

# ROW SPACING AND THE TERM OF HARVEST OF FLOWER HEADS AS DETERMINANTS OF CROP YIELD AND CHEMICAL COMPOSITION OF RAW MATERIAL OF CHAMISSO ARNICA (*Arnica chamissonis* LESS.)

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## ABSTRACT

**Background.** The surface area of chamisso arnica cultivation in Poland is very small; however, due to a decline in the natural resources of mountain arnica, and the resulting problems with the acquisition of raw material, the pharmaceutical market has become interested in raw material from chamisso arnica, which could be a substitute for the endangered species *A. montana*. This fact has underlined the need to conduct research on the cultivation of chamisso arnica so as to be able to refine individual elements of agricultural technology.

**Material and methods.** In 2015–2017 a field experiment was carried out to assess the impact of row spacing systems (traditional with single rows every 40 cm and a belt cultivation system with four rows with a spacing of 40 cm between each row alternating with an 80 cm wide spacing every four rows; in the case of both spacing systems the distance between plants within a row was 20 cm) and the harvest term (A – yellow bud stage, B – beginning of flowering, C – full flowering, D – end of flowering) on the yield and chemical composition of raw material. Sesquiterpene lactones were determined using the chromatographic method described in Farmakopea Polska VIII (URPLWMPB, 2008). The flavonoids content was determined as quercetin equivalents with the spectrophotometric method and hydrodistillation was applied to determine the percentage of essential oils, as described in Farmakopea Polska VI (URPLWMPB, 2002).

**Results.** The highest raw material yield was obtained in objects with the traditional spacing system and flower heads harvested at the end of flowering. The harvest term had a significant impact on the concentration and yield of the analysed metabolites. The content and yield of sesquiterpene lactones systematically increased between the yellow bud stage and the end of flowering. The highest contents of flavonoids and essential oils as well as the highest yields of these metabolites were determined in heads harvested at the full flowering stage. The lowest content of all analysed active substances was found in inflorescences harvested in the yellow bud stage.

**Conclusion.** Row spacing and the date of harvesting flower heads had a significant impact on raw material yields and the content of active substances. Chamisso arnica cultivation in traditional spacing was more favorable due to higher yields of flower heads and yields of sesquiterpene lactones, flavonoids and essential oils.

**Key words:** *Arnica chamissonis*, essential oils, flavonoids, harvest term, row spacing, sesquiterpene lactones

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## INTRODUCTION

Chamisso arnica (*Arnica chamissonis* Less.) is a North American perennial plant with a natural occurrence range extending from the Alaskan Archipelago to New Mexico and California. It prefers moist sites located on deep soils (Maguire, 1943). The species grows in meadows, along stream banks, and occasionally in forests in the Alpine and subalpine zones (Wenk, 2015; Denver Botanic Gardens, 2018). The plant produces long, thick, unbranched, light brown rhizomes with numerous fine adventitious roots. Above the soil surface the plant develops a leaf rosette and 20–90 cm high inflorescence shoots branching in the upper part. The stem leaves gathered in 5–10 pairs are smaller in the upper part of the shoot. They are 2–4 cm wide and 10–15 cm long and have a lanceolate or lanceolate-ovate shape. At the branching apices of the stems, chamisso arnica plants produce from 3 to 15 inflorescences composed of 2–4 cm diam. bright yellow heads with 8–20 ray florets (at the margins) and more numerous disc florets (Maguire, 1943; Wenk, 2015; Denver Botanic Gardens, 2018). In Poland chamisso arnica plants usually begin flowering at the turn of May and June and bloom for approximately 3 weeks (Sugier, 2007).

Inflorescences containing lactones, flavonoids, essential oils, and polyphenols (Kowalski *et al.*, 2015; Zheleva-Dimitrova *et al.*, 2015; Sugier *et al.*, 2019) are the most common raw material from the chamisso arnica plant (*Arnicae anthodium*, syn. *Arnicae flos*). The sesquiterpene lactones contained in this raw material have been classified into the pseudoguaianolide type dominated by helenalin and 11,13-dihydrohelenalin with their esters, arnicolides, as well as chamissonolides and arnifolines, which are characteristic for chamisso arnica (Nichterlein, 1995; Matławska, 2008). As reported by Roki *et al.* (2008), luteolin and luteolin-7-O-glucoside are the dominant flavonoid compounds in *A. chamissonis* flower heads. Additionally, the presence of isoquercetin and hyperoside has been demonstrated. The essential oil of the species contains high levels of  $\alpha$ -pinene, cumene, p-cymene, decanal, germacrene D, and caryophyllene oxide (Sugier *et al.*, 2017; 2019). In addition to inflorescences, arnica herb (*Arnicae herba*), leaves

(*Arnicae folium*), rhizomes (*Arnicae rhizoma*), and achenes can be used as raw material (Kaur *et al.*, 2006; Gawlik-Dziki *et al.*, 2009). The substances contained in arnica have antioxidant, anti-inflammatory, antiseptic, and analgesic properties. They also accelerate granulation and absorption of ecchymoses, strengthen capillary walls, and reduce swelling (Ekenäs, 2008; Gawlik-Dziki *et al.*, 2010). Scientific reports (Wszelaki *et al.*, 2010; Ghasemali *et al.*, 2013) indicate a potential application of arnica in the treatment of cancer and Alzheimer's disease.

