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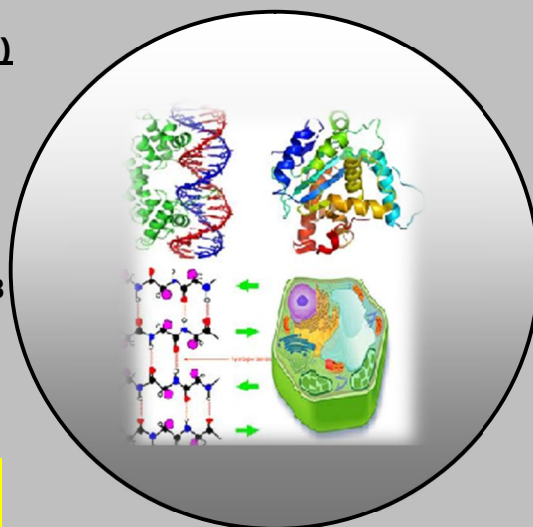
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RESEARCH PAPER

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Pre and Post-Harvest Fungi Associated with Fruit Rot Diseases of *Dialium guineenses* Wild and their Effects on the Nutrients of the Fruit Pulp

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ABSTRACT

*Isolation and identification of fungi associated with pre and post-harvest soft rot of the fruits of *Dialium guineense* was carried out. Investigated also is the proximate analysis and the mineral composition of the fruit pulp, and the effect of the fungi isolated on these food and mineral nutrients. The fungi isolated from the fruits included: No fungus was isolated from the fruit collected from the field. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus japonicum*, *Penicillium chrisogenum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Penicillium italicum*, *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus* and *ochraceus* were isolated from the post-harvest fruits. The result of the proximate analysis and investigation of the mineral composition of the fruit pulp showed that carbohydrate, protein, Ash, lipid, vitamin A, vitamin C and minerals such as calcium, phosphorus iron, sodium, zinc, magnesium, lead, copper, and nitrate were contained in the fruits. With the exception of moisture and ash, the food value of the apparently healthy fruit pulp was higher than that of the infected. Also the value of the mineral nutrients in the healthy fruit except potassium was higher in the apparently healthy fruit pulp.*

Key words: Post-Harvest, Fruit Rot, Fungi, Pre-Harvest and Diseases.

INTRODUCTION

Moulds cause extensive damage on foods, feeds, and other agricultural commodities in the field, during transportation, storage, and processing, leading to post-harvest losses (Agrois, 1997).

About 10 to 30% of the total yield of crops and more than 30% of perishable crops in developing countries are destroyed by reducing their quality and/or quantity (Cleveland *et al*, 2003). They also produce mycotoxins that can cause morbidity and mortality of the consumers (Smith *et al*, 1994). Mycotoxicosis causes acute liver damage, liver cirrhosis, tumor and attack on the central nervous system, skin disorder and hormonal effects.

Aspergillus species are worldwide in distribution. They are responsible for several disorders in various plants and plant products (Bilal, 1988; Kozakiewicz, 1989), and is often isolated from soil and plant materials, especially, in tropical and sub-tropical region (Bennet and Klich, 1992 and Leong *et al*, 2007). They secrete acids and enzymes into the surrounding environment, breaking down polymeric molecules into simpler ones that are then absorbed back into the fungal cell (Pitt, 2000). Gaining access to nutrients is aided by mechanical forces whereby fungal hyphal tips grow into and through food substrates, (Nielsen, *et al*, 2009). *Aspergillus* and other moulds play important roles which include: recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some are capable of degrading more refractory compounds, such as fats, oils, chitin and keratin. Maximum decomposition occurs when there is sufficient nitrogen, phosphorus and other essential inorganic nutrients. (Agrois, 1997). *Aspergillus* species range in growth from relatively slow growing to fast growing, and the spores are common components of aerosols where they drift on air current, dispersing them in short and long distances depending on the environment (Klich, 2002). When the spores come in contact with a solid or liquid surface, they are deposited, and if the conditions of moisture are right, they germinate, (Hawker (2002) and Klich (2002).

