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Verification of the effectiveness of AZORHIZ + F inoculant fertilizer and AZOTER L foliar fertilizer in soybean crop

Report on results observed in the pilot experiment from 2022

evaluating the effect of the soil treated with soil adjuvant in the form of concentrated Azorhiz bio-fertilizer (containing non-symbiotic bacteria *Azotobacter chroococcum, Azospirillum brasilense* and *Bacillus megaterium* and symbiotic inoculative bacteria *Rhizobium japonicum* and *Rhizobium leguminosarum*) with F additive (containing *Trichoderma atroviride* mycoparasitic fungi) and evaluating the effect of the plant biostimulant with Azoter L fertilizer (containing *Herbaspirillum seropedicae* bacteria) in the cultivation technology of soybean crop.

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Characteristics of the experiment: Verification of **Azorhiz** bio-fertilizer at a dose of 10 l/ha with an inoculation effect for the root system of soybean plants and supplemented **F additive** at a dose of 0.1 l/ha with a phytopathogenic effect was established in a drier area in Lhota pod Přeloučí, located in the flat terrain with an altitude of 207 m. The pilot experiment was established by a method of simple long plots with a size of 0.54 ha/each plot, in a total number of 8 plots including the untreated control variant. The soil on the experimental plot was classified as medium-heavy (sandy loam soil), with fluvial genetic origin of a slightly reddish color, in mostly good or moderately drying moisture conditions. Winter wheat was the pre-crop for soybean. The pre-crop straw was left on the plot and plowed in. The soil after applying pre-crop (winter wheat) showed a good content of available phosphorus, potassium, and magnesium. The content of available calcium on the plot was high and it contributed to the stabilization of the optimal and neutral soil pH (Table 1).

Indicator	P	K	Ca	Mg	pH
	mg/kg (used extraction method by Mehlich 3)				
Content:	111	227	3641	226	6,93
Evaluation:	Good	Good	High	Good	Neutral

Tab. 1. Basic agrochemical properties of the soil on the plot before establishing the experiment (30 March 2022)

Azorhiz bio-fertilizer contains non-symbiotic bacterial diazotrophs that bind atmospheric nitrogen: *Azotobacter chroococcum, Azospirillum brasilense*, bacteria involved in making phosphorus available in the soil: *Bacillus megaterium* and symbiotic inoculative bacteria (*Rhizobium japonicum and Rhizobium leguminosarum*) that occur on the roots of soybeans and bind most of the plants' nitrogen that they absorb from the air (N2). The product was enriched with F additive in the spray solution containing *Trichoderma atroviride* mycoparasite, which limits the development of spores of *Fusarium* (also it reduces *Pythium, Rhizoctonia* and *Botrytis*) and strengthens the effect of the product converting hemicelluloses and cellulose contained in post-harvest residues. Therefor it additionally makes nutrients available for cultivated crops.

Azorhiz bio-fertilizer was applied on 4.5.2022 with an AMAZONE UX 4200 trailer sprayer just before pre-sowing soil treatment, at a dose of 10 l/ha together with F additive at a dose of 0.1 l/ha. The dose of spraying agent was 300 l/ha. The system pressure of the sprayer did not exceed the limit of 2.5 bars for bio-fertilizer application with vital content. Incorporation of the applied fertilizer was carried out immediately with a pre-sowing compactor to a depth of 4 cm. After that, Alzon Neo nitrogen fertilizer (stabilization with urea, 46% N) was applied in variants up to a total dose of 0, 25 and 80 kg N/ha. Next, the soil was slightly processed with Horsch seeder, and at the same time, Amofos fertilizer at a dose of 30 kg/ha was applied between rows (it was refilled to the total dose with an extra 4 kg of N and supplied with 16 kg of P2O5/ha). The plot was sown on 5.5.2022 at a seed rate of 115 kg/ha (15 cm row spacing) at the seed depth of 3.5 cm. The stand was established with Brunensis (early) soybean variety. Soybean seed inoculation with HiStick Soy was applied by a dry method, at a stationary mixing plant just before the sowing date (contains spores of *Bradyrhizobium japonicum* sp. nodule bacteria) at the manufacturer's recommended dose (400 g/115 kg of seed). Seed inoculation was not applied on one of the experimental plots (var. no. 6) and the plot was sown as first.

Azoter L bio-fertilizer in a dose of 10 l/ha was applied on 15.06.2022. The application was performed on the emerged soybean stand in the developed 3rd-4th leaf layers on the stem. The application was carried out in a dose of spraying agent (300 l/ha). In variant No. 8, a hydrogel (polyacrylate) was added to the fertilizer at a dose of 1.0 l/ha to verify the extension of the leaf wetness and the penetration time of the fertilizer containing *Herbaspirillum seropedicae* vital bacterium through the plant cuticle. The system pressure of the sprayer did not exceed the limit of 2.5 bars during application. In addition to endophytic diazotrophic bacteria, the fertilizer also contained boron (micronutrient) in a dose of 25 g/ha, potassium in a dose of 26 g/ha and nitrogen in a dose of 91 g/ha.

The aim of the pilot experiment was to verify the effectiveness of soil inoculation with isolated strains of nodule bacteria living on soybean roots, together with the delivery of non-symbiotic bacteria fixing atmospheric nitrogen (N2) and phytopathogenic fungi for plant protection to the soil, and to compare this effect with the standard technology of seed inoculation with nodule bacteria including assessment the possibility of saving nitrogen and phosphorus fertilizers in the growing technology of soybean crops. The soil before sowing and during sowing was fertilized with nitrogen and phosphorus to support the nutrition and growth of the stands in the first half period of the vegetation, in which the galls of nodule bacteria on the roots are beginning to form (Table 2).

Variant (No.)	Fertilizing the soil before sowing		Pre-sowing seed treatment	Pre-sowing soil treatment	Treatment of the stand after emergence
	Fertilizer	Dose of nutrients (kg/ha)			
1)	Amofos Alzon Neo	25 N, 16 P_2O_5	HiStick Soy inoculation	---	---
2)	Amofos Alzon Neo	**80** N, 16 P_2O_5	HiStick Soy inoculation	---	---
3)	Amofos Alzon Neo	25 N, 16 P_2O_5	HiStick Soy inoculation	Azorhiz +F 10 l + 0,1 l/ha	---
4)	Amofos Alzon Neo	25 N, 16 P_2O_5	HiStick Soy inoculation	Azorhiz +F 10 l + 0,1 l/ha	Azoter L 10 l/ha
5)	---	**0** N, **0** P_2O_5	HiStick Soy inoculation	Azorhiz +F 10 l + 0,1 l/ha	---
6)	Amofos Alzon Neo	25 N, 16 P_2O_5	---	Azorhiz +F 10 l + 0,1 l/ha	---
7)	Amofos Alzon Neo	25 N, 16 P_2O_5	HiStick Soy inoculation	---	Azoter L 10 l/ha
8)	Amofos Alzon Neo	25 N, 16 P_2O_5	HiStick Soy inoculation	---	Azoter L + hydrogel 10 l/ha + 1 l/ha

Tab. 2. Design of the pilot experiment to verify the effectiveness of biological fertilizers (variants)

Results with Azorhiz + F soil inoculation and the effectiveness of Azoter L foliar fertilizer in soybeans stands

Using diagnostic methods, the nutritional status of plants during the vegetation and the harvest, including yield-generating elements and soybean seed quality was evaluated. At the same time, the evaluation of the basic agrochemical properties of the soil before and after the application of the verified fertilizers was carried out to get a comprehensive assessment of the effect, including the effect for the subsequent crop (usually for winter wheat).

1) The effect of Azorhiz + F bio-fertlizer on the agrochemical properties of the soil

In the soybean stand established after the cereal pre-crop, the soil showed an average supply of available mineral nitrogen (N_{min}.) in the amount of 41 kg/ha. The soil was subsequently fertilized with Amofos fertilizer applied between the rows. The remaining dose of nitrogen was applied earlier, before actual sowing, in the form of Alzon Neo stabilized urea. During the vegetation, N_{min} supply in soil of 147 kg/ha (good supply) was found on 14. 6. at the stand where the fertilizer was applied by a standard method (var. 3). It appears that the residual supply of N_{min}. in the soil during the soybean harvest was different only minimally, in the range of 27 – 35 kg/ha (in the category of small safe supply) using the standard application of fertilizers containing nitrogen (and phosphorus). Also, intensively fertilized soil (var. 2) with nitrogen for soybeans stands did not show increased (dangerous) N_{min} residues in the soil. The need for basic soil fertilization with a reduced (recommended) dose of nitrogen proved to be suitable and usable for the soybean crop.

The soil under the stand without fertilizer application (var. 5) before sowing showed the lowest residual N_{min} supply (22 kg/ha) (very low or low supply). The stand, without direct nitrogen and phosphorus soil fertilization and only with using Azorhiz treatment, used more soil nitrogen supply up to a reduced and very low (natural) organic residue. On the contrary, pre-sowing soil treatment with Azorhiz fertilizer together with standard seed inoculation and nitrogen and phosphorus fertilization, showed a greater availability of nitrogen in the soil for the stands, and so the highest residual N_{min} content was kept in the soil (34 – 35 kg/ha). The retained higher but adequate residue of $N_{min.}$ supply in the soil can be available for the subsequent winter wheat crop (Fig. 1).