The surface area of arnica cultivation in Poland is very small; however, due to the decline in the natural resources of mountain arnica, and the resulting problems with acquisition of raw material, the pharmaceutical market has become interested in raw material from the chamisso arnica plant, which could be a substitute for the protected and endangered mountain arnica. This fact has underlined the need to conduct research on the cultivation of chamisso arnica so as to be able to refine individual elements of agricultural technology. The sparse and fragmentary investigations conducted to date (Roki *et al.*, 2008; Sugier and Gawlik-Dziki *et al.*, 2009; Kowalski *et al.*, 2015; Sugier *et al.*, 2017; 2019) are insufficient for the development of a comprehensive agricultural technology for this species that would ensure high yields of raw material with the desired chemical composition. The important agrotechnical factors determining the yield size include row spacing and plant density (Kołodziej and Zejdan, 2000; Gruszczyk, 2001; Mordalski *et al.*, 2014; Lee *et al.*, 2015; Król, 2017) as well as the harvest date (Sugier, 2013). The available literature does not provide scientific reports on the issues discussed in the present study. Cultivation of chamisso arnica in the belt system is only indicated in practical recommendations (Sugier, 2018). The presence of a wide belt gives the possibility of easy harvesting of inflorescences without plant losses caused by trampling. Such a cultivation system is recommended, as chamisso arnica plants grow intensively and form a very compact crop after the second year, which impedes manual collection of flower heads. Bearing this in mind the following research hypothesis was proposed: the development of individual agricultural engineering elements,

including row spacing and harvest time, will ensure satisfactory yields of raw arnica characterized by a high content of biologically active substances. We undertook investigations to assess the traditional and belt spacing systems and the term of flower head harvesting (in the stages of development: yellow bud stage, beginning of flowering, full flowering, end of flowering) on the yield and chemical composition of raw material from the chamisso arnica plant.

## MATERIAL AND METHODS

The field experiment was carried out in 2015–2017 in a two-, three- and four-year old chamisso arnica plantation in Brzeźnica Bychawska (51°31' N; 22°45' E). The investigations were carried out on sandy soil with the granular structure of strong loamy sand. It was characterised by moderate humus content (1.52%; PB-34 – Tiurin's method), moderate phosphorus content (P – 60.2 mg·kg<sup>-1</sup>; PN-R-04023: 1996), low potassium content (K – 65.6 mg·kg<sup>-1</sup>; PN-R-04022: 1996 + Az1: 2002), very low magnesium content (Mg – 13 mg·kg<sup>-1</sup>; PN-R-04020: 1994 + Az1: 2004), and highly acidic reaction (pH KCl – 4.3; PN-ISO10390: 1997). Potatoes grown on a full manure dose (30 t·ha<sup>-1</sup>) were the forecrop of the chamisso arnica.

The field experiment was established at the end of the second ten days of April 2014. In autumn 2013, after harvesting the potatoes, the field was deeply ploughed, and a tillage set was used to cultivate the soil and level the field before setting up the plantation in spring 2014. 9–10 cm long stolon seedlings were used to set up the experiment. Immediately after collection from the stock plantation (Department of Industrial and Medicinal Plants of the University of Life Sciences in Lublin) the stolons were planted in previously prepared furrows. They were placed at a depth of 8–10 cm at 20 cm spacing within each row and covered with soil. This first year was regarded as a preliminary stage of the experiment as the yield of the flower heads was very low because the plants produced stolons and roots at the expense of the aboveground parts. The years 2015–2017 were regarded as productive and were included in the factorial system.

The experiment was established in a split-plot design in four replications on 10 m<sup>2</sup> plots. The first

experimental factor was the variable row spacing and plant density per unit area:

- a single-row system with spacing every 40 cm between rows regarded as the traditional system with a density of 125 000·ha<sup>-1</sup> of planted stolon seedlings;
- a belt of rows with four rows (with spacing every 40 cm between rows) alternating with an 80 cm wide spacing, which allowed for the planting of 100 000·ha<sup>-1</sup> seedlings.

The other experimental factor was the variable term of flower head harvesting at four stages of development:

- A – yellow bud (fully developed flower buds, ray florets closed, disc florets closed);
- B – beginning of flowering (disc florets opened in two inflorescence rows, ray florets opened);
- C – full flowering (half of disc florets opened);
- D – end of flowering (all disc florets opened, withering ray florets).

In autumn each year all plots were fertilised with phosphorus (single superphosphate) and potassium (60% potassium salt) at 24.0 kg P and 66.4 kg K per 1 ha. Nitrogen fertiliser (ammonium nitrate) at a total dose of 40 kg N·ha<sup>-1</sup> was applied twice: in spring before the beginning of vegetation (20 kg) and after flower head harvesting (20 kg). During the plant vegetation period necessary agricultural treatments, i.e. threefold manual weeding and loosening the inter-rows, were carried out.

Flower heads were collected from the plots successively following the experiment design. After each harvest the fresh weight of the heads was determined. Next, they were dried in a drying room at a temperature of 40°C. After drying the samples were weighed to determine the dry mass of the raw material, which was subsequently subjected to chemical analyses.

Sesquiterpene lactones were determined using the chromatographic method described in the Farmakopea Polska VIII (URPLWMPB, 2008). The results are shown as the percentage of the sum of sesquiterpene lactones calculated as dihydrohelenalin tiginate equivalents. The flavonoids content was determined as quercetin equivalents with the spectrophotometric method according to the Farmakopea Polska VI

(URPLWMPB, 2002). Hydrodistillation was applied using the Dering apparatus to determine the percentage of essential oils as described in the *Farmakopea Polska VI* (URPLWMPB, 2002), using method II (with m-xylene). Each analysis was performed in 3 replicates.

Based on the results the yield of sesquiterpene lactones, flavonoids, and essential oil was calculated per 1 ha (yield of flower heads × content of individual metabolites).

