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South Asian Journal of BIOLOGICAL RESEARCH

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To cite the article: Paul Omaye Joseph, Adewale Adebayo and Frank Ojochegbe Ojomah (2018). Assessment of microorganisms associated with the production of amaranth, *South Asian Journal of Biological Research*, 1(1): 12-27

Link to this article:
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ASSESSMENT OF MICROORGANISMS ASSOCIATED WITH THE PRODUCTION OF AMARANTH

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ARTICLE INFO

Article Type: Research
Received: 06, Sep. 2018.
Accepted: 05, Oct. 2018.
Published: 06, Oct. 2018.

Keywords:
Amaranth, contamination, irrigation water, microorganisms, soil, pathogen

ABSTRACT

The objective of the present study was to determine the correlation of microorganisms isolated from amaranth to that isolated from soil and irrigation water. Samples of amaranth, soil and irrigation water were collected in dry and wet seasons of 2016 from farm sites in Koriko, Bassa LGA, Kogi State for microbial analyses. Following sample preparation and serial dilutions, three dilutions of each sample were plated out in duplicates on nutrient agar using the pour plate and streak methods. Pure cultures of each observed microorganism were purified using sub-culturing techniques, then cultural, microscopic and biochemical characteristics were used for identification of specific isolates. A total of eighteen microorganisms namely *Bacillus* spp, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus mycoides*, *Lactobacillus* spp, *Leuconostic* spp, *Micrococcus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Escherichia coli*, *Enterobacter* spp, *Azotobacter* spp, *Staphylococcus* spp, *Salmonella* spp, *Serratia* spp, *Citrobacter* spp and *Flavobacterium* spp were isolated. The results of this study revealed that all organisms in amaranth were present in the analysed soil and irrigation water. Several pathogenic bacteria such as *Escherichia coli* and *Salmonella* spp were involved in the contamination of amaranth on the farm, and this is of a high potential health hazard to consumers. Contaminated irrigation water and soil were possibly the sources of contamination of amaranth growing on the field. Farmers should be educated on the risk involved in the use of contaminated water for irrigation.

1. Introduction

Food safety is a major public health concern worldwide. During the last decades, the increasing demand for food safety has stimulated research regarding the risks associated with consumption of food stuffs contaminated with pathogenic microorganisms. Several studies have revealed that the contamination of vegetables with pathogens poses a threat to consumers (D'Mello, 2003; Zandstra and De Kryger, 2007). Vegetables are produced in significant quantities both in urban and peri urban areas.

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked

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(Dhellot *et al*., 2006). Acoording to Mohammed and Sharif (2011), green leafy vegetables are valuable sources of nutrients for growth in man and animals especially in rural areas where they contribute substantially to protein, minerals, vitamins, fibres and other nutrients which are usually in short supply in daily diets. Problems linked with pathogens in fresh produce, including the associated public health and trade implications, have been reported in some countries worldwide (CAC, 2006). The inner tissues of healthy plants and animals are free of microorganisms, however, the surfaces of raw vegetables and meat are contaminated with a variety of microorganisms and this depends on the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling, the time and conditions of storage (Pelczar *et al.*, 2006). Contamination may also occur during post-harvest handling, including at points of preparation by street vendors, in food service establishments, home and also with viruses or parasites can result from contact with faeces, sewage and irrigation water (Cliver, 1997; Speer, 1997).

Most of the reported outbreaks of gastrointestinal diseases linked to the fresh produce have been associated with microbial contamination, particularly with members of the *Enterobacteriaceae* family (Hamilton *et al.*, 2006; Tyler and Triplett, 2008).

The growth of microbial populations and their action on soils are dependent on the interaction between plant species and soil (Grayston *et al.*, 1998). According to Marschner *et al.* (2001), bacterial community composition results from the interaction between soil type, plant species and its rhizosphere localization. Similar reports have shown that the size and structure of microbial populations are affected by soil type and plant species (Wieland *et al.*, 2001). However, a study made on three soils in England showed that the primary determinant of composition in bacterial communities was the soil type (Girvan *et al.*, 2003).

