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Influence of agrotextil foil on *Arnica montana* flower yield

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Abstract

Arnica montana is endemic medicinal plant species that, due to its biological activity, is widely used in medicine, pharmacy, and cosmetics, which is why it has a high commercial value. According to legal regulations prohibiting the exploitation of *A. montana* from natural habitats and the limitation of human resources in its production, there is a constant need to improve the technology of its cultivation. Therefore, this study aimed to investigate the effect of agrotextile mulch film on the flower yield of *A. montana* in the second year of cultivation. *A. montana* seedlings produced at the "Dr. Josif Pančić" Institute from autumn 2021 to spring 2022 were used for the experiment. In the middle of May 2022, a demonstration experiment was set up on Tara Mountain (43°54'31.4''N 19°32'22.6''E) on the district cambisol on an area of 10 m^2 with and without the application of mulch at a density of 24 plants/m^2. Due to the successive flowering of *A. montana*, seven harvests were conducted out in the period from the beginning to the end of June 2023. The average flower yield per plant differed significantly in harvests and treatments (with and without agrotextiles), but the highest number of flowers (1.20; 1.18), as well as the yield (30.03; 29.58 g/m^2), was obtained during the fourth harvest. The average yield of air-dried flowers without the use of agrotextiles ranged from 97.15 g/m^2 in the first harvest to the last, whereas a slightly higher yield was achieved with the use of agrotextiles, 105.9 g/m^2.

Key words: growth, yield, mulch, harvest, distric cambisol

INTRODUCTION

Arnica montana L. is a herbaceous plant species from the Asteraceae family. It is native to the Alps, Europe, the Pyrenees, the Balkan mountains, Scandinavia and the Baltic states. *A. montana* is a perennial herbaceous plant with a height of 30 to 60 cm. It develops short, cylindrical rhizomes, and above the ground forms a rosette of leaves from which flower-bearing stems with flowers from 5 to 8 cm wide are formed. The leaves in the rosette are large, elongated egg-shaped, and on the stem they are smaller and more numerous. The tree branches, developing from two to four lateral branches that end in yellow zygomorphic female tongue-shaped flowers. It blooms successively in the period from May to August. The flowers are characterized by yellow-orange tongue-shaped petals 2-3 cm long. Fertile tubular flowers and a small bulging inflorescence are located inside the flower. It houses cylindrical achenes (seeds) with a white and firm pappus (flying apparatus). The seeds are gray-black in color, elongated in shape with relatively good germination (Pljevljakušić et al. 2014).

The flower head - *Arnicae* flos, which is enriched with sesquiterpene lactones, flavonoids, carotenoids, pyrrolizidine alkaloids, polyacetylenes, phenolic acids and essential oils, has the greatest use value in this medicinal plant species. The effect is antimicrobial, antiphlogistic, analgesic, antirheumatic. *A. montana* is widely used in European folk medicine as an effective ointment for hematomas, bruises, sprains and muscle pain and against inflammation. It is used to make a large number of pharmaceutical preparations in the form of tinctures for internal use or preparations for external use such as ointments, creams, balms, gels, etc. (Kriplani et al. 2017).

In earlier years, meeting the market demand for *A. montana* raw materials (mainly flower heads) was from natural populations. Overexploitation of natural populations of *A. montana* in some countries such as Germany, Switzerland and Great Britain has caused the degradation of natural populations (Kathe, 2006). Because of this, the species became strictly protected in France, Germany and other Balkan countries. For the aforementioned reasons, the introduction of this plant species in the territory of the Republic of Serbia began in 2004 on Mount Tara (Radanović et al., 2006), while the first cultivation of its relative *A. chamissonis* began in 2000 on Mount Suvobor (Roki et al., 2001). According to Radanović et al. (2006), the establishment of plantation production can begin generatively from seeds and vegetatively by dividing the tiller of plants from 3 - 4 years of cultivation. Taking into account the morpho-physiology of the seeds, the establishment of production begins with the winter or summer production of seedlings under controlled conditions. It is preferable that the temperature of the substrate during the cultivation of seedlings is between 15-20ºC. Produced seedlings of *A. montana* are grown at altitudes above 900 m. *A. montana* is not sensitive to increased insolation, tolerates shady and semi-shaded places well and is tolerant of low temperatures. It is desirable that the average annual temperature be in the range of 5-7ºC, and the average during the growing season 11-13ºC with the sum of annual precipitation of 800 to 1100 mm. It is sensitive to waterlogged and carbonate soils. Loose, humus, acidic soils (pH = 5-6) are most suitable for it. During plantation cultivation, it is recommended to carry out certain care measures such as hoeing, fertilizing, irrigation and weed removal (Pljevljakušić et al. 2012).

