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Narva mnt 7-634, Tallinn, Eesti
Harju maakond, 10117
Tel. + 372 602-7570
e-mail: info@eu-jr.eu
Website: http://eu-jr.eu

CONTENT

FORMATION OF PRODUCTIVITY OF SOWING PEAS DEPENDING ON TECHNOLOGY MEASURES OF CULTIVATION IN THE CONDITIONS OF THE WESTERN FOREST-STEPPE

Mykola Bakhmat[1]
StepanchenkoV@i.ua

Oleksandr Chynchyk
Department of Ecology, Quarantine and Plant Protection[2]
chinchik1978@gmail.com

Kateryna Nebaba[1]
agronebaba@gmail.com

[1]*Department of Crop and Fodder Production2*
[2]*State Agrarian and Engineering University in Podilya*
13 Shevchenko str., Kamianets-Podilskyi, Khmelnytskyi rgn. Ukraine, 32316

Abstract

In the conditions of the western Forest-steppe, leguminous crops, including sowing peas, are the main and most important source of vegetable protein, which solve the biological and ecological problems of modern agriculture in Ukraine.

The article presents the main research results on the study of the effect of mineral fertilizers and growth regulators on the formation of the sowing peas productivity in the conditions of the western Forest-steppe.

The field trifactor experiment was laid in the ten-field crop rotation in Podillia Research Center of State Agrarian and Engineering University in Podilya, during 2016–2018. The field experiment was laid down in the research ten-digit crop rotation. In microstages VVSN 55-65 (budding – flowering) crops were sprayed with growth regulators PlantaPeg, Emistim C and Vympel.

The studies carried out showed that the individual productivity of plants of the Chekbek variety was the best of all the varieties that were studied. It was found, that high indices of the mass of 1000 seeds were in the variants, where mineral fertilizers were used at a dose of $N_{30}P_{30}K_{45}$ with the plant growth regulator Vympel. According to this fertilizer composition, the mass indices of 1000 seeds for peas of the Chekbek variety were 266.4 g, and for the Hotivskyi and Fargus varieties – 260.6 g and 238.4 g, respectively.

The grain yield of peas is an integrated indicator of the action of all life factors on the plant organism during its growth and development. To a large extent, it depends on the biological characteristics of the variety, the supply of moisture and nutrients to the plant, technological methods of cultivation, as well as natural and climatic conditions. In our studies, the crops, fed with mineral fertilizers and growth regulators, were significantly less exposed to adverse factors, and the studied technology elements had a positive effect on the productivity of pea grain. The maximum biological yield was for the application of mineral fertilizers in doses of $N_{30}P_{30}K_{45}$ and the plant growth regulator Vympel. For the varieties of Hotivskyi peas, these indicators were 3.79 t/ha, Chekbek 4.32 t/ha, and Fargus 3.30 t/ha.

Keywords: peas, variety, mineral fertilizers, growth regulators, yield, grain quality, cultivation technology.

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1. Introduction

One of the important directions of the successful development of the latest agricultural technologies in crop production is the creation of highly productive agrocenoses of leguminous crops, which make the most of the bioclimatic resources of the region [1, 2]. Legumes are the main and very important source of vegetable protein and solve the biological and ecological problems of modern agriculture in Ukraine [3].

Peas have high nutritional and fodder qualities. Pea grain contains from 16 to 36 % of protein, starch, sugar, fat, vitamins (A, B_1, B_2, B_6, C, PP, K, E), carotene, minerals (salts of potassium, calcium, manganese, iron, phosphorus) up to 54 % of carbohydrates [4]. The amount of ash in pea seeds varies considerably and depends on the soil and agricultural practices of their processing,

climate. The average ash content is from 2 to 5 %, as in cereals, 75 % ash consists of phosphorus and potassium. Unlike cereals, pea ash contains less magnesium, but more calcium and especially sulfur. The fat content is small within 2–3 % and varies slightly in different varieties of crops. In the seeds, the fat is mainly found in the embryo [5, 6].

The problem of growing peas in the last decade has been associated with its harvesting, when it was separate and was carried out with a large expenditure of time and energy, and the losses reached about 80 % [7]. But new varieties of foreign and national selection have appeared, suitable for direct combining, which is carried out by conventional combine harvesters with minimal losses. These are the so-called erect or half leafless varieties of peas [8–10]. Their main feature is that the upper leaves are morphologically transformed into fake whiskers, which cause additional adhesion between neighboring plants on the upper tier. However, plants, saturated with so many economically valuable traits, need proper care. First of all, special requirements for sowing material. Only the use of original seeds of high reproduction can provide the yield that was programmed by breeders [11, 12].

The introduction of new varieties into production allows more efficient use of material and technical resources and improves the quality of marketable and seed products [13]. The elements of the technology for growing peas should be aimed at creating optimal conditions for the growth and development of plants at each stage of ontogenesis, and otherwise will lead to a decrease in yield. Compliance with the basic technological conditions for growing peas will allow realizing the genetic potential of new varieties and obtaining high and stable yields and high-quality grain [14–16].

Favorable weather conditions and temperature regime, present during the growing season and especially from the beginning of the laying of generative organs to flowering, have the greatest influence on the productivity and quality of the sowing pea grain [17].

Compared to other legumes, peas have good grain quality and a short growing season. It is one of the best precursors for winter cereals [18–20].

The purpose of the research was to study the peculiarities of the formation of the yield of sowing pea, depending on the effect of mineral fertilizers and growth regulators in the conditions of the western Forest-steppe.

2. Materials and methods

2. 1. Agrochemical characteristics of the experimental site soil

Laboratory analyzes of soil were carried out in Khmelnytskyi Regional State Technological Center for Soil Fertility Protection and Product Quality according to the following methods: pH of aqueous and saline suspensions and hydrolytic acidity by the Kappen method; the number of absorbed bases by the Kappen-Gilkowitz method; humus content according to Tiurin; alkaline hydrolyzed nitrogen according to Cornfield; mobile compounds of phosphorus and potassium by the modified Chyrikov method [21–24].

The soil of the experimental field is typical black soil (chernozem), deep low-humus heavy loam on loess-like loam. According to the research results of the Department of Agriculture, Soil Science and Plant Protection of State Agrarian and Engineering University in Podilya, it was established, that the experimental site is characterized by the following agrophysical and agrochemical soil properties: the density of the solid phase of the 0–30 cm soil layer is 2.55–2.62 g/m^3; pH of aqueous and salt suspensions and hydrolytic acidity according to the Kappen method in the modification of TsINAO (GOST 26212-91). Thus, the aqueous pH in the upper layer is: 6.8 a, hydrolytic acidity is 0.70 mg-eq./100 g of soil. The content of humus according to Tiurin in the modification of TsINAO (GOST 26213-84) in the upper horizon is 3.39 %. Density of folding – 1.17–1.25 g/m^3; total porosity – 51.6–54.7 %, nitrogen content (according to Cornfield) – 13.6–14.2, phosphorus and potassium according to Chyrikov (DSTU-4115-2002) – 15.7–16.4 and 22.4–26.3 mg per 100 g of soil, respectively. Absorption capacity at the level of 20–25 mg-eq./100 g of soil.

2. 2. The scheme of the experiment and research methods

The field research envisaged the study of the growth, development, and productivity of sowing peas varieties, depending on fertilization with mineral fertilizers and growth regulators in the conditions of the western Forest-steppe.

The field experience was laid in the ten-field research crop rotation of the Podillia Research Center during 2016–2018.

The experiment studied the effect and interaction of three factors: A – varieties; B – mineral fertilizers; C – growth regulators (**Table 1**).

Table 1

The scheme of the experiment

Factor *A*: variety	Factor *B*: fertilizers	Factor *C*: growth regulators
A_1 – Hotivskyi (control)	B_1 – $P_{30}K_{45}$ (control)	C_1 – without growth regulators (control)
	B_2 – $N_{15}P_{30}K_{45}$	C_2 – Emistim C
A_2 –Chekbek	B_3 – $N_{30}P_{30}K_{45}$	C_3 – PlantaPeg
A_3 – Fargus	B_4 – $N_{45}P_{30}K_{45}$	C_4 – Vympel

Our research aimed to investigate and compare intensive varieties of sowing peas (factor A), recommended for the Forest-steppe zone.

The originator of the Hotivskyi variety, which was taken for control, is the company "Axial Eximpo Prague" (Czech Republic), added to the State Register of Ukrainian varieties in 2006. The variety is a high-yielding, intensive type, suitable for mechanized harvesting. By ripeness group – mid-season. According to the applicant, the recommended seeding rate is 1.0–1.2 ml/ha of germinating seeds.

The mid-ripening pea variety Chekbek was added to the State Register of Varieties in 2009. The originator of the variety is the Plant Production Institute nd. a. V. Ya. National Academy of Agrarian Sciences. This variety is suitable for direct harvesting. The recommended seeding rate, depending on the growing area of this variety, is 1.2–1.4 mln of germinating seeds per hectare.

In 2007, the pea variety Fargus was added to the State Register of Varieties of Ukraine, the applicant of which is the Research and Production Corporation "Stepova" LLC (Dnipro). The intensive type variety is suitable for mechanized harvesting. According to the applicant, the sowing rate is from 1.0 to 1.2 ml/ha of similar seeds.

Phosphorus and potassium fertilizers were applied to the main tillage in autumn, and nitrogen fertilizers were applied in spring under pre-sowing tillage.

Pea crops of all three studied varieties were sprayed with plant growth regulators (factor *C*) in the micro stage BBCH 55–65 (the first flower buds appear, but the flowers are still closed – full flowering, 50 % of the flowers are open). Emistim C growth bioregulator with a broad spectrum of action – a product of biotechnological cultivation of epiphytic fungi from the root system of medicinal plants was used at a dose of 30 ml/ha. Plant growth regulator PlantaPeg (active substance polyethylene glycol (PEG) – 400 and polyethylene glycol (PEG) – 1500, 800 g/l, fulvic acids and salts of humic acids, 4 g/l) was used with the recommended application rate of 25 g/ha. Vympel – a contact-systemic growth regulator, intended for the treatment of seeds and vegetative plants, the active ingredient is polyethylene oxide (PEO) – 770 g/l, washed salts of humic acids up to 30 g/l for the solution, used at a dose of 30 ml/ha.

The sown area of the elementary plot was 0.50 m^2, the accounting area – 0.48 m^2. The predecessor is winter wheat. The soil cultivation was carried out due to generally accepted for the Forest-steppe zone of Ukraine mode.

The seeds were sown with a grain seeder, in the usual line method with a row spacing of 15 cm, with a seeding depth of 5–6 cm and a seeding rate of 1.2 mln/ha of germinating seeds for all studied varieties of sowing peas. After sowing on the 2nd day, the sowing area was rolled with a ring roller. The studies were carried out according to the scheme in a trifactor field experiment by the method of randomized split areas. The variants are repeated four times.

The grain yield was determined on the counting part of the plots by the method of continuous collection and weighing the grain of each plot, followed by the determination of moisture and debris. To determine the mass of 1000 seeds from a grain of peas, two replicates of 500 seeds were manually deducted without selection and weighed with an accuracy of one-hundredth of a gram. In the case, when the actual discrepancy exceeds the allowable, the third repetition was taken [25–27].

3. Results

The maximum indices of the mass of 1000 seeds were in the peas of the Chekbek variety in the feeding areas of $N_{30}P_{30}K_{45}$ in combination with the Vympel growth regulator and amounted to 266.4 g, for the same dose of mineral fertilizers and growth regulators Emistim C and PlantaPeg, respectively 265.7 g and 264. 9 g. This fertilizer composition also had a positive effect for varieties Hotivskyi and Fargus, these indicators were 257.2–260.6 g and 235.1–238.4 g, respectively.

Yields on feeding options of $N_{30}P_{30}K_{45}$ in combination with growth regulators Emistim C and Vympel were respectively 3.71–3.79 t/ha for the variety Hotivskyi, 4.15–4.32 t/ha for the variety Chekbek, and the lowest yield was in variety Fargus 3.22–3.30 t/ha. The effect of PlantaPeg growth regulator had a smaller influence, but the biological productivity of peas was higher than the option without treatment with growth regulators and ranged from 3.13 to 4.0 t/ha depending on the variety (**Table 2**).

Table 2

Influence of mineral fertilizers and growth regulators on the individual productivity of peas (average for 2016–2018)

Factor *B* (mineral fertilizers doses)	Factor *C* (growth regulators)	Factor *A* (variety)					
		Hotivskyi		Chekbek		Fargus	
		Mass of 1000 seeds, g	Yield	Mass of 1000 seeds, g	Yield	Mass of 1000 seeds, g	Yield
$P_{30}K_{45}$ (c)*	Without processing (c)	249.5	2.11	249.5	2.68	231.4	1.82
	PlantaPeg	252.4	2.55	252.4	3.05	233.4	2.42
	Emistim C	253.9	2.74	253.9	3.18	234.0	2.51
	Vympel	254.3	2.85	254.3	3.31	235.1	2.64
$N_{15}P_{30}K_{45}$	Without processing	251.1	2.67	251.1	3.23	232.9	2.50
	PlantaPeg	254.7	3.17	254.7	3.75	234.7	2.95
	Emistim C	256.2	3.34	256.2	3.87	235.9	3.06
	Vympel	257.3	3.53	257.3	3.97	236.6	3.15
$N_{30}P_{30}K_{45}$	Without processing	253.0	3.08	253.0	3.47	234.0	2.84
	PlantaPeg	257.2	3.60	257.2	4.00	235.1	3.13
	Emistim C	259.2	3.71	259.2	4.15	236.8	3.22
	Vympel	260.6	3.79	260.6	4.32	238.4	3.30
$N_{45}P_{30}K_{45}$	Without processing	250.3	2.98	250.3	3.00	232.3	2.48
	PlantaPeg	253.6	3.28	253.6	3.34	234.0	3.01
	Emistim C	255.8	3.42	255.8	3.60	235.2	3.13
	Vympel	258.3	3.52	258.3	3.70	236.4	3.21
$LSD_{0.05}$ mass of 1000 seeds for factor *A* – 0.71							
$LSD_{0.05}$ mass of 1000 seeds for factor B – 0.83							
$LSD_{0.05}$ mass of 1000 seeds for factor C – 0.83							
$LSD_{0.05}$ yield for factor A – 0.03							
$LSD_{0.05}$ yield for factor B – 0.04							
$LSD_{0.05}$ yield for factor C – 0.04							

*Note: Footnote * – (c) – control.*

4. Discussions

Harvest structure analysis is an important method for assessing crop development. The main elements of the structure of the pea yield include the number of plants, preserved for harvesting, the number of beans per plant, the number of seeds in a bean, and the mass of 1000 seeds [28–30].

One of the important indicators of the yield structure is the mass of 1000 seeds, which in our studies depended on the varietal characteristics of the crop, the introduction of various doses of mineral fertilizers, and growth regulators. In the Hotivskyi pea variety, the mass of 1000 grains ranged from 249.5–260.6 g, Chekbek 261.1–266.4 g, and Fargus 231.4–238.4 g, depending on the applied cultivation techniques.

In the $P_{30}K_{45}$ fertilizer variants without spraying the plants with growth regulators, the Hotivskyi pea variety provided a mass of 1000 grains of 249.5 g, Chekbek – 261.1 g, and Fargus – 231.4 g.

After spraying the crops with PlantaPeg, Emistim C and Vympel growth regulators, the mass of 1000 seeds did not increase significantly, on average by 2.8–4.8 g, depending on the variety. With the addition of nitrogen in the norm N_{15}, N_{30}, and N_{45}, but without growth regulators, the mass increased for the variety Hotivskyi by 1.6–3.5 g, for the variety Chekbek by 1.4–2.7 g, and Fargus by 1.5–2.6 g.

Grain yield is an integrated indicator of the effect of all life factors on a plant organism during its growth and development. In our studies, it largely depended on the biological characteristics of the variety, the supply of moisture and nutrients to the plant, the technological methods of cultivation, as well as the natural and climatic conditions. The pea varieties Hotivskyi, Chekbek, and Fargus, intensive type, have a high yield potential, adapted to growing conditions in the western Forest-steppe.

Favorable conditions for the growth and development and realization of the biological productivity of peas were created with the introduction of mineral fertilizers in doses of $N_{30}P_{30}K_{45}$, with the finishing of crops with growth regulators, which, in extremely low concentrations, significantly changed the processes of their vital activity in plants and contributed to an increase in the yield of pea grain.

On the $N_{45}P_{30}K_{45}$ fertilizer variants without treatment with growth regulators, the average grain yield for three years in Hotivskyi, Chekbek, and Fargus pea varieties was 2.98 t/ha, 3,00 and 2.48 t/ha, respectively. After spraying the plants with regulators, the yield increased by 18–19 % compared to variant $P_{30}K_{45}$, while in the variants of fertilizers, where mineral fertilizers were applied in doses of $N_{30}P_{30}K_{45}$ and growth regulators, these figures increased by 23–25 %. Due to this, only mineral fertilizers in doses of $N_{45}P_{30}K_{45}$ were less effective.

5. Conclusions

Over the years of research, study, and detailed analysis of the grain of the sowing pea varieties Hotivskyi, Chekbek, and Fargus, it was established, that the mass of 1000 seeds depended on the varietal characteristics of the crop, the introduction of various doses of mineral fertilizers and growth regulators. In the Hotivskyi pea variety, the mass of 1000 grains ranged from 249.5 to 260.6 g, Chekbek 261.1 to 266.4 g, and Fargus 231.4 to 238.4 g.

The application of mineral fertilizers in doses of $N_{30}P_{30}K_{45}$ in combination with growth regulators Emistim C and Vympel had the best effect on the biological productivity of peas. Yields in these variants were 3.71–3.79 t/ha for Hotivskyi, 4.15–4.32 t/ha for Chekbek, and 3.22–3.30 t/ha for Fargus.

The introduction of higher norms of nitrogen fertilizers $N_{45}P_{30}K_{45}$ less effectively influenced the biological productivity of pea grain since the introduction of mineral nitrogen in a higher dose led to the suppression of the symbiotic and photosynthetic apparatus, these are the main indicators for the formation of the yield of leguminous crops.

Conflicts of interest

There is no conflict of interest

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THE CHARACTERISTIC OF ECONOMICALLY IMPORTANT TRAITS OF DAIRY COWS DEPENDING ON TYPE OF BODY CONSTITUTION

Ruslana Stavetska
Department of Genetics, Breeding and Selection of Animals
Bila Tserkva National Agrarian University
8/1 Soborna sq., Bila Tserkva, Ukraine, 09117
rstavetska@gmail.com

Yurii Dynko
Institute of Animal Breeding and Genetics nd. a. M. V. Zubets of National Academy of Agrarian Science of Ukraine
1 Pogrebniaka str., Chubynske, Kyiv reg., Ukraine, 08321
yura.dynko@gmail.com

Abstract

This study focuses on research of economically important traits of Ukrainian black-and-white dairy cows with different types of body constitution. The aim of the study was to differentiate dairy cows into constitution types and to identify the best types in terms of growth, exterior, milk productivity and reproductive capacity. The cows were differentiated into low-, mid- and high-capacity types of body constitution. Depending on their type the features of growth, exterior, milk productivity and reproduction capacity of primiparous have been studied.

It has been established, that the intensity of growth of heifers from birth to 18 months depended on the type of their body constitution. Higher live weight, absolute and average daily gains were characteristic of heifers with low-capacity body constitution. Their live weight at the age of 18 months on average was 388.5 kg, it was on 30.9 kg and 60.3 kg ($P<0.01$) more than live weight of mid- and high-capacity heifers. The advantages of low-capacity heifers in average daily gain during the growing period were 60 g and 117 g, respectively.

The results of the exterior evaluation have shown that primiparous with the high-capacity type of body constitution had greater size and larger measurements primarily of the chest and barrel ($P<0.05–0.001$).The higher values of linear evaluation were also observed in cows with the high-capacity type, which were characterized by well-developed chest, wide rump, desired rear view of rear legs, firm udder attachment and strong central ligament. The power of influence of the type of body constitution on linear type traits ranged from 0.5 % (rear teat placement) to 46.2 % (chest width).

Higher milk productivity was a characteristic of mid-capacity cows (an advantage of 305-d milk yield – 340–662 kg, milk fat yield – 9.0–21.0 kg, milk protein yield – 9.8–19.8 kg).The best results of reproduction capacity have been observed in cows with the high-capacity type of body constitution (calving percentage – 87.5 %).

Keywords: dairy cows, type of body constitution, growth, exterior, milk productivity, reproductive capacity.

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1. Introduction

Until recently, the most important traits for dairy cows were considered milk yield and milk composition [1]. However, one-sided selection for high yield has led to decrease the resistance of animals to disease, deterioration in reproductive performance and reduction of productive life of dairy cows [2, 3]. Nowadays the range of interest of milk producers has significantly expanded and the selection process takes into account reproductive traits, live weight, exterior, longevity, health of animals etc. Exterior as well as body constitution belong to the functional traits, they are elements of selection indices worldwide. Their share in the most widespread indices varies from 17 to 40 % [4–6]. The importance of studying the body constitution is that only animals with good constitution can be healthy, high producing, they have a high level of reproductive performance and longevity. Taking into account the types of body constitution in the selection process contributes to more objective and informative assessment of dairy cows.

Numerous studies of scientists were devoted to the types of body constitution. Their teachings were based to the ratio of tissues and organs in the body of animals [7], the intensity of the redox processes and gas exchange[8], the adaptability of species [9], the density of the animal body [10], the Golden Ratio of Pythagorean [11] etc.

For evaluation of the body constitution of dairy cattle, the shape of chest and its cross-sectional area was assessed [12]. Later this method was developed and it was proposed to assess the capacity of the thoracic cage [13]. Three types of body constitution was identified – with large medium and small volume. This method of determining the body constitution was further developed. The aim of this research was in developing a fundamentally new method of differentiation of dairy cows into low-, mid- and high-capacity types. The author proposed to determine the type of body constitution as a ratio of thoracic conditional capacity and live weight and he called it – the capacity-weight coefficient [14]. It was proposed to implement the capacity-weight coefficient into linear evaluation of dairy cows, which was recognized by ICAR [15] and was effective from selection and production standpoints.

The research aim was to investigate the economically important traits of dairy cows depending on their body constitution and establish the types of body constitution with the expected rate of growth, exterior, milk productivity and reproduction capacity. In order to achieve this goal primiparous were divided into three types of body constitution and depending on the type their growth, exterior, milk productivity and reproduction capacity have been studied.

2. Materials and methods

The study was carried out on the commercial dairy farm LLC "nd. a. Shchorsa", which located in Kyiv region, Ukraine. The study was conducted in 2016–2018. The farm is rearing more than 1500 dairy cattle of Ukrainian black-and-white dairy breed with 600 lactating cows. The differentiation of cows by types of body constitution included measurements of the chest depth and chest width behind the shoulder blades and last rib, length of the thoracic cage, area of the thoracic cage behind the shoulder blades and last rib, conditional capacity of the thoracic cage and live weight. Individual constitutional characteristics of animals were determined by the integrated capacity-weight coefficient (CWC), which characterized the liters of thoracic capacity per kilogram of live weight [14], (n=101).

$$CWC = \frac{h \times \left(S_1 + \sqrt{S_1 S_2} + S_2\right)}{LW \times 3,000}, \quad (1)$$

where CWC – capacity-weight coefficient, L/kg; h – length of the thoracic cage, cm; S_1 – cross-sectional area of the thoracic cage behind the shoulder blades, cm^2; S_2– cross-sectional area of the thoracic cage behind the last false rib,cm^2; LW – liveweight, kg; 3,000 – a constant value, obtained as a result of mathematical structuring of the formula (3×1,000).

In the studied group of cows CWC was 0.72 L/kg. Cows were divided into three types of body constitution based on the deviation CWC 0.67σ: low-capacity type (n=26, CWC=0.67 L/kg or less), mid-capacity type (n=52, CWC=0.68–0.75 L/kg) and high-capacity type (n=23, CWC=0.76 L/kg or more).

The intensity of growth of repair heifers was estimated by live weight, absolute growth rate and average daily gain from birth to 18 months. The exterior of cows with different types of body constitution was assessed through body measurements and linear evaluation. Milk productivity of primiparous (milk yield, fat content and protein content in milk, milk fat yield, milk protein yield, duration of lactation, milk yield per day of lactation) was estimated for 305-day and per lactation. Reproduction capacity of cows was studied by age and weight at first mating and first calving, days open, calving interval, services per conception and calving percentage.

Statistical analyses were performed using Microsoft Excel 2010.

3. Discussion of research results

It has been found, that the biggest newborn heifers had the high-capacity type of body constitution, but the highest live weight from birth to the age of 18 months was characterized for heifers with the low-capacity type. The live weight of low-capacity heifers at 18 months was by 30.9 kg ($P<0.05$) and 60.3 kg ($P<0.01$) more than the live weight of mid- and high-capacity heifers (Table 1).

