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MICROBIOLOGICAL QUALITY OF ORGANIC VEGETABLES PRODUCED IN SOIL TREATED WITH DIFFERENT TYPES OF MANURE AND MINERAL FERTILIZER

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ABSTRACT

An attempt was made to evaluate microbiological quality of horticultural crops grown organically. Three species of vegetables were used, lettuce (*Lactuva sativa*), radish (*Raphanus sativus*) and spinach (*Tetragonia expansa*), grown organically, in fertile soil. Six different treatments were applied: mineral fertilizer, chicken, cow, and pig manure, chicken litter and cow manure, in association with a liquid foliar biofertilizer. These crops were handled according to correct agronomic practices for growing crops organically for commercial purposes. Samples were examined for the Most Probable Number (MPN/g/mL) of total and fecal coliforms and to detect the presence of *Escherichia coli* and *Salmonella* spp. All analyzed samples were considered acceptable for consumption, as *Salmonella* spp. was not detected. However, 63.3%, 50.0%, and 23.3% of the samples of lettuce, radish and spinach, respectively, contained ≥10^2 total coliforms/g of product. None of the samples of spinach or radish presented ≥10^2 fecal coliforms/g, and only 6.6% of lettuce samples contained >10^2 fecal coliforms/g. The presence of *E. coli* was confirmed in one sample of spinach, cultivated with cow manure.

Key words: organic vegetables, animal manure, coliforms, *Escherichia coli*, *Salmonella*

INTRODUCTION

Western nations, following the successful example of oriental regions, are consuming "natural" or "organically" grown foods with increasing quantity and frequency. These terms are applied to unprocessed agricultural crop products or minimally processed foods, and products that have not received excessive amounts of chemicals. However, this change in dietary practices to minimally processed foods without chemical preservatives calls for adapted quality control (1,18). The increasing consumption of raw foods of vegetable origin gains importance, as they are an important source of vitamins, fiber, and minerals (7,32,41). Meanwhile, their harvesting, distribution, and commercialization brought to light a microbiological risk associated with these products (5,6,9,27).

Organic production, as an alternative to conventional agricultural, relies on the incorporation of organic material in soil, and not infrequently, by the use of animal manure as fertilizer (23,46). Animal manure is a good source of macro- and micronutrient, so using it as fertilizer is an important disposal method (39). This contributes to diminishing the environmental pollution from manure disposal (11). However, animal manure is a well-known source of foodborne pathogenic bacteria (32,33,35,37,46,51,61,63,65), and its inappropriate use in vegetable crops, especially organic ones, contributes a risk to consumer health. Research data and regulations recommend evaluating and normalizing the use of animal manure at the agricultural production (20,21).

As a consequence of the lack of information concerning the microbiological quality of organic vegetables, despite the

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increased consumption, the present investigation evaluated whether the use of animal manure (bovine, swine and chicken), chicken litter and matured cow manure, in association with a liquid foliar biofertilizer resulted in microbial contamination in the cultivation of lettuce, spinach, and radish.

MATERIALS AND METHODS

Vegetable production

The cultivation of lettuce (*Lactuva sativa*), spinach (*Tetragonia expansa*), and radish (*Raphanus sativus*) was conducted between April and July 2001 at the Department of Horticulture of the Food Engineering College of the Federal University of Goiás, Brazil. Six different treatments: bovine, chicken, and swine fresh manure (≤3 days old), chicken litter, bovine manure in association with a liquid foliar biofertilizer in the soil, and conventional chemical fertilizer were studied. The experimental design consisted of randomly assigned blocks with five repetitions (26).

Soil management

Soil was prepared by plowing, harrowing, and removal of damaging plants. Beds measured 1.0 x 3.0 m, each. Before planting, one soil sample (100 g) was taken for microbiological examination according to Clark's guidelines (15). Afterwards, the manure was incorporated with a shovel and draw hoe. Swine manure was distributed in the rows with a manual sprinkler.

Use of organic fertilizers

Manure piles were kept on cement floors in confined areas, protected with plastic from direct precipitation and sun but were exposed to climatic changes, for a period of 90 days, being churred and sifted. Swine manure was stored in plastic containers (33,58). Before incorporation, a sample (100 g) was collected from each manure type for microbiological examination, as described below. The liquid biofertilizer, not industrialized, had a composition of bovine manure, milk, molasses, calcium chloride, boric acid, copper, manganese, magnesium and zinc sulphates, chlofermol (a mixture containing cobalt, iron, and molybdenum salts), and water, at a pH of 4.0. The doses by square meter of the used fertilizers were: mineral, 375g; bovine manure and biofertilizer, 6.070g; chicken manure, 2.460g; swine manure, 4L, and chicken litter 5.143g.

