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AB: study idea and design, analysis of results, and writing the manuscript; MKa: conducting the field experiment; MKr: laboratory and statistical analyses

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# **Competing interests**

AB is a member of the Scientific Council of the *Acta Agrobotanica* journal; other authors: no competing interests have been declared

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# **ORIGINAL RESEARCH PAPER**

# Impact of effective microorganisms on weed infestation and yield of peppermint cultivated on muck-peat soil

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# Abstract

Peppermint (Mentha × piperita L.) rootstock cuttings with 9-11 internodes were planted on April 10, 2014 in rows 50 cm apart and with 25-cm distance in the row, on well fertilized muck-peat soil containing 82.1% of organic matter with a pH of 5.9. Peppermint plants were sprayed once with an activated EM-1 preparation, then on two or three further occasions as follows: at 10 cm height (May 10), at branching stage (May 29), and during rapid growth (June 19). EM did not affect peppermint growth or yield. Yields of the fresh and dry herb were high (means: 15,563 and 2,661 kg ha<sup>-1</sup>, respectively) and characterized by a medium (1.85–1.90%) essential oil content in the dry herb. Twenty-nine compounds were identified in the oil and its main components were menthol (53.1-58.5%), menthone (14.6-16.8%), isomenthone (6.3-6.7%), menthyl acetate (4.0-5.0%), germacrene D (2.3-3.4%), ß-caryophyllene (1.8–2.4%), viridiflorol (1.5–2.3%), and 1,8-cyneole (0.3–3.7%). EM did not affect the content of essential oil in the dry herb or the oil composition (except for 1,8-cyneole). Thirty-four days after planting, 22 weed species grew in the experimental plots and the dominant were common meadow grass (Poa pratensis L.) accounting for 20% of total weed population, annual meadow grass (Poa annua L.) 17%, common chickweed [Stellaria media (L.) Vill.] 20%, creeping yellowcress [Rorippa sylvestris (L.) Besser] 8%, hairy galinsoga [Galinsoga ciliata (Raf.) S. F. Blake] 7%, gallant soldiers (Galinsoga parviflora Cav.) 6%, Canadian horseweed [Conyza canadensis (L.) Cronq.] 6%, common groundsel (Senecio vulgaris L.) 5%, and annual nettle (Urtica urens L.) 5%. Other species occurred sporadically. The total number and fresh weight of weeds growing on 1 m<sup>2</sup> were 412 and 246 g on plots treated with EM and 389 and 227 g on control plots, respectively, but the differences were not statistically significant.

#### Keywords

fresh and dry herb; essential oil content and composition

# Introduction

The concept of "effective microorganisms" (EM) was developed by Higa in Japan in the 1970s [1–3]. Today, EM have been adopted in over 100 countries in all continents for commercial production and environmental management. EM comprise a mixture of live natural cultures of microorganisms (mainly lactic acid bacteria, photosynthetic bacteria, and yeasts) that can be applied to improve soil quality and the growth, yield, and quality of crops [3]. In some soils, a single application may be sufficient to produce expected results, whereas for other soils, even repeated applications may appear to be ineffective [4]. There are many articles presenting different aspects of EM application on cultivated plants. Among 22 reports on the effects of EM on the yields of vegetables

reviewed by Olle and Williams [5], 84% were positive, 4% were negative, and 12% showed no significant influence. In the opinion of these authors, EM can improve the quality and yield of vegetables by reducing the incidence of pests and diseases, and by protecting against weed infestation. However, according to Cóndor et al. [6], the studies conducted so far did not prove a significant effect of EM on the yield of cultivated plants, with the exception of those in tropical regions. In a greenhouse experiment conducted by Muthaura et al. [7], soil inoculation with EM did not affect the shoot length and diameter or the number of leaves developed by amaranth (Amaranthus dubians), which is cultivated as a leafy vegetable in African countries. In another greenhouse experiment curried out by Wolna-Maruwka et al. [8], EM did not affect plant height or inflorescence length of French marigold (Tagetes patula L.) grown in a peat substrate. Among nine EM treatments, only substrate watering with EM concentrated at 1:100 and plant spraying at 1:50 increased the number of shoots per plant. Any literature showing the reaction of medicinal plants to EM treatment is scarce. Based upon a complex study curried out on sweet basil (Ocimum basilicum L.) grown in a peat substrate in growth chambers, Frąszczak et al. [9] found that the application of EM resulted in the inhibition of plant growth dynamics, among others, in reduction of plant height and fresh mass. In a 2-year field experiment, Filipović et al. [10] observed usually positive, however, a mostly insignificant and weather-dependent influence of soil and foliar EM application on basil plant height and width, root length, number of inflorescences, yield of fresh and dry biomass, content and yield of essential oil.

