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Peculiarities Of Formation *Leonurus Quinquelobatus L.* Seeds In Ontogenesis.

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ABSTRACT

Scientific institutions of the Crimea are working on the creation varieties of these crops, studying certain elements of the technology of growing raw materials. However, the issues of the developmental biology and the formation of seeds have not been studied sufficiently. One of the most promising medicinal crops of the Russian Federation is motherwort five-blade. In medicine, preparations based on motherwort are used for treatment diseases of the nervous and cardiovascular systems. The purpose of our study the features of accumulation dry matter in the seeds at the period of physiological maturity and to determine the most optimal time and methods of harvesting. After the end of the flowering phase and every week were cut 4 sheaves. In the laboratory, each sheaf was taken 2 samples of seeds weighing 1-2 g, leaves - 2-5 g, stems 3-5 g. After they were dried in a drying oven at t ° 105°C for 3 hours, then samples were weighed again and the moisture content was determined. To determine 1000-seeds weight, 500 seeds from each sheaf were counted in two replications, weighed and recalculated in proportion. The intensity of reutilization in the dairy state was 30.7%, in the dough – 19.0%, wax – 13.2%, hard – 6.5%. Thus, harvesting of seeds of motherwort five-blade must be done separately, beginning with mowing the plants in sheaves in the wax ripeness phase with a seed moisture content of about 30% and a vegetative mass of 60%.

Keywords: medicinal plants, phases of seeds ripening, 1000-seeds weight, reutilization, dates and methods of seed harvesting.

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INTRODUCTION

Today it is known that medicinal plants are important in the treatment and prevention of various diseases [1]. Despite the favourable climatic conditions for the medicinal crops cultivation, the pharmaceutical industry of the Russian Federation is experiencing a significant shortage of medicinal plant raw materials. While in Europe the percentage of pharmaceuticals based on medicinal plants is about 10 %, in Russia this product takes only 0.5-1.5 % of the total pharmaceutical market. This is due to the lack of scientific and technical support for this branch of agricultural production [2].

The Republic of Crimea is the traditional region for cultivation medicinal, essential and ornamental plants. Motherwort five-blade, purple coneflower, medicinal sage, spotted milk thistle, chamomile, etc. are successfully grown here. Conditions of the peninsula allow to obtain not only high-quality medicinal raw materials, but also seed material. Scientific institutions of the Crimea are working on the creation varieties of these crops, studying certain elements of the technology of growing raw materials [3]. However, the issues of the developmental biology and the formation of seeds have not been studied sufficiently.

One of the most promising medicinal crops of the Russian Federation is motherwort five-blade. It is a perennial herbaceous plant in the family *Lamiaceae* or *Labiatae* with high adaptive properties, widespread almost everywhere in Europe, central part of Russia, in Western and Eastern Siberia, in the Caucasus [4, 5].

The grass of the motherwort of the five-lobed contains various compounds that belong to the groups of monoterpenes, diterpenes, triterpenes, flavonoids, phenylpropanoids, phenolic acids and nitrogen-containing compounds [6].

In medicine, preparations based on motherwort are used for treatment diseases of the nervous and cardiovascular systems [4, 5]. They are also effective in treating asthma, menopausal symptoms, and amenorrhea [6]. Two main alkaloids in motherwort extract are known to have a pronounced vasodilating and neuroprotective effect: leonurine и stachydrine [7] In addition, leonurine reduces synovial inflammation and prevents joint damage [8]. Sadowska in their studies revealed immunomodulatory activity of polyphenolic compounds of this plant, which prevents the development of chronic inflammatory processes in infected tissues [9].

In experiments Popescu revealed an increase in the roots of *Triticum aestivum* L. under the influence of aqueous extracts of motherwort. These studies have confirmed the possibility of using this plant not only in pharmacology but also in agriculture [10]

Scientists from Kazakhstan have developed a technology for growing motherwort to produce medicinal plant raw materials, which includes optimal soil, the use of fertilizers, harvesting time, etc. [11]. Bondarenko M. I. in his research described the methods of cultivation of soil and the optimal density of plants per unit area [12]. At the same time in the scientific literature there is little information about the technology of cultivation of motherwort to obtain high-quality seed.

