The Pharma Innovation Journal 2017; 6(11): 245-250

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 245-250

www.thepharmajournal.com
Received: 12-09-2017
Accepted: 14-10-2017

Amandeep Kaur
Dolphin College of Life Sciences, Chunni Kalan, Fatehgarh Sahib, Punjab, India

Priyanka bhowate
Dolphin College of Life Sciences, Chunni Kalan, Fatehgarh Sahib, Punjab, India

Correspondence
Amandeep Kaur
Dolphin College of Life Sciences, Chunni Kalan, Fatehgarh Sahib, Punjab, India

Bacteriological anaylsis of fruits and vegetables from local market of Chunni Kalan, Fatehgarh Sahib Punjab

Amandeep Kaur and Priyanka bhowate

Abstract
Fruits and vegetables are one of the essential diets of human being, which they are consumed widely. But, they are contaminated with different bacteria that may cause food borne illness. Bacteriological survey of 20 samples of fresh vegetables & fruits were done to study the density of microorganisms by standard plate count (SPC) method. The colony forming units per gram (cfu/g) of the vegetables were much less ($7.2x10^5$ to $30.4x10^5$) in local market sample as compared to super market sample ($12.0x10^5$ to $20.6x10^5$) and field sample ($8.0x10^5$ to $18.0x10^5$). But in case of fruits higher cfu were obtained for the field sample ($8x10^5$) as compared to local market sample ($4x10^5$ to $5.5x10^5$) and super market sample ($2.5x10^5$). Of the various pathogens identified from the surface of these samples *Escherichia coli, Staphylococcus aureus, Bacillus cerous* were found to be the pre-dominant species in most of the samples. it was observed that the *bacillus, enterobacter and Staphylococcus* were the dominant genera in vegetables. This chapter will address characteristics of spoilage microorganisms associated with each of these fruit and vegetable categories including spoilage mechanisms, spoilage defects, prevention and control of spoilage, and methods for detecting spoilage microorganisms *Escherichia coli, Staphylococcus aureus, Bacillus cerous* were found to be the pre-dominant species in most of the samples were identified on the basis of morphology (Gram's staining and negative-staining), Biochemical test and selective cum differential culture media (such as MacConkey Agar, Bile esculin agar, Bacillus cereus agar, EMB Agar, Mannitol salt agar media). The antibiotic sensitivity test (MIC Value) of clindamycin, ciprofloxacin, erythromycin, methicillin, penicillin-G, vancomycin, rifamycin, amoxicillin, were determined against identified bacteria. It was observed that the *E. coli* strains were resistant to all the antibiotics tested in the present study (Ciprofloxacin, Penicillin-G, Rifamycin, Amoxicillin) whereas, 75 per cent of the Gram positive strains showed resistance to all the four drugs (Clindamycin, Vaomycin, Erthomycin and Methicillin. The micro flora of fruits and vegetables are one of the great concerns in our society as they can be of great risk for human health. Proper handling, hygiene, transportation and proper storage is necessary to avoid microbial food spoilage and related health risks.

Keywords: fruits, vegetables, food pathogens, microbiological load, bacteriological analysis

Introduction
Fresh fruits and vegetables are an important source of nourishment, especially vitamins, and a vital ingredient in healthy and balanced diets. Fruits and vegetables are vital sources of Nutrients to human beings. They give the body the necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development. Fruits and vegetables however, have serious challenges to their existence. These include changes in climatic condition, pests and microbial attack. Food spoilage refers to various changes in which the food becomes less taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the types of microorganisms responsible for their deterioration (Akinmusire 2011) [2].

Over the years, there has been an increase in the need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it (Akinyele and Akinkunmi, 2012) [1]. Fruits and vegetables normally carry a non-pathogenic epiphytic microflora2. The inner tissues of healthy plants and fruits are free of microorganisms, however, the surfaces of raw vegetables and fruits 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. Fresh produce is highly recommended in any diet, virtually without quantitative restriction (Silva *et al*., 2007) [12]. Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents are associated with greater predisposition to microbial spoilage.

