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Microbiological analysis of fresh amaranthus from organic and conventional production

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Abstract

Globally, area under organic farming is increasing substantially over the years. There are certain apprehensions regarding microbial load of organically produced and conventionally produced leafy vegetables. The aim of this study was to analyze fresh amaranthus samples for the presence of aerobic mesophilic bacteria and coliforms. The aerobic mesophilic counts ranged between 7.15 – 8.54 $\log_{10}$ CFU/g and 7.83 $\log_{10}$ CFU/g from organic and conventional amaranthus, respectively. The total coliform counts varied from 0 to 7.5 $\log_{10}$ CFU/g and nil in organic and conventional amaranthus, respectively. This study determined no significant differences in aerobic mesophilic bacteria and total coliforms in organic and conventional systems of amaranthus production. Improvement in soil organic carbon (%) was recorded in organic treatments in comparison to inorganic treatment.

Introduction

Fruits and vegetables are very often consumed uncooked or with minimal preparation (Sehgal and Mehta, 2014). They are rich source of dietary fibre, vitamins and minerals (Eni *et al.*, 2010). India is the second largest producer of vegetables with a production of 162.8 mt (Indian horticulture database, 2014). Nowadays, there is a surge in the consumption of fresh fruits and vegetables/ ready-to-eat (RTE) produce in many parts of the world (Jeddi *et al.*, 2014). Advancement in standard of life coupled with availability of fresh produce throughout the year in India has led to the diversification of food requirements (Mathur *et al.*, 2014). Despite health benefits, they have been closely associated with growing number of food-borne illness (Sivapalasingam *et al.*, 2004). Raw vegetables may harbour potential foodborne pathogens (Sago *et al.*, 2003), *Salmonella* (Sagoo *et al.*, 2003) and *Escherichia coli* O157:H7 (Friesema *et al.*, 2007). Problems linked with pathogens in fresh produce including trade implications, have been reported worldwide (WHO, 2008). Contamination of vegetables with pathogenic organisms may happen directly or indirectly via animals, insects, water, soil, dirty equipment and human handling during crop production (Oliveira *et al.*, 2010).

Microbiological risk assessment is necessary to know the safety of food and water supplies. It is essential to take suitable measures to contain the incidents of food-borne illness due to consumption of contaminated foods. Generally the coliforms group has been used widely as an indicator of microbiological quality of food and water. Their presence indicates improper treatment or post-disinfection contamination (Chaturvedi *et al.*, 2013).

Use of organic methods in farming is environment friendly largely because of harmonious cooperation with nature and absence of synthetic chemicals (Matt *et al.*, 2011). Globally, there is a substantial increase in the area under organic cultivation. At present it is 43.1 m ha, which is 291.81% increase since 1999. About 72b US dollar worth organic products are sold internationally in the year 2013, with an annual growth rate of more than 10%. In Asia, 3.4 m ha area is under organic farming, leading countries include china (2.1 m ha) and India (0.5 m ha). India is home to largest number of organic producers (0.65 million out of 2 million) in the world (FiBL Survey, 2015). Organic industry is being growing continuously in India in the last few years.

Amaranthus is an herbaceous leafy vegetable produced throughout the year. This is one of the low-cost leafy vegetables in tropical markets, having rich nutritional value and has carotene, calcium, vitamin C, folic acid and other micronutrients in high amounts. Amaranthus leaves combined with condiments are used to prepare soup (Mepba *et al.*, 2007). In India,

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Table 1. Microorganism counts for different organic and inorganic systems amaranthus samples

Treatments		Total Aerobic mesophilic count (Log10 CFU/ g)	Total Coliforms (Log10 CFU/ g)
T1	FYM (100%) @ 16.6 t/ha	8.03	7.50
T2	Vermicompost (100%) @ 5.8 t/ha	8.12	3.65
T3	FYM (75%) + biofertilizer(*Azotobacter* @2g/kg seed)	7.69	3.80
T4	Vermicompost (75%) + biofertilizer (*Azotobacter* @2g/kg seed)	7.15	3.65
T5	FYM(50%)+jeevamrit+biofertilizer(*Azotobacter* @2g/kg seed)	8.44	3.74
T6	Vermicompost (50%) + Jeevamrit +biofertilizer (*Azotobacter* @2g/kg seed)	7.30	0.00
T7	FYM(50%)+panchagavya+biofertilizer (*Azotobacter* @2g/kg seed)	8.05	0.00
T8	Vermicompost (50%)+panchagavya+biofertilizer (*Azotobacter* @2g/kg seed)	8.54	0.00
T9	Inorganic control (80:60:50 Kg N P_2O_5 and K_2O/ha)	7.83	0.00
	SEM	0.30	2.48
	CD @ 5 %	0.99	8.07

