

## 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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***Hector Alfredo Ortiz-Ramirez***

“Universidad Autónoma de Zacatecas”,  
Academic Unit of Agronomy  
Zacatecas, Mexico  
<https://orcid.org/0000-0006-1129-6697>

***Alejandro Alarcon***

Postgraduate College, Microbiology Area  
of the Pedology Postgraduate, Montecillos,  
Texcoco, Edo. From Mexico, Mexico  
<https://orcid.org/0000-0002-7212-7751>

***Rodolfo de la Rosa-Rodriguez***

“Universidad Autónoma de Zacatecas”,  
Academic Unit of Electrical Engineering  
Zacatecas, Mexico  
<https://orcid.org/0000-0002-0795-5147>

***Martha Patricia Spain-Moon***

“Universidad Autónoma de Zacatecas”,  
Academic Unit of Agronomy  
Zacatecas, Mexico  
<https://orcid.org/0000-0002-7797-3375>

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**Julio Lozano-Gutierrez**

“Universidad Autónoma de Zacatecas”,  
Academic Unit of Agronomy  
Zacatecas, Mexico  
<https://orcid.org/0009-0009-9760-6749>

**Alfredo Lara-Herrera**

‘Universidad Autónoma de Zacatecas’,  
Academic Unit of Agronomy  
Zacatecas, Mexico  
Orcid : 0000-0002-3961-2608

**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 (CMB), the combination of CMB with chemical control (CQ) and only CQ 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 CMB applied to the soil weekly and biweekly by foliar application + CQ presented the best results. There were no significant differences in yield, only the treatment where the CMB + CQ 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 CMB in the soil and in the foliage and without the CQ, the best performance of the crop was obtained.

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

## 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 (CMB) it can be achieved to improve the physical, chemical, biological properties of the soil and the suppression of diseases. A CMB 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 CMB against the chemical control (CQ), 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.

### **CHARITABLE MICROBIAL CONSORTIUM (CMB):**

The CMB 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 longum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus delbrecki*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Micromonospora thermoactinomyces*, *Micromonospora frankia*, *Micromonospora actinomyces*, *Rhodobacter spaeroides*, *Rhodopseudomonas palustris*, *Streptococcus thermophilus*, *Streptococcus lactis*, *Streptococcus fecallis*, *Streptomyces albus*, *Sacharomyces cerevicae*, *Mucor hiemalis* and *Protoionobacterium freudenreichil* (Bacteria), *Mucor hiemalis*. *Aspergillus orizea*, and *Candida you use* (Fungi), as well as the yeast *Sacharomyces cerevicae*. The microbial load is 1,500,000 CFU/ mL of sample.

### **TREATMENTS AND MANAGEMENT OF THE EXPERIMENT:**

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

- (1) CMB applied weekly to soil and foliage,
- (2) CMB applied every week to the soil and to the foliage every two weeks,
- (3) CMB applied every two weeks to the soil and foliage,
- (4) CMB applied weekly to the soil and foliage + chemical control,
- (5) CMB 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 CMB 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 CMB 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 CMB for every 10 L of water [up to 43, 85, and 127 days after transplantation (ddt), respectively], in each of the sprays made. The doses were the same for all the treatments that included the CMB.

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 (Nave 7) in which only the CMB was applied, within the same shed the three beds per treatment were selected. In another module (Nave 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 (Naves 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 CMB 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 Warehouse 7, a product based on soybean vegetable oil was used, and for Warehouse 9 only commercial chemical products were used for the different pests that occurred.

For agrochemical applications via 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], Ran man (Ciazofamide), 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 (LH) : 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 (LRA) and flowering stem length (LTR) 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  $kg\ cm^{-2}$ . 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 mx 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, LCT and DT were the most sensitive variables to the applied treatments and only LH was not affected. Treatment 5 presented the highest growth, contrasted with Treatment 3 with the lowest growth. The LCT was more affected in treatments 3 and 4, which differed from the other treatments; LH did not present differences, DT was higher in Treatment 5, the greatest contrast was with respect to Treatment 4; AKI was higher in Treatment 5, which only differed from Treatment 3; Treatment 5 had the highest LTR 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 CMB in the soil and every fortnight to the leaves + the CQ, the highest growth was obtained (LCT, DT, LRA, LTR), with respect to Treatments 3 and 4 for LCT, with respect to all other treatments for DT, with respect to Treatment 3 in LRA, and

with respect to Treatments 2, 3 and 6 for LTR (Table 1).

### IN THE FRUIT

The variables that were taken into account for the production of fruits were the number (NF), weight (PF) and size of fruits (polar diameter, DP and equatorial diameter, DE) per plant, as well as the fruit yield in kilograms per plant. square meter; Of these variables, only the PD was not sensitive to the tested treatments; in the other variables, Treatment 4, consisting of the CQ and the weekly application of the CMB, presented the most adverse condition. The highest NF 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 FP was higher in Treatments 1, 2 and 3, consisting of the application of CMB 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 CQ was not used. The DE was higher in Treatments 2 and 3, statistically it differed from Treatment 4. Fruit yield was lower in Treatment 4 (CQ and weekly application of CMB), 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 CMB without the use of the CQ (Table 2).

In general, the application of the CMB 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* and *Streptomyces*. However, the beneficial effect of CMB decreased when combined with CQ, mainly when CMB was applied weekly to the

Treatments	TBI (cm)	LH (cm)	DT (mm)	LRA (cm)	LTR (cm)
1	26,769 to †	34.323 to	8,554 b	21,895 ab	3686 ab
2	25,624 ab	33,803 to	7,967 BC	19,552 ab	3,374 b
3	23.135c	33,629 to	7893 BC	17,990 b	3,242 b
4	24,599 BC	32.114 to	7,705c	20,369 ab	3,578 ab
5	26,803 to	33,470 to	9433 to	22,420 to	3991 to
6	26,037 ab	31,679 to	7,840 BC	18,301 ab	3,475 b

† Equal letters followed by each number in each column are statistically equal ( $p \leq 0.05$ ).

