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IDENTIFICATION AND INTENSITY OF OCCURRENCE OF FUNGI ASSOCIATED WITH SPOILAGE OF TOMATO FRUITS IN SOME MAJOR MARKETS IN OGUN STATE NIGERIA

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill*)* fruit has high moisture content and very soft exocarp which predisposes it to mechanical damage and subsequent microbial infections that lead to spoilage. The condition is worsened with the postharvest and marketing/retail practices in the open markets; hence the experiments were carried out to isolate and identify the fungi causing deterioration of tomato fruits in major markets of selected Local Government Areas in Ogun State. Partially-deteriorated tomato fruits were collected from the markets for laboratory analysis using serial dilutions and pour plate method to isolate, identify and determine the frequency of occurrence of the fungi species present. Presence of *Penicillium notatum, Aspergillus flavus, Alternaria spp* and *Fusarium oxysporum* was established. Frequency of occurrence ranged between 17.14 and 65.71% while average fungi load count was 2.0×10^3. Occurrence was highest in metropolitan open daily market and least percentage in village weekly market.

Keywords: Deterioration, Fruits, Health, Fungi load, Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) originated from South America and belongs to the family Solanaceae. It is a widely grown fruit vegetable with China being the leading producer, followed by India, Turkey, USA, Egypt, Italy, Iran and Spain (FAOSTAT, 2019). Principally, it provides vitamins and minerals for humans (Smith, 1994) and also serves as a raw material for many agro-based and agro-allied industries for value-added products like paste, puree, ketchup etc. In Nigeria, the bulk of fresh tomato production is in the northern part. However, the consumption and utilization is nationwide as it is an important commodity for the preparation of many local dishes and fresh consumption. Large quantities of tomato are produced all year round in Nigeria and production tonnage is highest between August and November (Obikwe and Obaseki-Ebor, 1987). Tomato has nature and peculiarities

of a very high moisture content, tender texture and active metabolism that are characteristics of most horticultural produce. They are therefore prone to postharvest damage if poorly handled. Inappropriate handling down the value chains further compounds the damages that thereafter result in pathogenic attacks and subsequent spoilage (Chun-Ta, 2010). There are incoherent studies addressing the poor handling of horticultural produce in open markets in this region, although fungi spoilage has been recognized as a source of potential health hazard to humans and animals. Postharvest losses due to fungi and bacterial soft rot have been estimated to vary between 15 and 30% of the harvested crop. Opadokun (1987) earlier reported that 21% of tomato harvested in Nigeria was lost to rot in the field and additional 20% to poor storage system, transportation and marketing. This was further buttressed by Kutama, *et al*, 2007, who found that estimated total loss in Nigeria due to these constraints is about 60%.

Produce deterioration or spoilage is the depletion in value, reduction of the physical and chemical composition or any form of alteration in the normal produce that ranges from scratches to absolute spoilage (Muhammad *et al*., 2004; Dogondaji*et al*., 2005). There is possibility of contamination of horticultural produce as a result of poor postharvest or harvest handling from fungi spoilage. Fungi infection can lead to production mycotoxins which are capable of causing mycotoxicoses in man following ingestion or inhalation (Onuorah, 2015).
The objective of this experiment was to identify and quantify fungi associated with the spoilage of tomato fruits in some major open markets in Ogun State.

Materials and Methods

The study was conducted at the Federal University of Agriculture Abeokuta, Nigeria. The study area covered some major markets in selected Local Government Areas in Ogun State, Nigeria. The State is situated within latitude 6°20' S and 7° 58' N; Longitude 2°40' W and 4°30' E. Ogun State has a total of 20 Local Government Areas, with a total population of 4,054,272. The State is bordered by Osun to the North, Ondo to the West, Lagos to the South and Republic of Benin to the West (Aminu *et al*., 2015). Ogun State is a major centre, considering the production, trading and consumption of horticultural produce, especially tomato. The specific study areas in 2017 were: Abeokuta South (Kuto market which is both daily and weekly), Abeokuta North (Lafenwa market which is both daily and weekly), Obafemi/ Owode (Siun market weekly) and Sagamu (Saabo market which is both daily and weekly) Local Government Areas (LGAs) while in 2018, the LGAs covered were Abeokuta South, Abeokuta North, Obafemi / Owode, Sagamu and Remo north.

