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EFFECT OF INOCULATES ON ABUNDANCE OF ACTINOMYCETES IN ALFALFA RHISOSPHERE

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Abstract. This paper presents the results of the impact of microbial inoculants on the number of actinomycetes in the rhizosphere soil of alfalfa (*Medicago sativa L.*). This plant species in addition to high yield potential and quality of biomass, is characterized by an intense process of nitrogen fixation. The rhizosphere of alfalfa abounds in numerous microorganisms. The aim of the research was to investigate the effect of inoculating with two nitrogen-fixing bacteria (*Sinorhizobium meliloti* and *Azotobacter chroococcum*) and two isolates (CC657 and Coll11) of the phytopathogen fungus *Colletotrichum destructivum* on the number actinomycetes in the rhizospheric soil of alfalfa varieties (Affinity, Perry and K-28). The highest number of actinomycetes was determined in rhisosphere of cultivar Affinity which was inoculated with *A. chroococcum* + isolate Coll-11, while the lowest number was found in rhisosphere of cultivar K-28 inoculate with CC657. Results of Fisher test shows the absence of statistically significant differences in abundance of actinomycetes between treatments, meaning there were four homogenous groups: 1. treatments of cultivar K-28 with Coll-11 and CC657 + *S. meliloti*; 2. treatments of cultivar K-28 with Coll 11 + S. meliloti and control treatment (without inoculation) in cultivar Perry; 3. treatments with Coll 11 + *A. chroococcum* in cultivar K-28 and CC657 + *A. chroococcum* in cultivar Perry; 4. inoculation with CC657 + *A. chroococcum* in K-28 and Coll 11 + *A. chroococcum* in cultivar Perry.

Keywords: Rhizosphere, fungi, actinomycetes, alfalfa.

INTRODUCTION

Alfalfa (*Medicago sativa L.*) is, economically, the most important forage legume. In addition to high potential for yield and quality of biomass, this species is characterized by intensive process of biological nitrogen fixation. This plant species can fix 100-400 kg N ha^{-1} per year in association with *Sinorhizobium meliloti* (Peoples et al., 1995). In the rhizosphere of alfalfa. there are numerous microorganisms which are directly influenced by root secretions (Macek et al. 2000). Also, these microorganisms can have different effects on the plant development, so that microorganisms and plant make one cohesive unit (Raaijmakers et al., 2009).

Rhizosphere microorganisms have participation in the mineralization of organic compounds, maintaining the soil structure, suppressing pathogens (Janvier et al.. 2007), stimulating plant growth (Jarak et al., 2012). They live on the root and in rhizosphere soil (azotobacter, actinomycetes), while rhizobia lives in the root tissue. Rhizobia provide macrosymbionts with nitrogen and, also, synthesize polysaccharides, vitamins B_{12}, B_1, B_2 (Denison., 2006) and biocontrol substances (Avis et al., 2008). The increase of nitrogen balance is achieved by the application of free nitrogen-fixers, primarily *Azotobacter spp.* which synthesize gibberellins, auxins, pyridoxine and nicotinic acid (Dobbelaere et al., 2003). The bacteria of *Azotobacter* genus give advantage to productive, neutral soils and are very succeptable to the moisture deficit. The acid reaction has negative effect on the number and activity of *Azotobacter spp.* (Andjelković et al., 2010). Due to their favorable impact, these microorganisms are used in the production of alfalfa.

Rhizosphere soil is rich by root secretions and therefore is a suitable environment for the growth of actinomycetes (Aldesuquy et al., 1998). They produce growth substances, antifungal and antibacterial substances. Actinomycetes are active decomposers of organic matter (Williams et al., 1984). They can break down lignin, pectin, the most resistant matter from humus, thus creating the necessary asimilatives for the plant (Nolan et al., 1988). Actinomycetes produce antibiotics, phytohormones and vita-

mins, which are beneficial for the growth of various plants (Fermino – Soares et al., 2007, Bredholt et al., 2008). Some are antagonists of phytopathogenic fungi (Gethaet al., 2005, Minutoet al., 2006) and inhibit growth of certain phytopathogenic bacteria, like *Erwinia amylovora* and *Agrobacterium tumefaciens* (Oskay et al., 2004).

One of the most important alfalfa diseases is anthracnose (Vasić et al., 2009). It is most commonly caused by *Colletotrichum trifolii* Bain et Essary but also by *Colletotrichum destructivum* O'Gara (Boland et al., 1989). These microorganisms cause weaker growth or death of plants and lower microbial activity in the rhizosphere of infested plants.

The aim of the research was to investigate the effect of inoculating alfalfa with two nitrogen-fixing bacteria (*Sinorhizobium meliloti* and *Azotobacter chroococcum*) and two isolates (CC657 and Coll11) of the phytopathogen fungus *Colletotrichum destructivum* on the number actinomycetes in the rhizospheric soil of alfalfa varieties (Affinity, Perry and K-28).