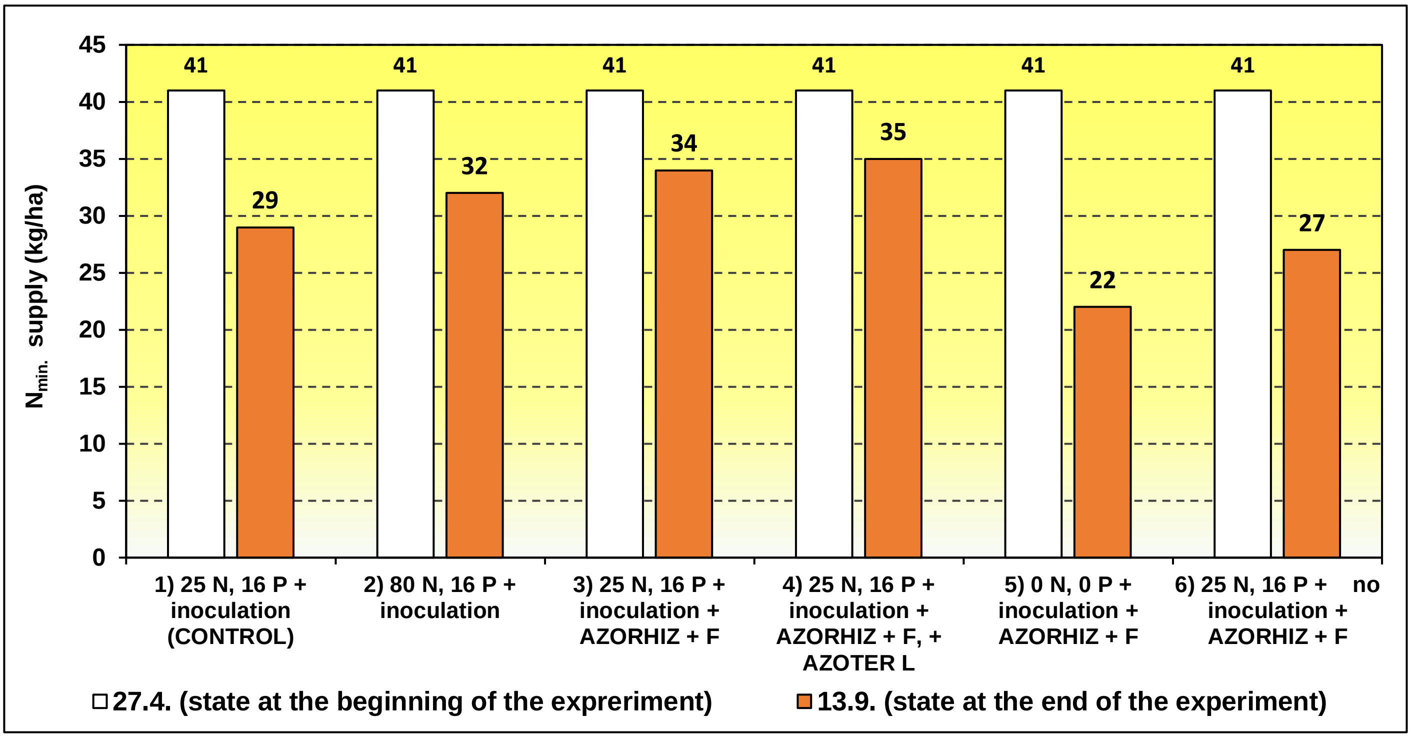

Fig. 1. Effect of pre-sowing treatment with Azorhiz +F biological fertilizer on the residual mineral nitrogen ($N_{min.}$) supply in the soil at harvest and soil supply for subsequent winter wheat (profile 0 – 30 cm)

Pre-sowing soil treatment with Azorhiz biological product showed an improved soil pH. The soil PH before the product application had a PH value of 6.25. The soil without Azorhiz treatment (var. 1) showed a drop in pH value of almost 0.2. After the application of an increased dose of nitrogen to 80 kg/ha before sowing (var. 2) and without Azorhiz application, the soil showed a slight increase in pH value by 0.03. After Azorhiz application at the standard fertilizer dose, the pH value of the soil increased by 0.24. After Azorhiz application to the soil and treating the stand with Azoter L fertilizer during the vegetation period, the pH of the soil increased by 0.09. Without fertilizer application and only after Azorhiz soil treatment, an increase in pH by 0.06 was detected. Without standard seed inoculation, with maintained standard fertilization and Azorhiz soil treatment, an increase in soil pH at soybean harvest by 0.07 was found. It is evident that the application of Azorhiz soil biological product does not cause soil acidification (reduction of pH). On the contrary, it supports its buffering capacity in the soil to resist acidification, e.g. due to the application of fertilizers etc. (Fig. 2).

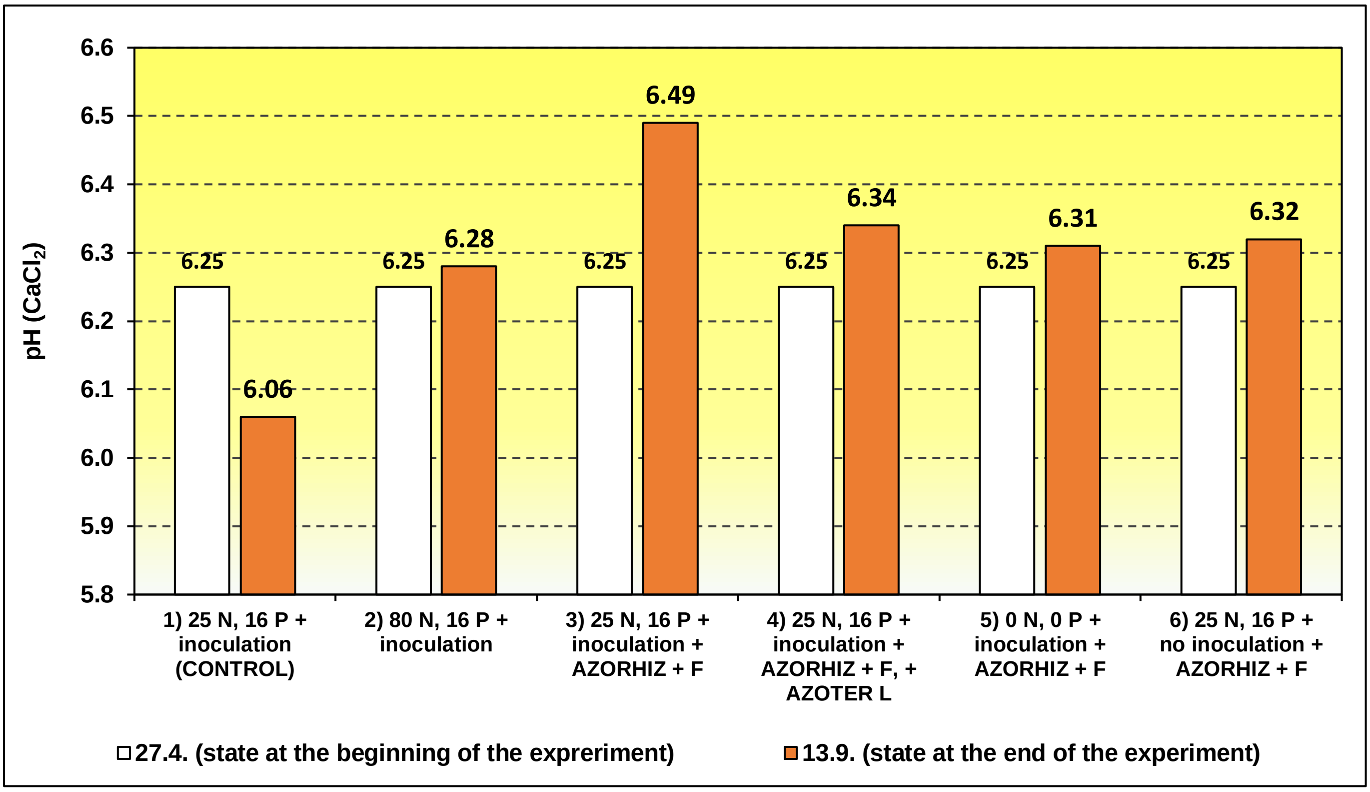

Fig. 2. Effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer on soil PH during soybean harvest (profile 0-30 cm)

2) The effect Azorhiz and Azoter L biological fertilizers on the vegetative phase and nutritional status of plants

After 40 days from sowing, the stands in the individual variants of soil and plant treatment, showed the formation of the 2nd - 4th leaf layers (height about 30 - 40 cm) with minimal differences. The soil on the control variant 1 that was not treated with Azorhiz fertilizer before sowing, the dry mass of the above-ground biomass was 1.6 t/ha. After the application of a high nitrogen dose of 80 kg/ha for the soybean stand (var. 2), the weight of the above-ground biomass was by 6% higher. After Azorhiz application (var. 3 and at that time also var. 4) the weight of above-ground biomass was by 25% and 31% higher than on the control plot. After omitting the basic dose of nitrogen and phosphorus at sowing (var. 5) and replacing plant nutrition with Azorhiz biological product application, the weight of the above-ground biomass was by 6% higher than on the control plot. After Azorhiz application and without parallel treatment of the seed with inoculation (var. 6), the stand showed by almost 13% lower increase of above-ground biomass than the control plot. It is evident that at the beginning of the first part of soybean vegetation, the stand in which the seed inoculation wasn´t performed before sowing, showed the worst dynamics increase of above-ground biomass. On the contrary, after the seed inoculation and soil treatment with inoculating and other bacteria in the form of Azorhiz bacterial fertilizer, the stand showed the best growth support already in the first half of the vegetation.

After intensive growth and the main setting of pods on the stems, there was an increase in above-ground biomass of 9.7 t/ha after 91 days from sowing on the control variant without soil and stand treatment. After the application of a high nitrogen dose of 80 kg/ha (var. 2), the stand showed a 37% higher increase in the weight of the above-ground biomass compared to the control variant. After pre-sowing soil treatment with Azorhiz fertilizer (var. 3), there was the highest increase in the above-ground biomass of plants. Biomass weight was by 73% higher than on the untreated control variant. The combination of soil treatment with Azorhiz fertilizer and treatment of the stand with Azoter L fertilizer after emergence on 15 June, resulted in a 12% greater weight of above-ground biomass compared to the control variant. Omission of the basic dose of nitrogen and phosphorus in fertilizers at sowing and after treating the soil with Azorhiz fertilizer, resulted in a 15% greater weight of above-ground biomass. Omission of the standard dry seed inoculation and after the applying Azorhiz fertilizer, the stand showed an increase of above-ground biomass by less than 18% compared to the control.

