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Botanical and morphological composition of artificial grassland of bird's–foot–trefoil (*Lotus Corniculatus* L.) treated with lumbrical and lumbrex

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Abstract. In the period of 2014–2016 at the Research Institute of Mountain Stockbreeding and Agriculture–Troyan, Lumbrical and Lumbrex bioproducts were tested in a field experiment on a grassland of bird's–foot–trefoil with 'Leo' cultivar. Organic fertilizers are high in humic and fulvo acids, macro and micro elements. They are a result of the processing of organic waste through the red earthworm (*Lumbricus rubellis*) and the application of modern biotechnology. The results show that the independent introduction of the studied preparations has a positive influence on the density of *Lotus corniculatus* L. in the grassland. The main crop in the harvested biomass prevails in the soil and leaf feeding variants with 3.9 and 1.2–2.7 percentage units, respectively. The weed vegetation is reduced more significantly mainly in Lumbrical variants, regardless of the fertilization level. The liquid fraction of Lumbrex, introduced in the bud–formation period – beginning of blossoming at a dose of 150 and 200 mL/da, had a slighter influence on the participation of bird's–foot–trefoil (89.8–91.4 %) in the total biomass as well as the level of weed infestation, while it increased more significantly the amount of leaf mass. The percentage share of variants with foliar treatment exceeded the control with 7.0 (Lumbrex 150 mL/da) to 10.4 (Lumbrex 200 mL/da), and those with soil nutrition with 1.5 (Lumbrical 150 mL/m^2) and 4.3 (Lumbrical 200 mL/m^2) percentage units.
Keyword: *Lotus corniculatus* L., Lumbrical, Lumbrex, bio–fertilization.

Introduction

The bird's–foot–trefoil is a fodder crop with a high productive potential and a good adaptability to environmental conditions [CHURKOVA, 2007, BERNES *et al*, 2000; PALLARES *et al.*, 2000; NAYDENOVA *et al.*, 2015].

The introduction of natural fertilizers improves the metabolism of the plant organism [VLAHOVA *et al.*, 2013], providing adequate moisture and temperature necessary to stimulate the activity of soil microorganisms.

Biofertilizers are a component that balances and stimulates the mineral nutrition of plants [PACHEV *et al.*, 2012, CAUNII *et al.*, 2015; IANCULOV *et al.*, 2004], improves the productivity and quality of fodder crops [SULEIMENOVA *et al.*, 2012; NAYDENOVA *et al.*, 2014], balances and increases the content of organic soil substance, creates ecological and economically sustainable biosystems [ANSARI and ISMAIL, 2001] in feed production.

Products of red earth worm (*Lumbricus rubellus*) are the subject of intensive research and foundations in the development of organic farming in Bulgaria. Cultivation and application of lumbriculture is a highly effective agro–technical event for the production of ecologically pure biomass, rich in protein and with better absorption and digestibility by ruminants.

Lumbrical stimulates plant production parameters [POPOV, 2014, BUTNARIU *et al.*, 2012, STOLERU *et al.*, 2012] and the liquid humatic form improves the biometric indices of individuals in plant grassland [GRAMATIKOV and KOTEVA, 2006] by increasing the germination, germinating energy and the absolute weight of the spring vetch seeds [VASILEVA and KERTIKOV, 2007].

According to Camberato and collab. an effective way to overcome macro and micro–elements deficiency in the soybean vegetation period is the use of leaf fertilizers that act simultaneously as a source of nutrients and a growth stimulant for plants [CAMBERATO et al., 2010].

The use of this type of bio–fertilizers in practice has not yet been supported by sufficient research in the field of fodder

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2018, IX(19)

production and grasslands. The purpose of the present study is to analyze the effects at different levels of fertilization with Lumbrical and Lumbrex on the botanical and morphological composition of the artificial grassland with bird's–foot–trefoil (*Lotus corniculatus* L.).

Material and methods

The survey was carried out at the Research Institute of Mountain Stockbreeding and Agriculture–Troyan in the period of 2014–2016. Lumbrical and Lumbrex biofertilizers were tested on a grassland of bird's–foot–trefoil, 'Leo' cultivar under non–irrigated conditions.

Organic products of Lumbrical and Lumbrex are ecologically pure products with high protein content [MARKOV, 2015, VARDANIAN *et al.*, 2018; STOLERU *et al.*, 2018], resulting from the processing of organic waste, through the red earth worm (*Lumbricus rubellis*) and the application of modern biotechnology.

