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EXPERIMENTAL PAPER

The effect of soil type and soil additives on the selected growth parameters and yield of flowerheads of *Calendula officinalis* L.

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S u m m a r y

Introduction: Soil additives, which usually contain nutrients and microorganism, can improve soil conditions for plants. There are still few papers dedicated to the application of soil additives in herb plants, especially in calendula. **Objective:** The aim of the study was to determine the effect of selected soil additives on growth and yield of calendula. **Methods:** In a pot experiment first order factor was a type of soil: mineral soil with straw, mineral soil and organic soil. The second order factor was soil additive: control, UG Max, EM1 and PRP SOL. **Results:** The application of UG Max and PRP SOL increased the yield of dry matter of flowerheads by respectively 48.1% and 46.3% in comparison with the control group. **Conclusions:** UG Max and PRP SOL proved to be the most useful soil additive for calendula growing. The effect of UG Max and PRP SOL was particularly good on organic soil.

Key words: *calendula, UG Max, EM1, PRP SOL, mineral soil, organic soil*

INTRODUCTION

Calendula (*Calendula officinalis* L.) has been cultivated since Roman times for its medicinal properties [1]. Recently it has come back into the spotlight as a medical herb with reports of anti-microbial [2], anti-viral [3], anti-tumor [4], and anti-inflammatory [5] effects. Calendula is grown for medicinal purposes indigenously throughout Europe and the Middle East [6-8]. Now it is produced in many countries such as France, Egypt, Algeria, Morocco, South Africa, China and Hungary. Many countries are important markets for calendula flower heads, namely Germany, Russia, the Netherlands, the UK and the USA [8]. In Poland, calendula is one of major medicinal plants grown on field plantations. The cultivation is stimulated not only by pharmaceutical industry, but also by the dynamic development of the manufacturing of cosmetics [9]. Crnobarac *et al.* [10] pointed out that similarly to other medicinal plants, the crop management of calendula has not been studied sufficiently. It particularly concerns the fertilization of calendula [7]. Apart from that, there are no papers about the application of soil additives in calendula cultivation. At the end of the twentieth century, environmental care and the impact of agriculture led to the division of the concept of fertilization into soil fertilization and plant nutrition or feeding [11]. The increasing significance of soil additives prepared by many manufacturers using different technologies can be observed. One of them, offered by Procedes Roland Pigeon Company, increases the enzymatic activity of soils and improves their physical properties. Trials carried out in France proved that this technology, named PRP SOL, contributed to the conversion of insoluble phosphorus, potassium and magnesium compounds into the forms available for plants [12]. According to the manufacturer, PRP SOL is a granular formulation containing the MIP (mineral inducer process) active substances, iron, zinc, boron, sodium and manganese compounds based on calcium carbonates and magnesium-related sticky substance: lignin sulfonate (galenical). There are also many soil additives based on beneficial microorganisms collected from natural environments. One of better known substances is Effective Microorganism (EM) developed by Prof. Dr Terou Higa at the University of the Ryukyus, Okinawa, Japan, in early 1980s. EM is a fermented mixed culture of naturally occurring species of coexisting microorganisms in an acidic medium (pH below 3.5) [13]. Some of the studies proved that the microorganisms in EM improve crop health and yield by increasing photosynthesis, producing bioactive substances, such as hormones and enzymes, accelerating the decomposition of organic materials and controlling soil-borne diseases [14]. Another soil additive named UG Max is a liquid concentrate containing microorganisms as well as macro- and microelements. It is used for increasing the soil fertility in vegetable, field crop and fruit growing. It initiates and accelerates the process of crop residue, straw, manure decomposition and along with soil minerals it is a part of humus – the natural soil life habitat and store of plant nutrients. In Poland, all tested soil additives are certified by the Ministry of Agriculture and Rural Development and can be used in conventional, integrated and ecological agriculture. Studies on interactions between plants, soil and microorganisms have already found practical application in sustainable and ecological agriculture, in the development of new cultivation and fertilization technologies. As a result, these studies can prevent the degradation caused by intensive agriculture. It particularly applies to plant material, which must meet certain quality standards to be allowed to further use in the industry. Currently, it is desirable to discuss this issue because in coming years, interest in growing this type of plants among farmers will be growing in order to balance the biodiversity, conservation and protection of resources with agricultural production [15]. Also, Polish farmers are encouraged to provide more flowering habitats to support pests' natural enemies and pollinators in the agro-ecosystems, especially by Directive 2009/128/EC, recommending Integrated Pest Management is being implemented.

