

Productivity and crop quality of *Salvia officinalis* L. in the conditions of the Southern steppe of Ukraine

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Abstract

One of the important elements of ecological production of medicinal plant raw materials is the development and implementation of innovative technologies for growing medicinal crops based on biologized approaches and minimizing anthropogenic pressure on the environment. Therefore, the research aimed to determine the effect of doses of mineral fertilizers and biopreparations Biocomplex-BTU on the yield of green mass of *Salvia officinalis* L. plants and the content of essential oil in it. Experimental studies were conducted in the ecological conditions of Mykolayiv National Agrarian University (Ukraine) from 2016 to 2021 years. It was found that biometric indicators of plants changed significantly depending on the years of vegetation and the factors studied. Thus, regardless of the year of vegetation, the greatest influence on the formation of biometric indicators of plants was influenced by the combination of applying mineral fertilizers at a dose of N₆₀P₉₀ and carrying out foliar top dressing of plants during the growing season with a Biocomplex-BTU. In this variant of the experiment, 4.04-24.26 t ha⁻¹ of green mass of plants was obtained, depending on the year of cultivation. The plant nutrition also affected the content of essential oil. Most of it was in plant leaves using N₆₀P₉₀ and Biocomplex-BTU - 2.68%. At the same time, it was determined that most of the essential oil was contained in *Salvia officinalis* L. plants in the budding phase.

Keywords: biopreparations; essential oil; yield; mineral fertilizers; *Salvia officinalis* L.

Introduction

One of the most popular medicinal plants in Ukraine and the world is sage (*Salvia officinalis* L.), which belongs to the family Lamiaceae of the genus *Salvia* L., which includes more than 900 species (Poulios *et al.*, 2020). *Salvia officinalis* L., known as sage, is an important medicinal and aromatic plant, whose leaves are commonly used as a condiment in food. But mostly leaves are used as raw materials in the pharmaceutical and perfume industries (Gericke *et al.*, 2018; Miguel *et al.*, 2011; Samani *et al.*, 2021; Soltanbeigi *et al.*, 2021).

The use of sage is diverse - the leaves are used as an infusion as an astringent, bactericidal and anti-inflammatory agent for rinsing the throat and mouth with chronic bronchitis, sore throat, laryngitis, are part of various herbal remedies. Externally, sage is used for inflammation of the skin, wounds, burns and frostbite. Furthermore, for some specialized metabolites occurring in sage, including carnosol, carnosic acid, rosmarinic acid, flavonoids and polysaccharides many biological activities such as antioxidant, anti-inflammatory and

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anticarcinogenic, antidiabetic, gastroprotective neuroprotective and immunomodulatory activity have been reported (Bauer *et al.*, 2012; Behradmanesh *et al.*, 2013; Lu *et al.*, 2001; Samani *et al.*, 2021). Essential oil is used for flavoring toothpastes, powders, also used in perfume compositions, soap making and cosmetics (Miguel *et al.*, 2011).

In Ukraine, medicinal plants are grown mainly in specialized farms of the central regions, but modern areas of sowing of these medicinal plants do not allow obtaining the volumes of raw materials that are necessary to provide the medical industry. One of the reasons for this is the lack of a proper amount of active temperatures in these regions for the period of formation of biologically active substances, which leads to a decrease in essential oil in raw materials. Therefore, it is important to develop a set of agrotechnical measures for growing clary sage under drip irrigation for the rational use of irrigation water, fertilizers and other resources (Ushkarenko *et al.*, 2018).

The quality of raw materials of medicinal and essential oil crops is primarily influenced by the genetic characteristics of the plant, climatic conditions of cultivation area, elements of cultivation technology, in particular fertilizer (Soltanbeigi, 2020). The macro elements such as N, P, and K have critical functions in plant development. Especially N has a vital role in cell division and enlargement, which increases plant height and branch number (Purbajanti *et al.*, 2019). The use of recommended standards for applying fertilizers, growth-regulating and biopreparations contributes to obtaining high-quality products of agricultural crops, including medicinal plants. This provides standard bioactive ingredients to the pharmaceutical industry (Abaas, 2014; Panfilova and Mohylnytska, 2019; Panfilova *et al.*, 2020).

