Article

Physical, chemical and natural alternatives to control *Meloidogyne* spp. in tomato in greenhouse

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Abstract

The objective of the present work was to evaluate the effectiveness of control of *Meloidogyne* spp., through the use of physical, chemical and natural alternatives, physics with solarization, chemistry with the nematode Vidate and the natural one by means of the interspersed plantation of cempasuchil (*Tagetes erecta*) between tomato plants, as well as the combination between the alternatives used. The experiment was carried out in a commercial greenhouse, located in the community of Chaparrosa, municipality of Villa de Cos, Zacatecas, in 2012. An experimental design was used in complete blocks at random with four repetitions and experimental units of a plant. Water, fertilizer and other agrochemicals were applied by drip, with the handling that the producer normally does. Twelve variables were measured, result of count of nematodes, bunches and fruit weight in different dates. The analysis of variance detected significant statistical differences in seven of the variables; with the results and the correlation analysis that was performed among the variables, it was concluded that, with the inclusion of a cempasuchil plant interspersed between tomato plants, as well as this combined treatment with solarization, the lowest number of nematodes and the greater yield of fruits.

Keywords: *Licopersicon esculentum* Mill., *Tagetes erecta*, cempasúchil, nematodes, solarization.

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Introduction

The tomato (*Lycopersicon esculentum* Mill.), is the most important vegetable in the world and in Mexico; in 2014, total world production was 5 023 819 t and an average yield of 55.1 t ha^{-1} (FAO, 2017). While in greenhouse only on a surface of 631 884 ha was obtained on average a yield of 200 t ha^{-1}, and a production of 126 376 800 t^{-1}. The countries with the largest area of tomato grown in the greenhouse are, in hectares: China 139 080, Italy 22 692, Spain 21 594, Japan 18 666, Turkey 5 124, Morocco 3 660, United States of America 3 385 France 3 367 and Mexico 3 251 (Castellanos, 2008).

In Mexico, the states with the largest area of tomato in protected environment are: Sinaloa, Baja California, Baja California Sur, Sonora, Jalisco, San Luis Potosí, Puebla and Zacatecas, the latter entity had an area of 230 ha in 2008 (Castellanos and Borbon, 2009) and 415.5 ha in 2015 (Padilla *et al*., 2016), the state of Zacatecas ranks eighth national in greenhouse tomato production, with an average yield of 176 t ha^{-1}, against 36 t ha^{-1} that are obtained in the open; under these conditions, tomato cultivation in the greenhouse represents an important option for agricultural producers; in addition, the climatic conditions in the state are difficult for open-pit cultivation due to the presence of frost during a long period of the year, strong winds and hailstorms, among others (Castellanos, 2008). It should be considered that tomato production in the greenhouse increases the cost of production and propitiates the appropriate conditions for the development and attack of pathogens, which requires taking control measures to reduce or eliminate the damage (Gullino *et al*., 2002).

The most important greenhouse tomato diseases in the state of Zacatecas are: bacterial cancer (*Clavibacter michiganensis*), late blight (*Phytopththora infestans*), botrytis (*Botryis cinerea*), ashtray (*Leveilula taurica*), early blight (*Alternaria solani*) and attack of root-knot nematodes of the *Meloidogyne* genus, the symptoms caused by the nematodes are: dwarfing of the plant, yellowing of the leaves, low production and the presence of galls in the root. Infected plants have symptoms of water deficiency in the hottest hours because the roots are damaged and absorb less water (Carrillo *et al*., 2000).

In Zacatecas and especially in greenhouse soils of the municipality of Chaparrosa, municipality of Villa de Cos, Zacatecas, where 32.5% of the surface of greenhouses of the state is located (SIAP, 2104), there have been severe problems due to the presence and attack of nematodes of the genus *Meloidogyne* spp., which have been tried to control through the application of chemical products (nematicides), which to date has not given favorable results. In addition, pesticides are an important factor in the contamination of soil, aquifers and crops, as well as damage to human health due to its high degree of toxicity, (Zavaleta *et al*., 2002; Wang *et al*., 2007; Padilla *et al*., 2015), due to its high capacity to create resistance, this form of control has not been effective, as its incidence and dissemination is increasing and its effects on production are economic considerations.

