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Gastrointestinal parasites and bacteria's in vegetables grown in soil treated with organic manure

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Abstract

An attempt was made to evaluate the parasitic load in vegetables grown organically is Majini Muhallah Gilgit, Pakistan. During this study Majini Muhallah was divided into three sectors. Six vegetables including Spinach (*Spinacia oleracea)*, Cabbage (*Brassica oleracea)*, Lettuce (*Lactuca sativa*), Radish (*Raphanus sativus*), Sonchual (*Malva verticillata)* and Pino (*Brassica rapa*) were selected for the study**.** Three samples of each vegetable were taken from each sector. The present study was carried out to assess the intestinal parasitic load (*Ascaris lumbricoid*, *Giardia lamblia*, *Tricuris tricuria*, and *cryptosporidium)*. Samples were identified on the basis of microscopy. In the sum total of three samples of each vegetable *Ascaris Lumbricoid* ranged maximum 18 (40.90 %) in spinach and minimum 7(25%) in sonchual. *Giardia lamblia* ranged maximum 14 (31.81 %) in spinach and minimum 7(24.13%) in pino. *Tricuris tricuria* ranged maximum 10(23.25%) in lettuce while minimum 7(24.13%) in pino. *Cryptosporidium* showed maximum load 9(25.71%) in radish where as minimum 3(6.81 %) in spinach respectively.

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Introduction

In developing countries an Intestinal parasitic disease are even now public health problem and threatens the lives of many people's (Joseph and David, 2011; Steketee, 2003; Curtale *et al*., 1998;). Almost one third of the world, more than two billion people is infected with intestinal parasites. Vegetables are necessary for good health; hence they make a major component of human diet in every family unit. They are important energy contributors that are dependent upon by all levels of human as diet supplement (Duckworth, 1996). As vegetables need a moist environment for their proper growth, which is also favors the development of infectious and transmissible parasites (Oliveira & Germano, 1992; Silva *et al.,* 1995). Almost all vegetables, particularly those who are normally eaten raw or without peeling can be major agents of transmission of protozoans (Porter *et al*., 1990; Al-Binali *et al*., 2006), and helminthes (Choi and Chang, 1967; Mesquita *et al*., 1999).

Vegetables usually become a potential reservoir of human parasitic infection by contamination during their production specially by applying organic manure, as the major sources of contamination are human feces, contaminated soil or water (*Slifko et al.,* 2000*; Damen et al., 2007).*

Many studies have revealed that there is a tough association between vegetables consumed raw and parasitic infections (*Cheesebrough,* 1991; Vuong *et al.,* 2007). Furthermore, various outbreaks of protozoans infections in humans have been tied to vegetables consumed raw (Mintz *et al*., 1993).

Contamination of vegetables can be minimized relaying on the application of good agricultural practices in cultivating the vegetables, well hygiene practices during harvesting, picking, transporting and processing mainly washing. The significance of washing vegetables properly particularly those eaten uncooked is to efficiently remove from the vegetables many pathogenic microorganisms which can cause infections upon ingestion. Solutions containing Chlorine or other antibacterial compound have been used to diminish the number of contaminating microorganisms in such vegetables. (Abdullahi and Abdulkareem, 2010).

Some food borne diseases have been identified due to field contamination before these greens are even harvested (APA, 2007).

This work is basically to establish the bacteriological load of a few fresh leafy vegetables that are generally not cooked before eating. Vegetable is the soft plant part which is not sweet in taste and may be flavored or spiced with ingredients before consumption (Okigbo, 1990). These plants or plant parts can be taken raw as salad or mixed to some cooked foods like rice. Vegetables are considered to be reaching in vitamins, iron, calcium, proteins, fats and minerals. Leafy green and yellow vegetables are consumed highly for their vitamin A and iron contents. Vegetables are useful in neutralizing the acid substances formed in the route of digestion of meat, cheese and other foods as they are considered as roughages which accelerate digestion and aid to avoid constipation (Oyenuga and Fetuga, 1985). While cultivating, vegetables may be exposed to lots of sources of contamination like polluted sewage used in watering the gardens from where these vegetables are grown. Salmonella species and other pathogens are studied from raw and treated sewage. (Schothorst *et al.,* 1999).

Vegetables are consumed either raw or cooked, in addition of its usage as an immense source of vitamins and minerals is a local tradition of Majini Muhallah Gilgit, Pakistan. Vegetables are eaten widely in many part of Pakistan, but unluckily people still don't know how to wash them appropriately.

There is lack of information so far to examine the prevalence of gastrointestinal bacteria & parasites in vegetables grown in soil treated with organic manure in Majini Muhallah Gilgit. Therefore, the present

investigation aimed to assess the intestinal parasitic load in some vegetables of the proposed study area.

