Evaluation of Effect of Aqueous Plant Extract in the Control of Storage Fungi

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Abstract — This study investigated in-vitro the control of fungal species found associated with the storage of four staple food crops at Ibadan in the humid forest of southern Nigeria using some indigenous plant extracts. Aspergillus flavus, Aspergillus niger, Penicillium expansum and Rhizopus stolonifer were found associated with the spoilage of horticultural crops in storage. The results of the investigation revealed that, Acalypha ciliata, Aloe vera, Azadirachta indica and Vernonia amygdalina were effective in the inhibition of Aspergillus flavus and Penicillium expansum. Annona squamosa effectively inhibited the mycelial growth and sporulation of Aspergillus niger and Rhizopus stolonifer. Aloe vera extract was equally effective in the reduction of mycelial growth and sporulation of Aspergillus niger. In the overall, a significant reduction in mycelial growth and sporulation of the pathogens was found associated with treatment with most of the plant extract tested.

Keywords: Aqueous plant extract, comparative effect, mycelial growth, sporulation, staple food, storage fungi.

1 INTRODUCTION

FOOD is man's primary need yet its production is limited both by man's ability to utilize the available land area and the presence of plant pests and diseases. Plant disease became a handicap right from the early attempt by man to feed and clothe himself [14]. Plants have always faced with potential deleterious organisms such as fungi, bacteria, viruses and nematodes responsible for crop losses worldwide [16] Fungi found world-wide, attacking and inducing diseases on many kinds of plants [15] has been reported to cause about 60 per cent of plant diseases [14]. They are responsible for billions of Naira worth of damage to crops in Nigeria [1]. Many countries are documented in history to have suffered famine and financial losses due to these diseases [17, 1]. The total estimates of losses due to fungal diseases are often enormous not only on the field and/or yield but also in storage. Production of staple food in Nigeria thus relies heavily on the use foliar sprays on the field and protectants in storage. However, the hazardous effects associated with the use of chemicals for plant disease control has recently received increasing attention worldwide and the pathogens continue to develop resistance to chemicals in use. Today, over 200 species of plant pathogens are reported to be resistant to chemical pesticides [20]. To a great extent therefore, the drawbacks of these chemicals and their hazardous effect on the environment have offset their benefits. Thus, there is a need for the development of alternative disease control materials that are both effective in plant disease control and at the same time environmentally friendly.

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It has been severally reported that farmers and local inhabitants especially in developing countries frequently use plants with medicinal properties to alleviate certain illnesses and sometimes to protect their farm produce from spoilage [13, 2]. These local herbs of natural origin offer cheap and safer control of diseases for those farmers who cannot afford the high cost of synthetic pesticides. This present study was initiated to find suitable natural plant extracts that are affordable and more environmentally friendly for fungal disease control as alternative to chemical control of plant produce in storage.

2 MATERIALS AND METHODS

2.1 Plant materials

Plant materials comprising of leaf and bark of Acalypha ciliata, Anonna squamosa, Azadirachta indica, Melia azedarach, Newbouldia laevis, Persea americana, Vernonia amygdalina, and Aloe vera (leaf only) were collected from Ibadan a lowland rain forest zone situated at latitude 7^{o} 23'N and longitude 3^{o} 55'E and 200 mm above sea level with annual rainfall of 1200 mm and mean daily temperature of between 24^{o}Celsius (minimum) and 34^{o}Celsius (maximum) lying between the humid forest and derived savannah agro-ecologies of Nigeria.
Cowpea, groundnuts, maize and melon seeds showing symptoms of fungal infection were collected from Apata, Bodeja and Dugbe markets in Ibadan, Nigeria. These were kept separately in sterile sampling bags and taken to the plant pathology laboratory of Nigeria Plant Quarantine Service, Moor Plantation at Ibadan, Nigeria. At the laboratory, the seeds were plated on moist filter papers in petri dishes and incubated for 7 days under room temperature that fluctuated between 25^{o}Celsius and 28^{o}Celsius under 65 to 90 per cent Relative humidity. After 7 days of incubation, isolated colonies were picked with sterile wire loop, streaked onto solidified Potato Dextrose Agar (PDA) in petri dishes and incubated for 5 days. Isolated colonies were re-inoculated into fresh petri dishes and incubated until pure cultures were obtained. The fungal isolates were examined by both visual examination and viewing under a stereo binocular microscopes. The identification of these fungi was based on cultural, morphological and description in existing publications [6, 10]. Adequate pathogenicity tests were conducted on the identified fungal isolates to confirm their pathogenesis in the spoilage of crops in storage.

2. 2 Preparation of plant extracts

Following [15] method, 10gm of each plant leaves or bark was washed and separately ground in a blender with 300ml of distilled water. The solutions were allowed to stand overnight and then strained through a 45µ mesh. The filtrates were dispensed into universal specimen bottle and stored in the Refrigerator. Before use, the filtrates were centrifuged at 1000rpm (revolution per minute) for 15 minutes.

