World Journal of Advanced Engineering Technology and Sciences
eISSN: 2582-8266
Cross Ref DOI: 10.30574/wjaets
Journal homepage: https://wjaets.com/

(RESEARCH ARTICLE)

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Fungal species associated with the surface of selected green leafy vegetables from Bwari market, Abuja - Nigeria: implications on consumer health

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World Journal of Advanced Engineering Technology and Sciences, 2024, 12(02), 208–213

Publication history: Received on 28 May 2024; revised on 11 July 2024; accepted on 13 July 2024

Article DOI: https://doi.org/10.30574/wjaets.2024.12.2.0283

Abstract

The growing demand for fresh vegetables has necessitated larger production and the substantial production of vegetables within the shortest possible time to meet the ever-increasing demand has placed them at a higher risk of contamination with the pathogenic microbes, making the safety of consumers uncertain. This study aimed to discover the fungal species found on the surface of green leafy vegetables and their implications on consumers' health. Four (4) species of green leafy vegetables (GLV) which include; waterleaf (*Talinum fruticosum* (L.) Juss.), African basil (*Ocimum gratissimum* L.), green amaranth (*Amaranthus viridis* L.) and fluted pumpkin leaves (*Telfairia occidentalis* Hook.f.) were randomly purchased from different parts of Bwari market in Abuja, Nigeria and analyzed using pour plate method. The studies discovered four different fungal species, including *Aspergillus* spp, *Candida albicans*, *Rhizopus* spp, and *Penicillium* spp. This confirmed that microorganisms naturally occur in every environment and can get food products contaminated. Since some of them are pathogenic, unhygienic handling of those vegetables can cause severe health effects on humans. Several measures have given positive hopes in controlling contamination caused by microbes which include washing vegetables with vinegar water. Washing vegetables with saltwater is also effective but adequate precautionary measures are necessary which include ensuring that the same water is not used to wash different vegetables from different sources that could be carrying different microbes.

Keywords: Green leafy vegetables; Consumer health; Contamination; Fungi; Microorganisms

1. Introduction

Vegetables contribute to more than 33% of world agricultural production and vegetable farming has employed about 800 million people [1]. Vegetables are among the 40 most cultivated plant species globally. African indigenous leafy vegetables (AILVs) are found in Sub-Saharan Africa (SSA) [2], where their market gardening represents an important sector of activity because of their nutritional composition, but also because they provide the farmers with a reasonable source of income. In Nigeria, the market gardening of vegetables represents on average, 27% of the gross national product (GDP).

The young shoots including the leaves, flowers, seeds, stems, tubers, and roots of vegetables are consumable and they have been part of the food systems for ages [2]. Vegetables contain high levels of nutrients and they could be an important source of nutrition in rural areas, where they can help curb the problem of malnutrition among poor populations [3]. Microorganisms are ubiquitous with a few exceptions, including sterilized surfaces. They include normal flora which is non-pathogenic, contributing to the larger percentage, and few pathogenic species. The activities of humans cannot be completely separated from them. Hence, most pathogenic microbes have found their way into fresh vegetables which are a great source of healthy diet for humans.

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The fungal kingdom includes as many as 6 million species [4] and is remarkable in terms of the broadness of its impact on several areas of human endeavours such as global health, agriculture, biodiversity, ecology, manufacturing, and biomedical research. More than 600 fungal species are associated with humans, either as commensals or as members of our microbiome, or as pathogens that cause some of the deadliest infectious diseases [5]. Individuals whose immune systems are already weak, are the most vulnerable. At the same time, healthy individuals are also at risk from well-known and emerging pathogens, especially in cases where the infection involves a large inoculum. With the current global hike in the incidence of fungal infections from invasive species of fungi and the spread of pathogenic fungi that are resistant to all current classes of antifungals, these organisms constitute serious threats to human health [6,7].

Also, the conditions of commercial washing and distribution of these vegetables may not be sufficient to reliably control human pathogens on fresh farm produce [8]. Since pathogens can adapt to dwelling on leaf surfaces and potentially penetrating internal leaf structures, this undermines the usefulness of chemical sanitation methods in preventing their transmission through contaminated produce. Generally, it is necessary to have a deeper understanding of how pathogens survive on vegetable surfaces to develop ways to reduce the risk to human health and the associated economic consequences [9].

