Avances
ISSN 1562-3297
SCIENTIFIC ARTICLE Vol.26 No 2, April-June, 2024

Vegetative growth of *Solanum lycopersicum* cv. L-43 with inoculation of arbuscular mycorrhizal fungi in an Arenosol soil

Crecimiento vegetativo de Solanum lycopersicum cv. L-43 con inoculación de hongos micorrízicos arbusculares en un suelo Arenosol

Crescimento vegetativo de Solanum lycopersicum cv. L-43 com inoculação de fungos micorrízicos arbusculares num solo Arenosol

Yoerlandy Santana-Baños
Agricultural Engineer, Master's in Agroecology and Sustainable Agriculture, Auxiliary Professor, University of Pinar del Río "Hermanos Saíz Montes de Oca". Faculty of Forestry and Agricultural Sciences, Department of Agricultural Sciences, Pinar del Río, Cuba, telephone: 48755452, :yoerlandy@upr.edu.cu; : https://orcid.org/0000-0003-3793-7828

Michel Ruiz Sánchez
Agricultural Engineer, Doctor in Agricultural Sciences, National Institute of Agricultural Sciences, Scientific and Technological Base Unit Los Palacios, Mayabeque, Cuba, :mich@inca.edu.cu; : https://orcid.org/0000-0002-7406-4715

Edenys Miranda Izquierdo
Agricultural Engineer, Master's in Agroecology and Sustainable Agriculture, Auxiliary Professor, University of Pinar del Río "Hermanos Saíz Montes de Oca". Faculty of Forestry and Agricultural Sciences, Department of Agricultural Sciences, Pinar del Río, Cuba, telephone: 48779662, :emiranda@upr.edu.cu; : https://orcid.org/0000-0002-5728-4601

Yosvel Sanjudo Ramos
Agricultural Engineer, Instructor Professor, University of Pinar del Río "Hermanos Saíz Montes de Oca". Faculty of Forestry and Agricultural Sciences, Department of Agricultural Sciences, Pinar del Río, Cuba, : yosvel.ramos@upr.edu.cu; :https://orcid.org/0009-0001-7668-6526

To reference this article / Para citar este artículo / Para citar este artigo

Santana-Baños, Y., Ruiz Sánchez, M., Miranda Izquierdo, E., & Sanjudo Ramos, Y. (2024). Vegetative growth of *Solanum lycopersicum* cv. L-43 with inoculation of arbuscular mycorrhizal fungi in an Arenosol soil. *Avances, 26*(2), 177-187, http://avances.pinar.cu/index.php/publicaciones/article/view/822

Received: September 12, 2023

Accepted: February 15, 2024

ABSTRACT
The use of mycorrhizal biofertilizers is promoted as an efficient and environmentally safe alternative in sustainable agricultural production. This research aimed to evaluate the response of *Solanum lycopersicum* L. cv. L-43 (tomato) to the inoculation of three strains of arbuscular mycorrhizal fungi (INCAM-2,

INCAM-4 and INCAM-11) in Arenosol soil from Pinar del Río, Cuba. The experiment was established on a completely randomized design with four treatments, including a control (without inoculation), and five replicates. Growth variables such as stem length, number of leaves, leaf length, leaf area and biomass were analyzed. Additionally, mycorrhizal colonization in the root system was also determined. The growth dynamics of tomato plants was similar in the treatments evaluated, although 35 days after transplanting, a slight increase in stem length (11.3 %), leaf area (17.7 %) and the values of fresh (16.5 %) and dry (13.9 %) biomass was achieved with the INCAM-4 strain. Mycorrhizal colonization was more effective when the INCAN-4 and INCAM-11 strains were inoculated, showinga differentiated effect on the biomass production of the plants.These results suggest the INCAM-4 strain as an alternative for biofertilization of tomato in Arenosol soil.
Keywords: Biofertilizer; biomass; colonization; tomato.

