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Microorganisms causing post-harvest tomato (*Solanum lycopersicum* L.) fruit decay in Nigeria

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Abstract
This study was carried out to isolate and identify pathogenic microorganisms associated with deterioration of tomato fruits. Fruit samples of infected and non-infected tomatoes were collected from two open markets, Oja-Oba and Sabo in Osogbo, Nigeria. Each of the tomato was cut and the liquid content inoculated on nutrient agar and potato dextrose agar, incubated at 37 ^{0}C and 25 ^{0}C, respectively, and observed from 24 hours to 5 days, after which different colonies obtained were identified using slide culture technique. Two bacteria, *Staphylococcus aureus* and *Bacillus* spp, as well as two fungi *Aspergillus flavus* and *Rhizopus stolonifer* were observed in the tomato samples in both markets. Prevalence indices revealed that isolated pathogens is higher at Sabo market than Oja-Oba market. Pathogenicity tests also revealed that both of bacteria and fungi caused fruit decay. Consumers' awareness on potential health hazards of consuming relatively cheaper and pathogen contaminated spoilt fruits should be intensified.

Keywords: *Solanum lycopersicum* L., fungi, bacteria, prevalence, deterioration.

1. Introduction
Food spoilage refers to several changes which make the food to be toxic and less palatable to consumers, and these could be associated with alterations in appearance, texture, taste or smell [1]. Tomato (*Solanum lycopersicum* L.) is the third most cultivated and world widely grown vegetable crop. The household consumption is on high increase worldwide. Tomato is of juicy flesh endocarp belonging to the fruit class, berry. Naturally, it is very rich in vitamins, minerals, dietary fibre and protein [2]. Tomato fruits are not only food but as medicine, nutrient supplement, flavoring ingredient, detoxificant and human system cleanser [3].
The microbial deterioration on tomato fruits causes reduction in market values and nutritional qualities, and at times rendered the fruits non-fit for consumption. This is due to contaminations with mycotoxins (naturally occurring toxic chemical usually of aromatic structure) that produces aflatoxins in human, following inhalation or ingestion and so resulting to food poisoning [4].
In the developing countries at the open markets, tomato fruits are often displayed in baskets and on benches for the prospective customers, thereby exposing them to opportunistic microbial infections especially mycotoxins [5]. Microbial fruit infections could occur during crop cultivation, harvesting, post-harvest handling at processing, storage, transportation and packaging and distribution (loading and offloading) at various channels and selling outlets of which bacteria and fungi are prevalence [6, 7, 8]. Adequate microbiological information and handling processes of the fruits could minimize wastage. Tomato fruit being succulent with about 80% water content, low pH, highly rich nutrients elements and sugars, served as suitable medium for microbial growth [9, 4,]. Research has also revealed that post-harvest loss of fruits due to microbial infections in Nigeria ranges between 50% and 90% [10, 11]. In view of the foregoing, there is need to isolate and identify microbes associated with tomato fruits spoilage with the view to proffering suitable solutions of controlling them before reaching the final consumers, to safeguard human health. The objective of this study therefore focused on isolation and identification of pathogenic microorganisms associated with tomato fruit spoilage in Osogbo, Osun State, Nigeria.

2. Materials and methods
Collection of samples
Materials used for this study included ripened healthy and decayed tomato fruits collected from two open markets (Oja-Oba and Sabo) in Osogbo, Osun State, South-Western Nigeria in

15th January 2011. The healthy tomato fruits were undamaged, fresh and firm, while the decayed fruits were rotten. The fruits were separately packaged, labeled and carefully transported immediately to Fountain University Microbiology Laboratory for microbial analyses. The healthy fruits were preserved from insects and dust at room temperature for 14 days to experience a natural spoilage.

