

Effect of vermicompost tea on yield and nitrate reductase enzyme activity in saladette tomato

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Abstract

In conventional agriculture, heavy doses of chemical fertilizers and pesticides are often used to improve the yield of various horticultural crops. These chemical fertilizers and pesticides cause health problems among consumers. Due to the adverse effects of chemical fertilizers, there is interest in the use of organic fertilizers. It has long been recognized that the rate-limiting step for nitrogen assimilation, the reduction of NO_3^- to NO_2^- catalyzed by nitrate reductase (NR), is highly regulated. An increase in the amount and activity of NR leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for general amino-acid synthesis, protein synthesis or total nitrogen assimilation. The aim of the current experiment was to determine the effect of organic and conventional fertilizers on yield and nitrate reductase activity in saladette tomato. Tomato plants were grown under controlled greenhouse conditions and treated with either organic or conventional fertilizer. We evaluated five treatments: F1, sand + inorganic fertilizer; F2, sand + vermicompost tea; F3, a 1:1 mixture of sand: compost + vermicompost tea; F4, a 1:1 mixture of sand: vermicompost + vermicompost tea; and F5, a 2:1:1 mixture of sand: compost: vermicompost + vermicompost tea. The evaluated variables were yield, fruit size, number of fruits, fruit quality, chlorophyll content, and in vivo nitrate reductase enzyme activity. Fertilizer type strongly influenced the yield, fruit size, and fruit quality. The best organic fertilizer for tomatoes was sand + vermicompost tea. Tomatoes in this treatment group produced the second highest yield, the best NO_3^- assimilation, the greatest nitrate reductase enzyme activity, and the second highest organic foliar nitrogen content.

Keywords: Nitrogen metabolism, organic fertilizer, organic waste, quality, *Solanum lycopersicum* L.

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1. Introduction

Livestock organic residues represent a serious ecological problem if they are not handled properly (Castillo *et al.*, 2010). Agriculture, particularly cattle farming, is an important economic activity in the state of Coahuila, México. According to official statistics, Coahuila contains 1,708,887 cattle; 79,050 hogs; 1,808,335 domestic fowls; 203,757 goats; 131,712 horses; and 86,621 sheep (INEGI., 2011), which generate significant quantity of waste. Although crop and livestock wastes are a potential source of contaminants causing human health problems, they can also provide the raw materials for an organic crop production and can have extensive applications.

One method for diminishing the environmental impact of organic waste materials is to compost or vermicompost them. The final products of these processes, composts and vermicomposts, can be used as sources of organic matter for soil amendment, sources of nutrients for soil fertilizer, or growing media constituents for soilless cultivation (González *et al.*, 2010). Vermicompost tea (VCT), a water-based vermicompost extract containing high levels of beneficial microbes and soluble nutrients (Edwards *et al.*, 2010), has attracted the attention of growers and researchers in recent years. The most important reason for applying vermicompost tea is to supply microbial biomass, fine particulate organic matter, and soluble chemical components of vermicompost to plant surfaces and soils in a way that is not possible with solid vermicompost.

Analysis of the nutrient content of plant tissues is a widely used technique for assessing the nutritional status of plants; however, in some cases, plants growing in the same location differ in production due to the levels of fertilizer used, without showing differences in the concentration of elements (Bourret *et al.*, 2009). The activity of the enzyme nitrate reductase (NR; EC 1.6.6.1), the first enzyme involved in the assimilation of N-NO_3^- , is crucial for the development of plants and plays a key role in the assimilation of N (De la Haba

et al., 2001). However, relatively little work has been undertaken to investigate the effect of vermicompost tea on the yield of vegetable crops in Mexico and on N-NO_3^- assimilation.

We hypothesized that vermicompost tea as a fertilizer type would interact with productivity and N-NO_3^- assimilation in saladette tomatoes. Accordingly, the specific objective of this study was to determine the effect of organic and conventional fertilizer on saladette tomato yield and on nitrate reductase enzyme activity.

