

EFFECT OF AQUEOUS AND n-HEXANE EXTRACTS OF *Khaya ivorensis* A Chev (Meliaceae) ON THE CONTROL OF RICE SEED-BORNE FUNGI

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ABSTRACT

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Experiments were carried out to test the effect of aqueous and n-Hexane extracts of *Khaya ivorensis* on the control of rice seed-borne fungi. Seed-borne fungi associated with the seeds of five rice varieties (NERICA 5, FARO 29, FARO 49, NERICA 18 and NERICA 36) were isolated using the agar plate incubation method. N-hexane and aqueous leaf extracts of *Khaya ivorensis* were tested at 5% v/v and 10% v/v of the culture medium on *Fusarium verticilloides*, one of the most predominant fungi, isolated from the seeds. Methanol alone applied at 5%v/v of the culture medium served as the control while Carbendazim (a benzimidazole fungicide) applied at 1g/litre served as a check. Inhibition of mycelia growth/diameter was recorded as a measure of the effectiveness of the extracts. The results of the experiment showed a significant reduction ($p > 0.05$) in the diameter of growth of the test fungus treated with the extracts compared to the control. N-hexane leaf extract of *khaya ivorensis* at both concentrations performed better than the aqueous extract in reducing the growth of the test fungus at the different days after plating studied. This portends great opportunities in the use of botanical pesticides as antimicrobial agent in seed-borne infection.

Keywords: *Khaya ivorensis*, *Fusarium verticilloides*, n-Hexane leaf extract, Anti-fungal property, seed-borne infection, Rice.

INTRODUCTION

Nigeria is one of the largest rice producing countries in Africa (Baksh, 2003). Production is estimated to be about three million tonnes of grains per year. This level of production falls short of annual domestic demand of about 5 million tonnes (FAO, 2006). Pests and diseases according to Imolehin and Wada, (2000) are an important constraints limiting rice production efforts in Nigeria. A number of economically important diseases affecting the crop are reported to be transmitted through the seeds (Mathur and Neergaard, 1970; Ibiam et al., 2006). Fungal pathogens are by far the most prevalent among rice seed-borne pathogens (Ou, 1985). The control of seed-borne pathogens as an integral part of rice disease management in realizing adequate rice production potential can therefore not be overemphasised. Apart from traditional cultural practices, application of chemicals (pesticides) is also widely used by farmers in developing countries like Nigeria. The indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Extensive use of these chemicals has been shown to pose a lot of threat to human health and the environment (Swaminathan, 1975). This has generated research interest into the use of alternative pesticides with no health implication on humans as well as little or no negative impact on the environment.

Some plants contain components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components are called botanical pesticides or botanicals. The determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure (Malkhan et al., 2012). Plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract (Parekh et al., 2005) and chloroform was found to be the best solvent for the extraction of non-polar biological active compounds (Harmala et al., 1992). The most commonly used solvents for preliminary investigations of antimicrobial activity in plants are methanol, ethanol and water. The other solvents used by researchers are dichloro-methane, acetone, and hexane while some authors use a combination of these solvents to obtain the best solvent systems for extraction (Eloff, 1998). Research on bioactive substances from plant sources could lead to the development of antifungal agents that can combat fungal resistance. This work was therefore carried out to evaluate the antifungal property of aqueous and n-hexane leaf extracts of *K. ivorensis* A. Chev. (*African Mahogany*) on rice seed-borne fungi.

MATERIALS AND METHODS

The seeds of the rice varieties used for this study were obtained from the National Cereal Research Institute (NCRI) Badegi, Niger State, Nigeria. Clean seeds free of physical damages were selected from the seed lots and kept in polythene bags for further use. Seed-borne mycoflora were isolated from the seeds using agar plate incubation procedure described in the International Rules for Seed Testing with slight modifications (ISTA,

1976). Rice seeds pre-treated with 1% NaOCl for 30 seconds were placed on acidified potato dextrose agar amended with streptomycin in 9cm diameter petri-dishes at the rate of ten seeds per variety per plate in three replicates.

