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Fungistatic Potential of Aqueous Extract from *Xylopia aethiopica* Against *Aspergillus niger* Isolated from Stored Onions (*Allium cepa*)

Nwolisa, C.N., Iheukwumere, I.H. and Chude, C.O.

Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Nigeria

Email of corresponding author: fortunelifas@yahoo.com

Abstract

Evaluation of fungistatic potential of extract from *Xylopia aethiopica* against *Aspergillus niger* isolated from stored onions (*Allium cepa)* was conducted. The phytochemical analysis of the plant material using hydrodistillation technique showed content of pharmaceutical constituents such as phenolics (1.51 ± 0.06), flavonoids (0.44 ± 0.75), glycosides (0.42 ± 0.01), saponins (0.22 ± 1.00), tannins (0.62 ± 0.00), steroids (0.14 ± 0.35) and alkaloids (1.94 ± 0.02). Using the radial growth technique, efficacy of the plant extract was tested against the mycelial growth of the isolate using quantities of 0.1 mL, 0.3 mL, 0.6 mL, 0.9 mL and 0.12 mL of the extract dried under anhydrous sodium sulphate. The analysis was observed for a period of 4 days which included the 3rd, 4th, 5th and 6th day of the fungal incubation. An enhancement in antifungal activity against *A. niger* was observed with increase in concentration and maximum inhibition was observed at 0.12 mg/mL with clear zone of 4.6 mm at the 6th day when the control plate showed maximum mycelia growth. An inhibitive activity of 4.3 mm was also observed for 0.9 mg/mL and 3.6 mm at 0.1 mg/mL concentration for the 6th day of study. However, the concentrations used in the treatment showed significantly ($p < 0.05$) clear zone of inhibition against *Aspergillus niger*. Hence, the result indicate that extract from *X. aethiopica* appear to be an effective alternative method of plant diseases control which are less harmful to human beings and environment.

Keywords: *Aspergillus niger*, *Xylopia aethiopica*, Preservation, *Allium cepa*, Eco-friendly

INTRODUCTION

The fungus *Aspergillus niger* is worldwide and considered to be one of the most important pathogens of stored onions (black mold). Symptoms of its presence are the black spores of the organism that are formed on and between the outer papery scales (Maude, 1983). The incidence may vary from a few clusters typically aligned along the veins of the bulb to a large part of the bulb being covered with conidia, giving the bulb a sooty appearance. The fungus may also advance into the storage scales below the papery scales and such invasions may be accompanied by introduction and development of secondary soft rot bacteria (Maude, 1983).

Several sources have been identified for the introduction of *A. niger* on onion plants and bulbs. These include seed, air, soil, and insects. Screening of onion seed samples showed that *A. niger*

was present on samples collected from a wide range of geographical locations (Hayden and Maude, 1992). Particularly high incidences were found on seeds produced in hot climates. A comparative study carried out in Sudan showed that in warm climates, the incidence of *A. niger* spores in soil around onion plants and in the air was higher than in temperate climates like in the UK (Hayden and Maude, 1992). However, spores of *Aspergillus niger* may also be introduced to the bulbs by mites and termites during storage (Onuegbu, 1994).

In essence, the incidence of *Aspergillus niger* on onion bulbs and the severity of the disease is a function of both preharvest factors (including climate, seed origin, crop rotation, and crop protection) and postharvest factors (including handling of the bulbs, and temperature and relative humidity during storage) (Hayden *et al*., 1997); thus, with the recent quest to curb the use of chemical preservatives in food storage, this possess huge challenges to food security and year-round availability of onions considering the characteristics of *A. niger*.

As an alternative to the use of chemical pesticides in food preservation, several plants has been evaluated and found to elicit varying degrees of antimicrobial effects (Swamy *et al.,* 2016) and also possess antioxidant properties (Beatovic *et al.,* 2015). The use of plant extracts as therapeutic agents and preservatives has become increasingly popular (Adaramonye *et al*., 2011) because it is believed to be eco-friendly and have fewer side effects as therapeutic agents.