Given the alternative of chemical control that has had little effectiveness to control the damage caused by nematodes, there are successful reports of physical alternatives, such as soil solarization (Candido *et al*., 2008; Vuelta *et al*., 2015), natural alternatives to base of the use of plants like cempasuchil which exudes chemical compounds that antagonize with the nematodes (Gómez and Zavaleta, 2001; Wang *et al*., 2007) or the use of extract of this same vegetable species (Natarajan *et al*., 2006).

The objective of this study was to evaluate the control of *Meloidogyne* spp., which infects tomato in the greenhouse, using strategies other than chemical control such as solarization (physical), cempasuchil (natural), the nematode Vidate (chemical) and the combinations of them. The working hypothesis was that *Meloidogyne* spp., can be controlled efficiently in greenhouse tomato cultivation, by using strategies different from chemical control.

Materials and methods

Study area

The present work was carried out in 2012, in a Batisierra type greenhouse, located in the Chaparrosa community of the municipality of Villa de Cos, Zacatecas. The community is located at 23º 06’ north latitude and 102º 22’ west longitude, at 1 900 meters above sea level. Its climate is subtropical arid temperate, with an average annual temperature of 17 ºC and frosts from the end of october to the beginning of april. The average annual precipitation varies from 400 to 500 mm (Medina *et al*., 2003). Predominantly flat soils of Feozem and Litosol type, whose chemical characteristics are: pH= 7.6, CE= 0.83 dS m^{-1}, N-NO_3= 62 mg kg^{-1}, MO= 1.51%.

Treatments, design and experimental unit

Eight treatments were tested: 1) plants of cempasuchil (*Tagetes erecta*) (C), located alternately with tomato plants; 2) solarization of the floor with transparent plastic gauge 620 gauge, equivalent to 155 μm thick, placed at the beginning of the work covering the width of the bed and 40 m in length (S); 3) application of the nematode Vidate (N) in the dose of 4 L ha^{-1}, applied at the moment of the transplant using the drip irrigation system; 4) S+C; 5) S+N; 6) N+C; 7) C+S+N; and 8) witness (without any treatment) (T). The cempasuchil plants were transplanted when they were 30 days old, on February 2 and seven days later the tomato was transplanted. An experimental design was used in complete blocks at random with four repetitions, the experimental unit consisted of a tomato plant.

Experiment management

The work was carried out in natural soil in a Batisierra type greenhouse, which has a drip irrigation system and fertilizer injection. The variety of tomato “El Cid” was used, with Saladette type fruits, because it is one of the varieties that are most cultivated in Zacatecas. A seedling developed in polystyrene trays with alveoli of 25 mL was used in the greenhouse; the transplant was performed on February 9, 2012.

Practices carried out

The population of plants that was established, in the ship where the experiment was developed, was 24 000 per hectare; the distance between beds was 1.40 m and between plants 30 cm. The cultivation practices that were carried out were the same in the experimental plot and in the commercial plantation and consisted mainly in the application of fertilizer from the transplant and then every week, by means of fertigation. When transplanting, 300 kg ha^{-1} of di-ammonium phosphate (18-46-00) were applied, after which the dose was applied in total: 280-00-200 in the following weeks, based on soluble fertilizers; 100 units of CaO and 50 units of MgO were also applied, based on calcium nitrate and magnesium sulfate, respectively.