Vegetable transplants

Radish planting and lettuce and spinach transplantation were carried out one day after the manure incorporation, using Plantmax® as substrate. Before transplanting, crops were maintained in the green house with automatic irrigation, and temperature control at 25ºC for 27 days. After that, rice husks were spread on the beds. Irrigation water (1,000 mL) and rice husks (100 g) were sampled for microbiological examination.

Crops and sampling

Radish, lettuce, and spinach were harvested at 26 days after planting, and 39 and 49 days of transplanting, respectively. Vegetables were randomly selected from the plants thinned or harvested, and the edible portions, roots for radish (three root samples) and leaves for lettuce and spinach (from each bed, 10 to 15 external, intermediate and internal leaves), without plant removal in different samples (statistically sampled) inside the bed. The vegetables went through a passive washing for 30 seconds under running tap water, were separated by using scissors or shears previously sprayed with 70% ethanol, placed in sterile stomacher plastic bags (Nasco®, Ft. Atkinson, Wisconsin, USA), transport to the laboratory, and refrigerated (5ºC) until analysis. In all vegetables samples the border locations were excluded. All samples were taken with bare hands.

Microbiological examination

Each manure pile, swine manure, chicken litter, liquid biofertilizer, rice husk, soil, and vegetables samples (approximately 100 g) were monthly analyzed to enumerate total and fecal coliforms, as well as to determine the presence of *Escherichia coli*, *E. coli* O157:H7, and *Salmonella* spp (2). For the water sample were conducted colony counts of heterotrophic bacteria, total and fecal coliforms and the search of *Escherichia coli* and *Salmonella* spp. (3).

Statistical analysis

The results were submitted for variance analysis, and the arithmetic medians were compared by the Duncan test. For the variation coefficient, dates were transformed using square root of X + 1 (26).

RESULTS

Table 1 presents the arithmetic means of the six treatments, in the organic vegetables tested. Statistical analysis demonstrated that there was a significant difference only in lettuce samples for total coliforms between the treatments applied. Treatments 2 (bovine manure) and 5 (chicken litter) showed a greater number of coliforms than those produced with mineral fertilizer (treatment 1) and chicken manure (treatment 3). In the radish and spinach samples no significant differences were observed in total and fecal coliform index.

The observed microbiological levels and results for *Escherichia coli* and *Salmonella* in manure, soil, water, and rice husks samples are presented in table 2. Bovine and swine manure (samples 1 and 3) showed the highest degree of total and fecal coliform contamination, and *E. coli* was only isolated from bovine manure. Nevertheless, untreated soil and rice husks samples (6 and 7) also showed similar levels of contamination to manure treated samples. Table 3 displays the variance analysis for coliform enumeration, revealing that fecal coliform

Table 1. Average values of total and fecal coliforms in organic lettuce, radish, and spinach samples, from April to July, 2001.

Treatment	Lettuce	Radish	Spinach			
	Total coliforms (MPN*/g)	Fecal coliforms (MPN/g)	Total coliforms (MPN/g)	Fecal coliforms (MPN/g)	Total coliforms (MPN/g)	Fecal coliforms (MPN/g)
1. Mineral fertilizer	169.0 b**	3.8 a	607.8 a*	6.4 a	289.6 a*	3 a
1. Mineral fertilizer	169.0 b**	3.8 a	607.8 a*	6.4 a	289.6 a*	3 a
2. Bovine manure	1,408.6 a	94.4 a	713.4 a	7.0 a	18.6 a	3.2 a
3. Chicken manure	300.8 b	6.0 a	761.2 a	7.2 a	233 a	3.2 a
4. Swine manure	460.6 ab	3.0 a	939.2 a	11.0 a	34.2 a	3 a
5. Chicken litter	1,400.8 a	3.4 a	705.4 a	3.0 a	797.2 a	3.2 a
6. Biofertilizer	686.6 ab	94.4 a	72.4 a	7.0 a	104.8 a	3 a

*MPN: Most Problable Number; **Averages followed by the same letters in columns, doesn't differ between then by Duncan test ($p<0.05$).

Table 2. Determination of total and fecal coliforms, and search of *Escherichia coli* and *Salmonella* spp. in organic fertilizers, untreated soil, rice husks, and water used in the vegetable production.

