# Pea yield and its quality depending on inoculation, nitrogen and molybdenum fertilization

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**ABSTRACT**

The influence of seed inoculation with a bio-preparation of nodule bacteria *Rhizobium leguminosarum*, fertilization of 0, 40, 80, 120 kg N/ha and molybdenum topdressing on the yield of green mass, dry matter, seed, average number of pods per plant, average number of seeds per pod, average nodule number per plant, nodule dry weight as well as plant and seed protein concentration were studied during the two years of investigations on two soils – Mollic Gleysols and Eutric Cambisols. The highest values of all investigated parameters were obtained in the inoculated seed variants with molybdenum application, except the average nodule number per plant where the highest values were achieved in variants without molybdenum. The effect of nitrogen fertilization depended on the soil type, i.e. its chemical properties. The largest number of the investigated parameters obtained the highest values as a result of fertilization with 40 kg N/ha on Mollic Gleysols (3.96% humus). Thus, seed yield was 4.02 t/ha, nodule dry matter 0.482 g per plant whereas seed protein concentration was 26.91%. The largest number of the investigated parameters on Eutric Cambisols (1.07% humus) obtained the highest values with fertilization of 80 kg N/ha where seed yield amounted to 3.65 t/ha, nodule dry matter 0.456 g per plant while seed protein concentration was 26.48%.

**Keywords:** inoculation; nitrogen fertilization; molybdenum; *Rhizobium leguminosarum*; field pea; seed yield; proteins; nodule dry weight

Apart from a reduction in production costs by using natural energy resources, agricultural production currently tries to avoid environment pollution. Since it is almost impossible to imagine crop production without fertilizer applications affecting the environment adversely, they are replaced by regenerated energy sources. In this aspect nitrogen plays a special role. It affects the production of plant organic matters and their yield to a large. However, it is the most expensive energy element. On the other hand, biological nitrogen fixation, one of the most important soil processes, is developed by means of microorganisms.

A special role in biological nitrogen fixation is attributed to symbiosis between nodule bacteria of the genus *Rhizobium* and *Bradyrhizobium* and legumes. Burns and Hardy (1975) reported they fixed about $175 \times 10^6$ tons of nitrogen per year. It is a many times higher amount of nitrogen in comparison with the world annual production of nitrogen fertilizers being $30 \times 10^6$ tons (Postgate 1982).

Inoculation of legume seeds with compatible strains of nodule bacteria brings about symbiosis development and host plant uptake of this yield-forming element from its symbiont. The required amount of fertilizers decreases in this way.

Many authors stated that yields could be increased by legume seed inoculation compared to nitrogen fertilizer variants (Kálalová and Šimon 1993, Dobereiner et al. 1995). It was found out that low doses of applied nitrogen had favourable effects on nodulation and nitrogen fixation whereas higher doses of mineral nitrogen resulted in nodule mass reduction. This directly affected the value reduction of all yield elements (Ergorov 1985, Waterer et al. 1992, Milaković et al. 2000). El Behidi (1985) reported that high rates of available soil nitrogen reduced nodulation and biological nitrogen fixation since plants did not require symbiosis with nodule bacteria.

Nitrogen fixation is catalysed by the enzyme nitrogenase that contains molybdenum, a vital microelement for the process of biological nitrogen fixation. Hageman and Burris (1978) reported that the enzyme nitrogenase contained two proteins: Mo-Fe protein (component containing molybdenum, iron and protein) and Fe protein (component containing iron and protein). Jongruaysup et al. (1993) stated that crops developing symbiosis with rhizobium bacteria had increased molybdenum requirements. Deficiency of this element causes a reduction of nodulation, leghaemoglobin concentration and nodule dry weight. Chahal et al. (1991) claimed that foliar applications of molybdenum stimulated nodulation and biological nitrogen fixation, thus increasing the legume yield. It was also proved by the results of many other authors (Jongruaysup et al. 1993, Srivastava and Ahlawat 1995).