The results of the yield and chemical composition of the chamisso arnica inflorescences were analysed statistically using Statistica 6.0 software (Stat. Soft, Inc., Kraków, Polska). The data were subjected to an analysis of variance (ANOVA) and the smallest significant differences were determined using the Tukey's test at a confidence level of 0.05.

## RESULTS AND DISCUSSION

The thermal conditions in the individual years of the four-year study (2014–2017) exhibited only slight fluctuations (Table 1). The mean air temperature in this period was 8.9°C, which was by 1.3°C higher than the multiyear mean (1963–2013). The highest mean annual air temperature (9.4°C) was recorded in the second year of the study, i.e. in 2015. In turn, 2017 with the average temperature of 8.4°C was the coldest year. The mean temperature during the plant vegetation period (April–October) in the years when flower heads were harvested (2015–2017) was 14.2°C, which was by 0.8°C higher than the multiyear mean value (Table 1).

The mean precipitation rate in the study years was 658.0 mm, i.e. by 71 mm higher than the multiyear mean value (Table 1). The highest precipitation was recorded in the first year of the study (789.9 mm) when the experiment was established. In turn, the lowest precipitation rate (531.8 mm) was recorded in the following year (2015). In the years of the flower head harvest (2015–2017), the individual arnica vegetation periods (April–October) were highly diverse in terms of the precipitation sum. The highest and the lowest precipitation during the growing season was recorded in 2017 (448.4 mm) and in 2015 (346.1 mm), respectively.

Favourable temperatures and precipitation prevailed during the establishment of the experiment

(2014), i.e. planting the arnica seedlings. May was characterised by a moderate air temperature (13.4°C) and an exceptionally high precipitation rate (239.9 mm), which promoted 100% survival of the seedlings. The thermal and water conditions prevailing during the flowering of chamisso arnica are important for the yield and quality of the raw material. Chamisso arnica blooms in Poland between the end of May and the end of the second ten days of June (Sugier, 2007). The weather during this phase in the three-year study period varied, but was generally favourable for the production of high yields of flower heads. May and June 2016 turned out to be the most favourable months with a mean temperature of 16.4°C. In turn, 2015 was the coldest year with a mean temperature of only 14.6°C in May and June; nevertheless, this value was by 0.3°C higher than the multiyear mean (Table 1).

The experimental factors significantly modified the dry matter yield of the chamisso arnica flower heads (Table 2). The highest raw material yield (139.8 g·m<sup>-2</sup>) was obtained in the third year of cultivation in objects with the traditional spacing system and head harvesting at the end of flowering. In turn, the lowest yields were obtained in the fourth year of cultivation in plots with the belt system and harvest of inflorescences at the yellow bud stage (63.6 g·m<sup>-2</sup>). Regardless of the harvest term the raw material yield from the plots with the traditional spacing was significantly higher in all years of the study, which can be explained by the higher plant density. The comparison of both cultivation systems demonstrated that the highest flower head yields were obtained in the third year in objects with the traditional row spacing (in the range from 117.1 to 139.8 g·m<sup>-2</sup>), whereas the yields in the belt system were by 11.5% lower (102.4–118.9 g·m<sup>-2</sup>). In the second year of cultivation, the yields from the two systems were by 12.4 and 11.9% lower, respectively. In the fourth experimental year, the yields drastically declined by 36.2 and 37.9%, respectively, in comparison with the year with the highest yields. Similar relationships were reported by Radanović *et al.* (2007) and Sugier and Gawlik-Dziki *et al.*, (2009). The yields obtained in the present study can be regarded as high and in line with the range previously reported in Poland (Sugier and Gawlik-Dziki, 2009; Sugier *et al.*, 2019).

The raw material yield depended significantly on

the term of flower head harvesting. It was found that the yield increased along with the delay in the harvest, regardless of the row spacing (Table 2). Compared to the weight of heads harvested at the end of flowering the yields of inflorescences harvested in the yellow bud stage, at the beginning of flowering, as well as at full flowering were lower by 14.3, 10.1, and 4.7%, respectively.

An increase in the inflorescence yield in line with harvest postponement from the yellow bud stage to the end of flowering was also found in mountain arnica (Sugier, 2013). The information on row spacing is not conclusive in the case of herbal plants. Studies on the density of black cumin (*Nigella sativa* L.) have shown that an increase in the width of inter-rows from 20 to 40 cm resulted in a nearly a 50% reduction in seed yields (Abdolrahimi *et al.*, 2011). A similar response was observed in the case of

safflower (*Carthamus tinctorius* L.) (Hamza, 2015) and tarragon (*Artemisia dracuncululus* L.) (Nurzyńska-Wierdak and Zawislak, 2014). These authors showed that a higher density of plants contributed to an increase in the mass of raw material obtained.

However, the belt cultivation system had a positive effect on the yields of St. John's wort (*Hypericum perforatum* L.) (Gruszczyk, 2001) and dandelion roots (*Taraxacum officinale* Web.) (Sugier, 2004). Similarly, wider inter-rows were found to increase the yield of chamomile (*Chamomilla recutita* (L.) Rausch.) heads (Kwiatkowski, 2015) as well as herbs and seeds (Surmacz-Magdziak, 2011). In contrast, there was no significant effect of different inter-row distances on the mass of seeds produced by fenugreek (*Trigonella foenum-graecum* L.) (Kołodziej and Zejdan, 2000) and on the weight of the basil herb (*Ocimum basilicum* L.) (Jadczak, 2007).

