THE EFFECT OF AZOTOBACTER AND ACTINOMYCETES ON THE GROWTH OF ENGLISH RYEGRASS AND MICROBIOLOGICAL ACTIVITY IN ITS RHIZOSPHERE

D. STAMENOV[1], Mirjana JARAK[1], Simonida ĐURIĆ[1], Timea HAJNAL-JAFARI[1], Snežana ANĐELKOVIĆ[2]

[1]Faculty of Agriculture, Dositeja Obradovića Square 8, 21000 Novi Sad, Serbia
[2]Institute of Forage Crops, Globoder bb, 37251 Kruševac, Serbia
E-mail: draganastamenov@yahoo.com

Abstract: *Microorganisms are still not widely used in forage grass production although higher and better quality yield is one of the goals of animal husbandry. The aim of this research was to investigate the effect of plant growth promoting microorganisms (Azotobacter chroococcum and Streptomyces sp.) on the growth of English ryegrass and microbiological activity in its rhizosphere. Two types of English ryegrass (Lolium perenne) were used - broad-leaved and narrow-leaved. The experiment was conducted in 10 l volume vegetation pots. Before sowing, 10 ml of inocula was introduced into the pots. Three Streptomyces sp. strains and three Azotobacter chroococcum strains were used for inoculation. The control variants were not inoculated. The number of microorganisms (the total number, azotobacter, actinomycetes and fungi), dehydrogenase activity, height and dry mass of the plant were determined thirty days after sowing. The results showed that microorganisms can have a positive effect in the production of English ryegrass. In broad-leaved ryegrass, the application of all six strains resulted in greater height and dry mass of the plant, whereas the effect was weaker in narrow-leaved ryegrass. In the rhizosphere of broad-leaved ryegrass, the total number of bacteria increased only with the application of one strain of azotobacter. In the rhizosphere of narrow-leaved ryegrass, the total number of bacteria increased with the application of all three strains of Streptomyces sp. and one strain of azotobacter. The greatest number of azotobacter in the rhizosphere of both types of English ryegrass was recorded in the variants inoculated with azotobacter. The number of fungi in the rhizosphere of broad-leaved ryegrass increased with the use of one strain of azotobacter and one strain of actinomycetes, whereas in narrow-leaved ryegrass, it increased with the use of two strains of actinomycetes and one strain of azotobacter. The number of actinomycetes and dehydrogenase activity in the inoculated variants did not significantly change in comparison with the control.*

Key words**:** *azotobacter, actinomycetes, English ryegrass, yield, microbiological activity*

INTRODUCTION

Plant growth promoting microorganisms exert a positive effect on the growth, development and yield of plants through various mechanisms such as biological nitrogen fixation, vitamin production, production of growth substances, providing plants with available phosphorus, increasing the amount of nutrients in rhizosphere, and some are used as biocontrol agents (DOBBELAERE et al., 2003; BERG, 2009). Introducing PGP microorganisms into rhizosphere changes microbiological activity and affects plant development. Many rhizospheric microorganisms, among which azotobacter and actinomycetes, have a PGP effect. BROWN (1974) achieved an increase in yield from 10% to 20% in field conditions using the combination of *Bacillus megatherium* and *Azotobacter chroococcum*. WI et al. (2005) achieved similar results. Inoculation with *Azotobacter chroococcum* has a positive effect on the increase in yield, seed germination and early growth of maize plants, as well as on the length of the part above ground and root (EGAMBERDIYEVA, 2007; GHOLAMI, 2009). KIZIKAYA (2008) recorded a positive effect of inoculation with azotobacter on wheat yield. Actinomycetes enhance plant

growth producing biologically active substances such as indole-3-acetic acid (IAA) to help growth of roots or produce siderophores to improve nutrient uptake (MERCK et al., 1987; SUZUKI et al., 2000). Peas inoculated with *Streptomyces sp*. had a greater dry mass, and higher nitrogen and protein content in the part above ground (JEVIĆ, 2006). Some actinomycetes inhibit the development of phytopathogenic fungi and are used as bioagents. BERG (2001) concluded that *Streptomyces sp*. strain isolated from the rhizosphere of strawberry had an antagonistic effect on several pathogenic fungi.