_____

RESUMEN

El uso de biofertilizantes micorrízicos se fomenta como una alternativa eficiente y ambientalmente segura en la producción agrícola sostenible. Esta investigación tuvo objetivo evaluar la respuesta de *Solanum lycopersicum* L. cv. L-43 (tomate) a la inoculación de tres cepas de hongos micorrízicos arbusculares (INCAM-2, INCAM-4 e INCAM-11) en suelo Arenosol de Pinar del Río, Cuba. El experimento se estableció sobre un diseño completamente al azar con cuatro tratamientos, incluyendo un control (sin inoculación), y cinco réplicas. Se analizaron las variables de crecimiento longitud del tallo, número de hojas, longitud de la hoja, área foliar y la biomasa. También se determinó la colonización micorrízica en el sistema radical. La dinámica de crecimiento de las plantas de tomate fue similar en los tratamientos evaluados, aunque a los 35 días después del trasplante, se alcanzó un ligero incremento en la longitud del tallo (11,3 %), el área foliar (17,7 %) y los valores de biomasa fresca (16,5 %) y seca (13,9 %) con la cepa INCAM-4. La colonización micorrízica fue más efectiva cuando se inocularon las cepas INCAN-4 e INCAM-11, mostrando un efecto diferenciado en la producción de biomasa de las plantas. Estos resultados sugieren que la cepa INCAM-4 podría ser una alternativa para la biofertilización del tomate en suelo Arenosol.
Palabras clave: Biofertilizante; biomasa; colonización; tomate.

_____

RESUMO

O uso de biofertilizantes micorrízicos é promovido como uma alternativa eficiente e ambientalmente segura na produção agrícola sustentável. Esta pesquisa teve como objetivo avaliar a resposta de *Solanum lycopersicum* L. cv. L-43 (tomate) à inoculação de três cepas de fungos micorrízicos arbusculares (INCAM-2, INCAM-4 e INCAM-11) em solo Arenosol de Pinar del Río, Cuba. O experimento foi instalado em delineamento inteiramente casualizado com quatro tratamentos, incluindo uma testemunha (sem inoculação), e cinco repetições. Foram analisadas as variáveis de crescimento comprimento do caule, número de folhas, comprimento foliar, área foliar e biomassa. Também foi determinada a colonização micorrízica no sistema radicular. A dinâmica de crescimento das plantas de tomate foi semelhante nos tratamentos avaliados, embora aos 35 dias após o transplante, tenha sido alcançado um ligeiro aumento no comprimento do caule (11,3 %), área foliar (17,7 %) e nos valores de fresco (16,5%) e biomassa seca (13,9 %) com a cepa INCAM-4. A colonização micorrízica foi mais eficaz quando as cepas INCAN-4 e INCAM-11 foram inoculadas, mostrando um efeito diferenciado na produção de biomassa das plantas.Estes resultados sugerem que a cepa INCAM-4 poderia ser uma alternativa para a biofertilização do tomate em solo Arenosol.
Palavras-chave: Biofertilizante; biomassa; colonização; tomate.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) constitutes a horticultural product cultivated in almost all latitudes (Colman et al., 2019). It is consumed in large quantities due to its commercial value and nutritional properties (Bayomi et al., 2020).

According to the most recent statistics available, world tomato production reaches186.1 million tons (FAO, 2022). In Cuba, it is a crop of great social importance, with production exceeding 317 thousand tons per year. However, the average agricultural yield is only 11 t/ha (ONEI, 2022). This value does not correspond to the potential of the commercial cultivars currently being used.

Chemical fertilizers are used to increase the production of these vegetables. However, there is a current priority to apply products that are environmentally compatible and have a positive influence on crop development. For this reason, the use of biostimulants in agriculture is the focus of attention of the international scientific community (Jerez-Mompie et al., 2023; Cabrera et al., 2017).

Arbuscular mycorrhizal fungi (AMF) represent a promising solution for sustainable agriculture, allowing the reduction of dependence on agrochemicals (Chafai et al., 2023; Reyes-Pérez et al., 2020). They also favor plant development, improve their nutritional status and protect them from biotic and abiotic stresses (Castañeda et al., 2020).