Several reports have been made in the literature about the extract of *Xylopia aethiopica* and have shown to have antimicrobial property against wide range of Gram negative and Gram positive bacteria. Hence, the present study aims to evaluate the possible anti-fungal efficacy of extracts from *Xylopia aethiopica* against *Aspergillus niger* and its possible use as organic preservative in the control of the fungus in onions during storage.

MATERIALS AND METHODS

Pre-extraction of the Plant Material

The fruits of *Xylopia aethiopica* were cleaned properly and all extraneous materials removed. Cleaning was done by hands. The plant material was dried, powdered using industrial grade blender and stored in an airtight polypropylene bag till needed for the extraction.

Extraction of the Crude Extract from *Xylopia aethiopica*

The aqueous extract from *X. aethiopica* was achieved by hydrodistillation using 100 g of the sample which was added to 800 ml of distilled water in a 2-liter flask. The setup was placed in a balloon heater for 3 hours. At the end of the distillation, the aqueous extract was collected, dried under anhydrous sodium sulphate and stored in sealed amber bottles at 4°C as described by Majda *et al*. (2019) to avoid photo-oxidation until used for analysis.

Characterization of the Extract from *Xylopia aethiopica*

The chemical composition of the aqueous extract was determined by gas chromatography coupled with mass spectrometry (GC/MS) as described by Majda *et al.*, 2019. The GC analysis was performed using a chromatography equipped with a flame ionization detector (FID) and two capillary columns of different polarities OV type: 101 (25 m x 0.22 mm x 0.25 mm) and Carbowax 20 M (25 m x 0.22 mm x 0.25 μm). The carrier gas was helium with a flow rate of 0.8 ml/min and the oven programming temperature between 50 and 200°C with a gradient of 5°C/min. The Mass spectroscopy (MS) was used in the detection of the constituents and to determine the molecular weight of the compounds and identify the presence of isotopes patterns. MS coupling was performed on a DB1-type fused silica capillary column (25 m x 0.23 mm x 0.25 μm) with helium as a carrier gas and temperature programming identical to that of the GC.

Preparation of Culture Media

In a 250mL conical flask, 10g of Potato Dextrose Agar was prepared following the manufacturers instruction and autoclaved at temperature of 121^{o}C at pressure of 15psi for 15 minutes. After cooling to 45^{0}C ± 2^{0}C using laboratory water bath, 7.6 mL of chloramphenicol was added into the medium in order not to allow the growth of bacteria, then it was dispensed in sterile petri plates. The agar medium was thoroughly and uniformly mixed by alternate rotations and back-and-forth motion of plates on flat level surface. The agar was allowed to solidify and stored in inverted position until used for inoculation.

Isolation of *A. niger*

Scraped portions of stored onions from different retail marketers were collected in a sterile petri dish. The samples was homogenized in sterile distilled water, serially diluted and was inoculated aseptically over the PDA prepared plates. The plates were checked for growth within the period

of 7days. After pure culture was obtained, the isolate was inoculated into Petri dishes containing the culture medium MEA (Malt Extract Agar 20.0 g, Peptone 1.0 g, Glucose 30.0 g, Agar 20.0 g, Distilled water 1 Liter) at 25 ºC and was used for the antifungal assay. Further identification of the morphological characteristics of the fungal isolate was carried out on czapek Dox agar. After 7 days of incubation, the microscopic and macroscopic characteristics were determined with fungal atlas of Kidd *et al*. (2016) and key features such as size of the conidial heads, nature of the biseriate, color and nature of the conidia and other features.

