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SIBIRSKII VESTNIK SEL'SKOKHOZYAISTVENNOI NAUKI

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ПРЕДПОСАДОЧНАЯ ОБРАБОТКА КЛУБНЕЙ КАРТОФЕЛЯ БИОПРЕПАРАТАМИ В УСЛОВИЯХ ЦЕНТРАЛЬНОЙ ЯКУТИИ

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Представлены результаты исследования влияния предпосадочной обработки клубней биопрепаратами на основе штаммов бактерий *Bacillus subtilis* на рост, фотосинтетическую деятельность растений, поражение болезнями и урожайность картофеля сорта Якутянка в условиях Центральной Якутии. Схема опыта включала контроль (клубни без обработки), обработку клубней перед посадкой биофунгицидом Фитоспорин-М и суспензией из равного соотношения штаммов бактерий *B. subtilis* ТНП-3 и *B. subtilis* ТНП-5, выделенных из мерзлотных почв Якутии. Установлено, что обработка клубней перед посадкой биопрепаратами ускоряла появление всходов на 2–4 дня, увеличивала биомассу и показатели фотосинтетической деятельности растений (на 6–19%), количество (11%) и массу (48–57%) клубней, снижала в 1,8–2,9 раза поражение растений комплексом болезней и повышала на 7,4–8,8 т/га (54–64%) урожайность. Показано, что доля влияния биопрепаратов в изменчивости хозяйственно ценных показателей и урожайности составляет 45–96%, погодных условий – 1–38%, взаимодействия факторов – 1–11%. Существенная положительная связь урожайности, количества и массы клубней выявлена с высотой, количеством и массой растений, площадью листьев, фотосинтетическим потенциалом (r = 0,81…0,98), отрицательная – с поражением ризоктониозом, обыкновенной паршой, обыкновенной мозаикой (r = –0,80…–0,96). Между распространенностью болезней и показателями роста и фотосинтетической деятельности растений наблюдается обратная зависимость (r = –0,33…–0,96). В засушливых условиях поражение обыкновенной паршой и ризоктониозом возрастает в 1,4–1,8 раза, черной ножкой, морщинистой и обыкновенной мозаикой снижается в 1,6–2,5 раза. Рассчитаны уравнения регрессии, позволяющие оперативно и с высокой точностью (R^2 = 0,85…0,95) прогнозировать площадь листьев, фотосинтетический потенциал и урожайность по массе растений. При увеличении массы куста в фазе цветения на 100 г площадь листьев растений повышалась на 2,3 тыс. м^2/га, фотосинтетический потенциал за вегетацию – на 120 тыс. м^2 · сут/га, урожайность – на 9,7 т/га. Разница между фактическими и рассчитанными значениями составила 2,7–4,7%.

Ключевые слова: картофель, обработка клубней, биопрепараты, фотосинтетическая деятельность, болезни, урожайность

PRE-PLANTING TREATMENT OF POTATO TUBERS WITH BIOPREPARATIONS IN CONDITIONS OF CENTRAL YAKUTIA

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The results of the study of the influence of pre-planting treatment of tubers with biopreparations based on *Bacillus subtilis* bacterial strains on the growth, photosynthetic activity of plants, disease damage and yield of the Yakutyanka potato variety under the conditions of Central Yakutia are presented. The experiment scheme included control (tubers without treatment), treatment of the tubers before planting with the biofungicide Phytosporin-M and the suspension of an equal ratio of *B. subtilis* TNP-3 and *B. subtilis* TNP-5 bacterial strains isolated from permafrost soils of Yakutia. It was found that treatment of tubers before planting with biopreparations accelerated sprouting by 2-4 days, increased biomass and photosynthetic activity of the plants (by 6-19%), the number (11) and weight (48–57%) of tubers, reduced the damage of the plants by a complex of diseases by 1.8–2.9 times and increased the yield by 7.4–8.8 t/ha (54–64%). The share of biopreparation influence in the variability of economically valuable indicators and yield was shown to be 45–96%, weather conditions 1–38%, factor interaction 1–11%. Significant positive correlation of the yield, number and weight of the tubers with the height, number and weight of the plants, leaf area, photosynthetic potential (r = 0.81...0.98), and negative correlation with rhizoctonia disease, potato scab, and common mosaic (r = –0.80...–0.96) were revealed. There was an inverse relationship between disease prevalence and indices of plant growth and photosynthetic activity (r = –0.33...–0.96). Damage by potato scab and rhizoctonia disease increases 1.4–1.8 times in arid conditions, while blackleg, rugose and common mosaic damage decreases 1.6–2.5 times. Regression equations were calculated, which allow to predict leaf area, photosynthetic potential and yield by plant mass promptly and with high accuracy (R^2 = 0.85...0.95). When the bush weight in the flowering phase increased by 100 g, the leaf area of the plants increased by 2.3 thousand m^2/ha, photosynthetic potential during the growing season by 120 thousand $m^2 \cdot$ day/ha, and the yield by 9.7 t/ha. The difference between actual and calculated values amounted to 2.7–4.7%.

Keywords: potato, tuber treatment, biopreparations, photosynthetic activity, diseases, yield

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INTRODUCTION

Potato growing is the most important branch of agriculture in the Republic of Sakha (Yakutia). The average annual volume of gross potato harvest in the region is 70–85 thousand tons with a yield of 10-11 tons/ha. To fully provide potatoes with local production, at least 150 thousand tons of tubers are needed annually. The solution to this problem is possible primarily due to an increase in yield, which in recent years in the re-publication does not significantly increase[1, 2].

One of the reasons for the low yield and profitability of the crop is the lack of adaptive agrotechnologies of cultivation using effective biopreparations, which are an integral part of organic farming. Strategic importance is now attached to this direction[3].

[1]Agriculture in the Republic of Sakha (Yakutia): stat. coll. Yakutsk, 2022, 145 p.
[2]*Okhlopkova P.P.* History of development and current state of potato breeding in Yakutia // Science and technology in Yakutia, 2017, N 1 (32), pp. 20–22.
[3]Strategy for the Development of Organic Production in the Russian Federation until 2030 // Order of the Government of the Russian Federation of July 4, 2023. N 1788-p. 91.

The use of bacterial preparations is based on the mechanism of antibiosis, which regulates the relationship between beneficial and harmful microorganisms[4]. Biopreparations for the suppression of the number of phytopathogenic microorganisms are an environmentally safe and effective alternative to chemical pesticides, since they are developed on the basis of natural regulators of the number of plant pathogens. Such regulators include entomopathogenic and antagonistic micro-organisms and their metabolites [1]. The most common agents of biological control of phytopathogenic fungi are bacteria of the genus Bacillus spp., which improve the growth of plant roots, facilitate access to them with trace elements, increase yields [5, 6] [2-4]. Of the aerobic spore-forming bacteria, Bacillus subtilis bacteria, whose strains have fungicidal activity, stimulate plant growth, and increase their yield, have the greatest importance as a biological agent for suppressing phytopathogen abundance[7, 8] [5, 6] The biofungicide Phytosporin -M, created on the basis of the bacterium Bacillus subtilis, strain 26D, has become widespread. The preparation increases the resistance of plants to diseases due to the antagonism of the bacterium in vitro to many phytopathogens and its ability to competitively occupy their niche in the internal tissues of plants [7]. The use of Phytosporin-M accelerated the growth and development of plants, increased resistance to biotic and abiotic factors of the external environment, the harvest and its quality, reduced disease damage and losses during storage of various crops, including potatoes[9-11] [8-10].

In 2006, a suspension was developed at the Yakutsk Research Institute of Agriculture (Yakutsk NIISKh) from an equal ratio of the strains of B. subtilis TNP-3 and B. subtilis TNP-5 bacteria isolated from permafrost soils in Yakutia and deposited at the All-Russian Research Institute of Agricultural Meteorology (VNIISKhM) under the numbers D149 and D150. The strains have an antagonistic and immunostimulatory effect against pathogenic microorganisms, which made it possible to develop biologics widely used in the northern animal husbandry of Yakutia against various animal diseases and for the growing stock weight gain[12] [11]. The addition of the strains of B. subtilis TNP-3 + TNP-5 bacteria during silage of feed crops ensured high quality, nutrition and safety of silage [12]. Spraying with a suspension of strawberry plant strains reduced damage to berries by gray rot, increased the number of peduncles and the mass of berries[13] and treatment of barley seeds and potato tubers reduced plant disease, increased the above-ground biomass, and increased the yields[14] [13].

The purpose of the study is to research the effect of pre-planting treatment of potato tubers

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[14]*Vlasenko N.G., Sleptsov S.S., Samsonova M.S.* Protection of barley from fungal diseases in Central Yakutia. Yakutsk: Publishing house of the Yakutsk Research Institute of Agriculture, 2012, 47 p.

with Bacillus subtilis bacteria-based biopreparations on the growth, photosynthetic activity of plants, disease damage and yield in Central Yakutia.

MATERIAL AND METHODS

The research was conducted at the station of the Yakutsk Research Institute of Agriculture "Kholbuya", located in the floodplain of the Lena River. The region is included in the zone of risky farming due to extremely low winter temperatures, large annual, seasonal and daily air temperature fluctuations, arid climate, short frost-free period, low-temperature permafrost rocks and cold soils with low fertility. The soil of the experimental plot is weakly alkaline (pH 7.8) cryosolic-alluvial soil, stratified sandy loam of mechanical composition. The arable layer is characterized by low content of humus (1.8-2.2%), total (0.23%) and nitrate (0.7-1.2 mg/100 g) nitrogen, high - mobile phosphorus (21-24 mg/100 g), exchangeable potassium (21-25 mg/100 g). The object of the study was potato plants and tubers of potato variety Yakutianka, a zoned medium-early variety. The experiment scheme included control (tubers without treatment), treatment of the tubers before planting with the biofungicide Phytosporin-M (10 g/0.5 l at the rate of 0.5 l/20 kg) and suspension of B. subtilis bacterial strains TNP-3 + TNP-5 (soaking before planting for 30 min at a dose of 1×10^9 CFU/ml at the rate of 300 ml/kg tubers).

Cultivation technology was prepared taking into account zonal recommendations[15]. The experiments were placed in a three-field crop rotation (oats for green manure crops - potatoes - potatoes). Planting was carried out by clonal planter SN-4BK on the scheme 70 × 35 cm (40.8 thousand tubers per 1 ha) on May 28 - June 3 at a soil temperature of 6 ... 8 °C at a depth of 10 cm. The study plot area was 24.5 m^2, the width of the protective strip was 5 m, the repetition was 4-fold, the placement of the variants randomized. Vegetation irrigation was carried out by a sprinkler system DDN-70 in the III ten-day period of June - I ten-day period of July at 350 m^3/ha. Potatoes were harvested manually on August 25-28.

Phenological observations, records and analyses were carried out according to the methodology of the All-Russian Research Institute of Potato Farming (VNIIKKh)[16]. Leaf area and photosynthetic potential (PP) of plants were calculated according to the method of A.A. Nichiporovich[17]. Moisture availability during the growing season was estimated by the hydrothermal coefficient Selyaninov (HTC)[18]. In the years of research, the sum of average daily air temperatures during the growing season (June-August) varied from 1451 to 1676° with the norm of 1443°, the sum of precipitation - from 60 to 187 mm with the norm of 127 mm, the conditions of moisture availability - from optimal (HTC = 1.29) to severe drought (HTC = 0.37). The experimental material was processed statistically according to the method of B.A. Dospekhov[19] using a package of applied programs[20].

RESULTS AND DISCUSSION

The duration of the period of sprouting - maturation of the potato variety Yakutyanka varied from 62 to 66 days at the sum of average daily air temperatures 1048-1227°, the period of planting - maturation - from 84 to 89 days at the sum of temperatures 1417-1615°. More significantly (V = 13%) varied the duration of the planting - sprouting period (from 18 to 27 days).

[15]The system of agro-industrial production of the Republic of Sakha (Yakutia) until 2005 / RAAS Siberian branch. Yakut. NIISKh, Novosibirsk, 1999, 304 p.

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In the variants with treatment of the tubers with Phytosporin-M and suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5, potato sprouts appeared 2-4 days earlier than in the control. Duration of other interphase periods varied insignificantly (V = 1-4%) and no significant differences were found. The greatest variability of heat availability of plants was observed from planting to flowering (V = 8-11%) in comparison with other periods (V = 2-6%).

Observations of plant biomass growth showed that when the tubers were treated with Phytosporin and suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 in all phases, it increased in comparison with the control by an average of 67-84 g/bush (16-19%). Biomass reached its maximum value in the flowering phase (451-535 g/bush), in the budding phase it was 89% of the maximum, in 10 days after flowering it decreased by 6%, in 20 days - by 9% (see Table 1). The greatest contribution to the variability of the index was made by biopreparations (64-74%) with minor influence of weather conditions and interaction of factors (1-9%). A strong direct correlation ($p < 0.01$) of plant weight with plant height ($r = 0.89...0.94$) and number in the bush ($r = 0.81...0.84$) was revealed. Regression analysis showed that when the height of plants in the flowering phase (x_1) increased by 1 cm, the bush weight (y_1) increased by 36.1 g. Equation (1) allows to calculate the mass of plants by their height promptly and with high accuracy (coefficient of determination $R^2 = 0.86$):

$$y_1 = 36{,}139x_1 - 1155{,}2,\ R^2 = 0{,}860. \quad (1)$$

Biopreparations also had a significant effect on the photosynthetic activity of plants. Leaf area when the tubers were treated with Phytosporin-M and suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 in all phases increased by 1.6-2.3 thousand m^2/ha (6-9%) compared to the control (see Table 2). It reached its maximum value in the flowering phase (28.3-30.5 thousand m^2/ha), in the budding phase it was 88% of the maximum, in 10 days after flowering it decreased by 1.7 thousand m^2/ha (6%), in 20 days - by 4.1 thousand m^2/ha (14%). The greatest contribution to its variability was made by the biopreparations (50-61%) with insignificant influence of weather conditions and interaction of factors (1-9%). A strong direct relationship ($p < 0.01$) between the leaf area and plant height and weight was found ($r = 0.82...0.95$). The regression coefficient indicates that with an increase in bush weight in the flowering phase (x_2) by 100 g leaf area (y_2) increased by 2.3 thousand m^2/ha:

$$y_2 = 0{,}0228x_2 + 18{,}108, \quad R^2 = 0{,}8490. \quad (2)$$

The difference between the actual and calculated leaf area using the equation (2) was not more than ±2.7%.

Табл. 1. Влияние предпосадочной обработки клубней картофеля биопрепаратами на динамику сырой массы растений (среднее за 2007–2009 гг.)

Table 1. Effect of pre-planting treatment of potato tubers with biopreparations on the dynamics of raw weight of plants (average for 2007–2009)

Option (factor)	Budding stage	Flowering stage	10 days after flowering	20 days after flowering
Growth of plant biomass, g/bush				
Control	401	451	425	409
Phytosporin-M	475	535	504	482
TNP-3 + TNP-5	468	529	494	480
Share of influence of factors and their interaction, %				
Preparation (factor A)	73,5**	71,0**	68,9**	64,1**
Year (factor B)	2,3	3,4	2,2	4,2
A × B	8,6*	1,3	0,5	1,1

Here and in Tables 2–4.
* Reliable at 5% significance level.
** Reliable at 1% significance level.

Табл. 2. Влияние биопрепаратов на динамику показателей фотосинтетической деятельности растений (среднее за 2007–2009 гг.)

Table 2. Effect of biopreparations on the dynamics of photosynthetic activity of plants (average for 2007–2009).

Option (factor)	Budding	Flowering	10 days after flowering	20 days after flowering	Sprouts - flowering	Flowering – 20 days after flowering	Amount per vegetation
	Leaf area, thous. m²/ha				PP, thous. m² · days/ha		
Control	25,0	28,3	26,5	24,3	459	528	987
Phytosporin-M	26,7	30,5	28,8	26,4	514	573	1087
TNP-3 + TNP-5	26,7	30,1	28,5	25,9	525	565	1090
Share of influence of factors and their interaction, %							
Preparation (factor A)	51,6**	61,1**	61,2**	49,6**	77,3**	69,4**	86,7**
Year (factor B)	9,4*	0,6	7,7*	8,3	3,5**	5,4	4,5**
A × B	8,1	5,0	2,8	0,9	11,1**	1,5	5,2**

Photosynthetic potential of crops (PP) in the variants with pre-planting treatment of the tubers with biofungicide Phytosporin-M and suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 in different periods of vegetation was higher by 37-103 thousand m² - day/ha (7-14%) than in the control (see Table 2). The greatest contribution to its variability was made by biopreparations (69-87%), the influence of weather conditions and factor interactions was significant in most cases, but less significant (2-11%). In all interphase periods, a direct correlation ($p < 0.01$) of the PP values with height, plant weight and leaf area was revealed ($r = 0.82...0.98$). Regression analysis showed that when plant weight in the flowering phase increased by 100 g (x_2), leaf area by 1 thousand m²/ha (x_3), PP during the growing season (y_3) increased by 120 thousand m² - day/ha (3) and 50 thousand m² - day/ha (4), respectively:

$$y_3 = 1{,}1972x_2 + 450{,}01, \quad R^2 = 0{,}8673; \quad (3)$$

$$y_3 = 49{,}689x_3 - 418{,}12, \quad R^2 = 0{,}9173. \quad (4)$$

The calculated equations allow to predict the PP value for vegetation by plant mass and leaf area in the flowering phase promptly and with high accuracy ($R^2 = 0.87 ... 0.92$).

In the years of research potato variety Yakutyanka during the growing season was affected by various diseases. The highest prevalence in the control variant was black leg (8.0% on average), common potato scab (7.9%), common mosaic (6.1%), the lowest - rhizoctoniose (2.0%), potato rugose mosaic (4.1%) (see Table 3). Pre-planting treatment of the tubers with Phytosporin-M reduced the plant damage by diseases by 1.8-6.8 times (2.9 times on average), by suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 by 1.5-2.7 times (1.8 times). Under drought conditions, the prevalence of common potato scab and rhizoctoniose increased 1.4-1.8 times, black leg, potato rugose mosaic and common mosaic decreased 1.6-2.5 times and more in the variants with the use of biopreparations. The share of the influence of preparations in the variability of disease prevalence was 45-97%, weather conditions 2-38%, interaction of factors 4-11%. The inverse correlation of potato disease incidence with height, weight and leaf area of plants, PP ($r = -0.33...-0.96$) was found, which indicates a lower degree of damage to more powerful and developed plants regardless of growing conditions. A significant direct correlation of common potato scab prevalence with the disease of plants by rhizoctoniose ($r = 0.84$) and common mosaic ($r = 0.76$); rhizoctoniose - with the disease of common mosaic ($r = 0.90$); black leg - with the disease of potato rugose mosaic and common mosaic ($r = 0.71...0.74$) was revealed. There was an average positive correlation between common and potato rugose mosaic disease ($r = 0.65$).

Табл. 3. Влияние предпосадочной обработки клубней биопрепаратами на распространенность болезней картофеля (среднее за 2007–2009 гг.), %

Table 3. Effect of pre-planting treatment of tubers with biopreparations on the prevalence of potato diseases (average for 2007–2009), %

Option (factor)	Common potato scab	Rhizoctoniose	Black leg	Potato rugose mosaic	Common mosaic
Plant lesions					
Control	7,9	2,0	8,0	4,3	6,1
Phytosporin-M	2,9	0,0	3,7	2,4	0,9
TNP-3 + TNP-5	5,4	0,0	4,0	4,1	2,3
Share of influence of factors and their interaction					
Preparation (factor A)	69,4**	87,9**	45,0**	48,1**	96,7**
Year (factor B)	22,4**	3,1**	38,2**	37,0**	1,6**
A × B	5,3**	6,2**	11,0**	3,5	0,4

According to the results of the research a new method of controlling the complex of potato diseases (rhizoctoniose, black leg, potato rugose mosaic and common mosaic) based on soaking tubers in a solution of suspension of bacteria strains *B. subtilis* TNP-3 and *B. subtilis* TNP-5 (patent No. 2428008 of the Russian Federation dated 10.09.2011) was developed.

Treatment of the tubers before planting with biopreparations significantly increased the number of the tubers by 11% by harvesting. The greatest contribution to the variability of the indicator was made by the preparations (41%), the influence of weather conditions and interaction of the factors was insignificant (1-8%) (see Table 4). A direct correlation ($p < 0.01$) of the number of the tubers in the bush with height, number and weight of the plants, leaf area, PP was revealed ($r = 0.84...0.97$). The number of the tubers significantly decreased when potatoes were affected by rhizoctoniose, common potato scab, and common mosaic ($r = -0.80...-0.91$).

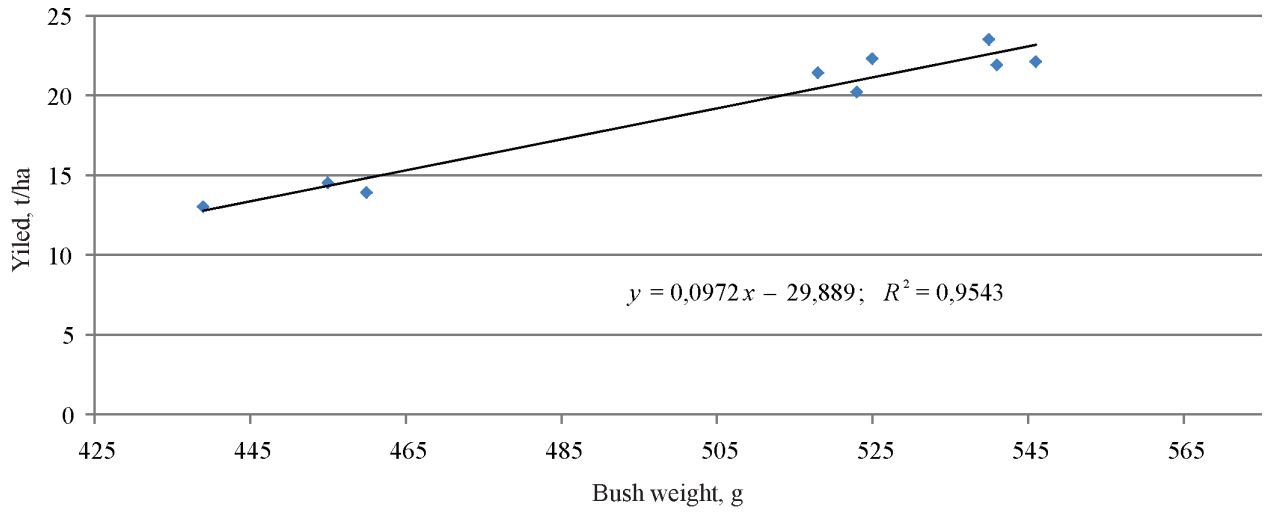
Pre-planting treatment of the tubers with biofungicide Phytosporin-M increased the tubers weight in the bush by 190 g (57%), by suspension of *B. subtilis* strains TNP-3 + TNP-5 by 161 g (48%) in comparison with the control (see Table 4). The main contribution to its variability was made by biopreparations (95%). A strong direct relationship ($p < 0.01$) of the tubers weight in the bush with height, number and weight of plants, leaf area, and PP ($r = 0.81...0.98$) was revealed. The tubers weight significantly decreased when potatoes were affected by rhizoctoniose, common mosaic, and common potato scab ($r = -0.85...-0.96$).

The yield of potato variety Yakutyanka varied in the years of research from 13.0 to 23.5 t/ha ($V = 22\%$). When the tubers were treated with biopreparations before planting, it increased in comparison with the control by 7.4-8.8 t/ha (54-64%), and in the variant with Phytosporin-M it was 1.4 t/ha (6.6%) more than when the tubers were treated with a suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 (see Table 4). The main influence on the yield variability was ex-

Табл. 4. Влияние биопрепаратов на элементы структуры и урожайность картофеля сорта Якутянка (среднее за 2007–2009 гг.)

Table 4. Influence of biopreparations on structure elements and yield of potato variety Yakutyanka (average for 2007–2009)

Option (factor)	Number of tubers, pcs./bush	Tuber weight, g/bush	Yield, t/ha
Productivity			
Control	8,4	334	13,8
Phytosporin-M	9,3	524	22,6
TNP-3 + TNP-5	9,4	495	21,2
Share of influence of factors and their interaction, %			
Preparation (factor A)	41,1**	94,5**	95,8**
Year (factor B)	8,3	3,1	2,4
A × B	0,7	1,0	0,9



Теоретическая линия регрессии при прямолинейной корреляции между урожайностью клубней и сырой массой растений в фазе цветения

Theoretical regression line for a straight-line correlation between tuber yield and raw weight of plants in the flowering phase

erted by the biopreparations (96%). There was a reliable direct correlation of the yield with plant height and weight, leaf area, PP (r = 0.82...0.98), and an inverse correlation with common potato scab, rhizoctoniose, and common mosaic (r = -0.80...-0.96). The regression coefficient shows that when the weight of the bush in the flowering phase increased by 100 g tuber yield increased by 9.7 t/ha (R^2 = 0.95) (see the figure). The difference between the actual yield and the yield calculated on the basis of the raw weight of plants in the flowering phase averaged 0.90 t/ha (4.7%).

CONCLUSIONS

1. In the conditions of Central Yakutia the duration of the period of sprouting - maturation of the potato variety Yakutyanka varies from 62 to 66 days at the sum of average daily air temperatures 1048-1227°, the period of planting - maturation - from 84 to 89 days at the sum of temperatures 1417-1615°. More significantly (V = 13%) varies the duration of the period of planting - sprouting (18-27 days). Pre-planting treatment of the tubers with Phytosporin-M and suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 accelerates the emergence of sprouts by 2-4 days.

2. Treatment of the tubers before planting with Phytosporin-M and suspension of *B. subtilis* bacterial strains TNP-3 + TNP-5 increases the weight and photosynthetic activity of plants (by 6-19%), the number (11%) and weight (48-57%) of the tubers, reduces 1.8-2.9 times the damage of the plants by a complex of diseases, increases the yield by 7.4- 8.8 t/ha (54-64%). The main contribution to the variability of indicators is made by biopreparations (45-96%), less significant - weather conditions (1-38%), interaction of the factors (1-11%).

3. A strong positive relationship ($p < 0.01$) of the yield, number and weight of the tubers with the height, number and weight of the plants, leaf area, photosynthetic potential (r = 0.81...0.98), negative - with rhizoctoniose, common potato scab, common mosaic (r = -0.80...-0.96) was revealed.

4. There is an inverse relationship (r = -0.33...-0.96) between the prevalence of diseases and indicators of growth and photosynthetic activity of plants. In arid conditions, the lesion of common potato scab and rhizoctoniose increases 1.4-1.8 times, black leg, potato rugose mosaic and common mosaic decreases 1.6-2.5 times.

5. Regression equations were calculated, allowing to determine the leaf area, photosynthetic potential and yield by plant mass promptly and

with high accuracy (R^2 = 0.85...0.95). When increasing the weight of the bush in the flowering phase by 100 g, the leaf area increased by 2.3 thousand m^2/ha, PP for vegetation by 120 thousand m^2 - day/ha, yield by 9.7 t/ha. The difference between actual and calculated values is 2.7-4.7%.

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ИСПОЛЬЗОВАНИЕ АЗОТА УДОБРЕНИЙ ЗЕРНОВЫМИ КУЛЬТУРАМИ В АРИДНЫХ УСЛОВИЯХ БУРЯТИИ

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Представлены результаты исследований в длительном (1982–2021 гг.) стационарном опыте по определению коэффициентов использования азота минеральных удобрений культурами зернопарового севооборота в условиях сухостепной зоны Бурятии. Каштановая супесчаная почва на изучаемых вариантах характеризовалась очень низким содержанием гумуса, общего азота и нитратов перед посевом культур. В период проведения исследований отмечено преобладание засушливых лет. Коэффициенты использования азота минеральных удобрений в условиях сухостепной зоны Бурятии в среднем за 39 лет исследований составляли для пшеницы 69%, овса – 54 и овса на зерносенаж – 90%. На вариацию коэффициентов значимо влияли условия увлажнения летнего периода. При экстремальной и сильной засухе они составляли 32%, при умеренной и слабой – 57% и значительно (до 124%) возрастали при благоприятном увлажнении. Использование азота туков было максимальным у третьей культуры (овса на зерносенаж) и более отзывчивым на атмосферное увлажнение. Наименьшие коэффициенты потребления и размах его изменений отмечены у овса на зерно. Первая культура севооборота (пшеница по пару) в этой оценке занимала промежуточное положение. Получены данные о влиянии почвенного увлажнения и гидротермических условий отдельных критических периодов вегетации на вариацию коэффициента использования азота минеральных туков (N_{40}) пшеницей, овсом и овсом на зерносенаж. Корреляционный анализ показал, что наиболее критичным периодом по потреблению азота пшеницей является июнь (r = 0,61), у овса – июль (r = 0,51) и июль – август для овса на зерносенаж (r = 0,50–0,52). Построены множественные линейные модели зависимости коэффициентов использования азотных удобрений от содержания продуктивной влаги и гидротермических условий.

Ключевые слова: коэффициент использования азотных удобрений, сухостепная зона

FERTILIZER NITROGEN USE BY CEREAL CROPS IN ARID CONDITIONS OF BURYATIA

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The results of research in a long-term (1982–2021) stationary experiment to determine the coefficients of nitrogen use of mineral fertilizers by the crops of grain and fallow crop rotation in the conditions of the dry-steppe zone of Buryatia are presented. Chestnut loamy sand soil on the studied variants was characterized by very low content of humus, total nitrogen and nitrates before sowing of the crops. During the research period, the prevalence of dry years was noted. Nitrogen use coefficients of mineral fertilizers in the conditions of the dry-steppe zone of Buryatia on average for 39 years of research were 69% for wheat, 54% for oats and 90% for oats for grain haylage. The variation of the coefficients was significantly influenced by summer moistening conditions. They were 32% under extreme and severe drought, 57% under moderate and weak drought, and increased significantly (up to 124%) under favorable moisture conditions. The utilization of nitrogen of solid mineral fertilizers was maximum in the third crop (oats for grain haylage) and more responsive to the atmospheric moistening. The smallest consumption coefficients and the range of its changes were observed in oats for grain. The first crop of the rotation (fallow wheat) occupied an intermediate position in this

assessment. The data on the influence of soil moisture and hydrothermal conditions of separate critical periods of vegetation on the variation of the coefficient of mineral fertilizer nitrogen utilization (N_{40}) by wheat, oats, and oats for grain haylage were obtained. The correlation analysis showed that the most critical period for nitrogen consumption in wheat is June (r = 0.61), in oats – July (r = 0.51) and July–August for oats for grain haylage (r = 0.50–0.52). Multiple linear models of dependence of N fertilizer use coefficients on productive moisture content and hydrothermal conditions were made.

Keywords: nitrogen fertilizer utilization rate, dry steppe zone

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INTRODUCTION

The coefficient of nutrient utilization of fertilizers is one of the main criteria of efficiency and environmental rationality of their use. The intensity of utilization of applied fertilizers is determined not only by biological characteristics of the plants, but also depends on a complex of edaphic and meteorological factors that determine crop yields. Their joint action forms a significant variation of soil-climatic conditions, but it is difficult to distinguish the role of individual factors on mineral nutrition of plants in short-term experiments. In this regard, mathematical processing of data from long-term field studies allows us to more accurately establish the coefficients of fertilizer use in a certain area and soil depending on climatic conditions of crop vegetation [1-4].

The determination of fertilizer nutrient use coefficients in chestnut soils of Transbaikalia under their systematic application in crop rotation was carried out in the period 1968-1997 in the Agrochemistry Department of the Buryat Research Institute of Agriculture. It was found that in the six-field grain-fallow-row crop rotation (1968-1981) the nitrogen utilization from ammonium nitrate (N_{40}) averaged 34%, under the crops of four-field crop rotation of fallow - wheat - oats - oats for grain haylage (1982-1997) increased to 89% with a very high variability of values. The main factors forming this variability were the hydrothermal conditions of crop vegetation. Nitrogen is the most deficient element, the efficiency of its application on chestnut soils of Buryatia was manifested both in conditions of moderate drought and under good moisture[1] [4-9].

The purpose of the research is to generalize the experimental material on the study of the peculiarities of the use of mineral fertilizer nitrogen by crops of grain-fallow crop rotation with the determination of the influence of hydrothermal conditions of vegetation on the variation of the coefficients of its use from mineral fertilizers in the dry-steppe zone of Buryatia.

MATERIAL AND METHODS

The experiment took place in the experimental field of the Buryat Research Institute of Agriculture in the central dry steppe of the Republic of Buryatia (Ivolginsk settlement) on zonal chestnut soils (included in the system of the Geographical Network of experiments with fertilizers). The experiment was laid in 1967 and consisted of 13 variants of mineral, organic and organic-mineral fertilizer systems. However, in 1982, the scheme of the experiment was changed to reduce the doses, because high rates did not provide the planned productivity in rainfed conditions of the dry steppe of Buryatia.

[1] *Revensky V.A.* Efficiency of nitrogen fertilizers on chestnut soils of Buryatia. Novosibirsk: Nauka, 1985, 149 p.

The most optimal dose of mineral fertilizers is $N_{40}P_{40}K_{40}$. Accordingly, to study the coefficient of nitrogen fertilizer use, the variant $P_{40}K_{40}$ was also introduced.

Fertility of sandy loamy mealy carbonate low humus sabulous chestnut soil on the studied variants was characterized by very low content of humus, total nitrogen and nitrates. The content of mobile P_2O_5 increased from high content in the control to very high in the variants with fertilizers, the content of exchangeable K_2O also increased from medium to high content in these variants (see Table 1).

The climate of the dry steppe of Buryatia is conditioned by the mountain-kettle character of the area and remoteness from the oceans. The sum of average annual precipitation was within 220- 340 mm, of which 150-240 mm fell during the growing season. The sum of active temperatures was 1600-1800°. Meteorological conditions during the study period were evaluated by HTC for June-August according to Selyaninov in the classification of E.S. Ulanova[2]. Correlation analysis revealed the highest dependence of the coefficient of nitrogen fertilizer use on the hydrothermal conditions of the calendar summer (r = 0.69). Extreme and severe droughts during the experiment (1982-2021) were observed in 11 out of 39 years, or in 28% of cases. Note that the impact of soil drought had a prolonged effect, especially with periodic onset. Seasons with favorable moistening were observed in 46% of the cases (see Table 2).

