

**Salahaddin University**

**Collage of science**

 **Environmental health and science department**

 **The**

 **Study Of Microorganisms (Bacteria and Fungi)**

 **In Fresh Vegetables**

 Research project

 Submitted to department environmental science and health in partial

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 In environmental science and health

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 **ABSTRACT**

This study was applied to isolate microorganisms (bacteria and fungi) in three different vegetables which are Lettuce, Arugula, and Celery. Power plate method by using Potato Dextrose Agar (PDA) was used for isolate and identifies different species of fungi and same procedure for isolation of bacteria in studied samples. The results show the identity and the total colony forming units (CFU) of fungi in three vegetables on PDA medium. The most frequently isolated fungi *were Rhizopus sp., Aspergillus Sp, Alternaria, Yeast, Penicillium, Alternaria,*and *Rhodotorula*. While the less frequently detected fungi species were*, Mucor*, and *Demaceous.* The higher number of counted bacteria found on celery in the first grocery which was 268 CFU. The higher number of bacteria in the second grocery was for lettuce which was 250 CFU. And the higher number of bacteria in the third grocery counted on celery which was 280 CFU.

**Keywords:**  Vegetables, Fungus species, food borne disease

 **INTRODUCTION**

Vegetables remain one of the most important portions of a balanced diet, because of the many attendant nutritional benefits that inure to their consumption. Vegetables are rich di­etary sources of micronutrients, minerals, vita­mins and, most importantly, antioxidants and fibre, all of which are vital to human health, wellbeing and disease prevention (Eni *et al*., 2010).

Overall public awareness of healthy eating habits has been intensified, prompting an increasing demand and incorporation of fresh vegetables in diets, especially because of their convenience, freshness, taste and health benefits (Olaimat & Holley, 2012).

The Food and Agriculture Organization and the World Health Organization strongly recommend more than 400 g/day intake of fruits and veg­etables in diets to promote good health (FAO/ WHO, 2014).

Despite the health benefits derived from consuming fresh vegetables, the risk of microbiological contaminations in vege­tables is of public concern due to the possi­bility of vegetable contaminations along the food chain, beginning from the vegetable farm to the dinner table (Kuan *et al*., 2017); this concern is compounded by the fact that these vegetables are mostly eaten fresh (not cooked) and washing may not guarantee decontamina­tion, so that any resident microorganism easily enter the alimentary canal.

Some studies in Ghana suggest that vegetables sold at the groceries in the mar­kets are contaminated with microorganisms, mostly bacteria (Amoah, 2014)

Fungi belong to kingdom Myceteae. The distinguishing characteristics of this group as a whole are that, they are eukaryotic, non-photosynthetic, lack tissue differentiation, have a cell wall of chitin or other polysaccharides and propagate by spores (sexual and /or asexual) (Benson, 2002).

Several factors may contribute to contamination of vegetables. Vegetables may get exposed to bacterial contaminants in the pre-harvest and post-harvest handling. Use of insufficiently treated wastewater for irrigation is an important risk factor for contamination of vegetables cultivated using irrigation in many of developing countries. Contamination of soil with animal wastes and increased application of improperly composted manures to soil has also similar role (Erkan *et al*.,2008)

Determining microbiological quality of fresh, leafy salad vegetables that are usually not cooked, but are consumed raw is very important. The present study, therefore, aimed to investigate the microbiological con­taminations of Lettuce, Arugula, and Celery.

 **MATERIAL AND METHOD**

 **Sampling:**

Samples were taken in three different places of selected grocery shops then samples cultured on the PDA media by power plate method and incubated for 3-5 days in 25℃ for fungal study and samples were cultured on nutrient agar and incubated for 24hrs in 37℃ for bacterial study.

Good quality fresh samples of studied vegetable (commonly eaten), were collected from nearer farmlands during period of study. Sample of vegetables from farms were selected by using random sampling procedures. The studied leafy vegetables were collected by hand and carefully packed into polyethylene bags, and returned back to laboratory as soon as possible.

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Media used for isolation and identification of fungi Potato Dextrose Agar (PDA) [Onuorah, 1982].

**Chemical compound Quantity**

Dextrose………………………20 g.

Potato………………………...200 g.

Agar…………………………..20 g.

Distill water (D.W.)…………1 L

 **Identification of the fungal genera**

The fungal isolates were transferred to sterilize plates for purification and identification. The grown fungi were mounted on a slide, stained with lactophenol-cotton blue to detect fungal structures, covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics based on atlas books (Rajankar *et al.*, 2007).

