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Effects of Fungi on The Spoilage and Nutritional Composition of Coconuts (*Cocus nucifera*) Harvested in Yenagoa Local Government Area and Its Relative Health Implications

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Abstract:

Background and Objective: Fungal inversion of coconut endosperm is a factor that grossly reduces the economic, consumption value and the proximate composition of the coconut meat. The proximate composition of fresh and spoilt coconut endosperm and the most pathogenic fungi associated with the spoilage of coconut endosperm were evaluated in this study. Materials: Three mature fresh Coconut fruits were harvested from a subsistence coconut farm in Yenagoa city. Result: This study revealed that the proximate content of protein (9.80±0.02), lipids (38.75±0.02) and dry matter (92.72±0.02) in the fresh coconut are higher than the proximate content of protein (8.75±0.02), lipids (36.84±1.16) and dry matter (92.18±0.02) of the spoilt coconut endosperm. Minerals such as Ca (2.74±0.01), Mg (3.68±0.02), Na (3.75±0.02), K (6.76±0.02), Mn (0.250±0.002), Cu (0.58±0.02), Zn (5.85±0.02) and PO_4 (2.56 ±0.02) in the fresh coconut endosperm are higher than Ca (2.72±0.02), Mg (3.66±0.02), Na (3.57±0.02), K (6.72±0.02), Mn (0.244±0.002), Cu (0.54±0.02), Zn (5.77±0.03) and PO_4 (2.48±0.02) of the spoiled coconut endosperm. A significant difference between the proximate composition of fresh and spoiled coconut endosperm was observed. The endosperm (meat) of the coconuts were heavily invaded and decayed by a variety of fungi within 24 hours after cracking the shell and exposing the meats to open air. Fungal growth observed after exposure to open air are: Mucor, Rhizopus, Aspergillus and Penicillium. Conclusion: With the present of Aspergillus and penicillium in the samples evaluated in the laboratory, it implies that there are health implications associated with the consumption of spoilt coconut meat.

Keywords: *Effects of Fungi, Proximate Composition of Coconut, Relative Health Implications.*

Introduction

Coconut (*Cocus nucifera*) popularly known as tree of life, is one of the most extensively grown and used palms in Nigeria (Chuku, Kasiemobi, & Osakwe, 2007). It is widely grown in residence, parks, school premises and in most of the villages as ornamental and economic plant. It contributes significantly to the economy of Nigeria. It is the source of coconut oil which is widely used in cosmetics industry. It can be consumed as appetizers in raw form, roasted or fried chips. Tender coconut is valued both for its sweet water which is refreshing drink and delicious gummy meat. Chemical composition and volume of coconut water change during maturation (Haseena, Kasturi Bai, & Padmanabhan, 2010). The quality and quantity of coconut water as well as consumer acceptability of tender nut is more after seven (7) months of maturity (Sudarsana, et al., 2008).

The incidence of people consuming discoloured and half spoiled (fungi invaded) coconut endosperm has occurred in many cases and this consumption can lead to serious complication or mild health problems by the consumers. The disease caused by these fungi leads to destruction of coconuts and reduce their market value in local and global market. This study was carried within Yenagoa city, Bayelsa State. The study covers the effects of fungi on coconut meat, especially the nutritional value of the fungi infected coconut and its health implications.

Materials and Method

Sampling Location/ Study Area

The laboratory study was conducted at the Bayelsa State Medical University research laboratory. Yenagoa is the capital city of Bayelsa state. Yenagoa is geographically located between latitude 4° 47‟ 15‟ and 5°11‟ 55” Nothings and Long. 6°07‟35” and 6°24‟00” Eastings (Sridhar, et al., 2011; Ndiwari, 2013). The projected population of Yenagoa Local Government Area from the 2006 population census using the growth rate of 2.9% per annum to 2022 becomes 541,633 people. The climate of Yenagoa LGA is an equatorial type of climate (Iyorakpo, 2015). Rainfall occurs generally every month of the year. The mean monthly temperature is 25°C to 31°C. Relative humidity is high throughout the year and decreases slightly during the dry season (Olatunde, Andrew, & Theophilus, 2017). The soil texture ranges from medium to fine grains. The vegetation is composed of mangrove forests, freshwater swamp and lowland rain forests.