During the research period, there were 10 incomplete rotations of grain-fallow crop rotation fallow - wheat - oats - oats for grain haylage. Mineral fertilizers (Naa, Rsd, Kx) were applied in spring annually by a scattered method under plowing. The number of repetitions in the experiment four times, the area of plots 100 m^2. The coefficient of fertilizer nitrogen use by plants (CFNU, %) was calculated by the difference method between the removal of elements by the economic yield in the fertilized $N_{40}P_{40}K_{40}$ and background $P_{40}K_{40}$ variants with subsequent calculation of the percentage in relation to the

Табл. 1. Агрохимические показатели пахотного слоя каштановых почв опытного участка (среднее за 1982–2021 гг.)

Table 1. Agrochemical parameters of chestnut soils arable layer of the experimental plot (average for 1982–2021)

Option	pH water	Humus	N total	P_2O_5 mobile	K_2O exchangeable	$N–NO_3$, mg/kg before sowing		
		%		mg/kg		Wheat	Oats	Oats for grain haylage
Control	6,9	0,87	0,072	159 ± 12	79 ±8	3,5 ± 0,2	2,8± 0,4	3,9 ± 0,5
$P_{40}K_{40}$	6,9	0,95	0,077	236 ± 19	140 ±14	3,2 ± 0,2	3,0 ± 0,2	3,3 ± 0,4
$N_{40}P_{40}K_{40}$	6,7	1,05	0,081	240 ± 22	130 ±23	4,3 ± 0,4	3,3 ± 0,6	4,6 ± 0,5

Табл. 2. Гидротермические условия периода исследований

Table 2. Hydrothermal conditions of the research period

Characteristics of moisture conditions June - August		Years (n = 39)
Drought:		
extreme and strong	HTC ≤ 0,6	1987,1989, 2002, 2007, 2010, 2011, 2013, 2014, 2015, 2017, 2018 (n = 11)
moderate and weak	0,6 < HTC ≤ 1,0	1993, 1996, 1997, 2000, 2004, 2005, 2009, 2016, 2019, 2020 (n = 10)
Provided and excessive moisturizing	HTC > 1,0	1982, 1983, 1984, 1985, 1986, 1988, 1990, 1991, 1992, 1994, 1995, 1998, 2001, 2003, 2006, 2008, 2012, 2021 (n = 18)

[2]*Ulanova E.S.* Droughts in the USSR and their impact on grain production // Russian Meteorology and Hydrology, 1988, N 7, pp. 127–134.

amount of active substance of nitrogen (40 kg/ha) applied to the soil with fertilizer. Total removal was calculated according to the formula

$$P_{removal} = Y_o C_o + Y_p C_p,$$

where Y_o - wheat and oat grain yield at 14% moisture content, c/ha; Y_p - wheat and oat straw yield at 17% moisture content, c/ha; C_o and C_p - nitrogen content in main and by-products

For oats harvested for green mass and grain haylage, the formula was as follows

$$P_{removal} = Y_o C_o,$$

where Y_o - yield of green mass of oats, c/ha; C_o - nitrogen content in green mass, %.

In some years oats did not produce grain, so its green mass was taken into account. A reduction factor of 0.542[3] was applied to bring the green mass of oats and grain haylage from harvested (55%) to standard moisture content. Humus, total nitrogen, nitrate nitrogen, mobile compounds of phosphorus and potassium, pH were determined in the soils according to methodological recommendations[4].

The influence of climatic conditions on the change in the coefficients of nitrogen use from fertilizers was determined for each factor on the basis of correlation dependencies. Regression models were built on the basis of the least squares method for the most significant predictors[5].

RESULTS AND DISCUSSION

The use of nitrogen fertilizers is associated with both biological characteristics of crops and the purpose of products. The technology of cultivation of crops of grain and fallow crop rotation implied different sowing dates: I and III ten-day periods of May for wheat and oats for grain, respectively, II ten-day period of June - for oats for grain haylage mass. In accordance with this, plants passed critical phases of development in different periods of moisture availability.

The lowest consumption of nitrogen fertilizers by oats is associated with its low productivity relative to wheat and oats for grain haylage (see Table 3).

With 73% probability of dry season from the end of May to the second ten-day period of June, it is the second crop that passes the critical period from sprouting to tube emergence under the least favorable conditions of atmospheric and soil moisture. Wheat on fallow is more provided with soil moisture of fallow field than oats, which uses only its aftereffect. In this regard, the strategy of cultivation of the third crop was carried out by the type of annual grasses to obtain the maximum yield of grain haylage. Intensive growth of vegetative mass was technologically brought to the most moisture-supplied period - from the second ten-day period of July to the second ten-day period of August, so that the nitrogen of fertilizers was more fully used in syn-

Табл. 3. Коэффициент использования азотных удобрений культурами зернопарового севооборота (1982–2021 гг.), %

Table 3. Nitrogen fertilizer use coefficient by crops of grain and fallow crop rotation (1982–2021), %

Culture in crop rotation	Coefficient			
	HTC ≤ 0,6	0,6 < HTC ≤ 1,0	1,0 < HTC	Average
First (wheat)	38	44	126	69
Second (oats)	24	49	89	54
Third (oats for grain haylage)	33	78	158	90
In crop rotation	32	57	124	71

[3] *Yagodin B.A., Zhukov V.P., Kobzarenko V.I.* Agrochemistry / edited by B.A. Yagodin. Moscow: Kolos, 2002, 584 p.

[4] Methodological recommendations for studying soil fertility indicators, humus and nutrients balance in long-term experiments. Moscow: Soil Institute, 1987, 80 p.

[5] *Eliseeva I.I., Kurysheva S.V., Gordeyenko N.M., Babaeva I.V., Kosteeva T.V., Mikhailov B.A.* Practicum on econometrics. Moscow: Finance and Statistics, 2005, 192 p.

thetic processes during the period of maximum growth from the emergence into the tube to ear formation. Average annual values of CFNU for rotation crops increased in the row oats → wheat → oats for grain haylage.

Under different meteorological situations, the CFNU in the crop rotation was naturally the lowest (32%) in conditions of extreme and severe drought, increased to 57% under moderate and weak drought and reached the maximum (124%) under sufficient moisture. The increase in the CFNU in favorable years is due to both the strengthening of the priming effect and the increase in the aftereffect of annual application of mineral fertilizers after dry years[6] [10]. Regardless of the moisture conditions, the general tendency of the distribution of CFNU among crops in different moisture availability periods was maintained.

Nitrogen nutrition of crops is conditioned by physiological features of plant development, growth rates during the vegetation period. In this regard, it is of interest to determine the most critical environmental parameters and the periods of their influence on the CFNU. As sign-factors for determining correlation relations (r), the indicators of HTC in different periods of vegetation and productive moisture content in the soil at sowing (PM, mm) in the 0-50 cm layer were selected (see Table 4).

Wheat, using the reserves of moisture and mineralized nitrogen accumulated in the fallow field by fallow, was in the most privileged position. Correlation analysis showed that the most critical period for nitrogen consumption by this crop is June (r = 0,61).

In the I-II ten-day periods of June, the tillering phase takes place, the secondary root system and, in general, the plant habitus are established, while in the III ten-day period, the vegetative mass growth, formation of wheat reproductive organs (number of spikelets in the ear and flowers in the spikelets) and water consumption increase sharply[7] [11]. To a somewhat lesser extent, the nitrogen consumption was affected by the conditions of July (ear fineness) and August (grain filling). In this regard, the most significant predictor, determining wheat CFNU, was HTC of the period June-August ($HTC_{VI-VIII}$). The content of productive moisture at sowing in the half-meter layer (PM, mm) influenced the CFNU to a medium degree. The model built on these factors had the form of multiple linear regression:

$$CFNU\ (wheat) = 108{,}9\ HTC_{VI–VIII} + \\ + 2{,}2\ PM – 125{,}4,\ R^2 = 0{,}61.$$

The development of the second crop (oats) was usually delayed by more than 2 weeks from wheat. This is due to both the shift in the sowing dates and the longer duration of the initial phases of development under typical drought conditions. In the conditions of the experiment, the interphase period between tube emergence and ear formation took place from the II to III ten-day period of July. This period is considered to be the most critical for oats in dry steppe, because it consumes up to 55-65% of the total water con-

Табл. 4. Влияние условий увлажнения на коэффициент использования азота удобрений культурами зернопарового севооборота (n = 39)

Table 4. Influence of humidification conditions on the nitrogen utilization rate of fertilizers by crops of grain-fallow crop rotation (n = 39)

Culture	PM, mm	HTC (by Selyaninov)							
		May	June	July	August	May – August	June – July	June – August	July – August
Wheat	0,44	0,01	0,61	0,42	0,44	0,66	0,66	0,73	0,60
Oats	0,31	–0,14	0,28	0,51	0,40	0,47	0,52	0,61	0,63
Oats for grain haylage	0,37	–0,05	0,23	0,50	0,52	0,54	0,49	0,65	0,69

[6]*Semenov V.M.* Modern problems and prospects of agrochemistry of nitrogen // Agrochemistry and ecology problems, 2008, N 1, pp. 55–63.

[7]*Osipov V.I.* Grain crops in Buryatia. Ulan-Ude: Buryat book publishing house, 1982, 88 p.

sumption [12]. With a significant increase in vegetative mass, nitrogen nutrition also increased (r = 0.51). August precipitation (r = 0.40) also influenced nitrogen consumption by oats, which is associated with a number of reasons: oat grain filling usually took place in the third ten-day period of August; August is a determining month for oat productivity in those cases when it is not possible to obtain a grain harvest. Thus, during the research period oat yield in 20% of the cases was formed in the form of green mass of the second wave of sprouting and afterspring. This was promoted by the drought in the first half of summer and favorable moisture in the second half of summer. The influence of productive moisture in the soil at sowing was weak (r = 0.29). The model of dependence of fertilizer nitrogen use by oats on hydrothermal conditions in July (HTC_{VII}) and August (HTC_{VIII}), as well as on the content of productive moisture in the 0-50 cm layer at sowing was as follows

$$CFNU\ (oats) = 37{,}4\ HTC_{VII} + 34{,}4\ HTC_{VIII} + + 0{,}55\ PM - 40{,}3,\ R^2 = 0{,}40.$$

The third crop of the crop rotation (oats for grain haylage) passed the stages of tillering, tube emergence, ear formation and flowering in July-August. Conditions of heat and moisture availability of this period ($HTC_{VII-VIII}$) had a determining influence on nitrogen mineral nutrition of the crop (r = 0.50-0.52). The influence of moisture reserves at sowing (PM, mm) was less significant (r = 0.37). The dependence of the coefficient of nitrogen consumption of fertilizers on the conditions of the atmospheric and soil moistening was most adequately expressed by the function

$$CFNU\ (oats\ for\ grain\ haylage) = = 110{,}6\ HTC_{VII-VIII} + 2{,}3\ PM - 94{,}4,\ R^2 = 0{,}51.$$

The constructed models showed that variations in the coefficients of nitrogen consumption from mineral fertilizers by 40-61% were caused by the changes in atmospheric and soil moisture. The most significant was the effect of hydrothermal conditions on nitrogen nutrition of oats and wheat. The change of HTC by 0.1 caused the change of the CFNU by 11.1 and 10.8%, respectively. For oats, this indicator was much lower (3.7%). The similar influence of soil moistening before sowing of crops was much lower. Each millimeter of productive moisture corresponded to a 2.2-2.3% change in the CFNU for wheat and oats for grain dressing and 0.6% for oats.

CONCLUSION

In the system of chestnut soil - climate - plant - fertilizer, the intensity of nitrogen nutrition of crops of grain and fallow crop rotation is closely related to meteorological conditions in the most critical periods of nutrition. Differences of crops on nitrogen consumption of fertilizers are conditioned by the features of growth and development of crops, technology of their cultivation.

Average annual coefficients of nitrogen use of mineral fertilizers in the conditions of the dry-steppe zone of Buryatia are 69% for wheat, 54 for oats and 90% for oats for grain haylage. A high variation of these coefficients was noted, which is due to the significant variability of moisture conditions during critical periods of growth and development of crops. A relatively large sample of field experiment data allows to reveal the influence of hydrothermal conditions and soil moisture on the coefficients of nitrogen consumption of fertilizers. Nitrogen nutrition of wheat is more influenced by HTC for June - August (r = 0.73), oats and oats for grain haylage - for July - August (r = 0.63-0.69). The influence of soil moisture during the sowing period is much weaker (r = 0.31-0.44).

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ХОЗЯЙСТВЕННО-БИОЛОГИЧЕСКАЯ ОЦЕНКА СОРТОВ И ГИБРИДОВ РАПСА ЯРОВОГО В УСЛОВИЯХ КИРОВСКОЙ ОБЛАСТИ

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В последние годы в Российской Федерации отмечена тенденция увеличения посевных площадей под рапсом, ареал которого постоянно расширяется. В одном из хозяйств Кировской области проведены исследования по хозяйственно-биологической оценке сортов и гибридов рапса ярового. В сравнительном аспекте в производственных условиях исследованы перспективные сорта и гибриды. Изучена полевая всхожесть семян, определена сохранность растений к моменту уборки. Дана оценка продолжительности межфазных и вегетационного периодов перспективных сортов и гибридов ярового рапса, проведено сравнение урожайности их семян. Представлен анализ элементов структуры продуктивности данных сортов и гибридов. В эксперименте высеяны сорта ярового рапса Герос, Кампино, Ярило, полученные в условиях хозяйства и имеющие соответствующие документы о качестве, и гибриды первого поколения Джой, Джаз, Гефест, Джокер, Джером, Джерри. При оценке продолжительности вегетационного периода установлено, что все образцы по биологическим ритмам укладываются в вегетационный период Кировской области и относятся к группе среднеспелых. Полевая всхожесть изучаемых сортов и гибридов в среднем за годы исследования колебалась от 48,8 до 75,8%. В среднем за 2 года наиболее высокая полевая всхожесть отмечена у гибридов первого поколения Джокер и Гефест (75,8 и 69,1% соответственно). Сохранность растений к уборке выше, чем у контроля (84%), в среднем за 2 года отмечена у сортов Кампино и Ярило. По урожайности семян достоверно превзошли контроль (15,5 ц/га) гибриды Джерри F_1 (16,7) и Джокер F_1 (16,2), сорт Ярило (16,3 ц/га). По числу стручков на одном растении в среднем за 2 года достоверно превзошли контроль (358) гибриды Джой (450), Джокер (482) и сорт Ярило (459).

Ключевые слова: рапс яровой, урожайность, структура продуктивности, изучение сортов и гибридов, хозяйственно-биологическая оценка

ECONOMIC AND BIOLOGICAL EVALUATION OF SPRING RAPE VARIETIES AND HYBRIDS UNDER CONDITIONS OF THE KIROV REGION

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In recent years there has been a tendency in the Russian Federation to increase the area under rapeseed, the area of which is constantly expanding. Research on economic and biological evaluation of spring rape varieties and hybrids was carried out in one of the farms of the Kirov region. Promising varieties and hybrids were studied under comparative aspect in production conditions. The seeds field

germination was studied, the safety of the plants by the time of harvesting was determined. The duration of interphase and vegetation periods of the promising varieties and hybrids of spring rape was estimated, the yield of their seeds was compared. Analysis of the productivity structure elements of these varieties and hybrids is presented. In the experiment spring rape varieties Heros, Campino, Yarilo, obtained under farm conditions and having appropriate quality documents, and the first generation hybrids Joy, Jazz, Hephaestus, Joker, Jerome, Jerry were sown. When assessing the duration of the vegetation period, it was found that all the samples by biological rhythms fit into the vegetation period of the Kirov region and belong to the medium-maturing group. Field germination of the studied varieties and hybrids on average during the years of the study ranged from 48.8 to 75.8%. On average for 2 years, the highest field germination was observed in the first generation hybrids Joker and Hephaestus (75.8 and 69.1%, respectively). Preservation of plants for harvesting higher than the control (84%), on average for 2 years was observed in the varieties Campino and Yarilo. In terms of seed yield, hybrids Jerry F_1 (16.7 c/ha) and Joker F_1 (16.2) and the variety Yarilo (16.3 c/ha) significantly outperformed the control (15.5 c/ha). Hybrids Joy (450), Joker (482) and the variety Yarilo (459) reliably surpassed the control (358) by the number of pods on one plant on average for 2 years.

Keywords: spring rape, yield, productivity structure, study of varieties and hybrids, economic and biological assessment

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INTRODUCTION

In recent years in the Russian Federation the need for production of high quality vegetable oils for food and technical purposes is growing. Among oilseed crops, sunflower, soybean, rapeseed and oilseed flax are widespread on the territory of our country. Spring rapeseed is both an oilseed and a fodder crop. Rapeseed oil is on the fifth place after soybean, cotton, peanut and sunflower in terms of world consumption [1-3]. It is known that new modern varieties and hybrids are characterized by almost complete absence of erucic acid in oil and glucosinolates in meal. In this regard, rapeseed oil became suitable for food purposes, cake and meal are used for animal feed [4-7].

The fatty acid composition of rapeseed oil is quite close to olive oil. Rapeseed oil contains less saturated fatty acids and more monounsaturated and, what is especially valuable, polyunsaturated fatty acids. Omega-3 and omega-6 in rapeseed oil are in the optimal ratio of 1 : 2 for the human body [8-11]. Rapeseed green mass is quite nutritious. It is willingly eaten by many species of domestic animals. The protein content of rape green mass is 3.9%, which is 0.4-0.8% more than that of alfalfa and clover [12, 13].

Rapeseed is a good forecrop for grain crops. Due to the deep penetration of roots, rape plants move soil nutrients from the lower to the upper layers. This culture is conventionally called "vegetable plow". The soil after rape is structured and loose enough, which allows not to carry out the autumn main tillage for spring crops [14, 15].

In the Russian Federation in 2005 the area under rape crops amounted to 244 thousand hectares, by 2021 it increased to 1.7 million hectares due to the introduction of spring rape into crop rotation. In the Kirov Oblast the area sown with rape in 2022 increased and amounted to 16.7 thousand hectares, which is 34% more compared to 2021. Seed harvest in 2021 exceeded last year's result by more than 1.7 times. Half

of the regional harvest was grown by the farmers of three districts: Kumensky, Nemsky and Kirovo-Chepetsky[1].

As of 2021, 45 varieties and hybrids of spring rape are included in the State Register of breeding achievements in the Volga-Vyatka region, including only six varieties and one hybrid in the Kirov region. The direction of research is relevant and corresponds to the tasks formulated in the Federal Scientific and Technical Program of Agricultural Development for 2017-2030, subprogram "Development of breeding and seed production of oilseeds".

The purpose of the research is to study in a comparative aspect the promising varieties and hybrids of spring rape under production conditions.

Objectives of the study are to:

– study field germination of seeds, determine the safety of plants by the time of harvesting;

– evaluate the duration of interphase and vegetation periods of promising varieties and hybrids of spring rape;

– compare their seed yields;

– analyze the elements of productivity structure of these varieties and hybrids.

MATERIAL AND METHODS

The experiments were laid in the Karinka department of the JSC Multi-unit agricultural enterprise stud farm "Krasnogorsky", which is located in the central agroclimatic zone of the Kirov region. Sod-podzolic heavy loamy soils, the average humus content of 2% prevail on the territory of the farm. There are 30 kg of nitrogen, 249 kg of potassium, 382 kg of phosphorus on average per 1 ha of arable horizon. Soil acidity varies from strongly acidic to neutral (pH 4.5-6.0).

The climate is moderately continental. The vegetation period lasts 157 days, of which 120 days have average daily air temperature above 10 °C. Multiyear averages of precipitation and average air temperature for the growing season are presented in Table 1.

Weather conditions in 2021 and 2022 differed significantly in terms of precipitation and average air temperature. Thus, during the summer months of 2021 precipitation fell at the level of average indicators for 5 years, in 2022 precipitation was significantly more than the average for 2016-2022. Monthly average May temperatures in 2021 were nearly half the 5-year average. The September temperature for the years of research was also below the mean annual values.

In the experiment spring rape varieties Heros, Campino, Yarilo, obtained in the farm conditions and having appropriate quality documents, and the first generation hybrids Joy, Jazz, Hephaestus, Joker, Jerome, Jerry, which were purchased from the LLC "KVS RUS" (Lipetsk) were sown.

Табл. 1. Метеорологические условия за 2021, 2022 гг.
Table 1. Meteorological conditions for 2021, 2022

Indicator	May	June	July	August	September
Precipitation amount, mm					
2021	58,0	63,0	92,0	38,0	79,0
2022	53,0	118,0	130,0	18,0	81,0
Average for 2016–2022	61,2	84,6	128,0	62,4	78,2
Average air temperature, °C					
2021	15,8	19,9	19,2	18,8	7,3
2022	8,5	16,1	20,0	20,0	9,0
Average for 2016–2022	14,8	19,1	22,9	20,3	11,5

[1]Ministry of Agriculture of the Russian Federation (official website). URL: https://reestr.gossortrf.ru/ (accessed on 24.09.22).

Perennial grasses were the forecrop in the experiment. Agrotechnics corresponded to the cultivation technology used in the farm. Since autumn, deep chiseling to a depth of 40 cm was carried out. Pre-sowing cultivation of the field included harrowing the soil with a heavy tooth harrow at an angle to cheeseleving at a depth of 3-5 cm and cultivation of the soil at an angle to harrowing at a depth of 6-8 cm. Urea was applied under cultivation at a dose of 150 kg/ha in physical weight. Sowing was done at a depth of 2-3 cm, the method of sowing was row by row with row spacing of 10 cm. Diammophoska in the amount of 150 kg/ha in physical weight was applied simultaneously with sowing. Hybrid seeds were purchased encrusted (treated), own seeds were pre-treated with Celest Top, SC at a dosage of 15 l/t with the help of self-propelled dresser PS-10. The seeding rate of rape seeds is presented in Table 2.

In the phase of 3-6th true leaf, the crops were treated with herbicide Megalit AS (the preparation consumption rate was 0.35 l/ha). Vostorg SC (0.15 l/ha) was used against cruciferous flea beetle. At the beginning of rapeseed budding, crops were treated with insecticide Danadim Expert, EC (1.0 l/ha). When the seeds in the pods of the middle tier became brown (10 days before harvesting), the plants were subjected to desiccation with contact desiccant Regulate Super, AS (2 liters/ha). Harvesting was carried out by direct combining.

Табл. 2. Норма высева семян рапса, шт./м^2
Table 2. Rapeseed seeding rate, pcs./m^2

Option	Recommended rate (at 100% sowing suitability)	Actual rate taking into account the sowing suitability	
		2021	2022
Heros (control)	85	100	101
Campino	97	115	114
Yarilo	94	110	112
Joy F_1	75	86	86
Jazz F_1	75	86	86
Hephaestus F_1	75	86	86
Joker F_1	75	86	86
Jerome F_1	75	86	86
Jerry F_1	75	86	86

The plot area was 150 m^2, the repeatability of the experiment was fivefold, the placement of the plots was systematic[2, 3]. Mathematical processing of the data was carried out using statistical and variance analysis[4, 5] using Microsoft Office 2013 software.

RESULTS AND DISCUSSION

The yield of agricultural plants is determined by many factors. One of them is field germination of seeds and preservation of plants to harvesting. Not all germinated seeds are preserved for harvesting. They may be killed by diseases, damaged by pests, cannot withstand competition with weeds and other factors, so before harvesting it is customary to count the number of surviving plants. Field germination of seeds and the number of surviving plants of the studied varieties and hybrids to harvest are presented in Table 3.

Field germination of the varieties and hybrids on average for the years of study ranged from 48.8 to 75.8%. Seeds of the hybrids Joker and Hephaestus showed high field germination - 75.8 and 69.1%, respectively. Preservation of the plants for harvesting in the control was at the level of 84%. Above this value, the preservation of plants was observed in the varieties Campino and Yarilo.

As a result of phenological observations, the duration of inter-phase periods of spring rape

[2]*Lukomets V.M., Tishkov N.M., Semerenko S.A.* Methodology of agrotechnical research in experiments with the main field crops. Krasnodar: LLC "Prosveschenie-Yug", 2022, 538 p.

[3]Methods of conducting field agronomic experiments with oilseed crops / V.M. Lukomets, N.M. Tishkov, V.F. Baranov et al. / edited by V.M. Lukomets. Krasnodar: V.S. Pustovoit All-Russian Research Institute of Oil crops, 2010, 327 p.

[4]*Vaulin A.V.* Determination of reliability of long-term averages of short-term field experiments in the processing of research results by the method of dispersion analysis // Agrochemistry, 1998, N 12, pp. 71–75.

[5]*Dospekhov B.A.* Methodology of field experiment (with the basics of statistical processing of research results). Moscow: Alliance, 2011, 351 p.

was analyzed, which differed for 2 years of research. The duration of vegetation period in rape varieties was shorter by 7 days compared to the hybrids. Consequently, the duration of interphase periods in hybrid plants is 2-3 days longer. The results of phenorhythmics showed that the studied linear varieties and hybrids by biological rhythms fit into the growing season of the Kirov region and belong to the group of medium-maturing (see Table 4).

The value of yield is one of the important factors in selecting the suitability of a variety for cultivation. In 2021, seed yield of Heros variety was 17.2 c/ha, in 2022 -13.9 c/ha. In 2021, the yield was reliably higher than that of the control in the first generation hybrids Jerry, Joker and the variety Yarilo. In 2022, no reliable exceeding of the control in seed yield was revealed. On average for 2 years, Jerry F_1 hybrids (16.7 kg/ha) and Joker F_1 hybrids (16.2) and Yarilo variety (16.3 kg/ha) significantly exceeded the control in seed yield (15.5 kg/ha) (see Table 5).

Agricultural producers are mainly interested in the yield of useful products. Grain yield is determined by the elements of productivity structure. In rapeseed, seed yield consists of the number of fruits on the plant, the number of seeds in the pod, the weight of 1000 seeds.

In 2021, the average number of pods per plant in the variety Geros (control) amounted to 348. A significant excess of pods per plant was observed in the variety Yarilo (471), hybrids Joker (570) and Joy (482). In 2022, a reliable excess of the control in the number of pods per plant was also observed in the variety Yarilo (447) and in the hybrid Jerome (437).

On average for 2 years, hybrids Joy (450), Joker (482) and variety Yarilo (459) significantly surpassed the control in the number of pods (see Table 6).

Spring rape varieties had a higher number of seeds in the pod compared to the hybrids. In 2021, the average number of seeds in the pod of the Heros variety (control) was 25. No significant exceedances were observed. In 2022, the

Табл. 3. Полевая всхожесть и сохранность растений рапса к уборке (в среднем за 2021, 2022 гг.), %

Table 3. Field germination and preservation of plants for harvesting (on average for 2021, 2022), %

Option	Field germination	Capacity for survival
Heros (control)	64,3	84,0
Campino	65,8	95,0
Yarilo	65,0	91,4
Joy F_1	65,6	70,8
Jazz F_1	66,3	70,5
Hephaestus F_1	69,1	70,7
Joker F_1	75,8	69,6
Jerome F_1	67,2	70,4
Jerry F_1	48,8	68,0

Табл. 4. Средняя продолжительность межфазных периодов за 2021, 2022 гг., сут

Table 4. Average duration of interphase periods for 2021, 2022, days

Option	Sowing - sprouting	Sprouts - leaf rosette	Leaf rosette - flowering	Flowering and fruiting - green pod formation	Green pod formation - full ripeness	Vegetation period
Heros (control)	11	16	13	30	50	119
Campino	11	16	13	30	50	119
Yarilo	11	16	13	30	50	119
Joy F_1	13	19	16	32	47	126
Jazz F_1	13	19	16	32	47	126
Hephaestus F_1	13	19	16	32	47	126
Joker F_1	13	19	16	32	47	126
Jerome F_1	13	19	16	33	47	126
Jerry F_1	13	19	16	32	47	126

Табл. 5. Урожайность семян сортов и гибридов ярового рапса, ц/га

Table 5. Seed yield of spring rape varieties and hybrids, c/ha

Option	2021	2022	Average
Heros (control)	17,2	13,9	15,5
Campino	16,3	11,4***	13,9***
Yarilo	18,3***	14,2	16,3**
Joy F_1	15,0***	13,8	14,4***
Jazz F_1	16,4	14,0	15,2
Hephaestus F_1	16,9	14,2	15,6
Joker F_1	18,5***	14,0	16,2**
Jerome F_1	16,8	14,5	15,7
Jerry F_1	19,2***	14,2	16,7***
LSD_{05}	0,66	0,87	0,55
LSD_{01}	0,80	1,05	0,67
LSD_{001}	1,07	1,40	0,89

*$p > 0,95$;
**$p > 0,99$;
*** $p > 0,999$.

average number of seeds in the pod of the control was 24. A significant excess of the number of seeds in the pod was observed in the Campino variety (25). On average for 2 years of research on the number of seeds in the pod of rape plants no exceedances of the control were revealed (see Table 7).

As a result of two-year studies of 1000 seed weight it was found that no significant fluctuations by years in this trait in rape varieties and hybrids were revealed. The highest value of 1000 seeds weight was observed in 2021 in the hybrid Jerry (4.8 g). This value reliably exceeds the indicators of the control. On average for 2 years of research exceeds of the control sample on the mass of 1000 seeds in most variants were not noted. Jerry hybrid significantly exceeded the control in this indicator (see Table 8).

CONCLUSIONS

1. The varieties and hybrids of spring rape, which were in the experiment, by biological rhythms fit into the growing season of the Kirov region and belong to the group of medium-maturing. Field germination of the studied varieties and hybrids on average for the years of study ranged from 48.8 to 75.8%. On average, the highest field germination was observed in the first-generation hybrids Joker and Gefest (75.8 and 69.1%, respectively).
2. Preservation of plants for harvesting is higher than that of the control (84%), on average for 2 years it was observed in the varieties Campino and Yarilo.
3. On average for 2 years on seed yields the hybrids Jerry F_1 (16.7 c/ha) and Joker F_1 (16.2),

Табл. 6. Число стручков на растении сортов и гибридов ярового рапса в 2021, 2022 гг.

Table 6. Number of pods per plant of spring rape varieties and hybrids in 2021, 2022

Option	2021		2022		Average for 2 years	
	Number of pods per plant	Deviation from the control, %	Number of pods per plant	Deviation from the control, %	Number of pods per plant	Deviation from the control, %
Heros (control)	348 ± 18,47	–	368 ± 22,33	–	358 ± 14,24	–
Campino	212 ± 7,85	–39	244 ± 14,49	–33,7	228 ± 9,01	–36,3
Yarilo	471 ± 30,92	35	447 ± 20,56	21,4	459 ± 18,91*	28,2
Joy F_1	482 ± 17,47	38,5	418 ± 21,60	13,6	450 ± 14,95*	25,7
Jazz F_1	337 ± 17,68	–3,2	382 ± 18,33	3,8	359 ± 15,39	0,3
Hephaestus F_1	349 ± 13,48	0,3	385 ± 22,75	4,6	367 ± 14,17	2,5
Joker F_1	570 ± 37,67	64	393 ± 20,74	6,8	482 ± 25,74*	34,6
Jerome F_1	363 ± 19,52	4,3	437 ± 28,50	18,7	400 ± 20,22	11,7
Jerry F_1	412 ± 16,22	18,4	387 ± 27,70	5,2	400 ± 16,93	11,7

$p > 0,95$.

Табл. 7. Число семян в стручке сортов и гибридов ярового рапса
Table 7. Number of seeds per pod of spring rape varieties and hybrids

Option	2021		2022		Average for 2 years	
	Number of seeds per plant	Deviation from the control, %	Number of seeds per plant	Deviation from the control, %	Number of seeds per plant	Deviation from the control, %
Heros (control)	25 ± 0,2	–	24 ± 0,2	–	25 ± 0,2	–
Campino	26 ± 0,4	4	25 ± 0,3	4,2	25 ± 0,3	0
Yarilo	26 ± 0,3	4	24 ± 0,3	0	25 ± 0,2	0
Joy F_1	18 ± 0,2	–28	17 ± 0,2	–29,2	18 ± 0,1	–28
Jazz F_1	23 ± 0,4	–8	22 ± 0,4	–8,4	23 ± 0,3	–8
Hephaestus F_1	22 ± 0,3	–12	21 ± 0,3	–12,5	22 ± 0,2	–12
Joker F_1	20 ± 0,4	–20	19 ± 0,4	–20,8	20 ± 0,3	–20
Jerome F_1	17 ± 0,5	–32	20 ± 0,4	–16,6	19 ± 0,3	–24
Jerry F_1	21 ± 0,3	–16	20 ± 0,2	–16,6	21 ± 0,2	–16

Табл. 8. Масса 1000 семян сортов и гибридов ярового рапса
Table 8. Weight of 1000 seeds of spring rape varieties and hybrids

Option	2021		2022		Average for 2 years	
	Weight of 1000 seeds, g	Deviation from the control, %	Weight of 1000 seeds, g	Deviation from the control, %	Weight of 1000 seeds, g	Deviation from the control, %
Heros (control)	4,1 ± 0,1	–	4,0 ± 0,1	–	4,0 ± 0,1	–
Campino	3,6 ± 0,2	–12,2	3,9 ± 0,1	–2,5	3,7 ± 0,1	–7,5
Yarilo	3,7 ± 0,3	–9,7	3,9 ± 0,2	–2,5	3,8 ± 0,3	–5
Joy F_1	4,0 ± 0,2	–2,4	3,8 ± 0,2	–5	3,9 ± 0,2	–2,5
Jazz F_1	3,8 ± 0,5	–7,3	3,8 ± 0,5	–5	3,8 ± 0,5	–5
Hephaestus F_1	4,4 ± 0,3	7,3	4,3 ± 0,4	7,5	4,4 ± 0,4	10
Joker F_1	4,4 ± 0,5	7,3	4,2 ± 0,6	5	4,3 ± 0,6	7,5
Jerome F_1	4,0 ± 0,2	–2,4	4,0 ± 0,1	0	4,0 ± 0,2	0
Jerry F_1	4,8 ± 0,3*	17	4,5 ± 0,3*	12,5	4,6 ± 0,3*	15

$p > 0,95$.

and the variety Yarilo (16.3 c/ha) significantly surpassed the control (15.5 c/ha).