The consumption of fresh vegetables that are contaminated is connected to occurrences of human food poisoning [10,11]. Outbreaks of foodborne illnesses are caused by contamination of fresh vegetables that are exposed to faecally infected manure fertilizers, irrigation with faecally contaminated water, and/or contaminated ice washing during their handling and transport [12]. Some researchers also stated that disinfectant wash is essential to reduce the microbial loads on vegetables [13]. This study tried to identify the fungal species that are found on the surface of green leafy vegetables and the implications on consumers' health. From the study, four fungal species were discovered as either naturally occurring in the environment or from human flora. Therefore, it becomes essential to be more careful in the cultivation and handling methods exhibited by farmers and hawkers towards the vegetables to prevent the health problems that might arise after their consumption by consumers.

2. Materials and methods

2.1. Sample Collection

Four samples each of the four (4) species of green leafy vegetables (GLV) which include; waterleaf (*Talinum fruticosum* (L.) Juss.), African basil (*Ocimum gratissimum* L.), green amaranth (*Amaranthus viridis* L.) and fluted pumpkin leaves (*Telfairia occidentalis* Hook.f.) were randomly purchased from different hawkers at different parts of Bwari market in Abuja. All samples were collected in sterile polythene bag and transported to the Microbiology Laboratory at the Veritas University, Abuja where they were analyzed within one hour after purchase. Samples were rinsed with 100 ml distilled water and diluted 10-fold. After washing the surface of the GLVs, 10 ml of the aqueous suspension were obtained which were further inoculated to 90ml potato dextrose agar (PDA) and incubated at 37°C.

2.2. Cultivation and enumeration of fungi

Each sample (10g) was thoroughly mixed in 10ml of sterile distilled water. Aliquot (1.0ml) 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, 0.1ml aliquot were transferred aseptically onto freshly prepared potato dextrose agar plates to which 0.2ml 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.

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 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 [14] and observing sexual and asexual reproductive structures like sporangia, conidial head, arthrospores and the vegetative mycelium.

The complete identification of fungal isolates was done by comparing the result of their cultural, morphological and biochemical characteristics with those of known taxa [15,16].

2.5. Statistical analysis

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

3. Results

Table 1 Some fungal species discovered on the vegetables

Sample	GLV	Scientific names	*Aspergillus* spp	*Rhizopus* spp	*Penicillium* spp	*Candida albicans*
GLV 1	Green amaranth	*Amaranthus viridis*	+	+	+	-
GLV 2	African basil (scent leaf)	*Ocimum gratissimum*	+	+	-	-
GLV 3	Fluted pumpkin	*Telfairia occidentalis*	+	-	+	+
GLV 4	Water leaf	*Talinum fruticosum*	-	+	-	-

Table 1 above showed the four fungal species discovered on the green leafy vegetables. It was observed that *Aspergillus* spp were the most dominant fungal species found on the vegetable surfaces.

Table 2 Colony characteristics of the Fungi species discovered

Probable organisms	Colour	Reverse colour	Edge	Pigmentation	Surface characteristics
Aspergillus spp	Dark brown	white	Whitish irregular	Yes	Cottony/Powdery
Rhizopus oryzae	Dark-grey	white	Whitish irregular	Yes	Cottony
Candida albicans	white	Cream	Whitish irregular	Yes	Cottony
Penicillium spp	green	Pale green	Whitish regular	Yes	Powdery

Table 2 above shows the different isolated fungal species and their characteristics.

Table 3 Frequency and Percentage occurrence of the fungal species

Probable organisms	Number of occurrences	% frequency
Aspergillus spp	3	33.33
Rhizopus oryzae	3	33.33

Candida albicans	1	11.11
Penicillium spp	2	22.22

Table 3 above shows the frequency and the percentage occurrence of each isolated fungal species per sample

Table 4 Frequency and percentage frequency of the occurrence of the fungal isolates per four plates

Probable organisms	GLV1 Frequency (%)	GLV2 Frequency (%)	GLV3 Frequency (%)	GLV4 Frequency (%)	Total
Aspergillus spp	3 (42.86)	2 (50)	4 (40)	0 (0)	9 (39.13)
Rhizopus oryzae	3 (42.86)	2 (50)	0 (0)	2 (100)	7 (30.43)
Penicillium spp	1 (14.29)	0 (0)	3 (30)	0 (0)	4 (17.39)
Candida albicans	0 (0)	0 (0)	3 (30)	0 (0)	3 (13.04)
Total	7 (30.43)	4 (17.39)	10 (43.48)	2 (8.70)	23 (100)

