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(RESEARCH ARTICLE)

Health impacts of some fungal communities found on the surface of selected citrus fruits from Bwari market Abuja, Nigeria

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Abstract

Fruits are in great demand since they are nutrient-dense. For this reason, it becomes highly essential to create more in the shortest amount of time in order to keep up with the always-rising demand. As a result, there has been a significant chance that these fruits may become contaminated with harmful microbes, raising concerns about consumer safety. This study sought to ascertain the potential health effects of fruit contamination by fungal species found on the fruit surfaces, as there is currently insufficient information in this area. Three (3) species of Citrus (CF) which include; lemon (*Citrus limon* (L.) Burm. f.), Chinese orange or sweet orange (*Citrus sinensis* (L.) Osbeck), and key lime or lime (*Citrus aurantifolia* (Christm.) Swingle) were randomly bought from different hawkers at different parts of Bwari market in Abuja, Nigeria. From the studies, five different fungal species were identified and they include: *Penicillium* spp *Trichophyton* spp, *Aspergillus* spp, *Candida* spp and *Rhizopus* spp. This demonstrated that fungi exist in all environments and that handling fruits improperly can have serious negative health impacts on people since some of these fungi are pathogenic. A number of approaches, such as washing fruits in vinegar water, have been found to be very effective in reducing microbial infection. Other washing methods, such as using saltwater, also work well. Additionally, it is a good idea to regularly replace the water used to wash those fruits in order to prevent cross-contamination while handling.

Keywords: Citrus fruits; Health impacts; Surface contamination; Fungal species; Microbes

1. Introduction

The global production of Citrus is around 102 million tons each year [1]. This Citrus which is commonly grown in the tropics and subtropics, belong to the family, Rutaceae. It grows both as an evergreen tree and as a shrub with a height of between 3 and 15 metres. They are found in moderate to warm temperatures including the Mediterranean with a remarkable number of them being sensitive to frost [2]. All the fruits produced by the different citrus species are commonly called citrus fruits. Every citrus species has its unique colour, taste, flavour and aroma which consumers admire before going for them. Citrus fruits also contain great metabolites that are very healthy for the consumers [3]. Studies on citrus have been carried out in so many countries of the world such as Italy, [4] Nigeria, [5] and Thailand, [6]. These fruits naturally possess immense nutritional, cosmetic and pharmaceutical potentials. Out of the citrus fruits, the species that are highly utilized are *Citrus limon* (lemon), *Citrus sinensis* (orange) and *Citrus aurantifolia* (lime).

Citrus limon has great nutritional values, though its biological activities are not yet fully understood and so are underrated and underutilized in modern cosmetology and phytotherapy [7]. The juice of *C. limon* popularly known as lemon juice was a trusted solution to scurvy before vitamin C was discovered [2]. Ascorbate, L-ascorbic acid or simply Vitamin C, is a major vitamin found in the fruit peels and flesh of citrus fruits. Lemon juice has also been used in folk medicine, to treat irregular menstruation, common cold and high blood pressure. Further, the essential oil extracted from *C. limon* gives huge relief from cough [8, 9]. *C. limon* has anti-inflammatory ability [10], antibacterial ability [11], antifungal ability [11,12], and antiparasitic ability [13]. The most grown citrus worldwide are oranges followed by

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lemon and others; and oils, citric acids and juices are the major products of citrus fruits [14]. *Citrus sinensis* is made up of several useful compounds which include: thiamine, riboflavin and folic acid etc. [15]. Orange peel is a good source of soluble sugar, ascorbic acid, fiber and pectin [16]. Another most discarded waste is the seed, which contain nutrients, micro nutrients and fiber [17, 18].

C. aurantifolia (lime) has several traditional uses which include; antibacterial, antidiabetic [19], antifungal [20], anti-lipidemia [21], and anti-parasitic [22-24]. It is valuable in the treatment of urolithiasis [25]. It is also good in promoting fertility [26], and it can be used as insect repellent [27].