Table 1. Chemical properties of soils in experimental localities

| Investigated properties Layer (0–30 cm) | Locality | |
|---|---|---|
| | Mollic Gleysols | Eutric Cambisols |
| pH ($H_2O$) | 7.12 | 6.62 |
| pH (KCl) | 6.34 | 5.94 |
| Humus (%) | 3.96 | 1.07 |
| P (mg/100 g soil) | 14.70 | 12.51 |
| K (mg/100 g soil) | 27.47 | 21.17 |

For these reasons these investigations aimed to determine the influence of seed inoculation with compatible strains of nodule bacteria *Rhizobium leguminosarum* as well as nitrogen and molybdenum fertilization on yield elements and field pea quality.

## MATERIAL AND METHODS

The experiment was set up on two soils: Mollic Gleysols (FAO 1998) and Eutric Cambisols (Table 1). It was established in a completely randomised design with 4 replications and 16 variants of inoculation, nitrogen and molybdenum fertilization.

The Danish variety of field pea Sobel was used in both years of investigations (1999 and 2000) at both sites. Stand density was 1 000 000 plants/ha. Sowing was carried out by hand with row spacing of 20 cm, space within a row of about 5 cm and sowing depth 4–6 cm. The basic plot size was 10 $m^2$.

The research elements were as follows: a) seed inoculation ($a_1$ – non-inoculated seed, $a_2$ – inoculated seed); b) nitrogen fertilization ($b_1$ – control, $b_2$ – 40 kg N/ha, $b_3$ – 80 kg N/ha, $b_4$ – 120 kg N/ha); c) molybdenum fertilization ($c_1$ – control, $c_2$ – 250 g/ha molybdenum in sodium molybdate).

Pea seed inoculation was done with a biopreparation of the nodule bacteria *Rhizobium leguminosarum* (produced by the Faculty of Agronomy in Zagreb, count of rhizobium cells per 1 g of inoculant – $1 \times 10^{10}$) by dry procedure in the course of sowing. Nitrogen was applied by hand in the form of 27% calcium ammonium nitrate. Molybdenum foliar fertilization was applied at a plant height of 25 cm using molybdenum in the form of sodium molybdate (250 g/ha, conc. 0.042%). Regardless of the above-mentioned practices, phosphorus and potassium fertilizations were also applied in doses of 43.60 kg P/ha and 66.40 kg K/ha. Phosphorus was applied in the form of triple superphosphate at a concentration of 45% $P_2O_5$ and potassium in the form of 60% KCl.

Approximately in a month after sowing the experimental plot was treated with the herbicide Basagran (2 l/ha) due to infestation with mostly broadleaved weeds. At the very beginning of the flowering phase fungicide protection was carried out by application of Sandofan M–8 (2.5 kg/ha) to control blight and the insecticide Sonet 100 EC (0.2 l/ha) was applied as weevil protection.

Soil samples were taken from each site in early spring before the experiment was established. Soil analyses were carried out by standard methods (Table 1): organic matter content was determined by bichromate method (Vukadinović and Bertić 1988), pH in $H_2O$ and KCl, phosphorus and potassium content by ammonium-lactate method according to Egner-Riehm-Domingo (Page 1982).

Yield of plant green mass and dry matter, average nodule number per plant as well as nodule dry weight were determined in the flowering phase (when at least 80% of plants were in the flowering phase). In this case dry matter yield means the

Table 2. *LSD* test values for the investigated indicators

| Investigated parameters | Soil type | Seed inoculation | |
|---|---|---|---|
| | | $\alpha$ = 0.05 | $\alpha$ = 0.01 |
| Green mass yield | A | 0.028 | 0.030 |
| | B | 0.046 | 0.071 |
| Dry matter yield | A | 0.014 | 0.016 |
| | B | 0.011 | 0.014 |
| Seed yield | A | 0.015 | 0.029 |
| | B | 0.030 | 0.043 |
| Average number of pods per plant | A | 0.044 | 0.120 |
| | B | 0.019 | 0.033 |
| Average number of seeds per pod | A | 0.023 | 0.037 |
| | B | 0.010 | 0.017 |
| Average nodule number per plant | A | 0.487 | 0.703 |
| | B | 0.681 | 0.991 |
| Nodule dry weight | A | 0.004 | 0.007 |
| | B | 0.002 | 0.003 |
| Plant protein concentration | A | 0.160 | 0.202 |
| | B | n.s. | n.s. |
| Seed protein concentration | A | 0.337 | 0.4110 |
| | B | n.s. | n.s. |

