

THE COMPARATIVE ASSESSMENT OF THE EFFICACY OF SOME SELECTED SYNTHETIC FUNGICIDES WITH BOTANICAL AGENTS AGAINST SOME FUNGI ASSOCIATED WITH FOOD ROT

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**ABSTRACT**

Synthetic fungicides have been used to control fungal diseases. Although, synthetic fungicides are highly effective, their repeated use has led to problems such as environmental pollution, development of resistance and residual toxicity. Plant extracts have been known for their medicinal and antimicrobial properties since ancient times and the antifungal action of plant extracts has gained much attention. Nowadays, plants are being used against many pathogenic fungi. Green plants are huge reservoirs of various effective chemotherapeutic agents and could serve as an environmentally friendly natural alternative to most toxic synthetic agents. Six different fungal species belonging to four genera were repeatedly isolated: *Fusarium oxysporum*, *Aspergillus carbonarius*, *Aspergillus niger*, *Phytophthora infestans*, *Fusarium moniliforme*, *Phanerochate carnosus*. The zones of inhibition of the four synthetic fungicides (Nystatin, Ketoconazole, Fluconazole, Clotrinazole) ranged from 13mm to 40mm. Some fungal strains were resistant to some of them, with the exception of Nystatin. The oils (Eucalyptus, Olive, Castor) and plant extracts (Neem, Garlic, Ginger, Aloe) {botanicals} also showed impressive antifungal potentialities, with their zones of inhibition ranging from 17mm to 46mm, thus making them comparatively efficacious as their synthetic counterparts. The botanicals employed revealed the presence of different phytochemicals, including alkaloids and tannins, which could have been responsible for their potency. Prolonged usage of synthetic fungicides pose health problems as modern society is becoming health conscious; therefore, botanicals

could be more cheaply commercially produced and made available in presentable forms.

**Keywords:** Toxicity, chemotherapeutic, antimicrobial, environmentally friendly, plant extracts

## INTRODUCTION

Synthetic fungicides are manufactured using chemicals that are produced from other chemicals, they do not come from natural sources and are often more effective than the natural fungicides. Synthetic fungicides have been used to control plant diseases. Although, these fungicides are highly effective, their repeated use has led to problems such as environmental pollution, development of resistance, health risk and residual toxicity (Vermes and Dankert, 2009). Synthetic fungicides have effectively controlled plant pathogenic fungi. They have undesirable effects on non target organisms and foster environmental and human health concerns. Therefore, alternative measures have been developed for crop protection. In an attempt to reduce the use of synthetic fungicides, intensive investigations into the possible exploitation of bio-fungicides such as natural commercial products that are safe for human and the environment are being carried out (Bernett *et al.*, 1995).

Mechanism of action is referred to the specific biochemical interaction through which a drug substance produces its pharmacological effect (O'Neil *et al.*, 2006). The mechanism of action usually includes specific molecular targets to which the drug binds such as an enzyme or receptor. Receptor sites have specific affinities for drug based on the chemical structure of the drug, as well as the specific that occurs there. Drugs that do not bind to receptors produce their corresponding therapeutic effect by simply interacting with chemical or physical properties in the body. Mechanism of action describes functional or anatomical changes at the cellular level, resulting from the exposure of a living organism to a substance (Dan, 1999).

A botanical is a plant or plant part valued for its medicinal or therapeutic properties, flavour and or scent. Herbs are subset of botanicals. Products made from botanicals that are used to

maintain or improve health may be called herbal products, botanical products or phytomedicines. Some plant contains component that are toxic to pathogens when extracted from the plant and applied on infested crop (Hawksworth *et al.*, 2007). In naming botanicals, botanist use a Latin name made up of the genus and species of the plant. Commonly used botanicals are:

- Plant extracts: Neem (*Azadirachta indica*, A.Juss...,
- Garlic (*Allium sativum*, Linn...,
- Eucalyptus (*Eucalyptus globulus*, Labill...,
- Turmeric (*Curcuma longa*, Linn...,
- Ginger (*Zingiber officinale*, Rosc...,
- Gel and latex: *Aloe vera* (Tourn. Ex Linn...,

Fungi are any member of a large group of eukaryotic organism that includes microorganism such as yeasts and mold, as well as the more familiar mushrooms. These organisms are classified as a kingdom, fungi, which is separate from plants, animals, protest and bacteria. The organisms of the fungi lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, molds and yeasts, as well as many less- known organisms (Alexopoulos *et al.*, 1996). Fungi are non-photo synthetic and thus must absorb nutrients from organic matter formed by other organisms.