Weeds are dangerous competitors to peppermint reducing its herb and oil yield, especially in the first cultivation year [11]. Microorganisms are capable of suppressing weeds in the field and they should be included in alternative weed management strategies [12]. Marambe and Sangakkara [13] found that effective microorganisms enhanced both the number and biomass of weeds growing in tomato plots in the first year of EM application and then these declined in 2 later study years.

Peppermint is one of the most important medicinal plants cultivated in the world [14] and in Poland [15,16]. Lublin Province (central-eastern part of the country) is the main region of its cultivation in Poland [15]. Peppermint is grown mainly for its essential oil (oleum menthae piperitae), and in the natural conditions of Poland, dry mint herb contains from about 0.5% to 1.5% of this oil. The main component of the oil is menthol (>50%) followed by menthon, menthofuran, pulegone, phellandrene, pinene, cineole, piperitone, and other compounds [17].

Peppermint grows well on muck-peat soil [17] and considerable resources of this soil type occur in the Lublin region [18]. However, on muck-peat soil, weed infestation is usually heavier and its control is more difficult than on mineral soil [19]. Wolna-Maruwka et al. [8] stated that EM had a positive effect on the activity of acid phosphatases but not of urease and dehydrogenases in peat substrate.

Taking into consideration that there is no information in the literature about the responses of peppermint to effective microorganisms, the aim of this experiment was to determine the effect of EM preparation on growth and yield of peppermint cultivated on muck-peat soil in the central-eastern part of Poland (Lublin Province). Additionally, the effect of this preparation on weed infestation was evaluated.

### Material and methods

The experiment was carried out on a farm located in central-eastern part of Poland (51°52′ N, 22°49′ E) in 2012. The pepper mint was cultivated on a muck-peat soil developed from sedge/reed peat and utilized for different plant cultivation for 12 years. It contained 82.1% of organic matter and its pH (in H<sub>2</sub>O) was 5.9. The soil was ploughed in November 2013 and then fertilized with 60 kg N ha<sup>-1</sup> supplied as urea, 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (superphosphate), and 120 kg K<sub>2</sub>O ha<sup>-1</sup> (potassium salt) and cultivated at the beginning of April 2014. On April 10, the pepper mint (*Mentha* ×*piperita* L.) rootstock cuttings with 9–11 internodes were planted 5–8 cm deep in rows 50 cm apart and with 25-cm distance in the row. Seventy-two cuttings were planted on 3.0 × 3.0 m plots. The cuttings were obtained from 1-year old peppermint plants cultivated on another field on the farm.

During vegetative growth, the mint plants were sprayed with EM microbiological preparation produced by the authorized Greenland Technology EM, Poland. Directly before spraying, the EM-1 concentrate was activated in the following way retaining proportions referring to 1 ha: 1.0 L of EM-1 was mixed with 4.0 L of water at 30°C and with 60 mL of molasses serving as a substrate for microorganisms. Then, the preparation was maintained in darkness for 12 h at the temperature of 25°C and applied using a backpack sprayer and 400 L water ha<sup>-1</sup>. The plants were sprayed when they attained 10 cm height (May 10), at branching stage (May 29) and during intensive growth (June 19). The following treatments were applied: no spraying (control), one spraying (on May 10), two sprayings (on May 10 and 29), and three sprayings (on May 10 and 29 and on June 19). During spraying, the plots were protected against preparation drift by foil screens. No chemical plant protection against diseases and pests was applied in the experiment.

On May 14, the percentage of soil covering by peppermint plants and weeds and also the composition of the weed flora were determined. Subsequently, weeds growing in four  $25 \times 40$ -cm frames placed randomly between rows were removed, counted, and their fresh weight determined. The plots were then hand weeded. A second weeding was carried out 3 weeks later. The health condition of mint plants was monitored during the whole vegetation period.

