Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Article

Rhizobacteria for the control of *Meloidogyne* in *Capsicum annum* L. in the greenhouse

José Alonso Calvo-Araya[1,*]
Victoria Valverde-Juárez[1]
Martha Orozco-Aceves[2]
Arlette Orozco-Muñoz[1]

1 Laboratorio de Fitopatología-Escuela de Ciencias Agrarias (ECA)-Universidad Nacional. Apartado 86-3000. Heredia, Costa Rica. (alonso.calvo.araya@una.cr; vickto13@gmail.com; arlette.orozco.munoz@una.cr).

2 Instituto Regional de Estudios en Sustancias Tóxicas (IRET)-Universidad Nacional. Apartado 86-3000. Heredia, Costa Rica. (martha.orozco.aceves@una.cr).

Autor para correspondencia: alonso.calvo.araya@una.cr

Abstract

The following study aimed to evaluate the effect of three plant growth-promoting bacteria: *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens* and *Streptomyces griseoviridis*, and two types of fertilization: chemical and organic (vermicompost) in the soil for the control of root-knot nematode (*Meloidogyne* sp.), and their effect on the development of chili crops in the greenhouse. Four fertilizations, both chemical and organic, were carried out at the time of transplant and at 15, 30, and 45 days after transplant. The trial lasted five months. A total of nine treatments were established (three types of bacteria + one chemical control × two types of fertilization + one control with water), each of them with four replications, for a total of 36 plants. Measurements of galling percentage (GP) and galling index (GI) were made, and the efficiency of the treatments was determined. The dry and fresh weights of both the aerial part and the root of the plants were also determined. The highest degree of damage occurred in the control treatment with 71.2% GP, while the lowest GP was observed in the chemical fertilization × *B. amyloliquefaciens* treatment. The GI did not present significant differences between treatments because the negative effect of galling was counteracted by adequate root growth, which was especially visible in treatments such as chemical fertilization × *B. amyloliquefaciens* and organic fertilization × oxamyl.

Keywords:

biological control, nematode, organic fertilization.

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Introduction

Chili (*Capsicum annuum* L.) is the main cultivated species of the genus *Capsicum* in Costa Rica. This vegetable is of great importance nationally and globally, its production has grown in recent years, which is estimated at about 4.5 million tons of dry product and 36 million tons of fresh product (FAO, 2021). In 2019, this vegetable had a cultivated area of 3.8 million hectares (ha), distributed in 126 countries on all continents (FAO, 2021).

The root-knot nematode *Meloidogyne* (Chitwood, 1949) is one of the main pests that affects horticultural crops, including the sweet pepper. This nematode can cause production losses between $77 and $125 billion (Abawi and Widmer, 2000; Chitwood, 2003) in different crops. The losses associated with this nematode in chili crops worldwide correspond to more than 15% (Tola *et al*., 2023).

The control of root-knot nematodes in horticultural crops has been based on the use of broad-spectrum nematicides, such as chloropicrin, 1,3-dichloropropene, and other broad-spectrum pesticides (Calvo and Zapata, 2020). Increasingly nematicides are being restricted due to their toxicity or environmental impacts associated with their application (Kearn, 2014).

These nematicides are considered nematostatic at the recommended doses and the nematodes manage to regain mobility and probably become infective again (Oka *et al*., 2012), so their use is sometimes inefficient.

The use of vermicompost contributes to suppressing nematode populations, among the proposed mechanisms on how vermicompost suppresses *Meloidogyne* populations are: expression of extracellular enzymatic activities of rhizobacteria, induction of plant response, competitive exclusion of the microbial antagonist, and improving plant resistance (Zuhair *et al*., 2022).

However, alternatives must be evaluated and validated. Therefore, the present research aimed to evaluate the effect of three plant growth-promoting bacteria (*Bacillus amyloliquefaciens*, *Pseudomonas fluorescens*, and *Streptomyces griseoviridis*) and vermicompost applied to the soil on *Meloidogyne* and their effect on the development and production of the chili crops.

Materials and methods

Plant material

The hybrid used was Nathalie, developed by Syngenta. It has the following characteristics: it produces a green-red ripening fruit, high yield.

