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**FUNGI ASSOCIATED WITH FRUIT ROT OF *Carica papaya* L. AND THE EFFECT OF THE PATHOGENS ON THE NUTRITIONAL VALUE OF THE FRUIT**

**(PAPAYA FRUIT ROT AND THE EFFECT ON NUTRITIONAL VALUE) ٭Tawose, F.O.,1 Akintimehin, E.S.,2 Ojo, F.M.3 and Abolaji, R.4**

Department of Biological Sciences, Ondo State University of Science and technology, Okitipupa

2Department of Chemical Sciences, Ondo State University of Science and technology, Okitipupa

3Department of Biological Sciences, Ondo State University of Science and technology, Okitipupa

4Department of Biological Sciences, Ondo State University of Science and technology, Okitipupa

**٭**Corresponding author’s e-mail: oluwabamikoletawose@gmail.com Phone: +2348039224251

**ABSTRACT**

Pawpaw (*Carica papaya*) is one of the most important crops grown in Africa. The fruit is consumed fresh by many people and hence, source of essential mineral elements and nutrients. This study was conducted to investigate the fungi associated with fruit rot disease of pawpaw and the effect of the disease on nutritional value of the fruits. The fungi isolated from fruit rot of pawpaw got from Okitipupa, Ondo State, were grown on Potato Dextrose Agar (PDA) at (28±2°C) and observed macroscopically and microscopically for cultural and spore characteristics. The fungi were identified as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium italicum*, *Rhizophus stolonifer*, *Mucor mucedo*, *Penicillium notatum and Fusarium solani*. Healthy freshly harvested pawpaw fruits (wounded) were inoculated with the fungus responsible for fruit rot and incubated for some days (pathogenicity test). All isolated fungi were pathogenic to the fruits when pathogenicity test was carried out. Healthy fruits as well as infected ones were analysed for carbohydrate, fibre, moisture, protein, ash and fat content. Furthermore, the infected fruits showed relative decrease in nutrient composition as compared to healthy fruits but increase in moisture content and protein.

**Key words:** *Carica papaya*, fungi, Fruit rot, pathogens, nutritional value

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**INTRODUCTION**

Pawpaw (*Caria papaya* L.) is a member of *Caricaceae* family. This family comprises of 13 (thirteen) species in the four genera: three genera from America (*Carica, Jacaritia* and *Jarilla*) and one from Equatorial Africa (*Cylicomorpha*) (Boning, 2006). *Papaya* is an economically important fruit crop in Hawaii, Australia, India, Srilanka, Phiilipines and South-east Asia including Thailand (Boning, 2006).

The origin of *Carica papaya* is in Tropical America. Its seeds were distributed from the Caribbean to Malacca and India by travelers and botanists in the eighteenth century. The distribution was continued throughout Asia and Pacific (Chan, 2013). *Carica papaya* is grown in all tropical countries and many subtropical countries between 32 °North and South latitudes but the high commercial production is found between 23 °N and S latitudes (Bayewu and Amusa, 2005). It is widely cultivated throughout West Africa around habitations in the forest zone and may occasionally become sub spontaneous where conditions of adequate rainfall and free-draining fertile soil pertain. Full sunlight is necessary. The plant is native of Central Tropical America and is now spread by man to all warm countries.

*Carica papaya* is a fast-growing tree herbaceous like plant 5-7 meters in

height. *Papaya* normally has a monaxial stem without branching but it has multi stems when damaged. When the stem is wounded white milky latex oozed from the wound. The length of Carica papaya which may be up to 9 meters or longer makes the harvesting of this crop a difficult task (Bayewu and Amusa, 2005).

Papaya flowers are born in florescence which appears in the axils of leaves. It can be female, male or hermaphrodite flowers. Female flowers are held close against the stem as single flowers or in cluster of 2-3 flowers. Male flowers are smaller and more numerous. Hermaphrodite (perfect) flowers are intermediate between the female and male (Bayewu and Amusa, 2005).