2. Materials and Methods

2.1. Crop design

Saladette tomato (*Solanum lycopersicum* L.) cv. Anibal was seeded in cell flats (cell size $3 \times 3 \times 10$ cm) filled with peat-lite mixture, which were placed on benches under greenhouse conditions for 5 weeks. The seedlings were then transplanted and grown under controlled conditions in an experimental greenhouse at Antonio Narro Agrarian Autonomous University (Universidad Autónoma Agraria Antonio Narro, UAAAN), Torreón, Coahuila, México. The experiment was conducted from October 2011 to April 2012. In this area, the climate is arid and the lands are intensively used for agriculture. The relative humidity was 60–80% and the temperature range was 24 ± 4 °C, with extremes of 15 °C and 34 °C in the greenhouse. The experimental design was completely randomized with five treatments, the first of which served as the control group. Tomatoes were transplanted in individual 18-L pots. Each treatment was replicated 12 times.

The treatments consisted of five fertilizer types: F1 = sand + inorganic fertilizer; F2 = sand + vermicompost tea at 2.5% concentration; F3 = a 1:1 mixture of sand: compost + vermicompost tea at 2.5% concentration; F4 = a 1:1 mixture of sand: vermicompost + vermicompost

tea at 2.5% concentration; and F5 = a 2:1:1 mixture of sand: compost: vermicompost + vermicompost tea at 2.5% concentration. Table 1 shows the inorganic fertilizer used in the F1 treatment (Castellanos and Ojodeagua, 2009). For water supply we used drip irrigation in all treatments and the amount of water applied, according to the phenological stage of the crop, ranged from 0.35 to 1.9 L plant⁻¹ day⁻¹. The irrigation water was classified as low-salinity water and low sodium content (C₁S₁, with a sodium absorption ratio of 2.18); E.C: 1.05 dS m⁻¹, pH: 7.8; cations (mmol L⁻¹): Ca²⁺ = 3.51, Mg²⁺ = 0.48, K⁺ = 0.22, Na⁺ = 2.71 and anions (mmol L⁻¹): HCO₃³⁻ = 3.12, Cl⁻ = 2.3, and SO₄²⁻ = 2.62. The substrates and vermicompost tea chemical characteristics are summarized in Table 2.

The fertilizers were applied gradually in the irrigation water over the entire growth period of the plants. The pH value of the solutions (inorganic and organic) was adjusted to 5.5.

The manure-based vermicompost, which was provided by the vermicompost module of the UAAAN, consisted of separated horse and goat solids mixed in a 1:1 ratio by volume with alfalfa (*Medicagos ativa* L.), processed by earth worms (*Eisenias* pp.) in indoor beds for 90 days. The compost was provided by Max Compost®. To prepare the VCT at 2.5% concentration, we followed the method recommended by Edwards *et al.* (2010), with a variation (Márquez-Quiroz *et al.*, 2014). The final pH and E.C were adjusted to 5.5 and 2.5 dS m⁻¹, respectively.

Table 1. Nutrient solution for three developmental stages of tomato (Castellanos and Ojodeagua, 2009).

Ion	Stage 1	Stage 2	Stage 3
	1 st set	1 nd – 3 rd set	3 rd – 5 th set
	meq L ⁻¹		
NO ₃ ⁻	6.0	8.0	10.0
NH ₄ ⁺	0.5	0.5	0.5
H ₂ PO ₄ ⁻	1.5	1.5	1.5
K ⁺	3.5	5.5	7
Ca ²⁺	8.0	8.0	8.0
Mg ²⁺	2.0	3.0	4.0
SO ₄ ²⁻	3.0	4.0	6.0
HCO ₃ ⁻	1.0	1.0	1.0
Na ⁺	<5.0	<5.0	<5.0
Cl ⁻	1.0	1.0	1.0
EC (dS m ⁻¹)	1.4	1.8	2.2

EC = electrical conductivity

Table 2. Mineral elements provided by each fertilizer source during the production cycle.

