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Post-harvest Survey of Fungal Diseases Associated with Soursop (*Annona muricata* L.) Fruits

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Abstract:
Annona muricata L. (soursop) fruit, consumed globally, is highly susceptible to attack by fungal organisms. Fungal fruit rots' impact on Annona muricata is gradually increasing globally, with distribution in tropical areas, including southern Nigeria, resulting in reducing market availability and fruit quality. This study was aimed at surveying the post-harvest disease symptoms associated with Annona muricata fruits displayed for sales and the disease incidence-severity, which indicates the extent of the damage caused by the symptoms. Soursop fruit samples in Port Harcourt Local Government Area (PHALGA) and Obio-Apkor Local Government Area in Rivers State, Nigeria, were surveyed. Each fruit was scored on a disease severity scale of 0–4 (0% no disease), (1–25% trace of disease), (25–50% light disease), (50–75% moderate disease) and (75–100% severe disease). The data obtained were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level of probability using LSD (Least Significant Difference). The study revealed that Annona muricata fruits were dominated by fungal disease symptoms of dry rot, soft rot, anthracnose, powdery mildew and diplodia fruit rot. There was no significant difference between location pairs for all disease symptoms of incidence and severity of Annona muricata, respectively. Fungal diseases at the post-harvest stage pose a threat to storage durability, distribution and market availability of fruits.

Keywords: *Annona muricata, Disease incidence-severity, Post-harvest fungal disease, survey*

1. Introduction

Annona muricata L., commonly called soursop is of the Annonaceae family (Moreira *et al.,* 2018). It is recognised as one of nature's resources due to its medicinal value (Adeola & Aworth, 2010). Soursop, a popular fruit, which originated from the Caribbean and Central America, is spreading throughout many tropics and other parts of Africa, including the Southern part of Nigeria (Morton, 1987; Sulaiman *et al.,* 2012; Ajayi *et al.,* 2015). It was affirmed by Vwioko *et al.* (2013) as a rich powerhouse of nutrients containing a number of anti-oxidant compounds. Nevertheless, it is limited by post-harvest diseases due to the activities of micro-organisms, especially fungi, thereby leading to a reduction in market value (Umme *et al.*, 1999; Amusa *et al.*, 2005; Ikegwu & Ekwu, 2009; Chukwezi, 2010; Vwioko *et al.,* 2013).

Saranraj *et al.* (2016) estimated that about 20% of harvested fruits are lost through microbial spoilage, causing one or more of 50 market diseases associated with soursop. Hernandez-Guerrero *et al.* (2020) stated that fruits (especially tropical ones) are susceptible to microbial attack and diseases caused by fungi represent up to 70% of the total losses during post-harvest storage. Generally, the impact of diseases caused by microbes on agricultural food crops has increased worldwide, which has led to a loss of appreciable fruit quality.

A research by Baiyewu *et al.* (2007) revealed that between 15 and 40% of soursop fruits displayed for sale in Nigerian markets showed symptoms of microbial infection and these infected fruits, which are sold at lower prices, are preferred by low-income earners. Meanwhile, storage conditions are often seen as a major force that brings about the spread of fungal diseases, which leads to large-scale fruit spoilage, thereby affecting the availability of soursop fruits in Nigeria (Ogwu *et al.,* 2016). Several fungal symptoms, such as dry rot, soft, etc., that are caused by pathogens like *Colletotrichum, Fusarium, Botrytis, Rhizopus* and *Penicillium* that affect the quality of the fruits in storage have been reported in Nigeria and different parts of the world (Palemón & Alberto *et al*., 2019; Ntsoane *et al*., 2019; Cambero-Ayón, 2019; Hernandez-Guerrero *et al*., 2020). Quality of information regarding the incidence and severity of soursop fungal diseases and the extent of the damage caused by these diseases cannot be over-emphasised, as diseases have led to great losses to growers.

Thus, just as fruits are seen as an important component of the human diet which contains vital sources of nutrients and food value, they also have serious challenges to their existence, especially post-harvest losses that occur after harvest. Observations from market surveys have shown that consumers overlook several disease symptoms on fruit displayed for sale without considering the effect of these micro-organisms on human health. Therefore, the objectives of this study are:

- Carry out a survey of post-harvest disease symptoms associated with *Annona muricata* fruits displayed for sale,
- Calculate the disease incidence and disease severity using Wheeler's (1969) method.

