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EFFICIENCY OF *PSEUDOMONAS SPP.* FOR BIOCONTROL OF THE POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS* (WOLL.)

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Abstract

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The potential of *Pseudomonas spp.* as biocontrol agent against potato cyst nematode *Globodera rostochiensis* was tested in pot experiment under glasshouse conditions. The best control of nematode population was provided by the bacterial strains *Pseudomonas putida* 3 (2) and *Pseudomonas aurantiacea* 13 (2). The plant growing parameters in the treatments enhanced as compared with the inoculated with *Globodera rostochiensis.* The two bacterial strains significantly reduced nematode populations by 40.7% - 42.2 % compared to the control.

Key words: cyst nematode, biocontrol, bacterial strains

Introduction

Plant-parasitic nematodes are among the most destructive plant pest, causing substantial economic losses to crops worldwide. The golden potato nematode *Globodera rostochiensis* (Woll.) causes severe yield losses of potato (*Solanum tuberosum* L.) in many regions of Bulgaria (Trifonova, 1995, 2000). Soil treatment with nematicides has been an established practice for the control of cyst-forming nematodes of the genus *Globodera*, though it is very expensive for the farming community. In recent years, we have been observing an increasing trend to use alternative methods of pest control based on replacing chemicals pesticides by natural compounds.

The results of more recent studies showed that in many cases, endophytic microorganisms have an important role in host protection against pathogens. According Hallmann et al. (1998) some endophytic bacteria are associated with beneficial effects such as plant growth promotion and bio control potential against plant parasitic nematodes. Most *Pseudomonas* spp. is free-living saprophytic organisms in soil whereas they play an important role in decomposition, biodegradation and the carbon and nitrogen cycles. Barahona et al. (2011) showed that three strains of *Pseudomonas fluorescens* possess biocontrol activity against fungal root pathogens as *Fusarium oxysporum* f. sp. *radicis-licopersici* and *Phytophtora cactorum*. Endophytes colonize the same root tissues as sedentary plant-parasitic nematodes. Therefore, this association of endophytic bacteria with nematodes throughout the nematode life cycle makes these bacteria excellent candidates for biocontrol strategies. Most research on the interaction of endophytic bacteria with nematodes has been conducted on root knot nematode (*Meloidogyne* spp.), but the association of other nematode species – cyst nematodes with the endophytic bacteria is also of great interest. Three *Pseudomonas fluorescens* strains inhibited invasion of the plant-parasitic nematode *Radopholus simillis* and *Meloidogyne* spp. in banana, maize and tomato roots. (Aalten et al., 1998; Ashoub and Amara, 2010; Siddiqui et al., 2001). One of the difficulties in developing rhizobacteria as a control measure is that the performance of these agents is highly variable.

The objective of this research was to determine the efficiency of different bacterial isolates against the potato cyst nematode *G. rostochiensis* applied to potatoes plants.

Material and Methods

Nematode population

The population of *Globodera rostochiensis* was obtained from soil samples collected from heavily infested potato fields in Smolyan region. The cysts of the nematode were pro-

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duced on susceptible potato cultivars *S. tuberosum* cv. Focal in glasshouse. They were extracted from the soil by wet-sieve technique (Southey, 1986).

Bacterial isolates

The sample of the test soil from raspberry field was placed in a beaker with a different amount of sterile distilled water, 1/50, 1/100, 1/200 (g/ml water), and mixed with a mixer for 2 minutes and filtered through Whatman paper (Grade 1:11 µm). From soil suspension 1 ml was taken and pipette into small drops (40 drops from 1 ml; 10 in each plate) on the surface of a 2% water agar (Duschefa, Netherlands) in Petri dishes. The plates were cultured at 24°C for 18-24 hours. Determinations of bacteria were recorded under a microscope observation.

Treatments

The treatments were made in a glasshouse at 20 - 22°C and 13 h day length. Clay pots of 12 cm diameter were filled with 600 g autoclaved soil. Seed tubers cv. Nadejda used in all tests. Each pot contained one-potato seedling inoculated with 30 cysts of *G. rostochiensis* (put in muslin bags) poured in the root zone of the plants. They were irrigated to maintain optimum moisture level.

The bacterial strains were added to the soil at the time of planting. We used 35 ml aqueous cell suspension of the bacterial inoculums (10^8 cells/ml). Oxamyl (Vydate® 10 G, Du Pont™) at the rate of 1.5 mg/g soil was applied through irrigation before planting. There were four replicates of each treatment. Non-inoculated plants were used as a control. The experiments were terminated after 90 days or after completion of the nematodes life - cycle. Final cyst density was determined by the wet-sieve decantation technique. The effect of the treatments on reproduction of nematodes was determined by calculating a reproductive index (R = Pf/Pi - final/initial population densities). The plant and root length were recorded.

