Cultivos Tropicales, 2022, vol. 43, no. 2, e07
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

abril-junio
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

http://ediciones.inca.edu.cu

Original article

Influence of two biostimulants on the growth and development of tomato (*Solanum lycopersicum* L.) plants

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ABSTRACT

Biostimulant use in the world is increasing more and more, in this sense, this work was carried out at the Tecomatlan Institute, Puebla, Mexico, to evaluate the influence of the Quitomax® use and the inoculation with two strains of mycorrhizae on the growth and development of tomato plants. Seedlings were produced in a shade house, in trays of 200 cells, filled with a commercial substrate, under same treatments that would later be applied in field conditions, which consisted of a control where seeds were imbibed in water for three hours. The same time was used when mycorrhiza was used, while the two strains of mycorrhizae (Incam4 and Incam11) were applied by coating the seeds, so that six treatments were formed, since both biostimulants were also used in combination. A randomized block design with four replications was used for the distribution of treatments under field conditions. At transplanting, each inoculum was applied to the root system and Quitomax® was applied sprayed to the foliage at seven and 28 days, in the treatments that required it. In the postures, height growth was evaluated every seven days and stem diameter at the transplanting time. Under field conditions, growth and yield variables were evaluated. In the posturing phase, the treatments did not modify stem diameter with respect to the control, and had a greater effect on plant height. Under field conditions, the effect was more marked on yield than on the growth variables evaluated, highlighting the combined action of Quitomax® with the Incam4 strain.

Key words: chitosan, arbuscular mycorrhiza, yield

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a highly appreciated vegetable in Mexico and throughout the world, with a high demand, both for fresh and processed consumption (1). Among the sowing methods, the most used is the transplanting of seedlings, thus, the efficiency of this system depends to a great extent that the seeds germinate and emerge as quickly as possible and the seedlings obtained reach in the shortest possible time the adequate growth parameters for transplanting, mainly in terms of size and mass, in this sense, the use of biostimulants can contribute to this need (2).

Among these biostimulants are mycorrhizae based on Arbuscular Mycorrhizal Fungi (AMF), which together with plants have evolved in an intimate relationship since 460 million years ago (3).

Arbuscular mycorrhizal symbiosis (4) is the obvious result of the interaction between plant roots and a fungus, as well as an excellent example of the extensive morphological alterations that roots undergo in order to accommodate the presence of a symbiont (5). AMF receive photosynthates from the plant, while the plant enhances its ability to take up nutrients and water. Fungi form a link between plants and mineral nutrients in the soil and fulfill various functions in terrestrial ecosystems (6). The application of these symbionts in a liquid formulation is currently being evaluated, which would make their application much simpler (7).

Another biostimulant is Quitomax® (abbreviated in the text as QMax®), a liquid formulation based on chitosan, obtained in the Biochemistry laboratory of the Department of Plant Physiology and Biochemistry of the National Institute of Agricultural Sciences, which has shown a stimulating action on seed germination and growth of different crops, by accelerating plant metabolism (8), which has favored the growth of plants of different species compared to untreated plants (9), in addition to an increase in yields, due to the stimulus they cause in enzymatic systems and other metabolites involved in these processes (10).

Both biostimulants have been used in tomato cultivation, both for seedling production and planting, in different forms and concentrations, as well as other mycorrhizal strains. In this sense, Quitomax® has been used in applications to seeds of two tomato varieties (11), for the production of seedlings, and foliar applications to increase yields (12). Similarly, tomato plants have been inoculated with arbuscular mycorrhizae (13,14) to evaluate the production of seedlings and their growth and development, respectively.

In the literature review, no article was found that reported the application of both biostimulants in combination in the tomato crop, although in other crops (15), as is the case of a work developed in corn (*Zea mays* L.), while both Quitomax® and mycorrhizae have been used in combination, but with other biostimulants (16,17).

According to these results, there is a current tendency to combine different biostimulants in order to enhance their action on plants; on the other hand, it is not a common practice to use those used in this work in the region where the research was carried out, so the objective of this research was to evaluate the effect produced by inoculation with two strains of mycorrhizae and Quitomax® applications, alone and in combination, on tomato plants.

