RESEARCH ON THE OPTIMISATION OF *ARNICA MONTANA* L SEEDLING PRODUCTION

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Abstract

The production and commercialisation of medicinal herbs derived from spontaneous flora has become an area of vast economic activity, being a source of income for producers, merchants and processors. The sustainable use of natural resources is one of the great challenges of our epoch. This challenge is directly bound to local livelihoods and to their economic viability, absent these factors the utilisation of natural resources (conservation) cannot be managed. Sustainable use is only possible through the development of "models".

Key words: conservation, biodiversity, seedling production

INTRODUCTION

Arnica montana is a threatened species. It is listed on Annex V of the Habitats Directive and on Annex D of the Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein. It appears as "vulnerable" on the Red List of Romanian tracheophytes and on the "Aii" category, being threatened on a European level. *Arnica montana* is an emblematic species of the region, the inflorescences being used in traditional medicine for a wide variety of diseases. The pharmacological profile includes antibacterial, antifungal and anti-inflammatory properties. It appears on low productivity oligotrophic pastures extensively exploited through both grazing and mowing.

Pastures with a high degree of phytodiversity are being threatened by: intensification (especially fertilisation), abandonment due to migration to other areas and reforestation. Within the "Proiectul Apuseni", implemented in the Gârda-Gheţari area, Alba County, as well as during the period when monitoring of *Arnica montana* habitats was carried out (Michler et al, 2007), both overseeding and direct sowing have been attempted, both attempts however resulting in failure. As a result, the only option regarding the establishment of *Arnica montana* cultures is through seedlings.

MATERIAL AND METHOD

At the present time all *Arnica montana* raw material (*Arnicae flos*) is derived from spontaneous flora. In the Gârda–Gheţari area approximately 3 tonnes of inflorescences are harvested annually from their natural habitats

(pastures and hayfields) by the local population. Large scale harvesting directly from the spontaneous flora is preferred, resulting in ecological imbalances in specific spontaneous flora basins. The surface area of the Gârda commune is 87.4 km^2 in which 597 areas where *Arnica* appears have been identified (Barbara Michler, 2007). These measure a total of 550 ha and are usually located on forest edges or on the peaks of rocky hills, with the land use management activities of local farmers influencing the floral composition of the habitats and the abundance of *Arnica montana*.

Objectives:

- Optimisation of the rooting substrate for vigorous seedlings, with the goal of establishing an experimental field in the Gârda–Gheţari area, as an attempt to conserve the spontaneous flora.
- The use of some germination stimulation methods (removal of the pappus, sowing without seed dressing).

Taking into consideration that arnica is a strongly oligotrophic species, for the rooting substrate used in the seedling production, the experiment will use the terra rossa soil, a soil type that is predominant in the Gârda area. The experiment will use different mixture variants, with peat, terra rossa soil and sand being combined in different proportions.

For experimental use, conditioned *Arnica montana* seeds harvested in 2012 were employed. Following research it was found that arnica seeds remain in a period of seminal rest 4 months after being harvested, explaining the low germination percentage in this period (38%) (Vârban et al, 2012). Therefor, seeds harvested 1 year and 4 months ago have been used.

For the identification of the optimal soil mixture 4 variants were used: V1- peat, V2- peat: terra rossa 1:1, V3- peat: terra rossa 2:1 and V4- peat: terra rossa 1:2. For each substrate 3 sowing methods were used: (1) with uncoated seeds, (2) with seeds coated in a thin layer of soil and (3) with the pappus removed.

In every case the percentage of emerged plants (%) and duration of emergence (no. of days until emergence) was observed. Sowing was done on 2.04.2014 using seed cells, 2 seeds per cell. Each variant was observed through 3 repetitions with one repetition consisting of 58 seed cells, consisting of 2 seeds per cell.

RESULTS AND DISSCUSIONS

Using the V1 variant as a control sample (regardless of sowing method used) it is observed that variants V2 and V4 present a larger emergence percentage, significant differences being registered. Variant V4 also has an emergence percentage superior to that of the control sample, as

such the presence of Terra Rossa soil in the rooting substrate has beneficial effects on the emergence of arnica plants (Table 1).

Table 1

Influence of the soil substrate on the emergence percentage of *Arnica montana*

Variant / Mixture	**Emerged Plants**		**± Difference**	**Significance**
	% Emergence	%		
V1 (control) Peat	72.67	100	0	Mt.
V2 Peat: Terra Rossa (1 : 1)	79.22	109.0	6.56	xxx
V3 Peat: Terra Rossa (2 : 1)	82.67	113.8	10.0	xxx
V4 Peat: Terra Rossa (1 : 2)	77.00	106.0	4.33	xx
DL 5%= 1.92	DL 1%=2.91		DL 0.1%= 4.68	

Following the result analysis it emerged that the sowing manner influences the percentage of emergence by plants. Although some previous results recommend uncoated sowing, it was observed that coating the seeds in a thin layer of soil increases the percentage of emergences (significant differences compared to the control sample are registered) by all rooting substrates (Table 2).

Table 2

Influence of the sowing method on the percentage of emerged *Arnica montana* plants

Sowing Method	**Emerged Plants**		**± Difference**	**Significance**
	% Emergence	%		
Uncoated (Control)	77.25	100	0	Mt.
Coated	79.67	103.1	2.42	xx
Removed pappus	76.75	99.4.8	-0.50	-
DL 5%= 1.44	DL 1%=1.99		DL 0.1%= 2.74	

Analysing the interaction between the two factors, higher values were observed on the variant with coated seed at the majority of rooting substrates, an exception being the peat-terra rossa 1:2 substrate, where a decrease in the percentage of emerged plants was observed. (Table 3).