This study will reveal the distribution, prevalence and extent of the damage caused by the disease, thereby providing valuable information on their impact, which is of paramount importance to the design and development of effective management strategies.

2. Materials and Methods

2.1. Disease Survey and Sample Collection

The disease survey was conducted in some selected Local Government Areas in Rivers State, Nigeria (Figure 1). Surveys were carried out from different market locations in PHALGA and Obio-Apkor Local Government Areas, respectively. The symptoms of different diseases were observed and evaluated. The total number of diseased fruits, the disease incidence and disease severity were recorded. The mean value of the infected fruits was used to calculate the disease incidence and severity. The fruits of *Annona muricata* used in the study are presented in figure 2. The disease incidence and severity were calculated on a scale of 0-4, where 0% is no disease and 4 is 100%, using Wheeler's (1969) formula as shown below:

Disease incidence (DI) = n/N × 100%

$$DI = \frac{\text{Number of infected fruits (n)} \times 100\%}{\text{Total Number of fruits Observed (N)}}$$

Where; DI = Disease Incidence,

n = number of fruits showing symptoms, and

N = the total number of fruits observed

$$\text{Disease severity (DS)} = \frac{\text{Sum of all Disease rating} \times 100}{\text{Total Number of Fruits Observed} \times \text{maximum rating value}}$$

2.2. Data Analysis

The whole fruit was considered 100%, whereas visual or eye estimation was used for Disease severity (DS) calculation of the infected area of the fruit. Anthracnose, diplodia fruit rot, dry rot, powdery mildew and soft rot often associated with *Rhizopus* sp., *Aspergillus* sp., *Lasiodiopodia* sp. (Cambero-Ayon *et al*., 2019) were evaluated in the post-harvest condition observed in the markets locations. Data were analysed for descriptive statistics following Microsoft Excel package program and subjected to an Analysis of Variance (ANOVA) to determine and assess the statistically significant differences among the samples. The separated means were subjected to Least Significant Difference (LSD 0.05) for multiple comparisons.

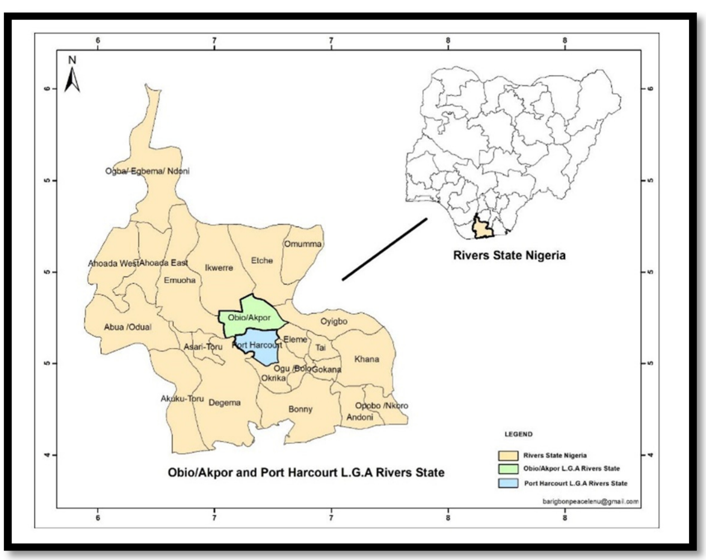

Figure 1: Map of Rivers State Showing the Local Government Area Samples

Figure 2: Soursop Fruits

3. Results

3.1. Major Fungi Disease Symptoms Observed on Annona muricta Fruits during Survey

A total of five fungal symptoms were identified on sampled *A. muricata* fruits in the study areas. The symptoms were dry rot, soft rot, powdery mildew, anthracnose and diplodia fruit rot (Figure 3).

