Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 31 Março 2024
https://doi.org/10.14295/CS.v15.3463

Organic fertilizer application combined with seed inoculation using rhizobacteria on tomato yield and quality

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Abstract

Organic fertilizer application combined with seed inoculation using rhizobacteria enables increases in industrial tomato production. The objective of this work was to assess the synergistic effect of organic fertilizer application and seed inoculation with rhizobacteria on tomato yield and quality. A randomized complete block experimental design with four replication was used, in a 6×3 factorial arrangement consisted of six fertilizer sources (mineral fertilizer; cattle manure; and organic composts based on tomato, sugarcane bagasse, tomato + sugarcane; and tomato + sugarcane + banana stalk) and three different seed inoculation with rhizobacteria (a control, without inoculation; inoculation with RZB 18; and inoculation with LEMB 17). The evaluated fruit characteristics were: commercial and non-commercial yields; firmness; soluble solids content (SSC); industrial yield; titratable acidity (TA); SSC to TA ratio, and pH. The rhizobacteria significantly affected all evaluated characteristics. The highest mean industrial yields were obtained with the application of mineral fertilizer (7.59), tomato-based compost (7.99), and sugarcane bagasse-based compost (7.95 Mg ha^{-1}). Although seed inoculation with rhizobacteria did not affect fruit yield and quality, organic composts based on tomato and sugarcane bagasse residues might be used as organic fertilizer for industrial tomato production.

Keywords: organic compost, productivity, post-harvest, rhizobacteria, Solanum lycopersicum

Introdution

Tomato (*Solanum lycopersicum*) is among the most consumed vegetables in the world, both in fresh natural and processed forms, presenting high economic importance (Luz et al., 2016).

Plant, animal, and agroindustrial residues have been applied as organic fertilizers to enhance soil recovery capacity by improving soil biological, physical, and chemical attributes, thus providing primary and secondary micro and macronutrients (Mueller et al., 2013).

According to Cherubin et al. (2015), the utilization of organic fertilizers increases the soil microbial activity. Zuba et al. (2011) reported that application of organic fertilizers reduces the incidence of diseases and pests in tomato crops compared to chemical fertilizers.

The effective utilization of natural resources is essential within organic systems to achieve ecological stability and sustainable production (Marouelli et al., 2011). The application of organic composts is a viable alternative in these systems. Composts can be formulated using plant and animal residues and are the main fertilizers utilized for vegetable crops (Sediyama et al., 2014). Silva et al. (2016) reported that the application of organic compost based on pequi (*Caryocar brasiliense*) and use of manure result in higher plant heights, and that the combination of organic fertilizer and rhizobacteria reduces nematode activity compared to the mineral fertilizer application.

Rhizobacteria can promote plant development through the production of growth hormones (Oliveira et al., 2012). They prevent the action of phytopathogenic agents and act as solubilizers of nutrients, making them available to plants; thus, they can be used as biofertilizers in several crops (CAMELO et al., 2011).

According to Szilagyi-Zecchin et al. (2015),

Received: 17 June 2020
Accepted: 10 August 2023

seed inoculation with a bacterium of the *Bacillus* genus promoted shoot growth in tomato seedlings. In addition, Fernandes et al. (2014) reported that inoculation of tomato seeds with *Bacillus subtilis* reduced the number of nematode eggs. Carrer Son et al. (2015), Podestá et al. (2013), and Rocha & Moura (2013) also successfully utilized rhizobacteria in studies with tomatoes.

The utilization of rhizobacteria and application of organic fertilizers have positive effects on crops, as they benefit plant development without causing damage to the environment and may affect post-harvest fruit quality. Considering this context and the scarcity of studies on this theme, the objective of this study was to assess the synergistic effect of organic fertilizer application and seed inoculation with rhizobacteria on the yield and quality of industrial tomatoes.