**Table 1.** Mean monthly temperatures and precipitation sums in 2014–2017 compared with 50-year means (1963–2013); data from Lublin–Radawiec meteorological station (Institute of Meteorology and Water Management – National Research Institute in Warsaw)

Year	Month												Mean
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Temperature, °C													
2014	-3.0	1.2	6.0	9.7	13.4	15.6	20.3	17.8	14.2	9.2	4.2	-0.2	9.0
2015	0.6	0.4	4.7	7.9	12.4	16.7	19.3	21.8	14.6	6.8	4.7	3.3	9.4
2016	-4.2	3.0	3.5	8.9	14.4	18.3	18.9	17.8	15.2	6.7	2.2	-0.2	8.7
2017	-5.7	-1.7	5.4	7.0	13.5	17.7	18.1	19.1	13.6	8.9	3.7	1.6	8.4
Mean 1963-2013	-3.5	-2.4	1.7	7.8	13.4	16.3	18.0	17.4	12.9	7.9	2.9	-1.5	7.6
Precipitation, mm													Total
2014	66.9	15	42.9	53.7	239.9	76.8	75.8	89.7	29.2	23.6	22.6	53.8	789.9
2015	51.0	13.6	45.4	27.9	113.7	18.0	42.9	7.1	89.7	46.8	48.2	27.5	531.8
2016	35.3	60.7	59.2	35.0	32.6	51.9	138.2	45.3	12.4	116.3	47.6	63.9	698.4
2017	14.7	39.6	33.8	55.8	40.6	30.2	86.7	55.1	87.6	92.4	46.2	29.3	612.0
Mean 1963-2013	32.1	30.6	32.7	41.8	61.1	70.5	80.4	66.4	55.6	42.0	39.7	34.1	587.0

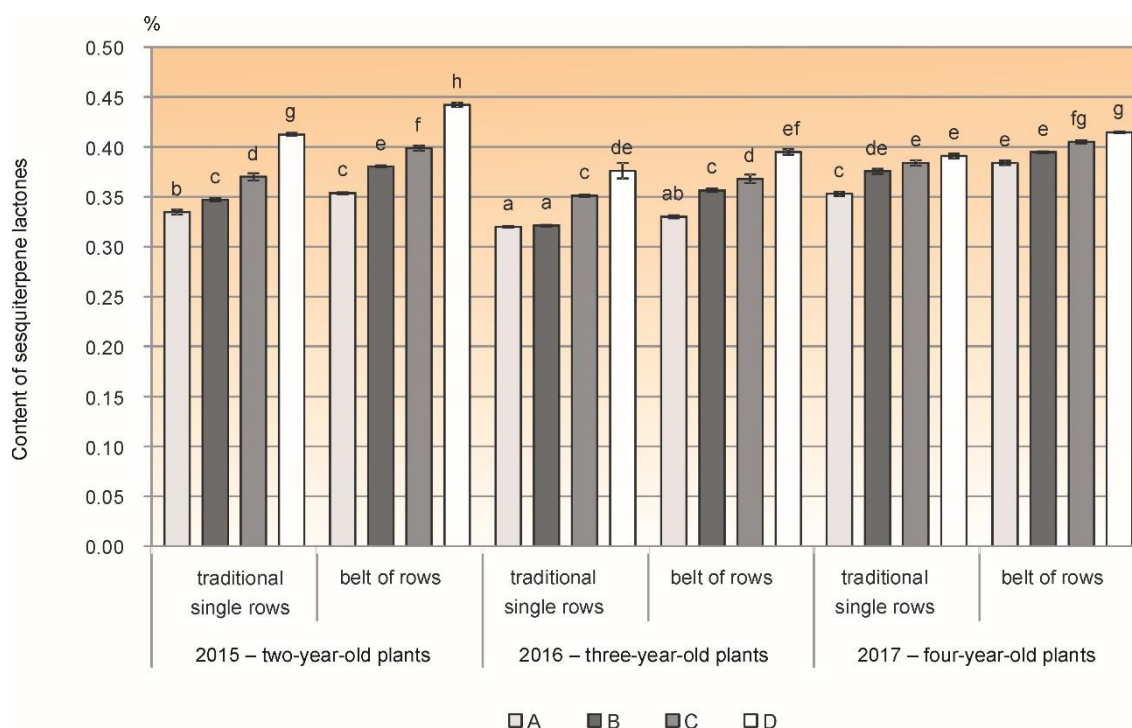
**Table 2.** Yield of dry mass of *Arnica chamissonis* flower heads ( $\text{g}\cdot\text{m}^{-2}$ ) depending on the experimental factors; A – yellow bud stage, B – beginning of flowering, C – full flowering, D – end of flowering. The values designated by different letters are significantly different (Tukey’s test,  $P < 0.05$ )

Harvest term	Traditional spacing			Belt of rows			Mean
	2015 II	2016 III	2017 IV	2015 II	2016 III	2017 IV	
A	102.3 <sup>cd</sup>	117.1 <sup>def</sup>	74.8 <sup>ab</sup>	91.3 <sup>c</sup>	102.4 <sup>cd</sup>	63.6 <sup>a</sup>	91.9 <sup>C</sup>
B	108.6 <sup>d</sup>	118.3 <sup>def</sup>	78.1 <sup>ab</sup>	95.9 <sup>cd</sup>	109.8 <sup>de</sup>	67.9 <sup>ab</sup>	96.4 <sup>D</sup>
C	112.2 <sup>de</sup>	129.5 <sup>ef</sup>	82.8 <sup>ab</sup>	101.6 <sup>cd</sup>	115.7 <sup>def</sup>	71.3 <sup>ab</sup>	102.2 <sup>DE</sup>
D	118.9 <sup>def</sup>	139.8 <sup>f</sup>	86.2 <sup>bc</sup>	104.9 <sup>cd</sup>	118.9 <sup>def</sup>	74.7 <sup>ab</sup>	107.2 <sup>E</sup>
Mean	110.5 <sup>E</sup>	126.2 <sup>G</sup>	80.46 <sup>B</sup>	98.43 <sup>D</sup>	111.7 <sup>F</sup>	69.36 <sup>A</sup>	