Cabbage (*Brassica oleracea* capitata), Brinjal (Solanum melongena L.), Tomato Lycopersicon esculentum, (Solanaceae), Cauliflower (Brassica oleracea var Botrytis) and Carrots (*Daucus carata* L.) are the important vegetables grown in India (Mansoor Hassan *et al* 2005, Wasim *et al* 2008, Felix and Mahendran 2009) [9, 14]. Ber (*Ziziphus mauritiana*), Grapes (Vitis) and Chickoo (Sapodilla) has been recognised as a useful edible fruits since antiquity in India (Einsett and Pratt, 1975; Asam Ali *et al*., 2006) [7]. Such and other fresh-cut vegetables are, therefore, offered as salads in more than 70 per cent of fast food establishments and family restaurants. These vegetables and fruits are, however, capable of causing human diseases while still on the plant in fields or orchids, or during harvesting, transport, processing, distribution and marketing, or in the home (FDA, 2000; and Joshi, 2008) [8, 11]. Salmonellae have been isolated from many types of raw fruits and vegetables (Beuchat, 1996; Wells and Butterfield, 1997) [4, 15]. Bacteria such as Clostridium botulinum, Bacillus cereus and Listeria monocytes, all capable of causing illness, are normal in habitants of many soils, whereas Salmonella, Shigella, Escherichia coli and Campylobacter reside in the intestinal tracts of animal, including humans and more likely to contaminate raw fruits and vegetables through contact with their faeces, sewage, untreated irrigation water or surface water (Cliver, 1997; Speer, 1997) [6, 13]. The incidence of food borne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years (Mukherjee *et al*., 2006) [10]. There is thus demand by the consumers to use only such fresh vegetables and fruits which are free from microbial contamination and safe for human consumption. In the developed world, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increased frequencies and dissemination of resistance genes into other ecosystem (Chikwendu et el., 2008) [5]. Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers. Keeping the above facts in mind the present study on "Bacterial population associated with fresh fruits and vegetables" was designed with the following objectives:-

a) Isolation and enumeration of bacteria in different samples of fresh fruits and vegetables.
b) Morphological, biochemical and physiological characterization of bacterial strains.
c) Antimicrobial profile of surface micro flora of fruits and vegetables.

Materials and Method

Collection of samples

Three fruits namely Ber, Grapes and Chickoo and five vegetables namely Cabbage, Carrot, Brinjal, Tomato and Cauliflower were selected in the present study. The samples were collected from Local market (LM), Super market(SM) and Field (F), thorough survey of market was carried out in the month of February and March, for the collection of the samples of fresh fruits and vegetables available in the market so as to check the variation in the superficial bacterial contamination and reduce the same through antimicrobial rinsing treatment. Following methodologies were used to check the variation in bacterial population load and its antimicrobial treatment.

1. Isolation and estimation of bacterial population

For the isolation of bacterial strain from different samples, serial dilutions of the samples were prepared. The respected sample was suspended in 90ml distilled water and serial dilution was prepared. 0.1 ml sample was taken, spread it on different selective media, and incubated the plates for 24 hours at 37°.

2. Calculation of colony forming unit

After the appearance of colonies on the plates the cfu of each soil sample were calculated using the formula:

CFU = Number of colonies appearing on the plate × Dilution factor
Volume of sample taken

3. Morphological characterization

The isolated strains were characterized on the basis of different morphological and physiological characterization as mentioned in Bergey's Manual of Systematic Bacteriology.

(a) Colony morphology

The colonies were examined for shape, size, colour and opacity. Morphological observation was done along with colony morphology.