Material and Methods

The experiment was conducted in a greenhouse under controlled-environment conditions (temperature of 25±3 °C and relative humidity above 60%), from June to November. A randomized block experimental design with four replications was used, in a 6×3 factorial arrangement consisting of application of six different fertilizers and three different types of seed inoculation with rhizobacteria.

The fertilizers used were: mineral fertilizer (1); aged cattle manure (2); and organic composts based on tomato (3), sugarcane bagasse (4), tomato + sugarcane bagasse (5), and tomato + sugarcane bagasse + banana stalk (6). The fertilizer applications were combined with three types of seed inoculation with rhizobacteria: control, without inoculation with rhizobacteria (1); and seeds inoculated with rhizobacteria RZB 18 (2) and LEMB 17 (3). The experimental units consisted of two pots containing one plant per 7.5-liter pot.

Soil was added to each pot together with the fertilizer treatments containing organic composts and manure, and to the control with application of mineral and manure fertilizers. The quantities of compost and manure were calculated based on a rate of 30 Mg ha^{-1} of organic material (IAC, 2014).

The soil used was classified as a Typic Hapludox (Latossolo Vermelho Eutrofico tipico; Santos et al., 2018) of mean texture. Soil samples of the 0-20 cm layer were collected for analysis, which presented the following characteristics: pH in water = 6.6; organic matter = 1.7 dag kg^{-1} (colorimetry); P = 5.6 mg dm^{-3} (Mehlich-1); K = 189 mg dm^{-3} (Mehlich-1); Ca = 3.5 cmol$_c$ dm^{-3} (KCl 1mol L^{-1}); Mg = 1.0 cmol$_c$ dm^{-3} (KCl 1mol L^{-1}); Al = 0.0 cmol$_c$ dm^{-3} (KCl 1mol L^{-1}); H+Al = 1.5 cmol$_c$ dm^{-3} (pH SMP); base saturation = 77%; aluminum saturation = 0%; P_{rem} = 42.7 mg L^{-1} (equilibrium solution P); sand = 66 dag kg^{-1}; silt = 9 dag kg^{-1}; and clay = dag kg^{-1}.

The organic composts were formulated using tomato residues from an agroindustry, sugarcane bagasse from an alcohol plant, and banana stalks from banana bunches.

Cattle manure was utilized as inoculant and the main source of nitrogen in the composting process; it was added between the layers of the organic materials. The proportion of each material for composting was adjusted to a C/N of 30/1 and 50% moisture. The piles were arranged with approximate dimensions of 2 × 2 m and a height of 1.5 m, as recommended by Souza and Alcântara (2008). The composting process concluded after a 90-day period.

Seed inoculation with rhizobacteria was carried out using a strain from okra roots, characterized as Gram positive, rod-shaped bacteria (LEBM 17), *Bacillus* sp.; and a strain described as *Pseudomonas fluorescens* (RZB 18).

The rhizobacteria were grown on cells preserved at -80 °C in Tryptic Soy Casein (TSB) broth supplemented with glycerol. Bacterial suspensions were prepared using a isotonic $MgSO_4$ solution (0.1 mol L^{-1}), obtained from 24-hour cultures in Tryptic Soy Agar (TSA) at 28 °C. The suspension concentrations were calibrated to approximately 108 CFU mL^{-1}, using a spectrophotometer (Ultrospec 1100 pro; Amersham Biosciences) (D.A. 540nm = 0.2).

Tomato seeds of the cultivar BRS Sena were cleaned by removing impurities, placed in an Erlenmeyer flask, and immersed in 4.68µL of bacterial suspensions for 1 hour under shaking at 150 rpm in an orbital benchtop shaker device (430/RDBP; Nova Etica). The seeds were dried for 2 hours, preserved in sterile paper bags, and immediately sent for planting.

The seeds were sown in the four organic compost treatments. Concerning the treatments with mineral fertilizer and aged cattle manure, the seeds were sown in a coconut fiber-based commercial substrate. All seedlings were transplanted 28 days after sowing, when the plants had developed 3 to 4 true leaves.

