

## CHEMICAL AND NONCHEMICAL CONTROL OF WEEDS IN THE CULTIVATION OF LEMON BALM FOR SEEDS

Marcela Krawiec<sup>1</sup>✉, Andrzej Borowy<sup>2</sup>, Katarzyna Dzida<sup>1</sup>

<sup>1</sup>Subdepartment of Plant Nutrition, University of Life Sciences, Głęboka 28, 20-612 Lublin, Poland

<sup>2</sup>Subdepartment of Pomology, Nursery and Enology, University of Life Sciences, Głęboka 28, 20-612 Lublin, Poland

### ABSTRACT

The fight against weeds in the organic cultivation of herbal plants, both raw and for seeds, is a big challenge for growers. The paper compares the chemical (bentazon, 960 g·ha<sup>-1</sup> and fluazifop-P-butyl, 150 g·ha<sup>-1</sup>) and nonchemical (scattering mustard seed meal in two doses 1.5 and 3.0 t·ha<sup>-1</sup>) control of weeds in the cultivation of lemon balm for seeds. Additionally, the influence of these factors on the yield of fresh herb and seeds of lemon balm and the sowing value of seeds was evaluated. The best method to control the weed infestation was to use mustard seed meal in an amount of 3.0 t·ha<sup>-1</sup>. Scattering of mustard seed meal in that dose reduced the number and weight of weeds on average from two years of research by 52.1 and 60.2% in relation to unweeded control. Following methods were slightly less effective: bentazon and application of mustard seed meal in quantity of 1.5 t·ha<sup>-1</sup>. The largest yield of fresh lemon balm herb was collected from plots where mustard seed meal was used in an amount of 3.0 t·ha<sup>-1</sup>. Seeds harvested from plots, on which mustard seed meal was used in a larger quantity was characterized by the lowest sowing value determined by the lowest energy and capacity of germination and the highest share of nongerminated seeds. Lowering the amount of used mustard seed meal to 1.5 t·ha<sup>-1</sup> significantly improved their sowing value. Although this method was less effective in counteracting weeds than using larger dose, it provided comparable effects of reducing the number and weight of weeds in relation to chemical protection.

**Key words:** lemon balm seed, mustard seed meal, herbicide, weed control, germination

### INTRODUCTION

In many countries of the world, organic cultivation of herbal plants is becoming more and more popular among growers due to higher profitability of production compared to conventional cultivation [Carruba and Militello 2013, Mihajlov et al. 2013]. Herbal raw material from organic cultivation is sought after on the market because it is free of pesticides and heavy metals and is valued due to higher content of biologically active substances [Kazimierzczak et al. 2014, Seidler-Łożykowska et al. 2015].

In the organic cultivation of herbal plants, a great challenge for growers is the fight against weeds [Bàr-

beri 2002, Kalinova 2010]. In cultivation applying this method, the use of synthetic herbicides is not allowed. The weeds of organic plantations are limited by the use of appropriate crop rotation, green manures, mulching, cover crops, balanced fertilization program, timely tillage and cultivation, mechanical and thermal weed control [Bond and Grungy 2001, Bàrberi 2002, Kalinova 2010]. Sometimes these methods are insufficient and it is necessary to introduce manual weeding, which increases the production costs and makes the cultivation less profitable. Weeds cause significant yield losses and reduce the commercial value of har-

✉ marcela.krawiec@up.lublin.pl

vested raw material. The average loss of weight of the crop that occurs when the weeding is given up reaches up to 30–90% [Carrubba et al. 2015]. Therefore, there is an urgent need to develop new methods of safe weed control, possible to use in organic farming.

Research indicates that one of such methods may be the use of seed meal of plants from the *Brassicaceae* family. In the literature there are available studies on the use of seed meal in the laboratory and in greenhouse conditions, while only a few have been carried out in field conditions [Rice et al. 2007, Snyder et al. 2009, Boydston et al. 2011].

Seed meal of the *Brassicaceae* family has a negative effect on seed germination and weed growth [Bialy et al. 1990, Vaughn et al. 2006, Boydston et al. 2008, 2011]. Their herbicidal action is attributed to the content of biologically active compounds with allelopathic properties. Substances responsible for the allelopathic potential are glucosinolates contained in seeds or their breakdown products [Oleszek 1987, Bialy et al. 1990]. Glucosinolates are organic anions containing glucose and sulfur located in cell vacuoles. As a result of the disruption of cell walls, glucosinolates are hydrolyzed by the enzyme myrosinase. Glycosinolate degradation products include isothiocyanates, thiocyanates, nitriles, epithionitriles and oxazolidinones. Many of them are biologically active. Some of them have lethal properties for living organisms, such as insects, fungi and bacteria. Others may have herbicidal activity [Vaughn 1999, Vaughn and Berhow 2005]. It was found that species of *Brassicaceae* family differ in their herbicidal properties, which is related to the content of various glucosinolates and their breakdown products. A review of available studies indicates that the most effective ones are Indian mustard (*Brassica juncea* L.) Czern., mustard (*Sinapis alba* L.), field pennycress (*Thlaspi arvense* L.), money plant (*Lunaria annua* L.) [Vaughn et al. 2006, Rice et al. 2007, Snyder et al. 2009, Handiseni et al. 2011].

Studies have shown that the use of seed meal of the *Brassicaceae* family as a soil additive affects the reduction in the number and weight of monocotyledonous and dicotyledonous weeds. Yu and Morishita [2014] as a result of the use of mustard seed meal found a decrease in emergence of kochia (*Kochia scoparia* L.), common lamb's quarters (*Chenopodium album* L.) and barnyardgrass (*Echinochloa crus-galli* L.) Beauv.), as well

as a decrease in the dry matter of aboveground parts of green foxtail (*Setaria viridis* L.) Beauv.) and common lamb's quarters. In research carried out by Boydston et al. [2008], mustard seed meal applied to the soil surface in the cultivation of container ornamental plants: rose, phlox and coreopsis, limited the number of annual bluegrass seedlings (*Poa annua* L.) and common chickweed (*Stellaria media* L.).

