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Research Article

Promotion of tomato growth by *Trichoderma* sp. under shade mesh conditions

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Abstract

Chemical fertilizers are frequently used in agriculture with harmful effects on ecological components, so the use of microorganisms as growth regulators is an agricultural practice increasingly used today. The aim of this research was to evaluate *Trichoderma* sp. as growth regulator in tomato plants. *Trichoderma* sp. isolated from soils was grown on solid PDA medium for morphological characterization of the fungus. An experiment to analyse the interaction between *Trichoderma* sp. and shade mesh conditions was established, where: T1 = *Trichoderma* sp.; T2 = without *Trichoderma* sp. (fertilization recommended for the crop was applied); T3 = shade mesh and T4 = without shade mesh. Several variables were evaluated in the plants and in the fruits. The macroscopic characteristics showed mycelium with a cottony morphology and a dark green coloration, and the microscopic characteristics of the fungus were conidiophores with a branch, phialides and ovoid to ellipsoid conidia. Interaction of *Trichoderma* sp. and shade mesh had a significant effect on plant height, number of flowers and number of fruits, with the greater values with *Trichoderma* sp. and shade mesh. Regarding the evaluation of the fruits significant differences were found in the weight, diameter, length, and colour (L and a* value) but not in b* value.

Keywords: chemical fertilizers; microorganism; morphological characterization; plant growth promotion; shade mesh

Introduction

Tomato is one of the most consumed vegetables worldwide, it belongs to the Solanaceae family (Lopes-Sobrinho *et al*., 2022); it is important for human health because the contents of micronutrients, potassium, beta-carotene, calcium, lycopene, folate, flavonoids, and ascorbic acid (Freeman and Reimers, 2011).

In Mexico for the autumn-winter agricultural cycle of 2022, 22,755 hectares (ha) of tomato were planted with a production of 862 thousand 557 tons (ton), being Sinaloa the main producer state, with a production

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volume of 536,829 tons, concentrating 62.2% of the national production, followed in importance for Baja California Sur with 51,988 tons, contributing 6.0%, Sonora 43,370 tons (5.0%) and Michoacán 36,855 tons that contributes 4.3%, these four states harvest more than half of the country's production with 77.5% (SIAP, 2022).

The nutrients that tomato plants need depend, mainly, of plant development stage, biotic and abiotic factors, such as crops systems, agricultural practices, water availability, and nutrient rates and sources (Fontes and Fontes, 1991; Silva *et al*., 2001). Nutrients are applied through chemical fertilization because plants growth fast and the yield can be higher, nevertheless the excessive use of these products affect not only the physical and chemical properties of soil, but also the water, mostly the subterranean (Singh *et al*., 2001).

The use of organic fertilizers by farmers is increasing and the balance between both types of fertilizers is necessary for the growth of plants with good quality and high yields. Plant growth promoting microbes are another alternative to reduce chemicals and this practice is eco-friendly and low cost (Bender *et al*., 2016; Toju *et al*., 2018).

The fungus *Trichoderma* sp. is found in different environmental conditions throughout the world. The genus includes more than 100 known species. This fungus is mainly used as a mycoparasite and antagonist, and some species have beneficial effects on seed germination, seedling emergence, grain growth and yield (Chagas *et al*., 2016), and stimulation of plant defences to achieve increased tolerance to disease and abiotic stress.

Trichoderma spp. as plant growth regulator can synthetize phytohormones (Jaroszuk-Ściseł *et al*., 2019; Cai *et al*., 2016) being the formation of these compounds essential for plant development. The fungus is frequently associated with plant roots and the genus is characterized by opportunistic, symbiotic, and avirulent microorganisms that can colonize the roots and stimulate plant growth including enhancement of root condition and structure, improvement of seed viability and germination, as well as increased flowering, photosynthesis efficiency, and yield quality (Halifu *et al*., 2019).

