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THE INCIDENCE OF SEED – BORNE MYCOFLORA OF SESAME (*Sesamum indicum* L.) AND ITS CONTROL USING *Anacadium occidentale* AND *Mangifera indica* BARK EXTRACTS

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ABSTRACT
Seeds of ten (10) Sesamme (Sesamum indicum L.) cultivars were tested for seed brone mycoflora in the laboratory. Phytopathogenic fungi viz: Aspergillus niger, Aspergillus flavus and Penicillium sp were found associated with these samples. The percentage of fungal incidence on seeds of ten sesame cultivars ranged from 66.50 - 97.50%, with E8 having recorded the highest percentage incidence, while Off-1 had the least. The different percentages of fungal infection on seeds by the isolated fungi were equally determined. It ranged form 0.00 – 15.50%. The efficacy of aqueous bark extracts of Anacadium occidentale and Mangifera indica to control the radial mycelial growth of the isolated fungi was evaluated. Results show that A. occidentale bark completely inhibited the radial growth of Aspergillus at 60% concentration; while A. indica bark extract significantly ($P \leq 0.05$) retarded the radial mycelial growth of Penicillium with highest inhibitory effect at 100% concentration. In vitro application of extracts for the control showed that Cashew (Anacadium occidentale) had more fungitoxic effect on mycelial growth of the fungi than was observed on mango (Mangifera indica). And the two extracts were generally showed more inhibitory effects on the mycelial growth of Aspergillus than was observed on Penicillium sp.

Key words: Seed borne, mycoflora, sesame (*Sesamum indicum* L.), extracts, percentage inhibition.

INTRODUCTION
Sesame (*Sesamum indicum* L.) is an important source of oil and protein. The plant is widely naturalized in tropical regions around the world and is cultivated for its edible seed which grow in pods (Bedigian, 2003). The quality and quantity of oil and protein is adversely affected by biological agents, which consequently influence the product manufactured by sesamum seed or its derivatives (Nasira *et al*., 2004).

The usage dates back to 300BC i.e. over 5000 years ago. It is an erect annual plant that grows to a height of 2m depending on the variety and growing conditions. Some varieties are highly branched, while others are unbranched (Mkamilo and Bedigian, 2007).

The health of *Sesamum* plant is affected by fungi by causing infections on roots, foliage and seeds. The products and by products of sesamum have tremendous value. The sesame seeds yield oil content of 46 – 52% (Metealfe and Donal, 1980). Sesame is an important oil seed drop of Nigeria and other parts of Africa (Schilling and Catan, 1991) its seeds are rich in oil (50 to 52%), carbohydrate 16 to 18% and protein (17 to 19%). Because of the high unsaturated fat and methionine contents, sesame seed and oil are in high demand in Nigeria as export materials.

Seed borne mycoflora are carried over by infected seeds. They cause deterioration in seed in storage and in soil before germination, causing seedling mortality and cause infection of foliage at adult stage. Such fungi include *Alternaria, Curvularia, Fusarium, Helminthosporium Memnoniella, Penicillium* and *Rhizopus* sp and have been found associated with sesame (Nasira *et al*, 2004). They equally reported that *Alternaria* is the most destructive pathogen of sesame, as it causes small brown sports on leaf ranging from 1–8mm in diameter. Infection of seeds, which reduces viability of seeds. *Alternaria* sesame produces brown necrotic sports on leaves, *Fusarium* sp which occurs in early stage of the crop growth, yellowing of the leaves is first noticeable symptom (Kumar *et al*, 1984) *Aspergillus* and *Fusarium* reduced the seed germination by causing seed rot. Many synthetic fungicides such as Benlate, Ridomil, Mancozeb had shown promise in the control of sesame disease; however, the high cost of these chemicals and its non degradable properly forbids their use by local farmers.

Seed – borne diseases are most disastrous as they reduce seed vigor, market value and weaken the plant at the initial stages of its growth. Seed – borne diseases caused by fungi are relatively difficult to control as the fungal hyphae may get established and become dormant. Little work had been done on seed – borne mycoflora of sesame and its control using plant extracts. Therefore the aim of this present study was to investigate the incidence of seed associated fungi, their frequency of association and also to determine the efficacy of the extracts of two tropical plant species on associated fungi.

MATERIALS AND METHODS

***Seed Samples*:** This study was carried out on seeds of 10 cultivars of sesamum from north central, Nigeria.

***Testing Procedure*:**

Seeds of all varieties were analyzed for their association of seed – borne mycoflora by standard blotter paper method, a modified method of (ISTA, 1985). Samples of 200 seeds were taken at random from each variety, plated on 9cm diameter sterilized Petri – dishes. In each of the dishes 20 seeds were placed on three – layered blotter paper soaked with sterilized distilled water. The seeds were disinfected with 0.1% Hypochlorite ($HgCl_2$) for 30 seconds and subjected to three washings with distilled sterilized water before plating.