Commencing on April 20, the primary shoot length of 20 randomly selected mint plants on each plot was measured every 10 days. The plants were cut by hand at soil surface on July 20 when first inflorescences appeared and the yield of the fresh mint herb was determined. The length and fresh weight of 20 plants from each plot was then measured. In addition, the diameter of the main shoot stem, the number of lateral branches developed on this shoot, the width of leaf blade, and the length of the largest leaf with petiole on the main shoot were all measured. Following this, the plants were dried in a shaded and airy place. On August 21, the weight of 20 dry mint plants and the yield of dry mint herb were determined. On August 25, the content of essential oil in the dry mint herb was determined by direct steam distillation, according to the Polish Pharmacopoeia VI [20]. Two weeks later, the qualitative composition of the mint oil was measured on an ITS-40 detector (system GC/IRMS of the Finningan MAT Co., Germany) using a GC/MS method. The detector was equipped with DB-5 column (J&W Scientific Co., USA) of 30 m length, 0.25 mm diameter, and 0.25 µm stationary phase film thickness. The injector temperature was 280°C. Initially, the 35°C temperature was maintained for 2 minutes and then it was gradually increased to 280°C, retaining an increase of 4°C per minute. Qualitative analysis was carried out by comparison of MS spectra obtained with NIST Atomic Spectra Database and with LIBR (TR) terpenes database delivered by Finnigan MAT. Compound identities were also confirmed on the basis of their retention indices as presented by Joulain and Kőnig [21] and Najda [22].

The field experiment was laid out as a one factor randomized blocks design with four replications. One plot served as one replication. The results were analyzed statistically by analysis of variance, and the significance of differences between means were tested by Tukey's test at 0.05 probability level.

# Results

Thirty-four days after planting, the peppermint plants were about 13 cm high and covered 28% of soil surface. The weeds were in the two-four leaf stage of growth and covered 71% of the soil surface. At that time, 22 weed species grew in the experiment and the dominants were common meadow grass (*Poa pratensis* L.) accounting for 20% of total weed population, annual meadow grass (*Poa annua* L.) 17%, common chickweed [*Stellaria media* (L.) Vill.] 20%, creeping yellowcress [*Rorippa sylvestris* (L.) Besser] 8%, hairy galinsoga [*Galinsoga ciliata* (Raf.) S. F. Blake] 7%, gallant soldier (*Galinsoga parviflora* Cav.) 6%, Canadian horseweed [*Conyza canadensis* (L.) Cronq.] 6%, common groundsel (*Senecio vulgaris* L.) 5%, and annual nettle (*Urtica urens* L.) 5% on average. Common dandelion (*Taraxacum officinale* F. H. Wigg.), creeping thistle [*Cirsium arvense* (L.) Scop.], small-flowered crane's-bill (*Geranium pusillum* L.), marsh

cudweed (*Gnaphalium uliginosum* L.), henbit dead-nettle (*Lamium amplexicaule* L.), purple loosestrife (*Lythrum salicaria* L.), wild chamomile (*Matricaria chamomilla* L.), lamb's quarters (*Chenopodium album* L.), pale smartweed (*Polygonum lapathifolium* L. ssp. *lapathifolium*), redshank (*Polygonum persicaria* L.), sheep's sorrel (*Rumex acetosella* L.), ribwort plantain (*Plantago lanceolata* L.), and common sowthistle (*Sonchus oleraceus* L.) also occurred sporadically. The total number and fresh weight of weeds growing on 1 m<sup>2</sup> were 412 and 246 g on plots treated with EM preparation and 389 and 227 g on control plots, respectively, with the differences being nonsignificant statistically. The plots were finally weeded, but soon afterwards new weed seedlings started to germinate. No effect of EM preparation on this secondary weed infestation was observed.

All mint cuttings rooted and the shoots sprouted 1 week after planting. From the beginning of the vegetative growth phase up to June 10, the length of main shoot increased from 33 to 63 mm on average during 10 days. In the next two 10-day periods, the growth was much more intensive and made 111.0 and 119.0 mm, respectively. At harvest, the mean length of main shoot ranged from 645 mm to 661 mm, and was not dependent on EM application (Tab. 1). At that time, the peppermint plants were well branched (Tab. 2) and covered 98% of the soil surface. There was no space for weeds to grow and their competition was therefore eliminated.

Mint plants were harvested 101 days after planting. At that time the length and the width of the largest leaf ranged from 75 to 78 mm and from 38 to 41 mm, respectively, and the stem diameter ranged from 4.5 to 4.7 mm depending on the treatment. The number of lateral branches developed by one plant ranged from 17.4 to 19.1. There was no effect of EM preparation on these traits (Tab. 2).

