ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 6 No. 3 September 2020*

Moulds Associated with Deterioration of Mango (*Mangifera indica* L.) and Proximate Analysis of Infected Fruits in Keffi, Nasarawa State, Nigeria

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Abstract
A study was carried out on moulds associated with deterioration of mango fruits and proximate analysis of infected fruits in Keffi, Nasarawa State. The disease survey covered four locations in Keffi. The locations include; Keffi Market, FMC Round about, Angwan Lambu and High Court. The four locations were visited four times and a total of 64 mango fruits were collected and sampled. Forty two (42) fruits were infected with different fungal diseases while twenty two (22) were free from fungal infection. The fungal species isolated and identified were *Aspergillus niger*, *Aspergillus oryzae*, *Mucor hiemalis* and *Rhizopus stolonifer*. Their frequencies of occurrence were 28.57%, 23.81%, 23.81% and 23.81% respectively. FMC Round About and High Court has the highest frequency of occurrence (18.75%) and Angwan Lambu has the least occurrence (12.50%). There was no significant difference ($P<0.05$) in the incidence of different isolates in relation to locations. Analysis of the nutritional contents of infected mango fruits showed increase in the moisture content and reduction in crude fibre, crude ash, crude protein and crude carbohydrates level compared with uninfected fruits. The result for pathogenicity test showed that *Aspergillus niger* was the most virulent, while *Mucor hiemalis* was the least virulent or may be mere contaminate. Consumption of deteriorated mango fruits should be avoided because of the health implication of swallowing these fungal isolates.

Keywords: Mould, Deterioration, Mango, Proximate analysis, Keffi.

INTRODUCTION
The mango (*Mangifera indica* L.) is a juicy stone fruit (drupe) from numerous species of tropical trees belonging to the flowering plant genus *Mangifera*, cultivated mostly for their edible fruit. The majority of these species are found in nature as wild mangoes. The genus belongs to the cashew family *Anacardiaceae* (Yong, 2016). Mangoes are native of South Asia, from where the "common mango" or "Indian mango", *Mangifera indica*, has been distributed

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worldwide to become one of the most widely cultivated fruits in the tropics. Other *Mangifera* species (e.g horse mongo, *Mangifera* foetida) are also grown on a more localized basis.
Mango is a large oval tropical fruit having smooth skin, juicy aromatic pulp, and a large hairly seed. It is a yellow-red, oblong tropical fruit with thick rind, somewhat acid, juicy pulp and a hard stone. It is eaten when ripe, or preserved or pickled when unripe.

India is the single largest producer of choicest varieties of mangos in the world (Schumann, 1991). Despite the fact that the country produces quality fruits, approximately 25-30% of total produce goes as waste due to improper handling and storage practices. All plant weather diseased or healthy is a host to a variety of fungi that can be categorized broadly in several biological groups (Alexopoulos *et al.,* 1996). Most fungi that affect mango plants are obligate parasite or biotrophs. These fungi spread from one plant to another and from one location to another in several ways. The pathogens producing spores, either through asexual or sexual reproduction that aid in the dissemination of the fungus. The spore may be moved by wind, human activities, insect or water, in which case, the disease generally spread over relatively short distance (Bandyopadhyay *et al*., 1998). Fungi have serious impact on the economical, social and environmental realms. They have also led to mango losses, increased cost of breeding program and scarcity of mango caused by epidermis of mango rot (Schumann, 1991). Therefore, this study is aimed at evaluating moulds associated with deterioration of mango and proximate analysis of infected fruits in Keffi, Nasarawa State.

MATERIALS AND METHODS

Study Area

The study was carried out in Plant Science and Biotechnology Department Laboratory, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi.

Survey and sample collection

A total of 64matured mango samples were bought in two visits (between March and April, 2018) from four different locations (16 each), in Keffi Local Government Area of Nasarawa State. Keffi is situated on Longitude 8.8558^{o}N and Latitude7.8694^{o}. All the fruits obtained were stored in a cold temperature at -4^{0}C until when required for the investigation. These locations include:

- Keffi market
- FMC round about
- Angwan Lambu
- High court

These selected locations form part of the guinea savannah region of Nigeria tropical climate.