In forage grass production, microorganisms are still not widely used although higher and better quality yield is one of the aims of animal husbandry. One of the best-known species of forage grass is English ryegrass. This plant is particularly convenient for making hay and silaging in mixtures with other plants and legumes. It is suitable for grazing, especially in spring, and in favourable conditions its yield can amount to 9-13 t/ha (ĐUKIĆ, 2002).

The aim of the research was to investigate the effect of plant growth promoting microorganisms (*Azotobacter chroococcum* and *Streptomyces sp*.) on the growth of English ryegrass and microbiological activity in its rhizosphere.

MATERIAL AND METHODS

Two types of English ryegrass (*Lolium perenne*) - broad-leaved and narrow-leaved (from the collection of the Institute for Forage Crops, Krusevac, Serbia) were used in this research.

The experiment was conducted in 10 l volume vegetation pots. The pots were filled with soil having optimum characteristics for plant production. Before sowing, 10 ml of the inocula was introduced into the pots. The bacteria used for inoculation were strains from the collection of the Faculty of Agriculture, Novi Sad: *Streptomyces sp*.5, *Streptomyces sp*. 7, *Streptomyces sp*. 9k, *Azotobacter chroococcum* SN, *Azotobacter chroococcum* Č and *Azotobacter chroococcum* RC.

Appropriate nutrient media (Hi Media Laboratories Pvt. Limited Mumbai, India) were used for multiplication of microorganisms: synthetic agar for the number of actinomycetes and Fjodorov medium for the number of azotobacter. The number of cells in 1 ml of the inoculum was $5x10^8$.

Thirty days after sowing, the following were determined: height and dry mass of the plant, total number of bacteria, number of actinomycetes, number of fungi, number of azotobacter and dehydrogenase activity.

The number of microorganisms was determined using the dilution method (TROLLDENIER, 1996) on appropriate nutrient media (Hi Media Laboratories Pvt. Limited Mumbai, India): nutrient agar for the total number of bacteria, synthetic agar for the number of actinomycetes, potato-dextrose agar for the number of fungi, meat-peptone agar for the number of aminoheterotrophs and Fjodorov medium for the number of azotobacter. Dehydrogenase activity was determined by LENHARD'S method (1956) modified by THALMANN (1968).

The data were statistically processed using STATISTICA 10 software.

RESULTS AND DISCUSSION

The effect of inoculation on the height and dry mass of the plant

The yield of English ryegrass, like the yield of all other plants, depends on the availability of nutrients in soil. One part of nutrients is provided in the process of microbiological activity and one part is introduced with fertilizers. Different strains of rhizospheric microorganisms have a stimulatory effect on the growth and development of plants (VESSEY et al., 2003). To what extent this effect will influence plant yield depends on the type of soil, effectiveness of indigenous and applied strains of microorganisms, plant

species, variety or hybrid (WALKER et al., 2003). The use of PGP microorganisms stimulates the early growth and development of plants (JARAK et al., 2007; UDOVIČKI and JARAK, 2005). In this research, it was observed that all six strains had a positive effect on the height and dry mass of broad-leaved English ryegrass, whereas such effect was not recorded in narrow-leaved English ryegrass (table 1).

Table 1.

The effect of inoculation on the height and dry mass of the plant

Variants		Broad-leaved ryegrass		Narrow-leaved ryegrass	
		Height (cm)	Dry mass (g)	Height (cm)	Dry mass (g)
Streptomyces sp. 5		20.50 **	0.042**	23.76	0.034**
*Streptomyces sp.*7		20.14**	0.042**	14.82**	0.025**
Streptomyces sp. 9k		19.34*	0.035	18.18*	0.028**
Azotobacter chroococcum SN		17.08	0.032	18.40*	0.030**
Azotobacter chroococcum Č		19.58*	0.041**	18,26*	0.030**
Azotobacter chroococcum RC		20.42**	0.040*	16.72**	0.027**
Control		14.44	0.031	22.92	0.040
LSD	1%	5.587	0.009	5.715	0.001
	5%	4.129	0.007	4.217	0.0003

*p<0.05; ** p<0.01

Streptomyces sp. 5 and 7 strains, as well as *Azotobacter chroococcum* RC had a statistically significant effect at the level p<0.01, whereas *Streptomyces sp*. 9k and *Azotobacter chroococcum* Č had a statistically significant effect at the level p<0.05. The best effect on the plant growth was recorded with the use of *Streptomyces sp*. 5 strain.