Development of cultivation technology for agricultural crops is based on the biological characteristics of plants, manifested in ontogenesis under different climatic conditions. Special attention is paid to phenology, morphogenesis, organogenesis, and fruit formation. Hence, the optimal sowing time, process of plant care, time and methods of harvesting are determined [13, 14]. At the same time, it should be taken into account that different cultivation technology can be used to obtain high-quality medicinal raw materials and sowing material, since the parent plants and seeds are at different stages of ontogenesis and their need for environmental factors may differ [15].

Purpose of research. Study the features of accumulation of dry matter in seeds at the period of physiological maturity and determine the most optimal time and methods of harvesting seed crops.

MATERIALS AND METHODS

The scheme of ontogenesis and vegetative period of *Leonurus quinquelobatus* was compiled on the basis of both phenological observations of plants, which were sown in spring, during vegetation period 2015-

2017, and the cyclic scheme of periodization ontogenesis and vegetative period developed by N. M. Makrushin for winter wheat (*Triticum aestivum* L.) [16].

Phenological observations were carried out daily throughout the year for each variant separately. The whole period of plant ontogenesis was recorded according to the phases of development in the field log with the date indicated. The first day of each phase registration in, at least, 10% of plants was considered as the beginning of the phase. If this was observed in 50-75% of plants – it was recorded as the mass occurrence of the phase.

To establish the optimal time and methods of harvesting motherwort seeds, 5 plants at a height of 10-15 cm in 4 replications were cut forming 4 sheaves after the end of the flowering phase and every week after. Two sheaves were taken to the laboratory on the day of harvesting, and the other two were left for ripening in a dry ventilated room. In the laboratory, the seeds were threshed from each sheaf and the leaves were separated from the stems. Two samples were taken with measuring devices of 1-2 g from the total weight of seeds: leaves - 2-5 g, stems 3-5 g. After they were dried in a drying oven at t ° 105°C for 3 hours, then samples were weighed again and the moisture content was determined.

To determine 1000-seeds weight, 500 seeds from each sheaf were counted in two replications, weighed and recalculated in proportion.

Two weeks later, similar experiments were carried out with sheaves that were left for ripening.

According to the moisture content and 1000-seeds weight, the optimal time and methods of harvesting were determined.

The data are processed according to the standard methods of statistical processing using the Microsoft Office software package (Excel) and "ANOVA" program.

RESULTS AND DISCUSSION

When motherwort five-bladed was sown in spring under the conditions of the foothill zone of the Crimea, first sprouts appeared in 16 days, and in 22 days – mass seedling growth was observed. The phase of the first three leaves is observed 4 days after germination. After 11 days, two more sufficiently developed leaves are formed. Mass stem elongation occurs in two weeks and ends with the beginning of the formation first buds in the upper part of the shoots in late July. The duration of this period is about 56 days.

In the first year of vegetation, the formation of full-fledged inflorescences and seeds is observed only on individual plants. Since the end of September, the death of vegetative mass begins. During this period, the motherwort is mowed to a height of 10-15 cm, since the lower part of the plant contains the renewal buds, which contribute to the spring re-growth for the next year of vegetation.

Favorable temperature conditions in the third decade of February (t° of air in 2016 – 7,4°C, in 2017 – 6,3°C, norm -2,4°C) and in the first decade of March (t° of air in 2016 – 10,3°C, in 2017 – 10,0°C, norm – 3,9°C) contributed to the active re-growth of vegetative mass in the first days of March. The beginning of the stem formation in the second and third year of vegetation was observed in 11 and 13 days after re-growth, respectively. Two weeks later, the beginning of the branching phase was observed on some plants, and a week later – mass. The duration of development of vegetative organs during the second and third year of vegetation is reduced by almost a week and is about 50 days. After 4 - 5 days mass budding phase starts, and in 9 – 10 days – flowering. The flowering period of the motherwort is quite long and is about 23 – 24 days. Since mid-July, the process of seed formation begins.

