

*Impact of foliar fertilization on the yield and bioproductive parameters of perennial ryegrass (*Lolium perenne* L.)*

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Abstract. The experiment was conducted at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria) with the aim of establishing the impact of the fertilizers Nano Sulfur and Panamin Agro on the height, productivity and composition of sown grass stand from perennial ryegrass (*Lolium perenne* L.). The perennial ryegrass plants have proven to form the highest grass stand with the application of Panamin Agro biofertilizer (250 g/da). The harvested biomass registered 4.05 cm (at $P < 0.05$) higher values of the trait compared to the control (67.18 cm). The highest average yield of fresh matter was registered in the grass stand of *Lolium perenne* L. treated with Panamin Agro - 250 g/da. The values of the trait significantly exceeded the unfertilized control by 16.87% ($P < 0.05$). No significant difference was proven in the amount of dry matter was found between the fertilized variants and the untreated control of perennial ryegrass. *Lolium perenne* L. occupied the highest relative share in the variants treated with Nano Sulfur (200 ml/da) and Panamin Agro (250 g/da). The percentage share of the grass crop was respectively 89.94% and 88.71% (in the control variant – 79.95%). The specified variants had the lowest degree of weed infestation.

Key words: *Lolium perenne* L., foliar fertilization, yield, heights, botanical composition.

Introduction

The application of conventional chemical fertilizers pollutes the environment and water resources, reduces soil fertility and accumulates toxic elements in it (Alori et al., 2017). Biofertilizers are an environmentally acceptable alternative to expensive and environmentally polluting chemical fertilizers that could provide satisfactory yields, preserving soil fertility and human/animal health (Markov, 2015; Bozhanska & Naidenova., 2020; Churkova, 2021; Khan et al., 2022). They are an environmentally friendly and reliable approach to increase crop productivity (Kour et al., 2020; Dasgupta et al., 2021; Gautam et al., 2021; Sansinenea, 2021).

The application of organic fertilizers in agricultural practices improves the qualitative and quantitative characteristics of fodder and contributes to the production of ecologically clean fodder production (Vasileva & Enchev 2018; Bozhanska, 2019a,b; Bozhanska et al., 2020; Churkova & Churkova, 2021). Biofertilizers, enriched with minerals, micro- and macroelements easily absorbed by plants, favor the biometric indicators of grass fodder crops. In modern agriculture, for the needs of organic agriculture, suspension fertilizers for foliar or soil application are increasingly used (Petrova & Kolev, 2019).

Perennial ryegrass (*Lolium perenne* L.) is an economically important and widespread

perennial grass species with multifunctional importance (for fodder, decorative and sports-technical purposes, etc.) (Naydenova et al., 2010; Katova, 2011; 2017). The application of humic acid at a low concentration (100 mg L⁻¹) increased the nitrogen (N) content and positively affected the development of the root system in perennial ryegrass, thereby facilitating nutrient uptake and plant resistance to drought (Maibodi et al., 2015).

Foliar application of biostimulants improves biomass composition in *Lolium perenne* L., as a result of which fodder is of high quality and the use of mineral fertilizers is limited (Olszewska, 2022). The introduction of a silicon biostimulator has a positive effect on the nutritional value of the treated plants. Cows fed with silage from them produced milk of higher quality and with higher protein and fat content (Radkowski et al., 2017).

The aim of the experiment is to determine the influence of the application of Nano Sulfur and Panamin Agro on the height, productivity and composition of *Lolium perenne* L., grown in the Central Balkan Mountain region.

Materials and Methods

The experiment was conducted in the period 2020-2022 at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), by the block method, in four replications, with 5 m² plot size. In autumn, plowing was carried out to a depth of 21-23 cm, and in spring the soil was disked (several times), milled and rolled before and after sowing. Perennial ryegrass (*Lolium perenne* L.) cultivar "Nui" was used for sowing the experimental plots. The sowing of the seeds was carried out manually, scattered, on a previously created firm bed at a depth of 0.5-1.0 cm. The sowing rate of fodder crops was calculated based on 100% seed germination, namely: perennial ryegrass - 2.5 kg/da;

Experimental variants are:

1. Control (Untreated)
2. Treatment with Nano sulfur (100 ml/da)
3. Treatment with Nano sulfur (200 ml/da)
4. Treatment with Panamin Agro (150 g/da)
5. Treatment with Panamin Agro (250 g/da).