This study tried to identify the fungal species that are found on the surface of some selected citrus fruits and the health impacts they pose. Five different fungal species were discovered from the surface of the fruits studied, and this is either because they were air borne, naturally occurring on the human flora or because of environmental or anthropogenic factors. Thus, it becomes very necessary to adopt proper practices from cultivation to harvesting and then, hawking of those fruits in order to prevent any health problems that might come from their consumption.

2. Materials and methods

2.1. Sample Collection

Five samples each of the three (3) citrus species which include; lemon (*Citrus limon* L.), orange (*Citrus sinensis* (L.) Osbeck), and lime (*Citrus aurantiifolia* (Christm.) Swingle) were randomly bought from different hawkers at different parts of Bwari market in Abuja. All samples were collected in a sterile polythene bag and transported to the Microbiology Laboratory at the Veritas University, Abuja where they were analyzed within one hour after procurement. Samples were rinsed with 100 ml distilled water and diluted 10-fold. After washing the surface of the citrus fruits (CF), 10 ml of the aqueous suspension were obtained which were further inoculated to 90ml potato dextrose agar (PDA), which was the media used for the study, and incubated at 37°C.

2.2. Cultivation and enumeration of fungi

Each sample (10 g) was thoroughly mixed in 10 ml of sterile distilled water. Aliquot (1.0 ml) of it were transferred into the next test tube and diluted serially in one-tenth stepwise to 10^{-4} dilution. From the dilution of 10^{-3} of each sample, 0.1 ml aliquot were transferred aseptically onto freshly prepared potato dextrose agar plates to which 0.2 ml of 0.01 % chloramphenicol were added to inhibit the growth of bacteria to allow only the growth of fungi. The inoculum was spread with a sterile bent glass rod. The dilution of 10^{-3} were used in plating for fungi because the dilution of 10^{4} gave fewer growths. The inoculated plates were inverted and incubated at 28^{o}C (room temperature) for 5 to 7 days. The colonies which developed were noted [28].

2.3. Preparation of Pure Culture

The fungal isolates which developed were sub-cultured onto agar slopes and incubated at 28^{o}C for 5 to 7 days. The isolates which developed were pure cultures which were stored in the refrigerator as stock cultures for subsequent characterization tests**.**

2.4. Characterization and Identification

The fungal isolates cultured on PDA were identified on following standard characterization tests which were performed in duplicates. Macroscopic examination of fungal growth was carried out by observing the colony morphology - diameter, colour (pigmentation), texture and surface appearance. Microscopic examination was done by needle mount method; staining with methylene blue and observing sexual and asexual reproductive structures like sporangia, conidial head, arthrospores and the vegetative mycelium. Sugar (Glucose, Fructose, Lactose, Sucrose, Galactose and Maltose) fermentation tests were also carried out for species identification [28].

The complete identification of fungal isolates was done by comparing the result of their cultural, morphological and biochemical characteristics with those of known taxa.

2.5. Statistical analysis

The frequency and percentage occurrence of the fungal isolates per plate and the fungal species per sample were recorded using SPSS version 26.

3. Results

Table 1 Fungal communities discovered on the studied citrus fruits

Sample	CF	Scientific names	*Candida* spp	*Aspergillus* spp	*Trichophyton* spp	*Rhizopus* spp	*Penicillium* spp
CF 1	Orange	*Citrus sinensis*	+	+	-	-	-
CF 2	Lime	*Citrus aurantifolia*	-	-	+	-	-
CF 3	Lemon	*Citrus limon*	-	+	-	+	+

Table 1 shows the five fungal communities which were discovered on the citrus fruits studied. It was observed that *Aspergillus* spp were found on the surface of the oranges and lemons studied, while other species were found on one citrus fruit at a time.