The data were subjected to factorial analysis of variance and treatments were compared using Duncan `s multiple range test (Steel and Torrie, 1980).

Results

The experimental results on the influence of different bacterial strains for control of *Globodera rostochiensis* on potato indicate that all treatments improved plant growth and decreased the nematode densities - from 8.3% to 42.7% compared with the untreated-inoculated plants (Table 1). The plants in treated

Table 1
Effect of soil application of *Pseudomonas spp.* strains on the growth of potato plants and reproduction of *G. rostochiensis*

Treatments	Length, cm			Means num.of cysts/ plant	Reduction over control %
	shoot	root	total		
1.	28.3	12.2	40.5 ^{ns}	115.1 ^{ns}	8.3
2.	26.6	11.6	38.2 ^{ns}	108.3 ^{ns}	10.7
3.	35.6	11.6	47.2 $^{+}$	94.6 $^{+}$	22.3
4.	37.1	12.1	49.2 $^{+}$	94.3 $^{+}$	22.4
5.	31.3	12.6	43.9 ^{ns}	101.2 ^{ns}	16.5
6.	37.3	11.1	48.4 $^{+}$	91.3 $^{+}$	24.7
7.	32.2	11.6	43.8 ^{ns}	88.1 $^{+}$	27.2
8.	50.3	12.6	62.9 $^{+}$	70.2 $^{+}$	42.2
9.	39.3	10.6	49.9 $^{+}$	74.6 $^{+}$	38.8
10.	39.6	11.6	51.2 $^{+}$	82.1 $^{+}$	32.2
11.	52.6	13.3	65.9 $^{+}$	72.2 $^{+}$	40.7
12.	60.2	13.6	73.8 $^{+}$	-	-
13.	30.3	12.1	42.4	121.6	-
14.	40.2	10.2	50.4 $^{+}$	20.1 $^{+}$	83.4
Se			2.7	1.1	

1. *Pseudomonas fluorescens* F 10 (1); 2. *Pseudomonas fluorescens* F 10 (2); 3. *Pseudomonas fluorescens* F 11 (1); 4. *Pseudomonas fluorescens* F 11 (2); 5. *Pseudomonas fluorescens* F 14 (1); 6. *Pseudomonas fluorescens* G 14 (4); 7. *Pseudomonas putida* 3 (1); 8. *Pseudomonas putida* 3 (2); 9. *Pseudomonas syxantha* 3 (3); 10. *Pseudomonas aurantiacea* 13 (1); 11. *Pseudomonas aurantiacea* 13 (2); 12. untreated; 13. nematode (N); 14. oxamyl.
+ significantly differences from the control at $P \leq 0.05$.

variants were higher compared to infested-untreated plants (N). Maximum plant height was recorded in the treatment with *Pseudomonas putida* 3 (2) and *Pseudomonas aurantiacea* 3 (2) (62. 9 and 65. 9 cm respectively). The plants were height means from 62.9 to 65.9 cm. Infection by *G. rostochiensis* caused significantly decreasing of the total length of potato shoot and root compared to the uninoculated control (C). The bacterial strains suppressed the nematode reproduction thus helping to increase plant length. Data in Table 1 showed that the root and shoot length of inoculated - treated with *Pseudomonas fluorescens* F 10 (1), F 10 (2), F 14 (1) and *Pseudomonas putida* 3 (1) plants were not significantly different from the untreated - inoculated plants (P≤0.05). The plants showed significantly lower value for these parameters in the range 38.2 - 43.9 cm.

Similar results were obtained in greenhouse test on mungbean (*Vigna radiate* L.) and tomato (*Lycopersicon esculentum* Mill.) treated with strains of fluorescent *pseudomonas* to control root-knot nematode (Siddiqui and Ehteshamul-Haque, 2000, a, b; Siddiqui and Shaukat, 2003). Among all treatments the greatest reduction over the control (N) in nematode multiplication occurred with oxamyl (83.4%), followed by strain *Pseudomonas putida* 3 (2) (42.2%) and *Pseudomonas aurantiacea* 13 (2) (40.7%).

