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EFFECT OF TWO BIOESTIMULANTS AND MYCORRHIZAL FUNGI ON TOMATO PLANTS SOWED AT HIGH TEMPERATURES

Efecto de dos bioestimulantes y hongos micorrízicos en plantas de tomate sembradas a altas temperaturas

Donaldo Morales Guevara[1✉], Luis Rodríguez Larramendi[2], José Dell'Amico Rodríguez[1], Eduardo Jerez Mompie[1] and Wilfredo Estrada Prado[2]

ABSTRACT. High temperatures is one of the main abiotic factors that influence the physiology and yield of crops, hence the present work has been conducted with the aim of evaluating the effect of two biostimulants and a biofertilizer applied to tomato seeds in conditions of high temperatures. The work was carried out in the National Institute of Agricultural Sciences and in the "Jorge Dimitrov" Research Institute under semi-controlled conditions. The seeds were subjected to the following treatments: imbibition for two hours in a solution of 5 mg L^{-1} of a mixture of oligogalacturonides, or a solution of 1 mg L^{-1} of chitosan. Another treatment with seeds pelleted with Arbuscular Mycorrhizal Fungi (AMF) and the control without seed treatment was also used. The variables evaluated were: relative water content, plant height, stem diameter, leaf surface, plant biomass, average number of fruits and average fruit mass. Both the height of the plants and the diameter of the stems did not reflect significant differences between the treatments used, while the other evaluated variables did differ from the control, with favorable responses in a general sense in those plants that were treated with the biostimulants. It can be concluded that both the biostimulants used and the biofertilizer based on mycorrhizal fungi are good alternatives to take into account for tomato planting under conditions of temperatures that exceed the optimum for the crop.

Key words: temperature, oligogalacturonides, mycorrhizae, chitosan, relative water content

RESUMEN. Las altas temperaturas es uno de los principales factores abióticos que influyen en la fisiología y en el rendimiento de los cultivos, de ahí que el presente trabajo se haya realizado con el objetivo de evaluar el efecto de dos bioestimulantes y un biofertilizante aplicados a semillas de tomate en condiciones de altas temperaturas. El trabajo se realizó en el Instituto Nacional de Ciencias Agrícolas y en el Instituto de Investigaciones "Jorge Dimitrov" en condiciones semicontroladas. Las semillas fueron sometidas a los siguientes tratamientos: imbibición durante dos horas en una solución de 5 mg L^{-1} de una mezcla de oligogalacturónidos, o de una solución de 1 mg L^{-1} de quitosana. También se utilizó otro tratamiento con semillas peletizadas con Hongos Micorrízicos Arbusculares (HMA) y el control sin tratamiento a las semillas. Las variables evaluadas fueron: contenido relativo de agua, altura de las plantas, diámetro de sus tallos, superficie foliar, biomasa de las plantas, número promedio de frutos y masa promedio de los frutos. Tanto la altura de las plantas como el diámetro de los tallos no reflejaron diferencias significativas entre los tratamientos utilizados, mientras que las demás variables evaluadas si se diferenciaron del control, con respuestas favorables en sentido general en aquellas plantas que fueron tratadas con los bioestimulantes. Se puede concluir que tanto los bioestimulantes utilizados como el biofertilizante a base de hongos micorrizógenos son buenas alternativas a tener en cuenta para la siembra del tomate en condiciones de temperaturas que superen las óptimas para el cultivo.

Palabras clave: temperatura, oligogalacturónidos, micorrizas, quitosano, contenido relativo de agua

[1] Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32700

[2] Instituto de Investigaciones "Jorge Dimitrov" (IIJD), Bayamo, Cuba

✉ dmorales@inca.edu.cu

INTRODUCTION

The global surface temperature is expected to increase by 1.5-2 gC by the end of this century (1) and this increase in temperature is likely to affect all areas of life, causing large negative impacts on agricultural productivity, thereby threatening global food production and security (2).

This increase in temperature is expected to affect crop productivity in several ways, including alterations in the biology of plants and their interaction with pathogens (3).