MATERIALS AND METHODS

The work was carried out in the experimental area of the Instituto Tecnológico de Tecomatlán, located in the south of Puebla State, Mexico, between parallels 17053'18" and 18007'24" North latitude and meridians 98012'42" and 98021'54" West longitude, at 960 m a.s.l. The tomato seedlings of the commercial variety Pony Express (F1) of the Saladette type were produced in a shade house. For this study, tomato seedlings of the commercial variety Pony Express (F1) of the Saladette type were produced in a shade house, using polystyrene trays with 200 cells, placing one seed in each one.

A commercial substrate (Peat-Moss Grow-mix) was used and for the AMF application, solid inoculants containing INCAM-4 (*Glomus cubense*), DAOM 241198 [18] and INCAM-11 (*Rhizoglomus irregulare*), DAOM 711363 [19], with a concentration of 30 spore 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.

Inocula were applied at the time of sowing by the seed coating method, in an amount equivalent to 10 % of its weight. For this purpose, a fluid paste was prepared with 6 mL of water for each gram of solid inoculant, in which the seeds were immersed. Seeds were then dried in the shade and sown.

In the case of QMax®, seeds were imbibed in the prepared solution at 0.1 g L^{-1} concentration, for three hours and when inoculation with the corresponding mycorrhizal strain was performed, seeds were first imbibed in water for the same time.

Six treatments were formed as follows:

T-1. Control (seeds imbibed in water).

T-2. QMax®.

T-3. Incam4

T-4. Incam4 + QMax® T-5.

T-5. Incam11

T-6. Incam11 + QMax®.

For the production of seedlings in a shade house, after sowing the seeds (September 11, 2019), the trays were placed one on top of the other and covered, to accelerate germination. After emergence, after three to four days, they were separated and distributed inside the house, without following a specific experimental design. At all times, irrigation was applied manually with a watering can, twice a day, up to the drip point, to guarantee the necessary humidity in the substrate.

The percentage of emergence was evaluated in each treatment and from 15 days after planting, 50 plants were measured at random for each treatment, periodically every seven days, their height from the stem base to the apical bud, with a tape graduated in mm, and up to the moment of transplanting (35 days), at which time the diameter of the stem was also evaluated at 5 cm from the soil with a King's Foot. Plant height data are included in the general dynamics of this variable.

The soil of the area selected to develop the experiment under field conditions is classified as Eutric Regosol [(20)] with a very low level of organic matter, alkaline pH, low level of assimilable phosphorus and low to very low exchangeable cations, except Ca which is classified as medium, according to its content [(21)].

Under field conditions, transplanting per treatment (the same as for seedling production) was performed on October 16, 2019, using a randomized block design with four replications. Beds covered with black polyethylene, were spaced 1 m apart and plants were placed at 0.5 m, each experimental plot had three furrows, two border furrows and a central evaluation furrow, with a 2 m wide plot size by 11 m long. The staking consisted of stakes placed at the beginning and end of the furrow and in the interior, at a distance of 2 m between each one, which were joined by rows of wires, to which the tomato plants were fastened with plastic twine. Prior to transplanting, a chemical fertilization was carried out in which the following doses were applied: 300 kg ha^{-1} of N, 250 k ha^{-1} of P_2O_5 and 600 kg ha^{-1} of K_2O, in each treatment.

Irrigation was applied using a localized irrigation system, with 8000-gauge tapes and drippers every 0.50 m, at a rate of 1.5 L per hour. Irrigation was applied with a frequency of two to three days and the time was between 2 and 2.5 h, depending on the water needs of the plants.

In treatments with mycorrhizae, this was applied to the root system by immersing it in a mixture of each inoculum for 10 minutes, prepared at a rate of 1 kg of each, in 600 mL of water, depending on the number of plants, after which time they were put to dry for a few minutes before planting.

Qmax® (10 mg L^{-1}concentration) was applied to the foliage at 300 mL ha^{-1}rate, to the treatments that required it, seven days after transplanting and at 28 days, the latter moment coinciding with the flowering beginning.

RESULTS AND DISCUSSION

Plant emergence occurred between three and four days after sowing and the percentage of emergence reached values between 92 and 94 %, but no significant differences were observed between treatments, perhaps because in the QMax® case, which has a greater effect in this phase, perhaps because in the case of QMax®, which has a greater effect in this phase than the mycorrhizal strains, since colonization has not taken place, the concentration used was very low, and according to the literature consulted [(8,23,24)], these authors used higher concentrations, which caused them to find significant differences with respect to the control used by them.

