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USE OF ENVIRONMENTALLY SAFE TECHNOLOGIES IN INCREASING BIOPRODUCT PRODUCTION

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Abstract

This article presents the results of experiments on the use of electrochemically activated w with alkaline (pH = 9.5–10.5) and acidic (pH = 3.0–3.5) media in biolaboratories for the breeding of e of the grain moth (*Sitotroga cerealella* Oliv.) and for the disinfection of production facilities, as wel the economic efficiency indicators of the new method.

In this regard, we obtained positive results by conducting a series of studies from 2016 to the present on the use of electrochemically activated water instead of conventional tap water to normalize grain moisture, improve the nutritional value of grain, and sanitize biolaboratory premises from harmful microorganisms during the reproduction stage of grain moth eggs (*Sitotroga cerealella* Oliv.) [5,6,7,8,9].

The study was carried out in the research laboratory of the Department of Chemical Engineering at Namangan State Technical University (formerly Namangan Institute of Engineering and Construction), in the central laboratories “Water Analysis” and the Namangan Regional Center for Sanitary Epidemiology and Public Health “Suvokava” (mutual cooperation agreement No. 2/2021), as well as at “Bioservice” LLC in Namangan.

INTRODUCTION

In our initial experiment, electrochemically activated water with an alkaline medium (pH = 9.5–10.5) was used to normalize the moisture content of barley grain damaged by larvae of

Sitotroga cerealella. The experiments were conducted in three replications with three variants. In this case, Variant 1 represented the conventional method, where barley grain was soaked in ordinary tap water and served as the control. In Variant 2, electrochemically activated alkaline water with pH = 9.0–9.5 was used for moistening the grain, while in Variant 3, electrochemically activated alkaline water with pH = 10.0–10.5 was applied.

Electrochemical activation of water was carried out in membrane electrolyzers made of membrane–canvas material. The anode was made of acid-resistant material, while the cathode was made of alkali-resistant material. The device is divided by a diaphragm into two sections; when a direct electric current is applied, positively charged cations in the water move toward the cathode, and negatively charged anions move toward the anode. In turn, oxidation processes occur at the anode, and reduction processes occur at the cathode.

The appearance and description of the electrochemical water activation device underlying this study are presented below (Figure 1 and Table 1).