They contain an organic substance of 45–50 %; humic acids up to 14 %; fulvoacids–7 %; ammonium nitrogen (NH_4–N)–33.0 ppm; nitric nitrogen (NO_3–N)–30.5 ppm; P_2O_5–1410 ppm; K_2O–1910 ppm; a useful microflora of 2 x 10^{12} pg / g and a large number of NFE.

The acidity of the product is 6.5–7.0 (pH in H_2O). The commercial products are a product of the organic farm–the village of Kostievo (Plovdiv, Bulgaria), intended for soil and foliar feeding of plants in organic farming, according to EU Regulation 889/2008.

Experimental variants are:1.1. Control /nontreated/; 2. Lumbrical–150 mL/m^2 (1 mL=0.58 g Lumbrical); 3. Lumbrical–200 mL/m^2 (1 mL=0.58 g Lumbrical); 4. Lumbrex–150 mL/da; 5. Lumbrex–200 mL/da. Prior to sowing, the necessary soil cultivation was applied to create artificial grassland.

The sowing of the independent crop was carried out in the second half of March, manually scattered by the blocking method in 4 replicates, with a plot size of 5 m^2 and a seed rate of 1.2 kg/da at 100 % purity and germination.

After sowing, the areas were rolled. During vegetation, Lumbrical fertilizer was introduced as a soil fertilizer (after mowing, the dark brown granular fraction is evenly dispersed and mixed with the top soil layer, then watered abundantly).

Spraying with Lumbrex was carried out in the bud–formation stage–beginning of blossoming.

The conventional technology for growing of grass meadows for fodder was applied. The following indicators are observed:

- Botanical composition of grassland (%)–determined by weight analysis of grass green mass samples taken at each mowing of each variation. Their weighing is carried out in an air–dry state, by weighing the percentage of sown grass species and motley grasses (in total).
- Morphological composition of grassland (%)–carried out in % by weight of 40 plants taken from each variant and each repetition of grass harvesting (experiments 1 and 2), by determining the quantity of stems, leaves and generative organs by the weighing method and it is based on their percentage of participation.

A variance analysis (ANOVA) was used for statistical data processing.

Results and discussion

Agrochemical soil analysis. The soils in the area of experiment are light gray, pseudopodzolic.

The humus content is about 1–1.5 %, with predominant fulvo acids, mostly concentrated in the fallow land.

The amount of total nitrogen is very low and does not exceed 0.10–0.12 %.

The reaction is strong and very acidic. According to Kachynski classification [KACHYNSKI, 1958, BONEA *et al.*, 2017, PENTEA et al., 2016, STOLERU *et al.*, 2012], the soils in the biological field where the experiment was derived are low in humus (0.96–1.44 %)–Table 1.

Soil stockpile in total and digestible phosphorus (1.2–2, 4 mg/100 g of soil) is very low, and slight for digestible potassium (5.9–9.9 mg / 100 g soil) and

poorly stockpiled with mobile nitrogen forms (8.6–20.2 mg/100 g soil).

The soil reaction is slightly acidic (pH in water of 5.2–5.3 and 4.3–4.4 in KCl). It is clear from the characterization that pseudopodzolic soils are poor and unfavorable for the cultivation of agricultural crops. Penkov and collab. found them suitable for growing forage grasses because they provide high crop productivity. The application of appropriate agrotechnical measures can overcome the negative properties of this type of soil and improve their composition and structure [PENKOV et al., 1992, BUTNARIU and SAMFIRA, 2012; IANCULOV *et al.*, 2005, STOLERU *et al.*, 2018].

Table 1.

Agrochemical analysis of light grey pseudo–podzolic soils (0–40 cm)

Soil layer	pH		Σ N [NH_4+NO_3]	P_2O_5	K_2O	Humus
	H_2O	KCl	mg/kg	mg/100		%
0–20	5.3	4.4	20.2	2.4	9.9	1.44
20–40	5.2	4.3	8.6	1.2	5.9	0.96

Botanical composition of grassland with bird's–foot–trefoil treated with Lumbrical and Lumbrex. The independent application of the biofertilizers included in the experiment, such as vegetative soil and leaf treatment, has a positive effect on the density of the crop and the degree of weed infestation in the bird's–foot–trefoil.

