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Article

Tomato yield and resistance to *Meloidogyne incognita* (Kofoid and White) Chitwood using mycorrhizae

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Abstract

This study evaluated the impact of five different doses of a commercial mycorrhizal consortium on tomato (*Solanum lycopersicum* L.) yield and its effect on plant resistance to nematodes of the genus *Meloidogyne incognita*. The following items were assessed biweekly: vegetative variables: plant height, number, length and width of leaves; reproductive variables: number and distance between clusters; and production variables: number of fruits, weight, length and yield of fruits; from 7 to 230 days after transplanting (dat). In addition, the effect on resistance was evaluated by performing taxonomic identification, quantifying nematodes, and determining the percentage of mycorrhization at 90, 160 and 230 dat. The analysis of variance indicated significant differences ($p \leq 0.05$) for the production variables: harvest yield, fruit length, and fruit weight; as well as for the quantification of nematodes and the mycorrhization percentage at the dose of 15 g L^{-1}. The 10-fold higher dose of the mycorrhizal consortium significantly increased yield (12.65 kg $plant^{-1}$), fruit weight (64 g) and fruit length (6.3 cm), as well as the percentage of mycorrhization (88.85%) and reduced root colonization by *Meloidogyne incognita* (3%) compared to the control.

Keywords:

Solanum lycopersicum, Meloidogyne incógnita, fruit weight, mycorrhizae.

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Introduction

Worldwide, Mexico stands out as one of the largest producers of tomatoes (*Solanum lycopersicum*), occupying an important place in global production (Orona-Castillo *et al*., 2022). However, tomato production faces serious challenges due to various diseases and pests that affect it, among which phytoparasitic nematodes, such as *Meloidogyne incognita*, are of particular concern. Nematodes reduce water absorption through plant roots and affect physiological functions, causing considerable yield losses (Guzmán-Piedrahita *et al*., 2020).

Arbuscular mycorrhizal fungi (AMF) have emerged as a promising alternative to improve plant health (Ramírez *et al*., 2021) and increase fruit quality (Vázquez *et al*., 2020). These fungi establish symbiosis with the roots, facilitating the exchange of nitrogen and phosphorus (Carrillo-Saucedo, 2022). In addition, arbuscular mycorrhizae may offer effective protection against other soil pathogens by competing for root space, modifying root exudates, and activating plant defense mechanisms (Harrier and Watson, 2004; Sousa *et al*., 2010).

AMF colonization improves plant nutrition and tolerance to water stress and activates defense mechanisms (Volpe *et al*., 2018). Understanding these interactions is crucial to developing effective biocontrol strategies against nematodes in tomato crops (Arias *et al*., 2009), where species such as *Glomus intraradices*, *F. mosseae*, and *Rhizophagus intraradices* can reduce nematode population and gall formation in tomato crops, providing an integrated management strategy (Sharma and Sharma, 2015).

In Mexico, the use of arbuscular mycorrhizae in tomato crops is still incipient, and more research is required to achieve their effectiveness in controlling *M. incognita* and other phytoparasitic nematodes. Based on the above, it was proposed to evaluate the impact of applying different doses of a mycorrhizal consortium on tomato crop yield and its ability to confer control *M. incognita*.

Material and methods

The research work was conducted from October 2023 to May 2024 under protected cultivation conditions in Culiacán, Sinaloa, located at 24.673352 north latitude and 107.49718 west longitude, using a shade net (anti-aphid mesh, Raschel shade 30%) and drip irrigation of 1 L h^{-1}.

Inoculation of AMF

A total of 600 seeds of indeterminate hybrid tomato, Saladette type, variety SVTJ7535 (Seminis®), were inoculated using polystyrene trays with 162 individual cavities. The seeds were sown on a BM 2 Euro® commercial substrate (designed for vegetable propagation) and then covered with VerLite® vermiculite to promote moisture retention and uniform germination.

For the study, each group of 100 seeds was inoculated with five different doses of spores of the commercial arbuscular mycorrhizal fungi (AMF) containing *Rhizophagus irregularis*, *Funneliformis mosseae*, *Glomus aggregatum*, and *Entrophospora etunicata* and one group remained without inoculation as a control. Finally, six treatments were established, corresponding to 1X, 2X, 4X, 6X and 10X of the recommended dose (Table 1), defined according to the manufacturer for irrigation applications, which indicates a standard dose of 1.5 g L^{-1} of water for vegetables in the transplantation phase (MycoApply®, 2020). This design allowed us to evaluate a possible response following criteria similar to those used in previous trials with mycorrhizal fungi in horticultural crops (Berruti *et al*., 2016).