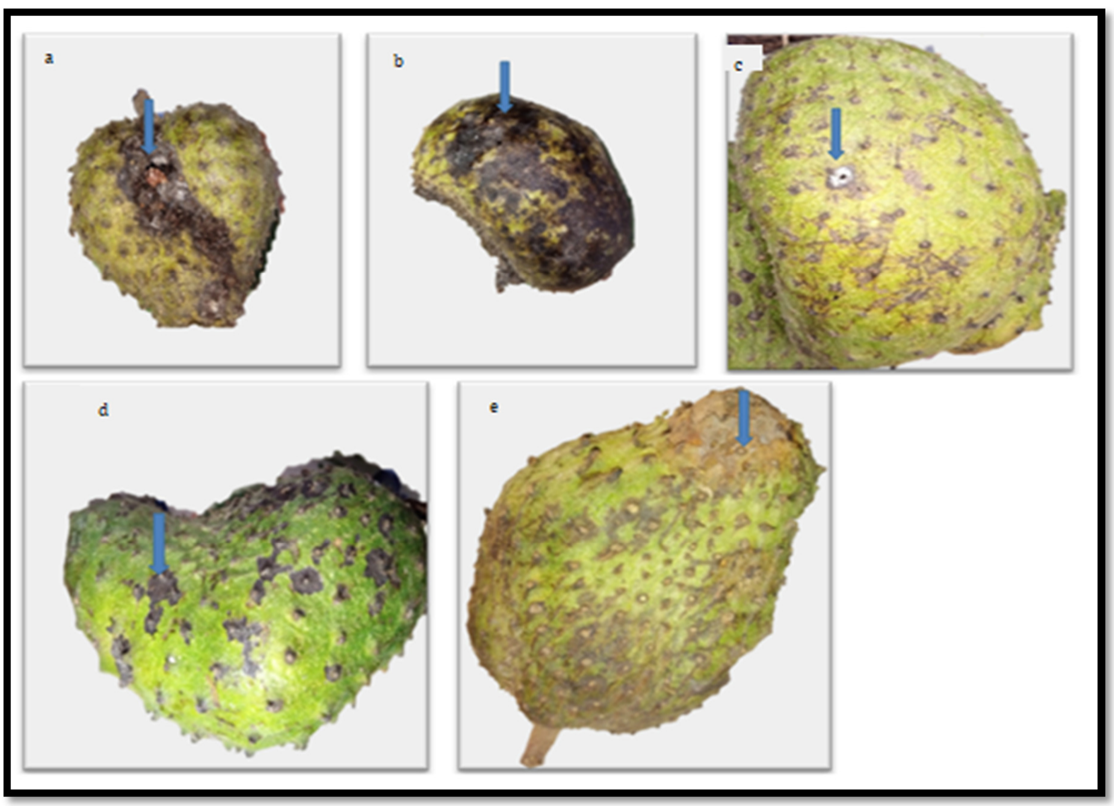

Figure 3: Symptoms Observed on Annona muricata Fruits
(a: Dry rot; b: Soft rot; c: Powdery mildew, d: Anthracnose, e: Diplodia fruit rot)

3.2. Disease Incidence and Severity of Annona muricata Fruits across the Market Locations in the Selected Local Government Areas in Rivers State

Across the different locations, the major disease incidence observed on *A. muricata* fruits were dry rot (20.28%), soft rot (16.02%), anthracnose (4.38%), powdery mildew (3.09%) and diplodia fruit rot (1.59%) as shown in table 1. A high disease Severity of Dry rot (12.84%) on the fruits was seen in Rumuo-Woji community, followed by (11.74%) in Ora-Abali community and 8.67% in Rumuo-Lumeni Community. Soft rot recorded a high severity of (11.88%), followed by powdery mildew (2.17%) in Rumuo-Woji and anthracnose (3.29%) in Rumuo-Masi community. Diplodia fruit rot dominated in Ogbunabali community, recording a disease severity of 1.59%, as shown in table 2. Disease incidence and severity revealed that the different locations are not significantly different at ($P > 0.05$) for all disease symptoms observed on *Annona muricata*, respectively. Data illustrating the statistically significant difference in incidence and severity for the different locations are presented in figures 4 & 5.

