IOŚ-PIB OCHRONA ŚRODOWISKA I ZASOBÓW NATURALNYCH
ENVIRONMENTAL PROTECTION AND NATURAL RESOURCES 2014
DE GRUYTER OPEN
VOL. 25 NO 3(61): 1–4
DOI 10.2478/oszn-2014-0010

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Evaluation of the impact of selected microbiological preparations on the development of the aboveground biomass of *Trifolium pratense* L.

Ocena wpływu wybranych preparatów mikrobiologicznych na kształtowanie się biomasy nadziemnej *Trifolium pratense* L.

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Keywords: microbiological preparations, red clover, dry matter, number of shoots, plant height
Słowa kluczowe: preparaty mikrobiologiczne, koniczyna czerwona, sucha masa, liczba pędów, wysokość roślin

Abstract
In order to determine the impact of improved microbiological preparations of soil on the development of the aboveground biomass of red clover (Parade variety) in the 2012–2013 year, field experiment with cultivation of this species was conducted. Experiment factors were three preparations containing composition of soil microorganisms (trade name: UGmax, Humus Active and Ekoużyźniacz). All study objects were carried out in triplicate. The analysed features were: green and dry matter of plant (kg•m^{-2}), plant height (cm), number of shoots (unit•m^{-2}), share of leaves and stems in biomass (%). The plants were cut three times, and collections of fresh weight were carried out at the beginning of flowering, i.e. when in 30–50% of the plants from plots the flower buds bloomed. The results were statistically analysed using analysis of variance. The study shows that the effects of biopreparations were dependent on the type of preparation and cut. Also, the meteorological conditions during the growing season of each research year were highly significant. The highest values of the analysed parameters were obtained for the plants that were sprayed using aqueous solutions of UGmax and Humus Active. There were no clear effects of soil fertilisers on the biomass structure expressed as the percentage of leaves and stems.

Streszczenie
W celu określenia wpływu mikrobiologicznych preparatów użyźniających glebę na kształtowanie się biomasy nadziemnej koniczyny czerwonej (odmiana Parada) w latach 2012-2013 przeprowadzono ścisłe doświadczenie polowe z uprawą tego gatunku. Czynnikiem doświadczenia były 3 preparaty zawierające w swoim składzie mikroorganizmy glebowe (nazwa handlowa: UGmax, Humus Active i Ekoużyźniacz). Wszystkie obiekty prowadzono w trzech powtórzeniach. Analizowane cechy to: wielkość wytworzonej zielonej i suchej masy rośliny (kg•m^{-2}), wysokość roślin (cm), liczba pędów (sztuka•m^{-2}), udział liści i łodyg w biomasie (%). Obiekty użytkowano trzyskośnie, a zbiory świeżej masy przeprowadzano na początku kwitnienia tj. gdy 30–50% roślin na poletku wybarwiło pąki kwiatowe. Uzyskane wyniki opracowano statystycznie stosując analizę wariancji. Z przeprowadzonych badań wynika, że efekty stosowania biopreparatów zależały od rodzaju preparatu i pokosu. Duże znaczenie miały również warunki meteorologiczne w sezonie wegetacyjnym w poszczególnych latach badań. Najwyższe wartości analizowanych cech uzyskano stosując oprysk roślin wodnymi roztworami UGmax i Humus Active. Nie wykazano jednoznacznego wpływu stosowania użyźniaczy glebowych na strukturę biomasy wyrażoną udziałem procentowym liści i łodyg.

