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ORIGINAL ARTICLE

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Irrigation with Magnetically Treated Water on Tomato (Solanum lycopersicum L.) Inoculated with Arbuscular Mycorrhizal Fungi

Riego con agua tratada magnéticamente sobre tomate (S*olanum lycopersicum* L.) inoculado con hongos micorrízicos arbusculares

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ABSTRACT: The aim of the study was to determine the effect of magnetically treated water (ATM) with a stationary magnetic field on the yield of tomato (*Solanum lycopersicum* L.) inoculated with three strains of arbuscular mycorrhizal fungi (AMF) infested with the nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. The investigation began in a soft brown soil without carbonate, in the “Campo Antena” Protected Cultivation Unit, belonging to “América Libre” Socialist State Enterprise; between November 2018 and February 2019. The inoculation of the microorganisms was carried out at the time of the transplant, in a proportion of 10% with respect to the volume of the root ball. The experimental design was completely randomized blocks, with 8 treatments and four replicas, with a control without AMF inoculation, a treatment without AMF inoculation and irrigation with magnetically treated water, 3 treatments with inoculation with *Glomus cubense*, *Rhizophagus irregularis*, *Fummeliformis mosseae*, and 3 treatments with the inoculation of these 3 AMF species combined with irrigation with magnetically treated water with an induction of 0.07 T. The data obtained were processed in the statistical package R Commander by means of a simple analysis of variance, applying Duncan's Test of mean multiple comparison for p≤ 5. The results obtained showed the efficiency of the treatments applied in tomato yield, highlighting the treatment with *G. cubense* and ATM, which yielded 112.9 $t \cdot ha^{-1}$ and provided greater protection to the plant against the nematode attack by reducing the galling index to 1.

Keywords: Protected Culture, Magnetic Induction, Mycorrhizae, Nematodes.

RESUMEN: El objetivo del estudio fue determinar el efecto del agua tratada magnéticamente (ATM) con campo magnético estacionario sobre el rendimiento del tomate (*Solanum lycopersicum* L.). inoculado con tres cepas de hongos micorrízicos arbusculares (HMA) infestado con el nematodo *Meloidogyne incognita* (Kofoid and White) Chitwood. La investigación se desarrolló en un suelo pardo mullido sin carbonato, en la Unidad de Cultivo Protegido “Campo Antena”, perteneciente a la Empresa Estatal Socialista América Libre; entre noviembre 2018 y febrero 2019. La inoculación de los microorganismos se realizó en el momento del trasplante, en una proporción del 10% con respecto al volumen del cepellón. El diseño experimental fue de bloques completamente al azar, con 8 tratamientos y cuatro réplicas, con un testigo sin inoculación de HMA, un tratamiento sin inoculación de HMA y riego con agua tratada magnéticamente, tres tratamientos con inoculación con *Glomus cubense, Rhizophagus irregularis*, *Fummeliformis mosseae,* y tres tratamientos con la inoculación de estas tres especies de HMA combinadas con riego con agua tratada magnéticamente con inducción de 0,07 T. Los datos obtenidos se procesaron en el paquete estadístico R Commander mediante un análisis de varianza simple, aplicándose la prueba de comparación múltiple de medias de Duncan para p≤ 5. Los resultados obtenidos mostraron la eficiencia de los tratamientos aplicados en el rendimiento del tomate, destacando el tratamiento con *G. cubense* y ATM logrando 112,9 $t \cdot ha^{-1}$, brindándole una mayor protección a la planta contra el ataque de los nematodos, reduciendo el índice de agallamiento a uno.

***Palabras clave*:** cultivo protegido, inducción magnética, micorrizas, nematodos.

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INTRODUCTION

MTW is called water that has been exposed to a magnetic field, when this occurs several physicochemical and molecular effects occur in it, for example: changes occur in the solidification and boiling points, changes in surface tension, viscosity, evaporation rate, dielectric constant and refractive index; there are also effects on the formation of cluster structures from hydrogen-bonded linear and ring chains of molecules. These effects are indifferent to nature (Krishnaraj *et al.*, 2017).

