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ANTI-INFLAMMATORY ACTIVITY OF ENDOPHYTIC FUNGI ASPERGILLUS SYDOWII STRAIN AV5L AND PENICILLIUM VERRUCOSUM STRAIN AV6L ISOLATED FROM ALOE VERA

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Abstract. *The aim of this study was to evaluate the anti-inflammatory activity of ethyl acetate extracts of the endophytic fungi Aspergillus sydowii strain AV5L and Penicillium verrucosum AV6L. Anti-inflammatory properties were studied in vitro in a models of inhibition of egg and bovine serum albumin denaturation, as well as in a model of erythrocyte membrane stabilization. The studied extracts effectively demonstrated inhibition of protein denaturation. In the system with ovalbumin, the highest activity was demonstrated by the extract of P. verrucosum AV6L, providing 65% inhibition at a concentration of 500 µg/ml (IC_{50} = 179.8 µg/ml), while for ascorbic acid this figure was 72% (IC_{50} = 215.9 µg/ml). In the system with bovine serum albumin, the most pronounced activity among the studied extracts was demonstrated by A. sydowii strain AV5L — 71% inhibition at 500 µg/ml (IC_{50} = 282.1 µg/ml), while ascorbic acid provided 78% inhibition (IC_{50} = 267.9 µg/ml). In a model of red blood cells membrane stabilization, extracts at concentrations of 200–800 µg/ml were found to inhibit thermally induced hemolysis in a concentration-dependent manner. The maximum inhibition for P. verrucosum AV6L was 52.40 ± 0.40% at a concentration of 600 µg/ml (IC_{50} = 360.80 µg/ml). Acetylsalicylic acid, used as a standard anti-inflammatory drug, provided 62.92 ± 0.75% inhibition at the same concentration (IC_{50} = 388.48 µg/ml). The obtained results indicate a pronounced anti-inflammatory activity of the studied endophytic fungi and confirm the promise of further study of their bioactive metabolites as potential sources of anti-inflammatory compounds.*

Keywords: *endophytic fungi, anti-inflammatory activity, inhibition of protein denaturation, stabilization of red blood cells membranes, biologically active metabolites.*

Introduction

Inflammation is a complex protective response of the body to infectious agents, toxic substances, and tissue damage. Despite the physiological role of inflammation, its chronic course is a key factor in the development of many pathological conditions, including gastrointestinal

diseases, autoimmune disorders, and metabolic disorders [1]. In recent years, inflammation induced by microbial enzymes, among which urease plays a significant role, has attracted particular interest. Urease is a nickel-dependent enzyme that catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. This enzyme is widespread among pathogenic microorganisms and plays a key role in their virulence. Specifically, *Helicobacter pylori* urease ensures the microorganism's survival in the acidic environment of the stomach by locally increasing pH, which promotes colonization of the mucosa and the development of inflammatory gastric diseases [2, 3].

Synthetic urease inhibitors, such as acetohydroxamic acid, are currently known, however, their use is limited due to significant side effects and toxicity. Similarly, the use of synthetic anti-inflammatory drugs is often accompanied by the development of side effects, sparking interest in natural sources of biologically active compounds [4,5].

Endophytic microorganisms that asymptomatically colonize the internal tissues of plants are considered as a promising source of biologically active secondary metabolites. According to a review by Strobel and Daisy (2003) [6], endophytes are capable of synthesizing a wide range of compounds with antimicrobial, antioxidant, and anti-inflammatory activity. Later, Kusari et al. (2012) [7] showed that many endophytic fungi produce metabolites identical or structurally similar to host plant compounds, emphasizing their importance for pharmacological and biotechnological research.

Among the metabolites of endophytic origin, phenolic compounds, flavonoids, terpenoids, alkaloids and peptides are of particular importance, characterized by the ability to inhibit enzymes, suppress inflammatory mediators and stabilize cell membranes by reducing the intensity of lipid peroxidation. We have previously established that ethyl acetate extracts of the endophytic fungi *Aspergillus sydowii strain* AV5L and *Penicillium verrucosum* AV6L possess pronounced urease-inhibiting activity [8]. Qualitative phytochemical analysis revealed the presence of flavonoids, terpenoids, tannins, cardiac glycosides, and peptides, which justifies the need for further study of their biological activity.

Taking into consideration the established phytochemical composition of the extracts and their pronounced ability to inhibit urease, the present study investigates the anti-inflammatory activity of secondary metabolites of the mentioned endophytes using *in vitro* models of protein denaturation inhibition and cell membrane stabilization. These methods are widely used for the initial assessment of the anti-inflammatory potential of natural compounds [9].

Materials and Methods

To evaluate the anti-inflammatory activity of the extracts, egg albumin (EA) and bovine serum albumin (BSA) were used, as well as a membrane stabilization test to confirm the universality and reproducibility of the inhibitory effects of the extracts on various proteins. The method was implemented according to Mizushima Y Kobayashi M (1968) with modifications proposed by C. Sree Kumari et al. (2015) [10,11]. All three experiments were performed in triplicate.

Inhibition of denaturation of ovalbumin. A 4 ml reaction mixture consisted of 0.2 ml of ovalbumin, 2.8 ml of phosphate buffer (pH 6.4), and 1 ml of extracts of varying concentrations. A similar volume of distilled water served as a control. The mixture was incubated at 37°C for 15 min and then heated at 65°C for 5 min. After the samples were cooled, the absorbance at 660 nm was measured spectrophotometrically. Acetylsalicylic acid served as a standard, from which a control was prepared at a concentration similar to the experimental extract. Protein denaturation inhibition (X) by the extract and standard was expressed as a percentage and calculated using the

formula: $X = \frac{A0 - At}{A0} \times 100$, where A_0 is the optical density of the control; A_t is the optical density of the test sample.

Inhibition of bovine albumin denaturation. The reaction mixture (4 ml) consisted of 2 ml of bovine serum albumin (1% aqueous solution), 0.4 ml of fungi extract of varying concentrations and 1.6 ml of phosphate-buffered saline (pH 6.4). Samples were incubated at 37°C for 30 minutes and then heated at 65^0C for 5min. After cooling absorbance was measured at 660 nm. 0.05 ml of distilled water was used instead of the extract as a control. The protein inhibition denaturation (X) by the extract and standard was expressed as a percentage and calculated using the formula: $X = \frac{A0 - At}{A0} \times 100$, where A_0 is the optical density of the control; A_t is the optical density of the test sample.

Membrane stabilizing activity. Preparation of Red Blood cells suspension. Fresh whole human blood (10 ml) was collected and transferred to tubes to centrifuge. The tubes were centrifuged at 3000 rpm for 10 minutes and washed three times with an equal volume of a normal saline. The blood volume was measured, and a 10% (v/v) suspension in 0,9 % sodium chloride solution was prepared.

Heat-induced hemolysis. The reaction mixture (2 ml) consisted of 1 ml of the test sample and 1 ml of a 10% RBCs suspension. For the control, the drug was replaced with a saline. Acetylsalicylic acid was used as a standard. The tubes were incubated in a water bath at 56°C for 30 minutes and then cooled under running water. The mixture was then centrifuged at 2500 rpm for 5 minutes, and the optical density of the supernatant was measured at 560 nm. The protein inhibition denaturation (X) by the extract and standard was expressed as a percentage and calculated using the formula: $X = \frac{A0 - At}{A0} \times 100$, where A_0 is the optical density of the control; A_t is the optical density of the test sample.

Results and Discussion

This study examined the anti-inflammatory activity of secondary metabolites of the endophytic fungi *A. sydowii strain* AV5L and *P. verrucosum* AV6L. Biological activity was assessed using in vitro models based on protein denaturation inhibition and membrane-stabilizing effects. These methods were chosen due to their ability to reflect key aspects of the inflammatory process, including damage to protein structures and disruption of cell membrane integrity.

Table 1.

Anti-inflammatory activity of Aspergillus sydowii strain AV5L and Penicillium verrucosum AV6L determined by inhibition of ovalbumin denaturation (n = 3).

№	Concentration µg/ml	Acetylsalicylic acid %	*A. sydowii strain* AV5L, %	*P.verrucosum* AV 6L %
1	100	37±0,33	43±0,45	33±0,23
2	200	40±0,41	45±0,65	45±0,45
3	300	58±0,68	50±0,33	49±0,23
4	400	50±1,33	30±1,44	51±0,68
5	500	72±0,45	42±0,35	65±0,40

Denaturation inhibition studies were conducted using egg albumin and bovine serum albumin models. Protein stabilization under these conditions is considered an indicator of anti-

inflammatory activity, as it prevents the formation of denatured forms that can induce an immune response and increase inflammation. The results showed that the extracts of the studied endophytic fungi effectively inhibit protein denaturation. The highest activity was demonstrated by *P. verrucosum* AV6L: at a concentration of 500 µg/ml, the inhibition rate of egg albumin denaturation was 65%, which is close to the activity of acetylsalicylic acid (72%) (Table 1).

Similar results were obtained in a study of the inhibition of bovine serum albumin denaturation, further confirming the anti-inflammatory properties of the secondary metabolites of the endophytic fungi studied. In this model, the standard anti-inflammatory drug acetylsalicylic acid demonstrated the highest activity among the control samples, with a denaturation inhibition rate of 78% at a concentration of 500 µg/ml. Among the studied fungi extracts, the most pronounced effect was observed with *A. sydowii strain* AV5L extract, which, at the same concentration, provided 71% inhibition of protein denaturation, close to the activity of the standard preparation. *P. verrucosum* AV6L extract also demonstrated significant anti-inflammatory activity, achieving 67% inhibition at a concentration of 500 µg/ml (Table 2). The obtained results indicate a pronounced ability of secondary metabolites of the studied endophytic fungi to suppress protein denaturation, which may indicate their potential as natural anti-inflammatory agents.

Table 2.

Anti-inflammatory activity of A. sydowii strain AV5L and P. verrucosum AV6L determined by inhibition of bovine serum albumin denaturation (n = 3).

№	Concentration µg/ml	Acetylsalicylic acid %	*A. sydowii strain* AV5L, %	*P. verrucosum* AV6L, %
1	100	25±0,60	23±0,45	31±1,05
2	200	20±0,55	29±0,32	34±0,45
3	300	45±0,33	44±0,60	40±0,60
4	400	50±0,45	49±0,55	54±0,33
5	500	78±0,60	71±0,32	67±0,32

The anti-inflammatory activity of the samples was assessed using IC_{50} values, which reflect the concentration required to achieve 50% inhibition of protein denaturation. Higher IC_{50} values indicate decreased anti-inflammatory activity.

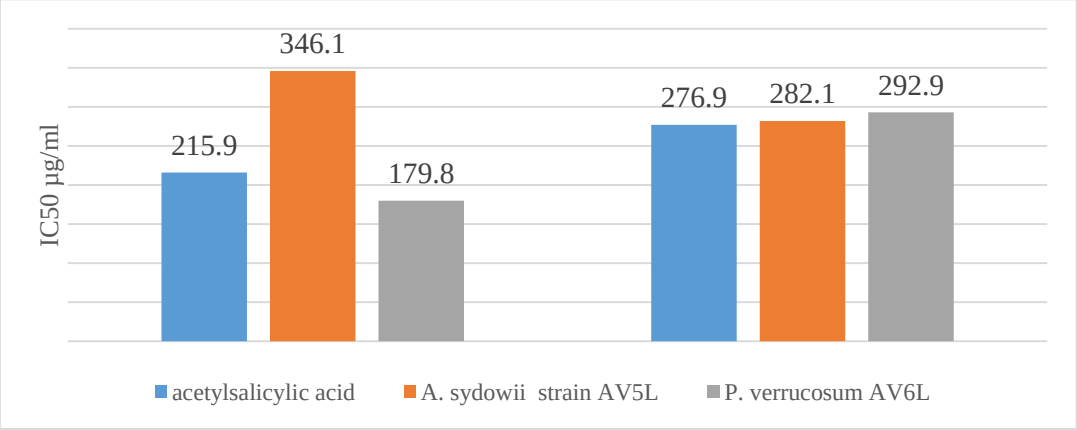


Figure 1. IC_{50} of ethyl acetate extracts of endophytic fungi A. sydowii strain AV5L and P. verrucosum AV6L for inhibition of denaturation of egg albumin (1) and bovine serum albumin (2).

Figure 1 shows the IC_{50} values of the ethyl acetate extracts of *A. sydowii strain* AV5L and *P. verrucosum* AV6L in the denaturation inhibition test of egg and bovine albumin. In the system with egg albumin, the lowest IC_{50} value was demonstrated by the extract of *P. verrucosum* AV6L (179.8 μg/ml), indicating a more pronounced anti-inflammatory activity compared to *A. sydowii strain* AV5L (346.1 μg/ml) and acetylsalicylic acid (215.9 μg/ml).

In the bovine albumin model, the IC_{50} values were 267.9 μg/mL for acetylsalicylic acid, 282.1 μg/mL for *A. sydowii* AV5L, and 292.9 μg/mL for *P. verrucosum* AV6L, demonstrating activity comparable to, though less pronounced than, that of the standard drug.

The obtained results are consistent with published data indicating the pronounced anti-inflammatory activity of secondary metabolites of endophytic fungi of the genus *Penicillium*. It has previously been shown that extracts of *Penicillium sp*. can effectively inhibit protein denaturation, stabilize biological membranes, and suppress the production of anti-inflammatory mediators, demonstrating activity comparable to standard nonsteroidal anti-inflammatory drugs (Govindappa et al., 2011; Kharwar et al., 2019; Zhang et al., 2020) [12-14].

Similar data on moderate anti-inflammatory activity were obtained for representatives of the genus Aspergillus, the metabolites of which are capable of inhibiting thermal denaturation of albumin and hemolysis of erythrocytes in a concentration-dependent manner (Mishra et al., 2017; El-Sayed et al., 2021) [15,16] (Table 1).

Overall, the obtained results indicate that both endophytic fungi, *A. sydowii strain* AV5L *and P. verrucosum* AV6L, have the ability to inhibit protein denaturation, confirming their anti-inflammatory properties. Moreover, *P. verrucosum* AV6L extract demonstrated higher efficacy in the ovalbumin denaturation inhibition model, while *A. sydowii strain* AV5L extract demonstrated slightly more pronounced activity in the bovine serum albumin system. The observed differences may be due to the peculiarities of the spatial structure of the substrate proteins used, as well as differences in the mechanisms of interaction of bioactive compounds of fungi extracts with protein molecules.

Table 3.

Anti-inflammatory activity of Aspergillus sydowii strain AV5L and Penicillium verrucosum AV6L in stabilizing erythrocyte membranes (inhibition of thermally induced hemolysis, %).

№	Concentration μg/ml	Acetylsalicylic acid %	*A. sydowii strain* AV5 L%	*P.verrucosum* AV6L %
1.	100	44,21 ± 1,10	15,64 ± 0,40	20,29 ± 1,33
2	200	54,57 ± 0,91	20,30 ± 0,38	22,79 ± 0,67
3	400	63,45 ± 0,64	35,50 ± 0,60	48,33 ± 1,63
4	600	72,56 ± 0,58	47,90 ± 1,20	52,40 ± 0,82
5	800	62,92 ± 0,75	46,90 ± 0,45	50,44 ± 0,40
	IC_{50} (μg/ml)	388.48	396.24	360.80

To further clarify the mechanism of anti-inflammatory action of the ethyl acetate extracts of *A.sydowii strain* AV5L and *P.verrucosum* AV6L, their effect on erythrocyte (RBC) membrane stabilization was examined. This method is widely used to evaluate the anti-inflammatory activity of natural compounds, as the ability to prevent thermally induced hemolysis correlates with membrane stabilization and potential anti-inflammatory activity.

