

## Cultivation experiment of *Origanum vulgare* L. in Northern Kazakhstan using nitrogen fertilizer

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### Abstract

The purpose of the research is to determine the effect of ammonium nitrate fertilizer on the growth and yield of *Origanum vulgare* L. essential oil grown in Northern Kazakhstan. One-factor analysis proved that the introduction of ammonium nitrate positively affected the growth and yield of *O. vulgare* L., the yield of essential oil and the content of its individual components. Plant height increased by 19.3%, bush size – by 17.4%, yield – by 11.8%. Essential oil output of *O. vulgare* L. at the experimental site, compared to the control site, increased by 20% and the yield of extraction reached 0.60% and 0.48%, respectively. The largest number of components was related to monoterpenes (52.57% in the experimental section, -50.10% in the control section), the most common were  $\gamma$ -Terpinene,  $\beta$ -Sabinene, Carvacrol and trans- $\beta$ -Ocimene. Sesquiterpene content in the experimental sample was 37.19%, compared to 38.5250% in the control sample, in which Germacrene D and Caryophyllene were the most common components. Cluster analysis showed that the essential oil composition of *O. vulgare* L. from Kazakhstan is similar to the composition of the essential oil of plants from Moldova, Poland, Turkey (Anatolia), Latvia and Russia. Germacrene D, Carvacrol and  $\beta$ -Sabinene were the binding components of plants from Kazakhstan and Moldova. The similarity between plants from Kazakhstan and other countries in the same cluster was due to the Germacrene D content (cluster's average value was 10.1%) and the content of Spathulenol (4.9%).

**Keywords:** cultivation; essential oil; growth; mineral fertilizer; *Origanum vulgare* L.

### Introduction

There are numerous research studies that determine pharmaceutical, therapeutic and nutritional use of *Origanum vulgare* L. and its essential oil, but there are very few reports about its cultivation. Cultivation of oregano is not widely carried out, although it can become a profitable business. Fundamentally, wild herbs are

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collected in the natural environment. However, uncontrolled harvest of medicinal and unsavory herbs has significantly reduced the growth range of oregano and biodiversity in Kazakhstan (Ajdarhanova *et al.*, 2019; Bulatova, 2011; Galaktionova, 2012). In order to preserve medicinal and unsavory herbs, action has been taken to prevent endangerment in those plantations (Bernth, 1997; Sarrou *et al.*, 2016).

In traditional medicine, *O. vulgare* L. it is used to treat asthma, diarrhea, colds (Li *et al.*, 2022), headaches, rheumatism, diabetes, cardiovascular and nervous system as well as immune deficiency (Akbari *et al.*, 2022; Singletary *et al.*, 2010; Ulupinar *et al.*, 2022; Sharifi-Rad *et al.*, 2021), it is also used as an antifungal agent (Manohar *et al.*, 2001; Andrade *et al.*, 2023; Al Kamaly *et al.*, 2023; Yue *et al.*, 2023), including agents against bacteria and pests that can lead to food spoilage (Özkan *et al.*, 2017; Yoncheva *et al.*, 2021; Alkan, 2023; Susurluk, 2023), and antibacterial food preservative (Yoncheva *et al.*, 2021). The presence of flavonoids and phenolic acids in *O. vulgare* L. reduces the risk of chronic and oncological diseases (Gutiérrez-Grijalva *et al.*, 2017; Paknejad *et al.*, 2017; Kryeziu *et al.*, 2022). In addition, the extract components, including rosmarinic acid and flavonoids, contribute to the beneficial consumption of phenolic antioxidants with food (Nurmi *et al.*, 2006; Özer *et al.*, 2020). It is believed that there is a potential for essential oils of medicinal herbs in the fight against viral diseases such as influenza, herpes, and HIV (Ma *et al.*, 2020). Comparative analysis of antibacterial and antifungal activity showed that essential oils of some culinary herbs, including *O. vulgare* L., have the strongest properties among the many herbs studied (Danuta and Kunicka-Styczyńska, 2003; O'Mahony *et al.*, 2006). The fungicidal activity of *O. vulgare* L. essential oil was revealed (Gutierrez *et al.*, 2023). The importance of the yield of the essential oil is its origin, place of cultivation and taxonomic characteristics of the oregano ecotype (Shamseddin *et al.*, 2020; Lombrea *et al.*, 2020).

