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# EFFECTS OF DIFFERENT STRAINS OF *Sinorhizobium meliloti* ON ALFALFA (*Medicago sativa* L.) BIOMASS YIELD**

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**Original scientific paper

**Abstract:** The effects of highly effective strains of *Sinorhizobium meliloti* on biomass and crude protein yields of alfalfa (*Medicago sativa* L.) were investigated in a three-year field experiment focusing on the advantage of alfalfa inoculation with N microbiological fertilizer over N mineral fertilization on chernozem soil. Strains 236 and 234 were found in this experiment to have significant effect on the yields of dry matter and proteins, by comparison to untreated control plants. In the third year of utilization, dry matter yield of alfalfa plants inoculated with strains 236 and 234 increased around 31% in the first cutting and 53% and 65% in the second one, compared to control plants. Strain 236 was found to increase the yield of crude proteins by 29.7% (second cutting) and 39.6% (third cutting) in the second year, and 27.5% (first cutting) and 50.3% (second cutting) in the third year of utilization, while strain 234 increased significantly the content of proteins in the third cutting of the second year (27%) and in the third year of utilization (51% in the first and 53% in the second cutting). The results indicate that effective strains of *S. meliloti* may be applied as N microbiological fertilizers on soils with optimal physical and chemical characteristics and autochthonous populations that may not have satisfactory effectiveness in nitrogen fixation.

**Key words:** alfalfa, *Sinorhizobium meliloti*, inoculation, yield, chernozem

## Introduction

Alfalfa (*Medicago sativa* L.) is a major legume in agriculture, especially

in animal husbandry. It provides high-quality fodder as its dry matter contains around 20% crude proteins. In an effort to enable increase in outputs of high-quality and, even more importantly, wholesome fodder, research has partly been focused on increasing the natural ability of alfalfa plants to uptake atmospheric nitrogen ($N_2$). That characteristic is based on its symbiotic association with the bacterium *Sinorhizobium meliloti*, which fixes 100-400 kg N $ha^{-1}$, *Peoples et al.* (1995). The presence, density and effectiveness of *S. meliloti* depend on the soil type and land use regime, *Vukmir-Delić et al.* (1994). In soils that have good physical and chemical characteristics, such as chernozem, *S. meliloti* strains are abundant (in the order of $10^5$-$10^6$), *Jarak et al.* (2002). Alfalfa is successfully cultivated on that type of soils, meaning that it achieves an effective nitrogen fixing process with the active strains typical for that soil. However, autochthonous strains are often insufficiently effective and occasions may arise when artificial inoculation of alfalfa is required as an important agricultural practice, *Milić et al.* (2001). The effects of some highly effective *S. meliloti* strains on biomass and crude proteins yields of alfalfa were examined in a three-year field trial with the aim of focusing on the need to apply alfalfa inoculation inoculation with N microbiological fertilizer over N mineral fertilization on chernozem soil in alfalfa production.

## Materials and method

The widely used alfalfa cultivar K-28 of the Institute for Forage Crops was used as a host plant in a three-year field trial (2005-2007). Several highly effective strains of *S. meliloti* (224, 234 and 236), originating from a collection of the Institute of Soil Science, were used as inocula. Alfalfa was hand-sown at high density in plots sized 0.2 x 5m (1 $m^2$) in an experimental field of the Institute for Animal Husbandry, Belgrade-Zemun, in 2005. N mineral fertilizers were not used before sowing. The plants were inoculated with pure culture suspensions of the investigated strains aged 72 h and grown on YMB ($10^9$ cells $ml^{-1}$). The trial included 4 inoculation treatments (3 with single strains and 1 with their mixture) and an untreated control (Θ) in a random block system with 5 replicates. Fresh plant weight (kg/plot) and dry weight ($Tha^{-1}$) were measured at the beginning of flowering after two cuttings in the year of sowing and the third year of utilisation, and three cuttings in the second year of utilisation. Based on the percentage of N measured by chemical analyses (Kjeldahl) in the dry matter of samples cut,the content and yield of proteins were calculated. The results were

statistically processed by LSD test.

## Results and Discussion

The experiment was conducted on a weakly calcareous chernozem. The chemical characteristics of soil, given in Table 1, show that the contents of humus and total nitrogen in the top arable horizon are within normal limits, while phosphorus and potassium contents are more than well supplied. Low $NO_3^-$ nitrogen content, expected for unfertilized soils, confirmed that the plots had not been fertilized before the trial in the initial year. Such content of nitrate N is favourable for the establishment of a symbiotic assotiation between alfalfa and *S. meliloti*, *Raun et al* (1999).