Increasing evidence of contamination of produce from irrigation water and increasing scarcity in water resources leave little doubt about the need to pay more attention to the fate and transport of pathogens in irrigation waters. Untreated water is most likely to transmit several microorganisms, which may include pathogenic strains of *Escherichia coli*, *Salmonella*, *Listeria*, protozoa and viruses (Díaz *et al*., 1999). Studies in different countries indicate that the use of untreated water for irrigation of vegetables is the practice most related to fresh produce safety issues (Díaz *et al*., 1999; Tyrrel and Quinton, 2003). According to Leifert *et al*. (2008), sources of irrigation water can be generally ranked by the microbial contamination hazard: in order of increasing risk these are potable or rain water, groundwater from deep wells, groundwater from shallow wells, surface water, and finally raw or inadequately treated wastewater.

The incidence of foodborne pathogens on fruits and vegetables varies by region and can be extremely high in some developing countries. However, substantial outbreaks continually occur in developed countries (Yakov *et al*., 2011).

Large-scale production of produce typically requires some form of irrigation during the growing season. Consequently, there is a rapidly growing body of research documenting and elucidating the pathways of produce contamination by water-borne pathogens. Excellent reviews by Steele and Odumeru (2004) and Gerba (2009) have recently been published. However, many gaps exist in our knowledge and understanding. The role of contaminated irrigation water, used in the production of vegetable crops, in the transmission of pathogens to humans is not clear (FAO/WHO, 2008). Studies on the sources of microorganisms on the surfaces of fresh vegetable produce in Southern Guinea

Savannah of Nigeria are rare if not completely absent. There the objective of this study was to determine the prevalence of microorganisms in the production of amaranth.

2. Materials and Methods

2.1 Sample Collection

Soil, amaranth and water samples were collected during the dry and wet seasons of 2016 from Koriko, Bassa LGA, Kogi State.

Soil samples were collected from the farm site for laboratory analyses of microorganisms using the pour plate method. Sub-samples (5) from the farm site were randomly collected and pooled to form composite samples. These samples were immediately transported to the laboratory for microbial analyses and then homogenized and spread in trays to be cleaned of extraneous materials (pieces of root, leaves, small stems, etc.). All the samples were collected in sterile universal containers and plastic bags.

Amaranth samples were randomly collected at the farm site and packed into sterile plastic containers and then transported to the laboratory for microbial analyses.

Water samples were collected in plastic bags from the source of water (Iteme River) used for irrigation and then transported to the laboratory for microbial analyses.

2.2 Processing of Samples for Culturing

Composite samples of 10 g of soils were collected from the farm site. Samples were placed into a sterile 250-ml Erlenmeyer flask, with 90 ml of sterile distilled water and thoroughly shaken. The supernatant was serially diluted ten times to a 10^{-10} dilution.

Duplicate composite samples of 10 g of fresh leaves were collected from the uppermost parts of amaranth. Samples were placed into a sterile 250-ml Erlenmeyer flask, with 90 ml of sterile 0.02 M phosphate-0.85% saline buffer (pH 7.0). The flask was shaken on a rotary shaker for 2 hours at 120 rpm at room temperature. The supernatant was decanted and serially diluted ten times with 0.02 M phosphate-0.85% saline buffer to a 10^{-10} dilution. The dilutions were used for most-probable-number (MPN) counting of bacteria.

Water samples were serially diluted ten times to a 10^{-10} dilution followed by plating in duplicate, using the pour plate technique. All inoculum were incubated at 30^{0}C for 24 to 48 hours.

2.3 Preparation of Dilution

Dilutions of 10^{-1} to 10^{-10} were prepared. A mass of 10 g of the sample was dispensed in 90 ml of sterile distilled water, mixed and allowed to settle. From this tube, 10^{-1} dilution was prepared by pipetting 1 ml into another test-tube containing 9 ml of sterile distilled water and homogenized. This gave a dilution of 10^{-2}. From this dilution, other dilutions were prepared serially.

2.4 Culture Media

The media used in this study included Nutrient Agar, MacConkey Agar, Desoxycholate Agar, Endo Agar, Hektoen Enteric Agar, Eosine Methylene Blue Agar, Xylose Lysine Desoxycholate Agar, and Phenylethyl Alcohol Agar.