Characteristics of the tested products

The commercial name of the fertilizer Nano Sulfur is AGROVIT NS. The manufacturer of the product is Sulfitek Innovative Enterprise, Russia (Certificate of Registration No. 0193/06.03.2017). The mineral product Nano Sulfur (AGROVIT NS) is harmless to the environment and living organisms and is suitable for use in organic agriculture. The substance is a mineral, inorganic fertilizer, in polysulfide form, extremely accessible for growing and nourishing plants during vegetation.

It is used as an improver of soil pH, neutralizes heavy metals in the soil, due to the innovative sulfur treatment process, where in the final product the particles have a size 1000 times smaller compared to conventional micronized sulfur (sulfur in powder form). It has a beneficial effect on plant immunity, and a secondary effect as a bio-fungicide and acaricide. It increases yields and protein content in grasses and legumes.

Chemical composition of the product: Sulfur (S) in the form of calcium polysulphide - CaS₅ (size 20 nm) - 230 g/l.

For fodder crops, the treatment is carried out at a plant height of 10-15 cm.

Panamin Agro (<https://panamin.bg/bg/>) is 100% organic product, permitted for organic farming according to EEC No 834/2007 and 889/2008. It is a complex foliar and soil fertilizer. Contains a mix of finely ground rock flours extracted from natural, Austrian volcanic rocks. Its action is expressed through remineralization of plants and soils.

Panamin Agro mainly contains three nutritional elements: calcium (Ca) and magnesium (Mg) - in the form of carbonates; silicon (Si) - in the form of silicon dioxide (SiO₂).

The application of Panamin Agro in the initial phase of crop development stimulates the development of the root system and the stem, increases the immunity of plants against various states of stress and diseases. The bioproduct is particularly effective in adverse climatic conditions, moreover it does not pollute the environment, which is important from an ecological point of view. The advantages of the biopreparation are also expressed in a reduction of the cost price, an increase in yields and the quality of the production (Fig. 1).



Fig. 1. *Lolium perenne* L. treated with Nano Sulfur (left) and Panamin AGRO (right).

The study indicators are:

- *Dry matter yield (kg/da)* was determined by regrowth's and years through mowing each harvest plot in replications. After that the plant samples were dried in laboratory conditions at 105°C and they were recalculated per an area of 1 da based on the dry matter content.

- *Botanical composition of the grass stands (%)* was determined by weight, by analyzing grass samples by groups (grasses and weeds), taken immediately before mowing.

- *Grass stand height (cm)* was measured by regrowths in the harvesting of the grass stand. On the diagonal of the plots, 10 (respectively 40 per variant) plants were measured from the soil surface to the top of the tallest stems. In mixed crops, the height was measured according to the crop type. 40 plants were measured from each variant.

Soil characteristic in the experimental area

Soil conditions are important for the growth, development and yields of fodder crops. The experimental area belongs to the pre-Balkan subzone of the North Bulgarian forest-steppe zone, which is characterized by light gray (pseudopodzolic) soils. According to different authors and taxonomic systems, these soils are also defined as: light gray, pseudopodzolic (Penkov, 1988), inceptisols,

dystrochrepts – metamorphic, unsaturated (Boyadzhiev, 1994) and planosols, distric (Pld) – planosols, unsaturated (Ninov, 1997). According to their usage (agricultural soils), they are infertile, superficially waterlogged, with poor drainage and an acidic soil reaction. The highly differentiated soil profile is typical for them with two main horizons - humus-eluvial, which contains much more humus in its upper part, whereas in its lower part it is purely eluvial and illuvial clay, which in its upper part is gley. The pH values for this soil type vary from 4.5 to 5.5 with base saturation of 20-40%, humus content: in wildlands – 2-2.5%, in arable land 1-1.5%, in the surface and illuvial horizon – 0.4-0.6%. Water properties and the functioning of water and air are their distinguishing characteristics. Nitrification and aeration processes are inhibited. High exchangeable acidity from exchangeable aluminum was observed.

