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## Biocontrol agents in the management of *Meloidogyne incognita* in tomato

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**ABSTRACT:** Recognized as prominent among the principal pests of tomato crop, the root-knot nematode (*Meloidogyne incognita*) has generated much study with regards to control methods. This study aimed to assess the efficiency of biological products in decreasing the *M. incognita* population in tomato. Four experiments were conducted under greenhouse conditions. Two of these experiments had the purpose to determine the efficiency of NemOut™ (*Bacillus subtilis* + *B. licheniformis* + *Trichoderma longibrachiatum*), and the other two to assess the efficiency of other different biological products in *M. incognita* management. Tomato plants of the cultivar Santa Cruz Kada Giant were inoculated using 2000 eggs and J2 of *M. incognita*. In the first two experiments doses corresponding to 0, 4, 6, 8 and 10 kg ha<sup>-1</sup> of NemOut™ were applied after inoculation. In the second study, the treatments consisted of the use of biological products containing different other biocontrol agents. Assessments were made at 45 and 65 days after inoculation (DAI) to evaluate plant height, fresh shoot and fresh root weight, gall index and egg mass index, population density and reproduction factor (RF). The NemOut™ doses reduced the *M. incognita* population density and RF at 45 DAI. The agent *Pochonia chlamydosporia* showed greater efficiency in controlling *M. incognita* on tomato. Biological products and the different doses of NemOut™ had no influence on the tomato plant development.

**Key words:** biological control, root-knot nematode, *Solanum lycopersicum*, opportunistic fungi, *Pochonia chlamydosporia*.

## Agentes de biocontrole no manejo de *Meloidogyne incognita* em tomateiro

**RESUMO:** O nematoide das galhas (*Meloidogyne incognita*) é relatado como uma das principais pragas da cultura do tomate, sendo importante o estudo de alternativas para o seu manejo. O presente estudo objetivou avaliar a eficiência de produtos biológicos na redução da população de *M. incognita* em tomate. Foram conduzidos quatro experimentos em casa de vegetação, dois visando avaliar a eficiência de doses de NemOut™ (*Bacillus subtilis* + *B. licheniformis* + *Trichoderma longibrachiatum*), e outros dois para avaliar a eficiência de diferentes produtos biológicos no controle de *M. incognita*. Plantas de tomate Santa Cruz Kada Gigante foram inoculadas com 2000 ovos e J2 de *M. incognita*. No primeiro estudo foram utilizadas doses equivalentes a 0, 4, 6, 8 e 10 kg ha<sup>-1</sup> de NemOut™, aplicadas após a inoculação. No segundo estudo os tratamentos constituíram de produtos biológicos com diferentes agentes de biocontrole. As avaliações foram realizadas 45 e 65 dias após a inoculação (DAI), determinando-se altura de plantas, massa fresca da parte aérea e da raiz, índice de galhas e de massas de ovos, densidade populacional e fator de reprodução (FR). Doses crescentes de NemOut™ reduziram a densidade populacional e o FR de *M. incognita*, aos 45 DAI. O agente *Pochonia chlamydosporia* mostrou-se mais eficiente no controle de *M. incognita* no tomateiro. Os produtos biológicos e as diferentes doses de NemOut™ não afetaram o desenvolvimento das plantas de tomate.

**Palavras-chave:** controle biológico; nematoide de galhas; *Solanum lycopersicum*, fungos oportunistas, *Pochonia chlamydosporia*.

## INTRODUCTION

The genus *Meloidogyne* (GOELDI, 1987) includes the plant nematodes most injurious to vegetables, particularly to tomato crops, causing high harvest losses, mostly in tropical and sub-tropical agriculture (KIEWNICK & SIKORA, 2006). Traditionally, the main control technique involves the use of chemical nematicides; however, the negative

consequences of these products to the environment and their inefficiency after long-term use have resulted in the prohibition or restrictions on various molecules employed. Therefore, there is an increasing requirement for developing non-chemical alternative methods for the management of the root-knot nematode (HUANG et al., 2016).

One beneficial alternative that is gaining popularity in nematode control is the biological

control, predominantly utilizing the microorganism groups like the fungi and bacteria already present in the soil biota (CRAWFORD & CLARDY, 2011). In fact, many microorganisms reveal the capacity to parasitize the egg and juvenile forms and sometimes even adult nematodes.

