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Effects of the Aqueous Extracts of *Azadirachta Indica* (Neem) and *Carica Papaya* (Pawpaw) on *Aspergillus Niger* isolated from Rice Grain

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Abstract

Aqueous extracts were obtained from the matured leaves of two medicinal plants: namely, neem (azadirachta indica) and pawpaw(carica papaya). They were evaluated for their antifungal activity over Apergillus niger, a pathogenic fungus of many economic crops. Crude methanolic extracts of A. Indica and C. Papaya at 40%, 60%, 80% and 100% concentrations were tested on potato dextrose agar (PDA) for activity against the mycelial growth. The replicated plates were incubated for 5 days at 27 $\pm$ 2oC. The results showed that the extracts have fungitoxic components that controlled the disease. The mean percentage inhibition of mycelia growth was highest in plates containing extract of C. Papaya at various concentrations tested, with the mean percentage inhibition value significantly (P=0.05) higher than in plates containing extract of A. Indica. A statistical evaluation of variance showed a significant difference between mycelia radial growth values recorded on the plant extracts concentrations used compared with the control. The fungitoxic nature of the extracts of these plants in controlling the mycelia growth of the fungus was appreciated as the plants are readily available, affordable, and easily biodegradable and

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environmentally friendly, could therefore, be preferable to synthetic fungicides.

Key words: *Aqueous leaf extracts. Aspergillus niger, concentration, inhibition, mycelia growth.*

Introduction

Rice (*Oryea sativa*), family poacea, is one of the leading food crops of the world. It is one of the most important stable foods of the world with several million people dependent on it. It is the second most important cereal crop in Nigeria in terms of area cultivated, output, consumption and nutritional value (FAO, 1998). Spoilage of stored rice is attributed to storage fungi which were introduced during the post harvest handling process (Khanzada and Jamil, 1987) and (Chiejina and Ulobo, 2006).

Medicinal plants are considerable in number and economically important, they contain active substances used in treatment of many human diseases, either as direct remedies as well as in pharmaceutical cosmetics, perfumery and food industries. The first treatment method for man from history is, making use of herbs which has been the first art of available treatment for man (Kafaru, 1994). A lot of plants have established healing power with varied examples.

Neem *(Azadirachta indica)* and pawpaw *(Carica papaya)* in the families meliacene and caricacaceae respectively are widely grown and used in different parts of Nigeria mainly for food, ornamental and in traditional health care services (Sofowora, 1997 and Iwu, 1993).

Recent reports show that there is an increase in the use of plant parts (leaf, root, stem and bark) in the control of fungal disease contrary to their ancient use in healing ailments by traditional medicine practitioners in Nigeria and other African countries (Bilgram *et al* 1979) Chin (1992) stated that those plants have effective broad-spectrum anti-fungal activities in laboratory studies over the years. The effectiveness has been confirmed by modern scientific studies. *Azadiracta indica* A. Juss has been under intensive study for the past decade (Schmutterer *et all,* 1981). It is a common

tropical tree, widely distributed in Africa and Asia. It's medicinal and shade uses have been known for several centuries. Fungicidal properties of neem extracts are promising. Lal *et al* (1980) showed that neem oil and neem seed cake extracts significantly reduced conidial germination in several fungi, especially *Sclerospora sacchari,* isolated from maize.

Due to identifiable problems (e.g. chemical residues, biodegradation, phytotoxicity, pollution, etc) associated with chemical control strategies; alternative control methods are employed. Also, since *Aspergillus* survives adverse environmental conditions and develops adaptive resistance to fungicides which are used for its control, it is necessary to attempt to find cheaper, environment friendly means of controlling the rot fungus using some medicinal plans. This could add to methods of control used by farmers, thereby reducing reliance on fungicides that are reported to predicate long term harmful consequences on environment, man and other wildlife.