(b) Gram staining

This is the most important widely used procedure for characterizing bacteria. It was first described by Christian Gram. This method divides the bacteria into two groups, Gram positive which is purple in colour and Gram negative which is pink in colour. This technique is based on the ability of bacteria to retain primary stain (crystal violet dye) during decolourisation with alcohol or acetone. Gram positive bacteria retain primary stain while Gram negative bacteria are decolourised by alcohol and takes up the red colour counter stain. A smear of an isolate was made on a clean slide and allowed to dry. It was then heat fixed by passing the smear through the bursen burner, this is done to enhance the sticking of the organism on the microscope slide. The smear was flooded with crystal violet and left for 60sec before washing off with water. Lugols iodine was added and allowed to stand for 60 sec before being washed off and decolourised with alcohol for 10 secs. The slide was then washed off, stained with safranin for 30 sec washed off and allowed to air dry. A drop of immersion oil was added to the slide which was then viewed under the microscope using the x 100 objective lens.

(c) Biochemical characterization

The isolated strains were characterized on the basis of different morphological and physiological characterization as mentioned in Bergey's Manual of Systematic Bacteriology.

(i) Catalase test

This test is used to detect the enzyme catalase which protects the bacteria from hydrogen peroxide accumulated which can occur during aerobic metabolism. Catalase breaks the hydrogen peroxide into oxygen and water. The organism was picked and emulsified on a clean slide. A drop of 3% hydrogen peroxide was added to the slide. The presence of sustained bubbles indicated a positive result while their absence indicated a negative result.

(ii) Carbohydrate Fermentation
This test is used to detect organism which utilize different sugar as sources of energy with the production of acid and or gas. The sugars used were glucose, maltose and lactose. To perform this experiment, four broth tubes amended with four different sugars i.e. each containing 0.5% sugar were used. Durham tubes were inverted in each tube. Every tube was inoculated with bacterial culture and incubated the culture at 637°C for 24 h. The tubes were examined for the development of acid or gas as shown by the change in colour.

(iii) Indole production
This test was carried out to determine the organism that break down the amino acid tryptophan into indole. The test organism was incubated in sterile test tubes containing peptone water and incubated at 37°C for 48h. 0.5ml of kovac reagent was added and mixed to stand for 10 min. The development of a pink color indicated a positive result.

(iv) Methyl Red (MR) and Voges Proskauer (VP) Test
MRVP broth tubes were prepared and 5 ml broth was poured in each tube and sterilized by autoclaving. Two test tubes were inoculated with bacterial culture while two tubes were kept as control. All test tubes were incubated at 27°C for 48 h. Drops of MR indicator were added to one tube and observed for the change in colour. MR indicator was also added to one of the control tube.

(v) Citric Acid Utilization
The medium used was simmone citrate agar. The test is used to identify each organism which of the organism can utilize citrate as the sole source of carbon for metabolism. It is used in the differentiation of the organism in the enterobacteriaceae and other genera. Bijou bottles were used for the test in saline preparation of the organism was inoculated inside the citrate medium and incubated at 37°C for 24h. A change in colour from green to blue indicates a positive result.

(vi) Oxygen requirement
Tubes of nutrient agar were prepared. Medium of deep tubes was melted in hot water bath at 100°C. The agar was cooled to 45°C and culture of bacteria was inoculated into the tubes and tubes were rotated to mix the inoculum with molten medium. Solidified the medium by placing the tubes in an upright position. Incubated the tubes at 35°C for 24 to 48 hours.

(4) Antibiotic Resistance Profile (Bauer *et al*., 1966)
Antibiotic resistance of selected pathogenic isolates was determined by disc diffusion assay on Mueller Hinton agar as per Kirby Bauer method.