The effect of AMF on plant growth is associated with physical, biochemical, and physiological changes in the roots that contribute to an overall improvement in plant health. Due to their numerous benefits, their utilization in Cuban agriculture is steadily increasing (Pérez-Ortega et al., 2022).

Given this context, it is necessary to conduct studies on the impact of AMF on tomato cultivars used in both industry production and fresh consumption. Such investigations facilitate the selection of more efficient strains based on their interaction with soil and plant. Therefore, this research aims to evaluate the response of *S. lycopersicum* cv. L-43 to the inoculation of three AMF strains in an Arenosol soil.

MATERIALS AND METHODS

Plant material and experimental conditions:

The research was carried under experimental conditions at the "Universidad de Pinar del Río "Hermanos Saíz Montes de Oca", Cuba (22° 24' 48.4'' N and 83° 41' 16.2'' W).Certified tomato seeds (*Solanum lycopersicum* L. cv. L-43) were used. The seedlings were obtained in trays and transplanted manually in pots with a capacity of 2.0 kg. The substrate consisted of soil (70 %) Arenosol (Hernández et al., 2015) mixed with organic matter (20 %) and rice husk (10 %). The soil had a pH (H_2O) of 7.9, organic matter content of 3.0 %, phosphorus (P_2O_5) of 168 cmol/ kg, calcium (Ca) content of 5.5 ppm, and magnesium (Mg) content of 2.5 ppm. Substrate sterilization was conducted in a vertical autoclave (LDZX-50KAS) at 127°C and 1.2 atm pressure for 30 min, five days prior to transplanting. Climatic conditions were characterized by temperatures ranging from 24.2 to 31.9°C, an average relative humidity of 73 %, and a photoperiod of 14 hours light and 10 hours dark.

Experimental design and treatments:

A completely randomized experimental design with four treatments and five replicates was utilized. The treatments comprised the AMF strains *Funneliformis mosseae* (T. H. Nicolson & Gerd.) C. Walker & A. Schüßler (INCAM-2), *Glomus cubense* (Y. Rodr. & Dalpé) (INCAM-4) and *Rhizophagus irregularis* (Blaszk, Wubet, Renker & Buscot) Walker & Shüβler (INCAM-11), sourced from the Mycorrhizal Laboratory collection at the National Institute of Agricultural Sciences (INCA) in Mayabeque, Cuba, along with a control group without AMF inoculation. Inoculation of the three AMF strains was conducted out at a rate of 150 spores per plant at the time of transplanting.

Evaluated growth variables:

Variables characterizing plant growth such as stem length (cm), number of leaves (u), leaf length (cm), leaf area (cm^2) and total fresh and dry biomass (g) were measured. Stem length was determined using a graduated ruler, measured from the base to the terminal bud. This variable was monitored every five days after transplanting until 35 days, at which point the plants were removed for further growth and biomass evaluations. Samples were weighed on an AdventurerTM Pro (OHAUS®) technical balance with a precision of 0.01 g. To obtain dry biomass, samples were dried in a Boxun Drying Oven (model BGZ-146) at 70°C for 48 hours.

Determination of mycorrhizal colonization:

Root samples from five plants per treatment were prepared. These were dried in the oven at 70°C for 48 hours and stained with 5 % ink in 2 % acetic acid (Rodriguez-Yon et al., 2015). Colonization frequency was determined using the intercept method (Giovannetti & Mosse, 1980).

Statistical analysis of the results:

Descriptive statistics, simple analysis of variance (ANOVA), and Duncan's Multiple Ranks test were used for the comparison of means, with a confidence level of 95 % ($p \leq 0.05$). The statistical software Minitab® 17.1.0 for Windows (Minitab, 2015) was used.

RESULTS AND DISCUSSION

The stem growth dynamics in L-43 tomato plants were similar across all treatments during the first 30 days after transplanting, with a higher growth rate observed between days 10 and 15. By days 35, a differentiation in stem length was observed in the INCAM-4 strain, with an average value of 50.1 cm (Figure 1). Therefore, in tomato plants established in an Arenosol soil, the significant biostimulant effect of AMF strains could be appreciated from 30 days after inoculation.