A daily watering of one hour was applied until the fifth bunch was taken (July 6, 2012); from which were applied two daily irrigations of 1.5 h each, until the end of the harvest. Weeding was done manually, three weeding was done: April 7, may 4 and June 8. At the time of the appearance of the pests whitefly (*Bemisia tabaci*), mite or red spider (*Tetranychus urticae*), paratrioza or jumping aphid (*Bactericera cockerelli*), soldier worm (*Spodoptera exigua*) and thrips (*Frankliniella occidentalis*), were controlled with application of agrochemicals, Cyromazine (Trigard), Abamectin, Imidacloprid (Confidor), Thiodicarb (larvin 375) and Abamectin, respectively in its commercial dose, as well as the control of crown rot by *Fusarium* (*Fusarium oxisporum*), *Verticillium* wilt (*Verticillium dahliae*), ashtray (*Leveillula taurica*), bacteria (*Clavibacter*, *Pseudomonas* and *Xanthomonas*), early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*), were controlled with the application of agrochemicals: Carbendazim (Derosal + Previcur), Cimoxanil + Mancozeb (Curathane), Bayleton, Dithane M-45, Carbendazin and Propamocarb+mancozeb (Bavistin 500 SC), respectively, in their commercial doses.

Plant management

Pruning of axillary and leaf buds was performed each time they were required; in a cycle in which 8 to 10 bunches were developed. The tutored was made with raffia and rings on the stem to avoid hanging it. The blunting was done when the plant had 8 to 10 clusters tied the thinning of fruits was not carried out.

Measured variables

Populations of nematodes and galling. Nematode populations in the soil (males and juveniles) were determined before starting the treatments and at the end of the crop cycle, for which soil samples were analyzed by centrifugation and sugar flotation technique, proposed by Zuckerman *et al*. (1985). The percentage of galling in plants attacked by *Meloidogyne* was also determined, according to the scale proposed by Bridge and Page (1980): 0= no gills; 1= small gills, hardly visible, the main roots, clean; 2= only small but clearly visible gills, with clean main roots; 3= some large gills, visible and clean main roots; 4= large gills predominate, but the main roots remain clean; 5= 50% of the roots infested, gills in parts of the main roots, reduced root system; 6= galling at the main roots; 7= most of the main roots grounded; 8= all the main roots grounded, few visible clean roots; 9= all roots severely gilled, the plant is usually dying; and 10= all severely rooted roots, radical system destroyed, the plant is dead.

Production evaluation. Approximately every week, as of June 9, a cluster was cut, the fruits produced by each experimental unit were weighed and quantified and classified by quality according to the following scale: First (more than 99 g $fruit^{-1}$), second (between 90 and 99 g) and third (less than 90 g). The cuts are made in the phase of grated maturation.

Statistical analysis

The measured variables were subjected to analysis of variance and in those that were statistically significant, the means test was applied by the minimum significant difference (DMS) with $p< 0.05$. A correlation analysis between the variables was also performed. The computer program Statistical Analysis System (SAS) version 2002 was used.

Acceptance of applied survey

To evaluate the acceptance or rejection of adoption of other control strategies and especially the presence of cempasuchil plants, together with tomato plants, a survey was applied, one month before the end of the crop cycle, to the technical manager of the greenhouse, as the workers and the producer, which consisted of the following points.

Survey for workers. 1) the work in the management of the cempasuchil plants was difficult; 2) The work in the treatments was more laborious than in the witness; and 3) how did you think having worked with these new treatments?

Survey for the person in charge of the personnel. 1) labor was increased in some of the treatments; 2) there were problems regarding the orders that were given to the staff; and 3) there were problems on the part of the workers in handling the treatments.

Survey for the producer. 1) how he thought the establishment of cempasuchil plants; 2) proposal to improve treatments; and 3) obstacles to working with cempasuchil.

The evaluation of results. At the beginning of the investigation soil tests were made (February 3, 2012), to know the population of nematodes, at the end of the investigation (august 10, 2012) the second soil test was made, which allowed to determine if the population of *Meloidogyne* spp., decreased or increased based on the treatments applied. The results obtained were analyzed by means of a variance analysis, DMS ($p> 0.05$) and a correlation test was performed with the SAS version 2002 program.