Materials and methods

A total of eighteen samples, three of each vegetable including Spinach (*Spinacia oleracea)*, Cabbage (*Brassica oleracea)*, Lettuce (*Lactuca sativa*), Radish (*Raphanus sativus*), Sonchual (*Malva verticillata)* and Pino (*Brassica rapa*) were collected from three different sectors of Majini Muhallah, Gilgit during Spring season period in May, 2010 (Fig.1).

The samples were taken to the laboratory and analysis carried out instantaneously.

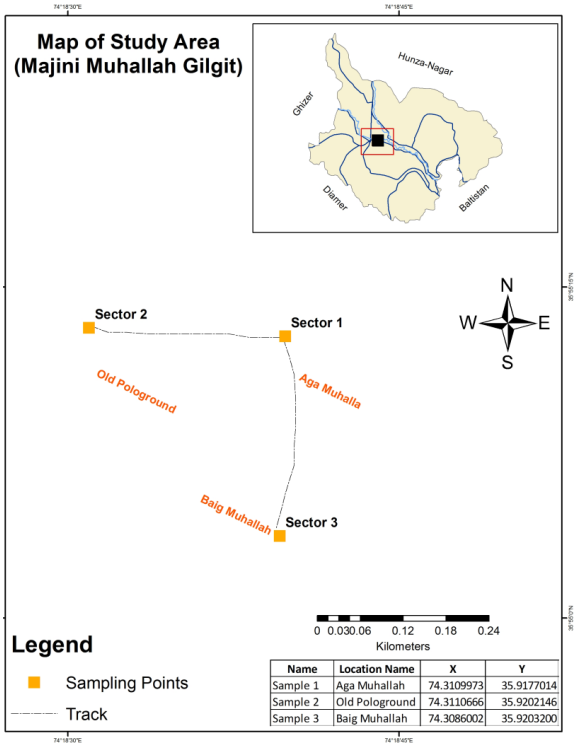

Fig.1. Map showing the sampling sites and their locations.

Total aerobic plate count of bacteria

Bacterial analyses were done according to standard method (Speck, 1988). Twenty-five grams of the vegetable was washed in sterile 223ml buffered peptone water from which 1ml was poured to the first test tube containing 9ml of the diluents. This was repeated for the other three sets of tubes to dilute to 10-5. From the last dilution 1ml was pipetted and dropped in pre- sterilized plates in duplicate. A molten plate count agar was poured into the plates and gently rocked to spread and cooled. The plates were packed and incubated at 370C for 24hrs at the end of incubation, the plates were removed and colonies counted and multiplied by the dilution factor.

Biochemical tests

Identification and characterization of isolates were done by carrying out biochemical tests that included coagulase, motility, indole, oxidase, citrate, malonate, methyl red, Voges Proskauer (MRVP), Triple iron sugar (TSI).

The coliform index

The coliform index of the vegetables was determined by using the plate count technique. Eosin MacConkey agar was inoculated with 0.1ml from the diluted sample and then incubated at 370C for 24 hrs after which the plate was removed and the colonies counted. The number of colonies was multiplied with the dilution factor to arrive at the index (FAO, 1979).

Statistical analysis

All the statistical computations were carried out using SPSS software version 16.0.

Results

Bacteria

This study was carried out to evaluate the bacteriological load in vegetables grown in soil amended with organic manure. Total six vegetables, three samples of each sector were taken.

Table 1 shows the total aerobic plate count and coliform index of six vegetables of Majini Muhallah, Gilgit. The results of bacteriological contaminations of vegetables in Majini Muhallah, Gilgit are shown in Fig. 2.

Table 1. Total aerobic plate count and coliform index of six vegetables in Majini Muhallah, Gilgit.

Sample	Vegetable	TAPC cfug^{-1}	CI cfu g^{-1}
1	Spinach	12×10^5	8×10^5
2		14×10^5	10×10^4
3		13×10^5	9×10^5
1	Lettuce	10×10^4	7×10^5
2		9×10^5	6×10^5
3		11×10^4	7×10^5
1	Cabbage	12×10^4	7.5×10^5
2		11×10^4	8.2×10^5
3		1.7×10^5	1.2×10^5
1	Radish	9×10^5	6×10^5
2		9.5×10^5	7×10^5
3		10×10^4	6×10^5
1	Pino	6×10^5	4.5×10^5
2		6.5×10^5	4×10^5
3		7×10^5	5.2×10^5
1	Sonchal	8×10^5	6.5×10^5
2		7.8×10^5	6×10^5
3		7×10^5	4.7×10^5