The studied extracts at concentrations of 200–800 μg/ml were found to inhibit thermally induced erythrocyte hemolysis in a concentration-dependent manner (Table 3). The maximum inhibition level for the *P. verrucosum* AV6L strain was 52.40 ± 0.40% at a concentration of 600 μg/ml, while the IC_{50} value was 360.80 μg/ml. Acetylsalicylic acid, used as a standard anti-inflammatory drug, demonstrated a higher inhibition level of 62.92 ± 0.75% at the same concentration (600 μg/ml), while the IC_{50} value was 388.48 μg/ml.

The obtained results indicate a pronounced membrane-stabilizing activity of the studied extracts, which indicates their potential anti-inflammatory effect comparable to acetylsalicylic acid at maximum concentration. The observed activity may be due to the presence of flavonoids, tannins, and terpene compounds in the extracts, detected during phytochemical analysis (Vakhabova N., et al., 2025) [17]. Flavonoids are natural polyphenolic compounds with pronounced antioxidant and anti-inflammatory properties. They can reduce the formation of proinflammatory mediators, suppress the production of nitric oxide and cytokines, and inhibit the activity of enzymes involved in the development of the inflammatory process. Due to their pronounced antioxidant activity, flavonoids reduce the intensity of lipid peroxidation and protect cellular structures from oxidative and inflammatory damage. Tannins, through their ability to form complexes with proteins and inhibit lipid peroxidation, help stabilize protein structures and cell membranes. Terpenoids, in turn, are known as inflammatory response modulators, capable of suppressing the synthesis of inflammatory mediators and reducing oxidative stress. Thus, the established membrane-stabilizing activity of the studied extracts may be associated with the complex action of polyphenolic and terpene metabolites, which provide protection of proteins and biological membranes under conditions of inflammatory damage. Thus, the established membrane-stabilizing activity of the studied extracts may be associated with the complex action of polyphenolic and terpene metabolites, which provide protection of proteins and biological membranes under conditions of inflammatory damage. The obtained results confirm the potential of endophytic fungi as a source of multifunctional natural compounds with anti-inflammatory potential and substantiate the need for further isolation, structural identification, and study of the mechanisms of action of individual active components.

Conclusion. This study demonstrated for the first time that ethyl acetate extracts of the endophytic fungi *A. sydowii strain* AV5L and *P. verrucosum* AV6L, isolated from the medicinal plant Aloe vera, exhibit pronounced anti-inflammatory activity in in vitro models of protein denaturation inhibition and membrane stabilization. *P. verrucosum* AV6L extract was shown to exhibit higher activity in the egg albumin model, while *A. sydowii strain* AV5L demonstrated a comparable effect in the bovine serum albumin system. The scientific novelty of this study lies in the identification and comparative evaluation of the anti-inflammatory potential of these endophytic strains, previously characterized as producers of biologically active metabolites with urease-inhibiting activity. For the first time, it has been demonstrated that these strains are capable of exhibiting a membrane-stabilizing effect comparable to a standard anti-inflammatory drug, expanding our understanding of the spectrum of their biological activity. The established activity is probably due to the complex action of polyphenolic and terpene compounds identified during phytochemical analysis, which provide protection of proteins and biological membranes under conditions of inflammatory damage. The obtained results confirm the potential of endophytic fungi as a source of multifunctional natural compounds with anti-inflammatory potential and substantiate the need for further isolation, structural identification, and study of the mechanisms of action of individual active components.

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PROBIOTIC MICROORGANISMS: METABOLITES, MECHANISMS OF ACTION, AND APPLICATIONS

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Abstract. *In this study, the amounts of organic acids produced in the culture liquids of various lactic acid bacteria strains belonging to the genera Lactobacillus and Pediococcus were determined. According to the results obtained, the Lactobacillus plantarum 3G strain was distinguished by higher levels of acetic (0.44 mg/mL) and succinic (0.32 mg/mL) acids, while Lactobacillus fermentum LF1 demonstrated the highest production of lactic acid (22.79 mg/mL). In Lactobacillus brevis 4G, Pediococcus acidilactici 1G, and Pediococcus acidilactici 2G strains, the concentrations of succinic, acetic, and lactic acids were 0.18, 0.28, and 11.37 mg/mL, respectively. In addition, the L. plantarum 3G strain exhibited a superior level of 3-phenyllactic acid production (1.85 mg/mL) compared to the other strains. The findings indicate that L. plantarum 3G and L. fermentum LF1 strains are promising probiotic microorganisms with a high capacity for organic acid production and pronounced antifungal properties and may be recommended for application in the pharmaceutical field.*

Keywords: *lactic acid bacteria, 3-phenyllactic acid, antifungal activity, antimicrobial activity.*

Introdcution

The primary probiotics are lactic acid-producing microorganisms, which are typical representatives of the normal human intestinal microflora [1].

Lactobacilli have been studied for their ability to produce acetic acid, lactic acid, reuterin, pyrrolidone-5-carboxylic acid, and 3-phenyllactate [2]. Lactic acid bacteria exhibit antagonistic activity against pathogenic and opportunistic microorganisms, as lactobacilli synthesize a very broad spectrum of antimicrobial compounds. Among these, lactic and acetic acids are of particular importance [3]. Another of their metabolites is hydrogen peroxide; in the presence of oxygen, lactic acid bacteria can produce through the action of NADH oxidase and superoxide dismutase. The antimicrobial property of hydrogen peroxide is associated with its strong oxidizing effect. By producing hydrogen peroxide, it increases oxidative stress and exerts an effect against pathogenic bacteria. This process targets the membranes of harmful microorganisms, leading to their destruction [4]. 3-phenyllactate is one of the metabolites of phenylalanine metabolism, produced from p-hydroxyphenyl-pyruvate in LAB cells. This end-metabolite demonstrates antibiotic activity against both Gram-positive and Gram-negative bacteria, and also affects a wide range of microscopic fungi [5]. 3-phenyllactate is an antimicrobial compound with a broad spectrum of activity against yeasts such as *Candida pulcherrima* and *Rhodotorula mucilaginosa*, as well as mycotoxigenic fungal species including *Aspergillus ochraceus*, *Penicillium verrucosum*,

and *Penicillium citrinum*. In the course of the research, *L. acidophilus*, *L. fermentum*, *L. brevis*, *L. plantarum*, *L. casei*, *L. delbrueckii*, and *L. jensenii* were isolated from the vaginas of a group of infected and healthy women. The synthesis of lactic acid, hydrogen peroxide, and diacetyl by these *Lactobacillus* strains was found to be 1.46 mg/l, 1.36 mmol/l, and 1.72 mg/l, respectively. In patients with vaginosis, the maximum levels were identified as 1.08 mg/l for lactic acid, 1.36 mmol/l for hydrogen peroxide, and 0.86 mg/l for diacetyl [6]. Quantitative analysis of lactic acid in the *L. fermentum* LF1 strain using the spectrophotometric method revealed a synthesis capacity of 40.91 g/l. It was confirmed that the ability to produce lactic acid depends on their genetic characteristics and cultivation conditions [7].

Lactic acid bacteria and their metabolites hold great significance in modern medicine as agents for prevention, treatment, and biotherapy. Their safety, natural origin, and broad biological activity make them a promising direction for the development of probiotic and postbiotic preparations.

Objectives: Modern microbiology aims to identify new types of probiotics, study their properties and antimicrobial metabolites, and explore the prospects for their practical implementation.

Materials and Methods. *Quantitative analysis of antimicrobial metabolites in the culture liquid was performed using High-Performance Liquid Chromatography (HPLC).*

Quantitative analysis of volatile fatty acids in the culture liquids of the strains was performed using an Agilent Technologies-1200 chromatograph (USA). Uninoculated MRS (de Man, Rogosa, and Sharpe) broth was used as a control. 50 µl of reconstituted Lactobacillus cultures were inoculated into 5 ml of MRS broth and incubated at 37°C for 18 hours. Standard samples of organic acids were weighed and dissolved in 1 ml of distilled water in specialized test tubes for quantitative analysis. To prevent column contamination and a rapid decrease in efficiency caused by mechanical and undissolved particles, the solutions were filtered through a 0.2 µm pore size filter. The sample solutions were then placed into the chromatograph's autosampler and analyzed according to the developed method. Centrifugation was carried out at 8,000 rpm for 10 minutes. The injection volume for each analysis was 5 µl.

Identification of metabolites using the Chip-Q-TOF mass spectrometry method. Antimicrobial metabolites of lactic acid bacteria were identified using an Agilent 6520B Series Chip-Q-TOF mass spectrometer. The culture liquid was fractionated using an Agilent Technologies 1200 series chromatograph. The following mobile phases were applied: Phase A – 0.1% formic acid + 5% acetonitrile; Phase B – acetonitrile + 0.1% formic acid + 10% deionized water. The sample was introduced at a flow rate of 4 µl/min. The gradient concentration for Phase B was measured as follows: 0% at 3 minutes, 60% at 12-18 minutes, and 0% at 20 minutes. Samples were injected into the column at a volume of 2 µl using a Micro WPS device. The obtained results were analyzed via mass spectrometry, accounting for the ionization source, drying gas flow and temperature, fragmentor voltage, ionization method, and mass range.

Analysis of the Results. The quantitative composition of fatty acids, such as succinic, acetic, propionic, and lactic acids, as well as 3-phenyllactate, in the culture liquids of the five studied strains was determined using the HPLC method. The acetic acid content in the culture liquid of *L. plantarum* 3G was the highest at 0.44 mg/ml, while the succinic acid content was 0.32 mg/ml. The *L. fermentum* LF1 strain was the leader in lactic acid production, with its culture liquid containing 22.79 mg/ml of lactic acid. In the culture liquid of *L. brevis* 4G, the succinic acid content was 0.18 mg/ml; for *P. acidilactici* 1G, the acetic acid was 0.28 mg/ml; and for the *P.*

acidilactici 2G strain, a lactic acid content of 11.37 mg/ml was identified. The *L. plantarum* 3G strain recorded the highest level of 3-phenyllactate at 1.85 mg/ml (Table 1).

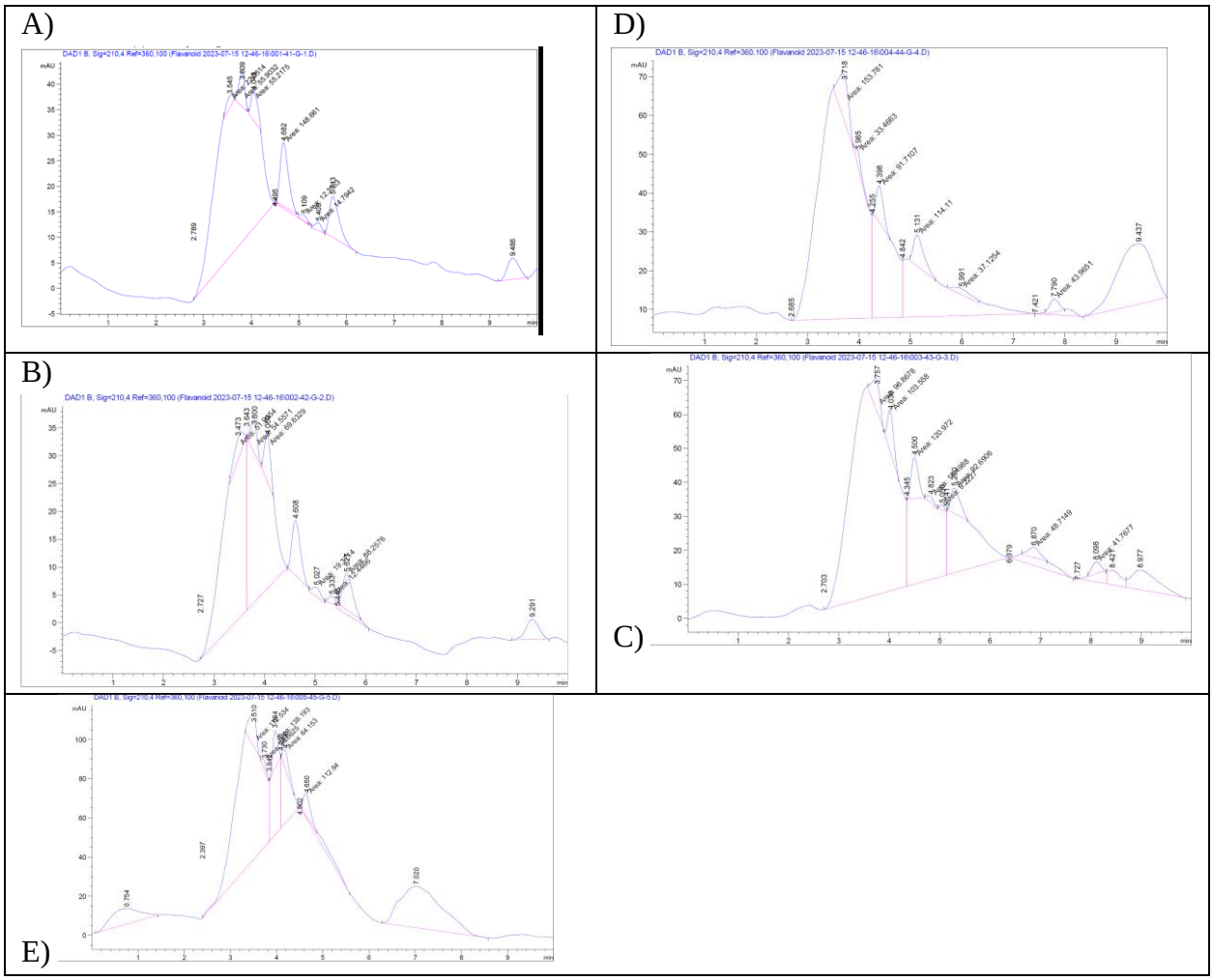


Figure 1. Quantitative analysis of antimicrobial metabolites in the culture liquid of LABs using the High-Performance Liquid Chromatography (HPLC) method.A) L.brevis 4G, B) L.fermentum L.F1, C) L.plantarum 3G, D) P.acidilactici 1G, E) P.acidilactici.

Table 1.