Measurement — date	Number of sprayings with EM preparation					
	0	1	2	3	Mean	
April 20	20	22	19	20	20.3	
April 30	62	66	60	62	62.5	
May 10	92	100	91	97	95.0	
May 20	159	161	153	160	158.3	
May 30	210	215	204	215	211.0	
June 10	275	271	265	269	270.0	
June 20	384	381	378	380	380.8	
June 30	503	501	497	498	499.8	
July 10	582	587	583	590	585.5	
July 20	645	659	648	661	653.3	

**Tab. 1** Effect of EM preparation on main shoot length (mm).

LSD<sub>0.05</sub> (for last measurement): 2.73.

**Tab. 2** Leaf length and width (mm), stem diameter (mm), and number of lateral branches on plant as affected by the number of applications with EM preparation.

Measured – feature	Numb	_			
	0	1	2	3	Mean
Leaf length	76.0	75.0	77.0	78.0	76.5
Leaf width	40.0	38.0	40.0	41.0	39.8
Stem diameter	4.7	4.5	4.7	4.6	4.6
Lateral branches	18.9	17.5	17.4	19.1	18.2

LSD<sub>0.05</sub> leaf length and width, stem diameter, lateral branches: not significant.

Mint root stock cuttings planted in the experiment were of good quality and therefore the shoots growing from them were healthy and in good condition. No pest or disease symptoms were observed on mint plants during the whole vegetation period.

The fresh weight of the aerial parts of the mint plants measured directly after harvest ranged from 186.7 to 204.5 g and did not depend on treatment with the EM preparation. Similarly, the yield of fresh mint herb (14,936.2–15,950.1 kg ha<sup>-1</sup>) and dry herb (2,558.4–2,759.1 kg ha<sup>-1</sup>) as well as the content of essential oil in dry herb (1.85–1.90%) and the oil yield (47.3–52.1 kg ha<sup>-1</sup>) were all not dependent on spraying with EM preparation (Tab. 3).

**Tab. 3** Plant fresh weight (g), yield of fresh and dry herb (kg  $ha^{-1}$ ), content of essential oils in the dry herb (%), and yield of essential oils (kg  $ha^{-1}$ ) as affected by the number of applications with EM preparation.

	Numb				
- Measured feature	0	1	2	3	Mean
Plant weight	193.5	186.7	197.5	199.8	194.4
Fresh herb yield	15,532.4	14,936.2	15,831.6	15,950.1	15,562.6
Dry herb yield	2,583.7	2,558.4	2,741.3	2,759.1	2,660.6
Oil content	1.86	1.85	1.90	1.88	1.87
Oil yield	48.1	47.3	52.1	51.9	49.9

LSD<sub>0.05</sub> plant weight, fresh and dry herb yield, content and yield of essential oil: not significant.

Twenty-nine compounds were identified in the essential oil obtained from the mint plants (Tab. 4). Menthol was the dominant one (53.1–58.5%) in all treatments. It was followed by menthone (14.6–16.8%), isomenthone (6.3–6.7%), menthyl acetate (4.0–5.0%), germacrene D (2.3–3.5%), ß-caryophyllene (1.8–2.4%), viridiflorol (1.5–2.3%), 1,8-cineole (0.3–3.7%), *trans*-sabinene hydrate (1.6–1.8%), 4-terpineol (0.9–1.1%), and isomenthone (0.9–1.0%). The contents of other components were <1%. The highest content of menthol in the essential oil was detected in plants treated with EM preparation two and three times: 57.3% and 58.5%, respectively. The lowest content of this component was in the essential oil obtained from control plants – 53.1%. Oil composition was not affected by EM treatment. Only the content of 1,8-cineole in the essential oil obtained from control plants much higher than that in the oil obtained from plants treated with EM preparation two or three times (0.3%) (Tab. 4).

# Discussion

The weed flora and density recorded in the experiment were similar to those observed by Borowy and Kossowski [19] in celery cultivated on muck-peat soil in the same region. Mechanical weeding of peppermint was labor-consuming. Weeds cut with a hoe had to be removed from the plots otherwise they rooted again. Moreover, frequent disturbance of peat-muck soil surface with weeding tools causes its drying and pulverization [18]. The results we obtained confirm the opinion of the above cited authors that weed control on muck-peat soil is more difficult than on mineral soil [18,19]. Hence, the possibility of using microorganisms [12] or effective microorganisms [13] for weed control would be of significant value. In our study, there was no effect of EM preparation on weed flora as well as on number and fresh weight of weeds in the first year after EM application which was contrary to the data collected by Marambe and Sangakkara [13]. Peppermint plants grew fast (Tab. 1) and competed fairly well with the weeds, especially in the second half of the vegetation period. According to Karkanis et al. [11], after the establishment year, peppermint plants become more competitive reducing the need for weed control.

