SCIENTIFIC NOTE

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Pochonia chlamydosporia var. *chlamydosporia* (Goddard) Zare & W. Gams for the management of lettuce infected with *Meloidogyne javanica* (Treub, 1885)

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The application of the nematophagous fungus *Pochonia chlamydosporia* var. *chlamydosporia* (Goddard) Zare & W. Gams during seedling production of vegetable crops can be an efficient approach to control root-knot nematode. The aim of this study was to evaluate the effect of treating seedlings and/or soil with bionematicide (wettable powder formulation) based on chlamydospores from isolate Pc-10 on the *Meloidogyne javanica* (Treub, 1885) control in lettuce (*Lactuca sativa* L.). Isolate Pc-10 was diluted in water and applied when watering the seedlings (0, 4.5, 9.0, 13.5, and 18.0 g L^{-1}) and/or to the potted soil (5000 chlamydospores g^{-1}) used for growing lettuce. The soil in each pot was infested with 3000 *M. javanica* eggs. The number of *M. javanica* eggs was reduced in lettuce roots when isolate Pc-10 was applied either to seedlings or soil; there was no interaction between application methods. The decrease in the number of eggs was proportional to the increase of isolate Pc-10 applied to seedlings with maximum reduction of 43.5% at the 18 g L^{-1} dose. When the fungus was applied to the soil, the number of eggs was reduced by 12.3%. Increasing doses of isolate Pc-10 reduced the number of galls up to 21% with the 18 g L^{-1} dose. Applying bionematicide based on *P. chlamydosporia* isolate Pc-10 at 18 g L^{-1} on seedlings controls *M. javanica* in lettuce.

Key words: Biological control, *Lactuca sativa*, nematophagous fungi, root-knot nematode.

INTRODUCTION

Sustainable management of root-knot nematode (*Meloidogyne* spp. Goeldi) can be accomplished by applying biological control agents such as *Pochonia chlamydosporia* (Goddard) Zare & Gams (Dallemole-Giaretta et al., 2013; Viggiano et al., 2014). This soil-borne fungus parasitizes eggs and exposed females of root-knot nematode, which can drastically reduce the inoculum of the pathogen in the soil. Additionally, it produces chlamydospores that persist in the soil for long periods of time (Manzanilla-López et al., 2013).

Applying cereal grains colonized by the fungus controls root-knot nematode in vegetable crops (Dallemole-Giaretta et al., 2010). However, this approach of delivering the antagonist can be cost-limiting. It is therefore important to evaluate strategies whose aim is to reduce the number of bioproducts based on *P. chlamydosporia* to be applied in the field without decreasing the efficiency of the biological control agent.

In Brazil, lettuce (*Lactuca sativa* L.) is transplanted in the field with seedlings grown in polystyrene trays filled with organo-mineral substrates. Fungus application during the lettuce seedling production period can be an alternative for the management of root-knot nematode. The aim of this study was to evaluate the effect of treating seedlings and/or soil with bionematicide based on chlamydospores of *P. chlamydosporia* var. *chlamydosporia* isolate Pc-10 (Pc-10) on the *Meloidogyne javanica* (Treub, 1885) control; this is one of the most important threats for lettuce production in tropical countries (Sikora and Fernández, 2005).

MATERIALS AND METHODS

The research study was carried out in a greenhouse with lettuce 'Regina 255' as the host plant and *M. javanica* as the pathogen to be controlled by Pc-10. A commercial bionematicide consisting of chlamydospores from the isolate Pc-10 (wettable powder formulation, mean concentration 3.1 × 10^8 viable chlamydospores g^{-1}, Rizotec, Rizoflora Biotecnologia S.A., Viçosa, Brazil) was used. The bionematicide was diluted and applied when watering the seedlings and/or to the potted soil used for growing lettuce.

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Lettuce seedlings were grown in polystyrene trays filled with organo-mineral substrate (pH 5.54, C/N 23.81, 5.5 g N kg^{-1}, 2.6 g P kg^{-1}, 2.2 g K kg^{-1}) previously moistened with 5% water (v:v). One seed was placed in each cell at a depth of 1 cm. Plants were irrigated daily with a hose fitted with a sprinkler nozzle. For seedling treatment, the bionematicide was diluted in water (at 0, 1.5, 3.0, 4.5, and 6.0 g L^{-1}), and 250 mL of the suspension was applied to the seedlings at 3, 7, and 12 d after sowing with a 500 mL polyethylene terephthalate bottle with a perforated lid. Seedlings were treated with 0, 4.5, 9.0, 13.5, or 18.0 g L^{-1} of the bionematicide suspension.

