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# MICROBIOLOGICAL AND CHEMICAL CONTROL OF Fusarium spp. AND Meloidogyne spp. IN THE PRODUCTION OF TOMATO (Solanum lycopersicum L.) IN SOIL UNDER GREENHOUSE

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Abstract: One of the main problems of tomato production (Solanum lycopersicum L.) in greenhouse soil is the damage caused by fungi and nematodes. The use of beneficial microorganisms for disease control is a biological alternative that has been intensifying, helping to reduce the use of agrochemicals and maintaining yield. The objective of the research was to know the effect of a beneficial microbial consortium (BMC), the combination of BMC with chemical control (CC) and only CC against the phytopathogens Fusarium oxysporum. and Meloidogyne spp. in the tomato crop, using the cultivar "El Cid". The research was carried out in 2019 in a company that produces vegetables in greenhouse soil in the state of Zacatecas. The growth of the plant was measured (length and diameter of the stem, length of the leaf, length of the bunch to the apex and length of the stem of the bunch); fruit production (number, size, weight and yield); fruit quality (total soluble solids and firmness); plant health index (damage by Meloidogyne and percentage of damage by Fusarium). In the growth variables, the combination of BMC applied to the soil weekly and biweekly by foliar application + CC presented the best results. There were no significant differences in yield, only the treatment where the BMC + CC was applied weekly presented lower yield, but better fruit quality. There were no significant differences in the plant health index. With the weekly application of the BMC in the soil and in the foliage and without the CC, the best performance of the crop was obtained.

**Keywords:** *Solanum lycopersicum* L., Nematode, microorganisms beneficial, microbial consortium.

#### INTRODUCTION

In Mexico, 70% of the crops produced in protected agriculture correspond to tomato

(Solanum lycopersicum L.) (SIAP, 2018). In the state of Zacatecas, 74.2% of the area produced in a protected environment is with tomato (Lara-Herrera et al., 2019). 80% of the horticultural crops that are produced in a protected environment in Mexico are grown in soil. Fusarium oxysporum f. is one of the microorganisms that most affect the tomato crop, which is responsible for yield losses of up to 60% (González et al., 2012) and nematodes represent one of the main pests of the crop, the genus Meloidogyne is the more distributed and harmful, causing yield losses of 20-33% worldwide (Hernández-Ochandía et al., 2015).

Monoculture and the indiscriminate use of agrochemicals have reduced the biodiversity of agroecosystems, causing a high incidence of pests and diseases in crops (Altieri and Nicholls, 2007). Also, with the inappropriate use of chemical products, pests and diseases become resistant (FAO, 2012).

It is of great interest to obtain alternatives to control pests and soil diseases that are effective, ecological and economical (Avelar - Mejía et *al.*, 2018). There are microorganisms through which it is possible to control phytopathogens. Through the use of a beneficial microbial consortium (BMC) it can be achieved to improve the physical, chemical, biological properties of the soil and the suppression of diseases. A BMC is a natural association of two or more microbial populations of different species where all benefit from each other's activities (Cadavid *et al.*, 2016).

The objective of the work was to compare the use of a BMC against the chemical control (CC), and the combination of these in the production and quality of tomato in greenhouse soil and the health of the plants with respect to *Fusarium spp.* and *Meloidogyne spp.* 

#### **MATERIALS AND METHODS**

### LOCATION AND ENVIRONMENTAL CONDITIONS WHERE THE RESEARCH WORK WAS ESTABLISHED:

The present work was carried out in two greenhouses with passive climate control, with a surface area of 7,800 m<sup>2</sup> in each greenhouse; during the months of February to July 2019. At Rancho Santa Rita, Panuco, Zacatecas, Mexico, at the coordinates: Latitude 23° 2′ 52.92″ N, Longitude 102° 36′ 4.42″ O.

#### BENEFICAL MICROBIAL CONSORTIUM (BMC):

The BMC that was used is a product that was obtained commercially and the microorganisms that it contains are: Aspergillus orizea. Candida utilis, Bacillus subtilis, (variety Natto). bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium Lactobacillus acidophilius, longum, Lactobacillus Lactobacillus bulgaricus, casei, Lactobacillus delbrecki, Lactobacillus plantarum, Lactococcus lactis, Micromonospora thermoactinomyces, Micromonospora frankia, Micromonospora actinomyces, Rhodobacter Rhodopseudomonas spaeroides, palustris, thermophilus, Streptococcus Streptococcus lactis, Streptococcus fecallis, Streptomyces albus, Sacharomyces cerevicae, Mucuor hiemalis and Protoionobacterium freudenreichil (Bacteria), Mucuor hiemalis. Aspergillus orizea, and Candida you use (Fungi), as well as the yeast Sacharomyces cerevicae. The microbial load was 1,500,000 CFU/ mL of sample.

