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THE INFLUENCE OF EFFECTIVE MICROORGANISMS APPLICATION ON THE CHEMICAL COMPOSITION IN LETTUCE GROWN UNDER COVER

WPŁYW ZASTOSOWANIA EFEKTYWNYCH MIKROORGANIZMÓW NA SKŁAD CHEMICZNY SAŁATY UPRAWIANEJ POD OSŁONAMI

Abstract: The aim of the study was to assess the influence of Effective Microorganisms (EM) on the content of nutrients in the leaves of lettuce (*Lactuca sativa* L. 'Sunny') grown under cover in the spring-summer season and in autumn. The influence of the following methods of EM application was assessed: root treatment, leaf treatment and seed inoculation. When EM was applied into the roots, plants were irrigated with a 1% solution of the EM-A preparation or with EM-5 (250 cm^3 of the liquid per plant), depending on the combination. The same preparations concentrated at 1% were sprayed on the leaves. Seeds were inoculated immediately before being sown (they were soaked for 30 minutes in a 10% solution of the EM-A preparation). During the growing season the plants were sprayed or irrigated four times at three-day intervals. Effective Microorganisms was not used in the control combination. The research proved the influence of EM on the content of nutrients in lettuce leaves. When the plants were irrigated and sprayed with EM-5, they had higher content of nitrogen. They were significantly better nourished with phosphorus, when the seeds were inoculated with EM-A, when the plants were sprayed with EM-5 at both terms of cultivation and when the plants were irrigated with EM-A in autumn. In comparison with the control combination the application of EM preparations significantly increased the content of potassium in the lettuce leaves. Simultaneously, the content of potassium generally tended to increase significantly when the plants were irrigated with both preparations and sprayed with EM-5 in the spring-summer season. The highest content of magnesium was noted when the plants were irrigated with EM. The inoculation of seeds resulted in the lowest content of this element. The tendencies were similar at both terms of cultivation. As far as the content of microelements is concerned, the application of EM at both terms of cultivation resulted in a significant increasing tendency in

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the content of iron and zinc in the lettuce leaves. The volume of increase depended on the method of application of the preparations.

Keywords: Effective Microorganism (EM), lettuce, nutrients, microelements, macroelements

Introduction

In recent years there has been growing interest in the possibility to apply biopreparations based on Effective Microorganisms (EM) for agricultural and horticultural production. These biopreparations were invented in Japan by Professor Terou Higa [1]. They contain coexisting species of different useful microorganisms and they are increasingly often recommended for organic cultivation. Many scientific centres all over the world are testing the effectiveness of these preparations. Researchers have found that the use of Effective Microorganisms may help to restore the lumpy structure of soil [2], increase the assimilability of macro- and microelements to plants [3], accelerate organic matter humification [4] and limit the process of decay [5]. In many cases the application of Effective Microorganisms increased the count of other useful microorganisms in soil [6]. EM may induce the photosynthetic process, increase plants' natural resistance to stress factors and neutralise the effects of drought [7]. Useful microorganisms can inhibit the development of different pathogenic factors, especially those developing in plants' roots, by releasing different substances, which are toxic to pathogens [8]. The application of Effective Microorganisms to seeds and planting material may accelerate seed germination [9, 10] and improve the development of roots [11]. Effective Microorganisms may positively influence the growth of plants and their florescence [12–14] and increase the yield, as was observed in studies on agricultural crops (peas, maize, wheat, potato) [3, 15–19] and horticultural plants (onion, apple-tree, tomato, saffron) [3, 7, 12, 20]. Better yield quality was also observed [7].

However, as results from studies conducted so far, the effects are not always replicable and they depend on numerous factors, such as the quality of the preparation with Effective Microorganisms, its form and frequency of use [21]. There are also reports on the ineffectiveness of Effective Microorganisms in plant cultivation [21–24].

Biopreparations with Effective Microorganisms may be applied in different ways, *eg* by inoculation of the substrate prepared for cultivation, soil irrigation and spraying. It is also possible to apply them directly to plants by spraying or seed inoculation.

The aim of the study was to assess the influence of different methods of application of Effective Microorganisms (EM) on the content of nutrients in the leaves of lettuce grown under cover.

Material and methods

The research on the influence of Effective Microorganisms (EM) on the content of nutrients (macro- and microelements) and sodium in butterhead lettuce leaves (*Lactuca sativa* 'Sunny') was conducted in 2013 (two independent cultivation cycles in the spring-summer season – May-June and in autumn – October) at the Experimental Station of Departments of the Faculty of Horticulture and Landscape Architecture, Poznan University of Life Sciences. The experiment was conducted in an unheated polytunnel, in a systemic arrangement with ten replicates. During the entire growing season we applied agrotechnical procedures according to current recommendations.