Content of organic acids in the culture liquid of the strains

Organic acids	*L.brevis 4G*	*L.fermentum L.F1*	*L.plantarum 3G*	*P.acidilactici 1G*	*P.acidilactici 2G*	Control MRS broth
	Concentration, mg/ml					
Succinic acid	0,18	0,23	0,32	0,11	0,10	0,31±0,05
Lactic acid	1,49	22,79	2,80	4,17	11,37	0,62±0,02
Acetic acid	0,005	0,04	0,44	0,28	0,03	0,34±0,03

Propionic acid	0,006	0,005	0,0070	0,002	0,006	0,72±0,09
3-phenyllactic acid	0.69	0.82	1.85	0.84	1,28	0.39±0,03

During the research, a qualitative analysis of antimicrobial metabolites, specifically succinic acid and 3-phenyllactate, found in the culture liquid of local LAB (Lactic Acid Bacteria) strains was performed using an Agilent 6520B Series Chip-Q-TOF mass spectrometer. In the analysis of metabolites from the *P. acidilactici* 1G strain, succinic acid was identified within the retention time range of 1.8–1.9 minutes. Given that the base peak in the spectrum was [m/z] = 118.11, it is highly probable that this represents the molecular ion ($M+H^+$) of succinic acid. During the fragmentation process (MS/MS), succinic acid dissociates into various fragments: m/z = 72, representing the fragment after the loss of a CO_2 group, and m/z = 102, a fragment characteristic of the carboxylic acid moiety (Figure 2).

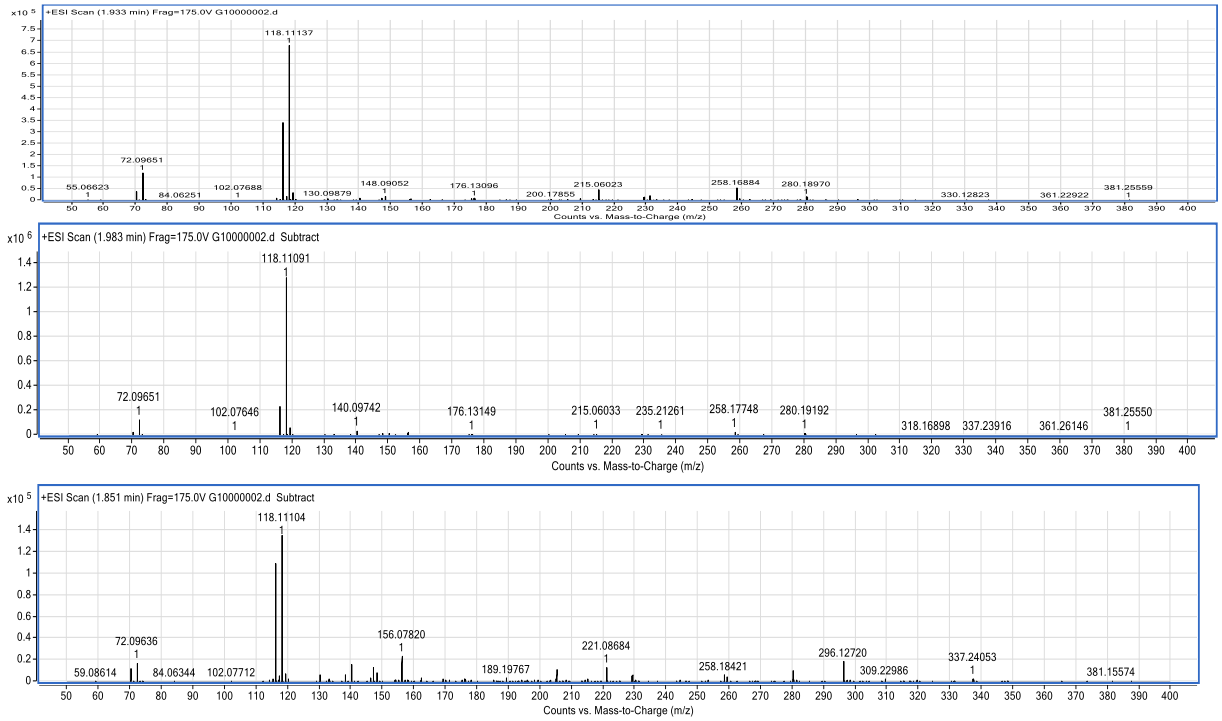
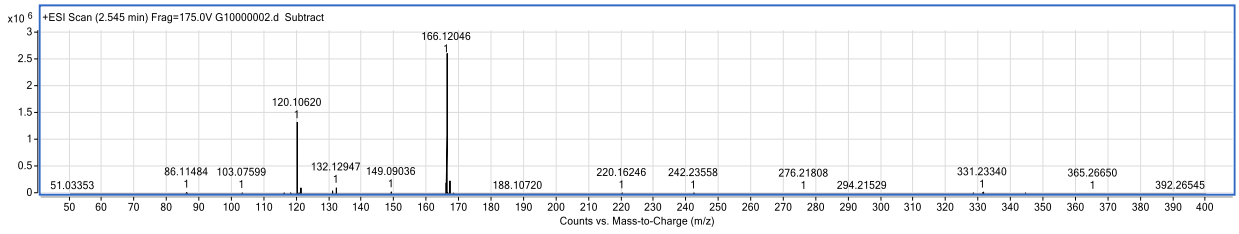


Figure 2. Qualitative analysis of succinic acid in the P. acidilactici 1G strain culture liquid using Chip-Q-TOF mass spectrometry.

Taking into account the presence of peaks at m/z 72.09651 and 102.07688, succinic acid was identified by comparison with the **HMDB (Human Metabolome Database)** online database (https://hmdb.ca/). In the process of identifying the **3-phenyllactate** metabolite of the *P. acidilactici* 2G strain, one of the main peaks detected between 2.5 and 2.8 minutes was found to be [m/z] = 166.12. Comparison with the HMDB database confirmed its consistency with 3-phenyllactate (Figure 3).



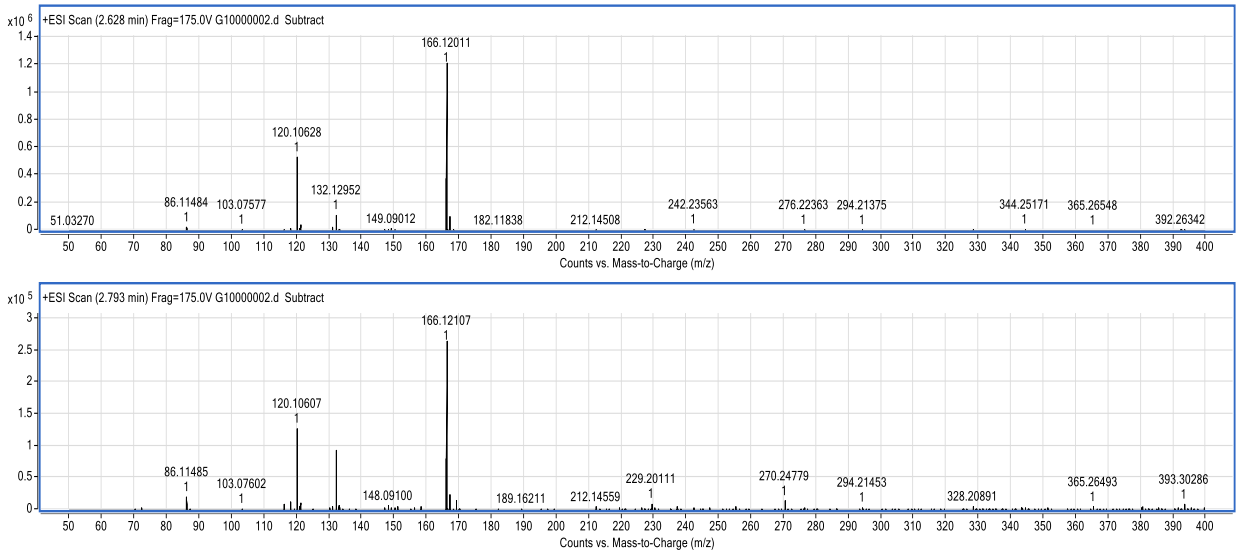
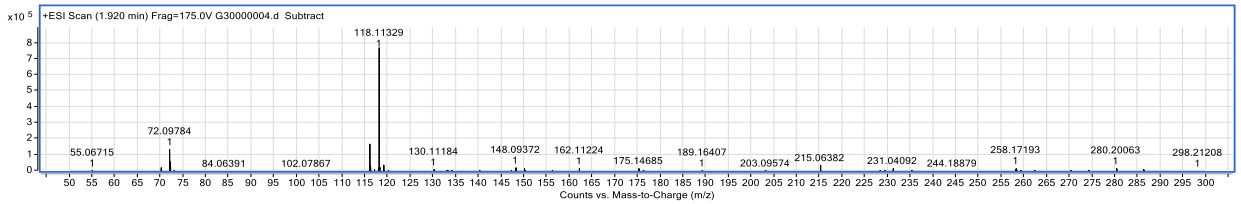


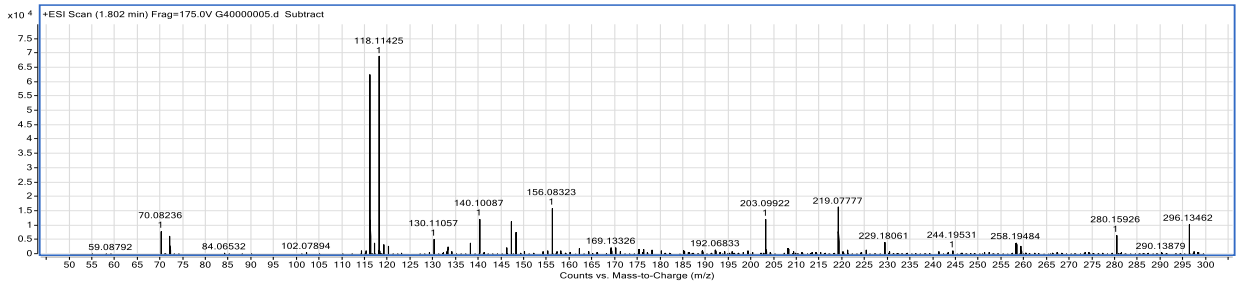
Figure 3. Chip-Q-TOF mass spectrometric analysis of 3-phenyllactate in the culture liquid of the P. acidilactici 2G strain.

Qualitative analysis of *L. plantarum* 3G, *L. brevis* 4G, and *L. fermentum* LF1 strains revealed a major spectral peak at [m/z] = 118.11 at a retention time of 1.9 minutes, similar to the *P. acidilactici* 1G strain. Based on the presence of mass fragments at m/z 72.09651 and 102.07688, the formation of succinic acid was confirmed. The results were verified by comparison with the **HMDB (Human Metabolome Database)** online database (https://hmdb.ca/) (Figure 4)

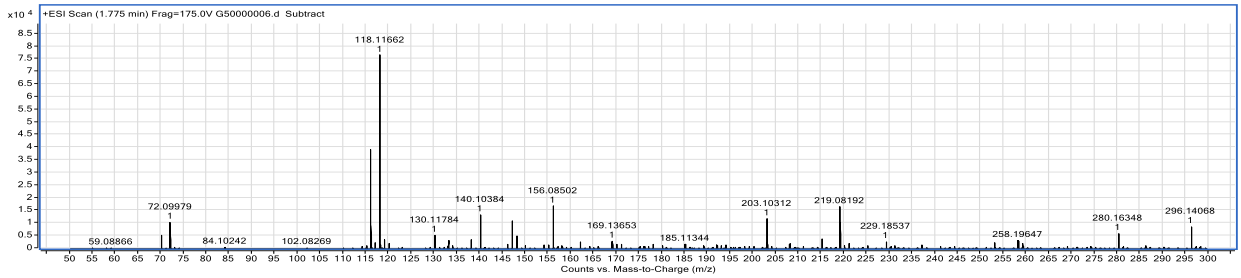
A)



B)



D)



4-pic. CHIP-Q-TOF mass spektrofotometr usulida A) L.plantarum 3G, B) L.brevis 4G, D) L fermentum LF1 shtammining qahrabo kislotasi sifat tahlili

Based on these findings, we concluded that all the studied strains produce high amounts of succinic acid, and the *P. acidilactici* 2G strain, in particular, contains 3-phenyllactic acid.

Conclusion.

As probiotics, these strains can be effective tools in maintaining the balance of human intestinal microbiota, eliminating dysbacteriosis, and restoring microflora after antibiotic use. Lactic and acetic acids create a natural barrier against pathogenic bacteria, specifically *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and fungi/yeasts such as *Candida albicans*, which is crucial in preventing infectious diseases. Furthermore, 3-phenyllactic acid exhibits potent antifungal and antioxidant effects, possessing the ability to reduce inflammatory processes, neutralize free radicals, and support cellular stress defense mechanisms. In this regard, these strains may also hold therapeutic significance in treating skin diseases (e.g., dermatitis, eczema), oral cavity infections, and maintaining women's urogenital health.

Additionally, the metabolites of *L. plantarum* 3G and *L. fermentum* LF1 exert immunomodulatory effects, activating the body's natural defense system, reducing allergic reactions, and enabling their use as a complex therapeutic agent in chronic inflammatory diseases (e.g., irritable bowel syndrome, metabolic syndrome). Consequently, the development of next-generation probiotic preparations, biotherapeutic agents, functional foods, and pharmaceutical dietary supplements based on these strains holds great practical importance in medicine, serving as a natural and safe alternative to antibiotics. The *L. plantarum* 3G and *L. fermentum* LF1 strains stood out as promising probiotic microorganisms producing high amounts of organic lactic and acetic acids. It was determined that their metabolites, especially 3-phenyllactic acid, along with the antimicrobial and antifungal properties of lactic and acetic acids, possess significant biotechnological value.

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FERNS OF THE KORATEPA RESERVOIR

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Abstract. *This study investigates the diversity, distribution, and ecological patterns of fern species in the Karatepa reservoir area of the Zarafshan Valley, Uzbekistan. Field research was conducted along the Amonkoton stream using vertical and horizontal transect methods during 2021–2022. A total of 490 fern individuals representing 7 genera, 6 families, and 13 species were identified and classified according to standard taxonomic systems. Biodiversity was assessed using ecological indices, including the Shannon–Wiener, Simpson, and Bray–Curtis similarity indices. Results reveal that fern diversity is strongly influenced by environmental factors such as altitude, humidity, and habitat structure. Species richness increases with elevation, particularly in humid, closed habitats, while dry and exposed areas support fewer species. Dominant species accounted for the majority of observed individuals. The findings highlight the sensitivity of ferns to ecological changes and emphasize their value as bioindicators in assessing environmental transformation under climate change and anthropogenic pressure in the region.*
Keywords: *fern diversity; transect method; biodiversity indices; Zarafshan Valley; altitude gradient; habitat conditions; ecological assessment.*

Introduction. Nowadays, as a result of climate change and increasing human influence on nature, the state of plants in the flora is changing. Zarafshan Valley is a region of special importance in terms of vegetation cover and diversity [2]. The study of plants of the Zarafshan Valley began several years ago, and a list of plants was formed [1,2]. Nowadays, as a result of increasing anthropogenic influences and climate change [10], the assessment of the current state of vegetation cover is becoming important. Plants belonging to the division Polyopodophyta appeared in ancient times [6]. Department representatives are very sensitive to environmental changes. The appearance or disappearance of these plants is closely related to the place of their growth. Amonkoton stream is located in the Urgut biogeographic region [4], and the main part of the stream is used by the population. For this purpose, a study was conducted in the area of the Amonko'ton creek to determine the current condition of the cuttlefish.