2.5 Culture Technique

Pour Plate Method: Sterile distilled water (9 ml) was dispensed into 10 labelled sterile test-tubes. Ten grams (10 g) of samples were added to 90 ml of sterile distilled water in a beaker. After thorough agitation, 1 ml was drawn with a sterile pipette into another test-tube containing 9 ml of sterile distilled water. This is dilution factor 10^{-2}. This serial dilution was repeated to produce dilutions of 10^{-1} through 10^{-9}. Aliquots of 1 ml from tubes 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were aseptically transferred into sterile petri-dishes, and molten nutrient agar (45^0C) was added and swirled thoroughly to allow even distribution. On setting, the plates were inverted and incubated aerobically at 30^0C for 24 to 48 hours. The colonies were counted using a colony counter (Stuart Scientific, UK).

2.6 Sterilization of Glassware

Glassware was sterilized using the autoclave at 121^0C for 15 minutes. The glassware include: pipettes, test-tubes, beakers, conical flasks, and Petri dishes. Pipettes and test-tubes were plugged with cotton wool and wrapped separately in aluminium foil, the beakers, and conical flasks were plugged with cotton wool before sterilization.

2.7 Preparation of Media

Media was prepared according to the manufacturer's specifications.

2.8 Isolation and Enumeration of microorganisms

Nutrient Agar (NA) was used for the isolation of bacteria. Media used were prepared according to the manufacturer's specifications. The Nutrient Agar plates were incubated aerobically at 30^0C for 24 to 48 hours. Ten grams 10 g of each of the samples, leaves and soil, were weighed into sterile test-tubes to which 90 ml of sterile distilled water was added and serially diluted. From an appropriate dilution of 10^{-4}, 1 ml was pipetted into NA plate which was labeled properly and incubated appropriately. The workbench was disinfected with 70% ethanol. The NA plates were incubated in an incubator at 30^0C for 24 to 48 hours.

2.9 Procedure for Sub-culturing

Pure isolates were obtained by selecting discrete colonies and having them sub-cultured onto Petri dishes containing freshly prepared NA media. The bacterial isolates were streaked out onto NA plates.

2.10 Preservation of Isolates

Pure isolates were inoculated onto freshly-prepared NA slants in McCartney bottles for bacteria. The NA cultures were incubated for 24 hours in an incubator at 30^0C and then stored in the refrigerator at 4^0C.

2.11 Identification and Characterization of Isolated Strains

2.11.1. Gram's staining

The pure bacterial isolates were stained according to Gram's techniques as described by Baker (1967).

2.11.2. Spore staining

A thin smear was prepared on a clean glass slide, air dried and heat fixed. The smear was flooded with Methylene Blue for 5 minutes. The smear was counter stained with carbon fuchsin for 30 seconds. This was then rinsed again with tap water and blotted with filter paper. The stained cells were then examined under the oil immersion objective.

2.11.3. Motility test

The stabbing technique was used to carry out this test. Test-tubes containing sterilized Sim Agar were prepared. The sterilized inoculating needle was used to pick up isolates from their pure cultures. Each test-tube was stabbed with the needle rubbed with each isolate in the middle. The test-tubes were then incubated at 30^0C for 24 hours. After 24 hours, the tubes were observed for the motility of the isolates. A motile isolate usually grows away from the point where the medium was stabbed.

2.12 Biochemical Tests

2.12.1. Catalase test

This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). A suspension of the bacterium colony was made on a clean glass slide with about 0.2 ml of sterile water and followed by the addition of 0.5 ml of hydrogen peroxide over it. The production of gas bubbles indicated a positive reaction.

$2H_2\ O_2 \rightarrow 2H_2O + O_2$

2.12.2. Citrate utilization test

This test demonstrates the use of citrate as a sole source of carbon by alkalinization of the medium by the bacteria. The inocula were inoculated into slants of sterilized Simmon's citrate agar and incubated at 30^0C for 72 hours. The positive result changed the green colour of the agar to blue colour, indicating the presence of citrate utilizing bacteria.

2.12.3. Triple Sugar Iron (TSI) agar test

Agar slant of TSI Agar was inoculated and stabbed with the isolates. Agar slants were inoculated for 24 hours. The slope and butt of the slants were observed for colour change, gas and hydrogen sulphide production.

2.12.4. Susceptibility to penicillin

Isolates with a cultural and microscopic appearance resembling *Bacillus* spp were subjected to Antimicrobial Susceptibility Tests with penicillin (36 mg/ml concentration) in broth and incubated at 35^oC for 18 hours. The turbidity of the broth was interpreted as resistance. An uninoculated broth with penicillin was incubated as a control.