However, AMF inoculation has been shown to improve plant growth and increase the quality of nutrient uptake (Schubert et al., 2020). Other advantages of AMF interaction include increased resistance against pathogens and improved tolerance to abiotic stresses (Miransari, 2017).

Recent studies with AMF strains indicated significant increases in the height of tomato plants when inoculated with *G. cubense* (Vuelta-Lozano et al., 2020; Gómez-Salazar et al., 2022), which could explain the observed differentiation 35 days after transplanting with strain INCAM-4 (*G. cubense*). Other authors propose that the application of mycorrhizal inoculants leads to significant differences in tomato growth variables compared to the control (Cabrera et al., 2016; Charles and Martín, 2015).

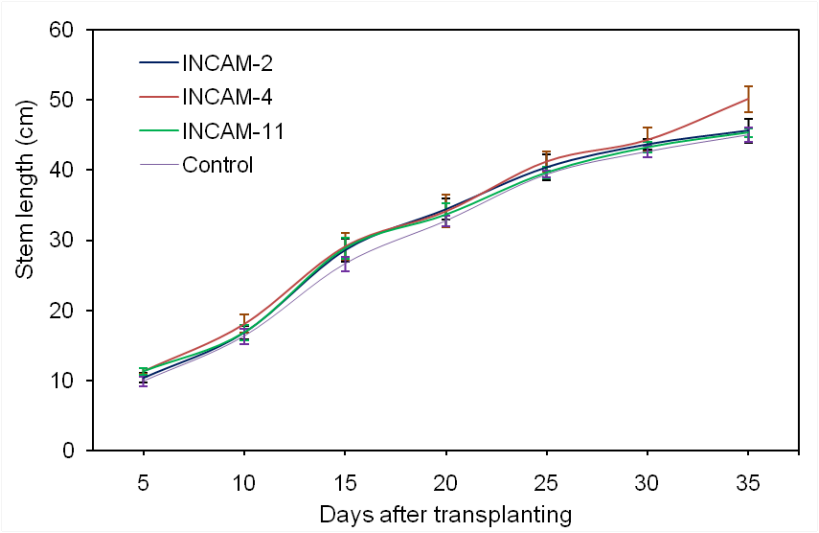

Figure 1. Effect of AMF strains on the growth dynamics of tomato plants. **Legend**: Bars above the lines indicate standard error of the mean (n=5, p≤0.05). **Source:** Own elaboration.

Leaf area and leaf length values did not show significant differences between treatments. However, leaf area in plants inoculated with INCAM-4 increased by 17.7 % compared to the control group (Table 1). This could justify one of the most well-known effects of AMF on the plants they colonize, which is related to an increase in the absorption and translocation of essential elements to the aerial part of the plants, thus favoring their vegetative development (Jerez-Mompie et al., 2023; Vuelta-Lozano et al., 2020).

Table 1. Morphological characteristics of tomato plants inoculated with mycorrhizae. **Legend:** ns - not significant, * - significant (p≤0.05). **Source:** Own elaboration.

Treatments	Leaf area (cm²)	Leaf length (cm)	Total fresh mass (g)	Total dry mass (g)
INCAM-2	1026,00	31,48	53,68 ab	6,90 ab
INCAM-4	1292,42	30,40	65,55 a	8,38 a
INCAM-11	1035,12	29,72	51,79 b	6,77 b
Control	1097,90	30,75	56,28 ab	7,36 ab
Standard Error (±)	65,61ns	0,92ns	2,30*	0,31*

In terms of biological productivity, a significant and differentiated increase was observed in the total fresh and dry mass of plants inoculated with the INCAM-4 strain compared to those inoculated with INCAM-11 (Table 1). These biomass values increased by more than 12 % in plants inoculated with the INCAM-4 strain

compared to the control group. Rivera et al. (2015) noted that EcoMic biofertilizer, which is based on AMF, leads to notable increases in plant productivity. Similarly, Ley-Rivas et al. (2015) and Cabrera et al. (2016) reported significant increases in aerial and root dry biomass, respectively.

