

Evaluation of the Storage Quality of Coconut Seed Using Extracts of *Mangifera indica* Against *Ceratocysis paradoxa*, the Bole Rot Fungus

Okwelle Austin Achinike, George Tubo Stephen

Biology Department, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Port Harcourt, Nigeria

Email address:

okwelleaa@yahoo.com (O. A. Achinike)

*Corresponding author

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Abstract: The coconut palm (*Cocos nucifera* Linn) is undoubtedly the most economically important species in the palm family of Areacaceae. Coconut meal is solely prepared from the coconut seed which, has been found to be attacked by several disease causing microorganisms especially *Ceratocysis paradoxa*, a fungus that causes the devastating Bole rot disease. The study is designed to assess the storage stability of coconut seed by applying bioorganic extracts of *Mangifera indica* on the bole rot pathogen, *Ceratocysis paradoxa*. Four different concentrations (3, 6, 9 and 12 %) of the stem and root extracts of *M. indica* were obtained using a fair-fold sterile cheese cloth and tested on the fungus cultured on plates of Sabouraud Dextrose Agar (SDA) medium. Data obtained was analysed using the two-way Analysis of Variance (ANOVA). The result showed significant fungal mycelial growth inhibition with the 9% root extract concentration than the stem extracts concentrations. The antifungal properties of *M. indica* root extract can be further harnessed, processed and packaged as one of the natural bioorganic alternatives to chemical fungicides.

Keywords: Coconut Seed, Spoilage Fungi, *Ceratocysis paradoxa*, *Mangifera indica* Extract, Antifungal Activity

1. Introduction

The Coconut palm (*Cocos nucifera* Linn) is a member of the palm family Arecaceae. It is undoubtedly the most economically important species in the family Arecaceae [1], and the most useful palm in the agrarian economy of many nations worldwide; providing food, drink, shelter and raw materials for industries [2]. It is also used as an ornament and food crop, providing oil, coconut milk, fiber from the husk, palm wine and timber for furniture and construction [3]. Finished products obtained from the coconut plant serves as a major means of export, such as desiccated coconut, copra meal, coco-chemical (fatty acids, alcohol, methyl ether) shell charcoal and activated carbon, fiber products, coconut cream and coconut milk powder [4].

Various species of bacteria, fungi viruses and other pests

attack the plants at different stages of their development, it reduces their productivity leading to huge food and economic loss to man. Parasitic fungi causes the greatest impact with regard to disease and crop production losses. It spreads readily through the soil by root contact between palms and probably by air-borne basidiospores (Jonathan et al., 2012). In Nigeria, the coconut bole rot disease is commonly caused by the fungus *Ceratocysis paradoxa* [5].

Coconut palm is solely propagated by seed [2] and seed borne diseases are the most disastrous as they reduce seed vigor, market value and weaken the plant at the initial stages of its growth. Seed borne diseases caused by fungi are relatively difficult to control as the fungal hyphae may get established and become dormant. More than 800 million people in the developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases. In Nigeria, coconut copra has been found to produce a good

number of products which is also a major means of export, but this copra is susceptible to fungal attack during storage. Most farmers who cultivate coconut palms stand at a loss during harvest as fungi and insects infect the copra [5].

The common method of protecting the plant (*Cocos nucifera*) against fungal attack is by the use of fungicides. However, many of the fungicides agent available in the market are phytotoxic and have undesirable effects on other organisms present in the environment. Over time, some fungal species can become resistant to the chemicals in fungicides, and higher rates or more frequent fungicide application may be ineffective [6]. Also, some fungicides can irritate the skin and eye, while others can cause throat irritation and coughing when inhaled. Fungicides such as ziram can cause neural and visual disturbance. Agricultural fungicide use may contribute to resistance against medications in humans with life threatening lung infection caused by the *sAspergillus* fungus. The use of chemical fungicides to control diseases is often associated with negative environmental impacts, potential human exposure to pesticides, and deposition of residues on the fruits [7]. It has also been noted that the fungicides chlorthalonic, the most commonly used synthetic fungicide in the United States is toxic to aquatic animals such as tadpoles, oysters and fish, when chemical ran off plant contaminates the nearby water or ground water source [8]. Therefore, it is desirable to use some eco-friendly measures for the management of fungal diseases. Plant metabolites and plant based fungicides appear to be one of the better alternatives in plant disease management, as they are known to have minimal harmful impact on the environment and to consumers in contrast to synthetic fungicides [9]. Studies have equally revealed that the extraction method of medicinal plants have profound effects on the isolation of antimicrobial chemical ingredients [10]. The leaf extracts of mango have been used to combat fungal infections. Mango leaf extract in the family Asteraceae are widely grown and used in different parts of Nigeria popularly as food ornamental and in traditional health care services [11]. They observed that the aqueous extracts of *Manifera indica*, retarded or inhibited mycelial growth of the fungi in vitro and concluded that water-soluble antifungal compounds in the plant are responsible for the antifungal activities. The bark of mango plant have scientifically been shown to contain a range of health given active chemical compounds [12]. Antifungal activity of plant extracts with the capacity to control pathogens in plant products such as in pineapple have been reported [13].

