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NEW APPROACHES IN GROWING TECHNOLOGY OF *Valeriana officinalis* L. UNDER THE CONDITIONS OF CLIMATE CHANGE

SUMMARY

There is an acute shortage of soil moisture in critical periods of growth and development of *Valeriana officinalis* L. on the territory of Ukraine. Classical technologies previously used for growing crops do not allow to obtain consistently high yields of roots under the conditions of climate change. Therefore, in order to find new effective methods of valerian growing, research studies have been conducted to establish the impact of drip irrigation, mineral nutrition, sowing dates on productivity and phytosanitary condition of valerian crops in Ukraine. The moisture content of the root layer of the soil was maintained at the level of 85% of the lowest moisture content, when irrigation was used. Mineral fertilizers in the dose of $N_{90}P_{90}K_{90}$ were applied for the main tillage and $N_{30}P_{30}K_{30}$ for fertilization. Sowing was carried out in two terms (spring and winter). The yield of valerian depended only on the weather conditions of the year, when irrigation was not used. In the first year of research, the natural soil moisture was the highest among all years of research. The yield of valerian raw materials was 19.4 – 26.3 quintal/ha for spring sowing of the first year. For the second year of research – it was only 5.4 – 7.9 quintal/ha due to summer drought. For the third year no seedlings were obtained due to the spring drought. On average over three years, the use of drip irrigation in combination with mineral fertilizers provided 26.3 – 36.6 quintal/ ha yield of dry roots. Prolongation of vegetation during winter sowing, application of irrigation and mineral fertilizers (with their application in the dose of $N_{90}P_{90}K_{90}$ mainly) provided a yield of 36.8 quintal/ha. The highest yield of valerian roots was 42.2 quintal/ha obtained for winter sowing when irrigation was used in combination with the application of basic fertilizer $N_{90}P_{90}K_{90}$ + fertilization $N_{30}P_{30}K_{30}$. Under the conditions of drip irrigation dangerous pathogens for valerian were *Erysiphe*

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cichoracearum f. valerianae, Ramularia valerianae, Uromyces valerianae, Septoria valerianae, Peronospora valerianae, and also fungus of the genus *Fusarium* and viruses. Stable yields of valerian raw materials under the conditions of climate change were obtained with the use of drip irrigation and mineral fertilizers with the extension of the growing season of valerian due for winter sowing.

Keywords: *Valeriana officinalis* L.*, drip, irrigation, yield, mineral fertilizers*

INTRODUCTION

Valeriana officinalis L. is one of the most widespread cultivated medicinal plants in Europe (Gholiloo et al., 2019; Gorban et al., 2004; Mishchenko et al., 2015; Romashchenko et al., 2015). According to the State Statistics Service of Ukraine, the in recent years area under this crop has decreased significantly. The decrease in the production of valerian raw materials is caused by a number of factors; climate change is one of the most important among them. Climate change includes long dry periods without rain. Such conditions, in combination with high average daily temperatures, lead to a significant decrease in the yield of agricultural crops, their infection by different pathogens, and often to the complete death of crops (Gholiloo et al., 2019; Filipovic and Kljajic, 2015; Mishchenko et al., 2019; Pryvedeniuk and Hlushchenko, 2020; El Bılalı et al., 2020). At the same time, the high quality raw materials, grown under the conditions of the Left-Bank Ukrainian Forest-Steppe zone, contain biologically active substances in concentration required by the Ukrainian and European standards. The shortage of valerian medicinal raw materials in the domestic pharmaceutical market is compensated by import of this plant (Pryvedeniuk et al., 2016). It is known that the medicinal plants raw materials quality can be significantly affected by viruses (Dunich and Mishchenko, 2015; Mishchenko et al., 2015).