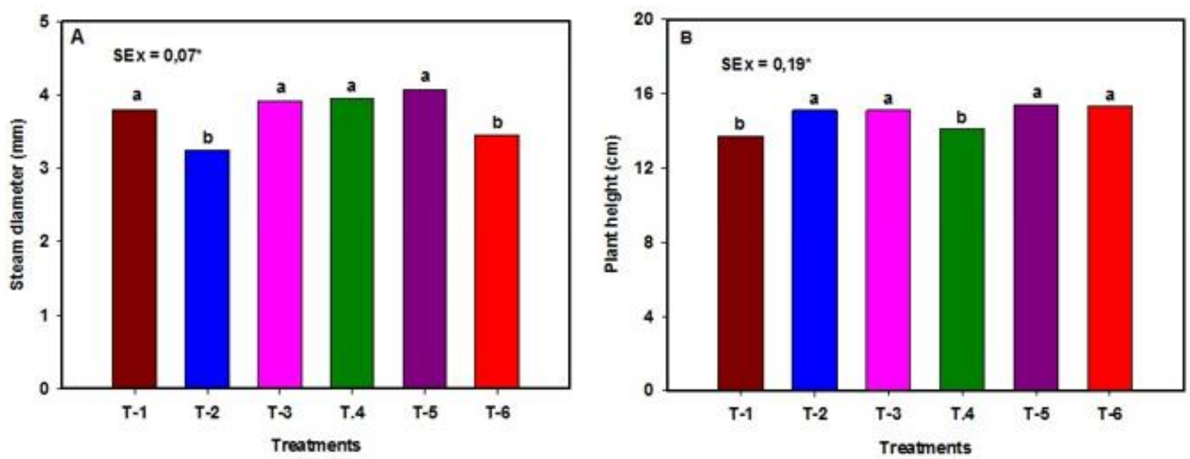
Figure 1 shows the results for stem diameter (A) and plant height (B) at the time of transplanting. According to the figure, in the case of stem diameter (A), the values were in correspondence with the age of the plants, but the treatments used did not cause increases in the variable, and were even lower when the seeds were soaked in QMax® and when inoculation with the Incam11 strain was added, with significant differences with respect to the control treatment and the rest of the treatments.

In relation to plant height, the behavior was different, and most of the treatments showed higher height values than the control plants, with significant differences with respect to the control, except when QMax® was combined with the Incam4 strain, since the values showed no differences with respect to the control treatment.

La emergencia de las plantas ocurrió entre tres y cuatro días después de la siembra y el porcentaje de la misma alcanzó valores entre 92 y 94 %, pero no se apreciaron diferencias significativas entre tratamientos, quizás porque en el caso del QMax®, que tiene un mayor efecto en esa fase, que las cepas de micorrizas, pues no ha tenido lugar la colonización, la concentración empleada fue muy baja, y de acuerdo con la literatura consultada [8,23,24], esos autores utilizaron concentraciones más altas, lo cual provocó que encontraran diferencias significativas, respecto al control por ellos empleado.

En la Figura 1 se presentan los resultados para el diámetro del tallo (A) y altura de las plantas (B) en el momento del trasplante. De acuerdo con la figura, en el caso del diámetro del tallo (A), los valores estuvieron en correspondencia con la edad de las plantas, pero los tratamientos empleados no provocaron incrementos en la variable, incluso fueron menores cuando las semillas fueron embebidas en QMax® y cuando se adicionó la inoculación con la cepa Incam11, con diferencias significativas respecto al tratamiento control y al resto de los tratamientos.

En relación con la altura de las plantas, el comportamiento fue diferente, y la mayoría de los tratamientos reflejaron valores de altura mayores a las plantas del control, con diferencias significativas respecto a este, excepto cuando el QMax® se unió con la cepa Incam4, pues los valores no mostraron diferencias respecto al tratamiento control.



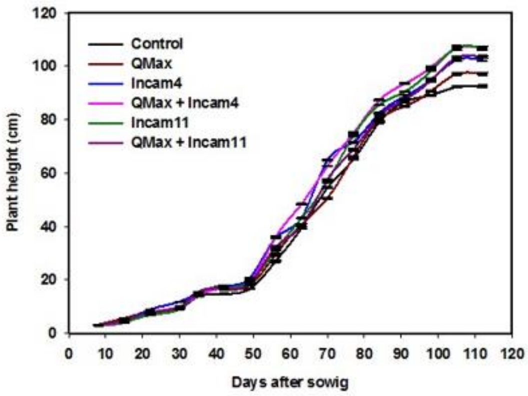
(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®).