The technologies that have been developed from non-polluting physical methods to stimulate plant growth and yield are one of the most promising solutions to the problem raised above and can contribute to the development of sustainable agriculture. The National Center for Applied Electromagnetism (CNEA), belonging to the Universidad de Oriente, Cuba, works on the application of magnetically treated water for agricultural purposes, demonstrating the benefits of the magnetic treatment of irrigation water in germination, photosynthesis and increase in agricultural yields (Elías-Vigaud *et al.*, 2020; Zamora-Oduardo *et al.*, 2020).

Tomato is the most important vegetable in the world, since it represents more than 30% of the horticultural production. The cultivation of tomato (*Solanum lycopersicum* L.) is of great importance in the world due to the volume of production, as well as its acceptance in fresh, sauces, soups and processed, among others (Reyes-Pérez *et al.*, 2020).

Traditionally, different control alternatives are used to reduce and/or eliminate nematode populations. For many years, a wide range of chemical nematicides has been used for their control, many of which are biocides, with negative effect on beneficial organisms present in the soil. The application of these products causes strong problems of environmental contamination (Álvarez *et al.*, 2016).

Current trends in the management of *Meloidogyne spp.* are based on the use of Integrated Nematode Management (MIN) involving soil microorganisms in which biological control agents (ACB) and arbuscular mycorrhizal fungi (AMF) are grouped (Benedetti *et al.*, 2021).

Arbuscular mycorrhizal fungi (AMF) constitute a functional group that is important in soil biota, since it favors the improvement of the structure, the multifunctionality of the ecosystem and the productive development of crops (Vallejos-Torres *et al.*, 2019). The importance of the study of AMF lies in the fact that there is evidence of association with more than 80% of plants, as well as its role in protecting the root system from phytopathogenic agents, promoting mineral nutrition and facilitating water absorption. , which contributes to the improvement of plant growth and survival (Urgiles-Gómez *et al.*, 2020).

AMF appear not to affect penetration or the infection process. The reductions in infestation and reproduction produced by AMF are evidenced in the density of nematodes or eggs per gram of soil. Good nutrition with phosphorus (P) would improve plant vigor, thus reducing losses caused by nematodes, especially in soils with low P content if mycorrhizae are established early in the host's life cycle, before planting infestation with nematodes (Nazareno-Saparrat *et al.*, 2020).

In studies carried out that propose the use of the magnetic field to fight pests, they report that plants subjected to magnetically treated water tend to behave tolerant to these pathogens (Quiala-Pérez *et al.*, 2011), although there is little information so far. The objective of this research was to determine the effect of irrigation with magnetically treated water plus the combination with AMF on the yield of tomato *(Solanum lycopersicum* L.) and populations of *Meloidogyne incognita* (Kofoid and White) Chitwood under protected cultivation conditions as a possible strategy within the management of *M. incognita*.

MATERIALS AND METHODS

The research was carried out in the protected cultivation unit "Campo Antena" belonging to “América Libre” Socialist State Enterprise; located on the Santiago de Cuba National Highway Km 3 ½ Santa María, with reference coordinate (X: 60757330 Y: 156332149), in Santiago de Cuba Municipality, Cuba. Tomato (*Solanum lycopersicum* L.) hybrid HA 3057 was used as a crop and the experiment was carried out in the period November / 2018 - February / 2019.

Strains from the AMF collection of the National Institute of Agricultural Sciences (INCA) were used as mycorrhizal inoculants. At the time of use, the inocula had an average title of 50 spores$\cdot$g^{-1} of fresh soil certified in INCA Mycorrhizal Laboratory.

Rhizophagus irregularis (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler)

Funneliformis mosseae (T.H. Nicolson & Gerd.) C.Walker & A.Schüßler)

Glomus cub ense (Y. Rodr. & Dalp)

A homogeneous paste was prepared in a proportion of 1 kg of each inoculum per 10 kg of seed, and the seed was covered with it until it was completely covered. Subsequently, they were put to dry in the shade for 5 to 10 minutes and sowed in alveolar trays in the plant nursery. The experimental design was completely randomized, with four replicas and eight treatments. For the evaluation of the studied indicators, 25 plants and 80 fruits were chosen at random for each treatment.