Treatment of the stand during vegetation with Azoter L biological product (var. 7), resulted in an almost 19% greater increase in above-ground biomass compared to the untreated control variant. Applying Azotel L fertilizer in a mixture (tank-mix) with an available hydrogel (var. 8), resulted in 24% higher increase in above-ground biomass compared to the control variant <u>50 days after application</u>. It is evident that the foliar treatment of the stand with Azoter L fertilizer containing the symbiotic bacterium *Herbaspirillum seropedicae* living in the leaf structures improves the growth of the above-ground biomass. The combined application of Azoter L with hydrogel to extend the leaf wetting and the penetration of the active ingredient of the fertilizer through the leaves cuticle, resulted in better growth of the above-ground biomass in the second half of the vegetation by 4% (Fig. 3).

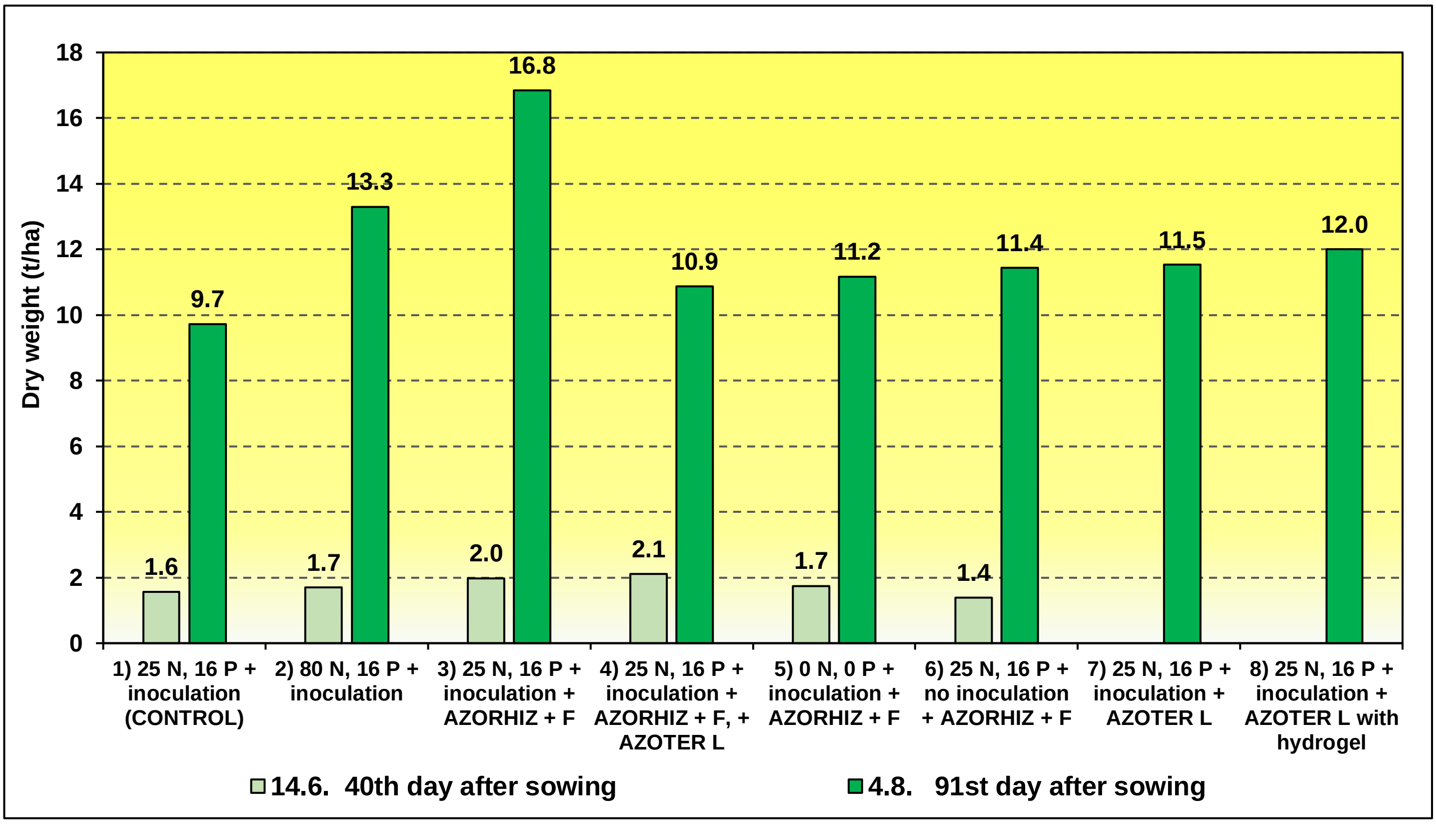

Fig. 3. The effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer and the treatment of the stand during vegetation period with Azoter L fertilizer on the dynamics of the increase in the dry weight of the above-ground biomass (average stand density: 56 plants per m^2)

On the 40^{th} day of vegetation, plant nutrition with the main macro-elements (N, P, K, Ca and Mg) was minimally different in individual variants. On the control untreated plot, the average nutrition was classified to fulfill 97% of the optimum, i.e. the optimal state of plant nutrition in the reached growth stage (2nd - 4th leaf layers formed). After the application of a high nitrogen dose of 80 kg/ha (var. 2), the average plant nutrition with macro-elements was at the level of 102% optimum (in the optimal level). The same was after applying Azorhiz with the application (var. 3) and without the application of the basic dose of nitrogen and phosphorus at sowing (var. 5). After applying Azorhiz fertilizer and at the beginning phase of Azoter L application (var. 4), the plant nutrition with the macro-elements was at the optimum level (99% fulfillment of the optimum).

Without seed inoculation at sowing and pre-sowing soil treatment with Azorhiz fertilizer (var. 6), plant nutrition with the main macronutrients was still at the level of 104% optimum (at optimal level). It is evident, that the nutrition of the stand was very balanced despite omitting the basic dose of fertilizer or the standard seed inoculation. The results also indicate the already ongoing effectiveness of soil treatment with Azorhiz fertilizer for plant nutrition in the first half of the vegetation, similarly to the usual support of plant nutrition by standard seed inoculation with symbiotic, nitrogen-fixing bacteria.

After 91 days from sowing, plant nutrition on the control variant was classified to fulfill 97% of the optimum for the observed macronutrients. Plant nutrition after applying a high dose of nitrogen 80 kg/ha (var. 2) was classified to fulfill the lowest optimum level of 94% (slight deficit in nutrition). After Azorhiz application (var. 3), the plant nutrition was at the level of 97% optimum (at optimal level). Plant nutrition after the soil treatment with Azorhiz and the application of Azoter L foliar fertilizer (var. 4) was at the level of 99% optimum (at optimal level). It is evident that the application of the foliar fertilizer improved plant nutrition minimally. Without using the basic fertilization with nitrogen and phosphorus and treating the soil with Azorhiz (var. 5), the plant nutrition at the end of intensive growth and setting the pods was still at the level of 98% optimum (at optimal level). Without the standard seed inoculation and soil treatment with Azorhiz (var. 6), the nutrition level was classified to fulfill 98% of the optimum (at optimal level).

50 days after the stand was treated with Azoter L fertilizer (var. 7) during the vegetation, the nutrition was at the level of 97% optimum (at optimal level). After treating the stand with Azoter L fertilizer in a mixture with available hydrogel, plant nutrition with the macro-elements was at the level of 98% optimum (at optimal level). It is evident, that during the vegetation, the foliar treatment of stands only with Azoter L fertilizer or in combination with Azorhiz soil fertilizer improved the plant nutrition minimally (Fig. 4).