In general, in conventional production, all the mentioned care measures are carried out in a mechanized way with the application of appropriate chemical agents, without additional difficulties. Taking into account that the production of medicinal plants takes place according to the principles of Good Agricultural Practice (GAP), where the use of chemical agents is not allowed, and the labor force is less and less, there are a large number of difficulties in implementing appropriate care measures. Primarily, weeding is a major difficulty in this production.

Solving this problem was started earlier by using polyethylene mulch film of different colors (Radanović et al. 2007). The mentioned research showed a great impact on the reduction of weeds as well as a positive impact on morphological characteristics and yield. Namely, according to the literature, the yield of flowers without the application of polyethylene film at a density of 70,000 to 80,000 plants per hectare was from 300 to 700 kg of dry flowers. However, the short duration of the used mulch material as well as the emergence of new mulching products represents a constant need to improve the existing *A. montana* growing technology. Therefore, the aim of this study was to investigate the effect of agrotextile film on the yield of arnica flower.

MATERIAL AND METHODS

The experiment was conducted from the beginning of 2022 to the middle of 2023. The first stage is the production of seedlings, and the second is cultivation and harvesting in the demonstration field with the registration of the yield.

Production of seedlings *A. montana*

The seeds (*A. montana*) used in this experiment come from the foreign commercial company "Jelitto", (Germany), where the weight of 1000 seeds is 1.08 g, and the germination rate is about 90%. The production of seedlings began at the end of January 2022 in the laboratory of the Department for Research and Development in Agriculture with its centre in Belgrade, R. Serbia (44°49′ N, 20°28′ E). The seeds were sown in styrofoam containers with 160 cells, previously filled with the commercial substrate "Cultivo I SF" (Gramoflor, Romania). According to the characteristics of the supplier, the properties of the substrate are: granulation 0 - 5 mm, fertilizer NPK 18:10:20+Mg+me in a dose of 1 kg/m3, RADIGEN® - Jost GmbH (reproductive micronutrient) in an amount of 50 g/m3, hydrogel (moisturizer) in a dose of 1 kg/m3. After sowing, the containers were placed in a polyethylene tent (Grow Box), under the following growing conditions: artificial lighting produced by cold fluorescent tubes with a photoperiod of 12 hours; air humidity from 40 to 60%; air temperature from 20 °C to 24

°C, and the substrate was constantly kept moist and its temperature was 21±2°C. Monitoring of air temperature and relative humidity in the Grove Box was provided using a HAXO-8 Data Logger and for substrate temperatures using a Testo 110 thermometer.

Figure 1. Production of seedlings *A. montana*

With the appearance and development of the first true leaves, in mid-February 2022, the adaptation process began for 3 weeks (Mrđan et al. 2022; Gordanić et al. 2023). The containers were taken outside the tent, and occasionally they were taken outside to ensure adaptation of the seedlings to lower air temperatures, reduced relative humidity and natural light with occasional watering. After acclimatization, the seedlings were placed in an unheated greenhouse, in 30% shade, at an average daily temperature of 24±2ºC and with irrigation as needed. At the beginning of March, seedlings were transplanted into styrofoam containers with 40 holes, and at the beginning of April, they were transplanted into plastic containers with 28 holes using the same substrate (Figure 1). At the end of April, the acclimatization process of the seedlings started again, which lasted until they were transplanted to the demonstration field.