Results and Discussions
The present study entitled Bacterial population associated with fruits and vegetables and its treatment using antimicrobial rinsing was carried out to study the micro flora associated with fruits and vegetables and reducing their superficial contamination with antimicrobial rinsing. A comparison among the total microbial load of fruits and vegetables from various markets and field sample was also carried out. Twenty samples were collected from local market, super market and field, and bacterial population was isolated from them. The bacterial strains were characterized on the basis of various morphological and biochemical tests. The strains were screened for antibiotic sensitivity against various antibiotics. Further, treatment of fruits and vegetables was also carried out to study the reduction in total bacterial load. The results of present study are summarized as under:
Data presented in Table 1showed that among vegetable samples the highest cfu (colony forming units) count was obtained for local market samples of carrot i.e. 30.4x105 on NAM as compared to super market (20.6x105) and field sample (18.0x105). Among fruits the cfu highest count was obtained for field sample i.e. 8x105 (Table 2) as compared to local market sample (5.5x105) and super market sample (2.5x105). Similar finding were observed by Ibrahim *et al*. (2009), Patel and Joshi (2008) [11] who observed high contamination and high number of pathogens on the surface of fresh vegetables and fruits in case of local market samples (log 9-10 cfu/ml) as compared to super market samples (log 5-6 cfu/ml).

Table 1: Colony forming unit (cfu) of vegetables samples collected from different markets

S. No	Vegetables	Cfu (x105)			
		NAM	MCA	EMB	MSA
Local market					
1	Cabbage	7.2	-	3.2	3.5
2	Cauliflower	13.6	1.1	1.3	2.3
3	Carrot	30.4	6.2	-	4.5
4	Tomato	13.2	6	1.5	2.5
5	Brinjal	23.2	3	-	3.0
Super market					
1	Cabbage	20.6	-	3.0	1.1
2	Cauliflower	12.0	1	-	1.2
3	Carrot	17.6	2.4	-	4.4
4	Tomato	16.8	1.4	2.1	1
5	Brinjl	12.0	-	2	1.0
Field					
1	Cabbage	16.0	2.5	-	1.4
2	Cauliflower	8.0	3.5	-	2.5
3	Carrot	14.0	3.4	-	3.0
4	Tomato	18.0	6.0	-	2.2
5	Brinjal	-	-	1.0	1.9

Where:
NAM= Nutrient agar
MCA= MacConkey agar
EMB= Eosine methylene blue Agar medium
MSA= Mannitol Salt Agar

Table 2: Colony forming unit (cfu) of fruit samples collected from different markets

S. No	Fruit Sample	Cfu (x105)			
		NAM	MCA	EMB	MSA
Local market					
1	Ber	4	-	3.4	-
2	Grapes	5.5	3.8	1.5	1.4
3	Chickho	1.9	4.0	1	2
Super market					
1	Grapes	2.5	-	2.2	1.0
Field sample					
1	Ber	8	-	-	9

Table 3: Colony morphology of bacterial strains

S. No	Strain	Colony morphology	Medium
1	VBL1	Yellow colour, entire, circular	MS
2	VCAL2	Abundant, circular with greenish margin	EMB
3	VCM3	Yellow colour, entire, circular	MS
4	VTL4	Abundant, circular with greenish margin	EMB
5	VTL5	Yellow colour, entire, circular	MS
6	VCM6	Yellow colour, entire, circular	MS
7	VCBL7	Yellow colour, entire, circular	MS
8	VTM8	Yellow colour, entire, circular	MS
9	VCL9	Yellow colour, entire, circular	MS
10	VCAM10	Yellow colour, entire, circular	MS
11	VCBM11	Yellow colour, entire, circular	MS
12	VCF12	Yellow colour, entire, circular	MS
13	VTF13	Yellow colour, entire, circular	MS
14	FCHL14	Yellow colour, entire, circular	MS
15	FGRL15	Abundant, circular, with greenish margin	EMB
16	FCHL16	Yellow colour, entire, circular	MS
17	FGRL17	Pinkish pigment, round entire,	MCA
18	FBL18	Pinkish pigment, round entire	MCA
19	FGRL19	Yellow colour, entire, circular	MS
20	FCHL20	White, oval, moist, round in shape	NA
21	FBL21	Abundant, circular, with greenish margin	EMB

Fig 1(a): citrate test (b) catalase test (c) A view of Growth of VBL1 on MSA (d) A view of Growth of FGRL15 on EMB