After the seedling stage, lettuce plants were grown in 80 plastic pots (2 L) filled with 2 kg of a soil:sand mixture (1:1, v:v). The mixture was previously fertilized with superphosphate (6 g kg^{-1}) and treated with methyl bromide (80 cm^3 m^{-3}). Soil in each pot was infested with 3 mL of aqueous suspension containing 3000 *M. javanica* eggs. Then, 20 mL Pc-10 aqueous suspension was applied to the soil in 40 pots to provide 5000 chlamydospores g^{-1} soil, while soil in the remaining 40 pots was not treated with the fungus. The nematode inoculum consisted of eggs obtained from pure populations and collected from potted tomato plant roots (*Solanum lycopersicum* L. 'Kada') kept in a greenhouse and extracted by the Hussey and Barker (1973) technique modified by Boneti and Ferraz (1981). A lettuce seedling was transplanted in each pot 7 d after the soil was infested with the nematode. Plants were grown in a greenhouse and irrigated daily to maintain soil moisture at approximately field capacity.

To determine the number of colony-forming units (CFU) of Pc-10 in seedlings, substrate adhering to the root systems of 10 seedlings was collected 23 d after sowing; this established a substrate sample per dose of Pc-10 applied in the seedlings. The number of CFU g^{-1} substrate was determined according to the method described by de Leij and Kerry (1991) using a semi-selective medium (Gaspard et al., 1990).

On day 43 after transplanting seedlings, the weight of fresh shoots and roots and number of galls and eggs per plant were evaluated. The average minimum and maximum temperatures during the development of lettuce plants were 23.4 and 36.5 ºC for air and 24.4 and 32.5 ºC for soil, respectively. Nematode eggs were extracted from lettuce roots according to Hussey and Barker (1973) and were counted under a dissecting microscope at 4X magnification. Root galls were counted with the naked eye. Rhizosphere soil samples were also collected to determine CFU g^{-1} soil of Pc-10 as described above.

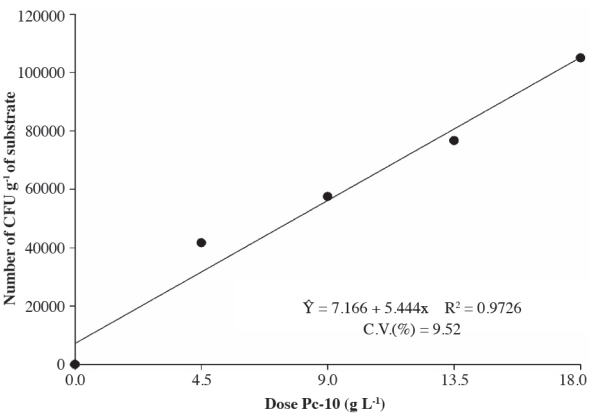
The experiment was repeated twice in a completely randomized design with a 5 × 2 factorial arrangement (Pc-10 doses applied to the seedlings × soil with or without Pc-10) and eight replicates. Each experimental unit consisted of a pot with a lettuce plant. The experiment-treatment interactions were not significant ($P > 0.05$). Data from the two experiments were pooled for further analysis. They were tested for normality of the error (Kolmogorov-Smirnov test), homogeneity of variances (Bartlett test), and subjected to ANOVA ($P < 0.05$). Data were analyzed by two-way ANOVA (Pc-10 doses in the seedlings × soil treatment and their interactions). Significant relationships between doses of bionematicide in seedlings and parameters were described by linear regression models.

RESULTS AND DISCUSSION

Increasing Pc-10 doses applied to seedlings increased fungus density in the substrate and varied from 4.2 × 10^4 to 10.5 × 10^4 CFU g^{-1} substrate at doses of 4.5 and 18.0 g L^{-1}, respectively (Figure 1). The fungus was not recovered from the untreated control, thus excluding the possibility of contamination among different treatments.

Lettuce root and shoot weight increased by 7.5% and 9.4%, respectively, when Pc-10 was mixed with the soil (26.87 g in Pc-10 × 24.99 g in control and 73.36 g in Pc-10 × 67.02 in control, respectively), regardless of the fungus application to seedlings. *Pochonia chlamydosporia* colonizes and produces chlamydospores in the rhizosphere of different plant species, such as tomato (*Solanum lycopersicum* L. 'Kada'), cabbage (*Brassica oleracea* L. var. *capitata* L.), kale (*Brassica oleracea* L.), corn (*Zea mays* L.), and sunn hemp (*Crotalaria juncea* L.) (Manzanilla-López et al., 2013). In addition, the fungus can promote plant growth (Maciá-Vicente et al., 2009). The present findings are consistent with those found in another study where the same fungus isolate was applied to lettuce (Viggiano et al., 2012) and suggest that Pc-10 colonizes the lettuce rhizosphere and enhances plant growth.