Materials and methods

Amonkoton stream begins in the southwestern part of the territory of Zarafshan valley, Urgut biogeographic region (Fig. 2) in the form of several small streams and flows into the Karatepa reservoir. Its starting point is 1860 m above sea level (39°18'12" N and 66° 53'57" N). The discharge point to the Karatepa reservoir is the lowest part of the stream and is located 930 m above sea level (39°24'34"N and 67°01'44"N). The total length of the stream is 24 km. Vegetation cover was analyzed using transect methods. Transects were placed vertically (vertically) and lengthwise (horizontally). A distance and a location were selected for placing 24 vertical (vertical) and longitudinal (horizontal) transects on the hillside (Fig. 1). 6 transects were randomly selected and set along the stream: steeply and longitudinally on the hillside (Table 1). The length of each horizontal transect is 100 m and the length of the vertical transect is 50 m. Based on the total length

of the transects, 20 1m x 1m mats were installed together with the transect. All samples were collected in April, May and June of 2021-2022. We used QGIS 3.30.0, Google Earth Pro software and data from https://www.diva-gis.org/gdata, https://mapcruzin.com databases to draw the research direction and map. Table 1. Location, altitude and orient of each transect.

Tr.	Location	Height above sea vel	Orient
I	39°24'20.02"N 67° 1'8.51"E	890-910 m	Latitudinal
II	39°24'12.68" N 67° 0'33.43" E	910-960 m	Altitudinal
III	39°22'40.87" N 66°59'41.26" E	970-1020 m	Altitudinal
IV	39°21'10.20" N 66°59'8.43" E	1060 m	Latitudinal
V	39°19'2.25" N 66°58'58.71" E	1200-1250 m	Altitudinal
VI	39°18'29.59" N 66°56'20.38" E	1385-1390	Latitudinal

Plots were 1 m × 1 m squared. All fern species in the sampling sites were investigated in spring and summer from March to August 2021 and 2022, periods when average monthly temperature and precipitation are mostly stable [7]. The nomenclature follows PPG I [8] and Flora Uzbekistan [9]. QGIS 3.30.0, Google Earth Pro programs, and data from the https://www.diva-gis.org/gdata, https://mapcruzin.com databases were used to draw the research direction and maps (Figure 1,2).

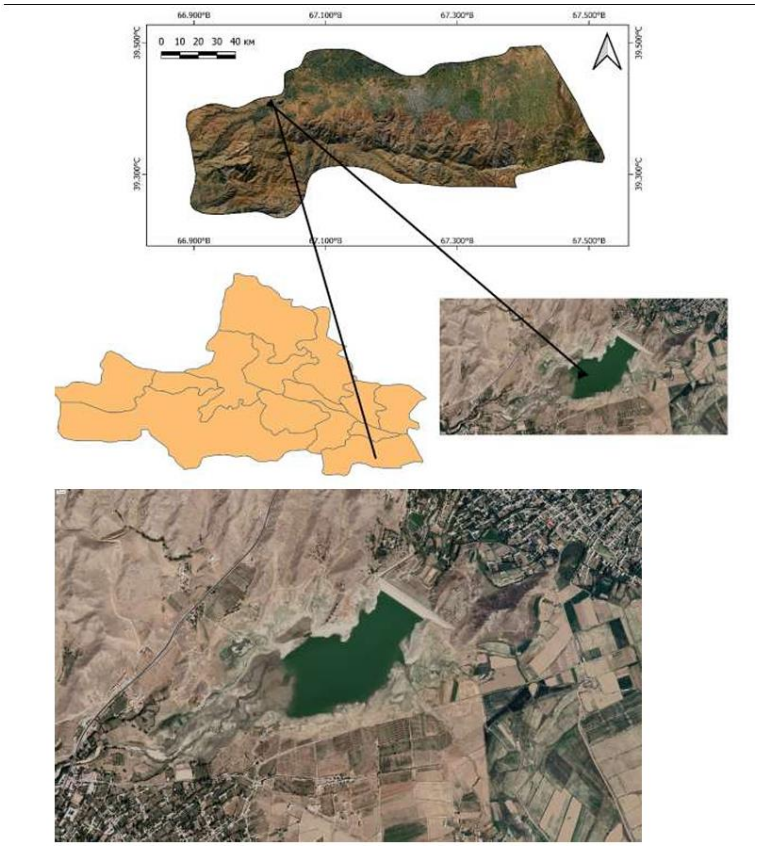


Figure 1,2. Karatepa reservoir

Statistical analyses In statistical analysis, we used Shannon-Wiener index (H), Simpson index (S), Bray-Curtis similarity index, Dominance, Evenness (J). [10, 11, 12].

Results

The analytical results are from 490 fern individuals belonging to 7 genera and 6 families (Table 2.)

Table 2. Species in transects

№	Family	Species	Tr. I	Tr. II	Tr. III	Tr. IV	Tr. V	Tr. VI
1	*Equisetaceae* Michx.	*Equisetum arvense* L.	20	56	40	34	0	10
2	C.	*Equisetum ramosissimum* Desf.	9	31	78	46	17	0
3	*Pteridaceae*	*Adiantum capillus-veneris* L.	5	14	20	0	0	0
4	M.Kirchn.	*Hemionitis persica* (Bory) stenh.	0	0	4	0	0	0
5		*Asplenium haussknechtii* Godet & . ex Milde	0	0	0	3	0	0
6	*Aspleniaceae* man	*Asplenium fontanum subsp. lofontanum* (Kossinsky) Reichst. & eller	0	0	0	0	4	0
7		*Asplenium ruta-muraria* L.	0	0	0	3	0	0
8		*Asplenium trichomanes* L.	0	0	2	0	0	0
9		*Asplenium ceterach* L.	0	0	5	0	0	0
10	*Cystopteridaceae* er) Shmakov	*Cystopteris fragilis* (L.) Bernh.	20	7	15	0	0	40
11	*Dryopteridaceae*	*Dryopteris filix-mas* (L.) Schott	0	0	0	0	0	4
12	er	*Dryopteris komarovii* Kossinsky	0	0	0	0	2	0
13	*Thelypteridaceae* g ex Pic.Serm.	*Thelypteris palustris* Schott	0	0	0	0	0	1

As a result of the study, 6 families belonging to the Pteridophytes department Equisetaceae, Pteridaceae, Aspleniaceae, Cystopteridaceae, Dryopteridaceae, Thelypteridaceae, 7 families: Equisetum, Adiantum, Hemionitis, Asplenium, Cystopteris, Dryopteris, Thelypteris and 13 plant species: Equisetum arvense, E. ramosissimum, Adiantum capillus-veneris, Hemionitis persica, Asplenium haussknechtii, A. fontanum subsp. pseudofontanum, A. ruta-muraria, A. trichomanes, A. trichomanes, A. ceterach (Ceterach officinarum), Cystopteris fragilis, Dryopteris filix-mas, D. komarovii, Thelypteris palustris were found to grow. The result obtained from the transects is as follows (Table 2), and their probability of meeting or distribution was significantly different from each other. It can be seen from the table that among the plants, Equisetum arvense, E. ramosissimum and Cystopteris fragilis are the most common plants, accounting for 76% of the plants found. Plots in transect are labeled 1–10 from the bottom to the top of the mountain. Transect II was a closed precipice although IV was open. Transect VI was a mountain ridge. There was a clear correlation between the H index and the altitude. It shows a tendency that fern became the mostly diverse as the altitude increased. Transect II had a telling correlation. It was a precipice with a closed habitat and vegetative plants. The humidity inside the precipice was relatively high due to good irrigating from the upper site. Most ferns grew well in this condition. Transect IV was also a precipice but aggregated by fragment rocks, and poverty vegetation. It could not keep groundwater, neither a close and humid habitat. Ferns were seldom discovered in this site. Transect VI was a mountain ridge which could not maintain water consistently, nor construct a humid habitat. Arid plants were well developed on the ridge compared to ferns. Plots in transects were labeled 1–10 from the upper to the lower across 100 meters. Transect I was an open river terrace while III and V were closed. The H index was nicely and considerably correlated along the river. Compared to altitudinal transects, the water supply of transects I/III/V seemed sufficient because

it was close to the river channel. Closed habitats were constructed in some areas in transect III and V due to developed shrubs and trees. However, transect I did not develop growing precipice vegetation.

The main reason would be lack of water in streams. Though all the six transects existed in a cramped valley of 24 km in length. Bray-Curtis index demonstrated a deviation altitudinally and latitudinally (Table 3). The maximum was 0,818 in latitudinal transect III and IV which defined similar living circumstances of fern and fern allies. The minimum was 0 in V and VI, which determined various circumstances.

Table 3. Similarity in transects

	I	II	III	IV	V	VI
I		0,49	0,546	0,532	0,766	0,516
II			0,308	0,31	0,74	0,791
III				0,36	0,818	0,771
IV					0,689	0,858
V						0
VI						

I, IV, and VI, latitudinal transects; II, III, and V, altitudinal transects

Bray-Curtis indices were calculated to defined in the mini environment in the plots of each transect. The values are listed separately (Table 4). Transects I, II, III, IV, and VI were like in the plots with Bray-Curtis, and the living circumstance of ferns was continuous. In contrast, Bray-Curtis in the plots of transects C and E was approaching 0. This is due to the poor records of target species and the same low humidity in these two places. The results match well with the field investigation.

Discussion

The findings of this study demonstrate that fern diversity in the Karatepa reservoir area is strongly influenced by environmental gradients, particularly altitude, humidity, and habitat structure. The observed increase in species richness with elevation supports patterns reported in previous ecological studies, where cooler temperatures and higher moisture availability create favorable conditions for pteridophyte development. Closed habitats, such as shaded precipices and areas with stable water supply, showed higher diversity values, confirming that ferns are highly dependent on microclimatic stability.

In contrast, open and arid habitats, including mountain ridges and fragmented rocky areas, exhibited significantly lower species richness. This pattern highlights the sensitivity of ferns to water availability and their limited adaptability to dry conditions. The dominance of a few species, such as *Equisetum arvense* and *Cystopteris fragilis*, further indicates that certain taxa possess greater ecological tolerance, allowing them to thrive across varied conditions.

The Bray–Curtis similarity analysis revealed clear spatial variation in species composition, suggesting that even within a relatively small geographical area, microhabitat differences significantly shape plant communities. Overall, these results reinforce the role of ferns as reliable bioindicators of environmental change and underline the ecological impact of both natural gradients and ongoing climate change in the Zarafshan Valley region.

Conclusion

Some studies have paid attention to altitudinal effect on vascular plants. In the tropical area, studies reveal a hump-shaped pattern with the highest diversity at midaltitudes and then decrease toward both high and low altitudes [13]. The altitude where maximum ferns diversity shows differs somehow among mountain ranges. For example, maximum diversity shows around 1,800 m in

both Costa Rica [35] and Mount Kinabalu, Borneo [14], 2,000 m in Bolivia [15], and 2,400 m on Mount Kilimanjaro, Tanzania [16]. Climatically, these gradients corresponded to the upper parts of tropical gradients where richness also lower. Species were comparable between these data sets at the same mean annual temperature. In temperate regions, richness was reported to fewer continuously with elevation or remains roughly constant, such as New Zealand or North America [17]. Last decades, climate change has been largely focused, while environmental disasters have occurred commonly. Powerful land degradation and climate events have been reported for more than recent years. Plants are actively affected by environmental changes too. In response to such ecological declines, this research demonstrates such changes in Zerafshan valley located in the river shared by Urgut region focusing on ferns affected by other environmental factors. From bottom to top of the mountain, precipitation increased while the temperature decreased. Fern and fern allies are positively correlated with height as a result of atmospheric conditions. Species richness increases further accompanied habitat heterogeneity in the valley. Even with no influence of human disturbance, these current appearances are not the original pattern of the areas, indicating another consequence of climate change

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ISOLATION OF MICROORGANISMS INVOLVED IN THE BIOSYNTHESIS OF CELLULASE ENZYMES AND THEIR BIOLOGICAL CHARACTERISTICS

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Abstract. *In this study, the bioconversion efficiency of various fungal strains in the production of feed protein from lignocellulosic raw materials was investigated. Substrates prepared from corn stover and wheat bran were used as experimental materials and inoculated with* Chaetomium globosum, Trichoderma asperellum, Trichoderma hamatum, *and* Penicillium canescens *under solid-state fermentation conditions. The results demonstrated significant variation in the protein (biomass) production capacity among the fungal strains. The highest level of protein synthesis was recorded for* Chaetomium globosum, *reaching a maximum of 71.2±2.87 mg/g.* Trichoderma asperellum *also exhibited high performance (59.3±2.24 mg/g) and was distinguished by its strong enzymatic activity.* Trichoderma hamatum *showed moderate results (46.5±2.17 mg/g), while* Penicillium canescens *demonstrated relatively lower values (40.3±1.81 mg/g). Microbiological treatment significantly improved the chemical composition of corn stover: protein content increased, crude fiber decreased, and both energy value and mineral content were enhanced. The highest nutritional parameters were observed in substrates treated with* Chaetomium globosum *(protein 18.50%, energy 3182 kcal/kg).* Trichoderma asperellum *was characterized by balanced bioconversion properties, whereas* Penicillium canescens, *despite its high enzymatic activity, showed comparatively lower biomass production.*

Keywords: *lignocellulose, bioconversion, solid-state fermentation, feed protein, micromycetes, Chaetomium globosum, Trichoderma asperellum, Trichoderma hamatum, Penicillium canescens, enzymatic activity, biomass synthesis, corn stover, wheat bran, nutritional value, protein, biotechnology.*

Introduction

At present, the growing global demand for food and feed products—particularly the sharply increasing need for high-quality protein sources—necessitates the development of alternative and sustainable technologies. Due to the limitations of conventional protein sources, including plant- and animal-derived proteins, as well as the associated economic and environmental challenges, the search for new and efficient sources has become an urgent issue.

Cellulose is one of the most abundant polysaccharides in nature and constitutes the primary structural component of plant biomass. Despite its widespread availability, its complex structure limits its direct utilization by most organisms. The biodegradation of cellulose is carried out by a specialized enzyme complex—cellulases. This enzyme system includes endoglucanases, exoglucanases, and β-glucosidases, which synergistically hydrolyze cellulose into simpler sugars, predominantly glucose.

The resulting simple carbohydrates are assimilated by microorganisms and serve as sources of energy and carbon for their growth and development. Consequently, microbial cell biomass

increases and the process of protein biosynthesis is intensified. Thus, the utilization of cellulose-based substrates enables the production of high-protein microbial biomass—feed protein.

The activity of cellulase enzymes is one of the key factors determining the rate and efficiency of cellulose degradation, which in turn directly influences the level of protein synthesis by microorganisms. Therefore, investigating the properties of cellulases, the factors affecting their activity, and their role in microbial protein production is of significant scientific and practical importance.

This article analyzes the activity of cellulase enzymes in cellulose degradation and their role in the biosynthesis of feed protein by microorganisms.

Literature Review

Globally, the demand for livestock meat exceeds 420 million kg per day. The primary sources of animal protein have traditionally included various types of farm animals and poultry, as well as milk, eggs, fish, and fish products. For the optimal functioning of physiological and biochemical processes in the human body, the recommended annual consumption levels are 70–75 kg of meat products, 320–340 kg of milk and dairy products, 260 eggs, and 18–22 kg of fish and fish products [1].