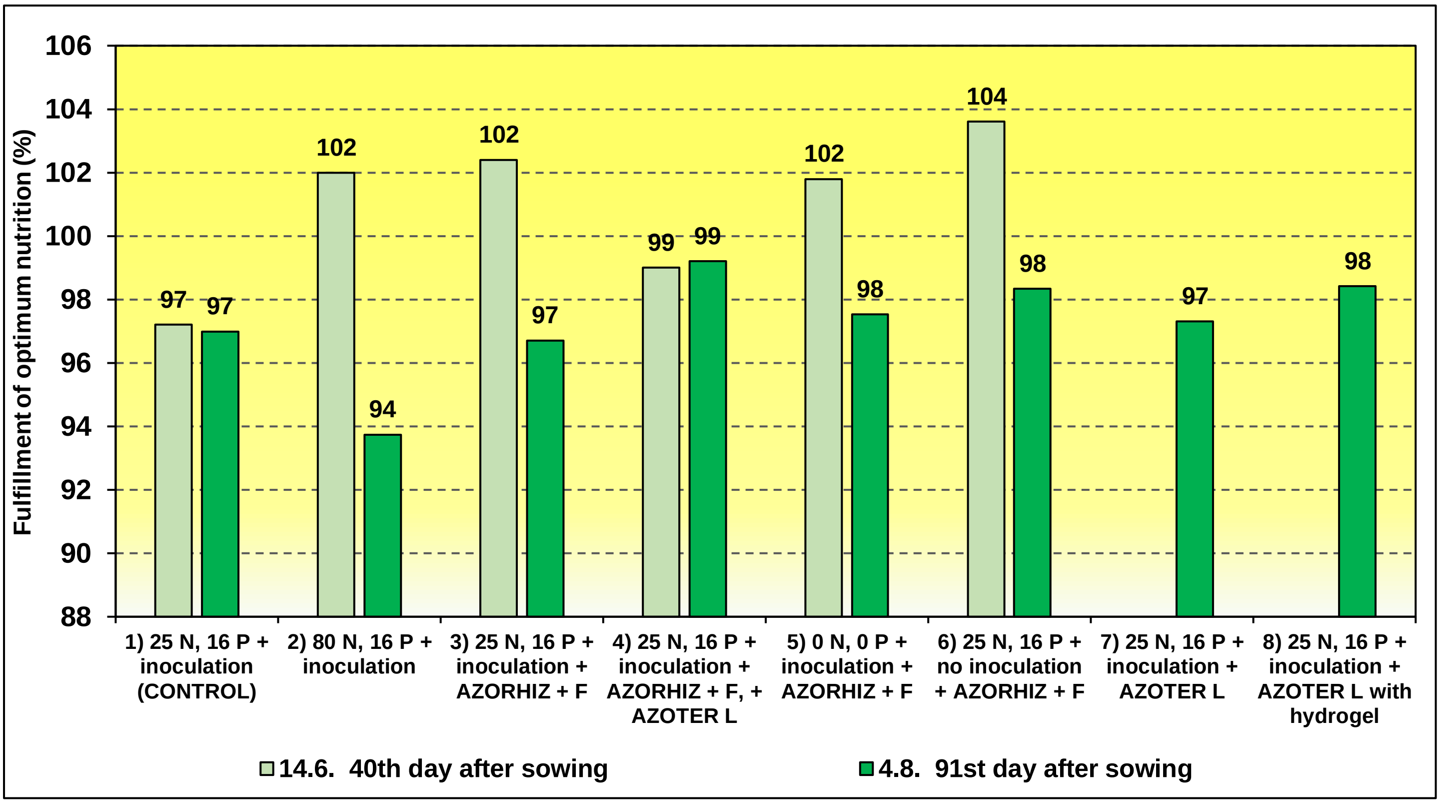

Fig. 4. Effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer and treatment of the stand with Azoter L fertilizer during the vegetation on the nutritional status of plants during vegetation (total N, P, K, Ca and Mg nutrition, fulfillment of the optimal nutritional status = 100%)

40 days from sowing, the nutritional status of the plants was very good, in an excess optimum and an excess of nitrogen, in a medium up to deep deficit of phosphorus, in an excess optimum of potassium, in a moderate deficit up to an optimum level of calcium, in a medium up to deep deficit of magnesium. Without treating the soil and the stand with the tested products, the nutrition of the plants with nitrogen was at the level of 118% optimum (excess optimum). After the application of a high dose of nitrogen of 80 kg/ha (var. 2), plant nutrition was at the level of 130% optimum (in excess level).

After applying Azorhiz (var. 3), plant nutrition was at the level of 134% optimum (in excess). After pre-sowing application of Azorhiz fertilizer to the soil and before the upcoming application of Azoter L during the vegetation (var. 4) the plant nutrition with nitrogen was at the level of 123% optimum (in excess). Plant nutrition with nitrogen without the application of the basic dose of nitrogen and phosphorus in fertilizers at sowing and after treating the soil with Azorhiz (var. 5) was at the level of 127% optimum (in excess). Without seed inoculation and soil treatment with Azorhiz (var. 6), the plant nutrition was at the level of 132% optimum (in excess).

The plant nutrition with phosphorus was balanced, in the range of 83 – 88% to fulfill the optimum. The exception was a deep phosphorus deficit (76% optimum) in plants after applying the combination of Azorhiz fertilizer to the soil before sowing and Azoter L fertilizer during the first half of the vegetation (var. 4). Plant nutrition with potassium was also very balanced in the stand variants. It was in the range of 110 – 113% to fulfill the optimum. The exception was a slightly lower nutritional status (106% optimum) without applying a basic dose of nitrogen and phosphorus at sowing (var. 5).

The plant nutrition with calcium was in the range of a slight deficit of 91-98% optimum on the control variant (var. 1), with the application of a high dose of nitrogen 80 kg/ha (var. 2), the application of Azorhiz fertilizer (var. 3) and with the application of both Azorhiz and Azoter L fertilizers (var. 4). When the soil was treated before applying Azorhis fertilizer, Calcium nutrition fulfilled the range of 102-103% of the optimum without applying a basic dose of nitrogen and phosphorus (var. 5) and without inoculation (var. 6). Plant nutrition with magnesium was in the range from 85 to 89% of optimum. There was only deep magnesium deficiency in plant nutrition (77% of the optimum) on the control variant. Plant nutrition with boron was improved by applying Azoter L foliar application, after which it fulfilled 127% of the optimum (excess). Stands that were not treated with Azoter L fertilizer showed the boron nutrition more than the optimum (115-116% of the optimum).

It is evident that pre-sowing soil treatment with Azorhiz fertilizer influenced and improved the plant nutrition before intensive biomass growth and pod formation mainly with nitrogen fixation.

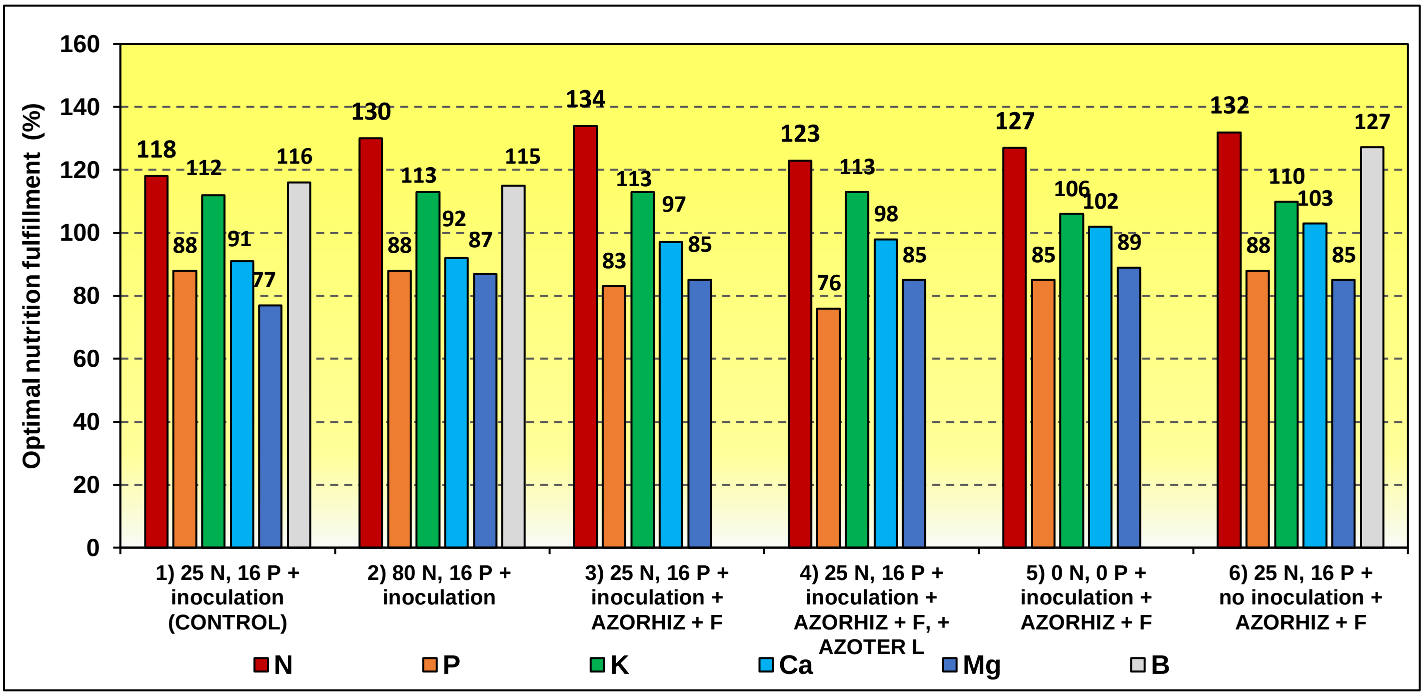

Fig. 5. Effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer and treatment of the stand with Azoter L fertilizer during the vegetation on the nutritional status of plants on 14/06/2022 to the 40th day after sowing, BBCH 12-14 (fulfillment of the optimal nutritional status = 100%)