Sample Collection

Three mature fresh Coconut fruits were harvested from a subsistence coconut farm in Yenagoa city. After collection, the samples were properly preserved to ensure that they are not contaminated before they are used for the experiment.

Determination Of Mineral Content in Coconut

2g of shredded coconut samples were weighed into 100ml Kjelahl flask and placed on the Kjeldahl digester block. 15ml of mixed acid was added to the sample (1ml perchloric acid, 4ml concentrated H_2SO_4, 3ml concentrated HNO_3 and 1ml HCl) [1:4:3:1] swirled to obtain a good mixture. The flask was then heated until a clear solution is obtained. After cooling the flask, 20ml of distilled water was added and stirred and then filled in 100ml volumetric flask. Sodium (Na), and Potassium (K), were analysed on the flame photometer. While Calcium (Ca), Maganessium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn) were analysed on the Atomic Absorption Spectrometer (AAS). Phosphorus Oxide PO_4 was analysed on the UV/VIS Spectrophotometer (AOAC, 1999).

Proximate Analyses of Coconut Meat

Determination of Moisture (%)

An empty evapourating dish was oven dried at 105°C for 1 hour to a constant weight. 5g of sample was placed in the oven at 105°C. the samples were weighed at interval until there is no more changes and constant weight is obtained.

Determination of Ash %

1g of moisture free sample was used at 550°C.

Determination of Crude Protein

0.5g of sample was weighed into a 100 ml kjeldahl flask, followed by 1g of mercury catalyst and 30 ml of concentrated H_2SO_4 was rapidly added from a burette. The amount of Nitrogen (N_2) was determined by filtrating the distillate with 0.01M of H_2SO_4.

Determination of Crude Lipid

Ether Extract AOAC (1999), 2g of moisture free sample was weighed into a thimble and placed in a SO_X wet extraction apparatus. The extraction lasted for 3 hours. The boiling flask with the extracted oil fat were placed in an oven at 100°C for 30 minutes to get rid of the solvent and cooled in a desiccate and later weighed.

Determination of Crude Fibre

2g of defatted dry sample was weighed into a 400 ml beaker. 200 ml of 1.25% H_2SO_4 was added and boiled for 30 minutes with constant stirring. The filter paper and residue were now placed in the oven set at 105°C for 12 hours. This is then cooled. The residue is placed in a furnace at 55°C for 3 hours.

Determination of the Pathogenic Fungi that Attack the Meat of Coconut

The coconut was extracted using a sterile knife. 20 grams of each of the coconut samples were weighed into a set of three sterile petri dishes, and inoculated for seven days. Pure culture of the fungi was isolated, using Saboured Dextrose Ager (SDA) the culture was also incubated for seven (7) to fourteen (14) days. At the end, the fungi were identified using cultural characteristics (colour, shape…), spore morphology and mycelia growth, with Lactos Phenol Cotton Blue.

Statistical Analysis

Data were based on the laboratory experimental results which were replicated thrice experimentally. The data were presented in the form of tables showing mean ± standard error. Analysis of Variance (ANOVA) was used to establish the relationship between sample A and sample B.

Results

Fresh coconut (sample A), spoilt coconut (sample B).

Table 1. Mean for Proximate Analysis of Sample A and Sample B

	% Moisture	% Ash	% Protein	% Lipids	% Fibre	% Dry Matter	% NFE
Sample A	7.28±0.02	0.96±0.02	9.80±0.02	38.75±0.02	7.35±0.02	92.72±0.02	43.14±0.04
Sample B	7.82±0.02	1.20±0.02	8.75±0.02	36.84±1.16	8.56±0.03	92.18±0.02	44.64±1.20

Note: Mean ± Standard error from three replicates.