Substrate	N	P	K	Ca	Mg	Na	Fe	Zn	Mn	OM	BD
	(g pot ⁻¹)						(mg pot ⁻¹)			(%)	(g cm ⁻³)
F3	151.8	75.0	211.7	551.9	59.2	35.9	37296.0	1638.0	1008.0	29.2	1.2
F4	80.0	9.4	55.4	436.0	37.8	6.3	44667.0	2079.0	1322.0	10.5	1.2
F5	115.9	42.2	133.6	493.9	48.5	21.1	40981.5	1858.5	1165.5	19.9	1.2

VCT	N	P	K	Ca	Mg	Na	Fe	Zn	Mn	VCT applied
	(g cycle ⁻¹)						(mg cycle ⁻¹)			(L cycle ⁻¹)
VCT	213.8	126.2	182.9	831.8	193.2	51.5	61.8	12.9	40.7	77.3

OM = organic matter; BD = bulkdensity; VCT = vermicompost tea.

2.2. Fruit sampling

Tomatoes were usually harvested weekly at the mature red stage. Yield was determined by counting and weighing all fruit on each plant. Fruit size was determined by measuring the equatorial diameter, using the Mexican standard norm (NMX-FF-031, 1997). The soluble solids concentration (SSC) of a composite juice sample was measured with a hand-held refractometer.

2.3. Plant sampling

Leaf samples were taken from plants with fully expanded leaves of the same size. Leaves were removed from a zone at approximately one-third of the total height of the plant, during flowering (77 days after planting, DAP) and 4 weeks later (105 DAP). Leaves were disinfected with nonionic detergent at 1% (Wolf, 1982), rinsed three times in distilled water, and blotted on filter paper. At each sampling, fresh leaf matter was used for the NR assay and analysis of foliar pigments; a subsample was dried in a forced-air oven at 70 °C

for 24 hours, ground in a Wiley mill, and placed in a plastic bag for further analysis of organic N.

2.4. Plant analysis

The *in vivo* NR activity was determined using an adaptation of the *in vivo* NR assay of Jaworski (1971). The NR activity was expressed as $\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ fresh weight (fw) h}^{-1}$. Chlorophyll was extracted and measured following Wellburn (1994). Chlorophyll content was expressed as $\mu\text{g pigment cm}^{-2}$. The organic N content was determined using the Kjeldahl method (Wolf, 1982).

2.5. Statistical analysis

Standard analysis-of-variance techniques were used to assess the significance of treatment means. Differences between treatment means were compared using the LSD at the 0.05 probability level. Levels of significance are represented by * at $p < 0.05$, ** at $p < 0.01$, *** at $p < 0.001$, and NS for not significant.

Table 3. Comparison of means for tomatoes developed with conventional and organic fertilizers.

Fertilizer	PD	ED	PT	SSC	FW	NF	Y
	(cm)			(°Bx)	(g)		(Mg ha ⁻¹)
F1	6.4 a	5.3 a	0.7 a	3.6 b	99.2 a	20 a	76.5 a
F2	6.5 a	5.1 ab	0.7 a	3.9 b	96.6 a	18 ab	66.9 ab
F3	6.0 b	5.0 bc	0.7 a	4.6 a	84.7 b	14 cd	48.9 cd
F4	6.2 b	5.0 bc	0.7 a	4.5 a	97.4 a	16 bc	54.3 bc
F5	6.1 b	4.9 c	0.7 a	4.7 a	83.1 b	12 d	37.0 d
Significance	**	*	NS	***	**	***	***

PD = polar diameter; ED = equatorial diameter; PT = pericarp thickness; SSC = soluble solids content; FW = fruit weight; NF = number of fruits; Y = yield; The results of LSD test is represented with lower case letters; *** _**_*_ significant differences at $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively, NS = not significant.

