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EFFECT OF PGPR ON THE GERMINATION AND GROWTH OF ENGLISH RYEGRASS AND MICROBIOLOGICAL ACTIVITY IN ITS RHIZOSPHERE*

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SUMMARY: The objective of this study was to examine the effect of plant growth promoting bacteria Pseudomonas sp., Bacillus sp., and Streptomyces sp. on germination and growth of English ryegrass and microbiological activity in its rhizosphere. One hundred seeds of perennial ryegrass inoculated with the appropriate bacteria were placed on filter paper in the dark at 22 C° until germination. Five and fourteen days after, hypocotyl and root length of seedlings were measured. The experiment was conducted in 10 l volume vegetation pots, with the following variants: 1. Pseudomonas sp. P12, 2. Bacillus sp. B1, 3. Streptomyces sp. A3, 4. control - no inoculation. Thirty and sixty days after sowing, length of stem and roots of plants (cm) were determined. Thirty days after sowing, the number of microorganisms was determined, using the dilution method. Application of Pseudomonas sp. P12 and Bacillus sp. B1 had a negative effect on ryegrass seed germination, while the use of Streptomyces sp. A3 gave positive results. On average, the application of Pseudomonas sp. P12 and Bacillus sp. B1 had the greatest effect on the stem and root length of English ryegrass. The number of the investigated groups of microorganisms increased in all variants in comparison to the control. The best effect was achieved with Bacillus sp. B1.

Key words: *microorganisms, germination, yield, English ryegrass.`*

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are originally defined as root-colonizing bacteria that can be found in the rhizosphere, at root surfaces and in association

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with roots, enhancing the growth of the plant, either directly, and/or indirectly. Treatment with PGPR has increased the germination percentage, seedling vigor, plant stand, root and shoot growth, total biomass of plants, seed weight, early flowering, increased grain, fodder, fruit yields etc. (Ramamoorthy et al., 2001).

On the other hand, after being introduced into the soil, PGPR may affect the indigenous microbial population. Likewise, the indigenous microorganisms can affect the inoculants. What kind of effect will be expressed depends on the conditions in soil, plant species, adaptation ability of the introduced microorganisms etc. (Egamberdiyeva, 2007). Some groups of microorganisms may be stimulated, some may be inhibited or there may be no effect of the introduced microorganisms on the structure of the indigenous population (Dobbelaere et al., 2003). Artursson et al. (2006) concluded that the use of PGPR in plant production results in the increase in the number and enzymatic activity of microorganisms, microbial biomass, microbial diversity in the rhizosphere and improved production characteristics of soil.

Different bacteria that have been reported as PGPR belong to the following genera: *Pseudomonas, Bacillus, Azospirillum, Agrobacterium, Azotobacter, Arthrobacter, Streptomyces, Trichoderma Variovovax, Xanthomonas and Phyllobacterium* (Berg, 2009). Most popular bacteria studied and exploited as biocontrol agents include the species of fluorescent *Pseudomonas*, *Bacillus* and actinomycetes *Streptomyces sp.* (Adesemoye et al., 2008).

Therefore, the objective of this study was to examine the effect of plant growth promoting bacteria *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. on the germination and growth of English ryegrass and microbiological activity in its rhizosphere.

MATERIAL AND METHODS

PGPR *Pseudomonas* sp. P12, *Bacillus* sp. B1, *Streptomyces* sp. A3 (from the collection of the Faculty of Agriculture, Novi Sad) were used as inoculants. English ryegrass (*Lolium perenne* L. Calibra) was taken from the collection of Institute of Forage Crops, Kruševac, Serbia.

The effect of PGPR on seed germination of English ryegrass was evaluated in controlled conditions. Before inoculation, the seed sterilization was performed by 70% ethanol and 0.1% $HgCl_2$ solution and rinsing with sterile water. After that, one hundred seeds of perennial ryegrass inoculated with the appropriate bacteria were placed on filter paper in the dark at 22 C°until germination. Five and fourteen days after, hypocotyl and root length of seedlings were measured.

The variants of the experiment were the following: 1. *Pseudomonas* sp. P12, 2. *Bacillus* sp. B1, 3. *Streptomyces* sp. A3, 4. control - no inoculation. The experiment was conducted in 10 l volume vegetation pots. The pots were filled with soil having optimum characteristics for plant production. Before sowing, 50 ml of the inocula was introduced into the pots. The number of cells in 1 ml of the inoculum was $5x10^8$CFU/ml. Thirty and sixty days after sowing, length of stem and roots of plants (cm) were determined.