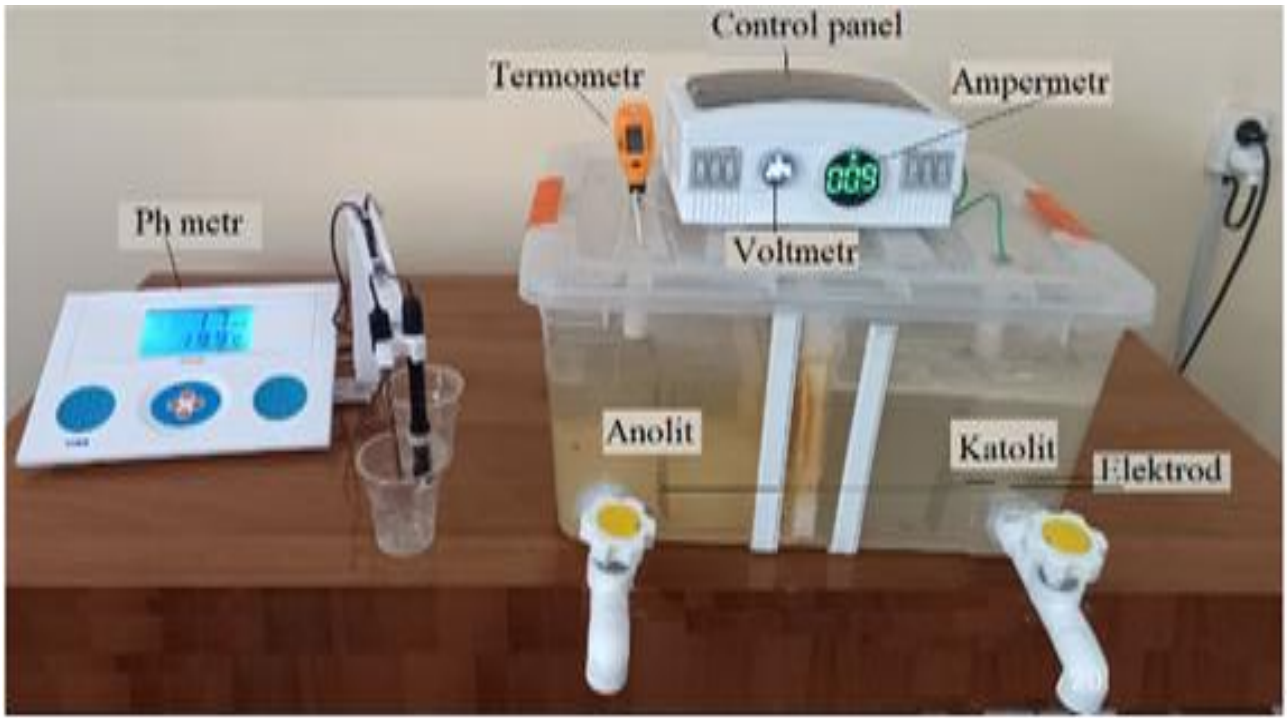

Fig. 1. Device for Electrochemical Activation of Water

Table 1.

Description of the Device for Electrochemical Activation of Water

№	Parameters	Values
1	Source voltage, V	220
2	Current consumption, A	1.6
3	Frequency, Hz	50
4	Energy consumption, kWh	0.17
5	Volume, L	20
6	Electrode surface area, cm^2	105.92

7	Electrode length, cm	16
8	Specific resistance of electrode material $\times 10^{-6}$ Ω·m	0.4
9	Number of electrodes, pcs	4
10	D112-10 marked diodes, quantity	4
11	RCD circuit breaker (UZO 40), quantity	1
12	Insulated cable entries, m	10

The composition of electrochemically activated water used in the experiment and its pH values were determined based on the requirements of the State Standard of the Republic of Uzbekistan for hygienic requirements and quality control of drinking water, in cooperation with the Central Laboratory of the Namangan State Enterprise "Suvokava".

According to the experimental results, when comparing certain physicochemical parameters of tap water with an alkaline medium to untreated tap water, the total hardness of untreated tap water in Variant 1 was 5.6 mg-eq/dm³, the content of Cl^- ions was 51.0 mg/dm³, and the SO_4^{2-} content was 162 mg/dm³. In Variant 2, the total hardness of electrochemically activated tap water with pH = 9.0–9.5 was 2.5 mg-eq/dm³, the Cl^- ion content was 32.8 mg/dm³, and the SO_4^{2-} content was 42 mg/dm³. In Variant 3, the total hardness of electrochemically activated tap water with pH = 10.0–10.5 was 2.3 mg-eq/dm³, the Cl^- ion content was 31.2 mg/dm³, and the SO_4^{2-} content was 38 mg/dm³. The analysis showed that the hardness, chloride, and sulfate content of electrochemically activated water in an alkaline medium are significantly lower than the normative indicators.

When studying the electrochemical properties of non-electrochemically treated tap water in an acidic medium, the total hardness of electrochemically activated tap water with pH = 3.0–3.5 in Variant 2 was 4.1 mg-eq/dm³, the Cl^- ion content was 50.2 mg/dm³, and the SO_4^{2-} content was 134 mg/dm³. In Variant 3, using electrochemically activated tap water with pH = 4.0–4.5, the total hardness was 3.8 mg-eq/dm³, the Cl^- ion content was 48.5 mg/dm³, and the SO_4^{2-} content was 131 mg/dm³. The analyses showed that the hardness of electrochemically activated water in the acidic (anolyte) medium is slightly higher than in the alkaline medium, while the levels of chlorides and sulfates differ significantly (Table 2).

Table 2.