For the analysis of the optimal rooting substrate the emergence duration was observed (no. of days).

Regarding the number of days until emergence, it was observed that the seeds germinate and sprout the fastest on the peat substrate (15.33 days), good results however were also registered at the V3 and V2 substrates. The longest emergence duration period was registered at the 1:2 peat-terra rossa substrate (21 days) - Table 4.

Table 3

Influence of the soil substrate and the sowing method on the percentage of emergences (interaction of the rooting substrate x sowing method)

Rooting substrate	**Sowing Method**	**Emerged Plants**		**± Difference**	**Significance**
		% Emergence	%		
V1 Peat	Uncoated (Control)	70	100	0	Mt.
	Coated	78	111.4	8.0	xxx
	Removed pappus	70	100	0	-
V2 Peat:Terra Rossa 1: 1	Uncoated (Control)	80	100	0	Mt.
	Coated	83	99.6	-0.33	-
	Removed pappus	78	97.5	-2.00	-
V3 Peat:Terra Rossa 2:1	Uncoated (Control)	81	100	0	Mt.
	Coated	86	106.2	5.00	xx
	Removed pappus	81	100	0	-
V4 Peat:Terra Rossa 1:2	Uncoated (Control)	78	100	0	Mt.
	Coated	75	96.2	-3.0	0
	Removed pappus	78	100	0	-

DL 5%= 2.88 DL 1%=3.97 DL 0.1%= 5.47

Table 4

Influence of the soil substrate on the emergence percentage of *Arnica montana* species

Mixture Variant	**Duration of emergence**		**± Difference**	**Significance**
	No of days	%		
V1 (control) Peat	15.33	100	0	Mt.
V2 Peat: Terra Rossa (1 : 1)	16.67	108.7	1.33	-
V3 Peat: Terra Rossa (2 : 1)	16.33	106.5	1.00	-
V4 Peat: Terra Rossa (1 : 2)	21.00	137.0	5.67	000

DL 5%= 1.92 DL 1%=2.91 DL 0.1%= 4.68

It was observed that the fastest emergence occured in the variants with coated and uncoated seeds. Regarding seeds with removed pappus a prolongation of the emergence period by all rooting substrates contrasted with the control sample (Table 5).

Table 5

Influence of the sowing variant used on the emergence percentages of Arnica montana

Sowing Method	**Duration of emergence**		**± Difference**	**Significance**
	No of days	%		
Uncoated (Control)	16	100	0	Mt.
Coated	17.5	109.4	1.5	-
Removed pappus	18.5	115.6	2.50	o

DL 5%= 2.18 DL 1%=3.00 DL 0.1%= 4.13

Observing the interaction of these 2 factors, we can observe the shortest emergence period by the variants with coated and uncoated seeds. At all substrate variants a prolongation of the duration of emergence by all seeds with removed pappus can be observed. We assume that the pappus has a role in the emergence of *Arnica montana* plants, contributing to the substrate seeding, especially in uncoated variants. (Table 6).

Table 6

Influence of the soil substrate and the sowing method on the percentage of emergences (interaction of the rooting substrate x sowing method)

Rooting substrate	**Sowing Method**	**No of days**	**%**	**± Difference**	**Significance**
V1 Peat	Uncoated (Control)	13	100	0	Mt.
	Coated	15	115.4	2.0	-
	Removed pappus	18	138.5	5.0	0
V2 Peat:Terra Rossa 1: 1	Uncoated (Control)	15	100	0	Mt.
	Coated	17	113.3	2.00	-
	Removed pappus	18	120.0	3.00	-
V3 Peat:Terra Rossa 2:1	Uncoated (Control)	14	100	0	Mt.
	Coated	17	121.4	3.00	-
	Removed pappus	18	128.6	4.00	-
V4 Peat:Terra Rossa 1:2	Uncoated (Control)	22	100	0	Mt.
	Coated	21	95.5	-1.00	-
	Removed pappus	20	90.9	-2.00	-

DL 5%= 3,95 DL 1%=5,53 DL 0,1%= 7, 85

Fig. 1 *Arnica montana* seedling- emergence

Fig. 2 Appearance of the basal rosette

CONCLUSIONS

Following the experiments and observations regarding the production of seeding stock for the establishment of *Arnica montana* cultures, we can formulate the following conclusions and recommendations:

- *Arnica montana* culture can be established only by seedling; direct seeding does not offer results, given the favourable areas this species presents;
- for sowing only older than 1 year, certified, conditioned, minimum 80% germination seed will be used (after 2 years of storage germination decreases); after harvesting the seeds enter seminal rest and emergence occurs only after a storage period (1 year after harvesting);
- for seedling production sowing in warmed greenhouses is recommended; sowing period: 20 January-20 February;
- for the obtainment of a vigorous seedling and for good emergence we recommend a soil substrate composed out of 1:1 or 1:2 peat and terra rossa soil. The proportional increase of terra rossa has no beneficial effects on the emergence and growth of *Arnica montana* plants. Likewise we recommend that the seeds are coated with a thin layer of soil right after sowing. Even though following data processing statistically relevant differences are not observed by all variants, the susequent seedling growth is better by variants with coated sowing. Even though seeds emerge the fastest from the peat-only substrate, we do not recommend the production of seedlings in this manner, due to the fact that in 1 month from emergence the seedlings weaken and many of them are extinguished. *Arnica montana* is a strongly oligotrophic species and the peat sortiments used were eutrophic (pH 7).

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