Fungal food rot also refers to food spoilage by fungi. Food decay as a natural phenomenon is an inevitable process that is rapid when adequate preventive measures are ensured. As long as nutrition and water are present, microbial growth would be promoted. Surprisingly, rotten food will not necessarily cause illnesses when consumed but the presence of pathogenic organisms will cause food-borne diseases (Omidbeygi *et al.*, 2007).

Antifungal susceptibility testing is done to develop standardized antifungal agents against fungal infections. These tests not only provide researchers with standardized methods for

testing but also with an understanding of the variables that affect inter-laboratory reproducibility. Antifungal susceptibility testing remains an area of exploration. Susceptibility testing can be used for drug discovery and epidemiology but this study will focus on the use of antifungal susceptibility test to predict the potency of some fungicides against some food-rot fungi (Lee *et al.*, 1991).

## **MATERIALS AND METHODS**

### **Sample Collection**

A healthy Yam tuber and a fresh loaf of unsliced bread were randomly purchased from a local market. They were then stored long enough for them to naturally rot. The two starchy food items were chosen because of their high susceptibility to fungal attack which includes their fairly high water of activity ( $a_w$ ).

### **Sterilization**

70% alcohol swab was used to disinfect the work surface to minimize contamination. Unwanted and indiscriminate movements were kept minimally to reduce air flow, thereby minimizing contamination by contaminated air currents. Beakers, test tubes and some other glass ware were sterilized using hot air-oven at a temperature of  $160^{\circ}\text{C}$  for one hour.

### **Isolation and Culture**

The method described by Chukwu *et al.* (2010) was employed to isolate fungi from the rotten five food samples. A sterile scalpel was used to cut off some infected portions of the samples. The cut infected portions were used to directly inoculate already prepared PDA plates with incorporated antibiotics. The plates were incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for four days. Distinct colonies were subcultured to obtain axenic cultures. The plated axenic cultures were then also incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for four days on PDA agar plates by repeatedly streaking. Agar slants for the fungal isolates were preserved in McCartney bottles and stored at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  until the

appearance of distinct and appreciable fungal colonies. The pure cultures were then recognized and kept refrigerated as stock cultures. Labeling of the sample containers was done accordingly.

### **Cultural Identification**

The isolates were identified on the basis of their morphological and cellular characteristics, as described by Barnett and Hunter (1999). The isolates were identified by first mounting each fungal mycelium on a glass slide then stained with Lactophenol-in-cotton blue dye, covered with a cover slip and then mounted on a compound microscope. The slides were viewed under x40 objective lens. The physical characteristics were compared to a Fungi Atlas for identification (Barnett and Hunter, 1999).

### **Pathogenicity Test**

The Test was conducted to determine the ability of each fungal isolate to cause disease on a fresh and apparently healthy version of yam tuber. To confirm the pathogenicity of the isolate, axenic cultures of the isolates were used to inoculate the sample. The healthy sample was washed in distilled water and surface sterilized with 95°C ethanol or 0.1% mercury chloride solution. A sterile scalpel was used to cut out portions of the healthy sample for inoculation with the axenic isolates onto the open cuts; the cuts were covered with sterile petroleum jelly at the point of inoculation and then kept for less than 21 days. The appearance of signs and symptoms on the healthy sample confirms the pathogenicity of the isolates which could be identical to those of the diseased sample. This confirms Koch's postulates (Okigbo *et al.*, 2009).

### **The Fungicides Employed**

Four different commercially purchased antifungal agents were used for this study, namely Nistatin, Fluconazole, Ketoconazole and Clotrimazole. Their efficacies were compared with botanicals such as commercially purchased refined Castor oil, Eucalyptus oil, Olive oil and extracts from *Aloe vera* leaves, *Azadirachta indica* leaves (Neem plant), *Zingiber officinale*

(Ginger cloves) and *Allium sativum* cloves (Garlic).