Continuous progress in research shows the competitiveness of the use of soil additives in comparison with conventional solutions. Therefore, the aim of this study was to determine the effect of selected soil additives on the growth and yield of calendula.

MATERIAL AND METHODS

In 2009–2010, a pot experiment involving calendula plants was carried out at Poznań University of Life Sciences, Poland. The experimental part of the research was designed according to the independent series method and set up in pots of 2 dm^3 volume and 0.029 m^2 surface placed in an unheated greenhouse. The first-order factor was the type of soil: M+S – mineral soil with straw (a dose of 12 g per pot), M – mineral soil, O – organic soil Athena. Mineral soil was collected from the field located in Złotniki, near Poznań (N 52° 29.193'; E 016° 20.569'). It contained luvisol soils with a sand texture (UG2) formed from glacial moraine clay. As far as soil quality is concerned, it is classified as a good rye complex consisting of: sand (17%), dust (12%), silt and clay fraction (17%) [16]. The soil was highly abundant in phosphorus, with medium content of potassium and low content of magnesium (18 mg $N \cdot kg^{-1}$, 270 mg $P \cdot kg^{-1}$, 189 mg $K \cdot kg^{-1}$, 72 mg $Mg \cdot kg^{-1}$) and with neutral pH – 6.5 in 1 M KCl). Main macronutrients contained in straw (82.2% dry matter content) were as follows: 0.73% total N, 0.30% P_2O_5, 1.19% K_2O. Organic soil (180 mg $N \cdot kg^{-1}$, 69.8 mg $P \cdot kg^{-1}$, 182.6 mg $K \cdot kg^{-1}$) was purchased at a garden center. The second-order factor was the soil additive (tab. 1): control, UG Max (a dose of 0.0026 ml per pot), EM1 (a dose of 0.28 ml per pot), PRP SOL (a dose of 1.2 g per pot). The pots were filled the soil types according to the experimental design. Then the established soil additives were applied either directly on the soil mixed with straw or on the soil with a Kwazar sprayer according to established doses. The soil in the pots was incubated for 14 days before calendula seeds were sown. Five calendula seeds were sown into each pot on 8th of July and after germination three similar plants were left. The pots were placed outside the greenhouse and they were regularly watered with distilled water when necessary during the vegetative season. Calendula inflorescences were harvested at the full blooming phase, every 3 days during the growing season. At the end of that period on 7th of October the unbloomed flower buds were also harvested. Before the harvest biometric measurements of the plants were taken. The greenness indicator of the plants, expressed in SPAD units was examined with a Hydro N-Tester pocket apparatus (Minolta, Japan). The roots were separated from the soil by gently washing the soil core with water. The root volume was determined by immersing the roots into a partially filled graduated cylinder and recording the resulting water displacement. The roots were dried and their air-dry mass was weighed. The fresh weight of the harvested inflorescences was determined and then the flowers were dried in a thermal drying chamber (at 30–35°C), according to Müller and Heindl's method [17], and their air-dry mass was also weighed.

Table 1.

Soil additives composition

Name	Content of macro- and micro- elements [mg·kg^{-1}]						Other [mg·kg^{-1}]	Microorganism
	N	P	K	Mg	Ca	Mn		
UG Max[1]	1200	500	3500	100	-	3	Na-200	Lactic acid bacteria, Photosynthetic bacteria, *Azotobacter, Pseudomonas*, yeast, Actinomycetes
EM1[2]	-	-	-	-	-	-	-	Photosynthetic bacteria, Lactic acid bacteria, yeast, Actinomycetes
PRP SOL[3]	-	-	-	48000	228600	-	Na-35000; 3-5% prefixes (48 trace elements)	-

[1][18]; [2][13]; [3][19]; – no data

The data were statistically analyzed with Statistica for Windows 10. The relationship between the type of soil, soil additives and the studied characteristics was determined with the analysis of variance for double factor experiments. The analysis of variance (ANOVA) was performed on the blocks to investigate the effect of the soil type and soil additives. The results for years 2009–2010 were analyzed. The assumption of the homogeneity of variance was examined with Levene's test. If the type of soil or soil additives had a statistically significant effect, Tukey's multiple comparison test was performed.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