Heifers with the low-capacity body constitution in the period of 0–3.0 months had by 6.4 kg higher absolute growth rate compared to the other types, in the period of 3.1–6.0 months – 3.2–4.0 kg, 6.1–9.0 months – 2.7–8.2 kg, 9.1–12.0 months – 3.9–11.9 kg, 12.1–15.0 months – 8.1–15.6 kg, 15–18 months – 8.1–17.3 kg. For average daily gain there was a trend similar to the absolute growth rate: the highest value was shown by the animals with the low-capacity type, the lowest – by animals with the high-capacity type of body constitution. The average daily gain of heifers with the low-capacity type from birth to 18 months was 657 g, mid-capacity – 597 g, high-capacity type – 540 g. Depending on growing periods, heifers with the low-capacity type of body constitution had an advantage in average daily gain over animals of the same age with the high-capacity type by 71–193 g, and with age the differences have been increased. The average daily gain of heifers with the low-capacity type was 29–90 g higher compared to the mid-capacity type.

Table 1

Growth rate of repair heifers with different types of body constitution, x±S.E

Parameters		Type of body constitution		
		low-capacity	mid-capacity	high-capacity
Birth weight, kg		33.5±0.74	35.0±0.43	36.6±0.48
Absolute growth rate during periods, kg	0–3.0	50.7±1.86	44.3±1.80	44.3±3.41
	3.1–6.0	59.9±3.29	56.7±2.36	55.9±4.71
	6.1–9.0	62.3±2.84	59.6±2.38	54.1±4.69
	9.1–12.0	64.9±3.18*	61.0±2.70	53.0±4.17
	12.1–15.0	61.1±3.34**	53.0±2.01	45.5±3.09
	15.1–18.0	56.1±3.92**	48.0±2.13	38.8±3.72
Live weight at 18 months, kg		388.5±11.76**	357.6±9.38	328.2±17.01

Note: $^{**}P<0.01$; $^{*}P<0.05$, level of significance compared to the high-capacity type

It has been claimed, that the higher live weight of newborn heifers, the higher their growth rate, and cows with higher live weight were characterized by higher milk productivity. In our study mixed results were obtained. In particular, newborn heifers with the low-capacity type of constitution had less live weight compared to heifers with the mid- and high-capacity types but at the age of 18 months heifers with the low-capacity type had an advantage in live weight [16, 17].

The optimal average daily gain of heifers from birth to the age of first mating was considered not less than 500 g [18] and 830 g at the age of 10–15 months [19, 20]. In our study the average daily gain from birth to 18 months exceeded 500 g, but at10–15 months was less than recommended [19, 20] and ranged from 506 g to 721 g depending on the type of body constitution.

Features of economically important traits of dairy cows, differentiated by the types of body constitution with *CWC*, were studied by the author of the method. The results of our research partially coincided with the data, provided earlier [21]. In particular, he reported the higher live weight at birth by 2.5 kg ($P>0.95$) in heifers with the low- and mid-capacity types of body constitution. In our study higher live weight was observed in newborn heifers with the high-capacity type.

Analyses of linear evaluation of cows with different types of body constitution on a 9-point scale have shown that animals with the high-capacity type compared to the low-capacity type had greater size, they were taller (+0.8 point), more width of chest (+1.7) and depth of barrel (+0.8), wider in the rump (+0.5), they had more correct shape of rear legs (+0.8, $P<0.05$), stronger fore and rear udder attachment (+0.4, $P<0.05$ and +0.9, respectively), stronger central ligament (+0.8, $P<0.05$) and longer teats (+0.5 point) (Table 2).

Table 2

Linear evaluation of dairy cows with different types of body constitution on a 9-point scale, $x \pm S.E$

Linear type trait	Type of body constitution		
	low-capacity	mid-capacity	high-capacity
Stature	4.5±0.36	4.8±0.26	5.3±0.44
Chest width	4.5±0.20	5.5±0.13	6.2±0.21
Body depth	7.3±0.17	7.5±0.11	8.1±0.14
Angularity	5.9±0.28	5.9±0.18	5.7±0.28
Rump angle	6.3±0.25	6.2±0.16	6.4±0.22
Rump width	6.9±0.19	7.2±0.16	7.4±0.21
Rear legs set	4.9±0.22	4.7±0.14	4.9±0.28
Rear legs rear view	4.4±0.21	4.6±0.14	5.2±0.21*
Foot angle	4.7±0.26	4.7±0.12	4.8±0.28
Fore udder attachment	4.4±0.10	4.2±0.09	4.8±0.19*
Rear udder height	5.2±0.36	5.3±0.24	6.1±0.31
Central ligament	5.8±0.23	6.1±0.19	6.6±0.23*
Udder depth	5.1±0.33*	4.7±0.21	4.0±0.26
Front teat placement	5.3±0.36	5.5±0.23*	5.4±0.36
Rear teat placement	4.7±0.21	4.7±0.19	4.9±0.23
Teat length	5.3±0.21	5.8±0.12	5.8±0.13
Body condition score	5.5±0.20*	4.8±0.22	4.8±0.25

*Note: *P<0.05, level of significance compared to the lowest value*

Simultaneously, cows with the low-capacity type had a deeper udder ($P<0.05$) and higher body condition score ($P<0.05$). The development of linear type traits of cows with the mid-capacity type of body constitution usually has taken an intermediate position between the low- and high-capacity types.

The strength and direction of relationship between the types of body constitution and linear type evaluation of dairy cows depended on the type of constitution and linear type trait. The correlation between them ranged from –0.22 to 0.58. There was a strong correlation between the type of body constitution and chest width ($r=0.58$, $P<0.001$), body depth ($r=0.48$, $P<0.001$); medium correlation – with rear legs set ($r=0.30$, $P<0.01$), central ligament ($r=0.26$, $P<0.05$), teat length ($r=0.25$, $P<0.05$) and udder depth ($r=–0.22$, $P<0.05$). It should also be noted the correlation between the type of body constitution and stature ($r=0.17$) and rump width ($r=0.19$). The power of influence of the types of constitution on linear type traits ranged from $\eta_x^2 = 0.5$ % (rear teat placement) to $\eta_x^2 = 46.2$ % (chest width).

The analyses of milk productivity of primiparous with different types of body constitution had shown the advantage of cows with the mid-capacity type in 305-d milk yield, milk fat yield and milk protein yield. Their advantage compared to the low- and high-capacity types in 305-d milk yield was 340 kg and 662 kg, respectively, milk fat yield – 9.0 kg and 21.0 kg, milk protein yield – 9.8 kg and 19.8 kg (Table 3).

Table 3

Milk productivity of primiparous with different types of body constitution, $x \pm S.E.$

Parameters	Type of body constitution		
	low-capacity	mid-capacity	high-capacity
Duration of lactation, days	404±23.5	387±16.0	358±25.9
305-d milk yield, kg	7,055±288.6	7,395±205.7	6,733±357.6
Milk yield per lactation, kg	9,338±782.6	9,239± 540.4	7,800±704.5
Fat content in milk, %	3.52±0.013*	3.48±0.007	3.51±0.010*
Milk fat yield, kg	328.7±17.09*	321.6±18.33*	273.7±13.51
Protein content in milk, %	3.11±0.004	3.10±0.003	3.11±0.004
Milk protein yield, kg	290.7±14.34*	286.3±10.62*	242.7±12.97
Milk yield per day of lactation, kg	23.1±0.85	23.9±0.73	21.8±1.17

*Note: *P<0.05, level of significance compared to the lowest value*

Prolonging lactation of cows with the low-capacity type has led to higher milk yield per lactation, milk fat yield and milk protein yield. The milk yield per lactation of cows with the low-capacity type of body constitution compared to the high-capacity type was 1538 kg higher, milk fat yield – 55.0 kg (P<0.05), milk protein yield – 48.0 kg higher (P<0.05). Cows with the mid-capacity type have also significantly prevailed over animals with the high-capacity type in milk yield per lactation (+1439 kg), milk fat yield (+47.9 kg, P<0.05) and milk protein yield (+43.6 kg, P<0.05). More objectively the milk yield per day of lactation has shown the efficiency of milk production. Given this trait, cows with the mid-capacity type of body constitution have shown higher efficiency of milk production. Their milk yield per day of lactation was 23.9 kg that was 0.8 more compared to the low-capacity type and 2.1 kg more compared to the high-capacity type.

It was found, that the higher milk productivity was observed in cows with the high-capacity type of body constitution [22]. The advantage of Holstein cows with the high-capacity type compared to the low-capacity type in 305-d milk yield was 1718 kg, milk fat yield – 64.84 kg, milk protein yield – 55.26 kg (P>0.999 in all cases). In our study Ukrainian black-and-white dairy cows with the mid-capacity type of body constitution have prevailed in 305-d milk productivity. The higher milk yield per lactation, milk fat yield and milk protein yield have been shown by cows with the low-capacity type of body constitution. Milk per day of lactation of cows with the mid-capacity type was 23.9 kg, low-capacity – 23.1 kg, high-capacity type – 21.8 kg.

The influence of body constitution type of primiparous on traits of milk productivity ranged from 2.4 to 34.8 % and it was significant for milk yield (η_x^2 = 24.5 %, P<0.05), milk fat yield (η_x^2 = 34.8 %, P<0.01) and milk protein yield (η_x^2 = 26.0 %, P<0.05).

It has been reported, that cows with higher average daily gain had prevailed in milk yield over animals with lower growth intensity [17]. The difference between them for the first lactation milk yield was 1093.0 kg or 28.06 % (P>0.999). The statement that animals with higher growth rate were characterized by higher milk productivity has not been confirmed in our study. If higher growth rate were observed in cows with the low-capacity type of body constitution, the highest 305-d milk yield was shown by primiparous with the mid-capacity type.

In our study we found some differences in the traits of reproduction capacity depending on the type of body constitution. The youngest age at first mating was typical for cows with the mid-capacity type of body constitution – 15.9 months, that was 2.8 months less compared to the high-capacity type (P<0.05) (Table 4).

Table 4

Reproduction capacity of primiparous with different types of body constitution, $x \pm S.E.$

Parameters	Type of body constitution		
	low-capacity	mid-capacity	high-capacity
Age at first mating, months	17.0±0.58	15.9±0.58	18.7±1.16*
Live weight at first mating, kg	367.4±12.95**	313.7±6.70	320.6±9.02
Age at first calving, months	26.3±0.55	26.3±0.67	28.8±1.33
Live weight at first calving, kg	503.0±6.30**	474.5±5.17	452.1±7.95
Days open	183±23.4	169±15.3	140±25.8
Calving interval, days	461±23.3	444±15.6	417±25.7
Services per conception	2.0±0.23	2.2±0.24	1.8±0.41
Calving percentage, %	79.1±3.61	82.2±2.56	87.5±2.56

*Note: *P<0.05, level of significance compared to the lowest value*

However, this did not have an impact on the age at first calving, which was the same for cows with the low- and mid-capacity types – 26.3 months. This was due to the higher value services per conception in cows with the high-capacity type (2.2), which was 0.2 higher compared to the low-capacity and 0.4 higher – high-capacity types. Since the cows with the low-capacity type of body constitution were characterized by the highest growth rate, their advantage in live weight at first mating and at first calving was evident. Their live weight at first mating was 46.8 kg and

53.7 kg higher, at first calving –28.5 kg and 50.9 kg higher compared to the high-capacity and mid-capacity types, respectively (P<0.01 in all cases).

An optimum age of dairy cows at first calving was not considered older than 24 months [19, 20, 23]. The recommended live weight at first mating was about 85 % of adult cows live weight, at which primiparous were characterized by high milk productivity and reproduction capacity. In our study an average age at first calving of primiparous was higher – 26.3–28.8 months with live weight 465–485 kg.

The less days open, shorter calving interval, higher calving percentage were recorded for cows with the high-capacity type of body constitution, the worst value of reproduction capacity traits was inherent to cows with the low-capacity type. Cows with the high-capacity type compared to the low- and mid-capacity types had 43 and 29 days open less, respectively, 44 and 27 days shorter calving interval, 8.4 and 5.3 % higher calving percentage.

Thus, respecting features of cows with different types of body constitution made it possible to optimize the selection process in a herd and to forward it into the required direction. It has been established, that cows with the low-capacity type had the higher live weight from birth to 18 months, higher growth rate and, as a result, higher age and live weight at first calving. Primiparous with the mid-capacity type had the higher 305-d milk productivity and milk yield per day of lactation. Cows with the high-capacity type were of the greater size, they have shown the best results of linear evaluation and reproduction capacity.

4. Conclusions

The obtained results of the study can be used in dairy herds to differentiate cows by types of body constitution. Since the type of body constitution of cows is associated with economically important traits (growth, exterior, milk productivity, reproductive capacity), it can be involved into the selection process in a herd, taking into account the desired characteristics of future offspring. The disadvantage of this study is the lack of results of differentiation of mature cows (second, third lactation) on types of body constitution and their relationship with economically important traits. This research has already been conducted and it will be presented in a future publication.

Differentiation of cows depending on the type of body constitution has enabled to identify the features of their growth, development, exterior, milk productivity, reproduction capacity. Taking into account the results could help to optimize the selection process in the herd.

It has been established, that growth intensity of heifers from birth to the age of 18 months depended on the type of body constitution. Higher live weight, absolute growth rate and average daily gain were characteristics of heifers with the low-capacity type. Primiparous with the high-capacity type were bigger, with higher measurements of chest and barrel (P<0.05–0.001). The influence of the low- mid- and high-capacity types of body constitution on the linear type traits ranged from $\eta_x^2 = 0.5$ % (rear teat placement) to $\eta_x^2 = 46.2$ % (chest width).

Higher milk productivity was inherent to the cows with the mid-capacity type. The influence of the type of body constitution on milk yield was $\eta_x^2 = 24.5$ % (P<0.05), milk fat yield – $\eta_x^2 = 34.8$ % (P<0.01), milk protein yield – $\eta_x^2 = 26.0$ % (P<0.05). Better reproductive performance was observed in cows with lower milk productivity– high-capacity type (calving percentage – 87.5 %).

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AGROECOLOGICAL INFLUENCE OF MICRONUTRIENT FETILIZERS AND SEED INOCULATION ON A SOYBEAN CROP

Inna Fedoruk[1]
fedoryk_i15@ukr.net

Oleg Bakhmat
Department of Ecology, Quarantine and Plant Protection[2]
qerbah@ukr.net

Yuri Khmelianchyshyn[1]
hmelya75@ukr.net

Olesia Gorodyska
Department of Agrochemistry, Chemical and General Biological Disciplines[2]
olesya_pv@ukr.net

[1]*Department of Crop Production and Forage Production*[2]
[2]*State Agrarian and Engineering University in Podilia*
13 Shevchenka str., Kamianets-Podilskyi, Ukraine, 32316

Abstract

The practical experience substantiates the need to treat soybean seeds with high-quality inoculants and VuksalKoMo 15 with the trace elements content of cobalt and molybdenum. The processes of inoculation of seeds in the form of rhizobial bacteria significantly improve the soy plants ability to fix atmospheric nitrogen in the early stages of development. We begin to observe the rhizobial formation on the corinium soybean system already at the stage of BBCH 12–13. This in turn will affect the yield and productivity of Rosin soybeans.

One of the important aspects of soybean cultivation is providing not only macroelements, NPK, Ca, S, but also microelements.

Carrying out experiments on the effect of seed inoculation on soybean yield, we combined an inoculant, VuksalKoMo preparation and Sdandak Top insecticidal fungicide preparation with a sowing period of up to 5–7 days in a tank mixture. One of the main requirements is the use of high-quality inoculants with a high content of viable nitrogen-fixing bacteria for processing soybean seeds. This, in turn, will ensure high yields of soybeans with optimal costs and the fastest return on investment, especially in today's conditions.

The research results are aimed at solving urgent problems in the technology of growing leguminous crops, namely: developing a version of the technology for growing soybeans for the selection of varieties, adapted to a given climatic zone, the use of inoculants and micronutrients in the conditions of climate change.

Keywords: variety, inoculation; trace elements; symbiotic nitrogen fixation; biological cultivation; grain yield.

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1. Introduction

Among crops, that recently occupy a leading place in the agricultural industry of Ukraine, a significant role belongs to soy. Wide possibilities of use, a consistently high demand in the market, including export, soil enrichment with nitrogen and, as a result, a favorable effect on crop rotation, as well as adaptability of cultivation stimulate the growth of crop areas to almost 2 million hectares.

Soybean is one of the most valuable oilseeds. The completeness of food products is determined by the protein content and its quality. A lack of protein leads to disorders in the physiological and functional functioning of the body. According to the FAO (Food and Agriculture Organization), the protein intake should be 12 % of the total calorie intake of a person's daily diet or 90–100 g, including 60–70 % of animal protein. In animals, the body cannot synthesize protein from inorganic substances, but creates it from plant protein.

The vegetable protein problem can be solved by growing legumes. Legumes in the farming system play an important role, which is associated with their ability to fix atmospheric nitrogen of the air with the help of nodule bacteria and enrich the soil with it.

The chemical composition of a soybean plant can vary significantly depending on the fertility of the soil and the balance of nutrients in it. Under optimal conditions, plants show the same composition regardless of the growing zone. Accordingly, 90 % of the dry matter of plants is carbon dioxide, hydrogen and oxygen from the air. However, they are not fully assimilated if the soil has an insufficient amount of other macro- and microelements.

The lack of trace elements reduces productivity, causes disease damage, affects the grain quality. Microelements are extremely important for the soybeans growth and development, since their presence in sufficient quantities is a prerequisite for intensive assimilation of nitrogen from the air [1].

Soya plays a significant role in biological farming. It fixes nitrogen from the air, providing them with 60–70 % of its needs and leaves it in the soil along with plant residues after harvest. The introduction of scientifically based technology for soybean cultivation allows to obtain 2.5–3.0 t/ha of seeds [2, 3].

However, a weighty argument, one of the main issues in technology, is the processing of high-inoculum seed inoculant.

Inoculation of the seeds of legumes with bacterial preparations (inoculant) has a significant biological potential of soils, in particular, due to legumes that form symbiotic bonds with microorganisms, it is in the soil. Bacteria (rhizobia) sprout in the form of thin hairs and become inflated. In the miscellaneous bacteria on the plant, males are assimilated by a bulbul, and in some cases bacteria are also multiplied. Cybacteria fix nitrogen from the diet, which converts to a gaseous one by assimilating the form of NH_4^+ ion ammonia for plants. Regardless of the bean inoculation, it is not necessary to remove nitrogen from the crop, but also to accumulate the nitrogen in the roots and early gratings, which will provide the successor plants with nitrogen, as well as the soil with organic nitrogen.

New environmental challenges, posed by nature to humanity, caused the need for a transition to steel-living and strong gratitude. The technology for growing soybeans with the strobing of a highly productive seed inoculum is a successful combination of the use of biological preparations and synthetic pesticides to increase economic efficiency and minimize negative environmental impacts.

First of all, soybean productivity depends on growing technology. But a weighty argument, one of the main issues in technology, is the processing of high-inoculum seed inoculant.

It is known, that rhizobia can enter the soil as part of commercial inoculants, spread by air, from seeds or cultivated as symbionts of local leguminous plants. When presowing inoculation of leguminous plants, the number of nodule bacteria entering the soil depends on the size of the seeds, the crop density, the ripeness group and the inoculation method. High-quality inoculants ensure the entry of at least 2×10^3 bacterial cells per seed, or at least 100 thousand bacteria per seed. With increasing frequency and soybean development, the number of rhizobia in the soil is growing rapidly due to their release from the vesicles, which die after the end of the growing season of legumes. In many cases, this ensures the dominance of inoculant strains within 5–15 years after the first, initial inoculation. Rhizobia make up a relatively small part of the soil microbiota – from 0.1 to 0.8 % of its total number.

Thus, inoculation is an important element in the technology of soybean cultivation along with the use of micronutrient fertilizers [4–8].

Trace elements are absorbed by soy in a smaller amount compared to nitrogen, phosphorus, potassium, and sometimes calcium, magnesium and sulfur. Despite this, their role is no less important, and the trace elements lack leads to a significant slowdown in growth rates and lower yields.

Zinc activates a number of enzymes, participates in nitrogen metabolism in the plant and the protein formation, and also plays a major role in the formation of vesicles in the production of tryptophan amino acids. Iron is a necessary component of chlorophyll and is necessary for respira-

tion and photosynthesis processes, the creation of light hemoglobin. Manganese plays an important role in metabolic processes, such as enzyme activation, chlorophyll synthesis, photosynthesis, and nitrate reduction. Copper plays an important role in the functioning of mitochondria and the photosynthesis improvement. Its deficiency can reduce the growth and yield of soybean plants by reducing the intensity of photosynthesis. Molybdenum is necessary for the activity of two important enzymes – nitroreductases and nitrogenases, which are necessary for the reduction of nitrates and atmospheric fixation of nitrogen, relieving the herbicidal load on the culture. Boron is necessary for the activity of the meristem and, consequently, for the growth of shoots, roots, flower organs. Cobalt plays an important role in the effective fixation of nitrogen, has a positive effect on the number and weight of nodule bacteria and the nitrogen content in the plant, which comes from the main application, as well as foliar nutrition [9–12].

The seed treatment with VuksalCoMo 15 positively affects the production of more friendly seedlings, promotes the growth and root system development. It is known the tuberculic load on the culture that activates the nitroreductase enzyme, which contributes to the biological fixation of atmospheric nitrogen.

An equally important element in soybean cultivation technology is its non-cinnamon micronutrient supplementation. Micronutrient deficiency leads to lower yields, increases the risk of disease damage, leads to a loss in seed quality. The key to effective nitrogen fixation is the balanced nutrition of roslins and their provision with micro elements boron, molybdenum, cobalt, sulfur, manganese – their balanced and plentiful selection outside cinnamon is a good harvest guarantee.

An analysis of studies and publications, in which the solution to the problem was started, shows us that micronutrients play a special role in improving the efficiency of the mineral nutrition of plants.

As noted [13], first of all, it is such microelements as: boron, molybdenum, copper, zinc, iron, manganese, cobalt, magnesium. In their absence, no plant can develop normally, since they are part of the most important enzymes, vitamins, hormones, and other physiologically active substances. Trace elements are involved in the synthesis of proteins, carbohydrates, fats, vitamins. Under their influence, the content of chlorophyll in the leaves increases, the assimilation activity of the plant increases, and the efficiency of the photosynthesis process increases.

At the initial stages of the development of the soidoid phase of budding and flowering, it consumes a small amount of NPK, but already from the flowering phase to the massive filling of beans, the time of maximum absorption of the main fertilizer elements begins. As you know, the best way to provide crops with micronutrients is foliar top dressing by spraying during the growing season in critical phases of crop development, namely: phases of 3–5 trigeminal leaves, budding and filling of the lower beans. It is in this way that we can ensure the need for crops in microelements at 100 % [14, 15].

The current state of the agricultural sector forms new approaches to the technology of growing crops. Growing technologies are becoming relevant, in which individual approaches to growing and providing the plant with the necessary elements during the growing season are formed in detail.

The purpose of the article: to reveal the growing technology for the formation of varietal productivity of soybeans depending on the inoculation of seeds and the micronutrient fertilizers introduction to obtain increased yield and seed quality. The basis of soybean cultivation technology using inoculants and micronutrient fertilizers is the southwestern part of the Forest-Steppe of Ukraine.

2. Research methods

The studies were carried out by the intensive planting technology for the western Forest-Steppe.

Agrotechnics is conventional for this zone. A precursor is winter wheat. For comprehensive evaluation of the obtained experimental results, the following observations, analyses and attendant studies were conducted according to conventional methods:

Phenological observations of main development and growth phases coming, plants density in the phase of sprouts and before harvesting, the analysis of structure elements of the harvest were conducted by testing sheaves, selected before harvesting in two non-adjacent iterations by

the "Method of state sort testing of agricultural crops" (2000); the leaf surface area in correspondent growth phases was determined by the method of "cuttings", photosynthetic potential (PP) and photosynthesis pure productivity (PPP) of soya plants were determined by the method of A. A. Nechiporovych; the use of PAR by plants was determined by the method of K. G. Tooming, B. I. Gulyaev; the number and mass of gray bubbles were determined by the method of G. S. Posipanov.

The biological grain yield was determined by the «test sheaves» method in the phase of crop full ripeness. When evaluating the quality of seeds, the following was determined: the grain nature, the "crude" protein content in the grain of soybeans and the «crude» fat content.

The yield structure was investigated in sheaf samples, which were taken at full ripeness, on plots of 0.25 m^2, in four repetitions. The sheaf mass, the number of plants, branches, beans on the main and lateral branches, seeds in a pod, the number and weight of seeds per plant, and the weight of 1000 seeds were determined. Counting of the yield was carried out from the entire accounting area of each plot. The grain harvest was brought to 100 % purity and 14 % moisture.

Mathematical and statistical studies of experimental data were carried out using the Microsoft Excel software package: yield data (by the analysis method of variance of multivariate complexes; correlation and regression analysis), quantitative characteristics of plants (by the method of variational series, difference, correlation, regression, etc.)