Research by Handiseni et al. [2011] showed that the species used for the control and the dose of used meal are of great importance for reducing the number and weight of weeds. The Indian mustard showed significantly better herbicidal effectiveness in relation to monocot weeds than mustard, which was more effective in controlling the number of dicotyledonous weeds. In limiting the emergence of wild oat (*Avena fatua* L.) seedlings, Italian ryegrass (*Lolium perenne* L.), prickly lettuce (*Lactuca serriola* L.) and redroot pigweed (*Amaranthus retroflexus* L.), a 2000 kg·ha<sup>-1</sup> dose than 1000 kg·ha<sup>-1</sup> was more effective. In all cases, the application of a dose of 1000 kg·ha<sup>-1</sup> meal of Indian mustard and mustard showed a greater herbicidal effect than the rape dose of 2000 kg·ha<sup>-1</sup>. In the studies of Vaughn et al. [2006], increased concentration of meal from seeds of 15 species from the *Brassicaceae* family from 0.1 to 1.0% in the substrate caused a reduction in germination of wheat (*Triticum* L.) and sicklepod (*Senna obtusifolia* L.) H.S.Irwin & Barneby) seeds.

The possibility of using *Brassicaceae* seed is dependent on its effect on crop plants. The seed meal with its herbicidal action must not have a destructive effect on the emergence and yield of crop plants. Research performed by Boydston et al. [2008] showed that mustard seed meal did not damage the rose, phlox and coreopsis plants in the container cultivation and did not affect their growth and flowering.

Effective use of seed meal requires knowledge of the time and environmental conditions necessary to reduce the hydrolysis products – glucosinolates to a non-toxic level. The use of *Brassica juncea* L.) Czern., *B. napus* L.) and *Sinapis alba* L. seed meal in a dose of 1 and 2 t·ha<sup>-1</sup> 36 days before sowing the carrot seeds did not worsen the emergence of seedlings. However, shortening the time from scattering the meal to sowing the carrot seeds to 15 days significantly influenced the reduction of carrot emergence. As the authors point out, the greater interval between

the application of seed meal and the sowing of carrot seeds combined with a higher amount of rain that year caused losses of compounds responsible for herbicidal activity of the meal due to volatilization and leaching from the germination zone [Snyder et al. 2009].

One of the medicinal plants cultivated with organic methods is lemon balm (*Melissa officinalis* L.) [Mihajlov et al. 2013, Seidler-Łożykowska et al. 2015]. It is a perennial bushy plant in the family *Lamiaceae* and native to southern Europe and the Mediterranean region. Lemon balm grows to a maximum height of about 1 m. The leaves with a mild lemon scent are used as a herb, in teas, and also as a flavouring. Lemon balm has been traditionally used for different medical purposes as antispasmodic, carminative, diaphoretic, sedative, but in modern pharmacology is value in the management of mild to moderate Alzheimer's, against migraine and rheumatism, antiviral and antioxidant activities [Moradkhani et al. 2010, Shakeri et al. 2016]. Lemon balm is widely cultivated in Europe and the United States [Moradkhani et al. 2010, Winarczyk et al. 2016]. The species is mainly propagated by seeds that are very small (1.5 to 2.0 mm long). The increase in the area of organic farming of herbal plants entails an increase in the demand for seed material. According to the principles of organic farming, only seeds or vegetative propagating material produced using organic methods may be used to establish organic plantations [Eu Reg. 2092/91].

Weeds in the cultivation of lemon balm not only reduce the yield of herb and deteriorate its quality. Additionally, weeds on seed plantations contribute to contamination of the reproduced sowing material of cultivated plant with weed seeds. In the case of such small seeds, which are lemon balm ones, the occurrence of weed seeds that are difficult to separate causes the necessity of repeating the cleaning process several times or using expensive devices. This often leads to large losses of the cleaned plant seeds, it is time- and cost-intensive. These treatments may cause an increase in mechanical damage to seeds that impair their sowing value [Tworkowski 2000]. Therefore, on lemon balm seed plantations should be counteracted with weeds.

The aim of presented study was to assess the effect of soil application of mustard seed meal on the weed infestation of lemon balm plantation, the yield of fresh

herb and seed of lemon balm and the sowing value of seeds. An interesting issue was the comparison of the effectiveness of this method of weed control with chemical protection using agents authorized in the cultivation of lemon balm.

## MATERIAL AND METHODS

The research was carried out in 2016–2017 on two-year lemon balm plantations located in Experimental Farm Felin – Lublin (51°23'N, 22°56'E). Plantations were established from sowing the seeds directly to permanent place in April 2015 and 2016. The experiment was set up on the podzolic soil developed from loess formations located on Cretaceous marls, containing 2.23% organic matter and pH 7.05. The seed sowing standard was 5 kg·ha<sup>-1</sup>. In the first year of cultivation, the plants were growing vegetatively. In the early spring of 2016 and 2017, mechanical soil loosening was carried out.

The experiment was planned as a single-factor, in three random blocks. The following methods were used to fight weeds:

- spraying the plantation with bentazon (Basagran 480 SL, BASF) at the dose of 960 g·ha<sup>-1</sup>, using water in the amount of 300 l·ha<sup>-1</sup>,
- spraying the plantation with fluazifop-P-butyl (Fusilade Forte 150 EC, Syngenta) at the dose of 150 g·ha<sup>-1</sup> using water in the amount of 300 l·ha<sup>-1</sup>,
- scattering the mustard seed meal (*Sinapis alba* L.) on the surface of plots in the amount of 1.5 t·ha<sup>-1</sup> and shallow mixing with the soil,
- scattering the mustard seed meal (*Sinapis alba* L.) on the surface of plots in the amount of 3.0 t·ha<sup>-1</sup> and shallow mixing with the soil.