The content of active compounds in a plant is the least dependent upon the edaphic conditions [20]. However, the author points that although the maximum content of the active substance in a plant is genetically conditioned, it may vary under the influence of such edaphic factors as irrigation, fertilization or appropriate selection of soil. Moreira *et. al.* [21] and Paim *et al.* [22] also point to the fact that vegetative development in medicinal species is one of the factors that can reduce the amounts of active substances produced by a given species. The selection of cultivation treatments and fertilizers can decide that the species are able to produce greater quantities of active substances due to the fact that many nutrients contained in the fertilizer are found to be involved in secondary metabolic pathways [23]. According to the recent research made by Fernandes *et al.* [24], the expression of metabolites from the plants grown under different soil treatments was not statistically significant, so they pointed that calendula is not affected by changes in the soil composition and it could be a source for phytomedicines with a constant content of polar metabolites.

This research proved that the vegetative development of plants was significantly influenced by the soil additives and soil type. The calendula plants were the tallest on mineral soil and on another soil types statistically shorter. The addition of rye straw decrease plants' height by 0.7 cm. When the plants grew on organic soil, their height was reduced by 3.7 cm, as compared with mineral soil (tab. 2).

Table 2.

Biometric characters of calendula – mean values from two years

Treatment	Plants height [cm]		Plants greenness indicator (SPAD)		Roots volume [cm^3]	
	mean±SD	*p* value	mean±SD	*p* value	mean±SD	*p* value
Soil type						
M+S	41.5±3.8 a	0.0041**	319.8±86.8 ab	0.0000**	9.5±4.2 a	0.0000**
M	42.2±3.4 a		337.5±54.9 a		8.7±3.9 ab	
O	38.8±4.0 b		281.5±60.5 b		6.4±2.7 b	
Soil additives						
Control	39.2±4.4 b	0.0319*	294.5±71.3	0.4318ns	8.9±4.7	0.0905ns
UG Max	41.7±3.2 a		346.2±62.4		9.2±3.8	
EM1	40.3±3.3 ab		314.4±67.9		8.4±3.5	
PRP SOL	42.1±4.4 ab		296.6±78.2		6.2±2.7	

* – statistically significant differences ($p < 0.05$), ** – statistically highly significant differences ($p < 0.01$), ns – no statistically significant effect on tested trait ($p > 0.05$), a, b, c – homogeneous groups (Tukey's test, $p < 0.05$), mean±SD of four observations

The PRP SOL application led to a significant increase in the height of plants, as compared with the control group. In the Paim *et al.* [22] study calendula plants positively react to liming. Authors obtained the tallest calendula plants (44.1 cm) when liming was applied in combination with an organic fertilizer. The plants grew taller when liming was applied or when it was applied in combination with a chemical fertilizer. Similarly, the PRP SOL application (32% CaO) caused plants' growth by 2.9 cm taller than in the control group and this increase was significant. In Bielski and Szwejkowska [9] study, magnesium fertilization did not have significant effect on the height of plants. However, taller plants tended to grow in the pots which had been enriched with magnesium. In our study, this component was not provided only in combination with EM1; this could be the reason that the plants grow shorter than in the combinations with UG Max and PRP SOL. The results of the experiments on mung bean made by Javaid and Bajwa [13] revealed that the effect of EM1 was insignificant to the shoot length. Hussein *et al.* [25] and Kumar and Singh [26] indicated that nitrogen had a strong effect on the height of calendula plants. Our study did not confirm the significant influence of nitrogen on the height of plants. When nitrogen was provided in the UG Max, the plants tended to reach the highest value of the leaf greenness indicator (SPAD) and they grew taller than in the control group. UG Max application in maize resulted in a statistically significant increase of grain and silage yield, although the influence of UG Max on leaf greenness indicator and plants height was not statistically significant [27].