Results and discussion

Variance analysis

The analysis of variance that was made to the 12 variables showed that there was no statistical significance ($p> 0.05$) for the variation factor in repetitions and if there were in seven variables of the treatments factor (Table 1). The non-statistical significance for repetitions is attributed to the fact that the experiment was carried out in a homogeneous environment, which is understandable, since it was carried out in the greenhouse.

Mean test of the variables that showed statistical significance

The greatest reduction in the number of nematodes occurred with the Cempasuchil treatment, although it was statistically the same as the treatments: cempasuchil + solarization + nematicide, solarization + cempasuchil, nemacidia, and solarization + nematicide (Table 2).

In all the variables where there were statistical differences, the treatment with cempasuchil was the most favorable, since it presented higher amount of fruits per cluster (R1 and R2), greater fruit weight (P1, P2 and P3), higher plant height, in addition to the lower population of nematodes, although in all the variables the treatment with cempasuchil was not different from other treatments in each variable (Table 2).

Table 1. Mean squares of the variables measured in the test experiment of different control forms of the *Meloidogyne* nematode in greenhouse tomato in Chaparrosa, Villa de Cos, Zacatecas (2012).

Factor of variation	GL	#1J_2	#2J_2	M1	M2	R1	R2	R3	R4	P1	P2	P3	AP
Repetitions	3	24.2 ns	6.9 ns	36.7 ns	1.58 ns	0.92 ns	0.53 ns	0.25 ns	0.2 ns	526 ns	209 ns	203 ns	426 ns
Treatments	7	10.3 ns	16.8 **	10.4 ns	4.43 ns	2.21 **	1 **	0.21 ns	1.14 ns	1658 **	435 **	1745 **	1745 **
Experimental error	21	13.1	2.8	14.5	4.08	0.46	0.46	0.46	0.75	333	136	217	160
CV (%)		65.4	34.6	78.1	107.8	22.7	9.48	34.1	12.1	16.6	10.5	14.4	14.2

GL= degrees of freedom; #1J2= number of juvenile nematodes in the first count; #2J2= number of juvenile nematodes in the second count; M1= number of adult nematodes in the first count; M2= number of adult nematodes in the second count; R1= number of bunches in the first count; R2= number of bunches in the second count; R3= number of bunches in the third count; R4= number of bunches in the fourth count; P1, P2 and P3= weight of the fruit in the first, second and third counts, respectively; AP= height of the plant; ns = not significant (p <0.05); *= significant with p< 0.05; **= significant with p< 0.01; CV= coefficient of variation.

Table 2. Averages of the variables measured in the test experiment of different control forms of the *Meloidogyne* spp. Nematode in greenhouse tomato in Chaparrosa, Villa de Cos, Zacatecas.

Treatment	J2	R1	R2	P1	P2	P3	AP
Solarization (S)	8.25 a	3.25 abc	7 bc	131 a	116 abc	95 b	89 bc
Control	6.5 ab	3 ab	7.25 abc	113 ab	109 bc	90 b	100 ab
C+N	6.5 ab	1.75 d	6.75 bc	84 c	103 c	103 ab	59 d
S+N	4.5 bc	3.25 abc	7.5 ab	111 ab	105 bc	99 ab	97 ab
Nematicide (N)	3.75 c	2.75 bcd	6.5 c	104 bc	104 c	101 ab	75 cd
S+C	3.5 c	3.75 ab	7.5 ab	124 ab	123 ab	103 ab	116 a
C+S+N	3.25 c	2.25 d	6.75 bc	80 c	100 c	109 ab	66 d
Cempasuchil (C)	2.25 c	4 a	8 a	135 a	129 a	120 a	113 a
DMS (0.005)	2.45	1	1	27	17	22	19

Values with the same letter in the same column are the same according to the DMS (p<0.05); J2= nematode in juvenile state 2; R1= number of bunches in the first count; R2= number of bunches in the second count; P1, P2 and P3 = weight per fruit in the first, second and third counts, respectively; AP= plant height.