According to the effect of mentioned factors on valerian growing, nowadays there is a necessity to improve technology of valerian production. One of the ways to solve this problem is improvement of water-nutrient regime. It could ensure the formation of high yields of appropriate quality for one year of cultivation. Extensive and successful implementation of drip irrigation in vegetable growing, potato farming and fruit harvesting show its effectiveness and prospects in medicinal plants cultivation (Filipovic, and Kljajic, 2015). Nowadays more attention is paid to study of the impacts of irrigation regimes on the spread and development of plant diseases. Irrigation affects several aspects of different cycles of plant diseases, such as sporulation, survival of the pathogen, their spread to new hosts, germination and infection. As follows, irrigation is the most important cultural practice that can protect from and reduce spread of plant diseases. The implement of drip irrigation was studied in order to solve mentioned problem. Research studies were held on valerian in the Research Station of Medicinal Plants IAE NAAS. Therefore, the aim of the study was to develop new approaches in cultivation technology of *Valeriana officinalis* L. under the conditions of climate change and control of virus damage.

MATERIAL AND METHODS

In order to improve the technology of valerian growing by applying drip irrigation and mineral fertilizers field studies were held in crop rotations of the Research Station of Medicinal Plants IAE NAAS in Poltava region in Ukraine during three years. The soil of the research field - strong chernozem, low-humus, thickness of the humus horizon 87 - 100 cm, light particle size distribution. The reaction of the soil solution is mildly acidic, in terms of metabolic acidity the soil is characterized as moderately acidic.

The supply of soil with basic nutrients: easily hydrolyzed nitrogen - low, mobile phosphorus - very high, exchangeable potassium - increased. According to the amount of salts, the soil is unsalted. The lowest moisture content of 0 – 100 cm layer is 18.2%, for 0 – 50 cm layer is 17.5%. The density of the composition of 0 – 100 cm layer is 1.27 g/cm^3, for 0 – 50 cm layer it is 1.32 g/cm^3.

Methodological approaches used in Ukrainian practice and in medicinal plant growing have been used in the scientific research. In particular, the development of experimental schemes was performed according to the methods of Dospekhov with the peculiarities of drip irrigation mentioned in the method of Romashchenko M.I. Sampling of plants, biometric measurements and phenological observations were carried out considering the medicinal crops characteristics according to the methods of Brykin (Brykin, 1981; Dospekhov, 1985; Romashchenko, 2014;).

The study of the viral particles morphology was carried out using the method of transmission electron microscopy. The research was carried out using the equipment of the Center for Collective Use at the Danylo Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The viral preparation was applied to copper grids with a substrate film consisting of 0.2% solution of formvar on dichloroethane. Negative contrast of the drugs was performed with 2% aqueous solution of phosphoric-tungstic acid for 2 minutes (Saliga and Snitinsky, 1999).

The method of solid-phase enzyme-linked immunosorbent assay in the modification of a double sandwich (DAS-ELISA) was used to determine the presence of viral antigens. The analysis was performed using commercial test systems for *Potato virus M, S, X, Watermelon mosaic 2 virus, Potato virus Y, Cucumber mosaic virus, Tomato spotted wilt virus, and Alfalfa mosaic virus* manufactured by Loewe (Germany) in three repeats. A commercial viral preparation (Loewe, Germany) was used as a positive control in the reaction, healthy valerian leaves – as negative. The reaction results were recorded on a reader of Termo Labsystems Opsis MR (USA) with Dynex Revelation Quicklink software at wavelengths of 405/630 nm. Values that exceeded the negative control at least three times were considered valid (Crowher, 1995).

Statistical processing of EM and ELISA results was performed taking into account the standard deviation: X = X ± Aσ; X = (X1 + X2 +… + Xi) / i; A = │Xmax - X│ = │Xmin -X│, where X - is the significant value of the virion length / value of the optical density of the reader at a wavelength of 405 nm; X - is the arithmetic mean of the measured values of the virion length / value of the optical density; X1 … Xi; σ - is the standard deviation (Lakin, 1980).