Antifungal Assay

The fungistatic activity of the aqueous extract of *X. aethiopica* was conducted by using the radial growth technique (Nene, 1971). The nutrient medium (PDA) was mixed with different concentrations (0.1 mL, 0.3 mL, 0.6 mL, 0.9 mL and 0.12 mL) of the plant extract per 15 mL of the medium. The mixture was poured in four sterilized Petri-dishes and was gently swirled and allowed to solidify. After solidification, 0.5 cm disc inoculum made by sterile cork borer of the isolated *A. niger* was taken from 7 day old culture and located in the center of the Petri-dish. The control was prepared as any treatment except the addition of the plant extract. The inoculated dishes were then incubated at 29 ± 1°C for 7 days and were maintained for observation of antifungal activity of the plant extract. The antifungal activity was evaluated by determining the zones of inhibition of fungal growth in the medium containing the plant extract. Diameter of the developed growth was measured at 3^{rd}, 4^{th} 5^{th} and 6^{th} day. The fungi-toxicity of the extracts in terms of inhibition of mycelial growth was calculated according to Singh and Tripathi (1999) by using the formula:

$$\text{Inhibition (\%)} = \frac{dc - dt}{dt} \times 100$$

Where:

dc = Average increase in mycelial growth in control

dt = Average increase in mycelial growth in treatment

Data Analysis

Each determination was analyzed in triplicate and the values was then be averaged. Data generated was analyzed using one-way analysis of variance and mean separation was done by Duncan's new multiple range test and paired t-tests. Significant difference was accepted at $p < 0.05$ confidence level.

RESULTS

Phytochemical Constituents of Extract from *X. aethiopica*

The results of phytochemical evaluation of the crude aqueous extract from *Xylopia aethiopica* showed content of pharmaceutical constituents such as phenolics (1.51 ± 0.06), flavonoids (0.44 ± 0.75), glycosides (0.42 ± 0.01), saponins (0.22 ± 1.00), tannins (0.62 ± 0.00), steroids (0.14 ± 0.35) and alkaloids (1.94 ± 0.02). Therefore, the efficacy of the plant extracts was tested against the mycelial growth of phytopathogenic fungi, *A. niger*.

Morphological Identification of *Aspergillus niger*

The morphological observation of the colonies of *Aspergillus niger* belonging to the genus *Aspergillus* Section *Nigri* characteristically present black conidia, with biseriate conidiophores, spherical vesicles and hyaline or lightly pigmented hyphae near the apex. The results showed a morphologic similarity between *Aspergillus niger* and *A. tubingiensis* and *A. foetidus*. The slide view of the *A. niger* isolate using digital electron microscope is shown in Plate 1 and the macroscopic and microscopic characteristics outlined in Table 1.

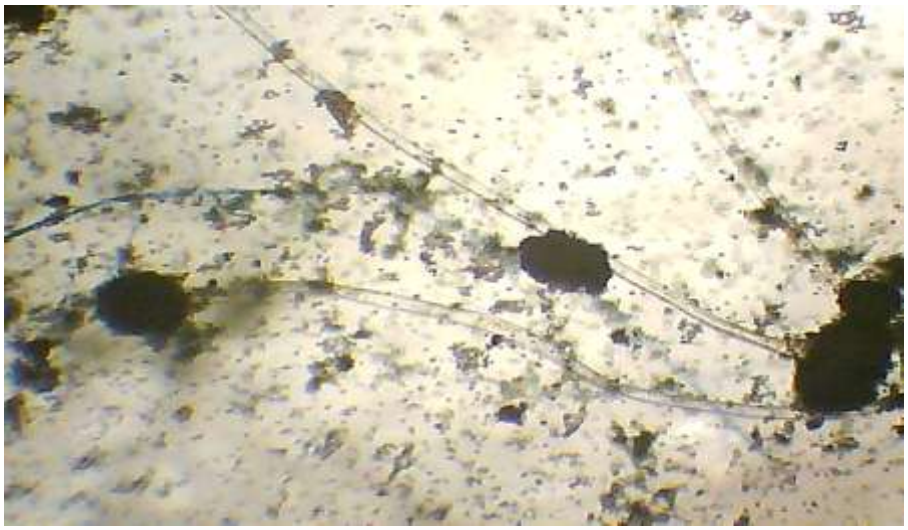

Plate 1: Slide view of *A. niger* using digital electron microscope

Table 1: Microscopic characteristics of *A. niger*

Attribute	Observation
Microscopic	
Diameter of Conidia (µm)	4 - 5
Texture of Conidia	finely wrinkled/wrinkled
Shape of Conidia	globular/ ellipsoidal
Diameter of Vesicles (mm)	20 - 73
Conidial Ornamentation	warty
Macroscopic	
Diameter of colony (µm)	53 - 69
Color	Black
Production of sclerotia	Absent