Species of *Bacillus* Gaertner interrupts the nematode life cycle by producing toxic metabolites which restrict their mobility and hinder the hatching and juvenile penetration into plant roots (KAVITHA et al., 2007). *Trichoderma longibrachiatum* Rifai, *Purpureocillium lilacinus* (Thom) Samson and *Pochonia chlamydosporia* Zare & Gams are well-recognized for their parasitic behavior on exposed eggs and females. They form the base of many commercial products available for the nematode control, as they reveal promising results as effective biological control agents (DALLEMOLE-GIARETTA et al., 2014). They also can release toxic metabolites that prevent the penetration and parasitic multiplication of the nematode. These opportunistic fungi parasitize some of nematode phases whenever they come into contact with them (ANASTASIADIS et al., 2008).

Although, several research studies are available on biological nematode control, there continues to be a dearth of registered biological nematicides. This study aimed at assessing the nematicidal potential of the commercial biological products available, which contain *Bacillus* spp., *Trichoderma longibrachiatum*, *Paecilomyces lilacinus* and *Pochonia chlamydosporia*, to control the *M. incognita* population in the tomato crop.

## MATERIALS AND METHODS

Under greenhouse conditions, we performed four experiments from March to May 2014 and from October to December 2014. Two of the experiments were done to examine the effect of the different doses of *Bacillus licheniformis* + *B. subtilis* + *Trichoderma longibrachiatum* (NemOut™) on decreasing the *M. incognita* population in tomato. The other two experiments were conducted to assess the efficiency of the different commercial products in controlling *M. incognita* in tomato.

The tomato cultivar Santa Cruz Kada Gigante seedlings were grown in a commercial nursery and transplanted 30 days after sowing to 5L polyethylene pots filled with substrate. The substrate was a previously sterilized soil and sand in a ratio of 1: 1. Each pot received one transplanted seedling. Five days later, each plant was inoculated with 2000 *M. incognita* eggs and J2, dispersed in two holes around

the plant collar. The inoculum used was obtained from Santa Cruz Kada Gigante tomato plants maintained in a greenhouse for inoculum production.

### *Effect of NemOut™ doses on M. incognita on tomato plants*

In the treatments the commercial product NemOut™ was used (*Bacillus licheniformis* + *B. subtilis* + *Trichoderma longibrachiatum*) in five doses of 0, 4, 6, 8 and 10 kg ha<sup>-1</sup> (corresponding to 0, 1.5, 2.25, 3 and 3.75x10<sup>12</sup>CFU). This product was mixed into the substrate one day after the nematode inoculation. The experimental design was completely randomized with five treatments and five replications. Each experimental plot involved two tomato plants, one in each pot.

At 45 and 65 days after inoculation (DAI), assessment was done on one plant for each period. Shoot length and shoots and roots fresh weigh were evaluated. Roots were staining with Floxin B and the gall index (GI) and egg mass index (EMI) were evaluated, in accordance with the method of TAYLOR & SASSER (1978).

The nematode population density was assessed after extraction of eggs and J2 based on the procedure of HUSSEY & BARKER (1973). Counting was performed under an optical microscope (40x magnification) using a Peters slide. The result was presented as number of eggs and J2/10g of roots. The reproduction factor (RF) was determined according to OOSTENBRINK (1966).

The findings of this study were submitted to the analysis of variance. Only when significance between the doses was observed, the regression analysis was done. The data were then adjusted for the linear, quadratic and cubic models. The best model was selected based on the significance of regression ( $P < 0.05$ ) and the coefficient of determination ( $R^2$ ).

### *Efficiency of the biological products for the M. incognita management in tomato*

Experiments were carried out in a completely randomized design with six treatments and with five replications. The treatments were five biological products plus one control as the treatments viz., *Purpureocillium lilacinus* (Paecilomyces JCO®, 400g tray<sup>-1</sup>), *Purpureocillium lilacinus* (Nemat® WP, 200g ha<sup>-1</sup>), *Bacillus subtilis* isolated QST 713 (Serenade® SC, ha<sup>-1</sup>), *Pochonia chlamydosporia* Pc-10 (Rizotec®, 2.0kg ha<sup>-1</sup>), and *Bacillus licheniformis* + *B. subtilis* + *Trichoderma longibrachiatum* (NemOut®, 7.0kg ha<sup>-1</sup>). The experimental plot included two plants, one per pot. The biological

products Nemat® and Paecilomyces JCO® were diluted prior to being applied onto the seedlings when they were still growing in the trays before being transplanted into the pots. The NemOut™, Serenade® and Rizotec® products were applied to the soil after dilution in water, close to the plant stem, one day after inoculation with the nematodes, according to manufacturer's recommendations. The treatments involved a single application of each product. One plant was removed on the 45<sup>th</sup> day and one on the 65<sup>th</sup> day after inoculation (DAI) and assessed for shoot length, fresh shoot and root weigh, gall and egg mass indexes, nematode population density and reproduction factor, as described before.