The present investigation was therefore undertaken to screen the crude extract of mango stem and root for their antifungal potency in laboratory conditions against an important pathogenic fungus *Ceratocysis paradoxa*.

2. Method

2.1. Source of Infected Coconut Seed

The infected coconut seed (*Cocos nucifera*) showing

symptoms of *Ceratocysis paradoxa* was collected from Deeyor Community in Gokana Local Government Area of Rivers state and brought to the Biology Research Laboratory, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt for isolation of the pathogen and fungi toxicity test.

2.2. Isolation and Identification of Pathogen / Subculture

The outer mesocarp of the infected coconut seed was removed using a clean cutlass. The hard inner endocarp was then also carefully broken with the cut-glass. With a sterile pair of forceps, small parts of the infected fruit were removed from different locations of the infected seed and inoculated into petri-dish plates containing prepared Sabourand Dextrose Agar Medium (SDA). The medium was incubated for three days at 37°C. The fungal mycelial growth on the medium was sub-cultured into freshly prepared SDA plates to obtain and maintain pure culture of the causal fungus. The colonies were mounted and viewed microscopically, under different magnifications to confirm the growth of *Ceratocysis paradoxa* using morphological features as described by Ojomo and Ekpo (1987).

2.3. Preparation of Stem and Root Extract

Mango (*Mangifera indica*) stem and root were collected separately, using a sterile machete for the stem and a hoe for the root.

The stem and root were surface sterilized by washing with 5% sodium hypo-chloride, rinsed in 500ml of distilled water and allowed to drain off for 10minutes by spreading them in a sterile tray placed in a slanting position. The stem and root pieces were later ground differently with a sterile grinder respectively to obtain 100g of the coconut powder respectively. This was adopted from the method used by Okwelle and George (2008^b).

The extract was prepared by weighing 3, 6, 9, and 12g of the ground coconut powder into different sterile 250ml conical flasks, 100ml of sterilized distilled hot water added to each conical flask and labeled accordingly. The mixture was allowed to stand for one hour. Hot water extract was obtained by filtration of the mixture through eight fold sterile cheese cloth into sterile test tubes bearing labels of the different concentrations. The filtrate gave 3, 6, 9, and 12% concentration of aqueous stem and root extract solutions. The test tubes were covered with cotton wool and foil paper.

2.4. Fungi Toxicity Test

The effect of the stem and root of mango (*Mangifera indica*) on mycelial growth extension of *Ceratocysis paradoxa* was determined by inoculating the fungus on SDA medium containing 3, 6, 9, and 12% concentration of the plant extract in petri-dish plates. The extract and SDA medium were prepared by adding 1ml of the aqueous extract solution into the molten SDA plates already marked with two perpendicular lines across the bottom. The extract -SDA medium was carefully swirled 10 seconds for homogeneity and allowed to solidify before inoculation. Sterile cork borer

(5mm) diameter was used to cut the fungal mycelium from the pure culture and a sterile forcep used to pick and place each of the cut inoculum on the extract-SDA medium at the centre of the two perpendicular lines. The inoculated plates were incubated at room temperature for 6 days and measurement of mycelial growth extension done for 6 days with a transparent meter rule. Four replicate plates per treatment were setup using a Completely Randomized Design (CRD). The experiment was done with four treatment and control under room temperature. Mycelial growth of the pathogen, *Ceratocysis paradoxa* was measured on each treatment and control plate for 6 days at the same time, and fungi toxicity was recorded in terms of growth inhibition.

$$\text{Percentage composition} = \frac{DC - DT}{DC} = 100$$

where

Table 1. Analysis of variance of stem extract measurement on *C. paradoxa* mycelial growth inhibition.