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Table 1. Treatments applied with doses of the arbuscular mycorrhizal (AMF) consortium and es timated concentration of spores per liter.

Treatment	Dose	Number of spores L^{-1}
T1, control without inoculation	0	0
T2, AMF in substrate	2.5 g kg^{-1}	2 408.33
T3, AMF half recommended in irrigation	0.75 g L^{-1}	722.5
T4, AMF recommended in irrigation	1.5 g L^{-1}	1 445
T5, AMF 10 times the dose recommended in irrigation	15 g L^{-1}	14 450
T6, AMF recommended irrigation + in substrate	1.5 ml L^{-1} + 2.5 g kg^{-1}	1 445 + 2 408.33

The application of AMF began with the development of the true leaf; that is, nine days after sowing (das), applying the solution on the base of the stem of the plant. Each treatment was applied individually and the inoculation was repeated every three days for 35 days until transplantation. At the same time, chemical fertilization was applied via drip irrigation, with a flow rate of 1 L h^{-1}. Granulated fertilizer with the formula N-P-K (5-10-5) was used, dissolving 5 g of fertilizer per treatment in 1 L of water. This solution was applied 10 days after sowing and repeated every three days for 230 days, covering the entire crop cycle, from the establishment phase to production.

The dose and method of application were determined as reported by Gill and Verma (2018). No additional micronutrients were applied, as soil analysis indicated sufficient levels for optimal crop development, which coincides with what was reported by Smith and Read (2008). As for the inoculations in the substrate, they were carried out only once, by manually mixing the peat with the product at sowing. During the sowing phase (45 days), the seedlings averaged 14 cm in height prior to transplanting.

Establishment of the experiment and experimental design

Mycorrhiza-colonized seedlings were transplanted into furrows covered with plastic mulch, with a distance of 1.96 m between the centers of one furrow and another, and a separation of 0.5 m between plants within each furrow in a greenhouse provided with a shade net structure and with a history of *Meloidogyne incognita* infestation. Once the transplantation was performed, the inoculation with AMF was reinforced at 15, 30 and 45 days after transplantation (dat), depositing the solution at the base of the plant stem at the respective doses by treatment. We worked for 230 dat using a traditional chemical fertilization of 20-10-10 N-P-K per plant, applied every two days until the final evaluation. The experimental unit by treatment consisted of three replications, with 14 plants per replication distributed under a completely randomized experimental design with six treatments.

Vegetative and production variables evaluated

Three plants per replication and 14 plants per treatment were evaluated biweekly. The vegetative variables were: 1) plant height (cm); 2) leaf width (5th leaf, cm); 3) leaf length (5th leaf, cm); 4) stem thickness (below 1st flowering, cm); 5) distance between clusters (5th-6th cluster, cm). The production variables were: 6) number of clusters; 7) number of fruits per cluster; 8) fruit weight (g); 9) fruit length (cm); and 10) fruit diameter (cm). Total fruit yield in kg. The measurements were made on the fully expanded fifth leaf, as this is considered physiologically representative of the plant's general state during the development stages. This leaf was selected for its morphological stability, lower interindividual variability, and more accurate reflection of the effects of nutritional or symbiotic treatments on leaf growth, as indicated by previous reports in nightshades (Lichtenthaler *et al*., 2005).

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The distance between the fifth and sixth clusters was considered to give uniformity and relevance in the vegetative development of the tomato, and the thickness of the stem was considered to provide a good representativeness to the data. To obtain the total yield, the weight of all the fruits harvested from each plant (kg) in each sampling period was summed, which allowed us to calculate the cumulative yield by treatment.

Mycorrhization percentage

At 90, 160, and 230 dat, the percentage of mycorrhization was evaluated using the staining method. To determine mycorrhizal colonization, the roots were extracted from the soil at the base of the plant to a depth of 30 cm, and washed with running water to remove excess soil. They were then cleared with a solution of 10% KOH and 10% H_2O_2 for 30 min at room temperature. Then, they were stained with trypan blue in 0.05% lactoglycerol for 24 h to facilitate the visualization of mycorrhizal structures. After staining, they were observed under a compound optical microscope with a 40X objective. The percentage of mycorrhizal colonization was determined in five equidistant visual fields per root segment. The presence or absence of mycorrhizal structures, such as coenocytic hyphae, vesicles, arbuscules, and spores, was recorded. The percentage of colonization was calculated by counting the interactions of hyphae and mycorrhizal structures within the roots and dividing this by the total number of segments observed.