Table 2 Frequency and percentage occurrence of the fungal communities

Probable organisms	No of citrus surfaces found	% frequency of occurrence
Candida spp	1	16.7
Aspergillus spp	2	33.33
Trichophyton spp	1	16.7
Rhizopus spp	1	16.7
Penicillium spp	1	16.7

Table 2 shows the number of citrus fruits that had fungal communities on their surfaces with their percentage frequency

Table 3 Frequency and percentage frequency of occurrence of the fungal isolates per five plates

Probable organisms	CF1 Frequency (%)	CF2 Frequency (%)	CF3 Frequency (%)	Total
Candida spp	1 (25)	0 (0)	0 (0)	1 (7.1)
Aspergillus spp	3 (75)	0 (0)	3 (37.5)	6 (42.9)
Trichophyton spp	0 (0)	2 (100)	0 (0)	2 (14.3)
Rhizopus spp	0 (0)	0 (0)	3 (37.5)	3 (21.4)
Penicillium spp	0 (0)	0 (0)	2 (25)	2 (14.3)
Total	4 (28.6)	2 (14.3)	8 (57.1)	14 (100)

Table 3 shows how many times different fungal communities were found in every five plates

4. Discussion

The result of this work showed that five fungal communities were found on the surfaces of the studied citrus fruits. The fungal species discovered were: *Candida albicans, Aspergillus* spp, *Trichophyton* spp, *Rhizopus* spp and *Penicillium* spp (Table 1). Among the fungal species isolated, *Aspergillus* spp had the highest prevalence rate of 33.3% followed by the other studied species which had 16.7 % prevalence rate each. From the study, it was recorded that *Candida albicans* and *Aspergillus* spp were found on *Citrus sinensis*, *Trichophyton* spp was found on *Citrus aurantifolia* while *Rhizopus* spp, *Penicillium* spp and *Aspergillus* spp were found on *Citrus limon*; (Table 1). Five plates were prepared for each of the

citrus samples and the number of plates where the fungal communities appeared were recorded. In some plates, fungal species were present while in some, they were absent (Table 3).

Shen [29] stated that the different fungal communities inhabiting fruit surfaces are closely associated with the control and preservation of fruits. From their studies, Tournas and Katsoudas [30] concluded that the most commonly found fungi in citrus fruits are *Penicillium* spp and *Rhizopus* spp. They also found *Aspergillus* spp present as they stated that *Penicillium* spp and *Aspergillus* spp have the ability to grow on fresh fruits [30], and this conforms with the present study. Liu et al [31] stated that *Rhizopus* spp has also been found on stored fruits after harvest. This can be due to environmental, and human factors such as handling. When fruits are contaminated, their food or nutrient values are reduced. This confirmed the conclusion made by Chanda et al [32] that the variable communities of microorganisms most especially fungi which are found on fruit surfaces have significant impact on the destruction of fruit quality, yield and their food values. This study also confirmed the conclusion made by Tournas and Katsoudas [30] that freshly harvested fruits are liable to get contaminated in the field, as well as during harvest, transportation and marketing of those fruits. That is to say that, every process the fruits undergo is capable of subjecting them to contamination. The implication of this is that some persistent fungal pathogens such as *Aspergillus* spp can cause aspergillosis, which can be lethal to humans [33]. Ezigbo and Makolo [33] also stated that as far as fungal species such as *Penicillium* spp and *Aspergillus* spp can produce mycotoxins, which can pose as a serious threat to both hawkers and consumers, adequate measures should be put in place to avoid fungal contamination.

Trichophyton spp which was found on lime surfaces has been known for ages to cause dermatophytosis. This fungal disease respects no age. Therefore, adequate awareness creation is important at all levels in order to let the rural, semi-urban and urban dwellers know the adverse health implications of some unhealthy practices they carry out, such as washing the fruits with contaminated water or eating them right away at the point of purchase without proper washing.

5. Conclusion

Microbes are naturally occurring and since some of them are pathogenic, adequate control measures are necessary from the harvesting to the marketing of those fruits to avoid contaminations and cross contaminations. Hawkers are advised to avoid using unclean and contaminated water such as one from the gutter or ditch to wash the harvested or the ready-to-be-sold fruits in order to prevent the health challenges that could come from such practices.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Mehl F, Marti G, Boccard J, Debrus B, Merle P, Delort E. et al. (2014). Differentiation of lemon essential oil based on volatile and non-volatile fractions with various analytical techniques: A metabolomic approach. Food Chemistry, 143, 325–335.

