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SHORT COMMUNICATION

Effect of Plant Extracts on Sporulation of *Aspergillus Niger* and *Penicillium chrysogenum*. from Sunflower (*Helianthus annuus* L.) Seeds

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Abstract

Ten plant extracts were evaluated *in vitro* against *Aspergillus niger* van Tieghem and *Penicillium chrysogenum* Thom. which cause seed rot and decay of sunflower seeds. Cold water extracts of Neem (*Azadirachta indica* L.); Mahogany *Khaya senegalensis* (Desr.) A. Juss. African bush tea (*Hyptis suaveolens* (L.*)* Poit*;* Acacia (*Acacia senegalensis* (Houtt.) Roberty and Eucalyptus (*Eucalyptus citriodora* (Hook) obtained from leaves and fruits were used to amend cheek hot potato dextrose agar prior to dispensing to petri dish. Cultures of *Aspergilus niger* van Tieghem and *Penicillium chrysogenum* Thom. from Sunflower (*Helianthus annus* L.) seed were aseptically transferred with No. 4 cork borer to the center of solidified media. These were incubated at ambient conditions and sporulation inhibition efficiency of amendment obtained. Petri dishes containing the different treatments were arranged in complete randomized design with five replications of each treatment. Number of spores harvested, 72 hours after incubation were significantly influenced by the type and source of extract used for the amendment. Spore counts of *A. niger* were inhibited with leaves of *H. suaveolens* (L.*)* Poit, followed by *K. senegalensis,* leaves while Acacia extracts amendment had least effect on the number of spores harvested. However, *P. chrysogenum* had the highest spore inhibition when incubated with media amended with *A. indica* seeds and leaves. The extracts tested in this study have the potency to inhibit the deterioration of stored Sunflower seeds.

Practical Applications

The tested extracts can be used to control the development of *Aspergillus niger* and *Penicillium chrysogenum* on Sunflower seeds.

Keywords: Plant extracts, Sunflower seeds, Sporulation, *A. niger*, *P. chrysogenum*

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1. Introduction

Sunflower, *Helianthus annus* (L.) belong to the Family of Compositae. It is highly valued for its oil, which has low cholesterol content. Ukraine and Eurasia are the world's largest producers of the crop (FAO, 2010). Olowe *et al.*, (2005) reported seed yield above 1 t ha^{-1} depending on cultivars used in Nigeria. Aduayi *et al. (*2002) stated that the crop can tolerate abiotic stress such as various temperature changes and does well in light textured, well drained sandy loam soils. The crop is a valuable indicator of environmental health providing sustainable ecological equilibrium as nectar copiously produced by its radiant flower attracts pollinating bees, *Aphis meliformis* (Purseglove, 1972). The oil extracted from the seeds can lose its quality and become contaminated with molds, which results in production of toxic oil probably due to influence of aflatoxin. In the drive for economic diversification and search for revenue source that is sustainable environmentally and economically, agriculture has proven to be an important sector to meet such need (Zarafi & Dauda, 2019). One major constrains to profitable crop production in Nigeria is pest, as well as disease and soil nutrition decline (Akpa *et al*., 2019). Among the major diseases listed by Emechebe *et al.* (1981) in Northern Nigeria are the leaf blight induced by *Alternaria heliathi (Hansf.) Tubaki & Nishih.,* seedling blights by *Penicillium* spp; and root rots by *Rhizoctonia* spp.