Collection of Samples

Samples of tomato fruits were collected during the dry season in 2017 from four open markets namely: Kuto market (Abeokuta South LGA), Lafenwa market (Abeokuta North LGA), Siun market (Obafemi/Owode LGA) and Saabo-Sagamu market in Sagamu LGA, for laboratory analysis. Three middle-sellers were randomly selected in each market. Twelve (12) sample bowls of 15 x 10 cm with lids were sourced locally and pre-labelled according to market location for easy identification. Tomato fruits with signs of decay or deterioration were collected into sample bowls and sealed with tight fitting lids in order to prevent contamination and subsequently moved to the laboratory. The

process was repeated in dry and wet seasons of 2018 and tomato samples were collected from the following markets: Kuto in Abeokuta South LGA, Lafenwa in Abeokuta North, Siun in Obafemi/Owode LGA, Saabo-Sagamu in Sagamu LGA and a Tomato depot in Ogere town in Remo North LGA. The laboratory analyses were carried out at the College of Veterinary Medicine Laboratory, Federal University of Agriculture Abeokuta using serial dilutions and pour plate method as described by Beauchat *et al.,* (1998).

Sample preparation Procedure:

i. One (1) g of the sample was weighed in weighing balance using watch glasses.

ii. One (1) g of the sample weighed was placed in a sterile flask containing 100 ml of sterile distilled water, to give $1:10^2$ dilution (tube A).

iii. The flask was shaken for 10 minutes, taking care not to wet the plug.

iv. It was allowed to settle for 30 minutes.

v. Five (5) tubes were arranged, each contained 9 ml sterile distilled water, in a stand.

vi. With a sterile pipette, 1 ml of diluted A was transferred to another 9 ml of sterile and mixed as before, giving $1: 10^3$ dilutions.

vii. With more test tubes, the dilution procedure was repeated, forming $1: 10^4$, $1: 10^5$ and $1: 10^6$dilutions

viii. 0.5 ml of ($1: 10^4$, $1: 10^5$ and $1: 10^6$) suspension was transferred into a sterile petridish using a sterile graduated pipette.

ix. A conical flask of melted nutrients agar and potatoes dextro agar (from water bath) which was not too hot was taken

x. The agar was quickly poured gently over the suspension inside the petri dish, and the suspension / agar were mixed evenly. The agar was thereafter allowed to gel.

xi. The petri dishes were incubated in an incubator, for bacterial at 37^0 C for 24 hours, while for fungi at 25^0 C at room temperature.

xii. With the aid of hand lens, the number of colonies was counted by multiplying the number of colonies by the degree of dilution to determine fungi load count.

Calculation: i.e.:

Using, $1: 10^4$ dilution.

With 0.5 ml of $1: 10^4$ dilution, 90 fungi were counted

1 ml of this dilution contained $90\times 2 = 180$ fungi

Therefore, total number of fungi cells = 180×10^4 cfu / ml or 1,800,000 cfu / ml.

In addition to sample collection and analysis, oral personal interview was conducted with the marketers to authenticate and validate possible outcomes of samples analyses. Questions relating to the source, duration and mode of transportation distance from produce source to markets and storage duration were asked.

Fungi Identification was done by staining the Fungi mycelia and the spores with lactophenol cotton blue and viewed under the microscope. Identification of the fungi present in the collected samples was based on recommendation of necessary management and storage techniques that reduces the rate of deterioration due to fungi attack. Data were subjected to analysis of variance (ANOVA) using Genstat 12th edition and significant treatment means separated using Least Significant Difference (LSD at $p\leq 0.05$).