Experiment location

This research was conducted in the greenhouse of the Santa Lucía Experimental Farm (FESL), for its acronym in Spanish of the National University (UNA), for its acronym in Spanish, located in Santa Lucía de Barva, Heredia, Costa Rica.

Soil collection, treatment, and characterization

The soil used was collected from the FESL. It belongs to the order of Andisols. A total of 400 kg was collected at a depth of 20 cm. It was screened and taken to the Phytopathology Laboratory of the UNA. Subsequently, it was subjected to a double sterilization process in an autoclave at 121 °C and a pressure of 15 psi, for 20 min (Hattori *et al*., 2015). Its characteristics were: pH 5.9, K 0.69 cmol (+)/L, Ca 7.4 cmol(+)/L, Mg 0.5 cmol(+)/L, acidity 0.3 cmol(+)/L, P 4 mg L, 10.93% organic matter.

Transplanting the seedlings

The seedlings were transplanted when they presented four true leaves, making a 5 cm deep hole in the center of each pot.

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v15i7.3544

Meloidogyne inoculum preparation and plant inoculation

The galled plants were transported in plastic bags in a cooler to carry out the extraction of eggs and juveniles in the Nematology Laboratory of the UNA. The extraction was carried out following methodology Manzanilla-López (2012); from the suspension of extracted nematodes, 10 aliquots of 5 ml were taken to perform counts. A 3.8 ml suspension per plant was used to inoculate 2 000 J_2 and eggs (inoculum units) (Zhao *et al*., 2021). Inoculation was performed seven days after transplantation (DAT) in the root zone.

Treatments evaluated

Two types of fertilization were evaluated: commercial and organic, and three rhizobacteria: *P. fluorescens*, *B. amyloliquefaciens*, and *S. griseoviridis* (Table 1).

Table 1. Description of the treatments established.

Treatments	Code	No. of replications
1. Chemical fertilization + commercial control (oxamyl)	CC	4
2. Chemical fertilization + *P. fluorescens*	CP	4
3. Chemical fertilization + *B. amyloliquefaciens*	CB	4
4. Chemical fertilization + *S. griseoviridis*	CS	4
5. Chemical fertilization + water	CW	4
6. Organic fertilization + commercial control (oxamyl)	OC	4
7. Organic fertilization + *P. fluorescens*	OP	4
8. Organic fertilization + *B. amyloliquefaciens*	OB	4
9. Organic fertilization + *S. griseoviridis* Total	OS	4 36

Application of treatments

Fertilization

Four fertilizations were carried out. In transplantation, a 20 cm hole was made and 20 g of commercial formula Abopac 10-30-10 (chemical) or 120 g of vermicompost (organic) was added to it, and then the seedling was placed.

At 15 dat, the second chemical and organic fertilization was carried out, which corresponded to 20 g of Abopac 10-30-10 together with 5 g of complete formula of the commercial fertilizer of Seracsa 18-5-15-6-0.2-7.3 and 125 g of commercial vermicompost (Puriscal, San José), respectively. The third chemical and organic fertilization was performed 30 days after the second fertilization and the amounts used were as follows: 2.5 g of ammonium nitrate (NH_4NO_3) along with 2.5 g of the formula 18-5-15-6-2 and 130 g of vermicompost.

Finally, the fourth fertilization was carried out 45 days after the last fertilization, the amounts applied were 5 g of NH_4NO_3 along with 2.5 g of the formula 18-5-15-6-2 and 140 g of vermicompost, respectively, for chemical and organic treatments.

Products evaluated

The rhizobacteria were inoculated by a 15 ml suspension of the commercial concentration (7.2 x 10^{10} CFU ml^{-1}). Both the bacteria and the nematicide oxamyl 24% SL at the recommended dose of 5 ml L-1 were applied five days after inoculation of Meloidogyne sp., as recommended by Oka et al. (2012).

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Variables evaluated

Galling percentage (GP)

It was determined at 90 dat, performing a destructive sampling of each experimental unit and for this purpose, the formula used by Zhao *et al*. (2021) was used.