Thirty days after sowing, the number of microorganisms was determined, using the dilution method (Trolldenier 1996). Appropriate nutrient media were used (Hi Media Laboratories Pvt. Limited, Mumbai, India): nutrient agar for the total number of bacteria, synthetic agar for actinomycetes, potato dextrose agar for fungi and medium

with mannitol for azotobacter.

The data were statistically processed using STATISTICA 12 software (Hamburg, Germany). The significance of the difference between the applied treatments was tested using Fisher's LSD test.

RESULTS AND DISCUSSION

In controlled conditions, the applied inoculants had different effects on the seed germination (Table 1).

Five days after inoculation, the highest percentage of germination (96%) was found in the variant in which *Streptomyces* sp. A3 was applied, while in the variant with *Pseudomonas* sp. P12, germination percentage was the lowest (11%). On hypocotyl and root length, the greatest impact had the application of *Streptomyces* sp. A3. Application of *Bacillus* sp. B1 and *Pseudomonas* sp. P12, compared to the control, had a negative effect on the hypocotyl and root length of seedling (Table 1).

Table 1. The percentage of seed germination, root and hypocotyl length

Variants	Seed germination (%)		Root length (mm)		Hypocotyl length (mm)	
Period	5days	14days	5days	14days	5days	14days
Pseudomonas sp. P12	11,0	89,0	1,0	6,0	13,0	90,0
Bacillus sp. B1	18,0	90,0	3,0	4,0	14,0	70,0
Streptomyces sp. A3	96,0	97,0	45,0	55,0	34,0	88,0
control	94,0	96,0	43,0	47,5	30,0	83,0

After 14 days, in the variant where *Streptomyces* sp. A3 was applied, the highest percentage of germination was determined. High percentage of seed germination was determined in other variants as well. *Streptomyces* sp. A3 had the highest effect on the hypocotil and root length of the seedling. Variants in which *Bacillus* sp. B1 and *Pseudomonas* sp. P12 were added negatively affected the root length. On the other hand, the application of *Pseudomonas* sp. P12 had a good influence on the hypocotyl length.

Negative effect of *Bacillus* and *Pseudomonas* on the seed germination of perennial ryegrass could be due to their ability to produce hydrogen cyanide gas, which in large quantities inhibits the germination and growth of plant roots (Stamenov, 2014). These results suggest that investigated isolates should be applied after plants emergence, but not during the sowing. This is in accordance with the studies of Heydari et al. (2008), who determined the inhibitory effect of the hydrogen cyanide producing *Pseudomonas* on germination of wheat and rye. Alstrom and Burns (1989) also studied the hydrogen cyanide producing *Pseudomonas* which inhibited the development of the beans. In contrast to these studies, Niranjan et al. (2004) reported that the application of *P. fluorescens* had a positive effect on germination of millet grains. Moreover, Shaukat et al. (2006) reported that the introduction of *Azospirillum*, *Azotobacter* and *Pseudomonas* in the rhizosphere of sunflower and wheat had a positive effect on the germination and the length of the seedlings. However, these isolates were not determined as a hydrogen cyanide producing bacteria.

After thirty days, all the applied treatments had a positive effect on the stem and root length of the plant, but the highest effect was achieved by using *Pseudomonas* sp.

P12 and *Bacillus* sp. B1 (Table 2). In these variants, increase of the stem and root length was statistically significant.

Table 2. The effect of inoculation on the stem and root length (cm)

Measured	Plant	control	*Pseudomonas* sp. P12	*Bacillus* sp. B1	*Streptomyces* sp. A3
30 days	Stem	$10,5^{b}$	$19,0^{a}$	$18,5^{a}$	$11,5^{b}$
	Root	3.0^{b}	$5,0^{a}$	$4,5^{ab}$	$3,75^{ab}$
60 days	Stem	$10,75^{a}$	$12,0^{a}$	$11,75^{a}$	$11,5^{a}$
	Root	$2,5^{a}$	$4,0^{a}$	$4,0^{a}$	$3,5^{a}$
Average	Stem	10,62	15,5	15,12	11,5
Average	Root	2,75	4,5	4,25	3,62

*The different superscripts indicate significant difference at $P<0.05$ according to Fisher`s test.

After sixty days, all applied treatments acted positively on the stem and root length of plants, but these changes were not statistically significant. On average, the stem and root length was the largest in the variant with *Pseudomonas* sp. P12 and lowest in the control.

Positive effect of inoculation on the grass yield was found in the research of Dragomir et al. (2007), who examined the effects of *Azospirillum* and *Beijerinckia* inoculation on the dry matter of cocksfoot (*Dactylis glomerata* L). Rennie et al. (1983) found that introduction of *Bacillus* sp. positively influenced the yield of grass, which is in accordance with the results of our study. El-Tarabily (2008) reported that several *Streptomyces* species have the ability to improve plant growth by increasing seed germination and root elongation, which is the case with our *Streptomyces* species. Similarly, Biswas et al. (1994) proved that the introduction of *Azotobacter* and *Azospirillum* positively affected the growth of annual and perennial grasses.