Serial dilution method was used as testing procedure. Ten (10) seeds of sesame were soaked in 10ml of distilled water in a test tube. To make a serial dilution of 10^{-1}, 1ml of 10^{1} serial dilutions was pipetted into 9ml of distilled water in a test tube to give 10^{-2} serial dilution according to Suleiman and Taiga, (2009). Similar method was carried out to give final concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and the stock (10^{0}). All the inoculated plates were incubated for seven days at 25^{0}C and observed for fungal growth. The emerging fungi were sub-cultured on Potato Dextrose Agar medium (PDA) and examined under compound microscope for specific identification. The platted

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seeds were classified as infected and non-infected to determine the fungal incidence.

The bark of *Mangifera indica* (mango) and *Anacardium occidentale* (cashew) were used for the preparation of the plant extracts. Fresh samples of each were used for the organic solvent (methanol) extractions following the method of Epidi and Alamene, (2005); Ojo and Olufolaji, (2005) respectively. Each of the plant samples was washed thoroughly in cold running tap water, sun – dried and kept in the laboratory. Five hundred grammes of each were pounded and homogenized using warring blender, and placed in 1000ml flasks containing 500ml methanol and thoroughly mixed together using glass rod. They were then placed in pots of water and heated to 100^0C for 30 minutes to allow for extraction of the active ingredients as hot organic solvent extraction. The filterates was concentrated using the vacuum evaporator so as to regenerate the methanol. It was filtered using Buckner funnel and the dried solidified extracts weighed. From the dry extracts, 15.g, 20g, 25g, 30g were weighed separately and dissolved in 50ml distilled water to give the final concentration of 40%, 60%, 80% and 100%, a modified method of Epidi and Alamene, 2005); Ojo and Olufolaji (2005). Streptomycin was added at the rate of $125mg^{-1}$ to each of the plant extracts to check bacterial contamination and kept for the *in vitro* assay.

In vitro assay of plant extracts

The bioassay of the plant extracts was carried out by determining the effects of their concentrations on radial growth inhibition as described by Amadioha (2003). The PDA/crude extract medium was prepared by spreading 1ml of the extract separately on the surface of the solidified PDA in the Petri dishes. The control was PDA on which 1ml of sterile distilled water was spread on the surface. With the aid of sterile cork borer, 5mm diameter discs of seven – day old culture were cut from the pathogen grown in PDA, each placed at the centre of the Petri dish containing the PDA/crude extract, and each treatment replicated three times.

The whole set-up was arranged in a completely randomized design. The incubation was carried out at 27^0C and terminated at 7 days when the control mycelia had reached the edge of the Petri dish. The growth rate was measured along four radii using the site of the inoculum as the centre, minus the inoculum. Two perpendicular lines intersecting at right angles were drawn at the bottom of each plate. Percentage inhibition of mycelia growth was calculated using the formula of Pandey *et al*., (1982) as follows:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where dc = average diameter of fungal colony in control plates, while dt = average diameter of fungal colony in treated plates.

RESULTS

From the survey in the four locations, E8 variety was more susceptible to fungal attack, while Off-1 had the least. Fungal indicidence ranged from 66.50 to 97.50% on cultivars of sesame; which are grouped into four categories. In category one, fungal incidence was more than 90% which include Any (94.00%), 01M (95.00%) and E8 (97.50%) respectively. More than 80% fungal incidence was found on seeds of Oke (88.00%) and 02A (87.50%) respectively.

Seeds of 03M, Off-2 and Ex-sudan showed 70 – 80% fungal infection. While seeds of Off – 1 and ILO showed fungal infection percentage between 60 – 70% (**Table 1**).

Table 1: Fungal Incidence on Seeds of ten sesame cultivars

Cultivars	Normal seeds*	Infected seeds*	Fungal incidence (%)
01 – M	10	190	95.00
02 – M	25	175	87.50
03 – M	53	147	73.50
E8	05	195	97.50
Ex sudan	41	159	79.50
Oke	24	176	88.00
Any	12	188	94.00
1Lo	61	139	69.50
Off – 1	67	133	66.50
Off – 2	44	156	78.00

***Out of 200 seeds of each cultivar**

Three (3) fungal species were found associated with sesame seed samples. They include *Aspergillus nigar, Aspergillus flavus* and *Penicillium* sp. The infection percentage of these fungi in seeds varied. *Aspergillus niger* and *Aspergillus flavus* were found almost on all cultivars. These fungi were found almost on all cultivars. These fungi were found on Oke (5%), 01 – M (15.5%), E8 (8.5%), Any (8.4%), Off-2 (6.3%), Ex sudan (7.5%) and ILO (9.25%) in *Aspergillus flavus* were found (**Table 2**). The result showed that 02-M, 03-M and Off-1 were resistant to fungal attack.