[2] Mabberley DJ. (2004). Citrus (Rutaceae): A review of recent advances in etymology, systematics and medical applications. Blumea – Journal of Plant Taxonomy and Plant Geography, 49, 481–498.

[3] Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O, et al. (2015). Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chemistry Central Journal, 9, 68.

[4] Costa R, Bisignano C, Filocamo A, Grasso E, Occhiuto F, Spadaro F. (2014). Antimicrobial activity and chemical composition of *Citrus aurantifolia* (Christm.) Swingle essential oil from Italian organic crops. Journal of Essential Oil Research, 26, 400-408.

[5] Lawal O, Ogunwande I, Owolabi M, Giwa-Ajeniya A, Kasali A, Abudu F, et al. (2014). Comparative analysis of essential oils of *Citrus aurantifolia* Swingle and Citrus reticulata Blanco, from two different localities of Lagos State, Nigeria. American Journal of Essential Oils and Natural Products, 2, 8 - 12.

[6] Kaewsuksaeng S, Tatmala N, Srilaong V, Pongprasert N. (2015). Postharvest heat treatment delays chlorophyll degradation and maintains quality in Thai lime (*Citrus aurantifolia* Swingle cv. Paan) fruit. Postharvest Biology and Technology, 100, 1-7.

[7] Goetz P. (2014). *Citrus limon* (L.) Burm. f. (Rutacées). Citronnier. Phytotherapie, 12, 116–121.

[8] Clement YN, Baksh-Comeau YS, Seaforth CE. (2015). An ethnobotanical survey of medicinal plants in Trinidad. Journal of Ethnobiology and Ethnomedicine, 11, 1–28

[9] Bhatia H, Pal Sharma Y, Manhas RK, Kumar K. (2015). Traditional phytoremedies for the treatment of menstrual disorders in district Udhampur, J&K, India. Journal of Ethnopharmacology, 160, 202–210

[10] Amorim JL, Simas DLR, Pinheiro MMG, Moreno DSA, Alviano CS, Da Silva AJR, Fernandes PD. (2016). Anti-inflammatory properties and chemical characterization of the essential oils of four Citrus species. PLoS ONE, 11, 1–18.

[11] Millet F. (2014). Huiles essentielles et essence de citronnier (*Citrus limon* (L.) Burm. f.). Phytotherapie, 12, 89–97.

[12] Guerrini A, Rossi D, Grandini A, Scalvenzi L, Rivera PFN, Andreotti E, Tacchini M, Spagnoletti A, Poppi I, Maietti S, et al. (2014). Biological and chemo-diverse characterization of Amazonian (Ecuador) Citrus petitgrains. Journal of Applied Botany and Food Quality, 87, 108–116.

[13] Aboelhadid SM, Mahrous LN, Hashem SA, Abdel-Kafy EM, Miller RJ. (2016). In vitro and in vivo effect of *Citrus limon* essential oil against sarcoptic mange in rabbits. Journal of Parasitology Research, 115, 3013–3020.

[14] Food and Agriculture Organization of the United Nations. (2022). Citrus. Markets and Trade. Available online at: https://www.fao.org/markets-and-trade/ commodities/citrus/en/ (accessed June 6, 2022).

[15] U.S. Department of Agriculture. (2019). Oranges, raw, navels. Available online at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/746771/nutrients (accessed April 14, 2022).

[16] Sathiyabama RG, Gandhi GR, Denadai M, Sridharan G, Jothi G, Sasikumar P, et al. (2018). Evidence of insulin-dependent signalling mechanisms produced by *Citrus sinensis* (L.) Osbeck fruit peel in an insulin resistant diabetic animal model. Food and Chemical Toxicology, 116, 86–99.

[17] Zayed EA, AinShoka AA, El Shazly KA, Abd El Latif HA. (2018). Improvement of insulin resistance via increase of GLUT4 and PPARγ in metabolic syndrome-induced rats treated with omega-3 fatty acid or L-carnitine. Journal of Biochemical and Molecular Toxicology, 32, e22218.