Applying the Kolmogorov-Smirnov test the normality of the residues was confirmed while the Levene test was used to ascertain the homogeneity of the variances. When the data failed to fulfill the assumptions of the analysis of variance (Anova), they were transformed using the method proposed by BOX & COX (1982). The data were submitted to Anova and when significance was observed the means were separated by the Tukey test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

No influence was observed in this study of the commercial product NemOut™ (with *Bacillus licheniformis* + *B. subtilis* + *Trichoderma longibrachiatum* as the constituents) on the variables connected with plant development (plant height, shoot and root fresh weigh). The *Bacillus* and *Trichoderma* are better known as plant growth promoters (RESENDE et al., 2004; FORTES et al.,

2007); although, in this study, concurring with the findings of FERNANDES et al. (2014) no increase in the fresh weigh of the tomato plants treated with *Trichoderma* spp. and *Bacillus* sp. was noted.

Increase in the *M. incognita* population was observed in Experiment 1; therefore, all the treatments were rated 5.0, according to TAYLOR & SASSER (1978), for the gall index (GI) and the egg mass index (EMI). Experiment 2 revealed that the GI was between 2.75 and 3.5 at 45 DAI and between 4.25 and 5.0 at 65 DAI while the EMI ranged from 2.12 to 3.0 at 45 DAI and from 2.87 to 4.28 at 65 DAI. These indexes indicated a nematode population rise during the second assessment period; however, no significant difference was observed among the treatments at either of the periods. This increase may be attributed to the plant development, because at 65 DAI the root weigh was higher than at 45 DAI.

The *M. incognita* population density and reproduction factor were affected by the doses of the commercial product constituted by *B. licheniformis* + *B. subtilis* + *T. longibrachiatum*. Significant regressions were noted only in the evaluations done at 45 DAI in Experiment 1, where the increased dosages of the product caused a linear decline of both the population density and RF (Figure 1). The increased *M. incognita* population development recorded in Experiment 1 may have improved the product efficiency in this experiment (Table 1). The linear reduction reported in the *M. incognita* population density as well as the RF, in response to the increasing doses of *B. licheniformis* + *B. subtilis* + *T. longibrachiatum* in only one experiment, is significant; however, it highlights the necessity for

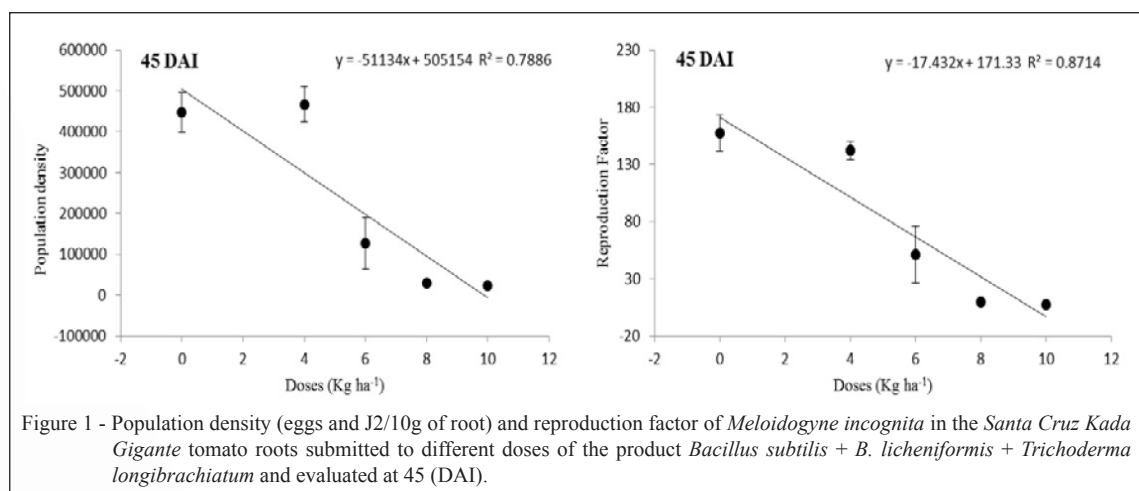




Table 1 - Population density (number of eggs and J2 / 10g of root) and reproduction factor (RF) of the tomato roots treated with different *Bacillus subtilis* + *B. licheniformis* + *Trichoderma longibrachiatum* (NemOut®) doses in two experiments. Evaluations performed at 45 and 65 days after inoculation (DAI) with *M. incognita*.