1. INTRODUCTION

With the growing interest in organic farms and their products, numerous biological preparations approved for use in these type of farms are created, fulfilling the role of fertiliser and plant protection product. These preparations are used not only in organic farms, but also in many traditional farms [Truba et al. 2012]. On the market there are many preparations that contain beneficial microorganisms, humus extract, an extract from the algae and seaweed. All these products, although their composition is different, have the same goal – to improve the physical, chemical and biological properties of the soil, which should be related to crop productivity. The study of potato yield effectiveness has shown that the use of microbial-based preparation has positive results in the form of a 7.1% of increase in yield compared with the controls [Trawczyński and Bogdanowicz 2007]. In turn, Zarzecka et al. [2011] showed an increase in the yield from 8.5% to 53.9% after applying UGmax and the reduction of infection bulbs and rizoctonioze with common scab. In contrast, the supporting of soil with microorganisms in maize silage cultivation resulted in a 15.1% yield increase [Sulewska et al. 2009]. Piskier [2006] and Wielgosz et al. [2009] after the application of microbiological preparations obtained the yield increase of grain and straw of spring wheat. The product has also increased the density of spikes and stalks per unit area. It should be noted, however, that many reports [Sosnowski and Jankowski 2010, Zarzecka et al. 2011, Sosnowski 2012, Zarzecka and Gugała 2012, Sosnowski and Jankowski 2013] demonstrated the impact of soil fertilisers to increase plant yields.

The aim of this study was to evaluate the effect of microbial preparations on the length and number of shoots, the volume of produced biomass and its structure in the cultivation of red clover.

2. MATERIAL AND METHODS

As the experimental objective of Grassland Department and the Shaping of Landscape Architecture in Siedlce in 2012–2013, field experiment with red clover – *Trifolium pratense* L (Parade variety) – was carried out. The soil on which the plots were located was included to order culture earth, hortisoli type. Based on the analysis performed at the Regional Chemical Station in Wesola, it was found that the soil was characterised by neutral reaction (pH in KCl = 7.2), high abundance in humus (3.78%), available phosphorus (17.25 mg P•100 g^{-1}) and magnesium (8.4 mg Mg•100 g^{-1} soil) and the average abundance nitrate (10.10 mg $N\text{-}NO_3$•kg^{-1} dry matter of soil), ammonium (7.74 mg $N\text{-}NH_4$•kg^{-1} dry matter of soil) and available potassium (13.11 mg K•100 g^{-1} soil). Single plot area was 3 m^2. The dose of seeds sowing was 15 kg•ha^{-1}. Before sowing and after mowings (three cuts), fertilisation with K_2O (120 kg•ha^{-1}) was used in three doses. Due to the very high phosphorus content in the soil, the use of this macrocomponent was abandoned.

The experiments factors were biopreparations containing in its composition the microorganisms. The preparations were applied as aqueous solutions (diluted at dose of 350 l of water) to the spring regrowth in the form of a spray. Methodological details and characteristics of biologicals used are shown in table 1. Each experimental objective was carried out in triplicate.

Collection of plant fresh matter was performed at the beginning of flowering (30–50% of plants has bloomed the buds). The analysed characteristics were the size of dry matter (kg•m^{-2}) produced by plants, plant height (cm), the number of shoots (unit•m^{-2}), share of leaves and stems in biomass (%). The results were statistically analysed using analysis of variance and $LSD_{0.05}$ according to Tukey's test.

Meteorological data were obtained from the Hydrological and Meteorological Stations in Siedlce. However, in order to determine the temporal variability of meteorological elements and their influence on plant growth, the coefficient of Sielianinov hydrothermic [Bac et al. 1993] was conducted. The data presented in table 2 show that the most favourable distribution and magnitude of rainfall, with optimum air temperatures during the growing period plants, was in April, May and June. For these months, the highest values were recorded (over 1), which show the optimal relation of air temperature to the volume of precipitation. Also, it should be noted that only in September strong drought was reported. However, in 2013 a very strong drought occurred in August.

3. RESULTS

In the first year, the use of biologicals improved the soil and resulted in a statistically significant average of 13% increase in the green weight of clover compared with the control (Table 3). In the next cuts (II and III), the amount of plant biomass produced on control objects was an average 70.7% of the yield collected from objects sprayed with UGmax, 77.2% with Humus Active and 85% with Ekoużyźniacz. In the second year, significant increases in the use of green mass were only in I and III cuts.