Table 1. Stem growth length (LCT), leaf length (LH), stem diameter (DT), bunch-to-apex length (LRA) and stem-to-bunch length (LTR) in flowering 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	NF	FAQ (g fruit <sup>-1</sup> )	PD (cm)	OF (cm)	YIELD (kg m <sup>-2</sup> )
1	203.40 to †	76.72 to	6.12 to	4:35 a.m.	13,508 to
2	197.00 ab	75.72 to	6.07 to	4.44 to	12,916 to
3	178.20 ab	77.30 a.m.	6.12 to	4.41 to	11,936 to
4	167.60b	63.59b	5.78 to	4.02b	9,205 b
5	207.20 a	69.85 ab	5.94 to	4.23 ab	12,552 to
6	186.20 ab	71.61 ab	6.10 a	4.29 ab	11,545 to

† Equal letters followed by each number in each column are statistically equal ( $p \leq 0.05$ ).

Table 2. Number (NF), weight (PF), polar diameter (DP) and equatorial diameter (DE) of tomato fruits in greenhouses due to the effect of root-soil disease control through the application of a beneficial bacterial consortium (CMB) and the CMB combined with the chemical control.

Treatments	FIR (kg cm <sup>-2</sup> )	OSH (°Brix)
1	2,938 ab †	5010 BC
2	2,903 b	5.177abc _
3	3063 ab	4,903c
4	3227 to	5,470 to
5	3008 ab	5,340 ab
6	2,980 ab	5.267abc _

† Equal letters followed by each number in each column are statistically equal ( $p \leq 0.05$ ).

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 (CMB) and the CMB combined with the control chemical.

soil and foliage (Treatment 4), which can be attributed to the inactivation of beneficial microorganisms with the chemicals., Rivera *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

The quality parameters 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 CMB with the CQ), 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-Rodríguez *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 SST) 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 CQ. In the treatments with the CMB, the PDF, although at the beginning it was low, was higher than with the CQ, this behavior was maintained throughout the cycle, however, with the weekly application of the CMB, both in soil and foliar form, the PDF was lower. that the biweekly applications of the WBC; in the final stage of the cycle, with the weekly application to both the soil and the foliage of the CMB, the PDF was similar to the treatments with CQ (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 CMB was applied without the CQ, 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 CMB was applied and not the CQ 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 (LCT, LH, LRA and LTR), in general, the treatment that presented the greatest growth was the application of the CMB to the soil every week and to the foliage every fortnight + the CQ, contrasted with Treatment 3 (application of the CMB every fifteen days on the ground and



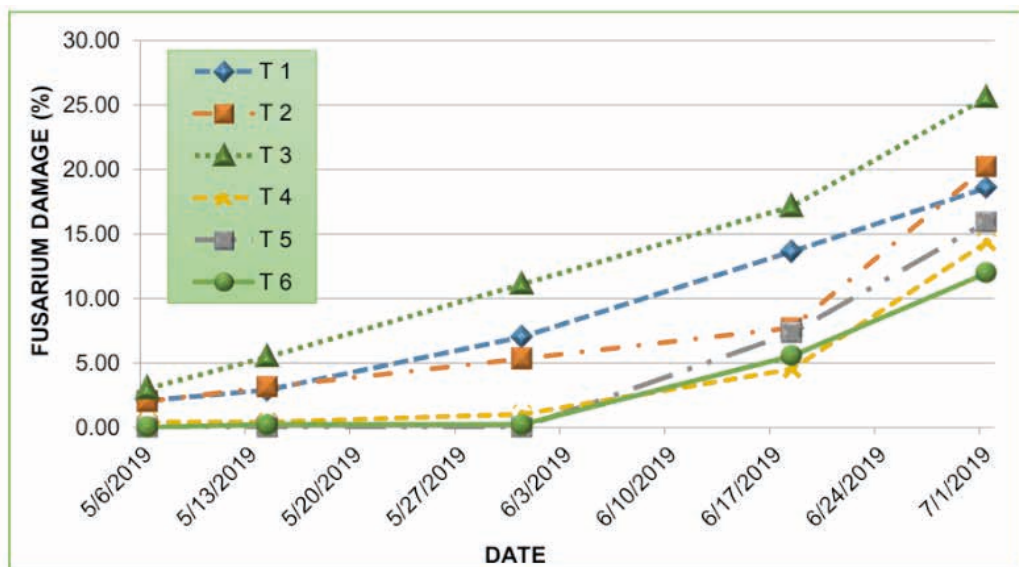


Figure 1. Damage caused by *Fusarium* spp. in tomato plants during the development of the greenhouse crop, due to the effect of root-soil disease control through the application of a beneficial bacterial consortium (CMB) and CMB combined with chemical control.

Treatments	DN
1	3.2 to †
2	3.2 to
3	3.6 to
4	3.8 to
5	3.8 to
6	4.2 to

† Equal letters followed by each number in each column are statistically equal ( $p \leq 0.05$ ).

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 (CMB) and the combined CMB with chemical control.

on the foliage).

With the exception of treatment 4 (weekly application of CMB with CQ), there were no differences in fruit yield. Therefore, with the use of the CMB the performance was equal to the use of CQ.

Fruit quality (FIR and SST) had better behavior in Treatment 4 (weekly CBB in soil and foliar + CQ) 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 CQ; In the

treatments with the CMB, although the PDF was low at the beginning, it was progressively higher than with the CQ; however, with the weekly application of the CMB, 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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