Sample	Total coliforms (MPN*/g/mL)	Fecal coliforms (MPN/g/mL)	*Escherichia coli* (P/A)**	*Salmonella* spp. (P/A)
1. Bovine manure	≥ 2,400	≥ 2,400	Presence/100 g	Absence/100 g
2. Chicken manure	23	43	Absence/100 g	Absence/100 g
3. Swine manure	460	≥ 2,400	Absence/100 mL	Absence/100 mL
4. Chicken litter	< 3	9	Absence/100 g	Absence/100 g
5. Biofertilizer	≤ 3	≤ 3	Absence/100 mL	Absence/100 mL
6. Soil	≥ 2,400	≥ 2,400	Absence/100 g	Absence/100 g
7. Rice husks	≥ 2,400	≥ 2,400	Presence/100 g	Absence/100 g
8. Water	$<1.0\times10^{-1}$ cfu/mL	$<1.0\times10^{-1}$ cfu/mL	Absence/1.000 mL	Absence/1.000 mL

*MPN: Most Probable Number; P/A: **Presence or absence.

Table 3. Variance analysis of total and fecal coliforms Most Probable Number of the organic vegetables.

Variation source	D.F.[a]	Average Square					
		Lettuce		Radish		Spinach	
		Total coliforms	Fecal coliforms	Total coliforms	Fecal coliforms	Total coliforms	Fecal coliforms
Fertilizer	5	5.7876	1.0133	2.7344	0.1750	4.5282	0.0049
Block	4	11.8838	2.0857	12.1915	0.8350	14.6568	0.0041
Residue	20	2.9552	1.7235	4.2934	0.7499	2.1286	0.0091
V.C.[b] (%)	32.48	83.94	43.44	59.16	41.69	8.46	

[a] D.F.: Degree of Freedom; [b] V.C.: Variation Coefficient.

enumeration in lettuce and spinach samples showed the highest (83.94) and lower (8.46) values of variation coefficient, respectively.

All vegetables examined were grown in close proximity of or in contact with the soil. *E. coli* (not *E. coli* O157:H7) was only isolated in one sample of bovine manure, one of rice husks, and in a sample of spinach that was fertilized with bovine manure (data not in table). *E. coli* O157:H7 and *Salmonella* were not detected (Table 2) in any of the samples examined (vegetables, manure, chicken litter, liquid foliar biofertilizer, rice husks, soil, and irrigation water).

DISCUSSION

This study has shown that raw ready-to-eat organic vegetables when produced according to correct agronomic practices were of acceptable microbiological quality (8).Unsatisfactory results were due to the presence of fecal coliforms in two (2.2%) samples of lettuce and one (1.1%) of radish (Table 4), probably because these vegetables were grown in contact with the soil.

Manure has a beneficial fertilizer value, however, it frequently contains enteric microorganisms (46) and land spreading can lead to pathogen entry into the food chain (29). Many infection outbreaks have been associated with processed vegetables, directly or indirectly contaminated with animal manure (12,13,60). Although competition with soil microorganisms and adverse environmental conditions can reduce the number of pathogens, there is little information regarding the degree to which these pathogens can survive in manure-amended soils (29).

In this study the presence of total and fecal coliforms in manure (mainly bovine and swine), as well as in soil and rice husks samples revealed that it could be a source of contamination. Even though it was not possible to ensure that manure was the source of coliforms in radish samples, because it was observed at different treatments, the samples fertilized with swine manure showed higher numbers of fecal coliforms than those fertilized with mineral fertilizer, both having equal soil and rice husks indicating that manure could indeed be a contaminating factor. The same was noted for lettuce samples, when they were analyzed with the same group of bacteria recovered from bovine manure and mineral fertilizer treatments. Also, it was observed that climate, soil conditions, and growing practices (e.g., irrigation water) failed to influence contamination. The period during which the samples were analyzed was not rainy months. Although the numbers of samples are low, these results suggest that the contamination rates for different vegetables may vary under identical growing conditions and that vegetables may become contaminated with soilborne pathogens early in growth. Several studies have suggested that colonization of plants is most likely when the plant is a seedling, and the emerging root is a key area of bacterial attachment (17,19). Subsequent internalization of soilborne pathogenic bacteria is also greatest when the plants are seedlings, and internalization is less likely as the plants mature (17).

The ability to colonize vegetable roots and shoots varies for different strains of a bacterial species (4,61) and for different bacterial species (4,14,19). Bacterial motility (17) and the ability to use seed exsudates as carbon sources (52) are related to the extent of colonization. Internalization of bacteria in seedlings is more likely in hydroponic systems than in soil (25,62), but internalization and transport throughout the plant can still occur in the latter growth medium (57). Collectively, the literature on bacterial colonization of plants and internalization suggests that the critical time for preventing vegetable contamination with manure fertilizer may be at the time of planting (28).

The public health hazard of fresh vegetables contaminated with feces used as fertilizer is a longstanding concern, there is a lack of information in microbiological status of organic products (42,53). However, much scientific information is produced in the study of detection, survival, fate, and spread of pathogenic microorganisms in animal manure (30,37,44,56,57). This concern is directed to *Salmonella* spp. (24,27,40,47,49) and *E. coli* O157:H7, because cattle is its main reservoir, explaining the risk of transmission by manure (22,34,43,50).