In plants, high temperatures not only affect basic physiological processes such as photosynthesis, respiration, membrane stability, but also modify endogenous hormones and primary and secondary metabolites (4).

In wheat it has been found that at temperatures above 30 gC the transport of assimilates from the flags leaves to the developing grains is reduced (5).

It has also been reported that in tomato anthers the synthesis of the enzyme sucrose phosphate synthase (SPS) modulates the partition of photo-assimilates and catalyzes the synthesis of sucrose contributing to the osmotic motive force for the translocation of the phloem, which was suppressed by heat stress (6).

There are several products that have been used to attenuate the effect of abiotic stress in plants, among which we can point out the case of chitosan or chitosan.

Chitosan has interesting properties that make them applicable in many fields, including agriculture, where they are used as biostimulants both to stimulate the growth of plants and to induce tolerance to abiotic stress or resistance to pathogens (7).

Recent work done by the author has shown the potential of this product as a stimulant of performance in different crops (8,9).

It has been reported that the coating of fruits and vegetables with chitosan has improved post-harvest life (10).

Another product that is interesting for agriculture is the Pectimorf® which is formed by a mixture of oligogalacturonides and is a biostimulant of vegetable origin composed of low molecular weight carbohydrates that functions as a hormonal chemical messenger that regulates the mechanisms of growth and differentiation in different crops, accelerating the growth process of the plants (11).

The oligogalacturonides derived from pectin (OGAs) are essential components to activate signaling pathways that induce rapid defense responses. Likewise, the OGAs showed that they regulate various processes related to development, such as the growth of the roots and the alteration of the formation of lateral roots, formation of adventitious roots and in particular the cellular differentiation (12).

Arbuscular mycorrhizal fungi (AMF) have been used in several crops exposed to abiotic stress conditions given their properties of mutually beneficial symbiosis with the root system of plants.

In this sense, we can point out the work done with mycorrhizal fungi as a strategy to improve the drought tolerance of datilifera palms (*Phoenix dactylifera* L.) (13) or the use of native mycorrhizae mixed with Bacillus thuringiensis bacteria in curly lavender plants or lavender (*Lavandula dentata* L.) which allowed increasing tolerance to drought and oxidative metabolism resulting in increased growth and biomass of plants (14).

Similarly, there are reports indicating the beneficial use of these fungi in the face of the effects of saline stress (15), in plants of wild laurel or durum (*Viburnum tinus* L) or in tomato plants (*Solanum lycopersicum* L) grown in conditions of high temperatures (16).

That is why the objective of this research has been to study the effect of two biostimulants and mycorrhizal fungi in tomato plants planted under conditions of temperatures that exceed the optimum for the crop.

MATERIALS AND METHODS

The investigations were carried out in the central area of the National Institute of Agricultural Sciences (INCA, Mayabeque province) and those of the “Jorge Dimitrov” Agricultural Research Institute (IIJD, Granma province). In the INCA, concrete channels of 2.40 m long, 0.60 m wide and 0.50 m deep were used in which a red Ferralitic soil mixed with cow dung was placed at a ratio of 2:1, while that in the IIJD concrete masons of 8 m long, one wide and 80 cm high were used. A brown type soil without carbonates (17) enriched with earthworm humus at a rate of 5 t ha^{-1} was used.

The experimental design was randomized blocks with four replications. Each plot had an area of 1.44 m^2 under the conditions of the INCA and 1.6 m^2 in the IIJD in which 20 and 12 plants were planted respectively at a distance of 0.25 m between plants and 0.40 and 0.8 m between rows respectively. The variety used was AMALIA from INCA.

For the treatment of the seeds in each of the treatments, the following procedure was followed:

We used a variant in which no treatment was applied to the seed and that was considered as the control treatment, the remaining treatments consisted of immersing the seeds for two hours in a solution of 5 mg L^{-1} of a mixture of oligogalacturonides, or a solution of 1 mg L^{-1} of chitosan.