Source of variance	Degree of freedom (df)	Sum of square (ss)	Mean of square (ms)	f-value for different	Tabulated of required f-value	
Total	24	20224.56			5%	1%
Treatment	4	184.56	46.14	0.50 ^{ns}	2.07	4.43
Error	20	1840.0	92.00			

Mean separation using the least significant difference (LSD)

$$LSD = \frac{t\sqrt{2(MSE)}}{r}$$

$$LSD = \frac{t\sqrt{2 \times 92.00}}{5}$$

$$\begin{aligned} LSD &= \frac{t\sqrt{184}}{5} \\ &= \frac{t\sqrt{36.8}}{5} = t(6.066) \\ &= 2.086 \times 6.066 \\ &= 12.65 \end{aligned}$$

DC = Average diameter of fungal colony with control

DT = Average diameter of fungal colony with treatment

2.5. Statistical Analysis of Data

Data obtained were analyzed using the two ways Analysis of Variance and the Least significant difference (LSD) to separate the means at 5% probability level ($p < 0.05$)

$$LSD = \frac{\sqrt{2(MSE)}}{r}$$

3. Result

The result of the test on the effectiveness of the use of mango stem and root extract on the growth of *Ceratocysis paradoxa* of coconut seed is presented below;

Table 2. Least significant difference of stem extract measurement of *C. paradoxa*.

Treatment	Mean	LSD	Inhibition %
3%	67.8 **	12.65	25.70%
6%	70.0**		26.53%
9%	64.0**		24.26%
12%	62.0**		23.50%

Key

ns = non significant

* = significant

** = highly significant

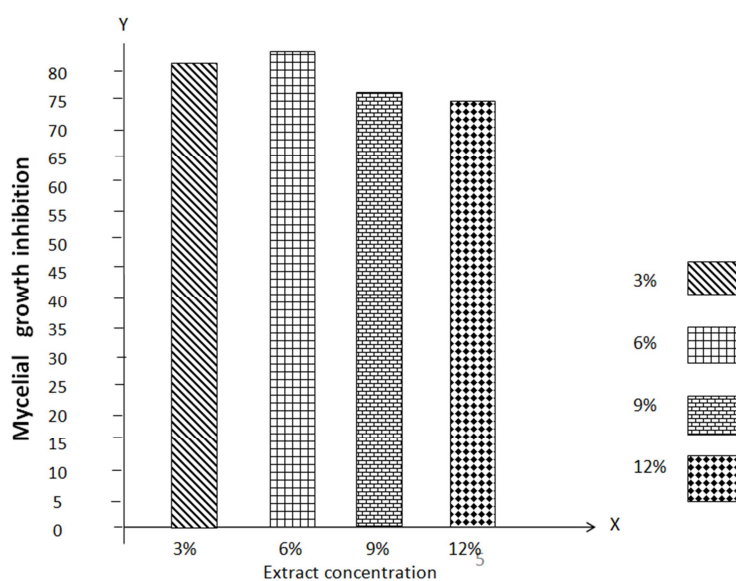


Figure 1. Effect of mango stem extract on *Ceratocysis paradoxa* mycelial growth.

Table 3. Analysis of Variance of Root extract inhibition on *C.ceratocysis*.

Source of variance	Degree of freedom (df)	Sum of square (ss)	Mean of square (ms)	f-value for different	Tabulated of required f-value	
Total	24	105.44			5%	1%
Treatment	4	57.84	46.46	6.07**	2.87	4.43
Error	20	47.6	2.38			

Mean separation using the least significant difference (LSD)

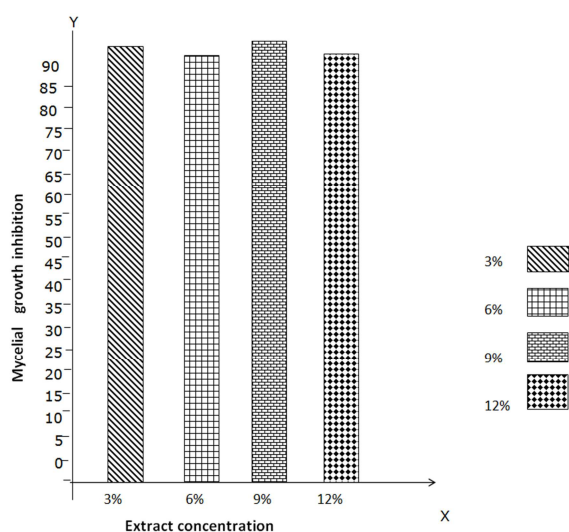
Mean separation using the least significant difference (LSD)

$$LSD = \frac{\sqrt{2(MSE)}}{r}$$

$$\begin{aligned}
 LSD &= \frac{\sqrt{2(2.38)}}{5} \\
 &= \frac{t\sqrt{4.76}}{5} \\
 &= t(0.436) \\
 &= 2.086 \times 0.436 \\
 &= 0.91
 \end{aligned}$$

Table 4. least significant difference of root extract of *C. paradoxa*.

