Meloidogyne javanica control by *Pochonia chlamydosporia, Gracilibacillus dipsosauri* and soil conditioner in tomato

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ABSTRACT

Podestá, G.S.; Freitas, L.G.; Dallemole-Giaretta, R.; Zooca, R.J.F.; Caixeta, L.B.; Ferraz, S. *Meloidogyne javanica* control by *Pochonia chlamydosporia, Gracilibacillus dipsosauri* and soil conditioner in tomato. ***Summa Phytopathologica,*** v.39, n.2, p.122-125, 2013.

Organic matter plays a fundamental role in the antagonistic activity of microorganisms against phytonematode populations on the soil. In this study, the compatibility between the fungus *Pochonia chlamydosporia* (Pc-12) and the rhizobacterium *Gracilibacillus dipsosauri* (MIC 14) was evaluated *in vitro*, as well as the effect of the fungus at the concentration of 5,000 chlamydospores per gram of soil, rhizobacterium at 4.65 x 10^9 cells/g of soil, and the soil conditioner Ribumin® at 10 g/pot, either alone or in combination, against *Meloidogyne javanica* population in tomato plants (3,000 eggs/pot). A suspension of water or Ribumin® alone was applied on the soil as negative control, while a suspension of nematode eggs was applied as positive control. The reduction in the number of galls in roots per plant was 48 and 41% for the treatments Ribumin + MIC 14 + Pc-12 and MIC 14 + Pc-12, respectively. Regarding to the number of eggs per plant, MIC 14 and Pc-12 + Ribumin led to a reduction by 26 and 21%, respectively, compared to the control treatment. Interaction between the nematophagous fungus and the rhizobacterium was positive for the nematode control, even though *G. dipsosauri* inhibited *P. chlamydosporia* growth by up to 30% in *in vitro* tests.

Additional keywords: Root-knot nematode, organic matter, rhizobacteria, alternative control.

RESUMO

Podestá, G.S.; Freitas, L.G.; Dallemole-Giaretta, R.; Zooca, R.J.F.; Caixeta, L.B.; Ferraz, S. *Pochonia chlamydosporia, Gracilibacillus dipsosauri* e condicionador de solo para o controle de *Meloidogyne javanica* em tomateiro. ***Summa Phytopathologica,*** v.39, n.2, p.122-125, 2013.

A matéria orgânica exerce papel fundamental na atividade antagonista de microrganismos sobre populações de fitonematoides no solo. Nesse trabalho foram avaliados a compatibilidade entre o fungo *Pochonia chlamydosporia* (Pc-12) e a rizobactéria *Gracilibacillus dipsosauri* (MIC 14) *in vitro* e o efeito deste fungo a 5000 clamidósporos/g de solo, da rizobactéria a 4,65 *x* 10^9 células/g de solo, e do condicionador de solo Ribumin® a 10 g/vaso, isoladamente ou em conjunto, sobre *Meloidogyne javanica* em tomateiro (3000 ovos/vaso). Uma suspensão de água ou apenas Ribumin®, foi aplicada ao solo como testemunhas negativas, enquanto uma suspensão de ovos do nematoide foi aplicada como controle positivo. A redução no número de galhas em raízes por planta foi de 48 e 41% nos tratamentos Ribumin + MIC 14 + Pc-12 e MIC 14 + Pc-12, respectivamente. Quanto ao número de ovos por planta, MIC 14 e Pc-12 + Ribumin proporcionaram redução de 26 e 21% respectivamente em relação ao tratamento testemunha. A interação entre o fungo nematófago e a rizobactéria foi positiva para o controle do nematoide, apesar do fato de *G. dipsosauri* ter inibido o crescimento do fungo *P. clamidosporia* em até 30% em teste *in vitro*.

Palavras-chave adicionais: Nematoide das galhas, matéria orgânica, rizobactéria, controle alternativo.

The fungus *Pochonia chlamydosporia* Zare & Gams (sin. *Verticillium chlamydosporium* Goddard) is considered one of the most promising agents for biological control of plant nematodes (8, 14). It is a facultative parasite of eggs and females of cyst and root-knot nematodes (12). Its application onto the soil is performed via chlamydospores, once the latter are survival structures of the fungus possessing nutritional reserves (14). Bacteria inhabiting the plant rhizosphere, also known as rhizobacteria, have been reported in scientific studies as potential agents to control plant nematodes. They act by producing toxic compounds (9, 17), by modifying root exudates, preventing recognition by the nematodes (18), or by inducing systemic resistance in plants (6, 26).