4. On average for 2 years, the hybrids Joy (450), Joker (482) and the variety Yarilo (459) reliably outperformed the control by the number of pods (358).

5. By the weight of 1000 seeds the hybrid Jerry stood out, which on average for 2 years reliably surpassed the control by 15%. In production conditions the Yarilo variety, the hybrids Jerry and Joker are recommended to be considered for cultivation.

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СОВЕРШЕНСТВОВАНИЕ ТЕХНОЛОГИЧЕСКОГО ПРОЦЕССА ПОЛУЧЕНИЯ ИСХОДНЫХ РАСТЕНИЙ ГРУШИ

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Рассмотрен процесс получения исходных растений груши. Технологический процесс включает выделение растений определенного сорта по помологическим, физиологическим качествам и продуктивности, проверку на наличие вирусов, фитоплазм и вредителей путем тестирования методами ИФА, ПЦР и на индикаторах; в случае отсутствия здоровых растений необходимо освобождение от патогенов методами термо- и хемотерапии, культуры *in vitro*, магнитотерапии с проведением повторного тестирования. После предварительного тестирования в условиях защищенного грунта получают растения-кандидаты в исходные растения, которые тестируют с использованием комплекса методов диагностики. Свободные от основных вредоносных вирусов и фитоплазмы растения переводят в категорию «исходные растения», в случае зараженности всех тестируемых образцов их подвергают оздоровлению. Суховоздушная термотерапия в сочетании с прививкой апексов на не зараженные вирусами подвои обеспечивает возможность получения здоровых растений в течение одного вегетационного периода. Для хемотерапии наряду с эталонными препаратами (рибавирин) перспективно применение фенольных соединений (салициловая, галловая кислоты), которые позволяют увеличить эффективность оздоровления растений от вирусов в среднем на 28–30%, снизить стоимость процесса оздоровления и повысить безопасность труда. Использование магнитно-импульсной обработки повышает экологическую безопасность технологии при отсутствии фитотоксического эффекта. После диагностики комплексом методов при отсутствии вирусов растения груши получают категорию «исходное растение», и в дальнейшем их размножают окулировкой или прививкой.

Ключевые слова: груша, исходные растения, диагностика, оздоровление от вирусов, технологический процесс

IMPROVEMENT OF THE TECHNOLOGICAL PROCESS OF OBTAINING INITIAL PEAR PLANTS

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The process of obtaining initial pear plants is considered. The technological process includes the selection of plants of a certain variety according to pomological, physiological qualities and productivity; diagnostics for the presence of viruses, phytoplasmas and other harmful organisms by ELISA, PCR and indicators testing; in the absence of healthy plants – release from pathogens by methods of thermotherapy, chemotherapy, *in vitro* culture, magnetotherapy with re-testing. After preliminary testing in greenhouse conditions, candidate plants for initial plants are obtained, which are then subjected to testing using a set of diagnostic methods. Plants free from the main harmful viruses and phytoplasma are transferred to the category of "initial plants", and if all the tested plants are infected, they are subjected to recovery. Dry air thermotherapy in combination with grafting of apexes on virus-free rootstocks provides the possibility of obtaining healthy plants during one growing season. For chemotherapy, along with reference drugs (ribavirin), the use of phenolic compounds (salicylic, gallic acids) is promising, which can increase the efficiency of plant recovery from viruses by an average of 28–30%, reduce the cost of the recovery process and improve workplace safety. The use of magnetic pulse processing increases the environmental safety of the technology in the absence of the phytotoxic

effect. After diagnosis by a complex of methods in the absence of viruses, pear plants receive the category “initial plant” and are further propagated by budding or grafting.

Keywords: pear, initial plants, diagnostics, recovery from viruses, technological process

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The subprogram "Development of horticulture and nursery farming" for 2017-2030[1] determines the need to increase the production of planting material of horticultural crops (including pear) of higher quality categories. Currently, in accordance with Article 10 of Federal Law No. 454 "On Seed Production" dated 22.12.2021, the following categories of planting material of fruit, berry plants and grapes are established:

1) initial planting material (initial plants);
2) basic planting material (basic plants);
3) tested planting material;
4) untested planting material.

The technological process of obtaining initial pear plants includes the following main stages:

1. Plant approbation with assessment of conformity to variety, physiological qualities, productivity and absence of regulated pests.

2. Checking candidate plants to the initial plants for the presence of viruses, phytoplasmas by enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and with the help of indicators. In the absence of healthy plants - liberation of candidate plants from pathogens and conducting retesting.

3. Keeping of initial plants in protected soil during regular retesting[2].

In accordance with GOST R 59653-2021[3] the following pests should be absent in pear planting material: viruses of chlorotic leaf spotting of apple trees, apple tree mosaic, furrowing, pitting and softening (pliability) of the apple tree wood; plants affected by phytoplasma of pear exhaustion - quarantine object. Not regulated by GOST R 59653-2021, but also must be free from virus-like diseases: bark cracking and necrosis, early bark buckling and pear bark bubble cancer viroid. It is not allowed to infect plants with pests and diseases transmitted with the planting material: crown gall (*Agrobacterium tumefaciens* Conn.), silver leaf (*Ghondrostereum purpureum* Fr.), common stem canker (*Nectria galligena* Bres.), late blight or crown rot (*Phytophthora cactorum*), bacterial gummosis of fruit trees

[1]Subprogram "Development of Horticulture and Nursery Production" of the Federal Scientific and Technical Program of Agricultural Development for 2017-2030 (additionally included in the program on 29.05.2022 by the Resolution of the Government of the Russian Federation No. 872 dated 13.05.2022).

[2]Technology of obtaining virus-free planting material of fruit and berry crops: methodical instructions. Moscow, 2013, 92 p.

[3]GOST R 59653-2021. Planting material of fruit and berry crops. Technical conditions. Moscow: Russian Institute of Standardization, 2021, 50 p.

(*Erwinia amylovora* Winsl.) – quarantine object, Californian scale (*Quadraspidiotus perniciosus* Comst.) – quarantine object, pear psylla (*Psylla pyri* L.), smaller pear sucker (*Cacopsylla pyricola* Foerster), larger pear sucker (*Cacopsylla pyrisuga* Foerster) – vectors of pear depletion phytoplasma.

Selection of candidates for initial plants is made by specialists-virologists together with specialists-pomologists (approbators) according to the established methods[4, 5]. After evaluation of plants for compliance with varietal characteristics, determination of productivity and physiological qualities, five to ten pear trees with typical varietal characteristics and high productivity are selected, which in optimal time (June) are tested by ELISA method for the presence of the main harmful viruses, by PCR method - for infection with phytoplasma of pear blight.

Cuttings or buds are taken from virus-free trees and grafted onto healthy rootstocks to produce at least 10 plants of one variety (at least 2 from each tree). The rootstocks are pre-grown in containers of 2-3 liters with peat-sand substrate (peat-sand volume ratio 3: 1) enriched with mineral fertilizers (N_{total} content - at least 150 mg/l, P_2O_5 - at least 150, K_2O - at least 250 mg/l) and neutralized by acidity (pH 5.5-6.5). Seedling rootstocks of all pear species are considered free from the main harmful viruses. Production of own-root plants or grafting onto virus-free clonal pear rootstocks is also acceptable.

Candidate source plants are transferred to quarantine, which can be a pest-proof vegetation (unheated) greenhouse or an insulated winter greenhouse box.

Candidate source plants are then subjected to basic testing which is carried out using several methods and covers all viral and virus-like diseases regulated for tested planting material (see the Table).

If diagnostic ELISA kits for all sap-tolerant viruses are available, the test on herbaceous indicators can be omitted. Since most forms of the woody indicators have low winter hardiness, a field test can be used in the southern regions of the Russian Federation, while in harsher conditions the greenhouse test on woody indicators should be used.

Field and greenhouse tests on woody indicators are carried out by grafting on annual indicator plants from four to six eyes or bark shields of the sample under study in 3- or 5-fold repetition (in accordance with the Table), leaving two or three uninoculated indicator plants for control purposes.

Plants that are found to be free from viruses and other regulated pathogens are categorized as "source plant". If pathogens are detected, plants are rejected or subjected to health improvement (when all tested plants of a certain variety are infected).

Depending on the type of the pathogen, the schemes of pear plants recovery may be different and require improvement. To increase the effectiveness of recovery, it is necessary to use a complex of different methods by mechanism of action, which is especially relevant in relation to viruses characterized by high thermotolerance. Thermotherapy, meristem culture, chemotherapy or magnetotherapy *in vitro*[6, 7] [1, 2] should be used as effective technologies of recovery.

The main method of freeing pear plants from phytopathogenic viruses is dry-air thermotherapy[8], the advantages of which include the possibility of obtaining virus-free plants during one growing season (see footnote 6) [3, 4].

Thermotherapy of vegetative plants in container culture is carried out in special thermo-chambers of different design, allowing to create the following treatment parameters: temperature in the chamber - 38 ± 1 °C, humidity

[4]Program and methodology of varietal study of fruit, berry and nut crops. Orel, 1999, 606 p.

[5]Pomology / ed. by E.N. Sedov. Orel, 2007, vol. 2: Pear. Quince, 436 p.

[6]*Prikhodko Y.N., Magomedov U.Sh.* Viruses of seed and stone fruit crops. Voronezh, 2011, 468 p.

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[8]Certification scheme fruit plants. Explanatory guide to top fruit Cydonia, Malus, Prunus, Pyrus Mother trees Pre-basic, Basic 1, Basic 2 and Certified categories, March 2021, 10 p. URL: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/985184/certification-scheme-top-fruit-mother-trees.pdf.

Методы выявления и идентификации вирусов и вирусоподобных агентов на груше
Virus detection and identification methods and virus-like agents on pear

Viruses and virus-like diseases	ELISA test	PCR	Greenhouse test on herbaceous indicators	Greenhouse test on woody indicators	Field test on woody indicators
Apple leaf chlorotic leaf spot virus	+	+	*C. quinoa*, 5-fold repetition, test duration – 20 days	*Pyrus communis* Nouveau Poiteau, 3-fold repetition, 1 year	*Cydonia oblonga* C 7/1, *P. communis* Beurre Hardy, 3-fold repetition, 2 years
Apple stem pitting virus	+	+	*C. quinoa*, 5-fold repetition, 20 days	*M. sylvestris* Virginia crab, 3-fold repetition, 1 year	*M. sylvestris* Virginia crab, 3-fold repetition, 2 years
Apple brown spot virus	+	+	*C. quinoa*, 5-fold repetition, 20 days	*M. sylvestris* Virginia crab, Pyronia veitchii, *P. communis* Nouveau Poiteau, Beurre Bosc, 3-fold repetition, 1 year	*M. sylvestris* Virginia crab, Pyronia veitchii, *P. communis* A20, *P. communis* Jules d'Airolles, Beurre Bosc, Durondeau, 3-fold repetition, 2 years
Apple mosaic virus	+	+	*C. quinoa*, 5-fold repetition, 20 days	*M. domestica* Lord Lambourne, 5-fold repetition, *M. sylvestris* Virginia crab, 3-fold repetition, 1 year	*M. domestica* Lord Lambourne, 5-fold repetition, 2 years, *M. sylvestris* Virginia crab, 3-fold repetition, 2 years
Apple wood softening (pliability) viruses	–	+	–	*C. oblonga* C 7/1, *M. domestica* Lord Lambourne, 5-fold repetition, 1 year	*C. oblonga* C 7/1, *M. domestica* Lord Lambourne, 5-fold repetition, 3 years
Phytoplasma of pear depletion	–	+	–	*P. communis* Doyenne du Comicem, 3-fold repetition, 1 year	*P. communis* Doyenne du Comicem, 3-fold repetition, 2 years
Cracking of pear bark, early peeling of bark, necrosis of pear bark	–	–	–	*P. communis* Beurre Hardy, *P. communis* Williams, 3-fold repetition, 1 year	*P. communis* Beurre Hardy, *P. communis* Williams, 3-fold repetition, 2 years
Viroids of vesicular bark cancer of pear trees	–	+	–	*P. communis* A20, 3-fold repetition, 1 year	*P. communis* A20, 3-fold repetition, 2 years

- 40-70%, illumination - not less than 3000 lux/m², duration of illumination - 16 hours per day, duration of the treatment - from 4 to 12 weeks. The mechanism of thermotherapy action is associated with slow replication of viruses in the tissues of the apex part of plants, disruption of their transport functions, increased degradation of viral particles, inactivation of viral enzymes and inhibition of nucleic acid synthesis (see footnote 7) [5-7].

Plants are kept from 6 months to 1 year in containers with a volume of 3-4 liters before being placed in the thermo-chamber, then after winter dormancy (February - April) transplanted into 5-liter containers and after 2-3 weeks transferred to the thermo-chamber. For recovery three to five plants of each variety are used.

Duration of thermotherapy is determined by the type of the virus. For destruction of thermolabile pathogens (apple mosaic virus, pliability, phytoplasma of pear exhaustion), thermotherapy with duration of 4-5 weeks is used. In the presence of thermotolerant viruses (chlorotic leaf spot disease of apple-tree leaves, apple-tree wood furrowing and pitting), heat treatment is increased up to 8-12 weeks or repeated the next year (see footnote 6) [8, 9].

In the process of thermotherapy, regular root fertilization with soluble mineral fertilizers and treatment with preparations against pests and

diseases are carried out. Fertilization is carried out with fertilizer Rastvorin (Crystaline) mark B (nitrogen, phosphorus, potassium in the ratio of 18 : 6 : 18) every 10 days. Pest treatment is carried out on the basis of regular inspections (once a week) and taking into account the pest threshold with various preparations (e.g. Fitoverm, EC, Vertimek, EC, Bitoxybacillin, TAB, etc.) at the rates recommended by the manufacturers.

After thermotherapy, apices of 1-2 cm in size were grafted into seed stocks (virus-free clonal rootstocks) or meristems were isolated and planted on a nutrient medium. Good results were achieved on Murashige-Skoog, Lee and de Fossard, and Quoirin & Lepoivre media[9]. As growth regulators, 6 BAP (concentration 0.25-0.50 mg/l) or CPPU (0.10-0.20 mg/l) in combination with IAA (0.05 mg/l) are used at the inoculation stage. At the propagation stage, 6 BAP (1.0-2.0 mg/l) or thidiazuron (0.2-0.5 mg/l) or CPPU (0.2-0.4 mg/l) in combination with IAA (0.1 mg/l) were applied. For rooting of obtained microshoots, diluted 2-fold mineral base of Murashige-Skoog medium with addition of IBA in concentration of 0.5-1.0 mg/l is used.

The obtained plants are tested for the presence of viruses using a set of methods (ELISA, PCR, indicators).

The advantages of *in vitro* chemotherapy include the absence of the need for thermotherapy, the possibility of introducing large explants (more than 1 mm) into culture, shortening the recovery time, high yield of plants free of harmful viruses [1].

The most commonly used drug in chemotherapy is ribavirin (virazole, 1 in D ribofuranosyl 1, 3, 5 triazole 3 carboxamide) at a concentration of 40-80 mg/l. Higher concentrations of ribavirin lead to severe phytotoxicity and death of explants [10, 11]. Ribavirin is added to the nutrient medium at the proliferation stage against the background of standard concentrations of cytokinins. In our experiments, ribavirin at a concentration of 40 mg/l in combination with *in vitro* thermotherapy provided recovery from ACLSV and ApMV viruses in 71% of pear rootstock explants of the Zagorievsky variety [1].

Compared with ribavirin, salicylic and gallic acids are safer in toxicological terms, and at a concentration of 1.5 × 10-4 M, they provided complete recovery of the Lada pear explants from viruses [1]. Salicylic acid at a concentration of 42 mg/l contributed to the yield of 50-100% of pear rootstock explants free from latent viruses. These preparations allow to increase the efficiency of plant recovery from viruses on average by 28-30%, reduce the cost of recovery process and protect the operator's labor. Taking into account the optimal concentrations of phenolic compounds, their use is about 25 times cheaper than that of 2-thiouracil.

To increase the yield of healthy pear plants and in case of low rooting ability of microshoots of a certain variety, micrografting is practiced, in which the tops of the recovered plants of 1-3 mm in size are grafted *in vitro* onto virus-free rootstocks characterized by a high ability to rhizogenesis. Grafting is carried out at the stage of rooting of microshoots. After formation of the roots and fusion of the components, the plants are adapted to non-sterile conditions. A variation of this method is the grafting of *in vitro*-grafted tops onto virus-free rootstocks cultivated in greenhouse conditions [12].

Magnetic pulse treatment (MPT) can be used as an alternative to chemotherapy, the use of which excludes inhibition of growth processes and increases environmental safety. Magnetotherapy of pear explants is carried out with the AMIS 8 device (design of the Federal Horticultural Center for Breeding, Agrotechnology and Nursery) or its analog with sequentially increasing and decreasing frequency of pulses in the range of 1-100 Hz. For example, the use of MPT with a continuously increasing pulse frequency in the range of 50-100 Hz provided the highest yield of explants free of virus complex (75%) on pear rootstock, which was accompanied by an increase in the number and length of shoots by 23 and 36% compared to the control (without treatment), by 60 and 150% - relative to the standard (ribavirin) [13].

After using *in vitro* culture methods, healthy plants obtained should be considered as candi-

[9]*Byadovsky I.A., Upadyshev M.T.* Clonal micropropagation of fruit crops: methodical recommendations. Moscow, 2020, 69 p.

dates for initial plants and subjected to comprehensive testing (at least 3-6 months after adaptation). In the absence of viruses, pear plants are categorized as "initial plants" and then propagated by butt grafting or inoculation.

Application of chemo- and magnetotherapy *in vitro* in the proposed modifications allows to reduce the cost price of initial pear plants by 1.8 times in comparison with *ex situ* thermotherapy [1].

The yield of virus-free pear plants using different methods of recovery averages from 53 to 83% depending on the variety and type of virus. The proposed technological process of obtaining initial pear plants can be implemented in the laboratories of scientific and commercial institutions engaged in the production of planting material of the highest quality categories.

CONCLUSION

The technological process of obtaining initial pear plants includes the following main stages: isolation of plants of a certain variety by pomological traits with evaluation for compliance with the variety, determination of productivity, physiological qualities; check for the presence of viruses, phytoplasmas and other pests by ELISA, PCR and indicator testing; in case of absence of healthy plants it is necessary to release from pathogens by methods of thermo- and chemotherapy, *in vitro* culture, magnetic therapy with repeated testing and inspection of the plants for pathogens, as well as by the use of the following methods. After preliminary testing under protected soil conditions, candidate plants are obtained as source plants, which are then tested using a set of diagnostic methods. Plants free from the main harmful viruses and phytoplasma are transferred to the category of "initial plants". In case of infection of all the tested plants, they are subjected to recovery. Dry-air thermotherapy in combination with grafting of the apexes onto virus-free rootstocks provides the possibility of obtaining healthy plants during one vegetation period. The use of phenolic compounds (salicylic, gallic acids) is promising for chemotherapy. Magnetic-pulse treatment increases environmental safety of the technology in the absence of phytotoxic effect.

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ВЛИЯНИЕ МЕТЕОУСЛОВИЙ НА УРОЖАЙНОСТЬ И СОДЕРЖАНИЕ БЕЛКА В ЗЕРНЕ ОЗИМОЙ ПШЕНИЦЫ

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Представлены результаты трехлетних исследований (2020–2022), проводившихся в лесостепной зоне Самарской области в агроценозе озимой пшеницы. Целью работы было изучение влияния природно-климатических условий района исследований на формирование показателей урожайности, содержание белка в зерне озимой пшеницы. Материалом служили десять сортов из коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР) и пять сортов селекции Поволжского научно-исследовательского института селекции и семеноводства им. П.Н. Константинова. Были определены урожайность, содержание белка в зерне и выход белка с гектара. Выявлены сорта, отличающиеся высокой урожайностью и большим количеством белка в зерне, показывающие максимальный выход белка в различные по метеоусловиям годы. Во время исследования самым благоприятным для формирования высокой урожайности оказался 2022 г. (в указанный год было собрано от 6,0 до 8,5 т/га), когда наблюдалось выпадение значительного объема осадков в мае – I декаде июня. Показатель содержания белка в зерне был максимален в 2021 г. (14–19%), когда в период налива зерна наблюдались высокие температуры воздуха на фоне отсутствия осадков. Для каждого сорта установлена корреляционная зависимость суммы активных температур и количества осадков в период весенне-летней вегетации с исследуемыми показателями. Отмечена отрицательная корреляция между суммой активных температур и урожайностью (от –0,553 до –0,981) и положительная зависимость с показателем «содержание белка» (от 0,605 до 0,984) в зависимости от сорта. Количество осадков находилось в положительной корреляционной зависимости с показателем урожайности, зависимость с содержанием белка в зерне была неоднозначна и зависела от сорта. На изучаемые показатели оказывали влияние не только природно-климатические условия, но и генотипические особенности изучаемых сортов, что и объясняет разноплановую корреляционную зависимость.

Ключевые слова: белок, урожайность, озимая пшеница, метеоусловия, вегетация

INFLUENCE OF WEATHER CONDITIONS ON YIELD AND PROTEIN CONTENT IN WINTER WHEAT GRAIN

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The results of three-year studies (2020–2022) conducted in the forest-steppe zone of the Samara region in the agrocenosis of winter wheat are presented. The purpose of the work was to study the influence of natural and climatic conditions of the research area on the formation of yield indicators, protein content in winter wheat grain. The material was 10 varieties from the Vavilov All-Russian Institute of Plant Genetic Resources (VIR) collection and 5 varieties of selection of the Povolzhsky Scientific Research Institute of Selection and Seed Growing named after P.N. Konstantinov. Yield, grain protein content and protein yield per hectare were determined. Varieties characterized by high yield and high amount of protein in grain, showing maximum protein yield in different weather conditions of the years were identified. Over the years of research, the year 2022 was the most favorable for the formation of high yields (between 6 and 8.5 t/ha were harvested in the indicated year), where a large amount of

precipitation was observed in May – the first ten-day period of June. The protein content in grain was the highest in 2021 (14–19%), when high temperatures were observed during the grain filling period in the absence of precipitation. For each variety, the correlation dependence of the sum of active temperatures and the amount of precipitation during the spring-summer vegetation period with the studied indicators was established. There was a negative correlation between the sum of active temperatures and yield (from –0.553 to –0.981) and a positive correlation with the protein content indicator (from 0.605 to 0.984) depending on the variety. The amount of precipitation was in a positive correlation with the yield index, the dependence on the protein content in the grain was ambiguous and depended on the variety. The studied indicators were influenced not only by natural and climatic conditions, but also by the genotypic features of the studied varieties, which explains the diverse correlation dependence.

Keywords: protein, yield, winter wheat, weather conditions, vegetation

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The authors declare no conflict of interest.

INTRODUCTION

Winter wheat is the main food and fodder crop in the Samara region. The area of winter wheat sowing in the region averages 500 thousand hectares annually and is gradually increasing. Winter wheat is an emergency crop that gives high yields even in drought years, so the interest in it is consistently high [1]. This property of wheat is especially important, because droughts during the grain filling period are not rare in the Samara region [2].

In the conditions of changing climate, varieties adapted to local climatic conditions are the determining innovative component of intensive wheat grain production technology [3]. As noted by I. Elahi et al. [4], climate change has the strongest impact in the areas of wheat cultivation on dry farming land.

Protein and gluten are considered the main indicators of wheat grain quality [5]. The level of grain production development in the country is evidenced by the quality of produced grain [6]. It is known that protein content is negatively correlated with yield. It is important to obtain not only high yields but also high-quality products in grain production [7]. Significant factors that can influence grain yield and quality include habitat, cultivation technique, and variety [8]. The conditions for obtaining high-quality grain depend on environmental factors (air temperature and precipitation). An important factor ensuring high yield of winter wheat is the temperature during the flowering - grain filling period (late May - June) [9]. A decrease in the amount of available moisture leads, on the one hand, to a decrease in yield, on the other hand, to an increase in grain quality (increased protein content) [10].

The purpose of the study was to investigate the effect of meteorological conditions on yield and protein content in wheat grain of the varieties from the collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) and selection of the Povolzhsky Scientific Research Institute of Selection and Seed Growing named after P.N. Konstantinov.

The research objectives are:

1) to determine the influence of the sum of active temperatures and precipitation on the yield and protein content in winter wheat grain;

2) to identify adaptive varieties showing high yields and high protein content in grain in different weather conditions in different years;

3) to conduct correlation analysis of the influence of the sum of active temperatures and the sum of precipitation during the spring-summer vegetation period on different varieties of winter wheat.

MATERIAL AND METHODS

The study was conducted in 2020-2022 at the breeding and seed production fields of the Povolzhsky Scientific Research Institute of Selection and Seed Growing named after P.N. Konstantinov (Laboratory of Breeding and Seed Production of Winter Wheat) under the conditions of ecological variety testing. Three varieties of local selection (Povolzhskaya niva, Povolzhskaya 30, Povolzhskoe zoloto), two promising lines (Erythrospermum 4287, Lutescens 3868), as well as ten varieties from the VIR collection were used: Skipetr (standard), Biryuza, Basis, Bonus, Victoria 95, Kapitan, Kipchak, Cepheus, Yunona, Shestopalivka. Analysis of protein content in grain was carried out in the laboratory of innovative technologies on infrared analyzer "Infralum FT" according to the method M 04 37 2009 (2014) according to GOST R 8.563-2009.

The area of experimental plots is 15 m^2, the location of the experiment variants is randomized, the repetition is fourfold. Forecrop - autumn fallow. Seeding rate was 4.5 million germinated seeds/ha. Agrotechnics was the same as for winter wheat. Harvesting was carried out by combine harvester Sampo 2010.

Meteorological conditions of the spring and summer growing season in the years of research had significant differences, which affected the realization of varietal potential in productivity and protein content (see Table 1).

In 2020-2022 on the territory of the Samara region the resumption of winter wheat vegetation fell on the third ten-day period of April. Before sowing in 2019, favorable weather conditions prevailed, when 99.7 mm of precipitation fell during the summer months. As a result, full-grown sprouts were obtained. Low September temperatures (5.8-14.3 ºC) and precipitation contributed to good plant development and hardening. During winter, 54.9 mm of precipitation fell on the background of high average monthly temperatures in January and February (-2.8 and -3.8 ºC, respectively). Overwintering conditions were mild and had no significant effect on plants. March was cool (2.2 ºC), snow melt was gradual. In April the first and second ten-day periods were warm, with temperatures reaching 5.5 ºC on some days.

Табл. 1. Гидротермические показатели за 2020–2022 гг.
Table 1. Hydrothermal indicators for 2020–2022

Month	Ten-day period	Sum of active temperatures				Amount of precipitation, mm				HTC			
		Long-run annual average	2020	2021	2022	Long-run annual average	2020	2021	2022	Long-run annual average	2020	2021	2022
April	III	63,0	65,1	12,2	104,2	11,1	13,9	18,2	12,6	1,76	–	–	1,21
May	I	135,0	170,1	162,9	60,1	8,7	2,8	2,8	22,5	0,64	0,16	0,17	–
	II	189,0	115,3	232,7	72,2	11,6	12,0	0,1	41,4	0,61	1,04	0,01	5,73
	III	176,0	190,8	230,8	103,0	13,5	2,8	17,9	19,6	0,77	0,15	0,78	1,90
For the month		500,0	476,2	626,4	235,3	33,8	17,6	20,8	83,5	0,68	0,37	0,33	3,55
June	I	178,0	183,9	190,4	179,3	15,5	45,2	34,5	42,6	0,87	2,45	1,81	2,37
	II	199,0	181,1	218,3	193,5	16,6	0,3	34,1	7,4	0,83	0,02	1,56	0,38
	III	207,0	168,7	278,2	196,6	22,5	2,8	3,7	3,9	1,09	0,17	0,13	0,20
For the month		584,0	533,7	686,9	569,4	54,6	48,3	72,3	53,9	0,93	0,91	1,05	0,95
July	I	210,0	248,1	213,8	205,8	22,2	0,9	6,4	3,9	1,06	0,04	0,30	0,19
	II	216,0	256,0	247,8	239,7	15,9	4,8	6,3	5,4	0,74	0,19	0,25	0,23
For the month		426,0	504,1	461,6	445,5	38,1	5,7	12,7	9,3	0,89	0,11	0,28	0,21
Total for the spring-summer vegetation period		1573,0	1579,0	1786,5	1354,4	137,6	85,5	124,0	159,3	0,87	0,54	0,69	1,18

In April - May 2020 insignificant oppression of plants, decrease of precipitation rate by 30.0% as compared to a multiyear average were observed. The sum of active temperatures was at the level of mean annual values. In the first ten-day period of June, a large amount of precipitation (45.2 mm) fell, which coincided with the period of mass grain filling. Precipitation deficit (15.0% of the mean annual value) was observed in the second and third ten-day periods of June and the first and second ten-day periods of July against the background of high temperatures. The spring-summer growing season of 2020 was dry, the hydrothermal coefficient (HTC) in this period amounted to 0.54 (according to G.T. Selyaninov).

In 2020 before sowing winter wheat there were favorable weather conditions. In June-August, 112.9 mm of precipitation fell, and full-grown sprouts were obtained. Precipitation in September and October on the background of low temperatures promoted good development and hardening of wheat. In November a small amount of snow fell. In December, January and February, 147.2 mm of precipitation fell, the snow cover height was 55 cm, which allowed winter wheat to be reliably protected from low temperatures. The average temperature during the winter months ranged from -10.4 to -13.9 ºC. March was cool and snow melt was gradual.

The period of spring vegetation 2021 was characterized as acutely dry. The sum of active temperatures in May exceeded the mean annual value by 25.0% with low precipitation (38.0% below the norm). Winter wheat plants were depressed and lagged in growth. In the third ten-day period of May, the first and the second ten-day periods of June a significant amount of precipitation fell, which coincided with the period of active grain filling. The sum of active temperatures in the summer months of vegetation exceeded the average annual value by 13.7% with a deficit of precipitation in July. Conditions in 2021 were difficult for growth and development of winter wheat. The HTC for spring and summer months was 0.69, i.e. this period can be characterized as a dry period.

In 2021 unfavorable weather conditions prevailed before sowing winter wheat. Insignificant precipitation in July (17.7 mm) and almost complete absence of precipitation in August (0.6 mm) against the background of high temperatures dried up the soil, sprouts were thinned. The situation was corrected by precipitation in the first ten-day period of September (31.0 mm). The leveling of crops was observed. October and November turned out to be warm, which allowed plants to bush additionally. In December, snow cover height reached 15 cm. January and February temperatures reached -23 ºC, snow cover height was 58 cm. March was cool and snow melt was gradual.

The spring-summer vegetation period of winter wheat 2022 differed from the previous years of the study by a large amount of precipitation against the background of a decrease in the sum of active temperatures in April-June. In May and the first ten-day period of June, a significant amount of precipitation was recorded - above the average annual values by 147.0 and 175.0%, respectively. There were optimal conditions for growth and development of winter wheat, which enabled the varieties under study to realize their genetic potential in terms of yield to the maximum. In the first and second ten-day periods of July there was an increase in temperatures against the background of low precipitation. The value of the HTC for 2022 amounted to 1.18 (slightly dry conditions).

In the autumn period of 2020-2022 conditions for growth and development of plants were different, full-grown seedlings were obtained, overwintering of which took place in mild winter conditions that did not have a significant impact on plants.

The years of research were characterized by contrasting growing conditions in terms of the sum of active temperatures and the sum of precipitation during the spring-summer vegetation period of winter wheat. Acutely dry periods were observed in 2020 and 2021, slightly dry periods - in 2022 the HTC ranged from 0.54 (2020) to 1.18 (2022). The main precipitation was recorded in early June, which coincided with the period of grain filling and allowed to obtain high yields.

The relationship of hydrothermal parameters with yield, protein content and protein yield per

unit area was studied. Mathematical processing was carried out according to B.A. Dospekhov* methods of correlation and dispersion analysis using Microsoft Exel computer program.

RESULTS AND DISCUSSION

Winter wheat is a high-yielding crop capable of forming high quality grain even in dry conditions. In our studies, the yield and protein content varied significantly by years (see Table 2).

In 2020, the yield of the studied varieties ranged from 3.2 (Biryuza) to 5.7 t/ha (Bonus), protein content - from 9.1 (Skipetr) to 13.8% (Shestopalivka), protein yield - from 0.36 (Skipetr) to 0.69 t/ha (Bonus).

In 2021, the minimum yield was observed in the varieties Basis and Kipchak (4.3 t/ha), the maximum - in the line Erythrospermum 4287 (6.2 t/ha). Protein content in wheat grain varied: the minimum was 14.2% (Skipetr) and the maximum was 18.9% (Yunona). The minimum protein yield was recorded in the variety Skipetr (0.69 t/ha), the maximum - in the line Erythrospermum 4287 (1.0 t/ha).

In 2022 the highest yield of winter wheat was observed during the study period. The minimum yield was observed in the variety Povolzhskaya Niva, referred to the steppe ecotype (6.1 t/ha), the maximum - in the variety Kipchak and the line Erythrospermum 4287 (8.5 t/ha). At the same time, low protein content in grain was recorded - from 11.0 (Cepheus) to 14.8% (Povolzhskaya Niva). Erythrospermum 4287 (1.1 t/ha) stood out in terms of the protein yield, the minimum was observed in the varieties Yunona, Cepheus and Volga 30 (0.81 t/ha).