II – two-year-old plants, III – three-year-old plants, IV – four-year-old plants

The row spacing and harvest term significantly modified the percentage content of sesquiterpene lactones in the analysed raw material. Their content in the chamisso arnica inflorescences ranged from 0.32 to 0.44% (Fig. 1). The highest concentration of lactones was determined in arnica heads collected at the end of flowering from plants growing in the belt system in the second year of cultivation, whereas the lowest content of the analysed compounds was detected in heads collected in the yellow bud stage from plots cultivated with traditional row spacing in the third year. The raw material obtained from plants grown in the belt system exhibited a greater concentration of lactones (on average by 6.2%) in all years of the study, regardless of the term of flower head harvesting. A positive effect of the harvest delay on the biosynthesis of lactones was demonstrated as well. Flower heads harvested in the end of flowering stage contained higher levels of these compounds than those collected in the yellow bud stage, at the beginning of flowering, and at full flowering (by approx. 15, 11, and 6%, respectively). These results are consistent with other reports demonstrating a systematic increase in the content of lactones in mountain arnica flower heads between the flower bud

stage and the end of flowering. Douglas *et al.* (2004) showed an almost twice as high content of sesquiterpene lactones in withered flowers (0.94%) as compared to buds (0.51%). Similarly, Sugier (2013) recorded the lowest lactone content in yellow buds of mountain arnica (on average 0.92%), which increased with the ripening of the inflorescences and reached an over 20% higher value (on average 1.17%) at the end of flowering. In turn, Mannan *et al.* (2011) compared plant material from *Artemisia* species obtained before and after the development of inflorescences and showed a higher content of artemisinin in leaves, stems, and roots harvested after flowering. However, Foster *et al.* (2011) showed a decrease in the lactone concentration in chicory leaves (*Cichorium intybus* L.) from late spring to autumn. It is also worth underlining that the flower heads collected at the end of flowering, especially from plants cultivated in the belt system in 2015 and 2017, met the guidelines for *Arnicae flos* contained in the Farmakopea Polska XI (URPLWMPB, 2017), which established a minimum content of lactones at 0.40%. The flower heads analysed in the present study had a significantly higher concentration of sesquiterpene lactones than those reported by Ivanova *et al.* (2018).

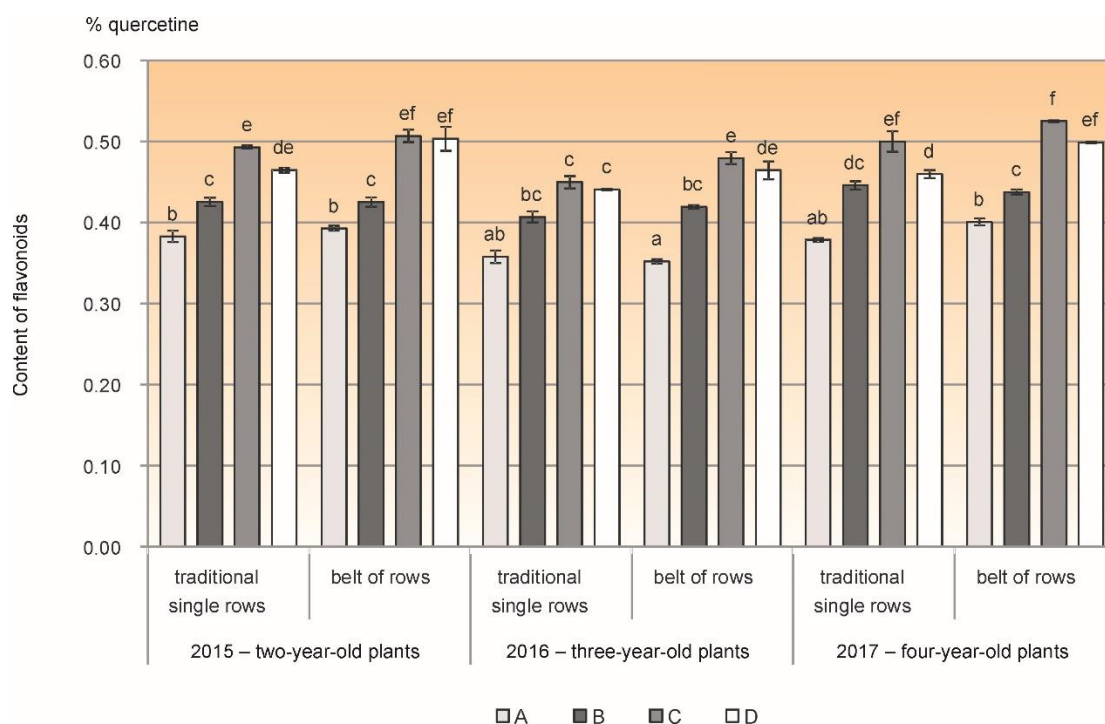


**Fig. 1.** Content of sesquiterpene lactones in *Arnica chamissonis* flower heads (%) depending on the experimental factors; A – yellow bud stage, B – beginning of flowering, C – full flowering, D – end of flowering. The values designated by different letters are significantly different (Tukey's test,  $P < 0.05$ )