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-Jafari, 2010). In this study, the quantity of systematic and physiological groups of microorganisms in the rhizosphere of ryegrass depended on the applied inoculants. The number of investigated microbial groups in relation to the control was increased in all of the variants (Table 3).

Table 3. The effect of inoculation on the number of microorganisms in the rhizosphere of English ryegrass

Variants	The number of microorganisms in 1 g of absolutely dry soil (CFU/g)			
	Total nu. (10^{6})	Fungi (10^{4})	Actinomycetes (10^{5})	Azotobacter (10^{2})
Pseudomonas sp. P12	37.16^{a}	11.87^{b}	6.69^{cb}	118.93^{b}
Bacillus sp. B1	30.84^{d}	27.6^{a}	8.29^{ab}	130.06^{a}
Streptomyces sp. A3	33.45^{b}	3.67^{c}	4.88^{dc}	55.07^{c}
Control	20.54^{c}	3.55^{c}	3.93^{d}	37.52^{e}

*The different superscripts indicate significant difference at $P<0.05$ according to Fisher`s test

Application of *Pseudomonas* sp. P12 led to the increase of the total number of microorganisms, while isolate *Bacillus* sp. B1 affected positively the number of fungi, actinomicetes and *Azotobacter*. Application of *Streptomyces* sp. isolates A3 increased the number of actinomycetes. On average, the best effect on the microbial abundance had the application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1. Applied inoculants affected the number of *Azotobacter* notably while the lowest effect was found on the total number of microorganisms. Similarly, Nannipieri et al. (2003) demonstrated the positive effect of *Pseudomonas* sp. on the total number of bacteria and enzymatic activity in the soil. The research of Stamenov et al. (2012) demonstrated a positive effect of *Pseudomonas fluorescens* and *Bacillus* sp. on the total number of microorganisms, fungi, *Azotobacter* in the rhizosphere of perennial ryegrass.

Microorganisms have not yet found significant practical use in the production of forage grasses, even though more qualitative yield has been sought in livestock production. The results of this study demonstrate that positive results in the production of perennial ryegrass can be achieved by the use of microorganisms. Therefore, it is very important to carry out further studies of the relationship between applied microorganisms and ryegrass under field conditions in order to optimize the method and time of application of inoculants.

CONCLUSION

Application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1 had a negative effect on ryegrass seed germination, while the use of *Streptomyces* sp. A3 gave positive results. On average, the application of *Pseudomonas* sp. P12 and *Bacillus* sp. B1 had the greatest effect on the stem and root length of English ryegrass. The number of the investigated groups of microorganisms increased in all variants in comparison to the control. The best effect was achieved with *Bacillus* sp. B1. 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.

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UTICAJ PRIMENE PGPR NA KLIJAVOST I RAST ENGLESKOG LJULJA I NA MIKROBIOLOŠKU AKTIVNOST U RIZOSFERI BILJKE

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Izvod

Cilj istraživanja je bio da se ispita efekat primene mikroorganizama promotora rasta *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. na klijavost i rast engleskog ljulja, kao i na mirkobiološku aktivnost u rizosferi biljke. Seme engleskog ljulja inokulisano određenom bakterijom, stavljeno je na filter papir na 22 C° na naklijavanje. Petog i četrnaestog dana, merena je dužina stabaoceta i korenka klice, kao i procenat klijavosti. Varijante ogleda su bile sledeće: 1. *Pseudomonas* sp. P12, 2. *Bacillus* sp. B1, 3. *Streptomyces* sp. A3, 4. Kontrola- bez inokulacije. Ogled je postavljen u posudama zapremine 10l. Trideset i šezdeset dana nakon setve, merena je dužina nadzemnog dela i korena biljke (cm). Trideset dana nakon setve, metodom agarnih ploča određivana je brojnost pojedinih grupa mikroorganizama. Primena *Pseudomonas* sp. P12 i *Bacillus* sp. B1 imala je negativan efekat na klijavost semena, dok je primena *Streptomyces* sp. A3 dala pozitivne rezultate. U proseku, primena *Pseudomonas* sp. P12 i *Bacillus* sp. B1 imala je najbolji efekat na dužinu nadzemnog dela i korena biljke. Brojnost ispitivanih grupa mikroorganizama u odnosu na kontrolu povećala se u svim varijantama. Najbolji efekat postignut je primenom *Bacillus* sp. B1.

Ključne reči: mikroorganizmi, klijavost, prinos, Engleski ljulj.

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