On average, the Bonus variety and Erythrospermum line 4287 (6.3 t/ha) stood out in terms of the yield over the three years of research, the minimum indicator was in the variety Povolzhskaya Niva (4.9 t/ha). The maximum protein content was characterized by the variety Victoria 95 (15.0%), the minimum - the variety Skipetr (11.9%). Erythrospermum 4287 (0.86 t/ha) stood out in terms of the protein yield, the minimum yield was recorded in the variety Skipetr (0.64 t/ha). It is worth noting that the varieties characterized by high yield had low protein content, and vice versa, the lower the yield, the higher was the protein content [11].

A close correlation was observed between the weather conditions and yield, protein content and yield (see Table 3). Negative correlation was observed between the sum of the active temperatures and yield; it ranged from average (-0.553) to high (-0.981). The dependence of protein content in grain on the sum of the active temperatures was positive and ranged from weak (0.065) to strong (0.984).

Under conditions optimal for growth and development of winter wheat, nutrients are converted to starch in the grain, which leads to a decrease in the amount of protein [12]. At high temperatures, there is a decrease in starch biosynthesis in the grain [13, 14]. Increased respiration and increased carbohydrate consumption lead to an increase in the amount of the protein in the grain [12].

The dependence of the indicator "protein yield" on the sum of active temperatures was versatile, depending on the variety and ranged from weakly positive (0.151 in the variety Cepheus) to strongly negative (-0.725 in the variety Bonus).

The yield of the studied varieties was in close positive correlation with the amount of precipitation. A similar result was obtained in the studies of A. Wegrzyn et al. [15] in Poland on winter wheat crops. The correlation dependence ranged from 0.656 (medium) to 0.997 (strong). The amount of precipitation had a versatile effect on the protein content: both weakly negative (-0.338 in the variety Shestopalivka) and strongly positive correlation dependence (0.833 in the variety Povolzhskaya niva) were observed in the studied varieties. High positive correlation dependence was observed for the indicators "protein yield" and "rainfall amount" - 0.781 and 0.999, respectively.

There is a clear correlation dependence for most of the studied indicators and meteorological conditions. It should be noted that the correlation coefficient is influenced by varietal features of winter wheat. Air temperature and pre-

* *Dospekhov B.A.* Methodology of field experiment. Moscow: Agropromizdat, 1985, 351 p.

Табл. 2. Генотипические особенности формирования урожайности и качества зерна озимой пшеницы
Table 2. Genotypic features of the formation of yield and quality of winter wheat grain

Variety, patent holder	2020			2021			2022			Average for years		
	Yield, t/ha	Protein content, %	Protein output, t/ha	Yield, t/ha	Protein content, %	Protein output, t/ha	Yield, t/ha	Protein content, %	Protein output, t/ha	Yield, t/ha	Protein content, %	Protein output, t/ha
Skipetr (standard), A.G. Poletaev, G.M. Poletaev	3,97	9,1	0,36	4,89	14,2	0,69	7,23	11,4	0,82	5,36	11,9	0,64
Biryuza, Samara SRIA	3,19	11,6	0,37	4,67	16,8	0,78	7,31	12,6	0,92	5,06	13,7	0,69
Basis, Samara SRIA	4,44	13,1	0,58	4,22	18,1	0,76	8,01	11,9	0,95	5,55	14,1	0,78
Yunona, National Grain Center P.P. Lukyanenko	5,33	12,1	0,64	4,45	18,9	0,84	7,31	11,1	0,81	5,70	14,0	0,80
Kapitan, “Donskoy” ASC	4,44	12,9	0,57	4,67	17,1	0,80	8,11	11,7	0,95	5,74	13,9	0,80
Bonus, “Donskoy” ASC	5,71	12,1	0,69	4,89	16,0	0,78	8,33	12,4	1,03	6,31	13,5	0,85
Victoria 95, Research Institute of Agriculture of South-East	4,89	13,7	0,67	5,11	17,7	0,90	6,70	13,5	0,90	5,57	15,0	0,84
Cepheus, EcoNiva – Seeds	4,67	12,6	0,59	4,67	18,4	0,86	7,39	11,0	0,81	5,58	14,0	0,78
Shestopalivka, Ukraine	4,00	13,8	0,55	4,67	16,4	0,77	8,31	12,3	1,02	5,66	14,2	0,80
Kipchak, “Donskoy” ASC	4,89	12,0	0,59	4,22	17,0	0,72	8,52	11,2	0,96	5,88	13,4	0,79
Povolzhskaya niva, Povolzhsky SRISSG	4,12	12,0	0,49	4,42	15,8	0,70	6,11	14,8	0,91	4,88	14,2	0,69
Povolzhskaya 30, Povolzhsky SRISSG	4,51	11,4	0,51	4,30	16,6	0,71	6,29	12,9	0,81	5,03	13,6	0,68
Povolzhskoe zoloto, Povolzhsky SRISSG	4,42	10,5	0,46	4,60	15,3	0,70	8,11	12,2	0,99	5,71	12,7	0,73
Erythrospermum 4287, Povolzhsky SRISSG	4,23	11,8	0,50	6,22	16,2	1,01	8,51	13,2	1,12	6,32	13,7	0,86
Lutescens 3868, Povolzhsky SRISSG	4,04	10,2	0,41	4,31	16,0	0,69	7,19	12,0	0,86	5,18	12,7	0,66
$LSD_{0,05}$	0,21	0,6	0,11	0,23	0,7	0,1	0,31	0,16	0,37	0,24	0,43	0,14

Табл. 3. Корреляционная зависимость метеоусловий и показателей «урожайность», «содержание белка», «выход белка» (2020–2022 гг.)

Table 3. Correlation of weather conditions with yield, protein content and protein yield (2020–2022)

Variety	Sum of active temperatures			Amount of precipitation		
	Yield	Protein content	Protein output	Yield	Protein content	Protein output
Skipetr (standard)	–0,710	0,605	–0,185	0,964	0,389	0,944
Biryuza	–0,649	0,746	–0,267	0,982	0,205	0,968
Basis	–0,900	0,933	–0,533	0,825	–0,154	0,999
Yunona	–0,981	0,909	0,116	0,656	–0,092	0,803
Kapitan	–0,849	0,944	–0,412	0,881	–0,187	0,995
Bonus	–0,963	0,816	–0,725	0,712	0,094	0,958
Victoria 95	–0,818	0,876	–0,022	0906	–0,017	0,878
Cepheus	–0,877	0,943	0,151	0,853	–0,181	0,781
Shestopalivka	–0,798	0,984	–0,551	0,919	–0,338	0,998
Kipchak	–0,937	0,913	–0,657	0,770	–0,102	0,981
Povolzhskaya niva	–0,802	0,065	–0,601	0,917	0,833	0,992
Povolzhskaya 30	–0,917	0,675	–0,349	0,802	0,304	0,986
Povolzhskoe zoloto	–0,823	0,532	–0,634	0,877	0,468	0,986
Erythrospermum 4287	–0,553	0,532	–0,634	0,997	0,335	0,950
Lutescens 3868	–0,864	0,656	–0,464	0,867	0,327	0,999

cipitation have a strong multidirectional correlation with yield and protein amount. At the same time, this dependence can significantly differ by varieties [16].

Thus, meteorological conditions of the years of the study had an impact on the yield, the amount of protein in the grain and protein yield per hectare. It should also be noted that genetic features of the variety play a significant role here. The studied varieties reacted differently to the indicators of the sum of active temperatures and the precipitation amount.

CONCLUSION

The sum of active temperatures and the sum of precipitation during the study period had a significant effect on the yield and protein content of winter wheat grain. 2022 was the most favorable in terms of the yield and 2021 in terms of the protein content.

The Bonus variety (Donskoy ASC) and Erythrospermum 4287 line (Povolzhsky Scientific Research Institute of Selection and Seed Growing named after P.N. Konstantinov), which formed 6.3 tons/ha each, stood out during the study in terms of yield. The highest protein content was observed in the variety Victoria 95 (Research Institute of Agriculture of South-East) – 15,0%.

Yield and protein content of winter wheat grain were in close correlation with the sum of active temperatures and precipitation. The value of the correlation coefficient varied among the varieties.

The studied indicators are influenced not only by meteorological conditions of the year, but also by genotypic features of the studied varieties.

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К ВОПРОСУ О ВЫЖИВАЕМОСТИ ГРИБА *COLLETOTRICHUM LUPINI* VAR. *LUPINI*, ЯВЛЯЮЩЕГОСЯ ВОЗБУДИТЕЛЕМ АНТРАКНОЗА ЛЮПИНА, В УСЛОВИЯХ БРЯНСКОЙ ОБЛАСТИ

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Представлены результаты изучения жизнеспособности гриба *Colletotrichum lupini* var. *lupini*, являющегося возбудителем опасного заболевания люпина – антракноза. Цель исследования – определить жизнеспособность гриба при разных сроках и условиях хранения семян и растительных остатков люпина в климатических условиях Брянской области. Работу проводили в 2018–2021 гг. Объект исследования – семена, проростки и растительные остатки люпина белого сорта Мичуринский и люпина узколистного сорта Витязь, хранившиеся в различных условиях в течение четырех лет. Зараженность семян антракнозом перед закладкой на хранение и после определяли методом бумажных рулонов. Идентификацию гриба осуществляли по типу конидий с использованием светового микроскопа. Жизнеспособность гриба на растительных остатках (створки бобов, стебли) определяли методом выделения в чистые культуры на питательную среду. Установлено, что основным источником инфекции антракноза служат зараженные семена. В условиях зернохранилища с естественным режимом температуры и влажности за первый год хранения инфицированность семян люпина белого антракнозом снизилась с 8,1 до 4,0%, узколистного – с 4,8 до 1,7%. Через три года хранения количество семян с жизнеспособной инфекцией гриба составило 1,1 и 0,4% соответственно. Следовательно, семена люпина прошлых лет представляют меньшую опасность в качестве источника инфекции. На зараженных растительных остатках в комнатных условиях и под крышей зернохранилища жизнеспособность гриба начиная с первого года хранения уменьшилась. К весне 2021 г. она составила 12,0 и 19,0% соответственно. Жизнеспособность гриба на незапаханных растительных остатках снижалась быстрее. Весной 2020 г. она составила 2,0%, к осени – полностью нивелировалась. В почве разложение остатков растений происходит быстрее, чем на поверхности, и после перезимовки находящийся на них гриб полностью теряет жизнеспособность и не может являться источником инфекции.

Ключевые слова: люпин, антракноз, источник инфекции, семена, растительные остатки, условия хранения

TO THE QUESTION OF SURVIVAL OF THE FUNGI *COLLETOTRICHUM LUPINI* VAR. *LUPINI* – THE LUPIN ANTHRACNOSE PATHOGEN – IN THE CONDITIONS OF THE BRYANSK REGION

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The results of studying the viability of the fungus *Colletotrichum lupini* var. *lupini*, which is the causative agent of a dangerous disease of lupine – anthracnose, are presented. The purpose of the study was to determine the viability of the fungus under different terms and conditions of storage of lupin seeds and plant residues in climatic conditions of the Bryansk region. The work was done in 2018–2021.

The object of research were the seeds, seedlings and plant residues of the Michurinsky variety white lupine and the Vityaz variety narrow-leafed lupine stored under different conditions for four years. Seed anthracnose infestation before and after storage was determined by paper roll method. The fungus was identified by conidia type using a light microscope. The viability of the fungus on plant residues (bean leaflets, stems) was determined by isolation into pure cultures on nutrient medium. It was found that infected seeds were the main source of anthracnose infection. In the conditions of a granary with a natural regime of temperature and humidity for the first year of storage the infection of white lupine seeds with anthracnose decreased from 8.1 to 4.0%, and narrow-leafed lupine – from 4.8 to 1.7%. After three years of storage, the number of the seeds with viable infection of the fungus was 1.1 and 0.4%, respectively. Consequently, lupin seeds from previous years pose less risk as a source of infection. The viability of the fungus decreased on the infected crop residues under room conditions and under the roof of the granary from the first year of storage. By spring 2021, it was 12.0 and 19.0%, respectively. The viability of the fungus decreased faster on unplowed crop residues. It was 2.0% in the spring of 2020 and leveled off completely by the fall. Decomposition of plant residues in the soil is faster than on the surface, and after overwintering, the fungus on them completely loses its viability and cannot be the source of infection.

Keywords: lupin, anthracnose, infection source, seeds, plant residues, storage conditions

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INTRODUCTION

Lupine is used in agriculture not only as a concentrated protein fodder for animals, but also as a crop with the ability to increase soil fertility and enrich it with nitrogen. Currently, two annual species of lupine are cultivated in the Russian Federation: white (*Lupinus albus* L.) and narrow-leafed (*Lupinus angustifolius* L.). Sown areas of these species are small. For example, in 2021 they amounted to 150 thousand hectares, of which 80.0% is occupied by white lupine. One of the main factors preventing the expansion of sown areas and limiting the productivity of lupine is a fungal disease - anthracnose. Seed yield losses from this disease can reach 30.0-90.0% [1]. German researchers found that in Russia this disease on lupine is caused by the imperfect fungus *Colletotrichum lupini* var. *lupini*[1].

Increase in temperature and moisture availability in the summer period entails changes in the microclimate in crops. In this regard, there are prerequisites for increased development of many diseases in different crops. As the temperature rises, the number of pathogens, initially characteristic of southern regions, increases, which leads to the expansion of the area of heat-loving fungal species [2–4].

There was an increase in the average annual air temperature by 2.1 °C on the territory of the

[1]*Nirenberg H.I., Feiler U.* Description of *Colletotrichum lupini* comb. nov. in modern terms // Hagedorn GM Mycologia, 2002, vol. 94, N 2, pp. 307–320.

Bryansk region from 1976 to 2016. For example, in 1987 this indicator was recorded at 3.4 °C, in 2016 - 7.4 °C, while the average annual value amounted to 6.2 °C. Since 1996, there has been a steady increase in the average annual air temperature [5].

In the conditions of climate change and expansion of international trade in planting and seed materials, pathogenic microflora is spreading over long distances[2] [6, 7]. In the early 80s of the last century, domestic breeders brought anthracnose from South America with lupine seeds, which is one of the diseases that cause significant damage to the lupine crop in this region[3] [8].

Weather conditions favorable for pathogen development (air temperature 18-25°C, air humidity 75.0-90.0%) and the absence of resistant lupine varieties created ideal conditions for epiphytotics. On the territory of Russia, reproduction and spread of the disease during the vegetation period occurs by means of conidial sporulation (see footnote 3) [9, 10]. Under field conditions, the period of viability of conidia on the surface of the affected plant organs is short: they often die in dry weather, and due to prolonged exposure to sunlight and other biotic and abiotic factors[4].

Many studies have established that seeds are the main source of primary anthracnose infection in lupine under field conditions. The threshold of their infection is no more than 0.001-0.01%, exceeding which can lead to significant yield losses[5] [10, 11]. However, there is no consensus in the literature on the viability of the fungus after overwintering on lupine plant residues. Some scientists claim that the fungus does not overwinter in plant residues in the open air and does not persist in the soil [10]. Other authors point out that besides seeds, the remains of affected lupine plants are an additional source of anthracnose infection [11]. The fungus overwintering on perennial lupine is difficult, and no independent outbreaks of the disease have been observed so far. Because perennial lupin can be a source of infection, its crops should not be located near fields where annual fodder lupins are cultivated (see footnote 3) [11].

To effectively protect lupine crops from anthracnose under climate change conditions, it is necessary to have a full understanding of the sources of its occurrence.

The purpose of the study was to investigate the viability of the fungus *Colletotrichum lupini* var. *lupini* under different terms and conditions of storage of the seeds and plant residues of lupine in the climatic conditions of the Bryansk region.

MATERIAL AND METHODS

The study was conducted in 2018-2021 in the north-eastern part of the Bryansk region on the basis of the phytopathology laboratory of the All-Russian Research Institute of Lupin - branch of the Federal Williams Research Center of Forage Production & Agroecology. The object of the study were seeds, seedlings and plant residues of white lupine of the Michurinsky variety and narrow-leafed lupine of the Vityaz variety with signs of anthracnose. Seed samples for determination of the infection by the fungus *C. lupini* var. *lupini* were taken after their drying and sorting in granaries. For each storage mode, three samples of seeds weighing 5 kg were taken and placed in bags. Before storing the seeds, anthracnose infection was determined in each sample. No seed sterilization was performed to determine external infection, while for internal infection the seeds were immersed in 96% alcohol for 2 min. Seeds were germinated in pa-

[2]*Magan N., Medina A., Aldred D.* Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest // Plant Pathology, 2011, vol. 45, N 4, pp. 451–458.

[3]*Korneichuk N.S.* Lupine fungal diseases. Kiev, 2010, 374 p.

[4]*Kungurtseva O.V.* Bioecological features of the causative agent of anthracnose of lupine: Extended abstract of candidate's thesis. St. Petersburg: Pushkin, 2006, 19 p.

[5]*Rutskaya V.I., Minyailo V.A., Minyailo A.K.* Development of lupin protection system against anthracnose depending on the biological characteristics of the pathogen // New lupin varieties, technology of their cultivation and processing, adaptation in the system of farming and animal husbandry: Proceedings of the International Scientific and Practical Conference, dedicated to the 30th anniversary of the All-Russian Research Institute of Lupin. Bryansk, 2017, pp. 226-236.

per-polyethylene rolls at the optimal temperature for pathogen development (22-24 ºC) for 7 days[6, 7]. The sample was 300 seeds (six rolls of 50 seeds each). Identification of the pathogen was carried out by the conidia type using a light microscope [9, 11]. The average percentage of the seed infection was determined by the number of affected seedlings among those grown in paper rolls.

Then the lupin seeds were stored under room conditions and in a typical storage room. Phytopathologic analysis of the seeds was carried out several times - after 12 (in October 2018), 24 (in October 2019), 36 (in October 2020) and 46 months (in August 2021).

The study of overwintering of conidia and mycelium of the fungus was carried out on the stems and leaves of lupine beans affected by anthracnose. These plant parts were collected at the phase of full ripeness. Bean leaves and stem parts with the size from 10 to 15 cm were placed in kapron nets and in September some of them were buried in gray forest light loamy soil in the experimental field of the laboratory (plowing depth - 22 cm, cultivation - 16 cm, seed sowing - 3 cm), others were left in room conditions, in the open air under the roof of the granary and on the soil surface. Each sample contained 50 bean leaves and 50 stems in four replications. Plant parts for laboratory study were taken twice a year - in May and September. The pathogen was isolated and studied from the residues of the stored plant parts in accordance with the methods accepted in mycology and phytopathology[8, 9].

RESULTS AND DISCUSSION

Analysis of the seeds collected from the beans infected with the fungus *C. lupini* var. *lupini* showed that the seed condition depends on the location relative to the pathogen introduction. The seed located directly in the zone of introduction is non-viable. Its surface is covered with fungus. Such seeds are stubby or rotted and are separated when sorted by machines and do not make it into the seed. Nearby seeds are viable, have visible signs of seed coat damage in the form of dark or light brown spots of different shape and size, or have no obvious signs of damage at all and do not differ from healthy seeds (see Figure 1). Such seeds serve as the carriers of infection.

It was required to determine the period of viability of the fungus *C. lupini* var. *lupini* in the infected seeds of white and narrow-leafed lupin, taking into account the duration and storage conditions. There is information in the literature that the anthracnose pathogen loses viability in yellow lupin seeds after about 32 months, and in narrow-leafed lupin - after 18 months[10].

Weather conditions in the years of research were different. In December and January 2018, the lowest air temperature was -10.8...-15.8 ºC. The coldest was February - the air temperature during 8 days was within -11.1 ... -19.9 ºC. The snow cover was 50 cm high. Soil froze to a depth of 110 cm. Relative air humidity varied from 67.0 to 90.0%. In June, July and August, the maximum air temperature was 31 ºC, and the minimum - 5.5 ºC. Relative humidity ranged from 58.0 to 79.0%. Precipitation during the summer period amounted to 188 mm (39.7% of the total amount for the year).

In 2019, the largest decrease in air temperature (to -11.5...-17.1 ºC) was observed in January during 7 days. The snow cover height reached 41 cm. Relative air humidity in December, January and February ranged from 83.0 to 92.0%. The depth of soil freezing amounted to 80 cm. During the vegetation period June was

[6]GOST 12044-93. Seeds of agricultural crops. Methods for determining disease infestation. Moscow: Standards Publishing House, 2011, 55 p.

[7]*Gadzhieva G.I., Gutkovskaya N.S.* Methodical guidelines for determining the infestation of lupine seeds with anthracnose. Minsk, 2013, 20 p.

[8]*Khokhryakov M.K.* Methodical instructions for experimental study of phytopathogenic fungi. Leningrad, 1974, 69 p.

[9]*Kirai Z., Clement Z., Shoimoshi F., Veresz Yu.* Methods of phytopathology. Moscow: Kolos, 1974, 342 p.

[10]*Lindebeck K., Moore K., Richards M., O'Connor G.* GRDC Update Papers: The Watch outs for pulse diseases in 2017. URL: https://grdc.com.au/Research-and-Development/GRDC-UpdatePapers/2017/02/The-watch-outs-for-pulse-diseases-in-2017.

Рис. 1. Проявления заражения антракнозом:
а – бобы люпина белого сорта Мичуринский, пораженные грибом *C. lupini* var. *lupini*; *б* – семена с видимыми признаками поражения этим грибом; *в* – семена без видимых признаков поражения

Fig. 1. Manifestations of anthracnose infestation:
а – Michurinsky variety white lupine beans affected by the fungus *C. lupini* var. *lupini*; *б* – the seeds with visible signs of infection by this fungus; *в* – the seeds with no visible signs of infestation

the warmest and driest. The air temperature rose to 31 ºC. Relative air humidity was 59.0%.

In 2020, December was the coldest. The air temperature was within -12...-20 ºC. Relative air humidity amounted to 89.0%. Snow cover height reached 20 cm, soil froze to a depth of 70 cm. During the growing season, June was the warmest (10.3...31 ºC) and the rainiest (141 mm of precipitation). Air humidity was 69.0%.

In 2021, the largest air temperature decrease (-11.8...-20.1 ºC) was observed in February. This period lasted for 19 days. The snow cover height reached 48 cm. Relative air humidity was 82.0%. The average daily temperature in December and January was recorded at -4.5 and -5 ºC, respectively. The depth of soil freezing amounted to 50 cm. August was the warmest (11...32 ºC) in the summer period. Relative air humidity was 55.0%. No water standing was observed during snowmelt in the experimental plots.

In conditions of granary after a year the infection rate of white lupine seeds of the Michurinsky variety was higher than when stored in room conditions. Compared to the initial amount (8.1%), the proportion of infection decreased to 4.0% on average, while in room conditions - to 3.1% (see Table 1).

After two years of storage, the infestation amounted to 2.5 and 1.1%. In the granary after four years, the infestation significantly (LSD_{05} = 0.75) decreased to 0.4%; in room conditions, white lupin seeds were free from infection.

The initial infection rate of seeds of the narrow-leafed Vityaz lupin variety was almost 2 times less (4.8%) than that of the white lupin. This species of lupine differs from others by its greater resistance to anthracnose (see footnote 1) [11].

Under granary conditions after 12 months of storage, seed infestation amounted to 1.7%, while under room conditions it was 0.9% (see Table 1).

After 46 months of storage in the granary conditions, the proportion of the seeds with anthracnose infection decreased significantly (LSD_{05} = 0.49) to 0.1%, while in the room conditions the seeds were free from infection. Obviously, the active reduction of seed infection by this pathogen in room conditions was due to lower air humidity, which reduced the moisture content of the seeds. Significant reduction of fungal viability occurred already after 36 months of storage in the seeds of both white (LSD_{05} = 0.72) and narrow-leafed lupin (LSD_{05} = 0.38). Thus, when the seeds were put into storage, their moisture content was 14.0%, while during storage for four years it decreased to 7.0%. Similar results were obtained during storage of the yellow lupine seeds of the Fast Growing 4 variety (see footnote 3).

The available data indicate that the viability of anthracnose pathogen in the infected seeds of

Табл. 1. Количество семян люпина сортов Мичуринский и Витязь, содержащих инфекцию возбудителя антракноза, в зависимости от продолжительности и условий хранения

Table 1. The number of white lupine seeds of the Michurinsky and Vityaz varieties containing anthracnose pathogen infection depending on duration and storage conditions

Storage conditions	Sample number	Initial infestation, %	Seed infestation at different storage durations, %			
			12 months	24 months	36 months	46 months
Michurinsky variety						
At room temperature, in the laboratory	1	8,8	3,6	2,0	0,4	0
	2	7,2	2,4	1,2	0	0
	3	8,4	3,2	1,6	0,4	0
	Average	8,1	3,1	1,6	0,3	0
At varying temperature, in a storage room	1	8,8	4,8	3,2	1,6	0,8
	2	7,2	3,2	2,0	0,4	0
	3	8,4	4,0	2,4	1,2	0,4
	Average	8,1	4,0	2,5	1,1	0,4
Vityaz variety						
At room temperature, in the laboratory	1	4,8	0,8	0,4	0	0
	2	5,2	1,2	0,8	0,4	0
	3	4,4	0,8	0,4	0	0
	Average	4,8	0,9	0,5	0,1	0
At varying temperature, in a storage room	1	4,8	1,6	0,8	0,4	0
	2	5,2	2,4	1,2	0,8	0,4
	3	4,4	1,2	0,4	0	0
	Average	4,8	1,7	0,8	0,4	0,1

white and narrow-leafed lupin persists for a long time. At the same time, the number of the seeds with viable infection significantly decreases starting from the first year of storage. Therefore, seeds of previous years compared to freshly harvested seeds are less dangerous as a source of anthracnose infection in the field.

Phytopathological analysis of the seeds showed that in the first year of storage the number of seeds with surface infection of the fungus decreases, which on lupine seedlings manifests itself on the root neck in the form of dark brown spots, forming tangles, which leads to the death of the seedling. Internal infection of the pathogen persists for a long time and begins to die from the second year of storage. It is manifested on the cotyledons and the growing point in the form of pink spots with a large number of conidia of the fungus, which are washed out by rain in the field, spread by wind through the crop and infect healthy plants (see Fig. 2).

At the end of the third and fourth years of storage of the lupine seeds in granaries, only internal infection of the fungus remained. In room conditions this infection died in the fourth year of storage. Fig. 3 shows a colony of the fungus *C. lupini* var. *lupini* cultured on potato glucose agar (PGA) in Petri dishes and its conidia isolated in pure culture from the seeds after three years of storage in the granary.

It was found that the fungus forms an aerial ashy-gray mycelium on the nutrient medium

а *б*

Рис. 2. Признаки пораженности 7-суточных проростков люпина белого грибом *C. lupini* var. *lupini*: *а* – на гипокотиле; *б* – на семядолях

Fig. 2. Infection symptoms of the 7-day old white lupin seedlings with the fungi *C. lupini* var. *lupini*: *а* – on the hypocotyl; *б* – on the cotyledon

(potato-glucose agar). Cylindrical conidia with rounded ends develop abundantly on the aerial mycelium. The size of conidia is highly variable, 7.0-21.5 × 3.5-7.0 μm (see footnote 1) [11]. The colony of the fungus obtained by us fully corresponds in morphological features to the colonies described earlier by other researchers, which confirms its belonging to the causative agent of lupine anthracnose.

The fungus persists on lupin plant residues in the form of mycelium and conidia pads, glued together as a gelatinous mass containing promenium, which protects spores from the negative effects of abiotic and biotic environmental factors (see footnote 3) [11].

In studying the role of lupine plant debris as an additional source of disease under field conditions, it was found that the period of viability of the fungus depends on the conditions in which it is located.

According to the results of the study of the fungus isolates from lupine plant material stored in room conditions for different times, it can be noted that during storage there is a loss of viability of anthracnose pathogen spores. Thus, from four samples of 2011 it was not possible to obtain a single isolate, in 2013 three isolates from four samples were obtained. Consequently, after three years, the vast majority of the spores lose viability, and after five years there is a complete loss of their viability [9].

In our studies, the fungus on plant residues stored in room conditions and in the open air under the roof of the granary kept viability for a long time, while it constantly decreased. Parts of the stored plants with signs of anthracnose lesions were transferred to the PGA nutrient medium. The results of the experiment are presented in Table 2.

During the fall-winter-spring period, the viability of the fungus on plant residues in room conditions and in the open air under the roof of the granary was detected in 76.0 and 83.0% of samples, respectively, after one year (spring 2021) only in 12.0 and 19.0%.

Viable fungi were found on the plant residues of lupine with signs of anthracnose lesions, decomposed on the soil surface, in the autumn-winter-spring period (from 05.09.2019 to 15.05.2020) only in 2.0% of cases. During the summer period, the fungus completely lost viability (data for September 2020). The fungus on plant debris in the soil at 22, 16, and 3 cm depths was non-viable during the autumn-winter-spring period. By the end of May 2021, these plant residues had almost completely decomposed and were no longer suitable for analysis.

а *б*

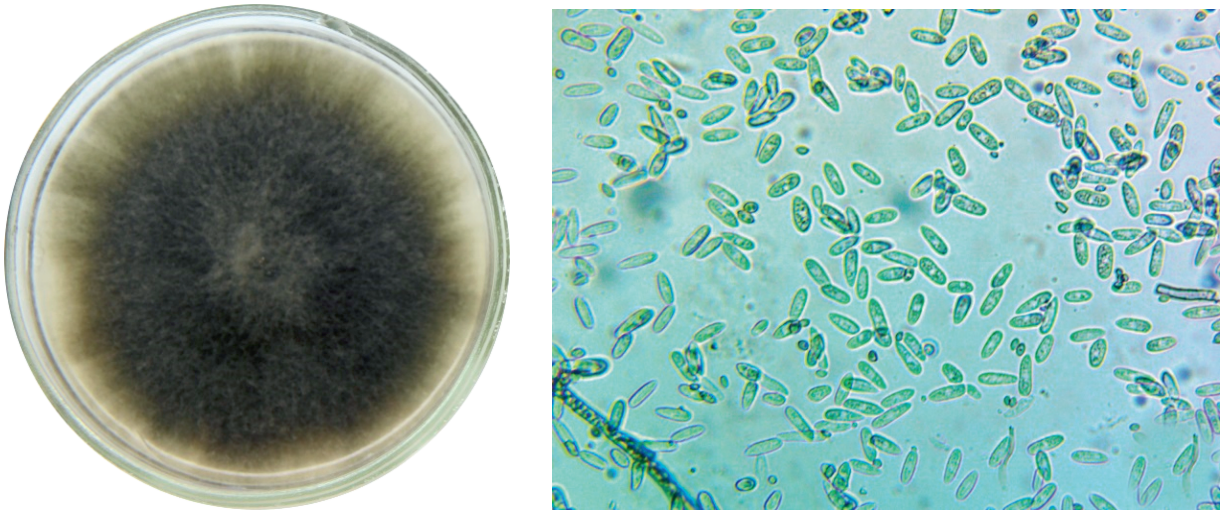

Рис. 3. Возбудитель антракноза люпина гриб *C. lupini* var. *lupini*:
а – внешний вид колонии; *б* – конидии гриба

Fig. 3. Lupin anthracnose pathogen – the fungi *C. lupini* var. *lupini*:
a – colony appearance; *б* – fungi conidia

Табл. 2. Жизнеспособность гриба *C. lupini* var. *lupini* на растительных остатках в зависимости от времени и способа хранения (опыт заложен 05.09.2019 г.)

Table 2. Viability of the fungi *C. lupini* var. *lupini* on plant residue depending on the period and storage conditions (experiment started 05.09.2019)

Timing of the tests	Percentage of the samples with viable fungus under different storage options, %					
	indoors	under the roof of the granary	on the soil surface	in the soil at depth, cm		
				22	16	3
15–21.05.2020	76	83	2	0	0	0
07–13.09.2020	49	54	0	0	0	0
23–29.05.2021	12	19	0	0	0	0

The results show that on infected plant residues in room conditions and under the roof of the granary the fungus remains viable, but from the first year of storage it decreases.

The viability of the fungus on unplowed plant residues in spring-summer period is preserved and can be the source of infection during re-sowing of lupine in this field. However, by the autumn its viability is completely lost. Affected lupine residues in the soil from the fall cannot be a source of lupine plant disease, as the anthracnose pathogen completely loses its viability in the spring.

Green mass of the yellow and narrow-leafed lupines, plowed into the soil in the blue-gray bean phase, in 8.5 months (by April of the next year) decomposed by 74.0-79.2%. Stubble and roots as more inert mass for microorganisms' activity mineralized slower and after 2.0-2.5 months decomposed by 46.9-53.0%, by spring - by 65.1% (see footnote 3).

When cultivating lupine according to the classical technology in the zone of sufficient moisture, soil treatment involves the following stages:

1) stubble shallow plowing with disc harrows to a depth of 10-12 cm after harvesting the forecrop;

2) underwinter plowing to a depth of 20-22 cm after 10-14 days.

If shallow plowing is not carried out, then underwinter plowing is carried out earlier. In spring (just before sowing) the soil is cultivated to a depth of 16 cm. The depth of sowing seeds is 2-3 cm. In crop rotation lupine should be returned to the same field not earlier than in 2-3 years [1]. When cultivating lupine with such a system of soil tillage and observing the terms of crop rotation, plant residues infected with anthracnose cannot serve as an additional source of pathogen infection. This is confirmed by our experience, in which the seeds of white and narrow-leafed lupine without signs of anthracnose pathogen damage were sown on the plot where in the previous year there was a sowing of lupine with the signs of anthracnose damage in plants (65.0%) and beans (82.0%). After harvesting, the plant residues of lupine were plowed into the soil to a depth of 22 cm. In spring, lupine was sown in this field. From sprouting to harvesting, no signs of disease damage were observed on the plants and beans of white and narrow-leafed lupin. Phytopathological analysis of the collected seeds did not reveal anthracnose infection. This fact indicates that the fungus completely loses its viability on plant residues decomposed during the autumn-winter-spring period.

CONCLUSION

The studies have shown that in climatic conditions of the Bryansk region the main source of anthracnose infection in lupine crops is infected seeds. The fungus on the seeds retains viability in conditions of granaries with natural temperature and humidity regime up to four years. Since the first year of storage the number of white lupine seeds with viable infection decreased from 8.1 to 4.0%, narrow-leafed lupine - from 4.8 to 1.7%. Therefore, it is advisable to use the seeds of the previous years' harvests for sowing.