The biological activity of pseudopodzolic soils is weak. They have low natural fertility, which necessitates the implementation of a number of measures in agriculture to improve this indicator (Gyurov & Artinova, 2001).

The humus content of the soil in the experimental area is low considering the classification of Katschinski (1958) – Table 1. The values of the indicator vary from 1.40% (in the zone 0-20) to 1.24% (in the zone 0-40 cm).

Table 1. Nutrient stock in the soil.

Site	pH		Σ N - NH ₄ +NO ₃ mg/kg	P ₂ O ₅ mg/100	K ₂ O	Humus %
	H ₂ O	KCL				
0-20	4.00	3.90	21.31	1.14	20.97	1.40
0-40	4.00	3.90	10.94	0.26	20.32	1.24

Regarding the content of digestible phosphorus and potassium, the values of the indicators range from 0.26 (0-40) to 1.14 mg/100 (0-20) for phosphorus and from 20.32 (0-40) to 20.97 mg/100 (0-20) for potassium. The stock of available nitrogen in the soil is low. In the soil horizon from 0-20 cm, where the root system of the plants is mainly located, the amount of the macroelement is 21.31 mg/100, whereas in the horizon is from 0-40 cm – 10.94 mg/100. The reaction of the soil is acidic at $pH_{H_2O} = 4.00$ и $pH_{KCl} = 3.90$.

The characterization of the pseudopodzolic soils that they are one of the poorest in the country, which makes them unfavorable for agricultural crops. According to Penkov et al. (1985) these soils are suitable for growing fodder crops because they provide a relatively high productivity of fresh and dry matter necessary for feeding ruminants in mountain regions.

The following software products were used for statistical data processing: Analysis Toolpak for Microsoft Excel 2010, Statgraphics Plus v.2.1 and STATSOFT Statistics for Windows 10.

Results and Discussion

Plant height of perennial ryegrass after applied foliar feeding

Over the years of study, *Lolium perenne* L. plants formed the highest grass stand in the variants with Panamin Agro (250 g/da) - Table 2. The harvested biomass had proven ($P < 0.05$) higher values of the indicator (by 4.05 cm) compared to the control (67.18 cm). For the study period, plant height in the variant ranged from 48.13 cm (2020) to 98.13 cm (2021).

In the year of sowing, the growth of *Lolium perenne* L. (47.30 cm) treated with a higher dose of Nano Sulfur (200 ml/da) was significantly different ($P < 0.05$) compared to the untreated control (45.03 cm). The values of the indicator exceed by 0.97 cm the height of the plants in the

variants with a lower dose (100 ml/da) of the same preparation.

The data obtained with the application of the bioproduct Panamin Agro are also similar. Grass stands enriched with a higher dose of biopreparation (250 g/da) formed proven ($P < 0.05$) taller plant stems (48.13 cm) compared to the untreated control (45.03 cm). The biomass height of the lower dose variant (150 g/da) was 1.7 cm less compared to that of the higher dose variant. The coefficient of variation for the tested variants of the first experimental year is in the range from 9.83 to 11.80%. For the second experimental year, only the height of the plants in the variant with the applied lower dose of the foliar fertilizer Nano Sulfur (100 ml/da) did not exceed the unfertilized control. The values of the indicator are lower by 2.65 cm. Treatment with a higher dose of mineral fertilizer stimulated to a higher extent the growth of *Lolium perenne* L. The height of the plants in the variant were higher by 4.87 cm (compared to those treated with a lower dose) and 2.22 cm (compared to the control). In the variants treated with the bioproduct Panamin Agro, the trend is maintained. Variants with a higher dose (250 g/da) formed a higher grass stand 3.25 cm ($P < 0.05$) at a coefficient of variation $CV = 6.56\%$.

In the third experimental year, grass stands of *Lolium perenne* L. treated with lower doses (100 ml/da and 150 g/da) of the tested preparations registered respectively 1.00 cm and 0.73 cm lower grass stands compared to the control. Only the variant with foliar feeding with Panamin Agro at a dose of 250 g/da has a proven excess regarding the height of the plants. The values of the indicator exceeded the control by 6.0% at $CV = 9.99\%$.