### **Extract Preparation**

The leaves and cloves were separately collected and washed with sterile distilled water. A warring industrial blender was used to crush them and then kept in labeled airtight containers before use. About 100g each was weighed out, soaked and stirred with a glass rod in 100ml of the solvent used (ethanol) for the extraction to give rise to the corresponding 100% extract concentration, for four days. Ethanol only without the crushed leaves was used as a control. The sample mixtures were filtered using clean filter papers (Whatman filter paper 1) into clean beakers and the filtrates were used as the extracts. The extracts were kept in sterile bottles until further use; they were concentrated to dryness by evaporation using a steam bath at 90<sup>0</sup>C for 48hours in order to enable the organic solvent to serve as an excipient (Joseph and Raj, 2010).

### **Extract and Oil Purity**

The plant extracts and oils used in this study were evaluated for their purity. This was done by aseptically streaking each onto sterile PDA plates; the plates were incubated at a temperature of 27<sup>0</sup>C for four days and examined for possible growth of contaminants. The absence of growth confirms the purity of the test plant extracts and oils (Cheesebrough, 2000).

### **Agar Diffusion Assay**

The inocula for this test were prepared using a 5-day old culture of the pure fungal isolates. Fresh PDA plates were prepared for this assay. The plates were then heavily inoculated with each axenic culture respectively and a sterile 5mm cork borer was used to make a well at the centre of each plate. 1ml at 100% concentration of each synthetic fungicide suspension, oils and the extracts was introduced into each well for comparison. The plates were then incubated at 28<sup>0</sup>C  $\pm$  2<sup>0</sup>C for four days. The zones of inhibition were marked by using a pair of venier calipers and then placed on a transparent ruler to take the exact readings. The zone of inhibition

readings were taken daily for three consecutive days from the fourth day of incubation. A control without any fungicide but instead distilled water was setup. The ability of the fungicide to inhibit the growth of the isolates was indicated by the appearance of clear zones within the medium for sensitivity, otherwise resistance.

### Phytochemical Screening

The phytochemical screenings were carried out in order to know the active ingredients of the extracts and oils which are responsible for the antifungal effect. The phytochemical tests were: Tannins, which are plants poly phenolic substances with a molecular weight of about 500. It has been suggested that tannins play a major role in plants' defense against fungi. Another phytochemicals are the Saponins, which are steroids or glycosides characterized by astringent taste and foaming properties (Ogbobe and Akamo, 1998). The other phytochemical tests conducted were Phlobatannins, Salkowski, Anthraquinone and Alkaloids tests.

### RESULTS AND DISCUSSION

The fungi isolated from these food samples, their colonial features and microscopic attributes are shown in Table 1. Six different fungal species belonging to four genera were isolated. The fungi isolated are: *Fusarium oxysporum*, *Aspergillus carbonarius*, *Aspergillus niger*, *Phytophthora infestans*, *Fusarium moniliforme*, *Phanerochate carnos*.

Table 1: Fungi isolated from the food samples

Isolates	Colonial appearance	Microscopy	Organisms
1.	Black mold	Colonies are jet black, spherical conidia	<i>Aspergillus niger</i>
2	White rot mold	Monolytic hyphal, simple septate, clavate	<i>Phanerochate</i>

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		basidia	<i>carnosa</i>
3	White mold	Multicellular distinctive sickle-shaped macroconidia, aseptate, floccose with sparse mycelia	<i>Fusarium oxysporum</i>
4	Brown mold	Lumpy appearance, aplerotic Conidiospore tall and brownish, aseptate,	<i>Phytophthora infestans</i>
5	Filamentous black mold	mostly globose Macro conidia with	<i>Aspergillus carbonarius</i>
6	Green mold	septation	<i>Fusarium monoliforme</i>

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Upon artificial inoculation onto a healthy yam tuber sample, all the six fungi produced characteristic symptoms on the inoculated sample after five days. The control sample did not show any disease symptoms. Some of the extracts and oils used in this study, in a bid to ascertain their purity, were not so very sterile, probably due to mild contamination from the laboratory environment or during the handling of the botanical agents by the experimenter. The synthetic fungicides used for the antifungal assay test that was carried out on each isolated fungus were effective and Nystatin was more effective than the others. The order of hierarchy



from the most effective to the least effective is as follows:-Nystain, Ketoconazole, Fluconazole and Clotrimazole respectively. Table 2 shows the result for the antifungal susceptibility or resistance of the fungi to the synthetic antifungal agents, as mean values for the readings taken for the three consecutive days, after the fourth day of incubation.