The viability of the fungus on lupine plant residues located on the soil surface decreased to 2.0% during the autumn-winter-spring period. During the summer period, the fungus completely lost viability. Conidia and mycelium of the fungus overwintering on plant residues left on the soil surface are viable during summer and can serve as a source of anthracnose infection. The viability of the fungus was not preserved by the beginning of the next summer on the plant residues of lupine, which were in the soil from the autumn at a depth of 22, 16 and 3 cm.

If the basic tillage system in the zone of sufficient moisture includes autumn plowing, then lupine plant residues cannot become the source of infection.

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ПРИМЕНЕНИЕ МЕТОДОВ БИОИНФОРМАТИКИ ПРИ ИССЛЕДОВАНИИ И СОЗДАНИИ ПЕСТИЦИДОВ

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Применение пестицидов сопровождается рядом проблем экологического и агротехнологического характера. Многие пестициды не деградируют и годами находятся в почве, имеют низкую избирательность. Массированное применение пестицидов с неизбирательным характером действия вызвало у вредителей постепенный рост резистентности в связи со стойкими наследуемыми изменениями их ДНК. Это сказывается на эффективности выращивания сельскохозяйственных растений и загрязнении окружающей среды и продуктов питания. В разрешении этой проблемы могут помочь методы компьютерной биологии, которые активно развиваются во всем мире. Несмотря на то, что в России методы биоинформатики применяют для изучения генов растений животных, метагеномов микроорганизмов, собственные базы данных и специализированные компьютерные приложения для подобных исследований и модернизации пестицидов отсутствуют. Разработка отечественных аналогичных инструментов биоинформатики также является актуальной задачей. В статье освещена проблема создания новых эффективных и экологически безопасных пестицидов. Приведены методы биоинформатики, которые можно применять при исследовании и разработке пестицидов. Рассмотрены этапы создания новых пестицидов методами биоинформатики (обзор баз данных, моделирование молекул, моделирование взаимодействия пестицида с мишенью, прогнозирование биологической активности). Дано описание методов оптимизации молекулярного каркаса пестицидов, представляющей собой изменение углеродного скелета с целью поиска новых активных соединений и отсеивания множества похожих соединений в химическом пространстве. Приведены зарубежные веб-ресурсы, применяемые для оценки наличия у веществ пестицидных свойств, таких как токсичность, метаболизм и физико-химические свойства, и последующей регистрации их как пестицидов.

Ключевые слова: пестициды, биоинформатика, виртуальный скрининг, докинг, моделирование

APPLICATION OF BIOINFORMATICS METHODS IN PESTICIDE RESEARCH AND DEVELOPMENT

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The use of pesticides is accompanied by a number of environmental and agrotechnological problems. Many pesticides do not degrade and stay in the soil for years and have low selectivity. Massive application of pesticides with non-selective nature of action caused a gradual increase of resistance in pests due to persistent inherited changes in their DNA. This affects the efficiency of growing agricultural plants and pollution of the environment and food. Computational biology methods, which are actively developing all over the world, can help to solve this problem. Despite the fact that in Russia bioinformatics methods are used to study plant genes of animals, metagenomes of microorganisms, there are no own databases and specialized computer applications for such research and pesticide modernization. Development of domestic similar bioinformatics tools is also an urgent task. The ar-

ticle highlights the problem of creating new effective and environmentally friendly pesticides. The methods of bioinformatics that can be used in the research and development of pesticides are given. The stages of creating new pesticides by bioinformatics methods (review of databases, modeling of molecules, modeling of the interaction of a pesticide with a target, prediction of biological activity) are considered. A description is given of the methods for optimizing the molecular framework of pesticides, which is a change in the carbon skeleton in order to search for new active compounds and screen out many similar compounds in the chemical space. Foreign web resources used to assess the presence of pesticidal properties in substances, such as toxicity, metabolism and physico-chemical properties, and their subsequent registration as pesticides are given.

Keywords: pesticides, bioinformatics, virtual screening, docking, modeling

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Initially, the properties of pesticides were unknown, and the name "pesticides" ("pestis" - contagion and "cido" - killing) was given to them after the discovery of their ability to cause the death of various organisms. Gradually, pesticides began to be used in large quantities against pests of agricultural crops and products (harmful insects, weeds, phytopathogenic fungi, etc.). In the XX century, despite regulatory laws, mass application of pesticides became practically uncontrolled, so in the XXI century the problem of pesticide accumulation is highly relevant for the national economy.

It is known that many pesticides due to their pronounced toxicity are capable of disrupting the vital activity of many species of organisms, including humans and animals[1]. Active study of pesticide toxicity and development of the methods of their analysis in biological objects has been conducted since 1950[2] Pesticides are able to persist for a long time in environmental objects (plants, soil, water, etc.) and in the organisms of warm-blooded animals eating pesticide-treated plants, which leads to human poisoning by inhalation, oral, transdermal and other routes. In addition, pests develop resistance to pesticides.

Nowadays, more and more effective pesticides are required to increase crop yields and preserve agricultural products. At the same time, they should be safe for people and the environment.

Pesticide development is a complex process that requires a lot of time and money. Each new pesticide must be tested against more than 140,000 different compounds, which takes more than 10 years and is estimated to cost more than $286 million. [1]. To ensure safety, as well as to save time, labor and other resources, it is neces-

[1] *Kramarenko V.F.* Toxicological chemistry. Kiev: Vyshcha Shkola. Golovnoe Publishing House, 1989, 393 p.
[2] *Vergeichik T.Kh.* Toxicological chemistry. Moscow: MEDpress-Inform, 2009, 350 p.

sary to look for alternative ways to create pesticides. One of such ways is the use of computer methods - bioinformatics [2].

Bioinformatics methods

Bioinformatics is an interdisciplinary field that combines general biology, molecular biology, cybernetics, genetics, chemistry, computer science, mathematics and statistics. The application of its methods makes a meaningful contribution to the study of bioactive substances because it is based on the rapid analysis of large data sets, including those obtained through extensive empirical experience.

Modeling a pesticide is directly related to the design of its structure [3]. This process consists of four steps:

– studying molecular databases that contain information on structures and ligand binding sites[3];

– molecular docking;

– molecular framework optimization;

– pesticide likeness assessments.

Protein and low molecular weight databases

Protein databases are important as a source of information for structural design purposes and can give researchers a better understanding of pathogenic mechanisms [3] by providing data on target structure, active sites, protein sequence, etc. Databases of low molecular weight substances allow to limit the choice of molecules due to confirmed physicochemical properties, energetic parameters (electrostatic potential, dipole moment, etc.) and biological activity. The choice of a suitable library of molecular compounds significantly increases the efficiency of work. The largest is the Protein Data Bank (PDB)[4]. It contains more than 200 000 records, information on fundamental, secondary and crystal structures of biological macromolecules[5]. The Uniport[6] protein sequence database also provides high-level annotation (translational modifications, domain structure, protein function, etc.)[7, 8]. A sought-after diversified compound library is the ChemBridge[9] database based on 3D pharmacophore design. Over 28 years, it has accumulated more than 1,300,000 compounds[10].

There are significant shortcomings of the databases: insufficient thermodynamic parameters related to proteins, as well as information on protein-pesticide interactions.

Molecular docking (virtual screening)

Molecular docking is a virtual modeling of molecule docking. It is a suitable choice for modeling the 3D structure of the receptor-ligand complex and assessing the stability of the complex. Virtual screening is an effective strategy for the identification of bioactive molecules and has the potential to significantly accelerate pesticide discovery[11]. The main task of docking is to explore the conformational space of ligands binding to target molecules and to rank this space according to the estimated binding affinity.

Molecular docking methods can be roughly divided into four categories:

– shape-matching methods (e.g., SANDOCK). This category does not take into account the intrinsic flexibility of molecules;

– systematic methods (e.g., DOCK, FlexX). DOCK shapes the core segment through spatial complementarity by systematically exploring the pose space of each key. FlexX places hard

[3]*López Pazos S.A., Cerón Salamanca J.A.* Minireview and hypothesis: homology modelling of Spodoptera litura (Lepidoptera: Noctuidae) am inopeptidase N receptor // Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales, 2008, vol. 32 (123), pp. 139–144.

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[6]https://www.uniprot.org.

[7]*Boutet E., Lieberherr D., Tognolli M., Schneider M., Bairoch A.* UniProtKB/Swiss-Prot // Methods in molecular biology (Clifton, N.J.), 2007, vol. 406, pp. 89–112. DOI:10.1007/978-1-59745-535-0_4.

[8]*Bairoch A., Apweiler R.* The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. Nucleic Acids Research, 2000, vol. 28 (1), pp. 45–48. DOI: 10.1093/nar/28.1.45.

[9]https://chembridge.com.

[10]*Facts K.* Chembridge screening libraries. Oct. 2022. https://chembridge.com/.

[11]*Wermuth C.G., Villoutreix B., Grisoni S., Olivier A., Rocher J-P.* Chapter 4 – strategies in the search for new lead compounds or original working hypotheses. In: Wermuth C.G., Aldous D., Raboisson P., Rognan D., eds. The Practice of Medicinal Chemistry. fourth ed. San Diego: Academic Press, 2015, pp. 73–99. DOI: 10.1021/acsomega.8b00778.

core fragments based on interaction groups between the fragments and receptors;

– stochastic methods (e.g., Monte Carlo);

– methods of molecular dynamics modeling.

Valuation techniques can be roughly divided into three categories:

– physics-based (such as DOCK, GOLD, Ledock);

– regression-based (for example, Autodock, Vina, Surflex, Glide);

– knowledge-based.

Improvements in high-throughput screening procedures using quantitative structure-activity relationship (QSAR) methods allow faster screening of large numbers of compounds under identical test conditions, reducing the risk of repeating variable test data from many sources. Both docking and QSAR methods help to identify a suitable candidate molecule for a pesticide[12].

A number of problems currently exist in the use of virtual screening approaches: the difficulty in locating allosteric binding pockets; the complexity of calculations and the corresponding construction of the protein model makes it difficult to take into account its flexibility; the strong influence of desolvation, entropic penalty, conformational stress, etc.; it is still difficult to predict the binding affinity of various small molecules with high accuracy by existing estimation methods. The resolution of these problems is necessary for the further development of virtual screening.

Optimization of molecular framework

Optimization of the molecular framework is necessary to obtain basic molecular compounds. The methods used at this stage of structural molecular design simplify the modification of the carbon skeleton, which reduces the synthesis of many similar compounds and increases the efficiency of the search for new active compounds.

Different scenarios require suitable molecular framework optimization tools. In 2020, an automated *in silico* ligand evolution (AILDE) control using one-step FEP and MD modeling strategies was developed for rapid identification of basic compounds in drug development in the available chemical space [4].

Fragment-based design is another optimization method. In 2016, Auto Core Fragment in silico Screening (ACFIS) server was released to find leading connections based on fragments. It includes computational modules PARA_GEN, CORE_ GEN and CAND_GEN, and uses the fragment deconstruction analysis method to obtain the key structure where fragment growth occurs[13].

There are also a number of challenges in using molecular framework optimization tools:

– the main problem is the choice of the key structure of the molecular framework;

– due to the poor pharmacological characteristics of the available compounds, the current fragment libraries need to be replaced with more extensive ones;

– shape descriptors, multidimensional structure parameters and molecular topology of the compounds are insufficiently studied [5];

– it is necessary to take into account the complexity parameters of the compounds, especially of chiral ones[14];

– it is difficult to take into account the photostability, permeability, solubility and ecotoxicology of the pesticide molecules[15, 16] [6].

Determination of pesticide likeliness

The process of pesticide development can be accelerated by the web resource ADMET[17],

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[17]https://admetmesh.scbdd.com.

which provides a database and models that filter a large number of compounds before testing for bioavailability [7]. Pesticide Info, a web searchable database based on the Pesticide Action Network (PAN)[18], is also used. Another example is the ECOTOX knowledge base[19], which combines three previously independent databases - AQUIRE, PHYTOTOX and TERRETOX - into a unique system in which 12,281 chemical toxicity data are derived primarily from peer-reviewed literature.

Pesticide similarity analysis models are used to identify pesticide similarity. Thus, over the last 10 years, the relevant tools have been continuously improved. In 2014, the QEP method was established, which is implemented by calculating exponential desirability functions of several descriptors and then their conditional summation[20]. Relative Drug Similarity (RDL) is a quantitative function used to predict pesticide likeliness[21]. In 2019 and 2021, based on the known molecular structures of pesticides, molecular descriptors were calculated, data were fitted, key parameter values for quantitative pesticide similarity assessment, relative chemical compound class and Gaussian function were obtained, and 9 evaluation functions of pesticide classes were fitted [7–9].

Another FungiPAD method is also used for rapid prediction of bioavailability, physicochemical properties and fungicide similarity. InsectiPAD provides comprehensive insecticide similarity analysis for any compound. In addition, the Agricultural and Environmental Research Group at the University of Hertfordshire has developed a database of pesticide properties[22].

Examples of in silico pesticide creation

Example 1. nhibitor of mitogen-activated protein kinase FgGpmk1.

Mitogen-activated protein kinase (MAPK) plays an important role in pathogenicity, differentiation and cell growth of phytopathogenic fungi[23]. The MAPK enzyme FgGpmk1 is known to be closely related to invasion and virulence in *Fusarium graminearum*[24, 25][10]. For this reason, FgGpmk1 is considered an attractive target for inhibiting Fusarium rot. In 2021, a multidisciplinary pesticide development strategy was applied to develop FgGpmk1 inhibitors (see footnote 24). The structural optimization process is shown in Figure 1.

By virtual screening, compound 21 was obtained, which showed optimal activity (EC_{50} = 13.01 μg-mL^{-1}). Further structural optimization, represented by the addition of the Schiff base, showed that the resulting compound 94 had a higher activity (EC_{50} = 3.46 μg - mL^{-1}). Observing the molecular design simulation, it is suggested that the Schiff base forms a vital hydrogen bond with Glu69 to stabilize the conformation of the compound 94. This hydrogen bond is quite different from that formed between the pyrazolpyrimidine group of the compound 21 and Glu31 (see Figure 1). This explains why compound 94 is more active than compound 21. However, the potential for the MAPK enzyme FgGpmk1 to be affected still requires further investigation [10]. The role of MAPK FgGpmk1 in the interactions of phytopathogenic fungi with the plants that are crops should be established [11]. The FgGpmk1 transcription factors regulating *Fusarium graminearum*, about which little is known, also require further study.

[18]https://pan-international.org/pan-regional/.

[19]https://cfpub.epa.gov/ecotox/.

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Example 2. Chitinase inhibitor OfChi-h

Chitin is a key component of the peritrophic membrane of insects and the exoskeleton of the midgut[26, 27] [12]. Since chitin is absent in higher plants and mammals, it can be considered as a convenient target for the action of pesticides in their development [13]. ZINC database molecules were used to obtain the compound VS-5 by virtual screening. The structural optimization process is shown in Fig. 2.

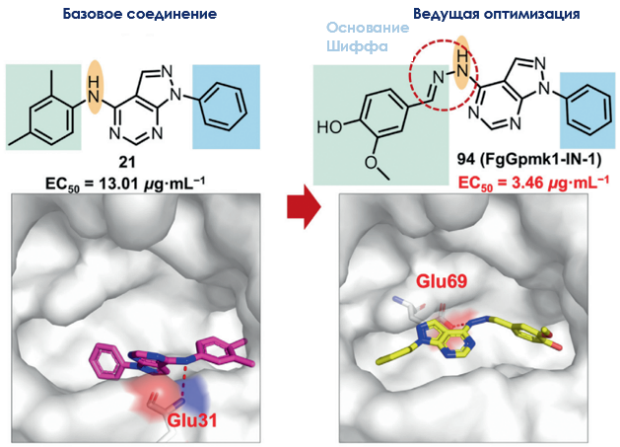

Рис. 1. Структурный молекулярный дизайн ингибитора митоген-активируемой протеинкиназы *Fusarium graminearum* [3]

Fig. 1. Structural molecular design of *Fusarium graminearum* mitogen-activated protein kinase inhibitor [3]

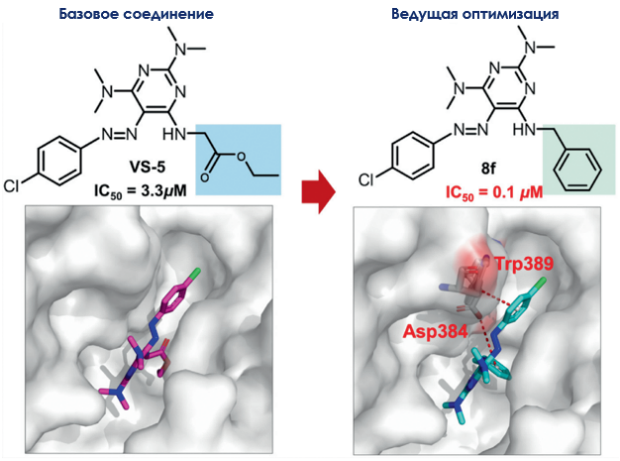

Рис. 2. Структурный молекулярный дизайн ингибитора хитиназы OfChi-h [3]

Fig. 2. Structural molecular design of the chitinase inhibitor OfChi-h [3]

In the binding mode, it can be seen that the 4-chlorophenyl fragment 8f moves slightly toward the entrance of the OfChi-h pocket (see Figure 2) and forms a strong π-π interaction with Trp389. In addition, modification of the ethyl ester group to a benzyl group led to H-π-π interactions with Asp384. These results explain why the inhibitory activity of compound 8f (IC_{50} = 0.1 μM) is higher than that of VS-5 (IC_{50} = 0,33 μM) [14].

ЗАКЛЮЧЕНИЕ

The use of pesticides is accompanied by a number of environmental and agrotechnological problems. Many pesticides do not degrade and remain in the soil for years, they have low selectivity. Massive use of pesticides with non-selective nature of action has caused gradual growth of resistance in pests due to persistent inherited changes in their DNA[28], which affects the efficiency of growing agricultural plants and contamination of the environment and foodstuffs. This requires the development of new targeting and biodegradable pesticides. The methods of computational biology, which are being actively developed all over the world, can help here. It should be noted that despite the fact that in Russia bioinformatics methods are actively used to study plant genes of animals, metagenomes of microorganisms, there are no own databases and specialized computer applications for such studies and modernization of pesticides. The development of domestic analogous bioinformatics tools is also an urgent task.

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ВЛИЯНИЕ КОМПЛЕКСНЫХ БИОДОБАВОК ФИТОГЕННОГО ПРОИСХОЖДЕНИЯ НА ПРОДУКТИВНЫЕ ПОКАЗАТЕЛИ ПЕРЕПЕЛОВ

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Проведены сравнительные исследования по изучению влияния фитобиодобавок из натурального растительного сырья и с синтетическим адаптогеном ауролом в комплексе с арабиногалактаном на продуктивные показатели перепелов. Ежедневное включение биодобавок в рационы перепелов с 5-суточного и до 107-дневного возраста по-разному влияло на их сохранность и продуктивные показатели. Контрольная группа получала основной рацион, 1-я опытная группа – основной рацион с выпаиванием водного раствора аурола с арабиногалактаном; во 2-й опытной группе к 99,9% основного рациона добавляли 0,1% биодобавки № 1, состоящей из порошка корня родиолы розовой с арабиногалактаном; в 3-й опытной группе в первый период выращивания – 98,4% основного рациона + 1,6% биодобавки № 2, состоящей из порошка корня родиолы розовой, скорлупы кедрового ореха и хвои сосны с арабиногалактаном, во второй период – 98,3% основного рациона + 1,7% биодобавки № 2а. Изучен биохимический состав крови, мышечной ткани тушек перепелов, яиц перепелок. В период выращивания перепелов включение в основной состав рациона опытных групп комплексных биодобавок растительного происхождения с арабиногалактаном № 1 и № 2 способствовало увеличению их живой массы на 7,6 и 11,1%, среднесуточного прироста на 8,6 и 12,4%, убойного выхода потрошеной тушки на 0,9 и 1,5%, повышению сохранности на 5,7 и 1,4% по сравнению с контрольной группой. В начале яйценоскости лучшие результаты были при использовании натуральных комплексных биодобавок. У несушек 2-й и 3-й опытных групп проброс яиц начался на 2 дня раньше, они имели наивысшую интенсивность яйцекладки (66,98 и 61,67%). По сравнению с контрольной группой более высокая средняя масса одного яйца отмечена во 2-й опытной группе – на 5,7%, интенсивность яйцекладки выше на 15,0, яйцемасса – на 38,9%.

Ключевые слова: перепела, аурол, арабиногалактан, биодобавки растительного происхождения, продуктивность, сохранность, яйценоскость

EFFECT OF COMPLEX BIOADDITIVES OF PHYTOGENIC ORIGIN ON PRODUCTIVE PERFORMANCE OF QUAILS

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Comparative research on studying the influence of phytobioadditives from natural plant raw materials and with synthetic adaptogen Aurol in complex with arabinogalactan on quail productive indices

were carried out. Daily inclusion of bio-additives in the diets of quails, from 5 days to 107 days of age, had a different effect on the safety and their productive performance. The control group received the basic diet, the 1st experimental group – the basic diet with drinking aqueous solution of aurol with arabinogalactan; in the 2nd experimental group 0.1% of bio-additive No. 1 consisting of roseroot powder with arabinogalactan was added to 99.9% of the basic diet; in the 3rd experimental group – 98.4% of the basic diet + 1.6% of bio-additive No. 2 consisting of roseroot powder, pine nut shell and pine needles with arabinogalactan in the first period of growing; in the second period – 98.3% of the basic diet + 1.7% of bio-additive No. 2a. Biochemical composition of blood, muscle tissue of quail carcasses, quail eggs was studied. In the period of quails growing, inclusion of complex bio-additives of plant origin with arabinogalactan No. 1 and No. 2 in the main composition of the diet of experimental groups increased their live weight by 7.6 and 11.1%; average daily gain by 8.6 and 12.4%; slaughter yield of gutted carcass by 0.9 and 1.5%; increased safety by 5.7 and 1.4% compared to the control group. At the beginning of egg production, the best results were with natural complex bio-additives. The laying hens of the 2nd and 3rd experimental groups started egg laying 2 days earlier, they had the highest egg laying intensity (66,98 and 61,67%). In comparison with the control group, a higher average weight of one egg was observed in the 2nd experimental group – by 5.7%, the intensity of oviposition was higher – by 15.0%, egg mass – by 38.9.

Keywords: quail, aurol, arabinogalactan, bio-additives of plant origin, productivity, safety, egg production

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INTRODUCTION

Effective development of quail breeding requires optimization of poultry feeding in order to obtain the maximum amount of products at the lowest cost [1]. One of the ways to solve this problem is the use of natural biostimulants and adaptogens from vegetable raw materials and medicinal plants[1].

Rhodiola rosea L., or golden root, is a source of vitamins, trace elements and various metabolites [2]. Biologically active substances of roots and rhizomes of golden root have antioxidant, adaptogenic, immunotropic, hepatoprotective, anti-inflammatory and other properties [3-5]. As a phytosupplement preparations of roots and rhizomes of golden root are applied to poultry at a dosage of 1 g per 1 kg of mixed fodder[2].

Two compounds of rhodiola rosea have the greatest adaptogenic activity: n-tyrosol and its glucoside - salidroside[3].

Synthetic adaptogen Aurol is an analog of plant adaptogen n-tyrosol[4]. Application of the

[1] *Vakhrusheva T.I.* Experience in the use of adaptogens of plant origin in industrial poultry farming // Principles and technologies of ecologization of production in agriculture, forestry and fisheries: Proceedings of the 68th international scientific and practical conference. Ryazan: Publishing House of Ryazan SAU, 2017, P. 3, pp. 37–41.

[2] *Vakhrusheva T.I.* Morphometric indicators of thymus in chickens at the age of 1-40 days under the influence of Rhodiola rosea // Problems of Modern Agrarian Science: Proceedings of the international extramural scientific conference / edited by A.A. Kondrashev, J.N. Shmeleva, 2015, pp. 35–37.

[3] *Kurkin V.A.* Rhodiola rosea (Golden root): standardization and creation of medicinal preparations. Samara: LLC "Ofort", 2015, 240 p.

4 Pat. № RU 2063395 C 07 C 39/06, C 07 C 37/52. Method for preparation of 4-(hydroxyalkyl) phenols / A.P. Krysin, T.G. Egorova, V.S. Kobrin; applied 18.07.1994, published 18.07.1996, Bulletin No. 19.

drug in poultry farming has shown a positive effect, but it is used only in aqueous solution at the rate of 1 mg/kg of live weight, which is not always possible in the technological line [6].

A natural polymer (class of polysaccharides) arabinogalactan consisting of galactose and arabinose was isolated from sawmill waste and biomass processing of Siberian larch (*Lárix sibírica* L.). Arabinogalactan has low toxicity, shows immunomodulatory, antioxidant and prebiotic activity, positively affects the safety, productivity and reproductive qualities of poultry at doses from 3.6 to 6.0 mg/kg live weight[5] [7, 8]. It is known that arabinogalactan has the property to bind various hydrophobic, biologically active molecules, which leads to a significant increase in their solubility, bioavailability and stability during storage [9].

When processing Siberian pine nuts (*Pinus sibirica* Du Tour), the shell is a waste, but the positive effect of its use in poultry feeding is widely known when included in the composition of the diet up to 3.0 wt.% [10]. Pine nut shell contains extractive substances with tannin, antioxidant, anti-inflammatory, disinfectant properties [11].

Phenolic compounds, carboxylic acids, polyprenols, terpenoids, vitamins, minerals and other components contained in pine needles (*Pinus sylvestris* L.) have antimicrobial, immunostimulant, antioxidant, anti-inflammatory, hematopoietic effects, positively affect poultry productivity and feed conversion [12]. It is recommended to introduce pine needle meal into poultry diet up to 3%[6].

Proceeding from the fact that all nutrients included in the composition of plant components are harmless, are in non-antagonistic combinations, have a similar effect on the bird's body and have not been previously used in quail diets, we have developed complex phytobiotic supplements of the following composition:

– aqueous solution of aurole and arabinogalactan;

– bioadditive No. 1 - powder from the underground part of *R. rosea* L. and arabinogalactan;

– bioadditives No. 2 and No. 2a - powder of underground part of *R. rosea* L., powder of pine nut shells of *Pinus sibirica* Du Tour, flour from pine needles of *Pinus sylvestris* L. and arabinogalactan.

The purpose of the study is to determine the optimal variant of complex bioadditive of phytogenic origin and with synthetic adaptogen Aurole in complex with arabinogalactan.

MATERIAL AND METHODS

The 103-day experiment was conducted in 2021 in the physiological yard of the SFSCA RAS on Japanese quails formed at 5 days of age into four groups of 70 quails each (see Table 1).

Intergroup differences were as follows: quails of the control group received the basic diet (BD); in the 1st experimental group - BD and aqueous solution of Aurol with arabinogalactan in equal amounts (1 g/liter of water each); in the 2nd experimental group, 0.1% of bioadditive No. 1 consisting of Rhodiola rosea root powder (93.0 wt.%) and arabinogalactan (7.0 wt.%) was added to 99.9% of BD; in the 2nd experimental group, 0.1% of bioadditive No. 1 consisting of Rhodiola rosea root powder (93.0 wt.%) and arabinogalactan (7.0 wt.%) was added to 99.9% of BD; in the 3rd experimental group up to 34 days of age of young animals the diet consisted of 98.4% BD and 1.6% of bioadditive No. 2 (Rhodiola rosea root powder (1.0 g) + pine coniferous meal (5.0 g) + pine nut shell meal (10, 0 g)), in the next period - 98.3% of BD and 1.7% of bioadditive No. 2a (Rhodiola rosea root powder (2.0 g) + pine coniferous meal (5.0 g) + pine nut shell meal (10.0 g)).

The studies were conducted in accordance with the requirements of the generally accepted

[5]*Medvedeva E.N., Babkin V.A., Ostroukhova LA* Larch arabinogalactan - properties and prospects for use (review) // Chemistry of plant raw materials, 2003, N 1, pp. 27–37.

[6]*Artemiev V.I., Eliseev O.A.* Homestead poultry farming; 2nd ed., suppl. and revised. L.: Agropromizdat, 1988, 96 p.

[7]Recommendations on feeding poultry / edited by V.I. Fisinin, Sh.A. Imangulov, I.A. Egorov, T.M. Okolelova. Sergiev Posad: RAAS, ISTC "Plemptitsa", VNITIP, 2003, 142 p.

Табл. 1. Схема опыта (n = 70)
Table 1. Experience scheme (n = 70)

Group	Feeding conditions
Control	BD*
Experimenta:	
1st	BD + with water aurole + arabinogalactan
2nd	BD (99,9%) + bioadditive № 1 (0,1%) (rhodiola rosea root powder and arabinogalactan)
3rd	First period – BD (98,4%) + bioadditive № 2 (1,6%); second period – BD (98,3%) + bioadditive № 2a (1,7%)

*BD – basic diet balanced according to VNIITIP norms (2003) taking into account age requirements of quails[7].

methods[8]. Daily accounting of feed and additives consumption, monitoring of behavior and health status of quails were carried out. Individual control weighing of birds was carried out at the time of experimentation (at the age of 5 days), as well as at the age of 34 and 64 days.

The influence of bio-additives on productivity and safety of young quails up to 64 days of age was studied, blood sampling and control slaughter of male quails (3 birds from each group) with sampling of muscle tissue for biochemical studies were carried out.

The study of the biochemical composition of quail blood serum samples and biochemical composition of muscle tissue samples was carried out by classical methods using modern biochemical analyzers.

The scientific experiment was continued according to the scheme of the experiment on the remaining quail stock in order to study egg productivity up to 107 days of age.

Aurole (n-tyrosol) [2-(4-hydroxyphenyl) ethyl alcohol] is an analog of the plant adaptogen *R. rosea* L. - a synthetic drug represented by a white to light yellow crystalline powder with a faint phenol odor (synthesized at the Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences).

Arabinogalactan (lavinol) - food additive, obtained from the pith of Dahurian larch wood [*Larix dahurica Laws*. (*L. dahurica* Turcz. et Trautv., *L. gmelinii* (Rupr.) Rupr.)], the manufacturer CJSC "Ametis" (Blagoveshchensk). It is a crystalline powder of white, pale gray or pale cream color with a slight pine odor. Rhodiola rosea root powder and pine needles powder provided by a third-party organization.

Siberian pine nut shells were dried, crushed on a hammer crusher, then the fractions up to 1 mm were separated under physiological yard conditions.

The following zootechnical indicators of quails were determined during the research: live weight gain, feed consumption, safety, carcass slaughter yield, egg production, egg weight.

Economic efficiency (conditional economic effect) was calculated by the difference in costs for the purchase of fodder means and preparations, the amount of feed costs per unit of production, taking into account the price of its realization.

The numerical material obtained during the experiment was processed by the method of variation statistics on a personal computer using Microsoft Excel software and according to N.A. Plokhinsky (1970).

RESULTS AND DISCUSSION

The inclusion of bioadditives in the basic diet or feeding water to experimental quails during rearing had a positive effect on the safety and growth rate of the birds (see Table 2). Safety of the quails in the experimental groups amounted from 90.0 to 94.3% (88.6% in the control). The best indicators were observed in the 2nd experimental group.

In the first month of growing the quails of the 1st experimental group were at the same level with the control group in terms of live weight (76.64 g each), in the 2nd and 3rd experimental groups they exceeded the control and reached 85.65 and 85.93 g ($p \leq 0.01$). For the second month the best results were also noted in the

[8]Methodology of scientific and production research on poultry feeding / edited by V.I. Fisinin and Sh.A. Imangulov. Sergiev Posad: Russian Academy of Agricultural Sciences, ISTC "Plemptitsa", VNITIP, 2000, 33 p.

Табл. 2. Показатели сохранности, интенсивности роста, расхода корма на прирост перепелят за период выращивания

Table 2. Indices of safety, growth intensity, feed consumption per gain of quails during the growing period

Indicator	Group			
	control	experimental		
		1st	2nd	3rd
Number of heads:				
at the beginning of the experiment	70	70	70	70
at the end of the experiment	62	63	66	65
Livability, %	88,6	90,0	94,3	92,9
Live weight, g:				
when put on the experiment	17,98 ± 0,41	17,97 ± 0,32	17,75 ± 0,28	18,50 ± 0,31
34 days	76,64 ± 1,94	76,64 ± 1,37	85,65 ± 2,04**	85,93 ± 1,80**
64 days	178,42 ± 3,16	186,63 ± 1,76	191,98 ± 1,33**	198,25 ± 1,99**
Average daily live weight gain at the age, g:				
5–34 days	1,97 ± 0,14	1,96 ± 0,12	2,26 ± 0,13**	2,25 ± 0,12 **
34–64 days	3,39 ± 0,22	3,67 ± 0,17	3,54 ± 0,11	3,74 ± 0,18*
5–64 days	2,67 ± 0,16	2,81 ± 0,21	2,90 ± 0,09*	3,00 ± 0,16*
Average daily feed intake, g:				
5-34 days	2,60	2,53	2,45	2,49
35–64 days	42,15	37,25	33,38	32,36
5–64 days	34,10	31,25	28,95	28,62
Feed consumption per 1 g of gain, g	12,77	11,12	9,98	9,54

Note. Here and in Tables 3–7: the difference is significant in comparison with the control group:
*$p \leq 0,05$,
**$p \leq 0,01$,
***$p \leq 0,001$.

quails of the 2nd and 3rd experimental groups - 191,98 and 198,25 g ($p \leq 0,01$), more by 7,6 and 11,1% than in the control group (178,42 g). During the record period, the average daily gain of the quails fed bioadditives No. 1 and No. 2-2a were 8.6 and 12.4% higher than the young of the control group and amounted to 2.90 and 3.00 g ($p \leq 0.05$).