It is necessary to apply optimal agricultural practices to improve soil conditions for the plant growth and to obtain a phytomass of medicinal herbs on the cultivated area. To accomplish this goal, various methods are used, including the application of fertilizers. Currently, there is a worldwide concern about the negative impact of chemicals on medicinal herbs (Bushkovskaya *et al.*, 2011; Malik *et al.*, 2013; Rekha *et al.*, 2018), especially heavy metals, which have an extremely negative impact on human health (Locatelli *et al.*, 2014; Soyлак *et al.*, 2004; Gasser *et al.*, 2009; Pereira and Dantas, 2016; Tokalioglu, 2012; Glavač *et al.*, 2017; Deepa *et al.*, 2009). Furthermore, the contents of pesticides, heavy metals, and traces of mineral fertilizers were found, which affect the quality of raw organic matter. There are opposing opinions about maintaining soil fertility and the use of mineral and organic fertilizers. According to some recommendations, the use of mineral fertilizers in the cultivation of medicinal herbs, in which roots and rhizomes are an important raw material, is justified and they can be amended with potash fertilizers. Some establishments do not use fertilizers at all. Other authors suggest fertilizing medicinal plants only with organic fertilizers which improve soil fertility and affect the acceleration of plant growth (Vityaz *et al.*, 2019; Patel and Rabari, 2022; El-Hennawy, 2018; Kazimierczak *et al.*, 2014; Nagar *et al.*, 2017; Tariyal and Ansari, 2021). The introduction of nitrogen fertilizers and Litovit nano-fertilizers in Northern Sinai is recommended to improve the growth and conditions of oregano (Hamed, 2018). To increase the yield and antimicrobial activity of oregano, it is proposed to introduce humic acid (Aytak *et al.*, 2022). There are interesting ways to increase the content of essential oil, antioxidants and flavonoids - by treating the green mass with a liquid culture of spores of the *B. thuringiensis* 888 strain (Kryzhko *et al.*, 2021).

Oregano growth studies on agricultural practices as well as irrigation methods (with or without irrigation), revealed that the yield of oregano was the same, and a small water stress has increased the content of essential oil (Morshedloo *et al.*, 2017). In addition, the experiment on irrigation of oregano with fresh water versus treated wastewater showed that the water source does not play a role in the content of essential oil and the yield of the green mass of the plant, and it does not change the chemical composition of the soil (Virga *et al.*, 2020). An experiment was conducted on irrigating oregano with fish effluents from fisheries and aquaculture production, which resulted in the increase of the plant growth yield and the contents of essential

oil (Kimura *et al.*, 2021). Information on medicinal plant irrigation emphasizes on oregano as an unpretentious plant which can easily withstand the absence of irrigation reducing the cost of cultivation practice.

In addition to these obvious benefits of medicinal herbs, there are also studies of honey from oregano, which is obtained in the mountainous area of Epirus, Greece. It has been determined that honey contains therapeutic properties against gastric diseases and *Helicobacter pylori* strains, unfortunately, it is considered to be a rare product (Voidarou *et al.*, 2021). In addition to the use of the medicinal green mass of oregano, it was proposed to use the plant material with poor qualities and unsuitable parts of the plant. Plant by-products have similar chemical composition, which will allow them to be used as food additives on a par with the green mass of plants (Dina *et al.*, 2022).

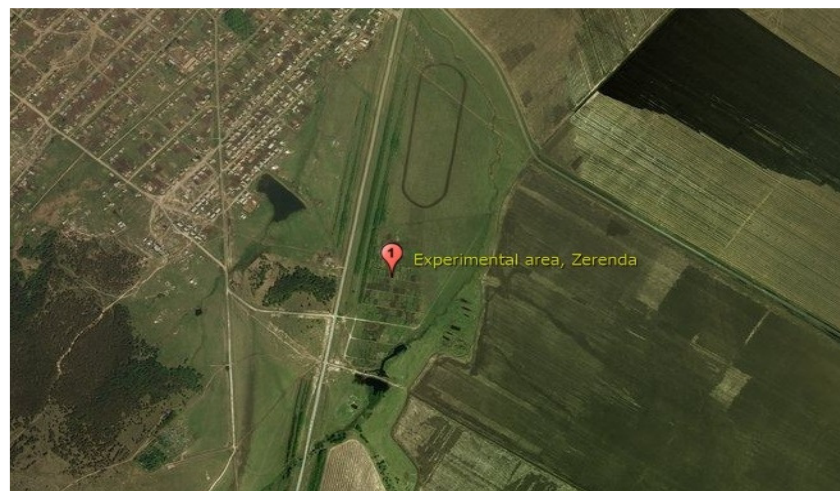
*O. vulgare* L. It is found in Akmola, Aktobe and East Kazakhstan regions, Altai, Tarbagatai, Zhetysu Alatau and Kungei Alatau. Currently, the distribution area of wild *O. vulgare* L. is significantly reduced, its cultivation on plantation areas is not carried out. Having studied scientific sources devoted to the artificial cultivation of *O. vulgare* L., we concluded that it is necessary to develop scientifically based recommendations for the cultivation for each specific region to increase the yield of phytomass without reducing the yield and composition of essential oil. For the conditions of Kazakhstan, such work is carried out for the first time which indicates significant importance of the work, which will be used in the cultivation practices of medicinal herbs.