According to Hoyos-Carvajal *et al*. (2009) the application of *Trichoderma* strains to the soil increases the productivity and quality of several monocotyledon and dicotyledon crops such as tomatoes, cucumbers, beans, carrots, cotton, and corn. An example of *Trichoderma* as plant growth regulator is that the inoculation of tomato seedlings with *T. harzianum* under drought stress and salinity conditions caused the maintenance of photosynthetic efficiency (Azad and Kaminskyj, 2016) and the same species inoculated in tomato plants reduced the effect of cold stress with an increment in fresh and dry weight of roots and shoots (Ghorbanpour *et al*., 2018).

The enhancement of plant growth because of the action of plant growth regulators induced by *Trichoderma* species has been also reported. Bader *et al*. (2020) reported the induction of Indole 3 acetic acid (IAA) produced by the action of *Trichoderma* species. *T. koningiopsis* was applied to tomato plants and the growth was significantly enhanced may be because gibberellins action (You *et al*., 2016).

The aim of this research was to evaluate *Trichoderma* sp. as growth regulator in tomato plants cv. 'Rio Grande' grown under greenhouse and open field conditions, compared to plants treated with chemical fertilizers.

Materials and Methods

Location of the research

This research was carried out at the facilities of the Faculty of Agricultural and Forestry Sciences (FCAyF) of the Autonomous University of Chihuahua (UACH), Chihuahua, Mexico.

Isolation and purification of the fungus

The isolate used for the preparation of the bioproduct was collected from an area of 1 ha at the FCAyF. The sample (≈ 500 g) was taken at a depth of 20 cm and close to the roots and stored in plastic bags under refrigeration at approximately 18 °C until processing (Sadeghian, 2018).

Sterile distilled water (1 g in 100 mL^{-1}) was added to each sample, of which 1 mL was diluted to 10^{-3}, then 100 µL were placed in Petri dishes with PDA dextrose agar medium (Potato Dextrose Agar medium). After spreading the suspension, the plates were incubated at 26 °C for 7 days until colonies of *Trichoderma* sp. appeared. Subsequently, colonies were purified and transferred to Petri dishes with PDA medium (Maniscalco and Dorta, 2015).

Morphological characterization of Trichoderma sp.

Macroscopic identification

The morphological characterization was done after obtaining a pure isolate of *Trichoderma* sp. following the method of Kubicek and Harman (1998), considering conidia development and pigmentation, and mycelium texture.

Microscopic identification

The microscopic identification was implemented through the observation of structures in culture, such as phialides hyphae, shape of conidia and number of conidiophores. Once the fungal structures were visualized, microcultures were done on slides with agar water at 2%, and they were incubated at 25 °C. Subsequently at 48–72 h, micro-cultures were observed with an optical microscope (VELAB x100 Scale bars: 10 µm). The *Trichoderma* sp. isolate was characterized using the codes and descriptions of Samuels and Hebbar (2015) for fungi genera and species.

Trichoderma sp. production

Different cereal grains can be used, in this work 300 g of rice per bag were used (Sivila and Álvarez, 2013). Rice was precooked between 5-7 min in water at 80 °C (Sandoval and Noelting, 2011), placed in trays for drying at room temperature and packed in polyethylene bags without exceeding a third of their capacity (Troya and Vaca-Granda, 2014), closing with a paper tape (Sivila and Álvarez, 2013) and sterilizing in an autoclave at 121 °C for 10 minutes. The bags were inoculated with the pure culture of the fungus, at a rate of 1 cm^2 of the culture medium colonized by *Trichoderma* sp.

The inoculated bags were placed in an oven at 26 °C and 12 hours of photoperiod for 8-9 days, every two days they were gently shaken to prevent the formation of lumps and facilitate the colonization of the substrate. After 9 days, the bags where the substrate was totally colonized by *Trichoderma* sp. were chosen, the colonization was visualized through the development of green coloration on the rice grains. From this moment on, the bioproduct was ready for its application (Sivila and Álvarez, 2013).

