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IDENTIFICATION OF FUNGAL SPECIES ASSOCIATED WITH THE SPOILAGE OF FRUIT IN DUTSIN-MA METROPOLIS, KATSINA STATE

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ABSTRACT

The aim of this study was to isolate and identify the fungi species associated with spoilage of orange (*Citrus sinensis*); Banana (*Musa acuminate*); and Mango (*Mangifera indica*) fruit. A total of 30 fruit samples were collected from market in Dutsin-ma metropolis, the study was carried out between June to August, 2023. The samples were surfaced sterilized with ethanol and the homogenates were cultured on Potato Dextrose Agar and incubated aerobically at room temperature for 5days at 30°C. The pure cultures obtained were identified morphologically and microscopically using a standard mircrobial Atlas for verification. The fungal species identified from the isolates are *Aspergillusspp*, *Candidaspp*, *Alternariaspp*, *Fusariumspp*, *Mucorspp*, *Rhizopusspp*, *Collectoricumspp*, and *Peniciliumspp*. *Aspergillusspp* was the most predominant fungal specie isolated from orange *(citrus sinensis)* having a frequency of 60% followed by *Candidaspp* and *Alternaira* with 20% prevalence. *Fusariumspp* was the most predominant fungal specie isolated from banana *(Musaacuminata)*, having a frequency of (44%), followed by *Mucorspp* (33%) and *Rhizopusspp* (22%). *Aspergillusspp* was the most predominant fungal specie isolated from mango *(Mangifera indica)* having a frequency of (70%) followed by *Collectoricumspp*(20%) and *Peniciliumspp*(10%). *Aspergillusniger* was the most predominant isolates from all the fruits under study. These findings are indicative that fungal species are associated with the spoilage of orange, *(Citrus sinensis)*, banana *(Musa acuminata)*, and mango *(Mangifera indica)* fruit leading to economic loss and possible human health hazard as a result of consumption of spoilt fruits.

Keywords: Fungi, Fruit, *Musa acuminate, Citrus sinensis, Mangifera indica*

INTRODUCTION

Fruits are the consumable part of mature ovary of flowering plants which are normally eaten raw and they include many structures that are not commonly called fruits such as bean pods, corn kernels, tomatoes, and wheat grains (Ikhiwili, 2012). The importance of fruit in human nutrition cannot be overestimated as it provides vitamins and minerals necessary for proper body metabolism (Al-Hindi *et al.,* 2011). Humans and many animals have become dependent on fruits as a source of food (Lewis, 2011). Fruits and vegetables generally give the body every necessary vitamin, fats, minerals and oil in the right proportion to maintain growth and development in humans (Mojtaba*et al*., 2019). However, thereare serious challenges that threaten the production of fruits such as changes in climatic conditions, pests, inadequate rainfall and fungal attack (Gullino *et al*., 2022; Amusa *et al.,* 2002). Over the years, there has been an increase in the reported spoilage of fruit resulting in change in the condition of fruit in which the fruit becomes less palatable, or even toxic, these changes may be accompanied by alteration in taste, smell, appearance or texture (Singh and Sharma, 2007). The high concentration of various sugars, minerals, vitamins, amino acids, and low pH enhance the successful growth and survival of various parasitic and saprophytic forms of fungi on the fruits (Droby, 2006). However, fruits are easily spoilt and usually have active metabolism during the storage stage (Oluwadara *et al*., 2022; Singh and Subali,2007).

Annual reports have shown that 20% of fruits and vegetables produced are lost to spoilage especially during post-harvest stages and this has been associated pathogenic fungi (Singh and Subali,2007).Toxin-producing fungi have been identified and isolated from spoilt fruits and have been reported in cases of infections or allergies (Thiyam and Sharma,2013). *Aspergillus* spp. produces mycotoxins and other toxic metabolites which can be harmful to humans and animals globally (Arauz, 2000).Micro-organisms, especially fungi, are known to destroy fruits, thereby reducing the quantity for consumption and the profits obtained from sales of fruits. There is need to identify these micro-organisms especially those that are pathogenic to humans so as to reduce the risk of contamination and infection arising from handling and consumption of fruits (Barth *et al*., 2009). The need for mycological examination of fruits is very significant as it an important contributor to the economic development of the region. Therefore, this study was undertaken to isolate and identify fungi associated with spoilt fruits commonly sold in Dutsin-ma market, Katsina state.