## Materials and Methods

### *General information*

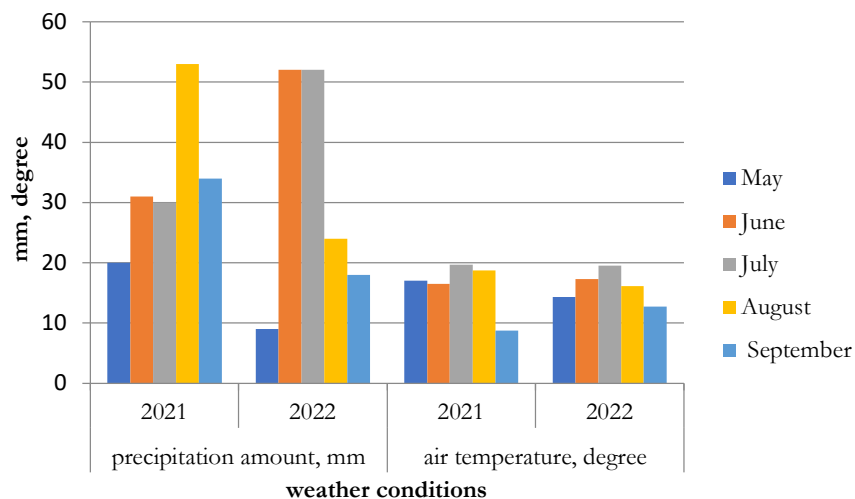
The purpose of the research is to determine the effect of mineral fertilizer on the growth and yield of *O. vulgare* L. essential oil growing on a cultivated area in Northern Kazakhstan.

The objects of research are biennial plants of *O. vulgare* L. The research was carried out on a site located in Northern Kazakhstan, Akmola region, Zerendi District, belonging to the Kokshetau-Munchaktinsky subdistrict of pine and birch-aspen forests (Figure 1). The climate of the region is sharply continental, the average annual temperature ranges from 0.8 to 2.6 °C. The area belongs to the zone of insufficient moisture. The annual precipitation of the plain area averages to 303 mm. The unfavorable factors of the region's climate include frequent droughts, dry winds, dust storms, late spring and early autumn frosts as well as severe frosts in winter. Winter is characterized by stable frosty weather. The average temperature of the coldest months (January and February) is – 15 °C. The absolute minimum air temperature reaches – 56 °C.



**Figure 1.** Location of the pilot site (Copyright © 2007 Free Software Foundation, <http://sasqis.org>)

Weather conditions in the year of sowing (2021) were not quite optimal for the active growth of *O. vulgare* L. The average air temperature of the growing season (May-September) was 16.5 °C, the average precipitation was 42 mm. In 2022, the average air temperature was 16.7 °C, and the amount of precipitation was 34 mm during the growing season.



**Figure 2.** Weather conditions of the growing season 2021-2022

*O. vulgare* L. seed sowing was carried out on two sites having approximately the same chemical composition of the soil. The soils are characterized by medium humus (4.3 and 5.2%), soil alkalinity (pH 8.07 and 8.48), nitrogen content was in the range of 32.6-39.6 mg kg, phosphorus – 105.01-105.02 mg kg, potassium – 82.05-80.01 mg kg. Consequently, there was an acute shortage of easily hydrolyzable nitrogen in the soil, and the average contents of potassium and phosphorus. Before sowing the seeds into the soil, mineral fertilizer was introduced into the soil of one site. To increase the growth, we decided to use the recommendations of scientists from Poland (Krol *et al.*, 2020) and add ammonium nitrate in the amount of 120 kg N ha<sup>-1</sup>. The composition of the fertilizer is N – 33.3%, Ca – 1%. Fertilizer was not applied to the soil of another site, as it was used as a control.

Seeds of *O. vulgare* L. were purchased from Aelita, Russia. Laboratory germination of seeds was high – 89.9%, field germination – 84.1%. The seeds were sown on May 9, 2021 in rows with a row spacing of 30 cm. During the growing seasons of 2021-2022, soil was softened, weeding was performed, along with the irrigation practice.

Measurements of quantitative indicators of plants were carried out on the plots of 10 x 10 m in diameter, where the number of studied plants was at least 100 plants. Biometric indicators (diameter and height of shoots, bush width, and leaf size) were measured with a ruler with an accuracy of 0.1 cm. The cut plants were weighed immediately after cutting on high-precision scales, then dried in a drying cabinet and re-weighed to determine the air-dry mass. The yield was determined by weighing all the cut plants on the accounting sites with a size of 1x1 m with an accuracy of ± 5 g, followed by the transfer of the resulting plant mass to 1 ha.

#### *Extraction, qualitative and quantitative analyses of the essential oil*

Essential oil from air-dry raw materials of phytomass of plants was obtained by hydrodistillation from air-dry raw materials for 3 hours, using a laboratory installation of a Clevenger. To study the composition of the essential oil, the chromatography-mass spectrometry method was used using an Agilent Technologies 7890 A gas chromatograph with an MSD 5975 C quadrupole mass spectrometer as a detector. The HP-5MS

capillary column had a size of 30 m x 0.25 mm (film thickness 0.25 microns). The temperature of the evaporator is 230 °C. The gas chromatographic column was kept at a temperature of 40 °C for 10 minutes; with temperature programming up to 240 °C with a temperature change rate of 2 °C/min, and then kept in isothermal mode for 20 min. The sample input mode is 100:1 flow division. The sample volume is 0.2 µl. The conditions for recording mass spectra are 70 eV, the mass range is m/z 10-360. MSD ChemStation software supplied by Agilent Technologies was used for data processing in combination with the Automated Mass spectrum Deconvolution and Identification System (software version: AMDIS 2.7) and mass spectral library with search program National Institute of Standards and Technology (software version: NIST 2.4).