Doses (kg/ha)	-----Experiment 1-----		-----Experiment 2-----	
	45 DAI	65 DAI	45 DAI	65 DAI
-----Population density-----				
0	446754.87	27598.61	324.11	3070.24
4	467323.26	31387.13	625.61	13566.02
6	127587.48	24964.02	473.52	12592.29
8	29486.75	39088.25	191.95	4598.13
10	22864.23	34408.56	372.22	23125.42
CV (%)	52.54	56.51	58.65	63.96
-----RF-----				
0	157.54	18.27	0.13	1.71
4	142.14	19.94	0.24	7.87
6	51.27	23.36	0.15	8.94
8	10.11	17.98	0.06	2.75
10	7.48	19.75	0.19	6.10
CV (%)	52.78	37.59	12.17	48.13

more investigations on the product efficiency in nematode control on tomato crop.

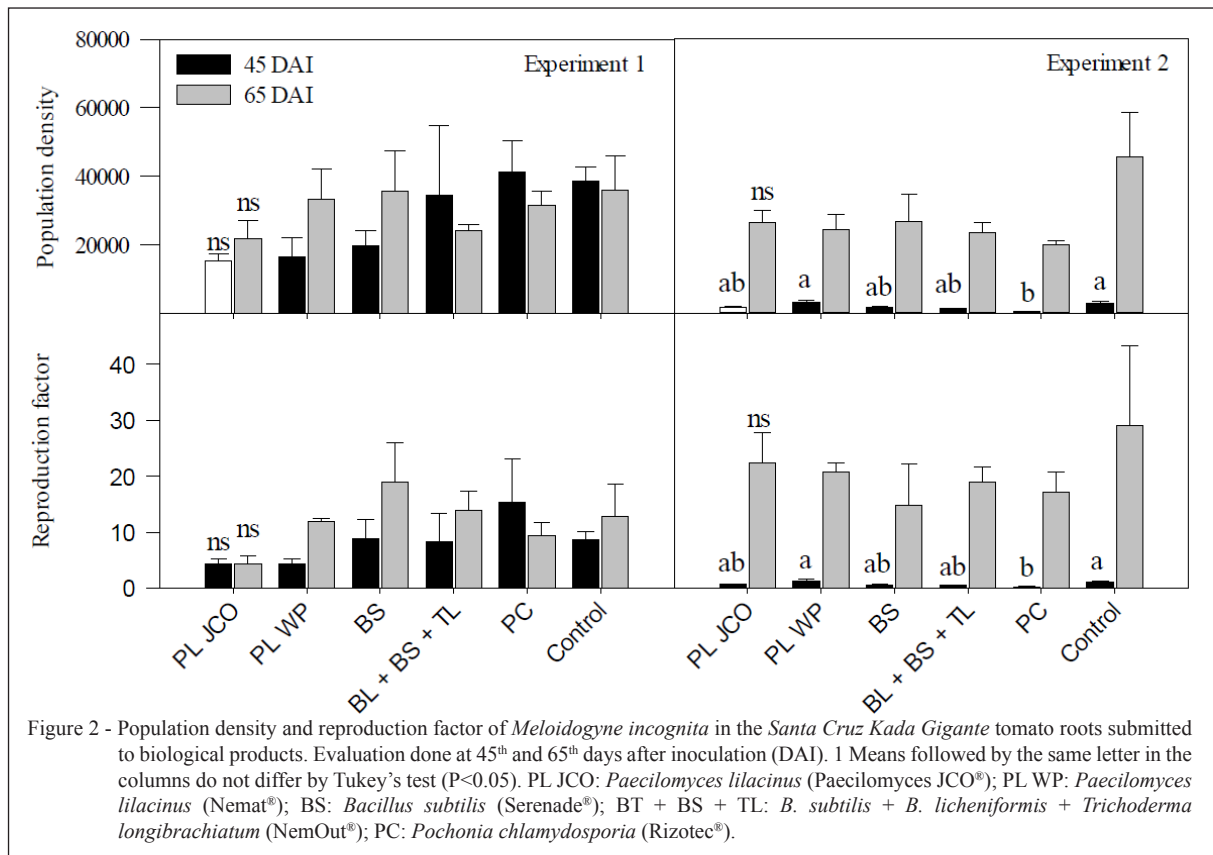
Products containing a combination of biocontrol agents have become an accepted and more efficient method of pathogen control and; therefore, improved plant growth, because it is probable that in sites where natural biological control occurs, it happens as a consequence of different combinations of many microorganisms (ROBERTS et al., 2005). The biocontrol agents of *B. subtilis* + *B. licheniformis* + *T. longibrachiatum*, that constitute the product used in this study, may have promoted the reduction in the *M. incognita* population recorded in this study. This is because different agents possess different action mechanisms, which are anticipated to complement nematode control.

