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ORIGINAL PAPER

INFLUENCE OF EFFECTIVE MICROORGANISMS ON THE DRY MATTER YIELD AND CHEMICAL COMPOSITION OF MEADOW VEGETATION*

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Abstract

Reports on the use of effective microorganisms in plant fertilization are ambiguous, as some authors claim it is beneficial to yield and plant quality and other studies do not confirm these findings. However, only few of the numerous studies investigating this topic both in Poland and abroad have focused on the fertilization of meadows. The aim of the study was to assess the influence of effective microorganisms on production capacity of meadow plants, their content of micro- and macronutrients and the level of absorbable forms of micronutrients in the soil. The study was carried out in 2014-2016, on a farm located in Małopolska, in the Kraków district. A univariate field experiment in a randomized block design with four replicates was established on a permanent meadow, with experimental plots of 10 m^2. The soil under the experimental meadow was Haplic Phaeozem developed from loess, which belonged to class I in the Polish soil classification system. The experimental factor comprised a spray application of the microbiological formulation ProBios fertilizer, tested at two doses: 20.0 and 40.0 dm^3 ha^{-1}. Foliar fertilization with the higher dose of effective microorganisms (40.0 dm^3 ha^{-1}) significantly ($p \leq 0.05$) increased dry weight yield, as compared with the control, and the biggest difference of 1.90 t ha^{-1} was observed in the first year of the study. Moreover, plant consumption of nutrients such as phosphorus, magnesium and calcium, but except sodium, was higher. The higher dose of effective microorganisms also improved the plant's uptake of zinc, copper, manganese and iron. The application of microorganisms contained in the preparation enhanced the content of absorbable phosphorus and potassium in the soil. In conclusion, the addition of effective microorganisms considerably improved plant development (aerial mass), which resulted in improved yields of meadow plants and more effective use of nutrients.

Keywords: effective microorganisms, meadow plants, dry matter yield, micronutrients, macronutrients.

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INTRODUCTION

Plant growth and development depend on multiple factors, including microorganisms, which are components of microbiological preparations. Their application facilitates restoration of the microbial balance, which may have been disturbed by environmental pressure in ecosystems used for feed production purposes. It favours the formation of beneficial probiotic communities in the environment that can ensure a proper growth and development of plants and inhibit adverse activities of pathogenic microorganisms by their effective isolation (WATERS, BASSLER 2005, BASSLER 2006, BASSLER, LOSICK 2006). The impact of probiotic bacteria is extremely versatile, as they strengthen natural plant resistance to pests and fungal diseases, accelerate decomposition of organic matter and humus formation, control air-water balance, eliminate decay processes, limit the presence of pathogens and pests, improve availability of some poorly available micro- and macronutrients to plants, contribute to considerable growth of microflora that is beneficial for biological activity of the soil, increase the number of microorganisms in the rhizosphere and optimize carbon to nitrogen ratio (IWAISHI 2001, XU 2001).

Formulations based on the Effective Microorganisms (EM) technology comprise a mixture of about 80 positive organisms, such as photosynthetic bacteria, lactic acid fermentation bacteria, actinomycetes, yeasts, fungi and anaerobic microbes. These are compositions of microorganisms carefully selected for their physiological properties that support plant development on poor and degraded soils (HIGA, PARR 1994, BADURA 2004, JAVAID 2006, KHALIQ et al. 2006, SCHULZ et al. 2013). Agricultural sciences and practice are often challenged with poor soil quality and effective measures for restoring biological activity of the soil are constantly sought for. One of them may be using microbial formulations that improve soil properties (FRĄSZCZAK et al. 2012). Opportunities for applying these formulations in agricultural practice are still not fully recognized. Their supporters advocate beneficial effects of microbial formulations on plant and soil condition manifested by improved quantity and quality of yield, while the opponents indicate low reliability of available research that are mostly short-term and local (SHAH et al. 2001, PISKIER 2006, VLIET et al. 2006, BOLIGŁOWA, GLEŃ 2008).

The aim of this study was to determine the effects of a microbiological soil conditioner on productivity and mineral composition of meadow vegetation and the presence of available forms of macronutrients in the soil.

MATERIAL AND METHODS

Study site and soil analysis

The study was conducted at an individual farm located in the Province of Małopolska, the Kraków district. A univariate field experiment in a randomized block design with four replicates was established on a permanent meadow, with experimental plots of 10 m^2. The soil under the experimental meadow was Haplic Phaeozem developed from loess, classified as class I in the Polish soil classification system. It was moderately rich in absorbable forms of phosphorus (55.2 mg P kg^{-1} soil), potassium (134.3 mg K kg^{-1} soil) and magnesium (80.6 mg kg^{-1} soil).