Growing high-quality products of *Salvia officinalis* L. in the conditions of the south of Ukraine today, it is impossible without the use of mineral fertilizers and modern biopreparations. It should be noted that irrigation during the growing season of the crop also plays an important role (Soltanbeigi *et al.*, 2021). Therefore, the aim of our study was to increase the productivity of *Salvia officinalis* L. by determining the optimal dose of fertilizers in combination with a biological product that reduces material and energy costs for growing crops.

Materials and Methods

The field experiments were carried out during 2016-2021 yrs. on the experimental field of the Mykolayiv National Agrarian University, Ukraine (GPS 46°56'18.4"N 31°39'32.1"E). The technology of its cultivation, except for the studied factors, was generally accepted to the existing zonal recommendations for the southern steppe of Ukraine. The soil of experimental sites was represented by the southern, resiliently weakly sunny, heavy-sooty black soil on the loess. The reaction of the soil solution was neutral (pH 6.8–7.2). The content of humus in the 0–30 cm layer was 123–125 g kg⁻¹. The arable layer of soil contained moving forms of nutrients on average: nitrates as 15–25 mg kg⁻¹, mobile phosphorus as 41–46 mg kg⁻¹, exchangeable potassium as 389–425 mg kg⁻¹ of soil.

Weather conditions varied slightly in temperature and especially in rainfall, but they were typical of the study area. Thus, the highest precipitation fell in 2016 and 2021 - 368.8 and 396.6 mm. The average daily air temperature in these years of research was +11.3 - + 11.5 °C. The least favourable for plant growth and development was 2017. 211.5 mm of precipitation fell during the year, with an average daily air temperature of + 12.7 °C.

The object of research was *Salvia officinalis* variety 'Medea'. The variety has been included in the State Register of Plant Varieties Suitable for Distribution in Ukraine since 2002. Direction of use - pharmaceutical industry, medicine. Recommended for growing in the steppe zone. Medium-ripe variety. It is a herbaceous plant with a quadrangular stem. The leaves are simple, without stipules, placed crosswise opposite. In the epidermis of the aboveground organs there are hairs and essential oil glands. The flowers are zygomorphic,

bisexual. Androcea is strong, gynoecium is cenocarpic. The average yield of raw materials (leaves) is 2.5 t ha^{-1} , the content of essential oil in raw materials is 1.3%. The essential oil contains more than 20% camphor. Weight of 1000 seeds is 8.0 g.

The scheme of experience included the following variants:

Factor A-nutrition background: 1. without fertilizers (control); 2. $\text{N}_{60}\text{P}_{30}$; 3. $\text{N}_{60}\text{P}_{60}$; 4. $\text{N}_{60}\text{P}_{90}$.

Factor B – foliar top dressing of plants: 1. without biopreparations; Biocomplex-BTU.

Mineral fertilizers were applied in the form of granulated superphosphate of ammonium nitrate on the plots and they applied manually, under the main tillage.

Biocomplex-BTU was used twice for processing plants during the growing season (in the phase of the beginning of vegetation and stemming). The consumption rate of the biological product is 0.3 l ha^{-1} . Biocomplex-BTU contains live cells and spores of *Bacillus subtilis* bacteria, nitrogen-fixing, phosphorus- and potassium-mobilizing bacteria (*Azotobacter* and *Paenibacillus (Bacillus) polymyxa*), lactic acid bacteria *Enterococcus*, *Lactobacillus*, phytohormones, vitamins, amino acids, macro- and microelements. The total titer is not less than $1 \times 10^9 \text{ CFU/cm}^3$.

Irrigation of crops during the growing season of garden sage was carried out using drip irrigation. Depending on weather conditions, during the spring-summer vegetation of garden sage plants, 2-4 vegetative watering was carried out. The humidity of the calculated soil layer (0-50 cm) was maintained by drip method of artificial moistening in the range of 70-75% HB.

The dynamics of vegetative mass growth was determined in each variant on permanently fixed sections with an area of 0.25 m^2 on two non-contiguous repetitions. Harvesting of garden sage at the accounting sites was carried out manually with immediate weighing and delivery of raw materials to the place of its processing. The content of essential oil was determined according to the state standard DSTU 14618.11-78.