Ontogenesis is the individual development of the organism, in which structural and functional changes are taking place that differentiate and specialize cells, tissues and organs through the realization of genetic information [17, 18].

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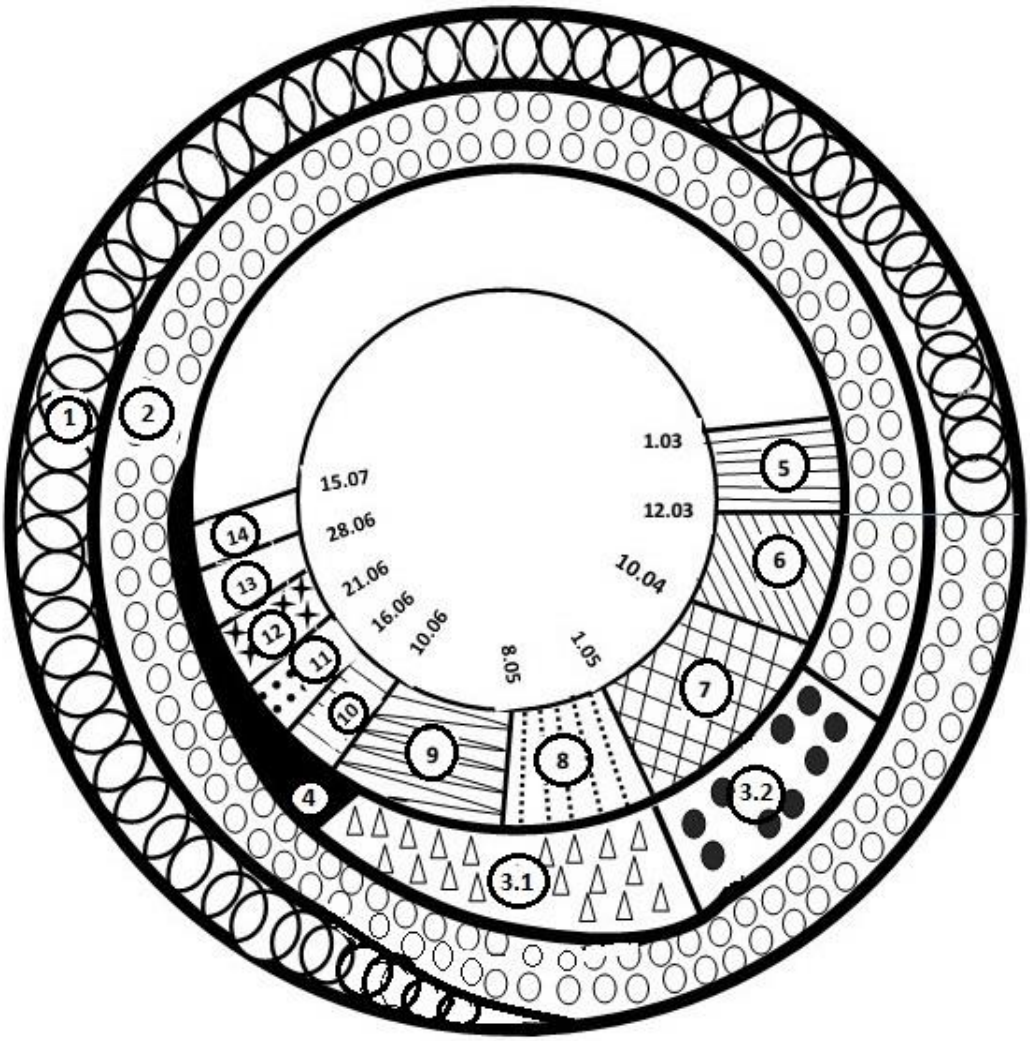
By the methodology of N. M. Makrushin we have made a cyclic scheme of ontogenesis and vegetative period of *Leonurus quinquelobatus*, which begins with the formation of a zygote and ends with natural death of the plant (Figure 1).