Oil components	IR	0 (%)	1 (%)	2 (%)	3 (%)	 Mean (%)
a-Pinene	931	0.3	0.2	0.1	0.1	0.18
Sabinene	971	0.1	0.1	0.1	0.2	0.13
ß-Pinene	975	0.4	0.2	0.3	0.1	0.25
Limonene	1,027	0.3	0.2	0.1	0.2	0.20
1,8-Cineole	1,029	3.7	3.7	1.3	0.3	2.25
γ-Terpinene	1,057	0.3	0.2	0.1	0.0	0.15
trans-Sabinene hydrate	1,066	1.8	1.6	1.7	1.8	1.73
Linalol	1,099	0.3	0.3	0.5	0.4	0.38
Menthone	1,154	16.8	16.7	15.1	14.6	15.80
Isomenthone	1,165	6.4	6.3	6.5	6.7	6.48
ß-Terpineol	1,169	0.2	0.3	0.3	0.4	0.30
Menhol	1,175	53.1	55.4	57.3	58.5	56.08
4-Terpineol	1,179	0.9	1.1	1.1	0.9	1.00
Isomenthone	1,185	1.0	0.9	0.9	1.0	0.95
a-Terpineol	1,192	0.2	0.3	0.3	0.3	0.28
Piperitone	1,257	0.5	0.6	0.5	0.7	0.58
Neomenthyl acetate	1,275	0.2	0.1	0.3	0.2	0.20
Menthyl acetate	1,293	4.1	4.2	5.0	4.1	4.35
Isomenthyl acetate	1,309	0.2	0.1	0.2	0.2	0.18
ß-Bourbonene	1,386	0.3	0.4	0.3	0.2	0.3
ß-Caryophyllene	1,421	2.2	1.8	2.2	2.4	2.15
cis-ß-Farnesene	1,456	0.2	0.2	0.3	0.4	0.28
Germacrene D	1,484	2.8	2.3	2.6	3.5	2.80
Bicyclogermacrene	1,500	0.5	0.4	0.4	0.5	0.45
δ-Cadinene	1,526	0.1	0.2	0.2	0.1	0.15
Spathulenol	1,583	0.3	0.3	0.2	0.2	0.25
Caryophyllene oxide	1,588	0.2	0.2	0.1	0.3	0.20
Viridiflorol	1,597	2.3	1.6	1.6	1.5	1.75
Caryophyllene oxide isomer	1,659	0.3	0.2	0.3	0.2	0.25

Tab. 4 Composition of peppermint essential oils as affected by the number of applications with EM preparation.

Different authors stated positive, negative, or a nonsignificant influence of effective microorganisms on plant growth and yield depending on the species, cultivation method, EM application method, and the prevailing environmental conditions [5-10,13]. In the our study, EM preparation did not affect the growth and yield of peppermint plants cultivated in the field on peat-muck soil (Tab. 1-Tab. 3). Similarly, Muthaura et al. [7] did not find a significant influence of EM on amaranth shoot length and diameter and Wolna-Maruwka et al. [8] for several growth traits of French marigold plants grown in a peat substrate under controlled greenhouse conditions. In our experiment, main shoot length, leaf length and width, plant weight, essential oil content, yield of oil and yield of fresh and the dry herb were higher in response to double EM spraying than in control plots. However, these differences were not significant (Tab. 1-Tab. 3). Furthermore, Filipović et al. [10] usually observed an insignificant positive influence of EM application on basil height, width, and yield of fresh and dry biomass under field cultivation. In this study, peppermint was planted on well-fertilized peat-muck soil containing 82.1% of organic matter and was harvested 100 days later. According to Frąszczak et al. [9], application of EM to spice plants grown on substrates rich in humus and macroelements and characterized by a short cultivation period failed to have any positive effects in the form of improved yield. In some soils it takes longer for

the introduced microorganisms to adapt to a new suite of ecological and environmental conditions and to become well established as a stable, effective, and predominant part of the indigenous soil microflora [4]. Fresh weight of peppermint plants and the yield of the fresh and dry herb were higher (Tab. 3) than those obtained by Najda [16] and Węglarz and Załęcki [23] and a little lower than the fresh and dry herb yield obtained by Rosłon et al. [24] on mineral soils. This confirmed the opinion of Rumpel [18] about the high yielding of temperate climate plants in cultivation on muck-peat soil. The yield of the dry herb and essential oils (Tab. 3) were similar to those recorded by Karkanis et al. [11] in Greece and a little lower than those obtained by Zhelyazkov et al. [25] in the hot, humid environment of the Southeastern United States. Yield of essential oils was higher than that obtained by Węglarz and Załęcki [23] in the natural conditions of Poland.