The fruit usually elongated and oval in shape superficially resembles a melon puriform. The fruits range in size from 7-30 cm (Heywood *et al*., 2007). The fruit is normally consists of 5 carpels. Fruits from female trees are spherical whereas the shape of fruits from hermaphrodite trees are been modified because they are affected by environmental factors and which in turn affects the floral morphology during early development of the inflorescence (Chan, 2013). Green fruits contain an abundance of milky latex. Ripe fruits have yellow-orange coloured skin.

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**Scientific classification of Pawpaw** Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Order: Brassicales

Family: Caricaceae

Genus: Carica

Species: *C. papaya*

Binomial name: *Carica papaya* L. SOURCE: Barro *et al.*, (2006)

**Production of Pawpaw by Country in 2014 (Millions of tonnes)**

**Health benefits of papayas**

Christopher Columbus, an Italian voyager once referred to papayas as the fruit of the angels. The fruit which is extremely rich in Vitamin C has a wide range of health benefits making it a great fruit option to include in your diet. The major health benefits of papaya include the following (Effiuvweywere and Oyelabi, 2000).

1. Lowers cholesterol

Papaya is rich in fibre, Vitamin C and antioxidants which prevent cholesterol build up in your arteries. Too much cholesterol build-up can lead to several heart diseases including heart attack and hypertension (Effiuvweywere and Oyelabi, 2000).

2. Protects against arthritis

Arthritis can be a really debilitating disease and people who have it may find their quality of life reduced significantly.

Eating papayas makes the bone healthy because of its anti-inflammatory properties and also contains Vitamin C which helps in preventing arthritis (Khali and Mazher, 2015).

3. Improves digestion

In today’s times, it is near impossible to avoid eating foods that are bad for your digestive system. Often we find ourselves eating junk food or restaurant food prepared in excessive quantities of oil. Eating a papaya daily can make up for such occasional mistakes, (Sarbhoy, 2000) as it has a digestive enzyme known as papain along with fibre which helps improve your digestive health.

**Aim and objective of this course** The aim of this study is;

➢ To isolate and identify the fungi associated with pawpaw Fruit rot disease

➢ To determine the pathogenicity effect of the different isolates on healthy pawpaw fruits to ascer tain that the fungi isolated were very much responsible for the spoilage and

➢ To determine the effect of the pathogen on the nutritional val ue of the fruit.

**MATERIALS AND METHODS Fruits collection**

Healthy ripe pawpaw and infected ripe pawpaw fruits were collected in Okitipupa, Ondo State Nigeria. The

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fruits were collected into a sterile polythene bag and brought to the laboratory for the study.

**Sterilization of materials**

All glasswares and slides were washed in ‘Omo’ detergent solution, rinsed under running tap water and finally with distilled water and allowed to dry. They were sterilized in electric oven at a temperature of 60°C for 24hrs.

**Detection of Fruit Rot**

Pawpaw fruits were examined externally and also by disserting the fruit with a clean knife after which the symptoms were described.

**Plating of the medium**

Potato Dextrose Agar (PDA) was used in this study and was prepared according to the manufacturer’s specification. Bacterial contamination was inhibited by aseptically adding of chloramphenicol to the sterile medium prior to pouring into sterile Petridishes. Surface sterilization of the infected portion of the fruits was done using alcohol. The fruits were then rinsed with sterile distilled water. Small portion of both healthy and diseased parts of the fruits were carefully cut with sterile 4mm cork borer and then transferred aseptically onto solidified PDA. The poured plates were then incubated at (28±2°C) for 72 to 96 hour.

**Isolation of the pathogens**

The numbers and types of colonies on the plates were observed and noted at the end of incubation. Representative colonies were randomly picked and sub-cultured until pure cultures of the isolates were obtained.