Ontogenesis (external circle, duration - 439 days): 1 – embryonic period; 2 – juvenile period; 3 – generative period: 3.1 – phase of puberty; 3.2 – phase of reproduction; 4 – senile period.
Vegetation period (inner circle, duration - 200 days). Growth stages: 5 – re-growth of vegetative mass; 6 – stem elongation; 7 – tillering; 8 – booting; 9 – anthesis (flowering); 10 – seed watery ripe (pre-milk); 11 – dairy; 12 – dough; 13 – wax ripeness; 14 – seed hard.

Figure 1: The scheme of the periodization of ontogenesis and vegetation period of *Leonuru squinquelobatus* L.

It includes four periods: embryonic, juvenile, generative and senile. The embryonic period begins with the formation of a zygote and is fixed by the average date of the flowering period, and ends with the formation seeds with maximum germination. Its duration is about 120 days. The juvenile period lasts from the moment of germinating seeds in the laboratory or from field germination to the formation generative organs (budding, sporogenesis) and lasts 220 days. The generative period includes two phases: puberty and reproduction. Sexual maturity begins from the beginning of the formation of male and female gametes and ends with the fertilization. In the phase of reproduction the whole process of formation of seeds prior to their seed-shattering is presented. The duration of the generative period is 69 days. The senile period is characterized by the gradual dying off of the vegetative mass of the parent plant and the completion of the seeds formation of the next generation. The duration of this period is about 30 days.

Thus, the total duration of ontogenesis of motherwort five-bladed is 439 days.

Vegetation period of this plant begins from the beginning of re-growth of the vegetative mass in spring, includes all phenological phases and ends with the maturation seeds of the child generation. According to the scheme, its duration is about 200 days.

Similar cyclic schemes for the periodization ontogeny and vegetative period for some medicinal and aromatic crops were compiled. E. A. Esoyan [19] developed such scheme for the perennial medicinal plant - *Echinacea purpurea*, the duration of ontogenesis of which is 315 days, and the growing season lasts 158. R. Yu. Shabanov [15] made a cyclic scheme of ontogenesis and vegetation period for coriander (variety Nectar), studying the individual development of plants at different sowing dates. The duration of ontogenesis when sown in autumn was 426 days, when sown in spring – 407. The duration of the growing season in the first case was 328 days, in the second – 109.

In our studies, we consider the ontogenesis of motherwort five-bladed from the formation of the zygote to natural death. In the scientific community there is no consensus on the beginning and the end of individual plant development. Some scientists say that ontogenesis is the complex of irreversible anatomical, morphological and physiological changes from the moment of zygote formation to natural death [20, 21]. Other scientists consider the beginning of individual development to be the moment of seed germination, and the end – its maturation on the parent plant [22, 23]. In this case, two concepts are identified – “ontogenesis” and “vegetation period of plants”.

However, this approach distorts the true periodization of ontogenesis as an individual development of the organism, since in this case, two generations are combined: “the mother's”, which begins with the seed germination and ends with the natural death of the plant and the “child’s” – from zygote to ripening seeds of a new generation [16]. Therefore, in order to obtain a high-quality seed crop, it is necessary to know a certain physiological state of the parent plant and seeds, which corresponds to specific terms and methods of harvesting.

Some researchers consider the stages of ontogenesis "from embryo to embryo" or "from seed to seed", thereby identifying individual development with the life cycle of plants [24, 25]. Of course, these two processes are organically linked: in the process of individual development, the body goes through all periods of the life cycle. However, ontogenesis is not cyclical, it begins with the zygote formation and ends with the natural death of the plant, while the life cycle ends with the formation of new zygote and represents a genetic link between the mother and daughter organism [16]. This ensures the continuation of life, not only of the individual, but also of the whole species.

1000-seeds weight of motherwort five-blade increased from the beginning of harvesting in the phase of dairy state to seed-hard ripeness: from 0.615 g to 0.944 g in 2015, from 0.301 g to 0.755 g in 2016 and from 0.294 g to 0.742 g in 2017 (Table 1).

