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Original article

Compost application and biofertilization with arbuscular mycorrhizal fungi in cuatomate (*Solanum glauscescens* Zucc) nursery

Aplicación de compost y biofertilización con hongos micorrizógenos arbusculares en vivero decuatomate (*Solanum glauscescens* Zucc)

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ABSTRACT: Cuatomate (*Solanum glauscescens* Zucc) is an important crop for the Mixteca Poblana, and the availability of technologies to increase its productivity is a priority for producers in the region. An experiment was conducted to evaluate the effect of compost addition and biofertilization with arbuscular mycorrhizal fungi (AMF) on cuatomate during the nursery stage. Twelve treatments, resulting from the combination of four substrates prepared with different soil:compost ratios and inoculation with AMF strains *Glomus cubense*, *Rhizoglomusi rregulare* and a control without inoculation, were studied in a completely randomized design, with factorial arrangement and ten replications, and indicators of mycorrhizal performance and plant growth were evaluated. No interaction was found between soil:compost ratios and biofertilization with AMF for mycorrhizal and growth variables. No response to AMF inoculation on plant growth was observed either. The greatest height, stem diameter and number of leaves were achieved with soil:compost ratios 0.50:0.50 and 0.25:0.75 v:v. Second order regression equations with high R^2 values were found between N-NO_3, assimilable P and exchangeable K contents of the substrates and plant height and leaf number. It is concluded that the mixture of soil and compost in a 0.50:0.50 ratio is a suitable substrate for cuatomate cultivation during the nursery stage. Further studies on the mycorrhization of the cuatomate crop are recommended.

Key words: organic fertilizer, nutrients, growth.

RESUMEN: El cuatomate (*Solanum glauscescens* Zucc) constituye un cultivo importante para la Mixteca Poblana, y disponer de tecnologías para aumentar su productividad es una prioridad para los productores de la región. Se realizó un experimento para evaluar el efecto de la adición de compost y la biofertilización con hongos micorrizógenosarbusculares (HMA), en cuatomate durante la etapa de vivero. Se estudiaron 12 tratamientos, resultantes de la combinación de cuatro sustratos elaborados mediante diferentes relaciones suelo:compost, y la inoculación con las cepas de HMA *Glomus cubense, Rhizoglomus irregulare*y un testigo sin inocular, en un diseño completamente aleatorizado, con arreglo factorial y diez repeticiones, y se evaluaron indicadores del funcionamiento micorrízico y el crecimiento de las plantas. No se encontró interacción entre las relaciones suelo: compost y la biofertilización con HMA para las variables micorrízicas ni del crecimiento. Tampoco se observó respuesta a la inoculación con HMA en el crecimiento de las plantas. La mayor altura, diámetro del tallo y número de hojas se alcanzaron con las relaciones suelo: compost 0,50:0,50 y 0,25:0,75 v:v. Se encontraron ecuaciones de regresión de segundo orden con altos valores de R^2, entre los contenidos de N-NO_3, P asimilable y K intercambiable de los sustratos y la altura y el número de hoja de las plantas. Se concluye que la mezcla de suelo y compost en relación 0,50:0,50, resulta un sustrato adecuado para el cultivo de cuatomate, durante la etapa de vivero. Se recomienda profundizar en los estudios sobre la micorrización del cultivo de cuatomate.

Palabras clave: abono orgánico, crecimiento, nutrientes.

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INTRODUCTION

Cuatomate (*Solanum glauscescens* Zucc) is a wild plant with a semi-woody stem and climbing growth habit, belonging to the Solanaceae family, which inhabits the deciduous forests of the Mixteca Baja Poblana region, Mexico. This species is a very valuable phytogenetic resource, intimately linked to the culture and knowledge of the farmers.

Its fruits are highly valued by the inhabitants of the region, who use it for the preparation of different culinary dishes and for commercialization, which is spreading rapidly, starting with an incipient potential market among the Mexican population living in the USA (1).

This has stimulated its domestication with the purpose of increasing production levels (2); however, the lack of an integral technology for the agronomic management of cuatomate limits its potential to reach yields that satisfy the growing demand for consumption and commercialization.