In comparison with the control, the best effect on the growth of narrow-leaved English ryegrass was recorded with *Streptomyces sp*. 5 strain (Table 1). In all other variants, the plants were shorter than in the control. *Streptomyces sp*. 7 and *Azotobacter chroococcum* RC led to a statistically significant decrease in plant height at the level p<0.01 and *Streptomyces sp*. 9k, *Azotobacter chroococcum* SN and *Azotobacter chroococcum* Č at the level p<0.05.

In broad-leaved English ryegrass, inoculation with all the applied strains had a positive effect on the dry mass of the plant. The greatest dry mass was recorded in the plants whose seed was inoculated with *Streptomyces sp*. 5 and 7 and *Azotobacter chroococcum* Č and that effect was statistically significant at the level p<0.01, whereas *Azotobacter chroococcum* RC had a statistically significant effect at the level p<0.05.

In the inoculated variants, the dry mass of the narrow-leaved ryegrass decreased at the level p<0.01 (Table 1).

The effect of inoculation on the number of microorganisms in the rhizosphere of English ryegrass

Microbiological processes can additionally be stimulated by introducing biofertilizers. These microorganisms reproduce in soil and with their enzymatic activity raise and maintain the appropriate level of organic matter in soil (HAJNAL et al., 2005; JARAK et al., 2009). The effect of bacterial inoculation on the change of microbiological activity in soil depends on soil conditions, plant species, adaptability of introduced microorganisms etc. (VAN OVERBEEK et al., 1997; DOBBELAERE et al., 2003; EGAMBERDIYEVA, 2007).

In this research, the total number of bacteria in the rhizosphere of the investigated plants was large and typical of fertile soils (Table 2). *Streptomyces sp*. 5, *Streptomyces sp*. 9k and *Azotobacter chroococcum* Č had a negative effect on the total number of bacteria while the effect of *Azotobacter chroococcum* RC on these was positive (p<0.01)(table 2).

In narrow-leaved ryegrass, the greatest positive effect was recorded with *Streptomyces sp*. 5 and 9k strains (table 2). *Streptomyces sp*.7 and *Azotobacter chroococcum* Č also had a

positive effect on the total number of bacteria at the level p<0.05.

Table 2.

The effect of inoculation on the total number of bacteria and number of azotobacter in the rhizosphere of English ryegrass

Variants		Broad-leaved ryegrass		Narrow-leaved ryegrass	
		Total number 10^6 g^{-1} soil	Azotobacter 10^2 g^{-1} soil	Total number 10^6 g^{-1} soil	Azotobacter 10^2 g^{-1} soil
Streptomyces sp. 5		26.65**	2.50	92.44**	1.30
*Streptomyces sp.*7		74.25	1.37	52.87*	1.57
Streptomyces sp. 9k		24.04**	1.09	78.12**	1.42
Azotobacter chroococcum SN		68.48	26.0**	20.31	1400.0**
Azotobacter chroococcum Č		44.22**	3000.0**	52.32*	510.0**
Azotobacter chroococcum RC		104.46**	70.0**	19.77	3600.0**
Control		75.88	1.29	31.82	1.26
LSD	1%	25.426	12.494	25.776	198.679
	5%	18.135	8.912	18.385	141.709

*p<0.05; ** p<0.01

The number of azotobacter in the rhizosphere of both types of English ryegrass increased several times in the variants with azotobacter (Table 2). In the variants with actinomycetes, the number of azotobacter was similar to its number in the control.

Unlike azotobacter, *Streptomyces sp*. strains did not increase the number of actinomycetes in the rhizosphere (table 3). The only stastistically significant increase in the number of actinomycetes at the level p<0.05 was recorded in the rhizosphere of narrow-leaved ryegrass where *Streptomyces sp*. 5 was applied (table 3).

Table 3.