Communities	Dry Rot	Soft Rot	Powdery Mildrew	Anthrancose	Diplodia Fruit Rot
ORA-ABALI	17.35±4.74a	9.52±5.79a	0.95±0.95a	3.7±3.7a	0±0a
RUMUO-WOJI	20.28±7.29a	16.02±7.29a	2.9±2.9a	1.19±1.19a	0±0a
OGBUNABALI	13.09±3.99a	6.38±1.59a	0±0a	1.75±1.75a	1.59±1.59a
RUMUO-MEME	7.07±2.04a	3.03±3.03a	3.09±1.63a	3.7±3.7a	0±0a
RUMUO-LUMENI	16.62±1.19a	5.93±2.97a	1.59±1.59a	1.45±1.45a	0±0a
RUMUO-MASI	8.21±4.26a	7.56±1a	2.08±2.08a	4.38±2.2a	0±0a
LSD Value	9.6	9.3	3.9	5.6	1.4
F-Statistic	1.453	1.091	0.442	0.296	1
p-value	0.275	0.414	0.811	0.906	0.458
Decision	Not Significant	Not Significant	Not Significant	Not Significant	Not Significant

Table 1: Diseases Incidence of Annona Muricata at Different Locations
Row Mean ± Standard Error with Same Alphabet Is Not Significant According to Duncan's Multiple Range Test (DMRT) At 5% Level of Probability. LSD-Least Significant Difference

Communities	Dry Rot	Soft Rot	Powdery Mildrew	Anthrancose	Diplodia Fruit Rot
ORA-ABALI	11.74±3.29a	6.03±3.37ab	0.71±0.71a	2.78±2.78a	0±0a
RUMUO-WOJI	12.84±3.58a	11.88±4.72b	2.17±2.17a	0.89±0.89a	0±0a
OGBUNABALI	8.47±1.75a	5.18±1.59ab	0±0a	1.75±1.75a	1.59±1.59a
RUMUO-MEME	4.84±1.75a	1.52±1.52a	1.08±0.56a	2.31±2.31a	0±0a
RUMUO-LUMENI	8.67±0.79a	3.36±1.97ab	0.52±0.52a	0.72±0.72a	0±0a
RUMUO-MASI	5.76±2.92a	4.87±0.15ab	1.04±1.04a	3.29±1.65a	0±0a
LSD Value	5.6	5.8	2.3	4.0	1.4
F-Statistic	1.539	1.753	0.463	0.313	1
p-value	0.250	0.197	0.797	0.896	0.458
Decision	Not Significant	Not Significant	Not Significant	Not Significant	Not Significant

Table 2: Diseases Severity of Annona Muricata at Different Locations
Row Mean ± Standard Error with Same Alphabet Is Not Significant According to Duncan's Multiple Range Test (DMRT) at 5% Level of Probability. LSD- Least Significant Difference