The flavonoid content in the chamisso arnica heads depended on the row spacing and harvest term (Fig. 2). Probably greater light access with lower plant density has a positive effect on flavonoid accumulation. The positive effect of the belt system on the flavonoid content was evident in all years of the study. The highest quercetin content was detected in inflorescences harvested in the full flowering stage from plants grown in the belt system in the fourth year of cultivation (on average 0.52%). The lowest concentration of flavonoids was detected in flower heads collected in the yellow bud stage in the third year of cultivation from plots with the traditional spacing (on average 0.36%) and from objects with the belt system (on average 0.35%). As in the case of the biosynthesis of lactones, delayed harvest had a positive effect on the content of quercetin in the heads. The flower heads collected in the yellow bud stage accumulated on average 0.38% of quercetin, whereas those collected in the full flowering stage contained significantly higher levels of the flavonoid,

i.e. 0.49%. In turn, the delay in the harvest to the end of flowering reduced its content to 0.47%. Similar relationships were found by Sugier (2013) in studies of the mountain arnica, where the content of flavonoids increased between the flower bud stage and full flowering. The content of quercetin shown in the present study ranged from 0.35 to 0.52%, which is clearly lower than the values (0.57-0.76%) reported by Sugier and Gawlik-Dziki (2009).

Increased row spacing has been found to stimulate the synthesis of flavonoid compounds not only in chamisso arnica. A similar plant response was also noted in chamomile and artichoke (*Cynara scolymus* L.) (Winiarska, 2006; Kwiatkowski, 2015). However, no significant effect of the distance between plants on the content of flavonoids was noted in the case of the tarragon herb (Nurzyńska-Wierdak and Zawislak, 2014), hyssop (*Hyssopus officinalis* L.) (Zawislak, 2011) and the Moldavian dragonhead (*Dracocephalum moldavica* L.) (Yousefzadeh and Sabaghnia, 2016).



**Fig. 2.** Content of flavonoids in *Arnica chamissonis* flower heads (%) expressed as quercetin equivalents depending on the experimental factors; A – yellow bud stage, B – beginning of flowering, C – full flowering, D – end of flowering. The values designated by different letters are significantly different (Tukey's test,  $P < 0.05$ )

In the present study the content of essential oils in the chamisso arnica flower heads ranged from 0.145 to 0.223% (Fig. 3). As in the case of quercetin and lactones the heads harvested from plants grown in the belt system were characterised by a higher average level of essential oils. The harvest term substantially modified the oil content in the raw material. In all years of the study the full flowering stage and the end of flowering turned out to be the most beneficial terms for essential oil concentrations in the flower heads (0.200 and 0.193%, respectively). The flower heads collected in the yellow bud stage and at the beginning of flowering had significantly lower content of essential oils (0.152 and 0.159%, respectively). Compared with the available literature the present values are substantially higher than the content reported by Sugier *et al.* (2019), who analysed inflorescences collected in full flowering stage in plots with similar soil conditions and fertilisation (0.146%).

The impact of the harvest term on the content of essential oil in chamisso arnica heads was also

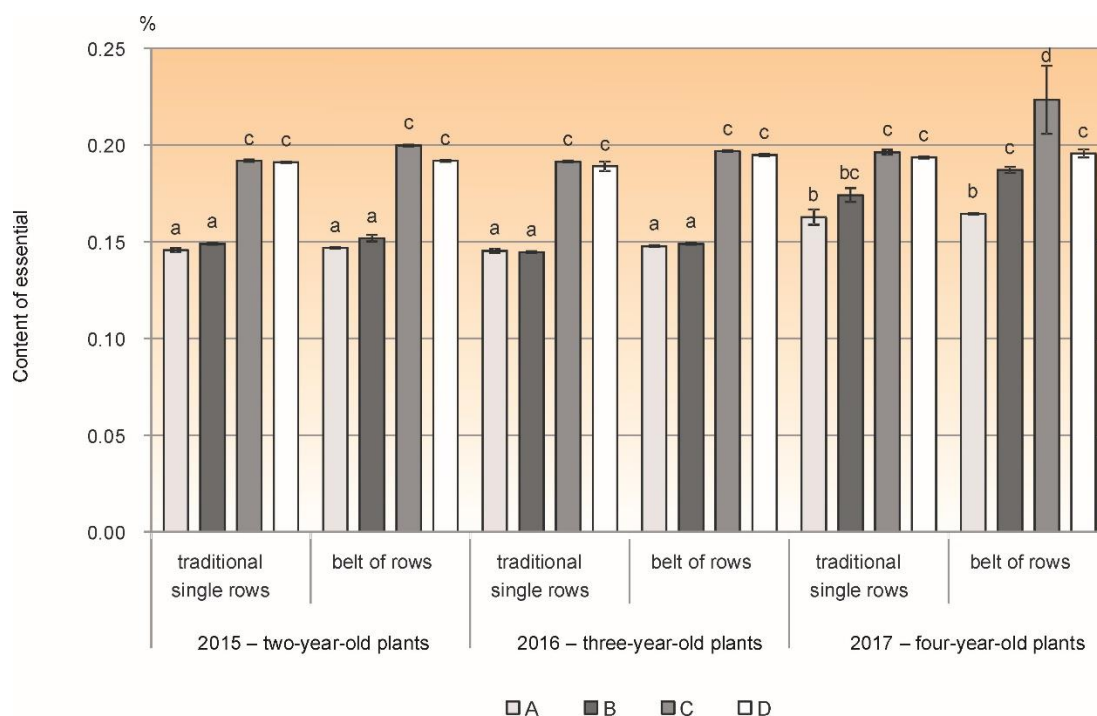
demonstrated by Kowalski *et al.* (2015). Their results indicate a systematic increase in the concentration of essential oil with a delay in flower head harvesting; the content ranged from 0.137 (beginning of flowering) to 0.194% (full flowering). Similarly, a significant effect of the harvest term on the essential oil content was demonstrated in a study of mountain arnica, where the content increased regularly from the yellow bud stage to full flowering and then declined (Sugier, 2013; Kowalski *et al.*, 2015). A similar relationship was noted by Zawislak (2011) in a study of hyssop plants.