Morphological identification of the isolate on czapek Dox agar showed white colonies covered by a dense layer of black conidial heads. The conidial heads measured 3 mm by 16 µm in diameter. The conidiophores were smooth-walled, while the conidial heads were biseriate and measured 4-5 µm in diameter.

Fungistatic Activity of *X. aethiopica* Extract against *A. niger*

The analysis for fungistatic activity was observed for a period of 4 days which included the 3rd, 4th, 5th and 6th day of the fungal incubation and the ability of the plant extract to inhibit mycelia growth was determined based on growth reduction and antifungal effect. Thus, an increase in antifungal activity against *A. niger* was observed with increase in concentration of the extract and the results presented in Table 2.

The extract showed fungistatic activity against the *A. niger* in all the concentrations studied and maximum inhibition was observed at 1.2 mg/mL with clear zone formation of 4.6 mm at the 6th day when the control plate showed maximum mycelia growth. An inhibitive activity of 4.3 mm was also observed for 0.9 mg/mL concentration for day 6. The study had a least inhibition value of 3.6 mm at 0.1 mg/mL concentration for the same day of observation. However, the

concentrations used in the treatment showed significantly ($p < 0.05$) clearer zone of inhibition against *Aspergillus niger*.

Table 2: Fungistatic activity of the essential oil of *X. aethiopica* against *A. niger*

Concentration (mg/mL)	Inhibition of Mycelial Growth			
	3rd day	4th day	5th day	6th day
0.1	$1.9.50^{bc} \pm 0.03$	$2.1.60^{d} \pm 1.00$	$2.9.50^{c} \pm 0.01$	$3.6.20d^{e} \pm 1.00$
0.3	$2.0.55^{b} \pm 0.08$	$2.8.25^{cd} \pm 0.05$	$2.9.80^{c} \pm 0.00$	$3.7.20^{d} \pm 0.00$
0.6	$2.0.85^{b} \pm 0.07$	$2.9.30^{c} \pm 0.02$	$3.4.20^{b} \pm 0.02$	$3.9.60^{c} \pm 0.02$
0.9	$2.5.27^{a} \pm 0.00$	$3.2.50^{b} \pm 0.06$	$3.9.45^{a} \pm 0.06$	$4.3.20^{b} \pm 0.04$
0.12	$2.5.65^{a} \pm 0.05$	$3.5.20^{a} \pm 0.00$	$3.9.70^{a} \pm 0.03$	$4.6.20^{a} \pm 0.00$

Values are means ± SD (n = 3). Values on the same column with different superscripts are significantly ($p < 0.05$) different

DISCUSSION

Plants are rich source of bioactive compounds such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds, reported to have antifungal properties (Arif *et al*., 2009). The presence of these metabolites suggests great potential of *Xylopia aethiopica* as a source of useful preservative in stored food products. Therefore, these constituents detected in the extract be could be responsible for its antifungal activity.

According to Ezekwesili *et al*. (2010); the preliminary screening for the phytochemical constituents of the fruits of *Xylopia aethiopica* have also shown the presence of pharmaceutical constituents such as tannins, alkaloids, cineol, phytosterols, saponins, glycosides, carbohydrates, flavonoids, Campheme, γ-terpinene, Limonene, 3-carene, α-cardinol, Cuminyl alcohol, 1, 8-

cineole, Bisabolene, α-Terpinene, α–Phellandrene, Myreene. These bioactive components are known to be bactericidal, pesticidal or fungicidal in nature.