Nematode counting

At 90, 160, and 230 dat, the number of nematodes was counted using the centrifugation method. The roots were extracted from the soil at a depth of 30 cm from the base of the plant and washed under running water over a fine-mesh sieve. A total of 300 cc of the washed roots was taken and placed in a sterilized 50 ml ARBiotech® polypropylene tube for each treatment. Then, 25 ml of distilled water was added to each tube. The next step was to centrifuge the sample at 1 500 rpm for 10 min, using a Thermo Fisher Scientific® centrifuge. The sediment was carefully transferred to a nematode counting chamber, and the nematodes were counted under a light microscope with a 40X objective. To ensure the accuracy of the count, five random visual fields were counted inside the counting chamber to obtain a representative average of the nematode population per sample. The total nematode count per treatment was calculated by summing all the nematodes observed in the different visual fields and dividing by the number of fields counted. Nematode identification was carried out according to Hunt and Handoo (2012).

Statistical analysis

The analysis of all variables was performed using an analysis of variance and comparison of means (Tukey, $p = 0.05$) in the Minitab 2018 statistical package.

Results and discussion

Plant height, stem thickness and leaf length and width

The results obtained indicate that no significant differences were observed between the treatments applied in terms of stem thickness and the average length and width of leaves per plant. Stem thickness ranged from 7.7 to 8.23 mm, leaf length varied between 27.3 cm and 29.9 cm, and leaf width between 24 cm and 24.9 cm (Table 2).

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Table 2. Results of the evaluation of morphological variables.

Treatment	PH (cm)	ST (mm)	LL (cm)	LW (cm)	NC	DC (cm)	FC	FY (total kg)
T1	275.8a	7.7a	27.3a	24.2a	9a	30.1a	13a	817.9
T2	275.9a	7.8a	28.1a	24a	10a	30a	13a	934
T3	246.2a	7.8a	27.4a	24.5a	9a	28.3a	14a	801
T4	289.3a	7.5a	27.7a	24.1a	9a	28.6a	13a	781.2
T5	307.6a	8.2a	29.9a	24.9a	11a	27.1a	16a	987
T6	304.5a	8.1a	28.5a	24.7a	10a	27.9a	15a	949.2

T1= control without inoculation; T2= AMF in substrate; T3= AMF half recommended in irrigation; T4= AMF recommended in irrigation; T5= AMF 10 times the recommended dose in irrigation; T6= AMF recommended in irrigati on + in substrate; PH= plant height; ST= stem thickness; LL= leaf length; LW= leaf width; NC= number of clusters; DC= distance between clusters; FC= fruits per cluster; FY= fruit yield. Means with different letters within each column are significantly different (Tukey, $p \leq 0.05$).

These similarities suggest that, under the conditions of the present research, the treatments did not exert a differentiating effect on these morphological variables. This coincides with what was reported by Ramírez *et al*. (2019). It is possible that factors such as the genetic uniformity of plant material or homogeneous environmental conditions have contributed to the absence of differences, thus limiting the morphological response of plants to the applied treatments.

Number of clusters, distance between clusters, and fruits per cluster

The average number of clusters per plant varied between 9 and 11, the distance between clusters ranged from 27.1 cm to 30.1 cm and the number of fruits per cluster fluctuated between 13 and 16 (Table 2). No significant differences were found in the distance between clusters or in the number of fruits per cluster, indicating a homogeneous response between treatments.

Fruit weight

The average weight of the fruits (Figure 1) showed significant differences between treatments. With T1 (without inoculation), the fruits presented the lowest weight (59.38 g), whereas with treatments T2 (2.5 g kg^{-1} of substrate), T5 (15 g L^{-1}) and T6 (1.5 g L^{-1} + 2.5 g kg^{-1}), the fruits registered the highest values, with 82.37 g, 84.49 g and 88.36 g, respectively. On the other hand, treatments T3 (0.75 g L^{-1}) and T4 (1.5 g L^{-1}) showed similar and low values. Highly significant differences were observed between T2, T5 and T6 compared with the rest (Tukey, $p = 0.05$) at 230 days after transplantation.

