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# Dry Rot of *Raphia hookeri* and its Effect on Proximate Composition of the Fruits

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**Abstract**

**Postharvest dry rot of *Raphia hookeri* palm fruits was studied and found to be caused by *Xylaria feejeensis*. Also associated with this fungus were *Penicillium dierckxii, Botryodiplodia theobromae*, *Aspergillus niger* and *Trichoderma* sp. The disease caused dryness of the fruits, thereby affecting the testa and embryo and making the fruit unsuitable for planting. Proximate analyses of the healthy and infected mesocarps of the fruit was carried out using the methods described by the Association of Official Analytical Chemists, revealed the presence of food components such as moisture, ash, fibre, protein energy fat and minerals. The results of the proximate and mineral composition of infected fruits showed decreases in all the food and mineral components apart from ash and magnesium which increased from 4.33% to 6.10% and 395.6ppm to 1538.93ppm, respectively. There was also a significant decline in the phosphate and zinc content which decreased from 1165.6pp to 65.31ppm and from 10.45ppm to 0.825ppm, respectively. The decrease in the moisture content, food and mineral contents and increase in ash indicate the utilisation of these components by the microorganisms. The result of the proximate and mineral composition shows that *R. hookeri* fruit contains high amounts of mineral elements required for good health and fitness in man, therefore adequate and proper care of the fruit during harvesting and storage should be ensure for proper conservation of these nutritional components.**

**Key Words:**

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## Introduction

In Nigeria, *Raphia* palms grow wild in the lowland forest region and swamps of the South as well as river courses of the Savannah region of the North (Ndon, 2003). *Raphia* palms are peculiar for their hepaxanthic flowering and so a trunk usually flowers and fruits only once and dies after 3-35 years of vegetative growth (Otedoh, 1985). Economic products of *Raphia* palm include building materials such as bamboos, *Raphia* fibre, thatch, pissava and palm wine. Most of the species are tapped for wine by tapping the young terminal inflorescence (Otedoh, 1982). The wine is now successfully bottled for commercial purposes at the Nigerian Institute for Oil Palm Research (NIFOR) Benin-City and other places in Nigeria such as Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos. The palm wine is also used in distilling local dry gin. In a recent study, *Raphia* was strongly recommended for its good quality pulp for paper making as well as for producing soft tissue paper (Odeyemi, 1985).

The economic importance of *R. hookeri* fruits as reported by Ndon , (2003) includes its oil which can be used for cooking and making of confectionery, the mature and ripe fruit served as food for coastal people of Akwa Ibom state, Nigeria. Ndon , (2003) also reported that the fruit contains plant growth

regulators such as auxins, cytokinins, ethylene, gibberellins and other chemicals which can be used in tissue culture and also to stupefy fish.

*Chalara paradoxa* and *Xylaria feejensis* are the major fungi causing the postharvest black rot and dry rot diseases respectively, of *R. hookeri* fruits. These fungi have the ability to affects the scale, mesocarp and endocarp of the *Raphia* fruits, thus destroying the embryo and thereby making the seed unsuitable for planting (Oruade-Dimaro, 1989; Esiegbuya *et al*., 2013a). Other fungi associated with the *Raphia* fruits as reported by these authors include *Aspergillus niger, Fusarium* sp and *Botryodiplodia theobromae*.

*Aspergillus, Penicilium* and *Trichoderma* are common moulds which are found in the air and soil, and can easily contaminate fruits. It is not surprising then that they were associated with the fruits of *R. hookeri*. *X. feejeensis* on the other hand, is an ascomycete of Class Sordariomycetes, Family Xylariaceae and Order Xylariales. The ecophysiological features of the Xylariaceae indicate a xerophilous lifestyle of their ancestors (Rogers 2000) which partly explains the ability of *X. feejeensis* to cause dry rot and thrive well on the dry fruit. As pointed out by Whalley (1996), the Xylariaceae have long been considered to be wood-destroying saprobes, aside from a few facultative tree parasites (Ostry and Anderson, 2009). Saprophytic Xylariaceae are considered to be white-rot fungi, owing to their ability to degrade lignin, but they even can degrade cellulose very effectively (Wei *et al*., 1992).