MATERIAL AND METHODS

The experiment was carried out in 10 l volume vegetation pots in semi-controlled conditions at the Institute for Forage Crops in Kruševac. The soil chemical characteristics were the following: pH/KCl 5.90; pH/H_2O 6.44; total nitrogen 0.138 %; humus 2.62 %; P_2O_5 6.6 mg/100g; K_2O 24.05 mg/100g. The experiment was a two-factorial, random block design with 5 replicates, where the first factor was alfalfa cultivar and the second was the variant of microbial inoculation. Research was conducted on three alfalfa cultivars: K-28 (semi resistant), (highly resistant) and Perry (susceptible).

Before sowing, the seed was inoculated with *Sinorhizobium meliloti* and *Azotobacter chroococcum* (10 ml of inoculum per pot with 108 cells in 1 ml). The plants were mown after six-seven weeks and thereafter treated with *Colletotrichum destructivum*: Coll-11 isolate and CC 657 isolate conidia. The number of conidia was 4-6x104/ml. The number of conidia was determined by means of hemocytometer according to Tom.

The variants of the experiment were the following:

1. Coll-11+*S. meliloti*
2. Coll-11+*A. chroococcum*
3. Coll-11
4. CC 657+*S. meliloti*
5. CC 657+*A. chroococcum*
6. CC 657
7. Control

The effect of inoculation was determined at the end of the vegetation period. The number of microorganisms was determined by the method of agar plates, by introducing a diluted soil suspension into proper media and counted per one gram of absolutely dry soil. The number of actinomycetes was determined on synthetic agar according to Krasiljnikov (Krasiljnikov et al., 1965) (dilution 10-4) (Jarak et al., 2006). The results were processed by means of STATISTICS 8.0 programme. The significance of the difference between the investigated treatments was determined upon the analysis of variance, i.e. LSD test.

RESULTS AND DISCUSSION

In this research, inoculation of alfalfa with two nitrogen-fixing bacteria (*Sinorhizobium meliloti* and *Azotobacter chroococcum*) and two isolates (CC657 and Coll11) of the phytopathogen fungus *Colletotrichum destructivum* had a different effect on the number actinomycetes. The effect of inoculation on the change of microbiological activity in soil depends on soil conditions, plant species, adaptability of introduced microorganisms etc. (Egamberdiyeva et al., 2007).

The highest number of actinomycetes was determined in rhisosphere of cultivar Affinity which was inoculated with A. *chroococcum* + isolate Coll-11. Gharib et al. (2009) (Gharib et al., 2009) point out that the inoculation of *Phaseolus vulgaris L.* with

Rhizobium leguminoarum bv. phaseoli and *Azotobacter chroococcum* showed increase in the number of actinomycetes. The lowest number was found in rhisosphere of cultivar K-28 inoculate with CC657. Application of a large number of microorganisms in the soil they can change the number and composition of domestic microbial population (Jeon et al., 2003).

The effect of inoculation was dependant upon alfalfa variety and the applied inocula. According to results of Fisher test shows the absence of statistically significant differences in abundance of actinomycetes between treatments, meaning there were four homogenous groups. The first group consists treatments of cultivar K-28 with Coll-11 and CC657 + *S. meliloti*; In the second group are treatments of cultivar K-28 with Coll 11 + *S. meliloti* and control treatment (without inoculation) in cultivar Perry. In the third group are treatments with Coll 11 + *A. chroococcum* in cultivar K-28 and CC657 + *A. chroococcum* in cultivar Perry; The tretmeans of inoculation with CC657 + *A. chroococcum* in K-28 and Coll 11 + *A. chroococcum* in cultivar Perry are the fourth homogenous group.

In comparison to the control, in the cultivar K-28 in all inoculation treatments there were statistically significant decreases in the number of studied microorganisms. In the cultivar Affinity in inoculation treatment Coll-11 + *A. chroococcum* positive effect was noted. Similar was in the cultivar Perry inoculated with same isolate Coll 11 and *S. meliloti*, while in all other treatments the number of actinomycetes was significantly lower than in the control. In addition to the fact that phytopathogenic fungi have a negative impact on plants, there might be other possible consequences on microbial activity of rhisosphere plants (Andjelković et al., 2013).

Table 1 - The effect of inoculants on the number of actinomycetes in the rhizosphere of alfalfa (104/g)

Variant		K-28	Affinity	Perry
1.	Coll-11+ *S. meliloti*	13 ^{f}	9.06 ^{m}	19.2 ^{b}
2.	Coll-11+ *A. chroococcum*	12.2 ^{g}	23.6 ^{a}	10.2 ^{k}
3.	Coll-11	15.3 ^{d}	7.93 ^{p}	8.6 ^{n}
4.	CC 657+*S. meliloti*	15.2 ^{d}	8.34 ^{o}	9.9 ^{l}
5.	CC 657+*A. chroococcum*	10.1 ^{k}	11.1 ^{j}	12.3 ^{g}
6.	CC 657	7.7 ^{q}	11.28 ^{i}	11.8 ^{h}
10.	Control	16.3 ^{c}	13.7 ^{e}	13.1 ^{f}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test ($p < 0.05$)