As part of the integral technology for cuatomate cultivation, the production of seedlings in nurseries can be advisable, since it guarantees the obtaining of vigorous plants for transplanting, and consequently, a desired population in the future plantation; but to achieve success in this phase, it is essential to select a suitable bed for seed germination and plant growth in its early stages.

It is known that the preparation of substrates from the mixture of soil and organic fertilizers accelerates plant growth in the nursery, both by the supply of nutrients and beneficial microorganisms, as well as by the improvement of their physical properties (3,4), although the adequate ratio of both components depends on the properties of the soil, the nature of the organic fertilizer and the requirements of the crop (5,6).

On the other hand, biofertilization with beneficial microorganisms, including arbuscular mycorrhizal fungi (AMF), also produces positive results in the growth and increased vigor of plants grown in the nursery stage, due to their role in improving the absorption of nutrients and water from the substrate, as well as in protection against pathogens (7,8).

However, there are very few agronomic studies on the cultivation of cuatomate 1, and there are no reports in the literature related to the mycorrhization of this species.

Based on this background, as well as the need for effective agronomic practices to increase cuatomate production in the Mixteca Poblana, this study was carried out to determine the most appropriate soil-organic fertilizer ratio and the effect of inoculation with arbuscular mycorrhizal fungi on the growth of this species during the nursery stage.

MATERIALS AND METHODS

The experiment was conducted in the nursery of Tecomatlán Technological Institute located at 17º53'N and 98º21' W, at 950 m a.s.l, in the municipality of Tecomatlán, Puebla, Mexico. Table 1 shows the behavior of some meteorological variables during the period in which the experiment was conducted.

Twelve treatments were studied, resulting from the combination of four substrates (soil:compost mixtures, in ratios 1:0; 0.75:0.25; 0.50:0.50 and 0.25:0.75, v:v) and inoculation with AMF strains *Glomus cubense*, *Rhizoglomus irregular* and a control without inoculation, in a completely randomized design, with 4x3 factorial arrangement and ten replications. The experiment was conducted from August 5 to September 14, 2019 and from October 2 to November 15, 2020.

For the preparation of the substrates, 20 cm of the surface layer of a Regosol Eutric soil (9) and compost made from goat manure (80 %) and crop residues (20 %), both from the Instituto Tecnológico de Tecomatlán, were taken. The chemical characteristics and moisture content of the compost are presented in Table 2.

For the characterization of the compost, the following analytical methods were used, established in Fertilab's laboratories (10).

Tabla 1. Average values of maximum, minimum and average temperatures, relative humidity and precipitation, prevailing during the nursery stage of cuatomate inoculated with AMF and uninoculated, planted in different combinations of soil:compost

Experiment execution periods	Temperatures (°C)			Relative humidity (%)	Precipitations (mm)
	Maximum	Minimum	Mean		
August 5-September 14. 2019	34	19	26.5	68.0	6
October 2 - November 15. 2020	32	14	23.0	64.5	1

Data taken at the Tecomatlán Technology Institute

Table 2. Chemical characteristics (data expressed on a dry basis) and moisture content of the compost used in the preparation of substrates for cuatomate cultivation during the nursery stage

Periods	OM ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	C: N Ratio	P ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Na ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	pH	Humidity (%)
August-September 2019	520.8	18.7	16.5	5.1	40.3	5.2	2.5	11.3	8.0	36.1
CI (α = 0.05)	±8.7	±0.4	±1.5	±0.1	±0.5	±0.1	±0.1	±0.2	±0.2	±0.9
October-November 2020	532.7	20.5	18.7	4.1	38.4	5.6	3.2	12.2	8.1	34.9
CI (α = 0.05)	±9.8	±0.7	±1.9	±0.2	±0.7	±0.2	±0.1	±0.4	±0.2	±1.2

OM: organic matter. Averages of five samples taken in each period, at the time of substrate processing. CI: confidence interval

- Moisture: gravimetry.
- Organic matter: Walkley and Black.
- Nitrogen: Kjeldahl.
- pH: potentiometry, compost-water ratio 1:5.
- Incineration of the sample in the muffle for 2 hours and determination of P by colorimetry, Ca and Mg by atomic absorption spectrometry, and K and Na by flame photometry.