Variant placing in the experiments was random; repetition was three times. The results of the research were processed using the method of multivariate disperse analysis.

Results and Discussion

The influence of environmental conditions significantly affects the formation of the structure of agrophytocenoses, but it acts as an additional factor and its results depend on individual components. More variable are quantitative features, which include biometric indicators. In the process of plant ontogenesis, structural elements are formed that determine the yield of a particular crop. That is why the determination of biometric indicators of garden sage is of great importance for the formation of the yield level.

Studies have shown that in the first year of life, the mass of stems varied from 11.6 to 28.8 g per plant and, on average, it was 18.8 g, and the percentage of stems in the aboveground mass of raw materials was 31.1%. The mass of leaves in the raw material yield ranged from 20.6-79.1 g per plant (on average - 41.9 g). The percentage of leaves and stems in the aboveground mass, respectively, ranged from 68.9% up to 31.1% or it was expressed in a ratio of 2.2:1.

In the second year of life, there was a sharp increase in leaf mass to 183.8 g on average per plant, with a variation in the range of 120-215 g, which is 4.4 times more than in the first year of life. The mass of stems increased by 5.7 times and it varied in the range of 84.6-131.8 g. In this regard, the ratio of leaf mass and stems changed in the direction of increasing the mass of stems to 34.1% and reducing the aboveground mass of leaves to 57.6%. In the future, with increasing age of the plant, the specific weight of the aboveground mass and its components increased. So, in the third year of life, the mass of the bush increased to 346.6 g and it varied between 218.6-577.3 g. The mass of leaves was 55.9%, and mass of stems was 33.0%. The yield of leaves varied widely (from 120 to 300 g per plant) and it amounted to 193.9 g. The mass of stems was 114.5 g per plant.

In the future, with increasing plant age, the amount of biomass began to decrease. At the same time, the ratio of leaf mass to stems remained at the level of 2:1 or 60.6% to 29.7%.

It should be noted that the results of our research established that the biometric indicators of plants varied significantly depending on the years of vegetation and the factors studied. The results of studies by Nadjafi *et al.* (2014) also show that the highest plant height, plant diameter, dry grass mass and essential oil yield are observed in biennial plants of *Salvia officinalis* L.

Regardless of the year of vegetation, the greatest influence on the formation of biometric indicators of plants was influenced by the combination of applying mineral fertilizers at a dose of $N_{60}P_{90}$ and carrying out foliar top dressing of plants during the growing season with a Biocomplex-BTU.

The main indicator of the productivity of garden sage is its aboveground mass. In the first year of vegetation of garden sage, the yield of its green mass fluctuated over the years of research. So, in the version without applying mineral fertilizers, it was in the range of 1.97-2.12 t ha⁻¹, and against the background of applying $N_{60}P_{90}$ it was 3.72-4.04 t ha⁻¹, depending on the application variant of the biopreparation (Table 1).

Table 1. Average yield of green mass of garden sage plants depending on research factors and years of crop life, t ha⁻¹ (average for 2016-2021 yrs.)

Background of nutrition	Foliar nutrition of plants	Year of life				
		1st	2nd	3rd	4th	Average
Without fertilizers (control)	without biopreparation	1.97	14.46	16.31	12.52	11.32
$N_{60}P_{30}$		3.17	17.42	19.54	15.38	13.88
$N_{60}P_{60}$		2.49	18.82	21.23	16.82	14.84
$N_{60}P_{90}$		3.72	21.45	23.90	18.87	16.99
Without fertilizers (control)	Biocomplex – BTU	2.12	14.86	16.73	12.83	11.64
$N_{60}P_{30}$		2.37	17.85	19.95	15.69	14.22
$N_{60}P_{60}$		2.82	19.38	21.64	17.12	15.24
$N_{60}P_{90}$		4.04	22.08	24.26	19.28	17.42

LSD_{0.5}: Factor A (nutrition background): 0.32-1.45. Factor B (foliar top dressing of plants): 0.51-1.79

The highest yield of the studied crop was formed in 2020 yr., when the greatest amount of precipitation fell during the growing season (1.IV-31.X), in the first years of life of garden sage (2016-2018 yrs.), the air temperature for the year was the lowest – 15.9 °C, and its absolute maximum did not exceed 31.7 °C.