Experimental design and treatments to evaluate Trichoderma sp. as plant growth regulator

Tomato seeds (*Solanum licopersicum* L.) cv. 'Rio Grande' were sown in loamy-clay soil with shade mesh (greenhouse) and clay soil without shade mesh (open field). The application of the fungus to tomato plants started after the first pairs of leaves appeared, at 50 mL by plant every 15 days. The control in each culture condition were tomato plants growing without the fungus.

Chemical fertilizers were applied to the controls as follows: FLUID K (00-34-36) liquid (P_2O_5 at 33.89%; K_2O at 36.02%; MgO at 11.06 ppm; Fe at 76.23 ppm; Mn at 0.5 ppm; Zn at 9.97 ppm; Cu at 0.1 ppm and Na at 0.24%) and MAX FLUID (P_2O_5 at 430 g L^{-1}; K_2O at 300 g L^{-1}; MgO at 40 g L^{-1} and B at 20 g L^{-1}), at a rate of 200 mL in 20 L^{-1} of water every 15 days; FOSFI-K (foliar fertilizer): 120 mL in 20 L^{-1} of water was

applied every 15 days. The agronomic variables evaluated were: plant height, number of flowers, number of fruits and quality of the fruits (weight, dimension, colour, total soluble solids, titratable acidity and pH).

Fruits weight

An analytical balance was used, and the weight was recorded as grams (g), doing this by triplicate.

Fruits dimension

The dimensions (length, width and thickness) were determined using a Vernier of the Surtek brand, and were expressed in centimetres.

Fruits colour

Values of L, a and b of the CIE L*a*b* colour system were obtained with a Chroma Meter CR-400/410 (Konica Minolta) at two opposite points on the equatorial zone of the fruit.

Total soluble solids

This variable was determined from three drops of juice obtained from fruits cut longitudinally, the juice was placed in the cell of a digital refractometer (ATAGO PR-100, Tokyo, Japan) with a scale ranging from 0 to 32%, expressing the TSS value in percentage at 20 °C.

Titratable acidity

For the acidity test, a sample of 10 g was macerated, and then 40 ml of distilled water were added with three drops of phenolphthalein to finally be titrated with 0.1 N Sodium Hydroxide (NaOH) until a pH of 8.2 was obtained.

pH

For the determination of pH, 10 grams of sample were macerated with 40 ml of distilled water, and the measurement was made with a pH meter (Hanna instruments, Romania).

Statistical analysis

A 2^2 factorial completely randomized design with three replications was used and Duncan multiple range test was performed at 5% level of probability using the statistical program SAS version 9.0.

Results

Morphological characterization

Macroscopic identification of *Trichoderma* sp.

The isolate showed a mycelium with a cottony morphology and a dark green coloration (Figure 1). Another distinctive morphology includes bright green conidial pigments.

Microscopic morphological characterization of *Trichoderma* sp.

Microscopic observation showed conidiophores with a branched main axis ending in one or two 5-8 µm cylindrical phialides and ovoid to ellipsoid conidia 3.5-4.5 x 2.3-3.0 µm (Figure 2).

