Effects of Botanical Extracts on Fungal Load of Sesame (Sesamum indicum L.) Seeds

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Abstract: This study assessed the mycoflora load on pesticidal botanical materials and on infected sesame (Sesamum indicum L.) seeds. Seeds treated with antifungal botanicals in the laboratory using Agar dilution method. Six botanical materials were screened and baobab (Adansonia digitata), hot pepper (Capsicum annuum) and ordeal tree (Erythrophleum suaveolens) leaf extracts had no fungal load. The antifungal activity of the extracts of these botanicals (100% conc.) was evaluated against the isolated fungi from infected sesame seeds. The pathogenic fungi isolated from the sesame seeds were Aspergillus niger, A. flavus, Fusarium spp., Alternaria spp. and Penicillium spp. Baobab and ordeal leaf extracts (10% and 100% conc.) had maximal antifungal activity (p<0.05) on all the six assessed seed-borne fungi. Hot pepper extracts (10% and 100%) inhibited only the mycelial growth of A. flavus and Alternaria spp. This study confirmed the variation in the fungal load of some Nigerian plant materials and indicated the effectiveness of baobab (Adansonia digitata) leaves, pepper (Capsicum annuum) fruit and ordeal tree (Erythrophleum suaveolens) leaf extracts as antifungal agents on seed-borne fungi of sesame. The effect of these extracts on germinability of the seeds and seedling vigour deserve further investigation.

Keywords: fungal load, sesame seeds, ginger extract, hot pepper extract, baobab leaf extract, ordeal tree extract

1. Introduction

Sesame (Sesamum indicum L.) popularly known as beniseeds in English, ridi in Hausa or gorigo in Ebira is considered to be the oldest oil seed crop known to humanity. One of the constraints of sesame seed is the adverse effect of seed-borne infection which often results in poor quality seed and oil as well as transmission of seed-borne pathogens to mature plants.

Seed-borne fungi of sesame seed have been reported to include Alternaria brassicola, A. redicinca, A. alba, A. flavus, A. niger, A. viridus, Cephalosporium spp., Curvularia, Drechsler spp., Fusarium and Penicillium spp. in Pakistan [1]. Also in Sudan [2] Nasireeene et al., reported Macrophomina phaseolina, Aspergillus niger, A. flavus, Alternaria spp., Fusarium spp., Curvularia spp. and Dredoslerarostrata to be found in sesame seeds.

The use of botanicals in the control of plants pathogens is an alternative technique to the conventional handling with synthetic fungicides which causes various problems such as toxicity to users [3] Whalen et al.; impairment of beneficial organism and resistance to the active ingredients of some synthetic fungicide in response to selection pressure due to high dose and continuous application, which have all led to great economic losses [4]-[5]. An economical and efficient alternative for disease control is the use of natural products derived from plants (secondary metabolites) [6] Wilson et al., since it is environment-friendly and their residues are easily degradable.

The potential use of plant extracts to control plant pathogen has been reported in different laboratory [7]-[9], green house and field studies [10] (Hernandez et al. 2010), botanicals offer a more economical and efficient alternative for disease control.

[11] Al-samarrai et al., in their study evaluated botanicals prepared from neem, chilli, lemon grass and ginger among others and showed that neem and chilli were more effective at inhibiting the test fungi than ginger and lemon grass. Aspergillus spp. mycelia grown for 96 h in culture media containing 50% neem leaf and seed extracts was inhibited by 90 and 65%, respectively [12] Razzaghi-Abyaneh et al.. Also non-sterilized phyto extracts of Allium sativum and Sapindus trifoliata were inhibitory to Fusarium moniliforme the incitant of wilt of sugarcane [13]. Allium sativum was also reported to inhibit the development of fungal mycelium of Macrophomina phaseolina which is known to affect groundnut [14]. Due to problems caused by seed-borne fungi to the crop, which include poor establishment and productivity, the effectiveness of some botanicals in controlling the fungal load on sesame seeds when investigated. It specifically investigated the fungi load in ordeal tree stem bark and leaves, garlic, ginger, hot pepper, baobab marketed in Gwagwalada-Abuja, Nigeria and to quantify and identify fungi load on the infected sesame seeds treated with hot pepper, baobab and ordeal tree leaves.