During the first month of the study, feed intake by the quails of the experimental groups was lower than the control from 2.7 to 5.8%, during the second month - from 11.6 to 23.2%. On average during the growing period feed consumption by the quails of experimental groups was lower from 8.1 to 16.1%. More economical feed consumption per 1 g of live weight gain compared with the control was observed in the quails of the 2nd and 3rd experimental groups.

The representative of para-substituted hydro- xyalkylphenols - n-tyrosol (2-(4-hydroxyphenyl) ethyl alcohol), on the basis of which the domestic drug Aurol was created, has not only a pronounced stress-protective effect, but also a central stimulating effect. It is known that the main active substances of Rhodiola rosea - phenolglycosides rhodioloside, salidroside and rosavin are used as the components of functional nutrition[9]. Phenols and flavonoids not only reduce the effects of pathogens but also improve the utilization of nutrients in the gastrointestinal tract [13]. Apparently, the complex of biologically active compounds of Rhodiola rosea had a more active effect on the assimilation of nutrients of the diet, which was reflected in the reduction of feed costs per live weight gain of the 2nd and 3rd experimental groups of quails,

[9]*Nikolaev E.V., Pinchukova E.V., Belik S.N.* Functional properties of Rhodiola // Innovations in food production: from animal breeding to food production technology: proceedings of the scientific conference. Persianovsky settl: Publishing House of the Don State Agrarian University, 2018, pp. 121–127.

respectively, by 21.8 and 25.3% compared with the control and by 10.3 and 14.2% - with the 1st experimental group.

A similar effect on feed payment by poultry also has the inclusion of arabinogalactan in the composition of diets [14]. Our study shows the positive effect of a complex of natural phytonutrients with arabinogalactan. The results of control slaughter of the birds showed that the average live weight of 2-month-old male quails in the experimental groups was higher than the control group by 6.5-10.1% and ranged from 183.2 to 189.3 g ($p \leq 0.05$), gutted carcass weight reached 133.0-140.3 g ($p \leq 0.01$), which was higher by 6.7-12.5%, respectively. However, a significant difference with the control in the carcass slaughter yield by 0.9-1.5% ($p \leq 0.01$) was observed only in the 2nd and 3rd experimental groups of the quails (see Table 3).

For the main nutrient components of minced meat (muscle tissue) of quails the most visible changes were noted in the samples of the 3rd experimental group: there was a decrease in the protein level by 0.52% ($p \leq 0.05$), an increase in fatty tissue by 3.21% ($p \leq 0.05$) and a decrease in ash residue by 0.26% ($p \leq 0.01$) compared to the control group. In the 1st experimental group, the level of ash in minced meat was significantly increased by 0.42% ($p \leq 0.05$).

The use of phytoadditives in the diets of experimental quails had different effects on the biochemical composition of blood (see Table 4). The level of protein in blood serum of the quails of the 2nd experimental group was higher by 10.27 g/l ($p \leq 0.01$) compared with the control, by 11.17 g/l - with the 1st experimental group ($p \leq 0.01$) and by 8.00 g/l ($p \leq 0.01$) - with the 3rd experimental group ($p \leq 0.01$).

Increase of globulin level by 5,61 g/l ($p \leq 0,01$) in the quails of the 2nd experimental group in comparison with the control group, by 7,71 g/l - with the 1st experimental group and by 4,62 g/l - with the 3rd experimental group indicates the strengthening of their biological status. Ac-

Табл. 3. Результаты убоя и качественные показатели мяса-фарша перепелов ($n = 3$)
Table 3. Results of slaughter and quality indicators of minced meat of quails ($n = 3$)

Indicator	Group			
	control	experimental		
		1st	2nd	3rd
Pre-slaughter live weight, g	172,0 ± 0,46	183,2 ± 0,46*	184,3 ± 0,67*	189,3 ± 1,57*
Gutted carcass weight, g	124,7 ± 0,86	133,0 ± 0,71**	135,3 ± 0,82**	140,3 ± 1,70**
Carcass slaughter yield, %	72,5 ± 0,47	72,6 ± 0,29	73,4 ± 0,18**	74,0 ± 0,32**
Content in meat-minced meat, %:				
dry matter	33,36 ± 0,67	32,76 ± 0,42	31,93 ± 0,39	35,68 ± 1,04
protein	19,24 ± 0,002	19,31 ± 0,21	19,32 ± 0,20	18,72 ± 0,16*
fat	10,64 ± 0,66	9,64 ± 0,67	9,21 ± 0,75	13,85 ± 1,17*
ash	3,38 ± 0,05	3,80 ± 0,07*	3,41 ± 0,20	3,12 ± 0,05**
calcium, g	1,39 ± 0,24	1,46 ± 0,014	1,24 ± 0,26	1,41 ± 0,23

Табл. 4. Биохимические показатели сыворотки крови перепелов
Table 4. Biochemical parameters of blood serum of quails

Indicator	Group			
	control	Experimental		
		1st	2nd	3rd
Total protein, g/l	30,92 ± 0,99	29,98 ± 0,70	41,15 ± 0,59**	33,15 ± 0,87
Albumin, g/l	15,40 ± 0,89	16,55 ± 0,30	20,00 ± 0,48	16,63 ± 1,49
Globulin, g/L	15,53 ± 0,34	13,43 ± 0,84	21,14 ± 0,59**	16,52 ± 2,03
AST, unit/l	181,68 ± 9,95	188,61 ± 20,76	112,12 ± 9,59	117,79 ± 7,85
ALT, unit/l	37,44 ± 3,73	36,52 ± 3,37	31,35 ± 1,62	28,89 ± 1,27
Creatinine, μmol/L	31,33 ± 1,43	35,12 ± 4,88	30,41 ± 0,36	28,16 ± 1,69

cording to the results of other researchers, the inclusion of Rhodiola rosea in the diet of poultry promotes an increase in the content of globulin and globulin fractions in the blood, which suggests an increased effect on metabolism[10].

Markers of the liver and kidney status - amylotransferases (ALT and AST) and creatinine - were within the physiological parameters, indicating the absence of toxicity of the studied complexes of phytogenic origin and their substitute (Aurol).

During the experiment on young quails, the cost of mixed fodder (BD) for the first period in the control group amounted to 26.25 rubles/kg; taking into account the cost of the additive in the 1st experimental group the cost was 26.84 rubles /kg, in the 2nd experimental group - 26.95, in the 3rd experimental group - 36.82 rubles/kg. In the second period of cultivation, the cost of 1 kg of feed amounted to 21.92 rubles /kg in the control group, in the 1st experimental group - 22.63, in the 2nd experimental group - 23.11, in the 3rd experimental group - 32.87 rubles /kg. The cost of realization of 1 kg of quail meat was 536 rubles.

The economic effect is calculated by the sum of costs for feed and additives and the difference with the cost of obtained products. During the growing period, the conditional economic effect per 1 head was higher in the 1st experimental group by 6.74 rubles, in the 2nd experimental group - by 11.65 rubles. In the 3rd experimental group, it was negative due to the high cost of additives compared to the control (see Table 5).

To study egg production of quail laying hens, the experiment was continued up to 107 days of age (under the same housing and feeding conditions).

Quail laying hens receiving bio-additives had a live weight higher than the control group by 9.58 g ($p \leq 0.05$) or 5.3%, 13.56 g ($p \leq 0.001$) or 7.7% in the 1st experimental group, and 21.36 g ($p \leq 0.001$) or 11.9% in the 3rd experimental group by 64 days of age.

Egg laying by laying hens of the 2nd and 3rd experimental groups began at the age of 63 days, in the 1st experimental and control groups - 65. The most eggs for 43 days (average per 1 head) at the end of the experiment were obtained from laying hens of the 2nd experimental group - 28,80 pieces, or more than in the control group by 31,7%, 3rd experimental group - 26,52 pieces, more than in the control group by 21,3% (see Table 6).

Positive effect on egg production of quail laying hens, quality and weight of eggs was noted when including rhodiola rosea and arabinogalactan in their diets, which is consistent with the data of other researchers [15].

Average egg weight and egg production are the main indicators of productivity of quail laying hens. By the weight of one egg young quails of the 2nd experimental group surpassed the control group by 0.63 g ($p \leq 0.001$), or 5.7%, the 1st experimental group - by 0.15 g ($p \leq 0.05$), or 1.3%, the 3rd experimental group - by 0.25 g ($p \leq 0.05$), or 2.3%, by the intensity of egg production - by 14.96; 14.26 and 5.31%, respectively.

Табл. 5. Экономическая эффективность выращивания молодняка перепелов при убое в возрасте 64 дня

Table 5. Economic efficiency of growing young quails at slaughter at the age of 64 days

Indicator	Group			
	control	experimental		
		1st	2nd	3rd
Cost of fodder including bio-additives spent per 1 head, rubles	48,88	46,59	42,91	60,23
Cost of bio-additives, % of feed cost	–	3,14	5,17	34,38
Cost of production, rubles	66,84	71,29	72,52	75,20
Economic effect, rubles	17,96	24,70	29,61	14,97
± to the control group, rubles	–	+ 6,74	+11,65	–2,99

[10] *Vakhrusheva T.I.* Biochemical and morphological indices of blood of chickens under the influence of meal levzea, rhodiola rosea and enterophar // Bulletin of Krasnoyarsk State Agrarian University, 2014, N 6, pp. 206-210.

Табл. 6. Яичная продуктивность несушек перепелов
Table 6. Egg productivity of quail laying hens

Indicator	Group			
	control	Experimental		
		1st	2nd	3rd
Number of laying hens, heads	42	37	30	27
Live weight of laying hens at the age, g at 34 days	77,51 ± 2,67	78,34 ± 1,35	85,24 ± 2,74*	86,32 ± 2,78*
at 64 days	179,57 ± 2,72	189,05 ± 2,73*	193,13 ± 2,11***	200,93 ± 2,91***
Eggs obtained, pcs. total	918	839	864	716
per laying hen	21,86	22,68	28,80	26,52
Egg-laying intensity, %	52,02	52,72	66,98	61,67
Average egg weight, g	11,15 ± 0,15	11,63 ± 0,13*	11,78 ± 0,21***	11,52 ± 0,14*
Egg mass obtained, kg	0,244	0,264	0,339	0,306
Feed costs, kg: total	1,156	1,547	1,605	1,734
for 10 eggs	0,529	0,682	0,557	0,653

Feed costs for the production of 10 eggs in the experimental groups were higher compared to the control: in the 1st group by 28.9%, in the 2nd group by 5.3, in the 3rd group by 23.4%. However, these indicators were compensated by obtaining higher egg mass from quail laying hens in relation to the control.

Biochemical composition of egg white from laying hens of the 1st experimental group was characterized by a decrease in dry matter, fat and ash, in the 2nd experimental group was at the same level as the control (except for a decrease in ash residue), in the 3rd experimental group there was an increase in the level of fat (see Table 7).

The composition of yolk and egg shell was in all groups without significant differences.

Inclusion of bio-additives in the diets of experimental groups had no effect on egg shell

Табл. 7. Биохимические показатели яиц несушек перепелов, %
Table 7. Biochemical parameters of quail laying eggs, %

Indicator	Group			
	control	experimental		
		1st	2nd	3rd
Content in egg-white				
Dry matter	13,18 ± 0,14	12,16 ± 0,04*	13,14 ± 0,08	12,99 ± 0,08
Protein	12,32 ± 0,14	12,04 ± 0,31	12,33 ± 0,08	12,52 ± 0,11
Fat	0,097 ± 0,004	0,083 ± 0,003*	0,103 ± 0,002	0,113 ± 0,004 *
Ash	0,733 ± 0,004	0,710 ± 0,007*	0,713 ± 0,01*	0,693 ± 0,029*
Content in yolk				
Dry matter	38,96 ± 0,28	38,72 ± 0,45	39,12 ± 0,34	39,37 ± 0,45
Protein	18,94 ± 2,20	19,13 ± 1,48	19,26 ± 2,13	19,14 ± 3,38
Fat	19,25 ± 0,40	19,01 ± 4,01	19,22 ± 1,88	19,25 ± 3,45
Ash	0,77 ± 1,23	0,66 ± 0,06	0,68 ± 0,09	0,67 ± 0,06
Calcium	0,32 ± 0,09	0,35 ± 0,12	0,32 ± 0,09	0,31 ± 0,08
Content in egg shell				
Dry matter	59,20 ± 3,27	55,57 ± 12,10	54,39 ± 12,30	55,27 ±15,16
Ash	45,34 ± 2,60	42,29 ± 6,29	42,62 ±3,44	42,61 ± 5,36
Calcium	19,56 ± 7,11	18,13 ± 9,95	20,24 ± 10,47	12,85 ±9,40
Phosphorus	0,37 ± 0,08	0,34 ± 0,16	0,35 ± 0,12	0,34 ± 0,12

Табл. 8. Экономическая эффективность производства яиц в среднем на одну несушку перепелов
Table 8. Calculation of the economic efficiency of egg production on average per quail laying hen

Indicator	Group			
	control	experimental		
		1st	2nd	3rd
Realization value of edible eggs, rubles	96,18	99,79	126,72	116,69
Feed cost including bio-additives, rubles	25,34	35,01	37,09	57,00
Economic effect, rubles	70,84	64,78	89,63	59,69
% to the control group	–	–6,0 6	+18,79	–11,14

thickness, fluctuations in all groups were within 0.21-0.22 mm.

Economic effect is calculated similarly to the first period of experience. It was obtained only from laying hens of the 2nd experimental group - profit per one bird by 18.79% more than from laying hens of the control group, at the cost of 10 eggs 44 rubles for the period of the experiment (see Table 8).

CONCLUSIONS

1. Inclusion of complex phytogenic bio-additives No. 1 and 2 in the diet of the 2nd and 3rd experimental groups of young quails during their growing period from 5 to 64 days of age contributed to a decrease in feed consumption by 21, 8 and 25.1%, live weight 191.98 and 198.25 g (in the control group - 178.42 g), or more by 7.6 and 11.1%, average daily live weight gain 2.9 and 3.0 g (in the control group -2.67 g), or higher by 8.6 and 12.4 g, respectively.

2. Slaughter yield of the carcasses from male quails on average per 1 head of the 2nd and 3rd experimental groups was higher than in the control group by 0.9 and 1.5%.

3. The best indicators of quail preservation were obtained in the 2nd experimental group - 94.3%. In the quails of the 2nd experimental group on the strengthening of biological status with the inclusion bio-additive No. 1 in the diet indicates an increase in the level of protein in the blood by 10.27 g/l, globulin by 5.51 g/l compared to the control group, by 11.17 and 7.71 g/l - with the 1st experimental group, by 8.00 and 4.62 g/l - with the 3rd experimental group, respectively.

4. There was no significant effect on the performance of young quails of the 1st experimental group receiving aqueous solution of arabinogalactan with aurole.

5. Phytogenic bio-additive No. 1 was economically favorable for young quails - conditional profit per 1 head was 11.65 rubles higher than the control group.

6. Egg production of quail laying hens of the 2nd and 3rd experimental groups began 2 days earlier than in the control and 1st experimental groups. The use of bio-additive No. 1 increased the weight of one egg compared to the control to 11.78 g (by 5.7%), egg mass yield per laying hen by 95 g, the intensity of egg production by 14.78%.

7. The lowest effect on egg-laying intensity and egg mass yield of quail laying hens among the experimental groups was caused by application of aqueous solution of arabinogalactan and aurole (1st experimental group).

8. The application of phytogenic bio-additive No. 1, consisting of powder of underground part of Rhodiola rosea and arabinogalactan, in the amount of 0.1% of the mass of mixed fodder, which contributed to the increase of conditional profit by 18.95% per 1 head from the level of the control group, was economically justified for young laying hens of quails.

9. Negative conditional economic effect was obtained due to low zootechnical indicators in the 1st experimental group and due to high cost of plant components in the 3rd experimental group.

10. Application of natural phytocomplexes of bio-additives in combination with arabinoga-

lactan to quails shows better results in comparison with synthetic preparation Aurole.

Recommendations for production

We recommend bio-additive No. 1, consisting of 93.0% of the underground part of Rhodiola rosea and 7.0% of arabinogalactan with the inclusion of 0.1% of the mass of mixed fodder, for introduction into fodder production during the cultivation and productive use of quails.

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РЕЗУЛЬТАТЫ ДОКЛИНИЧЕСКОГО ИСПЫТАНИЯ ОПЫТНОГО ФИТОПРЕПАРАТА ПРИ ОСТРЫХ РАССТРОЙСТВАХ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА ТЕЛЯТ

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Представлены результаты апробации фитопрепарата для профилактики и лечения желудочно-кишечных расстройств у телят. Методика доклинического испытания опытного образца фитопрепарата соответствовала ГОСТ Р 53434–2009 и руководству по проведению доклинических исследований лекарственных средств. Для доклинического исследования опытного препарата по принципу аналогов сформированы две группы лабораторных животных (беспородные белые мыши) по 10 грызунов в каждой группе в возрасте 2–5 мес. В опытной группе животным выпаивали разработанный препарат (водные экстракты плодов шиповника – *surrexit coxis*, плодов черемухи – *cerasis fructus*, цветов ромашки – *pyrethri flores*, корневища амаранта – *amaranthus rhizomatis)*. Доза составила 3 мл/сут на одно лабораторное животное в течение 10 дней. Рацион кормления не меняли (зерно, овощи, сено). Получены положительные данные о физиологических показателях (температура, пульс, дыхание, мочеиспускание, состояние фекалий, двигательная активность животных), которые соответствовали физиологическим нормам для белых мышей. Препарат не обладает токсичностью для организма животных (отсутствие токсической дистрофии в органах животных); уровень радиации был в пределах нормативного значения по цезию 137 (440 Бк/кг) и стронцию 90 (110 Бк/кг) соответственно; Ph составил 6,7 ед. Фитопрепарат является благоприятной средой для желудочно-кишечного тракта животных и обладает антибактериальным эффектом, достоверно повышает число лимфоцитов на 23,8%, гематокрита на 8,6%, гемоглобина на 40% ($p < 0,01$) в крови опытных животных. Фитопрепарат по классификации, принятой в настоящее время Всемирной организацией здравоохранения, можно отнести к группе малотоксичных веществ и по степени токсичности – к IV классу опасности (вещества малоопасные). Препарат рекомендован для клинических испытаний на молодняке крупного рогатого скота.

Ключевые слова: фитопрепарат, лабораторные животные, доклинические исследования, гематология, патоморфология

RESULTS OF PRECLINICAL TESTING OF AN EXPERIMENTAL PHYTOPREPARATION FOR ACUTE GASTROINTESTINAL DISORDERS IN CALVES

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The results of approbation of a phytopreparation for prevention and treatment of gastrointestinal disorders in calves are presented. The methodology of preclinical testing of the phytopreparation prototype was in accordance with GOST R 53434-2009 and guidelines for preclinical testing of medicines. Two groups of laboratory animals (mongrel white mice) with 10 rodents in each group aged 2-5 months were formed for preclinical study of the experimental drug according to the principle of analogs. The developed preparation (aqueous extracts of rosehip fruit – *surrexit coxis*, cherry fruit – *cerasis fructus*, chamomile flowers – *pyrethri flores*, amarantus rootstock – *amaranthus rhizomatis*) was administered to the animals in the experimental group. The dose was 3 ml/day per laboratory animal for 10 days. The feeding ration remained unchanged (grain, vegetables, hay). Positive data on physiological indices (temperature, pulse, respiration, urination, fecal condition, motor activity of animals) were obtained, which corresponded to physiological norms for white mice. The preparation

has no toxicity for animal organism (absence of toxic dystrophy in animal organs); radiation level was within the normative value for cesium 137 (440 Bq/kg) and strontium 90 (110 Bq/kg), respectively; Ph was 6.7 units. The phytopreparation is a favorable environment for the gastrointestinal tract of animals and has an antibacterial effect, significantly increases the number of lymphocytes by 23.8%, hematocrit by 8.6%, hemoglobin by 40% ($p < 0.01$) in the blood of the experimental animals. According to the classification currently adopted by the World Health Organization, the phytopreparation can be attributed to the group of low-toxic substances and according to the degree of toxicity – to the IV class of hazard (substances of low hazard). The drug is recommended for clinical trials on young cattle.

Keywords: phytopreparation, laboratory animals, preclinical studies, hematology, pathomorphology

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INTRODUCTION

At present, the world pharmaceutical market is characterized by a trend towards the use of drugs of natural origin for the prevention and treatment of various human and animal diseases, including diseases of the digestive organs (gastritis, enteritis of various etiologies) of cattle. Practicing veterinarians enjoy popularity with medicines based on plant raw materials. There are data on the use of complex medicines derived from plant components, the effect of which is associated with the synergistic action of their constituent biologically active substances [1–5].

In order to conduct preclinical drug trials - the study of biological and pharmacological activity of a drug in *in vitro* and *in vivo* studies - the requirements of the GLP (Good Laboratory Practice) system are followed in the world practice [2–5].

An important criterion before preclinical tests is the principle of inadmissibility of carrying a number of pathogenic and opportunistic agents of infectious and invasive nature: viruses, bacteria, parasites. Many countries have developed standards for different categories of animal health quality. They include a list of pathogens, the carriage of which is prevented. The higher the quality category of the animal, the larger the list of inadmissible agents [6–8].

In this regard, it is important to confirm their safe effect on the animal body and positive impact on the physiological status of animals before clinical trials of newly developed drugs, means, BAS.

The purpose of the research is to study the safety of the developed phytopreparation of natural origin on laboratory animals.

MATERIALS AND MTHODS

The studies were conducted in the laboratory of the Research Institute of Veterinary Medicine of Eastern Siberia - branch of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences taking into account the developed rules[1].

The experimental sample of the preparation is an aqueous solution of brown color, the composition of which included phyto raw materials: rosehip fruit - *surrexit coxis*, bird cherry fruit - *cerasis fructus*, chamomile flowers - *pyrethri flores*, root of amaranth - *amaranthus rhizomatis*[2] [1, 2].

Preclinical tests of the prototype drug were conducted in accordance with the requirements

[1]Order of the Ministry of Health of the USSR "Rules for Conducting Work Using Experimental Animals" No. 755 of 12.08.1977.
[2]*Lysnyansky M.V.* Medicinal plants of the millennium // Provizor, 2004, N 19, pp. 27–37.

of GOST[3] and guidelines for preclinical studies of medicines[4].

Studies on radioactive substances of plant raw materials for cesium 137 and strontium 90 were carried out on the Progress device. Ph of the preparation was measured using a PH-meter multi-precision PH-410. Antibacterial efficacy of the preparation was determined by drop and disk method with observation of the zone of growth retardation of daily culture of *E. coli* [9 – 12].

Two groups of laboratory animals (white mice) of 10 rodents in each group at the age of 2-5 months weighing 25-32 g were formed to test the experimental preparation according to the principle of analogs. In the experimental group, the animals were given the developed preparation, which was freely available to the mice (the dose was 3 ml/day per one individual). In the control group, the animals were not given the phyto preparation. Feeding ration in all animals was not changed (grain, vegetables, hay).

Clinical status of animals (temperature, pulse, respiration, examination of visible mucous membranes, condition of feces, animal behavior) was evaluated daily in animals of these groups by conventional methods[5]. Feeding of animals was carried out in the usual way (vegetables, grain, hay).

To evaluate blood parameters during the drug application in rodents of experimental (n = 10) and control groups (n = 10) on the 10th day of the experiment blood samples were taken in Vacutainer vacuum tubes with K_2EDTA. A hematological analyzer PCE 90 Vet with a set of special reagents[6] was used for hematological analysis.

To assess the general toxic effect, the pathomorphologic picture of internal organs of experimental animals was taken into account using the method of isolated organ extraction with macroscopic experimental analysis[7] [13].

RESULTS AND DISCUSSION

The preclinical study stages of the developed preparations provide an opportunity to identify the advantages and disadvantages of both the constituent components of the preparation and the complex composition of phyto raw materials.

The pharmaceutical compatibility of the ingredients was determined at the beginning of the experiment[8]. For this purpose, the raw materials according to the formulation were dissolved in distilled water mixed with 40% lactic acid in the ratio of 6 : 1. It was found that all preparations were well dissolved in this mixture, forming a brown-colored solution.

According to the results of radiological analysis of phytopreparation the level of investigated elements (cesium-137, strontium-90) was within the permissible level (440 Bq/kg and 110 Bq/kg, respectively). Ph of the preparation was 6.7 units, which is a favorable environment for the gastrointestinal tract of animals.

Evaluation of physiological parameters of animals in preclinical studies showed that on the first day the experimental animals drank the phytopreparation with caution, the average dose was 1.0-1.5 ml per one individual. On the second day the solution was drunk well, at a dose of 3 ml per one individual.

Physiological parameters of temperature, heart rate and respiration rate in experimental animals during the whole period of the experiment were within normal limits. The state of the gastrointestinal tract (fecal consistency) also corresponded to normal homeostasis. No chang-

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es in the mucous membranes and hair coat of the experimental animals were observed. Motor activity in animals of the experimental group was more pronounced. We believe that this fact is explained by the content of natural sugar in amaranth rhizome, respectively, at its breakdown in the body more energy resource is formed.

By evaluating morphological indices of blood of experimental animals it is possible to objectively assess the physiological state of the animal organism. Data of hematological parameters are shown in the table.

The result of the general blood analysis showed that the use of phyto preparation has a favorable effect on the content of the main blood parameters - increase in the number of lymphocytes by 23.8%, hematocrit by 8.6%, hemoglobin by 40%. By the number of monocytes and eosinophils the difference between the animals of the studied groups had no reliable differences. The number of granulocytes was significantly higher in mice in the control group ($p < 0.05$). The shift in leukocytic formula was registered in rodents of the experimental group - the number of lymphocytes was increased and the number of granulocytes was decreased. Differences in the hemostasis system are noted in the number of platelets, their content is 4.3% higher than in animals of the control group. Blood color index, which allows to determine the degree of saturation of erythrocytes with hemoglobin, in experimental rodents was 20.8%. This corresponds to the normal physiological value. When studying the bactericidal efficiency of the preparation, the average level of the zone of growth retardation of daily culture of *E. coli* (1.5-2.9 mm) was observed.

At the end of the experiment the rodents from the experimental group were selectively ($n = 6$) euthanized (since no death of laboratory animals was observed). At estimation of morphologic picture of the experimental animals the parenchymatous organs and gastrointestinal tract had no pathomorphologic changes corresponding to toxic changes. In particular, in the liver - the most sensitive organ to the action of toxic substances

Сравнительная характеристика гематологических показателей белых мышей после дачи препарата через 10 дней применения ($M \pm m$; $n = 20$)
Comparative characteristics of hematological parameters of white mice after giving drugs after 10 days of use ($M \pm m$; $n = 20$)

Hematologic indicator	Regulatory range	Group of animals	
		experimental	control
Leukocytes (WBC, 10^9 g/l)	1,8–10,7	9,8 ± 1,70	9,4 ± 1,10
Lymphocytes (LYM, 10^9 g/l)	1,0–9,8	8,0 ± 1,25	6,1 ± 0,5*
Monocytes, eosinophils (MIN, 10^9 g/l)	0–1,1	0,1 ± 0,07	0,3 ± 0,21
Granulocytes (GRA, 10^9 g/l)	0,1–4,1	0,7 ± 0,34	0,3 ± 0,55
Lymphocytes (LYM, %)	55,8–91,6	89,8 ± 6,65	72,2 ± 5,11
Monocytes, eosinophils (MON, %)	1,5–6,2	1,7 ± 0,65	4,6 ± 3,43*
Granulocytes (GRAN, %)	6,6–38,9	1,9 ± 8,42	8,5 ± 0,75*
Erythrocytes (RBC, 10^{12}/l)	6,36–9,42	6,5 ± 1,21	5,2 ± 0,32
Hemoglobin (HGB, g/l)	110–151	135,0 ± 24,50	95,0 ± 25,0**
Hematocrit (HCT, %)	35,1–45,4	37,73 ± 4,65	29,1 ± 3,41*
Average erythrocyte volume (MCV, fl)	45,4–60,3	65,0 ± 0,75	62,0 ± 0,75
Average hemoglobin content in the erythrocyte (MCH, pg)	14,1–19,3	18,5 ± 1,12	14,2 ± 1,15
Average hemoglobin concentration in the erythrocytes (MCHC, g/l)	302–342	311,0 ± 27,10	295,0 ± 22,52
Platelets (PLT, 10^9/l)	592–641	564,1 ± 76,21	588,0 ± 48,10

*$p < 0,05$.
**$p < 0,01$.

- macroscopic picture corresponded to the norm (the organ parenchyma was homogeneous, no structural and dystrophic changes were found in the organ).

CONCLUSION

Preclinical tests with application of experimental and comparative analysis methods, hematological and pathomorphological methods of the developed experimental sample of phytopreparation (rosehip fruit - *surrexit coxis*, bird cherry fruit - *cerasis fructus*, chamomile flowers - *pyrethri flores*, amaranth root - *amaranthus rhizomatis*) were carried out. Application of the phytopreparation at a dose of 3 ml per one individual administered per os to laboratory animals had a positive effect on their physiological state and did not adversely affect their behavior. The preparation does not possess toxicity for animal organism (absence of toxic dystrophy in animal organs); it is not radioactive (MAC within the normative value for cesium (137) and strontium (90)); Ph is 6.7 units; it has antibacterial effect; it significantly increases the number of lymphocytes by 23.8%, hematocrit by 8.6%, hemoglobin by 40% ($p < 0.01$) in the blood of the experimental animals.

Thus, guided by the classification currently adopted by the World Health Organization, the developed phytopreparation can be attributed to the group of low-toxic substances, and, in accordance with GOST 12.1.007-76, according to the degree of toxicity - to the IV class of hazard (substances of low hazard). The drug is recommended for clinical trials on young cattle.

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БИОРЕСУРСНЫЙ ПОТЕНЦИАЛ МЕДОНОСНЫХ УГОДИЙ В ХОДЕ СУКЦЕССИИ ЛЕСНОЙ РАСТИТЕЛЬНОСТИ

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Представлены результаты исследований медоносных угодий на лесных и нелесных землях лесного фонда Кемеровской области. Проведена оценка медоносных ресурсов по категориям земель лесного фонда и биоресурсного потенциала угодий для медосбора в процессе сукцессии лесной растительности. Изучение видового состава медоносных растений выполняли в 2018–2022 гг. на различных категориях земель. Исследования проводили в соответствии с общепринятыми методами проведения научно-исследовательских работ в пчеловодстве. Учет медоносных растений проводили на трансектах и учетных площадках, используя методику таксации леса. Установлено, что в процессе сукцессии лесной растительности изменяются микроклиматические и лесорастительные условия медоносных угодий. На вырубках высокой медовой продуктивностью отмечены акация желтая, ивовые, жимолость татарская и съедобная, малина лесная и представители травянистых фитоценозов – дягиль сибирский, кипрей узколистный. Медовая продуктивность угодий, не покрытых лесной растительностью, на вырубках, в таежных лесах составляет 229,4 кг/га. На лесных землях, не покрытых лесной растительностью в радиусе пасеки, на площади 414 га биоресурсный потенциал угодий для медосбора составил 68 464 кг. Медовый запас 88 600 кг позволяет содержать на учетной территории 738 пчелиных семей. Полученные достоверные научные сведения с практической стороны необходимы для корректировки лесных планов территорий и для своевременного планирования и организации территории пасек.

Ключевые слова: медоносные угодья, биоресурсный потенциал, лесные и нелесные земли, медовый запас, пчелиные семьи

BIORESOURCE POTENTIAL OF HONEY-MAKING LANDS DURING THE SUCCESSION OF FOREST VEGETATION

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The results of the studies of honey-making lands on forest and non-forest lands of the forest fund of the Kemerovo region are presented. The assessment of honey- making resources by categories of forest lands for honey collection and bioresource potential of lands for honey collection in the process of succession of forest vegetation was carried out. The study of the species composition of honey plants was carried out in 2018–2022 on various categories of lands. The research was carried out in accordance with the generally accepted methods of conducting research in beekeeping. The accounting of honey plants was carried out on transects and discount areas using the methodology of forest taxation. It is established that microclimatic and forest-growing conditions of honey- making lands change during the succession of forest vegetation. Siberian pea-tree, willow family, Tartarian honey-

suckle and edible honeysuckle, forest raspberry and the representatives of herbaceous phytocenoses - Siberian garden angelica, rosebay willowherb - are noted on clearings with high honey productivity. Honey productivity of the lands not covered with forest vegetation, on clearings, and in taiga forests is 229.4 kg/ha. The bioresource potential of the lands for honey collection amounted to 68,464 kg on forest lands not covered with forest vegetation within the radius of the apiary on the area of 414 hectares. The honey stock of 88,600 kg makes it possible to keep 738 bee colonies on the registration plot. The obtained reliable scientific information from the practical side is necessary for the adjustment of forest plans of the territories and for the timely planning and organization of the territory of apiaries.

Keywords: honey-making lands, bioresource potential, forest and non-forest lands, honey stock, bee families

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INTRODUCTION

Significant areas are occupied by honey-making lands which are characterized by a rich species diversity of honey-bearing flora on the territory of the Russian Federation. Among natural lands, forest lands are of interest as pastures for bees. Plants growing under the forest canopy and on forest edges, as well as on the lands not covered with forest vegetation and non-forest lands are characterized by their honey-bearing value. The composition of honey-bearing resources, their ecological and biological properties depend on natural and climatic factors. In different geographical zones, forest conditions are formed, which affect the growth and development of melliferous plants, physiological processes, in particular, the intensity of secretion of biological substances - nectar and pollen [1–3]. During the beekeeping season, the area where the apiary is based is characterized by peculiarities of honey collection conditions. The species diversity of honey bee flora, ecological and biological features and flowering dates depend on the microclimate: light, moisture, temperature and soil nutrition [4, 5].

Determination of the bioresource potential of forest lands is based on observations and registration of wild honeybees in all components of forest phytocenosis [6-8]. Forest pastures for bees are a source of valuable bee products, contribute to the conservation of natural populations in forests by pollination of entomophilous plants by bees[1] [9]. Therefore, clarification of honey productivity of the main honeybees of forest lands is of great importance for timely planning and organization of apiaries for the beekeeping season.

