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Use of *Aazadirachta indica* aqueous leaf extracts for the control of mycotic deterioration of yam tubers (*D. rotundata* poir)

Abstract

Considering the recurrent yam tuber rot in the tropical region of the world, all hands in crop protection need to be on deck to contain the menace, as it portends great danger to food security. Hence, cheap, accessible, easy to formulate and eco-friendly control measures need to be fully explored**.** *B. theobromae, A. niger*, *A. flavus* , *A. glaucus* were isolated from the rotten tissues and identified. The radial growth of *B. theobromae* was most reduced by 92.57% at 50g/100ml of hot water extract of *A. indica*. Similarly, hot and cold water extract of *A. indica* at 50g/100ml was most effective on *A. flavus* by 94.98% and 97.30% respectively. *A. glaucus* (100%) and *A. niger* (90.17%) were respectively inhibited at 50g/100ml of cold water extract of *A. indica*. The antimicrobial effects of hot and cold water extract of *A. indica* on the fungal rot organisms differed significantly from the control and standard. The efficacies of both cold and hot water extracts of *A. indica* on the rot organisms increased with increase in concentration.

Keywords: *A. indica*, local plant, rot organisms

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Introduction

Yam is the common name for some species in the family Dioscoreaceae, order *Liliflorae*, and genus *Dioscorea.* The most economically important species are: yellow yam **(***Dioscorea cayenensis* Lam.), water yam **(***Dioscorea alata* L.), *D. dumentorum* (Knuth pax), aerial yam (*Dioscorea bulbifera),* Chinese yam (*Dioscorea esculenta* (Lour), bitter yam **(***Dioscorea dumentorum* and white yam *(Dioscorea rotundata* Poir). It is a staple crop for over millions of people in both tropical and sub-tropical countries of West Africa, Caribbean, the Northern and Central part of East Asia, Malaysia, Japan and Oceania.[1–3] Some species of yams are used as pharmaceutical excipients and the toxic bye products are used for hunting and insecticides.[4] In addition, there are many medicinal uses of yam in dermatology and gastroenterology in Asia, Africa and the Americas. Yam is a source of diosgenin, a precursor of progesterone, cortisone and other medically important steroids.[1] Storage losses by microbes occur when the tubers are stored for long period post harvest.[5–7] Losses of yam tubers in storage due to rot are considered heavy in Nigeria,[8] the evaluation of rot in various parts of Nigeria showed that the extent of rot ranged from 0.5-18% at harvesting while storage rot ranged from 3-25%.[9,10] The aim of this study is to identify the tubers rot fungi and to investigate the efficacy of local plant extracts in protecting yam tubers in storage and in transit.

Materials and methods

Collection of yam and plant material

Softness of tubers tissue was used as a rot identity, while tubers freshness was used as healthy identity. Rotten and healthy tubers were randomly collected from the barn, placed polythene bag, and conveyed to the laboratory for assay. Also, plant materials were collected from the forest area in Ado-Ekiti. Authentication of plant identity was done in the herbarium unit of the Department of Plant Science, Ekiti State University.

Preparation of potato dextrose agar (PDA) and isolation of fungal rot organisms

Potato dextrose agar (PDA) for the culturing of respective fungus was prepared according to the manufacturer's instruction. Infected yam tubers were washed in a running tap water; rinsed in sterile distilled water; sterilized with 70% ethanol and air dried for few minutes to remove external contaminants. Sections were cut with sterilized scalpel; surface sterilized with 30% ethanol and rinsed with several changes of sterile distilled water. Infected portions of the yam tuber were cut under aseptic condition into small bits into a sterile dish with the aid of sterile scapel and dipped inside methylated spirit.[11] The cut infected bits were sterilized with 70% ethanol and placed on Petri dishes containing solidified PDA. Thin sections of the surface sterilized-rotten portion of yam were plated out on PDA incorporated with streptomycin in Petri dishes. These were incubated at room temperature (28°C) in the dark for 72hours and were observed daily for fungal growth.[12] Pure cultures were obtained and stored in McCartney bottles for further use.

Pathogenicity test

Healthy yam tubers were washed in running water; rinsed with distilled water and surface sterilized with 70% ethanol. Cylindrical cores of 3cm deep were removed from four different parts of an healthy yam tuber with sterile cork borer, one disc (3mm diameter) of 5-day-old culture of the pathogens was inoculated into each of five Petri dishes (1cm diameter) with 170ml PDA medium disc was taken from the colony of the initially isolated sub-cultured organisms and placed downward into the holes of yam with the aid of sterile forceps, The bored portions of 5mm pieces of yam tubers were replaced after they had been cut off to compensate for the thickness of the fungal culture. The wounded surface was sealed by placing petroleum jelly on a piece of cotton wool on separate, moistened, sterile and air tight polyethylene bags and incubated for 72hrs at room temperature (28°C) in 3 replications. The inoculated yam tubers were observed for

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infection after 14 days, 21days and 32 days.[13] The control experiment also followed the same process only that the discs of inoculated PDA were placed in the holes created on the tubers without organism, these were both incubated for fourteen days, observations and readings were taken accordingly.