The comparative study of the proximate and mineral composition of healthy and black rot infected *R. hookeri* fruits caused by *C. paradoxa* shows a significant decrease in all the food components analyzed for, except for ash which shows a slight increase. The decrease in the moisture, fat, protein, energy and mineral contents and increase in the ash content indicates utilization of these components by the *C. paradoxa* (Esiegbuya *et al*., 2013b).

The mineral contents of fruits have been shown to vary tremendously with changes in infectivity caused by microbial organisms (Pathak, 1997). The Ascomycetes are able to degrade mineral elements on substrates in which they grow (Lawal, 2011; Lawal *et al*., 2012). Previous study (Esiegbuya, et al., 2013a), has reported on the adverse effect of the black rot disease on the nutrient and mineral composition of *R. hookeri* fruits, there is therefore the need to study the effect of the dry rot disease caused by *X. feejensis* on *R. hookeri* fruits.

This study will also pave way for further research into prevention and control of this pathogen as well as protection of the fruits for its economic benefits.

## Materials and Methods

*Source of Plant Materials*: *Raphia* fruits used in this investigation were obtained from the gene pools harvested from wild grooves plantation in Bayelsa (Onuebum) and Delta (Otegbo) States of Nigeria. The species examined was *R. hookeri* (Man and Wendle 1864). Four bunches containing about five hundred fruits were harvested; the healthy ones were separated from the infected ones. The average weight of the fruit was 10.75 g.

*Detection of fruit rot*: This was carried out by visual examination. This is according to Oruade-Dimaro (1992) who reports that *R. hookeri* fruits are usually examined visually and by exerting little pressure on the fruits. Since the fungi deterioration usually causes the scales of the fruits to be loosely attached on application of little pressure.

*Isolation of fungi associated with fruit rot*: Small portions (including rotted and healthy portions) of 5 mm diameter were cut with a flamed scalpel blade from the mesocarp and scale. These were sterilised in 0.1% mercuric chloride solution for 2 minutes and rinsed in three changes of sterile distilled water, dried with sterile tissue paper and crushed before plating in Petri dishes containing potato dextrose agar (PDA) medium. Petri dishes were incubated on a laboratory bench at laboratory temperature of 28 ± $2^0$C for 7 days. After the period of incubation, different colonies of fungi associated with the fruit rot were each aseptically subcultured using a flamed inoculating loop, into a sterile plate fresh PDA.

*Pathogenicity test*: The method used was similar to that described by Oruade-Dimaro (1989) for *Raphia* fruits. The isolated fungi were tested for pathogenicity by using ripe and freshly harvested fruits soaked in 0.1% mercuric chloride for 3 minutes and then washed in five changes of sterile distilled water, to remove surface contaminants. Fruits were wounded by either beating surface of fruits with a mallet fitted with sharp nails to create wound about 1.5 mm in diameter, or scales removed to expose part of

the mesocarp under aseptic condition. Ten ripe wounded and unwounded fruits were inoculated by using a sterile inoculating loop to transfer a 7 day old pure fresh growing mycelium from a Petri dish. Each batch was transferred into a moistened transparent sterile beaker (500 ml). After incubating for 14 days, isolation was carried out on the inoculated fruits. A third batch of the fruits was treated as above except that they were soaked in sterile distilled water before transferring into the moistened beaker. This served as the control. This experiment was done twice.

*Identification of fungal isolates*: Isolated fungi were identified macroscopically and microscopically by comparison with the descriptions and illustrations of Barnet and Hunter (1998). The fungus responsible for the dry rot (after pathogenicity test) was sent to the Commonwealth Mycological Institute, UK for identity confirmation. The fungus was identified as *Xylaria feejensis* with IMI No: 501772

*Proximate composition*: Proximate composition of the fruits was determined by the official method of the Association of Official Analytical Chemists as follows: Moisture (section 926.08 and 925.09), Protein (section 955.04C and 979.09), Fat (section 922.06 and 954.02), ash (section 923.03), Energy (section 2003.09) and crude fiber (section 962.09) (A.O.A.C., 1990).