Table 1 shows the Mean ± Standard error of the proximate composition of fresh (sample A) and spoilt (sample B) coconut meat.

The result indicated that content of moisture (7.28±0.02), ash (0.96±0.02), fibre (7.35±0.02) and NFE (43.14±0.04) of the fresh coconut meat are lower than the content of moisture (7.82±0.02), ash (1.20±0.02), fibre (8.56±0.03) and NFE (44.64±1.20) of the spoilt coconut meat. While the content of protein (9.80±0.02), lipids (38.75±0.02) and dry matter (92.72±0.02) in the fresh coconut are higher than the proximate content of protein (8.75±0.02), lipids (36.84±1.16) and dry matter (92.18±0.02) of the spoilt coconut meat.

Table 2. Mean for Mineral Content of Sample A and Sample B

	Ca	Mg	Na	K	Fe	Mn	Cu	Zn	PO_4
Sample A	2.74 ±0.01	3.68 ±0.02	3.75 ±0.02	6.76 ±0.02	19.67 ±1.53	0.250 ±0.002	0.58 ±0.02	5.85 ±0.02	2.56 ±0.02
Sample B	2.72 ±0.02	3.66 ±0.02	3.57 ±0.02	6.72 ±0.02	20.84 ±0.02	0.244 ±0.002	0.54 ±0.02	5.77 ±0.03	2.48 ±0.02

Note: Mean ± Standard Deviation from three replicates. Source: Researcher (2022)

Table 2 shows the Mean ± Standard error of the mineral composition of fresh (sample A) and spoiled (sample B) coconut meat.

The results as shown in the table above shows that minerals such as Ca (2.74±0.01), Mg (3.68±0.02), Na (3.75±0.02), K (6.76±0.02), Mn (0.250±0.002), Cu (0.58±0.02), Zn (5.85±0.02) and PO_4 (2.56 ±0.02) in the fresh coconut meat are higher than Ca (2.72±0.02), Mg (3.66±0.02), Na (3.57±0.02), K (6.72±0.02), Mn (0.244±0.002), Cu (0.54±0.02), Zn (5.77±0.03) and PO_4 (2.48±0.02) of the spoiled coconut meat. While the Fe (20.84±0.02) content in the spoiled coconut is higher than the Fe (19.67±1.53) content of the fresh coconut.

The ANOVA result shows that there is a significant difference between the proximate composition of fresh and spoiled coconut.

Table 3. ANOVA Single Factor Analysis

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	12790.55	6	2131.758	3277.646	1.05E-11	3.865969
Within Groups	4.55275	7	0.650393			
Total	12795.1	13				