Identification of yam rot fungal organisms

The developing fungi were characterized and identified using a sterile needle to take a little portion of the hyphae containing spores on the sterile glass slide stained with lacto phenol-cotton-blue and examined under the microscope for fungal structures. The morphology and cultural characteristics observed were compared with structures in.[14]

Preparation of cold and hot water

Fresh leaves of *A. indica* were collected from the parent plant; air dried; pulverised and weighed into 10-50g each of the weighed sample was added to 100ml of hot water in separate flask and vigorously stirred for 15-20 minutes, this was allowed to cool for few minutes. The samples were filtered with a cheese cloth and filtrate used as the extract was used to poison the PDA. The same procedure was used for cold water extraction by replacing hot water with cold water.

Effect of plant extracts on rot development

Drawing two perpendicular lines on the plates created four equal sections on each plate with and warm plant extract in PDA was poured into plates, this was allowed to cool and solidify for 30 minutes. Isolated fungi were introduced into the Petri dishes using inoculating needle. The Petri dishes were immediately covered and incubated. The medium was checked daily for fungal growth. Diameter of the radial growth of fungi was measured perpendicularly using metre rule. The control experiment was set up using extract-free PDA media by Amadioha and Obi. The fungitoxicity levels of aqueous plant extract were determined using percentage inhibition method of Okigbo et al.,[15] thus:

$$Percentage\ growth\ inhibition\left(\%\right) = \frac{LC - LT}{LC} * \frac{100}{1}$$

Where LC = average length of healthy portion of control

LT = average length of healthy portion with treatment

Results

The following fungi were isolated and identified: *A. niger, A. glaucus, B.theobromae* and *A. flavus*. The antimicrobial effects of hot and cold water extract of *A. indica* on the fungal rot organisms differed significantly ($p<0.05$) from the untreated control and standard. The efficacies of both cold and hot water extracts of *A. indica* on the rot organisms increased with the increase in concentration.

Effects of aqueous leaf extract (hot and cold) of *A. indica* on mycelial growth of fungal rot organisms

The antimicrobial effects of both the cold and hot water leaf extracts of *A. indica* on the fungal pathogens are presented in Table 1. Hot water leaf extracts of *A. indica* at 10-50g/100ml was inhibitive on the rot parasite ranging between 69.24% and 92.57%. The mycelial growth of *B. theobromae* was most reduced to 92.57% by hot water leaf extract of *A. indica* at 50g/100ml, followed by exhibition of 92.57%, 83.53% and 73.81% by hot water leaf extracts of *A. indica* at 40g, 30g and 20g/100ml on *B. theobromae* respectively. Hot water leaf extract of *A. indica* at 10g/100ml elicited least inhibition on *B. theobromae* (69.24%). Cold water leaf extracts of *A. indica* at 10-50g/100ml had antimycotic effects on *B. theobromae* that ranged from 49.53% to 87.33%. The radial mycelial growth of *B. theobromae* (87.33%) was the most reduced by cold water leaf extract of *A. indica* at 50g/100ml. Similarly, cold water leaf extracts of *A. indica* at 40g and 30g/100ml manifested antifungal effects of 85.43% and 83.30% on *B. theobromae*. Inhibition of 80.95% was against *B. theobromae* by cold water extract of *A. indica* at 20g/100ml, while the lowest antimycotic ability was induced on *B. theobromae* (49.53%) by cold water leaf extract of *A. indica* at 10g/100ml. Hot water leaf extracts of *A. indica* at 10g-50g/100ml showed high phytotoxic effects on *A. flavus* ranging from 54.18% to 94.98%. Hot water leaf extract of *A. indica* at 50g/100ml was the most effective on *A. flavus*, exhibiting 94.98%, closely followed by hot water leaf extract of *A. indica* at 40g, 30g and 20g/100ml, eliciting antimycotic effects of 94.29%, 79.60% and 66.60% on *A. flavus* respectively. Cold water leaf extract of *A. indica* at 10-50g/100ml had fungitoxic effects against *A. flavus* that ranged from 79.93% to 97.30%. Cold water leaf extract of *A. indica* at 50g/100ml was most effective on *A. flavus* by 97.30%, followed by 91.86% and 91.31% by cold water leaf extract of *A. indica* at 40g and 30g/100ml against *A. flavus* respectively, cold water leaf extract of *A. indica* at 20g/100ml caused antimycotic effect of 84.28% on *A. flavus*.