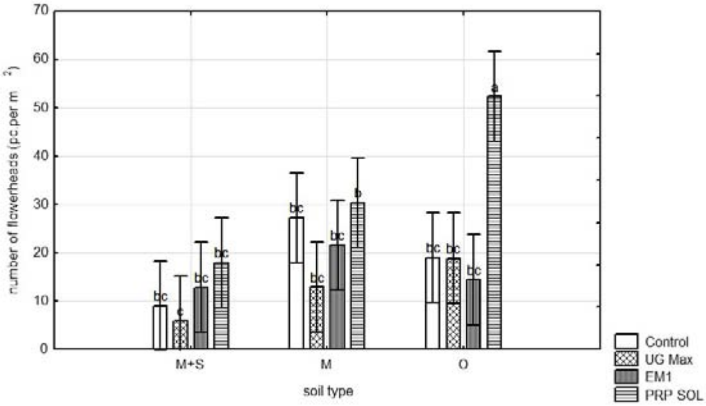
The type of the soil affected the value of leaf greenness indicator and the highest value was obtained on mineral soil (337.5 SPAD), whereas it was the lowest when calendula was grown on organic soil (281.5 SPAD). Each tested soil additive application tended to increase the SPAD value. In comparison with the control group the increase in the SPAD value was the largest after UG Max (51.7) and the lowest when PRP SOL (2.1) was applied.

In our study, the roots reached the greatest volume in the plants grown on mineral soil with straw. The reduction of roots volume on mineral soil without straw amounted to 0.8 cm^3 and on organic soil amounted to 3.1 cm^3 and this one was statistically significant. Among tested soil additives, UG Max application gave the best results in roots volume. In comparison with control group, the roots volume increased by 0.3 cm^3, but it was not statistically significant. When EM1 or PRP SOL were applied, in comparison with control group the roots volume was limited by 0.5 cm^3 and by 2.7 cm^3, respectively.

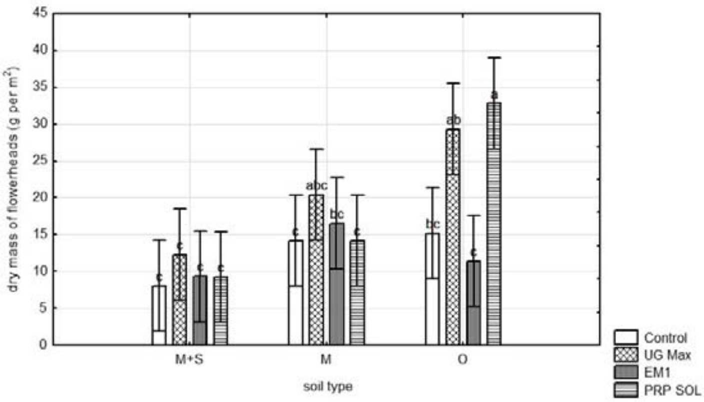
For calendula flowerheads and buds number and flowerheads dry matter significant interactions between studied factors were found. When UG Max and PRP SOL were applied, the number and dry matter of flowerheads were higher on organic soil (O) than on mineral and mineral with straw. The differences in flowerheads number after UG Max application were not statistically significant but after PRP SOL amounted 22.0 $pc.\cdot m^{-2}$ (M) and 34.3 $pc.\cdot m^{-2}$ (M+S), respectively. Also it was statistically proved that after PRP SOL application on organic soil flowerheads dry matter increased by 18.6 (M) and 23.5 $g\cdot m^{-2}$ (M+S), respectively (fig. 1a, b). Paim *et al.* [22] also obtained the highest flowers number from the combination with the Liming+organic fertilizer, but on the other hand, the flowers obtained from the combination with Liming were larger. They noted that the analyzed treatments did not significantly influenced the flowers biomass.

Contrary to our study, the results obtained by Acosta Da Luz *et al.* [28] indicated that calendula exhibits hardiness and adapts well to the soils that are relatively poor in organic material, but when organic material was applied, it could prolongate the vegetative development of this species. However, the organic fertilizer releases nutrients to the plants slower and in a higher constant manner, bringing the benefits of chemical and physical properties of the soil. The organic material improves the soil structure, aeration, drainage and water retention [29].

a)



b)



c)