These results are evidence of the positive effect that the cempasuchil had on the control of nematodes, on the development of the plant and on the production of fruits. In the treatment with cempasuchil, regarding the control treatment, the population of nematodes was reduced 188%, the clusters per plant increased 33 and 10% for the first and second counting, in the weights per fruit the increase was 18, 33 and 13% for the first, second and third evaluations, respectively. The average weight of the fruits of the three evaluations where there were differences was 128 g in the treatment with cempasuchil and 104 g in the control.

With cempasuchil, in addition to reducing the number of nematodes in the evaluation at the end of the crop cycle, it also reduced the presence of root galls in a greater degree compared to the other treatments, the cempasuchil and the combinations C + S + N and S + C obtained a degree of galling of 3 according to the scale of Bridge and Page (1980) compared to the treatments solarization, vidate, N + C and S + N that presented a degree of galling of 4 and the control of 7, which it is attributed, according to Gomez and Zavaleta (2001); Natarajan *et al*. (2006); Wang *et al*. (2007), to which the plant exudes thiophenol compounds that scare away phytopathogenic nematodes.

By sharing the space of soil, water and nutrients, the roots of the tomato plants with those of cempasuchil, there was no negative effect on the growth or production of the plants, on the contrary, there was a favorable effect for the tomato, which is confirmed by Gómez and Zavaleta (2001) and Natarajan *et al*. (2006), it cannot be said that synergism was present because the biomass of the cempasuchil plants was not evaluated, because the main objective was to evaluate the effect on the tomato plants. Therefore, when these two species coexist during development in nematode infested soil under greenhouse conditions was expressed according to the evaluated variables, a positive effect on the development of the tomato plants.

In the control treatment, the number of nematodes remained high, possibly due to favorable conditions for the development of the nematodes in the soil; even under these conditions, the plants remained productive, which can be attributed to the adequate availability of nutrients and water; since the tomato plants affected with *Meloidogyne* spp., can produce well when the plant is not subjected to a stress condition (Salazar and Guzman, 2013), although infested soils without any type of control gradually become infested until they are made unproductive.

The variables of juvenile nematodes 2, adult 1 and adult 2, gave the same result referring to the population presented in the seven treatments. The treatments of cempasuchil and cempasuchil + solarization were the lowest in terms of the number of nematodes (Table 3), this indicates that these two treatments are the best, both to have low populations of the *Meloidogyne* spp. nematode, and to obtain a high yield of fruits. This is confirmed based on the results presented in Table 3, since the correlation coefficients between variable J2 and P3 are negative, as well as between M2 and P3, in both cases the coefficients are highly significant. These cases show the tendency that a higher fruit weight corresponds to a lower number of nematodes.

The results obtained in the present work agree with those obtained by Castro *et al*. (1990), Zavaleta *et al*. (2002), Natarajan *et al*. (2006), due to the effect of the compounds exuded by allelopathic plant species such as cempasuchil on *Meloidogyne* in the tomato crop, as well as when the cempasuchil is combined with plastic for solarization. Castro *et al*. (1990) indicate that the population of nematodes decreases when there are cempasuchil plants interspersed between tomato plants, meanwhile, Katan (1981), Pullman *et al*. (1989), Devay (1992) and Candido *et al*. (2008), point out that the plastic gives rise to temperatures above 40 °C in the soil, which are lethal for nematodes, however, it was not possible to reach those temperatures in the place where the experiment was carried out. that inside the greenhouse the solar rays do not enter directly and the temperature only increased to 30 °C in the soil, in these conditions it is not possible for the solarization treatment to perform the correct function because the climatological conditions in which the experiment was established were not they allowed to reach temperatures higher than 30 °C, possibly there could also be an increase in moisture retention and that favored that the plant was not stressed and had no nematode attack.

Table 3. Correlation coefficient between the variables measured in the test experiment of different control forms of the *Meloidogyne* nematode in greenhouse tomato in Chaparrosa, Villa de Cos, Zacatecas.