Physicochemical Parameters of Electrochemically Activated Water with an Alkaline Medium (2017)

№	Варианты	Общая жесткость, мг. экв/дм3	Хлориды Cl-, мг/дм3	Сульфаты SO4, мг/дм3
В щелочной среде				
1	Текущий метод (водопро водная вода, контроль)	5,6	51,0	162
2	Электрохимически активи рованная вода (pH=9-9,5, эксперимент)	2,5	32,8	42

3	Электрохимически активи рованная вода (pH=10-10,5, эксперимент)	2,3	31,2	38
В кислой среде				
5	Электрохимически активи рованная вода (pH=3-3,5, эксперимент)	4,1	50,2	134
6	Электрохимически активи рованная вода (pH=4-4,5, эксперимент)	3,8	48,5	131
№	Variants	Total hardness, mg-eq/dm³	Chlorides Cl^-, mg/dm³	Sulfates SO_4^{2-}, mg/dm³
Alkaline medium				
1	Conventional method (tap water, control)	5.6	51.0	162
2	Electrochemically activated water (pH = 9.0–9.5, experimental)	2.5	32.8	42
3	Electrochemically activated water (pH = 10.0–10.5, experimental)	2.3	31.2	38
Acidic medium				
4	Electrochemically activated water (pH = 3.0–3.5, experimental)	4.1	50.2	134
5	Electrochemically activated water (pH = 4.0–4.5, experimental)	3.8	48.5	131

The experiments with electrochemically activated water were mainly conducted at the stage of barley grain infestation using larvae of the grain moth (*Sitotroga cerealella*) according to the established technology. In all variants, cuvettes (previously cleaned of additives, washed, and soaked under standard conditions) were filled with barley grain at a weight of 13 kg per cuvette, with a layer thickness of 3–4 cm. In total, 104 kg of grain was used in each variant, calculated as 13 kg per 8 cuvettes for the experiment. The experiment began on 01.03.2017.

The barley grain in all variants was kept on shelves for 24 hours (at a temperature of 24–25°C and humidity of 80–85%) until 02.03.2017 in the cuvettes, after which the grain moisture was measured using the "Kolos-1" device. The average air humidity was 16–18%. On that day, barley grain in all variants was uniformly inoculated on shelves using a sieve with a diameter of 2 mm with 1 g of test material (grain moth eggs aged 1 day, activated at 24–25°C for 24 hours) without damaging the grain.

The moisture content of the grain in all variants was measured on 12.03.2017 after grain infestation. To adjust moisture to the standard level, in Variant 1 the grain was moistened with an average of 250–300 ml of ordinary tap water per 10 kg of grain. The same amount of electrochemically activated water was used in the experimental variants.

From 15.03.2017, the grain in all variants began heating. Initially, 500 grains were taken as samples from each cuvette and examined by dissection, after which grain quality was determined. In this case, grain damage was 65% in the control (Variant 1), 72% in Variant 2, and 78% in Variant 3.

From 16.03.2017, until the observation of the initial emergence of adult moths, barley grain in Variant 1 was soaked in ordinary water

once every two days, while the other variants were treated with electrochemically activated water. In this case, the first emergence of moths in Variant 1 was observed on 28.03.2017 and 31.03.2017; in Variant 2 on 26.03.2017 and 29.03.2017; and in Variant 3 the first moths were observed on 25.03.2017 and were captured on 28.03.2017.

The results of the experiment are presented in the table below (Table 3).

Table 3.

Effect of the Use of Electrochemically Activated Water in an Alkaline Medium at the Stage of Barley Grain Infestation by *Sitotroga cerealella* Larvae on Grain Damage Quality and Larval Development (2017)

№	Variants	Grain consumption, kg	Sitotroga consumption, g	Grain infestation date	Water consumption, ml	Grain damage quality, %	Date of first moth emergence	Date of box installation
1	Conventional method (tap water, control)	104	104	01.03.2017	15,600	65	28.03.2017	31.03.2017
2	Electrochemically activated water (pH = 9.0–9.5, experimental)	104	104	01.03.2017	15,600	72	26.03.2017	29.03.2017
3	Electrochemically activated water (pH = 10.0–10.5, experimental)	104	104	01.03.2017	15,600	78	25.03.2017	28.03.2017

The process of collecting moths from the rearing boxes and obtaining eggs from them was carried out based on the existing method while maintaining the required hygrometric regime. The experimental results show that 633 g of barley was obtained in packaged form in Variant 1 (control), 655 g of barley in Variant 2 (pH = 9.0–9.5), and 686 g of barley in Variant 3 (pH = 10.0–10.5) (Table 4).