The objectives of the study is therefore to determine the efficacy of crude methanolic extracts of neem (*Azadirachta indica*) and pawpaw (*Carica papaya),* at different concentrations on mycelia growth of *Aspergillus niger* isolated from rice grain (*Oryza sativa*)

Methodolody

Sample Collection

Infected grain samples of three (3) local varieties of rice grown in Benue State namely: "China", "Mars" and "Turn-2" were collected from six suppliers in Makurdi metropolis namely: North Bank, Wurukum and Wadata markets. Samples were disinfected in 1% sodium hypochloride (NaOCL) solution for 3 minutes, rinsed in several changes of distilled water, dried in-between sheets of sterile filter paper and then plated on fresh potato dextrose agar (PDA) medium in 9.0cm petridishes. The dishes were incubated at 28°C for seven days. Three sub-cultures were made to obtain pure cultures of the pathogen. Plant materials used were leaves of *Azadirachta indica* (neem) and *Carrica papaya* (pawpaw). They were obtained in the University premises, Kogi State University.

Sample processing and extractions

From the fresh samples of each, crude methanolic extractions were used according to (Amadioha, 2002; Epidi and Alamene, 2005; Ojo and Olufolaji, 2005 and). Each of the plant leaf samples were washed thoroughly in cold running tap water allowed drying for seven days. 500g of each was homogenized using warring blender, and each of them placed in 1000ml flasks containing 500ml methanol and thoroughly mixed together using glass rod and left for 24 hours for proper extraction of the active ingredients as described by Wokocha and Okereke (2005). On the other hand, hot organic solvent extraction was carried out by weighing the same quantity of samples (500g), washed and soaked in 500ml of methanol in a 1000 ml conical flask. The filtrates was concentrated using the vacuum evaporator so as to regenerate the methanol. It was filtered using Buckner funnel and dried solidified extracts weighed. Percentage yield of extracts in percentage (%) was determined using the formula;

$$\frac{\text{Solid extracts}}{\text{Samples}} \times \frac{100}{1}$$ (Taiga et al, 2008)

From the crystal samples, 100g was weighed and dissolved in 100ml distilled water to give the final concentrations of 100% (stock), subsequent concentrations of 80%, 60% and 40% were prepared by serial dilution a modified method of (Epidi and Alamene, 2005). Potato Dextrose agar was prepared according to manufacturer's specifications and sterilized at 1.2 kg/cm3 pressure for 15 minutes in the autoclave. Six millilitres (0.1%) of streptomycin was added to the 1 litre of the sterile medium just before pouring into Petri-dishes, to prevent the growth of bacteria.

Determination of antifungal activity

One millilitre of each plant extract at 40%, 60%, 80% and 100% concentration was pipette separately and aseptically into 10ml of cool, molten potato dextrose agar (PDA) medium in each of the Petri-dishes. Each medium was thoroughly homogenized by gentle circular rotations in order to achieve uniform dispersal of the extract.

The media were allowed to solidify and after 24 hrs each plate was inoculated by placing a 5mm disc taken from the advancing edges of 6-day old cultures of the isolate, in the centre of each petri-dish, that is, disc diffusion method (Amadioha, 2002). Six-day old isolate previously inoculated into fresh PDA – extract media at different concentration stated above were inoculated onto fresh PDA – extract media at different concentration stated above were inoculated. Similarly, plant extract-free PDA plates inoculated with mycelia discs served as controls. All dishes were incubated for five days at 27 ± 2oC. All experiments were repeated three times with three replications each. Percentage inhibition of mycelia growth at different concentrations was calculated by a modification of the formular of Pandey *et al,* (1982).

$$\%\text{Inhibitions} = \frac{\textit{Diam, control plates} - \textit{Diam, in treated plates}}{\textit{Dim, control plates}} \times \frac{100}{1}$$

where dc = Average diameter of fungal colony in control plates;

dt = average diameter of fungal colony in treated plates.

Analysis of Results

All results obtained were analyzed using Simple Descriptive Statistics such as mean and standard error Means were separated using analysis of variance. Honestly Significant Difference (HSD) was used for inferential statistical analysis while standard error was used for descriptive statistics.