Figure 1. The Fresh Unshelled Coconut

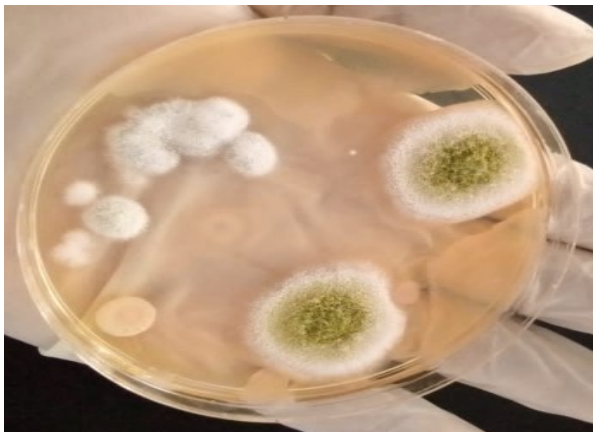

Figure 2. 48 Hours Old Aspergillus spp

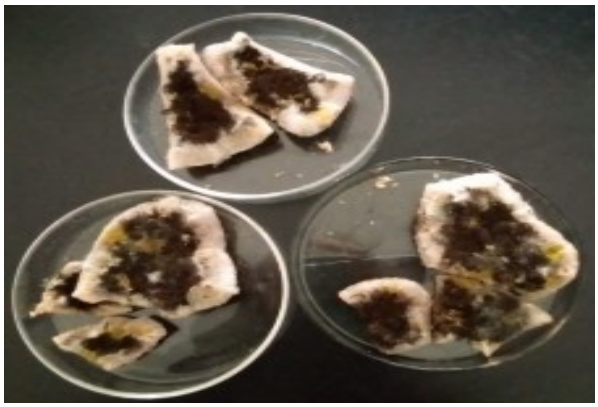

Figure 3. 2 Weeks Exposure of Sample to open Air

Figure 4. 24 Hours Old Aspergillus spp and Rhizopus spp

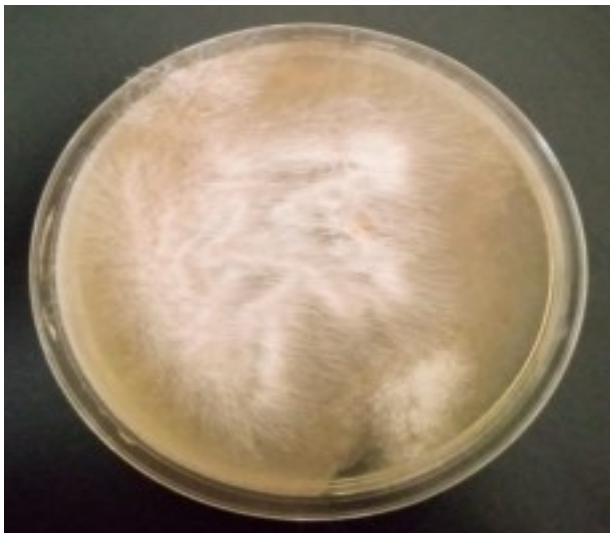

Figure 5. 24 Hours Culture of Mucor spp

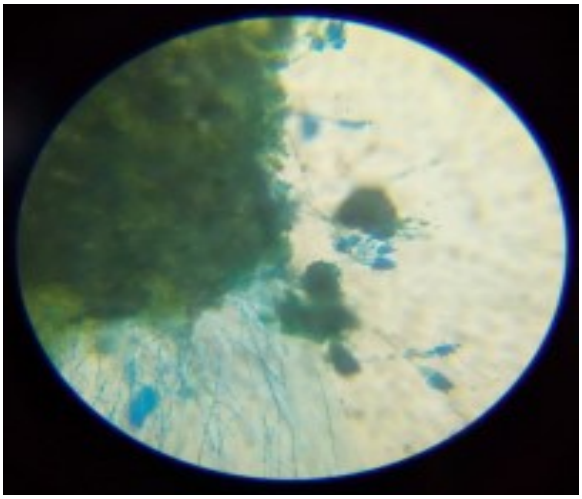

Figure 6. Penicillium spp with Hyphae Septate and Branched Brush-like cluster of Phialides Viewed under the Microscope

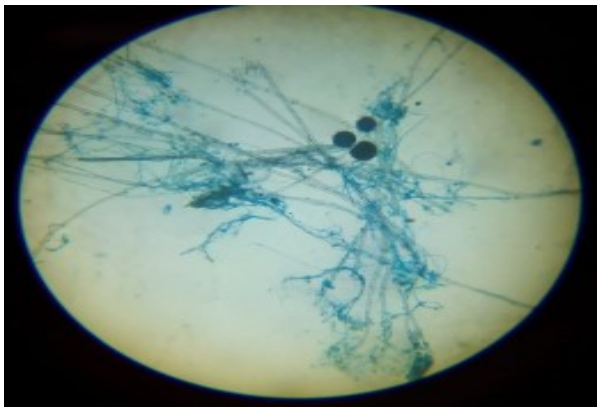

Figure 7. Rhizopus spp Showing Sporangiospores and Sporangium, with Rhizoids Viewed under the Microscope