MATERIALS AND METHODS

Study Area

This study was carried out between June to August, 2023. The study area is Dutsin-ma which is among the local government of Katsina State. The occupation of the city inhabitants includes farming, trading, fishing and leather works. The Dutsin-ma town is a dry Sudan savannah surrounded by Sandy terrain and isolated hills. Rainfall starts by June and ends early September but sometime extend to October. The average annual rainfall is 550m with peak in the month of August.

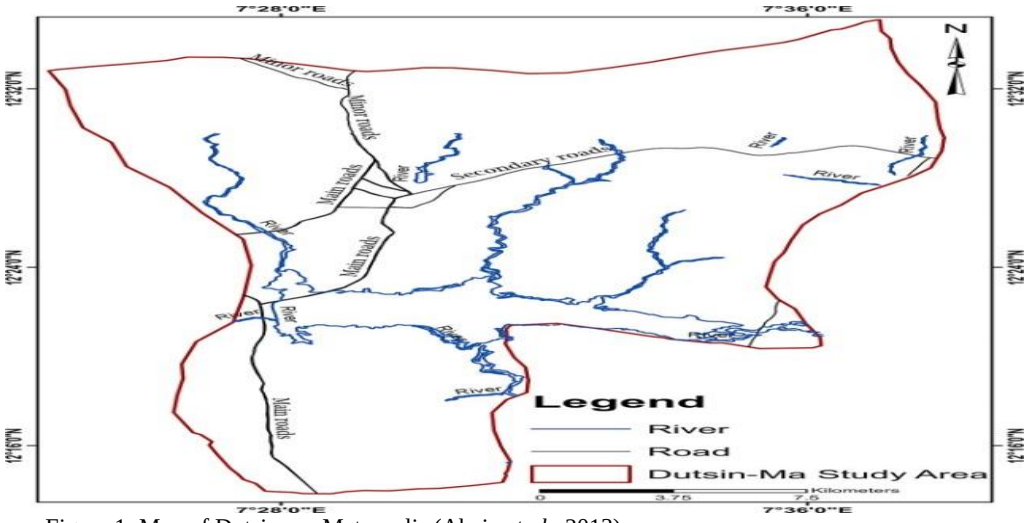

Figure 1: Map of Dutsin-ma Metropolis (Abaje *et al.*, 2013)

Sample Collections

Fruits samples were collected from Wednesday market within the metropolis of Dutsin-ma Local Government Area. Fruits collected include Orange *(Citrus sinensis),* Banana *(Musa acuminata),* and Mango *(Mangifera indica),* five (5) fresh fruits each and five spoiled fruit from the same locations were collected, making a total of 30 fruit samples collected. The fresh healthy samples were kept in a polythene bag for some days to observe any changes due to infection carrying out microbial assay while the spoilt fruits were immediately assayed. The spoilage parameters were carefully noted; completely rotten fruits were avoided for isolation as they contained mostly secondary pathogens. The collected fruits were taken to Biological Science Laboratory at federal university Dutsin-ma for analysis.

Media Preparation.

Potato Dextrose Agar used were weighed, prepared and sterilized according to the manufacturer's Instruction.

Sample Preparation and Inoculation.

Isolation of the fungi was carried out as described by (Baiyewu*et al.*,2007) 8 Segment (3–5cm) of tissues from the spoilt fruits was cut with sterile scalpel and placed on potato dextrose agar containing streptomycin (to prevent growth of bacteria) in petri dishes and incubated at room temperature for 5-7 days. Pure cultures were obtained from the isolation.

Incubation of the Inoculated Plates.

The inoculated plates were incubated at room temperature for 5-7 days under light to enhance fungal growth and sporulation (Sani *et al.,* 2023).

Purification of the Fungal Isolates.

After the appearance of a mixed growth, each spore was sub-cultured in a fresh Potato Dextrose Agar in order to obtain a pure culture using streak method as describe by (Sani *et al.,* 2023).

Macro Identification of the Isolates.

The fungal isolates were subjected to certain comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas contained in the Department of Biological Sciences, federal university Dutsin-ma. This was followed by a slide mount of each isolate. The characteristics observed were matched with those available in the aforementioned mycological atlas. They were then identified accordingly (Sani *et al.,* 2023).

