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

[a]Vinelina YANKOVA[*], [a]Dima MARKOVA, [b]Mladen NAIDENOV, [a]Boyan ARNAOUDOV
[a]Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria
[b]Agricultural University, Plovdiv, Bulgaria
*Corresponding author: vinelina@abv.bg

Abstract

The environmental pollution is one of the major problems in the world. The decrease of agro-chemicals 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 - *Artrhrobotrys 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*

significantly reduced *M. incognita* soil and root populations and increased yield of tomato.

BioAct WG is made up of the spores of the naturally occurring fungus *P. lilacinus*, strain 251. It was initially marketed as "Biocon" in Philippines, but was later manufactured by Prophyta Ltd. (Germany) and is registered as MeloCon in the USA. It is currently registered for sale for control of nematodes in several countries (Atkins et al., 2005; Kiewnick, 2004).
This strain, originally isolated from a *Meloidogyne* egg mass in the Philippines, has been shown to be highly effective in controlling *Meloidogyne* spp. on tomato and *Globodera rostochiensis* on potato (Davide and Zorrila, 1983; Davide, 1985).

Trichoderma spp. has also been described as biocontrol agents against plant-parasitic nematodes. Affokpon et al. (2011) tested different isolates of *Trichoderma* sp. against *Meloidogyne* spp. in tomato. They reported that *Trichoderma* isolates provided significant inhibition of nematode reproduction, suppression of root galling and an increase of tomato yield.

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).

Experiment with bioagents *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* и *Meloidogyne arenaria* were performed in tomato (Zaki, 1994; Ekanayake and Jayasundara,1994; Kiewnick and Sikora, 2004).

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 (*Paecilomices 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

   Bionematicide 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

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

4. Control – untreated

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

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

*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.

Treatments	Nematode population density (juveniles per 100 g soil)			Degree of root galling (0-5)
	Initial population (Pi)	Final population (Pf)	Reproduction factor (Rf= Pf/Pi)	
BioAct WG	372±80.74 b	187±41.31 c	0.54 b	1.9±0.88 bc
T. viride	517±39.32 a	328±56.00 b	0.63 b	2.2±0.74 b
BioAct WG+*T. viride*	428±85.48 b	176±50.49 c	0.41 c	1.7±0.96 c
Control	508±54.56 a	674±83.05 a	1.36 a	3.1±0.81 a

*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

Treatments	Yield (t ha^{-1})		
	I quality	II quality	Total
BioAct WG	40.150±0.840 b	9.080±1.530 b	49.230±1.350 b
T. viride	39.710±1.160 b	14.920±2.790 a	54.630±1.990 a
BioAct WG+ *T. viride*	43.510±2.180 a	10.680±2.570 b	54.190±0.790 a
Control	36.030±2.990 c	10.020±1.240 b	46.040±2.990 c

*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 *T. 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_2 100 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_2/100 g soil respectively as compared to *T. viride* strain T6 (328 J_2/100 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 *P. 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.

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