#### TREATMENTS AND MANAGEMENT OF THE EXPERIMENT:

The biological control was evaluated through the application of the BMC, the chemical control and the combination of both; Six treatments resulted, which consisted of:

- (1) BMC applied weekly to soil and foliage,
- (2) BMC applied every week to the soil and to the foliage every two weeks,
- (3) BMC applied every two weeks to the soil and foliage,
- (4) BMC applied weekly to the soil and foliage + chemical control,
- (5) BMC applied every week to the soil and to the foliage every two weeks + chemical control, and
- (6) chemical control, as control treatment.

In the treatments where the BMC was applied, for the applications to the soil they were carried out by means of drip irrigation, the applied dose was 0.51 L of BMC diluted in 5.1 L of water for each experimental unit. For foliar applications, the doses were 0.1, 0.5, and 1.0 L of BMC for every 10 L of water [up to 43, 85, and 127 days after transplantation (dat), respectively], in each of the sprays made. The doses were the same for all the treatments that included the BMC.

The experimental unit consisted of three beds with plastic mulch, each one 30 m long and 0.60 m wide, planted in double rows, at a density of 2.6 plants m<sup>-2</sup>. In each treatment, five repetitions were carried out in a randomized complete block experimental design, the useful plot was the central bed.

Treatments 1, 2 and 3 were established in a module (Greenhouse 7) in which only the BMC was applied, within the same shed the three beds per treatment were selected. In another module (Greenhouse 9) the three beds of each of the remaining treatments (4, 5 and 6) were selected.

Management (irrigation, nutrition, management, leaf pruning, lateral shoot pruning, weeding, pollination, and harvest) was the same for the six treatments.

The plant material that was established was a hybrid variety called "El Cid" (Harris Moran") of saladette type with indeterminate

growth, although for the evaluation of this work only ten bunches were determined.

The soil in which the plants were developed (Greenhouses 7 and 9) is sandy clay loam (51.48% sand, 28.52% clay and 20.00% silt) with a pH of 7.65. The soil was previously disinfected, 21 days before transplanting, with 40% metam sodium and a dose of 400 L ha<sup>-1</sup>.

The application of the BMC in the soil began one day after the transplant, from then on, the applications were weekly and biweekly, according to the frequency of application of the corresponding treatments. Foliar applications began at 30 dat and thereafter every week and biweekly, depending on the frequency of each treatment. Both soil and foliar applications were always made in the afternoon. To control some pests that occurred during production in Greenhouse 7, a product based on soybean vegetable oil was used, and for Greenhouse 9 only commercial chemical products were used for the different pests that occurred.

applications agrochemical drip irrigation, since the place where the experiment was established are commercial production greenhouses, there was only a record of the applications made during production for the control of phytopathogens. In addition, foliar applications were made to control some pests and diseases that occurred during the development of the crop, as well as some biostimulants, this for Treatments 4, 5 and 6. The products used were: Movento (Spirotetramat), Beleaf (Flonicamid) [Insecticides], (Ciazofamide), Ran man Curzate (Cymoxanil + chlorothalonil) [Fungicides] and Bionare, Maxi Grow [ Biostimulants ].

#### **MEASURED VARIABLES**

Three plants were selected from each experimental unit, where the central bed was taken and from this three plants located in the center of it were selected.

#### IN THE PLANT:

Stem growth length (SGL): It was taken using the mark of a previous week up to the point of growth. Leaf length (LL): The recently ripe leaf was used, the one between the cluster that is setting and the flowering cluster was taken as reference.

Flower cluster length to apex (FCLA) and flowering stem length (FSL) were also measured. All the mentioned variables were measured with a tape measure, once a week from the transplant to the apical sprouting of the plants, the sprouting was determined by the number of bunches, which was ten. Stem diameter (SD): Whose measurement was made with a vernier, the place that was taken as a reference to carry out this measurement was the point marked a week before as the growth point and taking the thinnest or flattest part of the stem.

