

Quality control of industrial hemp seed products, varietal responsiveness of hemp seeds to bioregulator action

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ABSTRACT

The article presents the data on the chemical composition of seeds and oil of industrial hemp four varieties of the Central Russian ecotype, registered for cultivation in Russia: Surskaya, Yuliana, Vera and Nadezhda. The largest weight of 1000 seeds was for the Surskaya variety (17.6 g), while the smallest for the Yuliana variety (16.5 g). The content of lipids in seeds was 33 - 35%, while that of proteins was 22 - 25%, which makes it possible to use all four varieties in the food industry. On average, all varieties had a high seed oil yield of 29.9 - 32.2%. The maximum oil yield was observed in Vera and Nadezhda (32.2 - 32.9%). A low content of all saturated fatty acids in the oil was noted for the hemp of Nadezhda (8.55%), while a higher content (91.5%) for other varieties. It was noted that, on average, the content of Omega-3 fatty acids was 19.5-21.1% in the oil of all studied varieties of industrial hemp. In the Surskaya and Vera, the content was 0.8-1.6% lower than in the Yuliana and Nadezhda. The content of Omega-6 fatty acids in hemp oil was 57.5-58.9%, and there was no significant difference between the varieties. The content of Omega-9 was 11.6-12.8% on average. The Nadezhda in oil exhibited the decreased Omega-9 by 0.5-1.3% than the other varieties. Using scanning electron microscopy, the structure of the hemp seed was established, which has a dense, thickened shell with high strength. The structure of the seed shell is ribbed with pores for the embryo respiration. According to the total elemental composition, hemp seeds contained (in % of wt): carbon 48 - 49%, oxygen 45 - 48%, silicon 0.1 - 0.4%, calcium 0.3 - 4.7%, potassium 0.1 - 1.0%, magnesium 0.1 - 0.7%, sodium 0.1 - 0.2%, sulfur 0.1 - 0.3%, phosphorus 0.1 - 0.2%, chlorine less than 0.1 %. The membrane of the seed contains up to 4.7% of calcium and up to 1% of potassium, while the kernel of the seed contained no higher than 0.1 - 0.3% of these elements. For pre-sowing seed treatment, it is recommended to use the following preparations: Zircon 4 g ton⁻¹, Epin-Extra 10 g ton⁻¹, EcoFus 0.5 kg ton⁻¹, and working fluid consumption 10 L ton⁻¹. The drugs in laboratory studies have shown the effectiveness of action on germination energy and seed germination increase.

Key words: Industrial hemp, Seeds, Oil, Plant growth and development regulators, Fertilizers.

Article type: Research Article.

INTRODUCTION

Industrial hemp is a promising agricultural crop for many countries around the world, including the Russian Federation. Industrial hemp has a wide growing area of different varieties in our country, as well as the possibility of a large range of products obtaining for the national economy, which increases every year. This culture experiences a rebirth in the modern world (Ellison 2021). Hemp has one important feature related to the fact that narcotic substances can be accumulated in the plant organs, therefore it was banned for cultivation in Russia for a long time, as well as in European countries, and the USA. Cultivation of hemp was allowed only in China (Carus & Sarmiento 2017), and today China is the leader by technical hemp cultivation, its processing and production of