The mustard seeds of variety 'Borowska' from Małopolska Hodowla Roślin Sp. z o.o. were used. The seeds were ground in a universal chopper "Bak" H-111/1 on sieves with a diameter of 1.5 mm. Chemical protection was carried out using agents authorized in the conventional cultivation of lemon balm in Poland [IPP 2016]. The aforementioned methods of combating the weeds were compared with untreated control. The area of each plot was 8 m<sup>2</sup>. There were 10 rows of plants 2 m long at 0.4 m spacing.

On April 19, 2016 and on April 21, 2017, mustard seed meal was sprinkled on randomly selected plots.

Herbicides (bentazon and fluazifop-P-butyl) were applied on May 19, 2016 and on May 22, 2017, when the majority of weeds were in the 2-4 leaf phase. Herbicides were sprayed using a backpack sprayer mounted with XR TeeJet® 80° nozzle at 1.5 bars pressure and 300 l of water · ha<sup>-1</sup>. Weed assessment was carried out on May 31, 2016 and June 2, 2017. For this purpose, two frames with dimensions of 0.2 × 0.5 m were placed on a randomly separated place on each plot. This means that the number of weeds, their species structure and fresh weight were determined on each plot on the area of 0.2 m<sup>2</sup>.

At the beginning of flowering period, the lemon balm herb was collected by hand, from the area of 1 m<sup>2</sup> of each plot. The yield of fresh herb was estimated. At the beginning of September, when the plants began to dry up, 15 measurements of the height of shoots were made in each plot. When most of the seeds on plants were ripe, a harvest of seed shoots was carried out. On September 15, 2016 and on September 18, 2017, seed shoots were cut out from the area of 4 m<sup>2</sup> of each plot and placed in a ventilated area for drying. When the plants were dried, threshing was carried out using a threshing machine. The seed yield from the plot was determined. A month after the threshing, the sowing value of seeds was determined: 1000 seeds weight, germination energy and capacity, share of abnormal seedlings and share of nongerminated seeds depending on the applied method of weed control. Seed germination parameters were determined using a standard germination test [ISTA 2004]. The test was performed in 4 replicates of 100 seeds. Seeds germinated on the filter paper in rolls in a thermostat at 20°C in the light for 21 days. Seven days after planting the seeds on the paper, the energy of germination was recorded (so-called “first counting”). It is the percentage of normally germinated seeds 7 days after planting relative to the total number of seeds tested. After 21 days, the final number of normally germinated seeds was measured (so-called “last counting”). It allowed to determine the germination capacity [Kubisz et al. 2012]. When evaluating the germination of seeds, abnormal seedlings and nongerminated seeds were measured.

In order to determine the weight of 1000 seeds, 8 × 100 seeds from each method of weed control were counted. Each replicate was weighed on the analyti-

cal balance with an accuracy of 0.0001 g. The mass of 1000 seeds was the average mass of 100 seeds from 8 repetitions multiplied by 10.

The results obtained were statistically processed using the analysis of variance ANOVA (Statistica ver. 13.1). Confidence intervals were determined by Tukey’s test at the significance level 0.05.

## RESULTS

The results presented indicate that in both years of research, the use of mustard seed meal and spraying with herbicide bentazon had a significant effect on reducing the number of weeds in relation to the unweeded control. The number of weeds in plots treated with fluazifop-P-butyl herbicide was smaller than on control plots, but the difference was insignificant.

In 2016 and 2017, the largest number of weeds was collected on unweeded plots (466 and 398 pcs · m<sup>-2</sup>, respectively). The smallest number of weeds was recorded in plots, where the mustard seed meal was used in amount of 3.0 t · ha<sup>-1</sup> (193 and 217 pcs · m<sup>-2</sup>, respectively). In comparison with the control, significantly less weeds were also found on the plots where bentazon was used (225 and 256 pcs · m<sup>-2</sup>) and where the mustard seed meal was spread in quantity of 1.5 t · ha<sup>-1</sup> (284 and 278 pcs · m<sup>-2</sup>) (Tab. 1).

In 2016, no significant effect of the weeding method on the fresh weed weight was recorded, which was related to large differences in the weight of weeds between repetitions. Weeds harvested from untreated plots had the largest weight (on average 400 g · m<sup>-2</sup>). The weeds of the lowest weight were collected from plots where 3 tons of mustard seed meal per 1 ha were spread. It was on average 110 g · m<sup>-2</sup>. In the second year of research, a significant effect of the weeding method on the fresh weed weight was found. The largest weight of weeds was recorded on unweeded plots (303.3 g · m<sup>-2</sup>). The use of bentazon and a double dose of mustard seed meal had significant effect on the reduction in fresh weight of weeds relative to the control (Tab. 1).