**Table 2: Antifungal Susceptibility or Resistance of the Isolates to Synthetic Fungicides**

Isolates	Synthetic Antifungal agents (mm)			
	Nyst	Clot	Keto	Flu
<i>Aspergillus niger</i>	32	R	15	29
<i>Aspergillus carbonarius</i>	31	20	30	23
<i>Fusarium oxysporum</i>	40	20	17	24
<i>Fusarium moniliforme</i>	27	25	30	R
<i>Phanerochaete carnososa</i>	16	13	R	15
<i>Phytophthora infestans</i>	35	23	28	21

Keys: R =Resistant, Nyst =Nystatin, Clot =Clotrimazole, Keto =Ketoconazole, Flu = Fluconzole

The result for the antifungal susceptibility or resistance of the fungi to the natural antifungal agents i.e. both the extracts and oils, as mean values for the readings taken for the three consecutive days, after the fourth day of incubation is shown in table 3.

**Table 3: Antifungal Susceptibility or Resistance of the Isolates to Natural Fungicides**

Natural Antifungal agents (mm)	
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Isolates	Euca	Olive	Cas	Neem	Garlic	Ginger	Aloe
<i>A. niger</i>	39	38	40	25	30	26	30
<i>A. carbonarius</i>	28	31	38	29	37	34	24
<i>F. oxysporum</i>	33	39	33	22	38	24	21
<i>F. moniliforme</i>	30	40	35	17	17	20	25
<i>P. carnosus</i>	36	42	19	18	15	19	26
<i>P. infestans</i>	39	46	26	29	24	28	22

Keys: Euca = Eucalyptus oil, Olive = Olive oil, Cas = Castor oil, Neem = *Azadirachta indica*, Garlic = *Allium sativum*, Ginger = *Zingiber officinale*, Aloe = *Aloe vera*

Comparatively, between table 2 and 3, the results showed that the natural fungicides, especially the refined oils, were equally as effective as Nystatin; the most potent of the synthetic fungicides. Although, between the refined oils and the plant extracts, the refined oils fared better, perhaps, as a result of the good manufacturing practices and quality control checks employed in their refinement or even a higher concentration of their phytochemicals and the intrinsic parameters associated with oils.

Table 4 below shows the result for the phytochemical screening test of the botanical fungicides.

**Table 4: Phytochemical screening of the *Zingiber officinale* extract**

Phytochemical	Euca	Olive	Cas	Neem	Garlic	Ginger	Aloe
Alkaloids	+	+	+	+	-	+	+
Tannins	+	+	+	+	+	-	+
Saponin	-	+	+	+	+	-	-
Anthraquinone	-	+	+	-	-	+	+
Phlobatannin	-	+	+	-	-	+	-
Salkowski	+	+	+	+	+	+	+

Keys: + =Present, - =Absent, Euca =Eucalyptus oil, Olive =Olive oil, Cas =Castor oil, Neem = *Azadirachta indica*, Garlic =*Allium sativum*, Ginger =*Zingiber officinale*, Aloe =*Aloe vera*

The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several treatments have also stated various types of contamination of herbal medicines which includes micro-organisms and toxins produced by micro-organisms, pesticides and toxic heavy metals. As a result, sterilization is needed especially for liquid extracts before use to get rid of these contaminations (Talaly and Talaly, 2001). According to Omidboygi *et al.* (2007) and Rota *et al.* (2009), the composition of an essential oil plays an important role in determining its antimicrobial activity. The susceptibility of the fungal isolates might have been due to their genetic make-up, the presence of phytochemical compounds and also incubatory substances.

## CONCLUSION

By this investigation, it can be deduced that the extracts and oils are equally as potent as the commercial fungicides i.e. they are equally fungitoxic. Commercial fungicides in the treatment of medical mycoses are still a source of health concern mainly because of the attendant side effects associated with their usage, besides the cost but the use of botanicals somewhat poses less worry.

## **RECOMMENDATIONS**

Botanicals could be more cheaply commercially produced and made available in presentable forms such as tablets, gel, capsules, soft gel capsules and syrup. Further investigation could be carried out to determine the long-term side effects, if any, of the prolonged use of botanicals as medical fungicides for oral administration.

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