Results

The fungal pathogen was isolated and identified as *Aspergillus niger.* From the results, it was observed that the two aqueous leaf plants extracts screened, *in vitro,* showed varying levels of toxicity to *Aspergillus niger,* expressed as mean percentage inhibitions of mycelia growth. Results in Tables I and II showed that the mean percentage inhibitions of mycelia growth in the fungus was highest (93.59%) in plates containing *C, papaya* leaf extracts. Analysing the tables, there is substantial variation in the level of radial mycelia growth inhibition values of *Aspergillus niger* in pawpaw leaf extract and that of neem leaf extract at 40% and 100% concentrations. The

inhibitory action of the extracts on mycelia growth increased with increasing concentrations, giving a toxicity profile of 100% > 80% > 60% > 40% with no significant (P=0.5) differences between them. There was however a significant difference in statistical test at (p=0.5) between mycelia radial growths values recorded on the various plant extracts concentrations used compared with the control.

Table 1: Inhibitory effect of the leaf extracts of *C. Papaya* on *Aspergillus niger.*

Concentrations(%)	Average (cm) (%)	Percentage inhibition
Control plate	4.50	0.00
40	2.25	50.0
60	1.95	56.6
80	1.12	75.1
100	0.29	93.5

Table II: Inhibitory effect of the leaf extract of *A indica* on *Aspergillus niger*

Concentrations(%)	Average (cm) (%)	Percentage inhibition
Control plate	4.50	0.00
40	2.62	41.7
60	2.17	51.7
80	1.63	63.7
100	0.83	81.5

Discussion

The outcome of this study showed that leaf extract of *C. Papaya* and *A. Indica* inhibited mycelia growth of *Aspergillus niger in vitro.* It was also observed that fungitoxicity of the extracts was higher at increased concentration, it equally varies with the plant leaf extracts used. These suggest that there is difference in the water soluble

antifungal element in the respective leaves extracts as reported by Iwu, (1993) and Sofowora, (1997). Neem leaf extract (*A. Indica*) was used for the study of control of plant diseases being ecologically friendly (Amadioha, 2002). Study shown that the neem, *A. Indica* as a medicinal plant posses' potent antifungal properties in the leaves which inhibit mycelia growth. It contains phytochemical compounds such as *azadirachtin, betasiterol,* 6-desacety1 nimbinene and 3-desciacetyl alamine which show antifungal properties. The ability of neem leaf extract to reduce the mycelia growth of *Curvularia lunata* isolated from neem itself, it's own endophytic mycoflora also proved that, neem has high antifungal properties (Venna and Kharwar, 2006). *The results obtained in this study on A. Niger agrees with these reports.*

Pawpaw leaf extract (*C.papaya*) equally used for the control of plant diseases in the present study proved that mycelia growth was inhibited at higher concentrations. Pawpaw leaf extract has also been reported as being used as fungicide in seed treatment of African yam bean. Pawpaw leaf extracts had been used to control incidence of foliar myco-pathgens of groundnut (*Arachis hypogeal*) due to its prophylactic effect (Ogwulumba *et al,* 2008).

Conclusions

This study has revealed the potential of plant extracts on *Asprgillus niger* isolated from rice grain. From the forgoing, pawpaw leaf, *Carica papaya* and neem, *Azadirachia indica* posses antifungal elements that inhibit *Aspergillus* mycelia growth at various concentrations. However, it is pertinent to note that this finding is one among many possibilities of utilizing plant parts in disease control, this required more efforts in integrating the study of other related findings. The use of plant product could reduce reliance on one source of agricultural chemical to the farmer, as well as cut down cost production.

Recommendations

In view of this remarkable report, one could confidently suggest that neem (*Azadirachta indica*) be used as medicinal plants in the control of certain microbial causative agents. The facts that plants

used in this study are easily available, with easy method of extraction, they can be exploited in the control of *Aspergillus niger* on rice grain.