Table 1: 1000-*Leonurusquinquelobatus* L. seeds weight during different phases of seed maturation (on the day of harvesting and after drying the sheaves)

Harvesting time	The state of ripeness of the seed	2015			2016			2017		
		1000-seeds weight, g	Increase relative to previous period,		1000-seeds weight, g	Increase relative to previous period,		1000-seeds weight, g	Increase relative to previous period,	
			g	%		g	%		g	%
		On the day of harvesting								
1	Dairy	0,615	-	-	0,301			0,294		
2	Dough	0,829	0,214	25,81	0,436	0,135	31,00	0,358	0,064	17,88
3	Wax	0,887	0,058	6,54	0,654	0,218	33,33	0,651	0,293	45,01
4	Hard	0,944	0,057	6,04	0,755	0,101	13,38	0,742	0,091	12,26
ISD_{05}		0,001			0,002			0,004		

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		After drying the sheaves								
1	Dairy	0,765	-	-	0,417			0,399		
2	Dough	0,921	0,165	17,92	0,543	0,126	23,20	0,467	0,068	14,56
3	Wax	0,963	0,042	4,36	0,761	0,218	28,65	0,756	0,289	38,23
4	Hard	1,004	0,041	4,08	0,809	0,048	5,93	0,787	0,031	3,94
ISD_{05}		0,002			0,004			0,006		

The most active increase in dry matter was observed at the initial stage of seed maturation. In the first year of vegetation from the phase of dairy to the dough-like state, 1000-seeds weight increased by 0,214 g or 25,81 %, and from the dough-like state to wax ripeness and from wax to hard-seed, this figure decreased and amounted 0.06 g or 6 %. During the second and third year of vegetation, the most dramatic increase in the seed mass was revealed in the period from the dough to the wax state: in 2016 it was 0,218 g (33,33%), and in 2017 – 0,293 g (45,01 %). In hard ripeness, the dry matter growth decreased to 0.101 g (13.38 %) and 0.091 g (12.26 %) in 2016 and 2017, respectively.

To determine the most optimal time for harvesting seed crops, some scientists take as a basis the qualitative changes in plants. So, Padilla et al., while studying the process of seed maturation of *Tithonia heterophyllous*, considers the phenological stage of the flower head as a determining factor for harvesting seed material [26]; Kasatkina, N. I. for red clover tetraploid - the percentage of reddish heads of plants [27].

According N. M. Makrushin, the most accurate criterion for determining the optimal time for harvest is the level of seed moisture. Using joint research conducted by I. E. Illi and N. B. Scherbatyuk [16] he found that the number of cells in the wheat germ increases up to the dough-like state. It was also noted that in the phase of milk ripeness during the ripening seeds in the rolls there is a longer process of reutilization of organic substances from the vegetative organs to the grain, as a result of which the embryo mass increases more intensively. It was promoted by high moisture content (dairy state – 68, dough – 52, wax – 41, hard – 24%). Thus, 1000-seeds weight after ripening in the rolls was higher than when threshing on the day of harvesting [15]. Similar results were obtained in our studies. The mass of the seeds of the motherwort of five-bladed increases up to a hard state and was greater with separate harvesting, since due to the high moisture content of vegetative organs (stems – about 57%, leaves – 73-56 %) and seeds (47-26 %) in the period from the dough to the wax state, their physiological maturation and weight increase continues.

During separate harvesting, the weight of seeds increased significantly during all phases of maturation, and the activity of dry matter growth changed similarly (Table 1).

In the dairy state, 1000-seeds weight was 0.765 g, 0.417 g and 0.399 g, and in the hard state-1.004 g, 0.809 g and 0.787 g in 2015, 2016 and 2017, respectively. At the same time, the weight of 1000 seeds when threshing plants on the day of harvesting in the phase of hard-seed ripeness and after drying in sheaves harvested in a wax state did not differ much and amounted 0.750 and 0.760 g, respectively. This was facilitated by the high intensity of the process of reutilization, the highest percentage of which was noted in the first period of harvesting – 31 %. From the phase of milk to the hard state of seeds, this figure gradually decreased to 7 % (Figure 2).