Another treatment was also used in which the seeds were pelleted before sowing with arbuscular mycorrhizal fungi (AMF) and one to which no product was added and constituted the control treatment.

The works began in both places in the month of August coinciding with the period of maximum temperatures.

The evaluations made at the beginning of flowering were: relative water content; the growth expressed by the height of the plants; the diameter of the stems; the leaf surface and the accumulation of biomass of the aerial part, as well as the number of fruits per plant and the average mass of the fruits.

The behavior of temperatures during the experimental period was recorded.

The cultural tasks were carried out as established for the crop. The irrigation was applied by means of micro-sprinklers with frequency of three days according to the demand of the crop, trying that this was not a cause of variation when analyzing the results.

The data was processed in a combined manner using the office packages MS Excell, SigmaPlot and STATISTICA (StatSoft, 2003). The hypothesis tests used were the ANOVA and the multiple comparisons of measures. For the last one, the Tukey test was used for p≤0.05.

RESULT AND DISCUSSION

Figure 1 shows the maximum, average and minimum temperatures for the months in which the experiments were developed, note the high values mainly during the period of growth and flowering (August and September), which undoubtedly had an impact on the growth and fructification of the plants, more markedly in Granma, where temperatures were significantly higher.

The behavior of the relative water content in response to the different treatments used appears in Figure 2.

In the first place it can be seen that both the treatments with the biostimulants and the biofertilizer used showed a favorable response of the hydric state of the plant, with respect to the control treatment.

The response shown by the plants treated with the mixture of oligogalacturonides or with AMF could be explained by the fact that the former, as has been pointed out, stimulates the growth and formation of roots (11) which allows the plants to perform a greater absorption of water favoring its hydric state. Likewise, mycorrhizal fungi, in symbiosis with the roots of plants, emit hyphae that allow them to increase the absorption of water and mineral nutrients from an increase in the volume of soil explored (16).

On the other hand, chitosan could be associated to what was proposed by other authors who have found when using this product in foliar applications in pepper (*Capsicum* sp.) The presence of stomatal closure, which suggested that the stimulating effect of growth, after stomatal closure, could be related to an antiperspirant effect in the plant (18), as well as there is evidence that foliar application of chitosan in potato reduced the effects of water stress (19).

Figure 3 shows the results obtained when evaluating the growth of plants expressed by the length of their stems.