2. Materials and Methods

Collection of botanical materials and sesame seeds

Dry ordeal tree stem bark and leave, hot pepper fruits, garlic bulbs, ginger rhizomes, baobab leaf powder, and sesame seeds were purchased from Gwagwalada, Abuja market and kept safe in a polythene bags and shade dried on the laboratory benches for 3 weeks.

The sample of ordeal tree stem bark and leaves, ginger rhizomes, garlic bulbs, hot pepper fruits and baobab were all ground into powder with a pestle and mortar in the laboratory.

Preparation of the sesame seeds

Two grams of sesame seeds were surface sterilized for 2(mins) in 80% ethanol and rinsed twice in sterile distilled
water and was later grounded into fine powder with a pestle and mortar in the laboratory; the essence of sterilization is to kill microorganisms.

**Determination of fungal load on botanical materials and sesame seeds**

One gram of each hot pepper, garlic, ginger, baobab, ordeal tree stem bark and leaf extract and sesame seeds sample that were ground into fine powder was added to 9ml portion of sterile water in a test-tube and was shaken very well. Four fold serial dilutions 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, were then prepared. 19.5g of Potato Dextrose Agar (PDA) was introduced into a conical flask containing 500 ml of distilled water and autoclaved at 121°C for 15 mins. To this, 13 mg each of streptomycin and penicillin were added and shaken thoroughly. After the media preparation, it was allowed to cool for some minutes, after which it was poured into Petri dishes and was further allowed to solidify. 1 ml portion of suitable dilutions of the samples were used to inoculate the Petri dishes containing PDA. The plates were incubated at 28°C for 2-5 days and examined for the growth of fungi and the Colony Forming Unit (CFU).

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\text{cfu} = \frac{\text{colony counted}}{\text{inoculum volume (ml) plated}} \times \text{dilution factor}
\]

**Treatment of sesame seeds with plant extracts of hot pepper, baobab and ordeal tree leaves**

Sesame seeds were surface sterilized for 2 mins in 80% ethanol and rinsed twice in sterile distilled water. One gram of sesame seeds were placed in separate Petri dishes and were ground into fine powder with a pestle and mortar. One gram of the ground sesame seeds which were preserved with different plant materials were introduced into 9ml portion of sterile water in a test tube and was shaken very well. Four fold serial dilutions were then prepared, then 1ml of the stock was introduced into the first test tube and shaken, then 1ml was transferred from the first test tube (10⁻¹) into the second test tube (10⁻²) and so on and finally 1ml was decanted from the last test tube (10⁻⁴). The potato dextrose agar used for the culture of fungi was prepared with an autoclave at 121°C for 15mins. After the media preparation, it was allowed to cool for some mins. After which it was further poured into Petri dishes and was allowed to solidify. Approximately 1 ml portion of suitable dilutions were used to inoculate the Petri dishes containing the culture media. The plates were incubated at 28°C for 2-5 days and examined for the growth of molds and CFU. Experimental set up was arranged accordingly in a complete randomized design (CRD) replicated three times.

**Identification of fungi**

After incubation for 5 days colonies of different shapes and colours were observed on the plates and number of colonies on each plate was multiplied with the dilution factor. A pure culture of each colony type on each plate was obtained by sub-culturing each of the different colonies onto PDA plates and then incubated at room temperature. The sub-cultured colonies were fully grown as pure culture after 5 days. The identification of fungi was done using light microscope. With the aid of a flamed and cooled needle a small portion of the mycelium was picked form the edge along with its spores and placed into the drop of lactophenol-blue on a clean glass slide. Another needle was used to tease the inoculum gently and used to mix it with the stain. A cover glass was placed over the preparation and care was taken to avoid trapping of air bubbles in the stain. The identification of fungi colonies was also done macroscopically using a hand lens. The observed structures were compared with the scheme of [15] (Barnmet, 2002) for identification as stated in Table 1.

**Data collection and analysis**

The incidence of fungi species and their colony forming units in each of the plant extracts and in sesame seeds preserved with it were recorded after 5 days.