#### *Statistical analysis*

The obtained data were processed by statistical methods using the Tibco Statistica program. According to the plant growth indicators, the average value and its error, and coefficient of variation (V, %) were determined. Cluster analysis was carried out between the main components of plant essential oil from Kazakhstan (control) and other countries to determine similarity in composition. Experimentally, the most optimal measure of proximity was chosen – Euclidean distance, the rule of unification – the method of single communication. The reliability of the differences between experimental and control plants was concluded using variance analysis.

## **Results and Discussion**

#### *Cultivation O. vulgare L.*

Table 1 shows quantitative growth indicators of *O. vulgare L.*, it can be observed that the use of nitrogen fertilizer increased the growth characteristics of plants – by 11.9-43.7%. Only the size of the leaves in the control exceeded the same indicator in the experimental group. The coefficient of variation in plant height was 21.7% in the experimental group and 32.5% in the control, the number of inflorescences per plant varied at 22.4 and 25.6%, respectively, and the average number of shoots was 26.4 and 21.5%, respectively. Such value of the coefficient of variation indicates increased variability of indicators.

**Table 1.** Quantitative growth indicators of *O. vulgare L.*

Group	Shoot diameter, cm	Shoot height, cm	Bush width, cm		Number of inflorescences, pieces	Number of shoots, pieces	Leaf size, cm	
			along	across			length	width
experiment	0.2 ± 0.01	52.2 ± 1.5	60.4 ± 2.4	72.9 ± 2.0	19.9 ± 1.1	46.8 ± 3.7	1.4 ± 0.1	1.0 ± 0.1
control	0.2 ± 0.02	42.1 ± 2.3	49.9 ± 3.1	64.2 ± 3.0	11.2 ± 1.4	41.4 ± 3.0	2.0 ± 0.2	1.3 ± 0.2
excess of experience over control, %	0	+19.3	+17.4	+11.9	+43.7	+11.5	-30.0	-23.1

The yield of plant phytomass on the site using fertilizers was also 28.3% higher in the raw mass and 26.4% higher in the dry mass, the overall yield was 11.8% higher (Table 2). The greatest variability was observed in the raw mass of one plant (V = 23.7-32.1%).

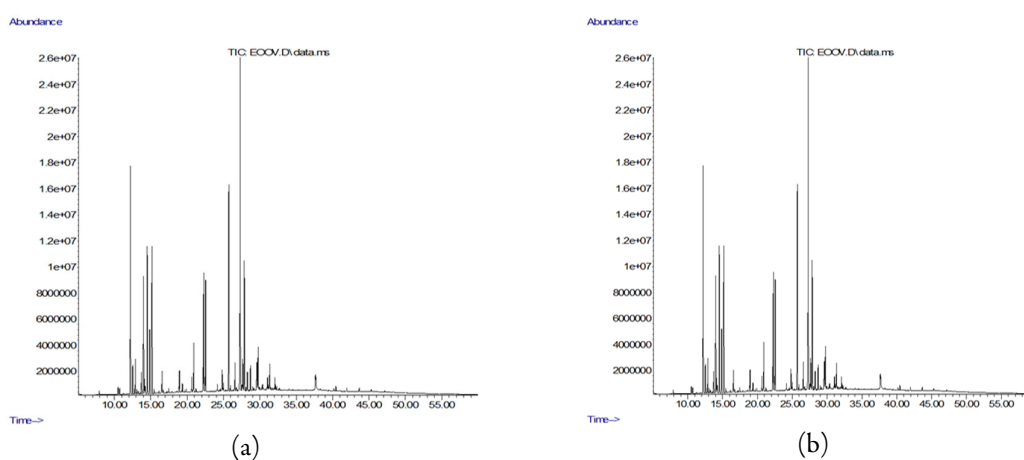
**Table 2.** Mass of aboveground parts and productivity of *O. vulgare* L

Group	The raw mass of one plant, g		Dry weight of one plant, g		Mass fraction of moisture, %		Yield, t ha
	X ± m	V (%)	X ± m	V (%)	X ± m	V (%)	
Experiment	168.8 ± 0.3	32.1	55.0 ± 0.4	28.9	49.0 ± 0.2	13.8	37.4 ± 0.2
Control	121.1 ± 0.4	23.7	40.5 ± 0.3	26.9	66.7 ± 0.7	12.9	33.0 ± 0.4

One-factor analysis of variance revealed significant differences in plant growth indicators between the experimental group and the control ( $p < 0.001$ ).

#### Chemical composition of the essential oil

The yield of *O. vulgare* L. essential oil grown with and without nitrogen fertilizer was studied (Figure 3, Table 3). It was revealed that the essential oil yield of *O. vulgare* L. on the fertilized plot (0.60%) increased in comparison to the non-fertilized plot (0.48%), and the composition of the main components has not changed. Essential oil of *O. vulgare* L. displays light yellow color with a pleasant smell.