A novel technology with the potential to reduce the environmental impact of protein production is fermentation-based “cellular agriculture,” in which microorganisms are utilized for the production of food products [2].

Most feeds used in livestock production do not contain sufficient amounts of protein and vitamins. Consequently, a significant deficiency of feed protein is observed in many countries. These shortcomings and challenges are primarily addressed by increasing the content of plant-derived proteins—particularly those present in grains—that are essential for animal nutrition [3].

Fungi are widely used in the production of valuable feed for ruminants (cattle). This is due to their ability to convert low-nutritional, and even non-proteinaceous substrates into protein by utilizing the nitrogen present in their composition, as reported in several studies. In addition, alternative plant biomass or concentrates can also be used as protein sources for ruminants [4].

In addition to proteins, microbial cells synthesize other valuable nutritional components, including easily digestible carbohydrates, lipids rich in unsaturated fatty acids, vitamins, as well as macro- and microelements [5].

The use of microorganisms enables the industrial-scale production of large quantities of feed concentrates within a limited area, irrespective of seasonal variations. Microbial cells are capable of synthesizing proteins from agricultural and industrial wastes and, at the same time, possess the ability to recycle these wastes, thereby contributing to environmental protection [6].

Microbial synthesis is characterized by a very rapid rate. For example, during the seed maturation stage, a soybean plant weighing 500 kg accumulates approximately 40 kg of protein per day, whereas a bull of the same weight produces only 0.5–1.5 kg, and 500 kg of yeast cells are capable of synthesizing up to 1.5 tons of protein. As sources of feed protein, various species of yeasts and bacteria, microscopic fungi, and unicellular algae are commonly utilized [7].

An important task is the search for active microbial strains capable of degrading lignin and exhibiting high resistance to microflora-mediated decomposition. At present, fast-growing fungal strains of the genera *Penicillium*, *Aspergillus*, *Fusarium*, and *Trichoderma* have been selected for industrial cultivation. These fungi consist of cells with relatively less rigid cell walls, a characteristic that positively influences the digestibility of mycelium and mycelium-based products [8].

Compared to yeast proteins, proteins of microscopic fungi are distinguished by a higher content of sulfur-containing amino acids and better digestibility. The concentration of nucleic acids in fungal mycelium (1–4% of dry weight) is nearly comparable to that of proteins in plant tissues. At the same time, fungal biomass contains relatively lower levels of protein (20–60% of dry weight) than yeasts, and their biomass grows at a comparatively slower rate. Lower filamentous fungi cultivated on cellulose- and lignin-containing plant wastes are capable of synthesizing complexes of hydrolytic enzymes, degrading cellulose and lignin into simpler compounds, and consequently producing amino acids and proteins [9].

The high digestibility of fungal protein in animals, along with its relatively low nucleic acid content, allows it to be used as a feed additive at significantly higher concentrations than feed yeast. Typically, fungal protein may be included at 15–20% of the total dietary protein when feeding young animals, and up to 50% in the rations of adult animals. To compensate for the shortage of protein raw materials, it is necessary to expand the use of secondary resources from the processing, food, microbiological, and chemical industries. This includes increasing the production of feed protein from plant residues and the rational utilization of fermentation by-products (molasses residues, brewer's grains, etc.), as well as wastes from the dairy and meat industries. It is also envisaged to utilize advances in biotechnology, particularly microbial synthesis products such as amino acids and feed yeast. Another important producer of feed proteins and enzymes is fungi of the genus *Trichoderma*. Currently, the most widely used method for commercial cellulase production is submerged cultivation (involving the preparation of inoculum followed by transfer into a nutrient medium). At the same time, surface cultivation provides more favorable conditions for the growth of aerobic microscopic fungi [10].

Currently, there are two principal approaches to the use of enzyme preparations in the feeding of agricultural animals: their direct inclusion in feed and the pre-treatment of feed with enzyme preparations. Since the main components of animal diets consist of plant proteins, cellulose, and starch-containing substances, the application of agents capable of degrading these components is considered appropriate. Enzymes such as proteases, amylases, and cellulases, as well as their complexes, hydrolyze proteins, starch, and fiber, thereby enhancing their digestibility and utilization by the animal organism. In livestock production, it is also advisable to employ enzymes synthesized by microorganisms inhabiting the animal gut. These enzymes are naturally selected and are capable of hydrolyzing dietary components incorporated into the ration [11].

Materials and Methods

Bioconversion processes of plant residues by selected micromycete strains were investigated. In studying the bioconversion of cellulose-containing plant raw materials, the enzymatic activity of selected micromycete strains (*Trichoderma asperellum* and *Chaetomium globosum*) was utilized. During the course of the study, enzyme complexes isolated from the culture liquids of these fungi were applied in the degradation of cellulosic substrates.

The fungal culture grown in a liquid nutrient medium was centrifuged at 3000 rpm for 15 minutes, and the mycelium was separated from the culture liquid. Under solid-state fermentation conditions, after completion of fermentation, either the entire mass of the culture medium or an aqueous extract obtained by pressing (filtrate) was used. In this process, the following ratio was applied: 10 units of cellulolytic activity per 1 g of treated substrate.

In developing the solid-state fermentation regime, pre-prepared aboveground biomass of amaranth and winter wheat straw were used as raw materials. These substrates were dried to a moisture content of 6–7% and subsequently ground to a particle size of 1.5–2.0 mm.

Fermentation under laboratory conditions was carried out in 1 L conical flasks. Under semi-industrial conditions, cultivation was performed in special trays measuring 30×40 cm, with a substrate layer not exceeding 50–55 mm in height. The nutrient medium was prepared based on the composition described by Mandels [12], consisting of ground wheat (50%) and amaranth biomass (50%), or straw moistened with a mineral medium. The initial pH was maintained within the range of 4.7–4.9.

During the enzymatic hydrolysis process, 10 mL of water was added per 1 g of substrate, and the mixture was heated to 100°C and boiled for 5 minutes. It was then cooled to room temperature, after which culture liquid with an enzymatic activity of 10 units per 1 g of substrate was added. The mixture was maintained at 29–33°C and continuously agitated at 150 rpm.

The efficiency of bioconversion was evaluated based on the amount of reducing sugars formed as a result of the degradation of cellulosic substrates. The determination of reducing sugars was carried out using Müller's alkaline solution. This method is based on the reduction of Cu^{2+} ions to Cu_2O under the influence of reducing substances. The resulting product was treated with an iodine solution, and the excess iodine was quantified by titration with sodium thiosulfate solution [11].

The protein content was determined using the Lowry method. In order to optimize the microbiological degradation of lignocellulosic substrates—amaranth biomass and wheat bran—micromycete strains (*Trichoderma asperellum* and *Chaetomium globosum*) were cultivated on the surface of the substrate. The experiments were conducted under sterile conditions in 250 mL glass flasks.

In the first variant, 10 g of amaranth stems ground to a particle size of 0.2–0.5 cm were placed into flasks. In the second variant, a mixture consisting of 5 g of ground amaranth stems and 5 g of wheat bran was prepared. In both cases, the substrates were moistened to 50–60% with Mandels mineral nutrient medium (pH 5.5–5.7), brought to a solid-state medium condition, and sterilized.

The prepared substrates were inoculated with 3 mL of a suspension of micromycete strains previously cultivated in liquid Mandels nutrient medium and thoroughly mixed. Control variants consisted of substrates of the same composition without the addition of fungal suspension. All flasks were incubated under thermostatic conditions at 29–33°C. The experiments were conducted over a period of 15 days, during which the total protein content formed in the substrate was determined on a daily basis.

For protein extraction, 90 mL of distilled water or 2 M NaOH solution was added to the solid substrates colonized by fungi, and the samples were agitated on a shaker at 200 rpm for 1 hour. The mixture was then filtered through filter paper and prepared for analysis. Protein content was determined using the Lowry method. This method is based on the reaction of aromatic amino acids in proteins with the Folin reagent, resulting in the formation of a colored complex.

During the analysis, 0.5 mL of the test solution was mixed with 2.5 mL of reagent C (a mixture of 50 mL of reagent B and 1 mL of reagent A) and incubated for 10 minutes. Subsequently, 0.25 mL of reagent D was added, the mixture was thoroughly homogenized, and kept in the dark for 30 minutes. The intensity of the resulting color was measured at a wavelength of 750 nm using a spectrophotometer. The protein content was calculated based on a previously constructed calibration curve [13].

The chemical composition of the prepared feed was determined in accordance with GOST standards: 13496.3–93, 13496.14–87, 13496.4–93, 13496.2–91, 26570–95, 26657–97, 13496.1–98, 13496.12–98, and 13496.7–97.

Results and Discussion

As a result of the conducted experiments, the protein (biomass) production capacities of different fungal strains under solid-state fermentation conditions were compared. The obtained results demonstrated significant differences in the levels of protein synthesis among the studied strains, which were found to depend on their physiological and biochemical characteristics as well as their ability to utilize the substrate.

The highest value was recorded for *Chaetomium globosum*, which produced 10.8 mg/mL of protein. This result indicates the strong ability of this fungus to extensively degrade lignocellulosic substrates, efficiently assimilate carbon sources, and synthesize biomass intensively. It is well established that fungi of the genus *Chaetomium* possess a pronounced capacity to hydrolyze cellulose and hemicellulose components, whereby the resulting simple carbohydrates are rapidly converted into cellular biomass. Therefore, *Chaetomium globosum* can be considered a promising microorganism for feed protein production.

The next highest result was observed for *Trichoderma asperellum*, which produced 8.5 mg/mL of protein. This strain is distinguished by its high enzymatic activity, particularly its active synthesis of cellulase and xylanase enzymes. This facilitates rapid substrate degradation and accelerates the microbial assimilation of organic compounds in the nutrient medium. As a result, the nutrients required for biomass formation are generated more quickly. However, its biomass production level was slightly lower than that of *Chaetomium globosum*, which may be explained by the allocation of a portion of energy toward enzyme synthesis (Fig. 1).

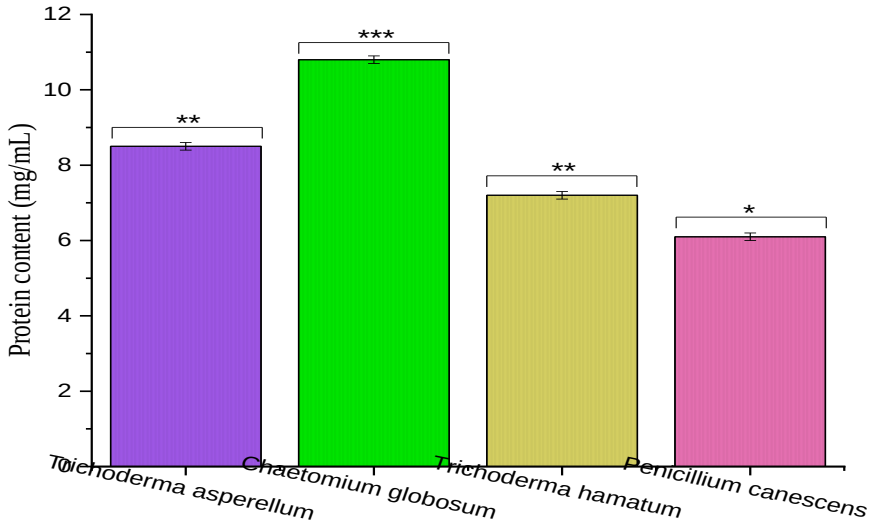


Figure 1. Protein synthesis by selected active strains, mg/mL (n = 4)

In *Trichoderma hamatum*, the protein content was 7.2 mg/mL, which can be considered a moderate level. Although this strain also exhibits cellulolytic properties, its enzymatic activity and biomass formation intensity were observed to be lower than those of *T. asperellum*. This may be associated with a relatively slower rate of substrate utilization and less intensive metabolic processes.

The lowest value was recorded for *Penicillium canescens*, which produced 6.1 mg/mL of protein. This strain is primarily known as a strong enzyme producer, particularly effective in the synthesis

of xylanase and cellulase enzymes. However, its capacity for biomass production is relatively low, which can be explained by the allocation of most of its energy toward enzyme synthesis. Therefore, *P. canescens* is more appropriately utilized not as a direct source of protein, but as an auxiliary microorganism for substrate degradation.

Overall, the obtained results indicate that the biological characteristics of fungi play a crucial role in the production of feed protein from lignocellulosic substrates under solid-state fermentation conditions. In particular, *Chaetomium globosum* is distinguished by its high biomass yield, whereas *Trichoderma asperellum* ensures rapid substrate degradation due to its strong enzymatic activity. Therefore, the combined application of these fungi may enhance bioconversion efficiency and enable the production of high-quality feed protein.

Degradation of Plant Residues by Fungi in Solid-State Media. Maize (*Zea mays* L.) is a widely cultivated agricultural crop characterized by high biomass production, and its stems and other residues represent lignocellulose-rich raw materials. The high content of cellulose, hemicellulose, and lignin in these residues limits their direct use as feed. Therefore, the enhancement of their nutritional value through microbiological processing is of significant scientific and practical importance.

In this study, for the purpose of feed protein production, a solid-state nutrient medium was prepared using ground corn stover mixed with wheat bran. The prepared substrates were inoculated with strains of *Trichoderma asperellum*, *Chaetomium globosum*, *Trichoderma hamatum*, and *Penicillium canescens*, previously cultivated in liquid Mandels medium at pH 5.5–6.5, and the fermentation process was carried out for 15 days.

During fermentation, the dynamics of protein synthesis were investigated, revealing that in all strains the protein content increased from the initial days of incubation, reached a maximum between days 5–10, and subsequently declined. According to the obtained results, the highest protein synthesis was recorded in *Chaetomium globosum*, with a maximum value of 71.2±2.87 mg/g. This indicates the strong capacity of this strain to efficiently utilize lignocellulosic substrates and produce a high biomass yield.

Trichoderma asperellum also demonstrated high performance, with a maximum protein yield of 59.3±2.24 mg/g. This strain exhibits strong enzymatic activity, and its ability to actively degrade cellulose and hemicellulose accelerates substrate utilization.

In *Trichoderma hamatum*, protein synthesis was at a moderate level, with a maximum value of 46.5±2.17 mg/g. This indicates that the metabolic activity of this strain is somewhat lower compared to the other strains.

In *Penicillium canescens*, protein synthesis was relatively low, with a maximum value of 40.3±1.81 mg/g. This can be explained by the fact that this fungus is primarily oriented toward enzyme production, while its capacity for biomass formation is limited.

It was also observed that protein synthesis was slightly higher in substrates prepared from a mixture of corn stover and wheat bran; however, no significant differences were recorded overall. This indicates that corn residues alone constitute a sufficient nutrient source for fungal growth and development. The strains produced 2.3–3 times more protein compared to the control (Table 1).