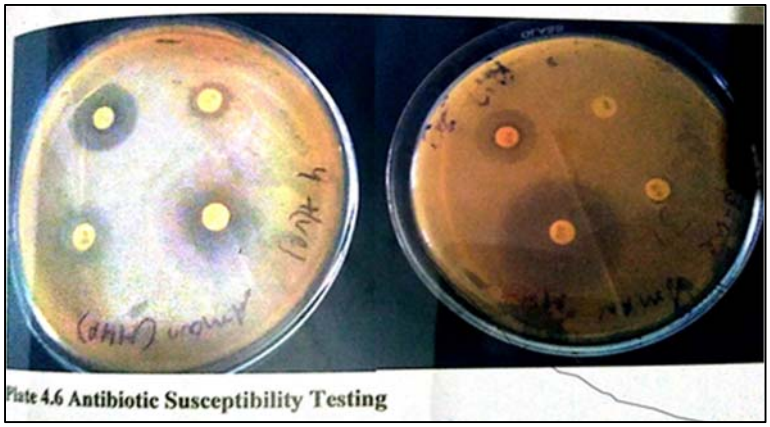
A total of twenty one bacterial strains were isolated in the present study. On the basis of morphological and biochemical tests ten strains namely VTL4, VCBL7, VTM8, VCL9, VCAM10, VCBM11, FBL18, FGRL17, FGRL15 and FBL21 strains isolated were identified as E.coli. Patel and Joshi (2008) [11] also detected the prevalence of the members of family Enterobacteriaceae among the bacterial population isolated from fruits and vegetables.While, VBL1, VTL5, VCM6, VTF13, FCHL14, FGRL19, VCF12 and FCHL16 were identified as Staphylococcus aureus and VCM3, VCAL2, FCHL20 were characterized as Bacillus cereus (Table 4). Viswanathan and Kaur (2001) also reported the presence of E. coli and Staphylococcus aureus in vegetables and fruits. Several other workers have also detected Staphylococcus aureus from carrot and ready-to-eat vegetable salads (Abdelnoor *et al* 1983, Houang *et al* 1991). Staphylococcus spp. and E. coli have been reported to be the major microbes associated with the contamination of the vegetables and fruits (Ibeyessie 2007, Ibrahim *et al* 2009). VCM3, VCAL2, FCHL20 were identified as Bacillus cereus. Portnoy *et al* (1976) observed the presence of Bacillus spp. while working with raw vegetables and fruits. Presence of spore formers can be attributed to the fact that spore-forming organisms can attach to vegetables grown near the soil for eg. Bacillus cereus (Linton, 2003).

Table 4: Morphological and Biochemical characterization of bacterial isolates from vegetable samples

Strain	Biochemical Tests											Identified as
	G.R	Shape	Lactose	Dextrose	Sucrose	Catalase	Indole	MR	VP	Citrate	O.R	
VBL1	+ve	Cocci	A	A	A	+	-	+	-	-	Aerobic	S.aureus
VCAL2	+ve	rods	-	A	A	-	-	-	-	-	Aerobic	B.cereus
VCM3	+ve	rods	-	A	A	-	-	-	+	-	Aerobic	B.cereus
VTL4	-ve	rods	AG	AG	A	+	+	+	+	-	F.A	E.coli
VTL5	+ve	Cocci	A	A	A	+	-	+	-	-	F.A	S.aureus
VCM6	+ve	Cocci	A	A	A	+	-	+	-	-	F.A	S.aureus
VCBL7	-ve	rods	AG	AG	A	+	+	+	-	-	F.A	E.coli
VTM8	-ve	rods	AG	AG	A	+	+	+	-	-	F.A	E.coli
VCL9	-ve	rods	AG	AG	A	+	+	+	-	-	F.A	E.coli
VCAM10	-ve	rods	AG	AG	AG	+	+	+	-	-	F.A	E.coli
VCBM11	-ve	rods	AG	AG	AG	+	+	+	-	-	F.A	E.coli
VCF12	+ve	Cocci	A	A	A	+	-	+	-	-	F.A	S.aureus