The granulating fraction of vermi–compost restrict the density and improves the water and air mode of the soil [SINGH *et al.*, 2004; EL–MAGD *et al.*, 2008, SAMFIRA *et al.*, 2014; BUTNARIU; 2012; BUTU *et al.*, 2014a, STOLERU *et al.*, 2016], as a result of which in the year of sowing (Figure 1) when legumes are most sensitive to weed infestation, the percentage share of the main crop in soil feeding variants exceeded the control with the minimum 0.2–0.4 percentage points.

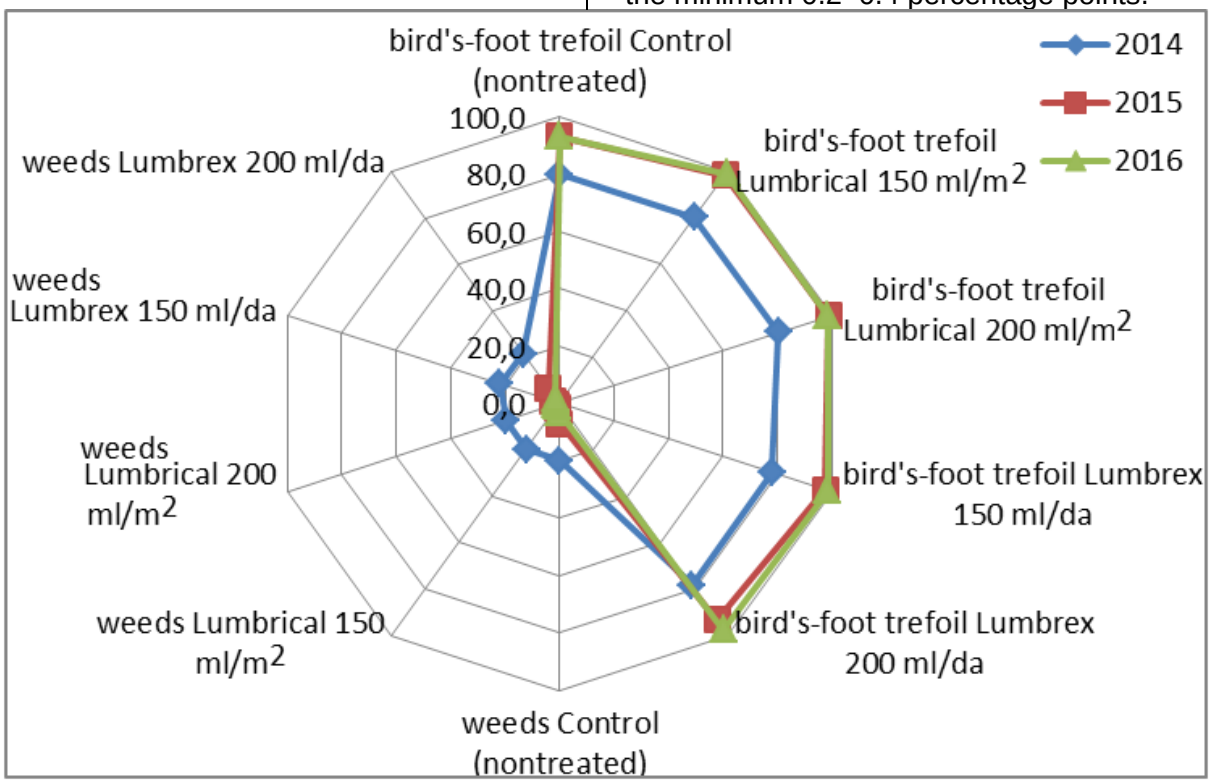

Figure 1. Botanical composition of grassland of bird's–foot–trefoil, treated with biofertilizers Lumbrical and Lumbrex during the years (%)

In the second (Lumbrical 200 mL/m^2) and third (Lumbrical 150 mL/m^2) vegetation, the rapid growth rate and development of bird's–foot–trefoil combined with additional soil nutrition increased the relative share of the legume

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Banat's Journal of Biotechnology
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fodder crop in the treated variants by 5.8–6.1 % relative to the control. In the second experimental year, the action of the reduced dose of granulated fraction (150 mL/m^2) affected less the share of the bird's–foot–trefoil, which corresponds to the higher presence of weed vegetation (1.6 %) in the treated variants.