**Characterization and Identification of fungal Isolates**

The fungal isolates were characterized based on their colonial and cellular morphology. The colonial morphology of the isolates was observed macroscopically and characteristics such as colour of mycelia and spores, shape and surface texture were noted. The microscopic examination was carried out using the lacto phenol cotton blue solution. A drop of the solution was placed on a clean slide and a fragment of the test fungus teased out and introduced into the stain. The fungus was properly spread on the slide with the aid of a sterile needle. A cover ship was gently placed on the slide to eliminate air bubbles. The slide was thereafter mounted and examined under the microscope at x100 objective lens.

**Pathogenicity Test of isolated organisms**

Healthy mature pawpaw fruits were washed and surface sterilized with alcohol. A sterile cork borer of 4mm diameter was used in punching out

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single fruit column from the healthy pawpaw fruits. Fungal mycelia punched from pure culture of each of the isolates using sterile inoculating pin, each disc was then removed using sterile inoculating loop and placed in the punched out space. The inoculated points were covered with the fruit skin columns and then sealed up with Vaseline gel to prevent contamination by other organisms. All the inoculated fruits were put in micro humidity chamber (Umoh, 2013). The pathogens were re-isolated from the inoculated fruit samples. This was done by cutting out small portion of spoilt fruit sample with sterile scapel and inoculated directly onto sterile PDA plates which were incubated thereafter. The resultant growths were sub--cultured to obtain pure culture of the isolates and the isolates were re-examined macroscopically and microscopically.

**Determination of proximate analysis** Proximate analysis was determined by AOAC (1990) and other standard procedure

**To determine moisture content** Procedure

❖ Grind sweet sample and weigh out 5g in to empty weighed ster ilized evaporating dish

❖ Place in the oven for 4hrs at 110°c

❖ Remove from the oven and al low cooling in the desiccator for few minutes

❖ Weigh as the beaker containing the sample and

❖ Calculate the % moisture con tent

Calculation

Weight of empty evaporating dish before = a

Weight of evaporating dish after = b

Difference in weight a-b =x

% moisture content = x/5g\*100

**To determine ash content**

❖ Weigh 5g out of a grinded sam ple in to a crucible

❖ Place in a furnace at 500°c - 600°c for 4hrs

❖ Remove and place in the desic cators to cool for 10minutes

❖ Weigh the crucible and deter mine the ash content.

Calculation

Weight of empty crucible = a

Weight of crucible after furnace = b

% ash content = a-b/5g\*100/1 Note: The sweet sample in the crucible turns to ash at the end of the 4hrs.

**To determine fat content**

❖ Dissolve 8g of sample containing 8.4cm3 of hydrochloride acid

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❖ Heated in a water bath for some minutes until colour change

❖ Allow it to cool and extract with 50ml of petroleum ether in a separating funnel

❖ After extraction the sample so lution was heated to dryness

❖ And then weigh after cooling

**Calculation**

% fat = weight loss of sample/weight of sample\*100

**Determination of crude fibre content** ❖ 5g milled fruit was weighed and put into beakers

❖ 50ml of 1.25% H2SO4 acid solution was added and made up to 200ml with distilled water and stirred.

❖ The mixture was heated with continuous stirring for thirty (30) minutes, allowed to cool and settle.

❖ Distilled water was added and allowed to settle, then decanted. ❖ Decantation was repeated for six (6) times consecutively to make the mixture acid free.

❖ 50ml of 1.25% NaOH was added to the mixture and made up to 200ml with distilled

 water a beaker, and

❖ Heated for thirty minutes with continuous stirring.

❖ It was allowed to cool and settled.

❖ Distilled water was added and decanted for six (6) times consecutively.

❖ The mixture was filtered with filter paper and

❖ Kept for about forty-five minutes for water to drain completely and weights taken.