The field experiment scheme is based on the action and interaction study of three factors: *A* – varieties (depending on the ripeness group); *B* – micronutrient fertilizers; *C* – inoculation.

3. Results

The studies were carried out at «Garant» LLC (Orynyn village, Kamianets-Podilskyi district, Khmelnytskyi region) in the field crop rotation during 2015–2018. The territorially experimental field is located in the southwestern forest-steppe part of the Khmelnytskyi region; according to the heat and humidification conditions, it belongs to the southern wet agroclimatic part of the region. The total area of the experimental plot was 198 m^2, accounting area – 150 m^2. Repeatability is fourfold. The way to place options in the repetition – by the method of a randomized Latin rectangle.

Having carried out studies on various ripeness groups of such soybean varieties: Maxus, Cordoba, Saska, positive results were obtained from micronutrient fertilizers. As well as from seed treatment with Standak Top 1 l/t and soybean seed treatment with inoculant and trace element with VuxalCoMo 15.

Advantages of treatment with Standak Top – extremely reliable protection against ground pests, as well as sprout flies. The use of this drug prevents the diseases development, such as fusarium, anthracnose, and moldy seeds. It promotes rooting of plants in the soil due to the accelerated development of the root system. An increase in the assimilation of the leaf apparatus surface promotes the nitroreductase activation, which, in turn, activates the photosynthesis processes work, manifests itself in the so-called AgCelence effect, plants have an intensely saturated dark green color, determines the elements of the structure of the grain yield from each experiment, plants are selected for the analysis. The main elements of the structure of the crop of soybean plants are presented in **Table 1**.

According to **Table 1**, the yield indicators of 2015 are quite high, a particularly high indicator in the experiment with seed treatment with inoculant HaiKot for varietal Saska (3.06 t/ha), and also in the experiment with seed treatment with inoculant Chi Stoke+Let Kot+Vuksal Boron in Cordoba varieties (3.14 t/ha), Saska (3.20 t/ha). High yields are shown by the experiment with seed treatment with the inoculant Let Kot+VuksalBoron+Bospholiarna varietal Cordoba (2.94 t/ha), Saska (3.28 t/ha), as well as the experience with seed treatment with the inoculant Chi Stoke+Let Kot+VuksalBoron+Bospholiarna varietal Cordoba (3.20 t/ha), Saska (33.5 t/ha).

When using inoculants in the conditions of the 2016 season, we observe a different effect on yield, depending on the ripeness group of varieties. Variety Maxus to the control gave an increase of 0.12 t/gado 0.72 t/ha, while Cordoba from 0.26 t/ha to 0.71 t/ha and the late-ripening Saska variety in conditions of moisture lack failed to fully form a potential crop and yield growth ranged from 0.06 t/ha to 0.34 t/ha.

Table 1

Grain yield of soybean varieties in the experiment, c/ha

Research option codes	Soybean yield by years, t/ha			
	2015	2016	2017	2018
Sort Maksus				
1	2	3	4	5
Control (without treatments)	1.50	1.32	1.75	3.78
Without inoculants+VuksalBoron	1.65	1.35	1.97	4.03
Without inoculants+VuksalBoron+Bosfoliar	1.87	1.36	2.18	4.31
Treatment with inoculant HiStick	2.07	1.44	2.27	4.08
Inoculant treatment HiKot Super+HighKot Super Extender	2.23	1.68	2.30	4.22
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender	2.12	1.80	2.63	4.72
Treatment with inoculant HiStick+VuksalBoron	2.21	1.48	2.39	4.59
Inoculant treatment HaiKot Super+HaiKot Super Extender+VuksalBoron	2.32	1.79	2.48	4.69
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender+VuxalBoron	2.29	1.97	2.77	5.05
Treatment with inoculantHiStick+VuksalBoron+Bosfoliar	2.33	1.55	2.58	4.41
Inoculant treatment HiKot Super+HighKot Super Extender+VuksalBoron+Bosfoliar	2.42	1.83	2.67	4.58
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender+VukxalBoron+Bosfoliar	2.38	2.04	2.96	4.53
Sort Cordoba				
Control (without treatments)	1.78	0.96	2.4	4.14
Without inoculants+VuksalBoron	1.97	1.01	2.45	4.33
Without inoculants+VuksalBoron+Bosfoliar	2.03	1.03	2.63	4.52
Treatment with inoculant HiStick	2.24	1.22	2.53	4.48
Inoculant treatment HiKot Super+HighKot Super Extender	2.70	1.41	2.67	4.61
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender	3.03	1.48	2.91	4.93
Treatment with inoculant HiStick+VuksalBoron	2.39	1.26	2.47	4.79
Inoculant treatment HaiKot Super+HaiKot Super Extender+VuksalBoron	2.83	1.47	2.86	4.91
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender+VuxalBoron	3.14	1.59	3.15	5.19
Treatment with inoculant HiStick+VuksalBoron+Bosfoliar	2.45	1.30	2.69	4.71
Inoculant treatment HiKot Super+HighKot Super Extender+VuksalBoron+Bosfoliar	2.94	1.52	3.15	4.84
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender+VukxalBoron+Bosfoliar	3.20	1.67	3.49	4.96
Sort Saska				
Control (without treatments)	1.71	0.83	2.27	3.28
Without inoculants+VuksalBoron	1.93	0.85	2.36	3.44
Without inoculants+VuksalBoron+Bosfoliar	2.01	0.86	2.48	3.51
Treatment with inoculant HiStick	2.25	0.89	2.59	3.46
Inoculant treatment HiKot Super+HighKot Super Extender	3.06	0.93	2.64	3.59
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender	3.16	1.05	2.73	3.85
Treatment with inoculant HiStick+VuksalBoron	2.45	0.93	2.88	4.25
Inoculant treatment HaiKot Super+HaiKot Super Extender+VuksalBoron	3.15	0.98	2.95	4.13
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender+VuxalBoron	3.20	1.14	2.92	4.37
Treatment with inoculant HiStick+VuksalBoron+Bosfoliar	2.59	0.94	2.64	3.62
Inoculant treatment HiKot Super+HighKot Super Extender+VuksalBoron+Bosfoliar	3.28	1.00	2.39	3.85
Inoculant treatment HiStick+HaiKot Super+HaiKot Super Extender+VukxalBoron+Bosfoliar	3.35	1.17	2.67	3.71
HIP05 The smallest significant difference	A	0.06	0.08	0.07	0.07
	B	0.06	0.08	0.07	0.07
	C	0.07	0.10	0.08	0.09
	AB	0.10	0.15	0.12	0.13
	AC	0.12	0.17	0.14	0.15
	BC	0.12	0.17	0.14	0.15
	ABC	0.03	0.05	0.04	0.04

As you can see, the largest increase in varieties Maxus and Cordoba was obtained from the use of the inoculant Hai Kot and an even better result was with the use of the minoculant Hai Kot and Chi Stoke, where we see a yield increase of 2.71 t/ha – 5.42 %, which is 0.63–0.65 t/ha (**Fig. 1**). In the late-ripening Saska cultivars, at the use of Vuksal Boron in areas, treated with the inoculant Chi Stoke yields, a yield increase is of 0.10 t/ha, while in the variants using the inoculant Hai Kot/ha 0.15 t/ha and Hai Kot+Chi Stoke, an increase from Vuksal Boron is 0.31 t/ha.

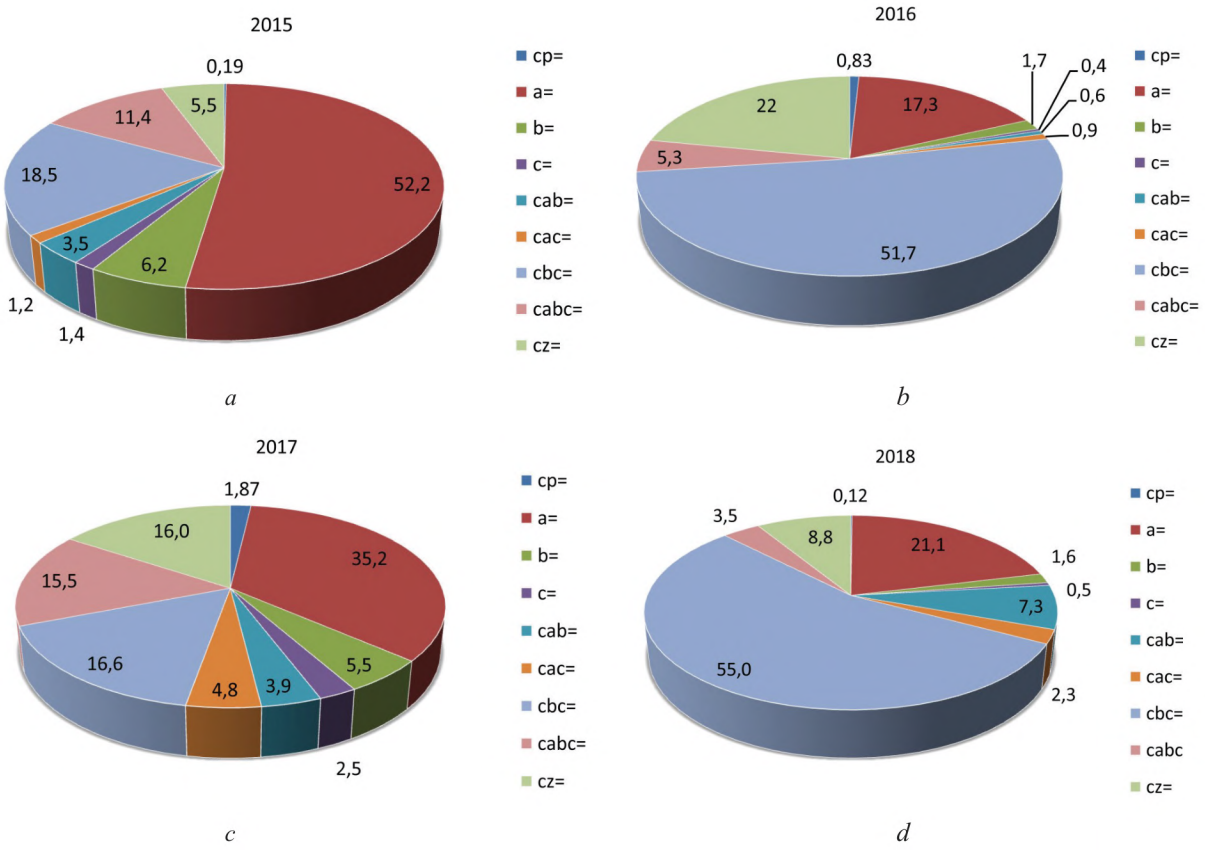

Fig. 1. Equity participation of the studied factors in the formation of soy bean yields, by 2015–2018 %: *a* – studied factors for 2015; *b* – studied factors for 2016; *c* – studied factors for 2017; *d* – studied factors for 2018

Coding in the figures:
– factor *a* – grade;
– factor *c* – inoculation;
– factor *b* – micronutrient fertilizers;
– factor *cbc* – two different inoculants, combined with micronutrient fertilization;
– factor *cac* – two different inoculants and variety;
– factor *cab* – inoculant, variety, micronutrient fertilizer;
– factor *cabc* – two different inoculants, variety, micronutrient fertilizer;
– factor *cp* – scattering (influence) of repetitions;
– factor *cz* – error dispersion.

Analysis of variance showed that in terms of factors, factor *a* (variety) turned out to be more influential in 2015 – by 52.2 %.The second largest was the indicator of *cbc* factors (two different inoculants in combination with micronutrient fertilizers) and amounted to 18.5 %, factor *c* (inoculation) influenced 1.4 %, the share of factor *cbc* (micronutrient fertilization) was 1.7 %.

Accordingly, in 2016, the analysis of variance showed that in the context of factors, the more influential factor – by 51.7 %, was the indicator of CBC factors (two different inoculants in combi-

nation with micronutrient fertilizers). Factor *a* (variety) was 17.3 factor *c* (inoculation) influenced by 0.4 %, the share of the factor *b* influence (micronutrient fertilizers) was 6.2 %.

The analysis of variance showed that in terms of factors, factor *a* (variety) turned out to be more influential in 2017 – by 35.2 %, the second largest was the indicator of *cbc* factors (two different inoculants in combination with micronutrient fertilizers) and amounted to 16.6 %. Factor *cbc* (inoculation) influenced 2.5 %, the share of factor *b* (micronutrient fertilization) was 5.5 %.

Accordingly, in 2018, the analysis of variance showed that in the context of factors, the more influential factor – by 55.5 % was the indicator of *cbc* factors (two different inoculants in combination with micronutrient fertilizers). Factor of (variety) was 21.1 factor *c* (inoculation) by 0.5 %, the share of influence of factor *b* (micronutrient fertilizers) was 1.6 %.

In the variants with repeated introduction of micronutrient fertilizers, the variety Maxus and Cordoba reacted in the best conditions under the conditions of the current drought and the increase is 0.23–0.72 t/ha before a single use of microfertilizer is 0.02–0.07 t/ha. In the variant with Saska varieties, where the application of micronutrient fertilizers with sulfur content, the yield increase was 0.01–0.03 t/ha, respectively, this is 0.11–0.20 – 0.26 %. Thus, the 2016 weather conditions made significant adjustments to the yield of soybean varieties. All varieties of experience responded positively to a much lesser extent to the use of inoculants, especially good indicators of the experiment variant with the inoculant HaiKot and mixtures HaiKot and High Kot+Chi Stik, the use of micronutrients gave an economically feasible increase in productivity. Weather and climatic conditions in 2016 did not allow all varieties to fully reveal their genetic potential

The weather and climatic conditions of 2017 compared with the last vegetation year (2016) were more favorable for growing crops. The moisture lack both in soil and in the air made adjustments to the yield of soybean varieties depending on the ripeness group. According to **Table 1**, yield indicators demonstrate that the early ripening variety Maxus and mid-ripening variety Cordoba, according to technological methods, carried out in accordance with the research scheme, provide yield growth. This is due to the fact that the early ripening variety Maxus and mid-ripening variety Cordoba were in conditions of insufficient moisture and its further decrease, starting from the second decade of July until the end of the third decade of August. Harvest formation by Maxors and Cordoba crops (laying of beans, filling) occurred with a moderate presence of moisture both in soil and in air. In the late-ripening varieties of Saska, flowering processes, laying of beans, poured them for the first and fourth tiers occurred under relatively favorable conditions. From the second decade of July until the third decade of August, the temperature rose to 30–40 °C, and the relative humidity dropped to 25–40 %, as a result of which the flowers and planted beans were aborted. This is due to the fact that the second application to the Bospholiaru, which took place at the beginning of the first decade of July, negatively affected the crop, as shown by the yield results in the table below.

In extreme weather and climatic conditions of 2017, the micronutrient fertilizers usage with low moisture reserves in soil, air and, accordingly, low sap flow in a plant of late-ripening soybean varieties negatively affected crop yields. On varieties of Saska variety, bean cracking was often seen for an hour, while on the variant with a single use of Vuksal Boron this did not happen. Therefore, this should be taken into account in the future, when growing soybean varieties of any ripeness group and long-term weather forecast. Processing soybean crops should be carried out in the presence of optimal productive moisture of the soil, plants should not be under stress.

After analyzing the data of **Table 1**, it can be seen, that in the variant without inoculants, but using VuksalBoron microfertilizer, regardless of the ripeness group of soybean varieties, a yield increase of 0.05 t/ha to 0.22 t/ha is obtained, which is 2.0–12.5 %, respectively. The reuse of Bospholiar made it possible to obtain an additional 0.11 t/ha to 0.21 t/ha, but the maximum result was on Maxus variety – 2.1 t/ha, while Saska variety provided an additional 0.11 t/ha.

The weather and climatic conditions of 2018 in comparison with the last vegetation year (2017) are more favorable for growing crops.

In 2018, the use of inoculants and Vuksal Boron on soy was fully justified. So in the budding phase, the beginning of flowering use of Vuksal Boron gave the following positive results. So, the early ripening variety Maxus added 0.81 t/ha to the control over the use of the Hi Stik drug, and let the Hai Kot Super+HaiKot Super Extender 0.91 t/ha with the use of the inoculant. The mid-season

Cordoba variety is 0.65 t/ha and 0.77 t/ha. Remarkable results were also obtained for late-ripening varieties, so using the Chi Stoke preparation was obtained in accordance with the control of 0.97 t/ha, and using the inoculant, HaiKot Super+HaiKot Super Extender 0.85 t/ha.

Processing soybean seeds with a complex of inoculants Hai Kot Super+Hai Kot Super Extender and Hi Stik and use them for vegetation during budding.The beginning of flowering Vuksal Boron received the following crop increases, the early ripening variety Maxus added 1.27 t/ha to the control. The mid-ripening Cordoba variety, respectively 1.05 t/ha, and the late-ripening variety Saska 1.09 t/ha.

The weather and climatic conditions of 2018 again made adjustments to the size of the crop of soybean varieties, regardless of the ripeness and reuse groups of Bospoliaru, all varieties reacted positively in yield increase. Reuse of microfertilizers in the bean loading phase reduced the yield increase from 33.6 % to 21.4 % with a single use of Vuksal Boron and up to 27.8 % with a maximum of 10.4 % with repeated use of Bospholiaru.

The weather and climatic conditions of 2018 allowed early and mid-ripening soybean varieties to reveal their genetic potential. The late-ripening Saska variety once again during the period of the experiments lacks productive soil moisture and air humidity, which negatively affects the flowering and setting of beans and their subsequent abortion. Ultimately, this is a shortage of crops and not stable economic efficiency of growing crops.

After conducting research on different ripeness groups from soybean varieties, such as Maxus, Cordoba, Sasuke, they received positive results from the use of inoculants.

Consequently, an increase in soybean production is possible only through the improvement of existing and the development of new elements of the technology for its cultivation, using new nutritional products.The use of inoculants, containing modern, highly effective, culture-specific strains of rhizobial bacteria with increased viability in high concentrations, ensures the formation of the maximum number of vesicles on the root system of plants.The combination of the inoculation process and the use of micronutrient fertilizers in the growing technology, as the results of the study show, give significant results in increasing yields.

4. Conclusions

An integral part of technological measures that make it possible to realize the potential of the soybean genotype is balanced mineral nutrition, not only the main elements (nitrogen, phosphorus, potassium), but also trace elements. One of the most effective measures to improve the growth and development of soybean plants, and, accordingly, its productivity, is the use of micronutrients.

The use of inoculants, containing modern, highly effective, culture-specific strains of rhizobial bacteria with increased viability in high concentrations, ensure the formation of the maximum number of bubbles on the root system of soy plants.

The combination of the inoculation process and the use of micronutrient fertilizers in growing technology give significant results and increased yields. The relative air humidity and productive soil moisture reserves should be considered.

Based on the results of the preliminary study, it is planned to develop a basic version of the technology for growing soybeans using inoculants and micronutrient fertilizers in the southwestern part of the Forest-Steppe of Ukraine.

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AFLATOXICOSIS OF CRUCIANS: EXPERIMENTAL TREATMENT AND BIOLOGICAL VALUE OF FISH

Roman Petrov[1]
romanpetrov1978@gmail.com

Oleksiy Pidlubniy[1]
o.pidlubniy@gmail.com

[1]*Department of virusology, patanatomy and bird diseases*
Sumy National Agrarian University
160 Herasym Kondratiev str., Sumy, Ukraine, 40021

Abstract

The aim of this study was to investigate a possibility to decrease a toxic influence of aflatoxin on the fish organism and veterinary-sanitary evaluation of fish, fed by a pure fodder, aflatoxin and ketoconazole+aflatoxin.

Fish aflatoxicoses cause essential losses at fish growing using industrial production technologies. It is characterized by decreasing weight gains and increasing kill of commodity fish, worsening fodder conversion. Farmers often use fodders of own production, without conducting laboratory studies, and don't know about aflatoxins. At the same time because of different reasons, first of all economic ones, they don't use adsorbents for decreasing the negative influence of aflatoxins on the fish organism. Their use doesn't guarantee 100 % fish resistance to micotoxicoses and correspondingly product safety for a consumer. Fish, received aflatoxins with food, is dangerous as a food product for humans and animals. Aflatoxins are very stable in the environment, so even thermal processing doesn't exceed risk of aflatoxin contamination.

The article presents a possibility of effective treatment of fish at aflatoxicosis. It is known, that aflatoxin beyond cells is not dangerous. Its activation takes place within a cell by the enzyme system cytochrome P-450, forming an epoxide, in which result the aflatoxin inclusion complex with DNA forms in the kernel. The veterinary preparation "Ketoconazole" inhibits cytochrome enzymes P-450, so aflatoxin activation within a cell doesn't take place, epoxides don't form, DNA cells are not injured, aflatoxicosis doesn't develop in fish that has been proved experimentally. The veterinary-sanitary mark of fish, treated for aflatoxicosis, is satisfactory.

The importance of this study is in fact that for today there is no developed effective method of fish aflatoxicosis treatment. An influence of aflatoxin on the crucian organism has not been studied experimentally.

Keywords: fish aflatoxicosis, micotoxins, toxic fodder, aflatoxins, cytochrome P-450, enzyme inhibitor.

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1. Introduction

According to FAO, 25 % of grains in the world are contaminated by micotoxins [1]. A loss, caused by microscopic fungi in the world, is estimated as 16 bil dollars annually [2].

Fodder contamination with microscopic fungi and micotoxins is often registered in Ukraine [3]. Microbiological studies have established that 21.5 % of fodders in Ukraine are contaminated by fungi of Aspergillus genus [4]. Aflatoxins are related to most dangerous poisons; penetrating the fish organism, they can provoke genetic disorders, resulting in cancer and death in 100 % of cases [5, 6]. Researchers described a case of carp aflatoxicosis, caused by fish feeding with a low-quality fodder of low toxicity [7]. Fish, consuming a fodder, containing aflatoxins, is potentially dangerous, because aflatoxin and its metabolites can be contained in fish liver and other organs. Aflatoxins are stable in the environment [8].

Aflatoxins are not dangerous beyond the organism. Its activation takes place in a cell by cytochrome enzymes P-450. The formed epoxide is chemically active; penetrating a cell kernel, it forms state chemical compounds – inclusion complexes of aflatoxins and DNA. As a result of cell kernel injury, there appear mutations that are triggers of oncological diseases [9]. Among all cells of the organism, liver ones are injured mostly, because hepatomas are typical for aflatoxicosis [10]. Clinical sings at the severe clinical course of aflatoxicosis are not expressed and include refusal of

food and unexpected death. At the chronic form, there are observed anemia, liver injuries, edema, body surface injuries, jaundice, blood coagulation disorders [8, 9].

Adsorbents based on aluminum silicates are used for preventing aflatoxicosis [11].

For treating fish for aflatoxicosis, a series of chemical substances was verified, but the results were ambiguous. Oxytetracycline D (10 mg/kg) at everyday intake with fodder decreases liver injuries and mortality. It is not recommended to use it with steroids. Activated carbon manifested its efficiency, especially used soon after the aflatoxin effect. According to the scientists, the combination of oxytetracycline and activated carbon is promising [9].

The aim of the work was to establish an influence of aflatoxin on the fish organism, to develop a possibility of prophylaxis and treatment of fish at aflatoxicosis.

2. Materials and Methods

The studies were conducted in the period from 10.11.2020 to 10.02.2021 at the department of veterinary expertise, microbiology, zoo hygiene and safety and quality of animal husbandry products of the Sumy national agrarian university and in the Sumy regional state laboratory of the State food consumption service (Ukraine). All studies were conducted in correspondence with ARRIVE recommendations and EU Guideline 2010/63.

For conducting the experiment, three groups of crucians were formed by the analogue principle: two experimental and one control of thirty individuals in each one (weight 40±16 g), grown at the LLC «Sumyribgosp», Ukraine, successful for main infectious diseases and micotoxicoses. All groups of fish were female. Fishes of control and experimental groups were placed in separate aquariums of 100 l. At temperature 18–20 °C, the oxygen concentration at level 7–10 g/m^3 was kept in water by artificial aeration. The acclimatization period in both groups was 21 days. A mixed fodder “Tetra sticks” was used for feeding all groups.

An individual diet was used for each group:

1. The mixed fodder with added aflatoxin in dose 0.4 mg/kg was used for the first group.

2. The mixed fodder, added with aflatoxin in dose 0.4 mg/kg and ketaconazole in dose 1 g/kg was used for the second group.

3. The pure mixed fodder was used for the third group.

For adding aflatoxin in the mixed fodder, 96 % ethyl alcohol solution, added with 1 ml of aflatoxin (10 mg/ml), was used. The mixed fodder was processed by aerosol, by the spraying method.

For preparing the solution “Ketoconazole”, produced by «MX and Gustav GEEC, Ukraine» LTD, batch number KNT/1910068, production date 10/2019, 96 % ethyl alcohol solution, added with 99,3 % “Ketoconazole”, was used. This solution was heated to boiling, after that few crystals of 99.5 % lemon acid were added to complete dissolution of ketoconazole. The fodder with aflatoxin was processed by this solution by the aerosol spraying method.