According to Barnett and Hunter (2000), the defining characteristics of the genus *Aspergillus* is the aspergillium-like spore-bearing structure. Species in the *Aspergillus niger* group bear black spores, while *Aspergillus flavus* are green. The most common species are *Aspergillus niger* and *Aspergillus flavus* followed by *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Aspergillus fumigatus*, which contaminate agricultural products at different stages including pre-harvest, harvest, processing and handling (Cleveland *et al*, 2003). *Aspergillus flavus* is the most common *Aspergillus* species associated with aflatoxin contamination of agricultural and wild crops (Cotty *et al*, 1994 and Cotty, 1997). Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which are among the economically most important mycotoxins. Aflatoxicosis causes death of several hundreds of people in Africa and Asia (Varga, *et al* 2009). *Alternaria alternata* also cause black rot disease in fruits of *Dialium guineense* (Bilal 1988; Lunn, 1977). *Aspergillus niger* causes black mould disease in the fruit of *Dialium guineense* (Cleveland *et al*, 2003). Various reports evidenced that members of *Aspergillus niger* coupled with *Aspergillus carbonarius* and *Aspergillus japonicum* are frequently responsible for post-harvest decay of fresh fruits and some vegetables (Battilami, *et al*, 2003 and Magnani *et al*, 2005). It is also among the fungi isolated from dried fruits, beans, oil seeds, black velvet and nuts (Chulze *et al*, 2006; Cleveland *et al*, 2003 and JEFCA, 2001).

Okegbile and Taiwo (1990), revealed that in many fruits of *Dialium guineense*, browning reactions occur when the tissue is injured, peeled or diseased, and this is due to the conversion of phenolic compounds to quinones by polyphenol oxidase. The quinones then undergo polymerization to impart brown coloration. Phenolases have also been implicated in the resistance of plants against various micro-organisms, and this resulted to lesser infestation and contamination of the fruits by many species of fungi, (Namrod and Montgomery, 1973). Achoba *et al*, (1992) analyzed fruits of *Dialium guineense* Wild for proximate composition, selected inorganic ions and ascorbic acid. Arogba *et al*, (1994), also reported that the edible pulp of *Dialium guineense* fruit is sweet but acidic (P^H 3.3), relatively poor in protein and oil, and fairly low level of ascorbic acid. Adepoju and Onasanya, (2008), reported the nutrient composition and anti-nutritional factors of *Dialium guineense* Wild fruit pulp. Nahar *et al*, (1990), reported that the fruits is an excellent source of nutrients such as minerals and vitamins and also contain carbohydrates in form of soluble sugars, cellulose and starch. Herzog *et al*, (1994), reported that wild fruits are important sources of vitamins and minerals. Okegbile and Taiwo, (1990), reported that there were appreciable physical changes in the texture and color of the fruit pulp when stored. According to them, in the pulp, the total ash, lipid, crude protein and fibre content were 58.4%, 0.1%, 0.3% and 0.4% respectively, while the vitamin C content of the seed was found to be 11% of that of the pulp. Their work also showed that, the seed is richer in minerals than the pulp, but the value of iron (1.82mg/100g) in the pulp, was higher than that of the seed (1.60mg/100g). Akinyele and Keshinro (1980), reported that the ascorbic acid content in the fruit pulp of *Dialium guineense* is higher than recorded in lime, pineapple, sweet banana and agbalumo (*Chrysophyllum albidum*). Mapson (1970), revealed that the relatively high amount of ascorbic acid in the pulp of *Dialium. guineense* compared to some other fruits may be as a result of its acidity, since ascorbic acid occurs more in acidic medium than at high pH values. Akande and Olowookere (1986), reported that the presence of sugar in the fruit, may be the reason why children frequent the trees for their fruits.

In Abakaliki metropolis of Ebonyi State of Nigeria, grownups, as well as children enjoy the fruit pulp so much. It has been observed that children complain of stomach ache after taking the fruit pulp, and that stored fruit degrade after some time. This work is therefore aimed at isolation and identification of pre and post-harvest fungi associated with the fruit pulp; the food nutrient and mineral composition, as well as and the effect of the fungi isolated on the fruit pulp.