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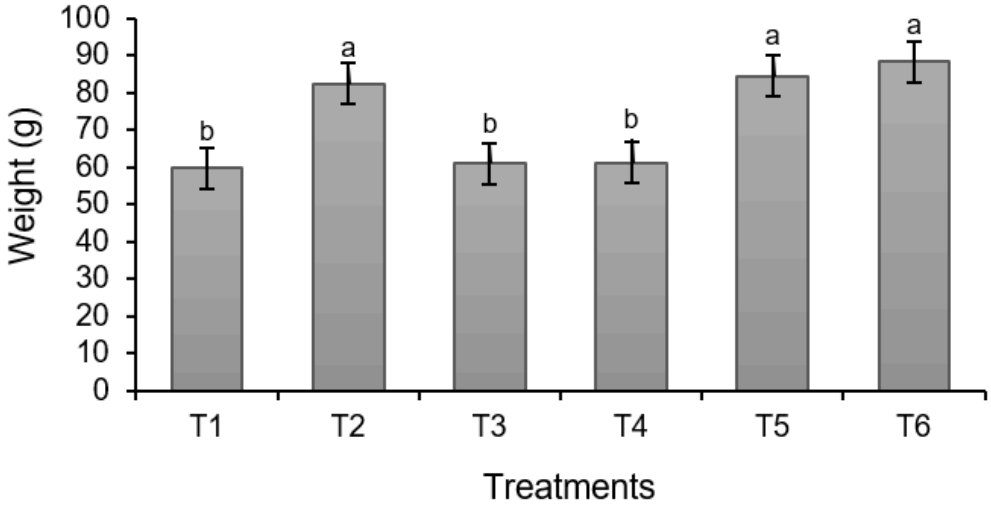
Figure 1. Weight of tomato fruits. T1= control without inoculation; T2= AMF in substrate; T3= AMF half recommended in irrigation; T4= AMF recommended in irrigation; T5= AMF 10 times the recommended dose in irrigation; T6= AMF recommended in irrigation + in substrate.



These results coincide with previous studies (Todeschini *et al*., 2018; Alvarado-Carrillo *et al*., 2014), which point out that AMF, such as *Rhizophagus irregularis* and *Funneliformis mosseae*, promote growth and improve yield through greater efficiency in nutrient absorption, which could explain the effects observed in treatments with higher levels of inoculation.

Fruit length and diameter

Fruit length ranged from 4.9 to 6.3 cm. Throughout the different evaluation periods, no significant differences were found (Tukey, $p = 0.05$) between the treatments applied, T2 (2.5 g kg^{-1} of substrate) and T6 (1.5 g L^{-1} + 2.5 g kg^{-1}) had 6.39 and 6.36 cm, respectively (Figure 2). The results for fruit diameter ranged from 4.4 to 5.4 cm, and there were no significant differences among treatments.

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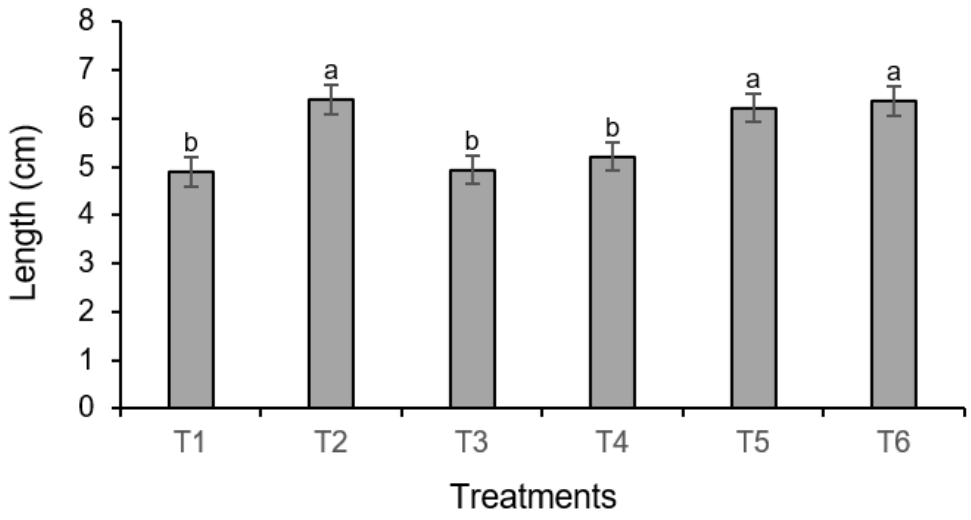
Figure 2. Fruit length. T1= control without inoculation; T2= AMF in substrate; T3= AMF half recommended in irrigation; T4= AMF recommended in irrigation; T5= AMF 10 times the recommended dose in irrigation; T6= AMF recommended in irrigation + in substrate. Means with different letters within each column are significantly different (Tukey, $p \leq 0.05$).