91 days after sowing, plant nutrition with nitrogen at the end of intensive growth and pod set was at the optimal level, i.e. in the range of 100-105% optimal nutrition fulfillment. The nutrition was not deficient in any variants of soil and stand treatment and even after the targeted omission of the basic dose of nitrogen and phosphorus in fertilizers or without standard seed inoculation. The best plant nutrition at the level of 105% optimal nutrition fulfillment was at the end of intensive growth, after applying Azorhiz fertilizer (var. 3), after applying Azorhiz fertilizer in combination with Azoter L foliar treatment (var. 4) and applying Azoter L foliar fertilizer in a mixture with available hydrogel (var. 8.). Plant nutrition with phosphorus showed an optimal nutrition fulfillment within the individual variants in the range of 97 – 103%. The best plant nutrition with phosphorus was found after applying Azorhiz soil treatment (var. 3, var. 4) without using the basic dose of nitrogen and phosphorus in fertilizers before sowing (var. 5) or without

the seed inoculation (var. 6). The best phosphorus nutrition was also with applying Azoter L foliar fertilizer in the mixture with hydrogel (var. 8). Plant nutrition with potassium varied widely between the stand variants within a range of 111–143% of optimal nutrition fulfillment. On the control variant (var. 1), plant nutrition with potassium was at the level of 118% of the optimal fulfillment (in excess optimum). The nutrition of plants with potassium was the same after applying a high dose of nitrogen before sowing (var. 2) and after the application of Azorhiz fertilizer without the seed inoculation (var. 6). Better nutrition with potassium, at the level of 136% of the optimal fulfillment (in excess optimum), was after applying Azorhiz fertilizer (var. 3) with the seed inoculation and the basic dose of fertilizers. After applying Azorhiz fertilizer to the soil before sowing and the subsequent application of Azoter L fertilizer during vegetation (var. 4), plant nutrition with potassium was at the level of 134% optimal fulfillment (in excess optimum). Plant nutrition with potassium after pre-sowing application of Azorhiz fertilizer without the basic dose of nitrogen and phosphorus in fertilizers (var. 5) was at the level of 143% optimal fulfillment (in high excess). Plant nutrition with calcium varied mainly between variants in the range of 71-79%, i.e. in a deep deficit. Better nutrition with calcium in the range of moderate deficit (90-91% of optimal fulfillment) was in the control stand (var. 1), in the stand after Azorhiz application and without parallel seed inoculation (var. 6) and after Azoter L foliar treatment of the stand during vegetation (var. 7). The plant nutrition with magnesium, at the end of the formation of yield-generating elements, was between the stand variants mainly in the range of 69 – 79% (in a very deep or deep deficit). Better magnesium nutrition, at the level of 86% optimal fulfillment (moderate deficit), occurred only after Azoter L application (var. 7). Plant nutrition with boron was found on the monitored variants mainly in the range of 77-79% optimal fulfillment (in a deep deficit). After Azoter L application during the vegetation, an improvement in plant nutrition with boron after 50 days of its implementation represented 84% of the optimal nutrition fulfillment (var. 7) and 91% of the optimal nutrition fulfillment (var. 8). In the case of boron, the addition of hydrogel to the Azoter L fertilizer did not result in better plant nutrition. Plant nutrition with manganese ranged from a deep to moderate deficit (79-88% of the optimal nutrition fulfillment). Plant nutrition with manganese was improved by soil treatment with Azorhiz fertilizer together with the fertilizer application and standard seed inoculation (Fig. 6).

Plants of soybean stands 33 days after sowing

Left: Control variant (var. 1)
Dry matter content of 1 plant=2,8 g

Right: Azorhiz soil treatment (var. 3)
Dry matter content of 1 plant = 3,5 g

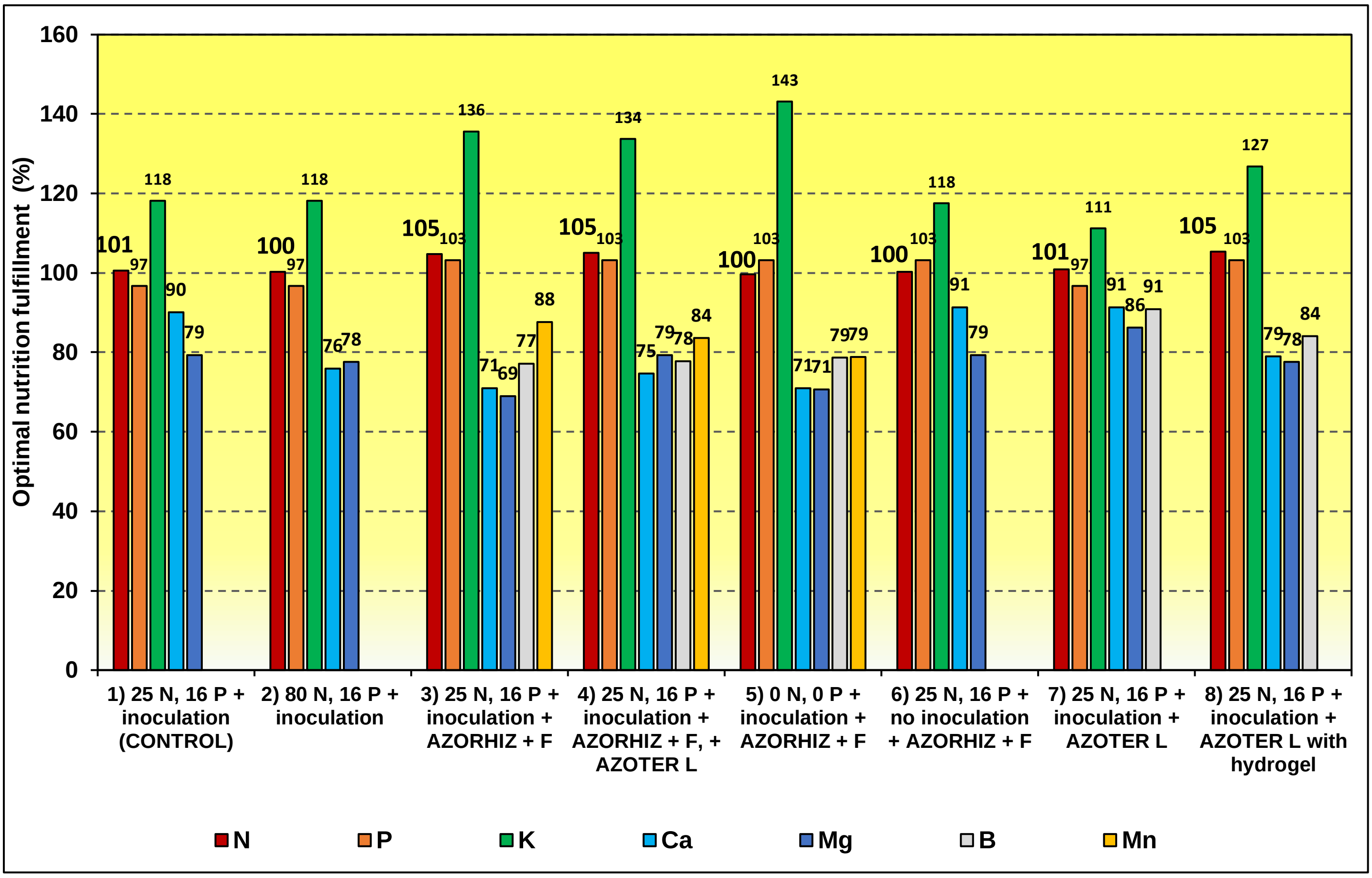

Fig. 6. Effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer and treatment of the stand with Azoter L fertilizer during the vegetation on the nutritional status of plants to 4.08.2022, the 91st day after sowing, BBCH 79-85 (fulfillment of the optimal nutritional status = 100%)

The specific Azoter L biological fertilizer used for the application during vegetation which, in addition to the direct implementation of nutrients, subsequently strengthens the uptake of nitrogen by the leaf surface of plants through the endophytic diazotrophic bacteria *Herbaspirillum seropedicae*, was experimentally applied with available hydrogel, extending the wetting of the leaves and the adherence of the fertilizer to the surface of the plants. The inspection of the stand, on the 2nd day after its application, showed an increased fertilizing effect of Azoter L with nitrogen and potassium after its application with hydrogel (at a dose of 1 l/ha). At this time, it was mainly about improving the plant nutrition with directly introduced nutrients. Soybean plants increased the growth of above-ground biomass, and the treatment with Azoter L fertilizer with hydrogel indicated a better (prolonged) penetration through the leaves cuticle for moistening and minimal drying (fertilizer salt deposits on the surface of the leaves, can be washed away with rain). After applying Azoter L treatment, without or in a mixture with hydrogel, stands significantly improved the growth rate and intensity of plant nutrition already on the 2nd day (Table 3).

At the end of the intensive growth, better nutritional status of plants with nitrogen, potassium, and other elements after applying Azoter L treatment in a mixture with hydrogel was also detected.

Azoter L (10 l/ha), 2nd day (slight leaf burn)

Azoter L (10 l/ha) + hydrogel (1 l/ha), 2nd day (no leaf burn)

Variant	Growth stage (BBCH)	Dry weight of above-ground biomass (kg/ha)	Nitrogen		Phosphorus		Potassium		Calcium	
			Dry matter content (%)	Uptake (kg/ha)	Dry matter content (%)	Uptake (kg/ha)	Dry matter content (%)	Uptake (kg/ha)	Dry matter content (%)	Uptake (kg/ha))
Before application (-2 days)	12-14	1568	3,99	82	0,36	7,4	2,57	53	1,69	35
7) 25 N, 16 P + inoculation + AZOTER L	14-51	2615	4,49	117	0,39	10,2	2,32	61	2,08	54
8) 25 N, 16 P + inoculation + AZOTER L with hydrogel	14-51	2755	4,34	120	0,35	9,6	2,48	68	1,82	50
Difference after tank-mix with hydrogel	-	**140**	-0,15	**2,2**	-0,04	-0,6	0,16	**7,7**	-0,26	-4,3

Variant	Growth stage (BBCH)	Dry weight of above-ground biomass (kg/ha)	Magnesium		Boron		Molybdenum		Sodium	
			Dry matter content (%)	Uptake (kg/ha)	Dry matter content (%)	Uptake (g/ha)	Dry matter content (%)	Uptake (g/ha)	Dry matter content (%)	Uptake (g/ha)
Before application (-2 days)	12-14	1568	0,48	9,9	31,4	64	1,1	2,3	-	-
7) 25 N, 16 P + inoculation + AZOTER L	14-51	2615	0,64	16,7	57,7	151	4,9	12,9	81	212
8) 25 N, 16 P + inoculation + AZOTER L with hydrogel	14-51	2755	0,54	14,9	51,7	142	2,5	6,8	124	342
Difference after tank-mix with hydrogel	-	**140**	-0,10	-1,9	-6,1	-9	-2,5	-6,1	43	**130**

Tab. 3. Effect of the soybean stand treatment during the vegetation with Azoter L fertilizer and the hydrogel supporting substance for extension the leaves wetting and improvement the penetration of the biological component of the product through the leaves cuticle on the dry weight of the above-ground biomass, the content of elements in the dry matter and their biological uptake by the stand to 17.6.2022, i.e.2 days after its application (after "foliar" treatment)