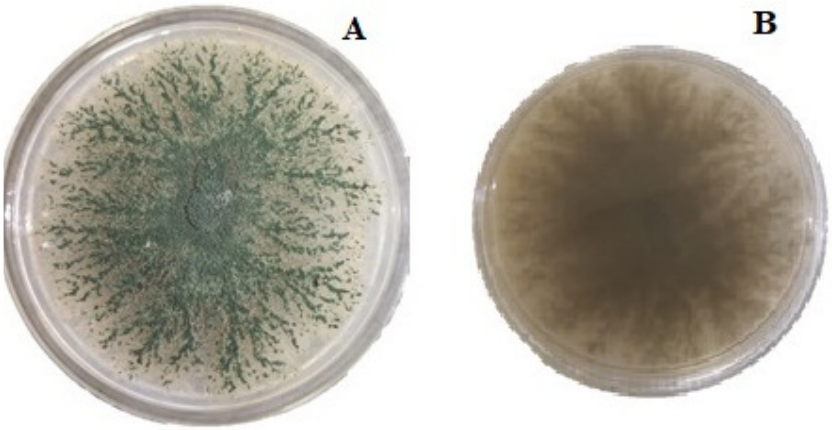

Figure 1. Colony of the native strain of *Trichoderma* sp. in PDA medium where A: Front, B: Back

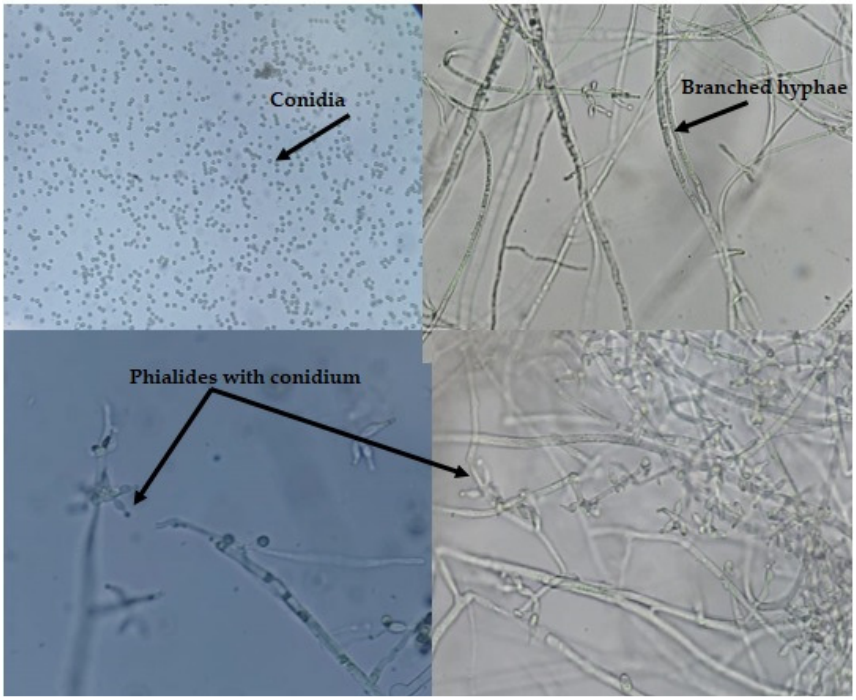

Figure 2. Microscopic structures of *Trichoderma* sp. (x100). Scale bars: 10 µm

Trichoderma sp. as plant growth regulator

The results of the effects of the application or not of *Trichoderma* sp. and the use or not of shade mesh for the agronomic variables (height of the plant, number of flowers and number of fruits) are shown in Table 1.

Table 1. Effect of treatments on agronomic variables

Treatments	Height of the plant (cm)	Number of flowers	Number of fruits
T*SM	67.6a	10.4a	5.7a
WT*SM	60.5b	10.4a	3.8b
T*WSM	57b	8.3a	2.2bc
WT*WSM	57b	5.1b	1.9c

T= With *Trichoderma* sp.; WT=Without *Trichoderma* sp.; SM=With Shade mesh; WSM=Without Shade mesh. Means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test at P ≤ 0.05.

The interaction between *Trichoderma* sp. and shade mesh had a significant effect on these variables. For plant height (p-value=0.0271), the higher value was achieved in the combination T*SM, surpassing the others.

Also, a significant interaction between factors was demonstrated for the number of flowers (p-value=0.0952). In this case, a similar statistical level was reached by the combinations including *Trichoderma* sp. or shade mesh (T*SM, WT*SM, and T*WSM) and a lesser value for the combination without fungus and without shade mesh (WT*WSM).