In both experimental years, the most effective method of combating the weeds was the use of mustard seed meal in the amount of 3.0 t · ha<sup>-1</sup>. Applying this method resulted in the reduction of the number of weeds in 2016 and 2017 by 58.6% and 45.5% respectively, and fresh weed weight by 72.5% and 47.8% in

**Table 1.** Effect of weed control method on number and fresh weight of weeds in the lemon balm field trial in 2016–2017

Treatments	Number of weeds per m <sup>2</sup>		Fresh weight of weeds (g·m <sup>-2</sup> )	
	2016	2017	2016	2017
Bentazon 960 g·ha <sup>-1</sup>	225 a	256 ab	208.3 a	173.0 a
Fluazifop-P-butyl 150 g·ha <sup>-1</sup>	295 ab	330 bc	203.3 a	263.3 ab
Mustard seed meal 1.5 t·ha <sup>-2</sup>	284 a	278 ab	210.0 a	216.7 ab
Mustard seed meal 3.0 t·ha <sup>-2</sup>	193 a	217 a	110.0 a	158.3 a
Control	466 b	398 c	400.0 a	303.3 b

Means followed by the same letter within a column do not differ significantly at P = 0.05

**Table 2.** Weed infestation in lemon balm cultivation in dependence on mustard seed meal application and spraying with herbicides in 2016

Weed species	Weed control method				
	Control	Bentazon 960 g·ha <sup>-1</sup>	Fluazifop-P-butyl 150 g·ha <sup>-1</sup>	Mustard seed meal 1.5 t·ha <sup>-1</sup>	Mustard seed meal 3.0 t·ha <sup>-1</sup>
			no. per m <sup>2</sup>		
<i>Amaranthus retroflexus</i> L.	18	–	10	8	3
<i>Anthemis arvensis</i> L.	2	–	2	–	–
<i>Artemisia vulgaris</i> L.	–	–	–	2	–
<i>Capsella bursa-pastoris</i> (L.) Medik.	193	27	112	75	47
<i>Chenopodium album</i> L.	27	5	10	10	–
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	45	75	10	65	83
<i>Elymus repens</i> (L.) Gould	–	1	–	–	3
<i>Epilobium roseum</i> Schreb.	1	–	2	–	–
<i>Erigeron annuus</i> L.	1	–	–	–	–
<i>Conyza canadensis</i> (L.) Cronq.	5	–	–	–	–
<i>Galinsoga quadriradiata</i> Ruiz et Pav.	78	18	48	23	18
<i>Gnaphalium uliginosum</i> L.	–	–	2	–	–
<i>Lamium amplexicaule</i> L.	10	–	5	–	–
<i>Myosorus minimus</i> L.	1	–	–	–	–
<i>Poa annua</i> L.	75	97	90	95	37
<i>Polygonum aviculare</i> L.	–	–	–	3	–
<i>Polygonum persicaria</i> L.	–	–	2	–	–
<i>Rorippa sylvestris</i> L.	2	–	–	–	–
<i>Senecio vulgaris</i> L.	2	–	–	–	–
<i>Sonchus arvensis</i> L.	1	–	–	1	–
<i>Stellaria media</i> (L.) Vill.	2	–	2	–	–
<i>Urtica urens</i> L.	–	–	–	–	2
<i>Veronica persica</i> Poir.	3	1	–	2	–
<i>Viola arvensis</i> Murr.	–	1	–	–	–

relation to the control. Slightly less effective methods of weed control was bentazon spraying and the use of mustard seed meal in the amount of 1.5 t·ha<sup>-1</sup> (Tab. 1).

In both years of research, the weeds occurring on the plots were mainly dicotyledonous (Tab. 2, 3). Among the monocotyledonous plants, the presence of annual bluegrass, barnyardgrass and couch grass (*Elymus repens* L.) was found. Species composition of weeds in individual experimental plots was similar in both years of research. The structure of weeds on control plots was dominated by: shepherd's purse (*Capsella bursa-pastoris* L.), shaggy soldier (*Galinsoga quadriradiata* Ruiz et Pav.), annual bluegrass. On the plots where bentazon was used, following weeds were in the highest number: annual bluegrass, barnyardgrass. Plots sprayed with fluazifop-P-butyl were dominated by: shepherd's purse, annual bluegrass, shaggy soldier. In plots, where the mustard seed meal was used in both doses, the following weeds occurred

most severely: barnyardgrass, shepherd's purse, annual bluegrass (Tabs 2, 3).

Compared to the control, the use of bentazon had the greatest impact on the reduction of the number of dicotyledonous weeds: shepherd's purse (in 2016 and 2017, respectively, 86.0% and 75.0%), common lamb's quarters – *Chenopodium album* L. (81.5% and 91.9%), shaggy soldier (76.9% and 59.6%). The application of this herbicide has also contributed to the elimination or reduction of the number of weeds present in control plots in lower intensity or individually (Tabs 2, 3). On the plots sprayed with this agent, an increase in the number of plants was noted: annual bluegrass (in 2016 and 2017 by 29.3% and 63.5%, respectively) and barnyardgrass (by 66.7% and 118.4%).

The species susceptible to the action of fluazifop-P-butyl are plants of the *Poaceae* family with the exception of annual bluegrass. The use of this prepara-

**Table 3.** Weed infestation in lemon balm cultivation in dependence on mustard seed meal application and spraying with herbicides in 2017

Weed species	Weed control method				
	Control	Bentazon 960 g·ha <sup>-1</sup>	Fluazifop-P-butyl 150 g·ha <sup>-1</sup>	Mustard seed meal 1.5 t·ha <sup>-1</sup>	Mustard seed meal 3.0 t·ha <sup>-1</sup>
	no. per m <sup>2</sup>				
<i>Amaranthus retroflexus</i> L.	15	-	22	5	5
<i>Anthemis arvensis</i> L.	-	-	2	-	-
<i>Artemisia vulgaris</i> L.	-	-	-	-	2
<i>Capsella bursa-pastoris</i> (L.) Medik.	168	42	83	92	77
<i>Chenopodium album</i> L.	37	3	31	12	4
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	38	83	5	62	55
<i>Elymus repens</i> (L.) Gould	3	-	-	1	-
<i>Conyza canadensis</i> (L.) Cronq.	5	-	-	-	-
<i>Galinsoga quadriradiata</i> Ruiz et Pav.	57	23	59	38	27
<i>Lamium amplexicaule</i> L.	5	-	3	2	-
<i>Poa annua</i> L.	63	103	113	62	43
<i>Senecio vulgaris</i> L.	-	-	-	-	2
<i>Sonchus arvensis</i> L.	2	-	-	-	-
<i>Stellaria media</i> (L.) Vill.	3	2	10	2	-
<i>Urtica urens</i> L.	-	-	2	2	-
<i>Veronica persica</i> Poir.	-	-	-	-	2
<i>Viola arvensis</i> Murr.	2	-	-	-	-