2.3 Activity of Plant extracts in-vitro

Following [7], 2ml of each plant extract and of a fungicide was incorporated into 15ml of potato Dextrose Agar (PDA) medium in different Petri dishes. One 5mm agar dish containing each fungal pathogen was placed at the center of each dish and incubated for 5 days under alternating 12 hour of light and dark periods at 25oCelsius. Adequate control was prepared by incorporating sterile distilled water into PDA as earlier described. Each treatment was replicated 3 times laid out in a Completely Randomized Design. After 5 days of incubation, mycelial development and sporulation were evaluated. To determine area covered by mycelial growth, with a measuring rule, diameters of the longest and shortest points were taken and area covered calculated. To collect conidia for sporulation count, Petri dish from each treatment was rinsed with 10ml of distilled water. One drop of the solution was diluted in 20ml of distilled water and with a loop; one drop from the dilution was placed on a haemacytometer counting slide and viewed under powerful stereo and compound electronic microscopes at 40 x magnification.

2.4 Statistical analysis

Data collected were subject to One-way ANOVA and means were separated by Duncan's Multiple Range Test (Pr < 0.05) using SPSS 14.0 version package.

3 RESULTS

The frequently observed symptom on stored seeds and grains was mold. The seeds or grains were observed caked or glued together by cottony conidia hyphae that maybe whitish, orange or greenish. The seeds were found to be bristle and easily crush under little pressure in between fingers. The pathogens found mainly associated with the seeds and grains were Aspergillus flavus, Aspergillus niger, Pennicillium expansum, and Rhizopus stolonifer. A significant loss of harvested crops was found associated with these pathogens. Evaluation of plant extract on the control of these isolated pathogens revealed that, the inhibitory effect of each of the nine plants tested varied with each of pathogens. Leaf extract of Vernonia amygdalina was as effective as benomyl in the inhibition of mycelial growth of Aspergillus flavus (Table 1) but were significantly different in the inhibition of its sporulation (Table 2). Leaf extract of Aloe vera, Annona squamosa, Azadirachta indica and Newbouldia laevis were equally effective in the inhibition of the mycelial growth of the pathogen. Bark extract of Acalypha ciliata, Annona squamosa, Azadirachta indica and Vernonia amygdalina were also effective in inhibiting the mycelial and sporulation of Aspergillus flavus. On mycelial growth of Aspergillus niger and Penicillium expansum the plant extracts showed only slight inhibitions of mycelial growth except Azadirachta indica that showed good inhibition of mycelial growth of Aspergillus niger whereas on sporulation, leaf extract of Aloe vera, Azadirachta indica and leaf and bark extract of Annona squamosa were effective in the reduction of Aspergillus niger spore development. Leaf and bark extract of Annona squamosa and the leaf extract of Azadirachta indica completely inhibited mycelial development and spread of Rhizopus stolonifer and were not significantly different from benomyl. The overall result revealed that leaf extracts had better in inhibitory effects on the pathogens than bark extracts.

Table 1: Effect of leaf and bark aqueous plant extract and benomyl on mycelial growth of stored product pathogens.

Control materials (Treatments)	*Aspergillus flavus*		*Aspergillius niger*		*Penicillium expansum*		*Rhizopus stolonifer*	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Sterile distilled water	14.14^{a}	14.14^{a}	14.14^{a}	14.14^{a}	14.14^{a}	14.14^{a}	14.14^{a}	14.14^{a}
Benomyl	0.78^{h}	0.78^{g}	0.13^{j}	0.13^{g}	0.00^{i}	0.00^{h}	0.00^{e}	0.00^{d}
Acalypha ciliata	2.09^{c}	1.57^{f}	10.32^{c}	11.52^{c}	12.99^{b}	10.60^{d}	12.99^{b}	0.73^{c}
Aloe vera	1.57^{e}	NA	4.03^{g}	NA	12.78^{c}	NA	14.14^{a}	NA
Annona squamosa	1.57^{e}	1.57^{f}	3.67^{h}	7.07^{e}	10.37^{d}	10.54^{f}	0.00^{e}	0.00^{d}
Azadirachta indica	1.15^{f}	1.57^{f}	0.79^{i}	13.35^{b}	12.77^{c}	11.10^{c}	0.00^{e}	4.29^{b}
Melia azedarach	0.89^{g}	6.81^{d}	7.60^{e}	7.07^{e}	9.74^{e}	10.91^{d}	14.14^{a}	14.14^{a}
Newbouldia laevis	1.83^{d}	8.11^{b}	8.59^{d}	7.60^{d}	9.43^{f}	14.04^{b}	14.14^{a}	14.14^{a}
Persea Americana	2.88^{b}	7.33^{c}	11.26^{b}	11.52^{c}	7.22^{g}	14.14^{a}	2.20^{d}	14.14^{a}
Vernonia amygdalina	0.79^{h}	2.10^{e}	6.13^{f}	6.24^{f}	6.50^{h}	9.32^{g}	10.68^{c}	14.14^{a}

Mean values in a column with same letter are not significantly different at 5% level of probability by Duncan Multiple Range Test.

