The main tuber crop yam in the tropical area

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Abstract: These days, consumers prefer agricultural produce that lack chemical traces. Therefore, bio-protection of yam tubers against microbial rot is very pivotal within the ambits of botanical approach due to its biodegradability and eco-friendliness. Ethanol of varied concentrations (10, 30 and 50%) were used to dissolve 10-50g of *Ocimum gratissimum* leaf powder, these mixtures were allowed to stay for 12hrs and filtered with cheese cloth, the filtrate served as extracts. The pathogenicity test showed *B. theobromae*, *A. glaucus, A. flavus* and *A. niger* as tuber rot pathogens. The growth of *A. flavus* was completely inhibited to 100% by 30% ethanol extracts of *O. gratissimum* at both 20g and 30g/100ml. 10% ethanol extract of *O. gratissimum* at 10g/100ml inhibited *A. flavus* to 96.88%. *A. flavus* (98.89%), *A. glaucus* (95.79%) and *A. niger* (97.11%) were most inhibited by 20% ethanol extract of *O. gratissimum* at 10g, 30g and 50g/100ml respectively, 50% ethanol extracts of *O. gratissimum* at 20g and 30g/100ml mostly inhibited *A. flavus* (92.25%) and *A. niger* (94.92%) respectively, 10% and 30% ethanol extract of *O. gratissimum* at 20g and 10g/100ml respectively inhibited *B. theobromae* to 97.76% and 95.52%. 10% ethanol extract of *O. gratissimum* at 30g/100ml inhibited *A. niger* (94.08%) mostly. 40% ethanol extract of *O. gratissimum* at 20g/100ml was mostly phytotoxic on *A. niger* (92.00%). Similarly, 50% ethanol extract of *O. gratissimum* at 40g/100ml was mostly phytotoxic on *A. niger* (92.92%), 30% ethanol extract of *O. gratissimum* at 30g/100ml against *A. glaucus* (100%) respectively, while *A. glaucus* (93.22%) was the most inhibited by 40% ethanol extract of *O. gratissimum* at 40g/100ml. Preservation of tubers using natural origin needs to be earnestly explored in other to save the teeming populace from acute food shortage. Hence, the use of *O. gratissimum* can serve as alternative inhibitory agent against all the rot fungi tested.
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Introduction

The main tuber crop in the tropical area is yam. However, yam tuber is bedeviled with post harvest deterioration as a result of physiological, physical and pathological effects (Ijato, 2011a). All these tissues degrading factors could combine to the detriments of yam tuber (Taiga, 2011). Perhaps, bacteria, viruses, and fungi are the main organisms responsible for bio-deterioration of yam tubers. *O. gratissimum* is an aromatic, perennial herb, 1-3m tall; stem erect, glabrous or pubescent round, quadrangular, much branched, and woody at the base, often with epidermis peeling in strips. Leaves strongly odoriferous and opposite; covered with minute hairs. Inflorescence a verticillaster, arranged in a terminal, simple or branched raceme 5-30 cm long; rachis lax, softly pubescent; bracts sessile. flowers in 6-10-flowered verticillasters, small, hermaphrodite; calyx 2-lipped, 2-3 mm long, in fruit 5-6 mm. ovary superior, consisting of 2 carpels, each 2-celled, style 2-fid. Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx (the lower lip closing the mouth of the fruiting calyx); varieties and form as are mainly based on differences in chemical content, the morphology of the fruiting calyx and on different degrees of hairiness, but the variation forms a continuum. The effectiveness of *O. gratissimum* necessitated its use as a bio-protector. This finding is aimed at isolating rot causing organisms of yam tubers and to evaluate the potency of this botanical against the degrading fungal microbes.

Materials And Methods

Source of material

Samples of infected and healthy yam tubers were obtained from Aba Ebira in Ado-Ekiti. *O. gratissimum* leaves were also obtained from forest reserve of Ado Ekiti.

Preparation of leaf extracts

Fresh leaves of *O. gratissimum* were air-dried for 30days, pulverized and weighed into 10-50g. Each weight was added to 100ml of 10, 30 and 50% ethanol in a 1000ml beaker stirred vigorously and left to stand for 12hrs, this was filtered with a sterile cheese cloth and the filtrate was used as the extract.

Preparation of potato dextrose agar (PDA)

PDA was prepared according to the manufacturer's instruction and following the techniques described by Arora and Arora (2008) for the purpose of obtaining pure culture.