The second cut did not show statistically significant differences in the discussed features. A similar correlation occurred in the dry matter of plants. Only Humus Active and UGmax caused a significant increase in plant height relative to the control object. This effect occurred only in the first cut in both years. However, in the second cut in the first year, the highest plants (78.8 cm) were obtained in the plots fertilised with UGmax. Both these formulations have also contributed to the increase (average 30%) in the number of shoots per unit area in the two years of cultivation, but only in the second regrowth of clover. According to Pisulewska et al. [2003], an important part of the productivity of plants is the crop structure determined as the percentage of leaves and stems. The authors point to a positive correlation between total protein content and the participation of the leaves. The study shows that only applying UGmax and Ekoużyźniacz resulted in significant variation of clover yield but only in the first year of the study (second and third cuts).

As shown by the data in Table 4, the annual yield of fresh and dry matter underwent a statistically significant differentiation under the

Table 1. Methodological details of applicable biopreparations

Objects		Dose l•ha^{-1}	Characteristic
Control	K	-	-
UGmax	UG	0.9	Improves soil preparation containing lactic acid bacteria, photosynthetic bacteria, *Azotobacter, Pseudomonas,* yeasts and *Actinomycetes* and micro- and macroelements
Ekoużyźniacz	E	5	Item breeding earthworms *Eisenia fetida,* improving the soil, it is the extract of vermicompost cattle in the form of a suspension – characterised by the presence and diversity of saprophytic fungi, including fungal, protects the root system from getting infected soil pathogens
Humus Active	HA	50	Improves soil preparation, it is composed of 90% organic matter in the form of humus and positive microorganisms (*Azotobakter, Nitrosomonas,* photosynthetic bacteria and fungi positive)

Table 2. The value of the hydrothermic coefficient by Sielianinov (K) in each month of the growing season

Year	Month						
	IV	V	VI	VII	VIII	IX	X
2012	1.12	1.22	1.56	0.69	0.94	0.27	1.32
2013	1.60	2.20	1.80	1.50	0.25	2.70	1.22

K < 0.5 – serve drought; 0.51 – 0.69 – drought; 0.70 – 0.99 – weak drought; K > 1 – no drought

Table 3. Some productive features of red clover depending on the applied biopreparation in each cut and research years

Trait	Object	Harvest year					
		2012			2013		
		Cut I	Cut II	Cut III	Cut I	Cut II	Cut III
Forage yield (kg•m^2)	K	6.32	3.87	2.01	2.84	1.94	0.93
	UG	7.74	5.32	3.00	4.02	2.56	1.44
	E	6.76	4.04	2.87	3.35	1.86	1.37
	HA	7.01	4.59	3.02	3.36	2.20	1.45
$LSD_{0.05}$		1.07	0.54	0.76	0.49	n.s.	0.43
Dry matter yield (kg•m^{-2})	K	1.70	1.10	0.70	1.13	0.67	0.44
	UG	2.58	1.90	1.07	1.65	1.15	0.68
	E	2.23	1.38	1.03	1.35	0.83	0.63
	HA	2.33	1.53	1.08	1.14	0.98	0.71
$LSD_{0.05}$		0.62	0.76	0.28	n.s.	0.47	n.s.
Plant height (cm)	K	73.3	65.8	53.8	65.7	54.8	48.9
	UG	90.1	78.8	61.3	80.9	64.4	55.7
	E	89.1	71.4	62,9	74.1	64.9	57.2
	HA	92.8	68.3	64,8	84.3	62.0	59.9
$LSD_{0.05}$		16.1	11.3	n.s.	14.1	n.s.	n.s.
Number of shoots (number•m^2)	K	680	408	340	606	364	375
	UG	689	523	408	613	513A	412
	E	701	478	437	687	491	421
	HA	677	563	418	664	502A	440
$LSD_{0.05}$		n.s.	112	n.s.	n.s.	134	n.s.
Percentage of leaves (%)	K	60.4	37.2	51.1	54.3	42.4	49.6
	UG	59.8	47.5	67.8	58.6	48.9	60.1
	E	60.9	49.0	63.0	61.0	43.6	56.3
	HA	63.8	39.7	61.7	60.8	41.8	57.4
$LSD_{0.05}$		n.s.	9.11	13.5	n.s.	n.s.	n.s.
Percentage of stems (%)	K	39.6	62.8	48.9	45.7	57.6	50.4
	UG	40.2	52.5	32.2	41.4	51.1	39.9
	E	39.1	51.0	37.0	39.0	56.4	43.7
	HA	36.2	60.3	38.3	39.2	58.2	42.6
$LSD_{0.05}$		n.s.	10.1	12.3	n.s.	n.s.	n.s.