3. Results

Table 1 shows the microbial isolates from soil, irrigation water and amaranth samples during the dry and wet seasons. *Bacillus* spp, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Leuconostic* spp, *Klebsiella* spp, *Pseudomonas* spp, *Escherichia coli*, *Enterobacter* spp *and Azotobacter* spp were isolated in the dry season. *Bacillus* spp, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus mycoides*, *Escherichia coli*, *Pseudomonas* spp and *Flavobacterium* spp were isolated in the wet season. Table 1 also shows the microbial isolates from irrigation water sample in both seasons. *Bacillus* spp, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus mycoides*, *Lactobacillus* spp, *Micrococcus* spp, *Pseudomonas* spp, *Escherichia coli*, *Enterobacter* spp, *Azotobacter* spp and *Staphylococcus* spp were isolated in the dry season. *Bacillus* spp, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter* spp, *Salmonella* spp, *Serratia* spp, *Klebsiella* spp, *Citrobacter* spp, *Escherichia coli*, *Flavobacterium* spp, *Azotobacter* spp and *Pseudomonas* spp were isolated in the wet season.

Table 1: Occurrence of microorganisms in soil, irrigation water and amaranth during the dry and wet seasons

Microbial isolates					
Soil		**Irrigation water**		**Amaranth**	
Dry season	**Wet season**	**Dry season**	**Wet season**	**Dry season**	**Wet season**
Bacillus spp	*Bacillus* spp	*Bacillus* spp	*Bacillus* spp	*Bacillus* spp	*Bacillus* spp
Bacillus cereus	*Bacillus cereus*	*Bacillus cereus*	*Bacillus cereus*	*Bacillus cereus*	*Bacillus cereus*
Bacillus subtilis	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Pseudomonas* spp
Bacillus megaterium	*Bacillus megaterium*	*Bacillus megaterium*	*Enterobacter* spp	*Bacillus megaterium*	*Escherichia coli*
Leuconostic spp	*Bacillus mycoides*	*Bacillus mycoides*	*Salmonella* spp	*Lactobacillus* spp	*Serratia* spp
Klebsiella spp	*Escherichia coli*	*Lactobacillus* spp	*Serratia* spp	*Micrococcus* spp	*Klebsiella* spp
Pseudomonas spp	*Pseudomonas* spp	*Micrococcus* spp	*Klebsiella* spp	*Pseudomonas* spp	*Enterobacter* spp
Escherichia coli	*Flavobacterium* spp	*Pseudomonas* spp	*Citrobacter* spp		*Salmonella* spp
Enterobacter spp		*Escherichia coli*	*Escherichia* spp		*Citrobacter* spp
Azotobacter spp		*Enterobacter* spp	*Flavobacterium* spp		*Azotobacter* spp
		Azotobacter spp	*Azotobacter* spp		
		Staphylococcus spp	*Pseudomonas* spp		

Source:

Table 2: Enterobacteriaceae and other Gram-negative bacteria flora present in the soil, irrigation water and amaranth

Isolates	***Soil***	***Irrigation water***	***Amaranth***
Enterobacter spp	+	+	+
Escherichia coli	+	+	+
Salmonella spp	-	+	+
Klebsiella spp	+	+	+
Citrobacter spp	-	+	+
Serratia spp	-	+	+
Others are;			
Flavobacterium spp	+	+	-
Pseudomonas spp	+	+	+
Azotobacter spp	+	+	+

Key: (+) indicates the presence of bacteria (-) indicates the absence of bacteria

Table 1 also shows the microbial isolates from samples of amaranth in both seasons. *Bacillus* spp,

Bacillus cereus, *Bacillus subtilis*, *Bacillus megaterium*, *Lactobacillus* spp, *Micrococcus* spp *and Pseudomonas* spp were isolated in the dry season. *Bacillus* spp, *Bacillus cereus*, *Pseudomonas* spp, *Escherichia coli*, *Serratia* spp, *Klebsiella* spp, *Enterobacter* spp, *Salmonella* spp, *Citrobacter* spp *and Azotobacter* spp were isolated in the wet season.