#### IN THE FRUIT

Number of fruits: The number of fruits harvested during the cycle was counted, only fruits that reached their physiological maturity were collected, which were in the state of: star or striped, orange and red.

Fruit weight. - By using a Santul digital scale with a precision of 1.0 g.

Fruit size: It was obtained at harvest time using a vernier, the measurements taken were equatorial and polar diameter, in cm.

performance. - The average fruit weight was taken based on the number of plants evaluated and the planting density (2.6 plants m  $^{-2)}$  to obtain the data in kilograms per square meter.

Fruit quality: To evaluate the quality of the fruit, the firmness of the fruit was determined, for which a penetrometer was used to measure the resistance of the fruit to penetration, the data obtained were expressed in kg cm<sup>-2</sup>. Total soluble solids (TSS), expressed in degrees Brix, were also evaluated using a refractometer with

a sensor, to which a sample (drop) obtained from the fruit juice was applied. The fruit that was used was the same as for the firmness measurement.

Two samples were taken at different stages of production with the same physiological maturity index for all of them, that is, the same color. For the first sample, fruits were taken from the fourth bunch and for the second evaluation from the eighth, considering the same position of fruits in the bunch and visually the same size. Five repetitions of each treatment, and three fruits of each repetition were evaluated.

#### PERCENTAGE OF DAMAGE BY FUSARIUM IN PLANT (PDF):

To measure the PDF, it was qualitative and quantitative, for the first case the level of incidence of the disease in leaves of the plant was taken as a reference, they were categorized from 1 to 5, where 1 was: healthy plant, 2: presence of the disease with some spots on only one of its compound leaves, 3: the presence of the disease manifests itself in no more than three of its leaves, 4: the disease occurs in more than 50% of the plant and 5: completely dead plant. For the quantitative measurement, the plants with the presence of damage were counted, removing the percentage of damaged plants from the total of each unit.

#### ROOT DAMAGE BY NEMATODES (DN):

Five repetitions of each treatment were taken at the end of the cycle, a block of soil with plant root of 0.40 m x 0.40 m and 0.40 m width x length and depth was extracted, after the extraction the soil from the root was removed and immediately a wash was done. To measure the level of damage, it was determined visually according to the presence of root nodules, the measurement being from

1 to 5, where 1 represented a healthy root and 5 roots were 100% damaged.

#### **EXPERIMENTAL DESIGN**

There were five repetitions for each treatment, in the same way the central bed was taken where the plants to be evaluated were located, the distance between each selected repetition was five meters.

A randomized complete block experimental design was used. The data was submitted to an analysis of variance (ANOVA), to the mean comparison test (Tukey,  $\alpha$ =0.05), and to the correlation analysis between the variables evaluated. The above using the program SAS (Statistical analysis System) v. 9.4.

#### RESULTS AND DISCUSSION IN THE PLANT

In general, SGL and SD were the most sensitive variables to the applied treatments and only LL was not affected. Treatment 5 presented the highest growth, contrasted with Treatment 3 with the lowest growth. The SGL was more affected in treatments 3 and 4, which differed from the other treatments; LL did not present differences, SD was higher in Treatment 5, the greatest contrast was with respect to Treatment 4; FCLA was higher in Treatment 5, which only differed from Treatment 3; Treatment 5 had the highest FSL than Treatments 2, 3 and 6.

Although there was no uniform behavior of the different growth variables, it is notorious that with Treatment 5, that is, with the weekly application of the BMC in the soil and every fortnight to the leaves + the CC, the highest growth was obtained (SGL, SD, FCLA, FSL), with respect to Treatments 3 and 4 for SGL, with respect to all other treatments for SD, with respect to Treatment 3 in FCLA, and with respect to Treatments 2, 3 and 6 for FSL (Table 1).

#### IN THE FRUIT

The variables that were taken into account for the production of fruits were the number (FN), weight (FW) and size of fruits (polar diameter, PD and equatorial diameter, ED) per plant, as well as the fruit yield in kilograms per square meter. Of these variables, only the PD was not sensitive to the tested treatments: in the other variables, Treatment 4, consisting of the CC and the weekly application of the BMC, presented the most adverse condition. The highest FN occurred in Treatments 1 (bacterial consortium applied weekly) and 5 (chemical control and weekly application to the soil and biweekly to the foliage of the bacterial consortium); the FW was higher in Treatments 1, 2 and 3, consisting of the application of BMC at different frequencies (1 weekly in soil and foliage. 2 weekly in soil and biweekly in foliage, and 3 biweekly in soil and foliage) in all these treatments the CC was not used. The ED was higher in Treatments 2 and 3, statistically it differed from Treatment 4. Fruit yield was lower in Treatment 4 (CC and weekly application of BMC), among all other treatments there were no statistical differences, however, a greater tendency can be seen with Treatment 1, that is, with the application of the BMC without the use of the CC (Table 2).