**Table 1. Chemical properties of soil in 2004 year.**

| Depth/(cm) | Acidity/pH) | | Org. mat.(%) | Humus ( %) | $CaCO_3$ ( %) | N mg/1000g | | | $P_2O_5$ mg/100g | $K_2O$ mg/100g |
|---|---|---|---|---|---|---|---|---|---|---|
| | $H_2O$ | KCl | | | | Total | $NH_4^+$ | $NO_3^-$ | | |
| 0-20 | 7,40 | 7,19 | 7,09 | 4,35 | 0,33 | 1975,0 | 0.00 | 10,5 | 66,32 | 40,0 |

Table 2 shows dry matter yields of alfalfa during the three-year monitoring of plants inoculated with *S. meliloti* strains.

**Table 2. Dry matter yield in inoculated and uninoculated alfalfa by strains of *Sinorhizobium meliloti* (2005-2006)**

| Treatments/ | 2005 (sowing year) Cuttings (C) (t/ha) | | 2006 Cuttings (C) (t/ha) | | | 2007 Cuttings (C) (t/ha) | |
|---|---|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_1$ | $C_2$ | $C_3$ | $C_1$ | $C_2$ |
| 236 | 0.554 | 1.562 | 4.565 | 5.220 | 3.400 | 6.895 | 7.500 |
| 234 | 0.426 | 1.235 | 4.205 | 4.590 | 3.175 | 8.645 | 8.100 |
| 224 | 0.284 | 1.076 | 4.195 | 4.370 | 2.600 | 7.350 | 5.800 |
| Mix | 0.385 | 1.000 | 4.050 | 4.295 | 2.125 | 5.005 | 4.500 |
| Ɵ | 0.234 | 0.897 | 3.765 | 4.150 | 2.400 | 5.250 | 4.900 |
| Lsd 0.05 | 0.047 | 0.314 | 0.206 | 0.493 | 0.386 | 0.631 | 0.812 |

As expected, the lowest alfalfa yield was recorded in the year of sowing (2005), *Radović et al.* (2005). Both fresh and dry matter yields were found to increase in the years of utilisation, which is congruent with the results reported by other authors, *Nešić et al.* (2005). It confirms that, regarding

profitability and cost-efficiency of production, it is desirable to keep a crop for several years. *Erić and Mišković* (1991) found alfalfa to be most productive in terms of biomass yield in the second year of utilisation, decreasing in the third year primarily due to reduced crop density, which is the result of low tolerance of various environmental factors. In our experiment, however, the yield was highest in the second cutting of the third year, which is indicative of good adaptability to environmental conditions. It was further contributed by heavy precipitation recorded in May of 2007 (www.hidmet.sr.gov.yu).

Of the inoculums applied, strains 236 and 234 had a significant effect on dry matter yield. The plants inoculated with strain 236 stood significantly above the others regarding dry matter values in the year of sowing and all cycles of the second year of utilisation (5 t/ha). In the third year, it achieved 6,895t/ha and 7,500 t/ha yields, which was 31% and 53% higher than the control plants. Strain 234 was significantly effective, compared to control plants, in the last cutting of the second year of utilisation (3,175 t/ha) and the two cuttings of the third year (8,645 and 8,100 t/ha), thus achieving the highest dry matter yields since sowing, i.e. 31-65% higher than control plants. The effective strain 224 and the mixture of three strains tested were found to have insignificant effect in most cuttings, compared to yields in control plots. It indicates that the compatibility of strains 236 and 234 with the cultivar K-28 secured higher effectiveness of nitrogen fixation than that of strain 224, *Barnes et al. (1984)*. Furthermore, the autochthonous population of *S. meliloti*, which is abundant and better adapted to chernozem soils, can prove more competitive than strain newly-introduced as inoculum, *Jarak et al. (2002).*

Chernozem enables high yields of agricultural crops by its optimal physical and chemical charcteristics. That, on the other hand, provides satisfactory abundance of the effective autochthonous populations of *S. meliloti*. However, autochthonous strains are not always effective enough in providing sufficient nitrogen for the plants, so that inoculation may be required with selected and highly-effective rhizobia strains, *Jarak et al.* (2002). The results of this study are indicative of that. The need for N mineral fertilization can thus be reduced, which enables a production of wholesome food and increases soil fertility.

The content and yield of crude proteins provided a basis for determining the quality of alfalfa dry matter. Employing a classification proposed by Ivanov (1980), we found that the inoculated and uninoculated alfalfa plants had a high percentage of crude proteins, i.e. 20-24% (except 19% in the first

and third cuttings of the second year). The content of crude proteins varied depending on the year of utilisation and cutting. The highest content was recorded in the third year (221.9-241.9 g/kg in the first cutting and 225-241.9 in the second), which is primarily the consequence of a greater leaf proportion in overall above-ground biomass in that cutting, *Radović et al.* (2007).

