FIELD STUDIES OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR BIOLOGICAL CONTROL OF *ROTYLENCHULUS RENIFORMIS* ON SOYBEAN

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Abstract

Two plant growth-promoting rhizobacteria (PGPR) strains previously identified to reduce soybean cyst and root-knot nematodes were evaluated for their biocontrol potential on reniform nematode on soybean at Tennessee Valley Research and Extension Center (TVREC) in Belle Mina, AL. The PGPR's were applied as in-furrow sprays at planting with the rate of 1×10^7 CFU/seed. Poncho/Votivo (Clothianidin plus *B. firmus* I-1582) and Avicta (Abamectin) seed were treated before planting as controls. Results indicated all the plant growth parameters were similar among the treatments at 45 days after planting (DAP). Strain Bmo3 (*Bacillus mojavensis*) significantly reduced *Rotylenchulus reniformis* eggs per gram of root at 45 DAP compared to the untreated control, which had an equivalent level of *R. reniformis* population density as the chemical standard Avicta ($P \leq 0.10$). Yield was similar among all the treatments ranging from 34 bu/a to 38 bu/a. However, Avicta supported the highest yield followed by strains Bmo3 and Bve2 (*B. velezensis*).

Introduction

Rotylenchulus reniformis (Linford & Oliveira), the reniform nematode, is the primary economic pathogen in cotton production (Castillo et al. 2013) and also causes significant economic losses on soybean production individually or collectively with soybean cyst and root-knot nematodes in the southern region of the United States (Wrather and Koenning 2009). Several species of *Bacillus* spp. have been reported to have biological control potential on *R. reniform*. *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 in commercial formulations applied separately or concomitantly were evaluated on *R. reniformis* in cotton. Seeds treated with either *B. firmus* (1.4×10^7 CFU/seed), or an application of *P. lilacinus* (0.3% v/v of water), or the combination of *B. firmus* and *P. lilacinus* reduced the population density of *R. reniformis* in the greenhouse, microplots, and field trials (Castillo et al. 2013). *Bacillus mojavensis* strain Bmo3 and *B. velezensis* strain Bve2 have been shown to reduce population densities of *M. incognita* in cotton (Xiang et al. 2016). The overall objective of this study was to evaluate two PGPR strains *B. mojavensis* (Bmo3) and *B. velezensis* (Bve2) for their ability to reduce *R. reniformis* population density on soybean and enhance yields in a field production system.

Materials and Methods

PGPR Strains

Two PGPR strains *B. mojavensis* (Bmo3) and *B. velezensis* (Bve2) previously found with the ability of managing soybean cyst nematode on soybean (Xiang et al. unpublished) and root-knot nematode on cotton (Xiang et al. 2016) were used in the study. The strains stored in 30% glycerol at -80 ˚C were transferred to tryptic soy agar (TSA) (VWR, Radnor, PA) plates, and incubated at 35˚C for 24 hours. Vegetative cells of each strain were suspended in water and the concentration was adjusted to 1×10^7 CFU/ml.

Plant Materials

Soybean (*Glycine max*) variety "Ag5935" (Monsanto, St. Louis, MO) known to be susceptible to *R. reniformis* (Lawrence et al. 2016) was used for the field experiment.

Field Experiment

The experiments were established at Tennessee Valley Research and Extension Center (TVREC), Belle Mina, AL in 2016. The field was infested with *R. reniformis* and the population density at planting was 5000 reniform per 100 cm^3 of soil. The experiments were arranged in a RCBD with 5 replications and the trial was repeated in different fields on the same station. The field trials were arranged in two-row plots that were 7 m long with 0.9 m row spacing. One hundred and seventy five soybean seeds were planted in each row with an Almaco plot planter. The PGPR

treatments were applied as in-furrow spray standardized to 1×10^7 CFU/seed and applied at 32.5 liter per hectare at planting. Seeds treated with Poncho/Votivo (0.424 mg ai/seed) and Avicta (0.15 mg ai/seed) were included as industry standard controls. Untreated seeds were used as untreated controls (CK). At 45 DAP, four random soybean plants were removed from each plot. Plant vigor, plant height, shoot and root fresh weights, and *R. reniformis* eggs/g root were evaluated by extracting eggs from the roots. Soybeans were harvested mechanically with a Almaco plot harvester at plant maturity approximately 160 DAP and yield recorded and adjusted to 13% moisture content.

Experimental Design and Statistical Analysis

All the experiments were arranged in a randomized complete block design with five replications and the trial was repeated. Data collected from the field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. LS-means were compared between the treatments, chemical standards Poncho/Votivo, Avicta, and the untreated control by Dunnett's method at significant level of $P \leq 0.05$. The LS-means are presented in the tables with adjusted *P*-values for statistical differences.