Fruit yield

Fruit yields (Table 2) showed that the dose used in T1 (without inoculation) presented a total weight of 817.9 kg; T2 (2.5 g kg^{-1} substrate) and T3 (0.75 g L^{-1}) had weights of 934 kg and 801 kg, respectively. T4 (1.5 g L^{-1}) reached 781.2 kg, whereas T5 (15 g L^{-1}) showed the highest average weight of 987 kg. Finally, T6 (1.5 g L^{-1} + 2.5 g kg^{-1}) had a weight of 949.2 kg. An average yield of 12.65 kg per plant was obtained in the treatment with the highest dose, far exceeding the control (8.3 kg), when considering total fruit yield divided by the number of plants per treatment (14 double-trained plants with three replications).

Nematode colonization and mycorrhization percentage

The number of nematodes per 300 cc of root showed significant differences between treatments (Table 3). The control, T1 (without inoculation), presented the highest colonization with 1 152 individuals. In contrast, treatment T5 (15 g L^{-1}) showed the lowest colonization with only 23 individuals, followed by T6 (1.5 g L^{-1} + 2.5 g kg^{-1}) with 24.3. Treatments T2, T3 and T4 registered 664, 176 and 949 individuals, respectively. A highly significant difference (Tukey, $p = 0.05$) was observed 90, 160 and 230 days after transplantation (dat), with T5 and T6 being significantly different from the rest at the end of the cycle, 230 dat.

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Table 3. Percentage of colonization (% M) of AMF in tomato and effect on control of *Meloidogyne* 90, 160, and 230 dat (days after transplantation).

Treatments	90 dat (% M)	IP3R	160 dat (% M)	IP3R	230 dat (% M)	IP3R
T1	n/m b	800 b	2.3 b	1201 b	2.1 b	1455 b
T2	49.95 a	331ab	41.34 a	769 ab	39.34 ab	892 ab
T3	16.9 b	564 b	10.5 ab	838 b	9.5 b	1336 b
T4	66.55 a	487 b	59.9 a	1120 b	56.7 ab	1239 ab
T5	83.3 a	n/n a	80.4 a	28 a	77.02 a	38 a
T6	88.85 a	n/n a	80.5 a	43 a	74.16 a	45 a

T1= control without inoculation; T2= AMF in substrate; T3= AMF half recommended in irrigation; T4= AMF recommended in irrigation; T5= AMF 10 times the recommended dose in irrigation; T6= AMF recommended in irrigati on + in substrate; n/n= no nematodes; n/m= no mycorrhization; IP3R= individuals per 300 cc/root. Different letters in the same column indicate significant differences, Tukey= 0.05

These results suggest that increased mycorrhization reduces nematode colonization, possibly by improving plant defense (Molinari *et al*., 2022). This coincides with reports that mycorrhizal fungi induce resistance mechanisms in the roots. In addition, the presence of nematodes negatively affected nodule formation (Hernández-Santiago *et al*., 2024), suggesting competition for space and resources between symbiotic microorganisms and parasites in the rhizosphere.

Conclusions

Inoculation with AMF consortia, especially at a dose of 15 g L^{-1} (14 450 spores L^{-1}), significantly improved the greenhouse crop yield of Saladette tomatoes. This dose had a direct effect on the average fruit weight, reaching 84.49 g, as well as on the total production per plant, with an estimated yield of 987 kg m^{-2}.

In addition, there was a 97% reduction in the presence of the nematode *Meloidogyne incognita* in the roots, an effect that persisted until 230 days after transplantation. In contrast, vegetative variables such as plant height, stem thickness, leaf length and width, number and distance between clusters, number of fruits per cluster and fruit diameter did not show statistically significant differences under the conditions of this experiment. This suggests that the main impact of AMF inoculation is manifested in productive and health variables, rather than in visible morphological characteristics.

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