Table 3: *Bacillus* spp and other Gram positive bacteria flora present in the soil, irrigation water and amaranth

Isolates	*Soil*	*Irrigation water*	*Amaranth*
Bacillus spp	+	+	+
Bacillus cereus	+	+	+
Bacillus megaterium	+	+	+
Bacillus subtilis	+	+	+
Bacillus mycoides	+	+	-
Others are;			
Lactobacillus spp	-	+	+
Leuconostic spp	+	-	-
Micrococcus spp	-	+	+
Staphylococcus spp	-	+	-

Key: (+) indicates the presence of bacteria

(-) indicates the absence of bacteria

The result of bacteria isolated from soil, irrigation water and amaranth samples in dry season (Figure 1) shows that *Bacillus* spp had occurrence rates of 23%, 18% and 33%, respectively, *Bacillus cereus* had occurrence rates of 14%, 13% and 22%, respectively, *Bacillus subtilis* had occurrence rates of 7%, 25% and 6%, respectively, *Bacillus megaterium* had occurrence rates of 7%, 8% and 16%, respectively, *Pseudomonas* spp had occurrence rates of 7%, 4% and 6%, respectively, *Escherichia coli* had 7% and 4% occurrence rates in soil and irrigation water, respectively, *Enterobacter* spp had 7% and 4% occurrence rates in soil and irrigation water, respectively, *Azotobacter* spp had 7% and 8% occurrence rates in soil and irrigation water, respectively, *Lactobacillus* spp had 4% and 6% occurrence rates in irrigation water and amaranth, respectively, *Micrococcus* spp had 4% and 11% occurrence rates in irrigation water and amaranth, respectively, *Leuconostic* spp had 14% occurrence rate in soil, *Klebsiella* spp had 7% occurrence rate in soil, *Staphylococcus* spp and *Bacillus mycoides* had 4% occurrence rates each in irrigation water.

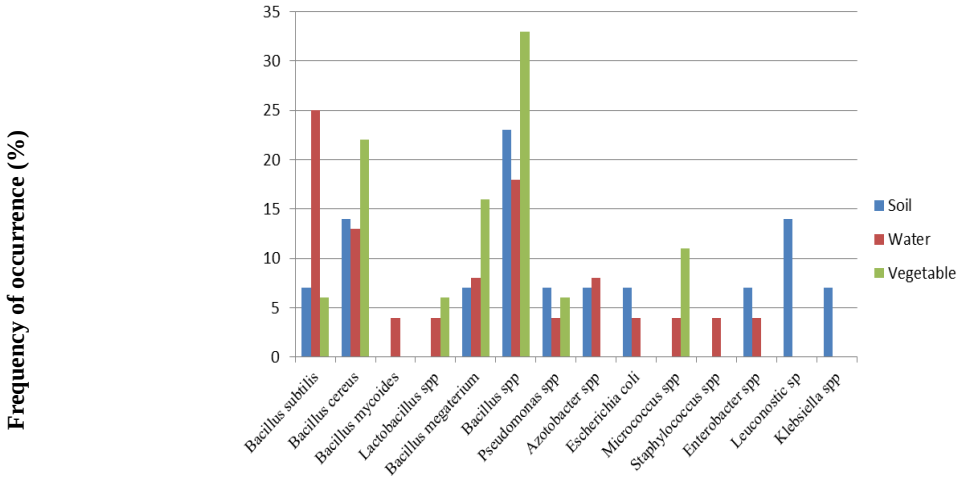

Figure 1: Frequency of occurrence of bacterial species isolated from soil, water and amaranth samples during the dry season

The result of bacteria isolated from soil, irrigation water and amaranth samples in rainy season were as shown in Figure 2. It shows that *Bacillus* spp had occurrence rates of 26%, 6% and 18%, respectively, *Bacillus cereus* had occurrence rates of 26%, 6% and 13%, respectively, *Pseudomonas* spp had occurrence rates of 13%, 6% and 13%, respectively, *Escherichia coli* had occurrence rates of 7%, 6% and 6%, respectively, *Bacillus subtilis* had occurrence rates of 7% and 6% in soil and irrigation water, respectively, *Flavobacterium* spp had occurrence rates of 7% and 6% in soil and irrigation water, respectively, *Enterobacter* spp had 13% occurrence rates each in irrigation water and amaranth, *Salmonella* spp had 6% occurrence rates each in irrigation water and amaranth, *Serratia* spp had occurrence rates of 20% and 6% in irrigation water and amaranth, respectively, *Klebsiella* spp had 6% occurrence rates each in irrigation water and amaranth, *Citrobacter* spp had occurrence rates of 6% and 13% in irrigation water and amaranth, respectively, *Azotobacter* spp had occurrence rates of 13% and 6% in irrigation water and amaranth, respectively, *Bacillus megaterium* and *Bacillus mycoides* had 6% occurrence rates each in soil.