Treatment	Mean	LSD	Inhibition %
3%	84.2 **	0.91	25.30%
6%	82.0**		24.63%
9%	84.8**		25.48%
12%	81.8**		24.57%

**Figure 2.** Effect of mango root extract on *C. paradoxa* mycelial growth.**Figure 3.** The root extract inhibition of the growth of *Ceratocysis paradoxa*.**Figure 4.** Plate of the fungi isolated from diseased coconut seed.

4. Discussion

There is food security when all people at all times have physical, social and economic access to sufficient, safe and nutritious food. Currently, the consequences of the application of fungicides in traditional agricultural production systems for the control of crop diseases have proved negative. Chemical fungicides has often been used to control food crop disease, but this conduct is associated with negative environmental impacts, potential human exposure to pesticides and deposition of residues on edible fruits and seeds. However, the effectiveness of synthetic fungicides have been reduced by the frequent development of resistance by the pathogens. Hence, there is a great demand for safer, alternative and effective chemotherapeutic agents [14].

Table 1 shows the analysis of variance of the activity of the stem aqueous extract, indicating no inhibition of the mycelial growth of *Ceratocysis paradoxa*, because the observed or calculated F-value which is (0.50) is not significant when compared to the tabulated or required f-value of (2.90) at 5% level of probability. In table 2, the means were separated using the least significant difference (LSD) which were obtained to be 12.65. This shows that there was a significant difference between the means of the different treatment concentrations (3, 6, 9 and 12%). The percentage of the fungi mycelial growth on each extract- PDA medium were also obtained to be 25.70% 26 -53%, 24-26% and 23.50% for 3, 6, 9 and 12 of treatment concentrations respectively, and reveals that the 6% concentration had the highest mycelial growth extension, while 12% had the least. This is represented graphically in figure 1.

Table 3 shows the analysis of variance of the root extract and indicates a very high rate of inhibition of the mycelial growth of *Ceratocysis paradoxa*, because the observed or calculated f-value, which is (6.07) is highly significant when compared to the tabulated f-value of (2.90). A similar significant activity in an in- vitro evaluation of the antioxidant efficacy of *Mangifera indica* extracts has been reported [15]. In table 4, the means of the treatment concentrations of the root extracts were separated using the least significant difference (LSD), which was obtained to be 0.91. This also shows that there was a significant difference between the mean of the 3, 6, 9 and 12% treatment

concentrations.

Figure 2 shows the percentage of fungi mycelia extension on each extract -PDA plate was also obtained to be 25.30%, 24.63% 25.48% and 24.57% for 3, 6, 9 and 12% concentration respectively, and it shows that 9% had the highest mycelial growth extension while 12% had the least. Comparatively, it can be deduced that the stem extract was not effective in controlling the growth of the fungus *Ceratocystis paradoxa*, the causal agent of bole rot of coconut seed, while, the root extract was very effective in controlling the growth of *Ceratocystis paradoxa*. This result also agrees with the report of on the evaluation of antifungal efficacy of some plant extracts on *Curularium lunata*, the causal pathogen of maize rot disease [16]. Some plant species such as *Aloe vera* (L.) Burm F. (*A. vera*) (aloe) *Glycyrrhiza glabra* L. (*G. glabra*) (licorice), and *Allium sativum* L. (*A. sativum*) (garlic) have been shown to consist of bioactive natural products with significant antifungal activity [17]. The inhibitory efficacy of the extract of Lime fruit bark (*Citrus aurantifolia*) on mycelial growth of the fungus, *Colletotrichum falcatum*, the causal organism of sugarcane red rot disease have been documented [18].

5. Conclusion

Based on the results of the statistical analysis of data from the study, it can be concluded that the stem (bark) of *Mangifera indica* had no effect on the growth of the fungus *Ceratocystis parathoxa*, while the root extract had a highly significant effect on the growth of the fungus. The positive effect of antifungal properties of *M. indica* root can be further harnessed, processed and packaged as one of natural bioorganic alternative for the use of synthetic fungicides. It is recommended that further research can be carried out using different other concentrations of the extracts to determine the best concentrations that could be applied. The method of application of the desired concentration of the extract and most suitable time also be assessed.

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