The plated seeds were then incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 7 days under alternating cycles of 12 hours of light and darkness. The plates were examined under a stereo binocular microscope for fungal growth and identification. The fungal isolates were then grown into pure cultures and slides were prepared from each isolate for observation of microscopic structures. Identification of the isolates was carried out by matching the cultural and microscopic characteristics with the descriptions in standard references. The references used in this study were Kulwant *et. al.*, (1991) and Barnett and Hunter, (1992). The percentage occurrence of each fungal isolates was recorded.

One fungal isolate with high frequency of occurrence was selected. Confirmation of identity of the fungus was carried out at the International Institute of tropical agriculture (IITA), Ibadan - Nigeria. This was then used for the test of antifungal property of the plant extracts.

Collection of plant material and preparation of plant extracts

Fresh leaves of *Khaya ivorensis* was collected from the plant where it was found growing naturally at Ilorin, Kwara State. The leaves were left in the air-dried for 2 days and then ground into fine powder using mortar and pestle. Five hundred grams of the leaves was soaked in 2.5 litres of N-Hexane for 5 days in 5 litres flask. The mixture was sieved on the sixth day to remove the extract from the leaves. This procedure was repeated for aqueous extraction. Steam bath distillation was later carried out to separate N-Hexane from the extract while water extract was air dried to get the extracts.

Test of antifungal property of the plant extracts (in-vitro)

One gram each of the plant solvent and aqueous extracts was dissolved in 10 ml of methanol. 500 μl and 1000 μl of the resulting mixtures was amended with Potato Dextrose Agar (PDA) to make 10ml of the growth medium before solidification in 9cm petri dishes giving 5%w/v and 10%w/v plant extract treatments. The medium amended only with methanol served as control and a standard fungicide carbendazim applied at 1g/litre served as a check. The plates were inoculated with 5mm diameter mycelia disk from 15-day-old culture of the test fungus (*Fusarium verticilloides*). The plates were incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under alternating cycles of 12 hours of light and darkness.

Measurement of mycelia growth was carried out on days 2, 4, 6, 8 and 10 after inoculation following two perpendicular lines passing through the centre of the dish. Percentage inhibition of mycelia growth was determined adapting formula by Wokocha and Okereke (2005) .

Percentage inhibition of mycelia growth = $\frac{dt - de}{dt} \times 100$

where dt (cm) = diameter of fungal growth in blank petri-dishes,

de (cm) = diameter of fungal growth in other petri-dishes.

The data collected were subjected to analysis of variance at 5% level of significance using Genstat (Discovery) Edition 3.

RESULTS AND DISCUSSION

Ten species of fungi were isolated from the five rice varieties. The isolated fungi were *Aspergillus flavus*, *A. Niger*, *A.fumigatus*, *Colletotrichum lindemuthianum*, *Cladosporium sp.*, *Fusarium oxysporum*, *Fusarium verticilloides*, *Macrophomina sp.*, *Penicillium chrysogenum*, and *Rhizopus stolonifer* (Table 1). The result showed a significant difference ($p > 0.05$) in the mean population of the different fungi isolated from the rice seeds used. Nearly all the fungal species have been reported earlier by other scientists to be associated with rice. *Aspergillus flavus*, *A. Fumigatus*, *A. Moniliforme*, *Cladosporium sp.* *Penicillium sp.* and *Rhizopus sp.* were isolated from 50 rice samples of 12 cultivars in Pakistan (Jamil Khan *et.al.*, 1988). Ibiam *et.al.*, (2008) reported *Aspergillus flavus*, *A. Niger*, *Fusarium moniliforme*, *F. Oxysporum*, *Penicillium sp.* and other fungi on three rice cultivars (FARO 12, 15 and 29) in Nigeria.