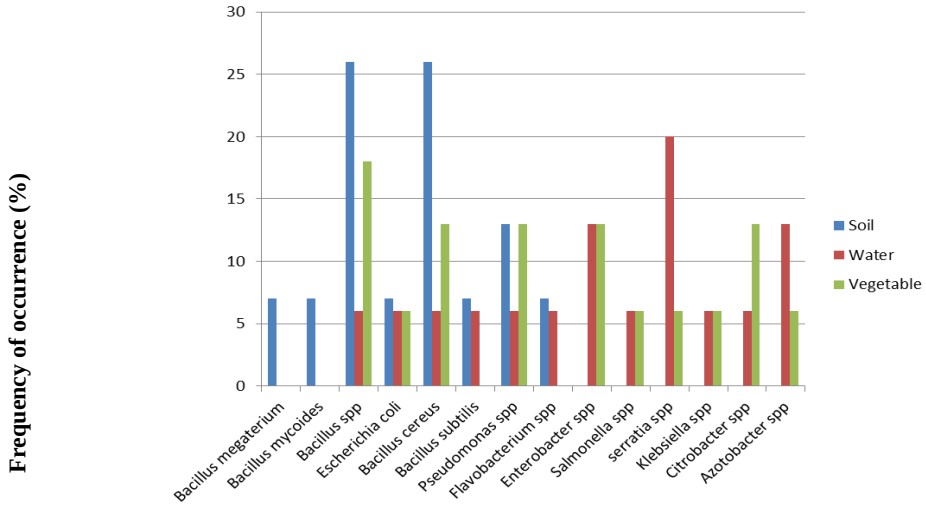

Figure 2: Frequency of occurrence of bacterial species isolated from soil, water and amaranth samples during the rainy season

4. Discussion

In the past two decades, consumption of fresh fruits and vegetables has significantly increased worldwide. At the same time the numbers of reported produce associated food-borne disease have increased. Several reports indicated that raw vegetables may harbour potential food-borne pathogens (Beuchat, 1996). Vegetables can be contaminated with such pathogenic organisms while growing, during harvest, in postharvest handling, or during distribution (McMahon and Wilson, 2001). Some of the organisms, such as *Escherichia coli* and *Salmonella* spp, detected in amaranth sample in this study constitute a potential health hazard for consumers. In order to determine the origin of these organisms in amaranth, soil and irrigation water were suspected and analysed. The results of this study revealed that all organisms in amaranth were present in the analysed soil and irrigation water. Indeed, Guo *et al.* (2002) demonstrated that soil and water are potential reservoirs of *Salmonella* that contaminates tomatoes. Moreover, results of the study of Islam *et al.* (2004) indicated that contaminated irrigation water might play an important role in contaminating the vegetables and the soil in which they grow. In addition, *E. coli* O157:H7 can be transmitted from manure - contaminated soil and irrigation water to lettuce plants and it can be introduced to the inner tissues of plants (Solomon *et al*., 2002). In this study, *E. coli* recovered from soil might have been transported by irrigation water because the farmers of this area did not apply animal manure. Therefore, irrigation water was the most probable source of the contamination of the soil and amaranth.

In this study, amaranths were contaminated with microorganisms capable of causing human diseases while still on the field. Bacteria such as *Bacillus* spp capable of causing illness are normal inhabitants of many soils, whereas *Salmonella* and *Escherichia coli* reside in the intestinal tracts of animals, including humans, and are most likely to contaminate raw amaranths through contact with faeces, sewage, untreated irrigation water or surface water. In other investigations, high percentages of samples have been found to contain bacteria capable of causing human diseases. Indeed, numerous outbreaks of illness caused by bacteria have been linked epidemiologically to the consumption of raw vegetables**.** Increased recognition of raw vegetables as suspected vehicles of human illness in industrialized countries (Altekruse *et al.,* 1997; *Bean et al.,* 1997) may probably result in increased numbers of associated or confirmed cases in the future. Improved diagnostic systems and surveillance programmes will enhance the prospect of identifying raw vegetables as sources of foodborne illness, thus possibly increasing in recorded numbers of outbreaks and cases of the disease.

