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Fungal pathogens of yam (*Dioscorea rotundata* Poir) and their bio-control.

Ijato J. Y

Department Of Plant Science, Faculty Of Science, Ekiti State University, Ado-Ekiti P.M.B 5363, Ekiti State, Nigeria.
E-mail: considerureternity@gmail.com; GSM: 08067335124

Abstract: Plant disease management should be ecologically based and should be undertaken within the purview of integrated pest and diseases management scheme. Therefore, the best method is to utilize environment-friendly concept. Really, the use of bio-fungicide of mainly plant based products has gained ground in the recent time. Moreover, plant biodiversity has provided a suitable platform and source of biologically active materials for use in traditional crop protection system. Investigation was carried out to test the efficacy of aqueous *Ocimum gratissimum* extracts for the control of yam tuber rot caused by *Botryodiplodia theobromae, Aspergillus glaucus, Aspergillus niger and Aspergillus flavus*. Hot and cold water extracts were obtained from leaves of *O. gratissimum* and were found to be fungicidal against the fungi. The extracts suppressed the growth of these fungi in culture and reduced rot development in yam tubers. The most phytotoxic capacity of hot water extract of *O. gratissimum* at 50g/100ml was found against *B. theobromae* (95.71%) and *A. flavus* (96.67%). Similarly, the most fungicidal values of 95.33% and 79.75% were respectively evoked against *A. glaucus* and *A. niger* by hot water extract of *O. gratissimum* at 50g/100ml. The most mycelial reduction effects of cold water extract of *O. gratissimum* at 50g/100ml was against *B. theobromae* and *A. flavus,* evoking 90.48% and100% respectively. Cold water extract of *O. gratissimum* at 50g/100ml performed most by causing inhibition on *A. glaucus* (100%) and *A. niger* (96.40%). The phytotoxic effects of aqueous extracts of *O. gratissimum* are without any known residual implication.
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Key words: *O. gratissimum,* rot pathogens, yam tuber rot.

Introduction

O. gratissimum (L) also known as scent leaf, belongs to *Lamiaceae* family, a vital multipurpose medicinal plant, and it is distributed in the tropical and warm regions. It is used for curing diarrhea, upper respiratory tract infection, fever, skin diseases and ophthalmic. It is also used as antimicrobial, antiprotozoic and pneumonia (Akujobi *et al*., 2010). It is an aromatic, perennial herb of 1-3m tall; stem erect, glabrous or pubescent round, quadrangular, much branched, and woody at the base, often with epidermis peeling in strips.

Materials and Methods

Two sets of experiments that involved isolation and identification of rot organisms together with inoculations on healthy yam tubers followed by applying aqueous plant extracts. **Collection of infected and healthy yam isolates**.

Infected tubers with signs of tissue softness were randomly acquired locally from Oja-Oba market in Ado-Ekiti. Five samples were collected from each selling point; these were placed in sterile polyethylene bags and taken to the laboratory for isolation and identification. The identified fungal isolates were used to infect healthy yam tubers to establish their pathogenicity.

Isolation of fungi

Diseased spots of the tubers were cut aseptically into small bits into a sterile dish using scapel which was flamed over a Bunsen's burner and cooled in methylated spirit (Fawole and Oso, 1988). The excised diseased bits were sterilized in 70% ethanol, placed in Petri dishes containing solidified PDA. The solidified plates were incubated at room temperature (28 ± 2^0C) in the dark for 72 hours. The fungal colonies grown on the incubated plates were sub-cultured into fresh media until pure culture was obtained. Microscopic examination was used after examining the colony characteristics. A sterile needle was used to take little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol-cotton-blue and examined under the microscope for fungal structures. The morphology and cultural characteristics observed were compared with structures in Snowdon (1990).

Pathogenecity Test

Disease-free yam tubers were surface sterilized in 0.1m of mercuric chloride ($HgCl_2$) for a minute and washed in five changes of distilled water. Five mm

cork borer was punched to a depth of 4mm into the healthy yam tubers and the bored tissues scooped out. Five mm diameter disc from pure culture was cut and placed back. The wound was sealed with prepared candle wax according to the method of Fawole and Oso (1988). The control was set up in the same manner only that sterile agar disc replaced the inocula. The inoculated yam tubers were placed in four (4) replications at room temperature (28±20C). The microbes were re-inoculated and identified using the same procedures described earlier.

Preparation of plant extracts.

O. gratissimum (leaves) were air dried and ground separately. Thirty grams of each sample was added to 15ml of distilled water in separate flasks. This was vehemently homogenised and left for 24 hrs. The sample was filtered with Whatman filter paper (No.1) and the filtrate used as extract.

Effect of plant extract on fungal growth.

Varied percentages of extracts were poured into separate flask containing sterilized potato dextrose broth with a sterile cork borer; different fungi were inoculated into separate flasks and incubated at room temperature (28±20 C) for seven days. After the incubation, mycelia from different broths were taken onto pre-weighed filter paper, oven dried at 85% and reweighed until a constant weight was obtained. The changes in weight were noted. For the control, no plant extract was added to the potato dextrose broth.