In general, the application of the BMC favored most of the production variables, mainly fruit weight and yield. Alfonso et al. (2005) also report benefits in tomato yield by improving the conditions for plant development with the help of beneficial microorganisms of the genera Pseudomonas, Azospirillum, Azotobacter, Bacillus Streptomyces. However, the beneficial effect of BMC decreased when combined with CC, mainly when BMC was applied weekly to the soil and foliage (Treatment 4), which can be attributed to the inactivation of beneficial microorganisms with the chemicals., Rivera

Treatments	SGL (cm)	LL (cm)	SD (mm)	FCLA (cm)	FSL (cm)
1	26,769 a †	34,323 a	8,554 b	21,895 ab	3,686 ab
2	25,624 ab	33,803 a	7,967 bc	19,552 ab	3,374 b
3	23,135c	33,629 a	7,893 bc	17,990 b	3,242 b
4	24,599 bc	32,114 a	7,705 c	20,369 ab	3,578 ab
5	26,803 a	33,470 a	9,433 a	22,420 a	3,991 a
6	26,037 ab	31,679 a	7,840 bc	18,301 ab	3,475 b

<sup>&</sup>lt;sup>†</sup> Equal letters followed by each number in each column are statistically equal ( $p \le 0.05$ ).

Table 1. Stem growth length (SGL), leaf length (LL), stem diameter (SD), flower cluster length to apex (FCLA) and flowering stem length (FSL) in tomato plants in greenhouse for the effect of controlling soil diseases through the use of a bacterial consortium and the combination of this with chemical control.

Treatments	FN	FW (g fruit <sup>-1</sup> )	PD (cm)	ED (cm)	Yield (kg m <sup>-2</sup> )
1	203,40 a $^{\dagger}$	76,72 a	6,12 a	4,35 a	13,508 a
2	197,00 ab	75,72 a	6,07 a	4,44 a	12,916 a
3	178,20 ab	77,30 a	6,12 a	4,41 a	11,936 a
4	167,60 b	63,59 b	5,78 a	4,02 b	9,205 b
5	207,20 a	69,85 ab	5,94 a	4,23 ab	12,552 a
6	186,20 ab	71,61 ab	6,10 a	4,29 ab	11,545 a

<sup>&</sup>lt;sup>†</sup> Equal letters followed by each number in each column are statistically equal (p ≤ 0.05).

Table 2. Number (FN), weight (FW), polar diameter (PD), equatorial diameter (ED) and yield of tomato fruits in greenhouses, due to effect of root-soil disease control through the application of a beneficial bacterial consortium (BMC) and the BMC combined with the chemical control.

Treatments	FIR (kg cm <sup>-2</sup> )	TSS (°Brix)
1	2,938 ab †	5,010 bc
2	2,903 b	5,177abc
3	3,063 ab	4,903 c
4	3,227 a	5,470 a
5	3,008 ab	5,340 ab
6	2,980 ab	5,267 abc

<sup>&</sup>lt;sup>†</sup> Equal letters followed by each number in each column are statistically equal ( $p \le 0.05$ ).

Table 3. Firmness (FIR) and total soluble solids (TSS) in tomato fruits grown in greenhouses due to the effect of root-soil disease control through the application of a beneficial bacterial consortium (BMC) and the BMC combined with the control chemical.

et al. (2010) mention that with the intensive use of agrochemicals the effectiveness of the microbiota in the soil can be negatively affected.

#### FRUIT QUALITY

Thequalityparameters of the evaluated fruits were firmness (FIR) and total soluble solids (TSS). The best behaviors in both variables occurred in Treatment 4 (Combination of the weekly application of the BMC with the CC), although with this treatment the lowest production and yield of fruits were obtained, these had greater consistency and concentration of soluble solids, the which provide favorable qualities such as flavor and shelf life. De la Rosa-Rodriguez et al. (2018) reported lower yield, but better quality of tomato fruits when subjecting the plants to water stress. The treatments that presented lower fruit quality (FIR and TSS) were 1, 2 and 3, that is, those that were treated with the bacterial consortium (Table 3).

