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Full Length Research Article

DECONTAMINATION EFFICACY OF TABLE SALT AND VINEGAR ON FUNGAL CONTAMINANTS OF FRESH VEGETABLES

*[1,2]Davou K.V., [1]Dapiya H.S., [1]Zumbes H.J., [1]Dul H.H., [1]Cirfat N.A., [2]Mathew C.L., [1]Tsok E.A.

[1]Department of Microbiology, University of Jos-Nigeria
[2]Department of Nutrition and Dietetics, College of Health Technology Zawan, Jos – Nigeria

*Corresponding Author Email Address: talk4torypam@gmail.com

ABSTRACT

Fresh vegetables harbor fungi species of substantial health risk, their removal is therefore crucial for public safety. This study determined the decontamination efficacy of table salt (NaCl) and vinegar (white vinegar) on fungal contaminants of fresh leafy vegetables sold in open markets of Farin Gada Jos, Nigeria. A total of 30 samples of cabbage heads and lettuce leaves were purchased and evaluated for mycological quality. Fungal decontamination effectiveness of table salt and vinegar on vegetables was assessed at 2.5%, 5.0%, 7.5%, and 10.0% for 2.30-minute intervals. The result showed total fungal counts of cabbage heads and lettuce leaves varied from 1.45 × 10^6 to 3.61 × 10^6 (CFU\g) respectively. Species of *Aspergillus niger, Candida tropicalis, Penicillium notatum, Trichophyton rubrum,* and *Mucor* spp. were dominant contaminants of the vegetables. Strains of *Aspergillus niger* 52 (20.16) were the most detected followed by *Penicillium notatum*50 (19.38), while *Candida tropicalis* 14 (5.43) were the least occurred. Data of this study revealed that salt and vinegar are both active decontaminating agents against fungi contaminants of fresh vegetables. The decontaminating efficacy of both salt and vinegar solutions were concentration and exposure time ($P < 0.05$) dependent. The result showed that vinegar solution exhibited higher (51.16 %; 2.10 ×10^6CFU/g) fungal decontamination efficacy than salt (46.51%; 2.30 ×10^6 CFU/g) in lettuce leaf at lower concentrations. At high concentrations (10%) however, salt and vinegar applied for 10 min reduced fungal load ($p > 0.05$) by 99.53 % (4.30 ×10^6 - 2.00 ×10^4 CFU/g) and 99.77 % (4.30 ×10^6 - 1.00 ×10^4 CFU/g) respectively in fresh lettuce. Similarly, a fungal load reduction of 96.00 % (1.50 ×10^6 - 60 ×10^4 CFU/g) and 100% was observed in salt and vinegar decontaminated cabbage respectively. Moreover, *Penicillium notatum, Aspergillus niger,* and *Candida tropicalis* were persistent at the end of the decontamination. The salt and vinegar solutions effectively reduced fungal contaminants of fresh vegetables, with their decontamination efficacy significantly influenced by both concentration and contact time.

Keywords: Decontamination Efficacy, Vinegar, Table Salt, Fungi, Fresh Vegetables.

INTRODUCTION

Leafy vegetables are consumed most commonly as minimally processed or uncooked to maintain their nutritional value and health benefits (Abadias *et al.*, 2008; Razzak *et al.*, 2023). In most developing countries such as Nigeria, the vegetables are cultivated under unrestricted or untreated irrigation water (Traoré *et al.*, 2013; Dia, 2017; Traoré *et al.*, 2018, Traoré, 2020). Owing to these practices, vegetables are susceptible to microbiological contamination by direct or indirect contact during cultivation and post-harvest handling (Eni *et al.*, 2010). Considering these, the possibility of fresh vegetables becoming contaminated along the food chain is increasingly becoming a health risk concern (Balali, *et al.*, 2020; Kłapeć *et al.*, 2022). This concern is heightened by the reason that vegetables are minimally cooked and could shoot foodborne disease outbreaks.

Typically, fungi pathogens of *Alternaria alternate*, *Absidia corymbifera*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium herbarum*, *Fusarium oxysporium*, *Geotrichum candidum*, *Mucur racemosus*, *Rhizopus stolonifer* and *Trichoderma harzianum* are common, postharvest contaminants of vegetables (Shehu *et al.*, 2014). Fungi species of *Penicillium*, *Botrytis cinerea*, and *Ceratocystis fimbriata* are also consistent contaminants of fresh vegetables (Maffei *et al.*, 2013; Tahar *et al.*, 2021; Uka-Okali *et al.*, 2023). Although the fungi naturally do not cause acute disease, however, species of *Fusarium*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Geotrichum*, and *Alternaria* are key potential mycotoxin producers and allergens (Skóra *et al.*, 2016; Kłapeć1 *et al.*, 2021).