Micro Identification of the isolates.

A drop of lactophenol cotton blue stain was placed on a clean slide and with the aid of a mounted needle, a small portion of the mycelium from the fungal cultures was removed and placed in the drop of the stain. The mycelium was spread very well on the slide with the aid of the two mounted needles and a cover slip was gently lowered on it. The observation was done at high power objective (×40) of the microscope. Morphological characteristics of the fungi such as type of hyphae and asexual reproductive structure were observed (Sani *et al.,* 2023).

Determination of Percentage Occurrence of the isolates.

This was done to determine the percentage occurrence of the different fungal isolates. The number of occurrence for each of the isolates were recorded and calculated as a ratio of the total number of occurrence and was then expressed using standard percentage formulae.

$$\text{percentage frequency} = \text{number of isolaedfungi} / \text{total number of isolates} * 100$$ (Sani *et al.,* 2011).

RESULTS AND DISCUSSION

The findings on the distribution of infected fruit samples among the samples collected (*Citrus sinensis; Musa acuminate* and *Mangifera indica*) as presented in table 1 revealed that out of the thirty fruits samples, 76% fungi infection was observed. *Citrus sinensis* and *Mangifera indica* had the highest infected percentage (100%), while *Musa acuminate* (40%) had the lowest infection. The findings on frequency of occurrence of various fungal isolates in spoilt oranges (*Citrus sinensis*); banana (*Musa acuminate*) and mango (*Mangifera indica*) as presented in table 2 revealed *Aspergillusniger, Candida tropicalis and Alternarianees* from *C. sinensis* spoilt samples. The number of isolate of *Aspergillus niger, Aspergillusflavus* were six (6), *Candida tropicalis* was two (2) and *Alternairanees* was two (2), the highest occurrence frequency was *Aspergillus niger* (60%), *Candida tropicalis* and *Alternairanees* had 20% frequency respectively. The fungi isolates from spoilt samples of *M. acuminate* revealed *Fusarium incarnatum, Mucor fragilis, Rhizopus stolonifer*. The number of isolates of *Fusarium incarnatum* was four (4), *Mucor fragilis* was three (3) and *Rhizopusstolonifer* was two (2), the highest occurrence frequency was *Fusarium incarnatum* (44%), *Mucor fragilis*

was 33% and *Rhizopus stolonifer* (22%) as presented in table 2. The spoilt samples of *Mangifera indica* observed revealed *Aspergillus niger, Colletoricum gloeosporioides, Penicillium chrysogenum*. The number of isolates of *Aspergillus niger* was seven (7), *Colletoricum gloeosporioides*, was two (2) and *Penicillium chrysogenum* was one (1). The highest frequency was *Aspergillus niger* (70%), *Colletoricum gloeosporiodes* was 20% and *Penicillium chrysogenum* was the least with 10%. The finding on the culture characteristic of fungal isolates from the fruit samples **(***Citrus sinensis,Musa acuminate* and*Mangifera indica*) as presented in table 3 revealed several fungi species identified by macroscopic and microscopic view with varying characteristic appearances and features differing from one specie to another.

Table 1: Distribution of infected fruit samples among the samples collected (*Citrus sinensis; Musa acuminate* and *Mangifera indica*) expressed in percentages

Sampling	Total collected samples	Total infected samples	Infected samples (%)
A1	5	3	60
A2	5	5	100
B1	5	2	40
B2	5	4	80
C1	5	4	80
C2	5	5	100
Total	**30**	**23**	**76**

Keys: A1 (Orange Fresh fruit), A2 (Orange Spoilt fruit), B1 (Banana Fresh fruit), B2 (Banana Spoilt fruit), C1 (Mango Fresh fruit), C2 (Mango Spoilt fruit)

Table 2: Frequency of occurrence of various fungal isolates in spoilt oranges (*Citrus sinensi*); banana (*Musa acuminate*) and mango (*Mangifera indica*)

Organisms identified	Number of isolate	Frequency (%)
Oranges (*Citrus sinensis)*		
Aspergillus niger	6	60
Candida tropicalis	2	20
Alternaria nees	2	20
Banana (*Musa acuminate*)		
Fusarium incarnatum	4	44
Mucor fragilis	3	33
Rhizopus stolonifer	2	23
Mango (*Mangifera indica*)		
Aspergillus niger	7	70
Colletoricum gloeosporioides	2	20
Penicillium chrysogenum	1	10
Total	**29**	**300**