For the study period, bio-fertilization with Panamin Agro (250 g/da) had the most significant effect on the height of *Lolium perenne* plants.

Table 2. Height of *Lolium perenne* L. with applied foliar fertilizing, by years (cm).

Variants	2020			2021			2022		
	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %
Control	45.03	5.31	11.80	94.88	6.84	7.21	67.18	8.56	12.74
Nano Sulfur-100 ml/da	46.33	4.63	10.00	92.23	6.42	6.96	66.18	9.38	14.18
Nano Sulfur-200 ml/da	47.30 ⁺	4.65	9.83	97.10	9.11	9.38	67.78	6.75	9.95
Panamin Agro-150 g/da	46.43	4.56	9.83	96.65	6.25	6.47	66.45	8.79	13.23
Panamin Agro-250 g/da	48.13 ⁺	5.15	10.70	98.13 ⁺	6.44	6.56	71.23 ⁺	7.12	9.99
LSD 0.05	2.15			3.13			3.61		

Fresh and dry matter yield of perennial ryegrass after foliar feeding

On average for the period with the highest yield of fresh matter are the grass stands of *Lolium perenne* L. treated with Panamin Agro - 250 g/da (2020.80 kg/da) – Table 3.

The values of the indicator exceeded the control by 16.87% ($P < 0.05$). In the variants with Nano Sulfur, a slight advantage (with 112.5-166.6 kg/da) was observed in the amount of fresh matter compared to the control (1729.20 kg/da), and in the grass stands treated with Panamin Agro (150 g/da) the values were lower compared to untreated.

For the study period, no proven difference in the amount of dry matter was found between the variants with fertilizers and the untreated control of *Lolium perenne* L.

In the first year (2020), the highest yield of fresh (1800.00 kg/da) and dry matter (608.04 kg/da) was registered in the grass stands

treated with Nano Sulfur (100 ml/da) followed by the variants with Panamin Agro-250 g/da (1700.00 – fresh matter and 565.42 – dry matter). The excess of indicators compared to the control is by 11.63% (fresh matter) and 7.58% (dry matter) in the variants with Nano Sulfur (100 ml/da) and by 5.43% (fresh matter) and 0.04% (dry matter) in the variants with Panamin Agro (250 g/da).

For the second year (2021), the applied fertilizing again did not significantly affect the productivity of *Lolium perenne* L. The yield of fresh and dry matter in the foliar feeding with Nano Sulfur - 100 and 200 ml/da was higher compared to the control by 50 to 100 kg/da, respectively – for the fresh matter and with 9.87 to 10.32 kg/da – for the dry matter. Grass stands of *Lolium perenne* L. with imported Panamin Agro have a lower productivity of grass matter compared to the unfertilized control.

Table 3. Yield of fresh and dry matter (kg/da) of *Lolium perenne* L. after foliar fertilizing, by year and average for the period 2020-2022.

Variants	2020		2021		2022		2020-2022	
	kg/da	%	kg/da	%	kg/da	%	kg/da	%
Fresh matter								
Control	1612.50	100.00	2350.00	100.00	1225.00	100.00	1729.20	100.00
Nano Sulfur-100 ml/da	1800.00	111.63	2450.00	104.26	1275.00	104.08	1841.70	106.51
Nano Sulfur-200 ml/da	1512.50	93.80	2400.00	102.13	1775.00	144.90	1895.80	109.64
Panamin Agro-150 g/da	1550.00	96.12	2275.00	96.81	1312.50	107.14	1712.50	99.04
Panamin Agro-250 g/da	1700.00	105.43	2275.00	96.81	2087.50	170.41	2020.80	116.87
LSD_{0.05}	466.55	28.93	334.78	14.25	332.33	27.13	268.93	15.48
LSD_{0.01}	654.88	40.61	469.92	20.00	466.48	38.08	377.49	21.73
LSD_{0.001}	924.54	57.34	663.42	28.23	658.56	53.76	532.93	30.67
Dry matter								
Control	565.18	100.00	756.00	100.00	485.10	100.00	602.50	100.00
Nano Sulfur-100 ml/da	608.04	107.58	765.87	101.31	491.26	101.27	621.25	103.11
Nano sulfur-200 ml/da	549.04	97.14	766.32	101.37	600.31	123.75	638.33	105.95
Panamin Agro-150 g/da	502.20	88.86	737.10	97.50	455.18	93.83	564.58	93.71
Panamin Agro-250 g/da	565.42	100.04	763.26	100.96	685.95	141.40	670.83	111.34
LSD_{0.05}	155.84	27.52	109.70	14.51	112.86	23.27	88.47	14.68
LSD_{0.01}	218.75	38.63	153.98	20.36	158.42	32.66	124.18	20.61
LSD_{0.001}	308.82	54.54	217.38	28.74	223.65	46.11	175.31	29.10