Fusarium verticilloides Syn *Fusarium moniliforme* was the most predominant species. In India, Lalitha *et. al.* (2011) reported isolation of *Fusarium moniliforme* and *Fusarium oxysporum* among other fungi from paddy collected from farmers fields, regulated markets and retail shops. The result of the test of antifungal property of the plant extracts on the test fungus showed that the plant extracts reduced growth of the test fungus significantly ($p > 0.05$) when compared to untreated fungal cultures (Table 2). The plant extracts were most effective at two days after plating. In the case of aqueous extract applied at 5%v/v, there was 13.26% reduction and at 10%v/v there was 37.67% reduction in the growth diameter of the test fungus compared with the control. For 5%v/v and 10%v/v of n-hexane extract, there was 36.05% and 47.87% reduction in the growth diameter of the test fungus respectively compared with the control. The effect of the extracts however reduced progressively as the number of days after plating increased.

Extracts from the stem bark of *Khaya ivorensis* has been reported to contain limonoids (Samir, et. al., 2005) which are believed to have a wide range of biological activities including antifungal property. In Nigeria, the leaves of *Khaya ivorensis* used in a mixture with those of *Piliostigma thonnigii* for parboiling yam was reported (Babajide and Atanda, 2008) to reduce the microbial load in the treated yams.

CONCLUSION AND RECOMMENDATION

In this experiment, the leaf extracts only suppressed the growth of the test fungus with better results at the higher concentrations in all cases. It was evident that *Khaya ivorensis* leaf extracts contains components which are active against the test fungus and n-hexane leaf extract performed better than aqueous leaf extract in suppressing the growth of the fungus. It is therefore suggested that more research be conducted that will further elucidate and characterize the active components and their mechanism of action. It will also be necessary to determine the most appropriate solvent for the extraction of the active component and the optimal level of application of the extracts for better results.

Table 1: Mean population of fungi isolated from rice seeds

Organisms	Mean incidence
<i>Aspergillus flavus</i>	0.4 ^a
<i>Fusarium verticilloides</i>	1.3 ^b
<i>Penicillium chrysogenum</i>	3.9 ^{bc}
<i>Colletotrichum lindemuthianum</i>	0.5 ^a
<i>Macrophomina phaseolina</i>	0.4 ^a
<i>Aspergillus niger</i>	0.2 ^a
<i>Rhizopus stolonifer</i>	0.1 ^a
<i>Cladosporium sp.</i>	0.4 ^a
<i>Fusarium oxysporium</i>	0.7 ^b
<i>Aspergillus fumigatus</i>	0.1 ^a
SE	0.2
LSD	0.6

Mean values followed by the same letter are not significantly different (p=0.05)

Table 2: Effect of treatments on growth (change in diameter) of the test organism (*Fusarium verticilloides*)

Treatment	2 (DAI)	4 (DAI)	6 (DAI)	8 (DAI)	10 (DAI)
Aqueous extract@ 0.05% w/v	3.73 ^{bd} (13.26)	4.30 ^{bd} (21.82)	5.10 ^{bd} (21.54)	6.00 ^{bc} (18.92)	6.20 ^b (27.06)
Aqueous extract@ 1.0% w/v	2.68 ^{bc} (37.67)	3.72 ^{bc} (32.36)	4.80 ^{bc} (26.15)	5.70 ^b (22.97)	6.15 ^b (27.65)
N-Hexane extract@ 0.5% w/v	2.75 ^{bc} (36.05)	3.68 ^{bc} (33.09)	4.75 ^{bc} (26.92)	5.50 ^b (25.68)	6.75 ^{bc} (20.59)
N-Hexane extract@ 1.0% w/v	2.25 ^b (47.67)	3.20 ^b (41.82)	4.25 ^b (34.62)	5.60 ^b (24.32)	6.80 ^{bc} (20.00)
Carbendazim @ 1g/litre	0.15 ^a (96.50)	0.10 ^a (98.18)	0.10 ^a (98.46)	0.10 ^a (98.65)	0.10 ^a (98.82)
Control (only methanol)	4.30 ^{be} (0)	5.50 ^{be} (0)	6.50 ^{be} (0)	7.40 ^{bc} (0)	8.50 ^{bd} (0)
LSD	0.13	0.27	0.12	0.73	0.14

Values are means of three replicates; Means in the same column with different superscript are significantly different at p=0.05; () percentage reduction in growth diameter of the test fungus; Note: DAI = Days after inoculation

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