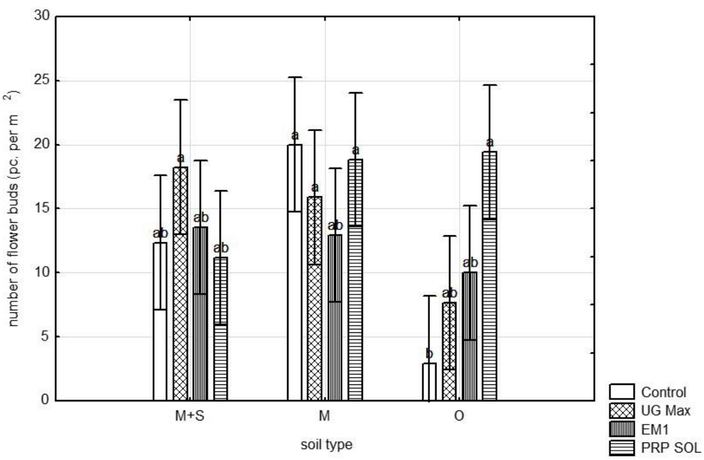


Figure 1.

Effect of soil type and soil additive with 95% confidence intervals (mean values from two years) on: a) number of flowerheads (pc.·m^{-2}); b) dry mass of calendula flowerheads (g·m^{-2}); c) number of flower buds (pc.·m^{-2}) which have not blossomed at the end of growing season

On the other hand, the biggest dry matter of flowerheads per 1 m^2 was obtained after EM1 application on mineral soil, but the differences between another soil types were not significant (fig. 1b).

When we consider the number of buds which did not bloom because of the end of the vegetation period, the highest effectiveness of UG Max was obtained on mineral soil with straw (18.2 pc.), but the differences in this trait between another tested soils were not significant. When EM1 was applied, the differences between buds number achieved on tested soil types were much smaller. On the other hand, after PRP SOL application the buds number was higher on organic soil (19.4 pc.) and mineral soil (18.8 pc.) than on mineral with straw (11.2 pc.) (fig. 1c).

Phosphorus is found to be abundant in the fruit and seeds of plants [30]. It is found that increasing dose of phosphorus fertilizer will promote reproductive yields [31] and inflorescence production [32], particularly when phosphorus is limited in natural systems [33]. Conversely, the limitation of phosphorus supply has been shown to decrease the production of floral structures [34]. In the combination with PRP SOL, phosphorus may have been more accesbile to the plants and therefore they produced the most flowers in that combination. In most of soils, phosphorus is present in a form unavailable to plants, as phosphates $FePO_4$ and $AlPO_4$, and potassium is blocked in clay minerals. According to Hüttl and Fischer [35], the application of PRP SOL to soil unlocks and provides plants with phosphorus and potassium. The organic forms of nutrients must be converted into inorganic forms by soil microbes before plants can use them [36]. This fact may have contributed to better development of calendula flowers grown on organic soil with PRP SOL. It should be emphasized that in our study the interactions showed that the calendula in the control sample on mineral soil with straw (M+S) produced about 82.8% less flower heads per 1 m^2 and 75.3% of its dry matter than those grown on organic soil (O) with PRP SOL applied. Also, Sulewska *et al.* [19] reported that the use of PRP SOL increased the fraction of potato seeds in the tuber yield. Similarly to that study, Krzywy-Gawrońska and Wołoszyk [12] found that the PRP SOL applied together with increasing doses of municipal sewage sludge compost induced a significant increase in the plant yields. This result is consistent with the study by Siebielec and Stuczyński [37]. They observed that when PRP SOL was applied to soil, it induced the conversion of unavailable forms of phosphorus and potassium into available ones. Stewart and Lovett-Doust [38] obtained contrary results, which challenged the general view in the literature that the high concentration of phosphorus can promote floral tissue development [31-33]. They found that the total biomass of calendula plants was significantly greater for the extra-low phosphorus (10 $mg \cdot l^{-1}$) treatment than after the highest phosphorus level (200 $mg \cdot l^{-1}$).

The results of analysis showed that the plants grown on mineral soil (M) produced a significantly higher dry mass of the roots and overground parts of plant than those grown on organic and mineral soil with straw (fig. 2a). The overground parts of plant obtained on organic soil were slightly heavier than on mineral soil with straw, and dry mass of roots was significantly heavier on mineral soil with straw than on organic one. However, the flower heads blossomed in a greater number and mass on organic soil, where the root system developed the worst. The worst development of vegetative parts on mineral soil with straw may have been caused by the immobilization of nitrogen resulting from the mineralization of wheat straw. In the laboratory experiment [39] the concentration of available nitrogen (straw nitrogen and soil mineral nitrogen) was only 0.63% of the added straw dry matter.