There are some studies on the antifungal activity of other plant extracts such as *Asparagus acutifolius, A. racemosus and A. officinalis*. Sautour *et al*. (2007) isolated six new steroidal saponins from the roots of *Asparagus acutifolius* which demonstrated antifungal activity against the human pathogenic yeasts, *Candida albicans, C. glabrata and C. tropicalis*. More so, Mathur *et al.* (2011) stated that, the hydro-alcoholic extract (50 % v/v) of *Asparagus racemosus* had antifungal activity against *Aspergillus niger* (MIC = 0.5 mg mL-1). Also, Sangvikar (2012) found that, all the extracts (cold water, hot water, ethyl acetate and alcohol) of *A. racemosus* roots were found to be inhibitory for *Alternaria solani* and *Fusarium moniliforme*. Wang and Ng (2001) isolated a Deoxy-ribonuclease from *Asparagus officinalis* seeds which had antifungal activity toward *Botrytis cinerea*. The crude saponin fractions of *A. officinalis* were ineffective against *Aspergillus niger* and *Rhizopus stolonifer* (Shimoyamada *et al*., 1990).

On the other hand, the method of extraction seem to influence the antifungal activity of plant extract as Sharma and Singh (2011) reported that, although *Cyperus rotundus rhizomes* contained several metabolites such as polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids and essential oil, but their ethanolic extract were ineffective against *Aspergillus niger*. While, Parekh and Chanda (2008) found that, the methanolic extract of *C. rotundus* exhibited antifungal activities against *A. candidus, A. flavus* and *A. niger*.

Several literatures has also reported the inhibitive potentials of some spice extracts such as aqueous extracts of *Syzygium aromaticum* (clove), *Allium sativum* (garlic), *Cinnamomum zeylanicum* (cinnamon), *Pipper nigrum* (black pepper) and *Trachyspermum ammi* (ajwoin) against the growth of *A. niger* (Garg and Siddiqui, 1992; Tewari and Dixit, 1994; Vazquez *et. al*., 2001). Thus, their results have shown great variability against the same test organism. This variability in antifungal potential in plant materials may be firstly due to the difference in the chemical compositions and secondly their solubility in water. This is also in agreement with the reports of Qasem and Abu-Blan (1996) and Amadioha (2000) who reported that the solubility potentials of phytochemical agents play a role in their ability to inhibit microbial population.

The findings of this experiment confirmed that plant extracts of *X. aethiopica* can be used as natural fungitoxicant to control the growth of phytopathogenic fungi (*A. niger*) and thus reduce the dependence on the synthetic fungicides. The *in-vitro* screening for sensitivity of the extract against *Aspergillus niger* observed mycelia growth reduction of the concentrations used for the study. Hence, the ability of this extract to be used as storage preservative of onions against its common pathogen (*A. niger*) by extending the shelf stability of the produce can go a long way to promote food security in temperate regions where black mold of onions is known to cause severe agricultural post-harvest loses.

CONCLUSION

Fungal contamination of stored commodities is a very serious problem in tropical warm regions of the world. Contamination by storage fungi and their mycotoxins is of great concern in food industry. Fungi, especially the species of *Aspergillus* are among the major reported genera having the ability to produce mycotoxins during storage. These fungi producing related mycotoxins reduce the quality of food products. The preservative actions of herbs and spices have received much attention in recent time where studies have been reported and showed that mycotoxin-producing molds may be inhibited by some herbs and spices. They generally produce many secondary metabolites such as alkaloid, flavonoids, tannins and phenolic compounds as observed in this study and these are the important sources of microbicides, pesticides and many pharmaceutical drugs.

There is an assumption that natural pesticides are safer, healthier, or more eco-friendly than synthetic products. Hence, this study has offered an alternative to the use of synthetic preservatives in the storage of onions. Availability of this perishable and seasonal agricultural product will greatly impact on the cost of the product and economic value of the farmers.

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