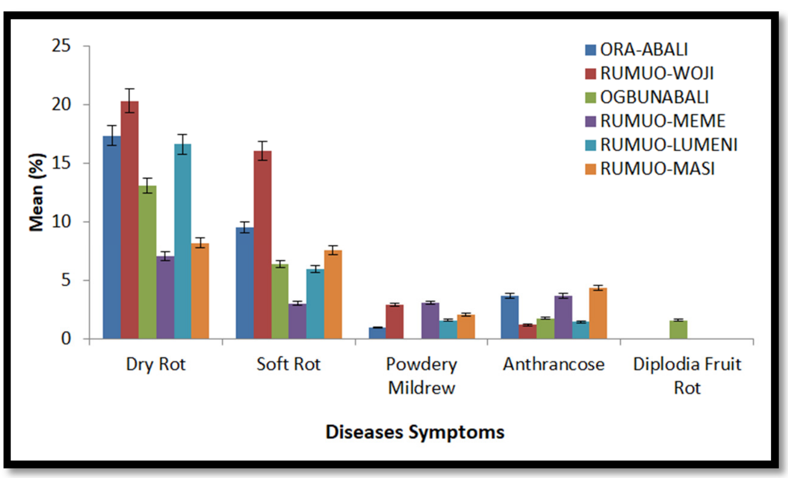

Figure 4: Diseases Incidence of Annona muricata Fruits at Different Locations

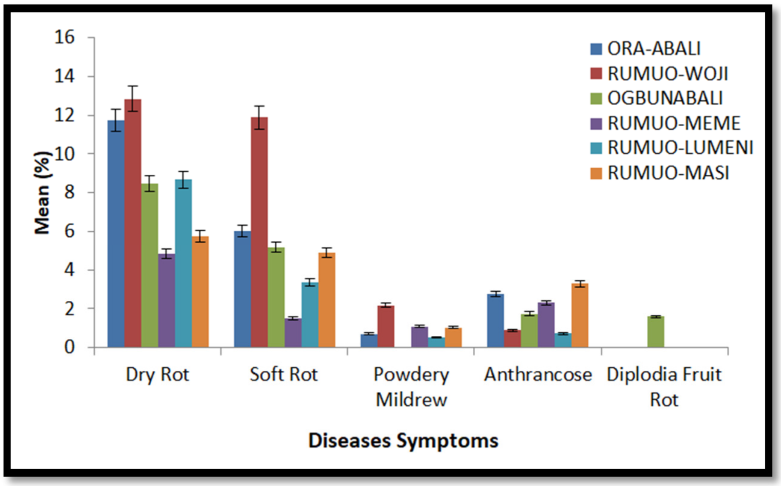

Figure 5: Diseases Severity of Annona muricata Fruits at Different Locations

4. Discussion

Fruits are recognised as high-value food because they contain nutrients such as amino acids, vitamins, dietary fibres, proteins (food energy), and minerals (Vwioko *et al*., 2013). Investigations in similar studies involving post-harvest spoilage and fruit deterioration due to microbial activities have been strongly documented, illustrating various severe economic losses significant to fruit rots (Youssef *et al.,* 2022; Ciofini *et al*., 2022). Analysis revealed disease symptoms of dry rot, soft rot, powdery mildew, anthracnose and diplodia fruit rot. Post-harvest symptoms of *Annona muricata* fruits in storage convey that rot occurrence is not significantly different at $P > 0.05$ despite the degrees of infection.

Anthracnose is seen as an essential primary disease affecting several fruits of soursop, leading to huge economic loss, loss of storage durability and availability (Kiprop *et al*., 2020; González-Ruíz *et al*., 2021; Ciofini *et al*., 2022). An investigation reported by Onyeani and Amusa (2015) showed that fruit decay and deterioration by Anthracnose is responsible for a serious threat to fruit availability and marketing in Nigeria. The symptoms of anthracnose observed in this study were characterised by occurrence in the form of sunken brown to black spots on the fruit surface. Its impact seems to be at its peak during post-harvest storage owing to serious fruit damage (Ciofini *et al*., 2022). Anthracnose has been reported to be associated with the disease of several fruits, amounting to 30-40% loss of marketable products (Maqbool *et al*., 2010; Darshan *et al*., 2019). Thus, anthracnose can present a severe post-harvest disease infection on fruits, especially in regions with favourable environmental conditions (Arauz, 2000; Ciofini *et al*., 2022).

Anthracnose, caused by *Colletotrichum* sp., was reported as a common disease of many medicinally important fruits, including banana, mango, avocado, passion fruit, citrus, coffee fruits, etc. (Sarkar, 2016; Serrato-Diaz, 2020). The anthracnose pathogen showcases a unique nature during infection due to its pathogenic variability (diversity), especially to ripe fruits after harvest, thus leading to a huge economic loss during storage and transport (Sarkar, 2016). Dembele *et al*. (2019) confirmed mango anthracnose as one of the most recorded important disease threats to production and marketing, indicating that infection was initiated in the field and remained latent until fruits were repining during storage. Kimaru *et al.* (2018) revealed that the anthracnose disease of avocado contributes to a huge loss of avocado fruits due to post-harvest rot limiting shelf life.

Powdery mildew, a commonly encountered fungal disease affecting fruits, strives mostly in a moist or warm environment (Subedi *et al*., 2019). Powdery mildew, seen as the most prevalent disease, is recognised easily on plants by the white to grey powdery mycelium and spore growth on the leaves or fruits. The growth of powdery mildew is unique in its spread due to the fact that it can strive under favourable dry weather conditions by utilizing the host nutrients (Mcfadden-Smith & Pickering, 2016). In a survey by Zhang *et al.* (2018), sixteen (16) pumpkin *(Cucurbita moschata*) samples that exhibited powdery mildew symptoms were evaluated and *Podosphaera* dominated as the key pathogen responsible for the pumpkin powdery mildew. Bellon-Gómez *et al.* (2011) discovered powdery mildew as a common fungal disease of cucurbits and the major cause of losses in cucurbit production worldwide. Furthermore, powdery mildew can be seen as one of the most aggressive diseases that affect leaves in cucurbits (McGrath, 2017).