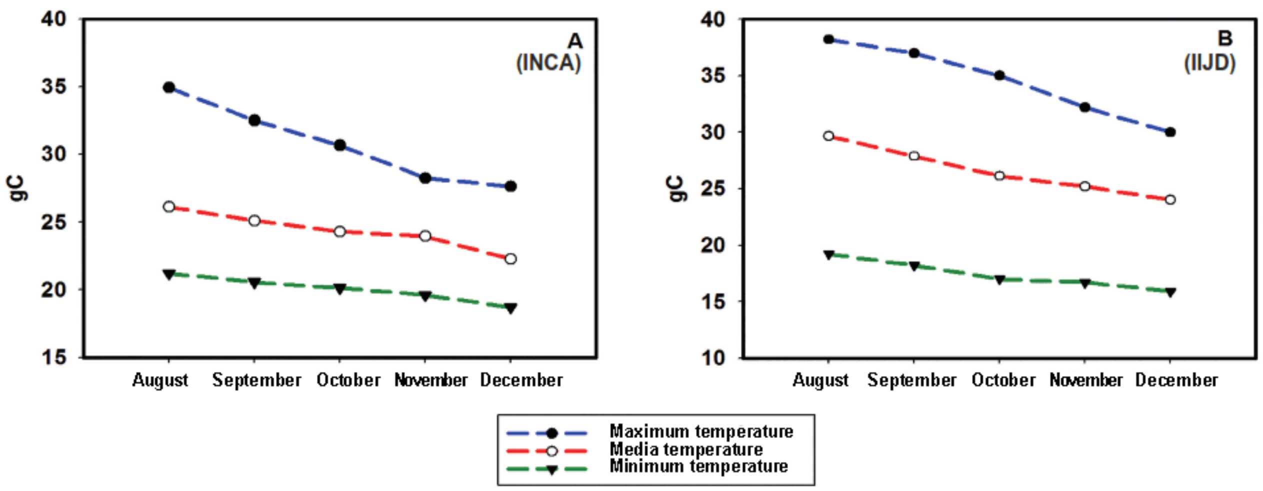
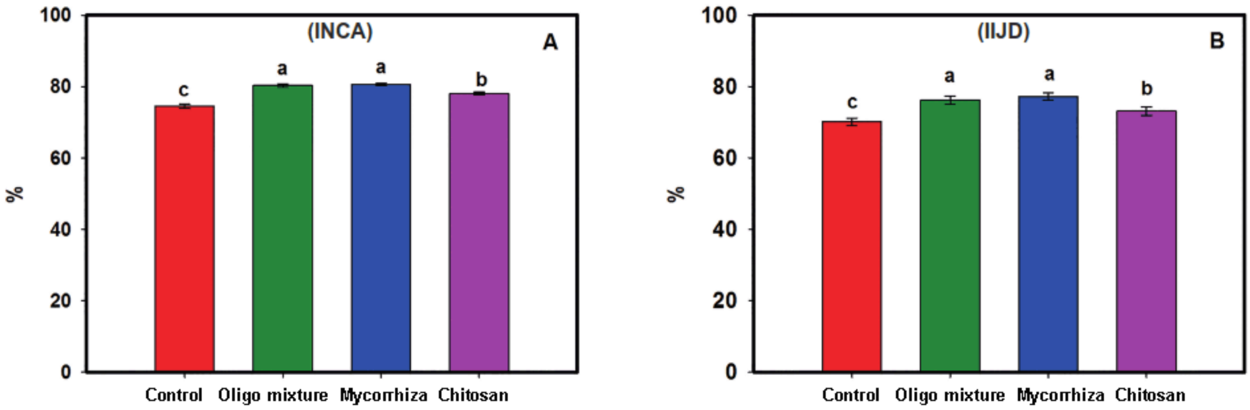


Figure 1. Maximum, average and minimum temperatures at the two sites where the experiments were carried out



Averages with different letters differ significantly according to Tukey for p≤0.05

Figure 2. Relative water content in tomato plants grown in gutters in the period of maximum temperatures pretreated with different bioactive products and biofertilizer