Preparation of potato dextrose agar (PDA)

Potato dextrose agar is a nutrient medium for growing fungi in test tubes and petri dishes. The laboratory preparation of the nutrient medium (PDA) was done by weighing 39g of the PDA in a weighing balance (Triple beam balance). This was dissolved in 300ml of distill water in a conical flask. Both the PDA and the distill water were mixed thoroughly using a stirrer. It was then covered and the medium was autoclave for 15 minutes, at 15 pound pressure. After 15 minutes, the autoclave power source was switched off and allowed to cool for 40 minutes, while cooling took place, the autoclave was unscrewed and the medium removed and allowed to cool at a minimum temperature of about 37^{0}C. (Ogaraku *et al*., 2017)

After the medium has cooled off and is ready for plating, 3ml of streptomycin sulphate was added to the medium and a glowing spirit lamp was used to sterilize the mouth of the conical flask before plating. Then 15ml of the solution (medium) was poured into each of the petri dish (Hamed *et al*., 2010) when the media completely solidified and cooled at room temperature, air bubbles present were removed using flame balls passing across the media.

Sample Preparation

The mango fruits were taken to the laboratory for disinfection. The mango fruits were washed with tap water to remove dirt. After which, they were placed on 70% ethanol for 5 minutes, it was also placed in 10% sodium hypochlorite for 20 minutes and rinsed with distilled water thrice in other to remove all microorganism. These procedures were carried out for all the mango fruits collected (Hamed *et al*., 2010). Then the disinfected mango fruits were kept in a sterilized bottle and covered for fungi growth and development for seven days. Finally, after four days, there was fungi structure and development on the mango fruit in the form of mycelium and spore indicating that they can be cultured. They were cut into small fragments since the mango fruit is large and cannot properly enter the plates.

Culturing of fungal isolates in the media (PDA)

To ensure a successful culturing of the fungi isolates, certain aseptic conditions were observed as follow;

- The scarpel used to pick small pieces of the infected portion of the rotten mango was dipped in 70% ethanol
- Spirit lamp flame was also used to flame these tools for further sterility, before inoculation into the medium, the medium was placed close to the spirit lamp flame before covering. The procedure was repeated for all until culturing was completed. Lastly, the medium was sealed using masking cello tape to prevent entry of other microorganisms. The method used in the isolation of these fungi is the direct surface agar plating method (Spack, 1976). Finally, the cultured media were kept in an incubator at a temperature of about 37^0C for five days, for further investigation.

Identification of fungal isolates

The identification of fungi was done by studying the cultural characteristics of each isolate (Domsh *et al*., 1980). Seven days after which the fungi structures have been well developed in each plate the fungi structures were collected, stained with lacto phenol on a slide, covered with cover slip and the mounted on a microscope for examination. An identification key was used to identify correctly the established fungus on the fruits in each of the media (Willensdorfer, 2009). The morphological features used for the identification of fungi species are as follows:

Shape of the hyphae
Type of conidia
Color characteristics of spores

Test for pathogenecity of fungal isolates

To establish that the fungal isolate causes the disease condition (i.e mango fruits deterioration) (Bengrson *et al*., 2017) methods was adopted. Five fresh mango fruits were washed with 10% sodium hypochlorite and rinsed in distilled water and allowed to dry. A hole 8-10mm in diameter was made on healthy mango fruits with a cork borer and each isolate inoculated into each hole. The samples incubated for the characteristics symptom to develop. Symptom of the disease were seen to develop on the fruits (mango) after five days of inoculation. The isolates were re-isolated and re-incubated (Dyer and O'Gorman, 2012).

Proximate composition of mango fruits

This analysis was carried out on infected and non-infected mango fruits in order to compare the major constituents of the two mango samples (Timmer, 2019). This method partitioned nutrients in the fruits into six (6) components: water, ash, crude, protein, crude fat, crude fibre and crude carbohydrate. Six samples each were used.

