Management of Root-Knot Nematodes (Meloidogyne spp.) in Greenhouse Cucumbers Using Microbial Products

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Abstract

The environmental pollution is one of the major problems in the world. The decrease of agrochemicals like chemical pesticides and fertilizers is important to protect human health and the environment. The use of biocontrol agents against some root-knot nematodes Meloidogyne spp. is an alternative option to reduce environmental pollution. Paecilomyces lilacinus and Trichoderma viride are biocontrol fungi with a potential range of activity to control plant parasitic nematodes. Greenhouse experiments were conducted in 2013-2014 at the Maritsa Vegetable Crops Research Institute, Plovdiv to establish the biological activity of BioAct WG (Paecilomyces lilacinus strain 251) and Trichoderma viride strain T6 applied alone and in combination against root-knot nematodes (Meloidogyne spp.) in cucumber variety Defense F1. Two treatments with bioagents were carried out in a natural nematode population density: the first is at transplanting and the second - six weeks after it. All tested variants suppressed nematode reproduction and root galling and result in plant growth improvement compared to the control. The lowest rate of infestation and the highest total yield were established in the combination BioAct WG and Trichoderma viride strain T6. The micro-bioagents could be an effective mean to control root-knot nematodes, which results in vegetable production free of pesticides.

Keywords: Meloidogyne spp., Paecilomyces lilacinus, Trichoderma viride, cucumber, biocontrol

Introduction

The root-knot nematodes (Meloidogyne spp.) are an important group of plant parasitic nematodes that have worldwide distributions, extensive host ranges that make the control difficult. Nematodes are a major constraint to successful vegetable production all over the world, causing severe damage that leads to yield losses (Sikora and Fernandez, 2005; Karssen and Moens, 2006).

The root-knot nematodes (Meloidogyne spp.) are soil pests frequently observed in Bulgaria in greenhouse cucumber growing. The intensive growing of vegetable crops in greenhouses promotes for their reproduction. The applied chemical products are not always with adequate effectiveness and often their use cause serious ecological problems. New ecological alternative methods are tried to be found. In this aspect, studies for establishment of the nematicide effect of different biological agents are conducted: bacteria Pseudomonas aeruginosa (Siddiqui et al., 2000); Bacillus firmus and Pasteuria penetrans (Lamovšek et al., 2013) and fungi - Arthrobotrys oligospora; Hirsutella rhossiliensis; Paecilomyces lilacinus (Amin, 2000); Trichoderma viride (Goswami and Mittal, 2004). There is information in the literature about the P. lilacinus as an effective bio-regulator of root-knot and cyst nematodes (Wang et al., 1999; Yang et al., 2000; Ganaie and Khan, 2010).

The egg-pathogenic fungus Paecilomyces lilacinus (Thom) Samson is one of the most widely tested biocontrol agent for the control of plant-parasitic nematodes (Atkins et al., 2005; Kiewnick and Sikora, 2006). Paecilomyces lilacinus is a soil-inhabiting fungus that colonize the plant roots and it is a pathogen of root-knot nematodes (Siddiqui et al., 2000). Lara et al. (1996) demonstrated that P. lilacinus
The increased demand of ecological resistant and safety methods for control of the root-knot nematodes is observed during the last years (Adegbite and Adesiyan, 2005). The microbioagents are an alternative for pure vegetables that are free of pesticides.

The aim of this study was to established the biological activity of the product BioAct WG (Paecilomyces lilacinus strain 251) and Trichoderma viride strain T6 used alone and in combination towards the root-knot nematodes (Meloidogyne spp.) in greenhouse cucumbers.

Materials and Methods

The study was conducted in the period 2013-2014 in the Maritsa Vegetable Crops Research Institute – Plovdiv.

Plant culture: Cucumber seed of variety Defense F1 were sown on 18-20 March in 0.5 l pots filled with peat-perlite substrate (1:1, v/v). Seedlings were transplanted on 18 April 2013 and 10 April 2014.

Greenhouse experiment: The experiments were conducted in an unheated greenhouse during the spring-summer growing season. The experiments were carried out in naturally infested soil by root-knot nematodes (Meloidogyne spp.). The treatments were done according to the following scheme:

1. BioAct WG

Bioematicide contains 1x10^10 vital spores per gram product of the fungus Paecilomyces lilacinus strain 251 dried on glucose. The commercial product is formulated as a water dispersible granule (WG). Producer: “Prophyta” – Germany. Treatment: first – 0.2 g/plant in transplanting; second – 0.2 g/plant 6 weeks after transplanting

2. Trichoderma viride strain T6

Bioagent Paecilomyces lilacinus and Trichoderma viride used separately and in combination were performed in order to control the root-knot nematodes. Better plant growth and smaller gall number was established in these variants (Goswami and Mittal, 2004; Goswami et al., 2006).