Both experiments conducted with the purpose to identify the influence of the different biological products, revealed that the plant growth (evident by the height, fresh shoot and root weigh) was not affected by the treatments. The increased growth induced by *Bacillus* spp. and *P. chlamydosporia* that has been reported in many studies (ARAÚJO & MARCHESI, 2009; MACIÁ-VICENTE et al., 2009) was not recorded in the present study.

In the tomato plants, the *M. incognita* population was affected in Experiment 2 by the different biological products. *P. chlamydosporia* Pc-10 caused a decline in the population density while the RF determined at 45 DAI, showed a significant difference from the control

and from the application of *P. lilacinus* WP (Figure 2). In the assessment done at 65 DAI, as well as in both the estimations of Experiment 1, no differences were visible among the treatments, with high population densities and RF values being recorded (Figure 2).

*P. chlamydosporia* is a fungus highly utilized in the biological control of nematodes, especially the root-knot nematodes, as it is a fungus that parasitizes females and eggs, the later being the most vulnerable stage of this pathogen life cycle. *P. chlamydosporia* caused a decline in the number of *M. javanica* galls and eggs, in tomato plants (DALLEMOLE-GIARETTA et al., 2014) by 40% and 72.83%, respectively. In the current study, the *M. incognita* in tomato roots was treated with the *P. chlamydosporia*, and its effect was compared with the control, as reported often by different authors (BOURNE & KERRY, 1999; ATKINS et al., 2003). However, the efficiency of the fungus in parasitizing the eggs present in the soil is dependent on two factors: temperature and developmental stage of the embryo within the egg. The fungus undergoes rapid colonization which occurs in the stages of cell multiplication and embryo development, and the juveniles hatch at temperatures approaching 30°C; they escape before the egg mass is colonized, because the fungus does not attack the mobile adult nematodes (DALLEMOLE-GIARETTA et al., 2008). With respect to the temperature conditions observed at the time the experiments were carried out (mean



minimum and maximum, about 21°C and 35°C, respectively), it is possible that the efficiency of the fungus in controlling the nematode population was reduced in the other evaluations that were performed.

The most important step is for the biocontrol agent to establish itself in the rhizosphere, in order to facilitate the interaction of the microorganism with the plant. The nutritional content and organic matter present in the soil can hamper this establishment; and consequently, root colonization will not occur. The soil contains organic compounds which form the substrate on which the antagonists can multiply and establish themselves (FREITAS et al., 2003). In the present experiments, the lack of organic matter in the substrate used may have exerted a negative effect on the development of the biocontrol agents tested.

For the biocontrol agents to be effective, the timing of the application is very significant. According to SILVEIRA & FREITAS (2007), these microorganisms must be added to the soil as early as possible, as the dynamics of the ecosystem that they attempt to invade may hinder their establishment.

RADWAN et al., (2012) reported that the *Trichoderma* isolates showed positive effects by minimizing the degree of damage caused by *M. arenaria* and *M. javanica*, respectively, when the fungus was applied at an early point in time to the soil. Thus, the biocontrol agent may not have been applied, in this study, at the ideal time to ensure its efficiency.

The establishment and maintenance of these beneficial microorganisms in the soil must be achieved year after year, because in this way biocontrol agents are established and reproducing, thus increasing the soil suppressiveness. Many authors reported that the results from studies using microorganisms are often inconsistent, as these microorganisms fail to always exhibit the same behavioral patterns, either as growth promoters or as pathogen controllers, even when they are subjected to identical testing conditions (CHANWAY et al., 2000; FREITAS et al., 2003).

## CONCLUSION

The tomato plants showed no response in their development to the biological products

tested. Increasing doses of the commercial product composed of *Bacillus licheniformis* + *B. subtilis* + *Trichoderma longibrachiatum* reduces the population of *M. incognita* in tomato. The agent *Pochonia chlamydosporia* Pc-10 caused the *M. incognita* population density and reproduction factor to decline in the tomato crop.

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