Isolation of microbes

The powered nutrient agar of 28g was dissolved in 1 litre of deionized water, allowed to soak for 10 minutes and then sterilized by autoclaving for 15 minutes at 121 ^{0}C. It was thereafter cooled and then poured into petri dishes. Potato dextrose agar of 39g was also dissolved in 1 litre of distilled water and boiled to dissolve the medium completely before sterilizing with autoclave at 121 ^{0}C for 15 minutes. The pH of the sample was adjusted to 3.5, after adding 10ml of lactic acid solution, to facilitate microbial growth. The medium was thereafter cooled to 55 ^{0}C and poured into petri dishes. The fruits were then rinsed with distilled water and serially diluted in 10 folds. The highest three dilutions were considered for microbial counts. The liquid content of each of the tomato cut was inoculated on nutrient agar and potato dextrose agar, incubated at 37 ^{0}C and 25 ^{0}C respectively and observed for 24 hours for 5 days, after which the different isolates were characterized using the slide culture technique. Microscopic examination was carried out after gram staining the bacteria isolates, while Lactophenol blue staining was carried out on fungi isolates [12].

3. Results and discussion

Fresh vegetable fruits including tomato have natural protective cover (epidermal layer) that effectively guide against most pathogenic microbes and plant spoilage. This protection however could be hindered and the fruits may be contaminated during field cultivation, harvesting, post-harvest handling and distribution [13]. Phenotypic characterization of isolated pathogens (Table 1) revealed that both bacteria (*Staphylococcus aureus* and *Bacillus spp*,) and fungi (*Aspergillus flavus* and *Rhizopus stolonifer*) were responsible for tomato fruits spoilage. *Rhizopus stolonifer* had highest number of isolates from healthy and decayed tomato fruits with 37.4% and 28.8% isolates, respectively, while *Bacillus spp* had least number of isolates from healthy and spoilt samples with 11.2% and 21.9% isolates, respectively, across the two markets. This observation was also similar to many researchers that fungi were the major source of tomato fruit spoilage than bacteria [14, 15, 2]. It is also reported that the isolation of soil bacteria, *Bacillus subtilis*, from tomato fruits, was an evidence of opportunistic contamination from human activity [2]. Some earlier researcher also suggested that occurrence of *Aspergillus spp*. (which are carcinogenic) in water might pose a serious health hazard to consumers as the microbes could be absorbed into the fruits through osmotic potential [16].

Table 2 reports pathogenicity of tomato fruits during the ten days deterioration processes, with fungi isolates recording higher magnitude compared with bacteria. Fungal pathogens, *Rhizopus stolonifer* and *Aspergillus flavus* had fast growth rate on the infected the tomato fruits, causing soft rot spoilage with necrotic patches on the surface of the fruits, two days of culturing. Bacteria pathogens, *Staphylococcus aureus* and *Bacillus spp*. on the other hand were recorded slow growth at the early days of culturing till the fourth day, after which the symptoms of fruit colour change appeared on the fruit skins, and followed by wrinkled textures. The occurrence of *Staphylococcus Aureus* in tomato fruits contamination has been reported to be associated with faecal matters through inadequate human handling processes [17]. Many researchers had earlier isolated various strains of bacteria and fungi in tomato fruits. Some earlier researcher observed bacteria *Bacillus megaterium* and *Bacillus Laterosporus*, while on the other hand some researchers found bacteria *Bacillus subtilis*, *Bacillus cereus*, *Bacillus Aureu, Lactobacillus fermenti, Pseudomonas stutzeri, Leuconostoc spp* and *Rothia spp*, as well as fungi *Aspergillus flavus, Aspergillus fumigatu, Penicillium expansum, Penicillium notatum, Escherichia coli, Mucor mucedo, Monilia spp, Klebsiella aerogenes, Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus, Rhizopus stolonifer, Botrytis cineria, Saccharomyces spp, Rhodotorula spp* and *Verticillium albo-atrum* from tomato fruits [13, 18, 19, 2]. The discoloration associated with bacterial infection on tomato fruits had been reported earlier by [20] Similar report was observed in sweet orange fruits damage from [21, 22]. Furthermore, *Rhizopus stolonifer* which possibly caused the most rapid rot on stored tomato fruits in Nigeria observed by [23].