Data were subjected to Analysis of Variance (ANOVA) and treatment means were separated by Duncan Multiple Range Test (DMRT) at (p<0.05) level of probability using Genstat,10th Edition statistical™ package. All the zeros were square root transformed using x + ½ so that the assumption of analysis of variance would not be violated

**3. Results and Discussion**

**Screening of botanical materials for fungi load**

The analysis of variance (ANOVA) showed that there was significant (p ≤0.05) difference between the incidence of fungal load in the botanical materials screened in the laboratory.

The *A.niger* load on the cultured ordeal tree stem bark extract was significantly (p<0.05) higher than in all other botanical materials (Table 1). There was no *A.niger* observed on the hot pepper, baobab and ordeal tree leaf extracts. The *Fusarium* spp. load on the ordeal tree stem and ginger extract was significantly (p<0.05) higher than in other investigated botanical materials. There was no fungal colony observed on the baobab and ordeal tree leaf extract. *A.flavus* load was observed only on garlic and ordeal tree stem extracts and this was significantly (p<0.05) higher than in all other botanical materials. *Mucor* spp. was the least species of fungi observed on all the six cultured botanicals material. The only fungi species observed on hot pepper extracts was *Mucor* spp. (Table 1). On ginger, *Fusarium* spp. had the highest number of colony (14.0) and closely followed by *Penicillium* spp. (12.0), while *A.flavus* and *Mucor* spp. had no colony. On hot pepper, *Mucor* spp. had one colony (1.0) while *A.niger*, *A.flavus*, *Penicillium* spp. and *Fusarium* spp. were absent. On garlic, *A.flavus* had the highest units of colony (8.0) and closely followed by *A.niger* while *Mucor* spp. had no colony unit. On ordeal tree stem extract *A.niger* had the highest units of colony (20.0) followed by *Fusarium* spp.
spp. (15.0) and A. flavus (8.0) while Penicillium spp. and Mucor spp. had no colony unit.

### Table 1: Mycoflora screening of some botanical material marketed in Gwagwalada Abuja Nigeria

| Extract treatment          | Aspergillus niger (cfu/ml) | Fusarium spp. (cfu/ml) | Aspergillusflavus (cfu/ml) | Penicillium spp. (cfu/ml) | Mucor spp. (cfu/ml) |
|----------------------------|---------------------------|------------------------|---------------------------|---------------------------|---------------------|
| Ginger                     | 5.00                      | 14.00                  | 0.00                      | 12.00                     | 0.00                |
| Hot pepper                 | 0.00<sup>a</sup>          | 0.00                   | 0.00                      | 0.00<sup>a</sup>          | 1.00<sup>b</sup>    |
| Garlic                     | 6.00<sup>a</sup>          | 3.00<sup>a</sup>       | 8.00<sup>b</sup>          | 1.00<sup>b</sup>          | 0.00<sup>b</sup>    |
| Ordeal tree bark extract   | 20.00<sup>a</sup>         | 15.00                  | 8.00<sup>b</sup>          | 0.00<sup>b</sup>          | 0.00<sup>b</sup>    |
| Ordeal tree leaf extract   | 0.00<sup>a</sup>          | 0.00                   | 0.00<sup>b</sup>          | 0.00<sup>b</sup>          | 0.00<sup>b</sup>    |
| Baobab leaf                | 0.00<sup>a</sup>          | 0.00<sup>b</sup>       | 0.00<sup>b</sup>         | 0.00<sup>b</sup>          | 0.00<sup>b</sup>    |
| Control (PDA)              | 0.00<sup>b</sup>          | 0.00<sup>b</sup>       | 0.00<sup>b</sup>         | 0.00<sup>b</sup>          | 0.00<sup>b</sup>    |

Means with same alphabet are not significantly different from one another on the same column using Duncan’s Multiple Range Test (DMRT) at 5% probability level.