Ichthyopathologic studies, ones of fish quality and safety were conducted at the department of veterinary expertise, microbiology, zoo hygiene and safety and quality of animal husbandry products of the Sumy national agrarian university by conventional methods [2, 12, 13].

Peripheral blood was taken from the caudal artery by cutting off the caudal peduncle near the pelvic fin. The blood was stabilized, adding 10 % EDTA, 1 drop for 1 ml of blood.

Hematological studies were conducted according to the conventional methods. The number of erythrocytes and leucocytes was calculated in the Goyaev’s chamber, hemoglobin concentration by Sahli using a hemometer. 2 smears were made for each individual. The leukocytic formula was derived by the results of differentiated calculation of 200 cells in smears, stained by Puppenheim under a microscope, using the immersion system [3].

The relative biological value was determined according to “Methodical recommendations for determining safety and biological value of fish using infusorians Tetrahymena pyriformis» (2009). The method is based on determination of the intensity of infusorians multiplication on a nutritive substrate, containing the studied samples as a source of protein and growth stimulators.

The obtained results were processed biometrically, using the program software Microsoft Excel 2007

3. Results

Group No. 1 on 5–7 day demonstrated clinical signs of aflatoxicosis, characterized with decelerated swimming movements, fish became to react weakly to stimuli, appetite worsened. Hemorrhages were observed on the body (**Fig. 1**), tail exfoliation (**Fig. 2**).

Fig. 1. Hemorrhages in the low part of the head (5 day of the experiment)

Fig. 2. Tail exfoliation (7 day of experiment)

On 21 day, two individuals died, changes were observed in their liver (**Fig. 3**), comparing with the norm (**Fig. 4**).

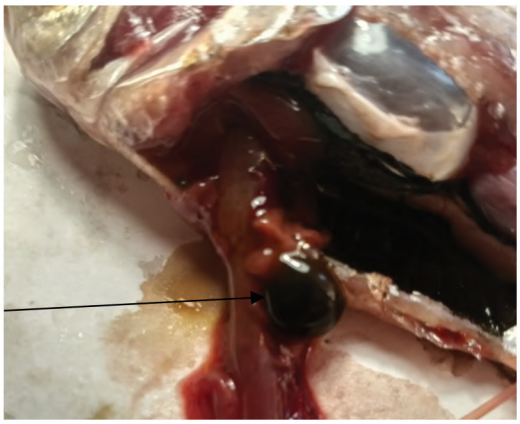

Fig. 3. Liver increase on 21 day of the experiment

On 28 day 17 individuals died. Liver structure disorders were observed at autopsy, the gall-bladder content was yellow (**Fig. 5**). It was impossible to identify kidneys in 50 % of individuals.

The content of the abdominal cavity was changed, and only the intestine was distinctly identified (**Fig. 6**).

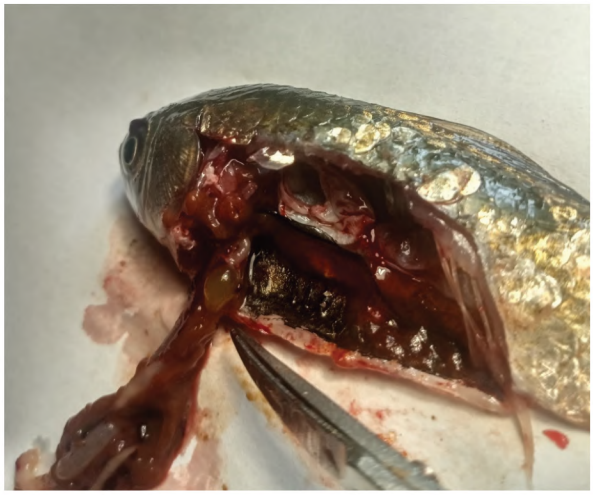

Fig. 4. Liver and gallbladder of the control group (norm)

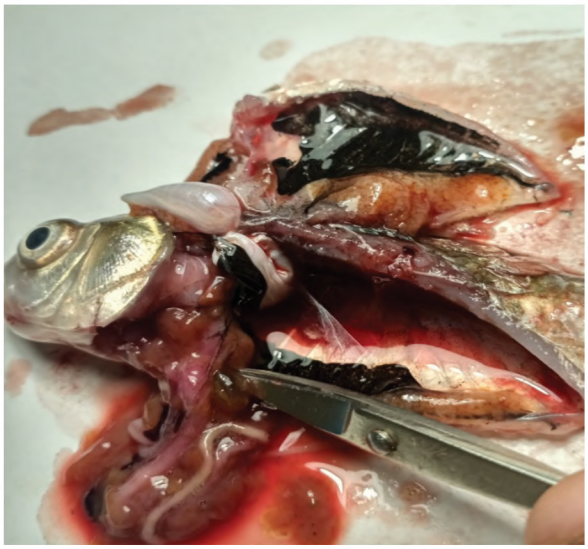

Fig. 5. Change of the gallbladder color, liver is light, flabby, its structure is weakly expressed. 28 day of the experiment

On 37 day of the experiment 5 fish individuals died and on 39 day of the experiment all fishes died, at that LD 50 is 0.44 mg/kg of fodder.

In group No. 2, received the fodder with aflatoxin and "Ketoconazole", fish death was not observed. Clinical signs of aflatoxicosis were not fixed.

In group No. 3, control, received the pure fodder, fish death was not observed. Clinical signs of aflatoxicosis were not fixed. Fish had also good appetite and a positive dynamics of weight gain was observed.

As it was testified by hematological studies, aflatoxin positively influences the blood picture of crucians. The data of hematological studies are presented in **Table 1.**

Table 1 demonstrates that in fishes from experimental group 1 that were not treated, the hemoglobin concentration decreased by 38.65 %, the number of erythrocytes also decreased by 33.89 %, comparing with the control. The total number of leucocytes increased by 21.69 %, and the increase of segment-kernel neutrophils is observed against this background. Changes of blood parameters in experimental group 2, received "Ketoconazole" preparation together with aflatoxin, were not reliable, comparing with the control group.

Fig. 6. Change in the abdominal cavity, filled with the jelly-like brown mass (indicated by the arrow on the left), where only intestine loops are identified (indicated by the arrow on the right); 37 day of the experiment

Table 1

Hematological indices of crucian blood on 30 day, *M*±*m* (*n*=30)

No. item	Parameters	Experimental group 1 (aflatoxin)	Experimental group 2 (aflatoxin+"Ketoconazole")	Control group
1	Hemoglobin, g/l	37.48±1.35***	60.55±2.78	61.1±0.59
2	Number of erythrocytes, mln/mcl	0.78±0.03	1.24±0.18	1.18±0.18
3	Number of leucocytes, thousand/ mcl	41.79±0.31***	32.44±2.10	34.34±0.29
4	Stab neutrophils, %	–	–	–
5	Segment-kernel neutrophils, %	2.13±0.19***	0.70±0.11	0.79±0.07
6	Eosinophils, %	3.58±1.05	–	–
7	Basophils, %	4.55±0.56***	0.50±0.07	0.63±0.07
8	Monocytes, %	4.01±0.58***	0.93±0.74	1.63±0.13
9	Lymphocytes, %	83.63±2.18***	98.13±1.48	96.95±1.31
10	Foamy cells, %	2.19±0.97	–	0.76±0.05

Note *** – *P*<0.001.

The number of changed erythrocytes in the total number of red blood cells in separate individuals (experimental group 1) varied from 10 % to 41 %. It is possible to separate the following disorders by character and manifestation degree: staining change (hypochromasia); vacuolization of the kernel and cytoplasm – high presence of kernel tissues. From 12 % to 25 % of all erythrocytes were hypochromized cells. At that cytoplasm parts, poor in hemoglobin, occupied the essential surface of erythrocyte, and only a little zone kept a possibility to be stained (**Fig. 7**).

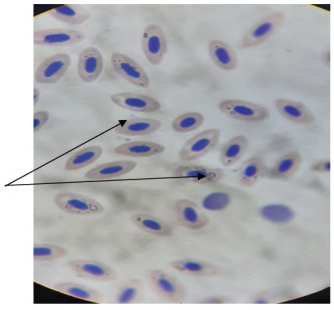

Fig. 7. Hypochromasia of erythrocytes, staining by Pappenheim ×900

Intracellular metabolism disorders were testified by vacuolization of both cytoplasm and kernel. Cytoplasm vacuolization (**Fig. 8**) was observed in 62.1 % of crucians, at that the intensity of its manifestation in separate individuals was high and reached 30.0 %. Despite the fact that kernel vacuolization was observed in the little number of fishes (4.5 %), its presence testifies to the pathological process severity.

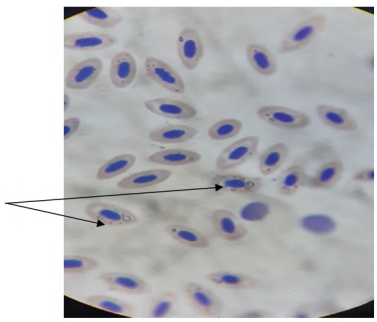

Fig. 8. Erythrocyte cytoplasm vacuolization, staining by Pappenheim ×900

Degenerative changes of erythrocytes (kernel shades), formed after complete disintegration of cytoplasm, were present in most smears of the experimental group (**Fig. 9**).

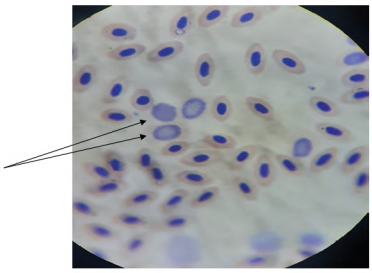

Fig. 9. Kernel shades, staining by Pappenheim ×900

Thus, the results of the conducted hematological study testify to deep and in several cases irreversible changes, taking place in the fish organism under the effect of aflatoxin.

Pathological changes of blood were not observed in groups No. 2 and No. 3.

The biological value of crucian of the different groups, taken part in the experiment, has been studied. The results are presented in **Table 2.**

Table 2

Biological value of crucian ($M \pm m$, n=20)

Crucian group	Number of infusorians, $\times 10^6/cm^3$ of the medium	Relative biological value, % of the control
Experimental group 1 (aflatoxin)	43.3±2.3***	57.5
Experimental group 2 (aflatoxin+Ketoconazole)	71.0±2.7	94.3
Control group (healthy fish)	75.3±2.5	100
Control (glucose)	75.6±1.7	100

*Note:****P<0.01.*

Table 2 testifies that the biological value of fish, treated by ketoconazole, doesn't reliably differ from the control group that testifies to the effectiveness and consumption safety of the proposed treatment.

4. Conclusions

Aflatoxin in dose 0.4 mg/kg results in 100 % death of crucians during 39 days. LD 50 of aflatoxin for crucians is 0.44 mg/kg of fodder.

Introduction of aflatoxin in the crucians' fodder in dose 0.4 mg/kg and "Ketaconazole" in dose 1 g for 1 kg of fodder improves fish preservation.

The biological value of crucian, treated by "Ketoconazole" is 94 %, comparing with the control.

Fish, received aflatoxin and treated by ketoconazole, can be used without limitations after the safety interval.

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ORNAMENTAL PLANTS IN THE SOUTHERN REGION OF ALBANIA CONTAMINATED BY ENTOMOPARASITES OF U/ ORDER COCCOINEA, INSECTA CLASS

Lavdi Hasani
Department of Biology
University "Eqrem Çabej"
30 Rruga Studenti, Gjirokastër, Albania, 6000
hasanilavdi@yahoo.com

Abstract

In this paper, one of the most specific groups of plant entomologists, that of the U/Order Coccoinea Class Insecta is taken into analysis. Below it is seen important to identify the problems of infections of a variety of ornamental plants, found in the Southern Region of Albania, precisely by this group of pests. The role of the quality of the natural environment in our health has recently taken tremendous priority in the context of the contamination of all forms in the wild nature and especially those in the air. Precisely, to increase the quality of our life, the establishment of major parks with a truly significant green space per unit of population, is one of the current main objectives of each country. In these conditions, those environments are pretty rich in a variety of plants that, in addition to the functional values of environmental creativity, also have many aesthetic values with relaxing power and positive effects on our physical and mental health This type of plants is represented not only by those that are cultivated directly in the nature, but also by those that are planted and cultivated in greenhouses, which from time to time we take them out to realize the required decorations and compositions, asked to cover the needs of the parks. The healthier these components of this environment are, the more effective is their role on our personal health. For this reason it is equally important to recognize the dangers that threaten this vegetation by disabling its main function, for which we, as citizens, are interested, due to the need of our health, to have it in the highest efficiency. On this basis arose the idea of a comprehensive study on the above group of insects, which in a form or another constitute some of its main pests. These insects, as a specific group of pests that feed on plant lymph, not only dry out the plant, but also transmit to it a series of viral diseases, leading the plant to complete degradation. Most of them spend the winter (one of the most delicate periods for their survival) as parasites on them. We show below, which of these entomophytes is found in this group of plants in the region in question. It is also shown, which of the analyzed plant species emerges as the most frequented by this group of pests. We also identify the prevalence of this pollution in both variety and percentage. On the basis of the analysis, the question is also what is the distribution of plant species encountered, at different altitudes above the sea level? The paper contains, figuratively, a series of morpho-defining characteristics of the representatives, mentioned in this paper. As will be seen in the following material, the truth is that this specific group of ornamental plants, part of the relaxing parks around the world, in our country turns out to be contaminated by a large number of these parasites. This shows that in order to carry out quality work in this direction, we must not leave without considering the role of these pests in this process. Thus, we will be able to control the quality and function of our recreational environments in the role, for which we realize them. For this reason, detailed data are given below. We have identified these parasites in about 15 (fifteen) species of these ornamental plants, taking into account that parks in our country are not valued for the size of the area, as in an inferior and small country. In this material we have identified the number and dynamics of parasites according to each plant and also according to their distribution in areas with different altitudes above the sea level. We also give a comparative report on the frequency of vulnerability of the various plants by representatives of these pests. Summarized in a table, we have given for each pest the plant variety that it frequents, noting, in which plant organ this parasite was most commonly found during our research. As it is a group with annual activity on the plants, we have data for each month that we have met them on this vegetation and for each plant organ, where they were met (in leaves, on stalks or even the fruit itself). Regarding the degree of the damage that they cause to the plants, given the many harmful valences they show, not all belong to the category of the very dangerous pests. Some enter the minor pests that are mostly caused by overlapping other diseases, some others into the normal pests and a more specific group enters the category of very powerful pests, the risk of which is maximized with other additional effects. These and other information are given in table Number 1 (one), with the required symbolism.

Since this group of plants is the environmental generator, where we live and work, the work in question takes on practical importance and value.

Keywords: Ornamental plants, parasites, pollution, pests, varieties, spread, micropreparations, region, damage, pigids.

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1. Introduction

With the group of insects, belonging to the Suborder Coccoinea, we have been dealing continuously by highlighting their role and "values" as pests. Working with them is a bit not easy for its spe-

cifics both in terms of nutrition as well as in terms of microscopic shape and size of their body. There are several strong and specialized species, associated with a certain type of plant, from which they get their name as: Lepidosaphes ficus Sign (affecting the fig) or Saisetia oleae Bern (affecting the olive) etc. There are also microscopic shapes (most parts) and shapes that can be easily observed [1–6].

In both cases the determination of the representatives of the U/Order is difficult, especially with microscopic forms. The damage they do to the plant consists in the reduction of their nutrient, in the deformation of the twigs and leaves of the plant, they reduce the photosynthetic profitability and they also transmit viral diseases to it.

In ornamental plants they become the main factor of various fungal plant diseases. In this way, contaminated plants are threatened by several pathogenic hazards simultaneously.

However, the level of damage is not the same for all types. There are strong direct pests, common pests and milder forms of parasitism (indirect pests) [7–15], etc.

By identifying the damage of these parasites on ornamental plants, we help the field specialists in their breeding in all our relaxing environments. So we also affect our quality of life as these plants are what beautify our living environments and relaxation parks.

2. Material and methods

The material, we collected from various plant organs, was stored directly in test tubes with alcohol above 75o. In the field we also carried out the labeling with all the required elements (**Fig. 1**). This canned material based on the methodologies, given in lit. [16], was further processed in the zoology laboratory of the Department of Biology of the University "E. Çabej ", Gjirokastër. We released samples from their wax coatings by treating them with 10 % NaOH solution and heating. Then we cleaned them with plenty of water, rinsing them five times and finally leaving them in it for 24 hours. Then leave them for 10–15 minutes in 70° alcohol and then put them for coloring in Fuchsia solution for 3–24 hours.

To fix their color, we put the samples in alcohol that gradually increases the concentration, for 10–20 min. time at each alcohol concentration, from 70° to 90° and finally to 96°. Carefully and under two eyes, the samples were extracted from there and based on the above methodologies, the material was processed until the formation of their permanent microscopic preparations. Based on the literature used, we decided which would be the main elements for their definition. For macroforms we have observed the complete morphology while for microscopic ones we have relied precisely on the composition of their pigids. By observing them under a microscope based on the determinant switches, given in [17, 19–24], we were able to realize their determination down to the species. At working binocularly, we also made a series of sketches on the construction of the pigids of each species, as well as in the more macro cases of the whole view of the living thing.

Fig. 1. Checkpoints, set up in the field

3. Results and discussions

As it is seen in **Fig. 2**, decorative plants, contaminated by this group of insects have been found in only four/six regions, taken into analysis. Among these checkpoints, as the map also shows, the most affected region is Saranda, followed by Vlora and then Gjirokastra. The amount of damaging Coccides, found in the whole region, in the morphologic determinative plan, is presented in **Table 1** and in **Fig. 5, 7, 8** as follows. Data, according to the possibility of these parasites of being found, are represented in **Fig. 4** as follows: abutilone and castor are mostly frequented by these parasites, seen from their spread spectrum in different heights above the sea level (4–220 m). The plant of laurel (4–200 m), rose etc. In restricted or low heights have been found the plants of greenhouses. In **Fig. 6** (down) it is obvious, that the plant, most frequented and preferred by many types of parasites, is rose and oleander with 18 % of all the parasites, encountered in the whole region. Then follows castor with 14 % of all the types, laurel with 12 % etc. less affected are the plants of aloe, lime (tilia), imiscus, pilea and palm. Graphically, this contamination is represented in **Fig. 3**. The whole gathered material in land, after being elaborated in lab according to the methods, described in literature, [25, 26], was formed in microscopic permanent preparations.

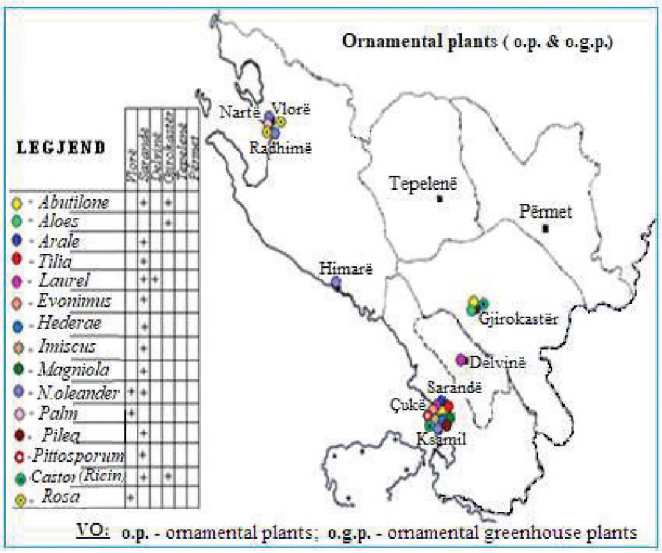

Fig. 2. Stages of infected plants

After that, with the help and definitions, used in literature [18, 25, 27, 28], the determination of the types was made possible. From the work under binoculars as a very difficult and important task, there were also compiled the morphological sketches of the animal itself, especially of the parts with special determinative importance, such as their pigid, where the majority of the waxen glands, anal rings, spikes in the marginal way, parts (*L*1, *L*2, etc), crests (*c*), hairs (*h*), denzarce (*d*), parafiza (*p*) etc. are placed **(Fig. 5).**

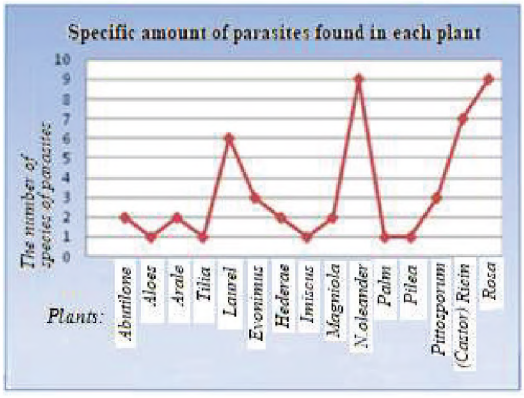

Fig. 3. The number of species of parasites, found in each plant

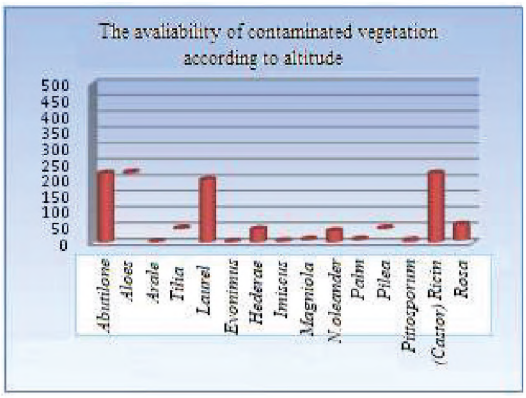

Fig. 4. Distribution of infected vegetation by altitude

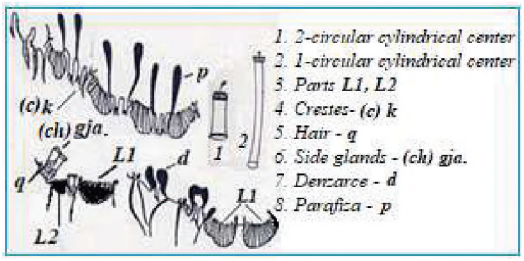

Fig. 5. Determinant of the pigid elements

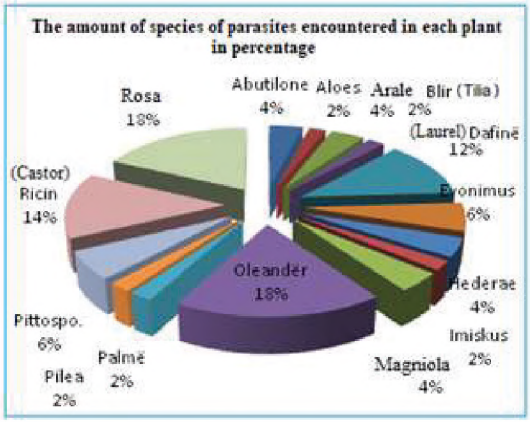

Fig. 6. Proportion of pests in each plant species

The elements, placed mainly in the area of pigid, have a special determinative importance not only for their forms variety that differ among others, but also for their placement way, symmetry preservation in both sides of the pigid, their measure and combination that create. The pigid glands can appear in cylindric but also fungal, tub, disc, bottle, multi cellar, three-five cellar forms etc. It is exactly this elements variety that should be taken into consideration for the realization of a determination as correct as possible. All these sketches that are carried out with binoculars are represented in the pages with images that associate the following **Table 1**.

VO:

A – The plant organ, where the harmer is found(*x*)
–(*s.*)=stem; –(*l.*)=leaf; –(*f.*)=fruit.

B – Explored regions
–*V.*=Vlorë ; –*GJ.*=Gjirokastër; –*T.*=Tepelenë; –*S.*=Sarandë; –*P.*=Përmet; –*D.*=Delvinë.

C – The months of the year [*x*]
–j, –f, –mch, –p, –m, –jn, –jl, –ag, –s, –o, –n, –d.

D - Damage
–(*Name*)=non-harming direct species;
–(**Name*)=harming direct species;
–(***Name*)=strong, harming direct species.