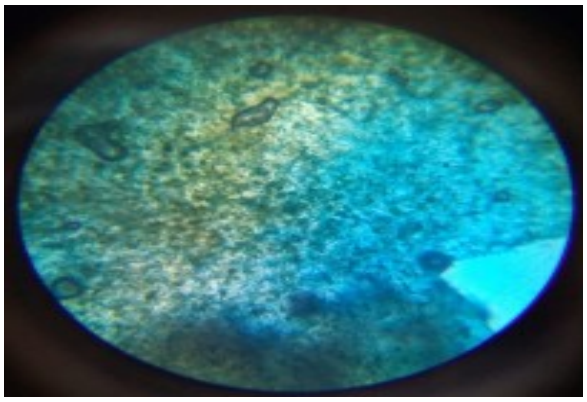

Figure 8. Mucor spp from 2 Days Old Culture Showing Sporangiospores, aerial Mycelia and Spores as it Appears under the Microscope

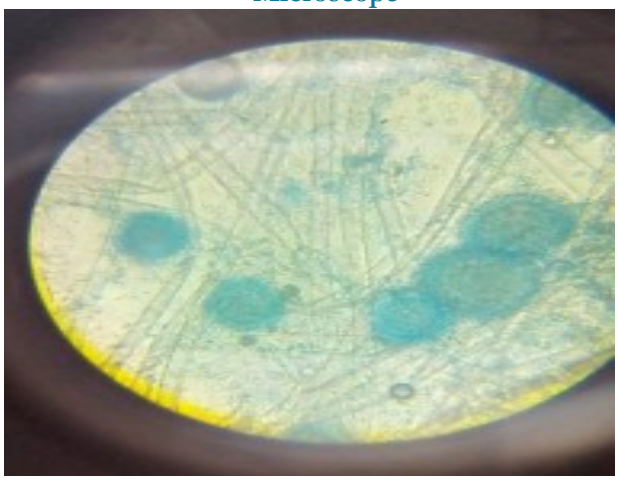

Figure 9. Aspergillus spp Showing Sporangiospores, sporangium and Spores. Stained with Lactose Phenol Cotton Blue as Seen under the Microscope

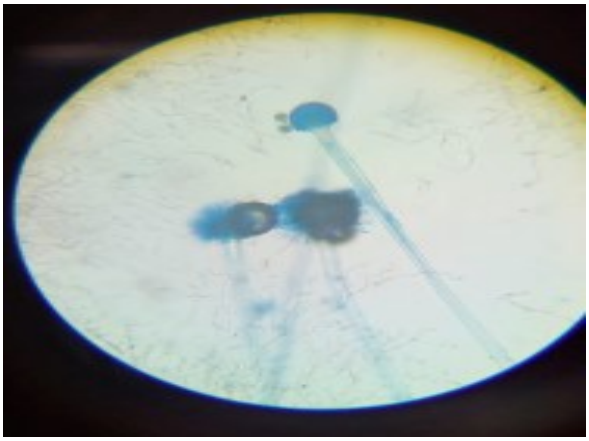

Figure 10. Microscopic Aspergillus spp Showing Open and Closed Sporangium, Sporangiospores and Spores Viewed under the Microscope