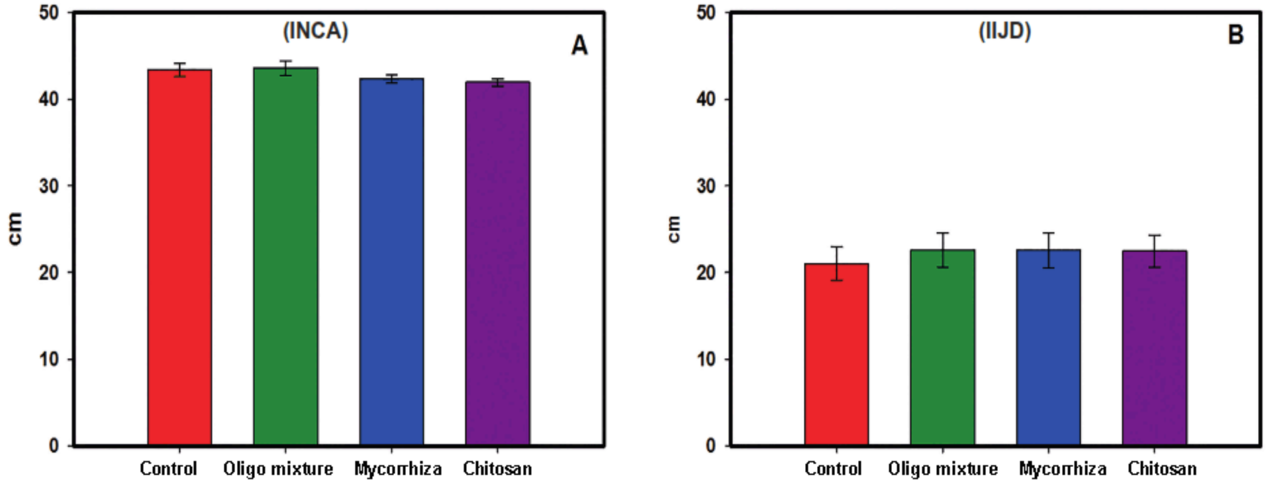


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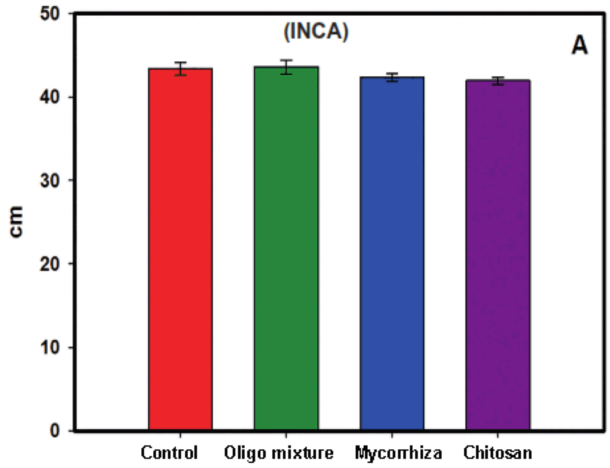
Figure 3. Height of tomato plants grown in gutters in the period of maximum temperatures and pretreated with different bioactive products and biofertilizer

As can be seen in Figure 3, the height of the plants showed a similar development in all the treatments, not finding significant differences between the different variants studied, which suggests that the products used did not have a favorable or harmful effect for the development of this variable.

Results reported from studies carried out with chitosan in plants of *Phaseolus vulgaris* under water deficiency conditions (9) or with mycorrhizae (16) in tomato plants (*Solanum lycopersicom*, L.) grown at temperatures above optimum for cultivation. indicated a more favorable growth of the plants treated with these products compared to those that did not receive them, which differs from these results and which could be explained by the fact that in the first case the results refer to another type of stress and in the second because the plants were planted under optimal temperature conditions and their cycles concluded when the temperatures were higher than the values considered adequate for the proper development of the crop; that is, in a reverse situation to this work.

The diameter of the stems (Figure 4) experienced a response similar to that shown by the height of the plants.



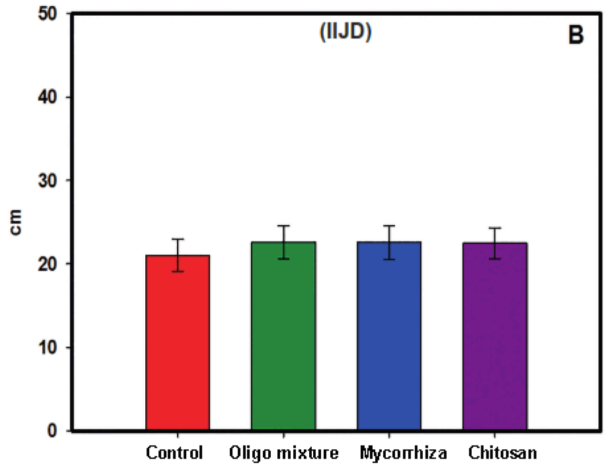


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Figure 4. Diameter of the stem of tomato plants grown in gutters in the period of maximum temperatures and pretreated with different bioactive products and biofertilizer

It is worth noting the differences shown by these variables in the different places where the experiments were developed, which could be a consequence of the higher temperatures that occurred in the IIJD, since as it is known the tomato from 30 gC begins to regulate its stomata as a mechanism to reduce the intensity of the different physiological processes, maintaining a better hydrological state between them that allows it to face adverse environmental conditions in better conditions.

In this regard, some authors have indicated that different types of stress influence differently the functioning of plants, either by inhibiting growth (20) and the case of high temperatures, among other effects, acting directly on the anatomy and integrity of cellular substructures (21).