Galling index (GI)

This indicator was obtained using the five-degree visual scale (Table 2) used by Zhao *et al*. (2021).

Table 2. Visual galling scale for the evaluation of the galling index in roots of chili under greenhouse conditions.

Scale	Galling percentage
0	0% galls (healthy root)
1	1-15% galls
2	16-25% galls
3	26-50% galls
4	51-75% galls
5	76-100% galls

Efficacy of products on *Meloidogyne* sp.

From the GP, visually determined, the biological effectiveness of the treatments for the management of *Meloidogyne* sp. was calculated utilizing the following formula used by Zhao *et al*. (2021); Calvo and Zapata (2020).

$$GP=\left(\frac{NGR}{TR}\right)*100$$

Where: GP= galling percentage; NGR= number of galled roots; and TR= total number of roots in the plant.

Variables to determine plant development

The fresh and dry weight of the root and aerial part was determined at 90 days dat. Fresh weight of the root and aerial part: the root systems of the four plants per treatment were separated manually. The roots were washed with water, drained, and weighed individually with a mechanical scale, as were the aerial parts.

Dry weight of the root and aerial part: the washed root system and the aerial part of the plants were subjected to a drying process in an oven at a temperature of 60 °C for a period of 72 h. After drying, the roots and aerial parts of the sampled plants were weighed again.

Experimental design

The structure of the experiment was bifactorial, the first factor was fertilization with two levels: chemical and organic fertilization. The second factor was the type of nematicide with five levels: three growth-promoting bacteria with nematicidal effect, a commercial chemical nematicide, and an absolute control of water. In total, nine treatments with four replications were established for a total of 36 experimental units under a completely randomized design.

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Statistical analysis

To determine the effect of the treatments on the determined response variables (GP, GI, fresh and dry weight of the aerial part of the plant, fresh and dry weight of the root), an analysis of variance (Anova) and mean comparisons by means of the Di Rienzo, Guzmán and Casanoves (DGC) test ($p \leq 0.05$) were performed through the statistical software of Infostat.

Results and discussion

Galling percentage

The results of the main Anova indicated that the GP was influenced by the two factors: type of fertilization ($p= 0.01$) and type of nematicide ($p= 0.01$); in addition, there was a significant interaction of these factors ($p= 0.01$). In the case of the type of fertilizer, an average GP of 23% was recorded under organic fertilization compared to 20.75% for plants fertilized with chemical fertilizer (Table 3).

Table 3. Average values of the percentage of galling (GP) caused by *Meloidogyne* sp., in sweet pepper roots.

Treatment	Code	GP	Standard deviation	Minimum	Maximum
1	CC	20.75b	2.22	18	23
2	CP	38.25b	1.26	37	40
3	CB	11.5a	1.29	10	13
4	CS	21.25b	1.5	20	23
5	CW	71.25c	2.5	70	75
6	OC	23a	2.45	20	25
7	OP	15.75a	1.5	15	18
8	OB	29.5b	1	28	30
9	OS	31.25b	2.5	30	35

Different letters associated with the GP indicate statistically significant differences ($p \leq 0.05$).

There was a reduction in the damage caused by *Meloidogyne* sp. due to the use of vermicompost, which had a positive effect on the reduction of galls and egg mass present in the root (up to 82.5% less). Additionally, Khairy *et al*. (2020) found that there was an improvement in the growth attributes of chili plants in terms of shoot length (18.5%) and total fresh weight (32.8%).

In the case of the type of nematicide, comparisons between pairs of means showed significant differences in all cases, except when comparing the GP of the chemical control vs *B. amyloliquefaciens* ($p= 0.148$) and *P. fluorescens* vs *S. griseoviridis* ($p= 0.409$). Based on the type of nematicide, the maximum average GP was recorded in the control to which distilled water was applied (71.25%).

The minimum average GP was recorded in the treatment of chemical fertilization × *B. amyloliquefaciens*, with 11.5%, followed by the plants of the treatment of organic fertilization × *P. fluorescens*, which had a percentage of GP of 15.75%, maintaining values well below the percentage obtained in the control treatment (Table 3).