The combination of biological control agents may enhance the efficiency of such agents, especially when these antagonists act on different stages of the pathogen's life cycle. Dube & Smart Jr (5) observed that soil applications of *Paecilomyces lilacinus* (Thom) Samson and *Pasteuria penetrans* (Thorne) Sayre and Starr resulted in higher control levels of *Meloidogyne incognita* (Kofoid and White) Chitwood population, when compared to control treatments or to the antagonist alone. Siddiqui & Ehteshamul-Haque (24) also observed similar results for the reduction in *M. javanica* (Treub) Chitwood when the biological control agents *P. chlamydosporia* and *Pseudomonas*

aeruginosa were simultaneously employed.

The inclusion of organic matter in the soils is also an alternative for the control of plant parasitic nematodes (23). The addition of organic matter increases the nutrient content and improves the soil texture, promoting the development of antagonistic microorganisms, while its breakdown produces compounds that are toxic to nematodes (1, 7, 22).

Utilization of different control strategies in a management system is a good alternative for growing tomatoes in places infested with root-knot nematodes. Thus, in this study the effect of the fungus *P. chlamydosporia* (isolate Pc-12), the rhizobacteria *Gracilibacillus dipsosauri* (Lawson et al.) Waino et al. (isolate MIC 14) and the peat-based soil conditioner, Ribumin®, was assessed alone or in combination, to control *M. javanica*, under greenhouse conditions.

MATERIAL AND METHODS

The biological control agents used in the experiments were the fungus *P. chlamydosporia* (Pc-12) and the rhizobacterium *G. dipsosauri* (MIC 14), previously known to reduce populations of *M. javanica* (9). Both antagonists were obtained from the microorganisms' collection of the Plant Nematode Biological Control Laboratory at "Universidade Federal de Viçosa". The commercial soil conditioning product Ribumin®, containing peat and calcium oxide, was obtained from Technes enterprise. It contained 30% organic matter, 35% maximum humidity, 90% minimum water retention capacity (WRC), 12% total organic carbon and 900 mmol/kg cation exchange capacity (CEC).

Compatibility tests

Petri plates containing medium Kado 523 (11) or potato dextrose agar (PDA) were used to set two culture disks of *P. chlamydosporia* in opposite positions in each plate, both located at 2.0 cm from the plate border. A microscope slide was submerged into the bacterial suspension of *G. dipsosauri* or water (control) and a scrape was made in the middle of the plate. Petri plates were kept inside a growing chamber at 26 ºC during five days before evaluating the fungal colony diameter. The experiment was performed in a completely randomized statistical design with tree replicates.

Inoculum production of antagonists

The fungus *P. chlamydosporia* was cultivated in sterilized milled corn substrate inside autoclavable polypropylene bags and kept in growing chamber in the dark, at 26 ºC, during 21 days, before chlamydospore extraction by means of substrate washing with tap water and filtering of the suspension in a double-layer fine cloth. Chlamydospores were counted with the aid of a light microscope and hemacytometer. A total of 5,000 chlamydospores/g of soil were applied for the tests. The bacterium *G. dipsosauri* was transferred to Petri plates containing Kado 523 medium (11) and kept for 24 hours in a growth chamber at 28 ºC. After that, 10 mL of tap water were added to each plate and the surface of the culture medium was scraped with the aid of a bent glass rod. The suspension was corrected for optical density (OD_{540}) = 0.53, containing 6.2 x 10^{11} colony forming unit (CFU)/mL. Each pot was inoculated with 3.75 mL of bacterial suspension.

In order to set the experiment in greenhouse, pots containing 0.5L of soil and sand 1:1 (v:v) as substrate, previously sterilized with methyl bromide, were used. The statistical design was completely randomized with eight replicates for each treatment. The treatments were: control; Ribumin®; nematode; nematode + Ribumin®; nematode + Ribumin® + MIC 14; nematode + MIC 14; nematode + MIC 14 + Pc-12; nematode + Pc-12; nematode + Ribumin® + Pc-12; nematode + Ribumin® + MIC 14 + Pc-12. Soil conditioner Ribumin® was used at 10 g/pot.