With the increasing number of vegetable-related disease outbreaks, several microbial decontamination approaches for fresh vegetables have emerged. Conventionally, the water washing method is traditionally used for pathogens decontamination from vegetables in many homes. The water washing technique, particularly when used alone, is a less efficient decontamination process for the inhabitant microbial flora of vegetables (Turantaş *et al.*, 2018; Subramanya *et al.*, 2018). In most developing countries, access to clean water is a herculean task and consequently, the vegetables are washed in conserved containers to enable water reuse, resulting in increased contamination risks (WHO, 2017a; Kumwenda, 2019). Although microbial decontamination of fresh produce using chemical sanitizers such as sodium hypochlorite, hydrogen peroxide, and peroxyacetic acid is effective (Banach *et al.*, 2021; Truchado *et al.*, 2020). These chemical sanitizers, however, are corrosive and can result in the production of potentially hazardous by-products (Coroneo *et al.*, 2017). For safety and consumer preference, organic acids such as citric, acetic, vinegar solutions, and lactic acids found application as an alternative decontamination agent for fresh vegetables (Temiz *et al.*, 2010). So far, most attention is focused on the bacteria and helminths decontamination in vegetables (Hajipour *et al.*, 2021; Maia *et al.*, 2022). Of concern, only a few attentions are placed on fungal decontamination in vegetables. This study determined the surface decontamination efficacy of table salt and vinegar on fungal contaminants in fresh leafy cabbage and lettuce.

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MATERIALS AND METHODS

Sample Area

This study was conducted on samples of fresh vegetables sold in the Farin Gada vegetable market in Plateau State, Nigeria. The Farin-Gada market is secluded vegetable markets for domestic consumption within the urban population of Jos and shipment to other parts of Nigeria. The plateau is situated in the middle belt between latitude 9°53" N and latitude 9° 56"N of the equatorial plane and longitude 8° 54"E and longitude 8° 52"E of the Greenwich Meridian (ZODML, 2013). Typically, the belt has a cool rainy climate with a mean annual rainfall of 1400 mm, characterized by mild climatic temperatures (10ºC to 32.2ºC) conducive for the growth of exotic vegetables all year round.

Collection of Fresh Vegetable

A total of 30 samples each of fresh cabbage (*Brassica oleracea*) heads and lettuce (*Lactuca sativa*) leaves were randomly purchased from vendors in the Farin Gada vegetable market in Jos, Nigeria. In this study, duplicate samples were collected randomly from five stratified areas in the market. The vegetable samples were labeled and packed in low-density polythene bags previously disinfected with 70% ethanol and analyzed immediately.

Preparation of Samples

Samples of cabbage and lettuce heads were shredded using a flame-sterilized stainless-steel knife into disinfected trays.

Preparation of Treatment Solutions

Stock salt and vinegar solutions were diluted to obtain concentrations of 2.5%, 5.0%, 7.5%, and 10.0% weight/volume (w/v). For the table salt solutions, 2.5g, 5.0g, 7.5g, and 10.0g of table salt were dissolved in 100 mL of sterile deionized water in separate conical flasks. The vinegar solutions were prepared by dissolving 2.5 mL 5.0 mL 7.5mL and 10.0 mL of vinegar in 97.5mL, 95.0 mL 92.5 mL, and 90 mL of sterile deionized water to achieve the desired volume/volume (v/v) concentrations.

Vegetable Surface Decontamination Procedure

The decontamination procedure involved immersing 400g of shredded vegetables in 2 liters of sterile distilled water for one minute. This was then followed by immersion in different concentrations of treatment solutions for 0, 2.30, 5.00, 7.30-, and 10.30-min contact time. Afterward, the disinfected vegetables were then rinsed in two liters of distilled water for 1 min and then strained in a 70 % alcohol-scrubbed cullender for 5 min. The control samples were not subjected to any surface decontamination method or rinsed with 2 liters of sterile deionized water.