Different letters above the bars mean significant differences between treatments at $p \leq 0.05$, according to Tukey's test.

Figure 1. Stem diameter (A) and plant height (B) at transplanting of tomato plants (Pony Expres variety) under the effect of two mycorrhizal strains, QMax® and their combinations

Nevertheless, it has been proved that QMax® applied to tomato seeds of Mara variety [23], caused increases in growth variables, such as plant height, root length and number of leaves, with respect to the control, which has also been corroborated by other authors [25]. These differences were more noticeable at the end of the cycle in this work and show the influence of this biostimulant on plant growth, although concentrations of the biostimulant were higher than those used in this work, which should be taken into account if the aim is to produce good quality seedlings.

When analyzing the dynamics of growth in height (Figure 2), three phases of growth can be distinguished: a slow phase, which corresponds to the initial stage (production of seedlings); a phase of rapid increase in the variable under analysis; and another slow phase, where a plateau is reached. Between 35 and 50 days there was a cessation in the increase in height, in all treatments, motivated by the stress caused by transplanting, as the plants had to adapt to field conditions, but from that moment on, the progression in the increase was rapid and the effect of the treatments imposed, which are a continuity of those established in the phase of production of seedlings, began to be differentiated.



(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®).

The bars signify the confidence interval of the means at 1-α≤0.5

Figure 2. Dynamics of tomato plant height (Pony Expres variety) under the effect of two mycorrhizal strains, QMax® and their combinations

The growth pattern was not modified by the effect of the treatments, but the moment in which the cessation in the increase of the variable was reached in the control treatment, where this instant was evidenced earlier (90 das), while this occurred after 100 das, in the rest of the treatments. It was in the control treatment where the smallest plant size was reached and, according to the confidence interval of means, showed significantly lower results with the rest of the treatments.

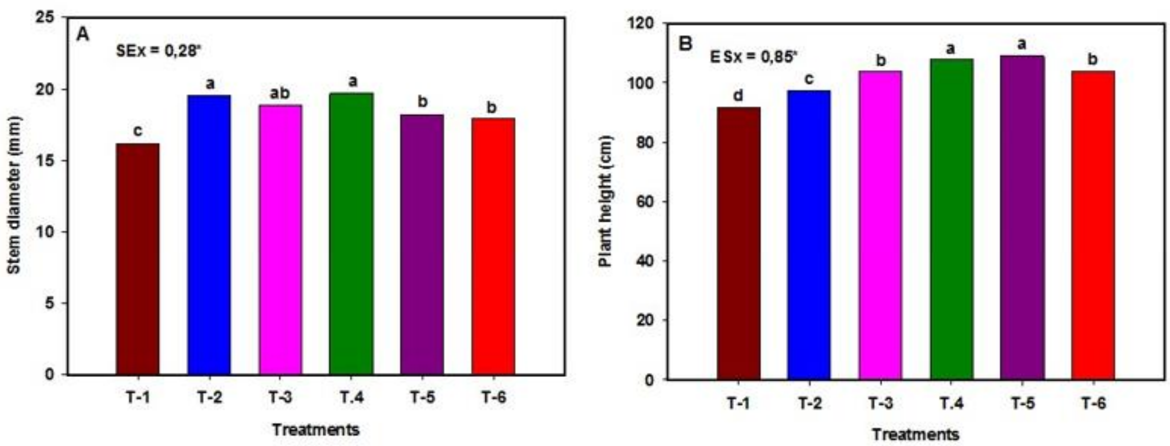
Both the imbibition of the seeds in QMax® and the foliar applications of this biostimulant after transplanting did not produce greater plant height compared to the rest of treatments, although it did produce greater plant height compared to the plants of the control treatment. However, its use combined with the inoculation of either of the two mycorrhizal strains used resulted in taller plants.

The use of two mycorrhizal strains alone, although both stimulated growth, the inoculation with the Incam11 strain produced plants of greater height, which shows that the efficiency of each strain is different, an aspect that has been recognized in other studies.