Table 3: Culture characteristic of fungal isolates from the fruit samples (*Citrus sinensis,Musa acuminate* and*Mangifera indica*)

Fungi identified	Macroscopic Examination	Microscopic Examination
Aspergillus flavus	White colony with light yellow-green later becoming dark- yellow-green	Radiate conidial later split to loose clumps. Rough conidia bone on vesicle
Candida tropicalis	Greenish white colonies, opaque, smooth and convex	Budding, spherical to elongate cells, forming pseudo mycelia
Rhizopusstolonifer	Colon compact and became darkening cotton after few days	Sporangia contains spores, have rhizoids
Aspergillusniger	Pick like black growth	Non-branched conidiophore with bulb and carries conidia like sun rays
Mucor fragilis	Greenish cotton like, colony like growth spotted with blackish color	Sporangia contain spore without have rhizoids.
Alternarianees	Colonies are fast growing, black to olivaceous-black or grayish, and are suede-like to floccose	Branched acropetal chains (blastocatenate) of multicellular conidia (Dictyoconidia) are produced
Penicilliumchrysogenumwere	Colonies are fast growing, in shades of green, sometimes white, mostly consisting ofdense like conidiophores	Chains of single-celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialide
*Fusarium*spp *inacarnatum*	Colonies are usually fast growing, pale or bright colored (depending on the species) with or without a cottony aerial mycelium	Microconidia one celled hyaline, smaller than macroconidia, pyriform, fusiform to ovoid, curved chlamydospores.
Rhizopusstolonifer	White cottony at firstbrownish grey then blackish-grey.	Sporangia are globose with a flattened base, grayish black powdery in appearance upto 175µm in diameter and many spores

