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FUNGI ASSOCIATED WITH SPOILAGE OF SOME EDIBLE VEGETABLES IN AND AROUND MINNA, NIGER STATE, NIGERIA

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Abstract

Fungi associated with spoilage of some edible - vegetables fluted pumpkin *Talfairia occidentalis,* Africa amaranth *Amranthus muricatus* and bitterleaf *Vernonia amegdalina* were examined. The result revealed that fluted pumpkin (*Talfairia occidentalis*), African amaranth (*Amranthus muricatus*) and bitterleaf (*Vernonia amegdalina*) grown on irrigated farms and open gardens around Minna and its environs favored the development of fungal infestation. The vegetables grown in urban and suburban open gardens were used for the test. The prevention of fungal pathogens was also examined to avoid drastic chemical treatment or minimize its application. Three major fungi - *Aspegillus sp.; Penicillum sp and Rizopours sp.* were isolated from the green vegetables. Suggestions were made on how to reduce the load of these fungi by educating farmers on the need for using hygienic water for irrigation in producing eatable vegetables.

Key Words: *Fluted pumpkin (Talfairia occidentalis) bitterleaf (Vernonia amegdalina), fungal disease.*

Introduction

Leafy vegetables are edible plant parts although other plant parts - stems, or even the roots can equally be consumed. The use of the word vegetables is however not scientific, and its meaning is largely based on culinary and cultural tradition. Vegetable is used in scientific and technical contexts to mean areal plants parts generally, edible or cooked. The meaning of "vegetable" as "plant grown for food" was not established until the 18th century.

Vegetables can be consumed raw, blenched, or cook. The report presented by this study suggests that caution should be taken when consuming any of these vegetables. According to Minhas and Samra (2004), vegetables eaten raw, can transmit pathogens to the consumers. Thus, the higher fungal load on vegetables calls for caution, as many people use bitter leaf and fluted pompkin for medicinal purposes where they only squeeze out the juice and drink it raw, as well as its use as salads. These can also be added to dishes to enhance flavor. A few vegetables are often used in desserts and other sweet dishes, such as rhubarb pies and carrot cakes. Some people consider mushrooms for example, as vegetables even though they are not plants but can add flavor to foods.

Vegetables make significant contribution to the diet of many people. Data on the consumption of contaminated vegetable and the level of vulnerability to fungal infection in Minna is scanty. Both traders and consumers often consume vegetables based on attributes such as freshness, color and how appealing they look before or after cooking, ignoring the level of contamination by microbes (Gutiérrez-Rodríguez, *et al.,* 2012). Similarly, there is no record of the effect of consumption of the contaminated vegetables on the consumers.

Lots of vegetables are known to be grown in Niger State, particularly along the existing water bodies that transverse the state. Such vegetables are the likes of bitter leaf; amaranth; fruits like garden egg, okra, water melon, sweet potato, carrots and fluted pumpkins - Talpharia sp. (Tsado *et al.*, 2013).

The objective of this study is to determine the fungal loads of some vegetables in both rural and urban environments of Minna. To identify the types of fungi in the different samples; locations and to try to proffer affordable solutions for the reduction of these fungal contamination of vegetables.

Materials and Method

The vegetable samples used in this study - fluted pumpkin (*Talfairia occidentalis*) Africa amaranth (*Amranthus muricatus*) bitterleaf (*Vernonia amegdalina*) were all collected from local farms in and around Minna, Niger State, Nigeria. These samples were then taken to Miocrobiology laboratory of School of Natural and Applied Sciences, Federal University of Technology, Minna, for analysis.

Media Preparation

The media used for the culturing the microorganisms were Saborad Dextrose Agar (SDA), commercially prepared and potato dextrose agar (PDA) locally prepared. A 31.5g of the SDA was weighed and dispensed in 500ml conical flask to make up the 500ml it was brought to heat to dissolve agar agent completely. Irish potato of 300g was pilled and sliced into pieces and put in 300ml of distilled water and cooked after which it was then filtered using white muslin cloth.

The filtrate 200mls of distilled water was added to make up to 500mls. Ten grams of glucose was then added ad stirred properly. To the samples were autoclaved for 15min at 121^0C as described by Cheesbrough (2003) and Oyeleke and Manga (2008), Chlorophenicol 0.25g was then incorporated to inhibit the growth of bacteria,.

Preparation of the Tenfold Serial Dilution

The preparation of the serial dilution was done by dispensing 9mls of distilled water into five tests to make a dilution factor of 10^{-5} for each sample, after dispensing the distilled water it was autoclaved as previously explain. One gram of each sample was introduced into the tubes of each sample, 1st tube was 1:10. This was shaken after which a milliliter was drawn into the 2nd tube, consequently up to the last tube. After serial dilutions a ml from 10^3 and 10^A of each sample was dispensed into the petri-dishes which had the label of each sample corresponding to test tubes. The cooled molten media was dispensed into each petri-dish containing 1ml of each sample, 20-25mls of media was introduced mixed properly by rotating the plates on the bench and allowing them to congeal before incubating them in the inoculating hood for 72hrs (at room temperature ± 28°C). The isolated colonies were then counted with a colony counter.