The purpose of the research is to identify honey bearing resources by categories of forest land for honey collection and to determine the bioresource potential of the lands for honey collection in the process of succession of forest vegetation.

MATERIAL AND METHODS

The territories of the Kemerovo region were chosen as the objects of the study:

Gornaya Shoria, Barzasskaya taiga (Kemerovo district), Chebulinsky, Izhmorsky, Krapivinsky districts of the Kemerovo region. Specification of the species composition of honey-bearing

[1]*Do Van Thao* Integrated assessment of honey-bearing plants of birch forests and bioresource potential of sites in the Baltic-Belozersky taiga forest region: Extended abstract of candidate's thesis, St. Petersburg, Russia, 2020. 24 c.

plants was carried out in 2018-2022 on different categories of lands. Registration of honey-bearing plants was carried out on transects and discount areas, using the methodology of forest taxation. Observations and collection of the data necessary to clarify the honey productivity of the main species were carried out according to the generally accepted methodology [10].

Bioresource potential was determined for the forest lands of the apiary of the Kemerovo region by the area of the radius of the effective flight radius of bees to obtain productive honey collection. Honey stock is 0.625 of the bioresource potential, as honey from the nectar of the main honey bees contains about 80% of sugars and uses half of the forest resource potential for honey collection [11].

RESULTS AND DISCUSSION

Observations have shown that after logging and forest fires in taiga conditions, the main valuable honey bee, great willowherb (*Epilobium angustifolium* L.), grows actively for the first 5-7 years and secretes nectar intensively. During the flowering period, the honey weight in the control hive of the apiary reached 18 kg.

Scientists have established that practically all forest areas of Siberia belong to the raspberry-willowherb type of honey collection. According to the assessment of the Kemerovo region lands, great willowherb is in the first place among other forest honeybees in terms of distribution area and honey-bearing value. However, the variation of control hives on the stands of willowherb from 400 g to 5-6 kg in the eastern districts of the region is associated with forest conditions [12].

The results of the studies of I.D. Samsonova [13] on morphobiological parameters and biological productivity of great willowherb allowed to reveal peculiarities in the process of growth, development of honey bee and nectar secretion. Comparing the density of species and flowering intensity on forest edges and burned areas, the advantage in the studied indicators on the lands after forest fires was established. This is due to the favorable ecological conditions of the burned areas, where there is no competition of other species for light, moisture and nutritional elements in the soil [13]. After 7 years, the potassium content in the soil decreases, which affects the nectar production of great willowherb. Honey bee thickets also actively develop, but at the same time they become less interesting for apiaries. The intensity of nectar secretion decreases, the control hive per day makes 0.5-1.8 kg of honey. Instead of willowherb, thickets of Siberian angelica (*Archangelica decurrens* Ledb.) and wood angelica (*Angelica sulvestris* L.) appear in the taiga. These mellifers provide honey yield of 10-14 kg per day. They grow for 10-16 years, and nectar secretion in these plants does not stop, as in willowherb. Felling in mature taiga stands creates favorable lighting conditions for honey-bearing shrubs Siberian pea-tree (*Saragana arborescens* Lam.), Tartarian honeysuckle (*Lonirera tatarika* L.), edible honeysuckle (*Lonirera edulis* Turcz.), European red raspberry (*Rubus idaeuns* L.), Tatarian dogwood (*Cornus alba* L.). In good light, these shrubs abundantly release nectar, the yield per day reaches 16.7 kg of honey from Siberian pea-tree [13]. These plants release nectar for 20-26 years. In 20-26 years after deforestation of taiga or forest fires, honeybee plants are replaced by poisonous perennial herbs: aconite (*Aconitum septentrionale* Koelle), bee larkspur (*Delphinium elatum* L.), field larkspur (*Delphinium consolida* L.), lance-shaped groundsel (*Senecio sagittatus* L.).), thickets of poplar aspen (*Populus tremula* L.) and white birch (*Betula alba* L.), rarely coniferous trees. At this time, there are no honey-bearing plants in the taiga and bees die en masse while collecting nectar and pollen from poisonous plant species [14]. In this regard, if the apiaries are not relocated from such an area, the existing forest conditions can negatively affect the vital activity of bee families. For a more accurate assessment of the forage base, forest surveys were conducted in the Kemerovo region (see Table 1).

As a result of the observations made in the chern fir-aspen taiga in forest glades, as well as on the lands not covered with forest vegetation (clearings and garlands), abundant growth of honey-bearing species is noted. Along the banks of mountain rivers there are honey-bearing for-

mations consisting of willow trees and shrubs. Large thickets of Siberian pea-tree are found on open slopes.

European red raspberry is widespread in 10-year thickets. Red currant (Ribes rubrum L.) adapts to favorable forest conditions in the lignified forests. There are valuable spring mellifers: Siberian mountain ash (*Sorbus sibirica* Hedl.), common bird cherry (*Padus avium* Mill.), cranberry tree (*Viburnum opulus* L.), Tatarian honeysuckle (*Lonicera tatarica* L.) and other shrubs. On forest glades and non-forested lands (meadows) herbaceous vegetation reaches its maximum height in summer. Among herbaceous mellifers, early-flowering species are widespread in taiga conditions: Siberian Trout Lily (*Erythronium sibiricum* Kryl.), Irkutsk anemone (*Anemonea ltaic* Fisch. Ex C.A. Mey.), Hairy Lungwort (*Pulmonaria mollissima* A. Kerner). There are also nectar-pollenos with a prolonged flowering period - woodland beakchervil (*Anthris cussylvestris* L.); late-flowering ones - Saussurea latifolia (*Saus-surea latifolia* Ledeb.), forest thistle (*Cirsium heterophyllum* L. All.) and other. In the first years on the burned areas great willowherb (*Epi-lobium angustifolium* L.), Greek valerian polemonium (*Polemonium ceruleum* L.), woodland angelica (*Angelica sylvestris* L.), Siberian angelica (*Archangelica decurrens* Ledb.) and summer honey bees - white clover (*Trifolium repens* L.) and pink clover (*Trifolium repens* L.) develop.), Siberian angelica (*Archangellica decurrens* Ledb.) and summer honey bees - white clover (*Trifolium repens* L.) and Swedish clover (*Trifolium hybridum* L.), which are rarely visited by bees.

Based on the data on the species composition and the areas of honey-bearing plants within the effective flight radius of bees, the honey balance of apiaries in the Kemerovo region was compiled (see Table 2).

Studies of the apiary area within the effective flight radius of bees have shown that 414 ha of the logged land in the Kemerovo region annually without sowing and maintenance release 68,464 kg of nectar (see Table 2). Bees deliver 50% of this bioresource potential of the lands to the hives. The remaining nectar cannot be utilized by bees due to bad weather, due to nectar collection by other insects, and not all plants are

Табл. 1. Медовая продуктивность не покрытых лесной растительностью земель Кемеровской области

Table 1. Honey productivity of non-forested lands in the Kemerovo region

Honey plant	Occurrence, %	Honey productivity, kg/ha
Clearings		
Siberian pea shrub (*Caragana arborescens* Lam.)	20	70
Tartarian honeysuckle (*Lonicera tatarica* L.)	1	2
European red raspberry (*Rubus idaeus* L.)	1,5	2,1
Willows of all kinds (*Salix* L.)	13	26
Common bird cherry (*Prunus padus* L.)	5	2
Siberian angelica (*Archangelica decurrens* Ledb.)	10	40
Forest glades		
Woodland angelica (*Angelica sylvestris* L.)	5	15
Greek-valerian polemonium (*Polemonium coeruleum* L.)	5,5	3,3
Common dandelion (*Taraxacum officinale* Wigg.)	21	21
White clover (*Trifolium repens* L.)	3	3
Great willowherb (*Epilobium angustifolium* L.)	15	45
Total		229,4

Табл. 2. Биоресурсный потенциал угодий и медовый баланс пасеки
Table 2. Bioresource potential of the lands and honey balance of the apiary

Honey collection period	Honey plant	Area, ha	Bioresource potential, kg	Honey supply, kg
	Forest lands (clearings)			
I (spring)	Goat willow (sallow) (*Salix caprea* L.)	108	25 920	16 200
	Siberian pea shrub (*Caragana arborescens* Lam.)	49	27 440	17 150
	European mountain ash (*Sorbus aucuparia* L.)	25	400	250
II (early summer)	Common bird cherry (*Padus avium* Mill.)	166	5312	3320
	Currant (*Ribes* L.)	41	4592	2870
III (summer)	European red raspberry (*Rubus idaeus* L.)	25	4800	3000
	Non-forest lands (meadow)			
I–II (spring and early summer)	Meadow mixed herbs	68 (20–30%)	3264	2040
III (summer)	Siberian angelica (*Archangelica decurrens* Ledb.)	32	11 776	7360
	Dissected cow parsnip (*Heracleum dissectum* Ledeb.)	31	5952	3720
	Great willowherb (*Epilobium angustifolium* L.)	43	24 080	15 050
	White and pink clover (*Trifolium repens* L., *Trifolium hubridum* L.)	44	7040	4400
	Dropwort (*Filipendula vulgaris* Moench.)	28	448	280
IV (late summer)	Melancholy thistle (*Cirsium heterophullum* (L.) Hill	40	11 520	7200
	Broad-leaved saussurea (*Saussurea latifolia* Ledeb.)	16	3072	1920
	Canada thistle (yellow thistle) (*Cirsium setosum*Willd.)	32	6144	3840
Total		748	141 760	88 600

visited by bees for nectar collection. According to our calculations, the honey stock on forest lands in this area reaches 88,600 kg. With apiary productivity of 30 kg of commercial honey from one family and 90 kg of forage honey, it is possible to keep 738 bee families and receive 22 tons of commercial honey only in the clearings of the Kemerovo region.

Taking into account that significant areas of forest felling only for one year give an opportunity to increase the number of bee families, the forest is cut down annually and almost as much is burned, then on these areas the number of bees can be increased by 7 times, the density of bee families can be increased to 51 hives/km^2.

CONCLUSIONS

1. In the process of succession of forest vegetation, microclimatic and forest conditions of honey lands change, which are reflected in their honey value.

2. Siberian pea shrub, willow family, Tatarian and edible honeysuckle, European red raspberry and representatives of herbaceous phytocenoses - Siberian angelica and great willowherb - are characterized by high honey productivity in clearcuts. Honey productivity of non-forested lands is 229.4 kg/ha.

3. The bioresource potential of the land for honey collection amounted to 68,464 kg on forest land not covered with forest vegetation within the apiary radius on the area of 414 ha. The

honey stock of 88,600 kg allows to keep 738 bee families on the discount area.

4. Reliable data on floristic composition of honey-bearing vegetation, honey reserve of forest lands, on abundance and territorial distribution of honey-bearing resources within different plant communities are necessary for adjustment of forest plans of the territories, development of forest exploration projects, for timely planning and organization of apiary territories.

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СРАВНЕНИЕ ЭФФЕКТИВНОСТИ ПРИМЕНЕНИЯ ПРЕПАРАТОВ ЗАМЕСТИТЕЛЬНОЙ ТЕРАПИИ ПРИ БРОНХОПНЕВМОНИИ ТЕЛЯТ

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Представлены результаты исследований методов лечения бронхопневмонии молодняка крупного рогатого скота. Отмечено, что заболевание связано с воздействием ряда различных этиологических условий (понижением температуры воздуха, увлажнением воздуха, а также его загрязнением и т.д.) на организм животного. Бронхопневмония телят регистрируется на животноводческих комплексах в холодное и сырое время года как сезонное заболевание. В связи с этим разработка эффективных схем лечения данной патологии является актуальной задачей. Изучена эффективность применения препаратов заместительной терапии при лечении бронхопневмонии телят. Исследование проведено в хозяйстве Алтайского края в осенний период. Для реализации данного опыта по принципу аналогов были сформированы две равные группы телят (опытная и контрольная) по 5 гол. в каждой. В схему лечения обеих групп входили препараты Ресфлор и Локсик 2%. Телята контрольной группы в качестве препарата заместительной терапии получали ВитОкей, опытной – Витам. Во время эксперимента проведена оценка клинических признаков и морфологический анализ крови. При клиническом исследовании телят отмечены следующие признаки: повышенная температура тела, хрипы, кашель, очаги притупления в легких в области передних и задних долей, апатичность, истечения из носа, потеря аппетита, эритропения (до $(4{,}6 \pm 0{,}2) \times 10^{12}$/л), лейкоцитоз (до $(16{,}5 \pm 0{,}6) \times 10^{9}$/л), повышение СОЭ (до 1,2 ± 0,1 мм/ч), нейтрофильный лейкоцитоз со сдвигом ядра влево и моноцитопения. К 5-м суткам лечения у телят опытной группы отсутствовали хрипы в легких, кашель, температура тела была в пределах нормы. На 7-е сутки отмечались повышение эритроцитов ($(6{,}4 \pm 0{,}3) \times 10^{12}$/л), снижение лейкоцитов ($(10{,}6 \pm 0{,}3) \times 10^{9}$/л) и СОЭ (0,4 ± 0,1 мм/ч). Применение витаминно-аминокислотного комплекса в комплексной терапии бронхопневмонии телят сокращает длительность и тяжесть течения заболевания.

Ключевые слова: ветеринария, бронхопневмония, телята, лечение, крупный рогатый скот, кровь

COMPARISON OF THE EFFECTIVENESS OF SUBSTITUTION THERAPY PREPARATIONS IN BRONCHOPNEUMONIA OF CALVES

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The results of research on the methods of bronchopneumonia treatment of young cattle are presented. It is noted that the disease is associated with the effect of a number of different etiological conditions (lower air temperature, air humidification, and air pollution, etc.) on the animal's body. Bronchopneumonia of calves is registered at livestock complexes in cold and wet seasons as a seasonal disease. In this regard, the development of effective treatment schemes for this pathology is an urgent task. The effectiveness of the use of substitution therapy preparations in the treatment of bronchopneumonia of calves was studied. The study was conducted at a farm in the Altai Territory in the autumn period. For realization of this experiment two equal groups (experimental and control) of 5 calves in each were formed according to the principle of analogies. The treatment regimen for both groups included Resflor and Loxic 2% preparations. Calves of the control group received VitOkey as a substitution therapy, the experimental group – Vitam. Clinical signs and blood morphologic analysis were evaluated during the experiment. Clinical examination of the calves showed the following signs: elevated body temperature, wheezing, coughing, foci of blunting in the lungs in the anterior and posterior lobes, apathy, nasal discharge, loss of appetite, erythropenia (up to $(4.6 \pm 0.2) \times 10^{12}$/l), leukocytosis (up to

(16.5 ± 0.6) × 10^9/l), increased ECR (up to 1.2 ± 0.1 mm/h), neutrophilic leukocytosis with a leftward shift of the nucleus, and monocytopenia. By the 5th day of treatment, the calves of the experimental group had no pulmonary rales, coughing, and the body temperature was within normal limits. On the 7th day, there was an increase in erythrocytes ((6.4 ± 0.3) × 10^{12}/l), a decrease in leukocytes ((10.6 ± 0.3) × 10^9/l) and ESR (0.4 ± 0.1 mm/h). The use of vitamin-amino acid complex in complex therapy of bronchopneumonia of calves reduces the duration and severity of the course of the disease.

Keywords: veterinary medicine, bronchopneumonia, calves, treatment, cattle, blood

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The authors declare no conflict of interest.

INTRODUCTION

One of the strategic objectives of the agricultural industry is to provide the population of the country with high-quality livestock products. The increase in production volumes is achieved, among other things, by accelerating metabolic processes in the organism of farmed animals. However, this leads to an increase in the risk of homeostasis disorders and the development of various pathologies in both adult cattle and young animals [1-3]. Diseases of respiratory organs occupy the second place among all pathologies in young farm animals after diseases of gastrointestinal tract [4, 5]. The most common among respiratory diseases is bronchopneumonia [6, 7]. Bronchopneumonia of calves is registered at livestock complexes in cold and wet seasons as a seasonal disease [8, 9]. It is a classical factor disease, which is caused by the combined effect of infectious pathogens and unfavorable environmental factors [10-12]. Diagnosis is made on the basis of clinical signs and blood tests [13-20]. In this regard, the development of effective treatment schemes for this pathology is an urgent task.

The purpose of the study was to investigate the effectiveness of substitution therapy drugs in the treatment of bronchopneumonia of calves.

MATERIAL AND METHODS

The study was conducted at the farm LLC "Michurinets" in the village of Altayskoye, Altai Territory, in the autumn period. For the experiment 10 calves of the Black-and-White breed of 3 months of age and live weight 102 ± 9.1 kg were selected. Bronchopneumonia was detected based on the clinical picture specific for this disease: increased body temperature, wheezing, coughing, foci of blunting in the lungs in the anterior and posterior lobes, apathy, nasal discharge, loss of appetite. All calves were in the same feeding and housing conditions. For the experiment the selected young cattle were divided into two groups: control and experimental. Calves of the control group were treated in accordance with the scheme of therapy used on the farm, including VitOkey, which was used at a dose of 2 ml/10 kg of animal body weight (once a day, intramuscularly, every 24 h). The treatment regimen of both groups included: antibacterial drug Resflor in a dose of 10 ml (once a day, subcutaneously, every 48 h); non-steroidal anti-inflammatory drug Loxic 2% in a dose of 2.5 ml/100 kg body weight of the animal (once a day subcutaneously every 24 h). In the treatment scheme of the experimental group the same therapy scheme was used, but VitOkey was replaced by Vitam at a dose of 3 ml/10 kg of animal body weight (once a day intramuscularly every 24 h).

VitOkey is a combined vitamin preparation for prevention and treatment of hypovitaminosis and diseases developing on their background in animals. VitOkey in 1 ml as active substances contains vitamin A (10 000 ME), vitamin D3 (2000 ME), vitamin E (10 mg), vitamin K (2), vitamin B1 (10), vitamin B2 (4), vitamin B6 (3 mg), vitamin B12 (10 mcg), nicotinamide (30 mg), calcium pantothenate (20), folic acid (0.2 mg), biotin (10 mcg), as well as auxiliary substances: Lactalbumin protein hydrolysate, nipagin, glucose, tween-80 and water for injection. Resflor is a medicinal product intended for treatment of diseases of bacterial etiology and management of inflammatory processes in cattle. As an active substance in 1 cm^3 the preparation contains 27.4 mg of flunixin meglumine (which corresponds to 16.5 mg of flunixin) and 300 mg of florfenicol, as excipients - N-methyl-2-pyrrolidone, propylene glycol, citric acid anhydride and polyethylene glycol. Loxik 2% is a medicinal product intended for treatment of inflammation of various etiologies in animals. 1 ml of Loxik 2% contains 20 mg of meloxicam as an active ingredient, as well as excipients (up to 1 ml). Meloxicam, included in the composition of the medicinal product, has pronounced anti-inflammatory and analgesic activity. Vitamin-amino acid complex Vitam refers to complex vitamin-containing products for farm animals. The preparation contains a complex of biologically active substances, thanks to which it optimizes metabolic processes in the body, normalizes blood formula, increases bactericidal activity of blood serum, has immunomodulatory and general biotonizing effect. As active ingredients, the preparation contains arginine hydrochloride (60 mg), lysine hydrochloride (60), isoleucine (17.3), leucine (52), methionine (13), phenylalanine (21.5), threonine (26), tryptophan (8.6), glutamine (86), valine (21.5), tyrosine (34.6), cystine hydrochloride (22.4), serine (21.5), glycine (43), alpha-alanine (21.5), proline (32.0), asparagic acid (26), oxyproline (8.6), glutamic acid (60 mg), and others. As excipients, the drug contains the following components: ascorbic acid (0.075 mg), sodium chloride (8000 mg), potassium chloride (400), magnesium chloride (106), sodium phosphoric acid (121), carbonic acid sodium (600), calcium chloride (276), magnesium sulfuric acid (100), sodium acetic acid (79.3), potassium phosphoric acid (60 mg), phenol red. Blood was taken from the calves of both groups for morphological analysis. In morphological blood examination, the quantitative content of erythrocytes, leukocytes and hemoglobin, erythrocyte sedimentation rate and percentage of leukocyte species were determined. Laboratory blood tests were performed on a veterinary hematology analyzer Mindray BC-2800 Vet (China) and on a biochemical analyzer Pointcare V3 (Japan). All clinical and laboratory tests were performed in 2022.

RESULTS AND DISCUSISON

At the beginning of the experiment, apathy, decreased appetite were observed in calves of both groups. The animals had hyperemia of visible mucous membranes, serous and mucous discharge from the nasal cavity. At auscultation of the lungs in calves vesicular breathing, moist wheezes were noted. Cough was dry at first, then became moist. Percussion of the lungs revealed foci of blunting of the anterior and posterior lobes. Breathing is rapid, difficult. The results of morphologic studies of blood are presented in Table 1.

The results of blood leukogram studies of calves of the studied groups are presented in Table 2.

The analysis of Tables 1, 2 shows that in the blood of the calves of both groups before the beginning of treatment there was a decrease in the number of erythrocytes in the blood and accelerated ESR, leukocytosis, hemoglobin was at the lower limit of the norm (see Table 1). The leukogram showed neutrophilic leukocytosis with a shift of the nucleus to the left, indicating an acute course of the disease. Monocytopenia was established in the animals of both groups (see Table 2). These changes, in our opinion, are associated with the impact on hematopoietic organs of various toxins and underoxidized metabolic products, which appeared in the process of pathogenesis of bronchopneumonia.

During treatment the animals were subjected to daily clinical examination. One of the efficacy

Табл.1. Морфологические показатели крови ($M \pm m$, $n = 10$)
Table 1. Morphological parameters of blood ($M \pm m$, $n = 10$)

Indicator	Group of animals	Before the treatment	On the 7th day of the treatment	Physiological norm according to A.P. Demidovich [1]
Erythrocytes, × 10^{12}/l	Experimental	4,7 ± 0,2	6,4 ± 0,3*.**	5–10
	Control	4,6 ± 0,2	5,2 ± 0,1	
Leukocytes, × 10^{9}/l	Experimental	15,8 ± 0,7	10,6 ± 0,3**	4–12
	Control	16,5 ± 0,6	11,7 ± 0,4**	
Hemoglobin, g/l	Experimental	109,6 ± 5,3	116,7 ± 5,8	80–150
	Control	107,4 ± 4,6	113,6 ± 5,6	
ESR, mm/h	Experimental	1,2 ± 0,1	0,4 ± 0,1**	0,1–0,6
	Control	1,1 ± 0,2	0,5 ± 0,2	

Here and in Table 2. *$p < 0.05$ between groups.
**$p < 0.05$ relative to baseline data.

Табл. 2. Лейкограмма телят, больных бронхопневмонией ($M \pm m$, $n = 10$), %
Table 2. Leukogram of the calves with bronchopneumonia ($M \pm m$, $n = 10$), %

Group	E	Neutrophils			L	M
		Y	BN	SN		
Physiological norm according to A.P. Demidovich (see footnote 1)						
	2–20	0–1	0–2	15–45	45–75	3–10
Before the treatment						
Experimental	2,6 ± 1,3	3,6 ± 1,1	1,2 ± 0,7	30,2 ± 0,4	55,4 ± 5,8	1,2 ± 1,2
Control	2,4 ± 0,5	4,0 ± 1,5	1,2 ± 0,8	29,4 ± 0,5	55,6 ± 4,7	1,6 ± 1,1
On the 7th day after the start of the treatment						
Experimental	5,0 ± 1,5*	0,2 ± 0,4	0,8 ± 1,1	34,2 ± 4,2	56,3 ± 8,2*	5,6 ± 0,8*
Control	3,4 ± 1,1	1,2 ± 1,3	1,4 ± 1,2	31,1 ± 3,6	57,4 ± 7,1	3,2 ± 1,8

indicators of the treatment regimen of the experimental group was the weakening of specific signs of the disease, as well as morphological blood parameters.

Thus, in the course of treatment the calves of the experimental group already on the 5th day had improved breathing, no rales were detected at auscultation, coughing was absent, body temperature varied within physiological limits, appetite appeared. According to the results of morphological blood examination on the 7th day after the beginning of treatment of bronchopneumonia in calves of the experimental group there was observed an increase in the content of erythrocytes relative to the initial data by 36% ($p < 0.05$), a decrease in the number of leukocytes - by 33% ($p < 0.05$), ESR decreased 3 times ($p < 0.05$) and reached physiological norms.

In calves of the control group, visible weakening of clinical signs began only on day 7. In control calves the number of erythrocytes relative to the first study increased by 13%, leukocyte content decreased by 29% ($p < 0,05$), ESR was at the upper physiological limit (0,5 ± 0,2). On the 10th day the animals of the control group were clinically healthy.

[1]*Demidovich A.P.* Diagnostic value of blood biochemical indicators (protein, carbohydrate, lipid metabolism), educational and methodological manual for students in the specialty "Veterinary Medicine" // Vitebsk State Academy of Veterinary Medicine. Vitebsk: VSAVM, 2019, p. 32.

The average group values by the 7th day of treatment were significantly higher in the experimental group relative to the control group in the number of erythrocytes by 23% ($p < 0.05$), eosinophils by 47% ($p < 0.05$), monocytes by 1.8 times ($p < 0.05$), and in the content of leukocytes, on the contrary, lower by 9.4% ($p < 0.05$). The number of segmented neutrophils was also in this period higher in the blood of experimental calves by 10%, hemoglobin by 3%, and the content of juvenile neutrophils, neutrophils, bacilli, ESR, lymphocytes was lower by 83.3; 9.4; 43.0; 20.0%, respectively, but there were no significant differences between the groups.

Thus, in the experimental group of the calves by the 7th day of the study not only clinical signs, but also the results of the general blood analysis testified to the effectiveness of Vitam in the complex therapy of bronchopneumonia of calves.

CONCLUSIONS

1. Clinically, bronchopneumonia of calves was manifested as follows: apathy, decreased appetite, serous and mucous discharge from the nasal passages, dry cough, moist wheezing and foci of blunting in the anterior and posterior lobes of the lungs.

2. In the blood in calf bronchopneumonia, erythropenia (up to (4.6 ± 0.2) × 1012/l), leukocytosis (up to (16.5 ± 0.6) × 109/l), increased ESR (up to 1.2 ± 0.1 mm/h), neutrophilic leukocytosis with leftward shift of the nucleus and monocytopenia are noted.

3. Application of vitamin-amino acid complex in the therapy of bronchopneumonia contributed to the improvement of clinical status on the 5th day (coughing, wheezing in the lungs were absent, appetite appeared), on the 7th day there was a positive trend in the changes of the main morphological blood parameters (increase in erythrocytes, decrease in the number of leukocytes, recovery to the physiological value of the ESR).

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ПОДХОДЫ К РЕДАКТИРОВАНИЮ ГЕНОМА СЕЛЬСКОХОЗЯЙСТВЕННЫХ ЖИВОТНЫХ

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Представлены результаты исследования генетических методов создания сельскохозяйственных животных с улучшенными характеристиками. В настоящее время получено значительное количество животных с отредактированным геномом. Методы модификации генома у крупного рогатого скота (КРС) постоянно совершенствуются. Изучены подходы генного редактирования эмбрионов крупного рогатого скота, доставки отредактированных конструкций, повышения выживаемости эмбрионов после внесения систем редактирования. Исследования проводили на эмбрионах КРС. Разработаны и апробированы системы редактирования генов BLG и СД209. Изучены варианты доставки системы редактирования в клетки КРС: микроинъекция в зиготу плазмидной ДНК с закодированной последовательностью с CRISPR/Cas9 с sgRNA, способ вирусных векторов (аденоассоциированные вирусы AAV, серотипы AAV1, AAV2, AAV6, AAV9, AAVDJ), совместное введение плазмидной ДНК и сперматозоида в ооцит на стадии MII, а также микроинъекции РНК Cas9 и гидовых РНК. Исследованы и усовершенствованы различные методики выполнения микроинъекций и опробованы различные варианты приготовления смеси РНК Cas9 и гидовых РНК. На основе полученных результатов оптимизирован протокол выполнения микроинъекции системы редактирования и проведен модельный эксперимент на 160 ооцитах, по 80 клеток на каждую конструкцию. В результате установили, что эффективность редактирования в целом повысилась. При инъекции гидовой РНК против гена BLG и мРНК spCas9 дробление начали 84% выживших клеток, бластуляция составила 20%, оказались с нокаутом по BLG – 69,2%. При инъекции против гена CD209 и мРНК spCas9 дробление начали 44,4% выживших эмбрионов, бластуляция составила 16,7%, с нокаутом по CD209 – 44,4%. Новизна работы заключается в получении данных о разработке систем редактирования с определенными генами-мишенями, в усовершенствовании системы доставки и культивирования эмбрионов КРС.

Ключевые слова: CRISPR/Cas9, доставка систем редактирования, вирусный вектор, микроинъекции РНК, доимплантационные эмбрионы КРС

APPROACHES TO GENOME EDITING IN AGRICULTURAL ANIMALS

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The results of research into genetic methods of breeding agricultural animals with improved characteristics are presented. By now a significant number of animals with an edited genome have been selected. Methods of genome modification in cattle are constantly improving. The approaches of gene editing of bovine embryos, delivery of edited constructs and improvement of embryo survival after introduction of editing systems have been studied. The studies were performed on cattle embryos. BLG and SD209 gene editing systems were developed and validated. Delivery options of the editing system into cattle cells were studied: microinjection into the zygote of plasmid DNA encoded sequence with CRISPR/Cas9 c sgRNA, the method of viral vectors (adeno-associated AAV viruses, serotypes AAV1, AAV2, AAV6, AAV9, AAVDJ), co-injection of plasmid DNA and sperm into the oocyte at the MII stage, and microinjection of Cas9 and guide RNAs. Different techniques for performing microinjections have been investigated and refined, and different preparation of Cas9 RNA and guide RNA

mixtures have been tested. Based on these results, the protocol for performing microinjection of the editing system was optimized and a model experiment was performed on 160 oocytes, with 80 cells per each construction. The findings have shown that the efficiency of editing has generally improved. When injected with guide RNA against BLG gene and spCas9 mRNA, 84% of the surviving cells initiated cleavage, blastulation was 20%, and BLG knockout was 69.2%. When injected against the CD209 gene and spCas9 mRNA, 44.4% of the surviving embryos started cleavage, blastulation was 16.7%, with CD209 knockout at 44.4%. The novelty of the work lies in obtaining data on the development of editing systems with specific target genes, in improving the delivery system and cultivation of bovine embryos.

Keywords: CRISPR/Cas9, delivery of editing systems, virus vector, microinjections of RNA, pre-implantation bovine embryos

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INTRODUCTION

Gene editing methods allow making precise and specified modifications in the genome of livestock. Genome editing in goats and pigs is currently performed quite efficiently using SCNT (somatic cell nuclear transfer) through CRISPR/Cas9-edited cells [1, 2]. The efficiency of genomic editing is about 60% [1, 3]. In mice, monkeys, dogs, and rabbits, another method is more often used, which consists in direct injection of the editing system into the cytoplasm of embryos at the single-cell zygote stage [4, 5]. It has been shown that the introduction of CRISPR/Cas9 constructs directly into zygotes can lead to the production of the offspring with specified traits. However, a serious problem in using this approach is genetic mosaicism arising in the offspring [6]. One of the proposed ways to eliminate mosaicism is the early introduction of CRISPR/Cas9 system at the metaphase II stage of the oocyte or at the early zygote stage immediately after pronucleus formation [7].

Effective microinjection of growth hormone and insulin-like growth factor I genes into pig zygotes has been reported. Later, two lines of transgenic pigs expressing growth hormone gained 11.1 and 13.7% more weight than the control pigs[1, 2].

Also targeted for research is the myostatin protein gene. Reduced expression of the myostatin gene (also known as GDF8 or growth differentiation factor 8) has been found to enhance

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muscle growth. Even single nucleotide polymorphisms in the myostatin gene trigger significant changes[3]. A large number of animals with edited genome have now been obtained, including pigs with edited MSTN (myostatin) gene, pigs with edited anti-PRRS (porcine reproductive and respiratory syndrome) gene and tuberculosis resistant transgenic cattle[4-6]. These genetically modified/edited farm animals have demonstrated improvements in weight gain or disease resistance and other beneficial traits.

Methods of genome modification (especially ZFN and TALEN) in cattle are constantly being improved[7]. TALEN technology has been used, for example, to insert the SP110 gene into the cattle genome, resulting in transgenic cattle resistant to tuberculosis[8].

In addition, using ZFN and TALEN approaches, the β-lactoglobulin (LGB) gene in bovine embryos was altered with good efficiency[9]. Using TALEN, cattle with a knockout of the MSTN gene were created. TALEN has also been used to eliminate the POLLED allele in Holstein cattle and to produce hornless dairy cattle[10].

A significant share of genome modification works was performed on cell cultures, in particular, inclusion of NRAMP1 using CRISPR/Cas9 in fetal fibroblasts to create genetically modified cattle resistant to tuberculosis[11]. In another study, knockout of the PRNP gene, which encodes the infectious protein PrPSc that causes diseases in cattle and humans (cattle spongiform encephalopathy, Creutzfeldt-Jakob disease and chronic wasting disease in deer) in fetal fibroblasts and early embryos was performed. In brucellosis, transduction of infected cells with lentiviral vectors containing CRISPR/Cas9 gene editing system was carried out to inactivate the gene involved in brucella replication in host cells; a significant increase in the resistance of culture cells to brucellosis infection was obtained[12] [8].

Currently, significant progress has been made in the methodology of genome editing using DNA nucleases. There are four main types of technologies based on programmed nucleases: meganucleases, zinc finger nucleases (ZFN), transcription activator-like endonucleases (TALEN), and clustered regularly spaced short palindromic repeats Cas9 (CRISPR/Cas9). These genome editing tools are capable of precisely cutting DNA in a given nucleotide sequence[13].

The CRISPR/Cas9 system is most attractive to use primarily because it is a relatively simple, efficient and economically the cheapest method

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of genome editing. Therefore, the use of CRISPR/Cas9 technology has made gene editing of farm animals more accessible, bringing it closer to application in practical animal husbandry.