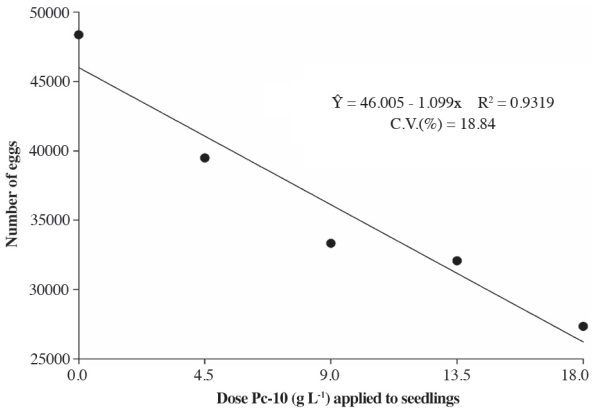
The number of *M. javanica* eggs was reduced in lettuce roots when Pc-10 was applied either to the seedlings or soil; there was no interaction between application methods. The reduction in the number of eggs was proportional to



CV: Coefficient of variation.

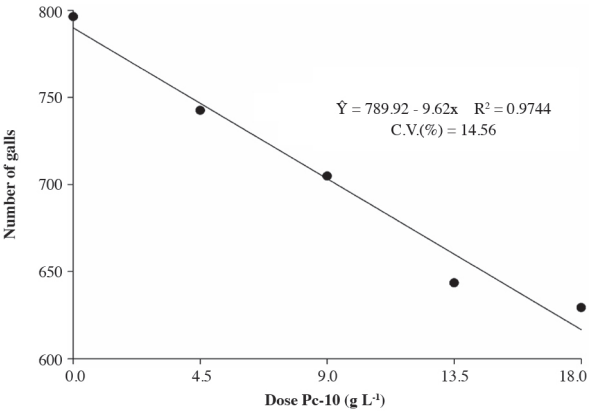
Figure 1. Number of colony forming units (CFU) of *Pochonia chlamydosporia* var. *chlamydosporia isolate* Pc-10 per gram of substrate in lettuce seedlings watered with different doses of a suspension of bionematicide based on Pc-10 chlamydospores.

the increase of Pc-10 applied to the seedlings (Figure 2), as observed in cucumber plants (Viggiano et al., 2014). The number of *M. javanica* eggs was 43.5% lower in plants treated with 18 g L^{-1} Pc-10. Applying the fungus to the soil reduced the number of nematode eggs by 12.3% (33 748 in Pc-10 × 38 471 in control). Increasing doses of Pc-10 reduced the number of galls up to 21% with the 18 g L^{-1} dose, regardless of the additional soil application (Figure 3).



CV: Coefficient of variation.

Figure 2. Number of *Meloidogyne javanica* eggs in lettuce roots treated with different doses of a suspension of bionematicide based on *Pochonia chlamydosporia* var. *chlamydosporia isolate* Pc-10.



CV: Coefficient of variation.

Figure 3. Number of galls induced by *Meloidogyne javanica* in lettuce roots after applying different doses of a suspension of bionematicide based on *Pochonia chlamydosporia* var. *chlamydosporia isolate* Pc-10.

The application of 18 g L^{-1} Pc-10 in seedlings was primarily responsible for reducing the number of *M. javanica* eggs and galls in lettuce. Seedling treatment may have favored the establishment of Pc-10 in the soil and rhizosphere. As a consequence, it is likely that nematode eggs in the soil were colonized by the fungus before infective second-stage juveniles hatched (J_2), which explains the decrease in the number of galls and eggs. An additional application of the fungus to the nematode-infested soil also reduced nematode reproduction, which corroborates findings of previous experiments involving Pc-10 and *M. javanica* in tomato (Dallemole-Giaretta et al., 2010; 2012). However, the overall effect on the *M. javanica* control was achieved when the fungus was applied to the seedlings, as observed in cucumber (Viggiano et al., 2014). It is likely that applying Pc-10 to seedlings of other vegetable crops can be an alternative for introducing the fungus in the substrate and in nematode-infested soils, especially for plants which allow extensive colonization of *P. chlamydosporia* in their rhizosphere, such as tomato, kale, and cabbage (Manzanilla-López et al., 2013).

CONCLUSIONS

Isolate PC-10 controls *Meloidogyne javanica* in lettuce by applying bionematicide based on *Pochonia chlamydosporia* at a 18 g L^{-1} dose on seedlings.

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