CONCLUSIONS

The results of the research show the effect of inoculation, reflected in the number of actinomycetes which varies depending on the alfalfa cultivar and inoculation treatments. The use of beneficial and phytopathogenic microorganisms showed different effects in all of the alfalfa cultivars. In the cultivar K-28, in all inoculation variants, number of actinomycetes decreased compared to the control. In this cultivar, the positive effect was achieved by using Coll 11 and *A. chroococcum* as well as Coll 11 and *S. meliloti* compared to the treatment where only Coll 11 was applied. The increase in the number of actinomycetes in comparison to the control (as well as in other treatments) was achieved in the rhizosphere of the cultivar Affinity only using the Coll-11 + *A. chroococcum*, and similar effect was achieved in the rhizosphere of cultivar Perry using the Coll-11 + *S. meliloti*. This is a preliminary study and to obtain better information on the effect of beneficial and phytopathogenic microor-

ganisms on the number and composition of alfalfa rhizosphere microbial community, research must be continued in this direction.

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РЕЗЮМЕ

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ВЛИЯНИЕ ПРИВИВОК НА ЧИСЛЕННОСТЬ АКТИНОМИЦЕТОВ В РИЗОСФЕРЕ ЛЮЦЕРНЫ

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В данной статье представлены результаты воздействия микробных биопрепаратов на число актиномицетов в ризосфере почвы под люцерну (*Medicago Sativa L.*). Этот вид растений в дополнение к высокому потенциалу урожайности и качества биомассы, характеризуется интенсивным процессом фиксации азота. Ризосфера люцерны изобилует многочисленными микроорганизмами. Целью исследования было изучение влияния прививки двумя азотфиксирующими бактериями *(Sinorhizobium meliloti* и *Azotobacter chroococcum)* и двух изолятов (CC657 и Coll11) фитопатоген гриба *Colletotrichum destructivum* на количество актиномицетов в ризосферной почве под сортами люцерны (Аффинити, Перри и К-28).Наибольшее количество актиномицетов было определено в ризосфере сорта Аффинити, в которую вносили *A. chroococcum* + isolate Coll-11, в то время как наименьшее количество было обнаружено в ризосфере сорта К-28 привитым с CC657. Результаты теста Фишера показывает отсутствие статистически значимых различий в изобилии актиномицетов между обработками, что означает, что там было четыре однородные группы: 1. обработки сорта К-28 с Coll-11 с применением CC657 + *S. meliloti*; 2. обработки сорта К-28 при помощи Coll 11 + *S. meliloti* и контрольная обработка (без прививки) сорта Перри; 3. обработки сорта Перри с применением Coll 11 + *A. chroococcum* сорта К-28 и CC657 + *A. chroococcum*; 4. прививка К-28 с CC657 + *A. chroococcum* и сорта Перри при помощи Сб 11 + *A. chroococcum.*

Ключевые слова: ризосфера, грибы, актиномицеты, люцерна.

ТҮЙІН

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ЖОҢЫШҚА РИЗОСФЕРАСЫНДАҒЫ АКТИНОМИҢЕТТЕРГЕ ҚАРСЫ ЕКПЕНІҢ ЫҚПАЛЫ

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Бұл мақалада микробтық биопрепараттардың жоңышқа (*Medicago Sativa L.*) өсетін топырақ ризосферасындағы актиномиңеттер санына ықпал етуінің нәтижелері берілген. Бұл өсімдік түрі жоғары өнім түсімділігінің әлеуеті мен биомасса сапасына қосымша азоты тиянақтау үрдісінің қарқындылығымен сипатталады. Жоңышқа ризосферасы алуан түрлі микроорганизмдерге бай.

Зерттеудің мақсаты екі азоттиянақтаушы бактериямен (*Sinorhizobium meliloti* және *Azotobacter chroococcum*) және екі изолят (СС657 және Coll11) *Colletotrichum destructivum* саңырауқұлағының фитопатогенмен жоңышқа түрлері (Affinity, Перри және К-28) өсетін ризосфералық топырақтағы актиномиңеттер санына екпе жүргізудің ықпалын зерттеу болды. Актиномиңеттердің көп мөлшері *A. chroococcum* + isolate Coll-11 екпесі жасалған Аффинити сұрыпты жоңышқа ризосферасынан анықталды, ал СС657 егілген К-28 сұрпының ризосферасында актиномиңеттер саны аз болды. Фишер тестінің нәтижелері өңдеу арасындағы актиномиңеттердің көп болуында статистикалық маңызды айырмашылықтар жоқтығын көрсетеді, бұл онда төрт біртекті топ болғанын білдіреді: 1. СС657 + *S. meliloti* пайдалану арқылы Coll-11 көмегімен К-28 сұрыпын өңдеу; 2. Coll 11 + S. meliloti көмегімен К-28 сұрыпты өңдеу және Перри сұрыпты (екпесіз) бақылау өңдеу; 3. Coll 11 + *A. chroococcum* пайдаланумен Перри сұрыпты, СС657 + *A. Chroococcum*-мен К-28 сұрыпты өңдеу; 4. СС657 + *A. chroococcum* көмегімен К-28 және Сб 11 + *A. chroococcum* көмегімен Перри сұрыпына екпе жүргізу.

Кілтті сөздер: ризосфера, саңырауқұлақтар, актиномиңеттер, жоңышқа