Table 1. Protein synthesis by active strains on plant residues (mg/g) (n = 6)

Days	*Trichoderma asperellum*	*Chaetomium globosum*	*Trichoderma hamatum*	*Penicillium canescens*
1	31,2±1,34	34,1±1,61	28,3±1,21	25,4±1,17

2	36,1±1,71	40,2±1,89	32,5±1,55	28,6±1,33
3	42,3±1,68	49,3±2,11	36,4±1,63	32,1±1,45
4	48,7±2,10	58,6±2,34	40,2±1,82	35,8±1,58
5	59,3±2,24	71,2±2,87	46,5±2,17	40,3±1,81
6	55,2±2,11	65,4±2,45	42,8±1,91	37,6±1,66
7	52,3±2,09	62,7±2,34	41,1±1,78	35,9±1,51
8	54,1±2,17	64,2±2,25	42,3±1,69	37,2±1,68
9	56,8±2,22	66,8±2,76	44,1±2,02	38,8±1,72
10	57,4±2,34	68,9±2,57	44,6±2,08	39,5±1,71
11	55,6±2,21	66,5±2,61	43,2±1,91	37,4±1,76
12	52,4±2,07	61,8±2,42	40,7±1,88	35,2±1,43
13	49,2±1,91	56,3±2,27	38,5±1,67	33,1±1,44
14	46,8±1,88	53,4±2,13	36,2±1,62	31,5±1,32
15	44,1±1,73	50,6±2,05	34,8±1,57	29,4±1,25
Control	22,3±0,95	24,7±1,19	20,8±0,76	18,9±0,72

The table below presents the dynamics of protein synthesis (mg/g) by active fungal strains on plant residues over a 15-day period, clearly reflecting their activity in the bioconversion process.

According to the results, protein synthesis in all strains increased progressively from the initial days of incubation, reached a maximum between days 5–10, and subsequently showed a gradual decline. This trend can be explained by the depletion of readily degradable nutrients in the substrate and a corresponding reduction in metabolic activity.

The highest values were recorded for *Chaetomium globosum*. In this strain, protein content increased from 34.1±1.61 mg/g on day 1 to 71.2±2.87 mg/g on day 5, followed by a gradual decline to 50.6±2.05 mg/g by day 15. These results indicate the strong ability of this fungus to efficiently utilize lignocellulosic substrates and produce a high biomass yield.

In *Trichoderma asperellum*, similarly high results were observed, with protein synthesis increasing from 31.2±1.34 mg/g on day 1 to a maximum of 59.3±2.24 mg/g on day 5, followed by a gradual decline to 44.1±1.73 mg/g by day 15. The high enzymatic activity of this strain facilitated rapid substrate degradation, thereby positively influencing biomass formation.

In *Trichoderma hamatum*, protein synthesis was at a moderate level, reaching a maximum of 46.5±2.17 mg/g on day 5. In the subsequent days, this value gradually declined. This indicates that the metabolic activity and substrate utilization rate of this strain are somewhat lower compared to the other strains.

In *Penicillium canescens*, the lowest level of protein synthesis was recorded, with a maximum value of 40.3±1.81 mg/g on day 5. This can be attributed to the fact that this strain is primarily oriented toward enzyme production, resulting in a comparatively lower capacity for biomass formation.

In the control variant, all values were significantly lower (18.9–24.7 mg/g), confirming that protein accumulation in the substrate proceeds at a much slower rate in the absence of fungal involvement.

The obtained results indicate that under solid-state fermentation conditions, *Chaetomium globosum* is the most efficient strain in terms of biomass (protein) production, while *Trichoderma*

asperellum is distinguished by its high enzymatic activity. Therefore, the combined application of these strains appears promising for the efficient bioconversion of lignocellulosic substrates and the production of high-quality feed protein.

Chemical Composition of Feed Obtained from Microbiologically Treated Corn Stover. Corn stover is a lignocellulose-rich raw material with low nutritional value. It contains high levels of cellulose, hemicellulose, and lignin, components that are not fully digestible in the animal organism. Therefore, improving the nutritional value of such plant residues through microbiological processing is of significant scientific and practical importance.

In this study, locally isolated and identified strains of *Trichoderma asperellum, Penicillium canescens,* and *Chaetomium globosum* were used. These strains were selected based on their ability to degrade lignocellulosic substrates and their enzymatic activity.

During the experiment, microbiological treatment was applied to substrates prepared from corn stover as well as mixtures of corn stover and wheat bran. The results demonstrated that all strains were capable of degrading cellulose and hemicellulose; however, their efficiencies differed.

In terms of enzymatic activity, *Penicillium canescens* exhibited the highest performance, particularly demonstrating superiority in the synthesis of xylanase and endo-1,4-β-glucanase enzymes. This indicates its strong capacity for the extensive degradation of lignocellulosic substrates.

Trichoderma asperellum was distinguished by the stability and complexity of its enzymatic activity, demonstrating the ability to efficiently hydrolyze both cellulose and hemicellulose simultaneously. This strain enables balanced substrate degradation and ensures the stable progression of the bioconversion process.

Although the enzymatic activity of *Chaetomium globosum* was relatively lower, it is notable for its strong ability to utilize lignocellulosic substrates and its high biomass production capacity. This indicates its important role in the process of feed protein production.

Overall, the obtained results indicate that each strain plays a specific role in the efficient bioconversion of lignocellulosic plant residues: *Penicillium canescens* ensures intensive enzymatic degradation, *Trichoderma asperellum* provides stable and complex hydrolysis, while *Chaetomium globosum* contributes to biomass accumulation and protein production.

Therefore, the application of these strains either individually or in combination ensures the efficient degradation of lignocellulosic substrates and enables the production of products with high nutritional value (Table 2).

Table 2. Chemical composition of feed obtained through microbiological treatment

Determined parameters	Trichoderma asperellum	Penicillium canescens	Chaetomium globosum	Control (corn stover)
Ash	12,30	13,40	10,60	9,86
Nitrogen	2,65	1,45	2,95	1,1
Protein	16,60	9,20	18,50	10,81
Fat	2,30	1,05	2,80	1,41
Fiber	33,80	40,50	25,90	34,12
Nitrogen-free extractives (NFE)	34,50	35,40	41,80	45,07
Feed units	0.62	0.52	0.86	0,45

Total energy value, kcal/kg	2880	2250	3182	2200
Calcium	2,35	1.77	1.61	1.25
Phosphorus	0.80	0.22	1,10	0.67
Acidity	0.8^0	3.4^0	0.7^0	5.8^0
Toxicity	Non-toxic	Non-toxic	Non-toxic	Non-toxic

Changes in the chemical composition of feed obtained after the microbiological treatment of corn stover using *Trichoderma asperellum*, *Penicillium canescens*, and *Chaetomium globosum* are presented. The results demonstrate that the microbiological bioconversion process significantly enhances the nutritional value of the feed.

According to the analysis results, an increase in protein content compared to the control was observed in all experimental variants. In particular, the highest value was recorded for *Chaetomium globosum*, with a protein content of 18.50%. This can be explained by its strong ability to efficiently utilize lignocellulosic substrates and produce high biomass. In *Trichoderma asperellum*, the protein content was also high (16.60%), indicating its capacity to effectively degrade the substrate through a complex enzymatic system. In contrast, *Penicillium canescens* showed a relatively lower protein content (9.20%), which can be attributed to its primary orientation toward enzyme production.

Significant differences were observed in fiber content. The lowest value was recorded in *Chaetomium globosum* (25.90%), confirming its strong ability to actively degrade lignocellulose. In *Trichoderma asperellum*, this parameter was at a moderate level (33.80%), whereas the highest value was observed in *Penicillium canescens* (40.50%). This can be explained by the specialization of this strain in producing enzymes that primarily degrade hemicellulose, while not fully decomposing lignin and complex cellulose components. The high fiber content in the control variant (34.12%) indicates the lack of degradation of the raw lignocellulosic substrate.

An increase in nitrogen-free extractives (NFE) also confirms the breakdown of complex polysaccharides into simple carbohydrates as a result of enzymatic hydrolysis. The highest NFE content was recorded in *Chaetomium globosum* (41.80%). Although the control variant showed a higher NFE value (45.07%), this is associated with the presence of soluble carbohydrates, while the overall nutritional value remains low.

The energy value and feed unit indicators were also significantly improved. The highest energy content was observed in *Chaetomium globosum* (3182 kcal/kg), demonstrating its superiority in enhancing feed quality. In *Trichoderma asperellum*, the energy value reached 2880 kcal/kg, indicating a favorable result. In contrast, *Penicillium canescens* showed a relatively lower energy value (2250 kcal/kg), which can be attributed to its lower biomass production capacity. The control variant had an energy value of 2200 kcal/kg, reflecting the low digestibility of lignocellulose.

An increase in mineral components, particularly calcium and phosphorus, indicates enhanced bioavailability as a result of microbiological treatment. Notably, a higher calcium content was recorded in *Trichoderma asperellum* (2.35%), while *Chaetomium globosum* showed the highest phosphorus content (1.10%).

Regarding acidity, a relatively higher value was observed in *Penicillium canescens* (3.40), which can be attributed to the formation of organic acids during the fermentation process. In the other strains, acidity remained at a lower level.

The absence of toxic substances in all variants indicates that the obtained product is safe for livestock use. The obtained results demonstrate that microbiological treatment significantly enhances the nutritional value of corn stover. In particular, *Chaetomium globosum* exhibited the highest efficiency in terms of protein and energy parameters, while *Trichoderma asperellum* was distinguished by its balanced bioconversion performance. Despite its high enzymatic activity, *Penicillium canescens* showed comparatively lower results in biomass production.

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ISOLATION OF MICROORGANISMS INVOLVED IN CELLULASE BIOSYNTHESIS AND THEIR BIOLOGICAL CHARACTERISTICS

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Abstract. *This article analyzes microorganisms involved in the biosynthesis of cellulase enzymes, as well as their biological and enzymatic characteristics. The study evaluates the significance of highly active micromycetes in the bioconversion of lignocellulosic plant residues, particularly* Trichoderma asperellum *and* Chaetomium globosum *strains. The results demonstrate that these fungi actively synthesize a complex of cellulolytic enzymes and are capable of efficiently degrading a variety of substrates.*

During the study, solid-state fermentation conditions were employed using wheat straw, corn stalk, and other lignocellulosic substrates, and an increase in reducing sugars as well as total protein content was recorded. Furthermore, the morphological and cultural characteristics, growth conditions, and enzymatic activity of the microorganisms were determined, and their potential application in biotechnological processes was substantiated.

The results indicate that cellulase-producing microorganisms represent promising biological agents for the processing of plant residues, as well as for the production of biofeed and enzyme preparations.

Keywords: *cellulase, lignocellulose, bioconversion, micromycetes,* Trichoderma asperellum, Chaetomium globosum, *enzymatic activity, solid-state fermentation, reducing sugars, biotechnology, plant residues.*

Introduction

Cellulose is one of the most abundant organic polymers in nature and constitutes the principal structural component of plant cell walls. Despite the annual generation of vast amounts of lignocellulosic biomass on Earth, a significant portion remains inefficiently utilized. For this reason, enzymes capable of biologically degrading cellulose—namely cellulases—are gaining increasing importance.

Cellulase enzymes are primarily synthesized by various microorganisms, particularly fungi and bacteria. They hydrolyze cellulose molecules into simple sugars, a process that plays a crucial role in the production of biofuels, feed additives, and other biotechnological products. In particular, micromycetes such as *Trichoderma asperellum* and *Chaetomium globosum* are distinguished by their high cellulolytic activity and are widely applied at the industrial scale.

In recent years, there has been a growing demand for environmentally friendly methods for the processing of lignocellulosic raw materials. Compared to chemical approaches, microbiological bioconversion methods are considered more economically and ecologically efficient. Therefore, the identification of microorganisms capable of active cellulase production, the study of their properties, and their application under optimal conditions constitute an important scientific objective.

From this perspective, investigating microorganisms involved in cellulase biosynthesis and their characteristics is of both scientific and practical relevance, and the present article is devoted to this issue.

Literature Review. If not properly managed in a timely manner, secondary plant products (i.e., residues) can cause environmental harm, contributing to greenhouse gas emissions or leading to soil and water contamination during decomposition. Numerous studies have demonstrated that biotechnological approaches can serve as effective solutions for utilizing such materials without causing environmental damage [1].

Microbial strains, particularly fungal strains, possess the ability to utilize cellulose as a source of carbon and energy. Fungi and bacteria with this capability must be able to synthesize enzymes that degrade these complex polymers. Cellulases are widely applied in various industries, including food and animal feed production, textiles, paper manufacturing, and wastewater treatment [2].

The use of plant residues as a carbon source in the composition of the culture medium has been shown in numerous studies to significantly enhance the cellulolytic activity of fungi belonging to the *Trichoderma* genus. For example, rice straw has been demonstrated to be a more effective carbon source than carboxymethyl cellulose (CMC) for cellulase enzyme production by the *T. harzianum* MTSS8230 strain [3].

Enzymes that degrade cellulose represent key tools for achieving the efficient reutilization of biomass. The fungus *T. harzianum* is capable of synthesizing cellulolytic enzymes responsible for cellulose degradation. However, one of the major components of the natural biocomplex—lignin—confers structural resistance to cellulose, rendering the complex resistant to hydrolysis. On average, plant biomass consists of approximately 23% lignin, 40% cellulose, and 33% hemicellulose by weight [4].

Plant cell walls constitute the most abundant renewable source of fermentable sugars on Earth, with plants producing more than 50×10^9 tons of cellulose annually. The total global reserves of cellulose exceed 10^{12} tons. The majority of cellulose is synthesized in plants as a result of photosynthesis [5].

Understanding the relationships between the genes responsible for enzymatic cellulose hydrolysis and the synergistic reactions involved is of critical importance for improving biomass biodegradation technologies. The synergistic interactions, transcriptomes, exoproteomes, and enzymatic activities of *T. harzianum*, *T. reesei*, and *T. atroviride* extracts have been characterized [6].

In order to better understand and reproduce the efficiency of these reactions, the scientific community has conducted numerous studies aimed at identifying and characterizing the enzymes required for the degradation of various components of lignocellulose, as well as determining the interactions between lignocellulosic components and the enzymes involved in their degradation [8].

Furthermore, the cultivation of producers on solid media is considered important for obtaining high yields of cellulase enzymes. However, the use of solid substrates also has certain limitations: the growth of producers under such conditions is more difficult to control. In addition, a portion of the enzymes synthesized by the producers may remain bound to residual solid substrates [9].

It has been established that the enzymatic system responsible for the conversion of cellulose into glucose includes at least three types of cellulases: endoglucanase (endo-1,4-β-D-glucanase,

EG, EC 3.2.1.4), cellobiohydrolase or exoglucanase (exo-1,4-β-D-glucanase, CBH, EC 3.2.1.91), and β-glucosidase (1,4-β-D-glucanase, BG, EC 3.2.1.21). Endoglucanase acts randomly along the cellulose chain to generate shorter cellulose fragments. Cellobiohydrolase functions as an exoglucanase, releasing the disaccharide cellobiose. Subsequently, β-glucosidase hydrolyzes cellobiose to produce glucose [5].

Numerous scientific studies have been conducted by various researchers on protein synthesis in *Trichoderma* fungi. In this review, we summarize findings since 2014 regarding proteins produced by *Trichoderma*, their origins, terminators, signal proteins, yields, and commonly applied fields, and provide an overview of the strategies used to advance this line of research [7].