Figure 11. 7 Days Old Aspergillus spp

Figure 12. 24- and 48-Hours Mixed Colonies

Figure 13. 48-Hours Penicillinum

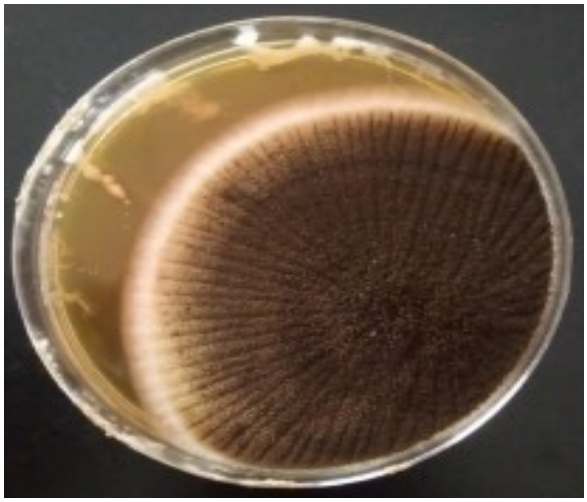

Figure 14. 48-Hours Aspergillus

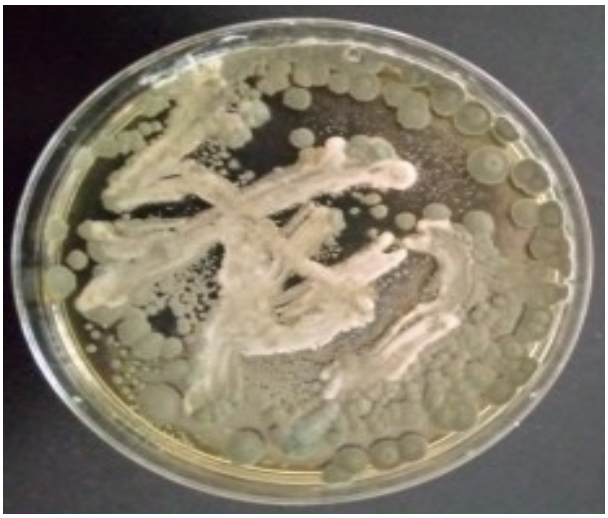

Figure 15. 7 Days Old Penicillinum

Figure 16. 4 Days Old Aspergillus

Figure 17. 7 Days Exposure of Samples to Open Air

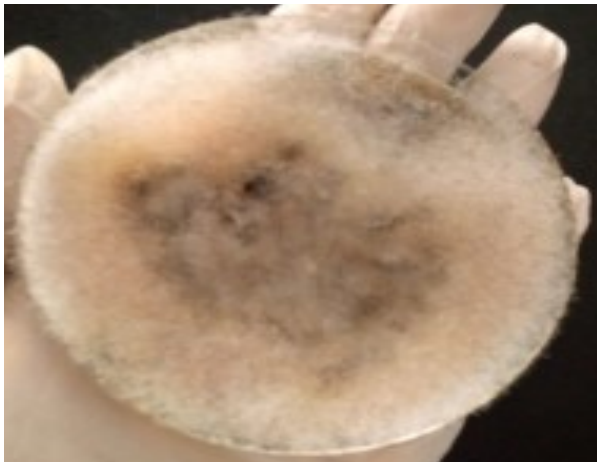

Figure 18. 48 Hours Mucor on the Sample

Figure 19. 4 Days Exposure of Sample to Open Air

Figure 20. 48 Hours Old Aspergillus and Rhizopus

In figure 1 to 20 above shows the laboratory evaluation of the samples. The laboratory evaluation revealed that the meat (endosperm) of the coconuts were heavily invaded and decayed by a variety of fungi after cracking the shell and exposing the meats. The fungi started to appear on the samples (meat of the coconut) within 24 hours after exposing the meat to open air. Fungal growth observed after exposure to open air are: Mucor, Rhizopus, Aspergillus and Penicillium.

Discussion

The proximate composition of plant products varies with age, cultural practices, environment, the season and the varieties (Singh, et al., 2008). Different plants and even different parts of some plant species differ widely in the amount of mineral elements which they absorb (Agbede, 2009). Mineral elements play different but important roles in the lives of human and other living organisms (Anoliefo, et al., 2006). Plant protein is an important source of food nutrient for the less privilege population in developing countries where the cost of animal protein is beyond their income per capita (Ekop, 2007).

lipids consitute part of the bulk of energy composition in plants (Shamina, & George, 2004). The results of this research show high content of moisture (7.28±0.02), Protein (9.80±0.02), lipids (38.75±0.02), fibre (7.35±0.02), dry matter (92.72±0.02) and NFE (43.14±0.04) in the meat of the coconut fruit. The mineral content in the meat of the coconut shows considerable high levels of Mg (3.68±0.02), Na (3.75±0.02), K (6.76±0.02), Fe (19.67±1.53), Zn (5.85±0.02) and PO_4 (2.56±0.02). Several researches have shown the importance of mineral nutrients in the food that we eat daily.