In the third year (2022), the effect of the foliar feeding affects to a higher degree the amount of grass stands formed by *Lolium perenne* L. The grass matter in the variants with Nano Sulfur (200 ml/da) and Panamin Agro (250 g/da) is significantly higher values regarding the productivity of fresh and dry matter. The excess in the values of the indicators is respectively by 44.90% and 23.75% (for the variants with Nano Sulfur - 200 ml/da) and by 70.41% and 41.40% (for the variants with Panamin Agro - 250 g/da).

Treatment with a lower dose of mineral, inorganic fertilizer (100 ml/da) increased the yield of fresh and dry matter by 4.08 and 1.27%, respectively, compared to the control.

The grass stands of *Lolium perenne* L. treated with Panamin Agro (150 g/da) also have a minimal difference in productivity compared to the control. The yield of fresh matter in the variant exceeds the control by 7.14%, and the yield of dry matter is with 6.17% less relative to the control which is 485.10 kg/da.

Botanical composition of pure crops with perennial ryegrass after foliar feeding

The botanical composition of the grass stand determines the quality and nutritional value of the fodder.

In the year of sowing (Fig. 2), the effect of the inorganic mineral fertilizer influenced to the highest extent the botanical composition of the grass stands. The percentage of *Lolium perenne* L. in the variants with Nano Sulfur varied from 72.09% (100 ml/da) to 74.07% (200 ml/da), and the excess compared to the control was 2.09 and 4.07%, respectively. In the variants with bio-fertilization, the share of *Lolium perenne* L. in the volume of the forage matter was from 63.89% (150 g/da) to 71.43% (200 g/da). A minimum excess of 1.43% compared to the control was observed in the variants with a higher dose of Panamin Agro. The values regarding the degree of weed infestation in the crops with applied mineral and organic fertilizing varied from 25.93% (Nano Sulfur - 200 ml/da) to 36.11% (Panamin Agro - 150 g/da).

In the second year (Fig. 3) *Lolium perenne* L. has a maximum share in the grass stand. Panamin Agro formulations (150 g/da) ensured the highest (98.77%) participation of *Lolium perenne* L. in the grass mass followed by the variants with added Nano Sulfur - 200 ml/da (98.04%) and 100 ml/da (97.56%) and Panamin Agro - 200 g/da (97.30%). The values of the indicator exceed the untreated control by 16.33%, 15.60%, 15.12% and 14.86%. A significant difference was found in the weed infestation degree in the variants treated with mineral and biofertilizers. The values of the studied trait vary from 1.23% (Panamin Agro - 150 g/da) to 17.64% (control).

In the year with the highest temperature and the lowest amount of precipitation (third experimental year), the proportion of weed vegetation in the treated variants varied from 2.30% (Nano Sulfur - 200 ml/da) to 12.60% (untreated control) - Fig. 4. *Lolium perenne* L. responded to the effect of the fertilizers and registered the highest share in the grass stands with foliar feeding by Nano Sulfur - 200 ml/da (97.70%), followed by the variants with Panamin Agro - 250 g/da (97.40%); 150 g/da (95.90%) and Nano Sulfur - 100 ml/da (94.90%).

Lolium perenne L., which shows a high sensitivity to soil and air humidity, had a relatively high share in the formed grass stands, which suggests that the fertilizing improves the resistance of the plants to the stress of prolonged drought in the experimental area and helps for optimal growth and development of the crop.