A – Mollic Gleysols, B – Eutric Cambisols
n.s. – not significant

yield of shoots taken in the flowering phase and dried in a drier at a temperature of 105°C until reaching the constant weight.

Pea harvest was followed by determination of seed yield, average number of pods per plant and average number of seeds per pod. Protein content in the aboveground plant mass and seed was determined by Kjeldahl method (AOAC 1984).

Results were processed by modern statistical methods (ANOVA) using the computer program StatSoft Inc. (2001) STATISTICA (data analysis software system), version 6.

**RESULTS AND DISCUSSION**

All investigated elements achieved highest values in the variants inoculated with compatible strains of the nodule bacteria *Rhizobium leguminosarum* and molybdenum topdressing (Table 3).

Mollic Gleysols were characterized by the highest green mass yield obtained by nitrogen fertilization of 40 kg/ha and Eutric Cambisols with 80 kg N/ha. This difference can be explained by different contents of organic matter in these soils. Namely, Mollic Gleysols contained 3.96% humus in its topsoil and Eutric Cambisols 1.07%. Thus the lower initial fertilization with this element in Mollic Gleysols brings about higher yield whereas Eutric Cambisols were known for more successful fertilization with higher nitrogen dosage.

Many authors claimed that a higher level of nitrogen fertilization along with seed inoculation with compatible nodule bacteria strains in soils with better humus supply reduced nodulation which brought about a reduction in yield parameters (Vargas et al. 1982, Redžepović et al. 1985, Milaković et al. 2000).

Green mass yield in non-inoculated plants was highly significantly lower (8.01–9.7%) compared to green mass yield in inoculated plants. Similar results were obtained by Carr et al. (1992) and Milaković et al. (2000). It is known that the synthesis of plant organic matter is promoted by inoculation, thus the mass of some plant parts as well as of the whole plant differs in inoculated compared to non-inoculated plants (Sarić and Fawzi 1983, Thies et al. 1995).

Molybdenum treatment resulted in an increase in green mass yield in both years of investigations. Variants treated with this element achieved a higher green mass yield by 12.5–15% compared to variants without molybdenum application. Since this element participates in the synthesis of nitrogenase – the enzyme catalysing the process of biological nitrogen fixation, increased requirements for this element are obvious in plants being in symbiosis with *rhizobium* bacteria. Similar results were reported by Chahal et al. (1991), who treated pea with molybdenum (30 μg/l) and achieved highly significantly higher yields of green mass and dry matter, nodule number and dry matter as well as nodule leghaemoglobin content compared to untreated variants.

Analogously to green mass yield, the highest dry matter yield on Mollic Gleysols was attained in the variant of inoculated seed treated with molybdenum and fertilization with 40 kg N/ha in both years of investigations. All other variants achieved statistically significantly lower dry matter yields (Table 2).

In both years of investigations the highest dry matter yield on Eutric Cambisols was recorded in the inoculated seed variant with nitrogen fertilization at a dose of 80 kg N/ha and foliar application of molybdenum.

The obtained results are in agreement with the results presented by Uribe et al. (1990) and Waterer et al. (1992), who reported positive effects of seed inoculation with compatible nodule bacteria strains, reduced applications of nitrogen fertilizers and molybdenum application on all yield and quality elements in soybean, pea and alfalfa crops.

Seed yield was in a significantly positive correlation with green mass yield ($r = 0.984^{**}$) and dry matter yield ($r = 0.985^{**}$).