In 2016 in the feed biomass enriched with Lumbrical 150 mL/m^2 we recorded maximum values (99.1 %) with respect to the participation of the main crop in the grassland and respectively the lowest weed infestation (<1 %) compared to the other variants in the experiment.

The high relative share of bird's–foot–trefoil in the second and third experimental years is the result of the specific biological features (fast growth rate, high growth energy, *etc*.) of the forage grass and the complementary effect of the preparations included in the experiment related to the overall mineral nutrition system in plants [PACHEV *et al.*, 2012, DIMITRIU *et al.*, 2016; GEORGIEVA *et al.*, 2018; BUTNARIU and CAUNII, 2013]. The biofertilizer helps soil aeration, retains moisture and allows excellent drainage for optimal root system development.

During the first year of the growth cycle, the proportion of legume crop in the cut forage mass was not affected in the variants with the independent **foliar treatment** by Lumbrex 150 and 200 mL/da. In the second and third vegetation, *Lotus corniculatus* L. retained a higher presence (97.7–98.4 %) in grasslands with a lower dose of introduced bioregulator than the control. The excess of the amount of bird's–foot–trefoil in these variants is 4.7 and 5.4 compared to the non–treated biomass [BAGIU *et al., 2012*; BUTNARIU and CORADINI, 2012].

From the above data, it was found that in the grasslands with soil and foliage treatment, the density of *Lotus corniculatus* L. was higher than the control by 3.9 and 1.2–2.7 percentage points. The quantity of weed vegetation is more strongly reduced (by 2.7 percentage points) in the variants with soil application of Lumbrical compared to the control variants. The liquid fraction of Lumbrex (150 and 200 mL/da) had a lower incidence of the main crop (89.8–91.4 %) in the total biomass and the extent of weed infestation in the treated variants.

Morphological composition of grassland with bird's–foot–trefoil treated with biofertilizers. The effect of Lumbrical and Lumbrex in the experiment is characterized by an increase in the quantitative parameters in the above–ground matter in bird's–foot–trefoil.

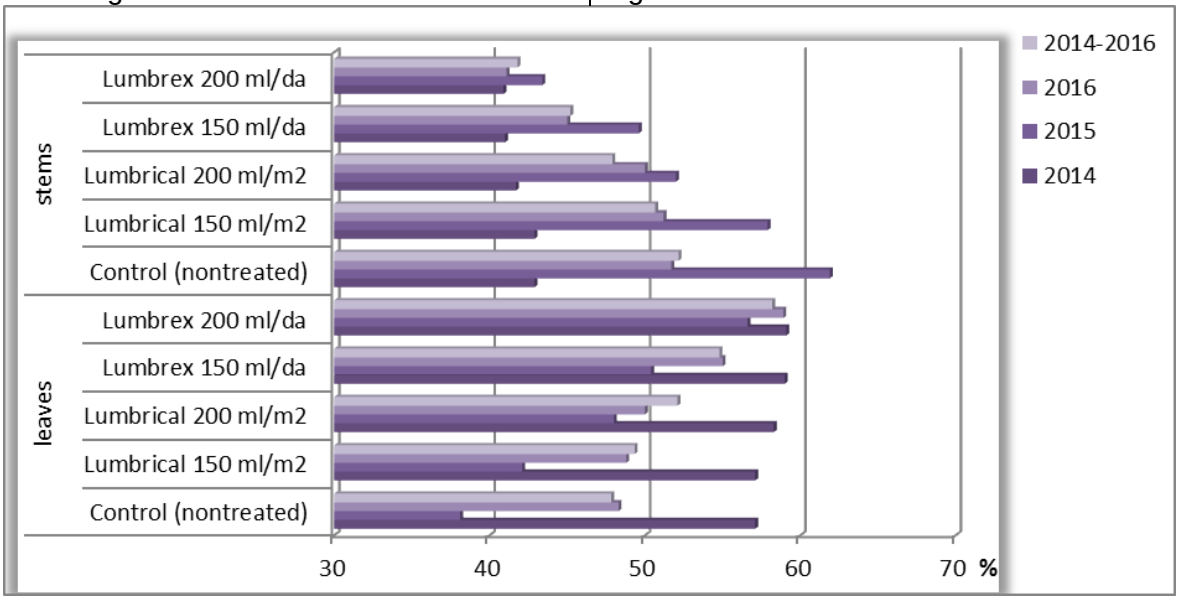

Figure 2. Morphological composition of bird's–foot–trefoil treated with Lumbrical and Lumbrex during the years and average for the period 2014–2016 (%)

The results indicate a significant increase in the relative share of the leaves (the basic element of the yield) in the harvested grassland of 1.5 (Lumbrical

150 mL/ m^2) to 10,4% (Lumbrex 200 mL/da)–Figure 2.