Regarding the response of the foliar area per plant (Figure 5), it was shown that plants from seeds treated with biostimulant products (oligogalacturonides, chitosan or with mycorrhizae) under INCA conditions, increase the leaf surface with respect to the control treatment while in the IIJD only the treatment with oligogalacturonides differed significantly from the control.

The low foliar surfaces obtained in the treatment without biostimulant confirm the results reported by the author when studying the behavior of this same species when subjected to higher than optimal temperatures under controlled condition (22).

Figure 6 describes the effect of biostimulants on the accumulation of biomass in tomato plants. It is appreciated as in both experimental sites; the treatment of the seed with the different biostimulants or mycorrhizae favored the accumulation of dry matter by the plants. The answer of the plants treated with chitosan stands out in this sense, as it presents in absolute terms the highest accumulation of dry matter.

In this regard, other authors found that the immersion of melon seeds (*Cucumis melo* L.) in a solution of chitosan stimulated the initial growth of the seedling and the increase of the dry mass of the aerial part of the melon seedlings (23), as well as it is indicated that the use of mycorrhizae in other types of stress have favored the accumulation of dry matter of the aerial part in date palms (13).

It can be pointed out that, in a general way, plants from seeds that did not receive any treatment were the ones that showed a less favorable behavior.

In this regard, it can be pointed out that under conditions of high temperatures there are reductions in foliar photosynthesis that are reflected in the production of dry matter, at the level of the whole plant due to decreases in the net rate of assimilation (24).

The average number of fruits per plant showed significant differences between the treatments in the two experimental sites (Figure 7), excelling in both experimental conditions plants treated with chitosan or with the mixture of oligogalacturonides. Likewise, plants from mycorrhized seeds significantly outperformed those that did not receive any treatment.

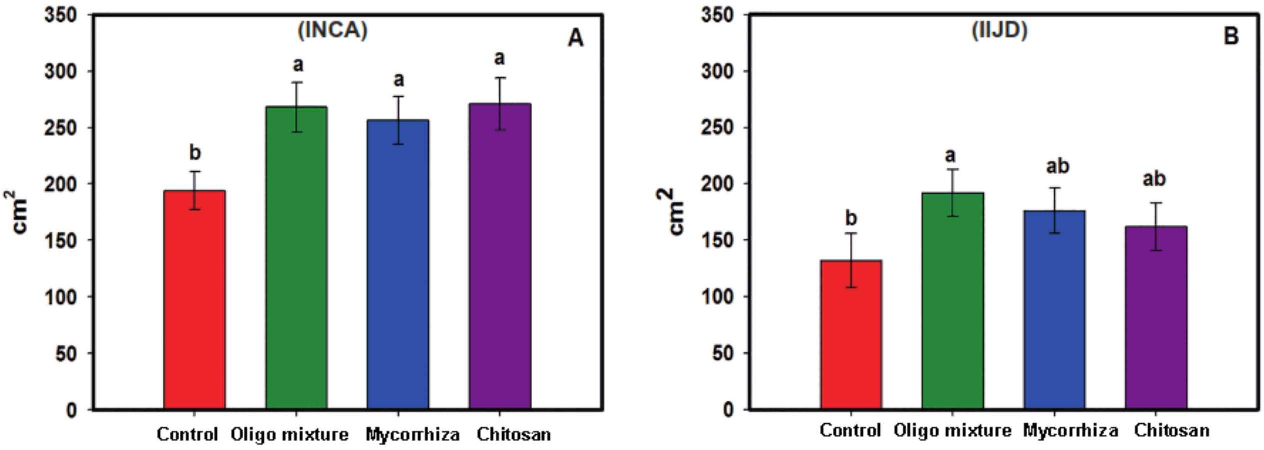

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Figure 5. Leaf area of tomato plants grown in gutters in the period of maximum temperatures and pretreated with different bioactive products and biofertilizer