In the third experimental years, *Lolium perenne* L. plants occupied the highest share in the above-ground matter in the variants treated with higher doses of Nano Sulfur (200 ml/da) and Panamin Agro (250 g/da). The percentage share of grasses is respectively 89.94% and 88.71% (in the case of the control - 79.95%). The specified variants are also with the lowest degree of weed infestation. The mineral treatment with a rate of 100 ml/da ensured an 88.18% share of *Lolium perenne* L. in the composition of the grass matter, whereas the biofertilization with Panamin Agro (150 g/da) took 86.18%.

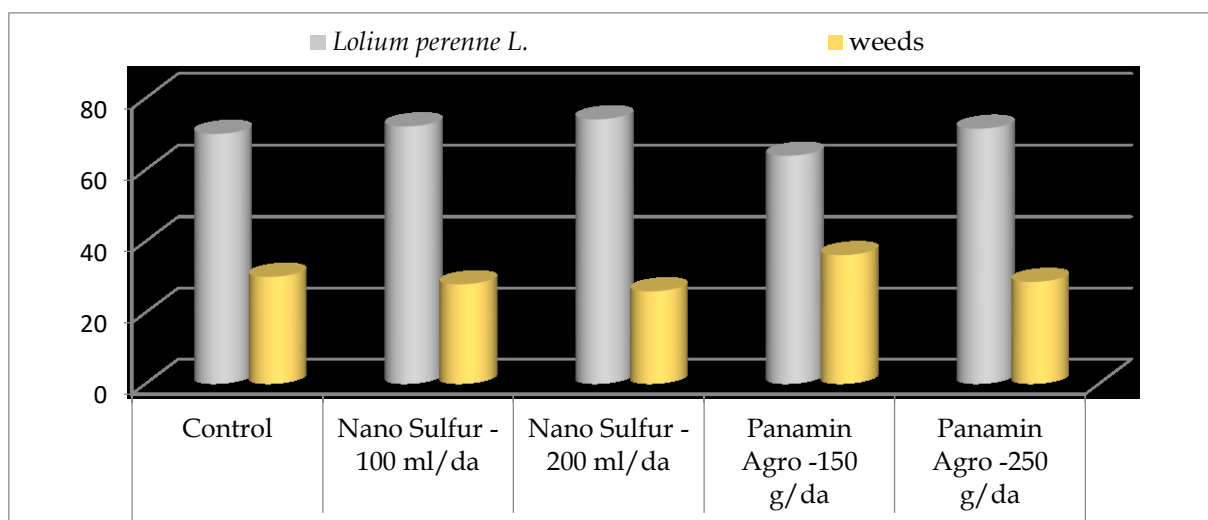


Fig. 2. Botanical composition (%) of grass stands of *Lolium perenne L.* after foliar feeding (first year).