The following methods of EM application were researched: root treatment (1), leaf treatment (2) and seed inoculation (3). The plants which were not treated with EM were used as the control combination. When EM was applied into the roots, plants were irrigated with a 1% solution of the EM-A preparation (Naturally Active EM) or with EM-5 (250 cm^3 of the liquid per plant), depending on the combination. The same preparations concentrated at 1% were sprayed on the leaves. They were applied with a hand-held sprayer 'Orion' equipped with a slot nozzle TeeJet XR 11003 at a constant pressure of 3 atm (conversion dose: 300 dm^3 of working liquid per ha). Seeds were soaked for 30 minutes in a 10% solution of the EM-A preparation immediately before being sown.

During the growing season the plants were sprayed or irrigated four times (in corresponding combinations) at three-day intervals. The preparations were applied on 24 May, 27 May, 30 May and 2 June 2013 in the spring-summer season and on 1 October, 4 October, 8 October and 11 October 2013 in the autumn cycle.

The plants grew in 5 dm^3 pots filled with peat substrate of the following chemical composition ($mg \cdot dm^{-3}$): N 150, P 150, K 175, Mg 150, pH 6.50. The plants were watered when necessary to maintain constant humidity of the substrate.

On the last day of each growing cycle the aerial parts of the plants were collected for chemical analyses. The collected material was dried at 45–50°C and ground. In order to assay the total forms of nitrogen, phosphorus, potassium, calcium, magnesium and sodium, the plant material (1 g) was digested in concentrated (96%, pure per analysis) sulphuric acid (20 cm^3) with the addition of 60 cm^3 of hydrogen peroxide (30%, pure per analysis). For analyses of total iron, manganese, zinc and copper the plant material (2.5 g) was digested in a 30 cm^3 mixture of concentrated nitric (ultra-pure) and perchloric acids (analytically pure) at a 3:1 ratio. After mineralisation of the plant material the following measurements were made: N-total – using the distillation method according to Kjeldahl in a Parnas Wagner apparatus; P – colorimetric analysis with ammonium molybdate; and K, Ca, Mg, Fe, Mn, Zn and Cu – using flame atomic absorption (on an AAS, Carl Zeiss Jena apparatus). The results of the chemical analyses were analysed statistically by means of Duncan's test, at a significance level $\alpha = 0.05$.

Results and discussion

Macroelements and sodium. Both cultivation cycles proved the multidirectional effect of EM on the content of nitrogen in the plants. It improved in the EM-5 combination (both after irrigation and spraying). However, there were no significant differences between the EM-A combination and the control variant (Tables 1 and 2). There was a significantly higher content of phosphorus in the plants when the seeds were inoculated with the EM-A preparation and when the plants were sprayed with the EM-5 in the spring-summer season. The same tendencies were observed in the autumn cycle, but there was significant improvement when the plants were irrigated with the

EM-A. The application of the EM preparations significantly increased the content of potassium in lettuce leaves (as compared with the control combination) and there was also a general increasing tendency for the content of calcium (it was significant after irrigation with both preparations and after spraying the EM-5 in the spring-summer season). There were significant differences in the content of magnesium between the combinations. The highest content was noted when the plants were irrigated with EM, the lowest – after seed inoculation (there were similar tendencies at both terms). The analyses proved significant differences in the content of sodium in lettuce leaves. The smallest content of this ballast ion was noted after irrigation with the EM-A preparation. It was higher in the other combinations.

Table 1

The influence of EM on the chemical composition of lettuce (the spring-summer season)

Combination	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
	[% in d.w.]						[mg · kg^{-1} d.w.]			
Control	2.18 ab	0.51 ab	4.10 a	1.85 a	0.31 ab	0.49 b	99.2 a	214.6 c	49.9 a	6.40 b
EM-A[1]	2.13 a	0.54 bcd	4.80 c	2.00 c	0.33 b	0.36 a	176.3 d	180.8 ab	81.0 cd	6.00 a
EM-A[2]	2.13 a	0.49 a	4.54 b	1.87 ab	0.32 ab	0.54 bc	150.3 c	232.7 d	76.7 bc	5.95 a
EM-5[1]	2.39 d	0.52 abc	4. 95 d	1.94 b	0.33 b	0.57 c	153.5 c	181.3 ab	83.7 d	6.10 a
EM-5[2]	2.31 c	0.55 cd	5.09 e	2.12 d	0.32 ab	0.60 c	179.6 d	175.3 a	74.7 b	5.80 a
EM-A[3]	2.23 b	0.57 d	5.06 e	1.89 ab	0.28 a	0.60 c	133.6 b	183.2 b	85.6 d	5.90 a

Explanation: EM application method: [1] irrigation, [2] spraying, [3] seed inoculation.