The effect of row spacing on the concentration of essential oils in the raw material is difficult to determine in a clear way. Literature data report changes in the content of essential oils in raw materials associated with a variable distance between plants in rows. A positive effect of wider spacing was demonstrated in the case of chamomile (Kwiatkowski, 2015) and mountain savory plants (*Satureja montana* L.) (Zawislak and Nurzyńska-



-Wierdak, 2017). However, also a positive reaction to increased plant density has been found in the case of dill (*Anethum graveolens* L.) (El-Zaeddi *et al.*, 2017) and valerian (*Valeriana officinalis* L.) (Moniuszko and Wiśniewski, 2011), both of which found higher

contents of oil at lower spacing between plants. No significant effect of the inter-row width on the essential oil concentration was observed in hyssop (Zawiślak, 2011) and tarragon plants (Nurzyńska-Wierdak and Zawiślak, 2014).



**Fig. 3.** Content of essential oils in *Arnica chamissonis* flower heads (%) depending on the experimental factors; A – yellow bud stage, B – beginning of flowering, C – full flowering, D – end of flowering. The values designated by different letters are significantly different (Tukey's test,  $P < 0.05$ )

Given the varying yields and contents of metabolites, the production of sesquiterpene lactones, flavonoids, and essential oils per 1 ha was determined to compare the individual plots. The mean dry matter yield from the heads per 1 ha was approximately 1000 kg (Table 2), with an average of 3.7 kg of lactones, 4.39 kg of quercetin, and 1.75 kg of oil (Table 3). The lowest yields of the analysed metabolites in the three years of study were found in the plots with the belt system of cultivation where the heads were harvested in the yellow bud stage (lactones – 3.03 kg, flavonoids – 3.25 kg, essential oil – 1.3 kg). In turn, the highest yields were obtained in the traditional spacing system where the heads were harvested at the end of

flowering (4.51 kg, 5.21 kg and 2.19 kg, respectively). Investigations conducted by Sugier *et al.* (2019) showed at the full flowering stage of chamisso arnica an essential oil yield of 1.71 kg·ha<sup>-1</sup>.

Plants in the third year of cultivation exhibited the most favourable results in terms of yield of the evaluated active compounds. In 2016 the theoretical yield of metabolites from 1 ha was on average 4.23 kg of sesquiterpene lactones, 5.04 kg of flavonoids, and 2.04 of essential oil. The two- and especially four-year-old plants were characterised by significantly lower yields of lactones, flavonoids, and essential oil.

Chamisso arnica has been introduced into field cultivation only in recent years; hence, there are few

reports on the impact of agrotechnical factors on plant yields and content of the main active compounds. In practice it is recommended that the species should be grown in both the traditional

spacing and belt systems. In the present experiment, considerably better results were obtained using the traditional spacing system, which ensured high yields of raw material and major metabolites.

**Table 3.** Yield of lactones, flavonoids, and essential oils per ha; A – yellow bud stage, B – beginning of flowering, C – full flowering, D – end of flowering. The values designated by different letters are significantly different (Tukey’s test,  $P < 0.05$ )

Harvest term	Traditional spacing			Belt of rows			Mean
	2015 II	2016 III	2017 IV	2015 II	2016 III	2017 IV	
Sesquiterpene lactones, kg dihydrohelenalin tiglate·ha <sup>-1</sup>							
A	3.42 <sup>f</sup>	3.75 <sup>gh</sup>	2.64 <sup>b</sup>	3.23 <sup>e</sup>	3.43 <sup>f</sup>	2.44 <sup>a</sup>	3.15 <sup>B</sup>
B	3.77 <sup>gh</sup>	3.80 <sup>h</sup>	2.93 <sup>c</sup>	3.65 <sup>g</sup>	3.81 <sup>h</sup>	2.68 <sup>b</sup>	3.44 <sup>C</sup>
C	4.15 <sup>i</sup>	4.55 <sup>l</sup>	3.18 <sup>de</sup>	4.05 <sup>i</sup>	4.28 <sup>j</sup>	2.89 <sup>c</sup>	3.85 <sup>D</sup>
D	4.90 <sup>m</sup>	5.256 <sup>n</sup>	3.37 <sup>f</sup>	4.63 <sup>k</sup>	4.91 <sup>m</sup>	3.10 <sup>d</sup>	4.36 <sup>F</sup>
Mean	4.06 <sup>DE</sup>	4.34 <sup>F</sup>	3.03 <sup>B</sup>	3.89 <sup>D</sup>	4.12 <sup>DE</sup>	2.78 <sup>A</sup>	
Flavonoids, kg quercetin·ha <sup>-1</sup>							
A	3.91 <sup>de</sup>	4.19 <sup>e</sup>	2.83 <sup>ab</sup>	3.59 <sup>c</sup>	3.61 <sup>c</sup>	2.55 <sup>a</sup>	3.44 <sup>A</sup>
B	4.62 <sup>f</sup>	4.81 <sup>f</sup>	3.48 <sup>c</sup>	4.08 <sup>e</sup>	4.60 <sup>f</sup>	2.97 <sup>b</sup>	4.09 <sup>C</sup>
C	5.53 <sup>hi</sup>	5.82 <sup>i</sup>	4.14 <sup>e</sup>	5.15 <sup>g</sup>	5.55 <sup>hi</sup>	3.74 <sup>cd</sup>	4.99 <sup>E</sup>
D	5.52 <sup>hi</sup>	6.16 <sup>j</sup>	3.96 <sup>de</sup>	5.28 <sup>gh</sup>	5.52 <sup>hi</sup>	3.723 <sup>cd</sup>	5.03 <sup>E</sup>
Mean	4.90 <sup>E</sup>	5.25 <sup>EF</sup>	3.60 <sup>B</sup>	4.52 <sup>D</sup>	4.82 <sup>D</sup>	3.25 <sup>A</sup>	
Essential oils, kg oil·ha <sup>-1</sup>							
A	1.49 <sup>d</sup>	1.70 <sup>f</sup>	1.22 <sup>b</sup>	1.34 <sup>bcd</sup>	1.51 <sup>de</sup>	1.05 <sup>a</sup>	1.38 <sup>A</sup>
B	1.62 <sup>ef</sup>	1.71 <sup>f</sup>	1.36 <sup>cd</sup>	1.46 <sup>d</sup>	1.64 <sup>ef</sup>	1.27 <sup>bc</sup>	1.51 <sup>B</sup>
C	2.15 <sup>hi</sup>	2.48 <sup>k</sup>	1.62 <sup>ef</sup>	2.03 <sup>gh</sup>	2.28 <sup>ij</sup>	1.59 <sup>ef</sup>	2.03 <sup>D</sup>
D	2.27 <sup>ij</sup>	2.64 <sup>l</sup>	1.67 <sup>f</sup>	2.01 <sup>g</sup>	2.32 <sup>j</sup>	1.46 <sup>d</sup>	2.06 <sup>DE</sup>
Mean	1.88 <sup>D</sup>	2.13 <sup>E</sup>	1.47 <sup>AB</sup>	1.71 <sup>C</sup>	1.94 <sup>D</sup>	1.34 <sup>A</sup>	