*Mineral content*: The ash of each sample obtained was digested by adding 5ml of 2M hydrochloric acid (HCL) to the ash in the crucible and heated to dryness on a heating mantle. Then 5ml of 2M hydrochloric acid (HCL) was added again, heated to boiling and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water, stoppered and made ready for reading of concentration of Calcium, Potassium and Sodium on the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element. Magnesium was read in a Perkinhelmer AAS Model 2300AA.

The concentration of each of the elements was calculated using the formula: %Ca or %K or %Na

$$= \frac{\text{Meter Reading (MR) x Slope x Dilution factor}}{1000}$$

NB: MR x slope x dilution factor gives concentration in parts per million (ppm) or mg/kg. The concentration in % is obtained by dividing by 1000.

Phosphorus was determined routinely by the vanado-molybdate colorimetric or spectrophotometric method. The ash of each sample obtained was treated with 2M HCL solution as described for calcium determination above. Then, 10 ml of the filtrate solution was pipetted into a 50 ml standard flask and 10 ml of vanadate yellow solution was added. The flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer at a wavelength of 470 nm. The percentage phosphorus was calculated using the formula:

$$\% \text{ Phosphorus} = \frac{\text{Absorbance x Slope x Dilution factor}}{1000}$$

The digest of the ash for each sample as for calcium and potassium determination was washed into 100 ml volumetric flask with deionised or distilled water and made up to mark. This diluent was aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements (magnesium and zinc) was read with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

## Results

*Result of pathogenicity test*: *Xylaria feejensis* and a *Penicillium* sp were the main fungi associated with the dry rot disease. However, the pathogenicity test showed that *X. feejensis* was responsible for the disease symptoms.

*Morphological identification of isolates*: The organism responsible for dry rot was *Xylaria feejeensis* which was identified by Commonwealth Mycological Institute (IMI Number 501772). Other

organisms' isolated included *Penicillium dierckxii* (IMI Number 500952), *Botryodiplodia theobromae*, *Aspergillus niger* and *Trichoderma* sp.

*Symptomatology*: *Xylaria feejeensis* was the first organism to appear on the fruit, suppressing the symptoms of the other (secondary) pathogens. After inoculation with the fresh mycelium, it was observed that the mycelium gradually grew round the entire fruit, forming a whitish mass. The effect of *X. feejeensis* led to a change in the colour of the ripe healthy mesocarp from orange to white and the shrinking of the affected fruits as a result of the dryness (Plates 1, 2). The symptoms of the dry fruit rot began with the growth of mycelial mat on the scale of the fruit which gradually penetrated the mesocarp, forming a weft of mycelia round it as shown in plate 3. The weft of mycelia prevented further manifestation of other secondary pathogens. The culture yielded a white cottony growth (Plate 4). The severe stage of the infection was characterized by dryness of fruit, with scales loosely attached. This infection damaged the testa and embryo as a result of the shrinking and dryness, thus making the fruit unsuitable for planting (Plates 5, 6).

Table 1 shows the proximate composition of the ripe healthy mesocarp of *R. hookeri* fruit and one infected by dry rot. The result revealed that there was decrease in all the food and mineral components apart from ash and magnesium which increased from 4.33% to 6.10% and from 395.6ppm to 1538.93ppm, respectively. There was also a significant decrease in the phosphate and zinc contents which dropped from 1165.6ppm to 65.31ppm and from 10.45ppm to 0.8.25ppm, respectively. The decrease in the moisture, food and mineral contents and increase in ash content indicate the utilisation of these components by the organisms.

Table 1: Proximate composition of healthy and dry rot infected mesocarps of *R. hookeri* fruits.

| Minerals | Fresh Samples | Dry rot infected fruit | % loss or gain | WHO RDA Standard |
|---|---|---|---|---|
| Ash (%) | 4.33 | 6.10 | 1.77% | |
| Moisture (%) | 9.87 | 8.88 | -0.99% | 3.7 |
| Fibre (%) | 56.15 | 35.33 | -20.82% | 38g/day |
| Fat (%) | 8.2 | 20.88 | 12.68% | 20-35g/day |
| Energy (J) | 20.45 | 16.091 | -4.359J | 130g/day |
| Protein (ppm) | 0.95 | 0.7 | -0.25ppm | 56g/day |
| Sodium (ppm) | 264 | 110 | -154ppm | 1500mg/day |
| Potassium (ppm) | 4861.2 | 4625 | -236.2ppm | 4700mg/day |
| Phosphate (ppm) | 1165.6 | 65.31 | -1100.29ppm | 700mg/day |
| Zinc (ppm) | 10.45 | 0.825 | -9.625ppm | 11mg/day |
| Manganese (ppm) | 623.18 | 374.83 | -248.35ppm | 350mg/day |
| Magnesium (ppm) | 395.6 | 1538.93 | 1143.33ppm | 2.3mg/day |
| Calcium (ppm) | 7891.1 | 4118.25 | -3772.85ppm | 1000mg/day |