**Table 4.** Effect of weed control method on dried herb yield, height of plants before seed harvesting, yield of lemon balm seeds, and weight of 1000 seeds in 2016–2017

Treatments	Fresh herb yield (g·m <sup>-2</sup> )		Height of plant (cm)		Yield of seeds (g·ha <sup>-1</sup> )		Weight of 1000 seeds (g)	
	2016	2017	2016	2017	2016	2017	2016	2017
Bentazon 960 g·ha <sup>-1</sup>	1553 ab	1374 ab	76.0 b	72.6 b	116.1 a	69.3 a	0.653 a	0.581 a
Fluazifop-P-butyl 150 g·ha <sup>-1</sup>	1515 b	1309 b	79.0 ab	74.5 ab	115.2 a	73.7 a	0.625 a	0.571 a
Mustard seed meal 1.5 t·ha <sup>-2</sup>	1587 ab	1394 ab	77.2 b	73.5 b	134.6 a	74.5 a	0.623 a	0.563 a
Mustard seed meal 3.0 t·ha <sup>-2</sup>	1776 a	1474 a	82.1 a	78.7 a	139.8 a	77.1 a	0.625 a	0.566 a
Control	1499 b	1321 b	78.5 ab	73.0 b	128.0 a	68.6 a	0.635 a	0.568 a

Means followed by the same letter within a column do not differ significantly at P = 0.05

tion reduced the number of barnyardgrass (in 2016 and 2017 by 77.8% and 86.8%, respectively) and increasing the number of annual bluegrass (20.0 and 79.4%). In 2016, compared to the control, a smaller number of dicotyledonous weeds were noted: redroot pigweed, shepherd's purse, lamb's quarters, shaggy soldier, horseweed (*Conyza canadensis* (L.) Cronq.) and henbit dead-nettle (*Lamium amplexicaule* L.). In 2017, on the plots sprayed with this herbicide, an increase in the number of annual bluegrass plants, redroot pigweed was found (Tabs 2, 3).

The use of mustard seed meal at a dose of 1.5 t·ha<sup>-1</sup> in comparison to the control resulted in a reduction in the number of lamb's quarters (in 2016 and 2017, respectively by 63.0% and 67.6%), shepherd's purse (61.1% and 45.2%), shaggy soldier (70.5% and 33.3%), redroot pigweed (55.6% and 66.7%) and an increase in the number of barnyardgrass (44.4% and 63.2%). In 2016, an increase in the number of annual bluegrass was also found (Tabs 2, 3).

The use of a double dose of mustard seed meal (3 t·ha<sup>-1</sup>) reduced the number of lamb's quarters (in 2016 and 2017 by 100% and 89.2%, respectively), redroot pigweed (83.3% and 66.7%), shepherd's purse (75.6% and 54.2%), annual bluegrass (50.7% and 31.7%) and shaggy soldier (76.9% and 52.6%) and increasing the number of barnyardgrass (84.4% and 44.7%) (Tabs 2, 3).

The yield of fresh herb of lemon balm was significantly modified by the method of weed control. The yield of fresh herb from plots, where a double dose of mustard seed meal was used, was the greatest (on average 1776 and 1474 g·m<sup>-2</sup> in 2016 and 2017 respectively). It was significantly higher compared to the control and application of the herbicide fluazifop-P-butyl (Tab. 4).

In both years of the study, a significant effect of the weed control method on the height of lemon balm shoots at the time of harvest was found. The longest shoots were produced by plants growing on plots where mustard seed meal was used in an amount of 3 t·ha<sup>-1</sup> (82.1 and 78.7 cm in 2016 and 2017, respectively). In the first year of research, they were significantly higher than plants from plots weeded with bentazon and a single dose of mustard seed meal, and in the second year significantly higher than plants from plots where bentazon, a single dose of mustard seed meal was used as well as control (Tab. 4).

No significant influence of the weed control method on the yield of lemon balm seeds was found. Despite the lack of significant differences between the weed regulation ways in both years, the highest yield of seeds was harvested from plots where mustard seed meal was spread at the rate of 3 t·ha<sup>-1</sup> (139.8 and 77.1 g·m<sup>-2</sup> in 2016 and 2017, respectively). The weight of 1000 seeds was not significantly dependent

**Table 5.** Effect of weed control method on sowing value of lemon balm seeds

Treatments	Germination energy (%)		Germination capacity (%)		Abnormal seedlings (%)		Nongerminated seeds (%)	
	2016	2017	2016	2017	2016	2017	2016	2017
Bentazon 960 g·ha <sup>-1</sup>	59.3 a	53.5 ab	94.0 a	90.5 a	1.5 ab	1.5 ab	4.5 a	8.0 a
Fluazifop-P-butyl 150 g·ha <sup>-1</sup>	61.3 a	60.3 a	94.8 a	89.8 a	2.5 ab	1.5 ab	2.8 a	8.8 a
Mustard seed meal 1.5 t·ha <sup>-2</sup>	64.0 a	59.5 a	92.5 a	87.5 ab	2.5 ab	2.5 b	5.0 a	10.0 ab
Mustard seed meal 3.0 t·ha <sup>-2</sup>	50.8 a	49.3 b	78.0 b	82.3 b	3.8 b	2.5 b	18.3 b	15.3 b
Control	58.8a	56.8 ab	95.5 a	89.3 a	0.5 a	0.5 a	4.0 a	10.3 ab

Means followed by the same letter within a column do not differ significantly at P = 0.05

on the weed control method. In 2016, the weight of 1000 lemon balm seeds was 0.623–0.653 g, while in 2017, it was 0.563–0.581 g (Tab. 4).