a wide range of various products from it (Fike 2016; Crini *et al.* 2020). In the last decade, there has been industrial hemp area increase all over the world. Hemp fiber is inferior to fiber flax in physical and mechanical quality, but modern technologies make it possible to obtain cottonized fiber for the manufacture of textiles. Besides, hemp is used to produce paper, cardboard, viscose, other artificial fibers and polymers, cellulose, organic substances - methanol, ethanol and others (Schlutenhofer & Yuan 2017). Hemp seeds are a valuable product for obtaining vegetable oil (Devi & Khanam 2019). During recent years, they noted the use of hemp seed core in the food industry for the production of confectionery (Farinon *et al.* 2020; Xu *et al.* 2021), hemp milk, hemp ice cream and other food products, since hemp seeds and oil are rich in polyunsaturated fatty acids (Taheri-Garavand *et al.* 2012; Potin & Saurel 2020). Hemp oil is used in the food and medical industries, as well as in cosmetology and for the synthesis of varnishes and paints (Zhou *et al.* 2018; Rupasinghe *et al.* 2020). The development of cannabis farming is one of the priority tasks of Russia. 28 varieties of technical hemp are registered in the RF State Register of Breeding Achievements (2019), most of them are of the Central Russian ecotype, for bilateral use for seeds and fiber. Since 2011 Russian State Agrarian University - Moscow Agricultural Academy named after KA Timiryazev has been involved in the development of agricultural technologies for new varieties of industrial hemp growing with a low content of tetrahydrocannabinol (THC), adapted for cultivation in the Non-Black Earth Zone of the Russian Federation. Taking into account the difficult agro-climatic conditions of this region, an important element in agricultural technologies is the development of improved systems of nutrition and plant protection (Smith *et al.* 2021). One of the essential elements of these systems is the use of modern highly efficient plant growth regulators for presowing seed treatment and plant treatment in different phases of the growing season (Chen *et al.* 2004). The result of such an impact is the yield increase and the quality of fiber and seed improvement which is especially important (Belopukhov *et al.* 2017; Ievinsh & Vikmane 2017).

A limited set of natural and synthetic plant growth regulators are used in many countries of the world that grow hemp (Belopukhov *et al.* 2017). For example, only one growth-regulating drug is approved for use in Russia. The purpose of our work was to assess the chemical composition of seeds and hemp oil of different varieties, to determine the effectiveness of new growth regulators and complex fertilizers for pre-sowing treatment of hemp seeds.

MATERIALS AND METHODS

In the present study, four varieties of industrial hemp of the Central Russian ecotype were used, which are grown to obtain fiber and seeds including Surskaya, Yuliana, Vera, Nadezhda. All selected varieties are registered in the State Register and allowed for production in Russia. Near infrared spectroscopy was used to determine the concentration of proteins and lipids in hemp seeds (SpectraStar 2600XT-R device model). Hemp oil is cold pressed. The qualitative and quantitative compositions of fatty acids in the oil were determined by gas chromatography (Clarus 600 C/D/S/T/MS). The microstructure of seeds and elemental composition were examined by scanning electron microscopy with an energy dispersive spectrometer (the device EM-30AX PLUS). Four preparations were used (Zircon 4 g ton⁻¹, Epin-Extra 10 g ton⁻¹, EcoFus 500 g ton⁻¹, Siliplant 30 g ton⁻¹ and working fluid consumption 10 L ton⁻¹ for pre-sowing seed treatment, which have shown the effectiveness of action on germination energy and seed germination in laboratory studies. Hemp seeds were pre-soaked for 5 hours in biological products; seeds were germinated on pure quartz sand; the seed germination energy was measured after 72 hours; and seed germination was measured after 120 hours. The main active ingredients of Zircon and Epin-Extra are the mixture of hydroxycinnamic acids and 24 epibrassinolide respectively (Belopukhov *et al.* 2017). EcoFus is an organic mineral fertilizer based on brown algae (*Fucus vesiculosus*), and contains the following trace elements (in g L⁻¹): Fe 1.8; Mg 0.5; Mn 1.2; Cu 0.3; B 0.4; Zn 0.3; Ca 0.25; Mo 0.2; Co 0.1; N 1.8%; P 1.0%; K 2.0%, as well as I, Se, Si, constituting over 40 elements in total. Besides, EcoFus contains proteins, amino acids, carbohydrates, vitamins, fiber, organic acids, carotenoids and other biologically active substances. Siliplant contains the following elements (in mg L⁻¹): Fe 300 chelates; Mg 100; Cu 240; Zn 80; Mn 150; Co 15; B 90; Si 7.0%; and K 1.0% (Zhou *et al.* 2018; Devi & Khanam 2019).