Macroelements. In both cultivation cycles after the application of EM the content of iron in lettuce leaves tended to increase, but there were significant differences between the application methods. There were multidirectional changes in the content of manganese – the tendencies were divergent in the research cycles. Like with iron, the content of zinc increased significantly after the application of EM. The tendency was observed in both research cycles. Simultaneously, the content of copper tended to decrease in the spring-summer season. There were multidirectional changes in the autumn cycle.

Table 2

The influence of EM on the chemical composition of lettuce (the autumn season)

Combination	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
	[% in d.w.]						[mg · kg^{-1} d.w.]			
Control	2.10 a	0.47 a	4.15 a	1.92 a	0.27 a	0.57 c	101.7 a	240.9 c	54.4 a	5.6 a
EM-A[1]	2.17 a	0.56 b	4.95 bc	1.95 a	0.32 b	0.42 a	194.1 d	155.9 a	93.9 d	6.3 b
EM-A[2]	2.12 a	0.51 a	4.45 ab	1.87 a	0.28 a	0.51 b	156.2 c	235.4 c	91.3 d	5.8 a
EM-5[1]	2.43 b	0.49 a	4.87 bc	1.89 a	0.34 b	0.49 b	147.3 c	187.6 b	78.9 b	5.7 a
EM-5[2]	2.28 ab	0.53 b	5.02 c	2.03 a	0.29 ab	0.59 c	193.8 d	179.1 b	71.5 b	6.1 b
EM-A[3]	2.21 a	0.56 b	5.07 c	1.99 a	0.27 a	0.56 c	124.7 b	173.4 b	82.6 c	6.2 b

Explanation: EM application method: [1] irrigation, [2] spraying, [3] seed inoculation.

The preparations containing Effective Microorganisms (EM) are mixtures of active microorganisms of biological origin. EM is composed of lactic acid bacteria (*Lactobacillus casei*, *Lactobacillus plantarum*, *Streptococcus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodobacter spae*), yeasts (*Saccharomyces albus*, *Candida utilis*), actinobacteria (*Streptomyces albus*, *S. griseus*) and moulds (*Aspergillus oryzae*, *Mucor hiemalis*) [3, 7]. The application of EM may influence the chemical composition of soil or the substrate in which plants grow. In consequence, plants' nutrition may be affected. According to Mayer et al [25], nitrifying bacteria increase the content of nitrogen, whereas actinobacteria affect the content of phosphorus. This thesis was generally confirmed in the study conducted by Gorski and Kleiber [13], but in many cases the changes observed by the authors were multidirectional.

The research proved the significant influence of Effective Microorganisms on the chemical composition of the aerial parts of lettuce. The research findings positively correspond to earlier data reported by Fraszczak et al [26], who investigated the possibility to apply EM to basil (*Ocimum basilicum* L.) grown in a peat substrate. Sahain *et al* [20] reported that apple-tree leaves collected from the plants treated with EM contained more N, P, K, Mn, Fe and Zn. The yield of plants also improved. Changes in the chemical composition of crops treated with EM may be caused by changes in the chemical composition of the substrate [13] Simultaneously, Gorski and Kleiber [13] applied EM to ornamental plants (roses and gerberas) and observed that their yield improved significantly. The highest yield of flowers was noted when EM was applied to the roots. Simultaneously, the diameter of rose flowers and the number of gerbera leaves increased. The positive effect of EM on the yield of horse-shoe pelargoniums was also proved [27]. The plants treated with EM had more buds and flowers and they bloomed earlier. Studies conducted by other authors [12, 28] also confirmed the positive effect of EM on the yield of other plans species, *ie* saffron and strawberry. Javaid [17] reported that EM applied to peas significantly affected the formation of root nodules. Available sources [29] also point to the stimulating effect of preparations containing EM on the utility parameters (vigour, germination capacity) of beetroot, carrot, tomato and cucumber seeds.

EM may have positive effect on plants' health. Boliglowa and Glen [30] claim that when winter wheat was sprayed with an EM solution, the plants were effectively protected from glume blotch (*Septoria nodorum*) and tan spot (*Drechslera tritici-repentis*). Stepien and Adamiak [31] reported that the EM-1 preparation applied to spring and winter wheat significantly inhibited the development of septoria leaf blotch (*Mycospharella tritici*), glume blotch (*Septoria nodorum*), wheat leaf rust (*Puccinia recondita*), barley powdery mildew (*Blumeria graminis*) and Fusarium ear blight (*Fusarium* spp.). Okorski and Majchrzak [32] proved that EM applied to peas significantly limited the occurrence of *Fusarium* fungi in seeds.