These diseases represent a serious threat to the production and biodiversity of this important crop in Nigeria (Dauda *et al.*, 2016). Farmers-saved seeds obtained from Kebbi State had varying degree of fungal infections by Fusarium spp., Aspergillus spp. and Mucor irregularis (Dauda *et al.*, 2017). Post-harvest infections of sunflower seed blights has contributed to discouragement faced by seed crushing industries leading to production of rancid oils and loss of market (Showemimo, 2009). The use of synthetic pesticides for control of pathogen of post-harvest origin are not encouraged, as they pollute the environment and are toxic for the consumers. Researches are ongoing in order to obtain alternative sources of pesticides from natural sources Abdullah & Al-Mosawi (2010). These are not only safe and available, but also biodegradable. This paper reports an investigation evaluating the effect of some extracts obtained from various plants against seed deteriorating fungi especially *Aspergillus* niger and *Penicillium chrysogenum.*

2. Material and methods

2.1. Material

The leaves and seeds of Neem (*Azadirachta indica* L.); Mahogany *Khaya senegalensis* (Desr.) A. Juss. African bush tea (*Hyptis suaveolens* (L.*)* Poit*;* Acacia (*Acacia senegalensis* (Houtt.) Roberty and Eucalyptus (*Eucalyptus citriodora* (Hook) K. D. Hill and L. A. S. Johnson were obtained from the Institute for Agricultural Research (IAR) farm and the University botanical gardens.

2.2. Methods

Each of these materials was air-dried for 2-weeks on laboratory bench, 2.5 g of each plant material was placed in 50 ml distilled water and ground using pestle and mortar. The suspension was left for 24 h before draining through double fold filter paper and placed on water bath for 30 mins to ensure sterilization (Shuklar & Dwivedi, 2012). 15 ml of the

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filtrate of each extract was pipetted into 85 ml PDA, swirled around and dispersed into 5-labeled Petri dishes. PDAs with no extract added served as negative control, while media amended with Benomyl as positive control. A total of 60 petri dishes were used.

Streptomycin sulfate 60 mgL^{-1} was added before dispensing. Solidified media had 0.4 mm diameter fungus picked from growing culture to center of plates which was incubated at room temperature for 7 days. At 72 hours incubation, fungal spores were harvested with 0.4 mm diameter cork borer used to pick portion of culture and dropped into 10 ml Sterile Distel Water (SDW) contained in McCartney bottle. This was agitated vigorously and 0.05 ml was pipetted on to a glass slide and cover slip placed on the slide. Slides were made from each 60 plates, viewed at 10x magnification and spore number counted.

2.3. Statistical analysis

Data collected were transformed using the square root transformation and subjected to analysis of variance (ANOVA). Significant means were separated with Student-Newman Keul (SNK) test at 5 % level of significance. A probability value at $p<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Results

It was observed that, *Aspergillus niger* conidiophores were upright bearing terminal clavate swellings with phialides at the apex, the conidiophores radiated from the entire surface and often variously coloured in mass. (Figure 1).

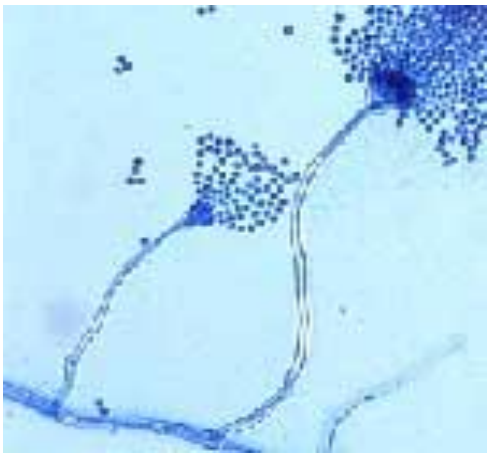

Figure 1: *Aspergillus niger* conidiophore and conidia

Media amended with the leaf extracts of *Hyptis suaveolens* and *Khaya senegalensis* gave significantly lower *Aspergilus niger* spore counts compared with un-amended media (Table 1). This observation agreed with earlier works of Moreira *et al. (*2010) who showed that leaf extracts of *Hyptis suaveolens* had inherent essential oil molecules that causes morphological alterations during spore production processes in *A. niger* including the loss of pigmentation, aberrant development of conidiophores as they become flattened, squashed and distorted. Shehu *et al.* (2016) also reported the *Aspergillus niger* mycelium growth inhibition plant extract of *K. senegalensis*.