RESULTS AND DISCUSSION

The chemical composition of seeds is an important characteristic for their further use. All four studied varieties had differences in the weight of 1000 seeds (Table 1). The largest weight of 1000 seeds was found in Surskaya (17.6 g), while the smallest in Juliana (16.5 g). The oil was obtained from hemp seeds by cold pressing. The



maximum oil yield was observed in Vera and Nadezhda (32.2-32.9%). On average, all varieties had a high yield of oil from seeds (29.9-32.2%), which makes it possible obtaining oil for food and industrial use. Hemp seeds are rich in proteins and lipids (Table 2). The lipid and protein contents were 33-35% and 22-25% respectively, which makes it possible to use all four varieties in the food industry. The fatty acid composition of the oil was determined by gas chromatography (Table 3).

Table 1. Indicators of hemp seed quality of different varieties.

Variety	Weight of 1000 seeds (g)	Oil yield (%)
Surskaya	17.6	31.2
Juliana	16.5	29.9
Vera	17.1	32.9
Nadezhda	17.0	32.2
HCP ₀₅	0.5	1.1

Table 2. The content of proteins and lipids in hemp seeds of different varieties, rate (%) on absolutely dry matter.

Hemp variety	lipids	protein
Surskaya	33.5 ± 1.1	24.5 ± 0.9
Juliana	33.0 ± 1.0	22.2 ± 0.8
Vera	34.1 ± 1.1	24.3 ± 0.9
Nadezhda	33.0 ± 1.1	22.4 ± 0.9

Table 3. Fatty acid composition of hemp oil (%).

Variety	Total fatty acid content (%)							Omega -6/Omega -3
	saturated	Unsaturated						
		mono-	poly-	total	Omega-3	Omega -6	Omega -9	
Surskaya	9.19	12.21	78.60	90.81	19.52	58.86	12.10	3.02
Juliana	9.10	12.60	78.30	90.90	20.30	57.79	12.49	2.85
Vera	9.03	12.88	78.09	90.97	19.50	58.43	12.81	3.00
Nadezhda	8.55	11.22	80.23	91.45	21.10	57.55	11.55	2.73
HCP ₀₅	0.3	0.4	2.5	2.9	0.6	1.8	0.4	-

Hemp oil is characterized by a high content of polyunsaturated fatty acids at the level of 90-91%, while a low content of saturated fatty acids at 8-9%. Saturated fatty acids of hemp oil are represented by a high content of palmitic (C 16:0) and stearic (C18:0) acids. The content of each of them is from 3 to 5%. A low content of saturated fatty acids in the oil was noted for Nadezhda (8.55%), while a higher content of unsaturated fatty acids (91.45%) for other varieties. Unsaturated fatty acids of hemp oil are represented by an increased contents of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids at the levels of 12-15%, 55-58% and 19-22% respectively. Unsaturated fatty acids are ranked according to their importance in the human diet according to the following order: Omega-3 > Omega-6 > Omega-7 > Omega-9. The division of unsaturated fatty acids into classes is based on the position of the double bond relative to the terminal methyl group of the fatty acid. All these four classes of fatty acids are found in hemp oil. The most important for the human diet are Omega-3 and Omega-6, which are involved in various biochemical processes of cells and in the body as a whole, many of them are essential. Oleic, linoleic and alpha-linolenic fatty acids belong to Omega-9, Omega-6, and Omega-3 respectively (Potin & Saurel 2020). When analyzing the fatty acid composition of hemp oil, it is necessary to take into account both the fatty acids themselves and their ratio. It was noted that, on average, the content of Omega-3 acids in the oil of all studied varieties of industrial hemp was 19.5-21.1%. However, in Surskaya and Vera (0.8-1.6%) was lower than in the Yuliana and

Nadezhda. The content of Omega-6 in hemp oil was 57.5-58.9%, and there was no significant difference between varieties. The content of Omega-9 made 11.6-12.8% on average. The oil of Nadezhda variety exhibits the decreased concentration of Omega-9 by 0.5-1.3%, relative to other varieties. The ratio of Omega-6 and Omega-3 fatty acids was 2.7:3, which is optimal for the human diet. The structure of the hemp seed was established by the method of scanning electron microscopy (Figs. 1-2).