MATERIAL AND METHODS

Collection of Fruits

The fruits of *Dialium guineense* were collected from the plants around Nkaliki Area of Abakaliki, in Ebonyi State, Nigeria. They were treated as soon as they were brought to the laboratory. Some were stored for seven days to determine the fungi that could attack them in storage.

Isolation of the Fungi

The standard blotter method of the International Seed Testing Association (I.S.T.A) 1976 was used for the isolation of the fungi. Seed samples were pre-treated with 1% Sodium hypochlorite solution for ten minutes before peeling the coat of the fruits to remove contaminants on the surface before plating ten seeds per Petri-dish; five at the outer side, followed by four seeds and one at the centre. They were incubated in an incubator at $25 \pm 2^{\circ}\text{C}$ for seven days. Four hundred seeds were treated for the pre-and post-harvest fruits each. Pure culture was prepared from the fungal growth on the fruits. Identification of the fungi was carried out using stereo-binocular microscope (6-50 x) based on their habit character and spore characteristics. Where necessary, spores of fungal mycelia were made on slides and viewed under compound microscope (x40) to confirm identification. The identification was done, following the fungal description by Barnett and Hunter, (1992) and Klich (2002).

Analyses of the Food and Mineral Nutrients of the Fruits of *Dialium guineense*

The freshly harvested fruits of *Dialium guineense* termed apparently healthy and those kept for several days, termed infected fruits were analyzed in triplicate for carbohydrate, crude fibre, moisture, protein, lipid (fat), and minerals using standard method of A.O.A.C. (2007). The vitamin A content of the fruit was also determined using Carr- price method and Vitamin c was also determined using 2, 6 – dichlorophenol indophenol method as described by A.O.A.C. (2007).

RESULTS AND DISCUSSION

Aspergillus niger, *Aspergillus flavus*, *Aspergillus japonicum*, *Penicillium chrisogenum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Penicillium italicum*, *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus ochraceus* and were isolated from the stored fruits of *Dialium guineense*, while none was isolated from the freshly harvested fruits. The work of Cleveland *et al*, (2003), stated that the most common species of fungi, such as *Aspergillus niger* and *Aspergillus flavus* contaminated agricultural and wild fruits at different stages such as pre-harvesting, harvesting, processing and handling. Chulze *et al*, (2006) stated that *Aspergillus niger* was among the commonest fungi associated with dried fruits, beans, oil seeds and nuts. Perrone *et al*, (2007), showed that *Aspergillus niger* caused black mould diseases on certain fruits such as grapes, onions, black velvet and pea nuts. Smith *et al* (1994) reported that post-harvest fungi produce mycotoxins that can cause morbidity and mortality of the consumers and Mycotoxicosis causes acute liver damage, liver cirrhosis, tumour and attack on the central nervous system, skin disorder and hormonal effects. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which are among the economically most important mycotoxins. Aflatoxicosis causes death of several hundreds of people in Africa and Asia (Varga, *et al* 2009).

Eating the fruit pulp of this plant infected by the organisms could lead to some of the health problems reported above. Hence, people are advised to litch the fruit pulp directly from the farm.

The work of Okegbile and Taiwo (1990) showed that not many micro-organisms could thrive on the fruits of *Dialium guineense* due to the presence of phenolases, which have been implicated in the resistance of the plant against these micro-organisms. This must have been the reason why no pathogenic fungi could not be isolated from the freshly harvested fruits of this plant before they were stored.

Table 1. The Nutrient Composition of Infected and Apparently healthy Fruit Pulp of *Dialium guineense*.