In contrast to the analysis carried out at the time of transplanting for the variables stem diameter and plant height, when a certain stability in growth was achieved, these variables showed a different behavior (Figure 3). Where biostimulants were applied, the results in the two variables analyzed were greater than the control, with significant differences with respect to the latter, while the use of Qmax® favored a greater stem diameter (Figure 3A), which did not occur in the presence of inoculation with the Incam11 strain. This did not occur in the presence of inoculation with the Incam11 strain, since it was with this strain that the lowest stem diameter values were found, even when combined with Qmax®, which indicates that both act differently on the different organs, but results with this strain were significantly higher than those of the control treatment.

Plant height (Figure 3B) was greater in all treatments, with significant differences with respect to the control; the highest values corresponded to treatments inoculated with the mycorrhizal strains evaluated, but the Incam4 strain also needed the application of QMax® to achieve this result, so that the Incam11 strain again showed lower efficiency in the performance of this variable. The results with the use of both strains showed that regardless of the fact that the soil is classified as alkaline, they caused stimulating effects on plant growth, regardless of the fact that their efficiency in action could have limitations due to this condition.

It is emphasized that the presence of mycorrhizae favors growth, largely due to the contribution they make to plant nutrition, by facilitating a greater absorption of nutrients, as well as improving the water status of the plants, even when there are no restrictions in their supply [26], as is the case in this work.



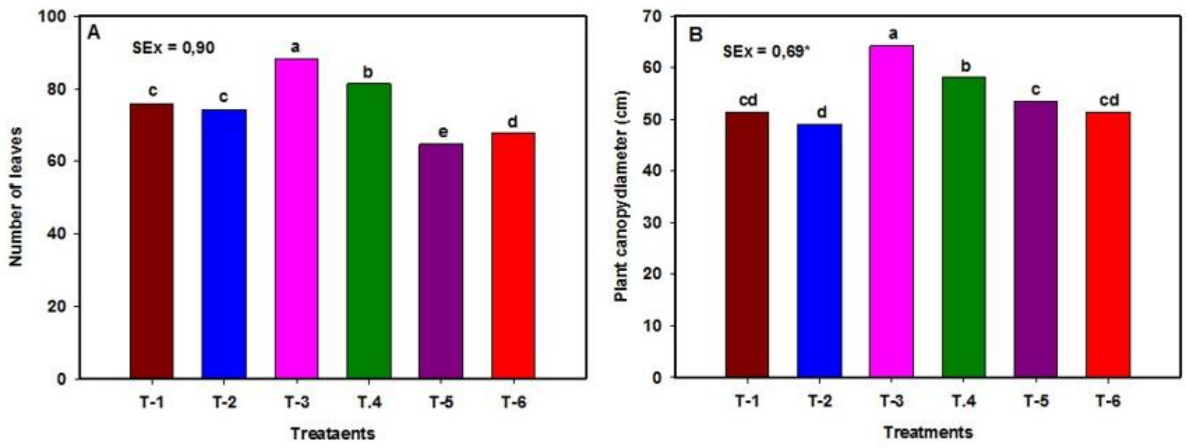
(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®).

Different letters above the bars mean significant differences between treatments at $p \leq 0.05$, according to Tukey's test

Figure 3. Stem diameter (A) and height (B) of tomato plants (Pony Express variety), when growth was stabilized under the effect of two mycorrhizal strains, QMax® and their combinations

However, it has been proven that chitosan applied through foliar applications increased stem diameter and plant height in the treatments in which it was applied, compared to the control without application [10], an aspect also proven by other authors [25], although the concentrations and imbibition time used in this case were different.

Other variables analyzed related to growth, at the time when height increments decreased, were the number of leaves and canopy diameter of the plants, Figure 4A and B, respectively.



T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®).
Different letters above the bars mean significant differences between treatments at $p \leq 0.05$, according to Tukey's test