For the number of fruits (p-value=0.0735), the combination of *Trichoderma* sp. and shade mesh was significantly superior to the remaining treatments.

Regarding to the evaluation of the fruits significant differences were found in their weight, diameter, and length (Table 2).

Table 2. Effects of treatments on fruit quality parameters

Treatments	Weight (g)	Diameter (mm)	Length (mm)
T*SM	173.3a	48.5a	64a
WT*SM	91.22b	40b	51.25b
T*WSM	179.95a	50.25a	63.5a
WT*WSM	158.95a	45.75a	61.5a

T= With *Trichoderma* sp.; WT=Without *Trichoderma* sp.; SM=With Shade mesh; WSM=Without Shade mesh. Means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test at P ≤ 0.05.

For weight, diameter and length of fruits, similar results were obtained for the combinations T*SM, T*WSM and WT*WSM; the treatment with shade mesh but without *Trichoderma* sp. (WT*SM) showed the lesser values for these variables. Values of total soluble solids (TTS), titratable acidity (TA), TA/TTS, and pH (not shown) displayed no significant differences between treatments.

The results of the interaction between factors analysed on fruits colour is showed in Table 3.

Table 3. Effect of treatments on colour parameters

Treatments	**L (Lightness)**	**a***	**b***
T*SM	51.35a	16.92c	27.68a
WT*SM	45.21b	24.61b	25.90a
T*WSM	48.13ab	25.05b	28.04a
WT*WSM	46.65b	46.65a	25.29a

T= With *Trichoderma* sp.; WT=Without *Trichoderma* sp.; SM=With Shade mesh; WSM=Without Shade mesh. Means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test at P ≤ 0.05.

The interaction of both factors (p-value=0.0680) had a significant effect on the L value, being the treatments to which *Trichoderma* sp. was added the one that presented the highest values, and significantly different from the other treatments. For the a* value (p-value=0.0010), significant differences were found, with

the highest value for WT*WSM and the lowest T*SM. The interaction of both factors (p-value=0.7607) did not provoke a significant effect on the b* value.

Discussion

In the past few years, *Trichoderma* has acquired great significance because the induction of rapid plant growth and production, an increase in rhizosphere change, nutrient absorption, and tolerance enhancement to both abiotic and biotic stresses (Hermosa *et al*., 2012; López-Bucio *et al*., 2015).

The macroscopic characteristics of the isolate are in agreement with the description of *Trichoderma* genus (Barnett and Hunter, 1972), that include rapid growth in culture medium, and development of conidia with green-yellow colour (García-Núñez *et al*., 2017). Typical growth characteristics observed included yellowish mycelium, white cottony hyphae, yellowish-greenish conidia and the production of a yellowish pigment that change the medium coloration (Gonzalez *et al*., 2020).

In this research the role of *Trichoderma* sp. as plant growth regulator have been proved. Several agronomics variables were evaluated and increases in some of them with the application of the fungus was observed.

Previous studies have shown the role of *Trichoderma* as plant growth regulator mainly because plants give sucrose to the fungi, so the fungi may induce rapid plant development by giving phytohormone to the plants, such as auxin and gibberellins, and also the fungus can promote the solubilization of magnesium, phosphates, manganese and iron in the soil (Lorito *et al*., 2010; Hermosa *et al*., 2012; López-Bucio *et al*., 2015; Sharma *et al*., 2017). Also, according to Halifu *et al*. (2019) and Sajeesh (2015) the application of *Trichoderma* spp. to plant rhizosphere induced the development of several plant morphological traits like plant height, number of leaves, number of flowers, etc, similar to that obtained in this study with the application of *Trichoderma* sp. to tomato plants. For example, Contreras-Cornejo *et al*. (2015) used *T. atroviride* for soil inoculation and the fungus improved root hair numbers as well as lateral roots in *Arabidopsis thaliana* L. In the same way, application of *T. harzianum* to cucumber roots (*Cucumis sativus* L.) increased plant biomass (Yedidia *et al*., 2001). Also, application of *T. longipile* and *T. tomentosum* to the roots of cabbage (*Brassica oleracea* L.) seedlings grown in a greenhouse significantly increased the total leaf area as well as fresh weight (Rabeendran *et al*., 2000).