Table 1
The variety of parasites encountered in each plant type

No.	Species name	The plant where it is found													
		Abutilone (*G. Abutilon*)	**Aloes (*G.Aloe*)**	**Arale (*G. Aralia*)**	**Blir (*G. Tilia*)**	**Dafinë (*Laurus nobilis L.*)**	**Evonimus (*G. Euonymus*)**	**Hederae (*G. Hederae*)**	**Imiscus (*G. Imiscus*)**	**Manjolë (*Magniola grandiflora L.*)**	**Oleandër (*Nerium oleander L.*)**	**Palmë (*G. Arecaceae*)**	**Pilea (*G. Pilea*)**	**Pittosporum (*G. Pittosporum*)**	**Ricin (*Ricinus communis L.*)**	**Trendafil (*Rosa centifolia L.*)**
1	*Coccus hesperidum L.* (k.gj) [sh, m, j, ms]	+	–	+	–	+	–	+	–	–	–	–	+	–	+	–
2	*Coccus pseudomagnoliarum Kuw.* (k.gj, f)[sh, m, j, ms]	–	+	+	–	–	–	+	–	–	+	–	–	–	+	–
3	**Saissetia oleae Bern.* (k.gj) [m, j, sh]	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–
4	**Parlatoria oleae Colv.* (k.gj) [m, p, ms]	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+
5	**Parlatoria cinerea Hadd.* (k.gj) [m, j]	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+
6	*Aonidiella aurantii Masc.* (k.gj) [m, ms]	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+
7	**Aonidiella aurantii rac citrina Coq.* (k) [p]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
8	**Aonidiella taxus Leone.* (k) [p]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
9	**Aonidia lauri Bouche* (gj) [m]	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
10	**Ephedraspis ephedrarum Indgr.* (gj) [m]	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
11	**Pinnaspis aspidistrae Sign.* (k.gj) [m]	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
12	***Unaspis euonymi Comst.* (k.gj.) [m]	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
13	**Unaspis citri Comst.* (k.gj) [m]	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
14	**Lepidosaphes beckii New.* (gj) [j]	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
15	*Aspidiotus spinosus Comst.* (gj) [j, ms]	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
16	**Pseudococcus citri Risso.* (gj) [m]	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
17	**Pseudococcus gahani Green.* (k.gj) [m]	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–
18	**Pseudococcus maritimus Ehr.* (k.gj) [m]	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–
19	**Pseudococcus adonidum L.* (k.gj) [m]	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–
20	**Pseudococcus rectus Borchs.*(k.gj) [j]	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
21	**Pseudococcus expressus Borchs.*(k.gj) [j]	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
22	**Aspidiotus nerii* (*hederae*) *Bouch.* (*Vall.*) (gj) [sh, m, j]	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–
23	**Hemiberlesia rapax Comst.* (k)[ms]	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
24	*Hemiberlesia lataniae Sign.* (k.gj) [ms]	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
25	**Hemiberlesia cyanophylli Sign.* (k) [ms]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
26	**Chrysomphalus aurantii Mask.* (gj) [m]	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
27	**Chrysomphalus ficus Ashm.* (k) [j]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
28	**Chrysomphalus dictyospermi Morg.* (k) [j]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
29	**Icerya purchasi Mask.* (k.gj) [m, j]	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
30	**Eriopeltis bichtensteinii Sign.* (k.gj) [j]	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
31	**Pseudaulecaspis pentagona Targ.* (k) [p]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+

Based on the symbols, represented in the end of this table, we see that from the group of plant parasites, only one of the species encountered is a strong direct harmer *(**Unaspis euonymi Comst.* (k.gj.) [m]), the others are direct harmers and a small amount are indirect harmers (*Coccus hesperidum L.* (k.gj.) [sh]), (*Coccus pseudomagnoliarum Kuw.* (gj.) [sh]), (*Aspidiotus spinosus Comst.* (gj.) [j]), *dhe* (*Hemiberlesia lataniae Sign.* (k.gj.) [ms]).

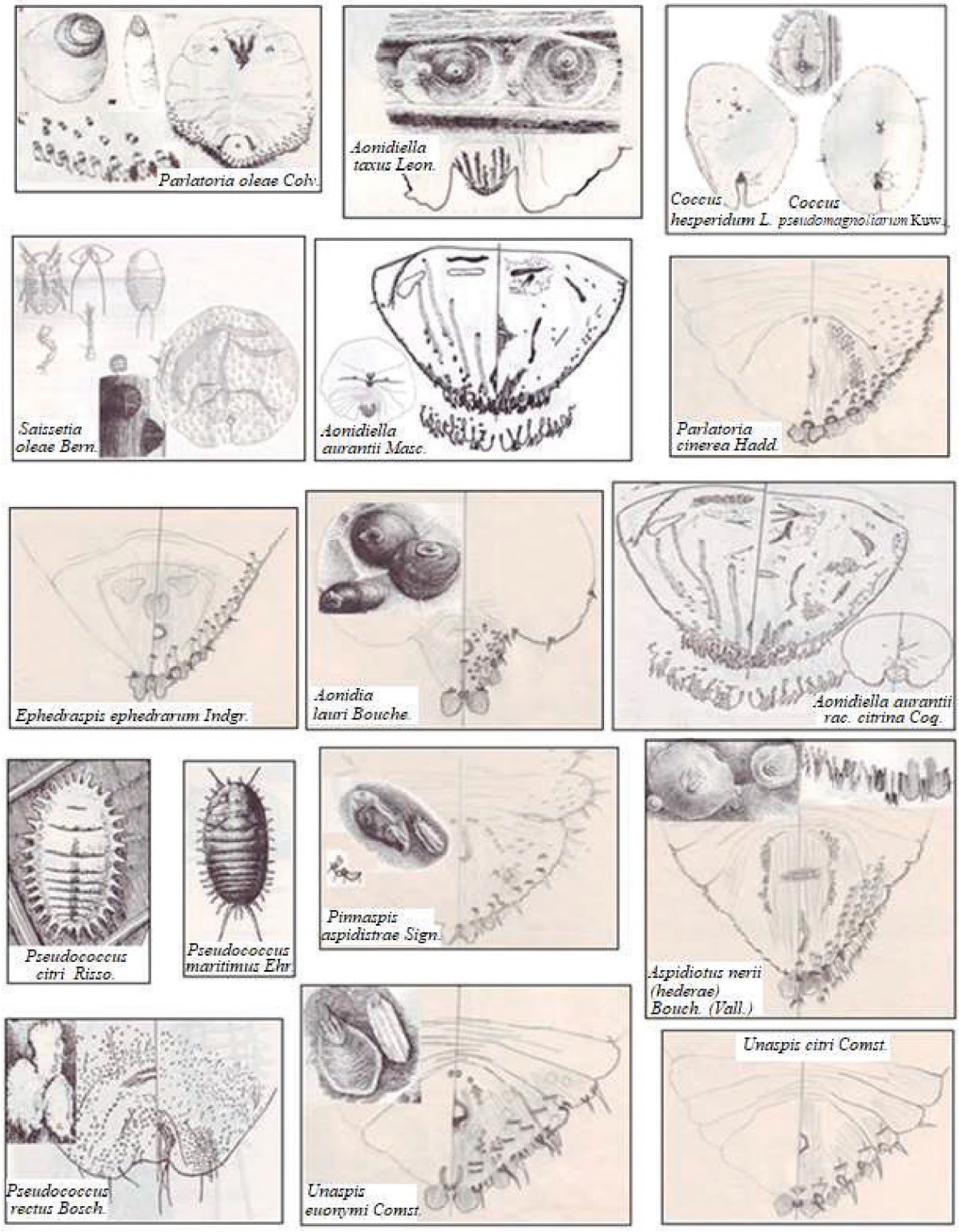

Fig. 7. Sub-ocular view of the pests in a microscope

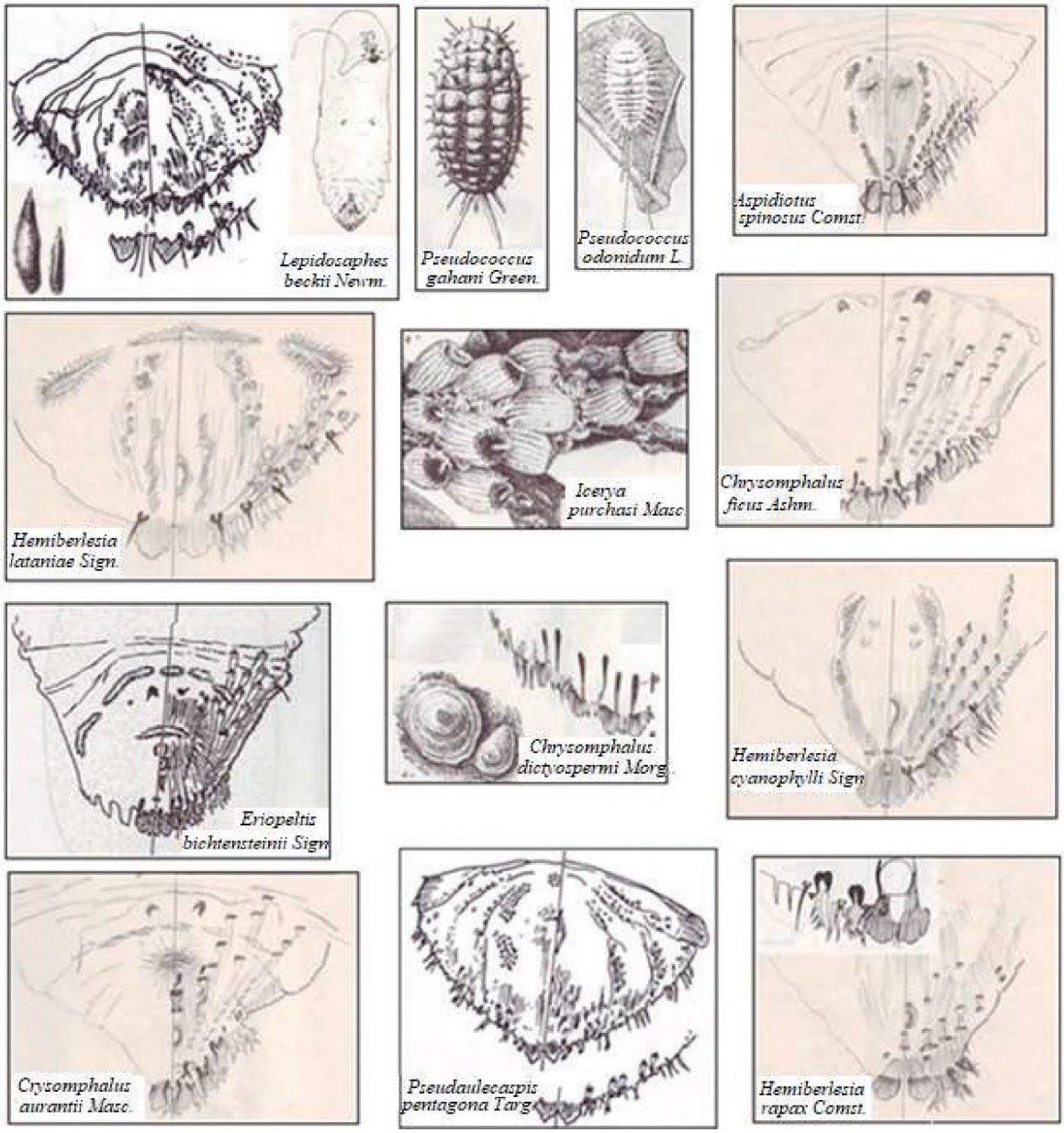

Fig. 8. Sub-ocular view of the pests in a microscope

4. Recommendations

1. As it was analyzed before, it must be taken a special care on the greenhouse plants, affected by parasites, as the microclimate, created in it, is in favor of the parasites despite the area with continental climate.

2. The plants must be held under control during winter even though we think that because of the cold the parasites are not active or fall in pause.

3. In the case of this plant group, the chemical fight is acceptable as it is not a consumable plant.

4. In some cases, the fight against consists in painting of the new stems with diesel, so the young larva will not have any chance to hook and winter there.

5. Conclusions

Based on the above data we reach these conclusions:

1. As it is also seen in **Table 1,** in the whole region being analyzed, we have found in total 31 kinds of parasites of N/Order Coccoinea, of which one represents a strong direct harmer ((**Un-

aspis euonymi Comst), 26 represent direct harmers (the majority) and only four kinds represent indirect harmers (*Coccus hesperidum L*, *Coccus pseudomagnoliarum Kuw.*, *Aspidiotus spinosus Comst.*, *dhe Hemiberlesia lataniae Sign.*))

2. All the decorative plants, both cultivated in nature and greenhouse, are contaminated by this group of harmer **(Fig. 2, 4).**

3. These parasites do not prefer the same plants. The most preferred by them are N.oleander and rose with 9 types, castor with 7 types, laurel with 6 types and so on as well as the plants with one type, such as palm, lime aralia etc. **(Fig. 3)**. This information is represented in percentage of the total of harmers in **Fig. 3**. The 9 types, found in the plants with the most infecting species, represent 18 % of the total and so on. This preference is thought to be caused by the preference for the food that these parasites have on the plants lymph.

4. From the whole region being analyzed we see that the most contaminated plants were found in Saranda, then in Vlora and further in Gjirokaster and Delvina. We do not have found any in the regions of Tepelena and Permeti. Given the fact that in Gjirokaster we have found contamination in greenhouse plants, we reach the conclusion that the continental spread of these parasites is much restricted. The possibility of non-cultivation of these kinds of plants is not excluded **(Fig. 1)**.

5. In some plants we find their contamination in a wider range of their spread in high levels above the sea and in some others we do not. Contaminated plants of the abutilone or castor have been found in territories 4 m above the sea level until 220 m above the sea level. These plants are followed by laurel, rose and hederae etc.

Being that the number of the parasites, found in these plants, is smaller than in the other plants, we reach the conclusion that the parasites, frequenting these plants, are the most adopted and connected to the plant and have great climate independence as far as their territorial spread is concerned **(Fig. 4)**.

6. The harmers affect in general all the plant organs (stem, leaf, fruit), but some parasites can be found only in one organ, such as the leaf, which is the most typical **(Table 1)**.

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ASSAY OF *BACILLUS CEREUS* EMETIC TOXIN PRODUCED IN ORANGE SQUASH

Sunita Singh
Division of Food Science and Post-harvest Technology
Indian Agricultural Research Institute
Pusa, New Delhi, 110012, India
sunitaiari@gmail.com

Prachi Lad
Department of Promotional & Medical Review Solutions (for AstraZeneca, USA.)
Indegene Pvt. Ltd.
Outer Ring Road, Nagawara, Bengaluru - 560 045, Karnataka, India.

Abstract

The contamination of squash by *B. cereus,* an enterotoxin producer, was found to range between 7.5×10^4 and 1.8×10^4 CFU/g in orange squash (during storage), that is hazardous. Orange squash is widely produced and consumed in India, but has a low rating of 3 on the scale of 10 (on feedback), mostly due to high sugars, not preferred these days. It can be preserved for >9 months due to added sugars and preservatives. During processing squash, if juice is not quickly cooled and/or squash is kept for long at temperatures <48 °C after processing, it can be a source of food poisoning. Reason, a large number of toxins can be produced by *B. cereus*. *B. cereus* strains, isolated from squash, produce heat stable toxin. Vacuolar assay confirmed them as emetic toxins, produced in squash. The toxin behaved like an ionophore in assay using mitochondria, extracted from liver cells of chicken with potassium ions in buffer. The toxicity of toxin by assay was 3200 IU/ng (BC IV strain) and 800 IU/ng (BC X strain). By the vacuolar expansions of mitochondria in assay, toxins of *B. cereus* demonstrated a toxic effect, in the range of 20.93 to 60.94 % by BC IV toxin and 43.28 to 45.02 % by BC X toxin, on the 3rd day growth of *B. cereus* in squash and toxin extraction for assay. It was also possible to produce antibodies against the *B. cereus* whole cell and toxin of BC IV, as an attempt to detect *B. cereus* contaminations in foods, by Ouchterlony's immune-diffusion test.

Keywords: *Bacillus cereus,* emetic toxin, chicken liver mitochondria, orange squash, vacuolar assay, antibodies.

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1. Introduction

Globally the total burden due to food borne diseases is not known [1]. *Bacillus cereus* is a ubiquitous contaminant of foods that cannot be completely eliminated and is also known to survive at temperatures as low as 4 °C to 6 °C [2]. It is an etiological agent of two distinct forms of food poisonings, caused by its toxins, showing syndromes of the emetic and diarrheal toxins [3] with >90 % of the poisonings by *B. cereus* being due to its emetic toxin [4], produced by growth of *B. cereus* in foods [5]. The rod shaped, aerobic gram-positive bacterium produces endospores in adverse conditions. The toxin, produced by emetic strains of *B. cereus*, has immune-modulating property in the human body [6]. This toxin is a low molecular weight- heat and acid-stable depsipeptide and can withstand intestinal proteolytic enzymes [7]. The organism is widely reported in foods like rice [8, 9] pasta, noodles [10], milk and milk products [11, 12], poultry products [13], cook chill meals [2], ready-to-serve meals [14], and various fruit products [15]. The toxin can maintain its activity after exposure to 126 °C for 90 min, and considered as one of the most stable enterotoxins [16]. It is thus advantageous to assay emetic toxins of *B. cereus* in contaminated foods.

The infective dose of *B. cereus* in foods is 10^5–10^8/g [17] that can lead to emetic poisoning if ingested [18, 19]. The toxin dose of ≤8 μg toxin kg^{-1} body weight, is a toxic dose in humans and none of the enzymes in the human body are known to detoxify this toxin [20]. If present in a dose as low as 0.01 to 1.28 μg emetic toxin /g food, it results in severe and acute poisoning symptoms [21].

To assay this emetic toxin, a positive vacuolar expansion (of mitochondria) confirms the toxin of *B. cereus,* related to emetic-syndrome (9) and is a good screening assay for toxin in foods. The vacuole activity is pH stable (2 to 11) and resistant to proteolytic enzymes [22]. On the other

hand, the toxin, a cereulide protein- a cyclic dodeca-depsipeptide [21, 23, 24] is tolerant and resistant to heat [10, 25] and highly stable with a low molecular size ~1.2 kDa [17]. Considering the ionophoretic property of emetic toxin [26], PIPES buffer containing K+, was used in this study. The vacuolar assays confirmed toxic effects of emetic toxins, by two strains BC IV and BC X of *B. cereus,* isolated from squash. The effectiveness of the toxins ranged between 2 % to 44 % for BC IV and 4 % to 9 % for BC X toxins, in the squash medium.

The antigenic potential of emetic toxin of *B. cereus* has been reported to be very poor in rhesus monkey, compared to Staphylococcal enterotoxin [16]. However still, an attempt, made to raise antibodies against *B. cereus* strain BC IV and its emetic toxin in rabbits (New Zealand White males), yielded positive results, presented herein.

2. Materials and Methods

Preparation of Squash: Orange squash, assessed for specific organism *Bacillus cereus* counts, was prepared (**Fig. 1**) with fruit juice using the standard protocol [27].

Fig. 1. Packed Orange Squash. Isolation and Enumeration of cultures (experimental works)

2. 1. Media and Solutions

Polymyxin Egg-yolk Thymol Blue Agar (PEMBA) (with Mannitol) (pH 7.2±0.2) [28]; Butterfield's phosphate buffered dilution water; Nutrient Broth and Nutrient Agar Medium (Hi-media); Plate count Agar (Hi-media) [29]. Solutions for Vacuolar assay: 0.25 M Sucrose solution 250 mL; 100 mL of Buffer [100 mM $KN0_3$+10 mM Piperazine N-N'-bis [2 ethanol sulphonic acid (PIPES)] pH 7.2.

2. 2. Enumeration of *B. cereus* counts

Spread Plate method was used to enumerate the microbial load (for required fruit products), on PEMB agar medium. If the quantity of food to be examined was large, uniformly distributed representative samples of the fruit product (50 g) were used. The samples of 10^2 to 10^6 dilutions were plated for enumeration. The enumeration of *B. cereus* in squash on PEMBA: Aliquots from the experimental flasks were diluted into 0.1 % Butterfield's phosphate buffered dilution water (10^2–10^7 dilutions), surface was plated (0.2 mL) on PEMBA medium in 90 mm dia petri plates and spread evenly, and incubated (30 °C) for 5 days growth and counted (CFU/mL) at 1, 3 and 5 days after inoculation. Arithmetic counts were converted to $\log_{10}$ CFU/mL values.

2. 3. Isolation and cultural characteristics of the strains

Standard procedures [29, 30] were followed to isolate *B. cereus* from squash. Preserved (60 days) squash sample(s) were serially diluted (10^{-2} to 10^{-5}) in autoclaved Butterfield's phosphate buffered dilution water. The dilutions were plated in replicates (0.2 mL), on PEMBA and incubated 24 h at 30 °C±2 °C. Colonies, presenting a peacock blue colour with precipitation zone/halo, due to egg yolk hydrolysis (lecithinase test) were considered positive and were enumerated [31]. The lecithinase positive and mannitol utilization negative colonies of *B. cereus* [32], were picked from plates, purified and transferred to nutrient agar slants. Care was taken not to pick colonies turning yellow (mannitol utilizing colonies) [28].

2. 4. Biochemical characteristics of the *B. cereus* strains

Standard procedures were followed for Voges-Proskauer Reaction (pH 7.5) in GP- (Glucose phosphate) broth [33, 34], Gram staining, hemolysin production and lecithinase tests [31, 34]. A known reference strain *B. cereus* NCIM 2185 served as the positive control for characterization of *B. cereus.*

The Emetic toxin Production, Extraction, Effective toxin assay, its Toxicity test and exploring it as an immuno diagnostic tool using the isolate strains BC IV and BC X, in the order of experimentation are mentioned (**Fig. 2**).

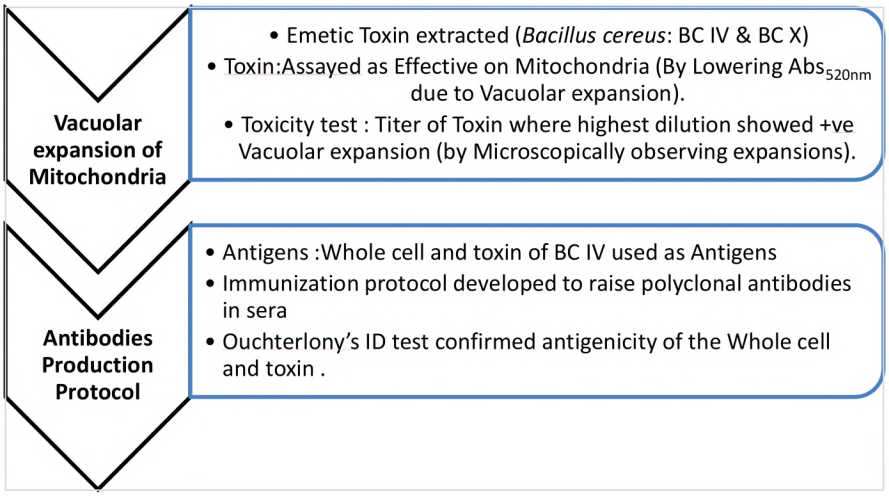


Fig. 2. Schematic showing of the steps of the *Bacillus cereus* Emetic toxin production and its detection

2. 5. Extraction of Mitochondria

A fresh, liver (from a broiler chicken ~1 kg weight, cut a BC IV and BC X fresh) was used for Extraction of Mitochondria [within an hour of procuring it] and a standard protocol [35] was followed for extraction (**Fig. 3**).

Chicken Liver tissue was washed in ice-cold sucrose (0.25 M pH 7.2) ▸ Cut into small pieces ▸ Homogenized in sucrose (0.25 M) (1 g /10 mL) ▸ Tissue was macerated gently in a pestle and mortar with 0.02 % EDTA and 0.25 % Trypsin solution. The resultant suspension was allowed to stabilize for 10 minutes in ice bath and centrifuged at 500 g for 1 minute and then again for 5 minutes ▸ Supernatant (Reject Sediments of Cell debris & Nuclei) ▸ Centrifuged at 8000 g for 10 minutes ▸ Pellet (Sediment mitochondria ($CLM^{\#}$) fraction) (0.05 g/mL) in 0.25M sucrose (Supernatant rejected) [# Chicken liver mitochondria].

Fig. 3. Extraction of mitochondria from chicken liver cells

2. 6. Buffered Mitochondrial suspension-

Chicken liver mitochondria (CLM) were suspended in 0.25 M sucrose solution (0.05 g/mL) (0.01, 0.02, 03, 04, 05, 0.1, 0.2 mL) in 4.0 mL buffered $KN0_3$ (100 mM $KN0_3$+10 mM Piperazine N-N'-bis [2 ethanol sulphonic acid (PIPES), pH 7.2. The final volume was made up to 4.4 mL.