WHO = World Health Organization; RDA = Recommended Daily Allowance

## Discussion

The moisture content of a sample determines the type of microorganism which can grow on it. The moisture content of any food is an index of its water activity (Frazier and Wwstoff, 1978) and is used as a measure of the stability and susceptibility to microbial contamination (Scott, 1980). The moisture contents of the ripe and infected mesocarps of *R. hookeri* fruit were 9.87% and 8.88%, respectively. (Inappropriate here. Logical sequence!!)Moulds are generally able to grow on foods with low moisture content, hence the other fungi could grow on the fruit. Moisture requirements of food borne moulds are relatively low; most species grow at a 0.85 *a*w or less, although yeasts generally require a higher water activity. Water activity 0.60 *a*w is considered the limit for cell growth, but spores of *Aspergillus* and *Penicillium* for example, are able to survive at lower *a*w for several years (Carlile and Watkinson, 1996). Reduction in the moisture, crude fibre and energy contents in fungi infected samples may be attributed to the fact that the fungi utilise the water and carbohydrate for metabolic activities (Burnett, 1976). Previous studies have indicated that pathogenic infection affects the overall level of nutritional components in a plant (Mba and Akueshi, 2001).

According to Igboh *et al*., (2009), consumption of fruits with high fibre content of *R.hookeri* fruit is of nutritional value because it has the ability to contribute to a reduction in the incidence of certain diseases like colon cancer, coronary heart diseases diabetes, high blood pressure, obesity and other digestive disorders. The high fibre content of *R. hookeri* fruits showed in this study was 56.15%. This suggests that consumption of *Raphia* fruits may contribute in reducing digestives disorders.

Fungi have been reported to increase the protein content of samples on which they grow (Rodolfo *et al.*, 2000). However, in this study there was decrease in the protein content from 0.95ppm to 0.7ppm which suggests that the protein content may have been utilized by these organisms associated with dry rot disease. The increase in the amount of fat in infected samples from 8.2% to 20.88% may be due to the possible transformation of carbohydrates to fat. Akindumila and Glatz (1998) has reported that certain fungi could produce microbial oil during growth on substrates.

Zinc (Zn) is an essential metal for proper body functioning such as normal growth and development, in human beings (Divrikli *et al*., 2006). The amount of zinc detected in the healthy fruit sample was 10.45ppm which decreased to 0.825ppm in the infected fruit sample. The maximum tolerable daily intake of Zn is 0.3–1 mg kg−1 (FAO/WHO, 1982). The uptake and accumulation of Mn in the healthy fruit sample was 623.18ppm which was reduced to 374.83ppm in the infected fruit sample. The WHO recommends 2-9 mg per day for an adult (WHO, 1994).

## Conclusion

This study shows that *R. hookeri* fruit contains high amounts of mineral elements required for good health and fitness in man. Adequate and proper care of the fruit during harvesting and storage will ensure the conservation of these nutritional components. Dry rot completely damages the fruit, making it unsuitable for sowing. Therefore, there is the need to provide adequate cost effective control measures for the preservation and storage of *R. hookeri* fruits against dry rot caused by *X. feejeensis*.

Plate 1: Healthy *R, hookeri* fruits

Plate 2: *R. hookeri* fruits with dry rot disease

Plate 3: Mycelia of the dry rot fungus on the scale of *Raphia hookeri* fruit

Plate 4: culture plate of the dry rot fungus grown on PDA medium at room temperature for 7 days

Plate 5: Healthy endocarp of *R. hookeri*

Plate 6:: Endocarp infected with dry rot

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