The values of crude protein yield followed those of dry matter yield (Table 3). As a result of high dry matter yield and high protein content, a high protein yield was achieved in plants inoculated with strains 236 and 234, particularly the latter. Strain 236 increased protein yield by 29.7% and 39.6% in the second year and 27.5% and 50.3% in the third year of utilisation, while strain 234 significantly raised protein yield in the third cutting of the second year (27%) and the first (51%) and second (53.8%) cuttings of the third year.

**Table3. Content and yield of crude protein in years of investigation of inoculated alfalfa by strains of *Sinorhizobium meliloti* (2005-2006)**

| Treatments/ | 2005 (god. setve) | | | | 2006 | | | | | | 2007 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cuttings (C) (t/ha) | | | | Cuttings (C) (t/ha) | | | | | | Cuttings (C) (t/ha) | | | |
| | $C_1$ | | $C_2$ | | $C_1$ | | $C_2$ | | $C_3$ | | $C_1$ | | $C_2$ | |
| | SP (g/kg) | PP (t/ha) | SP (g/kg) | PP (t/ha) | SP (g/kg) | PP (t/ha) | SP (g/kg) | PP (t/ha) | SP (g/kg) | PP (t/ha) | SP (g/kg) | PP (t/ha) | SP (g/kg) | PP (t/ha) |
| 236 | 201.2 | 0.111 | 215.0 | 0.336 | 185.0 | 0.844 | 226.3 | 1.181 | 198.1 | 0.674 | 234.4 | 1.616 | 237.5 | 1.781 |
| 234 | 213.1 | 0.091 | 202.5 | 0.250 | 197.5 | 0.830 | 230.0 | 1.056 | 191.3 | 0.607 | 221.9 | 1.918 | 225.0 | 1.822 |
| 224 | 220.6 | 0.063 | 223.7 | 0.241 | 195.6 | 0.821 | 219.4 | 0.959 | 194.3 | 0.505 | 236.3 | 1.736 | 238.7 | 1.385 |
| Mix | 216.9 | 0.083 | 209.4 | 0.209 | 196.9 | 0.797 | 218.8 | 0.942 | 194.8 | 0.413 | 238.8 | 1.195 | 241.9 | 1.088 |
| Ɵ | 220.6 | 0.052 | 209.7 | 0.187 | 218.1 | 0.821 | 219.4 | 0.910 | 201.3 | 0.483 | 241.9 | 1.253 | 232.5 | 1.185 |
| Lsd 0.05 | | 0.014 | | 0.065 | | 0.020 | | 0.042 | | 0.032 | | 0.147 | | 0.186 |

SP-crude protein content; PP-crude protein yield

# Conclusion

The application of some effective *S. meliloti* strains led to an increase in dry matter weight and protein yield of alfalfa plants by 27-53%. The data collected suggest a need to use them as N microbiological fertilizers in soils that have good physical and chemical characteristics since their autochthonous populations often fail to achieve satisfactory effectiveness in the nitrogen fixing process.

# EFEKAT SOJEVA *Sinorhizobium meliloti* NA PRINOS BILJNE MASE LUCERKE (*Medicago sativa* L.)

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## Rezime

Efekat sojeva *Sinorhizobium meliloti* na prinos lucerke, (*Mediscago sativa* L.) ispitan je u trogodišnjem poljskom ogledu. Kao inokulum su korišćeni visoko efikasni sojevi *S. meliloti*. Značajan uticaj na prinos suve materije i prinos proteina imali su sojevi 236 i 234. Najveći prinos suve materije oko 8 t/ha (pod uticajem soja 234) i oko 6.8 i 7.1 t/ha (pod uticajem soja 236) postignut je u terćoj godini iskorišćavanja. U odnosu na neinokulisanu kontrolu, soj 236 je uticao na povećanje prinosa sirovih proteina za 29.7% (drugi otkos) i 39.6% (treći otkos) u drugoj godini i 27.5% i 50.3% po otkosima u trećoj godini iskorišćavanja, dok je soj 234 značajno povećao prinos sirovih proteina u trećem otkosu druge godine (27 %) i u trećoj godini istraživanja (oko 50%). Rezultati ukazuju na potrebu primene efikasnih sojeva *S.meliloti* u formi N mikrobiološkog đubriva na černozemu čija autohtona populacija nije uvek zadovoljavajuće azotofiksacione efektivnosti.

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