Ten days after the substrates were prepared, they were placed in beakers of 8.5 and 5.5 cm in upper and lower diameter and 10 cm in height, with a capacity of 385 cm^3, which were previously opened with six small holes in the bottom to facilitate drainage and were filled to the surface.

Before filling the vessels, five samples of the substrates were taken in each period and the contents of organic matter, N-NO^3, assimilable P, exchangeable Ca, Mg, K and Na and pH-H_2O were determined, according to the analytical techniques used in the Fertilab laboratories (10).

For the application of AMF, solid inoculants containing INCAM-4 strains of the *Glomus cubense* species (DAOM 241198) (11) and INCAM-11 of the *Rhizoglomus irregulare* species (DAOM 711363) (12), with a concentration of 30 spores g^{-1} and abundant fragments of rootlets of the host plant (*Brachiaria decumbens*), were used. Both certified inocula came from the collection of the National Institute of Agricultural Sciences of Cuba.

In the upper center of each beaker a hole was opened to a depth of 1 cm, 5 grams of mycorrhizal inoculant were deposited and two cuatomate seeds were placed, which were then covered with the substrate. Five days after germination, one seedling was left in each glass. Irrigation was carried out every three days at a rate of 60 mL per beaker, from the beginning to the end of the experiment. The nursery was covered with Rashell type mesh, which guaranteed the passage of 50 % of the sunlight, taking into account the shade needs of the crop.

At the end of the experiment, the height of the plants was measured with a graduated ruler of 1 mm precision, the diameter of the stems using a caliper, and the number of leaves per plant was counted.

From each beaker, 50 g of substrate and 1 g of rootlets were extracted. The rootlets, after washing, were dried in an oven at 70 ºC for staining and clarification (13). The frequency of mycorrhizal colonization (14) and the visual density (DV) or intensity of colonization (15) were evaluated; in the 50 g of substrate, the number of spores in the rhizosphere was determined by wet sieving and decantation of these structures and their observation under a microscope (16).

The data, once normality and homogeneity of variances were verified, were statistically processed by analysis of variance and Tukey's test at P<0.05. Regression analyses were performed between the N-NO_3, P and K contents of the substrates and the height and number of leaves of the plants, and the best fitting equations were selected. In all cases, the statistical program SPSS 25 (17) was used.

RESULTS

A significant effect of compost addition on the organic matter and nutrient contents of the substrates was found (Table 3).

The substrate composed only of soil, although it presented an average content of exchangeable Ca, had low contents of organic matter and exchangeable Mg, and very low contents of assimilable P, K and N-exchangeable N (18). However, as the soil:compost ratio decreased, these, as well as the N-NO_3 content, increased until reaching the highest values with the mixture of 0.25 and 0.75 parts of soil and compost. With respect to the reaction of the substrate, no effect of the addition of organic fertilizer was observed, probably due to the fact that the soil had an alkaline pH, with values close to those of the compost.

In relation to the mycorrhizal variables, no interaction was found between the soil:compost ratio and the inoculation with AMF in either of the two moments in which the experiment was carried out. The substrates also had no effect on these variables, but both the frequency and intensity of colonization, as well as the number of spores in the rhizosphere, increased significantly with the inoculation of both AMF species in relation to the uninoculated control (Table 4).

In the two moments in which the experiment was carried out, no interaction was found between the soil:compost ratio and AMF inoculation on growth variables (Table 5), but plant height, stem diameter and number of leaves increased significantly as the amount of organic fertilizer in the substrate increased, reaching the highest values with the lowest soil:compost ratios (0.50:0.50 and 0.25:0.75), with no differences between the two, except for plant height,

Table 3. Effect of compost addition on the chemical characteristics of substrates prepared for cuatomate cultivation during the nursery stage