In some years of research, the average daily temperature background reached 38.5 °C, which negatively affected the formation of plant productivity. In addition, the experiments recorded a differentiated effect on the yield indicators of the studied agrotechnological techniques, and their effect changed over the years of life of sage. The obtained data indicated that the highest average yield of green mass of garden sage in all growing years was formed by applying mineral fertilizers at a dose of $N_{60}P_{90}$. At the same time, in the first year of plant life, 3.72-4.04 t ha⁻¹ green mass of garden sage was collected, depending on the use case of the biological product.

In the second and third years of life, there was an increase in the yield of raw aboveground mass of plants, while the best result was provided by a combination of applying mineral fertilizers in a dose of $N_{60}P_{90}$ and fertilizing crops with a biological product. Thus, in the third year of life, garden sage plants formed 24.26 t ha⁻¹ of aboveground raw mass. Studies by Ulugova and Ruzmetov (2021) found that the application of mineral fertilizers at a dose of $N_{90}P_{60}K_{40}$ under *Salvia officinalis* L. yield increased 1.9 times, ie by 195% compared to the control. The same trend was observed in the studies of Karimi *et al.* (2021) – high rates of mineral fertilizers contributed to increased yields of aboveground mass of plants and essential oil.

Essential oils are the main components of those essential oil plants that have a wide range of positive effects on various body systems. Being multicomponent combinations, they act differentially and multifaceted on various links of the pathological process (Ushkarenko *et al.*, 2018). By its chemical composition, it covers

many classes of organic compounds, among which there are fatty compounds, aromatic, heterocyclic and acyclic. More than 5,000 components have been identified and they identified from essential oils. In different plants the same chemical compounds are found in various combinations (Miguel *et al.*, 2011).

The maximum content of essential oil, in terms of both raw and dry weight, falls on the leaves. In the inflorescence, based on raw matter, the amount of it, compared to the leaves, was less by 0.02-0.32%, depending on the experiment variant, in the stems it was less by 0.12-0.50%, and in terms of dry matter it was less by 0.09-1.50 and 0.51-1.97%, respectively, less (Table 2).

Table 2. Essential oil content in the main organs of plants of the fourth year of vegetation (average for 2019-2021 yrs.)

Parts of plants	Essential oil content % in:			
	Raw mass		Dry substance	
	Without fertilizers (control)	N ₆₀ P ₉₀ + Biocomplex-BTU	Without fertilizers (control)	N ₆₀ P ₉₀ + Biocomplex-BTU
Aboveground mass	0.39	0.40	0.38	1.23
Leaf	0.20	0.65	0.74	2.68
Stem	0.08	0.15	0.23	0.71
Inflorescence	0.18	0.33	0.65	1.18

Mineral fertilizers and Biocomplex - BTU helped to increase the content of essential oil in all plant organs. The positive effect of fertilizer on the accumulation of essential oil by plants of *Salvia officinalis* L. was also observed in studies of Piccaglia *et al.* (2011).

The essential oil of garden sage is concentrated in the maximum amount in the leaves, and the least of it is contained in the stems. Observations have shown that at the beginning of the growing season of garden sage, the content of essential oil in the aboveground mass based on both Raw and dry matter was minimal (Table 3).

Table 3. Dynamics of essential oil accumulation in garden sage depending on growth phases, % (average for experimental variants and for 2017-2021 yrs.)

Phases of growth	Essential oil content in aboveground mass		Essential oil content in leaves	
	In raw mass	In dry substance	In raw mass	In dry substance
Beginning of vegetation	0.18±0.1	0.65±0.1	0.32±0.02	1.24±0.04
Stemming	0.29±0.1	1.16±0.2	0.43±0.02	1.56±0.08
Budding	0.61±0.2	2.15±0.2	0.61±0.03	2.52±0.08
Beginning of flowering	0.52±0.2	1.92±0.3	0.53±0.03	2.24±0.10
Full flowering	0.40±0.1	1.36±0.2	0.47±0.02	1.90±0.11
Fruiting	0.30±0.1	1.14±0.2	0.35±0.02	1.40±0.12

In the future it increased and reached a maximum in the phase budding. But already at the beginning of flowering, this indicator, in comparison with the budding phase, decreased by 14.8% for raw mass, and it decreased by 10.7% for dry mass, and in the fruiting phase it was 50.8 and 47.0%, respectively.