In the year of sowing, the data on the morphological composition of bird's–foot–trefoil indicates a maximum (59.0 %) amount of leaves in the grassland in variants of foliar treatment with Lumbrex (150 and 200 mL/da). Indicator values exceed the control by 3.5 %. Unlike the manure, in the first vegetation the solid fraction of Lumbrical stimulates predominantly the stem vegetative mass.

In the second and third experimental years, the positive difference in the relative share of leaves in the biomass of treated variants versus is from 4.0 to 18.5 % in 2015 and from 0.5 to 10.6 % in 2016 compared to the control. Over the same period, studies have shown that there is a decreasing trend in the amount of stems [PETRACHE *et al.,* 2014, BUTNARIU *et al.,* 2014, BARBAT 2013, BUTU *et al.,* 2015]. The amount of stem fraction in variants with applied bio–fertilization has lower values than those of the nontreated control. In the grasslands with soil fertilization (irrespective of the level of fertilization), the percentage share of the stems in the harvested fodder is higher than the variants with the leaf treatment by the bio–fertilizer [PUTNOKY *et al.,* 2013, BUTNARIU *et al.,* 2014; BUTNARIU and GIUCHICI, 2011]. In a second vegetation these differences, due to the way of introducing the same dose of vermi–compost, are 8.3 and 8.6 percentage points in favour of the soil application and in the third vegetation–respectively 6.2 and 8.9 percentage points [BUTU *et al.,* 2014c. SAMFIRA *et al.,* 2015, BUTNARIU *et al.,* 2015b; BUTU *et al.,* 2014b].

The nutritional impact of Lumbrical and Lumbrex preparations in the experiment, containing the basic micro and macro–elements in certain concentrations as well as the meliorative effect of the microorganisms on the soil influence the formation of the above–ground biomass in bird's–foot–trefoil.

Meanwhile, these characteristics show an increase in the quantitative parameters of *Lotus corniculatus* L., which affected both the yield value and the quality of the fodder crop. In other studies, with the same biofertilizers [ZLATEV and POPOV, 2013], an increase in the content of photosynthetic pigments and the functional activity of photosynthesis was reported as results.

Such an effect of biofertilizers can also be related to that observed in the present experiment [BONCIU *et al.,* 2018; BONEA *et al.,* 2018; GROZEA et al., 2017, STOLERU *et al.,* 2016].

The analyzed data confirms the stimulating effect of the bioproducts applied in the experiment, on the foliar share and their lesser impact on the quantity of stems in the grassland.

Leaves, as a major component in the feed composition, are a guarantee of higher feed quality and dry matter digestibility. The data from the analysis correspond to the findings of some authors [KOVAČEVIĆ, 2003; SILBERBUSH, 2002], namely: foliar and soil bio–fertilization increases leaf volume and corrects the access of nutrients to the root system of plants.

Conclusions

In the study period, the grasslands treated with Lumbrical and Lumbrex biofertilizers influence positively the density of *Lotus corniculatus* L. The contribution of the main crop in the harvested biomass in the soil and foliar feeding variants is higher by 3.9 and 1.2–2.7 percent units compared to the control. The composition of the granulated fraction of Lumbrical suppressed the growth of weed vegetation to a higher degree (by 2.7 percent) compared to the control.

The liquid fraction of Lumbrex, introduced at doses of 150 and 200 mL/da, had a slighter influence on bird's–foot–trefoil (89.8–91.4%) in the total biomass, respectively the level of weed infestation, but it increased the amount of foliage more significantly. Percentage share of leaves in the variants exceeded the control with 7.0 (Lumbrex 150 mL/da) to 10.4 (Lumbrex 200 mL/da) percentage units, and those with soil nutrition with 1.5 (Lumbrical 150 mL/m^2) and 4.3 (Lumbrical 200 mL/m^2) percentage units.

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