3) <u>Effect of Azorhiz and Azoter L biological fertilizer on seed yield and quality</u>

The stand without Azorhiz pre-sowing soil treatment and while maintaining the standard seed inoculation and application of the basic dose of nitrogen and phosphorus in fertilizers (var. 1 – control variant) before sowing showed a seed yield of 2.49 t/ha. After applying a high dose of nitrogen of 80 kg/ha before sowing and while maintaining the standard seed inoculation (var. 2), there was higher seed yield **by 9%** (0.23 t/ha) compared to the control variant. After the soil treatment with Azorhiz inoculation product, standard seed inoculation and application of the basic dose of nitrogen and phosphorus to the soil at sowing (var. 3), there was higher seed yield **by 11%** (0.28 t/ha) compared to the control variant. After treating the soil with Azorhiz fertilizer, maintaining the seed inoculation and applying a basic dose of nitrogen and phosphorus, and subsequently in combination with Azoter L foliar treatment of the stand during the vegetation (var. 4), there was an increase in seed yield by 9% (0.23 t/ha) compared to the control variant. Without applying the basic dose of nitrogen and phosphorus at sowing, while maintaining the standard seed inoculation and after treating the soil with Azorhiz fertilizer before sowing (var. 5), there was an increase in seed yield **by 19%** (by 0.47 t/ha) compared to the control variant. Without standard seed inoculation but after applying a basic dose of nitrogen and phosphorus and after pre-sowing soil treatment with Azorhiz fertilizer (var. 6), there was an increase in seed yield **by 13%** (0.33 t/ha) compared to the control variant. After treatment of the stand with Azoter L fertilizer during the vegetation, standard seed inoculation and applying the basic dose of nitrogen and phosphorus at sowing (var. 7), there was higher increase in seed yield **by 17%** (0.42 t/ha) compared to the control variant without foliar treatment. After Azoter L application in a mixture with available hydrogel (var. 8), there was higher seed yield **by 28%** (0.70 t/ha) compared to the control variant.

It is evident that individual Azoter L treatment of stands during the vegetation showed the highest intensification ability in seed production, especially after tank-mixing the product with available hydrogel to extend the wetting and penetration time of the product through the leaves cuticle to reduce the risk of burning soybean leaves. At the same time, intensive treatment of the stand, specifically the soil before sowing with Azorhiz fertilizer and the stand with Azoter L fertilizer, while maintaining standard inoculation and the basic dose of nitrogen and phosphorus in fertilizers at sowing, did not bring an adequate benefit in the achieved yield. The soybean stand did not utilize the set of improving treatments sufficiently for seed production. After treating the soil with Azorhiz, a very good yield was achieved without applying the basic dose of nitrogen and phosphorus at sowing. Similarly, it is evident that the standard seed inoculation can be omitted to achieve an increased seed yield, if the soil has been treated with Azorhiz inoculation preparation before sowing (Fig. 7).

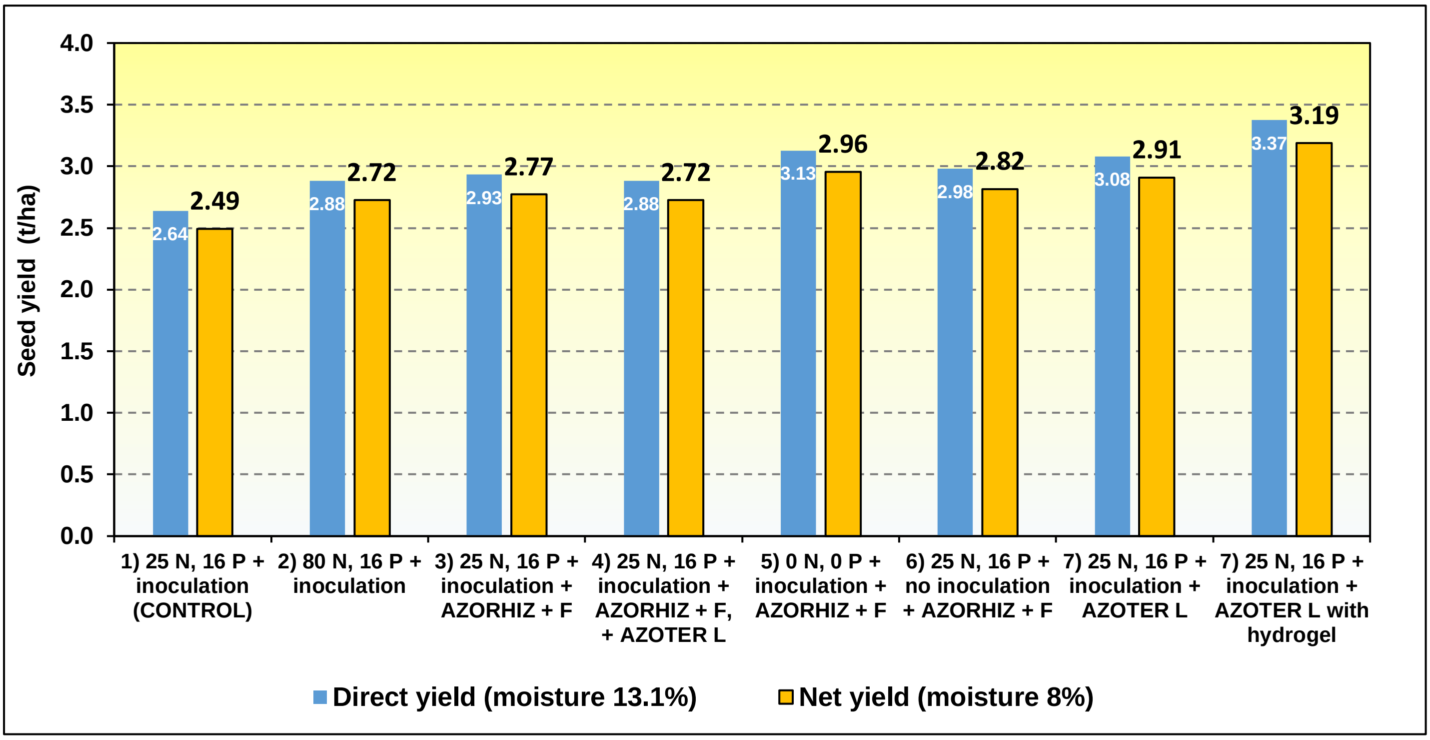

Fig. 7. Effect of pre-sowing soil treatment with Azorhiz + F biological fertilizer and treatment of the stand during the vegetation with Azoter L preparation on the achieved seed yield (harvested on 10.10.2022)

Soybean seed yield	1) Control variant	2) 80 N	3) Azorhiz	4) Azorhiz, Azoter L	5) 0 N, 0 P, Azorhiz	6) no inoculation, Azorhiz	7) Azoter L	8) Azoter L with hydrogel
t/ha	2,49	2,72	2,77	2,72	2,96	2,82	2,91	3,19
%	**100 %**	109 %	**111 %**	109 %	**119 %**	**113 %**	**117 %**	**128 %**

An increase in seed yield was achieved after pre-sowing application with Azorhiz fertilizer, Azoter L fertilizer during the vegetation and the application of increased nitrogen dose before sowing through higher number of plants at harvest, partly by higher number of layers with developed pods or partly by the total number of seeded pods on plants. Higher thousand seed weight was found at the highest achieved seed yields.

On the control plot (var. 1), there was an average number of 48 plants on an area of 1 m^2 at the harvest. After treating the soil with Azorhiz biological fertilizer (var. 3 to var. 6), there was a higher density of stands, in the range of 60-68 plants per m^2. Similarly, a greater density of stands was found at harvest after applying Azoter L fertilizer at the period of the 2nd - 4th leaf layer (var. 7 and var. 8), in the range of an average 64 - 72 plants per m^2. The density of the stands was also positively affected by increased fertilization with nitrogen before sowing at a dose of 80 kg N/ha (var. 2). The number of layers with developed or formed pods varied within all variants of the experiment in the range of 8.6 - 11.3 pieces per plant. The number of layers with more than 10 developed pods was found after pre-sowing soil treatment with Azorhiz fertilizer (var. 3), soil treatment with Azorhiz fertilizer in combination with Azoter L fertilizer applied on the stands during the vegetation (var. 4), soil treatment with Azorhiz without seed inoculation (var. 6) and after application of Azoter L foliar fertilizer in a mixture with available hydrogel (var. 8). The average number of soybean pods on the stem nodes of individual variants in the experiment was minimally different and it ranged from 1.6 to 1.8 pods per node stage. The average number of pods on the whole plant ranged from 12.8 to 18.4 pcs. The lowest number of pods per plant was found after omitting the basic dose of nitrogen and phosphorus with applying Azorhiz fertilizer into the soil (var. 5). On the contrary, a higher number of pods per plant (more than 17 pcs) occurred after Azorhiz application (var. 3), after applying Azorhiz and the subsequent application of Azoter L fertilizer (var. 4), after treating the soil with Azorhiz fertilizer and sowing seeds into the soil without inoculation (var. .6) and after treatment of the stand with Azoter L fertilizer in a mixture with hydrogel during the vegetation (var. 8). The average number of seeds in a pod varied in the range of 1.5 and 2.0 pcs. The lowest average number of seeds per pod was found after omitting the basic dose of nitrogen and phosphorus at sowing, maintaining the standard seed inoculation and pre-sowing soil treatment with Azorhiz fertilizer (var. 5). On the contrary, the highest number of seeds in a pod was found after applying a high dose of nitrogen before sowing (var. 2) and without the standard seed inoculation and pre-sowing soil treatment Azorhiz (var. 6). The average number of seeds on the whole plant ranged from 19.2 to 36.2 pcs. The lowest average number of seeds on the whole plant was found after omitting the basic application of nitrogen and phosphorus at sowing, maintaining the seed inoculation and soil treatment with Azorhiz fertilizer (var. 5). A higher number of seeds on plants (more than 30 pcs.) was found after treating the soil with Azorhiz fertilizer and parallelly with Azoter L fertilizer (var. 4), after applying a high dose of nitrogen of 80 kg/ha before sowing (var. 2), after applying Azoter L fertilizer in a mixture with available hydrogel (var. 8) and after Azorhiz application and sowing seeds without standard inoculation (var. 6). The thousand seed weight (TSW) ranged from 162 to 191 g within the individual stand variants. A higher TSW (more than 180 g) was found in the most productive stand variants. A higher TSW (more than 180 g) was found after pre-sowing soil treatment with Azorhiz fertilizer without basic application of nitrogen and phosphorus in fertilizers at sowing (var. 5), after application of Azoter L fertilizer during the vegetation without (var. 7) or in a mixture with hydrogel (var. 8). The highest TSW was found after pre-sowing soil treatment with Azorhiz fertilizer with sowing seed without standard inoculation treatment (var. 6).