It should be noted that the content of essential oil in the leaves of garden sage was also minimal at the beginning of the growing season, and it was maximum in the budding phase. In addition, during the flowering phase, this indicator, in comparison with the budding phase, decreased by 13.1% per raw substance, and per dry mass it decreased by 11.1; in the fruiting phase, the decrease was 42.6 and 44.5%. Thus, in garden sage plants, the maximum of essential oil is in the budding phase, and in the future this indicator decreases.

Long-term studies have shown that the mass fraction of essential oil in garden sage reached a maximum based on dry matter in the inflorescence, mass fraction of stem and leaves in the third year of life of the crop

were 1.36, 0.72 and 2.25%, respectively (Table 4). It should be noted that in the following years of vegetation, this indicator decreased.

Table 4. Mass fraction of essential oil in the main parts of the plant garden sage, % (average for experimental variants and for 2017-2021 yrs.)

Parts of plant	Type of raw material	Year of life			
		2nd	3rd	4th	Average
Inflorescence	Raw mass	0.31±0.03	0.36±0.03	0.30±0.03	0.32±0.03
	Dry substance	1.25±0.12	1.36±0.14	1.32±0.11	1.31±0.12
Stem	Raw mass	0.26±0.03	0.28±0.03	0.28±0.03	0.27±0.03
	Dry substance	0.56±0.03	0.72±0.04	0.67±0.04	0.65±0.04
Leaf	Raw mass	0.38±0.12	0.58±0.15	0.60±0.18	0.52±0.15
	Dry substance	1.72±0.14	2.25±0.32	2.17±0.18	2.05±0.21
Root	Raw mass	0.029±0.02	0.031±0.02	0.021±0.01	0.027±0.02
	Dry substance	0.048±0.03	0.041±0.03	0.042±0.01	0.044±0.02

The mass fraction of essential oil per dry matter in the inflorescence in the fourth year of life of the crop, compared to the third year, was less by 0.04%, and in the stem it was less by 0.05%. To a slightly greater extent, this indicator changes over the years of growing garden sage in the leaves.

The mass fraction of essential oil in garden sage was the highest in the leaves. Moreover, this indicator in different parts of plants, in comparison with the leaves, changed differently over the years of life of garden sage. In the stem, the mass fraction of essential oil per dry substance was lower, compared to the leaves, in the second and subsequent years of vegetation of the crop and it amounted to 0.56-0.72%, in the roots its content was 0.041-0.048%, and in the raw substance it was 0.26-0.28 and 0.021-0.031%, respectively.

The specific gravity of essential oil in the inflorescence of garden sage changed somewhat differently. A minimal decrease in this indicator, compared to the leaves, was observed in the second year of life of the crop. In subsequent years, based on both dry and raw matter, the mass fraction of essential oil reached a maximum in the third year as 0.36-1.36%.

Thus, the specific weight of essential oil in garden sage was the largest in the leaves, and the smallest one was in the roots. This indicator based on dry matter turned out to be the maximum in all growing years in the inflorescence, stem and leaves of the crop in the third year of its life, and in the roots it was in the fourth year.

Conclusions

The conducted studies made it possible to assess the formation of the clary sage crop in the period from 2016 up to 2021 yr. The plants of the studied crop were exposed to various weather and meteorological conditions during cultivation, which provided, on average, over the years of cultivation, an opportunity to assess the impact of the fertilizer system on the formation of the crop by year of use. The obtained data indicated that the highest average yield of green mass of garden sage in all growing years was formed by applying mineral fertilizers at a dose of N₆₀P₉₀ and double spraying of crops with Biocomplex-BTU biopreparations. At the same time, the highest yield of raw green mass of garden sage was obtained in the third year of plant life – 24.26 t ha⁻¹. This version of the experiment had a positive effect on the content of essential oil in plants. So, on average, over the years of research, the main organs of garden sage plants of the fourth year of vegetation contained 0.71-2.68% of essential oil in terms of dry matter. At the same time, it was determined that most of the essential oil was contained in garden sage plants in the budding phase.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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