Advances in the last decades produced quite a number of biocontrol products that are already marketed. Some of the well-accepted commercial products contain bacteria Bacillus firmus and Pasteuria penetrans, and fungus P. lilacinus (Lamovšek et al., 2013). Experiments for optimization of the schemes for dose application and for establishment of the effect of P. lilacinus towards Meloidogyne javanica, Meloidogyne incognita x Meloidogyne arenaria were performed in tomato (Zaki, 1994; Ekanayake and Jayasundara, 1994; Kiewnick and Sikora, 2004).

The potential of the fungal biocontrol agent Trichoderma harzianum was evaluated for control of the root-knot nematode M. javanica. In greenhouse experiments, root galling was reduced and top fresh weight increased in nematode-infected tomatoes following soil pretreatment with Trichoderma. All the Trichoderma strains showed the ability to colonize M. javanica-separated eggs and second-stage juveniles (J2) in sterile in vitro assays (Sharon et al., 2001).

Trichoderma harzianum and Trichoderma viride were tested for their ability to reduce the incidence of the root-knot nematode M. incognita. In vitro studies demonstrated that all tested isolates were effective and in causing J2 mortality compared with the control. The use of Trichoderma resulted in decrease of damages caused by root-knot nematode and improves the plant growth. The soil tillage with agents for biological control was made before and during the transplanting. Trichoderma isolates were re-isolated from the rhizosphere 45 days after fungal inoculation (Dababat and Sikora, 2007).

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Treatment: first - *Trichoderma viride* strain T6 $10^4$ spores/1 cm³ substrate in transplanting; second - *Trichoderma viride* strain T6 $10^4$ spores/1 cm³ substrate 6 weeks after transplanting.

3. **BioAct WG + *Trichoderma viride* strain T6**
   Treatment: first – BioAct WG 0.2 g/plant + *Trichoderma viride* strain T6 $10^4$ spores/1 cm³ substrate in transplanting; second – BioAct WG + *Trichoderma viride* strain T6 $10^4$ spores/1 cm³ substrate 6 weeks after transplanting.

**Table 1.** Effect of BioAct WG and *Trichoderma viride* strain T6 alone and in combination on plant growth of cucumber variety Defense F1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length (Stem (cm))</th>
<th>Diameter of stem (mm)</th>
<th>Root (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioAct WG</td>
<td>275.56±35.49 a</td>
<td>15.41±3.78 a</td>
<td>21.12±4.68 ab</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>293.68±36.23 a</td>
<td>14.03±3.14 a</td>
<td>25.64±4.61 a</td>
</tr>
<tr>
<td>BioAct WG + <em>T. viride</em></td>
<td>287.84±41.94 a</td>
<td>15.05±3.46 a</td>
<td>25.53±4.33 a</td>
</tr>
<tr>
<td>Control</td>
<td>243.30±41.94 b</td>
<td>10.86±3.17 b</td>
<td>19.76±3.79 b</td>
</tr>
</tbody>
</table>

*a, b, c ... – Duncan’s multiple range test (p < 0.05)*

**Table 2.** Effect of BioAct WG and *Trichoderma viride* strain T6 alone and in combination on nematode population, reproduction factor and root galling of *Meloidogyne* spp.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nematode population density (juveniles per 100 g soil)</th>
<th>Degree of root galling (0-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial population (Pi)</td>
<td>Final population (Pf)</td>
</tr>
<tr>
<td>BioAct WG</td>
<td>372±80.74 b</td>
<td>187±41.31 c</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>517±39.32 a</td>
<td>328±56.00 b</td>
</tr>
<tr>
<td>BioAct WG + <em>T. viride</em></td>
<td>428±85.48 b</td>
<td>176±50.49 c</td>
</tr>
<tr>
<td>Control</td>
<td>508±54.56 a</td>
<td>674±83.05 a</td>
</tr>
</tbody>
</table>