 **RESULTS AND DISCUSSION**

The result presented in table (1) shows the identity of fungi species in all three vegetables on PDA medium. The most frequently isolated fungi were *Aspergillus niger, Yeast, Penicillium, Alternaria, Rhodotorula.* While the less frequently detected were*, Mucor,* and *Demaceous.*

Figure (1) and table (2) shows the number of counted bacteria in three studied vegetables (Arugula, celery, and lettuce). Which the higher number of counted bacteria found on celery in the first grocery which was 268 CFU. The higher number of bacteria in the second grocery was for lettuce which was 250 CFU. And the higher number of bacteria in the third grocery counted on celery which was 280 CFU.

Figure (2) and table (3) shows the number of fungi in all three studied vegetables (Arugula, celery, and lettuce); which determined that the higher number of counted fungi in the first studied grocery was for celery which was 68 CFU.ml-1. And according to the second studied grocery the higher number of counted fungi was detected in lettuce which was 55 CFU.ml-1.

Figure (3) shows the total number of bacteria compared to total number of isolated fungi in all three studied vegetables, the results tell us that bacterial contaminations of the veg­etables are higher than fungal contaminations.

This was clear that samples from wet markets yielded a higher proportion of bacteria because it could be seen that the way vegetables were treated on the wet markets was less hygienic. The surroundings and places for vegetable displays on markets were not clean and tidy, and the handlers did not wear gloves while the vegetables were being handled. Contamination might be occurred through a contaminated container for transporting and improper handling (Chai et al., 2007).

Many pathogenic microorganisms can continue to live in the soil or on the surface of crops long enough to be transmitted to humans. The ability of microorganisms to persist for extend period depends of many factors, including structure and nature of soil, hygrometry, temperature, light, plant nature, and competition with natural plants and animals (Leff and Fierer,2013).

Considering high contamination found in our study, some measures should be taken to prevent the risk of illnesses due to the poor microbial quality of green leafy vegetable. In this regards, it has been shown that establishment of Hazard Analysis and Critical Control Points (HACCP) on the vegetable farms could be too effective for reduction of microbial risks of fresh‐cut produces (Mei Soon et al., 2012).

Dickin *et al*. (2015) are made a review about the risks related to the wastewater usage in the agriculture they showed that wastewater is increasingly being used in the agricultural sector to cope with the depletion of freshwater resources, as well as water stress linked to changing climate conditions. To provide a more comprehensive understanding of the health risks of wastewater use in agriculture, future research should consider multiple exposure routes, long-term health implications, and increase the range of contaminants studied, particularly in regions heavily dependent on wastewater irrigation.

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| **Table 1: Different fungi species in all three studied vegetables.** |
| **Grocery 1** | **Grocery 2** |  **Grocery 3** |
| ***Aspergillus niger*** | ***Penicillium sp.*** | ***Cladosporium sp.*** |
| ***Rhizopus sp.*** | ***Aspergillus sp.*** | ***Aspergillus niger*** |
| ***Alternaria sp.*** | ***Rhodotorula sp.*** | ***Alternaria sp.*** |
|  ***Fusarium*** | ***Rhizopus sp.*** | ***Penicillium sp.*** |
|  ***Penicillium sp.*** | ***Yeast*** | ***Rhodotorula sp.*** |
| ***Rhodotorula sp.*** | ***Aspergillus niger*** | ***Rhizopus sp.*** |
|  ***Yeast*** | ***Fusarium***  | ***Yeast*** |
| ***Aspergillus sp*** | ***Demaeous***  |  |
|  ***Mucor sp.*** |  |  |
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| **Table (2): Number of counted bacteria in three studied vegetables (CFU).** |
| **Vegetable** | **Grocery 1** | **Grocery 2** | **Grocery 3** |
| **Arugula** | **194** | **215** | **251** |
| **Celery** | **268** | **180** | **280** |
| **Lettuce** | **240** | **250** | **166** |
| **Total** | **702** | **645** | **697** |

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| **Table (3): Variation of fungi count (CFU.ml-1) in in three studied vegetables.** |
| **Vegetable** | **Grocery 1** | **Grocery 2** | **Grocery 3** |
| **Arugula** | **22** | **36** | **42** |
| **Celery** | **68** | **25** | **15** |
| **Lettuce** | **17** | **55** | **45** |
| **Total** | **107** | **116** | **102** |

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