In addition to that fungal infections percentage was generally high in *Aspergillus niger* on the infected seeds. *Penicillium* sp present on only five cultivars (01-M, 02-M, 03-M, E8 and Any) at 5.00%, 7.50%, 5.20%, 6.00% and Any 5.30% respectively. *Penicillium* infection range was highest on seeds of 02 – M and lowest on seeds of 01 – M (**Table 2**); while seeds of Ex sudan, Oke, ILO, Off – 1 and Off – 2 were free of this fungus.

Table 2: % of fungal infections on seeds of ten cultivars of sesame (%)

Fungus	**01-M**	**02-M**	**03-M**	**E8**	**Ex sudan**	**Oke**	**Any**	**ILO**	**Off-1**	**Off-2**
Aspergillus niger	15.50	0.00	0.00	8.50	7.50	5.00	8.40	9.25	0.00	6.30
Aspergillus flavus	10.30	0.00	0.00	7.20	6.50	6.50	8.00	8.40	0.00	6.10
Penicillium sp	5.00	7.50	5.20	6.00	0.00	0.00	5.30	0.00	0.00	0.00

Bark aqueous extract of cashew was found inhibitory to mycelial growth of *Aspergillus* during the first two days of inoculation. On the fourth day, however, their effects had reduced, especially at 40% concentration. There was mycelial growth at this concentration (**Table 3**), with a level of significant inhibition at 0.05% when compared with the control plates. Between 40% and other concentrations, there was a significant difference at ($P\ 0.00 < 0.05$), there was however no significant difference between 60% and 80% and 100% ($P\ 1.00 > 0.05$).

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Table 3: Inhibitory effect of cashew bark extract (*A. occidentale*) on mycelial growth of the fungi.

Concentration (%)	Mean percentage *Aspergillus*	Inhibition ± SE (%) *Penicillium*
Control (0)	0.00.0±0.0[a]	0.00.0±0.0[a]
40	98.87.0±0.4[b]	50.92.0±6.9[b]
60	100.0±0.0[c]	64.76±7.8[bc]
80	100.0±0.0[c]	76.64±5.4[cd]
100	100.0±0.0[c]	92.80±2.8[d]

In each fungus, means followed by the same letter are not significantly different (P ≤ 0.05).

The crude extract of cashew bark also showed significant reduction in mycelial growth of *Penicillium* sp at different levels of concentration. High fungitoxicity *in vitro* was observed at 80% and 100% concentrations but no significant difference between them (P 0.22 > 0.05). The result (table 3) shows that aqueous extracts at 80% and 100% concentration from cashew completely inhibited mycelial growth in *Penicillium* for the first three days of inoculation, but their inhibitory effects had worn off by the fourth day of inoculation. A highly significant difference was noticed in all the concentrations compared with the control. Between 40% and 60% there was no significant difference (P 0.37 > 0.05). A significant difference was noticed between 40% and 80% and between 40% and 100% at (P 0.01 < 0.05 and 0.00 < 0.05) respectively. Between 60% and 100% there was a significant difference (P 0.04 < 0.05) compared with 60% and 80% of no significant difference (P 0.57 > 0.05).

The bark crude extract from mango was effective in inhibiting the mycelial extension of the pathogen at all concentrations tested. Besides the inhibition of radial mycelial growth of *Aspergillus*, mango extract also affected the growth habit of the fungus. The inhibitory effects showed level of significance at 0.05% at all levels of concentrations compared with the control. The result between 40% and 60% showed no significant difference (P 0.24 > 0.05). Similarly, between 60%, 80% and 100% was not significant (P 0.31 > 0.05). The extract completely inhibited mycelial growth throughout the period of observation at 80% and 100% with no significant difference between them (P 1.00 > 0.05) (**Table 4**).

Table 4: Inhibitory effects of Mango bark extract (*Mangifera indica*) on mycelial growth of the fungi.

Concentration (%)	Mean percentage *Aspergillus*	Inhibition ± SE (%) *Penicillium*
Control (0)	0.00.0±0.0[a]	0.00.0±0.0[a]
40	98.3.0±0.6[b]	46.22.0±7.9[b]
60	99.2±0.3[bc]	50.82±7.5[bc]
80	100.0±0.0[c]	68.25±6.1[cd]
100	100.0±0.0[c]	81.15±5.4[d]

In each fungus, means followed by the same letter are not significantly different (P ≤ 0.05).