K – control, UG – UGmax, E – Ekoużyźniacz, HA – Humus Active

influence of the treated fertiliser preparations. The highest yields in both study years were obtained by using UGmax and Humus Active. UGmax resulted in an average 30% increase in fresh and dry matter of plants throughout the whole test cycle, but Active Humus an average 23% in the first study year and 19% in the next year. Using Ekoużyźniacz in clover cultivation contributed to an increase in crop yields but their mean did not differ significantly between those on control object. It should also be noted that the total annual yield of fresh and dry weight of clover in the second study year were on average 50% lower compared with the previous year. According to Wilczek [1984 a, b], Wilczek and Wilczek [2002], Buxton and Fales [1994] and Pisulewska et al. [2003], mass, composition and quality of the harvested biomass of red clover are mainly dependent on harvest date, and more specifically on the plant phenological stage at harvest, on the growth rate of each variety, as well as on soil and climate conditions and on harvest kind. Sufficient rainfall in 2012 (although unevenly distributed) and the right temperature during the growing season resulted in a high forage yield in relation to the specific experimental results of COBORU [Broniarz 1999]. A significant reduction in yields of green and dry matter of clover in the following year was the result of adverse weather conditions. Insufficient rainfall and high temperatures in August (0.25 Sielianinov coefficient expressing a very strong drought) resulted in a significant reduction in both yield and forage deterioration of its structure (the percentage of leaves and stems). A smaller crop of forage in all the objects in the first cut of the second year than in the first year causes probably the poor condition of plants after the winter, which affected the perennial crop also.

Broniarz [1999] pointed out that the hardiness of plants is crucial for legume crops. In the study by Peterson et al. [1992], harvested forage from first cut of red clover in the second year of its use was only 30% of the crop of the previous year. Also, Tomaszewski [1988] has found that the forage yield of clover in the second year of cultivation was lower by about 20% in diploid varieties and by about 15% in tetraploid varieties.

Table 4. The annual yield of green and dry matter red clover depending on the biopreparation in particular study years

Trait	Object	Harvest year	
		2012	2013
Forage yield (kg•m^{-2})	K	12.2	5.71
	UG	16.1	8.02
	E	13.7	6.58
	HA	14.6	7.01
$LSD_{0.05}$		1.98	1.18
Dry matter field (kg•m^{-2})	K	3.50	2.24
	UG	5.55	3.48
	E	4.64	2.81
	HA	4.94	2.83
$LSD_{0.05}$		1.33	0.58

K – control, UG – UGmax, E – Ekoużyźniacz, HA – Humus Active

4. CONCLUSIONS

- The effects of the biopreparations use containing microorganisms in the cultivation of red clover depend on the type of preparation, cut and meteorological conditions in the different study years.
- The highest values of the analysed parameters were obtained using UGmax and Humus Active. These preparations contributed to the increase in the volume of fresh and dry matter of plants and the length and number of shoots.
- Spraying the plants with UGmax resulted in an average of 30% and 20% with Humus Active increase of green and dry matter of red clover throughout the whole test cycle.
- There were no clear effects of soil fertiliser on the structure of plant biomass as a percentage of leaves and stems.

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