RESULTS

Identification and intensity of fungi causing deterioration in Tomato fruits in 2017 dry season

The identified fungi associated with Tomato fruits deterioration in the four open markets were *Penicillum spp, Fusarium spp and Mucor spp* in 2017. The three fungi species were found on Tomato fruits sold at Kuto market in Abeokuta South LGA, Lafenwa market in Abeokuta North LGA, Saabo-Sagamu market in Sagamu LGA and Siun market in Obafemi/Owode. Occurrence of *Penicillium spp, Fusarium spp* and *Mucor spp* was found in both daily (Kuto, Lafenwa, Saabo-Sagamu) and weekly (Siun, Kuto, Lafenwa, Saabo-Sagamu) open markets with average infections (Table 1).

Table 1: Identification and intensity of decay causing fungi on Tomatoes in selected markets in Ogun State in 2017 dry season

Market	*Penicillium spp*	*Fusarium spp*	*Mucor spp*
Kuto (Abeokuta South LGA)	++	++	++
Lafenwa (Abeokuta North LGA)	++	++	++
Siun (Obafemi/Owode LGA)	++	++	++
Saabo-Sagamu (Sagamu LGA)	++	++	++

Averagely infected ++

Load count and frequency of occurrence of Fungi causing deterioration in Tomato fruits in 2017 dry season

The fungi load count on Tomato fruits was significantly highest at Kuto market, followed by Saabo-Sagamu market, Lafenwa market while Siun had the least count (4.5, 3.6, 2.8 and 2.2CFU/g, respectively). The frequency of occurrence followed similar trend, with highest frequency at Kuto market 93.75%, Saabo-Sagamu with 75.0%, Lafenwa 31.25% and Siun had frequency of 12.5% (Figures 1 and 2).

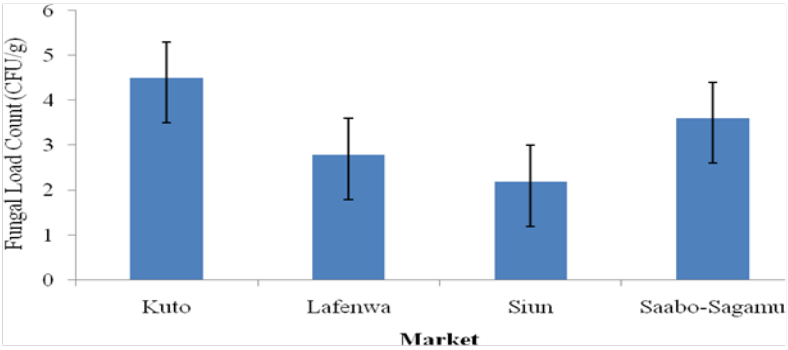

Figure 1: Fungi Load count on Tomato fruits in major markets of Ogun state in 2017 dry season

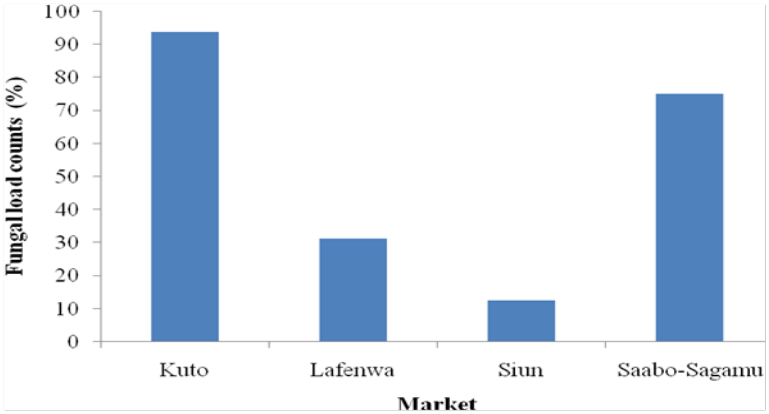

Figure 2: Fungi frequency of occurrence on Tomato fruits in major markets of Ogun state in 2017 dry season

Identification and intensity of fungi causing spoilage in Tomato fruits in the selected markets of Ogun State in 2018 dry season