Mycological Analysis

The mycological quality of fresh vegetables was determined following standard procedures described by Mcheik *et al*. (2018).

Fungal Load Determination

Twenty-five grams (25g) of the fresh vegetables were chopped aseptically using a sterile disposable scalpel and homogenized manually in 225 mL of sterile normal saline. The resultant homogenate was then tenfold serially diluted and pour-plated on Saboraud Dextrose Agar modified with 50 mg/L chloramphenicol. Afterward, the inoculated plates were incubated at room temperature (27± 2ºC) for 3 days. Discrete colonies formed were enumerated and expressed as colony-forming units per gram (CFU /g) of the sample.

Fungi Identification

Fungal isolates were identified as species based on cultural morphology and microscopic features following the procedure described by Bedasa *et al.* (2018). Distinct colonies formed were examined for colonial characteristics (colour, texture, and pigmentation) and microscopic features (vegetative and reproductive structures) compared to standard mycology atlas.

Data analysis

The average fungal count of duplicate vegetable samples was expressed as mean ± standard deviation. Decontamination efficacy of the agents was expressed as a % reduction compared to water washing and analyzed using SPSS version 11.5 to determine significant differences in mean values (P<0.05).

RESULTS

Mycological quality on Fresh Vegetable

Table 1 presents the total fungal counts of fresh cabbage and lettuce leaves sold in open markets of Farin Gada Jos, Nigeria. In this study, the total fungal counts varied from 1.45×10^6 to 3.61×10^6 (CFU\g) on cabbage heads and lettuce leaves respectively. The result revealed that lettuce leaves (3.61×10^6 CFU \g) were more contaminated ($p < 0.05$) than the cabbage heads (1.45×10^6 CFU \g).

Table 1: Total Fungal Count of Fresh Leafy Vegetables Sold in Open Market Farin-Gada Jos Nigeria

Vegetable Types	Number of Samples	Range (CFU/g)	Mean Count (CFU/g)
Cabbage	30	9.80×10^5 - 1.92×10^6	$1.45 \times 10^6 \pm 4.70 \times 10^5$
Lettuce	30	3.40×10^6 - 5.60×10^6	$4.50 \times 10^6 \pm 1.10 \times 10^5$

($p = 0.1255$, $t = 2.550$)

Species of *Aspergillus niger, Candida tropicalis, Penicillium notatum, Trichophyton rubrum, and Mucor* spp. were dominant fungal contaminants of the fresh vegetables sold in the open market of Frain Gada (Table 2). Strains of *Aspergillus niger* 52 (20.16%) were the most detected fungal contaminant followed by *Penicillium notatum* 50 (19.38%), while *Candida tropicalis* 14 (5.43%) were the least occurred. Comparatively, samples of lettuce leave 119 (46.12%) were more exposed than cabbage 102 (39.53%) to the fungal contaminants.

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Table 2: Occurrence of Fungal Contaminants of Fresh Vegetables Sold in Open Market Farin-Gada Jos, Nigeria

Fungi species	Occurrence (%)/ Vegetable types		Total Occurrence (%)
	Cabbage	Lettuce	
Candida tropicalis	5 (4.91)	9 (17.56)	14 (5.43)
Mucor racemosus	9(19.62)	19 (18.44)	42 (16.28)
Aspergillus niger	18 (11.76)	24 (11.76)	52 (20.16)
Aspergillus flavus	8 (13.73)	11 (15.13)	26 (10.09)
Trichophyton rubrum	7 (6.86)	8(6.72)	32 (12.40)
Penicillium notatum	15 (23.52)	23 (21.85)	50 (19.38)
Total	102 (39.53)	119 (46.12)	258