Isolation of fungi from rotten yam

D. rotundata tubers with signs of rot were obtained and used for the work were surface sterilized using cotton wool moistened in 70% ethanol, rinsed thrice in different changes of sterile distilled water, cut open using sterile scalpel to reveal the border area of rotten and healthy portions.

Four pieces of rotten yam tubers were placed at regular distance from one another with the aid of sterilized inoculating loop on the surface of prepared PDA in the sterile Petri-dishes. The inoculated plates were incubated at room temperature for 72hrs (3days) and the plates were examined daily for fungal growth.

Identification of fungal isolates

The fungal isolates were sub-cultured, inoculated on sterile PDA plates and incubated, when fungal growth was established, these were examined on the basis of uniformity as a mark of purity. The pure cultures obtained were identified on the basis of culture morphology as well as using Barnett and Hunters (1985).

Test for pathogenicity

Fresh and healthy yam tubers were washed with water and rinsed with several changes of sterile distilled water, surface sterilized by cleaning with 70% ethanol. A hole was made on the tuber with the aid of sterile cork borer and a 3-day old culture of each isolated rot fungi was transferred into the hole created on the yam tuber at equidistant site and scooped out tissues were replaced to cover the bored hole in the yam tuber. Petroleum jelly was used to completely seal each of the exposed tissue and the inoculated tubers were placed in separate moistened and sterile air-tight polythene bags at room temperature. The yam tubers were examined for infection and rot development.

Direct medium treatment

This method involved direct treatment of the medium with the extract before inoculation. One ml of ethanol extract of varied concentrations was added to sterile PDA and swirled very well; 15ml of the PDA was dispensed into each Petri dish, homogenized and allowed to cool down, and the isolates were inoculated on the surface of the medium, these were incubated for 5days. The plates were examined for growth, the absence of growth in any of the plates was indicative of the potency of the extract against the isolates.

Effect of the extract on fungal growth

Modified method of Sangoyomi (2004) was used to determine the effects of extracts on fungal growth. This was done by inoculating mycelia disc obtained from the colony edge of 8days old culture of fungi at the centre of the Petri plates, the control was setup using blank agar plates (no extracts). Three replicates of PDA extract per isolate were incubated at 20^{o}C and radial growth was measured after three (3) days. Colony diameter was taken as the means along two directions on two perpendicular lines drawn on the reverse of the plates. The percentage inhibition was calculated using this formula:

$$I = \frac{DC-DT}{DC} \times \frac{100}{1}$$

Where I = % inhibition (mm), DC = radial growth of fungus in the control, DT = radial growth of fungus in the treatment (extract).

Results

The fungitoxic potentials of 30% ethanol leaf extract of *O. gratissimum* on the fungal rot organisms differed significantly ($p<0.05$) from the untreated control and standard. The bio-efficacies of 30% ethanol leaf extract of *O. gratissimum* on the rot organisms increased with the increase in concentration.

Effects of 10% ethanol leaf extracts of *O. gratissimum* on mycelial growth of fungal rot organisms.

The antimycotic effects of 10% ethanol leaf extract of *O. gratissimum* on the fungal pathogens are presented in Table 1. Inhibitory effects of 10% ethanol leaf extract of *O. gratissimum* at 10-50g/100ml on *B. theobromae* ranged between 88.57% and 95.15%. The radial mycelial growth of *B. theobromae* was most reduced by 10% ethanol leaf extracts of *O. gratissimum* at 50g/100ml to 95.15%, followed by 10% ethanol leaf extract of *O. gratissimum* at 40g, 30g and 20g/100ml, manifesting 94.28%, 94.00% and 93.72% inhibitions on *B. theobromae* respectively.

High phytotoxic effects ranging from 74.03% to 96.88% were on *A. flavus* by 10% ethanol leaf extracts of *O. gratissimum* at 10-50g/100ml. Inhibitive effect of 10% ethanol leaf extract of *O. gratissimum* was on *A.* flavus at 50g/100ml, exhibiting 96.88%, 40g, 30g and 20g/100ml of 10% ethanol leaf extracts of *O. gratissimum* induced antimycotic effect of 94.31%, 94.09% and 91.30% against *A. flavus* respectively.

Fungicidal effects of 10% ethanol leaf extracts of *O. gratissimum* was induced on *A. glaucus* ranging from 63.11% to 93.78% at 10-50g/100ml. Antimycotic

effects of 93.78% was reflected against *A. glaucus* by10% ethanol leaf extract of *O. gratissimum* at 50g/100ml, while 10% ethanol leaf extract of *O. gratissimum* at 40g and 30g/100ml displayed phytotoxic effect of 93.55% and 92.45% against *A. glaucus* respectively. These were closely followed by antimicrobial effect of 91.87% on *A. glaucus* by10% ethanol leaf extract of *O. gratissimum* at 20g/100ml.