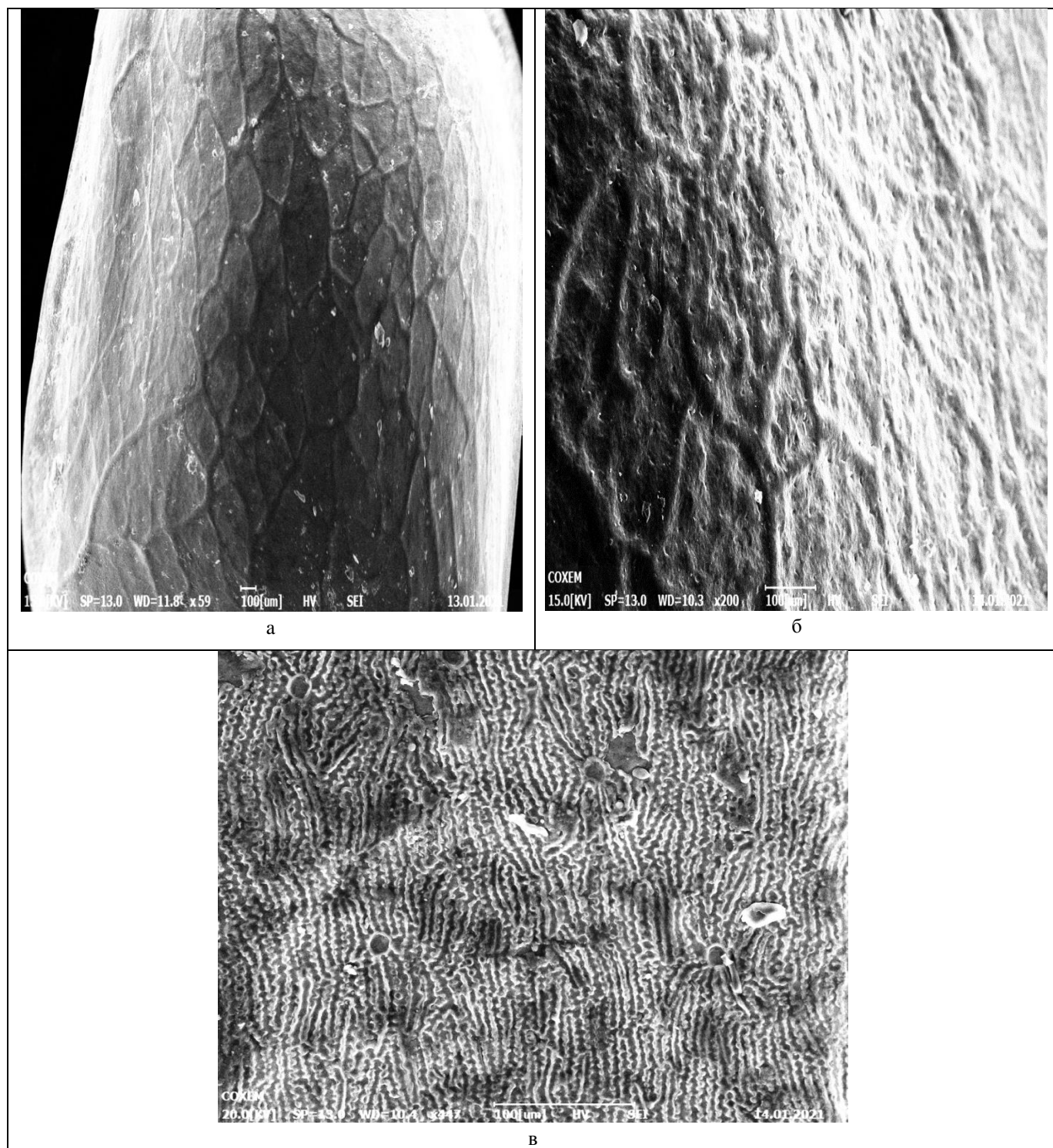


Fig. 1. Hemp seeds of the Nadezhda variety, (a): the outer structure of the seed, (b): the outer shell of the seed, (B): the membranous structure of the seed shell.

Hemp seeds have a dense, thick shell with high strength (Fig. 1a). The structure of the seed shell is ribbed. It has pores for the embryo respiration (Fig. 1 б). The outer shell of the seed has a protective film, consisting mostly of calcium ions, which protects