The microorganisms and the soil conditioner were integrated to the soil of each pot by manually mixing in order to promote substrate homogenization, and subsequently it was returned to its respective pot. Then, the soil of each pot was wetted and infested with 3,000 eggs of *M. javanica*. One week after soil infestation, 21-day old 'Santa Clara' tomato seedling was transplanted to each pot. After 65 days, height, mass of the fresh aerial plant portion, mass of the fresh roots and number of galls and eggs of *M. javanica* per root system of tomato were evaluated.

During the experimental setting and the data collection, soil samples of each treatment were obtained in order to determine the population of the fungus in the soil. Colony Forming Units (CFUs) were determined according to Kerry (13), in a semi-selective medium (10). Data underwent analysis of variance and differences between treatment means were compared based on Duncan's test of significant difference at $P < 0.05$ probability.

RESULTS AND DISCUSSION

The association of biological control organisms with organic matter was positive to control the nematode *M. javanica* in tomato, once the simultaneous application of the three agents resulted in the smallest number of galls, indicating less nematode penetration in the plant roots (Table 1), while the application of the fungal isolate Pc-12 alone did not differ from the control as to the number of galls. Reductions of 48.27% and 40.57% were observed for treatments with Ribumin + MIC 14 + Pc-12 and MIC 14 + Pc-12, respectively, when compared to the control.

Concerning the number of eggs produced per root system, only the treatment with the rhizobacterium MIC 14 and the treatment with the fungal isolate PC-12 applied together with the soil conditioner Ribumin® showed significant difference from the control treatment, reducing by 26.30% and 21.43%, respectively, the number of *M. javanica* eggs. The benefit of using the soil conditioner was obvious, increasing the efficiency of the fungus to reduce the number of eggs, but the association of the soil conditioner with the bacterium did not result in a similar effect, once the bacterium was more efficient when applied alone (Table 1). The efficiency of the fungus associated with the soil conditioner was higher in the absence of the bacterium. Association of the bacterium MIC 14 with the fungus PC-12 resulted in smaller reduction in the egg number when compared to the use of both separately and without Ribumin®. However, the association of both organisms, with or without soil conditioner, resulted in a more expressive reduction in the number of galls. The association of Ribumin® with the fungus was more efficient than its association with the bacterium to reduce the number of nematode eggs.

Previous studies have established that the mixture of biological control agents increases the potential to control phytopathogenic organisms (16, 20, 21), but each mixture has its own characteristics, which depend on the type of the involved microorganisms, their inhibition level, their form of action against the nematode, as well as their relationship with the soil organic matter and their capability of rhizosphere colonization or plant resistance induction. There was a slight reduction in fungal growth according to the compatibility test between the fungus PC-12 and the bacterium MIC 14 *in vitro* (Table

Table 1. Effect of *Pochonia chlamydosporia* (Pc-12), *Gracilibacillus dipsosauri* (MIC 14) and soil conditioner Ribumin® on *Meloidogyne javanica* and development of tomato plants, 70 days after seedling transplantation.

Treatments	Height (cm)	Mass of the aerial portion (g)	Mass of root (g)	Number of root galls	Number of nematode eggs	CFUs/ gsoil[1] (X 1,000)[1]
Negative control (without Mj*)	81.14 [ns]	40.55 [ns]	13.99 [ns]	-	-	-
Ribumin (without Mj)	85.57	48.16	13.47	-	-	-
Positive control (Mj)	85.00	39.71	15.38	1.191 a	431,053 a	-
Mj + Ribumin	71,71	42.62	16.80	906 bc	418,880 ab	-
Mj + Ribumin + MIC 14	73.86	43.79	16.72	765 cde	399,520 abc	-
Mj + MIC 14	75.57	37.08	13.74	822 cd	317,680 c	-
Mj + MIC 14 + Pc-12	80.71	38.99	14.05	703 de	409,493 ab	$6.6x10^2$
Mj + Pc-12	83.86	42.64	15.63	1040 ab	356,107 abc	$1.0x10^4$
Mj + Ribumin + Pc-12	73.86	40.86	15.02	955 bc	338,653 bc	$2.4x10^4$
Mj + Ribumin + MIC 14 + Pc-12	77.71	46.06	16.68	616 e	351,853 abc	$8.6x10^3$
CV (%)	15.64	22.20	14.52	18.79	18.71	-