#### PHYTOSANITARY INDICES

The incidence of diseases caused by Fusarium spp. (Percentage of Fusarium damage, PDF) was evaluated during the crop cycle. In general, from the beginning and during the cycle, there was a lower PDF in the treatments with CC. In the treatments with the BMC, the PDF, although at the beginning it was low, was higher than with the CC, this behavior was maintained throughout the cycle, however, with the weekly application of the BMC, both in soil and foliar form, the PDF was lower that the biweekly applications of the BMC; in the final stage of the cycle, with the weekly application to both the soil and the foliage of the BMC, the PDF was similar to the treatments with CC (Figure 1).

The damages by nematodes (DN) were evaluated at the end of the cycle, statistically there were no differences, although a tendency

is observed that in the treatments where the BMC was applied without the CC, that is, in treatments 1, 2 and 3, the damages tended to be minor (Table 4). At the end of the cycle, the ND were high in all treatments. Jonathan *et al.* (2000) report favorable effects of bacteria (*bacillus cereus*, *B. subtilis*, *B. sphaericus*, *Agrobacterium radiobacter*, *Pseudomonas fluorescens*, *P. chlororaphis*, and *Burkholderia cepacia*) against *Meloidogyne*, suppressing the development of galls on tomato roots and also these bacteria reduced the reproduction of the nematode.

The beneficial effect on the number, weight and yield of fruits with the treatments where the BMC was applied and not the CC was greater than those where this control was applied; this behavior may be related to the inactivation of microorganisms with chemical products. García-Gutiérrez and Rodríguez-Meza (2012) mention this effect due to the excessive use of chemical pesticides, mainly: herbicides (paraquat, glyphosate), followed by insecticides (organophosphates: methyl parathion, methamidophos, malathion) and fungicides (mancozeb and chlorothalonil) to control both pathogens and insect pests.

On the other hand, the presence of phosphate solubilizing bacteria did not differ between treatments, but it did differ in the percentage of solubilizing bacteria, with Treatment 1 being the most favored.

#### CONCLUSIONS

In the plant growth variables (SGL, LL, FCLA and FSL), in general, the treatment that presented the greatest growth was the application of the BMC to the soil every week and to the foliage every fortnight + the CC, contrasted with Treatment 3 (application of the BMC every fifteen days on the ground and on the foliage).

With exception of treatment 4 (weekly application of BMC with CC), there were no

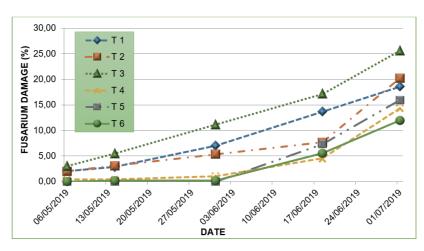


Figure 1. Damage caused by *Fusarium* spp. in tomato plants during the development of the greenhouse crop, due to the effect of root-soil disease control through the application of a beneficial bacterial consortium (BMC) and BMC combined with chemical control.

Treatments	DN	
1	3,2 a <sup>†</sup>	
2	3,2 a	
3	3,6 a	
4	3,8 a	
5	3,8 a	
6	4,2 a	

<sup>&</sup>lt;sup>†</sup> Equal letters followed by each number in each column are statistically equal (p ≤ 0.05).

Table 4. Index of damage caused by nematodes (DN) in tomato plants during the development of the greenhouse crop, due to the effect of controlling diseases in the root-soil through the application of a beneficial microbial consortium (BMC) and the combined BMC with chemical control.

differences in fruit yield. Therefore, with the use of the BMC the performance was equal to the use of CC.

Fruit quality (FIR and TSS) had better behavior in Treatment 4 (weekly BMC in soil and foliar + CC) although this was the one with the lowest production and yield, qualities such as flavor and shelf life favored it.

During the crop development cycle, there was a lower percentage of damage by *Fusarium* (PDF) in the treatments with CC; In the treatments with the BMC, although the PDF

was low at the beginning, it was progressively higher than with the CC; however, with the weekly application of the BMC, both in edaphic and foliar form, at the end of the cycle the PDF was lower than with the applications biweekly.

There was a significant incidence of nematodes in the intermediate stage of the cycle, however, in the final stage it was reduced, although the damage to the plant was high, there were no differences between treatments in this variable.

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