Figure 1 indicates that spoilt tomato fruit samples collected from Sabo market had higher number of isolates (86 isolates) than Oja-Oba (46 isolates). This indicated that tomato fruits sold at Sabo market had higher cases of microbial infections, than those sold at Oja-Oba in Osogbo. It was also observed that tomato sellers and retailers at Sabo market did not maintain proper sanitary and hygienic practices in the market, which probably enhanced microbial contamination of fruits, while Oja-Oba market showed better sanitation condition that could lessen the microbial deteriorations of fruits.

Table 1: Number of isolates and prevalence percentage of microorganisms obtained from healthy and decayed tomato fruits collected from Sabo and Oja-Oba markets in Osogbo, Nigeria

Pathogen	Microorganisms	Healthy tomato isolates	Prevalence (%)	Spoilt tomato isolates	Prevalence (%)
Bacteria	*Staphylococcus aureus*	23	21.4	33	25.0
	Bacillus spp	12	11.2	29	21.9
Fungi	*Rhizopus stolonifer*	40	37.4	38	28.8
	Aspergillus flavus	32	29.9	32	24.2
Total		107	100	132	100

Table 2: Four isolated microorganisms associated with tomato fruits spoilage collected from Sabo and Oja-Oba markets in Osogbo, Nigeria

Days after inoculation	*Aspergillus flavus*	*Rhizopus stolonifer*	*Staphylococcus aureus*	*Bacillus spp*
1	-	-	-	-
2	+	+	-	-
3	+	++	-	-
4	+	++	+	+
5	++	++	++	++
6	++	++	++	++
7	++	++	++	++
8	++	++	++	++
9	++	++	++	++
10	++	++	++	++

Keys: - = not infected, + = infected and = ++ high infections

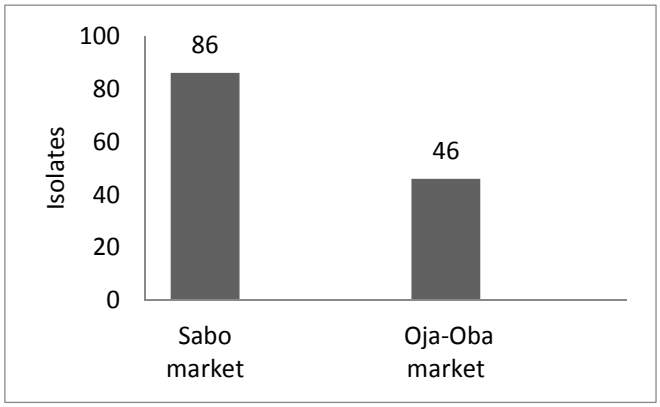

Fig 1: Total number of isolates of four pathogenic microorganisms obtained from spoilt tomato fruits collected from Sabo and Oja-Oba markets in Osogbo, Nigeria

4. Conclusion

Tomato fruits were more susceptible to fungal attack than bacteria damage, and *Rhizopus stolonifer* appeared to be the most active of all pathogens associated with tomato fruit spoilage. Therefore the general public should be enlightened on the potential health hazards bedevilling consumption of relatively cheaper ripen-spoilt tomato fruits, as these may be the mediators in food borne fungal and bacterial diseases. Market wastes and refuse should also be properly disposed of at designated sites to reduce microbial contaminations. Precautionary measures of preservation and handling processes of fruits such as proper sanitary conditions, treatment with antimicrobial agents (chlorinated water) and refrigeration are necessary not only in reducing microbial toxins deleterious to human health, but also enhanced the fruits' shelf-life.