Moisture Determination

Moisture content was determined when samples of fresh mango fruits were weighed into a silica dish. The samples were then dried in an oven for 65^0C for 36 hours, cooled into a silica dish. The drying and weighing continues until a constant weight was obtained.

Moisture content = $\frac{\text{Wt. of sample + dish before drying – weight of sample after drying x 100}}{\text{Wt. of sample taken}}$

Crude protein

The crude protein was determined by measuring the nitrogen content of the sample fruit and multiplying it by a factor of 6.25. This factor was used because most protein contains 16% nitrogen. Protein was determined by kjeidhl method. This method involves digestion, neutralization, distillation and titration.

- Digestion + conc. H_2SO_4+ catalyst nitrogen converter into ammonium
- Neutralized to get NH_3 and trap in boric acid
- Titration with hydrochloric

Gram of nitrogen/ gramof sample = (ml of sample – ml of blank) N standard acid x 0.014g/meq

Crude fat

About 90ml of an anhydrous diethyl ether of boiling point of 40 to 60^0C is placed in a flask. 2-4 of the mango sample was weighed into a thimble and the thimble was plugged with wool. The thimble with the content was placed into the extractor, the sample continues for at least 4 hours. The thimble was then removed and the solvent distilled from the flask into an extractor. The flask was then disconnected and placed in an oven at 65^0C for 4 hours, cooled in a desiccators and weighed,

Crude fat = Wt. of flask + extract– tare Wt. of flask x 100 Wt. of the sample

Crude Fiber

The organic residue left after the sequential extraction of the sample with ether was use to determine the crude fiber. The fat free material was then transferred into the flask and pre-heated 1.25 of H_2SO_4 was added and the solution gently boiled for 30 minutes, maintaining constant volume of acid by the addition of hot water. Finally the residue was transferred into the crucible and place in muffle furnace (400-60^0C)and ash for 24 hours and weighed.

%Crude fiber =$\frac{\text{Dry Wt. of residue before ashing– Wt. of residue after ashing x100}}{\text{Wt. of sample}}$

Crude Ash

Ash is the organic residue obtained by burning off the organic matter, the fruit stuff. At 400-600^0C in muffle furnace for 4 hours. 2g of the sample was weighed into a crucible. The crucible was then placed in the desiccators and weighed.

Ash= Wt. of crucible + ash – Wt. of crucible + Wt. of sample

Crude Carbohydrates

This determined by the difference after adding the percentage crude protein, moisture, ash, crude fiber and fat constant and subtracted from 100%

C:H:O= Crude protein + moisture + ash + crude fiber + fat – 100%
This analysis was done according to (Heitman, 2015; Pixton, 2015; Nguyen*et al*., 2017), to determine the significant differences in their composition.

RESULTS AND DISCUSSION

During the study, 64 mango fruits were collected and sampled for fungi species, 42 fruits had fungi species while 22 fruits samples were without fungi species. The fungal isolates were *Apergillus niger, Aspergillus oryzae, Mucor hiemalis and Rhizopus stolonifer*. Their percentage occurrences were 28.57, 23.81, 23.81 and 23.81 respectively (Table 1). From the four different locations (Keffi market, FMC Round About, Angwa Lambu and High Court), High court and FMC Roundabout has the highest incidence of fungal infection (18.75) while Angwa Lambu had least fungi infection (12.50) (Table 2). The pathogenecity test showed *Apergillus niger, Aspergillu soryzae,* to be the most virulent while *Mucor hiemalis*is the least virulent (Table 3). There was no significant difference (P<0.05) in the incidence of different isolates in relation to location (Table 4). The analysis of the nutritional content of infected mango fruits shows the reduction in the crude protein, crude fibre, crude ash and crude carbohydrate level compared to the uninfected mango fruits (Table 5).