II – two-year-old plants, III – three-year-old plants, IV – four-year-old plants

## CONCLUSIONS

1. The yield of chamisso arnica flower heads and the content of sesquiterpene lactones, flavonoids, and essential oils were significantly higher in plots where the plants were cultivated in the traditional spacing system compared with the belt system.
2. The raw material yield significantly depended on the term of flower head harvesting. The delay in the harvest from the yellow bud stage to the end of flowering was accompanied by a successive rise in the flower head yield.
3. The content and yield of metabolites depended significantly on the term of harvesting the flower heads. The content and yield of sesquiterpene lactones systematically increased between the yellow bud stage and the end of flowering. The highest content of flavonoids and essential oils was detected in flower heads harvested in the full flowering stage; the highest yields of these metabolites were obtained in this stage as well. The lowest content of all analysed active compounds was found in inflorescences harvested in the yellow bud stage.

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## ROZSTAWA RZĘDÓW I TERMIN ZBIORU KOSZYCZKÓW KWIATOWYCH JAKO CZYNNIKI KSZTAŁTUJĄCE PLON I SKŁAD CHEMICZNY SUROWCA ARNIKI ŁĄKOWEJ (*Arnica chamissonis* LESS.)

### Streszczenie

Powierzchnia uprawy *Arnica chamissonis* Less. w Polsce jest bardzo mała, jednak ze względu na kurczące się zasoby naturalne arniki górskiej i wynikające z tego problemy z pozyskiwaniem surowca należy ją powiększyć, gdyż rynek farmaceutyczny zainteresował się surowcem z arniki łąkowej jako substytutem zagrożonego gatunku *A. montana* L. Ten fakt przesądził o potrzebie prowadzenia badań nad uprawą arniki łąkowej w celu dopracowania poszczególnych elementów jej agrotechniki. W latach 2015–2017 przeprowadzono doświadczenie polowe, którego celem była ocena wpływu rozstawy rzędów (tradycyjnej – rzędy pojedyncze co 40 cm i pasowej – z czterema rzędami co 40 cm na przemian z szeroką rozstawą co 80 cm) oraz terminu zbioru koszyczków kwiatowych (A – faza żółtego pąka, B – początek kwitnienia, C – pełnia kwitnienia, D – koniec kwitnienia) na plon surowca oraz jego skład chemiczny. Laktony seskwiterpenowe oznaczono metodą chromatograficzną opisaną w Farmakopei Polskiej VIII (URPLWMPB, 2008). Flawonoidy w przeliczeniu na kwercetynę oznaczono metodą spektrofotometryczną, natomiast do oznaczenia ilości olejków eterycznych wykorzystano hydrodestylację, zgodnie z Farmakopeą Polską VI (URPLWMPB, 2002). Największy plon surowca uzyskano w obiektach, gdzie rośliny uprawiano w tradycyjnym układzie rzędów, a zbiór koszyczków prowadzono pod koniec kwitnienia. Termin zbioru miał istotny wpływ na koncentrację i wydajność metabolitów. Zawartość laktonów seskwiterpenowych i ich plon systematycznie zwiększał się od fazy żółtego pąka do końca kwitnienia. Najwięcej flawonoidów i olejków eterycznych stwierdzono w koszyczkach zbieranych w pełni kwitnienia, wówczas też uzyskano najwyższe plony tych metabolitów. Najmniejszą zawartością wszystkich analizowanych substancji czynnych charakteryzowały się kwiatostany pozyskane w fazie żółtego pąka. Rozstawa rzędów oraz termin zbioru koszyczków kwiatowych miały istotny wpływ na plony surowca i zawartość badanych substancji aktywnych. Uprawa arniki łąkowej w tradycyjnej rozstawie była korzystniejsza ze względu na większe plony koszyczków kwiatowych oraz plon laktonów seskwiterpenowych, flawonoidów i olejków eterycznych.

**Słowa kluczowe:** *Arnica chamissonis*, flawonoidy, laktony seskwiterpenowe, olejki eteryczne, rozstawa rzędów, termin zbioru