it from drying out and other external influences, and the shell is penetrated with rounded pores (Fig. 1 в). The elemental composition of cannabis seeds of the Nadezhda variety was determined by energy dispersive spectroscopy. The summarized spectrum-map of the chemical element distribution in hemp seeds is shown in Figs. 2-3.

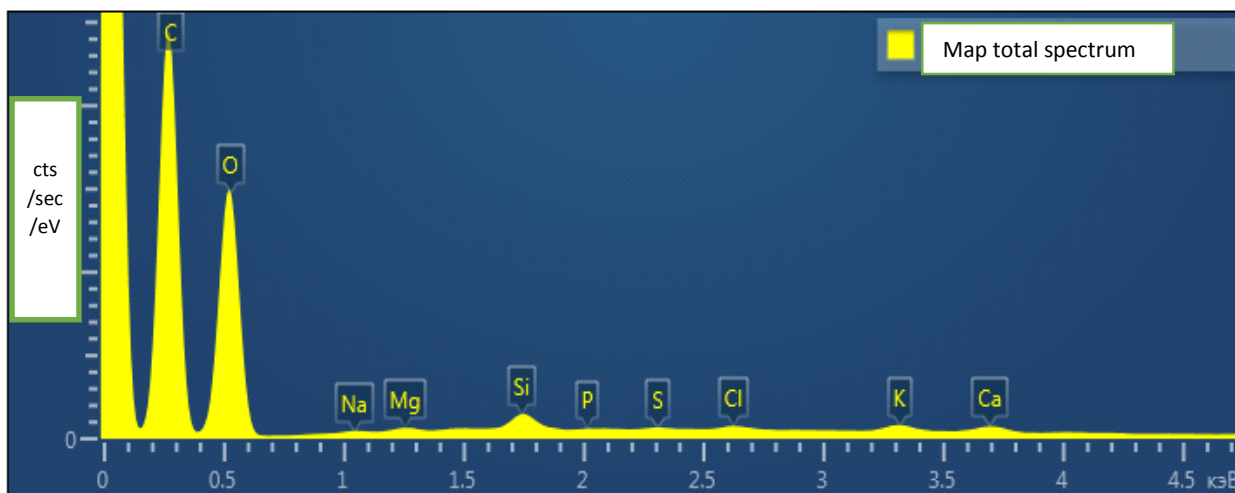


Fig. 2. Spectrum map of chemical element distribution in hemp seeds of the Nadezhda variety.