[18] Adubofuor J, Akyereko YG, Batsa V, Apeku O-JD, Amoah I, Diako C, et al. (2021). nutrient composition and physical properties of two orange seed varieties. International Journal of Food Science, 1–11.

[19] Karimi A, Nasab N. (2014). Effect of garlic extract and *Citrus aurantifolia* (lime) juice and on blood glucose level and activities of aminotransferase enzymes in streptozotocin-induced diabetic rats. World Journal of Pharmacy and Pharmaceutical Sciences, 2, 824-827

[20] Balamurugan S. (2014). In vitro antifungal activity of *Citrus aurantifolia* Linn plant extracts against phytopathogenic fungi *Macrophomina phaseolina*. International Letters of Natural Sciences, 13, 70-74.

[21] Ezekwesili-Ofili J, Gwacham N. (2015). Comparative effects of peel extract from Nigerian grown Citrus on body weight, liver weight and serum lipids in rats fed a high-fat diet. African Journal of Biochemistry Research, 9, 110-116.

[22] Enejoh S, Shuaibu K, Suleiman M, Ajanusi J. (2014). Evaluation of anthelmintic efficacy of extracts of *Citrus aurantifolia* fruit juice in mice experimentally infected with *Heligmosomoides bakeri*. International Journal of Biological Research 4, 241-246.

[23] Enejoh S, Suleiman M, Ajanusi J, Ambali S. (2015). In vitro anthelmintic efficacy of extracts of *Citrus aurantifolia* (Christm) Swingle fruit peels against Heligmosomoides bakeri ova and larvae. International Journal of Current Pharmaceutical Research, 7, 92-96.

[24] Domínguez-Vigil I, Camacho-Corona M, Heredia-Rojas J, Vargas-Villarreal J, Rodríguez-De la Fuente A, Heredia-Rodríguez O, et al. (2015). Anti-giardia activity of hexane extract of *Citrus aurantifolia* (Christim) Swingle and some of its constituents. African Journal of Traditional, Complementary and Alternative Medicines, 12, 55-59.

[25] Anggraini T. (2015). Potency of Citrus (*Citrus aurantium*) water as inhibitor calcium lithogenesis on urinary tract. Medical Journal of Lampung University, 4, 99-104.

[26] Okon U, Etim B. (2014). *Citrus aurantifolia* impairs fertility facilitators and indices in male albino wistar rats. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 3, 640-645.

[27] Soonwera M. (2015). Efficacy of essential oils from Citrus plants against mosquito vectors *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say). Journal of Agricultural Science and Technology, 11, 669-681.

[28] Agada EO, Ngwai YB, Ishaleku D. (2022). Molecular diversity among *Candida albicans* isolated from high vaginal swab (HVS) of patients attending health care in Nasarawa State, Nigeria*. Nigeria Annals of Pure and Applied Sciences,* 5(1), 1-11.

[29] Shen Y, Nie J, Li Z, Li H, Wu Y, Dong Y, Zhang J. (2018). Differentiated surface fungal communities at point of harvest on apple fruits from rural and peri-urban orchards. Scientific reports, 8, 2165.

[30] Tournas VH, Katsoudas E. (2005). Mould and Yeast flora in fresh berries, grapes and citrus fruits. International Journal of Food Microbiology, 105 (1), 11 – 17.

[31] Liu Q, Chen Q, Liu H, Du Y, Jiao W, Sun F, Fu M. (2024). *Rhizopus stolonifer* and related control strategies in postharvest fruit: A review. Heliyon, 10(8), e29522.

[32] Chanda D, Sharma GD, Dey M. (2023). Isolation and Characterization of surface fungi from local fruits of Meghalaya, India. Biological Forum - An International Journal, 15(2), 965 – 971.

[33] Ezigbo EC, Makolo D. (2024). Fungal species associated with the surface of selected green leafy vegetables from Bwari market, Abuja – Nigeria: implications on consumer health. World Journal of Advanced Engineering Technology and Sciences, 12(2), 208-213.