The three inoculated AMF strains demonstrated symbiotic capacity with the roots of the tomato cultivar L-43, although colonization percentages higher than 40 % were achieved in INCAM-4 and INCAM-11, significantly differing from INCAM-2 and the control (Figure 2). Recent results in tomato indicate more than 50 % mycorrhization (Tamayo-Aguilar et al., 2021), but values often vary depending on the soil or substrate conditions where the plants are established.

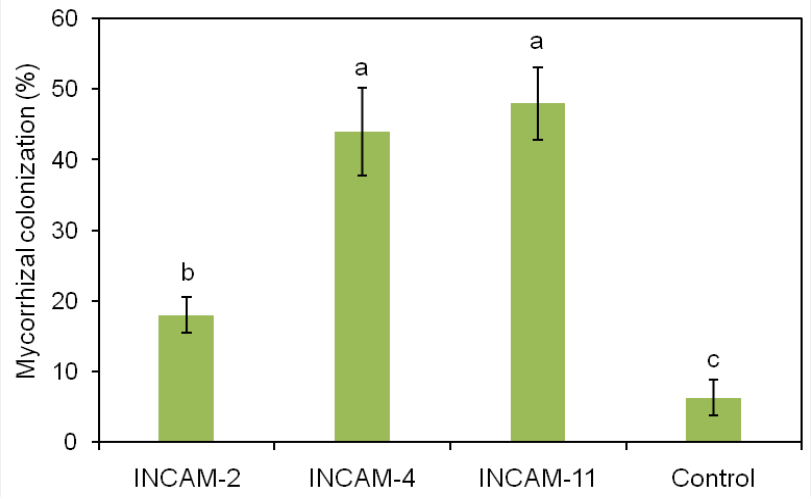

Figure 2. Percentage of colonization of mycorrhizal strains in the root system of tomato plants. **Legend**: Bars above columns indicate ± standard error of the mean (n=5, p≤0.05).
Source: Own elaboration.

Therefore, a determining factor in the effectiveness of mycorrhization is the edaphic environment, fundamentally the pH. However, the pH value (7.9) in the soil used could favor colonization, since the AMF strains *G. cubense* (INCAM-4) and *R. irregularis* (INCAM-11) performed well in soil with pH(H_2O)=7.2 (Pérez-Ortega et al., 2022).

Beyond the advantages of mycorrhization in tomato cultivation, these findings serve as a benchmark for local production conditions, aimed at fostering a more productive and sustainable agricultural system. The utilization of AMF demonstrates significant potential as an effective strategy for tomato production. This method not only enhances crop growth and nutritional quality but also diminishes reliance on chemical fertilizers (Chafai et al., 2023).

CONCLUSIONS

The growth dynamics of L-43 tomato plants is similar in the evaluated treatments, although 35 days after transplanting, a slight increase in stem length (11.3 %), leaf area (17.7 %) and fresh (16.5 %) and dry (13.9 %) biomass values is achieved with INCAM-4 strain. Mycorrhizal colonization is more effective when the INCAN-4 and INCAM-11 strains are inoculated, with a differentiated effect on the biomass production plants. These results suggest the INCAM-4 strain as an alternative for biofertilization of tomato in Arenosol soil.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Ministry of Science, Technology, and Environment (CITMA, Cuba) for funding the project "Strengthening capacities for the generalization of the EcoMic® biofertilizer and its interaction with other bioproducts in Pinar del Río province" (Sector Program: Agricultural Bioinputs and Veterinary Medicines, LABIOFAM). Additionally, they extend their gratitude to Engineer Santiago Enmanuel Reyes, Engineer Armando Acosta Hernández, and PhD Eduardo Pérez Ortega for their collaboration.