Where:
G.R- Gram Reaction
O.R- Oxygen Requirements
F.A- Facultative anaerobic
E.coli- Escherichia Colis
S. aureus- Staphylococcus cereus
B. cereus- Bacillus cereus

Almost 99 per cent of the E. coli strains were resistant to all the antibiotics tested in the present study (Ciprofloxacin, Penicillin-G, Rifamycin, Amoxicillin) whereas, 75 per cent of the Gram positive strains showed resistance to all the four drugs (Clindamycin, Vaomycin, Erthomycin and Methicillin) in figure 4.6. Overall, 95% isolates were resistance to antibiotics that were used in this study (Fig 2 and 3) Resistance of E. coli for Ciprofloxacin, Penicillin-G, Rifamycin and Amoxicillin has been reported earlier by Akond *et al.* (2009). Similar findings on multiple drug resistance of E. coli strains has been reported from in several parts of the world by several workers (Khan *et al*., 2002; Guerra *et al*., 2003; Zhao *et al*., 2005; Rahman *et al*., 2008). High resistance for Amoxycillin among E. coli have been also reported by Garba *et al*. (2009). Although, newer antibiotics are available, but emerging antimicrobial resistance is becoming an increasing problem in many pathogens throughout the world. The variation in the susceptibility of these organisms towards antibiotics may be connected to their previous exposure to the antibiotics and thereby varying the degree of resistance in addition to this the Gram reaction of the organisms also influences their susceptibility to the antibiotics used.



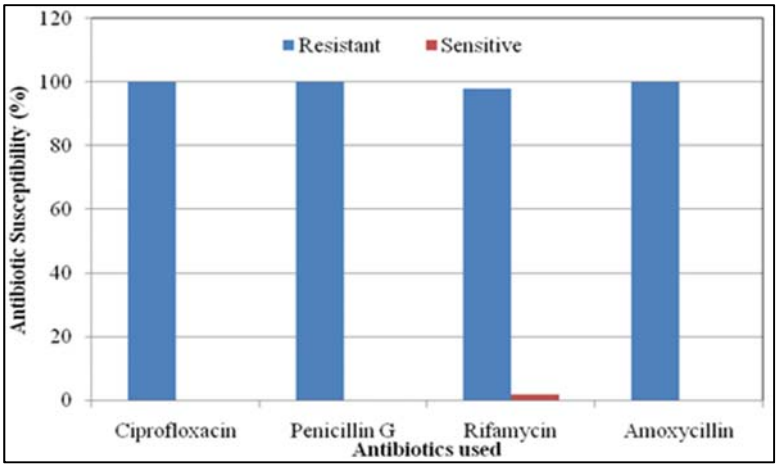


Fig 2: Antibiotic susceptibility patterns of E. coli strains isolated in the present study (figure-5)

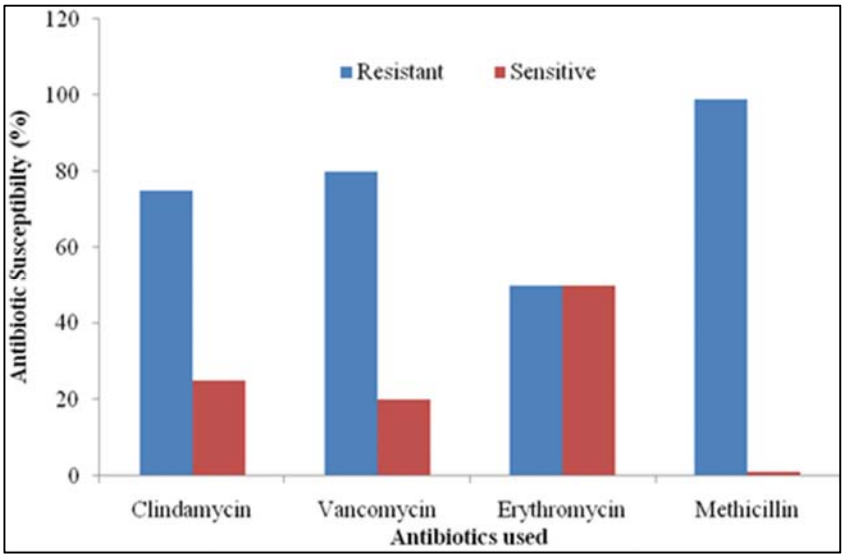


Fig 3: Antibiotic susceptibility patterns among Gram positive strains isolated in the present study