	J2	M1	M2	R1	R2	R3	R4	P1	P2	P3	AP
J1	-0.25	0.86*	-0.18	0.18	0.02	0.08	-0.13	0.04	0.19	-0.1	0.14
J2		-0.21	0.58**	-0.19	-0.01	0.04	0.14	-0.08	-0.22	-0.48**	0.21
M1			-0.15	0.13	-0.07	0.04	0.07	0.1	0.2	0.02	0.14
M2				-0.26	0.1	0.1	0.2	-0.1	-0.19	-0.5**	-0.21
R1					0.66**	0.22	0.24	0.58**	0.6**	0.14	0.8**
R2						0.07	0.15	0.44*	0.39*	0.04	0.68**
R3							0.18	0.2	0.21	-0.17	0.25
R4								0.13	0.14	0.21	0.11
P1									0.4*	0.1	0.66**
P2										0.18	0.58**
P3											0.1

Values without an asterisk are not significant with $p<0.05$ probability. *= significant with $p<0.05$, **= significant with $p<0.01$. J1; J2= nematode in juvenile state 1 and 2; M= adult nematodes in samples 1 and 2; R= number of bunches in sampling 1, 2, 3 and 4; P= weight of fruits in sampling 1, 2 and 3 respectively; AP= plant height.

It was not possible to determine the mechanism by which the combined effect of cempasuchil + solarization + nematicide, had a low yield of tomato fruits in the first and second cut dates, weight 1 and weight 2 (P1 and P2, Table 2), as well as few clusters, cluster 1 and cluster 2 (R1 and R2), although there was a low number of nematodes; Maybe there was some kind of antagonism between the components of the treatments that gave rise to those results. The explanation of the reasons why in the treatments of cempasuchil and cempasuchil + solarization there was a high yield of fruits and in the height of the plant (Table 2), is attributed to the effect of the exudates of the roots of the cempasuchil in the treatment with only this product, and in the treatment with the combination of cempasuchil + solarization, it can be due to the favorable effect that could be presented between the combination of the conditions generated by the plastic of the solarization, in the increase of the temperature and the soil moisture, and exudation of compounds of the tertienyl group, which have a nematicidal effect (Zavaleta *et al*., 2002; Wang *et al*., 2007; Salazar and

Guzmán, 2013). Another evidence obtained from the results reported in Table 2 is that the lowest values in these variables were obtained with the four treatments in which the nematicide is involved: cempasuchil + nematicide, cempasuchil + solarization + nematicide, solarization + nematicide, and nematicide, for which Vidate did not have a favorable effect on growth and fruit production.

Appreciation of the staff that helped in the work

The head of the staff that works in the greenhouse where the experiment was carried out and the worker who carried out the practices, indicated that it was not difficult for them to carry out the new practices, but it took them more hours of work, mainly where there was cempasuchil; the treatments seemed good because their observations confirmed the good yield and quality fruits. In addition, they mentioned that the best treatment was solarization + cempasuchil, which stood out in the height of the plant, in fruits of greater size and uniformity, since they are the variables that could be seen with the naked eye compared to the rest of the treatments.

In the same way, the producer agreed with the results obtained, although he did not agree with the fact of having intercalary plants. Given this situation, some alternatives to control the nematodes in the tomato crop without the need to grow the cempasuchil plants next to the tomato plants is to apply extracts from the cempasuchil plants (Natarajan *et al*., 2006), or the rotation between tomato crops with cempasuchil plants (Hooks *et al*., 2010), in these ways significant effects have been reported in the control of nematodes in the tomato crop compared to the control treatment and even with respect to the chemical treatment.

Conclusions

The greatest reduction in the number of nematodes occurred in the treatment with cempasuchil alone or in combination with the Vidate nemacticide, with solarization or with both.

In general, in the treatments where the population of nematodes was reduced the highest yield and quality of tomato was obtained.

The use of cempasuchil in commercial tomato plantations in the greenhouse does not represent problems of crop management and the producer has no objection to using it.

Control strategies different from chemical control are effective for the management of *Meloidogyne* spp., in greenhouse, for which reason, the proposed hypothesis is not rejected.

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