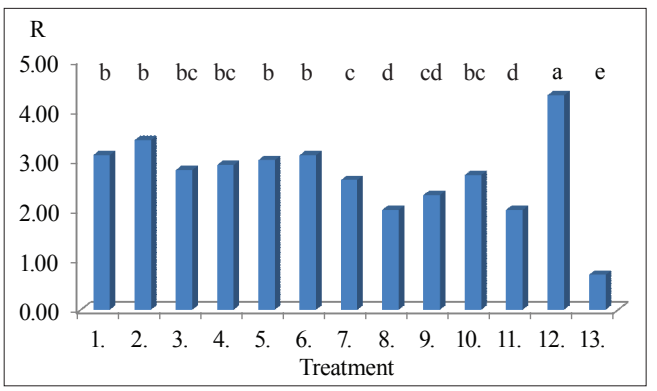

Fig. 1. Reproduction index (R) of *Globodera rostochiensis* in soil treated whit *Pseudomonas* strains

1.- *Pseudomonas fluorescens* F 10 (1); 2.- *Pseudomonas fluorescens* F 10 (2); 3.- *Pseudomonas fluorescens* F 11 (1); 4.- *Pseudomonas fluorescens* F 11 (2); 5.- *Pseudomonas fluorescens* F 14 (1); 6.- *Pseudomonas fluorescens* G 14 (4); 7.- *Pseudomonas putida* 3 (1); 8.- *Pseudomonas putida* 3 (2); 9.- *Pseudomonas syxantha* 3 (3); 10.- *Pseudomonas aurantiacea* 13 (1); 11.- *Pseudomonas aurantiacea* 13 (2); 12.- nematode (N); 13.- oxamyl.

Columns with the same letters are not significantly different (P ≤ 0.05) according to
Duncan `s Multiple Range Test.

The soil application of the bioagents significantly suppressed cyst formation (Figure 1). Population density of *G. rostochiensis* drastically declined compared with the control. It was decreased by 8.3% up to 42.2% over untreated-inoculated plants. The highest reproduction rate of *G. rostochiensis* was registered in the infected-untreated plants (R = 4.3). The greatest decreasing of the number of newly formed cyst was obtained in the oxamyl application (R = 0.7), followed by strain *Pseudomonas putida and Pseudomonas aurantiacea* (R = 2.0). *Pseudomonas fluorescens* F (10) 1 and F (10) 2 were significantly less effective (R = 3.1-3.4).

Discussion

Our results indicate that certain strains of fluorescent *pseudomonas* are able to suppress the nematode density and it could be used for the biological control of cyst nematode *Globodera rostochiensis*. The results are in agreement with those reported by Cronin et al. (1997). Hallmann et al. (2001), Siddiqui et al. (2000) and Ali et al. (2002) showed that endophytic bacteria produced specific metabolites, which can inhibit hatch of eggs and the mobility of the second-stage juveniles of nematodes. Metabolites of bacterial origin inside of the cells have significant importance because they could reduce the reproductive potential of the females (Siddiqui and Ehteshamul-Haque, 2000; Siddiqui et al., 2003).

The entomopathogenic bacterium *Pseudomonas putida* and its toxic secretions were used for the insect control that is an important component of integrated pest management against different insect pests (Mahar et al., 2005). The greatest increase in growth and enzyme activities of nematode-inoculated plants as well as the reduction in galling and nematode multiplication was observed with *P. putida* on root-knot nematode *M. incognita*. (Akhtar and Panwar, 2012; Siddiqui and Akhtar, 2008). Among plant growth-promoting rhizobacteria *P. putida* caused greater inhibitory effect on the hatching and penetration of *M. javanica* (Siddiqui et al., 2007).

There were a few reports examining the mechanisms of action. Three mechanisms of action are thought to be responsible for reduction in nematode infection: production of metabolites which reduce hatch and attraction, degradation of specific root exudates which control nematode behavior and enhancement of the defense mechanism in plants leading to the induction of systemic resistance (Sikora and Hoffmann-Hergarten, 1993; Hallmann et al., 2001; Siddiqui et al., 2001).

The level of control cannot be compared to those obtained by nematicides, but the rhizosphere bacteria offered very good biological plant protection. Although statistical significance, the level of nematode control achieved in the present study by the biocontrol bacteria is not substantially

high (42% in the most effective treatment) and thus there are reservation as to its possible exploitation under field conditions. Nonetheless, such *Pseudomonas fluorescens* strains can be exploited practically for the control of plant-parasitic nematodes through the application of integrated control strategies by combining their application along with soil organic amendments and nematophagous fungi.

Future research should be directed in order to find more strains that are effective antagonistic to nematodes by improving formulation and application techniques to enhance bacterial colonization of the root surface and to identify their mode-of-action.

Conclusion

The results obtained from the research of the soil treatments with the bacterial strains of *Pseudomonas* spp. for control of *G. rostochiensis* allowed us to make the following conclusions:

- The test bacterial strains caused significant inhibitory effect on multiplication of *G. rostochiensis*. Reproduction rates (R) of the nematode decreased from 3.4 to 2.0 in comparison with the control (4, 3).

- The percentages of reduction in the multiplication are from 40.7% to 42.2% over the control for the strain *P. aurantiacea* 13 (2) and *P. putida* 3 (2) respectively.

- All treatments improved the plant growth of nematode infected potato plants being greatest in variant *P. aurantiacea* 13 (2).

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