The research field was planted by direct sowing in the soil of valerian seeds of the medicinal variety Ukraine with a rate of 6 kg/ha, seed wrapping depth – 1.0 cm, row spacing width - 60 cm. Winter sowing was carried out in the third decade of August, spring sowing – in the second decade of April. An irrigation pipeline with a diameter of 16 mm with integrated water outlets every 20 cm with a water flow rate of 1.2 dm^3/h was used for irrigation, one pipeline was used to moisten one row of plants. During the vegetation period, soil moisture in the layer of 0 – 40 cm was maintained at 80-85% of the lowest moisture content. NPK mineral fertilizer containing the active substance $N_{16}P_{16}K_{16}$ was used for the basic application and fertilization. Valerian medicinal crops were recorded in the first decade of October: first the aboveground mass of plants was mowed, then the roots with rhizomes were dug up, separated from the soil residues and the soil was washed from the freshly dug roots for 15 minutes. The roots were dried at temperature +35 ^oC to humidity 10 –12%.

RESULTS AND DISCUSSION

The obtained research results show that the productivity of valerian in variants without use of irrigation depended on weather conditions, in particular the presence of soil moisture in critical periods of growth and development of the crop. Therefore, for the first year of research soil moisture during the growing season of valerian spring sowing was the highest among three years of research. This moisture level provided optimal seedling formation and, consequently, the yield of dry roots with rhizomes at 19.4 – 26.3 quintals/ha without irrigation. For the second year of research, soil moisture was sufficient for the growth and development of valerian, but during June-August there was an acute lack of moisture, so the yield of roots was only 5.4 – 7.9 quintals/ha. The spring period of the third year of research was characterized by the absence of precipitation with high average daily temperatures; as a result valerian seedlings were not obtained at all. (Table. 1). For the spring sowing of valerian, the best conditions for plant growth and development was when irrigation was used together with mineral fertilizers. Therefore, on average over three years, under conditions of drip irrigation, the yield of roots with rhizomes was in range of 26.3 – 36.6 quintals/ha. When irrigation was used without mineral fertilizers the yield was 26.3 quintals/ha, exceeding the variant without irrigation and without fertilizers (control) by 13.9 quintals/ha or 112%. The efficiency of mineral fertilizers application in the presence of soil moisture increases rapidly, which is confirmed by the additional yield of roots of 6.5 quintals/ha when watering and basic application of mineral fertilizers were applied compared to the variant with irrigation without fertilizers.

Table 1. Effect of soil water-nutrient regime on the yield of valerian dry roots with rhizomes during spring sowing.

Variants		Yield of dry roots, quintal/ha				% of control group
Factor A	Factor B	1st year	2nd year	3rd year	Average	
Without irrigation	Without fertilizers	19.4	5.4	-	12.4	100.0
	Basic application of $N_{90}P_{90}K_{90}$	21.8	7.2	-	14.5	116.9
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	26.3	7.9	-	17.1	137.9
Drip irrigation	Without fertilizers	26.5	28.4	24.1	26.3	212.0
	With basic application of $(NPK)_{90}$	31.6	36.2	30.7	32.8	268.5
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	36.3	39.4	34.1	36.6	295.2
$LSD_{0,5\ \text{main effects f. A}}$		1.4	1.1	1.6	2.8	-
$LSD_{0,5\ \text{main effects f.}}$		1.9	1.8	1.3	2.2	-
$LSD_{0,5\ \text{partial differences f.}}$		2.5	2.0	2.9	4.4	-
$LSD_{0,5\ \text{partial differences f.}}$		2.3	2.2	1.7	2.6	-

The highest yield of valerian roots 36.6 quintals/ ha during spring sowing was obtained when irrigation was used together with basic application of mineral fertilizers and additional fertilization, which exceeded the control by almost three times.