Prior to the experiment, dominant grass species were Perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*), false oat-grass (*Arrhenatherum elatius*), orchard grass (*Dactylis glomerata*), Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*) and timothy grass (*Phleum pratense*) – Table 1. The share of *Fabaceae* plants was 8% and they were represented by red clover (*Trifolium pratense*), white clover (*Trifolium repens* L.), and bush vetch (*Vicia sepium* L.). The experimental meadows also included 11 species of dicotyledonous plants, which accounted for 15% of its flora (Table 2).

Weather conditions

Annual rainfall in the study period (2014 - 2016) amounted to 684.7, 555.7 and 626.7 mm, respectively (Table 2). Mean rainfall during the plant growing period (April–September) was 508.0 mm in 2014, 317.7 mm in 2015, and 321.6 mm in 2016. Mean annual temperature in the years 2014 - 2016 was 6.1, 6.4 and 6.9°C, respectively, and between April and Septmeber it was 10.9, 12.1 and 12.1°C.

Materials and experimental design

The study included three variants: control (no treatments) and experimental spraying with ProBios® preparation at two doses: 20.0 and 40.0 dm^3 ha^{-1}. The first dose of 20.0 dm^3 ha^{-1} was applied at the beginning of spring plant growth, and the plots treated with the higher dose (40.0 dm^3 ha^{-1}) were divided into two parts and sprayed at the beginning of plant growth (20.0 dm^3 ha^{-1}) and after the first harvest (20.0 dm^3 ha^{-1}). The solution used for spraying was prepared by dissolving an appropriate amount of the microbial preparation in such amount of water so as to obtain 300 $dm^3 \cdot ha^{-1}$ of the working solution. ProBios contains live lactic acid bacteria, yeast, actinomycetes and phytothropic organisms enriched with extracts from 20 herb species. It is manufactured by PHU EKO-AGROTECH in Węgorzewo. According to the Council Regulation (EC No. 834/2007) and Commission Regulation (EC No. 889/2008), the product can be used in organic farming without special permits.

Table 1

Floristic composition of the experimental meadow prior to the experiment

Grasses	**77%**
Perennial ryegrass (*Lolium perenne* L.)	18%
Meadow fescue (*Festuca pratensis Huds.*)	17%
False oat-grass (*Arrhenatherum elatius* L.)	12%
Orchard grass (*Dactylis glomerata* L.)	10%
Kentucky bluegrass (*Poa pratensis* L.)	7%
Red fescue (*Festuca rubra* L.)	6%
Timothy grass (*Phleum pratense* L.)	5%
Yorkshire fog (*Holcus lanatus* L.)	1%
Rough bluegrass (*Poa trivialis* L.)	1%
Fabaceae	**8%**
Red clover (*Trifolium pratense* L.)	4%
White clover (*Trifolium repens* L.)	3%
Bush vetch (*Vicia sepium* L.)	1%
Dicotyledons	**15%**
English plantain (*Plantago lanceolata L.)*	1%
Broadleaf plantain (*Plantago major* L.)	1%
White campion (*Melandrium album* (Mill.))	+
Black knapweed (*Centaurea jacea* L.)	+
Chickweed (*Stellaria media* (L.) Vill.)	+
Red dead-nettle (*Lamium purpureum* L.)	1%
Common yarrow (*Achillea millefolium* L.)	2%
Common dandelion (*Taraxacum officinale* F. H. Wigg.)	2%
Germander speedwell (*Veronica chamaedrys* L.)	+
Hedge bedstraw (*Galium mollugo* L.)	+
Common knotgrass (*Polygonum aviculare* L.)	+
Common sorrel (*Rumex acetosa* L.)	1%
Shepherd's purse (*Capsella bursa pastoris*)	2%

During the study, basic mineral fertilization was applied in the form of 80 kg N ha^{-1} for the first crop and 60 kg N ha^{-1} as 34% N ammonium nitrate for the second crop. Phosphorus was applied once in the spring at 34.9 kg P ha^{-1} in the form of enriched superphosphate 17.4% P, and potassium was applied for the first and second cut at 49.8 kg K ha^{-1} as 49.8% K potassium salt.

The collected plant material was analyzed for its chemical composition and dry matter content (after oven-drying at 105°C). Following dry minerali-

Table 2

Rainfall and average air temperature at the Plant Breeding Station in Polanowice in the years 2014 - 2016.