BIBLIOGRAPHICAL REFERENCES

Bayomi, K., Abdel-Baset, A., Nassar, S. M. A., & Al-kady, A. (2020). Performance of some tomato genotypes under greenhouse conditions. *Egyptian Journal of Desert Research*, *70*(1), 1-10, http://dx.doi.org/10.21608/ejdr.2019.16947.1041

Cabrera, Y.L.; Miranda, E.; Santana, Y. (2016). Efectividad y momentos de aplicación del biofertilizante EcoMic® en la producción de *Solanum lycopersicum* L. var. Mamonal 21. *Avances*, *18*(1), 77-85, https://www.redalyc.org/articulo.oa?id=637867036004

Cabrera Romero, Y. L., Santana Baños, Y., & Miranda Izquierdo, E. (2017). Efecto de la inoculación de Rhizobium sobre el crecimiento de *Phaseolus vulgaris* (frijol) en condiciones semicontroladas. *Avances,* *19*(1), 66-74, https://www.redalyc.org/articulo.oa?id=637867024001

Castañeda, W., Toro, M., Solorzano, A. & Zúñiga-Dávila, D. (2020). Production

and Nutritional Quality of Tomatoes (*Solanum lycopersicum* var. Cerasiforme) Are Improved in the Presence of Biochar and Inoculation with Arbuscular Mycorrhizae. *American Journal of Plant Sciences*, *11*, 426-436, http://dx.doi.org/10.4236/ajps.2020.113031

Chafai, W., Haddioui, K., Serghini-Caid, H., Labazi, H., AlZain, M. N., Noman, O., Parvez, M. K., Addi, M. & Khalid, A. (2023). Impact of Arbuscular mycorrhizal Fungal Strains Isolated from Soil on the Growth, Yield, and Fruit Quality of Tomato Plants under Different Fertilization Regimens. *Horticulturae, 2023*(9), 973, http://dx.doi.org/10.3390/horticulturae9090973

Charles, N. J., & Martín, N. J. (2015). Uso y manejo de hongos micorrízicos arbusculares (HMA) y humus de lombriz en tomate (*Solanum lycopersicum* L.), bajo sistema protegido. *Cultivos Tropicales*, *36*(1), 53-62, http://scielo.sld.cu/pdf/ctr/v36n1/ctr07115.pdf

Colman, S. L., Salcedo, M. F., Mansilla, A. Y., Iglesias, M. J., Fiol, D. F., Martín-Saldaña, S., Alvarez, V. A., Chevalier, A. A., & Casalongué, C. A. (2019). Chitosan microparticles improve tomato seedling biomass and modulate hormonal, redox and defense pathways. *Plant Physiology and Biochemistry*, *143*, 203-211, http://dx.doi.org/10.1016/j.plaphy.2019.09.002

FAO. (2022). FAOSTAT. Roma, Italia, Recuperado de https://www.fao.org/statistics/es/

Giovannetti, M., & Mosse, B. (1980). An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *The New Phytologist*, *84*(3), 489-500, https://www.jstor.org/stable/2432123

Gómez-Salazar, A., López-Salvador, G., Jerez-Mompie, E., González-Cañizares, P., & Guerrero-Domínguez, L. (2022). Influencia de dos bioestimulantes en el crecimiento y desarrollo de plantas de jitomate (*Solanum lycopersicum* L.). *Cultivos Tropicales*, *43*(2), e07-e07, https://ediciones.inca.edu.cu/index.php/ediciones/article/view/1658/pdf

Hernández, A., Pérez, J., Bosch, D., & Castro, N. (2015). *Clasificación de los suelos de Cuba*. Mayabeque, Cuba: Ediciones INCA. 93 p.

Jerez-Mompie, E. I., Gómez-Salazar, A., López-Salvador, G. & González-Cañizares, P. J. (2023). Efecto de dos bioestimulantes en algunas variables del fruto de tomate (*Solanum lycopersicum* L.) cultivar Pony Express. *Cultivos Tropicales*, *44*(2), e04, https://ediciones.inca.edu.cu/index.php/ediciones/article/view/1725/3512

Kanwar, M. K., Bajguz, A., Zhou, J., & Bhardwaj, R. (2017). Analysis of brassinosteroids in plants. *Journal of Plant Growth Regulation*, *36*, 1002-1030, https://doi.org/10.1007/s00344-017-9732-4

Ley-Rivas, J. F., Sánchez, J. A., Ricardo, N. E., & Collazo, E. (2015). Efecto de cuatro especies de hongos micorrizógenos arbusculares en la producción de frutos de tomate. *Agronomía Costarricense*, *39*(1), 47-59, https://www.scielo.sa.cr/pdf/ac/v39n1/a04v39n1.pdf

Minitab. (2015). *Minitab 17*: gretting started with Minitab 17. Pennsylvania: Minitab Inc.