In 2018, the fungi species identified as causing tomato fruit deterioration in the selected markets were: *Aspergillus flavus, Fusarium oxysporum, Penicillium notatum* and *Alternaria spp* (Table 2). The four fungi isolates from Tomato fruits sourced from Kuto market in Abeokuta South LGA were: *Fusarium, Alternaria, Aspergillus* and *Penicillium* spp in decreasing order of dominance. Tomato fruits were heavily infected by *Fusarium oxysporum* in Kuto, Lafenwa, Sabo-Sagamu and Ogere markets with exception of Siun market. *Penicillium notatum* infection was consistently found in Tomato fruits from all the markets. At Lafenwa market in Abeokuta North LGA, the intensity of occurrence of Fusarium*, Aspergillus, Alternaria,* and *Penicillium* was in decreasing order. However at Ogere depot, *Aspergillus* infection was heavy, *Fusarium, Alternaria* infection was average and *Penicillium* infection was least. This depot is the sole point of consignment delivery from the north and operates non-stop with. Fungi identified in Saabo-Sagamu market were *Aspergillus, Fusarium* and *Penicillium* at low intensities. The fungi identified at Siun market in Obafemi/Owode LGA was *Penicillium* with very low intensity of infection. This market is farmers-marketers market where tomatoes covered little distance to get to sellers and buyers (Table 2).

Table 2: Identification and intensity of isolated fungi causing deterioration of Tomato fruit in selected markets in Ogun State in 2018 dry season

	Fungi Types			
Markets	*Aspergillus flavus*	*Fusarium oxysporum*	*Penicillium notatum*	*Alternaria spp*
Kuto	++	+++	+	++
Lafenwa	++	+++	+	++
Siun	-	-	+	-
Ogere depot	+++	++	+	++
Saabo-Sagamu	+	+	+	-

Key: Absent -, Infected +, Averagely infected ++, Heavily infected +++

Fungi load count from Tomato fruits from selected markets in Ogun State in 2018

The fungi load count was higher in the metropolitan markets (Kuto, Lafenwa, Ogere depot and Saabo-Sagamu) with average values of 2.07 $\times 10^3$CFU/ml, 2.0 $\times 10^3$CFU /ml and 1.6 $\times$ 10^3CFU /ml, 0.80 $\times 10^3$CFU /ml respectively but very light in Siun market with an average value of 0.12 $\times$ 10^3CFU /ml (Figure 3).

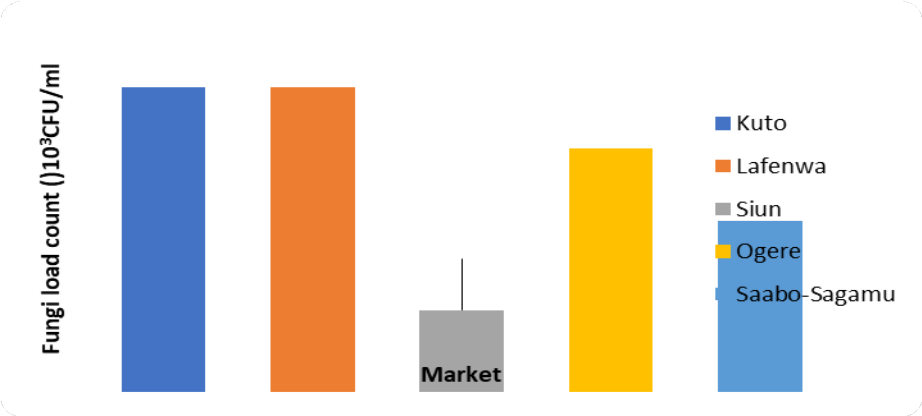

Figure 3: Fungi load count on decayed tomato fruits in selected markets in 2018 dry season

Effects of seasons on fungi load count and frequency of occurrence on decayed tomato fruits in selected markets in Ogun State in 2018

The intensity of fungi isolates on the tomato was between 4.8 and 82.8% with Siun market recording significantly lowest value compared to other markets (Figure 4). Season had significant effect on the fungi load count on Tomato from various markets. Samples collected during the wet season had significantly higher fungi load of 81.6% compared to those collected during the dry season with 29.6% (Figures 5 and 6)..