The microorganisms normally present on the surface of the raw vegetables may consist of chance contaminants from the soil or dust or bacteria that have grown and colonized by utilizing nutrients exuded from plant tissues. Among the groups of bacteria commonly found on plant, vegetation are those that test positive for coliforms or faecal coliforms such as *Klebsiella* and *Enterobacter* (Zhao *et al.*, 1997; Yakov *et al.,* 2011). Thus, the presence of coliforms or faecal coliforms on raw amaranths in this study does not necessarily provide an index of faecal contamination. Microorganisms capable of causing human disease can, however, be found on raw produce and should be viewed as a threat to public health (Beuchat, 1996; Doyle, 1990).

The presence of pathogenic and other microorganisms on raw amaranths in this study is dictated not so much by surface topography as by exposure to environmental factors that lead to contamination. However, rough, highly-textured surfaces with deep crevices would be more likely to harbour soil, with the possible consequences of increased numbers of microorganisms, than would smooth surfaced vegetables. Differences in microbial profiles result largely from unrelated factors such as resident

microflora in the soil, application of non-resident microflora via animal manures, sewage or irrigation water, rainfall and atmospheric humidity. Microorganisms that have become trapped on the inner leaves of certain vegetables can be particularly difficult to remove by routine cleansing practices.

Among the organisms encountered in this study, *Bacillus* spp, *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Klebsiella* spp, *Pseudomonas* spp, *Escherichia coli, Enterobacter* spp and *Azotobacter* spp occurred in all three samples. *Lactobacillus* spp, *Micrococcus* spp, *Salmonella* spp, *Serratia* spp and *Citrobacter* spp occurred in irrigation water and amaranth.

The occurrence of *Escherichia coli* in the soil, irrigation water and amaranth is in agreement with the findings of Ibenyassine *et al.* (2006) which investigated the possible similarity of strains of *Escherichia coli* isolated from soil and vegetables irrigated by treated wastewater. The occurrence of *Salmonella* spp in irrigation water and amaranth in this study was consistent with the findings of previous studies (Guchi and Ashenafi, 2010; Ijabadeniyi, 2010; Afolabi *et al.*, 2011; Desta and Diriba, 2016). The occurrence of *Escherichia coli, Salmonella* spp, *Klebsiella* spp and *Enterobacter* spp in irrigation water and amaranth in this study was consistent with the findings of Afolabi *et al.* (2011). This could be due to direct transfer of bacterial cells from irrigation water to soil thereby making both possible sources of the contamination of amaranth indicating serious consumer's health risk when consumed raw.

Irrigation water containing pathogens had been reported to be used in the production of leafy vegetables in some countries (Thurston-Enriquez *et al.*, 2002). Epidemiological evidence from specific outbreaks also points to the role of irrigation water in the introduction of pathogens to the production environment. For example, contamination of iceberg lettuce in a large outbreak caused by *E. coli* O157 in Sweden was linked to the use of contaminated irrigation water drawn from a small stream (Soderstrom *et al.*, 2005). Furthermore, experimental evidence confirms that water used for irrigation can transfer human pathogens to a variety of growing leafy vegetables (Song *et al.*, 2006; Melloul *et al*., 2001; Solomon *et al.*, 2002; Amoah *et al.*, 2005). For example, Okafo *et al.* (2003) reported the detection of *Salmonella* on lettuce and amaranthus when the irrigation water was contaminated. The risks associated with the contamination of leafy vegetables with enteric pathogens in irrigation water were quantified in several risk assessment exercises (Petterson *et al.*, 2001; Stine *et al.*, 2005; Hamilton *et al*., 2006). These collectively provide strong evidence that water quality is an important risk factor in the production of these foods.

A number of recent *Salmonella* and *E. coli* O157:H7 outbreaks have been linked to contaminated water. Furthermore, studies have demonstrated the ability of the pathogen to survive for extended periods in water (Wang and Doyle 1998; Chalmers *et al.* 2000; Solomon *et al*. 2003). The risk of contracting diseases resulting from pathogenic microorganisms present in irrigation water is influenced by the level of contamination, the persistence of pathogens in water, in soil, and on crops; and the route of exposure (Steele and Odumeru 2004). Another investigation showed that there is a high probability for soil contamination due mainly to the protection from solar radiation that leaves and other parts of the plants give to bacteria and other microorganisms such as permanent moisture, richness in organic matter, and a pH between 6 and 7, which are features that favour the reproduction of micro-organisms (Abdul-Raouf *et al.* 1993). Study of Islam *et al.* (2004) shows that *E. coli* O157:H7 in soil, regardless of source or crop type, persisted for >5 months after application of contaminated compost or contaminated irrigation water.