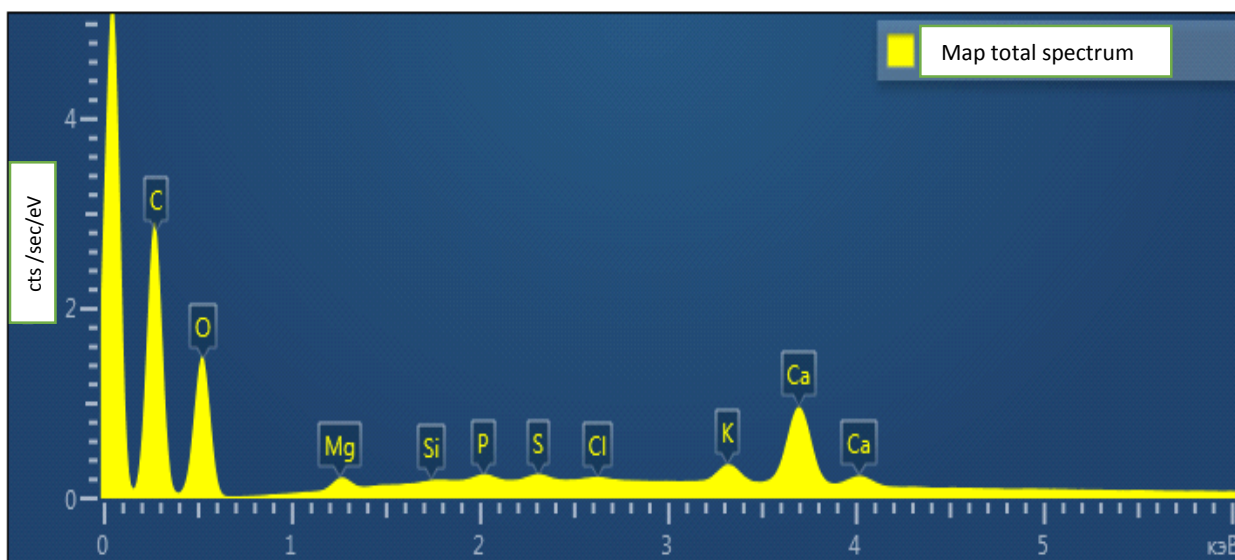


Fig. 3. Spectrum map of chemical element distribution in the membranous shell of the hemp seed variety Nadezhda.

It has been established that the seeds contain oxygen and carbon in macro quantities, since these elements are the main components of proteins and lipids in seeds, constituting 90 - 95%. The seeds contain sodium, magnesium, silicon, phosphorus, sulfur, potassium, calcium, and chlorine. According to the total elemental composition, hemp seeds contain the following elements (in % of wt): carbon 48 - 49%, oxygen 45 - 48%, silicon 0.1 - 0.4%, calcium 0.3 - 4.7%, potassium 0.1 - 1.0%, magnesium 0.1 - 0.7%, sodium 0.1 - 0.2%, sulfur 0.1 - 0.3%, phosphorus 0.1 - 0.2%, and chlorine less than 0.1%. At the same time, the membranous shell of the seed contained up to 4.7% calcium and up to 1% potassium, while the seed itself contained no higher than 0.1 - 0.3% of these elements.

In agricultural technologies for the cultivation of agricultural crops, it is important to adhere strictly to all technological operations during the growing season of plants: alternation of crops in crop rotation, correct and timely soil cultivation, application of mineral and organic fertilizers for crops, timely treatment of crops with

herbicides, fungicides and plant growth and development regulators. All these provide plants with normal growth and development, contribute to obtaining high yields of high-quality products (Chen *et al.* 2004; Glazova 2021). There are seven insecticides (Sumiju, Barguzin 600, Bishka, Binadin, Dimetus, Tod, Samurai Super) and one plant growth and development regulator (Artafit) in the List of pesticides and agrochemicals of the Russian Federation for industrial hemp, according to 2021 data. However, there is no herbicide and fungicide, which is insufficient to comply with the correct technological cycle of crop cultivation. Preparations of the ANO NEST M company (Russia, Moscow) including Zircon, Epin-Extra, Siliplant and EcoFus have been verified to be highly effective on many crops for many years. Zircon and Epin-Extra are registered in the List of pesticides and agrochemicals of the Russian Federation as the regulators of plant growth and development, while Siliplant and EcoFus are complex fertilizers (Andre *et al.* 2016; Belopukhov *et al.* 2017). In our study, these drugs were tested on the seeds of different varieties of industrial hemp for the first time. The seeds were soaked in effective concentrations (Zircon 4 g ton⁻¹, Epin-Extra 10 g ton⁻¹, EcoFus 0.5 kg ton⁻¹, Siliplant 30 g ton⁻¹, and working fluid consumption 10 L ton⁻¹). They performed the assessment of the germination energy (Table 4) and seed germination (Table 5).