Note: FYM –Farm Yard Manure

amaranthus is being grown from the foothills of The Himalayas to the coasts of south India. In the north, chaulai saag is made with amaranth leaves, garlic, onions and spices. Dal saag is prepared by cooking the leaves with lentils. In Andhra Pradesh, thotakura pappu is prepared with amaranthus and toor dal *(Cajanus cajan)*. In kerala, cheera thoran is prepared with amaranth leaves (Borah, 2015). The aim of the present study was to determine the effect of different organic production systems vis-a-vis conventional system on the microbial load of fresh Amaranthus.

Materials and Methods

Experimental site

The samples were obtained from organic and conventional plots that were located in the research farm of ICAR-Indian Institute of Vegetable Research, Varanasi, India (82.52 °E longitude; 25.10 °N latitude). The area receives an average rainfall of 1000 mm which is distributed over a period of more than 100 days with peak period between July and August. Scattered showers occur during winter months. In general, the temperatures ranges from 5^0C to 42^0C. The coldest month is January while the maximum temperature is observed during May and June. The crop (*Amaranthus cruentus*; variety VCP Gold) was grown during the summer season (April-June) of the year 2015. The treatment details of the experiment are included in Table 1. Colony Forming unit of *Azotobacter* used was 1 x 10^8 CFU/g.

Sampling of Amaranthus

Amaranthus samples were collected directly from the farm in surface sterilized polythene bags and were transported to the laboratory without being washed and immediately analyzed.

Microbiological analyses

Microbial analyses were carried out using the standard methodologies. Twenty-five grams of amaranthus were transferred in 225 mL of saline peptone solution (SP, 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone), in sterile blender. The samples were homogenized in a blender for 2 min. The blender was carefully disinfected to prevent any cross contamination. Further serial dilutions were made with the same diluents, 100 µl of each sample was inoculated on to the respective media for the analyses of aerobic mesophilic and coliforms. Total plate count in vegetables was determined by the procedure described in Indian Standards 5402:2002 (Chaturvedi *et al*., 2013). Total coliforms were determined by using EMB agar medium (HiMedia, India) (Tambekar and Mundhada, 2006). Typical colonies on the plates were enumerated and colony counts per 1 g sample were determined. Colony counts were converted into $\log_{10}$ CFU/ g.

Media used

All media used were of analytical reagent grade. Plate count agar (HiMedia, India) was used for detecting total aerobic bacterial count after incubation at 30°C for 48 h. EMB agar medium (HiMedia, India) was used for the enumeration of total coliforms.

Preparation of panchagavya and jeevamrit

Panchagavya was prepared by mixing the following ingredients; Cowdung slurry (4kg) + cowdung (1 kg) + Cow urine (3 L) +Cow's Curd (2 kg) +Cow ghee (1 kg). The mixture was allowed to ferment for 7 days and 3% solution was sprayed on soil.

Jeevamrit was prepared by mixing the following ingredients: Cowdung (10 kg) +Cow urine (10 L) +jaggery (2 kg) + pulse flour (2 kg) +soil (1 kg) + water (200 L).The mixture was fermented for 7days with intermittent stirring and sprayed on soil and this mixture would be sufficient for one acre area. The organic carbon of the soil, expressed as percentage of carbon was done following the methodology of Walkely and Black, 1934).

Statistical analyses

Analysis of the data was done using SPSS 16.0.

Results and Discussion

The present study was intended to provide some assessment on the microbiological load of Amaranthus produced through different organic and conventional systems in India. To our knowledge, this is the first report in Northern India that compares the microbiological load of amaranthus in two types of production system.

Aerobic mesophilic counts are generally utilized to determine the shelf-life and microbial quality of foods (Pianetti *et al.*, 2008). In the present study, the aerobic mesophilic count ranged from 7.15 to 8.54 log_{10} CFU/g in organic and 7.83 log_{10} CFU/g in inorganic treatment (Table 1). Maffei *et al*. (2013) reported the mesophilic aerobic bacteria, from 5 to >7 log_{10} CFU/g for organic vegetables and 3 to >7 log10 CFU/g for conventional vegetables. In the present investigation, the treatment T8 (vermicompost, panchagavya and biofertilizer) followed by T2, which received vermicompost and T7 (FYM, panchagavya and biofertilizer) recorded highest log_{10} CFU/g. Inorganic treatment recorded 7.83 log_{10} CFU/g. However, no significant differences were observed statistically within different organic treatments and inorganic treatment (Table 1).