Growth on a field

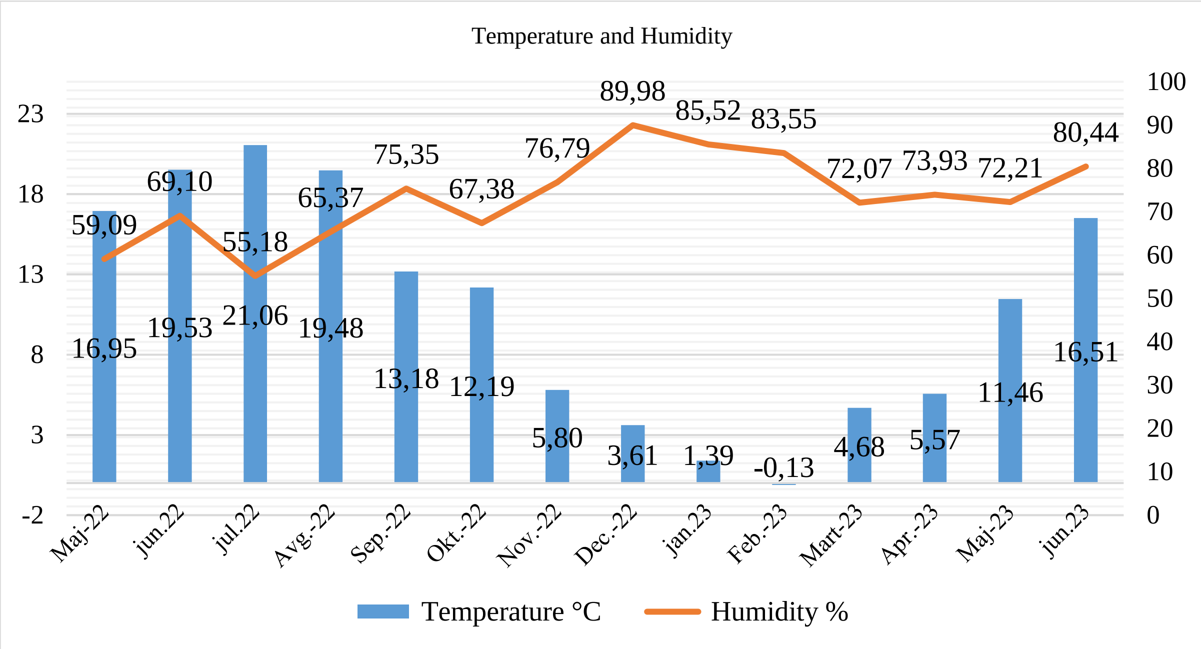
In mid-May 2022, the produced seedlings of *A. montana* were transplanted to the demonstration field of a private agricultural farm in Kaluđerski Bari - Tara (43°54'31.4"N 19°32'25.8"E). Planting was done on raised beds 1 m wide and 10 m long (0.1 ar or 0.001 ha) in two variants, with four replications (2x4). The first was with the application of mulch at a density of 24 plants/m^2 and the second, without the application of mulch at the same density. The soil on which the experiment was set up is District Cambisol, acidic reactions, well supplied with humus and nitrogen, poor in phosphorus and moderately provided with potassium (Table 1).

Table 1. Basic chemical properties of the surface soil layer on the demonstration field (0-25 cm)

pH		Humus (%)	Total N (%)	AL-mg/100 g	
H_2O	KCl			P_2O	K_2O
5.9	4.8	4.5	0.31	4.0	17.0