Conclusions

On the basis of above results it can be concluded that fresh vegetables & fruits mainly from local Market harbour many microbial contaminants and pathogens as compared to samples of super markets, indicating that these are protected from contamination while subsequent handling, packaging, storage and transit. As done by Ibrahim *et al*. (2009), Patel and Joshi (2008) [11] who observed high contamination and high number of pathogens on the surface of fresh vegetables and fruits in case of local market samples (log 9-10 cfu/ml) as compared to super market samples (log 5-6 cfu/ml). All the fruits and vegetables assessed bacteriologically had high viable counts even though within the tolerable limit.

References

1. Akinyele BJ, Akinkunmi CO. Fungi associated with the spoilage of berry and their reaction to magnetic field. Journal of Yeast and Fungal Research. 2012; 3(4):49-57.
2. Akinmusire OO. Fungal species associated with the spoilage of some edible fruits in Maiduguri, Northern Eastern Nigeria. Advances in Environmental Biology. 2011; 5(1):157-161.
3. Asam Ali S, Bonkoungou E, Bowe C, DeKock C, Godara A, Williams JT. Fruits for the Future 2- Revised edition – Ber and other Jujubes (Ziziphus species). Monograph. 2006, 289.
4. Beuchat LR. Pathogenic microorganisms associated with fresh produce. J Food Prot. 1996; 59:204-206.
5. Chikwendu CI, Nwabueze RN, Anyanwu BN. Antibiotic resistance profile of Escherichia coli from clinically healthy pigs and their commercial farm environments. African Journal of Microbiology Research. 2008; 2:012-017.
6. Cliver DO. Food borne viruses. In: Doyle, M.P, Beuchat, L.R. and Montville, T.J (eds.) Food Microbiology: Fundamentals and Frontiers, Washington, DC, American Society for Microbiology. 1997, 437-446.
7. Einsett J, Pratt C. Grapes. Janick J And Moore, J.N. (eds.), Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind. 1975, 130-153.
8. FDA. (Food and Drug Administration) Guide to minimize food safety hazards for fresh fruits and vegetables. Www. Cfsan fda. Gov/ html, 2000.
9. Mansoor-ul-Hassan F, Mohammed A, Sagheer M, Fareed I, Muhammad T. Residual persistence of chlorpyrifos, imidacloprid and acephate Brinjal fruit. Pak. Entomol. 2005; 27:53.
10. Mukherjee A, Speh D, Jones AT, Buesing KM, Diez-Gonzalez F. Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. Journal of Food Protection. 2006; 69:1928-1936.
11. Patel S, Joshi P. Microbiological analysis of fresh fruits and vegetables and effect of anti-microbial agents on microbial load. PhD thesis, University of Mumbai, 2008.
12. Silvia R, Silva P, Sylvia E, Frizzo Dariane C, Pereira A, Schatkoski MB, *et al*. Microbiology Quality of Minimally Processed Vegetables Sold In Porto Alegre, Brazil. Brazilian Journal of Microbiology. 2007; 38:594-598.
13. Speer CA. Protozoan parasites acquired from food and water. In: Doyle MP, Beuchat, L.R., Montville TJ (eds.) Food Microbiology: Fundamentals and Frontiers, Washington, DC, American Society for Microbiology. 1997, 478-493.
14. Wasim A, Paramasivam M, Sengupta D. Cost reduction in cabbage cultivation by weed control using oryzalin herbicide and hoeing technology. African Journal of Agricultural Research. 2008; 3:808-810.
15. Wells JM, Butterfield JE. Salmonella contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Dis. 1997; 81:867-872.