Mycelial extension of fungi *in vitro*

The method of Amadioha and Obi (1999) was used to determine the effects of extracts on mycelial extension of the fungi. This was obtained by placing one disc (3mm diameter) of 5-days-old culture of the microbes in each of the five Petri dishes (1cm diameter) with 170ml PDA medium and 1ml of leaf extract. The control experiments were set up with 1ml of sterile distilled water. Five replicated plates of leaf extract agar per isolate were incubated at room temperature (28±2 0C) for 7 days. Daily measurements of the mycelial extension of the cultures were determined by measuring culture along two diameters. Mycelial growth inhibitions were taken as percentage of growth on the PDA. Fungitoxicity was determined in form of percentage growth of colony inhibition and calculated according to this formula:

$$\frac{\mathrm{LT} - \mathrm{LC}}{\mathrm{LC}\ 1} \times 100$$

Results

Effects of aqueous extracts (hot and cold) of *O. gratissimum* on mycelial growth of fungal rot organisms

The antifungal effects of both cold and hot water leaf extracts of *O. gratissimum* on the fungal pathogens are studied in this work. Hot water extracts of *O. gratissimum* at 10-50g/100ml had antimycotic potentials that ranged from 46.52% to 95.71% on *B. theobromae*. The most phytotoxic capacity of hot water leaf extract of *O. gratissimum* at 50g/100ml was found against *B. theobromae* (95.71%), followed by mycelial growth reduction effects of 94.29%, 91.81% and 80.09% on *B. theobromae* by hot water leaf extracts of *O. gratissimum* at 40g, 30g and 20g/100ml respectively. Antifungal effects of cold water leaf extract of *O. gratissimum* at 10-50g/100ml on *B. theobromae* ranged from 51.14% to 90.48%. The most mycelial reduction effect of cold water leaf extract *O. gratissimum* at 50g/100ml was against *B. theobromae* by 90.48%, followed by antifungal effects of 85.23% and 81.14% against *B. theobromae* by cold water leaf extract *O. gratissimum* at 40g and 30g/100ml respectively. Also, cold water leaf extract of *O. gratissimum* at 20g/100ml caused antimicrobial effect of 77.00% on *B. theobromae*. The least mycelial reduction effect was exhibited by cold water leaf extract of *O. gratissimum* at 10g/100ml on *B. theobromae* (51.14%). Hot water extracts of *O. gratissimum* at 10-50g/100ml exhibited antimycotic capacities on *A. flavus* ranging from 11.00% to 96.67%. The fungitoxicty of hot water extract of *O. gratissimum* at 50g/100ml was highest on *A. flavus,* inducing 96.67%, followed by hot water leaf extracts of *O. gratissimum* at 40g, 30g and 20g/100ml, exhibiting inhibitory effects of 88.98%, 88.29% and 63.32% on *A. flavus* respectively. The antiparasitic effects of 30g and 40g/100ml of hot water leaf extract of *O. gratissimum*. Microbecidal effects of cold water leaf extract of *O. gratissimum* at 10-50g/100ml on *A. flavus* ranged between 76.50% and 100%. Cold water leaf extract of *O. gratissimum* at 50g/100ml recorded the most phytotoxic potential by causing 100% inhibition on *A. flavus*, this was closely followed by expression of antimycelial efficacies of 96.76% and 92.97% on *A. flavus* by cold water extract of *O. gratissimum* at 40g and 30g/100ml respectively; also, cold water leaf extract of *O. gratissimum* at 20g/100ml had antimicrobial effect of 91.63% against *A. flavus*. Hot water leaf extract of *O. gratissimum* at 10-50g/100ml had high phytotoxic effects on *A. glaucus* ranging between 36.33% and 95.33%. The most fungicidal value of 95.33% was against *A. glaucus* by hot water leaf extract of *O. gratissimum* at 50g/100ml, closely followed by induction of 94.11%, 69.00% and 57.45% by hot water leaf extract of *O. gratissimum* at

40g, 30g and 20g/100ml on *A. glaucus* respectively. Cold water leaf extracts of *O. gratissimum* at 10-50g/100ml had high biocidal effects on *A. glaucus* ranging from 77.44% to 100%. Cold water leaf extract of *O. gratissimum* at 50g/100ml performed most by causing 100% inhibition on *A. glaucus,* followed by cold water leaf extract of *O. gratissimum* at 40g and 30g/100ml by 88.00% and 86.66% effects on *A. glaucus* respectively. Cold water leaf extract of *O. gratissimum* at 20g/100ml reduced the mycelial growth of *A. glaucus* by 76.44%.

Hot water extracts of *O. gratissimum* at 10-50g/100ml showed high antimicrobial prospects on *A. niger* ranging from 39.33% to 79.75%. The greatest phytotoxic capacity of hot water extract of *O. gratissimum* at 50g/100ml was effected against *A. niger* (79.75%), followed by 75.00%, 72.50% and 47.48% by hot water leaf extracts of *O. gratissimum* at 40g, 30g and 20g/100ml on *A. niger* respectively. Cold water leaf extract of *O. gratissimum* at 10-50g/100ml had antifungal indications on *A. niger* that ranged between 33.23% and 96.40%. Cold water leaf extract of *O. gratissimum* at 50g/100ml exhibited the highest antimycotic performance of 96.40% on *A. niger*, followed by 92.91% and 90.53% inhibitions against *A. niger* by cold water extract of *O. gratissimum* at 40g and 30g/100ml respectively. Also, cold water extract of *O. gratissimum* at 20g/100ml induced antimicrobial effect of 68.00% against *A. niger*. *A. niger* was most resistant to cold water extract of *O. gratissimum* at 10g/100ml, eliciting antimycelial effect of 33.23%.

Conclusion

The outcome of this study has revealed the potentiality of aqueous extract from *O. gratissimum* leaves to control storage fungal decay of yam tubers. The effectiveness of *O. gratissimum* against rot microbes was earlier reported (Ijato, 2011b). It is relevant and laudable to adopt this result to ensure the prolongation of shelf life of yam tubers. It is hereby recommended that, subsequent research can be focused on assaying active phytochemicals in *O. gratissimum* and how their metabolic interaction on the fungi.

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