According to the content of the main component of nitrogenous substances, the quality of the soybean seed varied in a very narrow range within all variants of the experiment. The content of nitrogenous substances in the seeds ranged from 44.1 to 45.4% in dry matter (table 4). The content of nitrogenous substances (higher than 45%) was found after treating the soil with Azorhiz fertilizer without applying the basic dose of nitrogen and phosphorus at sowing (var. 5) and after applying Azoter L fertilizer in a mixture with available hydrogel (var. 8). On the contrary, the lowest content of nitrogenous substances in the seed was found after applying a high dose of nitrogen before sowing (var. 2), where neither the soil nor the vegetation was treated with the tested fertilizers.

Variant	Yield order	Achieved seed yield (t/ha)	No. of plants at harvest (ks/m^2)	No. of nodes with pods (pcs/plant)	No. of pods (pcs/node)	No. of pods (pcs/ plant)	No. of seeds (pcs/pod)	No. of seeds (pcs/ plant)	TSW (g)	Content of N-substances (%)
1) 25 N, 16 P + inoculation (CONTROL)	7.	2,49	48	9,3	1,7	15,8	1,8	28,5	**176**	44,6
2) 80 N, 16 P + inoculation	6.	2,72	**62**	9,2	1,8	16,6	2,0	**33,6**	166	44,1
3) 25 N, 16 P + inoculation + AZORHIZ + F	5.	2,77	**60**	**11,0**	1,6	**17,5**	1,7	29,6	162	44,6
4) 25 N, 16 P + inoculation + AZORHIZ + F, + AZOTER L	6.	2,72	**60**	**11,3**	1,6	**17,9**	1,7	**31,0**	**177**	44,9
5) 0 N, 0 P + inoculation + AZORHIZ + F	**2.**	**2,96**	**68**	8,6	1,6	12,8	1,5	19,2	**183**	45,3
6) 25 N, 16 P + no inoculation + AZORHIZ + F	**4.**	**2,82**	**60**	**10,6**	1,7	**18,1**	2,0	**36,2**	**191**	44,8
7) 25 N, 16 P + inoculation + AZOTER L	**3.**	**2,91**	**64**	9,9	1,6	15,8	1,8	28,4	**187**	44,6
8) 25 N, 16 P + inoculation + AZOTER L with hydrogel	**1.**	**3,19**	**72**	**10,8**	1,7	**18,4**	1,9	**34,7**	**181**	45,4

Tab. 4. The effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer and treatment of the stand with Azoter L fertilizer during the vegetation on the structure of yield-generating elements and seed quality (achieved yield from the plot on 10.10.2022, yield elements determined from harvest plant samples from 27 .9.2022)

After treating the soil with Azorhiz fertilizer, or after foliar treatment with Azoter L fertilizer during the vegetation and after applying a high dose of nitrogen before sowing, the stands showed higher seed production and significantly higher straw yield. The yield ratio of seed and straw within all variants of the experiment varied in the range 1: 1.7 – 2.5. The lowest yield ratio of seed and straw was found after treating the soil with Azorhiz fertilizer followed by Azoter L fertilizer during the vegetation (var. 4). The average yield ratio of seed and straw of 1:1.9 was found in the control variant (var. 1) and after applying a high nitrogen dose of 80 kg/ha (var. 2). A wider yield ration of seed and straw of 1:2.1 was at the stand treated with Azorhiz fertilizer with sowing seed without standard inoculation (var. 6). The stand showed a yield ratio of seed and straw of 1:2.2 after its treatment with Azoter L foliar fertilizer in a mixture with available hydrogel (var. 8). The stand showed wider yield ratio of seed and straw of 1:2.4 after Azorhiz application (var. 3). The stand showed the widest yield ratio of seed and straw of 1:2.5 without the application of the basic dose of nitrogen and phosphorus at sowing and with Azorhiz application (var. 5). Similarly, the widest yield ratio of seed and straw of 1:2.5 was found after Azoter L foliar application during the vegetation (var. 7).

It is evident that the highest achieved seed yields from the harvested area (with a threshing maschine) were related to the overall higher aboveground biomass production (higher straw yield). The highest straw yield with the potential for 9.3 t/ha was found after the application of a high nitrogen dose of 80 kg/ha (var. 2). Here, however, the harvested area didn´t show the highest seed yield and it is supposable that the high dose of nitrogen contributed to higher straw production (excessive size of the stand was observed). A high straw yield potential of 8.7 t/ha was found after the soil was treated with Azorhiz fertilizer with sowing seed without standard inoculation (var. 6). The achieved yield from the harvested area was the 4th highest one here. After Azoter L application in a mixture with hydrogel during the vegetation, the straw yield potential was 8.6 t/ha. The seed yield from the area achieved by the thresher was the highest one here. The results document the need for a gradual supply of nitrogen and other nutrients for soybean crops. A high single dose of nitrogen (80 kg/ha) promoted straw production, but the achieved seed yield was promoted less.

The seed yield potential ranged from 2.5 to 4.9 t/ha in all tested variants. The actual yield from the area, i.e. from harvesting with a thresher, ranged from 2.5 to 3.2 t/ha. First, the seed yield potential was not reached by full area harvesting after the application of a high nitrogen dose of 80 kg/ha (var. 2). In all variants, seed losses for late harvest in early October were reflected in the achieved yield per area (Fig. 8).

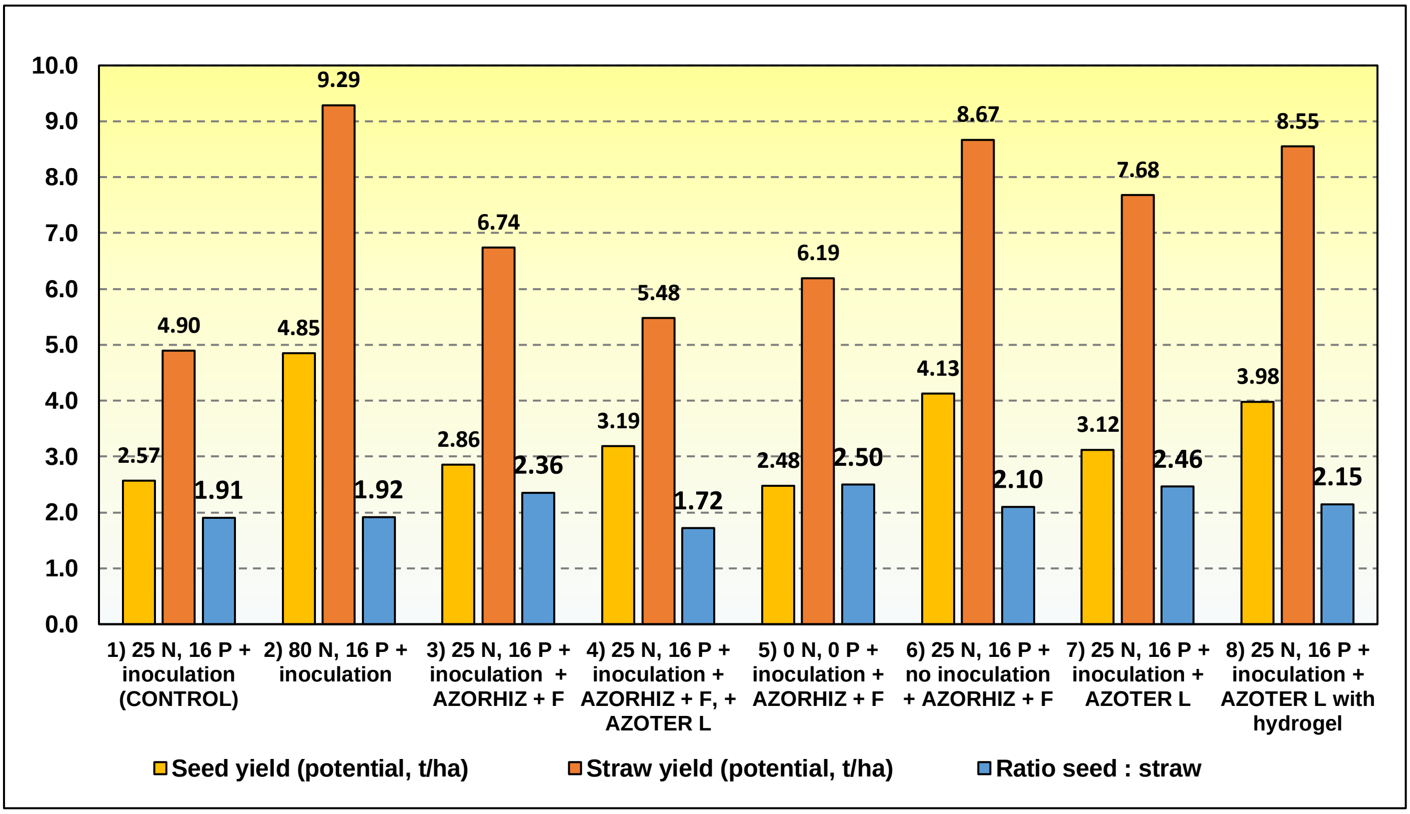

Fig. 8. Effect of pre-sowing soil treatment with Azorhiz +F biological fertilizer and treatment of the stand during the vegetation with Azoter L fertilizer on the main yield ratio (seed) and secondary yield ratio (straw) of soybean (harvest plant samples from 27.09.2022)