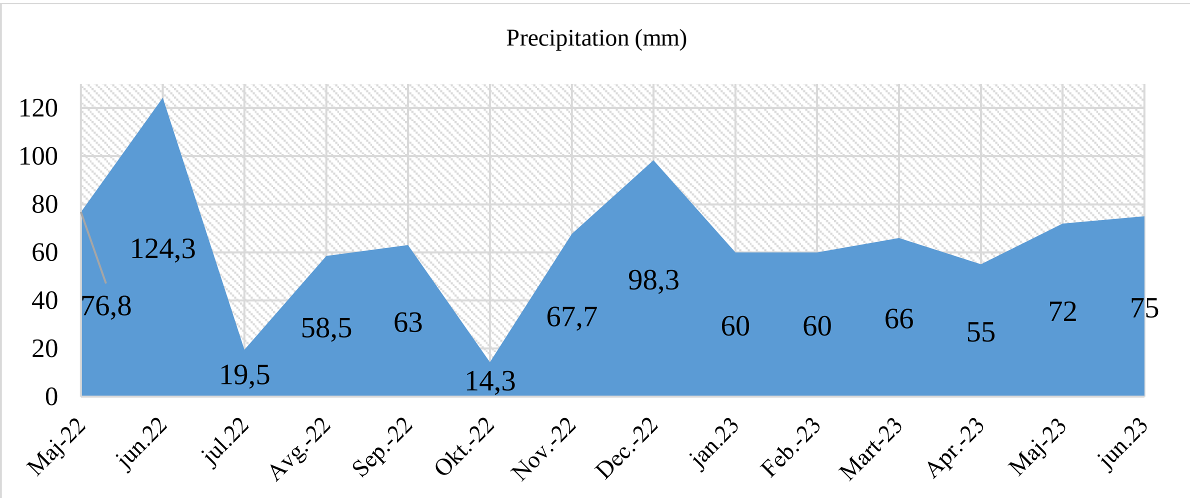
A commercial, synthetic, polypropylene material, photostable (against UV radiation), low mass and high elasticity, is used for mulching - an agrotextile film that does not retain or absorb water because water drops from rainfall and watering diffuse through its micropores.

During the experiment, climatic parameters (temperature and relative humidity of the air) were constantly monitored using a HAXO-8 data logger, and precipitation data were taken from the nearby automatic meteorological station - Zaovine - Tara (43° 53' 47" N 19° 23' 34" E), (Graph 1 and 2).



Graph 1. Temperature (°C) and Humidity (%) during vegetation

Since the beginning of cultivation of *A. montana* in the demonstration field, the highest recorded temperature (21.06 ºC) was in July 2022 and the lowest (-0.13 ºC) in February 2023 (Graph 1). The total temperature during cultivation in the demonstration field from the beginning of cultivation until harvest was 151.27 ºC, while the total precipitation was 910.40 mm. The average temperature was 10.80 ºC and the relative humidity was 73.28%. Taking into account the previous perennials (2008, 2009 and 2010), Pljevljakušić (2013) states about the climatic requirements of this plant species, more precisely about the average annual temperatures (11.44; 10.92 and 10.54 ºC) and precipitation (819; 806 and 936 mm), if we leave out the uneven distribution of precipitation (Graph 2), we can state that the research was conducted in optimal climatic conditions.



Graph 2. Precipitations (mm) during vegetation

Harvesting, yield registration and statistical analysis

Taking into account the physiology of the plant, the successive harvest was carried out during June 2023, according to the method described in the research of Pljevljakušić (2013). After harvesting the flower heads, they were dried according to the method of Prommersberger and Dapper, (1995); Bomme, (1991); Pljevljakušić (2013), and the comparison of yield mean values were statistically analyzed using SPSS software using Duncan's test ($p<0.05$).

RESULTS AND DISCUSSION

According to the obtained research results, it can be seen that there is a difference in yield parameters both in harvests and among treatments. Considering that *A. montana* is a perennial plant species whose yield in the first year is negligible or absent (Pljevljakušić 2012), it can be seen that during the second year of cultivation, a variable yield was achieved. The highest number of flowers in both treatments was recorded during the fourth harvest, among which there was no significant difference (Table 2). The lowest number of flowers when growing on agrotextile film was obtained in the first harvest, while without it the lowest number of flowers was recorded in the later stages of the harvest, which later reflected on the overall productivity of production. Different yield dynamics during multi-phase harvests in according to Dragumilo et al. (2021), can be attributed to the effect of mulch. We assume that the agrotextile foil, due to its physico-chemical composition, exerted a positive influence on the rational exploitation of soil moisture, which was later reflected in the yield parameters in the last harvests. The stated statement can be confirmed by the research of Vikana et al. (2019), conducted throughout Novreška, which points out that *A. montana* has the most pronounced requirements for precipitation, i.e. that it plays a key role in the physiological process of this species. Generally, similar yield parameters in cultivation without the use of agrotextiles were obtained in the multi-year research of Badalzadeh et al. (2022). Namely, when growing arnica on loamy type of soil, a similar number of flowers per plant (0.85) was obtained during the second year of cultivation as in several of our harvests (Table 2).