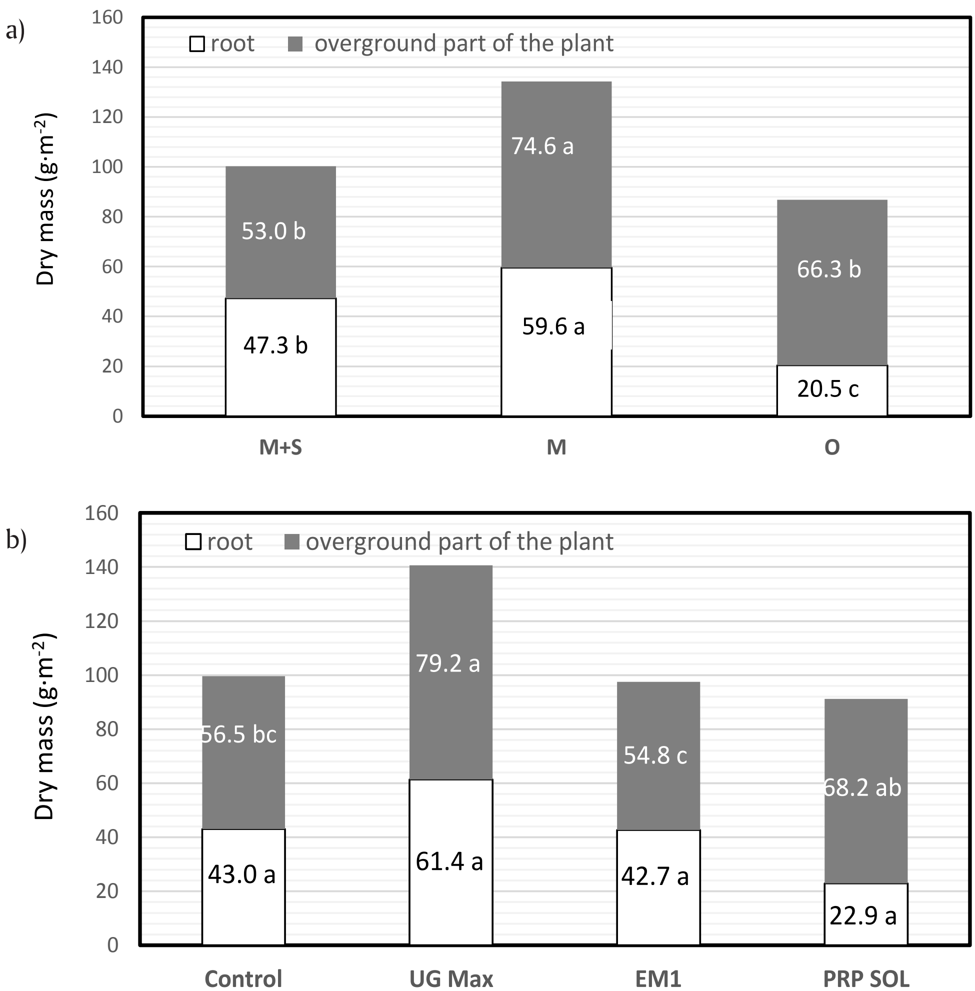


Figure 2.

The effect of: a) soil type treatment on the roots and dry mass of overground part of plant; b) soil additive treatment on the roots and dry mass of overground part of plant. Mean values that do not differ significantly have the same letter; a, b, c – homogeneous groups (Tukey's test)

Anders and Victoria [40] found that the biomass and leaf area of calendula was greater when it was grown on the soil amended with a source of high nutrients, but the flowering of plants in this high input system was retarded, probably because of the higher levels of inorganic nitrogen and greater vegetative growth. The results obtained by Chauhan and Kumar [41] revealed that the higher level of nitrogen (100 kg·ha^{-1}) had significant influence, increasing most of the growth, flowering and yield parameters studied on calendula plants.

Among tested soil additives after UG Max application, we observed the greatest influence on the development of both the overground part of the plant and its roots (fig. 2b). The application of UG Max contributed to an increase in the dry mass of overground part of plants, where the difference between the control group amounted to 22.7 g·m^{-2}. Our study did not reveal a significant interaction between the type of soil and applied soil additives on the yield of dry mass of overground part of calendula plants.