DISCUSSION

The findings on the distribution of infected fruit samples among the samples collected (*Citrus sinensis; Musa acuminate* and *Mangifera indica*) as presented in table 1 revealed 76% fungi infection. *C. sinensis* and *M. indica* had the highest infected percentage (100%), while *M. acuminate* (40%) had the lowest infection. This findings is similar with the report of Samuel *et al.* (2017) who reported that over 20% fruits produced are lost to spoilage due to fungi that are economically significant. The findings on frequency of occurrence of various fungal isolates in spoilt oranges (*Citrus sinensis*); banana (*Musa acuminate*) and mango (*Mangifera indica*) as presented in table 2 revealed *Aspergillus niger* (60%), *Candida tropicalis* (20%) *and Alternarianees* (20%) from *C. sinensis* spoilt samples. The number of isolate of *Aspergillus niger, Aspergillus flavus* were six (6), *Candida tropicalis* was two (2) and *Alternairanees* was two (2). The fungi isolates from spoilt samples of *M. acuminate* revealed *Fusarium incarnatum* (44%), *Mucor fragilis* (33%), *Rhizopus stolonifer* (22%). The number of isolates of *Fusarium incarnatum* was four (4); *Mucor fragilis* was three (3) and *Rhizopusstolonifer* was two (2) as presented in table 2. The spoilt samples of *Mangifera indica* observed revealed *Aspergillus niger* (70%); *Colletoricum gloeosporioides* (20%) and *Penicillium chrysogenum* (10%). The number of isolates of *Aspergillus niger* was seven (7), *Colletoricum gloeosporioides,* was two (2) and *Penicillium chrysogenum* was one (1). These findings fungal isolates frequency is in tandem with the report of Samuel *et al.* (2017) who reported *Aspergillus niger* to have the highest occurrence in spoilt orange, pineapple and pawpaw fruits and *Aspergillus flavus* and *Rhizopus stolonifer* with varying occurrence isolated from spoilt fruits. The finding on the culture characteristic of fungal isolates from the fruit samples **(***Citrus sinensis, Musa acuminate* and *Mangifera indica*) as presented in table 3 revealed several fungi species identified by macroscopic and microscopic view with varying characteristic appearances and features differing from one specie to another. This is in tandem with Oluwadara *et al*. (2022) who reported that *Citrus sinensis, Mannifera indica,* and *Musa acuminata*are consumed all over the world as an excellent source of vitamins and natural antioxidant that builds the body's immune system are however infested by various fungi that alters both the physiology and morphology of these fruits. The biologically active compounds in these fruits have been reported to prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health (Gullino *et al*., 2022). However, the impact of diverse diseases in this case fungi limits their production, nutritional value and market qualities. *Aspergillus niger, Alternarianees and Candida tropicalis* were the predominant fungal isolates identified from the deteriorated orange fruit with unique characteristics symptoms (dark yellow-green, grayish, greenish white), this is in correlation with the work of Chukwuka, *et al.* (2010) who reported that *Aspergillus niger*is the predominant organism associated with the spoilage of orange. The predominant fungal isolates from the spoilt mango samples was *Aspergillus niger, Aspergillus flavus, Colletoricum gloeosporioides,* and *Penicillium chrysogenum* with characteristics symptoms of black, dark yellow-green, pale color, green or white. This is in correlation with the report of Akinmusire (2011) and Baiyewu *et al.* (2007) who stated that all the organisms isolated from spoilt fruit samples were successfully taking part in the decay process and are thus confirmed as the causal organism of fruit decay. The fungi isolates from banana were *Fusarium incarnatum* (40%),*Mucor fragilis* (30%)and *Rhizopus stolonifer* (20%).This agrees with the work of Bello (2010) who reported that these fungi are responsible for fruit spoilage with certain unique characteristics symptoms such pale color, greenish, brownish grey similar to the microscopic observation of the spoilt fruits samples from this study. Similarly Chukwuka *et al.* (2010) reported that the organisms isolatedwere confirmed to cause spoilage of orange *(Citrus sinensis),* Banana *(Musa acuminate),* and Mango *(Magnifera indica)* fruit but in varying degrees. All the isolated fungi were found to be pathogenic to orange *(Citrus sinensis),* banana *(Musa acuminate),* and mango *(Mangifera indica)* fruit. *Aspergillus niger,Candida tropicalis, Fusarium incarnatum, Mucor fragilis, Rhizopus stolonifer, Colletoricum gloeosporioides, Penicillium chrysogenum,* and *Alternaira nees* was found to be responsible for the deterioration of these fruits and corroborated by Al-hindi *et al.* (2011) who reported that fungi that causes spoilage are considered toxigenic or pathogenic. The fungi isolates from the fruitsamples in this study have been reported to produce secondary metabolites in plant tissues that have potential toxic effect. This is in agreement with Chukwuka *et al.* (2010) who reported that these secondary metabolites produced by the fungi are potentially harmful to humans and animals. Tournas and Stack, (2001) similarly reported that some fungi produce mycotoxins such aflatoxins with significant impact on the fruit supply chain and as well as posing a health risk in the developing world. Akintoba, (2011) also reported that these fungi (*Aspergillus niger,Candida tropicalis, Fusarium incarnatum, Mucorfragilis, Rhizopus stolonifer, Colletoricum gloeosporioides*) on the other hand could also cause infection or allergies. Proper fruit handling and management practice by sellers as well as consumers are necessary to curb fruit spoilage associated with fungi and its consequent health impact.

CONCLUSION

In this study, *Aspergillus niger, Candida tropicalis* and *Alternaria nees* was identified in spoilt orange, *Fusarium incarnatum, Mucor fragilis,* and *Rhizopus stolonifer* was found in spoilt banana while *Aspergillus niger, Colletoricum gloeosporioides,* and *Penicillium chrysogenum* was found in spoilt mango. Some of these fungi species have been reported to contain potential mycotoxins which can lead to health complication for both human and animals. Therefore, the use of biological control agents, proper packaging, storage facilities and fruit handling by fruit sellers to ameliorate postharvest fruit spoilage is necessary to ensure the availability of safe and healthy fruits and reduce health risk due to consumption of fungi infested fruits.

RECOMMENDATION

Mature orange, mango and banana fruits are better eaten fresh to avoid long term exposure that lead to spoilage. Immediate and proper discarding of fruits with symptomatic features such as alteration in color or taste of the fruit is noticed as this can be hazardous to human health. The farmers who harvest the fruits into bags for transportation, the marketers and consumers should take necessary precautions in preventing contamination and also try to create an environment that would discourage the growth or multiplication of microorganisms. Since these fruits are contaminated easily by the activity of fungal pathogens, there is need to educate the growers, consumers and sellers to store these produce properly in clean, cool and dry environment with less humidity.

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