Table 4

Economic efficiency of applying EFS in an alkaline environment at the stage of barley grain infestation by Sitotroga larvae (based on 2017 prices)

№	Variants	Grain consumption (kg)	Sitotroga consumption (g)	ime spent on grain infestation (days)	Obtained Sitotroga eggs (g)	Product cost (sum)	Difference (+/– sum)
1	Current method (tap water, control)	104	104	31	633	633000	–
2	Electrochemically activated water (pH = 9–9.5, experiment)	104	104	29	655	655000	+22000
3	Electrochemically activated water (pH = 10–10.5, experiment)	104	104	28	686	686000	+53000

The obtained results show that, compared to the control variant, the 3rd variant—where barley grain is soaked in an alkaline electrochemically activated water with pH 10–10.5—makes it possible to obtain on average 53 g more Sitotroga eggs per 104 kg of grain. If it is assumed that the "biofactory" processes 120 tons of barley per year, this indicator corresponds to 61.15 kg, and production efficiency increases by 8–10%. Over one production season, the enterprise obtained 61 million UZS in revenue, while the profit amounted to 150,000 UZS. In addition, 2–3 days of work are saved at the grain spoilage stage.

Experiments on grain moisture normalization and decontamination of the biolaboratory premises from harmful microorganisms using acidic electrochemically activated water (pH 3–4.5) were conducted in three variants with three replications (room volume 72 m^3 and humidification area 24 m^2) in a laboratory facility. In this case, Variant 1 (control) represents the standard method used in Room 1, which includes air ventilation and grain moisture normalization using tap water. Variant 2 involves air decontamination in the room based on spraying electrochemically activated water (pH 3–3.5) together with grain moisture normalization. Variant 3 involves air decontamination in Room 3 based on spraying electrochemically activated water (pH 4–4.5) combined with grain moisture normalization. Barley grain of the "Scarlet" variety was used to obtain eggs of the grain moth (Sitotroga). The temperature and relative humidity in the rooms were maintained at standard levels (24–25°C and 80–85%).

The experiments were initiated by investigating the presence of harmful microorganisms in barley, which was initially

exposed to the laboratory air environment in order to assess potential grain spoilage. For this purpose, a direct experimental setup was established. The air quality of three laboratory rooms allocated for the study was analyzed, along with the contamination level of barley samples exposed to spoilage conditions. At the initial stage, Endo nutrient medium was prepared in the laboratory rooms for the detection of airborne pathogenic microorganisms. For the preparation of the Endo medium, 1 liter of distilled water was placed into a sterile container containing a pre-prepared powdered formulation and thoroughly mixed under heating conditions until boiling. After reaching the boiling point, the medium was removed from the heat source and cooled to 30–40°C. The cooled medium was subsequently dispensed in 25 g portions into 12 sterile Petri dishes with lids (four dishes per experimental room). The cooling process was carried out in a dedicated bacteriological laboratory chamber equipped with quartz ultraviolet lamps under sterile conditions. The prepared Endo agar plates were then placed at four corner points of each experimental room within an open exposure chamber. The exposure was initiated at 08:30 on 09.02.2021 and maintained for 60 minutes under controlled laboratory conditions.