Nutrients	Infected	Apparently Healthy
Moisture	11.65 ± 0.31	7.32 ± 0.26
Ash	4.33 ± 0.44	3.53 ± 0.5
Protein	5.56 ± 0.01	7.79 ± 0.42
Fat	2.67 ± 0.41	2.95 ± 0.42
Fibre	0.44 ± 0.05	0.70 ± 0.04
Carbohydrate	75.26 ± 0.70	80.81 ± 0.20

The result of the work on the food nutrient and mineral compositions of the infected and apparently healthy fruit pulp of *Dialium guineense* showed that the values of crude protein, fibre, carbohydrate, fats, minerals, vitamin A and vitamin C were relatively higher in the apparently healthy fruits, while the value of ash and moisture content were higher in the infected fruits relative to the apparently healthy ones $P > 0.5$ (Table 1). The relative increase in moisture content of the infected fruits might be attributed to the solubilization of the pulp by the fungal pathogens as a result of their actions on the infected fruits. Since vitamin C is an important growth factor to fungi, the relative decrease of vitamin C may be due to the utilization of the vitamin C content of the fruit by the fungi for its growth. More so, the conversion of the organic or biochemical compounds of the fruits into inorganic complexes might have resulted to the relative increase of the Ash content of the infected fruits used in this research. Haliwel (1996) showed that vitamin C played a key role in decreasing the incidence of degeneration diseases. Johnston *et al* (1998) reported that low ascorbic acid level has been associated with fatigue and increased severity of respiratory tract infections. The deficiency of vitamin C in man produces perifollicular hemorrhage, which leads to scurvy, while the deficiency of vitamin A result in Xerophthalmia, which is the keratinization of the ocular tissues and may progress to blindness.

Table 2. Some Vitamin and Mineral Content of Infected and Apparently healthy Fruits of *Dialium guineense*.

S. No.	Nutrients	Infected	Apparently healthy
1	Nitrates NO ₃	24. ± 0.47	30.43 ± 0.67
2	Phosphorus (pH)	13.04 ± 0.78	41 ± 0.52
3	Iron (Fe)	4.15 ± 0.39	7.42 ± 0.32
4	Lead (Pb)	0.72 ± 0.16	7.93 ± 0.05
5	Copper (Cu)	1.04 ± 0.12	1.04 ± 0.06
6	Calcium (Ca)	20. 2 ± 0.22	46.33 ± 0.39
7	Sodium (Na)	29.2 8 ± 1.92	38.6 ± 5.72
8	Zinc (Zn)	6. 55 ± 0.45	2. 86 ± 0.55
9	Potassium (K)	25.88 ± 0.85	23.25 ± 1.23
10	Magnesium (Mg)	11.67 ± 0.59	12.6 ± 1.20
11	Vitamin A	10.4. ± 3.38	51.48 ± 7.37
12	Vitamin C	9.55 ± 0.43	41.28 ± 2.21

Since there was a relative decrease in the mineral contents of the infected fruits, it could be suggested that these fungi must have utilized those minerals for their growth. Mineral deficiency has different impacts on the growth and metabolic activities of human body. Diribe and Elom (2002) stated that effects of deficiencies of minerals are as follows; Deficiency of calcium causes ricket in children and osteomalacia in adults, while low intake of sodium results in metabolic acidosis. Prolonged potassium deficiency may produce severe damage to the kidney. Iron deficiency leads to anaemia in man, while copper deficiency in infants is characterized by low copper and ceruloplasmin levels in blood. Failure of iron absorption leads to low white blood cell count and defective erythrocytes production, while zinc deficiency leads to dwarfed growth and severe retardation. It also leads to heptasplenomegaly (enlargement of liver and spleen) and severe iron deficiency resulting in anaemia. As a result of the heavy metals like copper and lead observed in the pulp of the fruit, this plant could play some role in phyto-remediation.

The deficiency of protein causes, kwashiorkor, and marasmus. The clinical feature of kwashiorkor include: Growth retardation, muscle wasting, some psychomotor disturbances and oedema; while clinical feature of marasmus include: Growth retardation, muscle wasting, and psychomotor disturbances (Diribe and Elom, 2002). Edem *et al* (1984) and Ishola *et al* (1990) reported that fruits in general are not usually considered as excellent sources of protein. Hence, the intake of infected fruits of *Dialium guineense* is deemed unfit as the nutrient values have been reduced by the actions of the fungi.

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