Efficacy of Disinfestation agents against fungal contaminants

The decontamination efficacy of salt and vinegar solutions on fungal load of fresh cabbage and lettuce leaves is presented in Tables 3 and 4. Data from this study revealed that both salt and vinegar are active decontaminating agents against fungi in fresh vegetables. More so, the efficacy of salt and vinegar on fungi was more drastic ($P < 0.05$) with increased concentration and exposure time. At low concentrations (2.50%.), fungal decontamination efficacy of vinegar solutions 51.16 % (2.10×10^6 CFU/g) was more than salt 46.51% (2.30×10^6 CFU/g) in lettuce leaves. On cabbage, the fungal load reduction efficacy was significantly higher ($p < 0.05$) in salt solution (2.50%) compared to vinegar solution (2.5%). High concentrations of salt and vinegar applied for 10 min reduced fungal load ($p > 0.05$) by 99.53 % (4.30×10^6 - 2.00×10^4 CFU/g) and 99.77 % (4.30×10^6 - 1.00×10^4 CFU/g) respectively in fresh lettuce. Similarly, a fungal load reduction of 96.00 % (1.50×10^6 - 60×10^4 CFU/g) and 100% was observed respectively in salt and vinegar decontaminated cabbage. In this study, species of *Penicillium notatum, Aspergillus niger,* and *Candida tropicalis* persisted throughout the decontamination process (Table 5).

Table 3: Fungal Count of Lettuce Leaf Decontaminated with Salt and Vinegar

Contact Time (min)	Salt / Vinegar Concentration (% w/v)	Salt Solution		Vinegar Solution	
		Total Fungi Count (cfu/g)	% Reduction	Total Fungi Count (cfu/g)	% Reduction
0.00	-	4.30×10^6	-	4.30×10^6	
2:30	2.5	3.10×10^6	27.91	2.50×10^6	41.86
5:00		2.60×10^6	39.53	2.30×10^6	46.51
7:30		2.30×10^6	46.51	2.10×10^6	51.16
10:00		2.20×10^6	48.84	1.90×10^6	55.81
2:30	5.0	2.00×10^6	53.49	1.70×10^6	60.47
5:00		1.80×10^6	58.14	1.30×10^6	69.77
7:00		1.70×10^6	60.47	1.20×10^6	72.09
10:00		1.50×10^6	65.12	1.10×10^6	74.42
2:30	7.5	1.30×10^6	76.92	6.80×10^5	84.19
5:00		9.80×10^5	77.91	4.40×10^5	89.77
7:30		7.00×10^5	83.72	2.70×10^5	93.72
10:00		3.80×10^5	91.16	1.60×10^5	96.28
2:30	10.0	1.10×10^5	97.44	9.00×10^4	97.91
5:00		2.00×10^4	99.53	2.00×10^4	99.53
7:30		2.00×10^4	99.53	3.00×10^4	99.30
10:00		2.00×10^4	99.53	1.00×10^4	99.77

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Table 4: Fungal Count of Cabbage Leaf Decontaminated with Salt and Vinegar

Contact Time (min)	Salt / Vinegar Concentration (% w/v)	Salt Solution		Vinegar Solution	
		Total Fungi Count (cfu/g)	% Reduction	Total Fungi Count (cfu/g)	% Reduction
0.00	-	1.50×10^6	-	1.50×10^6	-
2:30	2.5	9.80×10^5	33.67	1.50×10^6	0.00
5:00		8.10×10^5	46.00	1.40×10^6	6.66
7:30		7.90×10^5	47.33	1.20×10^6	20.00
10:00		7.00×10^5	53.33	1.10×10^6	26.67
2:30	5.0	6.20×10^5	58.67	1.02×10^6	48.00
5:00		5.80×10^5	61.33	7.60×10^5	49.33
7:00		5.10×10^5	66.00	6.80×10^5	54.67
10:00		5.00×10^5	66.67	4.10×10^5	72.67
2:30	7.5	4.20×10^5	72.00	3.80×10^5	74.67
5:00		3.50×10^5	76.67	2.80×10^5	81.33
7:30		3.00×10^5	80.00	2.50×10^5	83.33
10:00		2.70×10^5	82.00	1.90×10^5	87.33
2:30	10.0	2.20×10^5	85.33	1.10×10^5	92.67
5:00		1.80×10^5	88.00	3.00×10^4	98.00
7:30		1.80×10^5	88.00	NG	100.00
10:00		60×10^4	96.00	NG	100.00

KEY. NG = no growth

Table 5: Fungi Species Survived Salt and Vinegar Surface Decontamination of Cabbage and Lettuce Leaves

Fungi species	Occurrence /Type of Disinfectants			
	Cabbage		Lettuce	
	Salt	Vinegar	Salt	Vinegar
Aspergillus niger	-	-	+	+
Trichophyton rubrum	-	-	-	-
Penicillium notatum	-	-	+	+
Mucor racemosus	-	-	-	-
Candida tropicalis	+	-	-	-