This study used manure that was composted or stored in the soil before planting (passive manure treatment), because this practice is more common among vegetable producers in this region. Composting or active treatment is also an option for using the manure as a fertilizer. Composting and drying of

Table 4. Population intervals of positive samples of total and fecal coliforms in ready-to-eat organic vegetables.

Population Intervals (MPN*/g)	Total coliforms				Fecal coliforms			
	Lettuce MPN/g	Radish MPN/g	Spinach MPN/g	Total (%)	Lettuce MPN/g	Radish MPN/g	Spinach MPN/g	Total (%)
<30	6	10	18	34(37.7)	28	29	30	87(96.6)
30–100	5	5	5	15(16.6)	0	1	0	1 (1.1)
101–500	7	5	3	15(16.6)	2	0	0	2 (2.2)
501–1,000	0	0	0	0 (-)	0	0	0	0 (-)
1,001–2,000	7	5	3	15(16.6)	0	0	0	0 (-)
>2,000	5	5	1	11(12.2)	0	0	0	0 (-)

*MPN: Most Probable Number.

manure is known to reduce the number of viable pathogens like *E. coli* O157:H7 and *S. enteritidis* in bovine manure (38,46). Jiang et al. (31) observed that composting at 50ºC, in bovine manure inoculated with *E. coli* O157:H7 inactivated the bacterium within 7 to 14 days.

Reporting of foodborne disease associated with changes in agricultural practices or eating habits, as well as queries on how the pathogens spread from manure to environment; and, the relations between soil microbial population and with vegetal tissue are recent. Furthermore, the chance of antimicrobial resistance being transferred is of great relevance in pathogen emergence and re-emergence (10,16,45,48,55,57,59,64).

In an effort to determine the microbiological quality of organic vegetables, even though none of the practiced treatments had contributed to compromise the microbiological status, by using Pearson correlation we observed that the presence of fecal coliforms in 82%, 84%, and 99.1% of spinach (with bovine manure), radish (swine manure), and lettuce (bovine manure and biofertilizer) samples, respectively, warning to a situation of risk, since, only 6% of the people, rarely or even never wash fresh products before consumption (36). None of the samples examined were of unacceptable microbiological quality (Table 2) confirming earlier studies (42,53). They could be classified as satisfactory according to Brazilian microbiological guidelines that require absence of *Salmonella* spp. in 25g (8).

This is a pioneer study in Brazil to provide information on microbiological quality of raw ready-to-eat organic vegetables. It is not a comparative study and therefore does not aim to produce conclusive evidence on whether organically produced vegetables are microbiologically safer or not than those conventionally produced. Nevertheless, according to Brazilian law, this study suggests that the overall agricultural, hygiene, harvesting, and production practices were good, and consequently animal manure could be used in organic crops, if managed according to proper agronomic practices. This information, like Schlundt (54) can also help to formulate microbiological guidelines and to monitor demands for microbiological quality.

RESUMO

Qualidade microbiológica de vegetais orgânicos produzidos em solo tratado com diferentes tipos de esterco e fertilizante mineral

O objetivo do presente trabalho foi avaliar a qualidade microbiológica de hortaliças orgânicas produzidas sob diferentes condições. Três espécies de vegetais, alface (*Lactuva sativa*), rabanete (*Raphanus sativus*) e espinafre (*Tetragonia expansa*), foram cultivadas no sistema orgânico, em solo fertilizado com seis tratamentos diferentes: adubo mineral, estercos de galinha, bovino e suíno, cama de frango e esterco bovino associado com biofertilizante líquido de aplicação foliar. O cultivo das hortaliças foi feito de acordo com as práticas agronômicas recomendadas para o sistema orgânico em escala comercial. Das hortaliças cultivadas, foram coletadas amostras para a determinação do Número Mais Provável de coliformes totais e termotolerantes e detecção da presença de *Escherichia coli* e *Salmonella* spp. Todas as amostras analisadas foram consideradas apropriadas para o consumo humano, de acordo com a legislação brasileira em vigor, uma vez que, em nenhuma delas foi detectada a presença de *Salmonella* spp. Entretanto, 63,3%, 50,0% e 23,3%, respectivamente, das amostras de alface, rabanete e espinafre apresentaram contagens de coliformes totais ≥100/g. Em nenhuma das amostras de rabanete e espinafre o nível de coliformes termotolerantes foi ≥100/g e em somente 6,6% das amostras de alface foi maior que 100/g. *E. coli* foi detectada em uma amostra de espinafre produzido em solo fertilizado com esterco bovino.

Palavras-chave: hortaliças orgânicas, esterco animal, coliformes, *Escherichia coli*, *Salmonella*

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