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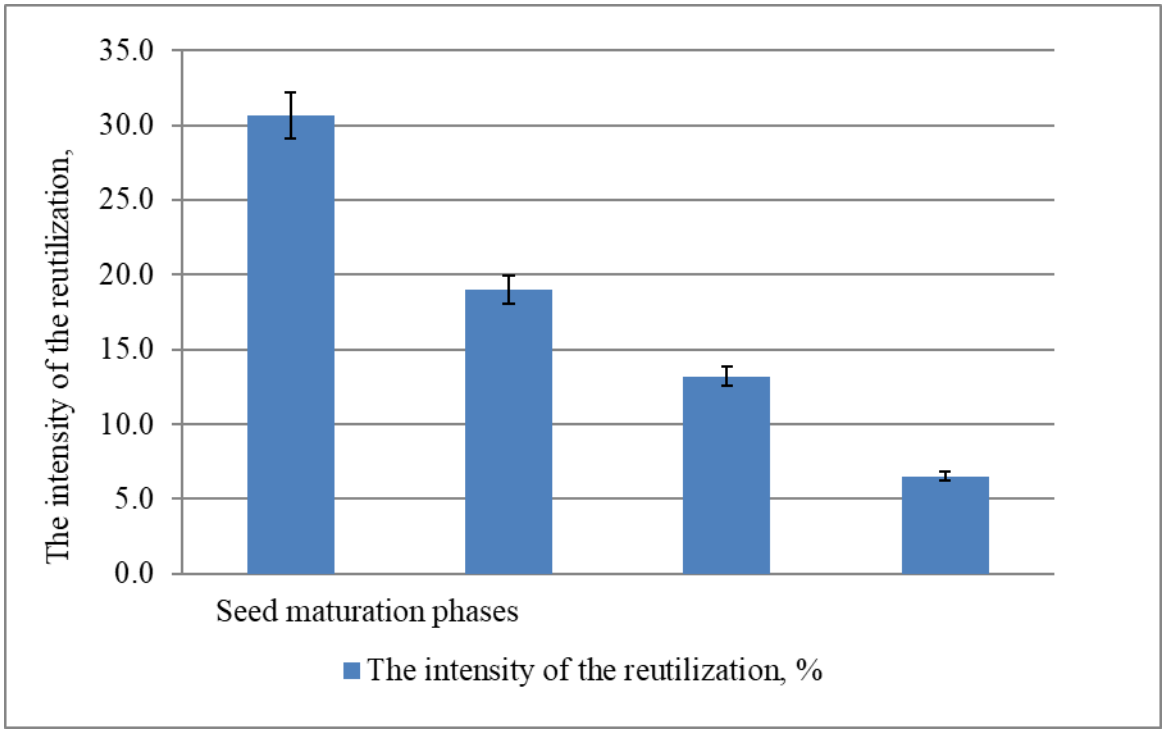

(1- dairy; 2-dough; 3-wax; 4- hard)

Figure 2: The intensity of the reutilization of *Leonurusquinquelobatus* during different phases of seed maturation on the day of harvesting and after ripening in the sheaf, %, in 2015 – 2017

The dynamics of organic matter accumulation and intensity of the process of reutilization during separate harvesting are closely related to the moisture content of seeds and vegetative organs. From dairy to hard-seed state, the moisture content of seeds on average for three years of research varied from 65% to 12 %. At the same time, the percentage of moisture in the vegetative organs remained sufficiently high throughout the growing season: stems – from 62% in dairy ripeness to 50% in hard-seed phase, leaves – from 70% to 52%, respectively. This explains the increase of 1000-seeds weight up to the hard-seed state (Table 2).

Table 2: The dynamics of the moisture content in vegetative organs and seeds of *Leonurusquinquelobatus* during different phases of seed maturation

Harvesting time	The state of seed ripeness	Moisture, %								
		2015			2016			2017		
		Seeds	Stems	Leaves	Seeds	Stems	Leaves	Seeds	Sems	Leaves
1	Dairy	61,95	54,96	57,70	67,95	65,75	74,80	67,48	65,36	74,64
2	Dough	46,20	50,80	51,66	40,05	56,16	73,50	53,59	60,92	73,94
3	Wax	31,10	49,31	51,25	26,85	55,83	56,20	26,45	55,86	56,57
4	Hard	16,00	47,82	50,84	11,08	51,11	52,50	10,10	51,37	52,89
ISD_{05}		2,02	0,59	0,66	0,47	0,35	0,35	0,53	0,30	0,25