Table 4. Energy of hemp seed germination (%).

Variety	control	Zircon	Epin-Extra	Siliplant	EcoFus
Surskaya	75.1	80.2	78.2	75.0	83.0
Juliana	79.0	85.3	80.1	76.1	85.1
Vera	76.2	85.1	81.0	76.2	86.2
Nadezhda	77.3	85.0	80.0	75.0	84.1
HCP ₀₅	2.5	2.7	2.6	2.4	2.7

Table 4 depicts that the germination energy of seeds of different varieties of hemp was 75 - 77% in the control variant. A significant difference between the varieties was noted for Yuliana variety compared to Surskaya. The plant growth and development regulators, Zircon and Epin-Extra, influenced elevating this indicator by 5-8% and 1-5%, respectively. Varietal responsiveness was higher in Yuliana, Vera and Nadezhda compared to Surskaya based on the effect of Zircon, and also in Vera compared to Surskaya, once employing Epin-Extra. Complex fertilizer Siliplant did not have a significant effect on the seed germination energy relative to the control. EcoFus influenced the increase of this indicator by 6-10%. It was noted that the seeds of the cannabis variety Vera responded better to the drug effect. Seed germination is an important indicator for further planning of crop sowing. In the present study, the germination rate of cannabis seeds of different varieties was 89-90% in the control (Table 5), no significant difference between the varieties was found.

Table 5. Germination of hemp seeds (%).

Variety	control	Bioregulators			
		Zircon	Epin-Extra	Siliplant	EcoFus
Surskaya	89.2	95.2	93.3	90.3	98.3
Juliana	90.3	96.1	93.0	91.4	95.0
Vera	90.0	98.0	95.2	90.0	95.2
Nadezhda	90.3	96.4	95.0	90.2	94.1
HCP ₀₅	2.8	2.9	2.8	2.8	2.8

Zircon and Epin - Extra increased the germination of hemp seeds by 6-8% and 3-5%, respectively. The Vera responded better to the action of these drugs relative to other varieties. No positive influence of Siliplant was established on all studied cannabis varieties in comparison with control group. EcoFus increased seed germination by 4–9% compared to control. The difference between cultivars on the drug effect was not established.

SUMMARY

The conducted studies allow us to conclude that the seeds of new varieties of Central Russian industrial hemp, i.e., Surskaya, Yuliana, Vera and Nadezhda contain a high concentrations of proteins (22 - 25%) and lipids (33 - 35%). They are rich in macro- and microelements (in % of wt.) including carbon (48 - 49%), oxygen (45 - 48%), silicon (0.1 - 0.4%), calcium (0.3 - 4.7%), potassium (0.1 - 1.0%), magnesium (0.1 - 0.7%), sodium (0.1 - 0.2%),



sulfur (0.1 - 0.3%), phosphorus (0.1 - 0.2%), chlorine less than 0.1%. The yield of hemp oil was up to 33%. Hemp oil obtained from the seeds of four varieties contains a large amount of polyunsaturated fatty acids Omega-3 (19.5-21.1%) and Omega-6 (57.5-58.9%), which makes it possible to recommend the seeds of these varieties for the food industry to obtain a diverse range of food products.

To improve the growth and development of industrial hemp during the growing season, it is recommended to use pre-sowing seed treatment with the preparations such as Zircon (4 g ton⁻¹), Epin-Extra (10 g ton⁻¹), EcoFus (0.5 kg ton⁻¹), and working fluid consumption (10 L ton⁻¹). These drugs have led to the effectiveness of action as well as elevating the germination energy and seed germination in laboratory studies.

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