The soil additives presented in this study have been the subject of different authors' research. Many of those studies point to the fact that there were no significant effects of the application of EM1 on the biological activity of soil and the yield of such plants as maize [42] or spring wheat [43]. Some studies even proved that soil additives negatively influenced the development of lettuce [44] and yield of tuber mass [45]. Also the application of UG Max can reduced biomass [45]. The studies by Sulewska *et al.* [11,46] proved that winter wheat, spring barley and winter rape reacted both positively and negatively to the application of PRP SOL, depending on the weather conditions. During the five years of the research the winter rape plants from the combinations fertilized with PRP SOL yielded 2.0 dt·ha^{-1} lower than in the control group. According to Martyniuk and Księżak [47] the application of such soil additives in farming practice cannot be economically justified.

It is also critical and major challenge in organic fertilizer management to find the methods optimizing the synchrony between nutrient mineralization and crop demand [48]. It is necessary to stress that all articles presented the results of other authors, which did not prove the positive influence of tested soil additives used in field conditions. This might have explained the slight plant reaction, because the mass of microorganisms and other components applied with the preparation was strongly diluted, because in 1 ha the arable layer of soil is about 3000 t, including 3.31 t of microorganisms. Apart from that, most soils in Poland have an acidic pH and introducing some bacteria into the soil where pH is under 6.0 does not led to the expected results. Some of the bacteria entered into the soil do not settle there and they only provide nourishment for native soil organisms [47].

There are no available results concerning the application of tested soil additives to plants grown in pots. Therefore, it is impossible to confront the results of our research more widely. For this reason it is necessary to conduct further research which will help to evaluate the usefulness of those additives in greenhouse and polytunnel plantations. Similarly, Bi and Evans [49] noted that different crops may respond differently to different organic fertilizers. Therefore, it is important for growers to test new soil additives before using them in the production practice.

In the era of promoting sustainable agriculture the application of soil additives with different components may be alternative to the use of industrial means of production. However, first it is necessary to investigate the dependence between the soil, additive and crop.

CONCLUSIONS

It was proved that organic soil is the best for calendula plants, because the largest number of flowerheads was set there and both the fresh and dry mass were the greatest.

UG Max and PRP SOL were more useful soil additive for growing calendula than EM1. The application of this additives increased the yield of dry matter of flowerheads by 48.1% (UG Max) and 46.3% (PRP SOL) in comparison with the control group. The effect of UG Max and PRP SOL was particularly good on organic soil.

Conflict of interest: Authors declare no conflict of interest.

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WPŁYW RODZAJU PODŁOŻA I DODATKÓW GLEBOWYCH NA WYBRANE PARAMETRY WZROSTU I PLON KOSZYCZKÓW *CALENDULA OFFICINALIS* L.

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S t r e s z c z e n i e

Wstęp: Dodatki glebowe, zwykle zawierające składniki mineralne i mikroorganizmy, mogą polepszać warunki glebowe dla roślin. Nadal jednak mało jest prac poświęconych zastosowaniu tego typu dodatków w roślinach zielarskich, szczególnie nagietku lekarskim. **Cel:** Celem badań była ocena wpływu wybranych dodatków glebowych na wzrost i plon nagietka lekarskiego. **Metoda:** W doświadczeniu wazonowym czynnikiem I rzędu był rodzaj podłoża: gleba mineralna z dodatkiem słomy, gleba mineralna i gleba organiczna. Czynnikiem II rzędu było stosowanie dodatków glebowych: kontrola, UG Max, EM1 i PRP SOL. **Wyniki:** Zastosowanie UG Max i PRP SOL pozwoliło na uzyskanie przyrostu plonu suchej masy koszyczków odpowiednio o 48,1% i 46,3% w porównaniu z obiektem kontrolnym. **Wnioski:** Spośród badanych dodatków glebowych, UG Max i PRP SOL okazały się najbardziej przydatne w uprawie nagietka. Szczególnie dobre działanie UG Max i PRP SOL uzyskano na podłożu organicznym.

Słowa kluczowe: *nagietek, UG Max, EM1, PRP SOL, gleba mineralna, gleba organiczna*