The experiments were initiated by investigating harmful microorganisms associated with barley grains that had been previously exposed to the laboratory air environment to evaluate potential spoilage. For this purpose, a direct experimental procedure was established. The air quality of three laboratory rooms allocated for the study was analyzed, along with the quantification of harmful microorganisms associated with barley samples subjected to spoilage conditions. At the initial stage, Endo nutrient medium was prepared in the laboratory rooms for the detection of airborne pathogenic microorganisms. For the preparation of the Endo medium, 1 liter of distilled water was placed in a sterile container containing a pre-prepared powdered formulation and thoroughly mixed under heating conditions until boiling. After reaching the boiling point, the medium was removed from the heat source and cooled to 30–40°C. The cooled medium was then dispensed in 25 g portions into 12 sterile containers with lids (four containers per experimental room). The cooling process was carried out in a dedicated chamber of the bacteriological laboratory equipped with quartz ultraviolet lamps under controlled conditions. The prepared Endo culture medium was subsequently placed in the four corners of each experimental room within an open exposure chamber with lids. Exposure was initiated at 08:30 on 09.02.2021 and maintained for 60 minutes under laboratory conditions.

After that, the lids of all samples in each experimental room were closed, and the containers were arranged according to the respective experimental variants. On the same day, the samples were transported to the “Bacteriology” laboratory affiliated with the Namangan Regional State Center for Animal Disease Vector and Food Safety. In the laboratory, microorganisms that had developed in the nutrient medium within the samples were

incubated in a thermostat for 24 hours at 37°C to ensure growth and proliferation.

Following incubation, the grown microorganisms were subjected to Gram staining in order to determine their taxonomic classification, and they were subsequently examined under a light microscope for morphological characterization.

The research results showed that the air of Laboratory Room 1, selected as the control, contained up to 25% Gram-positive cocci and 7% Gram-negative cocci. The air of Laboratory Room 2, used for experimental treatment, contained up to 24% Gram-positive cocci and 6% Gram-negative cocci, while the air of Laboratory Room 3 contained up to 25% Gram-positive cocci and 7% Gram-negative cocci.

Microorganisms present in the air were assumed to be transferred to barley grains placed in the laboratory for spoilage studies. To determine the level of microbial contamination, on 11.02.2021, 100 g samples of barley grains subjected to spoilage conditions in the three experimental laboratory rooms were collected, properly labeled, and transported directly to the "Bacteriology" laboratory. The collected samples were initially immersed in 200 mL of distilled water and incubated for 24 hours under laboratory conditions. Subsequently, using a Pasteur pipette, 5 mL of the resulting extract was taken from each sample and inoculated onto Endo nutrient medium under aseptic conditions in a laminar box using an спиртовая (alcohol) lamp in the bacteriological laboratory. The inoculated Endo medium was incubated in a TS-80 thermostat at 37°C for 24 hours. After incubation, Gram staining was performed to determine the taxonomic affiliation of the developed microorganisms. The grown colonies were then examined under a light microscope and morphologically described. It was observed that, on Endo medium in Petri dishes, colonies exhibited a pinkish coloration with air-like characteristics and consisted of numerous small, spherical, moss-like structures with smooth, round edges.

After incubation, microorganisms that had developed in the nutrient medium within the thermostat were subjected to Gram staining in order to determine their taxonomic classification, and were subsequently examined under a light microscope.

The results of the study showed that barley grain samples selected as the control in Laboratory Room 1 contained 26% Gram-positive cocci and 8% Gram-negative cocci. Barley grain samples from Laboratory Room 2, used for the experimental treatment, contained up to 25% Gram-positive cocci and 7% Gram-negative cocci, while barley grain samples from Laboratory Room 3 contained up to 26% Gram-positive cocci and 7% Gram-negative cocci. Based on the obtained results, it can be concluded that the level of microorganisms in barley grain prepared for spoilage across all experimental variants and laboratory rooms was practically similar.

The study was further continued to investigate the effect of electrochemically activated foamed water with an acidic medium on the existing microorganisms, following the detection of harmful microorganisms in the

laboratory air and in barley grains subjected to spoilage prior to the experimental production process.

The pH values of water fractions obtained through electrochemical activation in both acidic and alkaline media were determined using universal indicator paper.

The hardness, as well as the chloride and sulfate contents of water samples obtained for both control and experimental variants, were analyzed in accordance with the State Standard "Hygienic Requirements for Drinking Water."