Regarding the interaction of fertilizer × type of nematicide, the analyses showed that the type of fertilizer only influenced the type of nematicide when it was biological in nature; that is, when applying *P. fluorescens* ($p= 0.043$), *B. amyloliquefaciens* ($p= 0.032$), or *S. griseoviridis* ($p= 0.028$).

Research conducted by Khairy *et al*. (2020) found lower GPs in treatments fertilized with organic fertilizer, regardless of the type of biological nematicide used. Considering the two factors evaluated, the lowest average GP was observed in the treatment of chemical fertilization × *B. amyloliquefaciens* with a GP= 11.5, followed by the treatment of organic fertilization × *P. fluorescens* with 15.75%, and chemical fertilization × chemical nematicide with 20.7%.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i7.3544

The plants inoculated, when applying chemical fertilization with *B. amyloliquefaciens*, showed the lowest GP, which is consistent with what was reported by Mousa and Zawam (2010), who observed 87% of the inhibition of GP.

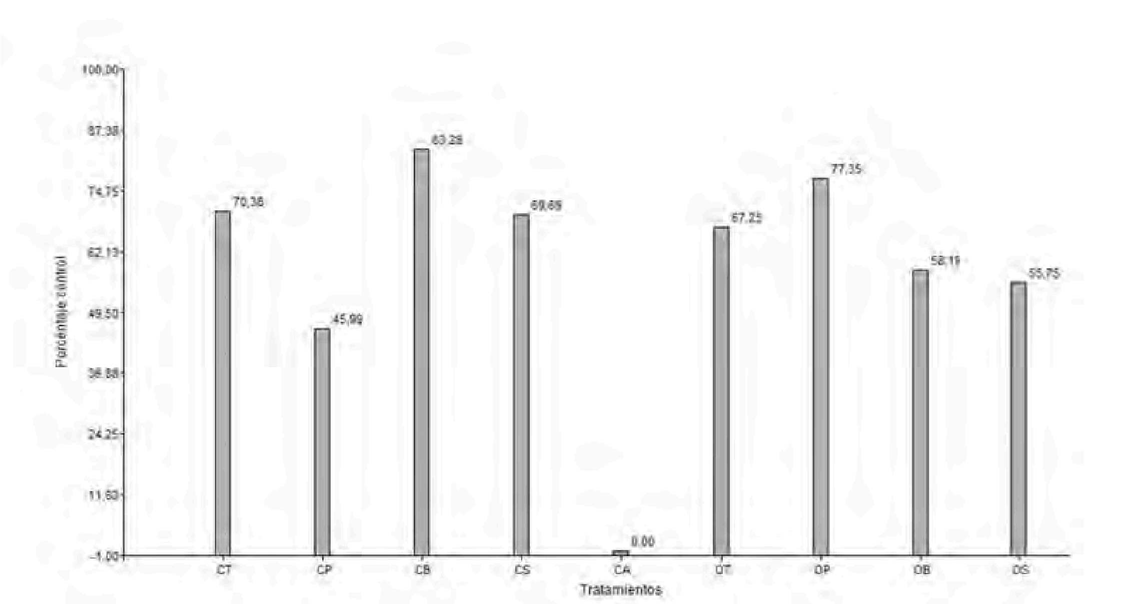
Galling index

The GI did not present statistically significant differences and showed a range of values from 1 to 4, with the highest indices being found in the treatment of chemical fertilization × water (4), chemical fertilization × *P. fluorescens* (3), organic fertilization × *B. amyloliquefaciens*, and organic fertilization × *S. Griseoviridis* (value of 3 in both).

Efficacy of treatments on *Meloidogyne* sp.

The bacteria *B. amyloliquefaciens* and *P. fluorescens*, with chemical and organic fertilization, respectively, presented high efficiency values (*p*= 0.409). The values obtained were found to be between 70% and 80% control (Figure 1).

Figure 1. Effect of treatment on control against *Meloidogyne* sp., in chili plants under greenhouse conditions.