Table 4 above showed how many times the fungal species occurred in every four plates analyzed

4. Discussion

The result of this work showed that four fungal species were associated with the contamination of the green leafy vegetables studied. The fungal species discovered were: *Aspergillus* spp, *Rhizopus* spp, *Penicillium* spp and *Candida albicans* (Table 1). Each fungal species isolated had a unique characteristic (Table 2). Among the fungal species isolated, both *Aspergillus* spp and *Rhizopus* spp had the highest occurrence with 33.33% rate followed by *Penicillium* spp which had 22.22% rate, with *Candida albicans* having the least rate of 11.11% occurrence. From the study, it was recorded that *Aspergillus* spp, *Rhizopus* spp and *Penicillium* spp were found on *Amaranthus viridis*; *Aspergillus* spp and *Rhizopus* spp were found on *Ocimum gratissimum*; *Aspergillus* spp, *Penicillium* spp and *Candida albicans* were found on *Telfairia occidentalis*, and only *Rhizopus* spp was found on *Talinum fruticosum* (Table 3). Four plates were prepared for each of the sample and the number of plates where the fungal species appeared was recorded. In some plates, fungal species were present while in some, they were absent (Table 4).

Kakde & Kakde [17] studied the fungal species associated with different types of vegetables and concluded that the dominant species of fungi found on vegetables were *Aspergillus* spp, *Penicillium* spp and *Rhizopus* spp which conforms with this present work. Yaradua et al. [18] also isolated some fungal species from selected vegetables in Katsina - Nigeria, and concluded that all the vegetables showed presence of fungi mostly *Aspergillus* spp, *Penicillium* spp and *Rhizopus* spp, which also agrees with this work. Abu et al. [19] worked on the microbial contamination of *Telfairia occidentalis* and *Amaranthus* spp in Ilorin – Nigeria, and noted that *Aspergillus* spp, *Penicillium* spp and *Rhizopus* spp were present in high numbers. From their work, they deduced that consuming unhygienic vegetables lead to the ingestion of reasonable number of human pathogenic fungi [19].

Tsado et al. [20] studied the fungi associated with *Amaranthus* spp and *Telfairia* spp from Minna – Nigeria, and also discovered a high occurrence of *Aspergillus* spp, *Rhizopus* spp and *Penicillium* spp. They stated that it will be worthwhile to get farmers educated on the need to use hygienic water for irrigation as this will help in the reduction of fungal loads [20]. Sani et al. [21] also worked on the isolation of fungi associated with the spoilage of vegetables sold in Dutse ultra-modern market Jigawa – Nigeria, and by discovering *Aspergillus* spp and *Penicillium* spp as the highest occurring fungal species, they concluded that it is necessary that vegetables be stored at appropriate temperature after washing them in order to minimize their level of contamination.

From this study, *Candida albicans* was discovered on *Telfairia occidentalis* and being one of the commonest and highly consumed vegetables, it is possible that through improper handling, the skin of a carrier must have gotten the vegetables contaminated. Although some fungal species such as *Penicillium* are generally non-pathogenic, *Aspergillus* spp, *Rhizopus* spp and *Candida albicans* are all pathogenic at different levels.

Since *Rhizopus* spp are naturally occurring and can settle on any surface including vegetables, if care is not taken, they can cause fatal diseases such as mucormycosis, as opportunistic pathogens.

On the other hand, *Aspergillus* spp can also cause a life-threatening disease known as aspergillosis on humans since they are equally opportunistic pathogens. *Candida albicans* which is a natural pathogenic fungus of man, can lead to severe health conditions in immunocompromised individuals. Also, fungal pathogens such as *Aspergillus* and *Penicillium* produce mycotoxins and some mycotoxins are considered carcinogenic and can pose a serious threat to the health of consumers.

5. Conclusion

Microorganisms are found everywhere in nature but their continuous transmission is dependent on several factors. For example, in the case of vegetables, if farmers adopt adequate methods of sanitation during farming by using clean irrigation water devoid of any contamination on the vegetables, and if the hawkers avoid using just any water such as one from gutters or some road-side pools to wash the vegetables that would be sold to the consumers, and also remain hygienic themselves, then microbial contamination will be minimal at all levels.

Thus, adequate precautionary measures are needed while handling edible green leafy vegetables to prevent contaminations and cross contaminations which can cause severe health challenges on the consumers.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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