Depending on the applied method of weed control, germination energy of lemon balm seeds was 50.8–64.0% in 2016 and 49.3–60.3% in 2017. In both years of research, the lowest germination energy was characteristic for seeds harvested from plots where mustard seed meal was used at 3.0 t·ha<sup>-1</sup> – 50.8% and 49.3%, respectively. In the first year of the study, no significant influence of the weed control method on germination energy of seeds was found. In contrast, in the second year, germination energy of seeds harvested from plots treated with mustard seed meal in an amount of 3.0 t·ha<sup>-1</sup> was significantly lower than those harvested from plots treated with fluazifop-P-butyl and a single dose of mustard seed meal (Tab. 5).

The method of weed control was a factor significantly differentiating the seed germination capacity. In both study years, the lowest germination capacity was found in seeds collected from plots, for which mustard seed meal was spread at 3.0 t·ha<sup>-1</sup> (in 2016 and 2017, respectively 78.0% and 82.3%). In 2016, the highest germination capacity characterized the seeds collected from control plots, and in 2017 from plots sprayed with bentazon (95.5% and 90.5%, respectively – Tab. 5).

The number of abnormal seedlings depended significantly on the method of weed control. In both years, significantly less abnormal seedlings developed seeds collected from untreated plots. In 2016, signifi-

cantly the most abnormal seedlings grew out of seeds obtained from plots using a double dose of mustard seed meal (3.8%). In 2017, significantly more abnormal seedlings in relation to the control were found for weed regulation method, where mustard seed meal was used in both doses (on average 2.5% – Tab. 5).

The proportion of nongerminated seeds was significantly related to the method of weed regulation. In 2016, the share of nongerminated seeds in the yield harvested from plots using mustard seed meal in the amount of 3.0 t·ha<sup>-1</sup> was significantly higher than in all other combinations and amounted to 18.3%. In 2017, the share of nongerminated seeds in the yield collected from the plots using mustard seed meal in the amount of 3.0 t·ha<sup>-1</sup> was significantly higher than in the plots sprayed with bentazon and fluzifop-P-butyl and it was 15.3% (Tab. 5).

## DISCUSSION

Results of presented work indicate that the use of mustard seed meal and shallow mixing with the soil has contributed to reducing the weed infestation on the lemon balm plantation. During the assessment of weed infestation carried out 6 weeks after application of the meal, a reduction in the number and weight of weeds in relation to the untreated control was found. From two applied doses: 1.5 and 3.0 t·ha<sup>-1</sup>, the application of meal in a larger amount proved to be more effective method of weed control. Scattering of mustard seed meal in this dose resulted in a reduction in the num-

ber and weight of weeds on average in two years of research by 52.1% and 60.2% in comparison to unweeded control. The application of mustard seed meal in an amount by half smaller reduced the number and weight of weeds by an average of 34.7% and 38.1%, respectively.

The obtained results confirm the studies of various authors on beneficial effects of mustard seed meal and other species from the *Brassicaceae* family on limiting germination of seeds, emergence and seed weight of various weed [Boydston et al. 2008, Handiseni et al. 2011, Yu and Morishita 2014]. Research carried out by Boydston et al. [2011] showed a reduction in the number of weeds in the cultivation of onions by 48% and 91% in two subsequent years as a result of the use of mustard seed meal. There was no effect of the increasing dose on the weed reduction. In another experiment by Boydston et al. [2008], mustard seed meal used in the container cultivation of ornamental plants in the amount of 113, 225, 450 g·m<sup>-2</sup> reduced the number and fresh weight of annual bluegrass and chickweed (*Stellaria media* L.) seedlings. The higher the dose, the greater the effect of reducing the weed infestation. In a greenhouse experiment conducted by Yu and Morishita [2014], the use of a growing dose of mustard seed meal from 2.240 to 6.720 kg·ha<sup>-1</sup> resulted in the limitation of redroot pigweed emergence. Handiseni et al. [2011] points to the high efficiency of mustard seed meal in controlling dicot weeds – redroot pigweed and prickly lettuce (*Lactuca serriola* L.).

In the experiment carried out, the use of mustard seed meal significantly reduced the number of several weed species found in high intensity on the control plots. These results are confirmed by studies of other authors with a negative impact of mustard seed meal on seed germination and seedlings weight of redroot pigweed [Rice et al. 2007, Handiseni et al. 2011, Yu and Morishita 2014], annual bluegrass [Boydston et al. 2008] and common lamb's quarters [Rice et al. 2007]. Results of this work also indicate the reducing effect of mustard seed meal on the number of shaggy soldier and shepherd's purse seedlings. The available literature lacks information on the inhibitory effect of mustard seed meal on the germination of these species. From these studies, it appears that mustard seed meal in the amount of

3.0 t·ha<sup>-1</sup> are combating both dicotyledonous and some monocotyledonous weeds.

The results of presented field experiment indicate that on plots where mustard seed meal was spread, a larger number of barnyardgrass plants were noted compared to the control. In this respect, there is some inconsistency with results of greenhouse research carried out by Yu and Morishita [2014], where the effect of this product was to limit the number of barnyardgrass plants by 47–94% compared to the control. Perhaps in own studies, the occurrence of seeds of this species on plots where the mustard seed meal were applied was much higher than on the control plots.