KEY= - is absent, + is present

DISCUSSION

This study confirms fungal contamination of fresh vegetables sold in open markets at levels that require effective decontamination processes to minimize the potential risk of microbial infection. In this study, the total fungal counts (1.45×10^6 CFU/g - 3.61×10^6 CFU/g) observed in the fresh vegetables at sale points indicate heavy contamination. This level of total fungal count in the vegetables corresponds with the reports of Yafetto *et al.* (2019). Moreover, Shehu, *et al.* (2014) reported 6.28×10^5 - 1.8×10^6 (CFU/g) fungal counts in the vegetables. The high fungal loads can be attributed to natural flora or contaminants resulting from the exposure of vegetables to untreated irrigation river water and inadequate postharvest handling processes (Kłapeć *et al.*, 2022; Olaitan *et al.*, 2022).

The diversity of *Aspergillus niger*, *Mucor* spp., *Penicillium notatum*, *Trichophyton rubrum*, and *Candida tropicalis* as dominant contaminants of fresh vegetables conform with several reports

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(Oladele and Olakunle, 2011; Shehu *et al.*, 2014). Similarly, *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor* spp., and *Aspergillus flavus* were reported as intuitive to the fresh vegetables in Akure, Nigeria (Akintobi *et al.*, 2011; Arienzo *et al.* 2020). In the Kano metropolis—Nigeria, *Aspergillus flavus*, *R. stolonifer*, and *Mucor* spp. were observed in the cabbage and lettuce leaves (Shu'aibu *et al.*, 2014). Typically, most of these fungal contaminants are mutual mycotoxin producers, and their occurrence does not necessarily assure the formation of mycotoxins. The presence of *Aspergillus* spp., *Mucor* spp., *Trichophyton rubrum*, and *Candida tropicalis* poses a risk to public health (Mohamed, 2013; Tsado *et al.*, 2013; Yaradua *et al.*, 2018). Some of these fungi species are producers of secondary metabolites potentially harmful to humans and animals (Baiyewu *et al.*, 2007).

In this study, both the table salt and vinegar significantly reduced fungal contamination levels on the cabbage and lettuce leaves. This is conformity with several reports that salt and vinegar effectively decontaminate fungi in fresh vegetables (Woldetsadik *et al.*, 2017; Bhilwadikar *et al.*, 2019; Pourzamani *et al.*, 2019). Although water washing reduces fungi on vegetables, the incorporation of disinfectants to the water improved the decontamination process (Dao *et al.*, 2018; Subramanya *et al.*, 2018; Bhilwadikar *et al.*, 2019).

Notably, the disinfection efficacy of table salt and vinegar solutions in vegetables increased with higher disinfectant concentration and longer exposure time. At 10 min contact time, the fungi load in the lettuce and cabbage reduced significantly regardless of the concentration. This decrease in fungal load with increased concentrations of the disinfection agents aligns with multiple research findings (Johnson *et al.*, 2017; Woldetsadik *et al.*, 2017). However, there is concern about the impact of high substance concentrations on vegetable quality (Lee *et al.*, 2018; Bhilwadikar *et al.*, 2019; Gehringer and Kaletunç, 2020). Appropriately, lower concentrations of decontaminating agents at extended contact time could, therefore, be a better approach to mitigate the degrading effect of disinfectants on the vegetable matrixes.

Comparatively, the vinegar was more active than the table salt solution during the fungal decontamination of fresh vegetables. This finding is consistent with the findings of Rahman *et al.* (2021), where vinegar was more effective than a sodium chloride solution in reducing fungal contamination on fresh vegetables. Moreover, vinegar had shown a better microbial inhibitory effect than sodium chlorine solution (Treesuwa *et al.*, 2023). At lower disinfectant concentrations, sodium chloride solution was more effective on the cabbage than vinegar. This drift is within the backdrop that high sodium chloride concentrations develop turgor pressure to disrupt the substrate transport of the cell. Moreover, the developed turgor pressure subsequently alters the cell membrane permeability and consequently causes leakage of the intracellular electrolytes and cell constituents (Wijnker *et al.*, 2006; Seok *et al.*, 2021).

Conclusion
This study confirms the fungal contamination of fresh vegetables sold in open markets at levels that present health risks to consumers. Accordingly, the salt and vinegar solutions exhibited a significant reduction in fungal contaminants of the fresh vegetables, with decontamination efficacy that is exposure time and dose-dependent.

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