Soil:compost ratio	N-NO_3	P	O. M	Ca Mg Na K				pH
	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})	(cmol$_c$ kg^{-1})				
1:0	19.7 d	3.27 d	16.1 d	14.24 d	1.19 d	0.13 c	0.22 d	7.9
0.75:0.25	25.4 c	4.21 c	90.8 c	17.88 c	2.15 c	0.15 bc	0.47 c	8.1
0.5:0.5	32.9 b	5.52 b	149.0 b	21.58 b	3.31 b	0.19 ab	0.69 b	8.0
0.25:0.75	40.3 a	7.28 a	187.5 a	25.62 a	4.05 a	0.22 a	0.92 a	8.1
SE $\overline{X}$	0.3**	0.10**	3.3**	0.6**	0.1**	0.1**	0.1**	0.2

Means with different letters in the same column differ significantly according to Tukey's test at P<0.05

Table 4. Effect of soil:compost ratio and AMF inoculation on mycorrhizal variables of cuatomate during the nursery stage

Soil:compost ratio	August 2019			October 2020		
	Colonization frequency (%)	Colonization intensity (%)	Spores/50 g	Colonization frequency (%)	Colonization intensity (%)	Spores / 50 g
Effect of soil:compost ratio						
1:0	6.76	0.15	38	10.75	0.18	43
0.75:0.25	7.14	0.13	34	11.32	0.21	46
0.50:0.50	7.47	0.14	36	10.57	0.18	53
0.25:0.75	6.71	0.15	39	11.40	0.22	48
SE $\bar{X}$	0.31	0.02	3	0.43	0.03	4
Effect of inoculation with AMF						
Without inoculation	5.13 b	0.06 b	25 b	6.87 b	0.13 b	32 b
G.cubense	8.10 a	0.17 a	42 a	12.81 a	0.22 a	52 a
R.irregulare	7.82 a	0.19 a	43 a	13.32 a	0.24 a	58 a
SE $\bar{X}$	0.29**	0.02**	2**	0.37**	0.02**	3**

Means with different letters in the same column differ significantly according to Tukey's test at P<0.05.

Table 5. Effect of soil:compost ratio and AMF inoculation on height, stem diameter and number of leaves of cuatomate plants during the nursery stage

Soil: compost ratio	August 2019			October 2020		
	Plant height (cm)	Stem diameter (mm)	Number of leaves	Plant height (cm)	Stem diameter (mm)	Number of leaves
Effect of soil:compost ratio						
1:0	6.25 c	1.60 c	5.61 c	4.61 c	2.45 c	9.68 c
0.75:0.25	8.71 b	1.88 b	6.72 b	6.02 b	3.06 b	11.20 bc
0.50:0.50	12.4 a	2.31 a	10.28 a	9.51 a	3.57 a	12.14 a
0.25:0.75	11.5 a	2.52 a	9.72 a	7.38 b	3.51 a	12.00 ab
SE $\bar{X}$	0.15**	0.08**	0.16**	0.19**	0.09**	0.22**
Effect of inoculation with AMF						
With inoculation	9.64	1.91	8.40	6.76	3.18	11.17
G.cubense	10.03	2.00	8.40	7.26	3.22	11.49
R.irregulare	10.36	2.02	8.13	6.66	3.35	11.55
SE $\bar{X}$	0.11	0.04	0.20	0.17	0.08	0.16

Means with different letters in the same column differ significantly according to Tukey's test at P<0.05

stem diameter and number of leaves: compost lower (0.50:0.50 and 0.25:0.75), with no differences between the two, except for plant height in October 2019. Inoculation with AMF had no effect on these indicators.

When the average values of plant height and number of leaves obtained on both dates of the experiment were related to the N-NO_3, P and K contents of the substrates, second order regression equations were found with high levels of adjustment (R^2 values higher than 0.90), as shown in Figure 1. As the contents of these nutrients in the substrates increased, plant height and number of leaves increased. The highest values were reached with values close to 35 mg kg^{-1} of N-NO_3, 6 mg kg^{-1} of P and 0.7 $cmol_c kg^{-1}$ of K, after which there was a tendency for both variables to decrease.