The ability of *Trichoderma* sp. to increase several agronomic variables in tomato plants in this research may be due to this strain can secrete ammonia and solubilize phosphate (Ikram *et al*., 2019).

Another study with similar results with this research was done in wheat growing in association with *T. reesei* where increases in shoot, root lengths, dried and fresh weight of shoots and roots were observed (Ikram *et al*., 2019). Uddin *et al*. (2015) also found in tomato plants treated with *Trichoderma* increases in plant height, number of flowers and number of fruits. The increment in plant height induced by *Trichoderma* application was also reported by McGovern *et al*. (1992) and Datnoff and Pernezny (1998).

Respect to the shade mesh, this is a temperature control technique that is increasingly widespread in protected horticulture, with which it is sought to reduce the intensity of radiation, to avoid high temperatures during hot periods (Valera et al., 2001). It induces reduction in leaf temperature and leaf transpiration rate, consequently improving tolerance to heat stress (Aberkani *et al*., 2008), so the effect of the shade mesh in the evaluated crop was to reduce transpiration in hot period when it was sown, which explains the greater number of flowers and fruits, which means greater productivity.

The tomato fruits analysis also showed differences in the weight, diameter and colour in this study when *Trichoderma* sp. was used corroborating the role of the fungus in tomato plants growth regulators. Uddin *et al*. (2015) results showed an increment in the weight and diameter of the tomato fruits like in this research. In the

same way Zaghloul *et al*. (2007) reported that application of selected *Trichoderma* increased the number of fruits/plants, weight of fruits and the total yield of tomato fruits.

The application of *Trichoderma* sp. to tomato plants in this study did not affect the TSS, TA, TA/TSS and pH. These results do not agree with other studies in tomato where the application of *Trichoderma* increased or decreased the values of TSS and TA (Palacios-Torres *et al*., 2019; Ruiz-Cisneros *et al*., 2018; Nzanza, 2011).

Lightness (L) indicates the presence of carotenoid pigments that are characteristics of fruits maturation stage. Respect to a* and b* values, the low values of the first are related to less formation of lycopene and the second showed no significant differences between the treatments with positive values, indicating little or no blue colour on the fruit. Ruiz-Cisneros *et al*. (2018) studied the effect of three *Trichoderma* species (*T. asperellum*, *T. harzianum* and *T. longibrachiatum*) on plant growth and tomato fruit quality and as results they found no significant differences between L, a* and b* values.

Conclusions

In this research the *Trichoderma* sp./shade mesh interaction showed a significant effect on plant height, the number of fruits and the number of flowers of the tomato plant. Respect to the quality of the fruits, the combination of the two factors has a significant effect on weight, diameter, and length of the fruits. Two of the colour parameters of the fruits (Lightness and a* value) were also affected by the two factors interaction, and no effect was found on the b* value.

In general, the use of *Trichoderma* sp. improved the variables and fruits parameters evaluated in this research so this fungus could be used as a tomato plant growth promotor.

Authors' Contributions

Conceptualization, C.U.G. and S.P.A.; methodology, C.U.G., S.P.A. and E.F.H. A; validation, S.P.A. and M.A.F.C.; formal analysis, E.F.H.A.; investigation, C.U.G. and S.P.A..; resources, S.P.A. and M.A.F.C.; data curation, S.P.A. and E.F.H.A; writing-original draft preparation, S.P.A. and E.F.H. A; writing-review and editing, C.U.G., S.P.A. and E.F.H.; supervision, S. P. A. and M.A.F.C. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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