In both years of research, the application of mustard seed meal turned out to be a bit more effective than bentazon chemical fight and definitely more effective than the fight with weeds using fluzifop-P-butyl. In our own research, it was found that bentazon in a dose of 960 g·ha<sup>-1</sup> fought on average for both years 80% of dicotyledonous weeds in relation to the control. These results are confirmed by the studies by Chmielowiec and Borowy [2004], in which the same preparation used in two divided doses of 480 g bentazon per 1 ha destroyed 57–95% of weeds depending on the year of research. After applying the first dose, bentazon destroyed 80–93% dicotyledonous weeds. Similarly in the experiment by Zheljzakov et al. [2006], the use of trifluralin and bentazon at a dose of 0.84 + 0.96 kg·ha<sup>-1</sup> in the cultivation of milk thistle resulted in a reduction in the number of dicotyledonous and monocotyledonous weeds by 70% and 78% in relation to the unweeded control.

In our own studies, the method of weed control significantly modified the yield of fresh lemon balm herb. The largest yield of fresh herb was obtained from plots on which mustard seed meal was used at the amount of 3.0 t·ha<sup>-1</sup>. The largest yield of seeds was also collected from these plots, although no significant differences were found between the different methods of weed control. Based on the available studies, it is difficult to clearly say whether the application of mustard seed meal has a positive effect on crop yields. Snyder et al. [2009] and Boydston et al. [2011] show different results in subsequent years of research regarding the proper cultivation of carrots and onions. In research by Boydston et al. [2011], in the first two years, the onion yield was not modified by the increasing dose of mus-

tard seed meal. However, in the following year, the total yield was reduced due to the increase and multiplication of the dose of mustard seed meal. In the cultivation of carrots, in the first year of research, no effect of the application of mustard seed meal on the field emergence and total carrot yield was found. However, in the following year, it was shown that this product significantly affected the reduction of the emergence, but contributed to the increase in total carrot yield [Snyder et al. 2009]. According to these authors, the increase in carrot yield was caused by the increasing dose of meal as a result of the increasing availability of nitrogen in the soil. In own research, the probable explanation of the increase in the yield of lemon balm seeds as a result of using the mustard seed meal in the amount of 3.0 t·ha<sup>-1</sup> in relation to other methods of weed control may also be an increase in the amount of available nutrients as a result of decomposition of mustard seed meal in soil and stimulation of plant growth. This thesis is confirmed by measurements documenting a more intense growth of shoots of these plants in length.

Results of the conducted research indicate that the weakest sowing value expressed by the lowest energy and capacity of germination and the highest share of non-germinated seeds had lemon balm seeds originating from plots where mustard seed meal at a dose of 3.0 t·ha<sup>-1</sup> was applied. The quality parameters of seeds harvested from plots weeded by the remaining methods were comparable. The poorer quality of lemon balm seeds collected from plots where larger dose of mustard seed meal was applied, may result from the greater amount of nutrients available in the soil. Some investigations showed that high level of nitrogen delayed flowering and seed maturation [George 1999]. For this reason, lemon balm seeds derived from plots, where a higher dose of mustard seed meal was used, could be immature somewhat affected lowering their quality.

Summing up the obtained results, it can be concluded that the best method of weed control, among tested ones, was the scattering of mustard seed meal in an amount of 3.0 t·ha<sup>-1</sup>. On the other hand, this method adversely affected the sowing value of lemon balm seeds. Lowering the amount of used mustard seed meal to half slightly decreased the seed yield and significantly improved its sowing value. Although this

method was less effective in counteracting weeds than using larger dose, it provided comparable effects of reducing the number and weight of weeds in relation to chemical protection. It seems, therefore, that it can be recommended for growing lemon balm for seeds using ecological methods.

## CONCLUSIONS

1. The most effective method of weed control on the lemon balm plantation was the application of mustard seed meal in the amount of 3.0 t·ha<sup>-1</sup>. It influenced the reduction in the number and weight of weeds on average from two years of research by 52.1 and 60.2% in relation to unweeded control.

2. Compared to other methods of weed control, the largest yield of fresh lemon balm herb was collected from plots where a double dose of mustard seed meal was used.

3. No significant influence of the method of weed control on the yield of lemon balm seeds was found.

4. Seeds harvested from plots, on which mustard seed meal was applied in an amount of 3.0 t·ha<sup>-1</sup> was characterized by the lowest sowing value determined by the lowest energy and capacity of germination and the highest share of non-germinated seeds.

## ACKNOWLEDGEMENTS

The research has been funded by the University of Life Sciences in Lublin, OIP/S/51/2019.

## REFERENCES

- Bàrberi, P. (2002). Weed management in organic agriculture: are we addressing the right issues? *Weed Res.*, 42, 177–192.
- Bialy, Z., Oleszek, W., Lewis, J., Fenwick G.R. (1990). Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. *Plant Soil*, 129, 277–281.
- Bond, W., Grundy, A.C. (2001). Non-chemical weed management in organic farming systems. *Weed Res.*, 41, 383–405.
- Boydston, R.A., Anderson, T., Vaughn, S.F. (2008). Mustard (*Sinapis alba*) seed meal suppresses weeds in container-grown ornamentals. *HortScience*, 43(3), 800–803.