5. References

1. Akinmusire OO. Fungal species associated with the spoilage of some edible fruits in Maiduguri, Northern Eastern Nigeria. Adv. Environ. Biol. 2011; 5(1):157-161.
2. Wogu MD, Ofuase O. Microorganisms responsible for the spoilage of tomato fruits, *Lycopersicum esculentum*, sold in markets in Benin City, Southern Nigeria. Sch. Acad. J. Biosci. 2014; 2(7):459-466.
3. Abhinaba G. Identification of microorganisms responsible for spoilage of tomato (*Solanum lycopersicum* L) fruit. J. Phytopath. 2009; 1(6):414-416.
4. Muhammad S, Shehu K, Amusa NA. Survey of the market diseases and aflatoxin contamination of tomato (*Solanum lycopersicum* MILL) fruits in Sokoto North Western Nigeria. Nutrit. Food Sci., 2004; 34:72-76. http://dx.doi.org/10.1108/00346650410529032
5. Baiyewu RA, Amusa NA, Ayoola OA, Babalola OO. Survey of the post-harvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya L*) in south western Nigeria. Afr. J. Agric. Sci. 2007; 2(4):178-181.
6. Barth M, Hankinson TR, Zhuang H, Breidt F. Microbiological Spoilage of Fruits and Vegetables. W.H. Sperber, M.P. Doyle (eds.), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety. C Springer Science Business Media, LLC, 2009, 135-183. http://dx.doi.org/10.1007/978-1-4419-0826-16
7. Fung DYC. Spoilage, preservation and quality control. Encyclopedia of Microbiology, 2009; 54-79.
8. Akinyele BJ, Akinkunmi CO. Fungi associated with the spoilage of berry and their reaction to magnetic field. J. Yeast and Fungal Res. 2012; 3(4):49-57.
9. Singh D, Sharma RR. Postharvest disease of fruit and vegetables and their management. In: Prasad, D. edition sustainable pest management. Daya Publishing House, New Delhi, India, 2007, 183-189.
10. Wokoma ECW. Preliminary report on disease of tomatoes in Choba, Rivers State. J. Appl. Sci. Environ. 2008; 12(3):178-121.
11. Eni AO, Ibokunoluwa O, Oranusi U. Microbial quality of fruits and vegetables. Afr. J Food Sci. 2010; (5):291-296.
12. Amusa NA, Kehinde IA, Ashaye OA. Biodeterioration of bread fruit in storage and its effects on the nutrient composition. African Journal of Biotechnology. 2002; 1(2):57-60.
13. Beuchat LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microb. Infect, 2002; 4:413-423.
14. Ibrahim AD, Musa K, Sani A, Aliero AA, Yusuf BS. Microorganisms associated with the production of volatile compounds in spoilt tomatoes. Res. Biotech, 2011; 2(2):82-89.
15. Matthew T. Post-harvest microbial deterioration of tomato (*Lycopersicon esculentum*) fruits. Report and Opinion, 2011; 3(4):52-57.

16. Ofor MO, Okorie VC, Ibeawuchi II, Ihejirika GO, Obilo OP, Dialoke SA. Microbial contaminants in fresh tomato wash water and food safety considerations in South-Eastern Nigeria. Life Sci. J. 2009; 6(3):80-82.
17. Ghosh A. Identification of microorganisms responsible for spoilage of tomato (*Lycopersicum esculentum*) fruit. J. Phytol. 2009; 1(6):414-416.
18. Chuku EC, Ogbonna DN, Onuegbu BA, Adeleke MTV. Comparative studies on the fungi and biochemical characteristics of snake guard *(Trichosanthes Curcumerina*linn) and Tomato *(Lycopersicon Esculentum Mill*) in Rivers State, Nigeria. J. Applied Sci. 2008; 8(1):168-172.
19. Chinedu SM, Enya E. Isolation of Microorganisms associated with deterioration of tomato (*Lycopersicon esculentum*) and pawpaw (*Carica papaya*) fruits. Int. J. Curr. Microbiol. App. Sci. 2014; 3(5):501-512.
20. Miedes E, Lorences EP. Apple (*Malusdomestica*) and tomato (*Lycopersicum*) fruits cell-wall hemicelluloses and xyloglucan degradation during penicillium expansum infection. J. Agric. Food Chem. 2004; 52:7957-8211.
21. Chuku EC. Spoilage of Avocado pear (*Persea americana*) in River State. J. Res. Biosc. 2005; 1(1):29-31.
22. Tafinta I, Abdulganiyyu H, Usman AR. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State. Nig. J. Basic Appl. Sci. 2013; 21(3):193-196.
23. Okoli CAN, Erinle ID. Comparative rate of rot induction by nine fungal pathogens on stored tomato fruits in Nigeria. J. Stored Prod. Res. 1990; 26:17-90.