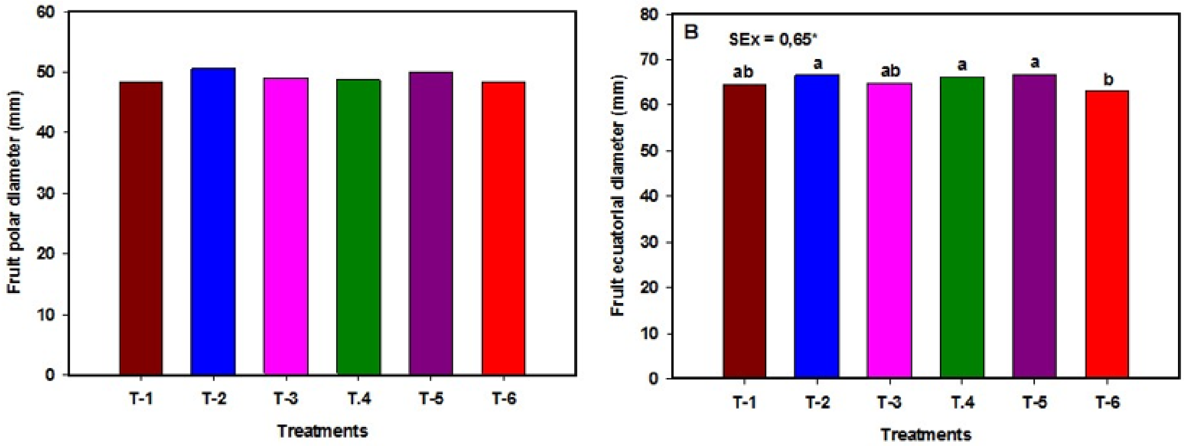
Figure 4. Number of leaves (A) and crown diameter (B) when the growth of tomato plants (Pony Express variety) was stabilized under the effect of two mycorrhizal strains, QMax® and their combinations

The influence of treatments on the behavior of both variables was similar, although the highest value was reached when inoculation was carried out with the Incam4 strain, but without the presence of Qmax®, with significant differences with respect to the rest of treatments. In the behavior of the two variables, the Incam4 strain stood out, with or without Qmax®, where higher values were reached, compared to the Incam11 strain, in spite of the efficiency that this strain had shown in other variables analyzed, all of which shows that not in all variables efficiency must follow a pattern, It depends on other factors and on those variables that have a greater relationship with yield, such as leaf area (not evaluated in this study), but it is to be expected that a greater number of leaves and a larger crown diameter are responsible for a greater capacity to absorb radiation. Notwithstanding what was found in this work, it was found that inoculated plants showed greater growth than non-inoculated plants, as well as greater leaf area than the control treatment [25], even though it was not the same strain as those used in this work, but it does demonstrate the importance of the use of this biostimulant. On the other hand, it has been proved that the effectiveness of strains used was shown in a different way, although those used stimulated growth and yield in a similar way [27], the recognition of symbiont presence in the medium by plants, although it occurs quickly, not all strains are recognized with the same speed [28], which can also cause delays in their effect on one or another variable under analysis.

The results in general in growth, evidenced a different effectiveness of the strains used, an aspect that had been proven before [29], which is also denoted in the interaction with QMax®, moreover, it has been proven that the presence of chitin, stimulated the development and sporulation of arbuscular mycorrhizal fungi [30].

In a review on the use of chitin and chitosan biopolymers in agriculture [31], emphasis was made on aspects related to their beneficial effects on plant growth and other important aspects that allow with their use an increase in agricultural production, all of which is of great importance when what is desired is an increase in yields.

Figure 5 shows the results of the analysis of the average dimensions of the fruit, through measurements of the polar diameter (A) and equatorial diameter (B).



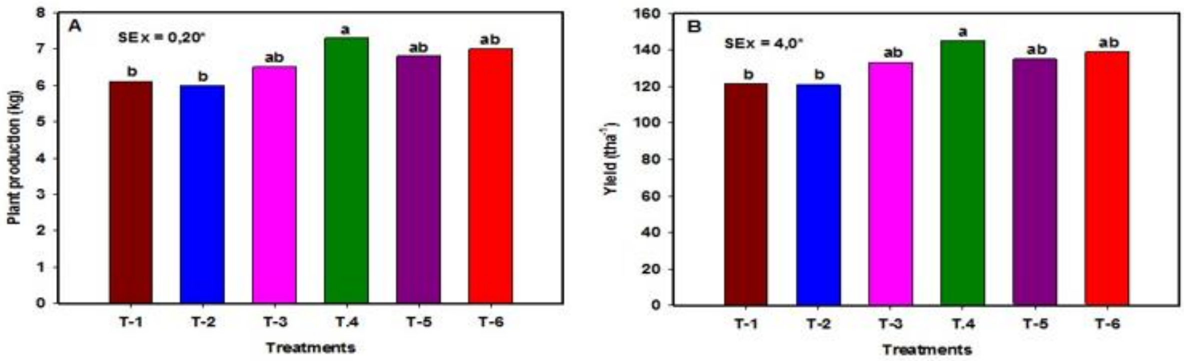
(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®).
Different letters above the bars mean significant differences between treatments at $p \leq 0.05$, according to Tukey's test