TAPC: Total aerobic plate count; Coliforms index; CFU: Colony forming unit

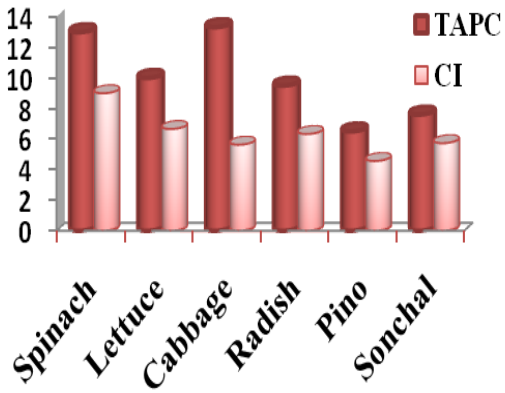

Fig.2. Bacteriological contamination of vegetables in Majini Muhallah, Gilgit.

This study showed that spinach (*S. oleracea*) had highest total aerobic count 14×10^5 cfu/g and highest coliforms index 10×10^4 Cfu/g in spinach sample 2. Lettuce (*L. sativa*) had highest total aerobic count 11×10^4 Cfu/g in sample 1 and coliform index was highest 7×10^5 in both samples 1 and 3. The total aerobic plate count was highest 12×10^4 in sample 1 and coliforms index was highest 8.2×10^5 in cabbage (*B. oleracea*) sample 2. In the same way radish (*R. sativus*) showed highest aerobic count 10×10^4 in sample 3 and coliforms index was observed to be highest 7×10^5 in sample two. Likewise for Pino (*B. rapa)* the total aerobic count was highest 7×10^5 and coliforms index was highest 5.2×10^5 in sample three. Both the aerobic plate count 8×10^5 and coliforms index 6.5×10^5 were highest in sample 1 of Sonchual (*M. verticillata).*

Parasites

During this study Majini Muhallah, Gilgit was divided into three sectors. Six vegetables including Spinach (*Spinacia oleracea)*, Cabbage (*Brassica oleracea)*, Lettuce (*Lactuca sativa*), Radish (*Raphanus sativus*), Sonchual (*Malva verticillata)*, Pino (*Brassica rapa*) were selected from each sector to study the parasitic contamination in vegetables grown in agriculture soil treated with organic manure. The results are shown in Table 2. Fig. 3 shows parasitic load of six vegetables in Majini Muhallah Gilgit.

Table 2. Prevalence of gastrointestinal parasites in vegetables from Majini Muhallah, Gilgit.

Sample Point	Vegetables	AL	GL	TT	Cryp.	
1	Spinach	8 (42.10 %)	5 (26.36 %)	4 (21.05 %)	2 (10.52 %)	19 (43.18 %)
2		4 (33.33 %)	5 (41.66 %)	2 (16.66 %)	1(8.33 %)	12 (27.27 %)
3		6 (46.15 %)	4 (33.33 %)	3 (25 %)	0 (0 %)	13 (29.54 %)
Total	44	18 (40.90 %)	14 (31.81 %)	9 (20.45 %)	3 (6.81 %)	
1	Cabbage	7(38.88%)	3(16.66%)	5(27.77%)	3(16.66%)	18(42.85%)
2		5(38.46%)	4(30.76%)	3(23.07%)	1(7.69%)	13(30.95%)
3		3(27.27%)	5(45.45%)	2(18.18%)	1(9.09%)	11(26.19%)
Total	(42)	15(35.7 1%)	12(28.57 %)	10 (23.09 %)	5(11.90%)	
1	Lettuce	3(23.07%)	4(30.76%)	3(23.07%)	3(23.07%)	13(30.23%)
2		5(27.77%)	5(27.77%)	5(27.77%)	3(16.66%)	18(41.86%)
3		7(58.33%)	2(16.66%)	2(16.66%)	1(8.33%)	12(27.90%)
Total)	(43)	15(34.88%)	11(25.58%)	10(23.25%)	7(16.27%)	
1	Radish	5(31.25%)	3(18.75%)	2(12.5%)	6(37.5%)	16(45.71%)
2		1(14.28%)	2(28.57%)	2(28.57%)	2(28.57%)	7(20%)
3		3(25%)	4(33.33%)	4(33.33%)	1(8.33%)	12(34.28%)
Total	(35)	9(25.71%)	9(25.71)	8(22.85%)	9(25.71%)	
1	Sonchual	2(16.66 %)	3(25%)	5(41.66%)	2(16.66%)	12(42.85%)
2		4(44.44%)	1(11.11%)	3(33.33%)	1(11.11%)	9 (32.14%)
3		1(14.28)	3(42.87)	1(14.28%)	2(28.57%)	7 (25%)
Total	(28)	7(25%)	7(25%)	9(32.14)	5(17.85%)	
1	Pino	4(40%)	3(30%)	2(20%)	1(10)	10 (34.48%)
2		2(16.66)	3(25%)	4(33.33%)	3(25%)	12(41.37%)
3		3(42.85%)	1(14.28%)	1(14.28%)	2(28.57%)	7(24.13%)
Total	(29)	9(31.03%)	7(24.13%)	7(24.13%)	6(20.68%)	
	AL:	GL:	TT	Crypto		