Month/Year	2014	2015	2016
	monthly rainfall (mm)		
January	21.0	42.5	23.0
February	23.2	38.0	94.0
March	33.5	55.0	42.5
April	39.5	11.0	67.0
May	76.0	89.0	38.3
June	102.0	34.0	35.8
July	163.0	93.0	80.6
August	117.0	43.0	82.0
September	10.5	47.7	18.0
October	51.0	56.0	99.1
November	26.0	40.5	33.5
December	22.0	6.0	13.0
Total	684.7	555.7	626.7
Total April-September	508.0	317.7	321.6
	Average monthly air temperature (°C)		
January	-2.8	-2.0	-1.3
February	-1.55	-1.9	3.0
March	2.57	0.8	2.0
April	5.1	4.7	5.3
May	10.3	10.3	10.9
June	13.65	14.2	15.5
July	9.6	16.9	17.0
August	16.7	16.1	14.0
September	10.1	10.6	10.1
October	7.27	4.5	5.5
November	2.88	0.8	1.3
December	-0.2	2.4	-0.6
Average	6.1	6.4	6.9
Average April-September	10.9	12.1	12.1

zation, material from each replication was used to determine the content of P, K, Ca, Mg, Na, Zn, Cu, Mn and Fe by inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma torch.

Statistical analysis

The significance of differences between the average yields of dry weight and content of individual elements was determined by the Duncan's test with Statistica 10 PL software (StatSoft, Inc., 2011). The analysis of variance and Duncan's test were performed at a significance level α = 0.05.

RESULTS

Three years of fertilization with the microbial formulation usually significantly increased soil levels of absorbable phosphorus as compared with the control plots not treated with the formulation (Table 3). The content of phosphorus differed in individual years and depended on the formulation dose. Mean levels of absorbable soil phosphorus rose over the three years by 12.7% for the double application and by 6.3% for the single application, as compared with the control plots. Fertilization with the formulation also improved the content of absorbable potassium in the soil (Table 3). The mean content of potassium on the plots treated with the higher dose of the formulation was significantly ($p \leq 0.05$) higher than in the control (by 10.5%). No differences in the soil pH and magnesium content were observed over the study period (Table 3).

Table 3

The content of available phosphorus, potassium and magnesium (mg 100 g^{-1} soil), and $pH_{(KCl)}$ in the soil (0-20 cm) at the end of the experiment

Microbial preparation dose (dm^3 ha^{-1})	Content (mg 100 g^{-1} soil)			$pH_{(KCl)}$
	P	K	Mg	
Control	5.7*b*	14.2*b*	9.0*ab*	6.0*a*
20	6.1*b*	14.7*b*	9.2*ab*	6.2*a*
40	6.8*a*	16.4*a*	10.3*a*	6.3*a*
Mean	6.2	15.1	9.5	6.2
CV(%)	8.5	7.8	7.4	2.5

a, *b* – values in columns marked with different letters differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test, CV – coefficient of variation

The meadow was harvested twice during each season. Crop productivity was highly variable depending on the experimental variant and dry weight yield ranged from 4.14 to 7.58 t ha^{-1}.

The largest dry weight yield was obtained from the plots fertilized with the higher dose of the microbial formulation (variant 3) – Table 4. In the first year of the study, the yield was by 15.5% higher than in the control, in the second year by 13.1%, and in the third by 14.2%. Abundant rainfall

Table 4

The effect of application of effective microorganisms on dry matter yield of meadow plants (t ha^{-1})

Microbial preparation dose (dm^3 ha^{-1})	2014			2015			2016		
	crop		total	crop		total	crop		total
	I	II		I	II		I	II	
Control	6.74*b*	5.52*b*	12.26*b*	5.16*b*	4.14*b*	9.30*b*	5.90*b*	4.60*b*	10.49*b*
20	6.81*ab*	6.38*ab*	13.19*ab*	5.46*ab*	4.48*a*	9.94*ab*	6.56*ab*	4.68*b*	11.24*ab*
40	7.58*a*	6.57*a*	14.16*a*	6.02*a*	4.50*a*	10.52*a*	7.05*a*	4.93*a*	11.98*a*
Mean	7.04	6.16	13.20	5.55	4.38	9.92	6.50	4.74	11.24
CV(%)	6.61	9.13	7.19	7.89	4.61	6.15	8.87	3.73	6.62

a, *b* – values in columns marked with different letters differ significantly ($P \le 0.05$) according to the Duncan's multiple range test, CV – coefficient of variation

occurring in the first year of the study contributed to intense productivity of the meadow, which resulted in a significant increase in the yield on the plots treated with effective microorganisms. Contrary to that, lower rainfall in the second year of the study, particularly in April, resulted in yield reduction for the first crop, which could not be balanced off by the use of effective microorganisms.