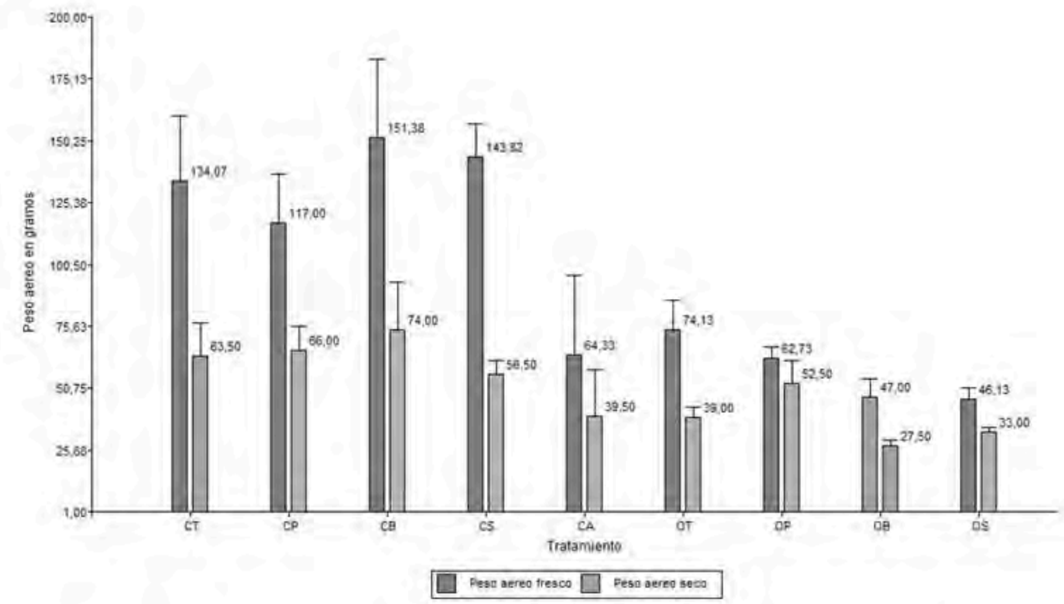
The use of bacteria, such as *P. fluorescens*, *B. amyloliquefaciens*, and *S. griseoviridis*, with different types of fertilization had a positive effect preventing root galling, these treatments being the ones that presented the highest efficiency values. These data are consistent with those obtained by Liu *et al*. (2020), who found that the use of bacteria suppressed the nematode *M. incognita*, demonstrating a reduction in the percentage of galling in the root.

Fresh and dry weight of the aerial part and root

The highest average dry aerial weight corresponded to chemical fertilization × *B. amyloliquefaciens* (74 g) and the lowest average dry aerial weight was organic fertilization × *B. amyloliquefaciens* (27.5 g) (Figure 2). The values of the aerial part and root had significant differences (*p*= 0.073).

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Figure 2. Average weight of the dry and fresh aerial part of the treatments applied for the control of *Meloidogyne* sp. in sweet pepper plants.