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References

Amadioha, A.C. (2002). Fungicidal activity of leaf extracts of four plants against pathogeni fungi of rice in Nigeria. *Archives of Phytopathology and Plant Protection 51:* 50-51.

Bilgram, S.K. Srivastava, L.M. and Shreemali, S.L. (1979). Fundamental of Botany p. 470-477.

Chiejina, N.V. and Ulobo, E.B. (2006). Fungi in soil cultivation with rice (*Oryza sativa*) to rotation with tubers, *Jour. Of Bio Research 4* (2): 78-84.

Epidi, T.T. Alamene A. and Onuegbu, B.A. (2005). Influence of some plant extracts on yield and Pests of Cowpea (*Vigna unguiculata (L.)* Walp), *Nigerian Journal of Plant Protection 2: 65-76.*

FAO (1998). Rice Milling in Developing Countries. FAO, Rome. Commodity Bulletin.

Iwu, M. (1993). Handbook of African Medical Plants. CRS Press.

Kafaru E. (1994). Immense Help from Natures Workship: Elikaf Health Services Publication, Ikeja, Lagos – Nigeria pp.1-10.

Khanzada, A.K. and Jamil Khan, S.A. (1987). Efficiency of Some Detection Methods for Seed Borne Fungi in Seed Health Testing Programme. *Pakistan Journal of Agriculture 8* (2): 74-86.

Lal, S.K. Nath and Saxena, S.C., (1980). Use of pesticides and Natural products in control of *Sclerospara sacchari* in maize. *Tropical Pest Management 26: 286-292.*

Ogwulumba, S.L. Ugwuoke, K.I. and Iloba, C. (2008). Prophatic effect of pawpaw leaf and bitter leaf extracts on the incidence of forliar mycopathogens of Groundnut (*Arachis hpogea*) in Ishiagu Nigeria. *African Journal of Biotechnology 7* (21): 240-262.

Ojo, B.A. and Olufolaji, D.B. (2005). Evaluation of the efficacy of crude Neem bark extracts in enhancing germination and seedling establishment of Anthracnose Diseased Soyabean seeds *Nigerian Journal of Plant Protec. 22:* 132-138.

Olufolaji, D.B. (1999). Control of Wet Rot Disease of *Amaranthus species* caused by *Choamephora cucurbitarium* with extract of *Azadirachta indica, Journal of Sustain. Agric Environ.* (1 & 2): 132-190.

Pandey, D.K. Tripath, N.N. Tripathi, R.D. and Dixit, S.N. (1982). Fungitoxic and Phytotoxic properties of essential oil of Hyptis suaveolens. Z. Pifkrankh. 89: 344-349.

Schmutterer, H. Ascher, K.R.S. and Rembold, H. (1981). Natural Pesticides from the neem tree (*Azadirachia indica A.* Juss). Federal Republic of Germany. 297 pp.

Sofowora, A. (1997). *Medicinal Plants used in Traditional Medicine in Africa.* First Ed. John Wiley and Sons. Chichester New York pp. 128 – 129.

Taiga, A. Suleiman, M.N. Sule, W.F. and Olufolaji, D.B. (2008). Comporative in vitro Inhibitory effects of cold extracts of some fungicidal plants on Fusarium oxysporum mycelium. *African Journal of Biotechnology 7* (18): 3306-3308.

Venna, V.C. and Kharwar, R.N. (2006). Efficacy of neem leaf extracts against his own fungi endophyte Curvularia lunata. *Jour of Agric. Technol. 2:* 329-353.

Wee Yeow Chin. (1992). A guide to medicinal plant, Singapore Science Centre. P.33

Wakocha, R.C. and Okereke, V.C. (2005). Fungitoxi activity of extracts of some medicinal plants on *Sclerothm rolfsii,* casual organism of the Basal stem rot diseases of Tomato. *Nig. Journ. of Pit. Protection 22: 106-110.*