According to the analytical results, the pH value of the tap water intended for use in Variant 1 (control) was 7–8. The total hardness was 5.8 mg/dm³, the chloride ion (Cl^-) concentration was 53.2 mg/dm³, and the sulfate ion (SO_4^{2-}) concentration was 158.5 mg/dm³.

The hydrogen ion index (pH) of electrochemically activated water in an acidic medium, intended for use in Variant 2 (experimental), was found to be pH 3–3.5, with a total hardness of 4.3 mg/dm³, chloride ion (Cl^-) content of 46.4 mg/dm³, and sulfate ion (SO_4^{2-}) content of 132.96 mg/dm³.

It was established that the electrochemically activated water planned for use in Variant 3 had a hydrogen ion index of pH 4–4.5, a total hardness of 3.6 mg/dm³, chloride ion (Cl^-) content of 42.3 mg/dm³, and sulfate ion (SO_4^{2-}) content of 130.9 mg/dm³. Variant 1, selected as the control based on the method applied in Experiment 1, involved laboratory air ventilation using a standard ventilation system and normalization of grain moisture using ordinary tap water. In Variant 2, electrochemically activated water with a strongly acidic medium (pH 3–3.5) was used for air decontamination in the laboratory room and for normalization of grain moisture. In Variant 3, electrochemically activated water with a weakly acidic medium (pH 4–4.5) was applied for laboratory air decontamination and for normalization of the moisture content of the treated grain. The treatment was carried out in two stages. In the first stage, air treatment of the laboratory room was performed by spraying electrochemically activated water for the purpose of protecting barley grain, without direct contact with the grain, for 10–12 days from the date of infestation. In this case, in a laboratory room with a volume of 72 m³, 720 mL of electrochemically activated water was used per application, based on a dosage of 10 mL per 1 m³. The total consumption per production cycle ranged from 3600 to 4320 mL.

In the second stage, treatment was carried out through moisture normalization by soaking barley grains every 4–5 days after larval hatching from deposited eggs used for infestation. This was performed using 300 mL portions of electrochemically activated water with an acidic medium per cuvette.

The results of the first stage of the experiment showed that the air of Laboratory Room 1, used as the control, contained 25% Gram-positive cocci and 6% Gram-negative cocci. The air of Laboratory Room 2, used for the experimental treatment, contained 14% Gram-positive cocci and 4% Gram-negative cocci, while the air of Laboratory Room 3 contained 16% Gram-positive cocci and 4%

Gram-negative cocci. These results indicate that Laboratory Room 2, treated with electrochemically activated water with a strongly acidic medium (pH 3–3.5), was more effectively decontaminated from harmful microorganisms compared to the other variants.

In order to control the existing microorganisms and simultaneously normalize grain moisture, barley grains used in the second stage of the experiment were treated with the acidic fraction of electrochemically activated water at a dosage of 300 mL per cuvette in the control variant, as well as in the experimental variants, starting from the onset of grain spoilage.

Variant 1 was selected as the control, in which ordinary tap water was used for disinfection of the laboratory air and for normalization of grain moisture. In Variant 2, electrochemically activated water with a strongly acidic medium (pH 3–3.5) was used for disinfection of grain and moisture regulation in the laboratory environment. In Variant 3, electrochemically activated water with a weakly acidic medium (pH 4–4.5) was applied for grain disinfection and moisture normalization. The treatment was carried out at a rate of 300 mL per cuvette, based on moisture monitoring of the grain after larval hatching from eggs used for infestation, at intervals of once every 4–5 days, covering the period from pupation to the emergence of adult moths.

Immediately after the emergence of primary adult moths from the infested barley, 100 g samples were collected from each experimental room to determine the level of associated harmful microorganisms. The samples were subsequently analyzed in a bacteriological laboratory.

The results of the analysis showed that barley grains from Laboratory Room 1 (control) contained up to 26% Gram-positive cocci and 8% Gram-negative cocci. Barley grains from Laboratory Room 2 (experimental treatment) contained up to 16% Gram-positive cocci and 4% Gram-negative cocci, while grains from Laboratory Room 3 contained up to 20% Gram-positive cocci and 5% Gram-negative cocci. These findings indicate that barley used for infestation in Laboratory Room 2, treated with electrochemically activated water with a strongly acidic medium (pH 3–3.5), was more effectively decontaminated from harmful microorganisms compared to the other experimental variants.