The combined content of essential oils in the peppermint dry herb ranged from 1.85% to 1.90% and was similar to that reported by Pourhadi et al. [26], but lower than that by Rosłon et al. [24], Karkanis et al. [11], and Lafmejani et al. [27] and much higher than that given by Zheljazkov et al. [25]. It was not dependent on any application of EM preparation (Tab. 3). Furthermore, the yield and composition of peppermint essential oils were not affected by EM application (except for 1,8-cyneole) (Tab. 3, Tab. 4). There is no information in the literature on this topic. The main component of the oil was menthol (Tab. 4) accounting for >50% of the oils, which agrees with the data of Kołodziej [17], and is more than that reported by several other authors [23,25–27]. Our results obtained in this experiment are in line with Higa and Parr's [4] statement that for some soils, even repeated application of effective microorganisms can be ineffective.

# Conclusions

Peppermint plants planted as rootstock cuttings on well-fertilized muck-peat soil grew vigorously and produced high yield of fresh and dry herb containing a medium amount of essential oils during 100 days of vegetation growth. Effective microorganisms applied once, twice, or three times did not affect the growth, the yield of fresh and dry herb, or the essential oil content and composition. Weed infestation occurring in the peppermint crop was not influenced by effective microorganisms.

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# Wpływ efektywnych mikroorganizmów na zachwaszczenie oraz plonowanie mięty pieprzowej uprawianej na glebie torfowej

#### Streszczenie

Sadzonki rozłogowe podziemne mięty pieprzowej (Mentha ×piperita L.) pozyskane z roślin jednorocznych i mające 9-11 międzywęźli, sadzono 10 kwietnia w rozstawie 25 × 50 cm na glebie torfowej, zawierającej 82,1% m. o. i o pH 5,9, nawiezionej nawozami mineralnymi. Rośliny mięty były opryskiwane aktywowanym preparatem EM-1 jeden raz, dwa razy lub trzy razy w następujących terminach: po osiągnięciu 10 cm wysokości (10 maja), w fazie rozgałęziania (29 maja) i podczas intensywnego wzrostu (19 czerwca). Nie stwierdzono wpływu preparatu EM na wzrost i plonowanie mięty. Uzyskane plony świeżego i suchego ziela były wysokie (odpowiednio średnio 15562,6 i 2660,6 kg ha-1) i charakteryzowały się średnią zawartością olejku eterycznego (1,85–1,90%) w suchym zielu. W olejku tym zidentyfikowano 29 składników, przy czym dominującymi były mentol (53,1-58,5%), menton (14,6-16,8%), izomenton (6,3-6,7%), octan mentylu (4,0-5,0%), germakren D (2,3-3,4%), ß-kariofilen (1,8-2,4%), wiridiflorol (1,5-2,3%) i 1,8-cyneol (0,3-3,7%). Preparat EM nie miał wpływu na zawartość olejku w suchym zielu, ani na jego skład (oprócz 1.8 cyneolu). W doświadczeniu wystapiły 22 gatunki chwastów, przy czym dominującymi były: wiechlina roczna (Poa annua L.) stanowiąca 20% całej populacji chwastów, wiechlina łąkowa (Poa pratensis L.) - 17%, gwiazdnica pospolita [Stellaria media (L.) Vill.] - 20%, rzepicha leśna [Rorippa sylvestris (L.) Besser] – 8%, żółtlica owłosiona [Galinsoga ciliata (Ref.) S. F. Blake] – 7%, żółtlica drobnokwiatowa (Galinsoga parviflora Cav.) – 6%, przymiotno kanadyjskie [Conyza canadensis (L.) Cronq.] - 6%, starzec zwyczajny (Senecio vulgaris L.) i pokrzywa żegawka (Urtica urens L.) - 5%. Pozostałe gatunki chwastów występowały sporadycznie. Liczba i świeża masa chwastów rosnących na 1 m² poletka traktowanego preparatem EM wynosiła odpowiednio 412 i 246 g w porównaniu do 389 i 227 g na poletkach kontrolnych, przy czym stwierdzone różnice były nieistotne.