Despite the advantage of separate harvesting, some scientists argue that direct harvesting is more rational method as it reduces energy costs and the cost of seeds [28, 29]. However, most scientists consider the method of mowing plants into rolls to be more effective. When harvesting seed crops in a separate way, the physiological ripening of seeds and increasing their weight continues, which allows to start harvesting some plants already in a wax state, when direct threshing is still impossible due to the high moisture content of the vegetative organs. In addition, it prevents injury and shedding of seeds [30, 31].

Studies to determine the optimal time and methods of seed harvesting were carried out on medicinal plants. Esoyan E. A. in her experiments, found that the harvesting of the seeds of *Echinacea purpurea* must remain separate: to mow plants in the phase of early-medium wax ripeness when seeds have a moisture content of 25-30%, and thresh when drying out to 14 – 16% [19]. In research of Astafieva, V. E., the study of the seed formation (*Nigella sativa* and *Plantago psyllium*) found that the best way to harvest nigella seeds is separate from the mowing plants in the swath when seed has a moisture content of 30 – 25 % and subsequent threshing it when drying to 12 – 14%, in the context of plantain the harvesting must be done during phase of wax ripeness, when seed moisture is 35 – 25% [32]

Thus, the most intensive increase in the mass of seeds of the motherwort five-bladed in the second and third year of vegetation is observed in the separate harvesting due to the process of reutilization in the period from the dough to the wax state at a seed moisture content of 47-26 %. The moisture content of the stems is 57 %, leaves – 73 – 56 %. Since the index of 1000-seeds weight when threshing plants on the day of harvesting in hard-seed phase and after drying in the sheaves, harvested during phase of wax, practically do not differ (0,750 and 0,760 g, respectively), and intensive seed-shattering is observed during hard-seed phase, which leads to considerable harvest loss, harvesting must be done separately, beginning with mowing the plants in sheaves in the wax ripeness phase with a seed moisture content of about 30% and a vegetative mass of 60%.

CONCLUSION

Ontogenesis is the individual development of the organism, which begins with the formation of a zygote, includes four periods-embryonic, juvenile, generative and senile and ends with natural death of the plant. Ontogenesis of motherwort of five-bladed is 439 days.

The most accurate criterion for determining the optimal time for harvest is the level of seed moisture. The mass of the seeds of the motherwort of five-bladed increases up to a hard state and was greater with separate harvesting, since due to the high moisture content of vegetative organs (stems – about 57%, leaves – 73-56 %) and seeds (47-26 %) in the period from the dough to the wax state, their physiological maturation and weight increase continues.

When harvesting seed crops in a separate way, the physiological ripening of seeds and increasing their weight continues, which allows to start harvesting some plants already in a wax state, when direct threshing is still impossible due to the high moisture content of the vegetative organs. In addition, it prevents injury and shedding of seeds. At motherwort five-bladed in the hard-seed state there is an intensive process of seed-shattering, which leads to a significant lost. As well as indicators of 1000-seeds weight when threshing plants on the day of harvesting in the phase of hard-seed and after drying in sheaves harvested during wax state do not differ, harvesting of the motherwort five-bladed seed material should be carried out separately, beginning with mowing plants in the sheaves in the wax ripeness phase with a seed moisture content of about 30% and a vegetative mass of 60%.

Thus, the correct understanding of the processes of individual development of plants and the peculiarities of their productivity formation is the basis of all agronomic sciences, creates a theoretical basis of agrotechnical systems aimed at increasing the yield and quality of agricultural products. Cyclic scheme of ontogenesis and vegetative period helps to identify in what phase of development specific physiological systems work and adjust the individual elements of technology of cultivation and harvesting of seed crops. The knowledge of the peculiarities of accumulation of organic substances in the seeds of motherwort five-bladed, as well as the optimal timing and methods of harvesting is necessary for the agronomists to obtain a stable and high-quality seed yields of this plant, which are the basis of obtaining high yield of medicinal raw materials.

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