DISCUSSION

One of the advantages of the elaboration of substrates from the addition of organic fertilizers for the cultivation of plants in the nursery phase, lies in the contribution of organic matter and nutrients (19,20), Judging by the response of the cuatomate plants, it can be inferred that the values reached in the substrate elaborated with the mixture of soil and compost in a 50:v ratio, were sufficient for them to reach their highest growth: This response is attributed to the low content of nutrients in the soil, especially those that could limit the growth and development of the plants.

This is confirmed by the high relationships obtained between the increase in N-NO_3, P and K contents in the substrate and the increase in height and number of leaves, three macronutrients essential to guarantee optimum growth and development of the plants from their early stages (21,22), and whose contents in the soil, already very low, were favored by the addition of compost.

The high correlations between the P content of the substrate and the height and number of leaves, which were demonstrated by regression equations with R^2 values of 0.99, indicate that this element plays a fundamental role in the growth of cuatomate, at least during the nursery stage, In this sense, some authors have observed that increasing the amount of compost in the substrate causes increases in P concentrations and, consequently, in the growth of aerial and root biomass of plants with high needs for this element during the nursery stage (19,20).

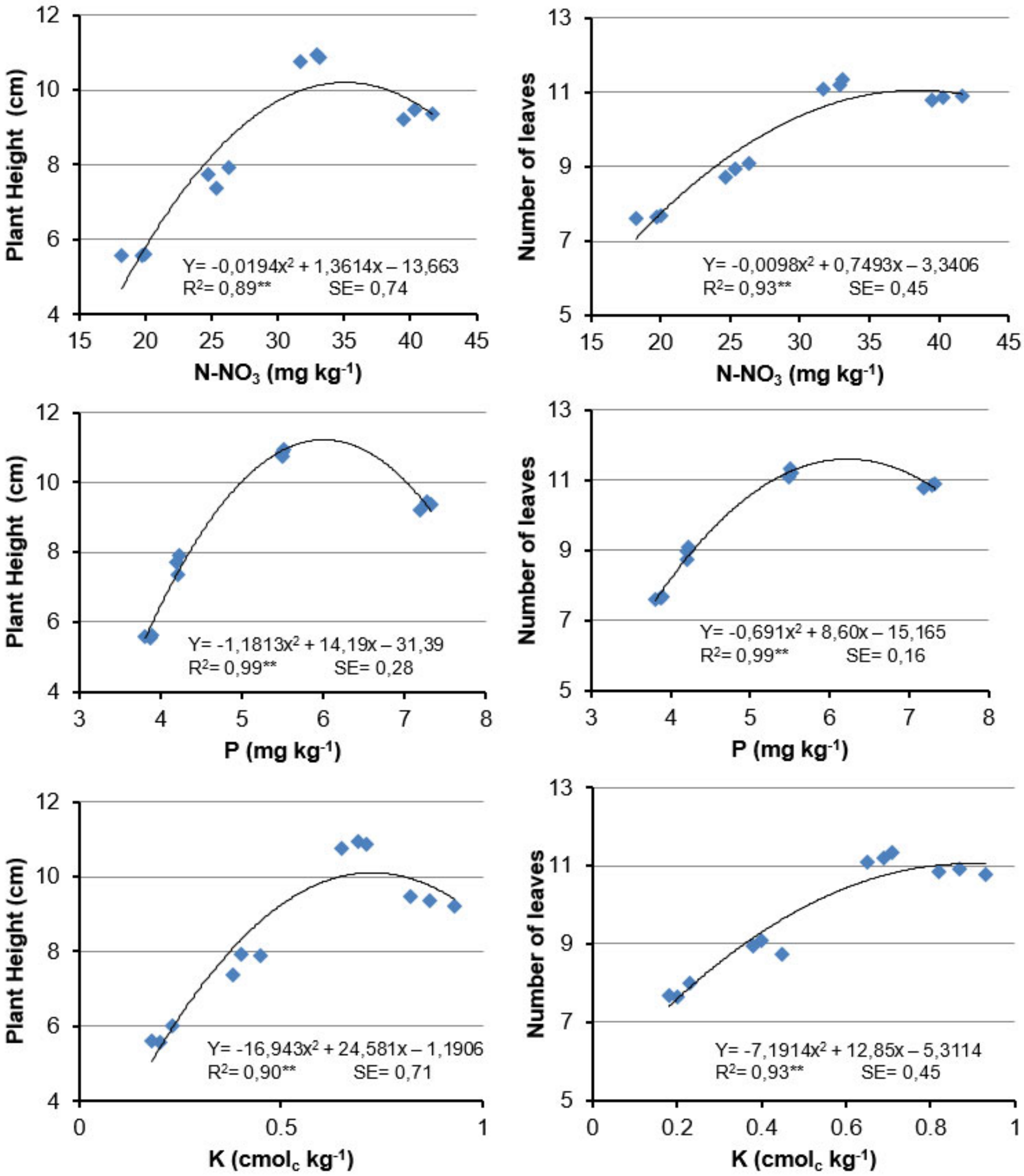

Figure 1. Ratios between macronutrient concentrations (N-NO3, P and K) in the substrate with the height and number of leaves of cuatomate plants

Benefits of compost in the growth of different plant species during the nursery stage have been indicated by several authors (6,22) and although the most adequate amounts of organic fertilizer depend on the needs of the crop, the properties of the rest of the materials that make up the substrate and the nature of the compost (23), they all state that they are related to the contribution of organic matter and nutrients that can be assimilated from the first stages.

But it cannot be ruled out that the benefits of compost may also have been related to the improvement of the physical properties of the substrate, such as bulk density and moisture retention capacity (19,24) and even to the improvement of its biological properties, due to the microorganisms present in the compost, which may have had a beneficial effect on plant growth (25,26).

In absolute terms, the plants that were planted in August reached, on average, greater height, smaller stem diameter and lower number of leaves than those observed in the October planting, which could be related to differences in the behavior of meteorological variables in each period in which the experiment was executed. Values of maximum, minimum and average temperature, and relative humidity were higher in August 2019 than in October 2020.