**Determination of crude protein** ❖ Samples were digested 5g each with 30ml of concentrated sulphuric acid using 2g of copper sulphate

❖ 16.0g of sodium sulphate salt until a clear green solution was obtained. ❖ This was dissolved in distilled water and made up to 100ml in a volumetric flask, 12.5ml of the digest was measured into a semi micro Kjeldhol Markham distillation apparatus and treated with 12.5ml of 1.25% of sodium hydroxide (NaOH) solution.

❖ This was distilled with 10ml of boric acid and double indicator.

❖ The distillate was then titrated with 0.1% HCl solution until a light pink end point was reached. Blank titration was also carried out in similar manner.

❖ Distillation was carried out in triplicate and

The percentage nitrogen obtained by appropriate calculation.

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% Nitrogen=Ml of HCl (blank) – Ml of HCl (sample) x 0.1M HCl x 14 x 100 x 100 Weight of sample x Ml of digest x 1000 6.25

**Statistical analysis**

The result from the proximate analysis of infected and uninfected pawpaw fruits was subjected to Duncan’s Multiple Range Test at P<0.05 to observe if there is any significant difference in the nutritional content of healthy and infected pawpaw fruits ( r)

**RESULTS**

**Characterization and Identification of Isolates**

Fungi were isolated from rotted *Papaya*

and identified based on the morphological characteristics (Table 3). A total of seven (7) fungi were isolated and identified, namely *Aspergillus niger*, *Aspergillus flavus* L., *Penicillium italicum*, *Rhizophus stolonifer*, *Mucor mucedo* L., *Penicillium notatum and Fusarium solani.* The macroscopic feature and microscopic features of the isolates are shown below:

**Macroscopic and microscopic features of the isolates**

**Pathogenicity Test of fungal isolate** In this test all the fungal isolates were observed positive for causing spoilage or rottening of pawpaw fruit

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Values are mean ± SD (S.E). Means followed by the same letter in the same column are not significant different by Duncan Multiple Range Test (DMRT) at P< 0.05.



**Plate 1:** Photomicrograph of *Fusarium solani* culture from diseased pawpaw fruit x100

**Plate 2:** Photomicrogragh of *Aspergillus niger* culture from diseased pawpaw fruit x100



**Plate 3:** Photomicrograph of *Aspergillus flavus* culture from diseased pawpaw fruit x100

**Plate 4:** Photomicrigraph of *Mucor mucedo* culture from diseased pawpaw fruit x100

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**Plate 5:** Photomicrigraph of *Rhizopus stolonifer* culture from diseased pawpaw fruit X100

**DISCUSSION**

Fungi have been documented to penetrate host tissue through natural openings such as lenticels, stomata and through the unbroken epidermis by means of appresorium or germ tube. For an organism to cause infection, it must have the ability to breakdown the natural defence mechanisms of the host. Most of these fungi cause rotting are spreads rapidly in the ripe fruit (Ilag *et al.*, 1994). The most common and well known post-harvest disease of the pawpaw fruit which is responsible for serious losses in fresh pawpaw fruit world industry is caused by *Rhizopus* soft rot fungus *Rhizopus stolonifer* and *Fusarium* fruit rot fungus *Fusarium solani.*

In this investigation, the fungi associated with the fruit rot disease of *Cacica papaya* were *Aspergilllus flavus,*

*Penicillium italicum, Penicillium notatum, Aspergillus niger, Mucor mucedo, Fusarium solani* and *Rhizopus stolonifer* (Table 4). This is in agreement with the findings of (Chan, 2013), (Chukwuka e*t al*., 2013) and (Bayewu and Amusa, 2005) that stated that *Aspergilli* have been found to be common pathogens of many crops and cause serious economic losses. Some of the pawpaw fruits have been perched by birds and destroyed by insects which reduced the quality of fruit and also creating openings for pathogen entry. *Aspergillus flavus* and *Aspergillus niger* are wide spread in nature being found on fruits, vegetable and other substrates that provide nutrients since they can tolerate high concentration of sugar and salt. *Rhizopus stolonifer* caused soft rot disease on pawpaw fruit and it is of considerable important post harvest

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decay organism.