Description of the Treatments

1. Control without AMF inoculation
2. Cultivation without AMF inoculation + irrigation with magnetically treated water
3. Inoculation with *Rhizophagus irregularis*
4. Inoculation with *Funneliformis mosseae*
5. Inoculation with *Glomus cubense*
6. Inoculation with *Rhizophagus irregularis* + irrigation with magnetically treated water
7. Inoculation with *Funneliformis mosseae* + irrigation with magnetically treated water
8. Inoculation with *Glomus cubense* + irrigation with magnetically treated water

For the magnetically treated water (MTW) a magnetic induction of 0.07 Tesla (T) was used. For the magnetic treatment, an external magnetizer with permanent magnets or a static magnetic field was used. The magnetizers were designed at the National Center for Applied Electromagnetism. (CNEA).

Soil preparation was carried out according to the requirements of this technology with tilling, subsoiling, harrowing and quarrying. The planting frame was 1.04 m x 0.30 m.

Prior to the transplant, irrigation was applied to the plantation area to guarantee adequate humidity in the soil and thus avoid the stress of the seedlings. Once planted, a light irrigation was applied without incorporating nutrients to guarantee adequate moisture around the roots and avoid air spaces between the root ball and the surrounding soil, so that the radical development of the seedlings can be benefited.

Healthy plants 25 to 28 days after sowing were used, with an average height of 12 cm, 5 true leaves and a stem thickness of 8 mm. The modification was of one row per bed.

This process was carried out in the early hours of the morning to avoid as much as possible the water stress of the plants, at the same time larger holes were made than the root ball of the posture, before placing it, the mycorrhizal inoculant was applied according to the methodology and once the posture was placed, the soil around it was slightly pressed in order to fix its root system.

Post-Transplant Water Stress

After the first irrigation, after the transplant, the plantation was subjected to water stress during the first 12 days, always monitoring the existing humidity in order to favor the radical development of the plant and its proper rooting. The fertirrigation with nutrients according to technology, began when the plantation had 75% of flower clusters emitted.

Retransplant

Between the 7th and 8th days, the entire plantation must be uniformly resealed and with 100% population.

Hilling or Stick Top

This activity is made to coincide with the retransplantation and the first background fertilization, with the aim of guaranteeing a greater root system.

Plantation Management

Tutoring

The plantation was carried out with three stems, two below the first cluster and one below the second cluster, achieving a total of 5,700 shoots, which were technically tutored, with five clusters per stem and four fruits per cluster in order to improve the general aeration of the plant and favor the solar radiation and the realization of cultural labors, which will affect the final production, fruit quality and disease control.

Defoliation

This work was carried out with the objective of eliminating the damaged, diseased or deciduous leaves of the plant throughout its entire vegetative cycle. The leaves opposite the cluster were folded so that it can photosynthesize. After each defoliation work, an application of fungicide was carried out.

Beheaded

After defining the crop cycle, from 20 to 30 days before its demolition, the decapitation of all the apical buds of the plant was carried out, in order to favor the weight and quality of the fruits.

Harvest and Postharvest

The optimal moment to carry out the harvests was determined, which is when the fruit has a good consistency and reaches technical maturity, which in the specific case of tomato is when it changes color from the apex of the fruit. The most favorable time for harvesting is early in the morning or late in the afternoon. It should be done with scissors or sharp knives, in order to avoid tearing or damaging fruits and plants. It is important that, after the harvest, the fruits are always handled with great care, to guarantee their commercial quality.

The Protected Cultivation Unit "Campo Antena" presents a high incidence of attack by gill nematodes, specifically, by *Meloydogine incognita.*

Determination of Nematode Infestation Degree

Soil analysis was done before transplanting and after harvesting (120 days), as well as counts of damaged plants with nodules to determine the degree and affectation of the crop.

The infestation index was determined through the indirect method of bioassay by indicator plant. The roots were extracted, washed carefully and the gall index was determined through the Taylor & Sasser Scale (1978) by counting galls under a stereomicroscope (Zeiss) with 160 magnification. This galling index was the indicator of the initial population of *Meloidogyne incognita*. The soil analysis to determine the infestation indices of the pest were carried out in the Provincial Laboratory of Plant Health.

Radical Colonization by AMF

Once the productive cycle of the crop was finished, the roots were carefully collected to protect as many rootlets as possible, in order to preserve the absorbent hairs and obtain as much information as possible. The samples were sent for processing to the National Institute of Agricultural Sciences (INCA).