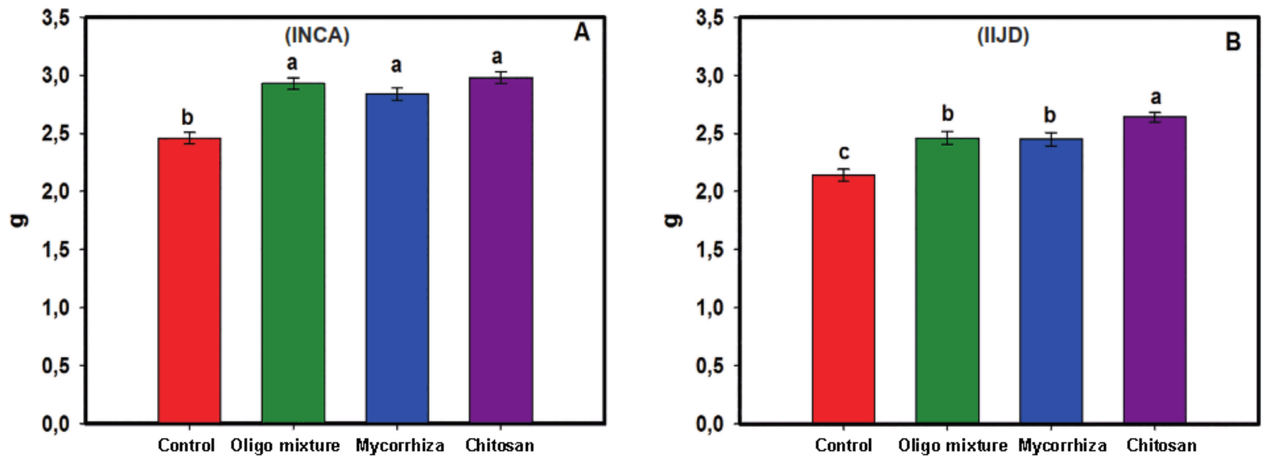

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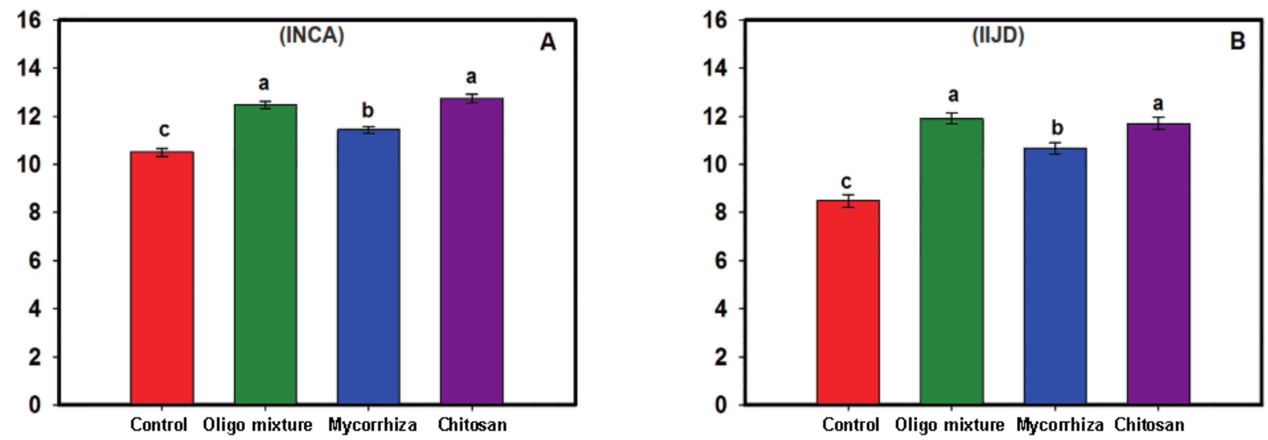
Figure 6. Dry mass of the aerial part of tomato plants grown in gutters in the period of maximum temperatures and pretreated with different bioactive products and biofertilizer



Averages with different letters differ significantly according to Tukey for p≤0.05