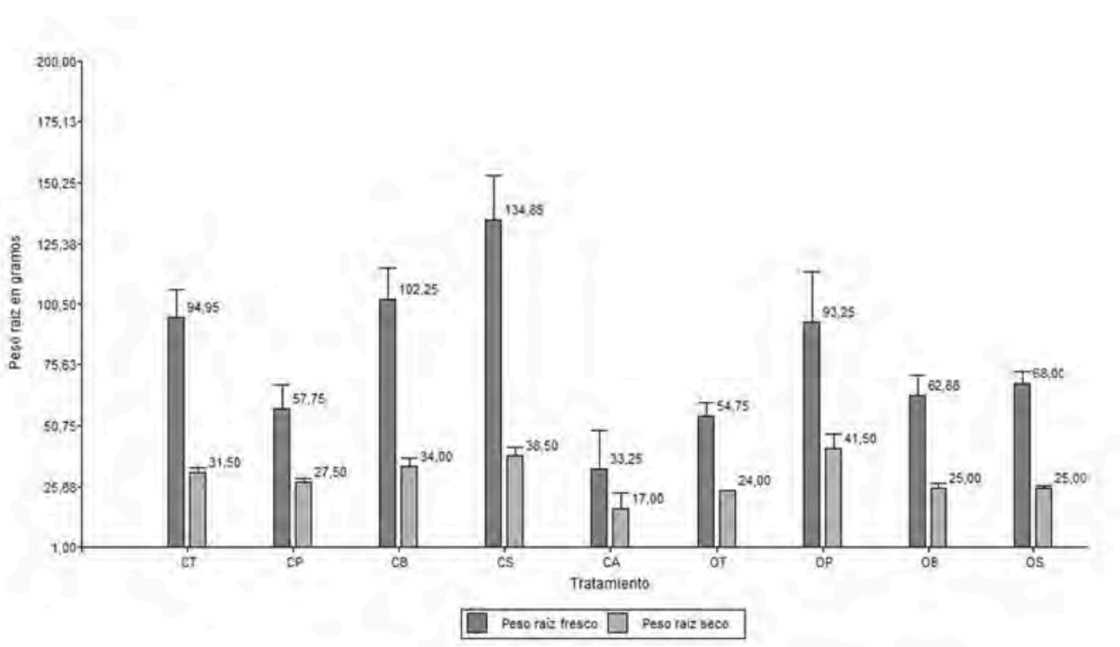


The dry weight of the aerial parts of the bacterial treatments showed lower values with respect to the chemical fertilization x oxamyl (63.5 g); in addition, with the treatment of chemical fertilization × *B. amyloliquefaciens*, a value of 74 g was obtained, being above the control treatment.

Regarding the weight of the roots, the *p*-values for fresh and dry weight were p= 0.0003 and 0.0005, respectively; in addition, the average results recorded in the treatments of chemical fertilization × *B. amyloliquefaciens* (CB) and organic fertilization × *P. fluorescens* (OP) were positive in both dry and fresh weight (Figure 3).

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Figure 3. Average fresh and dry root weight of treatments applied for the control of *Meloidogyne* sp., in sweet pepper plants.



The highest mean values of fresh and dry root weight corresponded to the treatment of chemical fertilization x *S. griseoviridis* (CS), with 134.85 g and 38.5 g, respectively.

Similar results were obtained by Ruanpanun *et al*. (2011), who determined a positive control of *Meloidogyne* spp. with the use of the bacterium *Streptomyces* sp., reporting a biocidal effect on eggs and J_2 of the nematode. The maximum value of root dry weight corresponded to the treatment of organic fertilization × *P. fluorescens* (OP).

The data on fresh and dry root weight of the treatments with organic fertilization and bacteria with nematicide effect were higher compared to what was obtained in the control treatment with this type of fertilizer and oxamyl.

Conclusions

The GP was influenced by the two factors: type of fertilization (p= 0.01) and type of nematicide (p= 0.001). In type of fertilizer, an average GP of 34.15% was recorded in the treatment with vermicompost against 22.9% with chemical fertilizer. The three bacteria evaluated had positive effects on the control of the nematode *Meloidogyne* sp. according to the galling percentages and control percentages, except for the combination of chemical fertilization × *P. fluorescens*.

The treatments that showed efficacy in fresh leaf weight and fresh and dry root weight corresponded to: chemical fertilization x *B. amyloliquefaciens*, chemical fertilization x oxamyl, chemical fertilization x *S. griseoviridis* and organic fertilization x *P. fluorescens*.

Bibliography

1 Abawi, G. S and Widmer, T. L. 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology.15(2000):37-47.

DOI: https://doi.org/10.29312/remexca.v15i7.3544

2 Calvo-Araya, J. A. and Zapata-Montes, N. 2020. Evaluation of nematicidal activity of fluensulfone against Meloidogyne incognita in Bell Pepper Crop. International Journal of Plant & Soil Science. 32(10):52-59.

3 Chitwood, D. J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. Pest Management Science. 59(6-7):748-753. 10.1002/ps.684.

4 FAO. 2021. Statistics Division of FAO (Online). http://faostat.fao.org.

5 Hattori, Y. H.; Kinouchi, T. A.; Fukutani, S. H.; Takahashi, T. F.; Fujiwara, K. A.; Iwata, K. H. and Takahashi, S. H. 2015. The Effect of Soil Sterilization on the 137Cs Transfer from Soil to Radish (*Raphanus sativus* var. Sativus). Jpn. J. Health Phys. 50(3):194-196.