Data Analysis

All the data generated were statistically analyzed using analysis of variance ANOVA test appropriate for randomized complete block design. When main and interaction effects were found to be significant, means were compared using LSD test at 0.05 level of probability (Steel and Torrie, 1981).

Results and Discussion

Differences in Fungal Load Observed On Vegetables from Rural Farms and Markets

Results of the fungal load on the three vegetables detected between rural farms and rural markets in Minna and its environs are presented in Fig. 1. This result show that fluted pumpkin had the highest fungal load of 5.0 $x10^4$cfu/g, while amaranth from the rural farm had the lowest fungal yield of $2.8x10^4$cfu/g. This result for the urban area is show in Fig 2. This showed a significant difference among the means, where means for Fluted pumpkin (FT), and bitter leaf (BT) were higher than that of African amaranth (AM) in urban markets.

This result also showed that Fluted pumpkin from the urban market had the highest fungal load of 6.6 x 10^4cfu/g followed by Amaranth from urban farm at $4.8x10^4$.cfu/g. Bitter leaf from urban farm had the lowest fungal yield of $3.2x10^5$cfu/g.

Comparisons for Fungal Load between Sampled Areas

Figure 3, shows the mean fungal load of the sampled areas (rural and urban) for the vegetables. This result showed no significant difference between the sampled sites although the loads of the markets were observed to be higher than the farms.

Fungi Isolated and Identified

Three major fungi were isolated and adduced to be associated with the green vegetables. This result agrees with the report of Amaike and Keller, (2011) who reported that that these organisms could be linked to the spoilage of raw vegetables. *Aspergillus flavous* has also being implicated in causing aspergillosis that infects and contaminates pre-harvest and post-harvest fruits and vegetable crops with the carcinogenic secondary metabolite aflatoxin (Amaike and Keller, 2011). These authors suggested that one of the more promising strategies in the field control of these fungi could involve the use of atoxic strains of *A. flavus* in competitive exclusion studies.

Rhizopus stolonifer was equally isolated in this current study. This organism has also been implicated in several human ailments (Schipper, 1984). This organism is most commonly found growing on soft fruits such as bananas, oranges and carrots.

Tab. 1 Fungi isolated from the green leafy vegetables studied

	Fungi	Fungi types
1	Aspegillus sp.	*Aspergillus flavous* *Aspergillus niger* *Aspergillus fumigatus*
2	Penicullum sp.	
3	Rhizopus sp.	*Rhizopus stolonifer var. stolonifer*

Discussion

Fungi associated with spoilage of fruits and vegetables in Minna area were established. The fungi isolated were Aspegillus sp. Penicullum sp. and Rhizopus sp. The level of pathogenicity in the two locations - rural and urban areas - was however not statistically significant. There presence of fungal load was however, more prominent in urban markets

than in the rural markets. This may not be unconnected with the number of inhabitants that move in and out of these markets. The higher rate of contaminants in urban settings compared to rural environs may also be associated. The continuous use of waste water (e.g. sewage water) for irrigation in vegetable production principally on urban farms means that fungi in most sewage can contaminate the vegetables. This is in agreement with the report of Beuchat and Ryu, (1997) and Tsado *et. al.*, (2013) who suggested that the presence of many pathogens in soils may major sources of microorganisms present on many vegetables. Also Mapanda *et al*., (2005) also shared the same view that pathogens existing in soils or water can be the source of both pre- and post-harvest contamination of several vegetables. Amadioha and Obi, (1998) have shown how most of the vegetables consumed are not produced by highly knowledgeable people therefore, there is the need to pay close attention to the type of vegetables we eat.

Fungal load present in vegetables in cities was higher than those from rural areas because rural farms may have been cleaner than urban farms. Of all the three vegetables, bitter leaf and fluted pumpkin consistently had the highest fungal load almost throughout the sampled areas in Minna. This could be due to organic fertilizer used in home gardening. The organisms isolated from the vegetables were fungi species except in fluted pumpkin which included also yeast cells.