The study demonstrated the greatest yield improvement on the plots fertilized with 40.0 dm^{-3} ha^{-1} of the microbial formulation. Total dry matter yield from the first and second crop was by 1.90, 1.22 and 1.49 t ha^{-1} DM greater than in the control for the first, second and third year, respectively.

The study presents weighted averages of the micronutrient content for the entire experimental period (2014 - 2016) – Table 5. The content of individual elements in the meadow plants varied and depending on the variant equalled: 2.83-3.24 g P kg^{-1} DM, 29.21-32.54 g K kg^{-1} DM, 8.12-9.30 g Ca kg^{-1} DM, 3.30-3.65 g Mg kg^{-1} DM, and 0.11 g Na kg^{-1} DM. The greatest

Table 5

The influence of effective microorganisms on the content of macronutrients in the meadow plants (g kg^{-1} DM) – mean content

Element	Microbial preparation dose (dm^3 ha^{-1})			CV(%)
	control	20	40	
P	2.83*b* ± 0.33	3.02*b* ± 0.35	3.24*a* ± 0.42	6.7
K	29.21*b* ± 2.54	30.05*b* ± 2.61	32.54*a* ± 2.83	5.7
Ca	8.12*b* ± 0.72	8.21*b* ± 0.70	9.30*a* ± 0.82	7.7
Mg	3.30*b* ± 0.30	3.35*b* ± 0.28	3.65*a* ± 0.32	5.5
Na	0.11*a* ± 0.02	0.11*a* ± 0.01	0.12*a* ± 0.03	3.3

a, *b* – values in columns marked with different letters differ significantly ($P \le 0.05$) according to the Duncan's multiple range test variability was found for Ca (coefficient of variation CV = 7.7%), and the smallest for Na (CV = 3.3%). The double application of effective microorganisms significantly ($p \leq 0.05$) enhanced the levels of the investigated macronutrients, except for sodium, while the single application caused no significant ($p \leq 0.05$) differences in the plant macronutrient content as compared with the control object. Two doses of effective microorganisms were the most effective in increasing the content of calcium but their effect was the weakest in the case of sodium and magnesium. An optimal content of macronutrients in plants (g kg^{-1} DM) is 3.0 for P, 7.0 for Ca, 2.0 for Mg, 17.0-20.0 for K, 1.5-2.5 for Na (FALKOWSKI et al. 2000). According to this benchmark, the content of potassium, calcium and magnesium was optimal in the meadow plants from all investigated variants but below the reference value for sodium.

Two doses of the effective microorganisms improved the levels of macronutrients (except for sodium) beyond the benchmark value.

Table 6 presents weighted averages of the micronutrient content for the entire experimental period (2014 - 2016).

Table 6

The influence of effective microorganisms on the content of micronutrients in the meadow plants (mg kg^{-1} DM) – mean content

Element	Microbial preparation dose (dm^3 ha^{-1})			CV(%)
	control	20	40	
Zn	78.91*b* ± 4.2	82.51*b* ± 5.3	90.32*a* ± 6.4	6.9
Cu	4.30*b* ± 0.6	4.46*b* ± 0.8	4.76*a* ± 0.9	5.1
Mn	45.24*b* ± 2.7	47.33*b* ± 3.4	50.48*a* ± 4.8	5.5
Fe	98.56*b* ± 6.3	101.40*b* ± 7.8	108.02*a* ± 9.2	4.7

a, *b* – values in columns marked with different letters differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test

Their levels determined in the meadow plants varied and depended on the fertilization variant. They were as follows: 4.30-4.76 mg Cu kg^{-1} DM, 45.2-50.5 mg Mn kg^{-1} DM, 98.6-108.0 mg Fe kg^{-1} DM, and 78.9-90.3 mg Zn kg^{-1} DM. The highest variability was observed for Zn (CV = 6.9%), and the lowest one occurred for Fe (CV = 4.7%). Among all the variants, a significantly ($p \leq 0.05$) higher content of Cu, Zn, Mn and Fe was determined in the plants fertilized with the higher dose of effective microorganisms. This rise was the most prominent for Zn and the least notable for Fe and Cu. The lowest acceptable levels of micronutrients in feed are as follows: 40-80 mg Fe, 40-70 mg Mn, 50-100 mg Zn, 7.1-10.0 mg Cu ha^{-1} (FALKOWSKI et al. 2000). The investigated plants had optimal levels of the micronutrients, except for copper, whose content was below the referential value.