The analysis of variance of mycoflora screening of sesame seeds treated with selected botanical materials showed that there was significant (p ≤ 0.05) difference due to the treatment effects. A. niger, Fusarium spp., A. flavus, Penicillium spp., Mucor spp. and Alternaria spp. were observed to be present on the untreated sesame seeds before and after storage (Table 2). The unit of colony of A. niger cultured sesame seeds before storage was significantly (p<0.05) higher than the seeds preserved with hot pepper at 100% and 10% level of concentration. The seeds treated with baobab and ordeal tree leaf extracts at 100% and 10% inhibited the growth of A. niger. The Fusarium spp. load on the sesame seeds before and after storage was significantly (p<0.05) higher than those preserved with hot pepper at 100% and 10% and the ordeal tree leaf extract at 10%. The A. flavus load on the untreated seeds before storage was significantly (p<0.05) higher than the seeds treated with hot pepper, baobab and ordeal tree leaf extract at 100% and 10%. The Penicillium spp. load on sesame seeds before and after treatment with hot pepper at 100% was significantly (p<0.05) higher than those seeds preserved with ordeal tree leaf extract at 10%. The Mucor spp. load on the seeds after storage was significant (p<0.05) higher but closely followed by sesame seeds only before storage and sesame seeds treated with hot pepper at 100% and 10%. The Alternaria spp. load on the seeds before storage was significantly (p>0.05) higher but closely followed by the seeds only after storage. On untreated sesame seed before storage A. niger had the highest unit of colonies (8.00) and closely followed by Fusarium spp. and Penicillium spp. (5.00) and A. flavus (4.00) while Mucor spp. had the least colony units. On untreated sesame seeds after storage A. niger and Fusarium spp. had the highest units of colony (5.00) and closely followed by Penicillium spp. (4.00) while A. flavus and Mucor spp. had the least units of colony (2.00). On Sesame seeds preserved with hot pepper at 10% level of concentration, A. niger had the highest units of colony (5.00) and followed by Fusarium spp. (2.00) and Mucor spp. (1.00). Those seeds preserved with hot pepper (100%) had the highest colony units (4.00) of A. niger and Penicillium spp. and followed by Fusarium spp. (2.00) and Mucor spp. (1.00) (Table 2). On the seeds preserved with ordeal leaf extract (10%) Fusarium and Penicillium spp. had a colony unit of one (1.00) respectively. All the six fungal species that were observed in the infected sesame seeds before and after storage were absent in sesame seeds preserved with baobab at 100% and 10%. This indicated that they were the most effective in inhibiting the infection of fungi in them.

| Sesame seeds treated with plant extracts | Aspergillus niger (cfu/ml) | Fusarium spp. (cfu/ml) | Aspergillus flavus (cfu/ml) | Penicillium spp. | Mucor Alternaria spp. |
|-----------------------------------------|---------------------------|------------------------|---------------------------|------------------|----------------------|
| Baobab leaf extract (100%)              | 0.00<sup>a</sup>          | 0.00<sup>b</sup>       | 0.00<sup>b</sup>         | 0.00<sup>b</sup> | 0.00<sup>b</sup>      |
| Baobab leaf extract (10%)               | 0.00<sup>a</sup>          | 0.00<sup>b</sup>       | 0.00<sup>b</sup>         | 0.00<sup>b</sup> | 0.00<sup>b</sup>      |
| Ordeal leaf extract (100%)              | 0.00<sup>a</sup>          | 0.00<sup>b</sup>       | 0.00<sup>b</sup>         | 0.00<sup>b</sup> | 0.00<sup>b</sup>      |
| Ordeal leaf extract (10%)               | 0.00<sup>a</sup>          | 1.00<sup>c</sup>       | 0.00<sup>b</sup>         | 1.00<sup>b</sup> | 0.00<sup>b</sup>      |
| Hot pepper fruit extract (100%)         | 4.00<sup>d</sup>          | 2.00<sup>b</sup>       | 0.00<sup>b</sup>         | 4.00<sup>d</sup> | 1.00<sup>e</sup>      |
| Hot pepper fruit extract (10%)          | 5.00<sup>a</sup>          | 2.00<sup>b</sup>       | 0.00<sup>b</sup>         | 0.00<sup>b</sup> | 1.00<sup>e</sup>      |
| Untreated sesame seed before storage    | 7.00<sup>a</sup>          | 5.00<sup>a</sup>       | 4.00<sup>a</sup>         | 5.00<sup>a</sup>  | 1.00<sup>e</sup>      |
| Untreated sesame seed after storage     | 5.00<sup>a</sup>          | 5.00<sup>a</sup>       | 2.00<sup>b</sup>         | 4.00<sup>a</sup>  | 2.00<sup>b</sup>      |

Means with same alphabet are not significantly different from one another on the same column using Duncan’s Multiple Range Test (DMRT) at 5% probability level.