The study was provided by studying to predict the period of valerian winter sowing study under the conditions of drip irrigation. This sowing period provides an increase in the growing season of the crop from sowing to harvest and promotes the formation of higher yields compared to spring sowing. For winter sowing, the research plots were laid in the third decade of August. The autumn period of the first year of research was characterized by an acute shortage of soil moisture with high temperatures, as a result valerian seedlings was not obtained without irrigation. When irrigation was used, the phase of full germination of valerian was observed on the 8th day after sowing, before the onset of persistent frosts, the plants formed 3-4 true leaves, which contributed to successful overwintering. The autumn period of the second research year was favorable for obtaining seedlings and their growth and development without irrigation, the yield of valerian roots at the end of the growing season was 17.3 – 20.3 quintals/ha (Table. 2).

Table 2. Effect of soil water-nutrient regime on the yield of valerian dry roots with rhizomes during winter sowing

Variants		Yield of dry roots, quintal/ha			% of control group
Factor A	Factor A	1st year	2nd year	Average	
Without irrigation	Without fertilizers	-	17.3	17.3	100.0
	With basic application of $N_{90}P_{90}K_{90}$	-	19.2	19.2	111.0
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	-	20.3	20.3	117.3
Drip irrigation	Without fertilizers	31.6	32.1	31.9	184.4
	With basic application of $N_{90}P_{90}K_{90}$	38.7	34.9	36.8	212.7
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	44.5	39.8	42.2	243.9
$LSD_{0,5\ \text{main effects f. A}}$		0.9	1.3	2.4	-
$LSD_{0,5\ \text{main effects f. B}}$		1.4	1.0	2.2	-
$LSD_{0,5\ \text{partial differences f. A}}$		1.5	2.3	3.6	-
$LSD_{0,5\ \text{partial differences f. B}}$		1.7	1.3	2.7	-

Under the conditions of drip irrigation, for winter sowing, the yield of valerian exceeded the variant without the use of irrigation by 14.6 quintals/ha. The basic fertilizer application under irrigation conditions increased the root yield by 4.9 quintals/ha compared to the variant without fertilizers when irrigation was used.

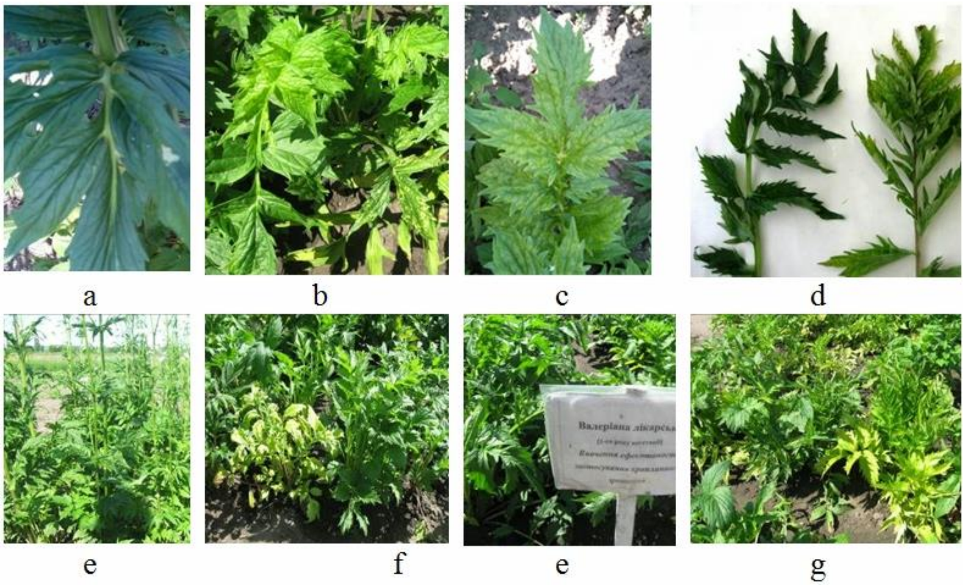
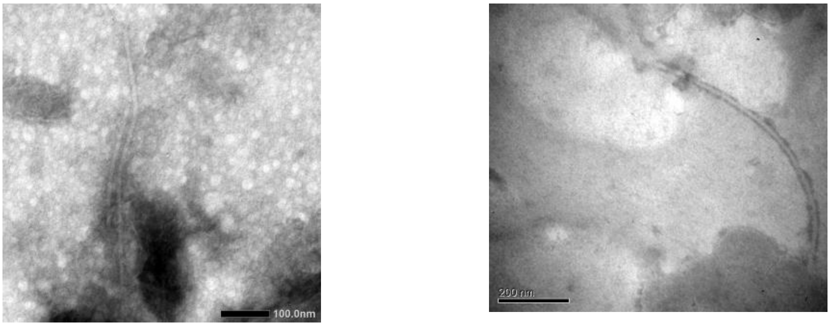