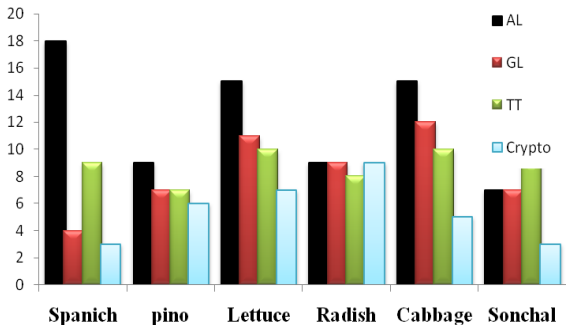

Fig. 3. Parasitic load of six vegetables in Majini Muhallah Gilgit.

The vegetables examined in this study showed contamination of many parasites including *Ascaris lumbricoid (AL), Gardia lamblia (GL), Trichuris trichuria (TT) and Cryptosporidium (Crypto)* respectively.

This study revealed that parasitic load of *A. lumbricoid* was maximum in Spinach 18 (40.90 %) and minimum in Sonchual 7(25%) while other vegetables showed intermediate contamination. In the same way *G. Lamblia* showed highest contamination in Spinach 14 (31.81 %) and lowest in Pino as it was examined that *T. trichuria* was highest in lettuce 10(23.25%) and lowest in Pino 7(24.13%) other vegetables showed intermediate contamination. *Cryptosporidium* showed highest contamination in radish and 9(25.71%) and lowest contamination in spinach 3 (6.81 %) While other vegetables showed intermediate contamination.

Discussions

The outcome of this study showed that bacteriological contamination is abundant in the vegetables grown in soil treated with organic manure. The aerobic plate count and coliform index showed that all six vegetable (Spinach, Lettuce, Cabbage, Radish, Pino, Sonchual) had contamination in different ranges. This contamination could be attributed to the unhygienic practices right from the excavation of human manure from pit latrine to the vegetables grown in the soil amended with human manure. Coliform are the indicators of intestinal contamination both from human and animals. It may not be amazing since water used for irrigation is contaminated with human waste. Our results are in agreement with the study of Frank-Peterside and Waribor (2006), where they

stated that contamination raises with time during storage of vegetables grown in soil applied human manure more than the vegetables grown in soil with inorganic fertilizers. If bacterial load increases than threshold then they are vulnerable to the consumers (Frank-Peterside and Waribor, 2006). A possible hazard exists for the constant pathogenic populations to be transferred to harvested vegetables indirectly through water contaminated with human feces or by straight contamination by nearness to animal farms improperly composted or from improperly composted animal manure and biosolids. The results obtained showed that contaminated vegetables or untreated vegetables can pose a huge risk to the consumers. That's why it is essential these vegetables be properly washed prior to consumption particularly when they are not cooked and eaten as a salad.

This study also revealed that parasitic contamination is inevitable in vegetables grown in soil treated with not properly composted organic manure. All the six vegetables studied during this study showed gastrointestinal parasitic contamination in different ranges. This indicates the heath status of the dwellers of the area and unhygienic practices of farmers applying organic manure as a fertilizer. Our results are in agreement with other researchers work carried out in different part of the world (Kloos and Tesfa, 1993), indicating a high prevalence of intestinal parasites and soil transmitted helminthes in vegetables grown with organic fertilizer. Such a high prevalence of intestinal parasites is largely due to poor personal hygiene practices and environmental sanitation, lake of supply of drinking water, poverty, ignorance of health promotion practices, and bad health services.

It is concluded that Pit latrines and organic manures are the main key determinants to groundwater contagion in Majini Muhallah, Gilgit. Groundwater with faecal bacteria has direct approach to the epidemic of waterborne diseases (diarrhea and typhoid). The competence of health facilities is inadequate to switch cases of these outbreaks thereby necessitate capacity building. Ecosan Toilet Technology might overturn the tendency if attached with suitable awareness and local institutional capacity building.

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