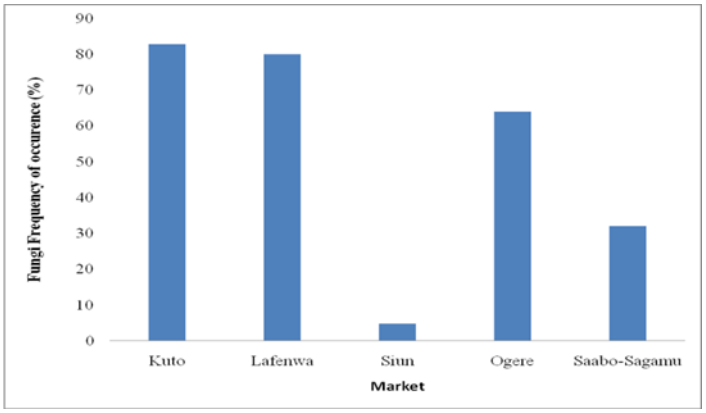

Figure 4: Fungi frequency of occurrence on decayed Tomato fruits in Selected Markets in 2018 dry season

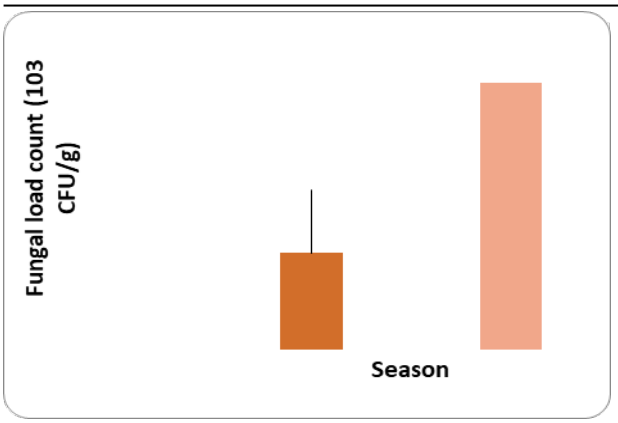

Figure 5: Effect of season on the fungi load count on decayed tomato in selected markets in 2018

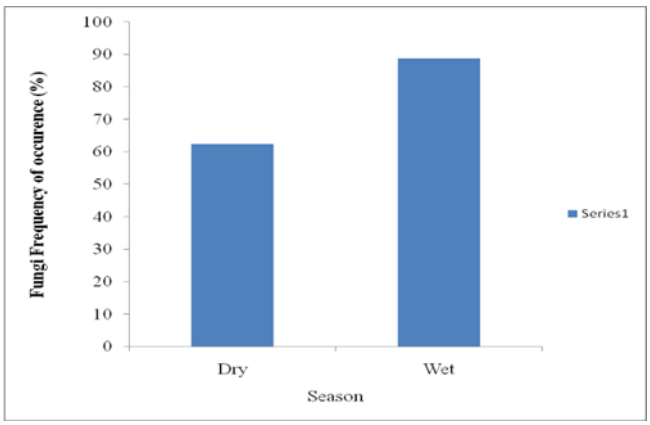

Figure 6: Effect of season on the fungi frequency on decayed tomato in selected markets in 2018

Interactive effects of seasons and markets on fungi load in tomato fruits from selected markets in Ogun State in 2018

The wet season also recorded significantly higher fungi loads counts in daily markets in the metropolis while lowest occurred in village market. The load ranged between 19.14 and 85.71% while the values for dry season were between (0.12 10^3cfu/g and 3.42% and (2.010^{3}cfu/g) 54.14% respectively (Figure 7).

Interactive effects of seasons and markets on fungi frequency in tomato fruits from selected markets in Ogun State in 2018

The interactive effects of season and market location were significant on the fungi frequency and the fungi load. The wet season had higher fungi occurrence, with the highest frequency at Lafenwa (97.14%) followed by Kuto, Ogere depot, Saabo-Sagamu and least (5.71%) at Siun. Fungi frequency of occurrence in dry season followed similar trends but with lower occurrence (0.85 to 51.42%) across markets (Figures 8 and 9).