The sampled rootlets were washed under running water to remove all soil and air-dried. The finest rootlets were taken and shredded. For the determinations, approximately 200 mg of rootlets were weighed, which were dried at 70 °C, to be stained according to the methodology described by Taylor y Sasser (1978). The evaluation was carried out using the Intercept Method, developed by Giovannetti & Mosse (1980), through which the percentage of mycorrhizal colonization or frequency of colonization was determined.

$$\%\ de\ infectión = \frac{\sum(1-5)}{\sum(0-5)} \cdot 100$$

Performance Variables and Their Components

For these evaluations, plant height and stem diameter (carried out 45 days after transplant), average fruit weight, fruit equatorial and polar diameter, and yield were considered.

Experimental data for each variable were subjected to simple classification analysis of variance, and mean comparisons were performed using Duncan's multiple range test for $p \leq 0.05$. The statistical program used was R Commander Version 4.1.1 for Windows.

RESULTS AND DISCUSSION

The results revealed that the use of AMF with inoculation prior to planting is a practice with positive results since the levels of infestation of the pest were reduced and the performance of the crop was even better when the AMF (*G. cubense* and *R. irregularis*) were combined with MTW (Table 1).

This seems to be due to the fact that to the effects exerted individually by both practices, it is added that jointly they show a greater potential for the development of the crop and a more effective response against the attack of nematodes.

The three AMF strains presented fungal functioning in relation to the control without inoculation and to the treatment without inoculation + MTW, leaving the tendency to higher values of % root colonization for *Glomus cubense* and *Rhizophagus irregularis* in combination with MTW, respectively, highlighting *Glomus cubense* + MTW.

This result could be determined by the pH-H_2O (5.8 to 7.3) in which this process was developed, since it was influenced by the neutralization of the bicarbonates contained in the irrigation water, which is more suitable for normal development of the strains. The values could be given by the ability of the efficient strains of AMF to establish a “molecular dialogue” with the macrosymbiont in close relationship with the edaphic environment and stimulate higher percentages of root occupation. Similar results were obtained by Rivera *et al.* (2015)

It can be seen that, in most cases of plants attacked by nematodes, AMF produce a decrease in the severity of the infestation, possibly because plant parasitic

TABLE 1. *Meloidogyne incognita* galling index in each treatment in tomato crop and % of root colonization by AMF

Treatments	Initial gall index	Final gall index	% of radical colonization
Control without AMF inoculation	2.8	3.8 a	7.75 g
Culture without inoculation of AMF + MTW	2.8	2.3 b	15 f
Rhizophagus irregularis	2.8	1.5 de	29.25 d
Funneliformis mosseae	2.8	1.9 c	25 e
Glomus cubense	2.8	1.4 e	46 b
Rhizophagus irregularis + MTW	2.8	1.4 e	35 c
Funneliformis mosseae + MTW	2.8	1.6 d	28.75 d
Glomus cubense + MTW	2.8	1.1 f	50.75 a
ESx	-	0.1029	0.2114

Same letters in the same column do not differ significantly for p≤0.05

nematodes are antagonists, biotroph obliged, just like AMF.

Likewise, it was verified that there is an antagonistic relationship caused by arbuscular mycorrhizal fungi towards pathogens and that the reduction of damage from attacks by these is not only based on a greater number of roots (Contreras & Mercado, 2019).

Numerous mechanisms are manifested when AMF promote biocontrol. They can be grouped into two: those that include a direct effect of the fungus on the pathogen, such as competition for nutrients, space, and infection/colonization sites, and those that include indirect effects on the pathogen, such as improved host nutrient uptake, changes in root architecture, changes in the interaction of rhizosphere microorganisms and activation of plant defense mechanisms (Dar & Reshi, 2017).

Trejo-Aguilar (2018) verified in the cultivation of coffee (*Coffea arabica*) that, with the inoculation of AMF, the root system increases and the susceptibility and damage caused by nematodes can be reduced. Forghani & Hajihassani (2020) mention the existence of mechanisms such as increased nutrient absorption, alteration of root morphology, competition for space and nutrients, and induction of systemic plant resistance.

Elaoud *et al.*(2019), state that mycorrhizal fungi used against nematodes as resistance inducers are capable of reducing the damage caused by plant parasitic nematodes, minimize damage, establish competition for space and resources, provide more nutrients and greater water absorption by the plant and modify the morphology of the root and/or the rhizosphere, which constitutes an advantage for the plant growth. In addition, fungi can induce resistance against nematodes by activating hormones (salicylic and jasmonic acid, strigolactones, among others) as a plant defense mechanism.