Production of Emetic Toxin:

a) in Orange squash: A 48 h culture growth of *Bacillus cereus* BC IV, was scrapped from two slants with 20 mL distilled water. The suspension was diluted to adjust OD_{600} of ~1.0. The inoculum of 100 µL was added into sterilized squash (150 mL). The culture growth

in squash was centrifuged (on day 1, 3&5) ~2000 ×g for 20 minutes to extract toxin (supernatant). The toxin was heated at 100 °C (7 min) to denature heat-labile diarrheal toxin of *B. cereus* before use for assay;

b) in Nutrient broth [Titer determination & Toxicity test of toxin] Cultures (BC IV&X), grown in agar slants were diluted in NB. A suspension (150 μL having ~1×10^3 CFU/mL, OD ~1.0) of *B. cereus* culture inoculum was added to make a total volume of 150 mL nutrient broth (in 500 mL Erlenmeyer flasks), incubated at 30 °C±2 °C (100 rpm in shaker) for 48 h. Cultures were diluted in NB to give final concentrations of approximately 1×10^3 CFU/ mL in triplicate [36, 37]. The growth in medium was centrifuged at ~2000 ×g for 30 minutes (4 °C). The molecular size of toxin (cereulide) is <1.2 kda. The cell pellet was separated and the supernatant (toxin extract) collected, filtered through 0.4 µm membrane filter, to produce toxin extract of *B. cereus* [23]. The toxin supernatant was heat-treated (100 °C for 5–7 minutes) to denature heat-labile diarrheal toxin of *B. cereus,* prior to assay for use as emetic toxin;

c) the maximum population density of *B. cereus* (MPD): The Maximum population density (MPD) in a 5 days growth of *B. cereus* strains (BCIV & BCX), during toxin production in squash, was recorded and expressed as $\log_{10}$ CFU/mL;

d) biochemical changes in squash, used for toxin production: After inoculation of *B. cereus* strains, to produce toxin in squash, Reducing sugars and total Titratable acidity were assessed [27].

Vacuolar assay for effect of emetic toxin. The toxin was assayed on mitochondria expansions (**Fig. 2**). The toxin, produced either in squash or in Nutrient broth, was used for spectrophotometric and microscopic observations, respectively. Since emetic toxin has the potassium ionophore property, it may then selectively help in the uptake of K+ by mitochondria in such a way so as to be a cause of swelling, or vacuoles [24, 38, 39]. Other changes with inter-membrane spaces of mitochondria are a result of toxicity [40], leading to expansions, recognized as emetic activity of toxin. The K+ containing PIPES buffer was thus used to suspend CLM.

Spectrophotometric assay & quantification of Mitochondrial Expansion by Emetic toxin [26, 36]. The main criterion to assay *B. cereus* emetic toxin [5, 41, 42], was to assess if it was effective in a buffer suspension chosen, in this study.

Mitochondria were extracted and suspended in sucrose solution along with heat treated toxin (in presence of K^+ cations in nitrate containing PIPES buffer solution pH 7.2) (**Fig. 3**). The vacuolar swellings in mitochondria in presence of toxin [26] were observed spectroscopically and reported as the effectiveness of toxin. The absorbance was observed with UV-VIS spectrophotometer (Model DIGSPEC 200 GL) using glass cuvettes. The swelling of mitochondria was observed as lowering of absorbance (λ_{520nm}) due to toxin, and compared to control (without toxin). The various combination- mixes, of mitochondrial fractions (0.05, 0.04, 0.03, 0.02, 0.01&0.1 mL) in KNO_3 buffer solution with emetic toxin (BC IV/BC X) supernatants (0.1 mL or 0.2 mL), and in a total volume of 4.0 mL were made up to 4.4 mL with distilled water. These expansions were assayed due to *B. cereus* strains BC IV and BC X on 1st, 3rd & 5th days of growth of organism using a mix of CLM with toxin volumes (0.1 mL or 0.2 mL), observed between 0 to 20 minutes exposure. The exposure to the toxin remained constant for experimental period of at least 4 hours (9, 21), in which the observations were conducted (at 30 °C). The Abs_{520nm} values were recorded in a 20 min period (with 5 min intervals, against blank without toxin). These trials, ascertained the toxic effects on mitochondria. All assays were run in duplicates.

Based on the results, it was observed, that the toxin was effective hence its titer (toxicity) also was determined as follows.

Toxicity test of Emetic toxin extracts for titer determination [9, 21, 36]. The toxin supernatants, filter sterilized and heated at 100 °C for 10 min, were used in the assay:

a) toxin extracts, 25 µL aliquots of BC IV&BC X, were diluted (two-fold) in buffered potassium nitrate (KNO_3) solution (100 mM KNO_3+10 mM PIPES) (pH 7.0) across 12 wells of a 96-well micro-titer plate {until 1: 4096 dilution level : (1/2, 1/4, 1/8, 1/16, 1/32, 1/ 64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096)}, in duplicate;

b) a 100 µL suspension of mitochondria (trypsinized) fraction from Chicken liver cells, [0.05 g cells /mL in 0.25 M sucrose medium (**Fig. 2**)], was added into each of the dilution volumes

(25 μL) of emetic toxin. After 10 minutes reaction, smears were prepared on clean glass slide from each dilution, air-dried, stained with indigo carmine solution (0.1 %) and then dried in air;

c) the morphological effect of toxin (mitochondrial expansion) was observed under binocular microscope (Leitz) (at 40 X and 100 X (Oil immersion lens) and also compared with the control (without toxin) slide;

d) the highest dilution that showed the Positive morphological effect (vacuolar expansion) was observed as a positive toxin effect on mitochondria. The inverse of the highest dilution of toxin that showed vacuoles was recorded as the titer of toxin. An average of 3 observed fields of the dilution, with a minimum 10 vacuoles/field was recorded. A positive vacuolar expansion in the assay, confirmed the emetic toxin activity [21] of the strains BC IV & BC X. The minimum dose of titer of 1 U was measured as 5 ng/mL [24, 43]. Based on 1 U value, the titer of *B. cereus* toxins of BC IV and BC X were calculated.

Production of polyclonal antibodies of the whole cell and emetic toxin of B. cereus strain BC IV.

The possibility to raise polyclonal antibodies (against the BC IV-whole cell antigen (WC-Ag) and emetic toxin Ag (Tx-Ag), a possible tool to detect *B. cereus* or its toxin in foods, was explored. The antigens (Ag) of *B. cereus* strain BC IV&BC X were prepared (**Table 1**).

Table 1

Preparation of Antigens – Whole cell (Ag) and Emetic toxn (Ag) for use in Immunizations [15]

Object	Description
Production of polyclonal antibodies of the whole cell and emetic toxin of *B. cereus* strain BC IV	*B. cereus* culture isolate BC IV, was grown in 250 mL Nutrient broth medium in four 500 mL Erlenmeyer flasks under shaking (100 rpm) at 30 °C±2 °C for 4 days to obtain cellular growth and toxin supernatant, for use as antigens.
Whole cell BC IV (Ag)	Cellular fraction was centrifuged and suspended in phosphate buffer saline (pH 7.0) [PBS] and stored (–20 °C) in small volumes in vials. The suspension was diluted to adjust to 10^6 CFU/mL (at Abs_{600nm} to ~1.0), with phosphate buffer saline (pH 7.0), before for use in immunization.
Toxin fraction BC IV (Ag)	The toxin fraction was also stored (–20 °C) in small volumes in vials. It was diluted to half its concentration with [PBS] before for use in immunization.
New Zealand White male rabbits as animal for experimentations	The schedule (**Table 2**) of intravenous and intramuscular immunization schedule was followed (**Fig. 4**) for raising antisera against: BC IV whole cell (WC-Ag)&BC IV toxin (Tx-Ag).
	New Zealand White male rabbits: 1) 2 kg by weight was used for WC-Ag; 2) 2.4 kg by weight was used for Tx-Ag, to raise polyclonal antibodies. Both rabbits were ~12 weeks old.

Antibodies Production Protocol in Rabbits.

After procuring the rabbits, they were initially fed and let to get use to the new environment for 10 days as well as to have a check on their proper health before immunizations. Antisera for *B. cereus* strain BC IV cellular and toxin fractions were raised in two separate rabbits as per schedule (**Table 2**). On day 1, pre-immunization blood was collected, (15 mL) for control rabbit serum. The schedule of intravenous (Iv) and intramuscular (I_M) immunizations was followed until booster doses. The immunization booster dose (I_M) for whole cell (*Ab*) at 90 days and for toxin (Ab) at 83 days was completed. On 7 days after completion, a high volume of blood was collected for sera (*Abs*).

For final bleeding, the rabbit was restrained and outer surface ear was cleaned with alcohol. The blood sample was taken from the auricular artery of the ear. After blood collection, direct pressure was applied to the ear with sterile cotton to prevent bruising and unnecessary bleeding. The ears were cleaned with alcohol and rabbit was allowed to relax.

An extra booster dose, after 15 days of blood collection, did not help to increase the titer of serum.

The rabbits were observed daily, and any lesions if present were taken care of. Blood collections were stored in smaller volumes (–20 °C).

The Ouchterlony's double diffusion test was used to study the banding patterns due to toxin sera interaction with different cellular antigens of different *Bacillus cereus* strains.

Table 2

Immunization Schedule (15) followed for Antibodies Production

Day	Injection	Amounts/Volumes**per Rabbit (**Serum volumes are approximate)	Blood Collection
0	Primary Injection $I_V^{\#}$	2.0 ml of Ag* (diluted with PBS1:1)+Conjugate solution: Freund's Complete adjuvant	Pre-bleed (blank sera) before injection
30	First $I_M^{\$}$	Ag* solution (1:1) containing 500 μg protein: Freund's Incomplete adjuvant	–
45	I_M	Ag* solution (1:1)	–
60	I_M	Ag* solution (1:1)	–
75	I_M	Ag* solution (1:1)	–
83	I_M	Ag* solution (1:1)	Toxin (Last dose)
90	I_M	Ag* solution (1:1)	Cellular (Last dose)
110	I_M	Ag* solution (1:1)	Final bleed of Toxin and Cellular Ag injected rabbits respectively

Note: $I_V^{\#}$ *– Intravenous injection;* $I_M^{\$}$ *– Intramuscular injection on thigh.*

3. Results and Discussion

Orange squash (Kissan) is one of the summer drinks in India. The high content of sugar and preservatives gives it a rating of 3 on a scale of 10 [44]. In spite of high sugar content, the drink can be hazardous, when a pathogen like *Bacillus cereus*, can inhabit/adapt to the medium, which can emerge as a specific ecotype [45] to *B. cereus*. Along the food chain, it is also very unclear as to how pathogens originate or are transmitted, that many pathogenic organisms show their presence in newer/processed- foods. In scanning microbial loads of processed products, *Bacillus cereus* was present in orange squash, sweet mango and papaya bars and tomato pulp (**Table 3**).

Table 3

Bacillus cereus load (CFU/g) in different heat-treated fruit products

Bacillus cereus counts (CFU/g)	Storage period (days)				
	0	30	45	60	90
Mango bar* ($\times 10^1$ CFU/g)	13.80±0.36	18.62±0.45	33.51±2.36	64.32±2.54	59.20±3.21
Papaya bar*$\times 10^1$ CFU/g	45.02±1.25	49.61±2.14	68.64±4.53	92.64±4.89	164.03±1.87
Tomato pulp* ($\times 10^1$ CFU/g)	2.31±0.21	1.65±0.30	5.63±0.27	4.36±0.54	2.66±0.68
Orange squash ($\times 10^3$ CFU/g)	75.2±5.23	62.0±0.25	17.90±0.32	–	–

*Note: *Source: [15].*

Other foods like Soymilk (overnight refrigerated), soy tofu, blanched soybeans, tomato pulp powder from blanched tomatoes (preserved >9 months), maize and soy blended extruded products also contain *B. cereus* [15] in appreciable numbers. A high population of *B. cereus* in orange squash resulted in a decision to examine it as a medium to produce toxin by inoculation of this organism and assay presence of emetic toxin, produced by two isolates of *B. cereus.*

3. 1. Morphological and Biochemical Characteristics of *Bacillus cereus* strains

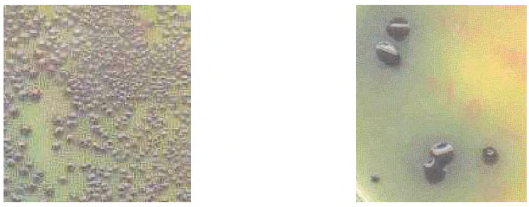
The bacterial colonies (BC IV and BC X, from pineapple and orange squash respectively) were enumerated and the typical peacock bluish colonies were isolated on PEMBA medium (**Fig. 4, 5**). They were characterized (**Fig. 4**, **5**, **Table 4**) and confirmed as *Bacillus cereus* strains [28–30, 34].

Table 4

Characteristics of *Bacillus cereus* strains

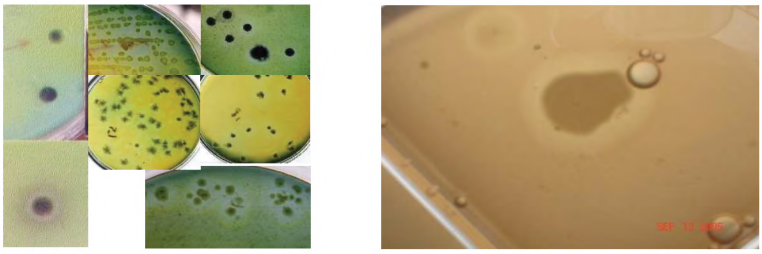
S. No	*B. cereus* strain	Colour (size-Dia., mm) of colony on NA medium	Smoothness Edges/margin of colony on PEMBA	Crystal inclusion	Blood AgarTest** Hemolytic [24–48 hrs] (+/–)	Voges Proskauer Test [24 hrs] (+/–)	Citrate Utilization [7 days] (+/–)
1	BC IV	Dull creamish (4–7)	Edges not Smooth	–	+	+	–
2	BC X	Creamish (1–5)	Edges not smooth	–	+	+	–
3	*B. cereus* NCIM 2185	Creamish (1–7)	Smooth edges	–	+	+ /–	–

*Note: ** ++: large clear zones; +: large zones, but not clear (Fig. 6); ±: zones are not clear*



a *b*

Fig 4. *Bacillus cereus* culture, plated for different dilutions on PEMBA medium, showing colonies, observed on plates with the sample: *a* – at dilution 10^{-3} *b* – at dilution 10^{-6}



a *b*

Fig 5. *B. cereus* strains isolates, on PEMBA medium (showing Mann-, Lecith+ colonies): *a* – BC IV (upper row right) & BC X (lower row right) isolated, among other isolates of *B. cereus strains*; *b* – *Bacillus cere*us BC IV, showing weak hemolysis on the Blood agar medium-

3. 2. Orange squash as a medium for toxin production by *B. cereus* strains:

The orange squash as a medium for *B. cereus* (BC IV & BC X) inoculation showed the increase in acidity by 28–30 % and total reducing sugars by 16–21 %, in 5 days (**Table 5**).

Table 5

Changes in total acidity (%TA) and total reducing sugars (TRS), of orange squash (inoculated with BC IV & BC X), in 5 days

Day	% TA		TRS (% mg)	
	BC IV	BC X	BC IV	BC X
0*	0.43	0.43	45.90	45.90
1	0.44	0.45	22.22	25.45
3	0.50	0.50	40.31	37.00
5	0.55	0.56	53.33	55.54

3. 3. Production and Extraction of *Bacillus cereus* Emetic Toxin

The presence of >10^3 CFU/g in squash can cause foodborne illness and hence it was important to understand the toxicity and effectiveness of a toxin, produced by *B. cereus*. In the vacuolar assay, a threshold concentration of the cereulide, can lead to the loss of mitochondrial membrane potential in human cells, which is similar to that, observed in boar sperm mitochondria during expansion, due to the cereulide (emetic toxin). Human killer cell mitochondria were reported as equivalent to boar semen [20] and that boar sperms were equally sensitive to cereulide [36]. This vacuolar assay thus establishes the toxin effect, as if it were to be present in a human body. However since boar semen was difficult to obtain, we assayed mitochondrial expansions using CLM in the assay, instead. The vacuolar assay of *B. cereus* emetic toxins showed, that the extracted heat treated toxins (BC IV and BC X) were effective (toxic) on CLM, reported as the extent of expansions, were also related with the extent of growth of the organism (Table 6). The results showed that:

1) the growth of *B. cereus* in squash reached the maximum population density (MPD) of log10 CFU/mL of 8.00 to 10.2, on 5th day;

2) that *B. cereus* produces toxins in the end of the logarithmic growth phase of the organism and is known to be independent of sporulation [46, 47]. The toxin effectiveness from 3rd day's growth (in late logarithmic growth stage it was high as compared to other days (1st or 5th day). The specific microbial load of *B. cereus* also reached the peak value of ~ $\log_{10}$ CFU/mL of 7.38–10;

3) the effectiveness of the toxin assay declined on 5th day. By neutralizing toxin or using un-neutralized toxin, of both strains, a decrease in effectiveness of 5th day's toxin extract as compared to 3rd day's extract was observed. However so BC X toxin (un- neutralized) was as effective (43–45 %) as the neutralized toxin (45 %) of 3rd day. On the other hand, the effect of un-neutralized BC IV toxin showed (~61 % lowering of the toxic effect) compared to its neutralized counterpart in assay conditions. The assay thus showed that the toxins of both strains did not have the same efficacy, even if produced in the same medium or are extracted at the same stage of growth of *B. cereus*. The production of toxin was definitely related to cell concentration of the organism in growth [21];

4) *B. cereus* strains could be differentiated on their toxicity levels based on extent of mitochondrial expansions due to toxin (neutralized or otherwise);

5) this assay may be extended to check toxicity of *B. cereus* emetic toxin directly from other foods.

The heat stable emetic toxin was effective and active (as un-neutralized toxin) at low pH also [16]. Thus toxin is active in stored foods, unlike the diahorreal toxin, where activity of the toxin can be eliminated or reduced under variable conditions. Thus, potential hazard of emetic toxin as shown by their toxicity when extracted cannot be ignored.

Table 6

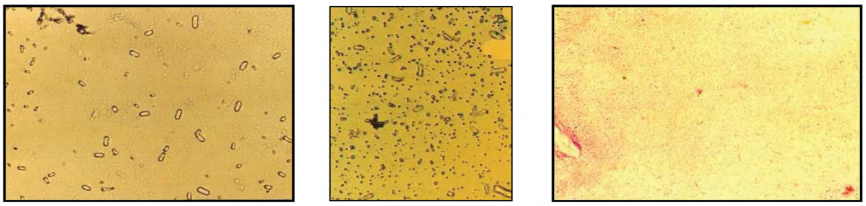
Toxic effects (mitochondria swelling) of *B. cereus* toxins of BC X and BC IV from squash after inoculation with 10^6 cfu mL^{-1} of cultures BC X and BC IV in orange squash

Day	Toxin[#] added (+/–)	CFU/mL in squash	Expansion time (min)	% Decrease in Abs by BC X Toxin	CFU/mL in squash	Expansion time (min)	% Decrease in Abs by BC IV Toxin
Neutralized Toxin (+)							
1	+	1.0×10^6 (6)	20	2.58 [0.04+0.1][$]	24.0×10^6 (7.38)	20	1.16 [0.04+0.1]
3	+	1.0×10^6 (6)	20	44.68 [0.01+0.2]	24.0×10^6 (7.38)	5	60.94 [0.04+0.1]
5	+	202.1×10^8 (8)	20	13.76 [0.01+0.2]	158×10^8 (10.20)	20	2.11 [0.04+0.2]
Un Neutralized Toxin –)							
1	–	1.0×10^6 (6)	15	19.35 [0.05+0.1]	24.0×10^6 (7.38)	20	14.45 [0.04+0.1]
1	–		20	12.07 [0.1+0.2]		20	12.07 [0.04+0.2]
3	–	179.0×10^8 (8)	15	43.28 [0.03+0.1]	123×10^8 (10.09)	5	7.04 [0.04+0.1]
3	–		10	45.02 [0.05+0.2]		20	20.93 [0.02+0.2]
5	–	202.1×10^8 (8)	20	5.17 [0.02+0.1]	158×10^8 (10.20)	20	13.60 [0.04+0.1]
5	–		20	31.48 [0.01+0.2]		15	6.45 [0.04+0.2]

Note: [#] Toxin neutralized (+) or used as such, Un-neutralized (–); [$] Figures in parenthesis are: M – Mitochondrial Solution (chicken liver)+ T – Toxin Extract (supernatant heat treated) in reaction mix to observe vacuolar expansion of Mitochondria (in mL); Abs_{520}: Percentage decrease in Absorbance at λ_{520nm}; CFU/mL squash figures in parenthesis are Log_{10} CFU/mL.

3. 4. Toxicity Test and Microscopic Examination of Titer of *B. cereus* Toxin:

The assay of emetic toxins (**Fig. 2**) showed high toxicity [21], with toxic concentration of BC IV>BC X. In the vacuolar assay with respect to *B. cereus* toxins BC IV and BC X were observed at highest toxin dilutions of 1/2048 and 1/512, respectively (**Fig 6**, **Table 9**). The concentration of toxin was calculated from the titer value in the total assay volume (125 µL) (**Table 7**). *B. cereus* BC IV and BC X toxins were toxic at 3200 IU/ng and 800 IU/ng respectively.



a *b* *c*

Fig. 6. Mitochondrial expansion seen (100X) at a different dilutions of toxins in the assay: *a* – BC IV; *b* – BC X under microscope; *c* – Liver cells only (control) (Bottom) (**Table 7**)

Table 7

Test of toxicity (by Vacuolar assay^) of Emetic toxins of *B. cereus* strains BC IV and BC X, produced in Nutrient broth

S. No.	*B. cereus* strain BC IV toxin extract (Volume µL)	Toxin Titer[@] in total assay volume (125 µL[$]) of Emetic toxin (25 µL[R])+CLM[#] (100 µL)	CLM solution in total assay volume (µL)	Titer/mL	Toxin concentration* (IU/ng)
1	BC IV	2048	100	16384	3200
2	BC X	512	100	4096	800

*Note: ^ Observed under Microscope (See, Fig 6); [@] Highest dilution (of a two-fold dilution series of 25 µL toxin) [9] showing+ve vacuolar expansion; [$] 100 µL CLM (0.05 g/mL in 0.25 M sucrose)+25 µL Toxin extract from 2 fold dilution series; [R] Toxin, diluted with buffered potassium nitrate (KNO_3) solution (100 mM KNO_3+10 mM PIPES-buffer); [#] CLM: Chicken liver mitochondria; * A titer of 1 U Natural cereulide, measured as 5 ng/mL [24, 43].*

3. 5. Immunization Protocol and Immunodiffusion test

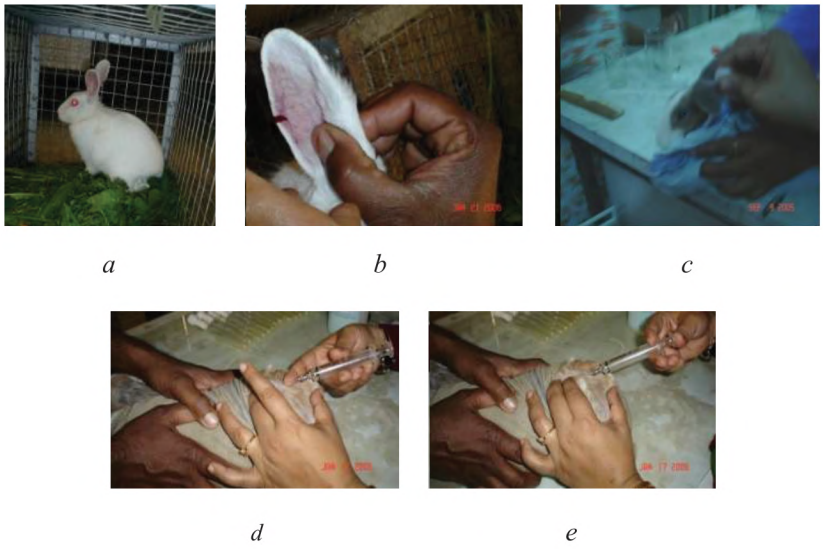
The sera booster dose on 83rd day (for toxin Ag) and 90th day (for cell Ag), of immunization schedule (**Tables 1, 2, Fig. 7**) was followed by blood collection and sera preparation for antibodies. The precipitin test on serum raised showed titers of 2048 and 1024, for WC-Ab and toxin-Ab respectively. The Ouchterlony's Immuno-diffusion test with the serum antibodies against Whole cells (WC-Ag) and Emetic toxin (Tx-Ag), of *B. cereus* BC IV strain showed clear banding patterns, confirming that the same single strain serum can be used to detect other *B. cereus* strains that may be present in contaminated foods (**Table 8, Fig. 8**). It is thus possible to use serum (with polyclonal antibodies) as an alternate tool to detect *B. cereus* in foods

Table 8

Immuno-diffusion test, showing Banding pattern in Ag-Ab reaction.