Conidiophores of *Penicillium chrysogenum* on amended media however emanated from substrate-adhered mycelium, mostly singly and branched near the apex forming brush-like conidia bearing apparatus that ends as phialides. The colonies were bluish-green to dark green in colour with velvety and sulcate surface, having numerous spores and a white border of 2 to 3 mm wide. (Figure 2).

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Table 1: Spore counts of *Aspergillus niger* growing on media amended with plant extracts

Plant extract	Spore counts (No.)
Eucalyptus seeds	36.00^{d}
Eucalyptus leaves	59.60^{b}
Neem seeds	52.40^{bc}
Neem leaves	41.00^{cd}
African bush Tea seeds	54.00^{b}
African bush Tea leaves	31.00^{d}
Acacia seeds	54.80^{b}
Acacia leaves	56.60^{b}
Mahogany seeds	34.00^{d}
Mahogany leaves	32.80^{d}
Chemical check (Benomyl)	0.00^{e}
Sterile Distilled Water	85.20^{a}

Means followed by the same letters on the column are not significantly different at P = 0.05 on Student-Newman-Keul(SNK) test.
[1]All extracts are used at 17.6% v/v concentration (extract/PDA)

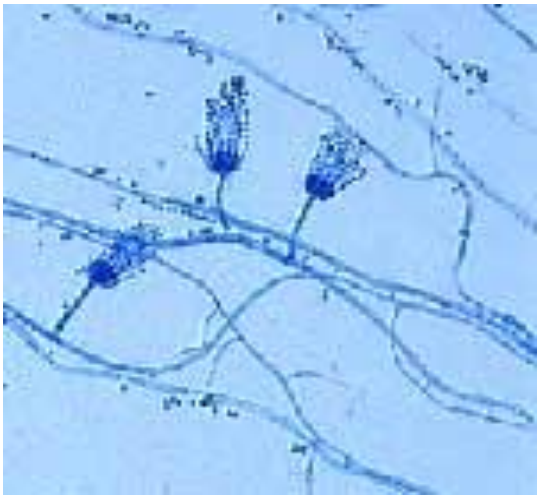

Figure 2: *Penicillium chrysogenum* conidiophore and conidia

Table 2 shows the counts of *Penicillium chrysogenum*, made after culture on media amended with various plant extracts. *P. chrysogenum* however had highest spore inhibition when incubated with media amended with *A. indica* seeds and leaves while *K. senegalensis* had least effect on the development of *Penicillium chrysogenum* spores. Nduaga *et al*. (2007) also showed that leave extracts of *Azadirachta indica* has fungicidal activity against *Penicillium coryloplium*. Taiga *et al.* (2008) had Neem extracts complete inhibition of fungal radial growth. Azadirachtin & Schmuttere (1990) showed that seed diffusates of Neem effected control of several fungi.

Table 2: Spore counts of *Penicillium chrysogenum* growing on media amended with plant extracts

Treatments	Spore counts (No.)
Eucalyptus seeds	103.30^{a}
Eucalyptus leaves	50.50^{de}
Neem seeds	45.30^{e}
Neem leaves	46.40^{de}
African bush Tea seeds	47.30^{de}
African bush Tea leaves	57.70^{dce}
Acacia seeds	85.00^{b}
Acacia leaves	85.00^{b}
Mahogany seeds	68.10^{c}
Mahogany leaves	60.20^{cd}
Chemical check(Benomyl)	8.50^{f}
Sterile Distilled Water	106.00^{a}

Means followed by the same letters on the column are not significantly different at P = 0.05 on Student-Newman-Keul (SNK) test.
[1]All extracts are used at 17.6% concentration (extract/PDA).

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Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

Conclusion

This study showed that leaf extracts of African bush tea, mahogany seeds and leaves and extracts of Eucalyptus seeds are potential antimicrobial agents to be used in the management of *Aspergilus niger*, while the extracts from seeds and leaves of Neem can be used on *Penicillium chrysogenum*. Theses plant parts are possible materials for biopesticides formulation, which justifies our search for alternative ecofriendly control agents against phytopathogenic fungi.

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