The stimulating effect of microorganisms on the growth of plants may be caused by their secretion of secondary metabolites, growth hormones, phytochelatins, organic acids and B vitamins [19]. Daly and Stewart [3] reported that microorganisms might stimulate some physiological processes in plants. This thesis was confirmed by Xu

Hui-lian et al [15], who proved that EM stimulated photosynthesis and influenced the content of vitamin C and sugars in tomato fruits. Vitamin C plays a key role in controlling the redox potential in plants and it acts directly as an antioxidant capturing reactive oxygen species and as a co-factor for many enzymes [33]. The study by Sahain et al [20] proved that the plants treated with EM had higher content of chlorophyll in leaves and better plant growth parameters (the emergence of new roots, the root length and diameter and the leaf area).

Conclusions

The research proved the influence of EM on the chemical composition of aerial parts of lettuce.

As far as macroelements are concerned, there was higher content of phosphorus after seed inoculation with EM-A and spraying with EM-5 in the spring-summer season. The tendencies were confirmed in the autumn cycle (there was significantly higher content after the irrigation of plants with EM-A).

There was significantly higher content of potassium in lettuce leaves (as compared with the control combination) and there was a simultaneous general increasing tendency in the content of calcium.

The highest content of magnesium was noted when the plants were irrigated with EM. The inoculation of seeds resulted in the lowest content of this element. There were similar tendencies at both terms.

As far as microelements are concerned, the content of iron (significant differences between the methods of application) and zinc tended to increase. Apart from that, there was significant diversification in the content of sodium in lettuce leaves. The smallest content was observed when the plants were irrigated with EM-A. The content was significantly higher in the other combinations. Changes in the content of nitrogen, manganese and copper were multidirectional.

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Abstrakt: Celem przeprowadzonych badań była ocena wpływu zastosowania Efektywnych Mikroorganizmów (EM) na zawartość składników pokarmowych w liściach sałaty (*Lactuca sativa* L. 'Sunny') uprawianej pod osłonami, w okresie wiosenno-letnim i jesiennym. Określono wpływ następujących form aplikowania EM: dokorzeniową, dolistną oraz poprzez zaprawianie nasion. Przy stosowaniu EM dokorzeniowo, rośliny podlewano w zależności od kombinacji 1% roztworem preparatu EM-A lub EM- 5 (250 ml cieczy na 1 roślinę). Do opryskiwania dolistnego wykorzystano wyżej wymienione środki, w stężeniu 1%. Zaprawianie nasion przeprowadzano bezpośrednio przed wysiewem (moczenie przez 30 minut w 10% roztworze preparatu EM-A). W okresie wegetacji przeprowadzono 4-krotnie zabiegi opryskiwania lub podlewania roślin, w odstępach 3 dniowych. W kombinacji kontrolnej nie stosowano Efektywnych Mikroorganizmów. W przeprowadzonych badaniach wykazano wpływ zastosowania EM na zawartość składników pokarmowych w liściach sałaty. Stwierdzono poprawę odżywienia roślin azotem po stosowaniu preparatu EM-5 poprzez podlewanie i opryskiwanie roślin. Jednocześnie wykazano istotną poprawę odżywienia roślin fosforem przy zaprawianiu nasion preparatem EM-A oraz opryskiwaniu roślin środkiem EM-5, w obu terminach uprawy i dodatkowo przy podlewaniu roślin (EM-A) w cyklu jesiennym. Zastosowanie preparatów EM wpływało istotnie na zwiększenie zawartości potasu w liściach sałaty (w relacji do kontroli), przy równoczesnej generalnej tendencji wzrostowej zawartości wapnia (istotnej dla podlewania obydwoma preparatami oraz opryskiwania środkiem EM-5 w terminie wiosenno-letnim). Największą zawartość magnezu oznaczono w przypadku podlewania roślin EM, a najmniejszą dla zaprawiania nasion (tendencje w obydwóch terminach uprawy były zbliżone). W przypadku mikroskładników w obu cyklach uprawowych po zastosowaniu EM zaobserwowano istotną tendencję wzrostową zawartości żelaza oraz cynku w liściach sałaty, a poziom wzrostu był zróżnicowany w zależności od sposobu aplikowania preparatów.

Słowa kluczowe: Efektywne Mikroorganizmy (EM), sałata, składniki pokarmowe, makroskładniki, mikroskładniki