Fungicidal activities of 10% ethanol leaf extracts of *O. gratissimum* at 10-50g /100ml on *A. niger* ranged between 74.75% and 95.83. The antimycotic effect of 50g/100ml of 10% ethanol leaf extract of *O. gratissimum* was greatest on *A. niger*, causing 95.83%, followed by 94.75%, 94.08% and 94.08% inhibitions against *A. niger* by 40g, 30g and 20g/100ml of 10% ethanol leaf extracts of *O. gratissimum* respectively.

Effects of 30% ethanol leaf extracts of *O. gratissimum* on mycelial growth of fungal rot organisms.

The antifungal effects of 30% ethanol leaf extract of *O. gratissimum* on the fungal pathogens are presented in Table 2. Fungicidal potentials of 30% ethanol leaf extract of *O. gratissimum* at 10-50g/100ml on *B. theobromae* ranged from 75.24% to 95.52%. The highest phytotoxic capacity of 30% ethanol leaf extract of *O. gratissimum* at 50g/100ml was recorded against *B. theobromae* (95.52%), this was followed by growth reduction effects of 90.57%, 87.99% and 78.09% on *B. theobromae* by 30% ethanol leaf extract of *O. gratissimum* at 40g, 30g and 20g/100ml respectively.

Table 1: Effects of 10% ethanol leaves extracts of *O. gratissimum* on mycelial growth of fungal rot organisms.

g/100ml	% inhibition of mycelial growth			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10	88.57[a]	74.03[b]	63.11[b]	74.75[b]
20	93.72[a]	91.30[a]	91.87[a]	94.08[a]
30	94.00[a]	94.09[a]	92.45[a]	94.08[a]
40	94.28[a]	94.31[b]	93.55[a]	94.75[a]
50	95.15[a]	96.88[a]	93.78[a]	95.83[a]
Standard	40.70[b]	30.20[c]	60.50[b]	50.00[c]
Control	0.00[c]	0.00[d]	0.00[c]	0.00[d]

Mean with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan multiple range test.

Antifungal reflections of 30% ethanol leaf extract of *O. gratissimum* at 10-50g/100ml on *A. flavus* ranged from 64.10% to 100%. Complete antimycotic effect of 100% was exhibited by 30% ethanol leaf extract of *O. gratissimum* at 50g/100ml against *A. flavus,* followed by 30% ethanol leaf extracts of *O.* gratissimum at 40g, 30g and 20g/100ml, exhibiting inhibitions of 86.62%, 74.35% and 64.55% on *A. flavus* respectively. Phytotoxic effects of 30% ethanol leaf extract of *O. gratissimum* at 10-50g/100ml on *A. glaucus* ranged from 43.11% to 100%. The most remarkable antimycotic capacity of 100% was against *A. glaucus* by 30% ethanol leaf extract of *O. gratissimum* at 50g/100ml, while 30% ethanol leaf extract of *O. gratissimum* elicited 74.44% and 73.33% inhibition on *A. glaucus* at 40g and 30g/100ml respectively. Radial growth reduction effect of 72.22% on *A. glaucus* was by 30% ethanol leaf extract of *O. gratissimum* at 20g/100ml. High antimicrobial prospects of 30% ethanol leaf extract of *O. gratissimum* at 10-50g/100ml on *A. niger* ranged from 46.41% to 90.00%. Harmful capacity of 30% ethanol leaf extract of *O. gratissimum* at 50g/100ml was exhibited on *A. niger* (90.00%), followed by 30% ethanol leaf extracts of *O. gratissimum* at 40g, 30g and 20g/100ml, exhibiting 83.33%, 81.00% and 80.83% against *A. niger* respectively.

Table 2: Effects of 30% ethanol leaf extracts of *O. gratissimum* on mycelial growth of fungal rot organisms.

g/100ml	% inhibition of mycelial growth			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10	75.24[c]	64.10[d]	43.11[d]	46.41[c]
20	78.09[c]	64.55[d]	72.22[b]	80.83[b]
30	87.99[b]	74.35[c]	73.33[b]	81.00[b]
40	90.57[b]	86.62[b]	74.44[b]	83.33[b]
50	95.52[a]	100[a]	100[a]	90.00[a]
Standard	40.70[d]	30.20[e]	60.50c	50.00[c]
Control	0.00[e]	0.00[f]	0.00[e]	0.00[d]

Mean with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan multiple range test.