Miransari, M. (2017). *Arbuscular mycorrhizal fungi and heavy metal tolerance in plants*. En Q. S. Wu (ed.), Arbuscular mycorrhizas and stress tolerance of plants (pp. 147-161). Springer, https://doi.org/10.1016/j.jhazmat.2020.123919

ONEI (2022). *Anuario Estadístico de Cuba 2021*. Capítulo 9: Agricultura, ganadería, silvicultura y pesca. Edición 2022. República de Cuba. http://www.onei.cu

Pérez-Ortega, E., Rivera-Espinosa, R. A., Saggin-Junior, O. J., & Fernández-Suárez, K. (2022). Efecto del pH en la bioprotección ejercida por algunas cepas de hongos micorrízicos arbusculares. *Cultivos Tropicales*, *43*(3), https://cu-id.com/2050/v43n3e06

Reyes-Pérez, J. J., Enríquez-Acosta, E. A., Ramírez-Arrebato, M. Á., Rodríguez-Pedroso, A. T., & Falcón-Rodríguez, A. (2020). Efecto de ácidos húmicos, micorrizas y quitosano en indicadores del crecimiento de dos cultivares de tomate (*Solanum lycopersicum* L.). *Terra Latinoamericana*, *38*(3), 653-666, https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-57792020000400653

Rivera, R., González, P.J., Hernández-Jiménez, A., Martín, G., Ruíz, L., & Fernández, K. (2015). La importancia del ambiente edáfico y del pH sobre la efectividad y la recomendación de cepas eficientes de HMA para la inoculación de los cultivos. *VIII Congreso de la Sociedad Cubana de Ciencia del Suelo*, del 2 al 5 de junio. La Habana, Cuba.

Rodríguez-Yon, Y., Árias Pérez, L. A., Medina Carmona, A., Mujica Pérez, Y., Medina García, L. R., Fernández Suárez, K., & Mena Echevarría, A. (2015). Alternative stainning technique to determine mycorrhizal colonization. *Cultivos Tropicales*, *36*(2), 18-21, https://ediciones.inca.edu.cu/index.php/ediciones/article/view/962/pdf_1

Schubert, R., Werner, S., Cirka, H., Rödel, P., Tandron, Y., Mock, H., Hutter, I., Kunze, G., & Hause, B. (2020). Effects of arbuscular mycorrhization on fruit quality in industrialized tomato production. *International Journal of Molecular Sciences*, *21*(19), 7029, http://dx.doi.org/10.3390/ijms21197029

Tamayo-Aguilar, Y., Martín-Alonso, G., Herrera-Altuve, J. A., Cesar-Gainza, A., Abad-Michael, M., Nápoles-García, M. C., ... & Juárez-López, P. (2021). Biofertilizantes en la sucesión *Canavalia ensiformis* - *Solanum lycopersicum*: Rendimiento y calidad en frutos de tomate. *Revista fitotecnia mexicana*, *44*(3), 341-348, http://dx.doi.org/10.35196/rfm.2021.3.341

Vuelta-Lorenzo, D. R., Mas-Diego, S. M., Montero-Limonta, G., & Rizo-Mustelier, M. (2020). Efecto de ocho especies de hongos micorrízicos arbusculares sobre el manejo de nematodos en el cultivo del tomate (*Solanum lycopersicum* L.) en condiciones protegidas. *Ciencia en su PC*, *1*(3), 108-124, https://www.redalyc.org/articulo.oa?id=181365138007

AUTHOR CONTRIBUTIONS

SBY, **RSM**, **MIE**, and **SRY** contributed to the research design, trial supervision, and data processing. All authors contributed to the drafting of the article submitted to the journal for publication.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this article.