**Figure 3.** Gas chromatograph of the essential oil *O. vulgare* L.: (a) – experiment, (b) – control**Table 3.** Percentage composition of the essential oil of *O. vulgare* L. according to the GC/MS data

No	RT (min)	RI lit	Compounds	Samples	
				Experiment	Control
1	7.9225	851	2-Hexenal	0.1972	0.1963
2	10.5272	911	$\alpha$ -Thujene	0.4567	0.4463
3	10.7365	929	$\alpha$ -Pinene	0.2987	0.3716
4	12.2156	943	$\beta$ -Pinene	1.3101	1.2742
5	12.7784	946	$\beta$ -Sabinene	8.9991	8.3898
6	12.8939	984	3-Octanone	0.4563	0.2495
7	13.2385	984	$\beta$ -Myrcene	0.9276	1.2839
8	13.5865	997	$\alpha$ -Phellandrene	0.3761	0.2026
9	13.7236	1003	3-Carene	0.53	0.4116
10	13.9978	1012	2,4-Heptadienal, (E,E)-	0.0999	0.1139
11	14.1349	1017	$\alpha$ -Terpinene	1.0086	0.9800
12	14.2503	1020	<i>o</i> -Cymene	6.1234	5.8489
13	14.5101	1028	$\beta$ -Terpinene	0.9671	0.8871
14	14.8492	1032	Eucalyptol	0.3967	0.4415
15	14.8781	1032	trans- $\beta$ -Ocimene	7.9673	7.7661
16	15.4769	1037	$\beta$ -Ocimene	1.5371	1.6042

17	16.1552	1038	cis- $\beta$ -Ocimene	2.0005	1.8303
18	16.5087	1060	$\gamma$ -Terpinene	9.9142	9.0100
19	16.5654	1087	Isoterpinolene	0.2198	0.2976
20	17.4756	1143	(4E,6E)-Allocimene	0.4612	0.3856
21	18.8970	1131	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	0.3331	0.2498
22	19.8855	1247	Thymol methyl ether	3.8712	3.2903
23	20.6503	1299	Carvacrol	8.9128	8.8226
24	22.2016	1303	Piperityl acetate	0.3345	0.3150
25	22.4686	1334	3-Methyl-4-isopropylphenol o-Cymen-5-ol; p-Thymo	0.5182	0.6265
26	21.1714	1377	(4aS,7S,7aR)-Nepetalactone	0.5981	0.6073
27	24.8007	1388	$\beta$ -Bourbonene	1.3654	1.2990
28	24.9939	1398	$\beta$ -Elemene	0.7121	0.7066
29	25.7299	1423	Caryophyllene	9.291	8.5329
30	25.9608	1433	$\alpha$ -Bergamotene	0.4523	0.4440
31	26.8194	1435	cis-Muurolo-4(15),5-diene	0.4312	0.2384
32	27.2956	1436	$\beta$ -Copaene-4 $\alpha$ -ol	0.1234	0.5850
33	27.5337	1439	Unidentified component	0.0176	1.1715
34	27.6564	1477	Unidentified component	1.6213	2.1724
35	27.7213	1481	Germacrene D	14.132	13.2511
36	27.8656	1481	$\gamma$ -Muurolole	1.4512	2.8348
37	28.0749	1501	Germacrene B	1.4911	1.5202
38	28.2841	1507	$\alpha$ -Muurolole	0.1982	0.2023
39	28.7098	1507	$\alpha$ -Farnesene	5.6123	5.2934
40	29.5540	1512	$\gamma$ -cadinene	0.4578	0.5037
41	29.6045	1519	$\delta$ -Cadinene	1.2312	1.2102
42	29.7488	1544	trans- $\alpha$ -Bisabolene	0.9781	1.2395
43	32.0649	1645	Eudesma-4(15),7-dien-1. $\beta$ -ol"	0.7618	0.8815
44	43.6599		Eicosane	0.8128	1.0011
			Total	99.9563	98.9901

More than ninety-nine percent of the essential oil components were identified in the experimental group and 98.9% in the control group. The main components were germacrene D (respectively 14.1320 and 13.2511%), carvacrol (respectively 8.9128 and 8.8226%), caryophyllene (respectively 9.2910 and 8.5329%),  $\gamma$ -terpinene (respectively 9.9142 and 9.0100%),  $\beta$ -sabinene (respectively 8.9991 and 8.3898%).

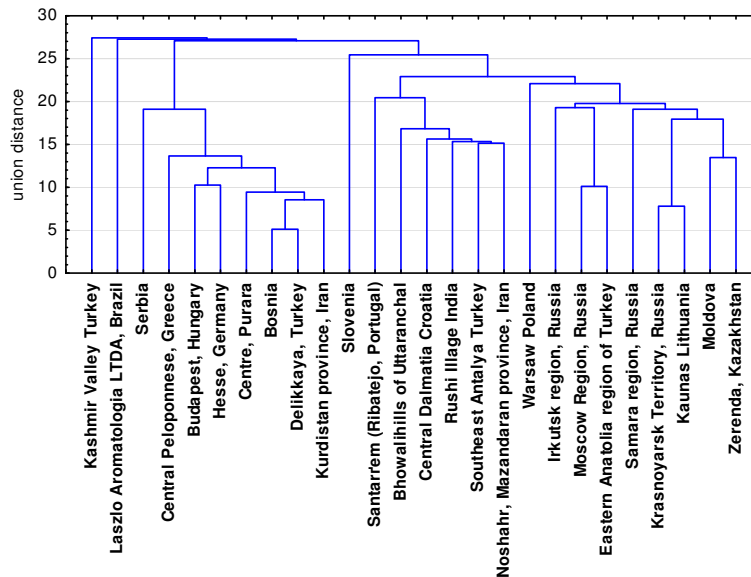
The total number of components in the experimental group that occur in more than 1% was 18 components, less than 1% - 26 components and 22 components, respectively. Table 4 shows the main components of samples (>1%) of *O. vulgare* L. essential oil, which are classified based on their chemical structure. Monoterpenes prevailed in the essential oil of experimental and control samples (52.5700-50.1000%), the largest amount:  $\gamma$ -terpinene (9.9142-9.0100%),  $\beta$ -sabinene (8.9991-8.3898%), carvacrol (8.9128-8.8226%) and trans- $\beta$ -ocimene (7.9673-7.7661%). The content of sesquiterpenes in the experimental sample was 37.1900%, in the control - 38.5250%. The leading compounds among them were germacrene D (14.1320-13.2511%) and caryophyllene (9.2910-8.5329%).