6 Khairy, D. O.; Refaei, A. R. and Mostafa, F. A. 2020. Management of *Meloidogyne incognita* infecting eggplant using moringa extracts, vermicompost and two commercial bio-products. Egypt. J. Agronematol. 20(1):1-16.

7 Kearn, J. J; Ludlow, E. E; Dillon, J. J.; O'Connor, V.; and Holden-Dye, L. 2014. Fluensulfone is a nematicide with a mode of action distinct from anticholinesterases and macrocyclic lactones. Pesticide Biochemistry and Physiology. 109(2014):44-57. 10.1016/ j.pestbp.2014.01.004

8 Liu, G. l.; Lin, X. C.; Xu, S. T.; Liu, G. A.; Liu, F. T. and Mu, W. A. 2020. Screening, identification and application of soil bacteria with nematicidal activity against root#knot nematode (*Meloidogyne incognita*) on tomato. Pest Management Science . 76(6):2217-2224.

9 Manzanilla-Lopez, R. H. 2012. Methodology and symptomatology. *In*: Manzanilla-Lopez, R. H. and Marban-Mendoza, N. Ed. Practical plant nematology. Biblioteca básica de Agricultura, Montecillo. 89-129 pp.

10 Mousa, L. A. and Zawam, H. S. 2010. Efficacy of some biocontrol agents on reproduction and development of *Meloidogyne incognita* infecting tomato. Journal of American Science. 6(11):495-509.

11 Oka, Y. S.; Shuker, N. N. and Tkachi, N. N. 2012. Systemic nematicidal activity of fluensulfone against the root knot nematode *Meloidogyne incognita* on pepper. Pest Management. Science. 68(2):268-275. https://doi.org/10.1002/ps.2256

12 Ruanpanun, P. P.; Laatsch, H. H.; Tangchitsomkid, N. N. and Lumyong, S. S. 2011. Nematicidal activity of fervenulin isolated from a nematicidal actinomycete, *Streptomyces* sp. CMU-MH021. On *Meloidogyne incognita*. World Journal of Microbiology and Biotechnology. 27(6):1373-1380. https://doi.org/10.1007/s11274-010-0588-z

13 Tola, S. D.; Muleta, D. D.; Assefa, F. and Meressa, B. H. 2023. Population dynamics and damage threshold of *Meloidogyne incognita* to the dinsire hot pepper variety. Pakistan Journal of Nematology. 41(2):108-117.

14 Zhao, J. J.; Wang, S. S.; Zhu, X. A.; Wang, Y. Y.; Liu, X. S.; Duan, Y. B.; Fan, H. H. and Chen, L. L. 2021. Isolation and characterization of nodules endophytic bacteria *Pseudomonas protegens* Sneb 1997 and *Serratia plymuthica* Sneb 2001 for the biological control of root knot nematode. Applied Soil Ecology . 164(103924):1-10. https://doi.org/10.1016/ j.apsoil.2021.103924.

15 Zuhair, R. A.; Moustafa, Y. T. A.; Mustafa, N. S.; El-Dahshouri, M. F.; Zhang, L. Z. and Ageba, M. F. 2022. Efficacy of amended vermicompost for bio control of root knot nematode (RKN) Meloidogyne incognita infesting tomato in Egypt. Environ. Technol. Innov. 27(102397):1-9.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i7.3544

Rhizobacteria for the control of *Meloidogyne* in *Capsicum annum* L. in the greenhouse

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 June 2024
Date accepted: 01 August 2024
Publication date: 04 November 2024
Publication date: Oct-Nov 2024
Volume: 15
Issue: 7
Electronic Location Identifier: e3544
DOI: 10.29312/remexca.v15i7.3544

Categories

Subject: Articles

Keywords:

Keywords:

biological control

nematode

organic fertilization

Counts

Figures: 3

Tables: 3

Equations: 2

References: 15

Pages: 0