Topdressing application started 25 days after transplanting (DAT) in the organic treatments, through weekly application of a biofertilizer consisted of organic compost, ground castor bean, plant ash, and water (200 mL per plant). Additionally, two commercial organic fertilizers were applied weekly: JK®, a liquid foliar compound that is source of Ca and N (1.42 mL plant^{-1}); and SOIL RICH®, a source of micronutrients (0.70 mL plant^{-1}).

The first application in the treatment with mineral fertilizer was carried out one day before transplanting, and topdressing was carried out 25 and 52 DAT. The fertilizers used were: urea (0.767 g pot^{-1}), simple superphosphate (10.42 g pot^{-1}), and potassium chloride (0.39 g pot^{-1}), as sources of N, P, and K respectively, applied at transplanting and as topdressing (nitrogen and potassium).

A fertilizer with calcium nitrate (100 kg ha^{-1}) was applied approximately 60 days after sowing, split over two consecutive weeks, due to symptoms of calcium deficiency in leaves of plants in the treatment with mineral fertilizer.

The fruits were harvested weekly as they ripened and exhibited a uniform reddish color. Harvesting of fruits from the treatments with organic composts started three months after sowing. One month after this initial harvest, the harvesting of fruits from the treatments with mineral fertilizer and aged cattle manure started, as these plants had a delayed cycle.

Ten harvests were carried out for each treatment; all fruits (ripe, green, deficient, or colorful) were harvested in the last harvest.

Chemical analysis of the organic composts and cattle manure was performed for determining pH and macro and micronutrient contents, which assisted in the analysis of the results (MAPA, 2013) (**Table 1**).

Commercial and non-commercial fruit yields were evaluated. Fruit quality analysis were carried out by determining titratable acidity (TA), soluble solids content (SSC), SSC to TA ratio (SS/TA), pH, firmness, and industrial yield of fruit pulp.

TA was determined by titration of 10 mL of pure juice from two ripe fruits with NaOH, under shaking, using 1% phenolphthalein as indicator. SSC to TA ratio was obtained dividing SSC by TA.

The pH was determined directly in the juice using a pH-meter (Reichert). Pulp firmness was determined by perforating the fruit at the equatorial region using a manual penetrometer with a 6-mm thick tip; the results were expressed as Newton. SSC was determined using a digital refractometer (Bel Engineering).

Industrial yield was obtained using the formula: *IY (Mg of pulp ha^{-1}) = [(FC (Mg ha^{-1}) × 0.95) × °Brix of the juice] / 28*, where *IY* is the industrial yield of concentrated pulp at 28 °Brix, and FC (Mg ha^{-1}) is the production of ripe fruits (Silva et al., 2006). Yield was calculated based on a population of 42,000 plants ha^{-1}.

The data were subjected to analysis of variance, and significant means were subjected to the Scott-Knott test at 5% probability level.

Results and Discussion

The rhizobacterial isolates RZB 18 and LEMB 17 had no significant effect on the analyzed characteristics, denoting the need for further studies to better detail their effects.

The applications of mineral fertilizer and organic fertilizers based on tomato, sugarcane bagasse, and tomato + sugarcane bagasse resulted in higher commercial yields, with 755, 695, 665, and 655 g plant^{-1}, respectively (**Table 2**).

The highest yield reached by the applications of organic fertilizers based on tomato and sugarcane bagasse is due to their high organic matter content (43.44%) (Table 1), among other factors, which benefits soil structuring and microbiological activity, improving the availability of nutrients (Ferreira et al., 2017).

The application of the compost based on tomato + sugarcane bagasse also resulted in high commercial fruit yield (Table 2). This fertilizer presented the lowest

Table 1. Chemical characterization of the aged cattle manure and organic composts utilized as organic fertilizers in industrial tomato crops.