Several authors have confirmed the antifungal properties of several plant parts and phytochemicals [16]. Extracts from many plant species have been found to be active against many phytopathogenic fungi without imposing ill side effects [17]. Aspergillus niger, Fusarium spp., A. flavus and Penicillium spp. were observed in garlic in this study. [18] Ghangaonkar reported more seed-borne fungi such as A. niger, A. flavus, Fusarium oxysporum, Macrophomina phaseolina, Botrytis alli, Penicillium cyclopium, Rhizopus stolonifer and Chaetomium globosum to have been isolated from the rhizome of Garlic. From ginger powder, A. niger, Fusarium spp. And Penicillium spp. were isolated. This observation is similar to the report of [19] Mandeel and [20] Paull and Moss that reported Aspergillus, Fusarium, Penicillium and Rhizopus spp. isolated from ginger rhizomes.

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In this study, Baobab and ordeal tree leaf extracts were generally observed to be the most tolerant or resistant to fungal infection [26] Adekunle et al., reported that E. suaveolens seed oil was fungitoxic and probably have a broad spectrum antifungal activity.[27] Cowan, (1999) associated the fungitoxic profile of botanicals to phytochemical compounds such as the essential oils (catechols and eugenol) in betel pepper, sulfoxide (allicin and ajoene) in garlic (Allium sativum), terpenoid in grapefruit peel, alkaloid (6,7-dimethylesculetin) in Luenna camara and phenethyleesculetin alkaloid in mesquite (Prosipipsilifloral).

Apart from the plant materials investigated in this study, [28] Bankole et al., (2006)and [29] Suleiman et al., (2010) in their findings reported that Azadirachtaindica and Morindalucidaextracts inhibited the growth of A. flavus. The activity of the steam-distillate and hot-water extracts of fresh leaves of Cymbopogon, Ocimungratissimum, Chromoleanadodara and fruits of Xyliaaethiopica against Ustilagomaydis, Ustilagovirens, Curvularialunata, and Rhizopuspp., reducing growth by 10-60%[30] (Awuah and Ellis, 2002), and the tomato fruit rot, which is commonly observed in local markets in many parts of Africa, can be significantly reduced with the extracts of a number of local plants such as Cassia alata, Alchorneacrofoidota and Moringaoleiferaas postharvest agents[31] (Enikuomehin, 2010). On sesame seeds,[32] Ezekiel et al.(2014) isolated Alternaria, Aspergillus, Fusarium and Penicilliumssp. from sesame seeds in Plateau state Nigeria.

[33] Ojiambyet al. (2000) reported the isolation of Alternariaesaesami in seeds obtained from Nairobi, Kenya and also [34] Gbodi et al. from Nigeria.[35] Mbah and Akueshi,[36] Afolagboyeye isolated A. flavus A. niger, A. nidulans, A. fumigatus, A. glaucus, Cercosporasesami, Curvularialunata and Rhizopusmiricicans from the seeds in Abeokuta of the south western Nigeria. [30] Awuahand Ellis reported the effective use of packed powders of leaves of O. grattissimum and cloves (Syzygiumaromaticum) in combination to protect groundnut kernels artificially inoculated with A. parasiticus. Similarly [37] Pandir and Jain studied the efficacy of 22 plant extracts against food associated fungi and found that ginger extract was more effective than other plant extracts.[38] Anjorinet al. reported that antifungal action of plant extracts has great potential as their fungicidal products are easy to prepare and they are safe and effective in view of being systemic in their action and easily biodegradable.

4. Conclusion

The results obtained from this study confirm the level of fungitoxicity of some Nigerian plant materials and indicated the effectiveness of baobab (AdansoniadrigitataL.) leaves, pepper (Capsicum annuum) fruit and ordeal tree (Erythrophleumsuaveolens) leaf extracts as antifungal agent on seed-borne fungi of sesame. It is imperative that further studies should determine the effect of those extracts on germination and mode of action of the extracts on the pathogenic fungi.

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