Table 1: Percentage occurrence of fungi species

Location	*Aspergillus niger*	*Aspergillu soryzae*	*Mucor hiemalis*	*Rhizopus stolonifer*	Total Frequency
Keffi Market	3	2	4	1	10
FMC Roundabout	4	5	-	3	12
Angwan lambu	3	2	1	2	8
High Court	2	1	5	4	12
Total	12	10	10	10	42
Percentage total	**28.57**	**23.81**	**23.81**	**23.81**	

Table 2: Incidence of fungal isolates with relation to locations

Location	Total No. of Mango fruits examined	No. With fungi species	No. Without fungi species
Keffi Market	16	10(15.63)*	6(9.38)*
FMC Roundabout	16	12(18.75)*	4(6.25)*
AngwanLambu	16	8(12.50)*	8(12.50)*
High Court	16	12(18.75)*	4.(6.25)*
Total	**64**	**42(65.63)***	**22(34.38)**

*values in parenthesis are in percentage.

Table 3: Percentage infection of mango fruits artificially inoculated with fungi diseased samples (Pathogenecity Test).

Fungi isolates	No. of Mango Fruits Inoculated	Percentage infection after 5 days
Aspergillus niger	4	100
Aspergillus oryzae	4	100
Mucor hiemalis	4	20
Rhizopus stolonifer	4	60
Total	16	

Table 4: Chi-square analysis on the incidence of fungi species in different locations

Location	No. with fungi species (negative)	No. with fungi species (positive)	Column Total
Keffi Market	10(10.50)*	6(5.50)*	16
FMC Roundabout	12(10.50)*	4(5.50)*	16
Angwan Lambu	8(10.50)*	8.(5.50)*	16
High Court	12(10.50)*	4(5.50)*	16
Total	**42**	**22**	**64**

*Number in parenthesis is the expected frequencies
H_0: There was no significant difference (P<0.05) in the incidence of isolates in relation to locations.

F. Tab 7.815>Cal 3.05, therefore we accept the hypothesis

Table 5: Proximate composition (in %) of Mango fruits.

Mango fruit Condition	Moisture Content	Crude Fibre	Crude Ash	Carbohydrate	Crude Fat	Crude Protein	Crude
Uninfected	78.41	0.94	0.66		0.06	1.38	19.60
Infected	83.00	0.52	0.31		0.06	0.21	16.01

This study revealed that a range of mycotoxic fungi are associated with the deterioration of mango fruits in Keffi Local Government Area of Nasarawa State, Nigeria. The prevalence of these fungi in this area may be due to their ability to produce proteolytic enzymes such as pectic methyl esterase (PME), polygalaturonase and protease (Thompson, 1999). Following their entry into the host tissue, the enzyme breaks down cells hence causing rot. The spoilt fruits become squashy, emit bad odour and become less in nutritional value as observed in this investigation. The bad odour experienced may be due to the action of enzymes produced by the fungi involved. The enzymes may have acted on the pectic acid into methyl alcohol (Bengrson *et al*.,2017). Relative humidity of the locations surveyed is very high and this may have aided in spread of the organisms in these areas (Ogaraku *et al*., 2012).

This study revealed that there was high incidence of *Aspergillus niger* in the different locations when compared to other fungi isolates in the same locations. There was no significant difference ($P<0.05$) in the incidence of different isolates in relation to location. This could be as a result of little or no variation in soil type, geographical and climatic conditions of the area since they are adjoined to each other.

The proximate determination result showed that the moisture content is low on uninfected mango samples when compared to the infected samples, also the crude fat showed an equal range of mean value 0.06, which means that these have no effect on the production of mould. Also the infected samples show high mean value in crude fibre, crude ash, crude protein and carbohydrate which could predisposed them to fungi infection, which agreed with Pixton (2015); and Nguyen *et al*., (2017). This study therefore recommend that farmers should have a thorough understanding of the farming system suitable for mango production for proper disease management (usually plantation).

CONCLUSION

The use of proper chemical control against fungus causing deterioration on field and store crops, adoption of natural cooling method during preservation of the mango fruits (the use of temperature that does not allow the growth of microbial as most of them are thermophillic) and also grafting of desirable but susceptible (scions) on resistance root stock to prevent diseases caused by pathogen which survive in the soil.

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