Coliforms were present in lesser numbers than bacteria. They ranged between 0 and 7.50 log_{10} CFU/g in both organic and inorganic treatments (Table 1). Maffei *et al.* 2013 reported total coliforms counts from 2 to 7 log_{10} CFU/g for organic vegetables and 1 to 7 log_{10} CFU/g for conventional vegetables. The treatments T1 recorded more counts followed by T3; inclusion of FYM in both the treatments might have

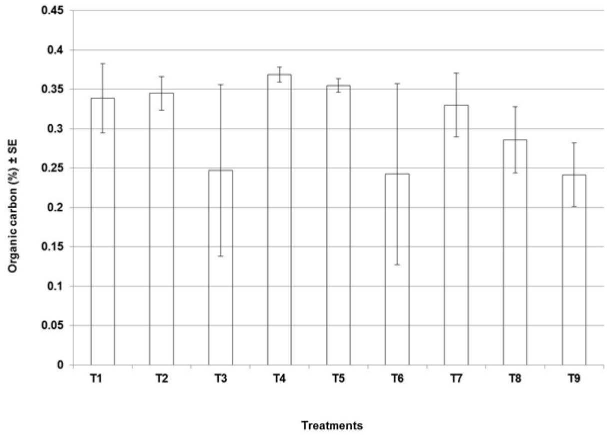

Figure 1. Influence of different treatments on soil organic carbon

Note: SE- Standard Error

resulted in relatively higher counts in these treatments. No coliform count was recorded in the inorganic treatment (Table 1). The coliforms are widespread in nature and most common in raw vegetables so, they are not correlated to faecal contamination (Brackett and Splittsesser, 1992; Doyle and Erickson, 2006). In the present investigation, no significant differences were observed among different organic and inorganic treatments.

Influence of different treatments on soil organic carbon was analyzed. Maximum soil organic carbon (%) of 0.3683 was observed in T4 which included vermicompost (75%) + biofertilizer (*Azotobacter* @ 2g/kg seed) followed by T5-FYM (50%) + jeevamrit+biofertilizer (*Azotobacter* @ 2g/kg seed), T2-vermicompost (100%) and T1-FYM (100%). Lowest organic carbon was observed in 0.2418 inorganic control (Figure 1). Jeevamrit has also been reported to help in preventing infestation of pests and diseases and increasing yield in chilli, potato and soybean when applied as seed/seedling treatment in organic cultivation (INHERE, 2006).

In the present study, it was noticed that, total aerobic mesophilic count was maximum in the treatment which received vermicompost 50%, panchagavya and biofertilizer *(Azotobacter)*. It was followed by vermicompost. The enhanced microbial count in these treatmens might be due to vermicompost, panchagavya which are good source of nutrients and are reported to improve the microbial population (Pathma and Sakthivel, 2012; Shubha *et al.*, 2014). Kumar *et al*. (2011) reported the panchagavya as a source of organic nutrient in blackgram *(Vigna mungo)*. Its foliar application resulted in significant enhancement in plant height, number of branches per plant, leaf area index (LAI), N content of root nodules, chlorophyll content and dry matter production when compared with NPK and control. Enhanced microbial population usually indicate food quality and shelf-life duration (Pianetti *et al*., 2008). Microbial contamination may occur at any point during producer to consumer value chain. Some of the researchers have been reported that, the produce derived from organic means is more responsible for transmitting food borne diseases than the one obtained from conventional farming (Avery, 2002). In contrast to this, Oliveira *et al.* (2010) reported that, consumption of organically produced lettuce is not related to increasing risk of food borne diseases. The present investigation also found no significant differences in microbial load between organic and inorganic treatments (Table 1).

Conclusion

The result of the present study shows that, there is no significant influence of different organic treatments and inorganic treatment on microbial load of amaranthus. There was an improvement in soil organic carbon status in organic treatments than the inorganic treatment. Consumption of organically produced amaranthus does not represent an increasing risk of food borne diseases to consumers. However, proper hygiene practices during farming, transit, marketing and during consumption should be maintained.

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