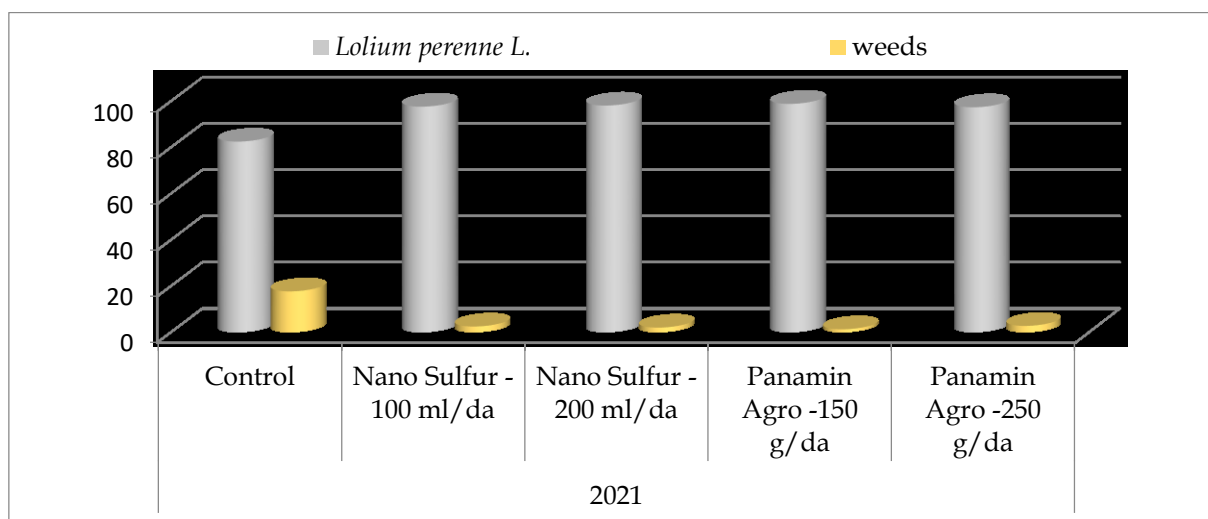


Fig. 3. Botanical composition (%) of grass stands of *Lolium perenne L.* after foliar feeding (second year).

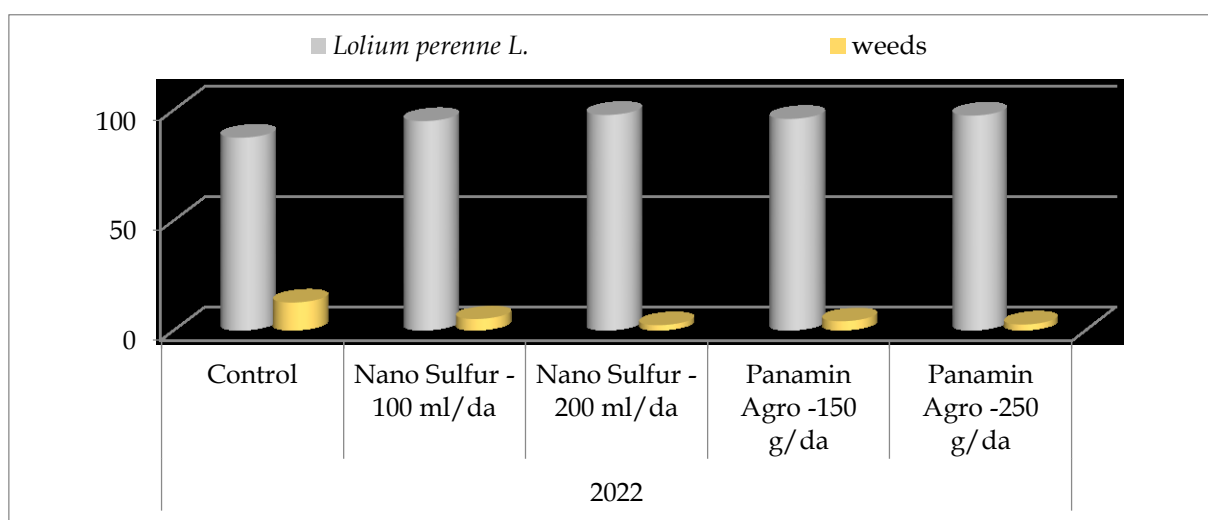


Fig. 4. Botanical composition (%) of grass stands of *Lolium perenne L.* after foliar feeding (third year).

Conclusions

For the experimental period, the plants of *Lolium perenne* L. marked the greatest height of the aboveground matter in the variants fertilized with Panamin Agro (250 g/da). Grass stands were found to be ($P < 0.05$) taller by 4.05 cm compared to the control (67.18 cm).

The highest yield of fresh matter was registered by the grass stands of *Lolium perenne* L. treated with Panamin Agro - 250 g/da, where the values of the indicator exceed the unfertilized control by 16.87% ($P < 0.05$). In the variants with Nano Sulfur, a slight advantage (by 112.5-166.6 kg/da) was observed in the amount of fresh matter compared to the control (1729.20 kg/da). For the study period, no proven difference in the amount of dry matter was found between the variants with applied fertilizers and the untreated control of *Lolium perenne* L.

Lolium perenne L. occupied the highest relative share in the variants treated with Nano Sulfur (200 ml/da) and Panamin Agro (250 g/da). The percentage share of this grass species in the grass stand is respectively 89.94% and 88.71% (in the control - 79.95%), and weed infestation is the lowest.

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