Table 2: Effect of leaf and bark aqueous plant extract and benomyl on the sporulation of stored product pathogens.

Control materials (Treatments)	*Aspergillus flavus*		*Aspergillius niger*		*Penicillium expansum*		*Rhizopus stolonifer*	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Sterile distilled water	1427.7^{a}	1427.7^{a}	898.8^{c}	898.8^{a}	528.7^{b}	528.7^{b}	599.7^{a}	599.7^{a}
Benomyl	27.7^{f}	27.7^{f}	6.33^{j}	6.33^{j}	7.83^{j}	7.83^{i}	10.17^{j}	10.17^{h}
Acalypha ciliata	48^{d}	10.33^{i}	920^{b}	83^{f}	142.33^{g}	91^{g}	221.33^{d}	225.33^{b}
Aloe vera	70^{b}	NA	15.33^{g}	NA	177.7^{e}	NA	196^{g}	NA
Annona squamosa	12.7^{h}	24.33^{g}	13.7^{h}	60.33^{i}	65.33^{i}	70.33^{h}	43.33^{h}	32^{d}
Azadirachta indica	8.33^{i}	47^{e}	10^{i}	891.33^{b}	681^{a}	725^{a}	24^{i}	67.33^{d}
Melia azedarach	57.7^{c}	314.7^{b}	87.7^{f}	164.33^{d}	218.7^{c}	154.7^{e}	288.33^{c}	46.33^{e}
Newbouldia laevis	33.7^{e}	234.7^{d}	363.33^{e}	282.33^{c}	207.33^{d}	187^{d}	554.33^{b}	139^{c}
Persea Americana	15.33^{g}	274.7^{c}	1461.7^{a}	87.7^{e}	132.33^{h}	92.33^{f}	204^{e}	32^{f}
Vernonia amygdalina	33^{e}	21.00^{h}	486.33^{d}	282.33^{c}	164^{f}	247.33^{c}	200^{f}	24.66^{g}

Mean values in a column with same letter are not significantly different at 5% level of significance by Duncan Multiple Range Tests.

4 DISCUSSIONS

Aspergillus flavus is a mycotoxigenic fungus infecting several crops including maize, moldy groundnuts, soyabean and other stored products in Africa [8, 4, 5]. Rhizopus stolonifer was found associated with soft rot of cashew and guava. He et al. [12] found Penicillium expansum associated with decay of fruits and vegetables. Bankole et al. [5] reported 23 and 25% loss in stored maize due to Aspergillus flavus and Aspergillus niger infection respectively. In this present study, these two pathogens were frequently recovered from maize grains. Azadirachta indica, Acalypha ciliata, Aloe vera, Annona squamosa and Vernonia amygdalina, showed great significant inhibition on Aspergillus flavus, both in mycelial growth and sporulation. This is in agreement with the results obtained by [19], where Azadirachata indica seed oil was found to have inhibitory effect on Aspergillus flavus and [15], who found Acalypha ciliata and Vernonia amygdalina to have inhibitory effects on Fusarium moniliforme. Similarly, [9] found ethanol extracts of ripe fruits of Melia azedarach to have fungistatic and fungicidal effect against Aspergillus flavus. In this study, the crude aqueous extracts of Melia azedarach showed significant inhibition against Aspergillus flavus, Aspergillus niger and Penicillium expansum. Singh, et al [18], reported the effective control of Aspergillus flavus with Azadirachta indica. In this study, Azedirachta indica was very effective in the control of Rhizopus stolonifer, Aspergillus flavus and Aspergillus niger. Ali, et al., [3], equally found Azedirachta indica to be as effective as thiabendazole in checking post-harvest fruit rotting fungi of tomato. Azedirachta indica in this study, compared favourably with benomyl in the control of Aspergillus flavus, Aspergillus niger and Rhizopus stolonifer.

In this study, Annona squamosa and Azadirachta indica completely inhibited the mycelial growth and sporulation of Rhizopus stolonifer. This is in agreement with the earlier report of [7] on the inhibitory effect of Annona species on Rhizopus stolonifer sporulation. Owolade, et al [15] reported the inability of Azadirachta indica to completely reduce the incidence of a fungal pathogen although it significantly reduced the rate of sporulation of the pathogen. In this present study, Azadirachta indica leaf extract completely inhibited mycelial growth of Rhizopus stolonifer but could not do so on its sporulation. Furthermore, the result of this study revealed that, Vernonia amygdalina was effective against all the fungal pathogens tested. This result is similar to [11] result on the reduction seed mycoflora of wheat when stored with extracts of Vernonia amygdalina.

5 CONCLUSIONS

Postharvest losses of durable crops in storage can only be reduced if seeds are protected with fungicides but the perceived harmful effects of synthetic fungicides currently in use on human and the environment no longer make them attractive to use. Based on the findings of this study, there are great potentials in the control of storage fungal diseases using naturally occurring substances that are both humanly and environmentally friendly and at the same time affordable at less cost to the users than the procurement and use of chemically formulated fungicides.

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