Hot water extracts of *A. indica* at 10-50g/100ml reflected potent fungicidal effects on *A. glaucus* ranging from 43.78% to 85.78%. The most fungicidal effect of 85.78% was evoked by hot water leaf extract of *A. indica* at 50g/100ml against *A. glaucus,* followed by exhibition of biocidal potential of 83.00%, 74.11% and 73.69% by hot water leaf extract of *A. indica* at 40g, 30g and 20g/100ml on *A. glaucus* respectively, while the least effect of the concentration of 43.78% was exhibited by hot water leaf extract of *A. indica* at 10g/100ml against *A. glaucus*. Cold water leaf extracts of *A. indica* at 10-50g/100ml showed high fungicidal effects on *A. glaucus* ranging from 80.99% to 100%. *A. glaucus* was highest inhibited by cold water leaf extract of *A. indica* at 50g/100ml by 100%, followed by 95.00% and 93.56% inhibition against *A. flavus* by cold water leaf extract of *A. indica* 40g and 30g/100ml respectively. Cold water leaf extract of *A. indica* at 20g/100ml inhibited *A. glaucus* to 92.33%. Hot water leaf extracts of *A. indica* at 10-50g/100ml exhibited high fungicidal effects on *A. niger* ranging between 48.50% and 91.33%. The antimicrobial activity of cold water leaf extract of *A. indica* at 50g/100ml was greatest on *A. niger* by 91.33%, followed by exhibiting inhibitory values of 75.50%, 73.66% and 63.50% against *A. niger* by hot water leaf extract of *A. indica* at 40g, 30g and 20g/100ml respectively. Cold water leaf extract of *A. indica* at 10-50g/100ml exhibited biocidal effects on *A. niger* ranged between 74.58% and 90.17%. The extract had most antimycotic effect of 90.17% on *A. niger*, followed by antimycelial effect of 89.50% and 86.67% by cold water leaf extract of *A. indica* at 40g and 30g/100ml of on *A. niger* respectively, cold water extract of *A. indica* at 20g/100ml elicited antimicrobial effect of 82.42% against *A. niger*, while *A. niger* proved most resistant to cold water leaf extract of *A. indica* at 10g/100ml, eliciting antimycotic effect of 74.58%.

Discussion and conclusion

Yam tuber rot in the yam producing part of the world has become a serious challenge that requires a collaborative effort of scientists with focus on biological approach due the adverse effect that inorganic agrochemicals has on ecosystem in general. *B. theobromae, A. niger, A. flavus*, *A. glaucus* were isolated and identified. All the concentrations of plant extracts reduced rot development by all the fungi. The results of aqueous plant extracts showed that *A. indica* extracts were

phytotoxic to the fungal pathogens. All the concentrations of tested plant extracts proved effective in controlling tuber rot. The biocidal effects of *A. indica* in this study also agreed with the report of Ogbebor and Adekunle,[16] that *A. indica* extract was very effective in inhibiting *F. moniliforme, A. flavus* and *A. niger*.

Moreover, Bokhari et al.,[17] reported significant biocidal effects of *A. indica* on black scurf which is one of the oldest and commonest diseases of potato stems and stolons, this study also corroborated the finding of Mondali et al.,[18] who reported the efficacy of water and ethanol leaves extracts of neem on seed borne fungi: *Aspergillus* spp and *Rhizopus* spp and chemical characterization of the neem leaf extracts were studied *in vitro* on the culture medium. Joshi subjected aqueous and ethanol extract of *A. indica* to *in vitro* antibacterial assay against human pathogenic bacteria viz: *Escherichia coli, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* employing cup diffusion method with high inhibitive potential. Obilo et al.,[19] reported that the wood ash from *A. indica* proved active in the control of *B. theobromae* that is responsible for most yam tuber rots. Report also had it that paste of wood ash from neem tree was able to prevent yam tubers from infection throughout twenty four months in storage.[19] Antifungal effects of *A. indica* in this study could be attributed to the presence of saponin and alkaloid: chemical components which have been identified as antifungal agents in plant by Kumar. More also, the inhibition of radial mycelial growth of rot pathogens in this study could be due to the presence of azardirachtin in *A. indica* extract by Okute and SaiRam; this was also reported earlier by). The antimycotic effects of the plant extracts could be ascribed to the presence of antimycotic substances.[20]

It seems that antifungal and the antimicrobial effects of the test plants are the outcome of many active principles working synergistically by Bangamolla. It could be inferred from this finding that *A. indica* could serve as a reliable antifungal agent.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest in this work.

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