

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 treated with antifungal botanicals in the laboratory using Agar dilution method. Six botanical materials were screened and baobab (*Adansonia digitata*), hot pepper (*Capsicum annum*) and ordeal tree (*Erythrophleum suaveolens*) leaf extracts had no fungal load. The antifungal activity of the extract of these botanicals (10% and 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*, *Mucor* spp., *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* L.) leaves, pepper (*Capsicum annum*) 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 Epira 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 brassicicola*, *A. radicina*, *A. alba*, *A. flavus*, *A. niger*, *A. viridus*, *Cephalosporium* spp., *Curvularia*, *Drechsler* spp., *Fusarium* and *Penicillium* spp. in Pakistan [1]. Also in Sudan [2] Nasiredeen *et al.*, reported *Macrophomina phaseolina*, *Aspergillus niger*, *A. flavus*, *Alternaria* spp., *Fusarium* spp., *Curvularia* spp. and *Drechsleria rostrata* 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 trifoliolate* 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 leaf, 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).

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

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

Sesame seeds were surface sterilized for 2mins in 80% ethanol and rinsed twice in sterile distilled water. One gram of sesame seeds were placed in separate Petri dishes containing ordeal tree leaf, baobab, hot pepper that were ground into fine powder diluted with distilled water at the ratio of 2:1(w/v) for 100% concentration and ratio 1:9(w/v) for 10% concentration, where 100% is highly concentrated and 10% is diluted [16] (Satish *et al.*, 2007). The sesame seeds which were placed into the Petri dishes containing the respective plant materials were later removed from the Petri dishes and were further preserved inside zip sachet for 2 weeks at room temperature. After the seeds preservation, it was ground into fine powder with a pestle and mortar. One gram of the ground sesame seeds which was preserved with different plant materials were introduced into 9ml portion (stock) 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 from 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] (Barnnet, 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 $x + \frac{1}{2}$ 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 ($p \leq 0.05$) 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 other botanical materials. The *Penicillium* spp. load on ginger extract 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 botanical 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. (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	<i>Aspergillus niger</i>	<i>Fusarium</i> spp.	<i>Aspergillusflavus</i> (cfug/ml)	<i>Penicillium</i> spp.	<i>Mucor</i> spp.
Ginger	5.00 ^c	14.00 ^a	0.00 ^b	12.00 ^a	0.00 ^b
Hot pepper	0.00 ^d	0.00 ^c	0.00 ^b	0.00 ^b	1.00 ^a
Garlic	6.00 ^c	3.00 ^b	8.00 ^a	1.00 ^b	0.00 ^b
Ordeal tree stem bark	20.00 ^a	15.00 ^a	8.00 ^a	0.00 ^b	0.00 ^b
Ordeal tree leaf extract	0.00 ^d	0.00 ^c	0.00 ^b	0.00 ^b	0.00 ^b
Baobableaf	0.00 ^d	0.00 ^c	0.00 ^b	0.00 ^b	0.00 ^b
Control(PDA)	0.00 ^d	0.00 ^c	0.00 ^b	0.00 ^b	0.00 ^b

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 ($p \leq 0.05$) difference due to the treatment effects. *A.niger*, *Fusarium* spp., *A.flavus*, *Penicillium* spp., *Mucor* spp. and *Alternariaspp.* were observed to be present on the untreated sesame seeds before and after storage (Table 2). The unit of colony of *A.niger* on untreated sesame seeds before storage was significantly ($p < 0.05$) higher than those 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 the seeds 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 the 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.