Fig. 1. *Valeriana officinalis* L. with symptoms of viral disease (b, c, d, f, g) and healthy (a, e); d - healthy on the left

The best conditions for the valerian growing and development were observed when irrigation was used together with basic fertilization and additional fertilization, the yield of dried roots with rhizomes under such conditions was 42.2 quintals/ha, exceeding the control by 24.9 quintals/ha.

Under the conditions of drip irrigation, fungal and viral diseases are dangerous pathogens for valerian. Viruses are insufficiently studied both in Ukraine and in the world in general. Our previous studies in 2006-2009 showed that under traditional growing conditions 15-20% of *Valeriana officinalis* L. plants had symptoms of a viral disease (yellow mosaic, stunting and peduncle reduction), in which filamentous viral particles 530 ± 20 × 10-12 nm were detected (Mishchenko et al., 2009). Under conditions of drip irrigation (since 2013), valerian plants with symptoms of stunting and yellow ringspot on the leaves were also detected, but their number was lower compared to traditional growing conditions of this crop and was 2-5% (Fig. 1).

Our attention was drawn to new symptoms for this culture – ring spot on the leaves (Fig. 1 b, c). We have detected filamentous viral particles with a size of 550-600 × 11-12 nm (Fig. 2 a) and 750-800 × 11-12 nm (Fig. 2 b) in valerian leaves with this symptom by TEM.



a b

Fig.2. Electron microscopy picture of viral particles detected in the leaves of *Valeriana officinalis:* a – 550-600 nm, b – 750-800 nm

The morphology of the revealed virions is similar to carlaviruses and potexviruses (Fig. 2 a), and potyviruses (Fig. 2b), respectively (King et al., 2012).

ELISA was used to identify these viruses. For this purpose, test systems for several viruses were selected based on literature data and morphology of the studied virions. It should be mentioned that the identification of the viruses infecting valerian is quite difficult, as they are polyphagous and infect a wide range of different plant species. So, most likely, they are transmitted to valerian from near growing wild and agricultural crops. Therefore, to identify virions with a size of 550-600×11-12 nm testing was performed using test systems for viruses from genus *Potexvirus* and *Carlavirus*, which are widespread in the agrocenoses of our country - *Potato virus M, S, X*. According to the literature (King et al., 2012) valerian is susceptible to infection with the filamentous *Watermelon mosaic 2 virus*, which belongs to the genus *Potyvirus* of the family *Potyviridae*. Therefore, in order to identify the virus with a size of 750-800 × 11-12 nm, test systems were used for potyviruses *Watermelon mosaic 2 virus* and *Potato virus Y*. In addition, testing was performed for the presence of spherical viruses *Cucumber mosaic virus, Tomato spotted wilt virus* and bacilliform *Alfalfa mosaic virus*, which are registered in Bulgaria and Italy on valerian plants and circulate in Ukraine on other crops. ELISA results showed absence of antigens of all of the mentioned above viruses in the valerian plants leaves (Fig. 3).