DISCUSSION

The study assessed the effects of the microbial formulation on plant productivity and content of micro- and macronutrients and the levels of absorbable forms of macronutrients in soil. Intensive cultivation based on large amounts of chemicals causes degradation, impoverishment and acidification of soils. The use of microbial preparations might be one of the methods to improve soil properties. Introduction of probiotic bacteria improves physical and chemical properties of soil but the actual effect depends greatly on the soil type and a preparation dose (Kaczmarek et al. 2008*a*,*b*). Kaczmarek et al. (2008*b*) indicated that the microorganisms they used induced development of soil bacteria, fungi, actinomycetes and copiotrophic microorganisms. They also inhibited the growth of oligotrophic microorganisms and enhanced the activity of soil dehydrogenases. However, Kucharski and Jastrzębska (2005) reported inhibited growth and development of fungi and other soil microorganisms following application of effective microorganisms. Badura (2004) claimed that microbial preparations were more effective in poor, damaged and degraded soil with disturbed microbial balance.

High effectiveness of the formulation in the first year of the study (2014) was stimulated by the advantageous weather conditions, including high rainfall, which was favourably spread between individual months and over the entire season. Our results showing a higher content of absorbable forms of phosphorus and potassium in the soil treated with the microbial preparation confirmed to some extent the findings published by Górski and Kleiber (2010), Kocoń and Jadczyszyn (2015). Górski and Kleiber (2010) reported a positive effect of applying an EM preparation prior to a plant growing season on the content of available and easily soluble forms of nutrients (e.g. P and K) in the substrate used for growing ornamental plants as compared with control objects. Kocoń and Jadczyszyn (2015) claimed that an application of microbial formulations increased the content of absorbable phosphorus and potassium in the soil after cereal crop. Gajda and Igras (2003) also observed enhanced levels of absorbable phosphorus in the soil treated with EM-A in a pot experiment. Contrary to that, Martyniuk and Księżak (2011) described the results of a one-year field study in corn crop that showed no beneficial effects of EM-Farming and UGmax microbial preparations on soil levels of absorbable P_2O_5, K_2O, Mg and an increase in soil pH. In addition, Zydlik and Zydlik (2008) demonstrated a reduction in the levels of absorbable forms of phosphorus, potassium and magnesium in the soil treated with effective microorganisms. The authors concluded that the nutrients were taken up by plants. An experiment conducted by Jakubus et al. (2010) indicated a relationship between the influence of effective microorganisms on the soil nutrient content and the type of soil. Incubation experiments including soil with no plant cover showed that increasing doses of EM-A preparation enhanced the levels of absorbable potassium, magnesium and sulfur in luvisols but reduced their content in chernozems. Kobayashi et al. (1996) and Mertens and Hess (1984) demonstrated beneficial effects of the presence of appropriately selected microorganisms in plant root zone on root development, uptake of water and nutrients, plant growth and condition, and finally crop yield. In this study, the microbial formulation considerably improved dry weight yield of meadow plants. Sosnowski (2011) described a 30% increase in *Festulolium brauni* biomass following mineral fertilization combined with a biopreparation. Positive effects of EM in spring wheat crop were reported by Piskier (2006) and Kołodziejczyk et al. (2012). Piskier (2006) concluded that soil application of EM biopreparation may increase grain yield by as much as 23% owing to its beneficial effects on yielding parameters and biometric traits of the plants. Kołodziejczyk et al. (2012) noticed the biggest growth in grain yield (by 0.4 t ha^{-1} on average) in variants treated with Proplantan AM and effective microorganisms EM. They observed favourable influence of these preparations in all years of the study. Both Polish and international research literature comprises papers highly critical of the use and effects of microbial preparations based on the technology of effective microorganisms in agricultural crops (Condor et al. 2006, Martyniuk, Księżak 2011).

Positive effects on the yield and concentration of the investigated micro- and macronutrients induced by effective microorganisms suggest advisability of foliar fertilization of meadows with this preparation, even at optimal nutrient availability.

CONCLUSIONS

1. The three-year study indicated that application of the tested microbial formulation enhanced the content of available phosphorus and potassium in the soil but no significant effects of this preparation on the content of available magnesium and soil pH were observed.

2. The higher dose of the preparation (40 dm^3 ha^{-1}) caused a significant ($p \leq 0.05$) increase in dry matter yield.

3. Fertilization with the microbial formulation improved the plant content of macronutrients, except for sodium, and the best results were achieved on the plots treated with the formulation twice in a plant growing season.

4. The application of the microbial preparation facilitated the plant uptake of Cu, Zn, Mn and Fe.

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