Antigen used (Ag)	Banding pattern of Antigen (Ag) with Antibody (Ab)	
	Cellular WC-Ab (serum) used in center well	Toxin Tx-Ab (serum) used in center well
BC IV Cellular WC-Ag	4 bands	5 bands
BC IV Toxin Tx-Ag	4 bands	5 bands

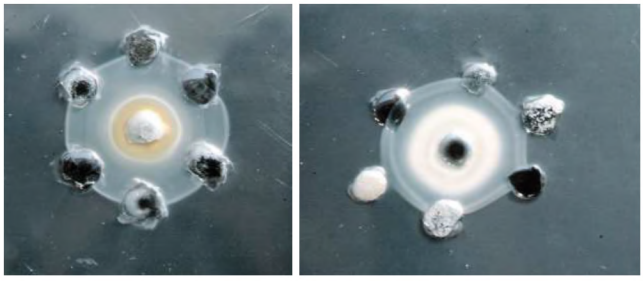
Toxin, used in a brown rabbit, Cellular Ag, used in a white rabbit (**Fig. 8**).



a *b* *c*

d *e*

Fig. 7. Few steps, showing Immunization of Himalayan rabbits: *a* – rabbit feed used; *b* – Pre-bleeding from lateral (auricular) artery (ear); *c* – stoppage of flow after bleeding; *d*, *e* – intramuscular immunization, as per schedule. All procedures were performed with care as per standard practices (**Table 1, 2**)



a *b*

Fig. 8. Immunodiffusion test of *B cereus* antigens: *a* – cellular Ag in side wells banding with its antisera (in center well); *b* – emetic toxin Ag in side well banding with its antisera (in center well)

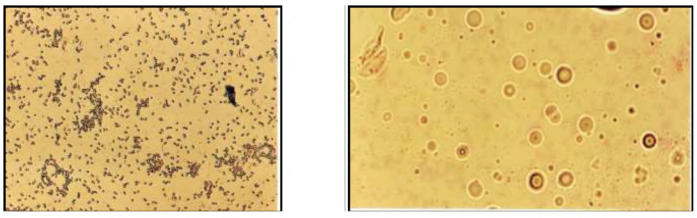
4. Conclusions

B. cereus toxin is known to be present in foods and fruit products [48–50]. In foods their viable counts ranged between 10 CFU mL^{-1}/ $g^{-1}$to 10^6 CFU mL^{-1} /g^{-1} [15, 48]. Under low pH (as in squash) there is a possibility of increase in the lag phase and generation times of this organism [51], to let the organism multiply later. Thus, the inoculum was added at higher active cell concentration for toxin production. *Bacillus cereus* emetic toxins in squash were effective and toxic. Its presence in squashes (acidic product) among other products [15]-is one of the first reports and is a signal on quality defects that can arise in acidic fruit products, if preserved at room temperature (≥30 °C). In preparing a fruit squash, the juice is not heat-treated, and thus it is possible for entry of *B. cereus* from a raw material. *B. cereus* strain(s) can thus survive in storage. Orange Pineapple squash usually has a long shelf life (9–12 months). Contamination levels of this organism can reach between 10^2–10^8 g^{-1}, in various foods [15, 52]. Due to heat and acid resistance of *B. cereus* and its toxin, the organism can survive and propagate in foods [52–54]. A multiplication of the organism to threshold levels can lead to emetic toxin production [47]. Thus, if not detected early in storage may cause serious hazard in foods. A prolonged storage of such products may contribute to sporulation until favorable conditions

return for growth and toxin production by this organism. Hence storage periods should be carefully recommended and good manufacturing practices in all Food Processing units are needed. The contamination level should not exceed 100 $CFU \cdot g^{-1}$or mL^{-1} of food, to avoid the heat resistant toxin [52], which is adverse to hygiene [55, 56].

This study used CLM and *B. cereus* toxin in assay of vacuolar expansions as a first report, to the best of our knowledge, on the use of chicken liver tissue for vacuolar assay. There is a possibility of using polyclonal antibodies (Cellular WC-Ab, Toxin Tx-Ab of *B. cereus*), as a tool, to detect *B. cereus* or occurrence of emetic toxin, where molecular diagnostic facilities may not be accessible in Labs. Improvements in immunization schedule can help to obtain higher titer of the sera. To mention, the sera, obtained for these two Ags, have shown the possibility to detect other strains of emetic strains of *B. cereus* (15 & not reported in this study). Their antigenicity can be used to further our knowledge and gain insights into *B. cereus* emetic toxin(s) and its antibodies. A large volume of high titer sera, required to detect large number of strains, is the only limitation.

The growth of *B. cereus* at temperatures <48 °C can cause food poisoning. To avoid this organism entering foods, a cook of 70 °C for 12s can help to achieve a 6 Log reduction of *B. cereus* [57] as shown by a study on D-values [(1 min (60 °C) to 33.2 min (50 °C)] of *B. cereus* vegetative cells]. In phosphate buffer the D-value was reported to be 10 min at 49–55 °C. A temperature of only 30 °C can form spores of *B. cereus* [46]. The spores of *B. cereus* BC IV germinated in presence of alanine (1 mM–100 mM), at 30 °C [15] (**Fig. 9**). Thus BC IV can propagate by spores at room temperature, in squash. The spores can allow the organism to escape pasteurization or sanitation procedures [58], due to its heat and acid resistance and can be problematic in convenience foods and mass catering. It is therefore important, to control *B. cereus* population [59] to avoid toxin production in foods. Hence the emetic toxin of *B. cereus* is certainly one of the most dangerous enterotoxins [60].



a *b*

Fig. 9. Morphological features of *Bacillus cereus* BC IV:
a – gram stained cells of *Bacillus cereus* BC IV; *b* – *Bacillus cereus* BC IV spores

The PCR method has shown promise to detect a cereulide (emetic toxin) in Germany [61]. It is one of the most recent ways to detect the toxin. However series of at least 18 cereulide variants of emetic toxin have been reported to exist [62]. In India the detection or assay of this organism is not at a good scale. Its diversity in various Indian foods also needs to be studied. Among the serious damages, caused by emetic toxins, fulminant liver failure can be fatal, wherein the toxin can inhibit hepatic mitochondria fatty acid oxidation [63, 64]. The loss of retinal structure and function [65, 66] is also a severe disorder due to emetic toxin of *B. cereus*. There is a need to create larger awareness on contaminated food(s) and food poisoning symptoms. Good manufacturing practices in food-processing units are again emphasized.

Bacillus cereus can be present on surfaces, sporulate and survive heat treatments (especially in dairy industry), where hygiene methods often are not able to control *B. cereus*. This is because the spores have high hydrophobicity and high adhesion ability for surfaces. An easy, fast and cheap detection method is also a limitation. To detect it by PCR method or by the use of antibodies requires skilled hands and may not always be available in the food industry.

The awareness of consumer preference for food safety, easy possibilities of detection shown, may not remain a limitation in future for its inclusion in critical control points in HACCP programs, where *B. cereus* is a problem.

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Conflict of interests.

The authors have no conflict of interest.

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LOW-MOLECULAR COMPONENTS OF COLOSTRUM AS A REGULATOR OF THE ORGANISM REDOX-SYSTEM AND BIOLOGICAL ANTIDOTE

Ievgen Ivanov
Department of Molecular Biology and Biotechnology[3]
E-mail: ivanovevg321@gmail.com

Valentyn Kozheshkurt[1]
E-mail: v.kozheshkurt@karazin.ua

Anatoly Bozhkov[2]
E-mail: bozhkov@univer.kharkov.ua

Anatolii Goltvjansky[2]
E-mail: profkom@karazin.ua

Victor Katrich[1]
E-mail: vkatrich@karazin.ua

Vadim Sidorov[2]
E-mail: visidorov@univer.kharkov.ua

Taras Gromovoy
Laboratory of Mass Spectrometry of Surface of Nanosystems
Chuiko Institute of Surface Chemistry
17 Henerala Naumova str., Kyiv, Ukraine, 03164
E-mail: grota@ukr.net

[1]Department of Physical and Biomedical Electronics and Complex Information Technologies[3]
[2]Research Institute of Biology[3]
[3]V. N. Karazin Kharkiv National University
4 Svobody sq., Kharkiv, Ukraine, 61022

Abstract

The protein composition in the diapason of molecular masses from 4800 to 9500 Da has been studied in colostrum, taken from different cows, and manifested the expressed biological activity. For this aim, an influence of low-molecular components of colostrum on some physiological parameters (change of body mass and temperature) at intoxication of animals (*Wistar* rats) by blue stone has been studied. An influence of colostrum low-molecular components on parameters of the organism redox-system (content of hyperperoxides of lipids and activity of glutathione peroxidase) in the blood serum of animals has been studied. For determining integral characteristics of colostrum components, electric conductivity of skim colostrum and one of colostrum with low-molecular proteins (less than 10 000 Da), taken from different cows, were used. The aim of this work is to study interconnections of an influence of colostrum low-molecular proteins on models of organism intoxication by cooper ions.

It is demonstrated, that the colostrum composition includes 25–35 different proteins with a molecular mass from 4800 to 9500 Da. The number and ratio between protein fractions depend on individual physiological-biochemical characteristics of producers. It has been revealed, that there is no direct dependence between the protein content in a measuring cell (2 mg/ml, 4 mg/ml and 10 mg/ml) with skim colostrum and electric conductivity change, and this dependence is different for skim colostrum, taken from different cows. Individual differences are manifested both at electric conductivity change and by the content of colostrum low-molecular proteins in a measuring cell. It is demonstrated, that colostrum low-molecular components can eliminate the toxic effect of blue stone on the organism, which mechanisms are connected with a balance shift in the sys-

tem "prooxidants↔antioxidants" towards antioxidants. The electric conductivity of colostrum components may be used as an express-method for evaluating biologically active substances of colostrum.

Keywords: colostrum, biologically active compounds, low-molecular proteins, electric conductivity, glutathione peroxidase, hydroeroxides of lipids.

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1. Introduction

It is known, that metabolism programming, determining not only peculiarities of ontogenesis, but also a risk of a series of pathologies, takes place in neonatal and early postnatal ontogenesis [1, 2]. It was demonstrated, that excessive nutrition in the early postnatal period formed specific metabolic patterns, preserved during the whole ontogenesis and correlated with lifespan [3]. Today active studies of an influence of animals' feeding peculiarities at early stages of ontogenesis on the mechanism of metabolic and immunologic programming and formation of epigenetic regulation processed are conducting [3].

Studies of colostrum and its influence on the biological systems functioning are of more interest in this case. It is explained by the fact that the placenta structure in ruminants prevents immunoglobulins transmission to a fetus, and newborn calves are deprived of so-called passive immunity [4, 5]. In the process of evolution, the transmission system of the molecules complex, supporting the immunity and metabolism programming by colostrum, formed.

Colostrum is a unique liquid, forming in mammary glands during only several days after delivery [6, 7] and is rich in immunoglobulins and other growth factors [8] with the extremely wide spectrum of biologically active substances [9]. It is demonstrated, that immunoglobulins transmission to newborns by colostrums is called colostrums immunity [5], and it is the most important survival factor of newborns in the early postnatal period [10]. Together with immunity, colostrum components provide metabolism formation and its epigenetic component. In this connection studies of the colostrum component are of great interest, because it favors peculiarities of postnatal development, protection from infectious agents and other negative environmental factors.

One of most spread environmental factors, negatively influencing the organism, is ions of heavy metals, especially, copper ones [11]. It is known, that cooper ions, penetrating the organism, can cause oxidative stress, and at the chronic influence – result in hepatic fibrosis [12]. In this connection it is interesting to study the influence of colostrum components on parameters of the organism redox-system at intoxication by blue stone.

Many works are devoted to studying the colostrum composition, and it is demonstrated, that it is very rich in proteins, and their composition depends on a "producer", contains relatively small amount of lipids [13], it is rich in microelements [14]. But the most important characteristic of colostrum may be considered its instability and composition dynamics. In this connection it is necessary to search and to develop methods, allowing to evaluate the dynamics of changing colostrum integral characteristics.

Such biophysical method as electric conductivity measuring may be used as integral parameters of multicomponent dynamic biological mixtures. This approach allows to evaluate the total number of charged ions and other molecules in colostrum [15]. The most biological activity in metabolism regulation mechanisms is inherent to peptides and low-molecular proteins. That is why it may be expected, that an index of colostrum components electric conductivity allows to evaluate the quality and potential biological activity of complex, multicomponent, dynamic mixtures fast. But "a contribution" of proteins, especially ones with different molecular masses in the electric conductivity index has not been studied.

It may be concluded, that colostrum in a unique biological product that can provide metabolism regulation, including at organism intoxication by heavy metal ions. But colostrum is not stable as a multicomponent mixture, and its composition depends on many parameters. In this connection, it is necessary to search for fast and effective methods of colostrum quality

evaluation. The aim of this was to study an interconnection between the content of low-molecular proteins with the electric conductivity and biological activity of colostrum on the model of organism intoxication by cooper ions. At that such organism redox-system parameters as content of hydroperoxides of lipids and glutathione peroxidase activity in the blood serum at animals' intoxication by cooper ions were determined.

2. Materials and methods

Colostrum was obtained at the Farm economy "Alfa" (Ukraine) from cows on the Ukrainian milky-pitted breed, second milk yield after calving. Skimming was conducted at colostrum centrifuging at 3000 *g* during 20 min at room temperature. After eliminating lipids, all proteins with a molecular mass more than 10 000 Da were removed from skim colostrum by membrane filtration. Electric conductivity was measured in the obtained fraction with low-molecular proteins by a vector analyzer ZNB 40 "Rohde & Schwarz" (Austria). Specific electric conductivity was measured in the frequency diapason as 100 kHz–10 MHz with interval 50 kHz. After that samples were dried in a rotation apparatus. Intoxication was conducted by threefold administration of blue stone to the experimental animals with interval 48 hours that was 5 days after the beginning of the experiment, in dose 0.1 mg/100 g of the body mass. The obtained samples of low-molecular proteins were dissolved in a physiological solution and administered to the experimental animals *per os* in dose 0.1 mg/100 g of the body mass. After 24 hours the animals were experimented. The blood serum was taken, and the content of hydroperoxides of lipids was determined in it by method [16] and glutathione peroxidase by [17]. The content of low-molecular proteins was determined on a mass-spectrometer Autoflex II LRF 20 "Bruker Daltonics" (Germany), equipped by an impulse nitrogen laser (λ=337 nm, impulse duration 3 ns). The obtained results were statistically processed by Mann-Whitney method using the program software Statistica 8.0 (StatSoft Inc., USA). Differences between the control and experimental groups were accepted reliable at $p<0.05$, comparing with a control variant.

3. Results

The composition of proteins with a molecular mass less than 10 kDa in different producers. The study of colostrum proteins with a molecular mass from 4800 Da to 9500 Da demonstrated that 27 fractions were revealed in the cow Aurora (**Fig. 1,** a). The content of these proteins was different, the most amount was of proteins with a molecular mass of 5500–6000 Da and 6500–7000 Da (**Fig. 1, *a***).

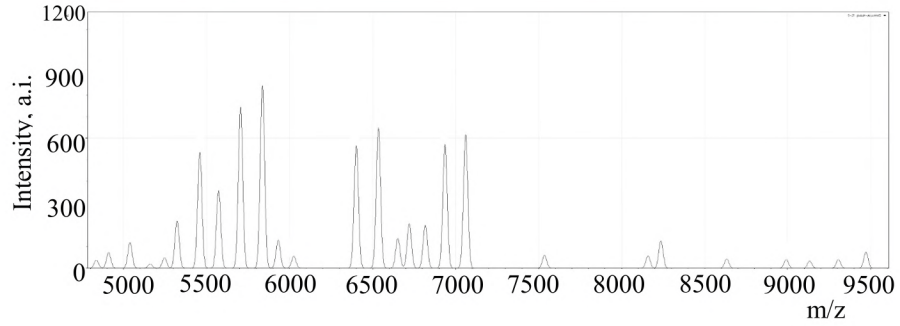
The content of colostrum proteins in this diapason of molecular masses in the cow Barinya was different. Thus, 31 fractions were identified by the method of mass-spectroscopy (**Fig. 1, *b***). If analyze coincidences of colostrum protein fractions, taken from the different cows, it may be concluded, that only 5 fractions were equal in the two cows (**Fig. 1, *c***).

The obtained results indicate that colostrum as most biological substances has a unique composition that is depends on genetic and epigenetic characteristics of a producer. These peculiarities must be taken into account at receiving and further standardization of multicomponent biological mixtures.

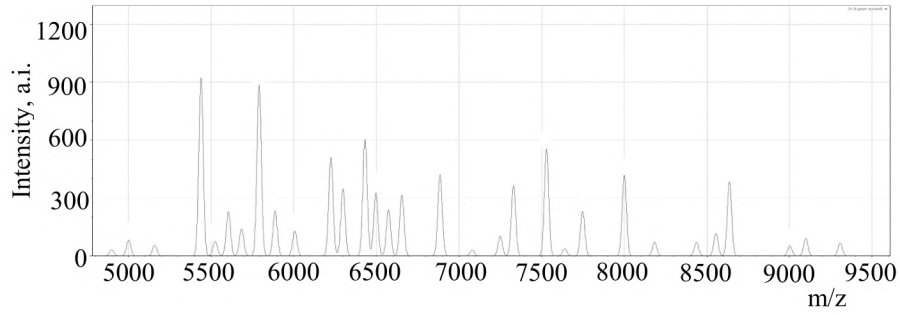
The electric conductivity of components of colostrum, taken from different producers. It is known, that lipids manifest dielectric properties [18], and their elimination is accompanied by an electric conductivity increase as it was demonstrated above [15]. Lipids elimination from colostrum demonstrated that 92 % of proteins, 7.69 % of carbohydrates and 0.31 % of vitamins and other substances, including ions, took place in skim milk counting for solid residue (**Fig. 2**). It may be stated, that skim colostrum is a concentrated solution of proteins with different molecular masses.

The determination of the dependence of specific electric conductivity on protein concentration, introduced in a measuring cell, demonstrated that at introducing 2 or 4 mg/ml the electric conductivity of skim colostrum, taken from the cow Aurora was the same. At introducing 10 mg/ml in the cell, the electric conductivity increased by 2 % (**Fig. 3,** a). If skim milk

was taken from Barinya, the concentration increase didn't result in an electric conductivity increase, the same is for Aurora's colostrum (**Fig. 3, *b***). These data testify to the absence of a direct mutual connection between protein content and electric conductivity.



a



b

Values diapason m/z, Da	Cow name	Protein groups by the ratio between mass and charge m/z, Da in the given diapasons						
4800–5300	Aurora	4835	4909			5037	5159	5245
	Barinya			4897	5000		5158	
5300–5700	Aurora	5321		5458		5571		
	Barinya		5438		5524		5603	5683
5700–6100	Aurora	5703		5835		5930		6024
	Barinya		5790		5886		6005	
6100–6600	Aurora			6400			6534	
	Barinya	6224	6297		6430	6496		6573
6600–7100	Aurora	6650	6720	6816		6934	7059	
	Barinya	6653			6884			7078
7100–8000	Aurora			7534				
	Barinya	7248	7329	7528	7639	7747	7999	
8000–9000	Aurora	8157		8235			8635	8990
	Barinya		8183		8436	8553	8631	
9000–9500	Aurora			9131	9304	9470		
	Barinya	9001	9097		9305			

c

Fig. 1. Composition of proteins of colostrum low-molecular fractions by the data of mass-spectrometry: *a* – colostrum, taken from the cow Aurora; *b* – colostrum, taken from the cow Barinya. Typical spectrums of proteins are presented; *c* – ratio between the molecular mass and the protein charge in Aurora and Barinya, the proteins, coinciding by the ratio between the molecular mass and the protein charge in Aurora and Barinya, are marked dark

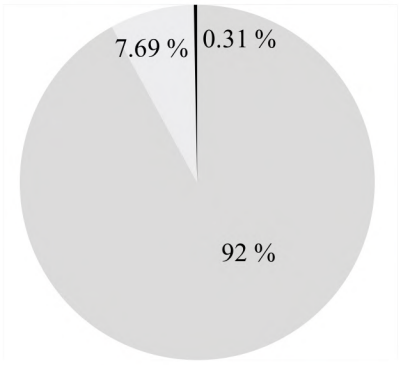


Fig. 2. Protein content ( ), sugars ( ) and other non-identified components ( ) in skim colostrum counting for solid residue

High-molecular proteins (more 10 kDa) that were the most part of proteins were eliminated from skim colostrum. In this case there was observed the linear dependence between the electric conductivity increase and the content of low-molecular proteins in a cell, if colostrum was taken from Barinya (**Fig. 3, *d***). If colostrum was taken from Aurora, the non-linear increase was observed (**Fig. 3, *b***).

So, the electric conductivity of both skim colostrum and colostrum low-molecular proteins demonstrate an individual character of change, because these characteristics were different for colostrum of the different cows. These results may testify to the fact that ratios of charged/neutral molecules are different in the different cows, and electric conductivity may serve an integral parameter of multicomponent mixtures. Electric conductivity depends not only and possibly not so much on molecules number, but is determined by the number of charged molecules and ions and ratio between molecules sizes in a mixture (low-molecular and high-molecular proteins).

Taking into account these expressed individual characteristics of colostrum, we offer to conduct further studies at the group level that is to combine colostrum of at least 5 producers at obtaining biologically active substances before processing for eliminating individual differences. At the next stage of the work colostrum, taken from 5 cows of the same breed, was combined, skimmed, and obtained low-molecular components were used as biologically active complexes.

The influence of colostrum low-molecular components on some parameters of organism redox-systems after intoxication by blue stone. Intoxication of the experimented animals was accompanied by their body mass loss during first 2–3 days (**Fig. 4, *a***). In further their mass remained stable up to 10–12 days, then they restored the body mass growth again, and didn't differ from the control group after 16 days of the experiment (**Fig. 4, *a***).

The body mass loss and the growth stop were observed at the reliable body temperature decrease (**Fig. 4, *b***). Thus, if the body temperature of the intact control group remained stable during the experiment as 37.5 °C, in the animals after intoxication it was by 1 °C lower than the control (**Fig. 4, *b***).

If the animals received colostrum low-molecular components (CLC) per os and blue stone was administered to them, their body mass growth, comparing with the control, stopped. Just after 6 days after the beginning of the experiment their growth had recovered, and by 20 days then didn't differ from the control group and even a bit exceeded it (**Fig. 4,** a). At that the body temperature of the animals, received CLC at intoxication, didn't differ from the control animals' one (**Fig. 4, *b***).

Consequently, CLC can eliminate the negative inhibiting effect of blue stone on metabolism and may be considered as a potential antidote. Mechanisms of this effect may be extremely diverse.

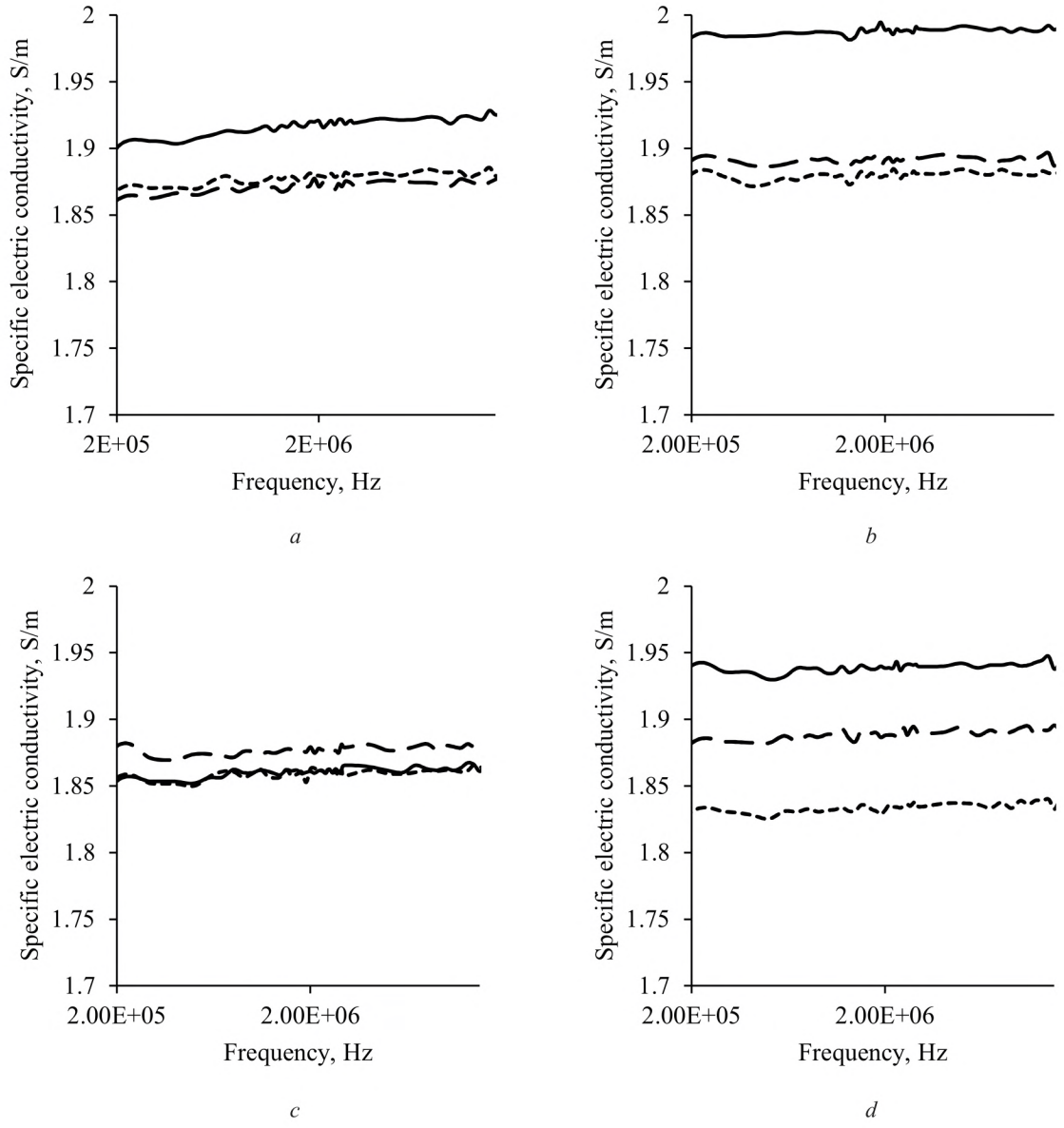


Fig. 3. Electric conductivity of colostrum components at introducing in a measuring cell 2 mg/l (- - - -), 4 mg/l (— —) and 10 mg/l (——) of proteins: *a* – electric conductivity of skim colostrum, taken from Aurora; *b* – electric conductivity of low-molecular components of colostrum, taken from Aurora; c – electric conductivity of skim colostrum, taken from Barinya; *d* – electric conductivity of low-molecular components of colostrum, taken from Barinya

It is known, that cooper ions may manifest the prooxidant effect, connected with increasing products of free radical reactions [19]. The next series of experiments demonstrated that after 24 hours after the last administration of blue stone to the animals, the content of hydroperoxides in the blood serum increased by 75 %, comparing with the control (**Fig. 5, *a***). Such increase of products of free radical reactions took place at inhibition (by 36 %) of one of "central" antioxidant enzymes – glutathione peroxidase (**Fig. 5, *b***).