Table 2. Yield parameter results

Harvest	Flower yield with the use of mulch (treatment)		Flower yield without the use of mulch (control)	
	Air-dry flower yield per plant (g)	Yield of air-dried flower (g/m^2)	Air-dry flower yield per plant (g)	Yield of air-dried flower (g/m^2)
1.	$0.17±0,82^{eB}$	$4.22±1,09^{fB}$	$0.26±1,30^{eA}$	$6.62±0,95^{dA}$
2.	$0.64±0,69^{cA}$	$16.12±2,18^{cA}$	$0.67±2,98^{cA}$	$16.73±2,23^{bA}$
3.	$0.76±0,23^{bA}$	$19.01±3,23^{bA}$	$0.71±0,96^{bA}$	$17.63±1,38^{bB}$
4.	$1.20±0,55^{aA}$	$30.03±2,08^{aA}$	$1.18±0,75^{aA}$	$29.58±3,29^{aA}$
5.	$0.79±0,42^{bA}$	$19.77±436^{bA}$	$0.56±0,92^{dB}$	$14.1±1,47^{cB}$
6.	$0.30±0,53^{dA}$	$7.4±2,42^{eA}$	$0.23±0,85^{fB}$	$5.86±0,59^{eB}$
7.	$0.37±0,44^{dA}$	$9.35±2,19^{dA}$	$0.27±0,96^{eB}$	$6.63±0,85^{dB}$
		∑ 105.9		∑ 97.15

*Means with the same small superscript letters within the same column between harvest are not significantly different ($p < 0.05$); Means with the same capital superscript letters between treatment are not significantly different ($p < 0.05$)

However, there was a difference in the flowering dynamics. The highest yield of flowers by Pljevljakušić et al. (2014), were achieved in the first harvests, which was not the case in this study, where the highest yield was achieved from the third to the fourth harvest. Earlier research by Radanović et al. (2007), indicates that the flowering dynamics of *A. montana* is largely influenced by climatic factors, which is probably the main cause of this case. Accordingly, taking into account the climatic characteristics during the research (Graph 1 and 2) that the plants during the growing season in the phase of regeneration and formation of the leaf rosette and the stem (April-May) were exposed to significantly lower temperatures than was the case in Radanović et al. (2007) and Pljevljakušić et al. (2014). In general, the flower yield of *A. montana* grown on Agrotextile was 8.3% higher than in the control, more precisely the total yield of the air-dried flower in the control was 97.5 g/m2, while in the treatment it was 105.9 g/m2, i.e. 975 kg and 1059 kg of flower per hectare. Comparing the obtained yields with yields from earlier research when growing on mulch foil (about 10 g/m2) by Radanović et al., 2007) and without it (from 3.2 to 38.65 g/m2) by Pljevljakušić et al. (2014) conducted in the same area, we can state that the results of this research are significantly better. Taking into account similar ecological conditions, we assume that the main reason is the density of cultivation, because in Radanović et al. (2007) and Pljevljakušić et al. (2014) *A. montana* was grown at a lower density. (24 and 22 plants per m2). Generally, the obtained yield results in the second year show the positive impact of agrotextile film and denser planting, but it should be continuously monitored through the yield and other parameters of its quality during the following years.

CONCLUSION

The obtained results showed a positive influence of the agrotextile film on the yield of flowers per plant, which was later reflected in the total yield of *A. montana*. The conducted research shows that A. montana can be successfully grown on this type of mulch, which achieves higher productivity with minimal labor input. Considering the physiology of *A. montana,* it remains to monitor the multi-year yield, which will be reflected in the quality of the obtained raw material.

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