Figure 5. Polar and equatorial fruit diameter of tomato plants (Pony Express variety) under the effect of two mycorrhizal strains, QMax® and their combinations

Treatments applied did not cause modifications in the polar diameter, and in the case of the equatorial diameter, only in the treatment in which QMax® was applied, plus inoculation with the Incam11 strain (T-6), the values were significantly lower than when the plants were inoculated with the same strain and when Qmax® was applied, both alone and together with the Incam4 strain.

The polar diameter/equatorial diameter ratio of the fruits was less than 1 in all treatments, so they were considered flattened fruits [22]; thus, the treatments used did not modify the shape of the fruits. This behavior evidenced the varietal character of this variable, since, as fruit production is responsible for yield, the treatments used could only have modified the mass or number of fruits, aspects not taken into account in this work.

The analysis of production per plant and yield is shown in Figure 6. Both variables showed similar behavior in terms of the effect of the treatments, and the results were favored when QMax® was applied together with the inoculation with the Incam4 strain, in an absolute manner. Neither production per plant nor yield was favored when this biostimulant was applied alone, perhaps because the seeds were imbibed at a low concentration (0.1 mg L^{-1}), although the foliar application was at the concentration with which satisfactory results were obtained (300 mL ha^{-1}).



(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®).
Different letters above the bars mean significant differences between treatments at $p \leq 0.05$, according to Tukey's test

Figure 6. Production per plant (A) and yield (B) of tomato (Pony Express variety) under the effect of two mycorrhizal strains, QMax® and their combinations

The use of the combination of both biostimulants favored production and in the case of QMax®, the results found can be attributed to the fact that its active principle is chitosan, which has been demonstrated to be a stimulator of plant metabolism [(32,33)], but in future works the use of other concentrations should be evaluated, since in general, in the reviewed works, these have been higher.

In a study in which two biostimulants were used, alone and combined, the results showed that simple inoculation with a rhizobacterium and a mycorrhizal fungus stimulated growth and fruiting indicators in both tomato hybrids used, but a superior response was found in the presence of co-inoculation, with significant increases in total production [(34)]. The use of both biostimulants tended to increase tomato production in a semi-hydroponic production system.

On the other hand, in another study [(35)], it was found that the imbibition of seeds with the highest concentration (1.0 g L^{-1}) of QMax® produced the greatest increases in growth at the seedling level; while, its combination with the subsequent foliar spraying of 300 mg ha^{-1}, also caused increases in crop yield by 55 % with respect to the production control [(23)], which is attributed to the effects produced by chitosans when applied to the plants, even though for the particular region in which the study is made, it is necessary to continue the studies in this variety, very common its use in the region, but not the use of biostimulants, as well as to evaluate other concentrations.

In the case of the use of arbuscular mycorrhizae [(36)], they proved different behaviors of tomato at the level of the cellular wall of roots, which is the result of different answers, regarding the effect of this biostimulant in plants, from which it is necessary to evaluate the behavior of other strains of arbuscular mycorrhizal fungi, especially for the region where the work was done, where this practice has not been generalized, besides the effect that they cause in the quality of fruits [37].

Yields in the present work were above 100 t ha-1 in all treatments, but in this case a highly productive commercial variety was used; however, in the evaluation of 100 native populations in Mexico [22], it was found that the most productive one reached a yield of 108 t ha^{-1}, with an average of 121 fruits per plant and a mass of 74.3 g, without the application of any biostimulant, thus proving the convenience of the use of the two biostimulants used, which also contribute to the care of the environment by not affecting its contamination.

CONCLUSIONS

- According to results, it was found that in the posturing phase, treatments did not modify the stem diameter with respect to the control and had a greater effect on plant height, while in field conditions the effect was more marked on yield than on the growth variables evaluated, highlighting the combined action of Quitomax® with the Incam4 strains.
- It is necessary to evaluate in future works the increase in the concentration of Quitomax®.

ACKNOWLEDGEMENTS

To the third year agronomy students of the Technological Institute of Tecomatlan, Iris and Mariluth, for their active participation in the attention, conduction and realization of the evaluations in the experiment.

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