➲ **Conclusions**

The cultivation process of the seemingly undemanding soybean crop can be purposefully intensified by complex biological principles of plant nutrition, providing the plants with a gradual supply of nitrogen and other nutrients. Treatment of the soil with bacteria living in symbiosis with the soybean root system and the targeted introduction of non-symbiotic diazotrophic bacteria into the soil that bind atmospheric nitrogen (N_2), intensified the increase of above-ground biomass, improved plant nutrition during the vegetation which resulted in a significant increase in seed yield. The achieved soybean seed yield increased by 0.23 - 0.47 t/ha after pre-sowing soil treatment with the concentrated **Azorhiz +F biological fertilizer at a dose of 10 l/ha (+0.1 l/ha of F additive**), which could replace the standard seed inoculation (dry method) at sowing.

Azorhiz application, which contains the specified vital bacteria, could replace the basic spring soil fertilization of soybeans with a “starting” dose of nitrogen and phosphorus (originally applied to ensure nutrition from the period of emergence to root nodulation by nodule bacteria). The replacement in the process of spring soil supply with mineral nitrogen ($N_{min.}$), a dose of nitrogen and phosphorus represents 10 mg/kg of soil (N_{min}. = 41 kg/ha) and in the process of good supply of soil with available phosphorus (P), a dose of nitrogen and phosphorus represents at least 85 mg/kg soil.

A very effective intensification measure was the treatment of soybean stands during the vegetation with the foliar (extra-root) Azoter L biological fertilizer at a dose of 10 l/ha. After the treatment, the stand showed better nutrition with potassium and nitrogen, which supported a more intensive dynamic of above-ground biomass growth. After the treatment of the stand with Azoter L fertilizer, the seed yield increased by 0.42 t/ha. After applying Azoter L in a mixture with available **hydrogel** to support the penetration of the vital bacterial component of the product into the leaves, the seed yield increased by 0.70 t/ha compared to the untreated control variant.

➲ Recommendations: 1) Azorhiz +F fertilizer for the soil treatment applied before sowing the soybeans must be immediately **incorporated into the soil with pre-sowing preparation**. If the application dose of 10 l/ha (0.1 l/ha of F additive) is maintained and incorporated into the soil before sowing in time, the fertilizer can replace the standard seed inoculation (in dry method) before sowing the soybean in structural, biologically active soils, soils with slightly acidic or soil with neutral pH and in soils where growing a series of different types of crops in the same area occured in the past, including this crop.

2) The basic "**starting" dose of nitrogen and phosphorus in fertilizers** (type Amofos, NPK, etc.) **can be replaced** by treating the soil with Azorhiz +F fertilizer before sowing the soybeans on well-supplied soils with phosphorus and mineral nitrogen (usually achieved after a mild winter in semi and arid regions), applied before or at sowing. At the beginning, stands without a "starting" dose of nutrients supplied by fertilizers may show poorer nutrition, but due to the long vegetation and the formation of the main yield-generating elements in the summer, the direct application of nitrogen and phosphorus did not have a significant effect on the achieved seed yield, when the soil was treated with Azorhiz + F soil inoculant before sowing.

3) **It appears to be an economically very effective treatment** of soybean stands during the beginning of vegetation (developed on the main stem of the 3rd - 4th leaf layers) with an auxiliary biological source of nitrogen in the Azoter L fertilizer. The application of the fertilizer is very suitable in a mixture with hydrogel (at a dose of 1 l (kg)/ha) to reduce the risks of burning

the leaves with the solution (stained soybean leaves have a minimal protective wax layer) and the extension of the leaves wetting is necessary to preserve the vitality of the active substance of the *Herbaspirillum seropedicae*. Deficient plant nutrition can be supplemented with **boron or molybdenum** in Azoter L fertilizer, to which the stands respond positively in terms of yield. Boron participates in the formation of buds, pollen and during flowering. Molybdenum participates in the metabolism of nitrogen obtained from fixation by nodule bacteria on plant roots.

➲ Economic evaluation:

Special pre-sowing treatment of the soil with Azorhiz + F biological fertilizer or specially performed treatment of the stands during the vegetation with Azoter L biological fertilizer applied with a sprayer completely covered the **additional costs incurred** and, in addition, **significantly increased the profitability of the soybean cultivation technology**:

Calculation (abbreviated)	1)	2)	3)	4)	5)	6)	7)	8)
	Control (standard)	80 kg N/ha	Azorhiz +F	Azorhiz +F, Azoter L	0N, 0P; Azorhiz	~~inoculation~~, Azorhiz	Azoter L	Azoter L + hydrogel
Market outpput								
Seed yield (t/ha)	2,49	2,72	2,77	2,72	2,96	2,82	2,91	3,19
Seed yield comparison	100 %	109 %	111 %	109 %	119 %	113 %	117 %	128 %
Purchase price valuation (CZK/t)	15 000	15 000	15 000	15 000	15 000	15 000	15 000	15 000
Sales (CZK/ha)	**37 350**	**40 800**	**41 550**	**40 800**	**44 400**	**42 300**	**43 650**	**47 850**
Variable costs (multiple costs of agricultural machinery)								
Fertilizer Azorhiz +F 10 l/ha (CZK/ha)	0	0	1 000	1 000	1 000	1 000	0	0
Application (300 l of water/ha) (CZK/ha)	0	0	400	400	400	400	0	0
Sub-total (CZK/ha)	***0***	***0***	***1 400***	***1 400***	***1 400***	***1 400***	***0***	***0***
Fertilizer Azoter L 10 l/ha (CZK/ha)	0	0	0	1 000	0	0	1 000	1 400
Application (300 l of water/ha) (CZK/ha)	0	0	0	400	0	0	400	400
Sub-total (CZK/ha)	***0***	***0***	***0***	***1 400***	***0***	***0***	***1 400***	***1 800***
Nitrogen fertilizer (Alzon Neo 46 % N)	0	3 300	0	0	0	0	0	0
Application (manure spreader)	0	300	0	0	0	0	0	0
Sub-total (CZK/ha)	***0***	***3 600***	***0***	***0***	***0***	***0***	***0***	***0***
Cost savings in agricultural technology								
Type of saving	-	-	-	-	NP fertilizer	Inoculant	-	-
Saving rate (CZK/ha)	0	0	0	0	2 430	570	0	0
Total variable cost (CZK/ha)	**0**	**3 600**	**1 400**	**2 800**	**1 400**	**1 400**	**1 400**	**1 800**
Contribution to cover other costs (Variable costs + Fixed costs)								
Contribution to pay (CZK/ha)	**37 350**	**37 200**	**40 150**	**38 000**	**43 000**	**40 900**	**42 250**	**46 050**
The difference in the payment contribution (CZK/ha)	x	-150	**2 800**	650	**5 650**	**3 550**	**4 900**	**8 700**

Note: The calculation includes the prices of fertilizers according to the price list valid in spring 2022, the price of work considers the increasement of inputs in 2022, price of auxiliary substance hydrogel 1 l = 400 CZK, price of inoculation 470 CZK + 100 CZK for work at the mixing plant = 570 CZK/ha, price for sowing the seed 110 kg/ha. Valuation of soybean seed based on the realized purchase price of 2022.

The results of the tested Azorhiz +F and Azoter L biological fertilizers were obtained in 2022, from the pilot experiment in the implemented agricultural company, for AZOTER Trading s.r.o. The results of the experiment were provided to the representatives of the implementing company.

In Žamberk on 13. 12. 2022

Photo documentation

A) Soybean stand at the beginning of long-lasting/term growth on 14.6.2022 (2nd - 4th leaf layer)

Soybean stand in the period before the treatment with Azoter L foliar fertilizer in the prepared dose of 10 l/ha

B) Soybean stands in their intensive growth and first flower on 17.6.2022

A soybean stand in its intensive growth after pre-sowing soil treatment with Azorhiz fertilizer at a dose of 10 l/ha and after the treatment of the stand with Azoter L fertilizer during the vegetation at a dose of 10 l/ha

C) <u>Soybean stand at the end of its intensive growth and flowering, on 4.8. 2022</u>

Soybean stand after pre-sowing soil treatment with Azorhiz fertilizer at a dose of 10 l/ha.

1)	2)	3)	4)	5)	6)	7)	8)
Control var.	80 kg N/ha	Azorhiz	Azorhiz	0N, 0P	No inoculation	Azoter L	Azoter L
			+ Azoter L	+Azorhiz	+Azorhiz		+hydrogel

Samples of average soybean plants taken from stands at the end of their intensive growth on 4.8.2022

Root nodulation by root nodule bacteria in plants <u>without seed inoculation</u>, after pre-sowing soil treatment with Azorhiz biological inoculant - at a dose of 10 l/ha, from 4 .8. 2022

Soybean stand without seed inoculation, after Azorhiz soil treatment at a dose of 10 l/ha before sowing

D) Soybean stand at the time of ripening on 13.09.2022

Soybean stands at the time of ripening on 13.9.2022

Soybean plants at full maturity on the control variant (without treating with the tested products)