The aqueous extracts of mango at all levels of concentrations significantly retarded mycelial growth of *Penicillium* sp. These results showed that the inhibitory effects of the extract on mycelial growth increased with increase in concentration (**Table 4**). The extract was inhibitory to mycelial growth in *Penicillium* during the first two days at 100% concentration, but their inhibitory effect had generally worn off by the third day and fifth day. However, their inhibitory effect at 40% concentration had reduced when mycelial growth in them and in the untreated control almost completely filled the culture plate. There was a significant difference between the control and the other concentrations. But some concentration showed no significant difference when compared; between 40%, 60% and 80%, at (P 0.98 > 0.05) and (P 0.90 > 0.05), and between 80% and 100% (P 0.57 > 0.05), respectively. However, 40% and 100% and between 60% and 100% were significant at (P 0.001 < 0.05) and (P 0.007 < 0.05) respectively.

DISCUSSION

The result obtained from this study revealed that *Aspergillus niger, Aspergillus flavus* and *Penicillium* sp are associated with spoilage of beniseed. They are either surface contaminants or seed borne which could cause biodeterioration and reduce the vigour (Bhat *et al*., 1999). The authors equally reported *Aspergillus* and *Penicillium* as the major storage fungi attacking beniseed in storage similar to the present reports. Seed borne mycoflora are carried over by infected seeds. They cause deterioration of seed in storage and in soil before germination, causing seedling mortality which may result in infections of foliage at adult stage. Nasira, *et al*., (2004) reported some of the seed borne fungi of beniseed as *Penicillium, Alternaria, Fusarium* and *Curvularia*.

In the present study, fungal incidence ranged from 66.50 to 97.50%. The highest incidence was in E8 cultivar. Other cultivars with more than 90% fungal incidence include 01 – M and Any. Percentage fungal incidence was 87.50% and 88.00% in 02 – M and Oke respectively. Slightly lower than those in 03 – M, Off – 2 and Ex-sudan as presented on **Table 1**. The results (**Table 2**) equally showed that the percentage of fungal infections on seeds of ten cultivars of sesame varied. The two species of *Aspergillus* were found almost on all cultivars, except 02-M, 03-M and Off-1. However, among the two fungi, *Aspergillus niger* generally showed comparatively high fungal infection percentage in seeds of ten cultivars of sesame. The highest was noticed on 01-M.

Many synthetic fungicides had shown promise in the control of sesame diseases (Shokalu *et al*., 2002), but the high cost of some of these chemicals had lead to the search of alternative, cheap and eco-friendly plant extracts. The present study showed that the plant extracts evaluated significantly reduced or inhibited the mycelial growth of the fungi *in vitro*, which could lead to reduction in the incidence of the disease in storage. The mean percentage inhibition of mycelial growth in *Aspergillus* in plates containing bark extract of cashew plant was 100% at 60% concentrations. The extract was inhibitory to mycelial growth of *Aspergillus* during the first few days of inoculation by the fourth day; however, their effects had reduced, especially at 40 percentage concentration. There was however a significant difference (P 0.00 < 0.05) between 40% and other concentration; suggesting high toxicity of bark extract of cashew plant against the mycelia growth of the fungus. Treatments containing cashew plant bark extract showed mean percentage inhibition of 92% in *Penicillium* sp. The crude extract showed significant reduction in mycelcial growth of *Penicillium* sp at different

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levels of concentration. High fungitoxicity *in vitro* was observed at 80% and 100% concentration but no significant difference between them (P 0.22 > 0.05). A highly significant difference was noticed in all the concentrations compared with the control and between 40% and 60%, there was no significant difference (P 0.37 > 0.05). Significant difference was noticed between 40% and 80%, between 40% and 100% at (P 0.01 < 0.05 and 0.00 < 0.05) respectively. Between 60% and 100% was a significant difference (P 0.04 < 0.05) compared with 60% and 80% of no significant difference (P 0.57 > 0.05).

The present investigation has shown that bark crude extract from mango tree progressively inhibited the radial mycelial growth by 99.2% compared to the control plates. And on the crude extract on *Penicillium* sp, the extracts seems to be the least effective among the extracts employed at various concentration on mycelial growth. The percentage level of inhibition at various concentration shows that there is a level of significant at 80% and 100% concentration, compared with control plates. These results show that the inhibitory effects of the extract on mycelial growth increased with increased in concentration. Some concentration showed no significant difference when compared; between 40%, 60% and 80%, at (P 0.98 > 0.05) and (P 0.90 > 0.05); and between 80% and 100% were (P0.57 > 0.05) respectively. However, 40% and 100% and between 60% and 100% were significant at (P 0.001 < 0.05) and (P 0.007 < 0.05) respectively.

CONCLUSION

This study has revealed the potential of botanicals in the control of seed-borne mycoflora of sesame caused by *Aspergillus* and *Penicillium.* This has gone a long way in providing better alternative to the over dependency on synthetic fungicides. The use of plant products in disease control could reduce over reliance on source of agricultural chemical to the farmer, as well as cut down cost production. The facts that cashew and mango bark used in this sudy are easily available, with easy method of extraction; it can be exploited in the control of seed-borne mycofloral of sesame.

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