There are also studies (Quiala-Pérez *et al.*, 2011) which show that MTW gives crop plants greater tolerance to pathogen attack. Quiala-Pérez *et al.* (2011) report a decrease in the degree of infestation by nematodes due to the influence of MTW which improves the aptitude of the host plant, since it presents good vigor due to a greater availability of nutrients.

Table 2 shows that, the highest yields and best results in terms of plant growth and development, were achieved in the treatments that received the combination of AMF (*Glomus cubense* and *Rhizophagus irregularis*) and irrigation with magnetized water, compared to the same treatments without irrigation. The best result was obtained with the use of *Glomus cubense* and MTW. This allows inferring that there is a synergistic effect between AMF and MTW that enhance the development and growth of the plant, as well as the productive parameters and yield, also promoting a better plant response to the attack of root phytopathogens.

The results shown in Table 2, may be due to a better nutrition of the plant because of the rapid assimilation of water and nutrients in a balanced way when it is subjected to magnetic induction. That shows that magnetic ionization prevents ionic imbalances, which solves soil nutrition problems and, consequently, increases the growth and yield of the harvest, manifesting a positive combined effect when joining the AMF, (Zhang *et al.*, 2017 and Reyes-Pérez *et al.*, 2020)..

The species *G. cubense* and *R. irregularis* showed a better behavior for this type of soil, which indicates a greater capacity to adapt to these soil conditions. In this sense, Calero *et al.* (2019) reported notable improvements in tomato production using microorganisms, so they recommend their use in this crop.

TABLE 2. Effects of AMF and MTW on growth and yield parameters of *S. lycopersicum* hybrid HA 3057 infested with *M. incognita*

Treatements	Plant height (cm)	Stem diameter (cm)	Average weight of the fruits (g)	Ecuatorial Diameter of the fruits (cm)	Pole Diameter of the fruits (cm)	Yields (t. ha^{-1})
Control without AMF inoculation	105.6 h	1.48 c	200.25 h	6.53 d	5.6 g	79.96 g
Culture without inoculation of AMF + MTW	109.3 g	1.55 bc	204.25 g	6.56 d	5.68 fg	81.7 fg
Rhizophagus irregularis	125.2 d	1.64 ab	247.25 d	6.9 bc	6.32 d	96.2 d
Funneliformis mosseae	113.0 f	1.60 abc	207.5 f	6.58 d	5.73 f	83.5 f
Glomus cubense	127.2 c	1.67 ab	284.75 b	7.24 a	6.82 b	109.5 b
Rhizophagus irregularis + MTW	128.4 b	1.71 a	263.2 c	7.12 ab	6.48 c	99.2 c
Funneliformis mosseae + MTW	115.2 e	1.68 ab	227.5 e	6.67 cd	5.89 e	87.5 e
Glomus cubense + MTW	129.5 a	1.74 a	293.45 a	7.29 a	6.98 a	112.9 a
ESx	0.0517	0.0412	0.2555	0.0763	0.0315	0.5809

Same letters in the same column do not differ significantly for $p \leq 0.05$

It is considered that magnetized water is better assimilated by cells, which favors the intensity of water flow into the plant by making it faster than under normal conditions due to the different mechanisms of osmosis and diffusion and includes the mechanisms of alteration of the membrane permeability, associated with nutrient transport mechanisms, proposed by Lasso (2019), which, consequently, gives the plant, greater resistance or tolerance against pest attacks (Boix *et al.*, 2019).

The AMF caused an increase in the growth and development of the plants and an improvement in their resistance against the nematode infestation, acting as an effective and alternative biocontrol agent against the nematode. Similar results were found by Sharma *et al.* (2021) in their research.

CONCLUSIONS

Irrigation with ATM enhances the positive effects of AMF in the management of *M. incognita* in tomato, which shows that their combined use can be a beneficial alternative for the management of this pest in the crop.

The AMF (mainly *G. cubense*) allowed a considerable reduction in the formation of galls and in the reproductive capacity of the nematode. In addition, the inoculation of AMF + MTW led to an increase in the growth and development indicators of the culture, increasing its yield.

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