1. The importance of mineral elements in human, animal and plant nutrition has been well recognized (Darby, 1976). Deficiencies or disturbances in the nutrition of humans or an animal cause a variety of diseases and can arise in several ways (Gordon, 1977). When a trace element is deficient, a characteristic syndrome is produced which reflects the specific functions of the nutrient in the metabolism of humans or animals (Simsek, & Aykut, 2007). The significance of the mineral elements in humans, animals and plants nutrition is well known in existing literatures (O' Connor, 1995). Mineral elements play important roles in health and disease states of humans and domestic animals (Wood, 2000; Agriopoulou, Stamatelopoulou, & Varzakas, 2020).

The laboratory analysis for the pathogenic fungi that attacks coconut meat after exposure to open air, revealed a variety of fungi. Most especially Aspergillus and penicillium were predominant amongst others as shown in the figures above. These fungi produce Mycotoxins which are toxic secondary metabolites. Fungal based mycotoxins were identified as causative agents of illness in humans and animals in the last century (CDC, 2020). In the 21st century, aflatoxins, produced by many species of the fungus Aspergillus, are considered inevitable contaminants in the global food supply(Gülhan, 2020).

Mycotoxins can readily enter the food supply chain through fungal infection of agricultural commodities in the field during harvest, postharvest storage, or processing and transportation (Matthews, Kniel, & Montville, 2017). The major producers of mycotoxins include certain species within three fungal genera: Aspergillus, Fusarium, and Penicillium (Bennett, & Klich, 2003). Consumption of fungal containing food or feed may induce adverse health effects in humans or animals. With more than 100 species, Aspergillus are capable of producing several mycotoxins, including aflatoxins, cyclopiazonic acid, ochratoxin A, and sterigmatocystin (Richard, 2007).

Aflatoxins fungi are considered to be the most toxic among the range of mycotoxin classes (Marin, et al., 2013). Ochratoxin A produced by A. ochraceus has immunosuppressant, immunotoxic, genotoxic, neurotoxic, teratogenic (reproductive) and carcinogenic effects. Research indicates a strong correlation between nephropathy (kidney disease) and ochratoxin A exposure in humans and animals (EFSA, 2020).

Among the Penicillium species, more than 80 are documented toxin producers. The Penicillium mycotoxins that affect liver or kidney function, by acute or chronic exposure, are usually asymptomatic in humans or animals. Those that affect nervous system function (i.e., neurotoxins) are characterized by continuous trembling in animals (CPCRI, 2007).

Conclusion

The effects of chemicals like pesticides, herbicides, fertilizers on the mineral contents of the soils where coconut are cultivated, genetic, location and environmental factors could also influence the levels of the mineral elements in coconut. Fungal inversion of coconut meat (endosperm) is a factor that grossly reduces the economic, consumption value and the proximate composition of the coconut meat when exposed to open air. The major fungal growth observed after exposure of the coconut meat to open air are: Mucor, Rhizopus, Aspergillus and Penicillium. Fungi commonly invade coconut and other commodities consumed by animals and humans. Due to their

growth on the commodities, they produce low molecular weight secondary metabolites. Environmental conditions such as temperature, water activity, and humidity impacts on fungal production and growth. Other factors such as pH, fungal strain, and substrate also play roles. These toxins cause many health conditions in animals and humans, including death. With the present of Aspergillus and penicillium in the samples evaluated in the laboratory as shown in figure 1 to 20 above, implies that there are health implications associated with the consumption of spoiled coconut meat. Different researches have proven the pathogenetic properties of Aspergillus and Penicillium consumed by humans and other animals (Hoda, et al., 2000; Jackson, et al., 2004; Mahajan, Sharma, & Dhall, 2009).

Significance Statement

This study will be helpful in identifying the means of preserving coconut in good quality for consumption, storage, and commercial purpose. It is important to identify and check fungal growth which are associated with the deterioration of coconut and suggests possible control measures. This research will be helpful to coconut producer, nutritionists, health agencies and the academia.

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