If at intoxication the animals received CLC threefold in dose 0.1 mg/100 g of the body mass, the content of lipid hydroperoxides in the blood serum didn't differ from the control. The activity of glutathione peroxidase in this case even exceeded such of the intact control elements by 30 % (**Fig. 5, *b***).

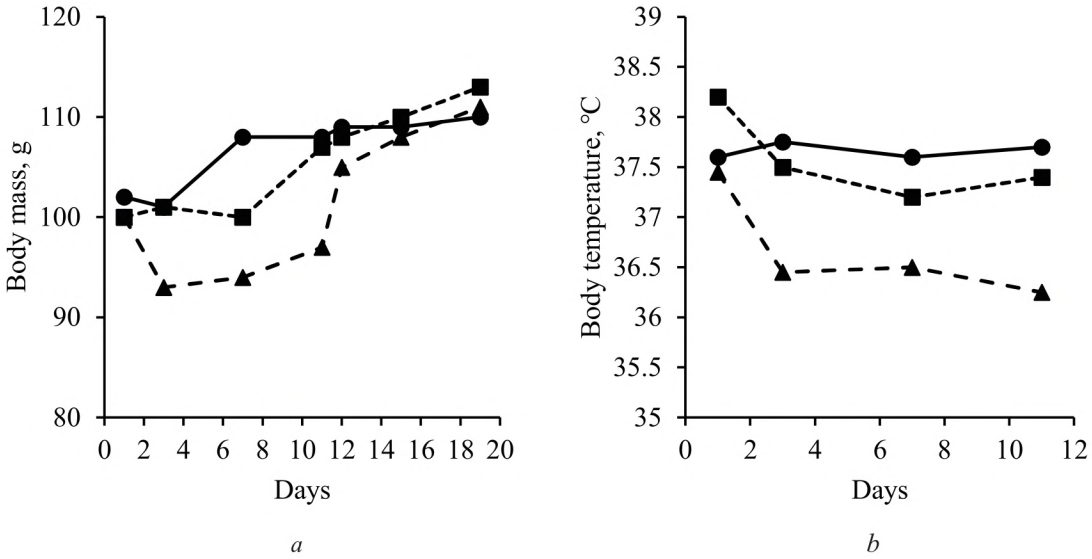


Fig. 4. Change of physiological parameters in the control group of animals (▲), in the animals after blue stone intake (■) and in the ones with toxicosis after taking low-molecular components of colostrum in dose 0.1 mg/100 g of the body mass (●): *a* – body mass; *b* – body temperature. The average values for 5 animals in each group are presented

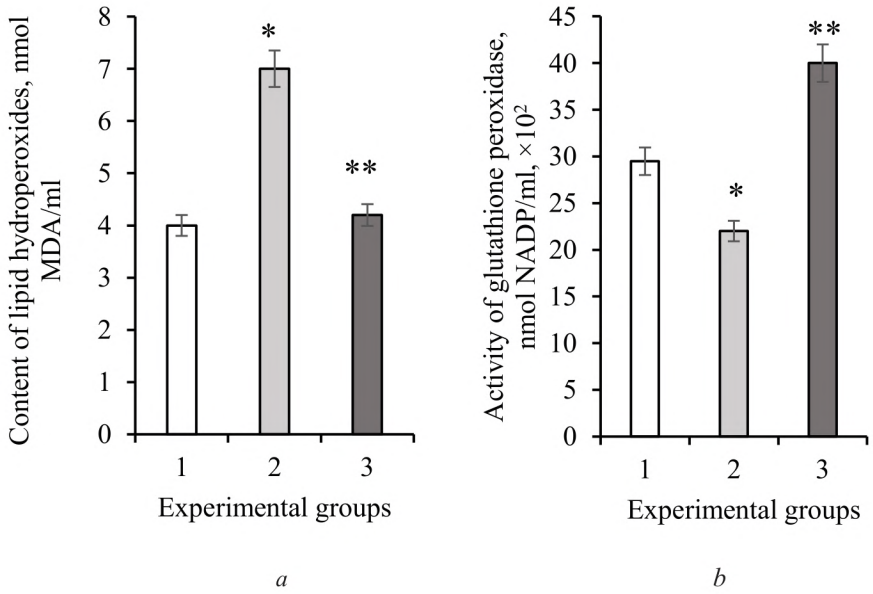


Fig. 5. Change of redox-system parameters of the experimental animals (1), animals with toxicosis (2) and animals, administered with colostrum low-molecular components in dose 0.1 mg/100 g (3): *a* – content of hydroperoxides of lipids in the blood serum; *b* – activity of glutathione peroxidase in the blood serum. The average values of 5 animals in each group are presented

So, elimination mechanisms of the toxic effect of cooper ions by colostrum low-molecular components are realized through regulation of the organism redox-system. Based on CLC, medical preparations, able to eliminate the toxic effect of heavy metal ions and possibly of other toxicants, may be developed, and the electric conductivity of colostrum substances may be used as standardization methods.

The "individual" composition character of proteins with a low molecular mass (less than 10 000 Da) is conditioned by several causes. Among them are genetic characteristics, physiological-biochemical status as a result of "interrelations and mutual influences" between the genome

and living conditions of the animals, age, number of calvings and also time and number of milk yields after calving [20]. At the same time the content of colostrum and proteins is influenced by season and a series of other factors [21]. Storage time of colostrum cannot be also excluded, because its composition includes diverse enzymes with protease activity. In this connection it may be stated, that by its composition colostrum is a unique, high-dynamic substance with the brightly expressed biological activity. These peculiarities complicate receiving of biological substances from colostrum. It is connected with a fact that it is impossible to get substances with equal standard characteristics, and control methods need great time and material costs.

These questions may be solved by combining colostrum, taken from five and more animals for eliminating brightly expressed individual characteristics. Electric conductivity evaluation may be used as a control method for both colostrum and components, obtained from it. Electric conductivity depends not only on ions content, but also on number of lipids and low-molecular proteins as it is demonstrated in this work. That is why this method allows to get data about the ratio of different molecules in the colostrum composition that is an important characteristic of biological activity of substances. It is testified by the obtained data on CLC influence on the organism protection from blue stone effects.

4. Conclusions

The colostrum composition includes 25–35 proteins with molecular masses from 4800 to 9500 Da. Number, content and ratio between fractions depend on animals.

A direct dependence between the electric conductivity of skim colostrum, low-molecular components of colostrum and concentrations in a measuring cell is absent. This dependence was different for colostrum from different animals and reflects their individual physiological-biochemical peculiarities.

Colostrum low-molecular components can eliminate the toxic effect of blue stone on the organism, which mechanisms are connected with regulation of the prooxidant-antioxidant system that is a balance shift towards antioxidants.

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INFLUENCE OF FORM AND SIZE OF A ROOT ON THE STORAGE LIFE OF TABLE BEET

Ludmila Pusik
Department of Technologies of Processing and Food Production[1]
Ludmilap@gmail.com

Vladimir Pusik
Department of Agrotechnology and Ecology[1]
kysmish@gmail.com

Veronika Bondarenko
Department of Agrotechnology and Ecology[1]
zim-hot@rambler.ru

Ludmila Gaevaya[2]
Gaevaaludmila9@gmail.com

Nina Lyubymova
Department of Forest Management and Life Safety[3]
nina.lioubimova@gmail.com

Galyna Sukhova
Department of Crop Production[3]
syhovagalinaiv@gmail.com

Nataliya Didukh[2]
Natasha_didukh@ukr.net

Galina Slobodianyk
Department of Vegetable Growing
Uman National University of Horticulture
Institutska str., 1, Uman, Ukraine, 20305
sgy123@i.ua

[1]Kharkiv Petro Vasylenko National Technical University of Agriculture
44 Alchevskih str., Kharkiv, Ukraine, 61002
[2]Department of Fruit and Vegetable and Storage[3]
[3]Kharkiv National Agrarian University named after V.V. Dokuchaiev
township Dokuchaevskoe, Kharkiv distr., Kharkiv reg., Ukraine, 62483

Abstract

Table beet have a series of high-value parameters: good taste properties, healing-prophylactic importance, ability to long-term storage. There are many sorts of table beet, different by root form. Most widespread are ones of the round and cylindrical forms. At the same time plants of table beet at growing form roots of different masses. The aim of the study was to investigate the storage life of table beet depending on form and root sizes. The conducted studies give a possibility to substantiate scientifically an influence of table beet' form and sizes on their storage life for determining its term.

It has been established, that roots of the round form of the Kharkiv Bordo sort lost moisture more intensively at the expanse of breath and evaporation – 4.4–5.4 %. In the Vital sort with roots of the cylindrical form, mass natural losses were 4.1–5.1 %. At that more natural mass losses were in small roots with mass 150–300 g.

Small roots were more inclined to sprouting at storage. Among sprouted roots, 1.6–1.8 % were small ones with mass 150–300 g. More percent of sprouted roots was for ones with mass 500–700 g as 2.3–2.5 %. At that less percent of sprouted roots was in

the Vital sort of the cylindrical form.

Small roots with mass 150–300 g were more damaged by rots at storage – 10.4–12.3 %. Among roots of middle sizes, 6.0–6.8 % were damaged by roots, among big ones – 4.5–4.7 %. It must be also noted, that cylindrical roots of the Vital sort were less damaged by rots at storage than round ones of the Kharkiv Bordo sort.

Keywords: table beet, sort, storage, root form, root mass, losses.

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1. Introduction

Consumption of fresh vegetables is extremely important for normal human life activity. It is explained by the fact that even with a small amount of dry substances, contained in juicy vegetable products, the human organism receives biologically active compounds, necessary for metabolism, life activity support, health and life prolongation. At the same time the modern production volume of fresh vegetables doesn't satisfy growing needs of the population. According to physiological norms, table beet consumption is 10 kg [1]. Continuous supply of consumers with fresh roots is complicated by the season character of production and long storage of the products. Scientists' researches demonstrate that long-term storage of vegetables is accompanied with quality and quantity losses, content decrease of biologically active substances. The world experience of vegetable growing testifies that at shortcomings at harvesting and without additional processing of products at passing through the logistic chain, 35 % or one third of the harvest of fresh vegetables is lost [2].

So, the problem of vegetables storage combines a wide circle of questions. It is important to know the complex of causes, conditioning products losses, namely planting, harvesting, transportation, sort peculiarities, optimal regimes and best storage methods [3, 4].

Many complex studies as to increasing the roots stability at storage were conducted, but several questions still unsolved. One of main factors, decreasing the commodity quality of roots and causing great losses at their transportation and storage, are elements of after-harvesting processing of products, namely sorting by root sizes.

At storing any juicy product, it is always necessary to base on its biological characteristic. All roots, excluding radish, are biennial crops. Their general biological peculiarity is to stay at a low temperature at rest that is not deep, but rather forced for roots. At satisfactory conditions their growth recovers. The biological function of the rest condition is differentiation of a cone of root buds growing, their preparation to reproductive development. The period, during which buds finish this preparation, determines the rest duration that is storage life of products [5].

Economic-botanic sorts of table beet differ by form, coloration and flesh structure, ripeness term, favorableness to ageing [6, 7]. Sorts belong to five sort-types (Egypt flat, Egypt round, Bordo, Eclipse and Erfurt), each of which combine several economic-botanic sorts [8].

The storage life of table beet depends on root form. The storage life of sorts with flat roots is satisfactory, they ripen fast, their flesh is mainly violet-red with more or less expressed white rings, taste is good, dry substances content is 8–11 %. At early spring sowings they are grown for summer consumption, and at ones at the end of May – for autumn and early winter one. These sorts are not much suitable for long-term storage. Ones with the round-flat and round root forms are most suitable for that. They ripen a bit later than flat ones, contain 10–12 % of dry substances, have good taste qualities, are well stored, are less sick, have less natural losses and are stored longer without sprouting. These sorts include the Bordo sort-type and other. Sorts with conic roots contain 12–16 % of dry substance, are stored well, but have a low quality, because contain much cellulose and have the fibrous flesh. They include Erfurt sort-type. These sorts are little spread [9].

Table beet sorts are recommended to be harvested at the stage of technical ripeness, coming in 84–150 days after sprouting. There are also recommendations for harvesting roots as

needed or at reaching necessary sizes [10]. It must be noted, that the storage life of juicy products of root plants depends on conditions and methods of storage [11–13]. For long-term storage it is recommended to use table beet with the most cross diameter from 5.0 to 14.0 cm [14].

Studies have established that vegetables, different by sizes of the product organ, are stored unequally. Thus, the storage life of bush pumpkin fruits depends on fruit size. Under conditions of raw material ground at daily temperature 26…30 °C fruits with a diameter more than 8 cm are stored during 13–18 days, diameter 4.5–6.0 cm – 2–6 days. At temperature 5±1 °C fruits with diameter 4.5–6.0 cm are stored during 5 days, diameter 6.1–8.0 cm – 13–16 days, the mixture of fruits with this size - 10–12 days, and ones with diameter 8.1–10.0 cm – 18–21 days. The mass loss of fruits at storage at temperature 5±1 °C by 42 % depends on their size. The output of commodity products by 22 % depends on fruit sizes [15].

Under usual refrigerator conditions cucumbers in an open box preserve their properties during two days, more natural losses are observed in fruits with length 91–110 mm than in the mixture. Cucumbers are stored almost two weeks practically without losses at temperature 5±1 °C in boxes with a polyethylene film or in polyethylene packets of 20 kg. Average daily losses of fruits at their storage in polyethylene packets don't exceed 0.08–0.10 % [16].

A harvested batch or one package may contain fruits, different by sizes within the same sort. Thus, their breath efficiency is different. The studies testify that more breath intensity is inherent to small bush pumpkin fruits, because metabolic processes are more intensive in them. With increasing the fruit size, the breath intensity decreases. The breath intensity of small fruits is five times higher than in big ones, and in middle – two times less than in ones with a less diameter [17].

Products of small bush pumpkin fruits must emit 4.3 times more heat than ones of middle sizes, and 8.1 times more than big ones; cucumbers with length 91–111 mm – in 1.3 times more than ones with length 111–140 mm. The cooling speed of pumpkin fruits to the storage temperature depends on their size. Small melon fruits are cooled to storage temperature 5±1 °C during 17 hours, bush pumpkin – 7, cucumber – 1.5 hours, middle melon fruits – 24 hours, bush pumpkin – 8.5 hours, big melon fruits – 25.5 hours, bush pumpkin – 14.5 hours [18, 19].

Any batch of table beet is a heterogenic mass of roots of different sizes, so of different ripeness. In this connection the intensity of physiological processes depends on root size. Such batch is difficult for storage.

For establishing the optimal storage temperature for table beet as 0±0.5 °C, it is necessary to eliminate heat, formed as a result of roots breath and their heat content.

For decreasing losses of stored roots, it is necessary to keep the optimal temperature regime at the expanse of heat removal from them, by regulating amounts of ventilating air. For example, lately ripening radish sorts of big fruits have almost 2 times more specific surface area, comparing with early ripening ones with small fruits, that positively influences the natural mass loss at their storage. The cooling speed of roots from 20 °C to the optimal storage temperature (+1 °C) depends on their diameter. Radish enthalpy doesn't depend on root size. Physical and thermal properties of roots are determining factors of their storage economic efficiency [20].

So, the research aim follows from the presented data – to study the table beet storage life depending on roots' form and size.

2. Materials and methods for studying the intensity of natural mass losses of table beet at storage

The studies were conducted with table beet sorts Kharkiv Bordo and Vital of middle ripeness, Ukrainian selection (Institute of Vegetable and Melon Growing of the National academy of agrarian sciences of Ukraine).

Kharkiv Bordo is a sort of universal destination, middle ripeness, the vegetation period is 130–133 days. A root is round-oval, with dark-cherry coloration, core without rings, with perfect taste qualities (**Fig. 1**). Roots are well stored till spring, are stable to white and gray rots, have a perfect commodity outlook.

Vital is a sort of universal destination, middle ripeness, vegetation period is 130–138 days (**Fig. 2**). Roots are of the cylindrical form with a small acute tip, their diameter reaches 5–7 cm. The flesh is of dark-red color with a dark-violet tint, sweet, juicy, without expressed white rings. Roots are characterized by good transport ability.

Fig. 1. Kharkiv Bordo

Fig. 2. Vital

Vital table beet are of the cylindrical oblong form with the form index (ratio between root length and diameter) from 4.2 to 4.5; Kharkiv Bordo – from 1 to 1.2. At the same diameter roots essentially differed by mass. Thus, at studying beetroots were divided by mass in three fractions: 500–700 g (big roots), 300–500 (middle) and 150–300 g (small roots). They were stored in nets (**Fig. 3**) at temperature 0± 0.5 °C.

After putting for storage, observations were conducted in dynamics with 1 month interval. A sample was excluded from storage, if natural mass losses reached 10 % and more, and products had signs of damage by diseases and physiological disorders. For determining mass losses at storage of beetroots, each accounting sample (mass 5 kg) [21] was weighted, numbered, recorded in a journal with a quality characteristic of roots.

There were determined: natural losses, root mass, damaged by diseases and sprouted ones; commodity products outlet at the end of storage.

Natural mass losses were determined by the formula:

$$X = \frac{A - B}{A} \cdot 100, \quad (1)$$

where X – mass loss, %; A – products' mass at putting for storage, g; *B* – products' mass at the end of storage, g.

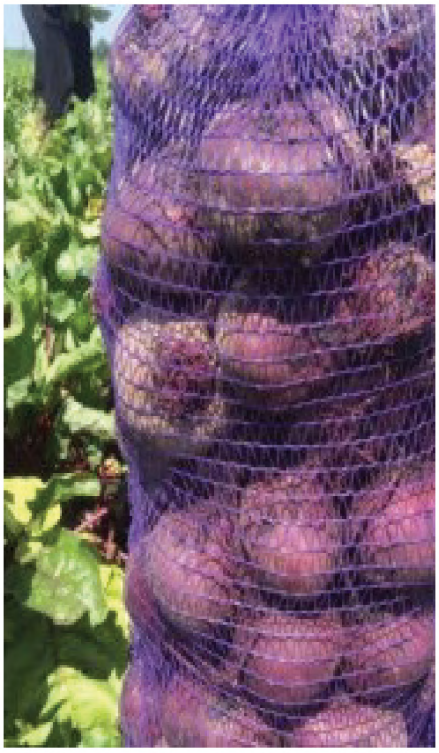

Fig. 3. Storage of table beet in nets

The statistical processing of the obtained results was conducted by the method of dispersion and correlation-regression analysis and using computer programs «Statistica 6» and MicrosoftExcel 2003.

The conducted studies were aimed at scientific substantiation of the influence of table beet sizes and forms on its storage life for determining its duration. The following tasks were set for attaining this aim:

– to determine natural mass losses of roots during storage;

– to determine the output of commodity products depending on root size.

3. Results of studying the intensity of natural mass losses of table beet at storage

Mass losses of roots at storage take place unevenly. At the beginning of storage, when the healing period has not finished yet, losses are rather high and are 1.3–1.7 % depending on sort

peculiarities. Less mass losses are in Vital table beet – 1.3–1.5 %. Small Kharkiv Bordo beetroots lost mass in the first month of storage by 6.6 % and 13.3 % faster than middle and big roots, respectively. The analogous regularity of mass loss is in Vital roots.

Then mass losses gradually decrease to 0.5–0.9 %. At the storage period day mass losses in small roots varied from 0,034 in the Vital sort to 0,036% in Kharkiv Bordo, in middle size roots – from 0,030 to 0,032%, in big – 0,027–0,029% according to the sort (**Table 1**).

Table 1

Influence of table beet mass on natural mass losses at storage, %

Roots mass, g	October	November	December	January	February	Totally
Kharkiv Bordo						
500–700	1.5	1.1	0.7	0.6	0.5	4.4
300–500	1.6	1.2	0.7	0.6	0.7	4.8
150–300	1.7	1.3	0.8	0.7	0.9	5.4
Vital						
500–700	1.3	1.0	0.6	0.6	0.6	4.1
300–500	1.5	1.1	0.7	0.6	0.7	4.6
150–300	1.6	1.2	0.8	0.7	0.8	5.1

As to sprouting, there were more sprouted roots among young small ones, than among more ripened. Already in January big and middle table beet became to sprout under conditions of the stationary artificially cooled warehouse, and small ones – no (**Table 2**).

Table 2

Storage life of table beet depending on their mass and sort peculiarities, %

Roots mass, g	Natural mass losses	Sprouted	Average sprout length, cm	sick
Kharkiv Bordo				
500–700	4.4	2.5	0.9	4.7
300–500	4.8	2.2	0.9	6.8
150–300	5.4	1.8	0.5	12.3
Vital				
500–700	4.1	2.3	0.9	4.5
300–500	4.6	2.0	0.9	6.0
150–300	5.1	1.6	0.5	10.4

Big and middle roots had longer sprouts than small ones. All not sick roots preserved the ability to sprout.

The studies have established that immature roots are less resistant to diseases, especially to rots [22]. At the storage period small roots were damaged by diseases (mainly gray rot) by 10.4%–12.3%, whereas biggest – only by 4.5–4.7 %, and middle – by 6.0–68 %. The disease of the tail part of a root was observed. The commodity products output for 150 days of storage was 80.5–89.1 % depending on root size (**Fig. 4**)

It has been established, that Vital table beet of the cylindrical form were stored better, than round Kharkiv Bordo ones: the commodity products output was by 0.7–2.9 % higher. The storage life of big table beet is higher than of middle and small ones. The highest ability to sprouting was observed at withering. Big and middle roots must be put for long-term storage. It is better to allow big roots in normative documents and to exclude smalls ones completely.

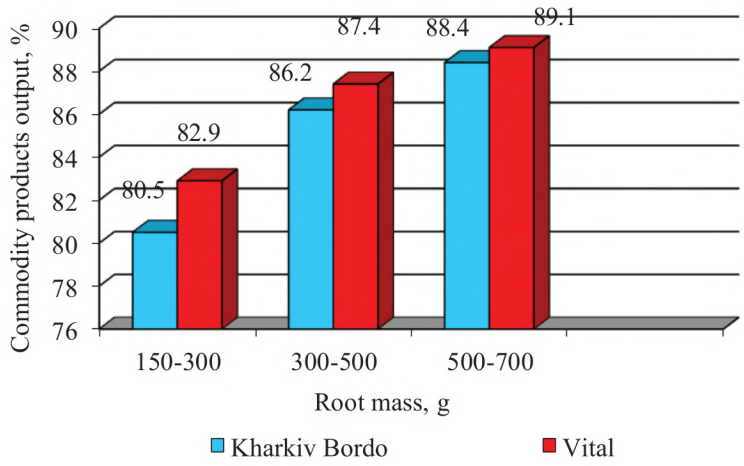

Fig. 4. Storage life of table beet depending on root size and sort peculiarities, %

4. Conclusions

Less natural mass losses are inherent to big roots of 500–700 g – 4.1–4.4 %, and more ones were in small roots (150–300 g) – 5.1–5.4 %. At that less natural mass losses were observed in the Vital sort with roots of the cylindrical form.

More commodity products output at the end of storage was in the fraction of big roots of 500–700 g as 88.4–891 %. The fraction of small roots was characterized by the least commodity products output as 80.5–82.9 %. It must be noted, that cylindrical roots of the Vital sort were stored better.

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