Effects of 50% ethanol leaf extracts of *O. gratissimum* on mycelial growth of fungal rot organisms.

The antimicrobial effects of 50% ethanol leaf extracts of *O. gratissimum* on the fungal pathogens are presented in Table 3. *B. theobromae* was inhibited by 50% ethanol leaf extracts of *O. gratissimum* at 10-50g/100ml ranging between 80.67% and 92.86%. The highest fungicidal effect of 50% ethanol leaf extract of *O. gratissimum* at 50g/100ml was against *B. theobromae,* exhibiting 92.86%, followed by 50% ethanol leaf extract of *O. gratissimum* at 40g and 30g/100ml, exhibiting antimycotic capacities of 92.09% and 87.05% on *B. theobromae* respectively. Similarly, 50% ethanol leaf extract of *O. gratissimum* at 20g/100ml had biocidal value of 81.56% on *B. theobromae*. Antifungal effects on *A. flavus* by 50% ethanol leaf extracts of *O. gratissimum* ranged from 30.88% to 91.31% at 10-50g/100ml. The fungicidal performance of 50% ethanol leaf extract of *O. gratissimum* at 50g/100ml was highest on *A. flavus* (91.31%), followed by 50% ethanol leaf extracts of *O.* gratissimum at 40g, 30g and 20g/100ml, exhibiting 91.19%, 90.42% and 88.41% on *A. flavus* respectively.

Antimycotic effects were exhibited on *A. glaucus* by 50% ethanol leaf extracts of *O. gratissimum* at 10-50g/100ml ranging between 59.50% and 94.33%. The highest antifungal effect of 50% ethanol leaf extract of *O. gratissimum* at 50g/100ml was exhibited against *A. niger,* inducing 94.33%, this was closely followed by 40g and 30g/100ml of 50% ethanol leaf extract of *O. gratissimum,* exhibiting antiparasitic capacities of 92.11% and 90.78% on *A. glaucus* respectively, while 50% ethanol leaf extract of *O.* gratissimum at 20g/100ml, eliciting 81.56% inhibition on *A. glaucus.*

Antipathogenic effects by 50% ethanol leaf extracts of *O. gratissimum* at 10-50g/100ml on *A. niger* ranged between 57.50% and 94.92%. Inhibitory effect of 94.92% was exhibited by 50% ethanol leaf extract of *O. gratissimum* at 50g/100ml on *A. niger*, similarly, antifungal effects of 92.92%, 92.25% and 86.41% were evoked by 50% ethanol leaf extracts of *O. gratissimum* at 40g, 30g and 20g/100ml against *A. niger* respectively.

Table 3: Effects of 50% ethanol leaf extracts of *O. gratissimum* on mycelial growth of fungal rot organisms.

g/100ml	% inhibition of mycelial growth			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10	80.67[b]	30.88[b]	59.50[c]	57.50[c]
20	81.56[b]	88.41[a]	81.56[b]	86.41[b]
30	87.05[ab]	90.42[a]	90.78[a]	92.25[a]
40	92.09[a]	91.19[a]	92.11[a]	92.92[a]
50	92.86[a]	91.31[a]	94.33[a]	94.92[e]
Standard	40.70[c]	30.20[c]	60.50[c]	50.00[d]
Control	0.00[d]	0.00[d]	0.00[d]	0.00[e]

Mean with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan multiple range test.

Conclusion

Disease management should be ecologically based and be undertaken within the framework of integrated crop management and integrated pest management and best method is to utilize eco-friendly approaches. The use of bio fungicide mainly plant based products has gained ground. Moreover, plant biodiversity has provided a suitable platform and source of biologically active materials for use in traditionally crop protection. Since the result of this study has revealed the potentiality of ethanol extracts from *O. gratissimum* leaves to control the post harvest fungal deterioration of yam tubers. The effectiveness of *O. gratissimum* against rot microbes has been earlier reported (Ijato, 2011b). It would be quiet relevant to adopt this result to ensure prolongation of yam tubers shelf life as the extracts are easy to prepare and without any known threat to the environment. This will indeed provide much required economic profit from yam tubers cultivation and production. It is hereby recommended that, in the subsequent research that the active phytochemicals (active principles) that are resident in this plant be explored.

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