Evaluated parameter	Organic compost				
	Manure	Tomato(T)	Sugarcane bagasse (SB)	T + SB	T + SB+Banana stalk
pH	7.3	7.8	7.5	7.7	8.5
N Kjeldahl (mg g^{-1})	9.4	8.40	5.60	8.12	7.7
P (%)	0.35	0.33	0.28	0.27	0.34
K (%)	*1.4	0.97	1.1	0.73	1.3
Ca (mg g^{-1})	26.9	40.08	28.06	16.03	40.08
Mg (mg g^{-1})	3.3	7.29	7.29	4.86	12.16
Cu (%)	-	0.002	0.002	0.002	0.002
Co (%)	-	<0.001	<0.001	<0.001	<0.001
Fe (%)	-	1.0	1.4	1.1	1.2
Mn (%)	0.02	0.03	0.05	0.03	0.04
Zn (%)	0.002	0.005	0.006	0.005	0.006
OM (%)	41.56	43.44	43.44	26.89	45.51
CT (g dm^{-3})	241	252	252	156	264
C/N (g dm^{-3})	17/1	30/1	45/1	19/1	34/1

* Approximate content, according to Ribeiro et al. (1999)

Table 2. Commercial (CY) and non-commercial (NCY) yields, titratable acidity (TA), soluble solids content (SSC), SSC to TA ratio (SS/TA), pH, firmness, and industrial yield (IY) of fruits from tomato plants subjected to different fertilizer applications.

Fertilizers	CY(g plant^{-1})	NCY(%)	TA(mg 100g^{-1})	SSC/TA
Mineral fertilizer	755.0 a	0.006 b	699.0 a	10.69 a
Aged cattle manure	580.0 b	0.084 b	648.0 a	11.53 a
Tomato compost (T)	695.0 a	0.721 a	610.0 a	12.57 a
Sugarcane bagasse compost (C)	665.0 a	0.774 a	643.0 a	12.34 a
T + C	655.0 a	0.858 a	594.0 a	11.67 a
T + C + Banana stalk	560.0 b	0.874 a	648.0 a	11.22 a
Mean	651.0	0.553	640.0	11.67
CV %	18.75	17.32	20.36	14.90
	pH	Firmness (Newton)	SSC (°Brix)	IY(Mg ha^{-1})
Mineral fertilizer	4.20 a	68.09 a	6.95 b	7.59 a
Aged cattle manure	4.14 a	68.56 a	7.43 a	6.85 b
Tomato compost (T)	4.40 a	56.79 b	7.70 a	7.99 a
Sugarcane bagasse compost (C)	4.27 a	52.97 b	7.94 a	7.95 a
T + C	4.25 a	50.70 b	6.82 b	6.43 b
T + C + Banana tree	4.29 a	49.14 b	7.19 b	6.48 b
Mean	4.26	57.51	7.34	7.22
CV %	8.35	24.49	12.46	16.81

CV = coefficient of variation. *Means followed by different letters are significantly different from each other by the Scott-Knott test at 5% probability level.

organic matter content among the analyzed fertilizers (Table 1), however, its C to N ratio (C/N = 19/1) may have favored its performance.

According to Kiehl (2012), organic composts reach bio-stabilization and maturity with C/N of approximately 18/1 and 10/1, respectively. Therefore, the results found in the present study denote a low degradation of organic matter in the composts after 90 days, with the compost based on tomato + sugarcane closer showing the closest proximity to the adequate value.

Non-commercial fruit yield (NCY) was lower than commercial yield (CY), regardless of the fertilizer applied, varying from 0.006% to 0.874% of the total fruit production (Table 2). The lowest NCY was found for the treatments subjected to application of mineral fertilizer (0.05 g plant^{-1} or 0.006%) and cattle manure (0.55 g plant^{-1} or 0.084%) (Table 2).

Titratable acidity (TA), SS/TA, and pH were not significantly affected by the applications of different fertilizers (Table 2).

The mean TA was 640 mg 100 g^{-1} of pulp (Table 2). Despite the non-significant variation, TA reached values within the required standard, i.e., none of the treatments had a mean lower than 350 mg 100 g^{-1} of pulp, which requires longer time and higher temperature for pulp processing, increasing the processing costs (Clemente & Boiteux, 2012).