Materials and Methods

Isolation and Screening of Cellulolytic Micromycetes from Natural Sources. To isolate cellulose-degrading micromycetes, samples were collected from various ecological sources. In particular, soil, plant residues (wheat straw and corn stalks), wood debris, and rhizosphere samples were obtained from different locations in Tashkent city as well as the Kashkadarya and Bukhara regions, and were stored at 4°C until transported to the laboratory.

Micromycetes were isolated using serial dilution and direct plating methods of soil samples. For cultivation, Czapek–Dox medium (g/L: sucrose – 30.0 or glucose – 20.0; $NaNO_3$ – 3.0; K_2HPO_4 – 1.0; $MgSO_4 \cdot 7H_2O$ – 0.5; KCl – 0.5; $FeSO_4 \cdot 7H_2O$ – 0.01) and Mandels mineral medium (g/L: KH_2PO_4 – 2.0; $(NH_4)_2HPO_4$ – 1.4; $MgSO_4$ – 0.5; $CaCl_2$ – 0.3; urea – 0.3; peptone – 1.0; sucrose – 2.0) were used. After sterilization, tetracycline was added to the media at a concentration of 300 µg/mL to prevent bacterial contamination. Incubation was carried out at 28–30°C for 3–6 days.

The resulting colonies were transferred to subculture, and purification was performed through repeated inoculation of spores or mycelial fragments. When necessary, Triton X-100 was added to the medium to facilitate the isolation of pure cultures. As a result, several micromycete isolates were obtained, from which strains exhibiting cellulolytic activity were selected.

The isolated micromycetes were characterized based on macromorphological features (colony color, structure, and growth rate) and micromorphological traits (structure of mycelium, conidiophores, and conidia). Microscopic observations were carried out using a light microscope with 400× magnification. The systematic position of the fungi was determined according to modern mycological identification keys and relevant literature.

Screening for cellulolytic activity was conducted in two stages. In the first stage, isolates were cultivated on filter paper moistened with Mandels medium and evaluated for their ability to degrade cellulose. The most active strains were then subjected to a second stage, where they were grown in a modified liquid Mandels medium (pH 5.5–5.8) supplemented with 2% wheat bran, corn stalk, and other cellulosic substrates, at 28–30°C on a rotary shaker at 200–220 rpm for 4 days.

During the study, the cellulase and xylanase activities of the strains, their protein synthesis capacity, and their ability to enrich cellulosic substrates with reducing sugars were determined, and the most active micromycete strains were selected. These strains were subsequently used as the primary objects for further investigations [10].

Genomic DNA Extraction Method. Genomic DNA from the selected cellulolytic micromycete strains was isolated using the classical phenol–chloroform extraction method. This approach enables the recovery of high-quality and purified DNA from fungal cells.

Fungal biomass grown in liquid culture medium was centrifuged at 2500 × g for 15 minutes, and the resulting cell pellet was collected. A 2 g sample of frozen mycelium was then ground in a mortar using liquid nitrogen to mechanically disrupt the cell wall.

For cell lysis, a specialized extraction solution preheated to 55°C was used. This solution contained triisopropylnaphthalene sulfonic acid (TNS), p-aminosalicylic acid (PAS), deionized water, Tris-HCl, NaCl, EDTA buffer, and phenol. These components function to disrupt the cell membrane and proteins, thereby facilitating the release of DNA into the solution.

The mixture was incubated at 55°C under continuous agitation, after which 5 mL of chloroform was added and incubation was continued. Subsequently, the mixture was centrifuged at 2500 × g for 15 minutes to achieve phase separation. The upper (aqueous) phase was carefully collected and transferred to a new tube.

For DNA purification, an equal volume of phenol–chloroform (1:1) was added to the aqueous phase, mixed using a vortex, and centrifuged again. This step ensured the removal of proteins and other impurities. In the subsequent step, chloroform alone was added, followed by centrifugation, and the resulting supernatant was transferred to a new tube.

To precipitate genomic DNA, 30 µL of 3.0 M sodium acetate (pH 5.2) and 25 µL of 96% ethanol were added, and the samples were incubated for approximately 18 hours. Thereafter, the samples were centrifuged at 14,000 × g for 30 minutes at 4°C, and the DNA pellet was collected.

The DNA pellet was washed with 70% ethanol and centrifuged again at 14,000 × g for 10 minutes at 4°C. Subsequently, the DNA was air-dried, dissolved in deionized water, and stored at 4°C for further molecular biological analyses [12].

Amplification of rDNA Gene Regions by PCR. For the purpose of molecular identification of micromycete strains, the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR) using the isolated genomic DNA as a template. The ITS region is a widely used universal molecular marker for species-level identification of fungi.

For amplification, the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were employed. These primers are complementary to conserved regions of the rDNA gene cluster and enable the amplification of a fragment encompassing the ITS1–5.8S–ITS2 regions.

The PCR reaction was prepared in a total volume of 100 µL, containing 25 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each of dNTPs (dATP, dCTP, dGTP, dTTP), 50 ng each of ITS1 and ITS4 primers, and 2.5 units of Taq DNA polymerase.

Amplification was performed in a thermal cycler under the following conditions: initial denaturation at 95–97°C for 3 minutes, followed by 30 cycles of denaturation (95°C for 30–35 s), annealing (52–55°C for 45–60 s), and elongation (72°C for 1 minute). A final elongation step was carried out at 72°C for 5–7 minutes.

The PCR products were analyzed by electrophoresis on a 1.4% agarose gel in 1×TBE (or TAE) buffer. The results were visualized under ultraviolet illumination, and the presence and size of the amplified fragments were evaluated [14].

Determination of the General Characteristics of Selected Micromycete Strains. To determine the morphological and cultural characteristics of selected cellulolytic micromycete strains (*Trichoderma asperellum* and *Chaetomium globosum*), as well as to establish their optimal growth conditions, studies were conducted using various culture media. For this purpose, Mandels,

Czapek, wort agar (diluted to 7° on the saccharometer scale), and potato dextrose agar (PDA) media were prepared.

The prepared media were sterilized at 1.0 atm for 30 minutes and poured into Petri dishes. Subsequently, fungal spores were inoculated and incubated at 29–33°C under thermostat conditions. The macromorphological characteristics of the colonies (color, structure, and growth rate) were observed, while micromorphological features were examined using XSP-136 B and LEICA DM750 light microscopes at 400× magnification.

Mandels medium was evaluated as a favorable substrate for the growth of cellulolytic and enzymatically active micromycetes. Based on this, experiments were conducted to determine the growth dynamics and radial growth rate of the selected strains.

To enhance the economic efficiency of the culture medium and to identify optimal substrates for fungal growth, various cellulose-containing materials were used as carbon sources at a concentration of 2%, including wheat straw, corn stalk, wheat bran, filter paper, and others. Mandels mineral medium was used as the control variant.

To determine the growth rate of fungal colonies, inoculation was performed at the center of Petri dishes, followed by incubation at 29–33°C. The colony radius was measured every 24 hours using a ruler, and average values were calculated.

The radial growth rate was determined using the following formula:

$$Kr = (r - r_0) / (t - t_0)$$

where:

- ***Kr***–radial growth rate (mm/h),
- r_0 – colony radius at the initial time,
- r – colony radius at a given time,
- t_0 and t – time points [10].

In addition, the developmental cycle of the selected micromycete strains was studied in liquid Mandels medium. Observations were carried out using a LEICA DM750 light microscope at 400× magnification. The vegetative and reproductive stages of fungal development were monitored at 30-minute intervals, and the corresponding morphological changes were recorded.

Bioconversion of Plant Residues by Selected Micromycete Strains. The enzymatic activity of selected micromycete strains (*Trichoderma asperellum* and *Chaetomium globosum*) was utilized to study the bioconversion processes of cellulose-containing plant raw materials. During the experiments, enzyme complexes isolated from the fungal culture liquid were applied to the degradation of cellulosic substrates.

Fungal cultures grown in liquid medium were centrifuged at 3000 rpm for 15 minutes, and the mycelium was separated from the culture liquid. Under solid-state fermentation conditions, following the completion of fermentation, either the entire mass of the culture medium mixture or an aqueous extract (filtrate) obtained by pressing was used. In this process, the following ratio was applied: 10 units of cellulolytic activity per 1 g of treated substrate.

For the development of the solid-state fermentation regime, pretreated aboveground biomass of corn stalk and winter wheat straw were used as raw materials. These substrates were dried to a moisture content of 6–7% and ground to a particle size of 1.5–2.0 mm.

Under laboratory conditions, the fermentation process was carried out in 1 L Erlenmeyer flasks. In semi-industrial conditions, cultivation was performed in specialized trays (30 × 40 cm) with a substrate layer not exceeding 50–55 mm in height. The culture medium was prepared based

on the Mandels formulation and supplemented with ground wheat (50%) and corn stalk (50%), or straw moistened with mineral medium. The initial pH was maintained within the range of 4.7–4.9.

As inoculum material, 5-day-old fungal cultures grown on Czapek–Dox agar and Mandels mineral medium were used. The inoculum itself consisted of a suspension of culture liquid obtained from growth on corn stalk and wheat-based substrates.

Under solid-state fermentation conditions, the degree of hydrolysis of wheat bran, corn stalk, and wheat straw using selected micromycete strains was investigated. As a result, the formation of reducing sugars and an increase in total protein content were observed, allowing the efficiency of the bioconversion process to be evaluated.

Determination of Reducing Sugars in the Enzyme Preparation. The content of reducing sugars was determined using the Somogyi–Nelson method. This method is based on the quantitative determination of reducing sugars formed as a result of cellulose hydrolysis under the action of cellulolytic enzymes.

Determination of Cellulolytic Enzyme Activity. The activity of cellulolytic enzymes was determined using a slightly modified Mandels & Weber method, based on measuring the amount of reducing products formed as a result of enzyme action on specific substrates (Na-CMC and filter paper).

Procedure. A mixture of substrate and enzyme (culture liquid) was incubated in 15–20 mL test tubes at 50°C in a water bath. After incubation, 1.0 mL of Somogyi reagent was added. The reaction mixture was then heated in a boiling water bath for 20 minutes, followed by cooling with cold water. Subsequently, 1.0 mL of Nelson reagent was added and mixed thoroughly. Each sample was transferred into a 25 mL volumetric flask and diluted to volume with distilled water. For the control, 1.0 mL of sodium acetate buffer (0.1 M, pH 5.5) was used.

The optical density of the reducing substances was measured at a wavelength of 610 nm using a Shimadzu UV-1800 spectrophotometer. The concentration of reducing sugars was determined based on a calibration curve constructed using glucose as a standard. One unit of cellulase activity was defined as the amount of enzyme required to produce 1 mg of reducing substances (expressed as glucose equivalents) under the experimental conditions.

The activity of exo-1,4-β-glucanase was determined using filter paper as a substrate. The filter paper was cut into 2–3 mm fragments, and 50 mg was accurately weighed and incubated with the enzyme at 50°C and pH 5.5 for 1 hour. Enzyme activity was then calculated based on the amount of glucose released.

The activity of endo-1,4-β-glucanase was determined by measuring the amount of reducing sugars formed in a reaction mixture containing 0.5 mL of 1.0% Na-CMC (degree of substitution – 0.86, degree of polymerization – 800) prepared in 0.1 M sodium acetate buffer (pH 5.5) and 0.5 mL of culture liquid. The mixture was incubated at 50°C, and the released reducing sugars were quantified. One unit of endo-1,4-β-glucanase activity was defined as the amount of enzyme that produces 1.0 mg of glucose under the conditions of 50°C and pH 5.5 within 30 minutes [12].

Determination of Xylanase Activity. Xylan obtained from the core of corn cobs was used as a substrate. Xylanase activity was determined based on the enzyme's action on a 1% xylan solution. The amount of reducing substances formed was measured using the Somogyi–Nelson method. A calibration curve was constructed using xylose as a standard. One unit of xylanase activity was defined as the amount of enzyme required to produce 1 mg of xylose under the experimental conditions [11].

Results and Discussion

Analysis of Morphological and Cultural Characteristics of Isolated Fungi. At present, micromycetes belonging to various taxonomic groups of fungi, particularly the genera *Trichoderma*, *Penicillium*, and *Chaetomium*, are widely distributed in nature and are distinguished by their high enzymatic activity. As with all microorganisms, the proper progression of physiological and biochemical processes in these fungi depends on the selection of optimal environmental conditions. Therefore, in the course of this study, optimal growth conditions were established for the isolated strains, and their morphological as well as biological characteristics were investigated.

To determine the structural and biological characteristics of the studied strains, they were analyzed using light microscopy. According to the results, colonies of the *Trichoderma asperellum* strain were initially white, gradually turning light green and subsequently dark green during incubation. The mycelium was hyaline, with a cotton-like or spider web–like appearance, and was characterized by rapid growth.

The conidiophores were tree-like and regularly branched, bearing flask-shaped phialides (2–3 μm in width and 5–7 μm in length), typically arranged in groups of 2–5 on the branches. The conidia were green, aggregated in mucilaginous heads, and exhibited a spherical to slightly ellipsoidal shape (2.5–3.2 × 2.7–3.0 μm). They contributed to the formation of a dark green coloration and, as a result of active sporulation, promoted the development of aerial hyphae.

Colonies of the *Penicillium canescens* strain were found to be typically light green to grayish-green in color, with a velvety or powdery surface appearance. The mycelium was hyaline, well developed, and densely spread over the substrate surface.

The conidiophores were branched and exhibited a characteristic three-part (penicillus-type) structure, consisting of sequentially arranged metulae and phialides. The phialides were cylindrical or slightly flask-shaped (2–3 μm in width and 6–9 μm in length), typically arranged in groups of 3–6 on the metulae. The conidia formed through these structures were arranged in chains, with a spherical to ellipsoidal shape (2.0–3.0 μm), imparting a powdery appearance to the colony.

Colonies of the *Chaetomium globosum* strain were initially white, gradually turning yellowish or grayish during incubation. The mycelium was dense and cottony in structure.

In this fungus, classical conidiophores were poorly developed, and reproduction predominantly occurred through the formation of perithecial-type fruiting bodies. The perithecia were spherical to oval in shape, with surfaces covered by hair-like structures (setae). Within these structures, asci were formed, in which ascospores developed. The ascospores were lemon-shaped or ellipsoidal, dark in color, and were released into the environment upon maturation.

Based on morphological and microscopic characteristics, the studied strains were clearly distinguished from one another. *Trichoderma asperellum* was characterized by its rapid growth and green-spored structure; *Penicillium canescens* by its brush-like conidiophores and chain-forming conidia; and *Chaetomium globosum* by its ability to form perithecia. These features not only confirm their taxonomic identity but also substantiate their potential applications in biotechnological processes (Figure 1).

As a result of studying the morphological and cultural characteristics, fungi belonging to the genera *Trichoderma* and *Chaetomium globosum* were identified among the isolates obtained during the study. These findings were confirmed based on their colonial, microscopic, and sporulation features.

According to modern classification, fungi of the genus *Trichoderma* belong to the phylum *Ascomycota*, class *Sordariomycetes*, order *Hypocreales*, and family *Hypocreaceae*.

Representatives of this genus are characterized by rapid growth, the formation of green-colored spores, and high enzymatic activity.