**Table 4.** Main compounds (>1%) of the essential oils from *O. vulgare* L.

Groups of components	Compound	Composition, %			
		Experimental	Total	Control	Total
Monoterpenes	$\beta$ -Pinene	1.3101	52.5700	1.2742	50.1000
	$\beta$ -Sabinene	8.9991		8.3898	
	$\beta$ -Myrcene	0.9276		1.2839	
	$\alpha$ -Terpinene	1.0086		0.9800	
	o-Cymene	6.1234		5.8489	
	trans- $\beta$ -Ocimene	7.9673		7.7661	
	$\beta$ -Ocimene	1.5371		1.6042	
	cis- $\beta$ -Ocimene	2.0005		1.8303	
	$\gamma$ -Terpinene	9.9142		9.0100	
	Thymol methyl ether	3.8712		3.2903	
	<i>Carvacrol</i>	8.9128		8.8226	
Sesquiterpenes	$\beta$ -Bourbonene	1.3654	37.1900	1.2990	38.5250
	Caryophyllene	9.2910		8.5329	
	Unidentified component	1.6213		2.1724	
	Germacrene D	14.1320		13.2511	
	$\gamma$ -Muurolene	1.4512		2.8348	
	Germacrene B	1.4911		1.5202	
	$\alpha$ -Farnesene	5.6123		5.2934	
	$\delta$ -Cadinene	1.2312		1.2102	
	Unidentified component	0.0176		1.1715	
	trans- $\alpha$ -Bisabolene	0.9781		1.2395	
Others	Eicosane	-	-	1.0011	1.0011

A cluster analysis was performed on the content of the components of *O. vulgare* L. essential oil on the samples of herbs grown in different countries (Table S1, Figure 4). The content of the components in the essential oil was very heterogeneous; therefore, the resulting dendrogram was conditionally divided into 7 main clusters. It was revealed that the 1st cluster included samples from Portugal and Slovenia, the 2nd cluster - from Kazakhstan, Moldova, Poland, Turkey (Anatolia), Latvia, Russia, the 3rd cluster - from Turkey (Kashmir) and Brazil, the 4th cluster - from Iran (Kurdistan), Turkey (Turkey), Greece, Bosnia, Serbia, India (Himalayas); the 5th cluster - from Croatia, India, the 6th cluster - from Iran (Mazandar), Turkey (Antalya), India (Tibet), and the 7th cluster - from Germany and Hungary.

After statistical evaluation, it was revealed that the clusters are divided by the size of the components contained in the essential oil. In the 1st cluster, the main elements were carvacrol (24.45) and thymol (8.1%), in the 2nd cluster - germacrene D (10.1%) and spathulenol (4.9%), in the 3rd cluster -  $\alpha$ -terpinene (34.4%) and carvacrol (8.3%), in the 4-th cluster - carvacrol (61.4%),  $\alpha$ -terpinene (10.7%) and p-cymene (8.6%), in the 5th cluster - thymol (32.4%) and carvacrol (26.5%), in the 6th cluster - thymol (39.0%),  $\alpha$ -terpinene (15.4%) and carvacrol (8.1%), in the 7th cluster - carvacrol (80.8%).



**Figure 4.** The results of hierarchical cluster analysis between the main components of essential oil of plants from Kazakhstan and other countries, obtained by a measure of proximity – Euclidean distance by the method of single connection

*O. vulgare* L. originates from the Mediterranean; therefore, it can be assumed that similar climatic and soil conditions are necessary for its successful cultivation (El-Alam *et al.*, 2019). Successful results of *O. vulgare* L. growth were achieved in a temperate cool climate (Kosakowska *et al.*, 2019; Węglarz *et al.*, 2020). Our research confirms that *O. vulgare* L. can be grown in the conditions of Northern Kazakhstan, and its climatic and soil conditions favor this plant's growth. During two growing seasons, the medicinal herb reached a height of over 50 cm, a bush width of over 70 cm, the yield of the phytomass was more than 30 t ha. In addition, *O. vulgare* L. is an unpretentious plant that requires a minimum amount of care, and the usefulness of this plant is undeniable. There is information about the falsification of *O. vulgare* L. raw materials on the market, which indicates a lack of green mass of the plant and its value. Methods are being developed for the determination of falsified herbal products by screening using chemometry (Black *et al.*, 2016), or DNA barcoding (Nazar *et al.*, 2022). Unfortunately, these methods have not yet been widely used to detect falsification. The decrease in the distribution area in nature, the difficulties of harvesting, the costs of cultivation of *O. vulgare* L. along with other factors makes it challenging for cultivation practices (Hoffman *et al.*, 2021). Based on these conclusions, *O. vulgare* L. can be recommended for plantation cultivation in Northern Kazakhstan.