The mean SS/TA was 11.67, which is consistent with the results obtained in a crop with ten hybrids evaluated by Schwarz et al. (2013), who found means between 9.6 and 13.2. Kader et al. (1978) reported that high-quality fruits should have SS/TA higher than 10 and that a high SS/TA denotes a proper balance between sugar and acid compounds, which is connected to a smooth fruit flavor.

The mean pulp pH was 4.26, which is consistent with the acidity required for fruits intended to industrial processing. A pulp pH below 4.5 is desirable, as it prevent proliferation of microorganisms in the final product, but pH below 4.0 would result in a pronounced acidity of the final product (Clemente & Boiteux, 2012).

Fruit firmness was significantly affected when using mineral fertilizer and cattle manure, presenting means of 68 and 69 N, respectively (Table 2). Both treatments resulted in a better Ca to Mg ratio, not inhibiting the translocation of Ca to the cell wall, which confirms the higher resistance and consequent fruit firmness.

Firmness can be affected by epidermis resistance, pericarp texture, and internal fruit structure (Silva et al., 2006). The higher the fruit firmness, the lower the losses caused during harvesting and transportation to the industry (Luz et al., 2016). Machado et al. (2018) found decreases of approximately 37.57% in fruit firmness after harvesting. Thus, the fruits from plants under applications of mineral fertilizer and cattle manure had a firmer pericarp wall, denoting better resistance to impacts.

Soluble solids content (SSC) was significantly higher in plants in the organic fertilizer treatments compared to those treated with mineral fertilizer, with 7.94, 7.70, and 7.43 °Brix for the treatments with sugarcane bagasse-based compost, tomato-based compost, and cattle manure, respectively (Table 2).

Some studies have reported low SSC in industrial tomato fruits; Schwarz et al. (2013) found SSC below 5.42 °Brix for ten cultivars, whereas Luz et al. (2016) found values lower than 4.60 °Brix for thirteen tomato genotypes.

The hybrid BRS Sena produces tomatoes with reasonable SS; however, Nascimento et al. (2013) reported that SSC in fruits may be a genetic characteristic of the cultivar, but can be affected by temperature, irrigation, and fertilizer application (Branches et al. 2013), as observed in the present study.

SSC is one of the most important characteristics to be determined in fruits, as it directly affects the yield of processed pulp, and fruits with high SSC result in lower production costs (Moreira et al., 2012). An increase in °Brix may result in an increase of 20% in industrial tomato yield (Rocco & Morobito, 2016).

The higher industrial yield was found for plants in the treatments with application of tomato-based compost, sugarcane bagasse-based compost, and mineral fertilizer, which presented means of 7.99, 7.95, and 7.59 Mg ha $^{-1}$ of pulp respectively (Table 2).

The treatment with cattle manure did not result in a high industrial yield, as it presented lower fruit production (item used in the calculations). The application of mineral fertilizer had an opposite effect, i.e., despite the low SSC, a higher industrial yield was found due to a high fruit production (Table 2).

The use of the compost based on tomato + sugarcane bagasse + banana stalk as organic fertilizer had no significant effect on the evaluated variables (Table 2), which may be attributed to its high C/N (34/1) or high pH (8.5) (Table 1). The pH and C/N of organic composts can directly affect the availability of nutrients. Silva & Giordano (2000) reported that the soil pH for industrial tomato crops should range between 5.5 and 6.5 for optimal nutrient absorption.

The fertilizers that contributed the most to the tomato crops evaluated in the present study were the composts based on tomato and sugarcane bagasse; however, their combination did not present a similar result.

Conclusions

Seed inoculation with rhizobacteria did not affect the evaluated tomato agronomic characteristics.

The application of organic fertilizers based on agroindustrial residues (tomato-based compost and sugarcane bagasse-based compost), resulted in increases in quality and yield of industrial tomato fruits.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.