The fact that with the use of compost the plants have reached the highest growth in only 45 days, may also have economic implications, since this means a shortening of the stay of plants in the nursery, a decrease in the use of water for irrigation during this period, and possibly, an earlier start of the harvest of fruits in the future plantation.

In relation to the inoculation with arbuscular mycorrhizal fungi to obtain seedlings in the nursery stage, several authors agree that this is a positive strategy to improve the indicators of plant growth and development (27,28), Although several factors could be involved in this behavior, it seems that 45 days after germination were not enough for plants to reach levels of colonization that could induce an adequate growth response.

In the literature reviewed, no studies on mycorrhization of cuatomate were found, so the dynamics of colonization of this plant species by AMF and its degree of mycorrhizal dependence are unknown; However, it can be inferred that both the frequency and intensity of colonization reached by seedlings at the transplanting time were low, if it is taken into account that in most of the studies where positive responses of plants to AMF inoculation were found, these reached colonization frequencies higher than 40 %, independently of plant species (29,30).

In this sense, some authors, when evaluating the effect of AMF inoculation in plants with low colonization levels during the nursery stage, obtained the best results with the mycorrhizal inoculant application at the transplanting moment (31).

On the other hand, AMF strains used in this experiment have shown high efficiency in different crops, which translates into high levels of colonization and increases of up to 40 % in yields, although their effectiveness has been associated to the edaphic environment in which the plants develop (32). In the specific case of *R, irregulare*, its inoculation in different crops has yielded positive results, in soils or substrates with chemical characteristics and pH values very similar to the one used in this work (33,34). However, further studies will be required to know the degree of mycorrhizal dependence of cuatomate and its response to inoculation with AMF.

CONCLUSIONS

No interaction was found between the soil:compost ratio in the substrate and AMF inoculation for cuatomate growth during the nursery stage. The increase in plant height and number of leaves was related to the increase in the N-NO_3, P and K contents of the substrate.

The mixture of soil and compost in a 0.50:0.50 ratio ensured optimum crop growth during this stage.

RECOMMENDATIONS

Further research is recommended in studies on mycorrhization of cuatomate crop.

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