Depending on the place of growth, soil composition and climatic conditions, plants need to be supplemented with nutrients. For medicinal plants, minimal accumulation of nitrates in the medicinal mass is important, but as experiments of many research studies show, the use of nitrogen fertilizers increases the plant growth and the yield of essential oil (Said-Al-Ahl *et al.*, 2009; Giannoulis *et al.*, 2020; Karamanos and Sotiropoulou, 2013). It can be assumed that in northern countries, the amount of nitrogen fertilizer applied to the soil increases. Thus, for the conditions of Poland, it is recommended to add 120 kg N ha<sup>-1</sup> (Krol *et al.*, 2020), for Greece 80 kg N ha<sup>-1</sup> (Ninou *et al.*, 2021), and for Argentina 40 kg ha<sup>-1</sup> (Barreyro *et al.*, 2005; Ozgüven *et al.*, 2006). For the experiments, we chose the recommendations on fertilization of Polish scientists due to similar soil and climatic conditions than in the previously mentioned countries. The results of our research showed that the introduction of 120 kg N ha<sup>-1</sup> of ammonium nitrate allowed us to increase the yield of biennial plants by 11.8% compared to the areas with no wind, height by 19.3%, the number of shoots in the bush - by 11.5%, the number of inflorescences – by 43.7%. The raw phytomass of plants in the experiment was 28.3% higher

than the control, and the dry mass was 26.4% higher than the control plot. In addition, fertilization played a significant role in increasing the yield of essential oil by 20%, which convincingly indicates the positive effect of fertilizer. In further studies, we plan to determine the optimal rate of application of mineral fertilizers and to identify the dosage of biological fertilizers for the conditions of Kazakhstan.

Research studies worldwide show that the yield and composition of *O. vulgare L.* essential oil varies significantly in different countries. As a result of plant breeding studies, genotypes of herbs with a high content of essential oil – more than 7% and a content of carvacrol up to 93% were obtained (Szabo *et al.*, 2010). However, the yield of essential oil ranges from 0.1 to 5% and the most common component of the essential oil of *O. vulgare L.* is carvacrol and thymol. This is typical for the conditions of Germany (Azizi *et al.*, 2009), Turkey (Kordali *et al.*, 2008), Portugal (Teixeira *et al.*, 2013), Greece (Aligiannis *et al.*, 2001), Montenegro (Stešević *et al.*, 2018), Hungary (Veres *et al.*, 2007), Bosnia (Stoilova *et al.*, 2008), Croatia (Kulisic *et al.*, 2004), Albania (Hodaj-Çeliku *et al.*, 2017), Slovenia (Kocić-Tanackov *et al.*, 2012), Slovakia (Kryvtsova *et al.*, 2020), Italy (Russo *et al.*, 1998), India (Raina and Negi, 2014), Iran (Vazirian *et al.*, 2015; Moradi *et al.*, 2014), Moldova (Gonceariuc *et al.*, 2014), Chile (Simirgiotis *et al.*, 2020), and Spain (Carrasco *et al.*, 2016). It should be noted that even in one country there are different chemotypes, in which the oil content and the main component differ. Thus, in Poland, the highest content of carvacrol in essential oil was noted (Kosakowska *et al.*, 2021). But it is also noted that the main component was sabinene and germacrene (Nurzyńska-Wierdak, 2009). Similar results were obtained in Italy (Martino *et al.*, 2009), where 3 chemotypes of plants were identified: with a predominance of carvacrol and thymol; thymol and  $\alpha$ -terpineol; linalyl acetate and linalool. In Turkey, of the 62 constituent elements of essential oil, caryophyllene and spatulenol were the main ones (Şahin *et al.*, 2004), other researchers point to carvacrol as the main component (Başer *et al.*, 1997; Toncer *et al.*, 2010), trans-sabine hydrate is the main component of plants from Jordan (Al-Kalalkeh *et al.*, 2010) and Argentina (Asensio *et al.*, 2015). In *O. vulgare L.* from Lithuania, the main component is sabinene (Radušienė *et al.*, 2005),  $\alpha$ -terpineol – in plants from Israel (Danin *et al.*, 1997) and Ukraine (Kryvtsova *et al.*, 2020),  $\gamma$ -terpinene – in plants from India (Chishti *et al.*, 2016), and E-citronellol and citronellol acetate – in plants from China and Pakistan (Gong *et al.*, 2014).