3. Results and Discussion

3.1. Yield

Vermicompost contains a substantial amount of humic acids that stimulate plant growth and yield (Arancon *et al.*, 2007). The highest total yield was recorded for the F1 treatment (Table 3); this treatment exceeded by 32.3% the overall average of 51.8 Mg ha⁻¹ obtained with organic fertilizers. The maximum yield obtained using inorganic fertilizers, compared with the organic fertilizers, was assessed by De la Cruz-Lazaro *et al.* (2010). Stanhill (1990), mentions that in organic agriculture the yield is 10% to 30% lower than the yield achieved by conventional farming. The lower yield can be offset by the higher price that organic tomatoes command, which is 5.8 times the price of tomatoes grown under conventional management (De la Cruz-Lazaro *et al.*, 2010). It means that the organic tomato production increases the cost-benefit relationship (Rodríguez *et al.*, 2008).

Table 3 shows that the F2 treatment provided the maximum organic fruit yield, 66.9 Mg ha⁻¹, followed

by the F4 treatment. (Arancon *et al.*, 2007) reported that humic, fulvic, and other organic acids extracted or produced by microorganisms in VCT may promote the vegetative development of plants and increase the yield. This finding may explain the results obtained by Martinez *et al.* (2002), who concluded that compost tea contains a compound with molecular structure and biological activity analogous to auxins. Leachate from well-decomposed compost has been shown to contain a cytokinin-like substance, derived from the hydrolysis of cyanogenic glucosides by the enzyme β -glucosidase, which is produced by microbes (Arthur *et al.*, 2001). Although phytohormones or growth regulators in VCT were not measured in this study, we suggest that they may play an important role in plant response.

The greatest number of fruits per plant was obtained in the F1 treatment group (Table 3). The numbers of fruits obtained using organic fertilizer were 10%, 30%, 20%, and 40% less under the F2, F3, F4, and F5 treatments, respectively, than the number of fruits obtained using the F1 treatment. Similar results have been reported by Rodríguez *et al.* (2008), who obtained 22.9% and 31.2% fewer fruits from two tomato hybrids using

mixtures of sand and vermicompost. Fruit size was based on the equatorial diameter, using the Mexican standard norm (NMX-FF-031, 1997).

The F3, F4, and F5 treatments produced small tomatoes (Table 3), whereas the F1 and F2 treatments resulted in medium fruit size. No statistical difference was detected for the thickness of the pericarp. The average weight of the fruits under the F2 and F3 treatments was not affected. Coletto (1995) mentions that after cell division, photoassimilates begin to accumulate, affecting growth and fruit weight. It is therefore likely that in the organic fertilizer groups (F2 and F4), there was reduced demand for assimilates and other growth hormones in apical tissues, which favored the content of cytokinins and

the accumulation of photoassimilates in fruit. The average SSC under organic fertilizer use increased 22.9% relative to the value obtained in the F1 treatment group. Increases in SSC in fruit of organic plants, in contrast, likely result from the interaction between reduced fruit water content, increased ion content, and maintained hexose accumulation. This result confirms that the use of organic fertilizers produces fruits with better quality in terms of soluble solids, as a tomato intended for fresh consumption must contain more than 4.0 °Bx (De la Cruz-Lazaro *et al.*, 2010). Diez (1995) mentioned that a tomato for processing or fresh consumption must have an SSC of at least 4.5 °Bx. The F3, F4, and F5 treatments produced tomatoes with over 4.5 °Bx that were suitable for fresh consumption and for use by industry.

Table 4. Effect of conventional and organic fertilizers on chlorophyll content, organic N content in leaves and in vivo endogenous NR activity and NR activity in presence of 50 mM NO₃⁻ (NR+NO₃⁻), 20 mM Mo (NR+Mo), 50 mM NO₃⁻, and 20 mM Mo (NR+NO₃⁻+Mo) in leaves of tomato.