However, some experts argue that altering the genomes of farm animals to improve production efficiency may lead to secondary effects. For example, accelerated muscle growth when the myostatin gene is mutated may lead to more caesarean sections, limb and respiratory system abnormalities[14]. Finally, genome editing by somatic cell nucleus transfer can have a negative impact on animal welfare: increased embryonic death, postnatal mortality and congenital disorders [9]. In addition, genome editing can lead to off-target mutations or unintended consequences that will accumulate and manifest themselves in subsequent generations[15]. However, given the many advantages of the technology, genome editing with CRISPR can be used to improve breeds of farm animals. Studying and improving any new technology takes time, but CRISPR technology has already proven to be an effective research tool for scientific endeavor that requires further improvement, especially in productive animals.

To realize the delivery of CRISPR/Cas9 system into the cell, transfection by different methods, and transduction by viruses are used; combinations and modifications of different methods are constantly studied and used in experimental works. For effective gene editing, CRISPR/Cas9 protein complexes must cross both cellular and nuclear membranes. Delivery of the CRISPR/Cas9 system inside a eukaryotic cell can be accomplished through viral or non-viral vectors and physical manipulation. The most common methods of physical delivery are liposome and microparticle transfection, microinjection and electroporation; other methods: hydrodynamic delivery, gene gun, and impalefaction are currently being actively studied and improved at the research stage.

Microinjection is considered the "gold standard" for introducing CRISPR components into cells. It is best suited for *in vitro* work with embryos or cell cultures. There are three main methods of microinjection: injecting DNA directly into the cell nucleus, injecting mRNA molecules transcribed *in vitro* into the nucleus, and injecting mRNA into the cytoplasm. These different methods have advantages and disadvantages (including significant off-target effects)[16]. Despite the traumatic nature of microinjections, in a number of cases they were used to achieve high editing efficiency, for example, simultaneous knockout of four genes using a single injection into rat zygotes[17]. With few exceptions, microinjection of CRISPR/Cas9 RNA components into cells results in a finite duration of the editing system due to natural mRNA decay within eukaryotic cells. Microinjection is a well-established technology and its use is widespread [10].

One of the physical methods of gene delivery to a population of cells is electroporation. This method uses high voltage pulsed electric currents to temporarily open nanometer-sized pores in the cell membrane of the cells suspended in buffer, allowing the components with diameters of tens of nanometers to penetrate the cell[18].

A method for delivery of an editing system using an adeno-associated virus (AAV) belonging to the genus *Dependovirus* and the family *Parvoviridae* has been developed. It represents a virus used for gene therapy with single-stranded

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DNA. The virus is able to efficiently infect the cells with little or no induction of innate or adaptive immune response or associated toxicity, at least when this serotype is first used [11]. The artificial deletion of sequences associated with pathogenicity factors from the viral genome and the size of the viral DNA allow AAV to be used as a container for atraumatic delivery of the editing system into the nucleus of target cells. Due to the multiple serotypes of the virus with broad tropism, the problem of immune response to the virus can often be circumvented. AAV viral particles can be used *in vitro*, *ex vivo* and *in vivo*, making them versatile delivery vehicles.

The purpose of the research is to study genetic methods of creating farm animals with improved characteristics.

MATERIAL AND METHODS

A complex of laboratory and clinical methods of donor biomaterial preparation, artificial maturation and fertilization of oocytes, cultivation of early pre-implantation mammalian embryos, cryopreservation thawing of oocytes and embryos, micromanipulations on oocytes, zygotes and early embryos of animals, as well as molecular-biological methods of development and testing the efficiency of editing systems were used in this work.

Ovaries of cows were selected postmortem and transported to the laboratory in a controlled environment (temperature +37,5 °C or +4 °C) within 3-4 hours after receipt. Follicle aspiration and all further work with oocytes and embryos were carried out in sterile conditions of the "clean zone" in laminar boxes with a heated surface. Commercial media were used for maturation of oocyte-cumulus complexes. The medium for IVM was a commercially produced Continuous Single Culture Complete human embryo culture medium (CSCC, Irvine Scientific, USA), developed for human in vitro fertilization procedures, with the addition of 50 μg/mL hCG (Human Chorionic Gonadotropin, RF) and 0.5 μg/mL FSH (Gonal, Serono). Vitrification Media kits (Kitazato, Japan) and Cryotop straws (Kitazato, Japan) were used for cryopreservation of cattle cells and embryos. Micromanipulations, including injection of mRNA into the cytoplasm of oocytes were performed on a micromanipulation unit Narishige (Japan). Visual analysis was performed using Nikon (Nikon Eclipse Ti-U, Japan) and Leica (DMI3000 B, Germany) inverted microscopes, OCTAX software (OCTAX EyeWare with ICSI Guard, Germany).

Recombinant AAVs for transduction were obtained by triple transfection [11]. The design and synthesis of guide RNAs (SgRNAs) for microinjection were performed using the CRISPOR online algorithm; the criteria for guide selection were high MIT with the smallest number of non-target sites and the absence of self-complementary sites. The second exon of the CD209 gene and the promoter of the BLG gene were selected as targets (no quality SgRNA could be found for the BLG gene itself)[19, 20]. CRISPR efficiency was analyzed by searching for cuts in the genes of interest in blastocysts using the Sanger sequencing method.

Statistical analysis of the obtained data was performed in MS Excel and Statistica 10.0 programs using parametric and nonparametric methods. In case of normal distribution Student's *t*-criterion was used, in other cases when analyzing independent samples - *U*-criterion (Mann-Whitney), when analyzing dependent samples - W-criterion (Wilcoxon).

RESULTS AND DISCUSSION

Genetic modification is a modern approach that allows to obtain organisms with specified characteristics. However, when working with

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large farm animals, the ability to detect the effects of genome editing is limited by the fact that the total period of prenatal development, onset of reproductive age, and subsequent pregnancy in cows exceeds 2 years. In addition, most economically important traits in cattle are quantitative in nature (controlled by many genes), and therefore, improving cattle through genetic manipulation almost always requires genome editing at multiple sites. The introduction of CRISPR/Cas9-associated RNA into zygotes often results in mosaicism[21-23]. In general, it can be seen that there are very few successful attempts at genome modifications in cattle that have yielded a low degree of mosaicism.

We have investigated several options for delivery of the editing system into cattle cells, including microinjection of plasmid DNA encoded with CRISPR/Cas9 c sgRNA sequence into the zygote, the method of viral vectors (adeno-associated AAV viruses), co-injection of plasmid DNA and sperm into the oocyte at the MII stage, and microinjection of Cas9 RNA and guide RNAs.

Species-specific features of the bovine oocyte cytoplasm structure - a large number of lipid inclusions - make visualization of pronuclei much more difficult, so the first method of introducing the editing system directly into the pronucleus of the zygote did not yield positive results. The delivery method using viral vectors proved to be more effective. Five different serotypes of recombinant AAV were pre-tested, with the green fluorescent protein (GFP) gene encoded in the sequence. Then serotypes AAV1, AAV2, AAV6, AAV9, AAVDJ were used to obtain GFP-positive cattle embryos.

A total of 116 embryos obtained after fertilization of oocytes from 114 cows were used for these experiments. Three independent experiments were conducted for each group of serotypes of AAV-infected embryos [12]. It was found that the AAV9 serotype showed minimal efficiency (38.10%) as a tool for transferring genetic material. Four other AAV serotypes (AAV1, AAV2, AAV6 and AAVDJ) showed very close transduction efficiency (52.94-58.33%). Based on the results of the experiments, AAV2 serotype was selected for knockout of the gene associated with the CD209 receptor. CD209 is a C-type lectin receptor located on the surface of macrophages and dendritic cells and recognizes a wide range of pathogens, including bovine leukemia virus. Potentially, knockout of this gene could allow animals to become resistant to a variety of infections. After editing of experimental cattle zygotes with delivery of adeno-associated virus (serotype AAV2), the resulting blastocysts were analyzed by Sanger sequencing: 3 out of 22 cases showed mosaic shifts of the reading frame of the CD209 region [12].

In our experiments, the most efficient method of gene editing in cattle was the use of an editing system based on spCas9 RNA and guide RNAs delivered into the cell using an improved and species-specific microinjection method (see footnotes 19, 20). Zygotes were placed in G-MOPS buffer (Vitrolife, Sweden) for the time of injection. Only oocytes that ejected the first and second polar bodies after *in vitro* fertilization were used for injection. Injection was performed using a retention pipette (Sooreg Surgical, USA) and a microinjection needle. Two independent experiments were performed for each editing system, and a total of 200 cells

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were injected. In the process of manipulations about 10% of cells died, which, in our opinion, is associated with high traumatization of microinjections as such. 98 cells initiated fragmentation, of which 67.6% reached the eight-cell stage, and 11 embryos reached the blastocyst stage, representing 5.5% of all cells used. In 32.3% of zygotes arrest occurred soon after the start of fragmentation. Sequencing of biopsy material from embryos showed the presence of characteristic changes in the genes of interest.

The obtained chromatograms of individual embryo samples after microinjections of genetic constructs for making a cut in BLG and CD209 genes showed the main sign of the presence of genetic modification - double sequences. Cutting was detected in 5 of 17 embryos (29.4%) after microinjections of guide RNA against BLG gene and spCas9 mRNA and in 2 of 9 embryos (22.2%) after microinjections of guide RNA against CD209 gene and spCas9 mRNA. Thus, the knockout rate in the experimental cell group did not exceed 30%, with about 5.5% of the embryos reaching the blastocyst stage, which is presumably due to the high traumatic nature of microinjection and the death of a significant number of early embryos at the initial stages of fragmentation. Despite this, in general, our data showed the success and efficiency of this delivery system, but further improvement of the execution technique was required to increase the survival rate of the embryos after manipulation.

The key criterion was to achieve the minimum possible traumatization of microinjections. We investigated different ways of performing microinjections, studied the influence of the needle shape on the manipulation efficiency, and tested different variants of preparing a mixture of Cas9 RNA and guide RNA, differing in viscosity and concentration of the components. Two types of needles were tested: those with a 45° bend, similar to the instruments used for ICSI (intracytoplasmic sperm injection), and straight needles, usually used for microinjections in small laboratory animals. Technical solutions were developed that allowed to perform microinjections with a straight needle using a retaining suction cup with a 45° bend, standard for ICSI, in a "semi-open" chamber for microinjections in a horizontal plane. The optimal concentration of Cas9 RNA and guide RNA in the mixture for microinjections was also determined. As a result of the studies, a method of gradual reduction of the mixture concentration was developed to obtain a solution of optimal viscosity and fluidity corresponding to the parameters of needle filling: 50 ng/μl of Cas9 RNA and 15 ng/μl of guide RNA.

Based on the obtained results, the protocol of microinjection of the editing system was optimized and a model experiment was conducted on 160 oocytes, 80 cells per each construct. As a result, it was found that despite increased initial cell death, the efficiency of the editing itself was higher. When the guide RNA against the BLG gene and spCas9 mRNA was injected, cell death was 68.8%, 84% of surviving cells initiated fragmentation, and the ratio of blastulation initiated to arrested zygotes was 20 to 80%. At injection of the editing system against the CD209 gene, cell death was 66.3%, 44.4% of surviving embryos started fragmentation, and blastulation was 16.7%. All developmentally arrested embryos were sent for sequencing: 13 and 10 (one was broken) in two groups, respectively. As a result, it was found that out of 13 embryos there were 9 with BLG knockout (69.2%), and out of 9 embryos - 4 with CD209 knockout (44.4%).

CONCLUSION

According to the studies [8], the efficiency of b-lactoglobulin gene knockout on the edited 1511 cattle zygotes has the following indicators: the level of growth to blastocyst - 15%, the number of embryos carrying the deletion - 21%. In the present studies using the CRISPR/Cas9 system, similar results were achieved: 20-30% knockout rate and 16% blastulation rate. The

frequency of blastocyst formation was comparable with the control (intact) group. Taking into account that cattle oocytes were obtained after the slaughter of animals culled from farms due to age changes and low milk yield, and without preliminary hormonal preparation, the low level of competence to develop such oocytes is quite understandable.

Our results showed that delivery of the editing system by micro-injection of a mixture of spCas9 and guide RNAs is quite effective and can be used to obtain knockouts by genes of interest. However, there is a problem of reducing the level of mosaicism after editing embryos. In this regard, it is promising to introduce editing reagents as early as possible (in MII oocytes) before the beginning of DNA synthesis.

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ИСПОЛЬЗОВАНИЕ СОРНЫХ РАСТЕНИЙ В КАЧЕСТВЕ ЗЕЛЕНОГО УДОБРЕНИЯ В СЕЛЬСКОМ ХОЗЯЙСТВЕ

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Рассмотрена проблема практического применения сорняков в сельском хозяйстве. Сорная флора так же, как и другая растительность, выполняет ряд экологических функций и, по определению биогеохимической науки, является фитомассой, несущей в себе большие запасы биофильных (C, O, N, H, Ca, P, S) и ряда других химических элементов. Остается не до конца изученной функция сорной растительности как составляющей экологической стабильности агроценозов. В связи с этим в статье представлены данные по результатам использования сорной растительности в адаптивно-биологическом земледелии в качестве зеленого удобрения. Приведены примеры положительного влияния сорняков на почвенное плодородие и урожайность возделываемых культур.

Ключевые слова: сорняки, фитомасса, органическое вещество почвы, сидераты, естественный фитоценоз

USE OF WEED PLANTS AS GREEN MANURE IN AGRICULTURE

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The problem of practical application of weeds in agriculture is considered. Weed flora, just like other vegetation, performs a number of ecological functions and, according to the definition of biogeochemical science, is a phytomass carrying large reserves of biophilic (C, O, N, H, Ca, P, S) and a number of other chemical elements. The function of weed vegetation as a component of ecological stability of agrocenoses remains incompletely studied. In this regard, the article presents data on the results of using weed vegetation in adaptive-biological farming as a green manure fertilizer. Examples of positive effects of weeds on soil fertility and yields of cultivated crops are given.

Keywords: weeds, phytomass, soil organic matter, green manure, natural phytocenosis

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INTRODUCTION

Weed-field plants are a group of plants historically formed as a result of human activity. The process of its formation does not stop at present. It should be noted that this group of vegetation is an integral part of agrophytocenoses, its phytomass in most cases does not exceed 20% of the value of the same indicator of the cultivated crop [1].

At this stage, agriculture is largely inherent in the negative perception of weeds, which is quite justified by science. Having a higher ecological plasticity, weeds survive in any conditions, so they compete very successfully with cultivated plants in agroecosystems and have a versatile negative impact on them[1, 2]. It is known that the degree of weed infestation of crops directly affects the quantitative and qualitative indicators of crop yields. In recent years, the phytosanitary situation in Russia has deteriorated significantly. Most of the crops are contaminated, about 70% of them in high and medium degree. Despite the fact that the volume of applied herbicides is growing, in our country there is still a significant infestation of grain crops [2]. Annual potential losses in irrigated crops due to the negative impact of weeds are estimated at about 15 million tons in terms of grain [3]. The average level of yield losses from weeds in the Russian Federation is 15% [4].

However, the scientific community has data on the positive impact of segetal vegetation on agroecosystems. Weeds are usually considered as competitors of crops in the use of water, nutrients or sunlight, but in most cases the fact that weeds are a natural component of biocenoses that performs the functions of maintaining their biodiversity and sustainability is not taken into account[3, 4]. All agrocenoses are considered to be unstable ecosystems, as the number of species in them is artificially limited by man. The inclusion of fields with weeds in agricultural use can contribute to the resilience of agrocenoses. Scientific studies have been conducted to prove the usefulness of weeds: it has been found that weeds contribute to the increase in the number of beneficial insects (pollinators) and the overall biodiversity of the ecosystem. The emergence of various hypotheses addressing this phenomenon and the success of some studies in proving this fact indicate that the subject described above deserves further study [5].

Cultivated plants, and above all row crops, have approximately the same root systems (as a rule, superficial) and for the most part effectively use nutrients only from the upper soil layers [1]. Therefore, nutrients in deeper layers are not available to cultivated plants and can be lost through leaching. Weeds with strongly developed root systems extract nutrient elements from deep soil layers and parent rock and are their reservoirs. Under conditions where competition for nutrients does not prove to be a limiting factor, weed growth can contribute to the retention of these substances in the agroecosystem [1]. In this regard, at present, agricultural science should shift from the paradigm of weed control to the paradigm of management of the weed component of agrophytocenosis.

The destruction of weed vegetation by chemical methods causes enormous damage to ecosystems. When using pesticides, soils are regularly polluted by a large group of chemicals of different classes. The normal function-

[1] *Korchagin A.A., Mazirov M.A., Shchukin I.M.* General agriculture: textbook. Vladimir: Vladimir State University Publishing House, 2021, 193 p.

[2] *Eryomin D.I., Konischeva V.A.* Biogenic removal of nutrients of wheat agrophytocenosis in the conditions of forest-steppe zone of Trans-Urals // Agrarian Bulletin of the Urals, 2014, N 1, pp. 9–13.

[3] *Sorokin I.B.* Application of weed plants as green manure crops // Plant Protection, 2008, N 7, pp. 34–35.

[4] *Petit S., Boursault A., Mélanie Le G., Munier-Jolain N.* Weeds in agricultural landscapes. A review // Agronomy for Sustainable Development, 2011, N 2, pp. 309–317.

ing of plants and soil biota is determined by the physical, chemical and biological processes that take place in the soil, and pesticides can be incorporated into these processes. Most pesticides are highly toxic and mutagenic compounds that can not only accumulate in soil, tissues of living organisms, but also migrate in the biosphere due to abiotic and biotic processes[5]. All this points to the need to reduce the pesticide load on ecosystems, so weed control should be carried out within the framework of biologization of farming. In this regard, it is worth thinking about the practical use of weeds in agriculture.

The purpose of the work is to present material reflecting the current state of the problem of using weed plants as green manure.

MATERIAL AND METHODS

The review is written based on published literature sources.

RESULTS AND DISCUSSION

The system of row spacing maintenance in fruit-bearing vineyards was studied in Dagestan at the enterprise "Caspiy", located in the Kayakentsky district. It was revealed that in recent years the soil was subjected to strong compaction under the action of agricultural machinery. This phenomenon is considered to be the main reason for obtaining low average yields of agricultural crops in the republic (5-8 tons/ha). In this regard, a number of authors have developed recommendations to improve soil condition[6]. It is proposed to use a grass deck of cut weeds to prevent direct contact of wheels, sunlight with the soil and to preserve moisture in the hot season. This layer also performs the function of organic fertilizer, enriching the soil with nutrients and creating favorable conditions for microorganisms and root systems of plants.

Research was conducted in the experimental-production farm "Tsentralnoe" (Krasnodar, OJSC "Agronom") on the role of weeds in the row spacing of apple-tree plantations. In the first year after the cessation of tillage, the most typical for the local flora weeds prevailed (lamb's quarters, barnyard grass, redroot amaranth, etc.), phytomass of which reached 30 tons/ha, in terms of dry mass up to 6.8 tons/ha. In the fifth and sixth years, loose-bush cereals with legume inclusions (field bromegrass, wall barley, annual meadow grass, etc.) prevailed, phytomass of which was about 33.8 t/ha, dry mass - 4.58 t/ha. Agrochemical properties of leached chernozem changed under grasses: the content of humus, mobile P_2O_5, exchangeable K_2O, the degree of saturation of bases and their sum increased, the acidity of the medium decreased. Also, in the soil under grasses various yeasts were more frequent, the largest reserves of microbial pool were recorded and an increase in the number of some soil animals was noted, which together indicates an improvement in soil fertility. The introduction of herbs into the ecosystem of apple orchard from 2-3 years of age of fruit plants contributed to their earlier onset of fruiting. The first four years from the beginning of fruiting, the yield in the variants with grasses was reliably higher than when the soil was kept under autumn fallow. The highest yield was obtained from the trees whose inter-row spacing were occupied by natural grasses[7].

At present, the scientific community has sufficient data on the balance of organic matter (OM) in soil. The main source of OM post-accumulation in the soil is phytomass. It determines the input and accumulation of OM in the form of plant residues in the upper soil horizons, which

[5]*Shilnikova N.V., Andriyashina T.V.* Influence of pesticides on soil biocenosis // Bulletin of the Technological University, 2012, N 7, pp. 140–144.

[6]*Chupanov M.A., Kaziev M.-R.A., Alichaev M.M*. Weed Vegetation to Enhance Soil Fertility in Vineyards // Agricultural Sciences, 2014, N 5, pp. 839–842.

[7]*Popova V.P., Chernyavskaya N.V.* Protective role of weeds in the garden ecosystem // Pomiculture and small fruits culture in Russia, 2010, vol. 24, N 2, pp. 329–337.

affects humus content, structure, absorption capacity and a number of other soil characteristics. The main cause of degradation of arable land is the loss of humus, which occurs as a result of mechanical cultivation and violation of the natural balance of OM. It is established that in natural phytocenoses, unlike agrocenoses, 5-7 times more plant residues remain annually [6]. In the context of this problem good results are shown by green manure. In the course of numerous studies, it has been revealed that green manure in various crop rotations have a beneficial effect on soil fertility, replenishing the reserves of nutrient elements and, at least, reducing the deficit of OM and humus [7-9]. In the Non-Chernozem zone of the Russian Federation, green manure crops are not inferior to traditional organic fertilizers in terms of their impact on yield. Their introduction into the fertilizer system increases the profitability of field crop rotations up to 30%[8]. It should be taken into account that communities of segetal flora, like other plants, according to the definition of biogeochemical science, are phytomass, but unlike cultural green manure the weed phytomass is given to the farmer for free.

Some authors recommend introducing sideral fallows of weed vegetation as natural sources of increasing plant productivity under conditions of small-scale production[9]. In their opinion, there are several reasons for this. First, these phytocenoses allow to obtain 25-30 tons/ha of organic matter with a narrow ratio of C: N, which is especially important for the beginning of active microbial activity. Secondly, weeds accumulate nutrition elements not only from the upper cultivated, but also from deep soil layers, which are practically inexhaustible sources of mineral substances. Thirdly, weed vegetation actively interacts with rhizosphere, endophytic and other types of microorganisms, as a result of which the biological cycle involves elements of nutrition, in normal conditions are not available to cultivated plants.

In conditions of Western Siberia (Tomsk region, Novoarkhangelskoye and Luchanovo villages) on gray forest soils in grain and fallow crop rotations the influence of green manure crops and straw on the balance of organic matter was studied. It was found that annual weeds (*Echinochloa crus galli* - 52-93% of mass) were not inferior in quantity and quality of phytomass to cultural green manure crops (22 t/ha). Their average absolutely dry mass reached 4.1 tons/ha. At plowing up of annual green manure crops in the III ten-day period of September (taking into account the growth of tallow after cutting in early July), the deficit of OM decreased to 2.55 t/ha, i.e. by 80% in comparison with complete fallow (deficit of OM - 12.8 t/ha), but the deficit-free balance was not achieved. In Novoarkhangelskoye village there was a reliable increase in grain yield by 16% (by 2.6 c/ha) on average for two years as a result of lupine application. In this variant the share of segetal flora in the green mass was more than 85%, so their total impact should be taken into account. On more fertile dark gray forest soil, the influence of sideral fallow from weeds on grain yield was at the level of complete fallow (more by 40-50%). But unlike it, sideral fallow increased grain yield due to decomposition of introduced green fertilizer, preserving soil OM[10].

The experience of using weeds as a green fertilizer in potato growing in Russia is not insignificant for science. Thus, in the Blagoveshchensky district of the Amur region in the peasant (farmer) farm "Shchegorets" a short-rotation crop rotation was used in potato cultivation:

[8]*Novikov M.N., Tamonov A.M., Frolov L.D., Ermakov L.I.* Green manure crops in farming of the Non-Chernozem zone // Agrochemical Herald, 2013, N 4, pp. 20–26.

[9]*Syumak A.V., Rusakov V.V., Mungalov V.A., Selin A.V., Tsyban A.A.* Production testing of resource-saving technological and technical system of production of environmentally friendly agricultural products in grain-soybean crop rotation for small-scale production // Far East Agrarian Herald, 2009, N 3, pp. 59–63.

[10]*Sorokin I.B.* Renewable bioresources for increasing the fertility of arable soils in the sub taiga zone of Western Siberia: Extended abstract of candidate's thesis, Omsk, 2013, 41 p.

sideral fallow - potato - potato. The natural phytocenosis of various weeds with dominance of ruderal hemp, lamb's quarters, redroot amaranth, barnyard millet was used as green manure crops. The phytomass consisting of weed plants was chopped by rotary mower KIR 1.5 to small segments and plowed to a depth of 10-15 cm no later than mid-July. High potential of ecological plasticity of weed vegetation allowed to form phytomass of sideral fallow up to 70 t/ha. Productivity of natural weeds exceeded this indicator of soybean green manure by 2-3 times. Dry matter in natural "crops" was from 5.7 to 37.8 t/ha and contained colossal amounts of nitrogen, phosphorus and potassium: N - 350-1659 kg/ha, P_2O_5 - 85-352 and K_2O - 455-956 kg/ha. The highest values were characteristic of ruderal hemp. The use of weedy plants as green manure crops showed their efficiency as excellent forecrops for potatoes - the soil received a large dose of organic and mineral substances, which allowed to harvest 30-45 t/ha (over 20 t/ha in the most unfavorable years). Under these conditions, green manure from weeds was the cheapest type of fertilizer that did not require costs for soil preparation, sowing, and care. The level of profitability of potato production at application of green manure crops from natural weeds amounted to 487%, which is 2 times higher than when using soybean as a green manure crop (212%)[11, 12]. Consequently, sideration with weeds increased the level of biologization of farming, which was also reflected in its sustainability.

In field experiments on meadow-chestnut heavy loamy soils in the Tarumovsky and Khasavyurtovsky districts of Dagestan the following alfalfa forecrops were studied: winter wheat (control), natural phytocenosis of weed-field vegetation grown after harvesting winter wheat, corn, sunflower. It was found that the best alfalfa forecrop was natural phytocenosis formed after harvesting wheat for green fertilizer or fodder. Phytomass of weed-field vegetation in both variants was harvested at the end of August and in the second ten-day period of October. It reached 5,50-5,75 t/ha, contained on average 52,95 kg/ha K_2O and 27,84 kg/ha P_2O_5, which was 2,1 (by K_2O) and 2,7 times (by P_2O_5) higher than in the control. The natural phytocenosis yielded 5.58 and 3.82 t/ha more plant matter inalienable from soil compared to the control (4.04 t/ha) and 4.48-6.34 t/ha more than after tilled forecrops (3.38 and 3.28 t/ha). Alfalfa hay yield (total for two years) increased by 1.7-4.1 and 3.6-6.2 t/ha relative to the control when this mass was plowed up compared to corn and sunflower [10, 11].

In conditions of irrigated agriculture of the Tersko-Sulaksky lowland of Dagestan the systems of light chestnut soil maintenance in the crop period were studied. Significant results were shown by "energy accumulation" system. After harvesting winter wheat in the first ten-day period of July, irrigation was carried out at the rate of moistening of the meter layer of soil to 100% MWC to provoke weed germination. The natural phytocenosis (NP) formed after that was used as a sideral fallow. Green mass of weeds in the phase of milk-wax ripeness of seeds (or 5-7 days before sowing the crop) was chopped with heavy disc harrows and plowed to a depth of 20-22 cm. As a result, this system allowed to obtain more than 20 t/ha of green mass containing 121.90 kg N, 26.87 P_2O_5 and 32.09 kg K_2O in the afterharvest period, without any cultivation costs, except for irrigation using the existing irrigation network. The incorporation of green mass of the green manure provided an increase in the yield of the cultivated crop by an average of 0.7

[11] *Shchegorets O.V.* Experience of biological farming in Priamurye // Agro-industrial complex: problems and prospects of development: materials of the international scientific and practical conference (Blagoveshchensk, April 5, 2017). Blagoveshchensk, 2017, pp. 85–90.

[12] *Shchegorets O.V.* System of fallow and sideral fallow treatment in potato crop rotation // Agro-industrial complex: problems and prospects of development: materials of the All-Russian scientific and practical conference (Blagoveshchensk, April 20-21, 2022). Blagoveshchensk, 2022, pp. 349–358.

t/ha (up to 4.78 t/ha) and 4.2 thousand p/ha of additional net income[13]. When applying mineral fertilizers in the rotation link "NP - winter wheat" fractionally in two terms (by $N_{45}P_{45}$ under NP and culture), the yield of green mass of NP increased by 1.75 t/ha, wheat grain - by 1.57 t/ha [12]. This allowed better utilization of nutrients, as they are gradually released during decomposition of organic mass of the green manure, evenly used by plants and there is no danger of nitrogen losses from the root-inhabited layer [13].

The results of the experiments with afterharvest natural phytocenoses of weeds are interesting from the point of view of production of early vegetables, when the fields after harvesting the crop for a long time (up to 2 months and more) remain without vegetation, and the soil is subjected to various types of erosion.

In India, the All India Coordinated Weed Research Program (AICRP) plays a major role in the study of weed vegetation. One of the key objectives of the program is to study the potential use of weeds for various economic purposes[14]. Also, some studies have revealed that certain weed genera are capable of forming large reserves of biomass with high nutrient content: parthenium - 50-200 t/ha, chromolena - 93, cassia - 30, lantana - 10-15, eichhornia - 100-115 t/ha[15]. According to some authors, many weed plants can be used for food, fodder, as green manure plants, for bioethanol production, for medicinal and other purposes (see footnote 15) [14, 15]. Such species as *Cassia sofera*, *Cassia tora*, *Tephrosia purpurea*, *Ipomoea carnea*, *Eichhornia crassipes*, *Vernonia*, *Calotropis gigantia*, *Water hyacinth* are used in agriculture as green manure crops. Weeds *Chromolaena ordorata*, *Cassia serecia* and genus *Parthenium* are recommended to be used for composting[16].

Studies conducted in the eastern Himalayas (Aruchal Pradesh, Basar) proved the increase in the yield of *Oryza sativa* rice and *Brassica rapa* thoria seeds when mixed weed biomass (WBM), rice crop residues (CRR), thoria (CR) and microbial inoculants were applied to the soil. With the combined application of WBM and CRR (or CR) at 2 t/ha each (under rice at 1 t/ha), rice grain and thoria seed yields reached 3.52- 3.90 and 0.90-1.11 t/ha, respectively. When only 4 t/ha CRR and 2 t/ha CR were applied, the yields of the above-described crops were lower at 2.83-3.22 and 0.76-0.84 t/ha. Yield after weed biomass application was slightly inferior to the variant that provided for the joint application of WBM and CRR (CR). Incorporation of WBM and crop residues into the soil jointly and separately increased the content of basic nutrients and carbon, reduced soil acidity [16].

CONCLUSION

The above material proves that weed vegetation can be used in the cultivation of agricultural crops as a green fertilizer. A promising direction is the green manuring of soil by small (one- and two-year) weeds, which allows to significantly save on the costs associated with the purchase of seeds of typical green manure crops and their sowing, to obtain phytomass, the size is not inferior to annual green manure crops (in some conditions both to perennial). For further development of this topic, it is necessary to conduct scientific research, clarifying the species composition of weeds suitable for green manuring, the value of the phytomass of weed communities in different natural zones of the Russian Federation, their influence on the balance of soil organic matter, methods and terms of cultivation. It

[13]*Hasanov G.N., Arslanov M.A.* About the systems of soil maintenance in the harvest period under irrigation conditions in the south of the Russian Federation and their classification // Zemledelie, 2017, N 1, pp. 21–24.

[14]*Surinder S.R., Rajinder K., Neelam Sh*. A Collocate of Publications on Weed Science (Under AICRP – Weed Management, Palampur Centre). Palampure, 2016, 49 p.

[15]*Priya H.R., Veena, Pavithra A.H., Divya J*. Prospects and Problems of Utilization of Weed Biomass: A Review // Research and Reviews: Journal of Agriculture and Allied Sciences. 2014, vol. 3, Is. 2, pp. 1–11.

[16]*Singh P., Srivastava D.* Exploitation of weed plants as beneficial purpose // Journal of Biological and Scientific Opinion. 2013, vol. 1 (2), pp. 123–127.

is important to study the potential for the use of weed communities as green manure crops in the conditions of the Non-Black Earth region, since the green manure crops, which are familiar to this region, are an effective organic fertilizer that increases the profitability of crop rotations.

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Periodical publication

Pakul A.L., Lapshinov N.A., Bozhanova G.V., Pakul V.N. Technological grain qualities of spring common wheat depending on the system of soil tillage // Siberian Herald of Agricultural Science. 2018. vol. 48. № 4. pp. 27-35. DOI: 10.26898/0370-8799-2018-4-4.

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Example: Author A.A., Author B.B., Author C.C. Title of article.

Transliteration of the authors. English title of the article.

Zaglavie jurnala = Title of Journal, 2012, vol. 10, no. 2, pp. 49–54.

Transliteration of the source = English name of the source

Monograph

Klimova E.V. *Field crops of Zabaikalya*. Chita, Poisk Publ., 2001, 392 p. (In Russian).

Part of a book

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FOOTNOTES:

Quated $text_1$.

1*Klimova E.V., Andreeva O.T., Temnikova G.P.* Ways to stabilize food production in Transbaikalia // Problems and prospects of perfecting zonal farming systems in modern conditions: materials of the scientific and practical conf. (Chita, October 16-17 2008). Chita, 2009, pp.36-39.

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Photos, screenshots and other non-drawn illustrations must be uploaded separately as *.jpeg files (*.doc and *.docx if the image has additional marks). The resolution of the image should be >300 dpi. The image files should be given a name corresponding to the figure number in the text. In the description of the file a caption should be given separately, which should correspond to the name of the picture placed in the text.

Attention should be paid to the spelling of formulas in the article. To avoid confusion, it is necessary to write Greek (α, β, π, etc.), Russian (A, a, B, b, etc.) letters and numbers in straight font, Latin letters in italics (*W, Z, m, n*, etc.). Mathematical signs and symbols should also be written in straight font. It is necessary to clearly indicate upper and lower superscript characters (W_1, F_1, etc.).

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