- Boydston, R.A., Morra, M.J., Borek, V., Clayton, L., Vaughn, F. (2011). Onion and weed response to mustard (*Sinapis alba*) seed meal. *Weed Sci.*, 59(4), 546–522.
- Carrubba, A., Militello, M. (2013). Nonchemical weeding of medicinal and aromatic plants. *Agron. Sustain. Dev.*, 33, 551–561. DOI: 10.1007/s13593-012-0122-9
- Carrubba, A., Lo Verde, G., Salamone, A. (2015). Sustainable weed, disease and pest management in medicinal and aromatic. In: Medicinal and aromatic plants of the world, Máthé, Á. (ed.), Springer Science+Business Media Dordrecht, 205–235. DOI: 10.1007/978-94-017-9810-5\_11
- Chmielowiec, P., Borowy, A. (2004). Evaluation of bentazon and metolachlor in common bean (*Phaseolus vulgaris* L.) 'Bona' crop. *Acta Sci. Pol. Hortorum Cultus*, 3(1), 75–87 [in Polish].
- Council Regulation (1991). (EEC) No 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs (with amendments). OJ L 198 (22.07.1991), 12. Available: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1991R2092:20060506:EN:PDF> [date of access 07.09.2018].
- George, R.A.T. (1999). Vegetable seed production. 2<sup>nd</sup> ed. CABI Publishing, New York, 38–47.
- Handiseni, M., Brown, J., Zemetra, R., Mazzola, M. (2011). Herbicidal activity of Brassicaceae seed meal on wild oat (*Avena fatua*), italian ryegrass (*Lolium multiflorum*), redroot pigweed (*Amaranthus retroflexus*), and prickly lettuce (*Lactuca serriola*). *Weed Technol.*, 25(1), 127–134.
- IPP [Institut of Plant Protection] (2016). Plant Protection Recommendations for 2016/17. Vol. IV. Ornamental plants, herbal plants. Institut of Plant Protection in Poznań.
- ISTA. (2004). International rules for seed testing. ISTA, Zürich.
- Kalinova, J. (2010). Allelopathy and organic farming. In: Sociology, organic farming, climate change and soil science, Lichtfouse, E. (ed.). Sustainable agriculture reviews 3. Springer Science+Business Media B.V., 379–418. DOI: 10.1007/978-90-481-3333-8\_14
- Kazimierczak, R., Hallmann, E., Rembiałkowska, E. (2015). Effect of organic and conventional production systems on the content of bioactive substances in four species of medicinal plants. *Biol. Agric. Hort.*, 31(2), 118–127. DOI: 10.1080/01448765.2014.977948
- Kubisz, L., Hołubowicz, R., Gauza, M., Li, H., Hojan-Jezińska, D., Jaroszyk, F. (2012). Effect of low frequency magnetic field on germination of onion (*Allium cepa* L.) seeds. *Acta Phys. Pol. A*, 121, 49–53.
- Mihajlov, L., Ilieva, V., Markova, N., Zlatkovski, V. (2013). Organic cultivation of lemon balm (*Melissa officinalis*) in Macedonia. *J. Agric. Sci. Technol. B*, 3, 769–775.
- Moradkhani, H., Sargsyan, E., Bibak, H., Naseri, B., Sadat-Hosseini, M., Fayazi-Barjin, A., Meftahizade, H. (2010). *Melissa officinalis* L., a valuable medicine plant. A review. *J. Med. Plant Res.*, 25, 2753–2759.
- Oleszek, W. (1987). Allelopathic effects of volatiles from some *Cruciferae* species on lettuce, barnyard grass and wheat growth. *Plant Soil.*, 102, 271–273.
- Rice, A.R., Johnson-Maynard, J.L., Thill, D.C., Morra, M.J. (2007). Vegetable crop emergence and weed control following amendment with different *Brassicaceae* seed meals. *Renew. Agric. Food Syst.*, 22(3), 204–212. DOI: 10.1017/S1742170507001743
- Seidler-Łożykowska, K., Mordalski, R., Kucharski, W., Kędzia, E., Nowosad, K., Bocianowski, J. (2015). Effect of organic cultivation on yield and quality of lemon balm herb (*Melissa officinalis* L.). *Acta Sci. Pol. Hortorum Cultus*, 14(5), 55–67.
- Snyder, A., Morra, M.J., Johnson-Maynard, J., Thill, D.C. (2009). Seed meals from *Brassicaceae* oilseed crops as soil amendments: influence on carrot growth, microbial biomass nitrogen, and nitrogen mineralization. *Hort. Science*, 44(2), 354–367.
- Shakeri, A., Sahebkar, A., Javadi, B. (2016). *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol.*, 188, 204–228.
- Tworowski, J. (2000). Zasady produkcji materiału siewnego. In: Nasiennictwo, Duczmal, K.W. Tucholska, H. (eds). Vol. I. PWRiL, Warszawa, 182–204.
- Vaughn, S.F. (1999). Glucosinolates as natural pesticides. In: Biologically active natural products: agrochemicals. Cutler, H.G., Cutler, S.J. (eds.). CRC Press, Boca Raton, 81–92.
- Vaughn, S.F., Berhow, M.A. (2005). Glucosinolate hydrolysis products from various plant sources: pH, effect, isolation, an purification. *Ind. Crops Prod.*, 21, 193–202. DOI: 10.1016/j.indcrop.2004.03.004
- Vaughn, S.F., Palmquist, D.E., Duval, S.M., Berhow, M.A. (2006). Herbicidal activity of glucosinolate-containing seed meals. *Weed Sci.*, 54, 743–748.
- Winiarczyk, K., Seidler-Łożykowska, K., Gębura, J., Bocianowski, J. 2016. Vitality and germination of lemon balm (*Melissa officinalis* L.) seeds. *J. Appl. Bot. Food Qual.*, 89, 156–162. DOI: 10.5073/JABFQ.2016.089.019
- Yu, J., Morishita, D.W. (2014). Response of seven weed species to corn gluten meal and white mustard (*Sinapis alba*) seed meal rates. *Weed Technol.*, 28(1), 259–265.
- Zheljzakov, V., Zhalnov, I., Nedkov, N.K. (2006). Herbicides for weed control in blessed thistle (*Silybum marianum*). *Weed Technol.*, 4(20), 1030–1034.