The result of the pathogenicity test showed that the fungi inoculated into the healthy pawpaw fruits had the same features as the ones re-isolated from them, indicating that the fungi were responsible for the spoilage of the pawpaw fruits.

The other isolated fungi, especially *Penicillium* spp. have the potential to induce rot on fresh fruits which might have a remarkable effect on the value of the fruit especially in food industry as well as on human health. This agrees with (Amusa *et al*. 2003) and (Vargea *et al*., 2009) who reported that *Aspergillus niger* produces mycotoxins which causes food intoxication in man and other animal.

The result from Table 5 shows that there was a significant difference in the Ash content of the infected fruits (0.72a±0.01) and healthy pawpaw (2.20b±0.26) i.e that was increase in the ash content level of healthy fruit compared to infected pawpaw fruit. This result agrees with report of (Ihekeronye and Ngoddy, 2002) who reported that the ash content tends to increase with the deterioration of fruits. From this study, there was a significant difference in the moisture content of a healthy fruit (88.61a ±0.25) and the infected fruit (95.66b±0.12). This shows there is increase in the water content of the infected fruit compared to the healthy

fruit. This result agrees with the report of (Vargea *et al*., 2009) that stated that the increase in water content of infected fruits accounts for the increase in the softness of the fruit during spoilage. Consequently, the taste of diseased fruit ranges from loss of good characteristic tastes to the development of objectionable tastes, thus a diseased fruit develops acidic taste which is often bitter.

There is no significant difference in the fibre content of infected fruit (0.56a±0.02) and healthy fruit (0.78a ±0.03) as reported from this study which also agrees with the findings of (Nwofia *et al*., 2012) that the spoilage of pawpaw fruit has little or no effect on the fibre content of the fruits.

From this study, there is a significant difference in the fat content of a healthy fruit (6.08b ±0.14) and the infected fruit (0.13a±0.02). This shows there is increase in the fat content of the healthy fruit compared to the infected fruit. This result agrees with the report of (Mensah *et al.*, 2002). He reported that the fat content of healthy fruit is usually higher than spoilt ones.

From this study, there is a significant difference in the protein of a healthy fruit (0.5a ±0.03) and the infected fruit (1.23b±0.02). This shows there is increase in the protein of the infected fruit compared to the healthy fruit. This result agrees with the report of

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(Heywood *et al*., 2007). They reported that, as the spoilage of the pawpaw fruit increases so the quantity of amino acid in free as well as bound forms increase in infected protein. The increase protein bound amino acids is due to proteolysis of fruits protein catalyzed by the fungal enzymes.

From this study, there is a significant difference in the carbohydrate of a healthy fruit (2.15b ±0.002) and the infected fruit (1.81a±0.01). This shows there is increase in the carbohydrate of the healthy fruit compared to the infected fruit. This result agrees with the report of (Chukwuka *et al.,* 2013). He reported that, the diseased pawpaw usually contain lower carbohydrate than the healthy ones.

**CONCLUSION**

Fruit rot disease is a universal fresh fruit problem which occurs in field when fruit is injured in poorly drained soil and in low acidic soil. The knowledge of survival and growth of fungi gotten from this study shows that fruit rot disease can be properly managed by careful handling of fruits so as to avoid bruises.

**Recommendations**

Every fruit tree has future potential for disease and insect damage. The degree of spoilage of papaya fruit can be reduced so as to enhance longer shelf life and greater income. There are

various treatments to control disease in papaya fruit, if available though, planting disease-resistant trees remains the best option. For easy care for all trees, proper maintenance (such as watering, fertilizing, pruning, spraying, weeding and fall clean up) can help keep most insects and diseases a bay. However, the best measure against papaya spoilage is through hygiene, quarantine treatment procedures and vector control.

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