The effect of inoculation on the number of actinomycetes and fungi in the rhizosphere of English ryegrass

Variants		Broad-leaved ryegrass		Narrow-leaved ryegrass	
		Number of actinomycetes 10^4/g soil	Number of fungi 10^4/g soil	Number of actinomycetes 10^4/g soil	Number of fungi 10^4/g soil
Streptomyces sp. 5		71.08	75.11	107.57*	92.44*
*Streptomyces sp.*7		164.83	102.81*	68.33	92.73*
Streptomyces sp. 9k		82.21	83.76	73.24	69.17
Azotobacter chroococcum SN		65.27	91.86*	82.86	83.67
Azotobacter chroococcum Č		72.88	58.96	35.71	88.86*
Azotobacter chroococcum RC		69.64	72.96	91.74	81.46
Control		96.22	61.02	62.09	65.97
LSD	1%	101.521	32.331	51.626	26.996
	5%	72.41	23.060	36.823	19.255

*p<0.05; ** p<0.01

The number of fungi in the rhizosphere of broad-leaved ryegrass ranged from 58.96 to 102.81 x 10^4/g of absolutely dry soil. A statistically significant increase in comparison with the control at the level p<0.05 was recorded in the variants with *Streptomyces sp.* 7 and *Azotobacter chroococcum* SN (table 3).

In the rhizosphere of narrow-leaved ryegrass the results were more even, with the number of fungi ranging from 69.17 to 92.73 x 10^4/g of absolutely dry soil. The largest number of fungi in comparison with the control was recorded in the variants with *Streptomyces sp*. 7 and 5 and *Azotobacter chroococcum* Č (table 3).

An increase in dehydrogenase activity in comparison with the control was recorded only in the rhizosphere of broad-leaved English ryegrass in the variant with *Streptomyces sp.* 5

at the level p<0.05 (table 4).

Table 4.

The effect of inoculation on dehydrogenase activity in the rhizosphere of English ryegrass (µg TPF/10 g of soil)

Variants		Broad-leaved ryegrass	Narrow-leaved ryegrass
Streptomyces sp. 5		215.233**	153.33
*Streptomyces sp.*7		80.967	97.133
Streptomyces sp. 9k		99.033	121.933
Azotobacter chroococcum SN		95.267	96.167
Azotobacter chroococcum Č		82.867	116.20
Azotobacter chroococcum RC		91.433	133.33
Control		80.00	103.833
LSD	1%	69.84	80.571
	5%	49.814	57.468

*p<0.05; ** p<0.01

Numerous investigations into the influence of inoculation on field and vegetable crops have proved its positive effects (GOVEDARICA et al., 2001; HAJNAL et al., 2004; KUZEVSKI et al., 2008). STAMENOV et al. (2011) concluded that *Trichoderma asperellum* fungus had a positive effect on the yield of English ryegrass and on the number of microorganisms in the rhizosphere of the plant. BISWAS et al. (1994) claimed that the use of azotobacter resulted in a higher yield of annual and perennial grasses. The results of our research confirmed that using specific species of microorganisms has positive effects in the production of English ryegrass which justifies the use of biofertilizers in the production of forage crops.

CONCLUSIONS

On the basis of the results of the research, it can be concluded that the effect of inoculation depends on the type of English ryegrass and applied microorganisms.

All six strains had a positive effect on the height and dry mass of the broad-leaved ryegrass, whereas the effect on the narrow-leaved ryegrass was weaker. The best effect on the growth of both types of English ryegrass was achieved with the use of *Streptomyces sp.* 5 strain.

The total number of bacteria in the rhizosphere of broad-leaved ryegrass increased only with the use of one azotobacter strain. In the rhizosphere of narrow-leaved ryegrass the total number of bacteria increased with the use of all three strains of *Streptomyces* sp. and one azotobacter strain.

The largest number of azotobacter in the rhizosphere of both types of English ryegrass was recorded in the variants inoculated with azotobacter.

The number of actinomycetes in the inoculated variants did not significantly change in comparison with the control.

The number of fungi in the rhizosphere of broad-leaved English ryegrass increased with the use of one strain of azotobacter and one strain of actinomycetes, whereas in narrow-leaved ryegrass, this number increased with the use of two strains of actinomycetes and one strain of azotobacter.

A significant increase in dehydrogenase activity was recorded only in the rhizosphere of broad-leaved ryegrass in the variant inoculated with *Streptomyces sp.* 5 strain.

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