The impacts of powdery mildew on crop production can range from reduced photosynthesis and impaired growth to a yield loss of the plant (Lebeda *et al.,* 2010). Similarly, *Podosphaera xanthii* was identified as the causal agent of the powdery mildew on the vegetable sponge (Elgamal & Khalil, 2020). Powdery mildewon *Manihot esculentus* Crantz and *Elaeis guineensis* Jacq investigated on a cultivated crop plant in Nigeria showed the predominance of *Erysiphe* species, indicating a rising trend in the disease which could result in low yield in plants (Bem *et al.,* 2013). It was reported that powdery mildew is among the most common diseases of vegetables and fruits favoured by warm and dry climates, which implicates a high relative humidity to spread (Glawe, 2008). Plants severely affected by powdery mildew reduce growth and production due to losses of the net photosynthetic area and the subtraction of nutrients (Sambucci *et al.,* 2019).

Dry rot is one of the post-harvest diseases that are capable of causing adverse effects and a drastic significant decrease in the nutritional components of a fruit (Esiegbuya *et al*., 2013). It exhibits symptoms of rough, firm brown to dark sunken areas on fruits during storage. Dry rot in storage fruit gives room for insect infestation due to the dry surface of the diseased area of the fruit, although the rot is hardly seen in the pulp. The widespread dry rot caused by *Fusarium* species is reported as a major threat to crop production globally (Tiwari *et al*., 2020). Dry rot damage aggravates when other symptoms like soft rot and late blight accompany dry rot during storage (Tiwari *et al*., 2020).

Globally, more than thirteen (13) *Fusarium* spp. are considered the most aggressive pathogen causing dry rot disease in potatoes (Cullen *et al*., 2005; Du *et al*., 2012). Similarly, Azil *et al.* (2021) identified thirteen (13) species of *Fusarium* associated with potato rots and wilt during storage. Dry rot also has a long history in citrus production, affecting it only when under stress by the soil fungus *Fusarium solani* (Adesemoye *et al.,* 2014). Esiegbuya *et al.* (2013) studied the post-harvest dry rot in palm fruits caused by *Xylaria feejeensis* and discovered that the disease caused dryness of the fruits, thereby making the fruit unsuitable for planting. Losses due to post-harvest dry rot caused by several genera of fungi, such as *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Collectotrichum* spp., *Fusarium,* etc., associated with storage decay of yam tubers are prevalent in Nigeria (Gwa & Nwankiti, 2017). It was estimated that between 20 and 39.5% of stored tubers might be lost to dry rot causal organisms (Okigbo & Ogbonnaya, 2006). Recently, there has been an estimated value of 40%. The frequency of occurrence and the destructive nature of the fungus depends upon the storage condition. Garlic crops colonized by *Fusarium* sp. remained a latent pathogen and developed rot during storage, especially during appropriate post-harvest environmental conditions (Gálvez & Palmero, 2022). Thus, *Fusarium* dry rot can remain dormant and then develop during post-harvest storage period, causing losses of up to 30% of the harvest yield of garlic (Tonti *et al.,* 2012; Gálvez & Palmero, 2022).

Diplodia fruit rot invades most fruits in tropical regions leading to significant losses (Zhang, 2014). Infection occurs via the development of soft brown to black decay symptoms at both ends of the fruit resulting in the highest post-harvest damage in soursop (González-Ruíz *et al*., 2021). Exposure through injuries during post-harvest handling leads to the rapid spread of decay observed in fruit during storage or on market arrival (Ismail & Zhang, 2004). Diplodia fruit rot caused by *Diplodia sapinea* was reported as one of the most severe opportunistic pathogens that accumulate in a healthy tree causing the disease diplodia tip blight (Blumenstein *et al*., 2021). *Diplodia* can live as an endophyte in its host plant before it breaks out and transforms into a sudden threat to the plant (Terhonen *et al.,* 2021). In combination with increased temperature and mechanical wound, *Diplodia sapinea* can rapidly become pathogenic, leading to sudden disease outbreaks (Langer *et al.,* 2011; Blumenstein *et al.,* 2021). Furthermore, it was revealed that with the help of suitable environmental conditions, the endophytic and latent pathogen can have a significant effect on the development of rot disease in a plant (Piškur & Jurc, 2011; Marsberg *et al*., 2017).