*a, b, c ... – Duncan’s multiple range test (p < 0.05)*

**Table 3.** Effect of BioAct WG and *Trichoderma viride* strain T6 alone and in combination on yield of cucumber variety Defense F1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (t ha⁻¹)</th>
<th>I quality</th>
<th>II quality</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioAct WG</td>
<td>40.15±0.840 b</td>
<td>9.08±1.530 b</td>
<td>49.23±1.350 b</td>
<td></td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>39.71±1.160 b</td>
<td>14.92±2.790 a</td>
<td>54.63±1.990 a</td>
<td></td>
</tr>
<tr>
<td>BioAct WG + <em>T. viride</em></td>
<td>43.51±2.180 a</td>
<td>10.68±2.570 b</td>
<td>54.19±0.790 a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>36.03±2.990 c</td>
<td>10.02±1.240 a</td>
<td>46.04±2.990 a</td>
<td></td>
</tr>
</tbody>
</table>

*a, b, c ... – Duncan’s multiple range test (p < 0.05)*

All treatments were replicated four times. Five plants in each replicate were uprooted at the end of the growing season (22 July 2013 and 21 July 2014).

**Parameters:** Soil samples for nematode analysis were collected from each plot. Five soil cores (3 cm diam. to 20 cm deep) were pooled to one sample per plot. Soil samples were taken at the beginning and end of each plot to estimate initial (Pi) and final population densities (Pf) of nematodes. Nematodes were extracted from 100 g subsamples for each plot, using a modified Baermann-funnel technique (Rodriguez-Kabana and Pope, 1981). The root gall severity was rated on a 0-5 scale, where: 0 = no galling, 1 = trace infections with a few small galls, 2 = <25% roots galled, 3 = 25–50% galling, 4 = 50–75% galling, and 5 = > 75% of roots galled (Hussey and Janssen, 2002).

In the end of vegetation were recorded the following parameters: length of stem and root (cm) and stem diameter (mm). The total yield (t ha⁻¹) was calculated and the treatments were grouped by quality.

The results from the experiments were processed mathematically. Comparison of means was made according to Duncan's
Multiple Range Test at P<0.05 levels (Duncan, 1955).

Results and Discussion

Effect on plant growth: Both bioagents BioAct WG and _Trichoderma viride_ strain T6 alone and in combination significantly promoted the growth of plants compared with the control. The stem length was greatest where plants were treated with _T. viride_ strain T6 (293.68 cm), followed by the treatments with BioAct WG + _T. viride_ strain T6 (287.84 cm) and BioAct WG (275.56 cm). There were no remarkable differences in diameter of stem in all treatments but significantly exceed those in the control. Root length was observed significantly different between the treatments. It was greatest in treatments _T. viride_ strain T6 alone and BioAct WG + _T. viride_ strain T6 25.64 cm and 25.53 cm respectively, as compared to untreated control (19.76 cm) (Table 1).

Effect on nematode population and root galling: Initial population densities (Pi) ranged from 372 to 508 J/100 g of soil (Table 2). Final population densities (Pf), however, were significantly different among treatments. As expected, populations were highest in untreated control. Differences between Pf for the other treatments were minor. Both the bioagents helped in reducing nematode population in the treatments i.e. alone or in combination. BioAct WG + _T. viride_ strain T6 and BioAct WG reduced Pf 176 and 187 J/100 g soil respectively as compared to _T. viride_ strain T6 (328 J/100 g soil). The combination of BioAct WG + _T. viride_ strain T6 showed least reproduction factor (0.41) as compared with untreated control (1.36). Maximum reduction in root galling as well as the soil population occurred in soil treated with BioAct WG + _T. viride_ strain T6 (1.7) as compared to the control (3.1).

The obtained results confirm the data established by Goswami and Mittal (2004) that the combined application of _Paecilomyces lilacinus_ and _T. viride_ reduced the nematode population and improves the plant growth.

Effect on yield: In all treatments with application of microbial products were observed significantly higher total yield compared with the control. The highest total yield was recorded in _T. viride_ strain T6 (54.630 t ha\(^{-1}\)) and in the combination BioAct WG + _T. viride_ (54.190 t ha\(^{-1}\)), followed by BioAct WG – 49.230 t ha\(^{-1}\). The highest yield of production of first quality was in plants treated with BioAct WG + _T. viride_ strain T6 (43.510 t ha\(^{-1}\)), which significantly exceeds the other treatments. In plants of the control were established the lowest values of production of first quality (Table 3).

Conclusion

The lowest root galling (1.7) was established in the combined application of BioAct WG + _Trichoderma viride_ strain T6 as well as relatively higher growing parameters.

In all studied treatments with application of microbial products (BioAct WG, _Trichoderma viride_ strain T6, BioAct WG + _Trichoderma viride_ strain T6) was observed demonstrated higher total yield compared with the control.

References


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incognita by *Paecilomyces lilacinus* strain 251. – Biological Control 38: 179-187.