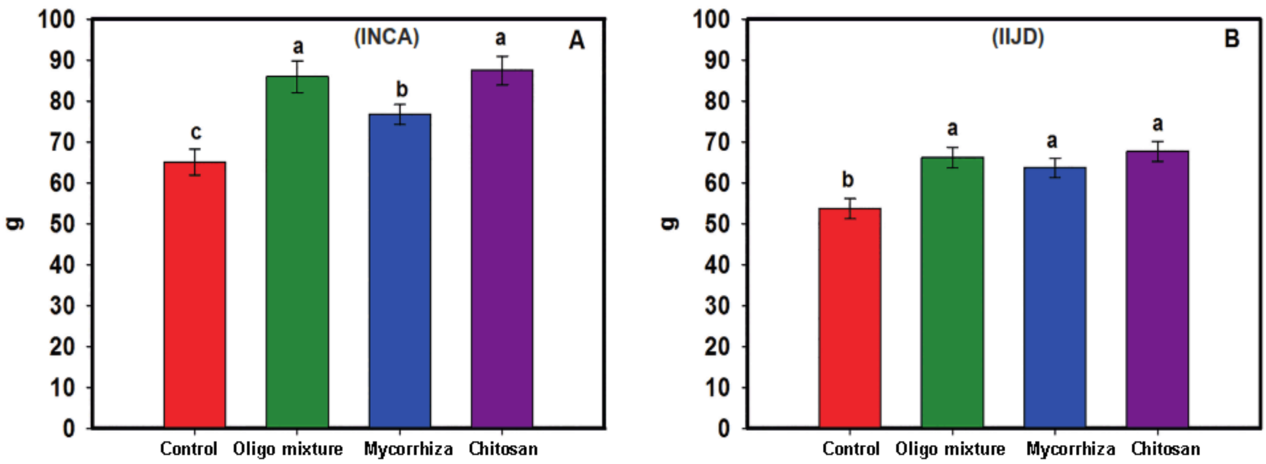
Figure 7. Average number of fruits in tomato plants grown in gutters in the period of maximum temperatures and pretreated with different bioactive products and biofertilizer

This answer could be explained by the fact that on the one hand, the mixture of oligogalacturonides has shown to have a favorable effect on the development of the root system, while the mycorrhizae once the symbiosis with the roots of the plants is established, they emit their hyphae which constitutes a lengthening of these, providing in both cases a more favorable hydric and nutritional status compared to the plants that did not receive these biostimulants. On the other hand, the chitosans, by exerting an antiperspirant effect, allows the plant to maintain a better hydric and even thermal status that helps it to face the stressful condition in a less drastic way.

The effect of the treatments used was evaluated when evaluating the accumulation of fresh mass of the fruits when significant differences were found with respect to the results shown by the control treatment (Figure 8).

CONCLUSIONS

As a conclusion, it can be stated that the results of this work demonstrate the feasibility of making tomato plantations in earlier stages, thus making it possible to obtain more than one crop in the same space, even though in the first one, yields are lower than those of the optimal time. On the other hand, it can be pointed out that the oligalacturonide mixture, the chitosan or the pelletizing of the seeds with mycorrhizae are alternatives that can be used to attenuate the effects of high temperatures, when the tomato is planted under temperature conditions that exceed the optimum for the crop.



Averages with different letters differ significantly according to Tukey for p≤0.05

Figure 8. Average mass of the fruits of tomato plants grown in gutters in the period of maximum temperatures and pretreated with different bioactive products and biofertilizer

The results obtained in the plants treated with the mycorrhizal biofertilizer, even though they presented superior results to those of the control plants, were lower in a general way than those obtained with the other biostimulants, which could be motivated because the substrates were fertilized organic with what improves its fertility and limits the beneficial action of these fungi.

Although there is no evidence of results where these biostimulants have been used in conditions of temperatures not optimal for the development of the tomato, if results are reported (15) with the use of mycorrhizae applied to plants of this crop in conditions of more elevated in the final stage of the plant cycle, in which results superior to the control were achieved in terms of the number of fruits per plant and the fresh mass of the fruits.

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