The predominant microflora of fresh amaranth in this study was generally *Bacillus* spp followed by members of the family Enterobateriaceae (e.g. *Escherichia*, *Salmonella*, and *Enterobacter*) and Pseudomonadaceae (e.g. *Pseudomonas* and *Azotobacter*). The predominance of *Bacillus* isolates among the Gram positive bacteria is in agreement with the findings of Guchi and Ashenafi (2010). The predominance of *Bacillus* spp was probably due to the presence of spores in irrigation water and soil as well as other environmental factors. The survival of *Bacillus* depends on several factors such as the nature of the organism, resistance to a new physical environment, and ability to form spores (Godon, 1977). The high number of *Bacillus* could cause food poisoning (Mead *et al.*, 1999; Desta and Diriba, 2016). Also, endospores of *Bacillus* are more resistant than the vegetative cells to harsh weather conditions and even to antimicrobial treatments (Codex, 2007). Therefore, the presence of a high percentage of *Bacillus* spp in fresh amaranth could have consumer's health risk.

In this study, Enterobacteriaceae were the dominant microflora among Gram-negative isolates unlike previous reports on lettuce and green pepper from Ethiopia which showed the predominance of *Pseudomonas* isolates (Guchi and Ashenafi, 2010). The occurrence of enterobacteriaceae in this study indicates that the water used for irrigation was contaminated with faecal matter and sewerage from diverse sources. In addition, fresh fruits and vegetables often carry high levels of enterobacteriaceae as part of their normal flora (Gilbert *et al.*, 2000). Members of enterobacteriaceae have been implicated for causing several foodborne illnesses (Mandrell, 2011).

The occurrence of *Micrococcus* spp in this study is consistent with the findings of previous studies (Santamaria *et al.*, 2003; Guchi and Ashenafi, 2010; Desta and Diriba, 2016). *Micrococcus* spp is common environmental bacteria that could be introduced into fresh vegetables through cross-contamination, for instance, from irrigation water used by the farmers during irrigation. *Micrococcus* is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with the compromised immune system, such as HIV patients (Smith *et al.*, 1999).

The occurrence of *Staphylococcus* spp in irrigation water but not on amaranth in this study is consistent with the findings of Ikpeme *et al.* (2011) which isolated *Staphylococcus* spp from two rivers that were used for irrigation of vegetable in South Africa but not on vegetable. Although *Staphylococcus* spp did not occur in soil and amaranth in this study, in the study of Obi (2014), *Staphylococcus* spp was recovered from soil and vegetable. *Staphylococcus* spp has been reported to remain the most prominent aetiological agent of pyogenic infections, and that staphylococcal infection leads to a worsening of some already existing superficial infections (Adegoke and Komolafe, 2009).

The numbers and types of bacteria isolated in this study were slightly uniform across seasons. This could be as a result of continuous cropping of amaranth on the same piece of land and the use of the same source of water for irrigation over the years. Of the 18 organisms encountered in this study, 14 each were isolated in both seasons; 10 of which were uniform and four each which occurred differently across seasons. The variation could be as a result of contamination which could occur indirectly through dust particles and runoff in dry and rainy seasons respectively from livestock production and other human activities (Nesse *et al.*, 2005).

5. Conclusion

The present study revealed the correlation of bacteria isolated from amaranth to that isolated from soil and irrigation water collected from Koriko, Bassa LGA, Kogi State. The results of this study revealed

that all organisms in amaranth were present in the analysed soil and irrigation water. Contaminated irrigation water and soil were possibly the sources of contamination of amaranth growing on the field. Several pathogenic bacteria were involved in the contamination of amaranth on the farm, and this is of high potential hazard to consumers especially the illiterate majority who are not aware of such risks and can go ahead to consume this vegetable without washing them thoroughly. The presence of total aerobic enteric bacteria such as *Escherichia coli* and *Salmonella* spp is a cause for concern because it could cause foodborne diseases. There is a need to educate farmers on the risk involved in the use of contaminated water for irrigation.

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