Average seed yield of inoculated variants in both years of investigations was 2.94 t/ha and average yield of non-inoculated variants was 2.67 t/ha, being lower by 9.2%. Significant differences in seed yield of inoculated and non-inoculated variants were determined by Redžepović et al. (1985) for soybean (17–39.5%) whereas many authors reported that the inoculation of legumes with compatible strains of the nodule bacteria *Rhizobium leguminosarum* considerably increased seed yield (Uribe et al. 1990, Salez et al. 1992, Dobereiner et al. 1995, Thies et al. 1995).

As for the inoculated and non-inoculated variants the highest seed yields were produced by foliar application of molybdenum. It was in accordance with the results of Sarić et al. (1983) and Srivastava and Ahlawat (1995).

In both years of investigations the highest average number of pods per plant and the highest average number of seeds per pod were achieved on Mollic Gleysols in the variant of inoculated seeds with compatible strains of nodule bacteria, treated with sodium molybdate and fertilized with 40 kg N/ha. All the other parameters were significantly lower.

As for Eutric Cambisols, the highest values of the given parameters were also attained for seed inoculation and molybdenum foliar application but using the higher dose of nitrogen fertilizers – 80 kg N/ha.

Table 3. Average values of investigated parameters (1999 and 2000)

| Investigated parameter | Soil type | Non-inoculated seed | | | | | | | | Inoculated seed | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | not treated with molybdenum | | | | treated with molybdenum | | | | not treated with molybdenum | | | | treated with molybdenum | | | |
| | | nitrogen fertilization | | | | | | | | | | | | | | | |
| | | 0 | 40 | 80 | 120 | 0 | 40 | 80 | 120 | 0 | 40 | 80 | 120 | 0 | 40 | 80 | 120 |
| Green mass yield (t/ha) | A | 4.43 | 4.64 | 5.41 | 4.94 | 4.96 | 5.02 | 6.31 | 5.13 | 5.05 | 6.44 | 6.00 | 4.57 | 5.55 | **7.29** | 6.31 | 4.76 |
| | B | 4.23 | 4.57 | 5.32 | 5.95 | 4.23 | 4.90 | 5.88 | 5.90 | 4.66 | 5.00 | 6.12 | 4.84 | 5.25 | 5.47 | **6.86** | 5.06 |
| Dry matter yield (t/ha) | A | 0.32 | 0.37 | 0.49 | 0.41 | 0.41 | 0.40 | 0.54 | 0.43 | 0.44 | 0.54 | 0.46 | 0.35 | 0.49 | **0.69** | 0.51 | 0.37 |