Fertilizer /77 DAP	N (%)	Chla+b (µg cm ⁻²)	NR endogenous			
			NR+NO ₃ ⁻	NR+Mo	NR+NO ₃ ⁻ +Mo	
			[µmol (nitrates) g ⁻¹ (f.w) h ⁻¹]			
F1	3.4 b*	0.033 a	3.4 c	26.7 a	20.9 e	29.8 c
F2	3.9 a	0.027 d	7.0 b	22.5 d	23.2 d	23.0 e
F3	3.3 c	0.026 e	11.7 a	23.5 c	28.6 c	25.1 d
F4	2.7 e	0.032 b	2.6 d	20.8 e	37.7 a	38.1 b
F5	3.1 d	0.029 c	0.3 e	23.9 b	31.8 b	38.7 a
Fertilizer /105 DAP						
F1	2.1 d	0.032 b	0.6 c	32.9 b	24.6 d	41.3 c
F2	2.6 b	0.033 a	1.0 b	35.7 a	22.8 e	32.4 e
F3	2.7 a	0.027 e	0.3 e	19.6 e	30.4 a	45.10 a
F4	2.5 c	0.030 c	0.4 d	22.9 d	28.1 c	33.8 d
F5	2.1 d	0.029 d	1.4 a	25.4 c	28.4 b	44.9 b

*The results of the LSD test are represented with lower case letters.

3.2. Organic N content and foliar pigments

Chlorophyll concentrations are related to N fertilizer and the N foliar content (Haboudane *et al.*, 2002). In the present study, the organic N leaf content in the F1, F2, F3, and F5 treatments was within the optimal level at 77 DAP, while at 105 DAP, only leaves from the F1 and F5 treatments showed low levels of organic N (Table 4) (Mills *et al.*, 1996). The total chlorophyll content (a + b) at 77 DAP was within the optimum level reported by Moreno *et al.* (1999) for the F1 and F4 treatments, whereas at 105 DAP, the optimum total chlorophyll content was recorded in the F1, F2, and F4 treatments.

3.3. In vivo NR activity

One of the major and limiting stages of NO_3^- assimilation is NR activity (De la Haba *et al.*, 2001). The highest in vivo assay values were found in the F2 and F3 treatments at 77 DAP, showing a 95.7% increase over the lowest values, which were found in the F5 treatment at 77 DAP (Table 4). At 105 DAP, the highest in vivo assay values were found in the F2 and F5 treatments, showing a 70% increase over the lowest values, which were recorded for the F3 treatment. One of the principal factors regulating both the increase in de novo NR synthesis and NR activity is the presence of NO_3^- (Crawford, 1995). With respect to the NR activity in the presence of NO_3^- and molybdenum (Mo), the plants require this ion and microelement; Mo is a cofactor for activating NR and for converting nitrates to nitrites (Zimmer and Mendel, 1999). Our results coincide with those obtained by Crawford (1995) and Villora *et al.* (2002), who found that the NR enzymatic activity was modified by altering the concentrations of NO_3^- and Mo in the growth medium. The values of NR activity induced by simultaneous presence of NO_3^- and Mo showed the general need of plants for both NO_3^- and for Mo, to activate the enzyme and make use of cellular N. The presence of flowers and fruits in some plants accelerates and increases the endogenous NR activity (Carelli *et al.*, 2006). At 105 DAP, NR activity produced higher induction of

NO_3^- , Mo, and the combination of both factors, while endogenous NR activity diminished (Table 4). This occurred presumably because the crop was in the maturation phase; furthermore, the NR activity varied according to the physiological age of the tissue, levels of water, light intensity, N source, temperature, and the levels of Mo, iron, and sodium (Tarakcioglu and Inal, 2002). An increase in the amount and activity of NR leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for general amino acid synthesis, protein synthesis, or total N assimilation (Ruiz *et al.*, 2000).

4. Conclusion

We conclude that the best source of organic fertilizer for tomatoes was the F2 treatment, sand + VCT. It produced the second highest yield, the maximum assimilation of NO_3^- , the highest level of NR activity, and the second highest organic N leaf content.

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