Conclusion

This study confirms the presence of fungi on vegetables in Minna and its environs and that the source is not limited to any particular location but that these fungi can also be found in all the sampled locations - urban or rural (farms or markets). The fungal load observed in the rural areas was lower compared to those of the urban areas. The isolated organisms bring to mind the health hazards attributable to raw vegetable consumption by people in and around Minna.
This study suggests that caution should be taken when consuming any of the vegetables grown locally as they may contain high levels of fungi. According to Minhas and Samra, (2004) vegetables eaten raw, can also transmit pathogens to the consumers, hence the higher fungal load on vegetables calls for caution, as many people use bitter leaf and fluted pumpkin for medicinal purposes where they express the juice for consumption, as well using the vegetable as salads (Ghimire,*et. al.* 2011). Vegetable farmers should hygienic water to irrigate their fields. In a similar vein vegetables should be properly washed in clean or iodized water before sending them to the markets for sale.

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Key:Fluted pumpkin'(FT), Bitter leaf (BT), African amaranth (AM)

Fig 1. Fungal load of leafy vegetables sampled in and around Minna.

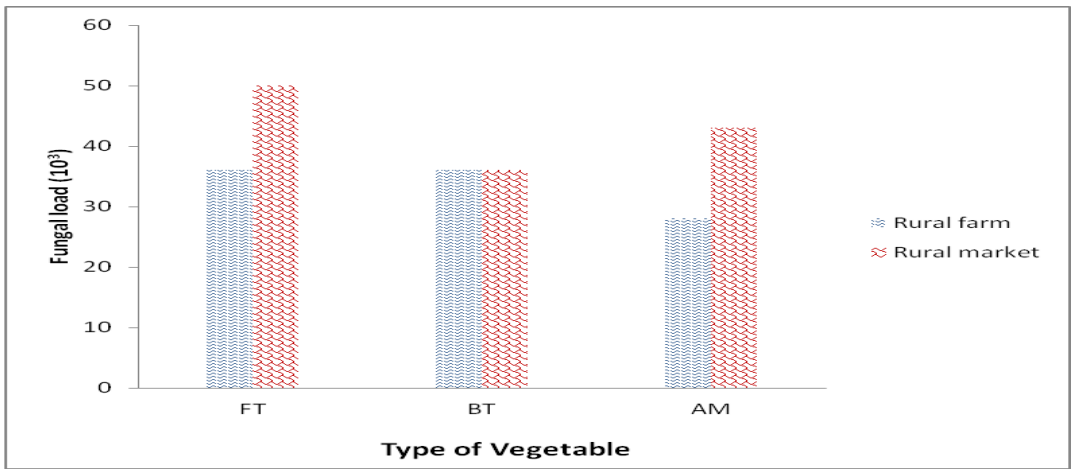

Fig 2. Fungal load of leafy vegetables sampled in and around Minna.

Key: Fluted pumpkin'(FT), Bitter leaf (BT), African amaranth (AM)

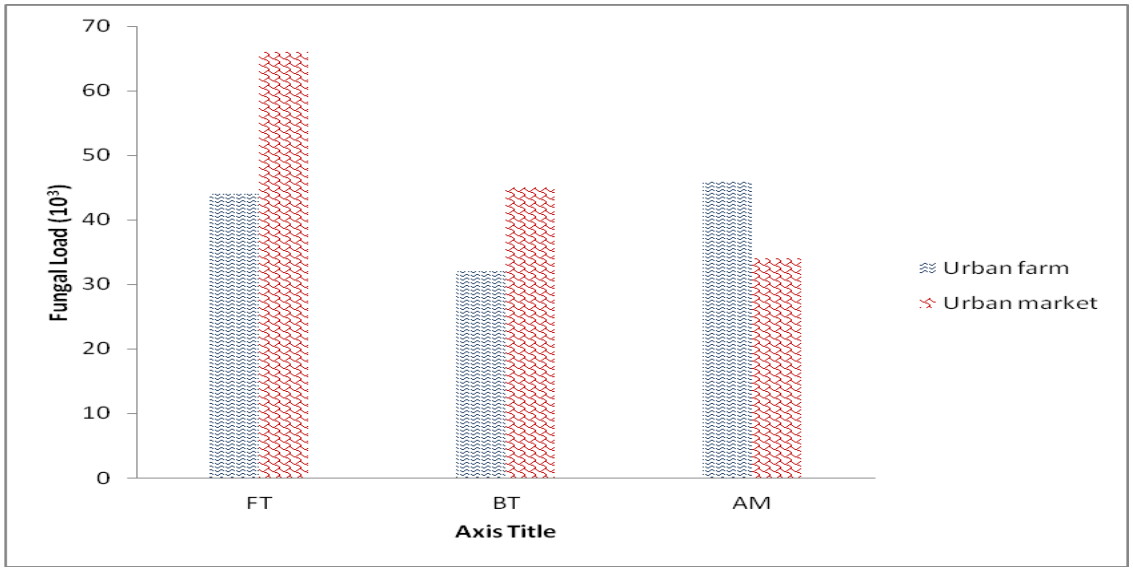

Fig 3: Difference in the fungal load between rural and urban areas.