| | B | 0.33 | 0.38 | 0.46 | 0.53 | 0.32 | 0.41 | 0.51 | 0.50 | 0.38 | 0.44 | 0.55 | 0.39 | 0.45 | 0.47 | **0.62** | 0.43 |
| Seed yield (t/ha) | A | 2.29 | 2.35 | 2.84 | 2.59 | 2.61 | 2.53 | 3.24 | 2.64 | 2.73 | 3.31 | 3.00 | 2.36 | 2.88 | **4.02** | 3.18 | 2.46 |
| | B | 2.17 | 2.54 | 2.81 | 3.13 | 2.19 | 2.65 | 3.00 | 2.96 | 2.50 | 2.70 | 3.20 | 2.57 | 2.80 | 2.97 | **3.65** | 2.74 |
| Average number of pods per plant | A | 6.04 | 6.28 | 6.90 | 6.49 | 6.52 | 6.66 | 7.45 | 6.46 | 6.66 | 7.94 | 7.44 | 6.19 | 7.14 | **8.83** | 7.74 | 6.32 |
| | B | 6.05 | 6.40 | 7.07 | 7.52 | 6.12 | 6.84 | 7.73 | 7.30 | 6.36 | 6.65 | 7.74 | 6.38 | 6.84 | 7.53 | **8.57** | 6.54 |
| Average number of seeds per pod | A | 4.11 | 4.29 | 5.45 | 4.70 | 4.46 | 4.52 | 5.84 | 4.70 | 4.56 | 6.09 | 5.42 | 4.22 | 5.21 | **6.89** | 5.93 | 4.35 |
| | B | 3.99 | 4.20 | 5.31 | 5.52 | 4.07 | 4.98 | 5.81 | 5.33 | 4.12 | 4.57 | 5.88 | 4.17 | 5.23 | 5.58 | **6.52** | 4.51 |
| Average nodule number per plant | A | 10.55 | 13.07 | 17.48 | 12.57 | 9.76 | 11.60 | 15.28 | 10.55 | 23.53 | **47.24** | 41.04 | 18.56 | 21.10 | 34.68 | 30.79 | 15.58 |
| | B | 9.36 | 10.85 | 15.62 | 13.92 | 8.43 | 10.05 | 10.02 | 11.54 | 22.43 | 26.01 | **43.04** | 35.77 | 21.04 | 23.69 | 31.06 | 26.45 |
| Nodule dry weight (g/plant) | A | 0.12 | 0.145 | 0.184 | 0.139 | 0.141 | 0.164 | 0.219 | 0.169 | 0.255 | 0.441 | 0.397 | 0.197 | 0.284 | **0.482** | 0.44 | 0.242 |
| | B | 0.106 | 0.129 | 0.176 | 0.149 | 0.135 | 0.151 | 0.206 | 0.159 | 0.238 | 0.309 | 0.420 | 0.362 | 0.258 | 0.363 | **0.456** | 0.376 |
| Plant protein concentration (%) | A | 14.99 | 15.83 | 16.50 | 15.96 | 16.79 | 16.67 | 20.14 | 17.31 | 16.40 | 19.83 | 19.02 | 15.78 | 17.25 | **21.91** | 19.71 | 16.21 |
| | B | 14.69 | 16.57 | 17.34 | 20.34 | 15.17 | 16.84 | 20.26 | 19.10 | 15.77 | 16.26 | 20.45 | 15.90 | 16.34 | 16.72 | **20.91** | 15.75 |
| Seed protein concentration (%) | A | 19.99 | 20.83 | 21.48 | 20.98 | 21.97 | 21.66 | 25.15 | 22.31 | 21.40 | 24.84 | 24.03 | 20.78 | 22.25 | **26.91** | 24.70 | 21.22 |
| | B | 19.52 | 21.52 | 21.28 | 25.12 | 19.75 | 21.86 | 25.27 | 24.94 | 20.77 | 21.41 | 25.45 | 20.87 | 21.31 | 21.72 | **26.48** | 21.01 |