In the next stage of the experiment, 500 grains were sampled from each cuvette and subjected to mechanical crushing using a mortar and pestle, and the grain damage coefficient was determined. The level of grain damage was 68% in the control variant, 82% in Variant 2, and 76% in Variant 3.

On 05.03.2021, the air in the laboratory room was disinfected by spraying electrochemically activated water with a strongly acidic medium, which was also used for grain moisture normalization. In Experimental Variant 2, the initial appearance of moth infestation was observed. In contrast, in the third and control variants, similar developmental stages were observed on 06.03.2021 and 08.03.2021, respectively. Damaged barley grains

in each variant were packaged two days after the emergence of the first adult moths.

The process of collecting adult moths and obtaining eggs from barley grains stored in containers was carried out using a standard method under controlled humidity conditions. The results showed that 845 g of Sitotroga eggs were obtained from barley in Variant 1 (control), 920 g from Variant 2, and 902 g from Variant 3. Compared with the control, these results demonstrate that an additional 75 g of Sitotroga eggs were obtained per 130 kg of barley in Variant 2, and 57 g more in Variant 3 (Table 7).

Table 7

Economic efficiency of electrochemical treatment against harmful microorganisms in barley grains damaged during rearing of the grain moth

№	Experimental variants	Grain consumption (kg)	Sitotroga consumption (g)	Grain infestation date (month, year)	Date of first moth emergence (month, year)	Grain infestation rate (%)	Packaging date (month, year)	Obtained Sitotroga eggs (g)	Product value (sum)	Difference (+/– sum)
1	Tap water used for air ventilation and grain moisture normalization (conventional method)	130	130	09.02.21	08.03.21	68	10.03.21	845	1,267,500	–
2	Air disinfection and grain moisture normalization using electrochemically activated water with acidic medium (pH 3–3.5)	130	130	09.02.21	05.03.21	82	07.03.21	920	1,380,000	+112,500
3	Air disinfection using electrochemically activated water with weak acidic medium (pH 4–4.5) and grain moisture normalization	130	130	09.02.21	06.03.21	76	08.03.21	902	1,353,000	+85,500

Based on the data presented in the table above, the most effective variant is the disinfection of room air through spraying electrochemically activated water with a strongly acidic medium (pH 3–3.5), combined with grain moisture normalization. In this variant, the barley infestation rate reached 82%. In addition, the initial emergence of moths

occurred three days earlier compared to the other variants, which contributed to a reduction in the production cycle and saved approximately one working day.

Based on the results of the study, the following conclusions were drawn:

1. When barley is soaked using a portion of electrochemically activated water with an alkaline medium (pH 10–10.5), on average more than 53 g of Sitotroga eggs are obtained per 104 kg of grain compared to the conventional method, and production efficiency increases by 8–10%.

2. During the reproduction process of the grain moth (Sitotroga) from barley, the optimal application of electrochemically activated water is 250–300 mL per 10 kg of grain under controlled conditions of 24–25°C and 80–85% relative humidity, ensuring that grain moisture does not exceed 16–18%.

3. The use of electrochemically activated water with an acidic medium (pH 3–3.5) for decontamination of laboratory rooms from harmful microorganisms is effective because it allows direct application within the production process, unlike conventional chemical treatments.

4. In the rearing of Sitotroga moths from barley grains, the anodic (strongly acidic) fraction of electrochemically activated water can be applied 10–12 days after grain infestation, up to the period of first adult moth emergence.

5. The optimal single application of electrochemically activated water for barley (approximately 300 mL per 13 kg of grain) ensures both microbial reduction of 40–50% and maintenance of moderate grain moisture (around 16%).

6. Treatment of both laboratory air and barley grains with electrochemically activated water with a strongly acidic medium (pH 3–3.5) during Sitotroga rearing increases production efficiency by 12–15% and reduces working time by approximately one day.

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