Table 2: Mycoflora screening of sesame seeds treated with antifungal botanical extracts at different concentrations

Sesame seeds treated with plant extracts	<i>Aspergillusniger</i>	<i>Fusarium</i> spp.	<i>Aspergillusflavus</i> (cfug/ml)	<i>Penicillium</i> spp.	<i>Mucor</i> / <i>Alternaria</i> spp. spp.
Baobab leaf extract (100%)	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^b	0.00 ^b 0.00 ^b
Baobab leaf extract (10%)	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^b	0.00 ^b 0.00 ^b
Ordeal leaf extract(100%)	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^b	0.00 ^b 0.00 ^b
Ordeal leaf extract(10%)	0.00 ^c	1.00 ^{bc}	0.00 ^c	1.00 ^b	0.00 ^b 0.00 ^b
Hot pepper fruit extract(100%)	4.00 ^b	2.00 ^b	0.00 ^c	4.00 ^a	1.00 ^{ab} 0.00 ^b
Hot pepper fruit extract(10%)	5.00 ^b	2.00 ^b	0.00 ^c	0.00 ^b	1.00 ^{ab} 0.00 ^b
Untreated sesame seed before storage	7.00 ^a	5.00 ^a	4.00 ^a	5.00 ^a	1.00 ^{ab} 2.00 ^a
Untreated sesame seed after storage	5.00 ^b	5.00 ^a	2.00 ^b	4.00 ^a	2.00 ^a 1.00 ^b

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

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 *phytopathogenicfungi* without imposing ill side effects [17]. *Aspergillusniger*, *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*, *Fusariumoxysporum*,

Macrophominaphaseolina, *Botrytis alli*, *Penicilliumcorymbiferum*, *Rhizopusstolonifer* and *Chaetomiumglobozum* 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.

Only *Mucor* spp. was observed on the hot pepper fruits. However, [21] Matthew *et al.*, reported that hot red pepper harboured, *Rhizopus* spp., *Colletotrichumcapsici* and *Goetrichumcandidium* in addition to *Mucorspp.* In addition, [22] Karapynar, [23] Galiet *et al.* and [24] Ito *et al.*, reported the inhibitory effect of red pepper fruit crude extract on the growth of *A. parasiticus* and *A. flavus*. *Erythrophleumsuaveolens* leaf and stem extract were found to be free from most fungi load. [25] Onuorah confirmed that the presence of erythrophleguine in the *E. suaveolens* is responsible for the antifungal activities of the seed.

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 fungitoxicity of botanicals to phytochemical compounds such as the essential oils (catechols and eugenol) in betel pepper, sulphoxide (allicin and ajoene) in garlic (*Allium sativum*), terpenoid in grapefruit peel, alkaloid (6,7-dimethylesculetin) in *Lantana camara* and phenethylesculetin alkaloid in mesquite (*Prosopisjuliflora*).

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 *Azadirachta indica* and *Morindalucida* extracts inhibited the growth of *A. flavus*. The activity of the steam-distillate and hot-water extracts of fresh leaves of *Cymbopogon*, *Ocimumgratissimum*, *Chromoleanaodorata* and fruits of *Xylopiiaethiopic* against *Ustilagomaydis*, *Ustilagovirens*, *Curvularialunata*, and *Rhizopus* spp., 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*, *Alchorneacordifolia* and *Moringaoleifera* as postharvest agents [31] (Enikuomehin, 2010). On sesame seeds, [32] Ezekiel *et al.* (2014) isolated *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* spp. from sesame seeds in Plateau state Nigeria.

[33] Ojiambet *et al.* (2000) reported the isolation of *Alternariasesami* in seeds obtained from Nairobi, Kenya and also [34] Gbodi *et al.* from Nigeria. [35] Mbah and Akueshi, [36] Afolagboye isolated *A. flavus*, *A. niger*, *A. nidulans*, *A. fumigatus*, *A. glaucus*, *Cercosporasesami*, *Curvularialunata* and *Rhizopusnigricans* from the seeds in Abeokuta of the south western Nigeria. [30] Awuah and Ellis reported the effective use of packaged powders of leaves of *O. grattissimum* and cloves (*Sizygiumaromaticum*) in combination to protect groundnut kernels artificially inoculated with *A. parasiticus*. Similarly [37] Pundir 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 *et 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 (*Adansoniadigitata*L.) leaves, pepper (*Capsicum annum*) 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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