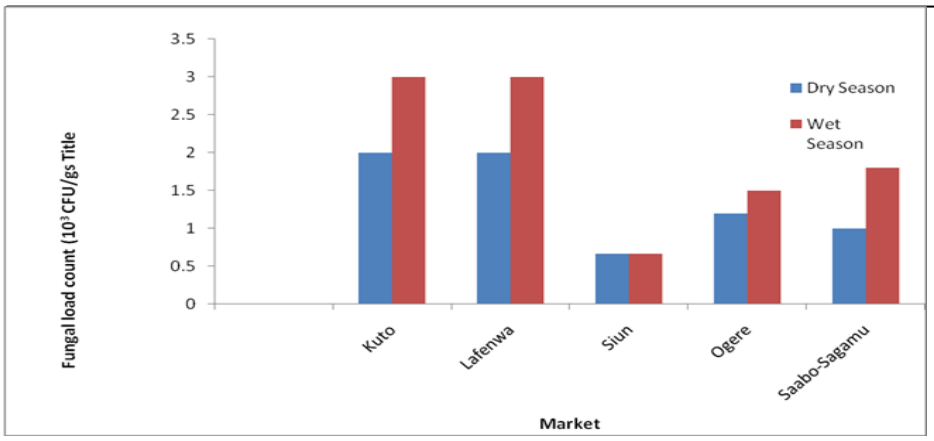

Figure 7: Effects of interaction of seasons and markets on fungi load of fungi isolates on Tomato fruits deterioration in selected markets in Ogun State in 2018

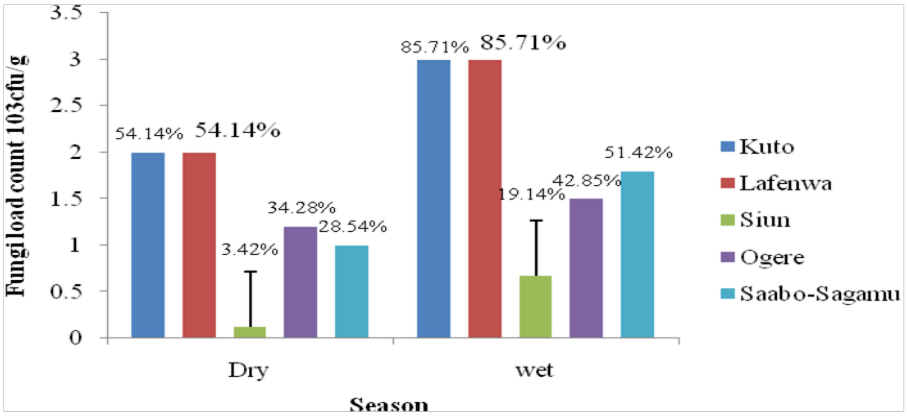

Figure 8 Interactive effects of seasons and markets on fungi load counts on spoilt Tomato fruits in selected markets in Ogun State in 2018

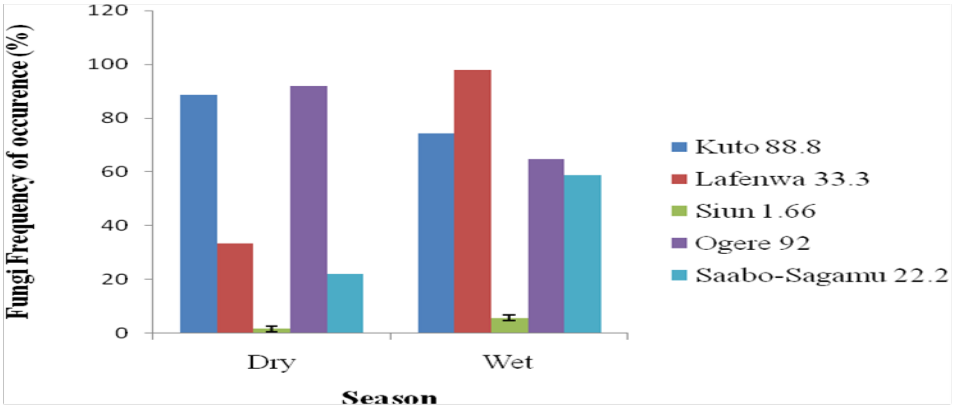

Figure 9: Effects of interaction of seasons and markets on frequency of fungi isolates on Tomato fruits deterioration in selected markets in Ogun State in 2018