*O. vulgare L.* provides beneficial properties due to its content of thymol, carvacrol,  $\beta$ -caryophyllene, rosemary acid, terpenes, flavonoids, phenolic acid, as well as trace elements and vitamins (Karaođlan *et al.*, 2017; Mohamad *et al.*, 2021). Carvacrol and thymol contained in the essential oil of *O. vulgare L.* exhibit antioxidant, antifungal and antimicrobial activity (Nostro and Papalia, 2011; Marchese *et al.*, 2018; Soltani *et al.*, 2021; Chun *et al.*, 2005). Our research has shown that for the conditions of Northern Kazakhstan, the main components of *O. vulgare L.* essential oil were germacrene D,  $\gamma$ -terpinene and caryophyllene. The contents of carvacrol and  $\beta$ -sabinene were slightly lower. Moreover, the content of components that occur in essential oil more than 1% was 41%, components with a content of less than 1-59%.

It was found that the application of nitrogen fertilizers reduces the content of carvacrol but increases the content of thymol (Braga *et al.*, 2006). In our studies, the content of ethyl thymol was low (in the experimental group - 3.29%, in the control – 2.81%), and carvacrol – 8.82% in the experimental group and 8.12% in the control. Consequently, the use of fertilizer in our study did not reduce the content of carvacrol, but its content was higher on the experimental plot.

The yield and composition of essential oil varies significantly depending on the soil and climatic conditions. The samples from Moldova were the closest in the composition of the essential oil of plants from Kazakhstan. In both countries, germacrene D was the leading component: essential oil from Moldova contained 17.01%, essential oil from Kazakhstan - 13.25%. Carvacrol content values were 11.65 and 8.82%, respectively, and  $\beta$ -sabinene (9.78 and 8.3%, respectively). The main difference was the fact that there was no  $\beta$ -caryophyllene in the essential oil of plants from Kazakhstan at all, whereas plants from Moldova had a large amount of it in the essential oil - 13.05%. More significant differences were between the composition of the

essential oil of plants from Kazakhstan and Latvia and the Krasnoyarsk Territory, which were part of the same cluster. The main component of *O. vulgare* L. from Latvia and the Krasnoyarsk Territory was  $\beta$ -caryophyllene (17.50 and 13.36%, respectively), which was not present in plants from Kazakhstan, germacrene D contents in oregano essential oil from Kazakhstan were higher than in plants of the previously mentioned countries. In plants from Latvia,  $\beta$ -ocimene was 12.11%, from the Krasnoyarsk Territory – 10.91%, in plants from Kazakhstan - only 1.6%. A separate part of the cluster was occupied by the samples from the Saratov region of Russia. Further, the cluster included samples from the Irkutsk and Moscow regions of Russia, Turkey (Anatolia province) and Poland (near Warsaw).

## Conclusions

The use of ammonium nitrate in the amount of 120 kg N ha<sup>-1</sup> significantly increased the phytomass (by 28.3%) and the yield of *O. vulgare* (by 11.8%) compared to the areas with no wind. One-factor analysis of variance revealed significant differences in plant growth indicators between the experiment and control, which proves the positive effect of fertilization practice. The yield of essential oil was 0.60% at the experimental site, 0.48% at the control site, therefore, the use of fertilizer leads to an increase in the yield of essential oil by 20%. Forty-four components were identified in the composition of the essential oil, the main component was sesquiterpene precursor - germacrene D (in the experiment - 14.1320 and in the control - 13.2511%). Eighteen components had a concentration of more than 1%, and the majority of components belonged to monoterpenes ( $\gamma$ -terpinene,  $\beta$ -sabinene, carvacrol and trans- $\beta$ -ocimene). According to the literature, it was revealed that the composition of the essential oil of *O. vulgare* L. varies greatly depending on the place of growth. Therefore, statistical method of cluster analysis was implemented to measure the components of the essential oil of plants from different countries and their various growing conditions.

The obtained data of the chemical composition of *O. vulgare* L. essential oil from Kazakhstan was compared to the data of essential oils composition of plants from Moldova, Poland, Turkey (Anatolia), Latvia and Russia. Evidently, the natural and climatic conditions of these countries are similar to the growing conditions of Northern Kazakhstan. It should be noted that the agrotechnics of cultivation of *O. vulgare* L. do not require large expenditures and labor efforts and given the undoubted versatility of such plants in terms of nutritional, medical, environmental, and economic value, it can be recommended for cultivation in the farming and other agricultural communities.

Summing up the above, we can say that the range of wild *O. vulgare* L. is declining and in order to preserve their populations, it is necessary to initiate oregano preservation in the environment and in cultivation areas. Studies by many authors from different countries have shown great differences in the requirements of medicinal herbs for soil fertility and climatic conditions. Consequently, for each growing area of *O. vulgare* L., it is necessary to develop scientific recommendations for agricultural techniques to grow and care for plants, provide rules for its harvest, processing and storage practices. Our further plans are to continue experimental work on the growth and cultivation of oregano and expanding the range of mineral fertilizers as well as testing organic fertilizers, and biological stimulants without reducing the content of biologically active substances.

## Authors' Contributions

S.K.: collection of plant material samples, description of collection sites, methodology, data analysis; P.S., V.B., M.D. and S.S.: methodology, literature review, writing and editing the manuscript; A.K., Y.K. and I.K.: planning of the research, literature review, participation in the experiment of this study, data analysis. All 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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