

Okigbo, R. N. and Emoghene, A. O. (2003). Effect of leaf extracts of three plant species on *Mycosphaerella fijiensis* Morolet, the causal organism of black sigatoka disease of banana. *Nig. Journal Plt protect.* 20:101-110.

Okoi, A. I. and Afuo C. O. (2009), Effect of leaf extracts of three plant species on *Cercospora arachidicola* Hori, the causal fungus of leaf spot disease of groundnut (*Arachis hypogea L*) Nig. Jour. *Plt. Protect 22. (*Special edition) 132-139.

Okujagu, T. F. (2008). Medicinal plants of Nigeria, South East Zone Vol. 1 Nigerian National Medicinal Development Agency, Federal Ministry of Science and Technology Lagos, Nigeria, p180.

Owolade, B. F., Amusa, F. A. N. and Osikanlu, Y. O. K. (2000). Efficacy of certain indigenous plant extract against seed-borne infection of Fusarium moniliforme on maize (Zea mays) in South western Nigeria. *Cereal Research Communication* 28:323-324.

Pritchard, M. K., D. E. Boese and Rimmer, S. R. (1992). Rapid Cooling and Field-applied fungicides for reducing losses in store carrots caused by cottony soft rot. *Canadian Jour. Plant Pathol.* 14:177-181

Sofowora, C. A. (1984). Medicinal plants and traditional medicine in Africa John Wiley and Sons, N. Y.

Whipp, J. M. (1987), Effects of media on growth interactions between a range of soil-borne glass pathogens and antagonistic fungi. *New Pathologist.* 107:127-142.

Wood, R. (1988). The Whole Foods Encyclopedia New York, Prentice-Hall Press; PMID: 1520.

Wokocha, R. C. and Okereke, V. C. (2005). Fungi toxic activity of extracts of some medicinal plants on Sclerotium rolfsii, causal organism of basal stem rot disease of tomato. *Nig. J. plt. Prot.* 25:106-111.

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