DISCUSSION

Occurrence of fungi in tomato fruits across the markets is a confirmation of earlier findings of Ibrahim *et al.,* (2014), Onuorah and Orji (2015), who reported presence of *Aspergillus, Fusarium, Alternaria*, *Penicillium* and *Rhizopus* in tomato samples. *Aspergillus* spp. which were consistently present in the samples collected from the markets have been reported to be heavy producers of carcinogenic aflatoxin especially *Aspergillus flavus* and all other species of *Aspergillus* which could predispose the consumer to the growth of cancerous cells (Rodriquez *et al*., 2007). Barkai-Golan and Paster, (2008) earlier stressed that *Aspergillus*, *Penicillium* and *Alternaria* species were the major contributors to fruit and vegetable decay while Zain (2011) affirmed the harmful outcome on human and animal health. The identification of fungi associated with tomato spoilage in the open markets is of importance as they affect the proximate and nutritive values of the fruit (Sanyaolu, 2016). The peculiarities of each market could have influenced the frequency of occurrence of identified fungi and fungi load count. Kuto, Saabo-Sagamu and Lafenwa are urban daily markets with tomato supply majorly from the North. Siun is a village market operated once in a week, with supply from local Cotonou and Igede farmers. The distances covered to bring the tomato down was shorter compared to those of daily markets in the metropolis that receive majorly from the North. The fact that the village market had break in operation i.e. market 'rest' after the week's sales followed by sanitation could have accounted for the minimal occurrence of fungi on the tomato fruits.

The low fungi occurrence in markets that receive supply from short distance had fairly fungi free, healthy and safe to consume tomato fruits. This is supported by findings of Abel-Malleke*t al.,* (1995) and Ugwu *et al* (2015) who reported that absence of fungi species in healthy tomato fruits. The occurrence of lesser fungi during the dry season and distance covered is in line with the assertion of Idah *et al*., (2007) that environmental conditions such as heat and drought, improper postharvest, handling and transportation over long distance and on bad road network can enhance wastages of produce as a result of damages. During collection of samples, marketers reported that the tomato fruits were procured from Ogere depot which receives consignment transported from Kano State by road over a distance of 1137.4 km to Ogun State. The tomato fruits were packed in woven baskets in trucks or hung across fuel tankers coming down southern Nigeria. The heavy fungi load on the tomato fruits from Ogere depot, Kuto and Lafenwa markets could be as a result of poor post-harvest handling such as over packing during the movement from the source to the market, use of woven basket with rough internal surface for storage and display compared to Siun market which is a farmers' village market. Sellers from the metropolitan markets cover additional 60 km to transport the fruits by road. The method of storage of the produce by markets was with the use of woven raffia baskets and polyethylene sheets since sales take couple of days. This could have accounted for higher fungi load observed in daily markets compared to the depot and weekly market which had lower loads. In Siun market, the intensity of fungi species was very low and inconsistent. Following a personal interview with the sellers, it was gathered that tomato being sold in the markets are being produced in farms in the locality. The produce passes through little or no transportation stress as they are transported from the farm gate to

the market. The higher occurrence of fungi during the wet season was due to higher moisture or relative humidity that characterizes the season.

The wet season is usually characterized by high relative humidity which favors microbial growth. Also wet condition is the appropriate or conducive environment for fungi growth as such the high fungi load counts. On the other hand, the dry season had lower fungi load counts compared to wet season. During the dry seasons, the dry environment due to little or no moisture prevents the fungi species from growing.

CONCLUSION

Tomato fruit deterioration was found to be associated with microbial pathogens especially fungi. Frequencies of fungi occurrence and fungi loads were much prevalent in daily open urban markets; and especially during the wet season. It is pertinent that high level of hygiene and marketers' enlightenment and sensitization of these conditions be put in place.

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