A – Mollic Gleysols, B – Eutric Cambisols, bold – significant at the level of 0.01

Our results are in accordance with the results obtained by Fageria and Carvalho (1996), who reported that seed yield in pea inoculated variants was in positive correlation with average number of pods per plant and average number of seeds per pod.

Molybdenum fertilization resulted in a statistically highly significant increase in these yield parameters compared to variants without molybdenum. Srivastava and Ahlawat (1995) also determined positive molybdenum effects on the average number of pods per plant.

The highest average number of nodules per plant was attained on Mollic Gleysols in the inoculated variant, not treated with molybdenum but fertilized with 40 kg N/ha.

Eutric Cambisols were known for the highest average of the mentioned parameter obtained in the inoculated variant without sodium molybdate application and with fertilization of 80 kg N/ha.

In both years of investigations the highest values of nodule dry mass on Mollic Gleysols were determined in inoculated seed variants with molybdenum and nitrogen fertilization at a dose of 40 kg/ha. All other variants attained statistically significantly lower averages of nodule dry mass.

Eutric Cambisols are known for the highest nodule dry weight averages also obtained in the inoculated variant with molybdenum fertilization at a nitrogen dose of 80 kg/ha.

Molybdenum application led to a reduction in the average number of nodules per plant and at the same time to a highly significant increase in nodule dry weight in both experimental years. Our results are in agreement with the results of investigations of some authors (Vojinović and Petrović 1967, Jongruaysup et al. 1993) who stated that the total number of nodules (being coarser and physiologically more active) per plant was reduced by molybdenum application. This is the reason why molybdenum treated legumes, regardless of the decrease in nodule number compared to untreated plants, take up a higher amount of nitrogen.

In both years of investigations protein content in plants and seeds was in positive correlation with nodule dry weight, being in accordance with the results of investigations carried out by Vikman and Vessey (1993), who stated that protein content depended on nitrogenase activity that is in direct relation with the activity and number of nodules.

Protein concentration in both years of investigations was higher in inoculated plants compared to the non-inoculated ones (2.80–4.07%). The same case was with the seed protein content where the above-mentioned difference ranged from 3.09 to 15.62%. Milaković et al. (2000) reported similar results for beans where seed proteins of inoculated plants were higher by 11.5–25% than protein concentration in non-inoculated plants. Hanus et al. (1981) reported that the inoculation of legumes with various $HUP^+$ (hydrogen uptake of positive strains possessing the enzyme hydrogenase that recycles developed hydrogen and includes it in the nitrogenase reaction again) nodule bacteria strains significantly increased total protein content (in both plant and seed) in long-term field experiments in Oregon (USA).

The highest plant and seed protein concentrations in non-inoculated plants were achieved in molybdenum treated variants at nitrogen fertilization of 80 kg N/ha, and without molybdenum using slightly higher nitrogen fertilization – 120 kg/ha. It is in accordance with the results of investigations conducted by Nygren et al. (2000), who found out that both yield elements and plant and seed protein content were increased by nitrogen fertilization.

Based on the obtained results it can be concluded that seed inoculation with compatible strains of the bacteria *Rhizobium leguminosarum* and molybdenum foliar application lead to nitrogen fertilization reduction and yield elements and field pea quality increase at the same time. It is of special importance from economic and ecological aspects.

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**ABSTRAKT**

**Výnos hrachu a jeho kvalita v závislosti na inokulaci semen a hnojení dusíkem a molybdenem**

V dvouletém pokusu byl na dvou půdách – mollických glejích a eutrických kambizemích sledován vliv inokulace semen biologickým preparátem obsahujícím hlízkové bakterie *Rhizobium leguminosarum*, dále vliv aplikace dávek 0, 40, 80 a 120 kg N/ha a vliv hnojení na list molybdenem na výnos zelené hmoty a sušiny, výnos semen, průměrný počet lusků na rostlinu, průměrný počet semen na lusk, průměrný počet hlízek na rostlinu, hmotnost sušiny hlízek a koncentraci bílkovin v celé rostlině a v semenech. Nejvyšší hodnoty všech sledovaných parametrů byly zaznamenány u variant inokulovaných semen s aplikací molybdenu s výjimkou průměrného počtu hlízek na rostlinu, kde nejvyšších hodnot dosáhly varianty bez molybdenu. Vliv dusíkatého hnojení závisel na půdním typu, tj. na chemických vlastnostech půd. Nejvyšší počet sledovaných parametrů dosáhl nejvyšších hodnot při hnojení dávkou 40 kg N/ha na mollických glejích (3,96 % organické hmoty). Výnos semen zde činil 4,02 t/ha, sušina hlízek dosahovala 0,482 g na rostlinu a koncentrace bílkovin v semenech 26,91 %. Nejvyšší počet sledovaných parametrů na eutrických kambizemích (1,07 % organické hmoty) zaznamenal nejvyšší hodnoty při hnojení dávkou 80 kg N/ha, kdy výnos semen dosahoval 3,65 t/ha, sušina hlízek činila 0,456 g na rostlinu a koncentrace bílkovin v semenech 26,48 %.

**Klíčová slova:** inokulace; hnojení dusíkem; molybden; *Rhizobium leguminosarum*; hrách setý; výnos semen; bílkoviny; sušina hlízek

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