Post-harvest fungi soft rot on *Annona muricata* fruit produces soft brown spots and, subsequently, a characteristic black, rotten, and sometimes shrunken fruit. Gottsberger (1988) confirmed that several post-harvest fungal soft rot pathogens are associated with *Annona muricata* owing to their presence in the air as a contaminant (Nweke & Ibiam, 2012). Hossain *et al*. (2013) affirmed that soft rot symptoms developed quickly on fruits in storage, especially those in very warm storage conditions. Disease surveys have shown that soft rot is an economically important disease affecting production and availability, which in turn causes great losses to whole sellers and retailers (Kumari, 2020). Djellout *et al*. (2020) explained further that the fruit's richness in nutrients makes it a target for soft rot. Improper storage system leads to the loss of up to 20% of harvested fruits to soft rot (Lee *et al*., 2013; Abdullah *et al*., 2016).

Decayed fruit with symptoms of soft rot is usually found in large numbers during post-harvest transportation and storage. In a post-harvest survey of citrus diseases, the fungal strain *Lasiodiplodia pseudotheobromaethe* was found to be associated with citrus rot (Chen *et al*., 2021). Interestingly, the strains could only infect a wounded citrus fruit causing decay within two days of post-inoculation (Chen *et al*., 2021). *Lasiodiplodia pseudotheobromaethe* is reported as a common pathogen of tropical and subtropical plants with a wide host range (Kwon *et al.,* 2017; Pipattanapuckdee *et al*., 2019). The organism was reported to be associated with citrus stem-end rot disease in Bangladesh and the post-harvest fruit rot of lemon in Turkey (Awan *et al*., 2016; Sultana *et al*., 2018). Sweet potato tuber with soft rot symptoms subjected to screening ascertain a high degree of pathogenicity susceptibility to soft rot disease caused mainly by fungi such as *Botryodiplodia theobromae, Cerato cystis, Rhizopus oryzae, Aspergillus flavus, Fusarium solani* and *Sclerotium rolfsii,* (Nsofor, 2020). Rotten fruits nourish various types of fungi and some of them produce mycotoxin, which may cause harm to the consumers (Bashar *et al*., 2012).

Lasiodiplodia pseudotheobromae was confirmed to be responsible for soft rots in soursop fruits in Mexico (Cambero-Ayón *et al.*, 2019). This corresponds with the results of Nweke and Ibiam (2012), who mentioned that rot in soursop fruit in Nigeria is due to *Colletotricum gloeosporioides* and *Rhizopus stolonifer*. Sandoval *et al.* (2013) associated *Lasiodiplodia pseudotheobromae* with the death of descendant branches and rot in mango peduncle (*Mangifera indica*) in the Pacific coast of Mexico. Awan and Akgül (2016) further revealed that *L. pseudotheobromae* is a highly virulent post-harvesting pathogen in lemon by damaging around 40 to 50 % of the area of the fruit. Cruz-Lachica *et al.* (2017) reported that the susceptibility of papaya (*Carica papaya* L.) to soft rot fungi causes post-harvest losses of up to 50%. Similarly, symptoms of soft rot caused by *Rhizopus oryzae* devastate a wide range of crops, including banana (*Musa sapientum*) fruits placed on local market shelves (Kwon *et al*., 2012).

The disease on fruits combined with progressive, quick severe rotting causes a loss in their nutritional value, thus, making them unfit for use. More so, the presence of the pathogens not only results in spoilage, but they are also detrimental to human health in the case of food poisoning. Interestingly, some fruits show no change in appearance even after contamination. Nevertheless, an in-depth investigation into the characterization of these micro-organisms can be realized using conventional microbiological methods or molecular methods.

5. Conclusion

Fruits are vulnerable to microbial attack mainly during post-harvest handling due to their food composition, thus an ideal medium of survival for various forms of fungi. The results from this study confirmed the activities of fungal micro-organisms in the deterioration of *Annona muricata* fruits. To a significant extent, fungal post-harvest diseases are the major contributing factor to loss after harvest and also in storage. The post-harvest survey of fungi diseases in *Annona muricata* fruits, in addition to its incidence and severity, is of paramount value and an essential means in strategic disease control measures and effective management practices. Furthermore, precautions relating to the fruit handling process should be in-cooperated as a necessary measure in reducing microbial disease expression and composition, which cause the deterioration of fruits and are detrimental to human health.

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