Mean of seven replicates. Mean values followed by the same letter are not statistically different based on Duncan's mean separation test at $p< 0.05$. Mj* = *Meloidogyne javanica*. [ns] = Not significant
[1]Population of *Pochonia chlamydosporia* Pc-12 on the soil at 70 days after transplantation of tomato seedlings, with the presence of *Meloidogyne javanica*. Mean values of three replicates.

2). There was also a reduction in the number of colony forming units for PC-12 in pot soil at the end of the experiment for treatments in which the bacterium was associated with the fungus, when compared to treatments in which the fungus was applied alone or with the conditioner, without the bacterium (Table 1).

The positive effect of the mixture of fungus and bacterium on the reduction in the number of galls, associated or not with Ribumin®, was probably due to the action of plant resistance induced by the bacteria. The fungus *P. chlamydosporia* has slower growth when compared to the bacterium and acts mainly on nematode eggs. This elucidates why the treatment with the fungus alone had no difference from the control treatment regarding the number of galls. The low control potential of the isolate Pc-12 without the addition of organic matter was previously observed by Dallemole-Giaretta (3), who reported the inferiority of this isolate compared to isolate PC-10 concerning nematode control, as well as the production of chlamydospores. Selection of a competent isolate is very important when the objective is to develop a biological control product, once there is a great difference among isolates of a single fungal or bacterial species (3, 4, 19).

Although tomato plants were transplanted at seven days after the application of the biological control agents into the soil, the fungus *P. chlamydosporia* had little effect on the destruction of the nematode eggs, reducing the number of galls by only 2.68 %. When the fungus was applied simultaneously with Ribumin®, a more expressive reduction in galls was observed. Nonetheless, this is due to the effect of this soil conditioner on the nematode, once the same result was obtained for the treatment with Ribumin® applied alone.

Table 2.Mean of colony radial growth (cm) of *Pochonia chlamydosporia* (Pc-12) at five days after incubation in culture medium PDA or MB1, compared with the bacterium *Gracilibacillus dipsosauri*, isolate (MIC 14).

Treatments	Growth (cm)	Reduction (%)
Pc-12 (Culture medium PDA)	2.90	-
Pc-12 + MIC 14 (Culture medium PDA)	2.47*	14.82
Pc-12 (Culture medium 523)	2.52	-
Pc-12 + MIC 14 (Culture medium 523)	1.76*	30.15

* Statistically significant according to the F test at $p < 0.05$.

The soil conditioner Ribumin® reduced the number of galls caused by the nematode when used alone or simultaneously to the fungus or the bacterium, yielding better results when applied together with the bacterium or with both organisms. The integration of organic matter with biological control agents may increase the control potential against plant nematodes, once organic matter may release compounds with nematicidal effect and support the increase in native antagonist population in the soil or serve as substrate for the development and the establishment of antagonists applied to the soil (2, 23). Besides that, substrates rich in organic matter may contain natural propagules of other antagonist organisms that may help in nematode control.

The prominent activity of the bacterium MIC 14 may be possible due to the different modes of action that rhizobacteria have on nematodes. They may produce toxic compounds that may influence nematode's hatching or motility, modify root exudates that may prevent recognition by nematodes or induce systemic resistance in plants. In addition, rhizobacteria may also promote plant growth (6, 9, 17, 18, 25). This study confirmed once again the great potential for biological control of this rhizobacterium against *M. javanica*, corroborating previous results obtained by Freitas et al. (9).

CONCLUSIONS

Combination of biological control agents with organic matter, such as soil conditioners, is a good alternative for the management of root-knot nematodes. The simultaneous use of the rhizobacterium *G. dipsosauri* (isolate MIC 14), the fungus *P. chlamydosporia* (isolate Pc-12) and the peat-based soil conditioner Ribumin® has a positive effect in reducing populations of the root-knot nematode *M. javanica*, but the different combinations among them result in differential reductions in the number of galls and eggs.

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