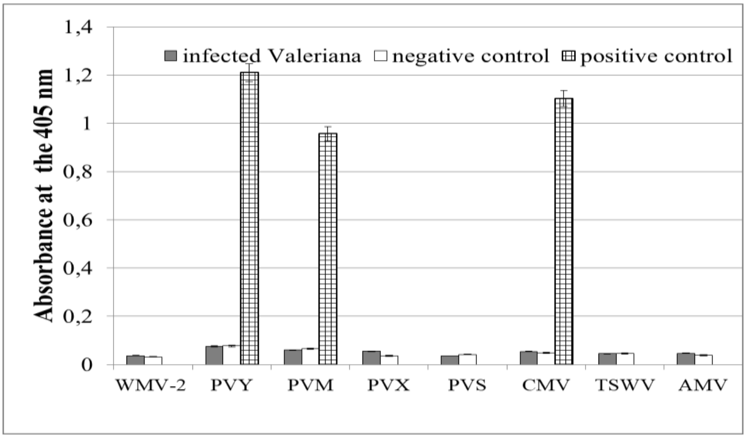


Fig. 3 Content of viruses` antigens in leaves of infected *Valeriana officinalis*

As mentioned above, the identification of viruses infecting valerian is difficult. This also applies to other medicinal plants, such as *Echinacea purpurea*, *Panax ginseng* and others. The literature data suggests that they do not have "own" highly specialized viruses and are affected by polyphagous viruses. This was the case with mint plants, but the development of modern molecular studies of plant viruses has allowed scientists to determine that peppermint plants still have their own highly specialized viruses, which were included in ICTV (Tzanetakis et al., 2005a, b). Therefore, we can assume that the viruses we have detected are also not yet described and are the new valerian viruses. At the time of this manuscript writing, the results of molecular research conducted by Japanese researchers (Uehara-Ichiki et al., 2020) on the first detection of two new carlaviruses on *Valeriana fauriei* plants - *Gaillardia latent virus* (GaILV), *Ligustrum necrotic ringspot virus* (LNRSV). Therefore, our further studies of viruses that infect valerian in Ukraine will include testing them for GaILV and LNRSV presence.

CONCLUSIONS

The obtained research results prove that the cultivation of valerian under the conditions of the Left-Bank Forest-Steppe zone of Ukraine without the use of irrigation is risky. Due to the deficit of soil moisture in the critical periods of growth and development of the crop, a low yield of valerian is formed in the range of 12.4 – 17.1 quintals/ha for spring sowing and 17.3 – 20.2 quintals/ha for winter sowing. For some years, due to drought, it is impossible to get seedlings of valerian. The application of drip irrigation allows to obtain guaranteed yields of dry roots with rhizomes of valerian in the range of 26.3 – 36.6 quintals/ ha for spring sowing and 31.9 – 42.2 quintals/ha for winter sowing. Due to the lack of soil moisture, the use of mineral fertilizers without irrigation is ineffective. Under the conditions of drip irrigation, the basic application of mineral fertilizers with additional fertilization increased the yield of raw materials by 10.3 quintals/ha compared to the variants without fertilizers.

The highest yield of valerian 42.2 quintals/ha was obtained for winter sowing when drip irrigation was used together with the basic application of mineral fertilizers and additional fertilization, which is explained by the creation of the most favorable water and nutrient conditions for growth and development of the crop. Drip irrigation together with mineral fertilizers and extension of the growing season for winter sowing of valerian provides stable yields of raw materials under the conditions of climate change

Knowing the pathogen is important to determine the type of irrigation, frequency and volume of water used to control one specific plant disease. It could be a key factor in achieving high yields. Viruses, transmitting with their vectors, can be controlled by sprinkle irrigation, which disrupts the contact of the insect with the plant» (Café-Filho and Lopes, 2018). Therefore, our data on a smaller number of virus-infected valerian plants under drip irrigation could be recommended for further use for valerian cultivation.

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