Results and Discussion

Field Results

Results indicated that plant vigor, plant height, and biomass including shoot/root fresh weights were statistically similar among all the treatment at 45 DAP (Table 1). However, visual difference were observed among treatments in some of the plots at 37 DAP (Fig. 1) with the Avicta and *B. mojavensis* strain Bmo3 treatment looking more vigorous than the untreated control (CK). These vigor differences were also observed at 80 DAP (Fig. 2) in the same Avicta and Bmo3 plots. *Bacillus mojavensis* strain Bmo3 significantly reduced *R. reniformis* eggs per gram of root at 45 DAP compared to the untreated control, which had an equivalent level of reniform population density as the chemical standard Avicta ($P \leq 0.10$) (Table 1). *R. reniformis* population levels ranged from 381 (Bmo3) to 900 (CK) eggs per gram of root over all biologicals and nematicides. Soybean yield at harvest ranged from 34 bu/a to 38 bu/a varying by 4 bu/a across all the treatments (Table 1). Ranking the soybean yields, Avicta supported the highest yield followed by *B. mojavensis* strain Bmo3 and *B. velezensis* strain Bve2, which supported numerically higher soybean yield by 12%, 3%, and 3% respectively as compared to untreated control (Table 1).

Figure 1. Soybean plants in the Untreated Control (CK left), Avicta (middle), and Biological Bmo3 (right) TVREC at 37 days after planting.

Table 1. Efficacy of two *Bacillus* strains on reduction of *Rotylenchulus reniformis* population density and plant growth promotion in soybean in the field ($P \leq 0.05$)[a].

Treatment	PH[b]	Plant height at 40 DAP			Bio[c]	Plant biomass at 40 DAP			Eggs/gr[d]	Reniform eggs/g root at 40 DAP			Yield[e]	Soybean yield in bu/a at 160 DAP		
		Dunnett's *P* vs.				Dunnett's *P* vs.				Dunnett's *P* vs.				Dunnett's *P* vs.		
		Poncho/ Votivo	Avicta	CK		Poncho/ Votivo[c]	Avicta	CK		Poncho/ Votivo[c]	Avicta	CK		Poncho/ Votivo[c]	Avicta	CK
Bve2	14.8	0.3140	0.1766	0.9145	20.0	0.1430	0.8380	1.0000	530.8	0.6725	0.8347	0.1332	34.9	1.0000	0.3683	0.9450
Bmo3	16.1	1.0000	0.9901	0.7484	24.2	0.9127	0.9340	0.4380	380.8	0.1901	1.0000	0.0189	34.8	1.0000	0.3423	0.9589
Poncho/Votivo	16.1	…	0.9925	0.7311	26.1	…	0.5384	0.1421	717.9	…	0.2048	0.6913	34.8	…	0.3449	0.9577
Avicta	16.3	0.9925	…	0.5075	22.4	0.5384	…	0.8360	387.9	0.2048	…	0.0209	38.2	0.3449	…	0.1338
CK	15.3	0.7311	0.5075	…	20.0	0.1421	0.8360	…	900.2	0.6913	0.0209	…	33.7	0.9577	0.1338	…

[a]Field trials were performed in Tennessee Valley Research and Extension Center in 2016. Data collected were repeated and analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of 0.05. Adjusted *P* values less than 0.05 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. The LS-means are presented in the tables with adjusted P values for statistical differences.
[b]PH = plant height (cm) at 60 DAP.
[c]Bio = plant biomass including shoot fresh weight and root fresh weight (g) at 60 DAP.
[d]Eggs/gr = *R. reniformis* eggs per gram of root.
[e]Yield = soybean yield (bu/a) obtained at 160 DAP and adjusted to 13% moisture content.

Figure 2. Soybean plants in the Untreated Control (CK left), Avicta (middle) and Biological Bmo3 (right) TVREC at 80 days after planting.

Summary

In summary, *B. mojavensis* strain Bmo3 did reduce *R. reniformis* eggs/g of root at 45 DAP and supported a yield similar to Avicta control. Strain Bmo3 is a promising biological control agent that should be further evaluated for the potential use to manage and reduce plant-parasitic nematode population densities.

References

Castillo, J. D., Lawrence, K. S., and Kloepper, J. W. 2013. Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and Paecilomyces lilacinus 251 on cotton. Plant Dis. 97:967 - 976.

Lawrence, K., Lawrence, G, Faske, T., Kemerait, R., Overstreet, C., Wheeler, T., Young, H., and Mehl, H. 2016. Beltwide nematode research and education committee 2015 nematode research report cotton varietal and nematicide responses in nematode soils. Proceedings of the 2016 Beltwide Conference Vol 1: 737-740. National Cotton Council of America, Memphis, TN. http://www.cotton.org/beltwide/proceedings/2005-2016/index.htm.

Wrather, J. A. and Koenning, S. R. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Plant Health Progress. http://www.plantmanagementnetwork.org/pub/php/research/2009/yields/.

Xiang, N., Lawrence, K. S., Kloepper, J.W., Donald, P. A, McInroy, J.A., and Lawrence, G.W. 2016. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton. Plant Dis. First Look. http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-09-16-1369-RE.