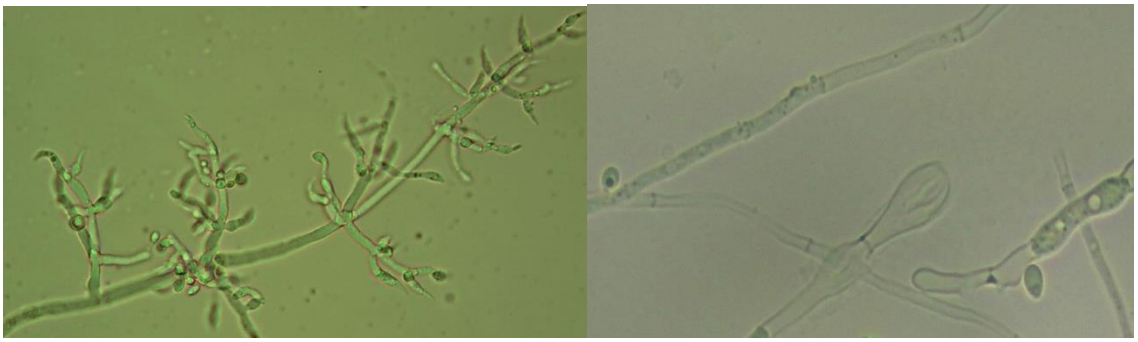


Figure 1. Light microscopic view of* Trichoderma *sp. S3 and* Chaetomium *sp. S18: 1 – conidia, 2 – phialide, 3 – conidiophore (Microscope: Leica DM 750; magnification 400×).

Chaetomium globosum belongs to the phylum *Ascomycota*, class *Sordariomycetes*, order *Sordariales*, and family *Chaetomiaceae*, and is distinguished by the formation of perithecial-type fruiting bodies. These fungi are notable for their ability to degrade lignocellulosic substrates and for their high biomass production capacity.

The morphological and cultural characteristics of the isolated strains—including growth rate, colony color and appearance, optimal temperature and pH values, as well as growth behavior on various solid agar media (Czapek, Mandels, potato dextrose agar, Sabouraud, and wort agar)—were investigated. Based on the experimental results, the optimal growth conditions were determined to be within the temperature range of 29–33°C and a pH range of 5–6.

During the study, different plant residues (wheat straw, wheat bran, other lignocellulosic substrates, and filter paper) were added to Mandels medium at a concentration of 2% as carbon sources. The rate of colony formation and the degree of substrate utilization by the fungal strains were subsequently evaluated.

According to the results, *Trichoderma* strains were distinguished by rapid and intensive growth on almost all culture media tested. The colonies typically developed from light green to dark green in color, exhibiting a granular and highly sporulating structure. In the central part of the colony, the mycelium was dense, cottony, and spider web–like in appearance, while increased sporulation over time resulted in a darker coloration.

In contrast, *Chaetomium* sp. strains formed relatively dense colonies with yellowish-brown to olive coloration. Although the mycelium was well developed, sporulation was comparatively less pronounced. These fungi were characterized by their ability to uniformly cover the substrate surface and their tendency toward substantial biomass production.

The results of the study demonstrated that the isolated fungi possess the ability to modify the structure of substrates prepared from various plant residues and effectively utilize them, actively participating in the hydrolysis of lignocellulosic compounds. In particular, *Trichoderma* strains were found to intensively synthesize cellulase enzyme complexes and rapidly degrade substrates, indicating their high efficiency in bioconversion processes.

It was also observed that, under identical temperature conditions, the growth rate of the fungi varied across different culture media. This variation is primarily associated with differences in the chemical composition of the substrates and the availability of carbon sources (Figure 2).

During the study, the macromorphological and cultural characteristics of the isolated micromycete strains were examined by cultivation in Petri dishes. The results showed that the isolates differed significantly in terms of colony color, shape, structure, degree of sporulation, and

radial growth rate. This diversity indicates that the isolated microorganisms belong to different taxonomic groups and exhibit distinct physiological characteristics.

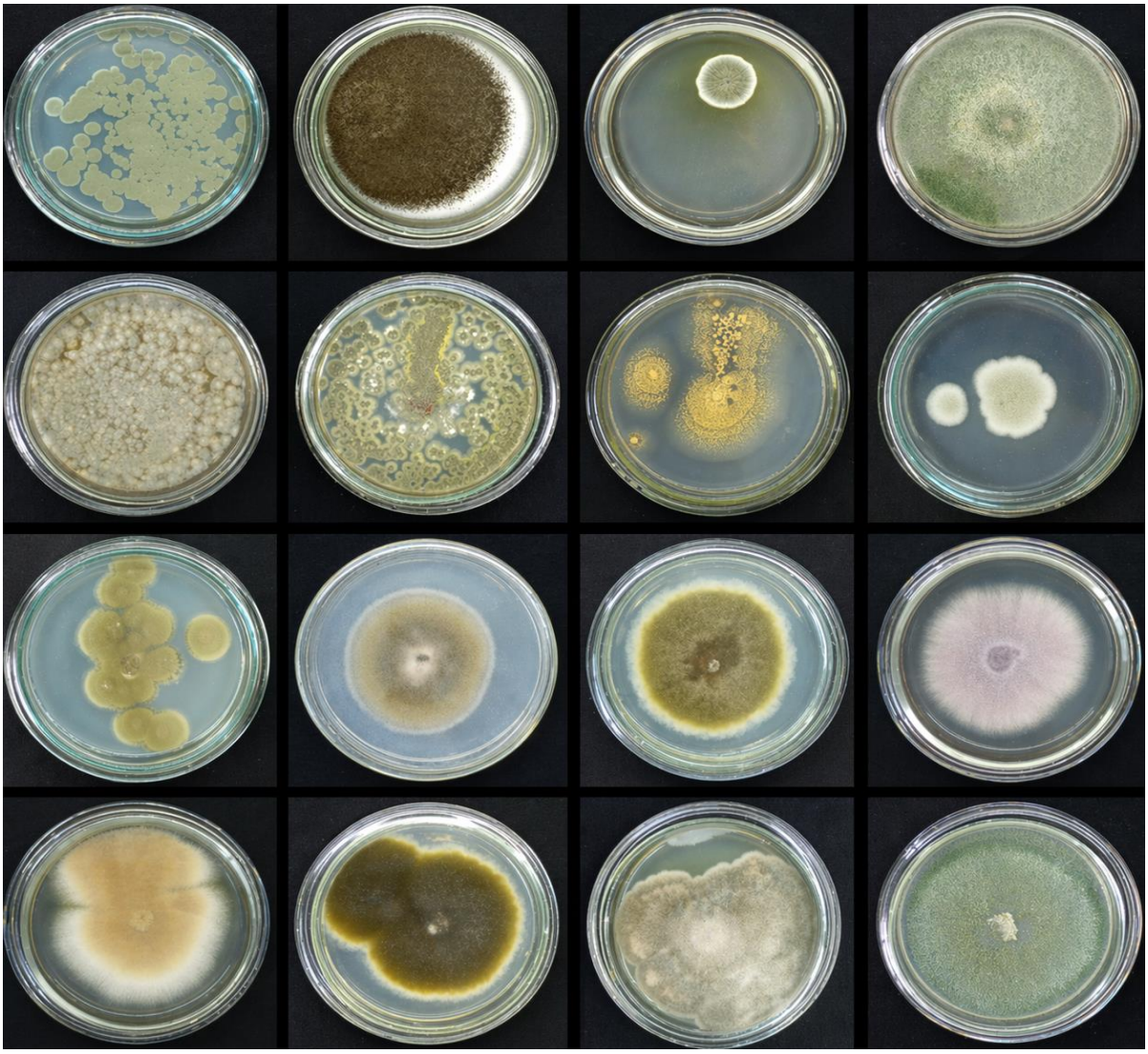


Figure 2. Colonies of the isolated micromycete isolates on Mandels medium (n = 5).

The green to dark green, powdery-textured, and rapidly growing colonies observed in the figure are characteristic of fungi belonging to the genus *Trichoderma*. Such colonies are distinguished by a high level of sporulation, rapid radial expansion from the center toward the periphery, and a granular surface structure. These features indicate their high cellulolytic activity and their ability to efficiently degrade lignocellulosic substrates.

In some colonies, yellowish or golden pigmentation, as well as concentric ring-like growth radiating from the center, was observed. These features may be characteristic of fungi belonging to the genera *Aspergillus* and *Penicillium*. These fungi are also capable of synthesizing cellulolytic enzymes and play an important role in biotechnological processes.

In addition, white, cottony or fluffy colonies exhibiting rapid growth but relatively low sporulation were also observed. Such characteristics may be typical of fungi belonging to the genera *Mucor* or *Rhizopus*, or may correspond to young colonies that have not yet reached the sporulation stage.

Overall, among the isolated micromycetes, fungi belonging to the genus *Trichoderma* were distinguished by their rapid growth and intensive sporulation, demonstrating their potential as promising producers for the bioconversion of cellulosic substrates. At the same time, fungi such

as *Chaetomium globosum* are of particular importance due to their capacity for substantial biomass production. It should be noted that these identifications were primarily based on macromorphological characteristics.

Potato dextrose agar (PDA), Czapek, and wort agar media were found to be favorable for the growth of *Trichoderma* sp. and *Chaetomium globosum* strains isolated in this study. On these media, the fungi exhibited rapid growth and formed circular colonies within a short period. In *Trichoderma* strains, colonies were predominantly light green, with a gradual transition to darker green shades during sporulation. On potato dextrose agar, initially white mycelia were formed, which later developed a green coloration.

On Mandels medium, *Trichoderma* strains formed uniformly growing, cottony colonies, and by 72 hours of incubation, granular mycelial structures appeared in the central region. The colony density on this medium was high, and overall development was more pronounced compared to other media. The colonies were initially whitish, gradually changing to light green and then dark green over time.

On Czapek medium, the growth of *Trichoderma* strains was relatively slower; colonies were initially white and later turned light green, with the mycelium forming ring-like structures.

In contrast, *Chaetomium globosum* strains produced relatively dense colonies with yellowish-brown to olive coloration. These fungi were characterized by well-developed mycelia that uniformly covered the substrate surface. Although sporulation was less pronounced compared to *Trichoderma*, a higher capacity for biomass production was observed.

In experiments conducted on Mandels medium supplemented with 2% plant residues (wheat straw, wheat bran, and filter paper), *Trichoderma* strains exhibited very rapid growth, covering the entire surface of the Petri dish within 72 hours of incubation. The colonies were circular, with a uniform granular and segmented structure, and a dense accumulation of mycelium was observed in the central region. By 96 hours, the colonies had developed a completely dark green coloration.

On the medium supplemented with wheat bran, the formation of light yellow mycelium in the central part of the colony was observed, indicating the influence of substrate composition on fungal metabolism.

It was also determined that the growth rate of fungal colonies varied depending on the type of culture medium, with significantly accelerated radial growth observed in variants where additional carbon sources were incorporated into Mandels medium. During the experiments, the strains were cultivated at 29–32°C, and the colony radius was measured every 24 hours.

The results demonstrated that *Trichoderma* strains exhibited a high growth rate, covering the entire surface of the Petri dish within 72 hours. In media supplemented with plant residues, the colony diameter reached up to 90 mm, whereas in the control variant it was 79 mm. This confirms that lignocellulosic substrates serve as important stimulatory factors for fungal growth. At the same time, although *Chaetomium globosum* strains showed relatively slower growth, they were distinguished by their high biomass production capacity.

In media supplemented with corn stalks and wheat bran, it was observed as early as the second day of incubation that the radial growth rate of colonies was higher compared to other variants. In *Trichoderma* strains, the growth rate reached 0.82 mm/h in corn stalk–based medium and 0.87 mm/h in wheat bran–supplemented medium. In contrast, the *Chaetomium globosum* strain exhibited growth rates of 0.87 mm/h in corn stalk–based medium and 0.92 mm/h in wheat bran–supplemented medium.

At the same time, *Chaetomium globosum* strains exhibited more intensive growth, with radial growth rates higher than those of *Trichoderma*. This indicates a strong capacity of this fungus for the effective utilization of lignocellulosic substrates and for substantial biomass production.

In addition, relatively high growth rates were also recorded for both fungal strains on media supplemented with wheat straw; however, these values were slightly lower compared to those observed in media based on corn stalks and wheat bran.

The obtained results confirm that the chemical composition of lignocellulosic substrates directly influences the growth rate of fungi. In particular, the faster growth and higher biomass production of *Chaetomium globosum* highlight its importance as a key organism in substrate bioconversion processes, while *Trichoderma* strains are distinguished by their high enzymatic activity.

Molecular Identification of the Selected Active Strains. Many taxa of micromycetes have traditionally been identified based on morphological criteria. To confirm or refute these identifications, nucleotide sequencing of specific genes has been employed. Therefore, genetic analysis is used as a complementary approach to morphological diagnostics of species.

As indicated in the literature review, it is often difficult to distinguish certain fungal species based solely on biological characteristics. For this reason, the genetic concept of phylogenetic species recognition (GCPSR), based on DNA sequence data, has been widely accepted as an effective method to complement or verify the results obtained through morphological approaches in species identification.

In our study, sequencing of the ITS1–ITS4 rRNA region of the active strain revealed the following nucleotide sequence.

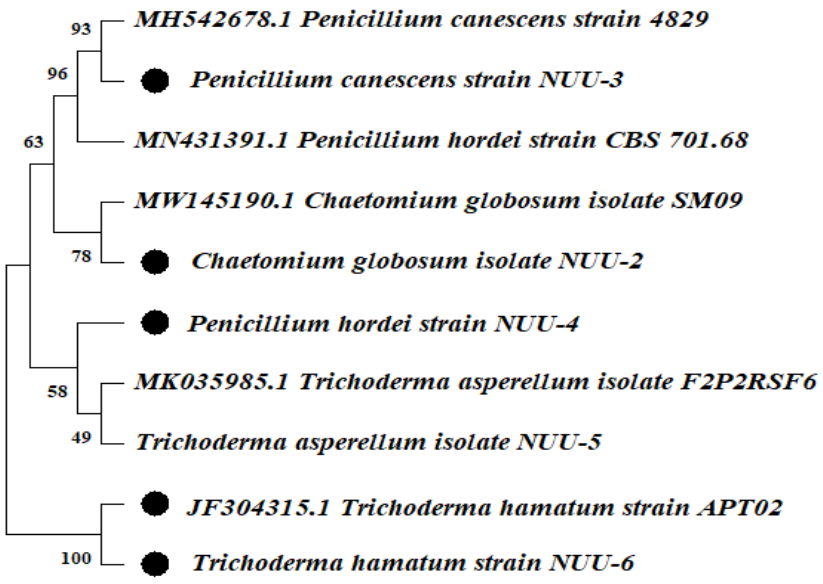


Figure 3. Phylogenetic tree constructed for the active strains.

Constructing a phylogenetic tree based on the analysis of multiple, independent genes is considered the most reliable approach for accurately determining the phylogenetic position of organisms. Based on the obtained nucleotide sequences, a phylogenetic tree of the studied strain was constructed (Figure 3).

Heterokaryosis plays a significant role in the life cycle of fungi. Many species possess haploid nuclei, allowing both dominant and recessive genes to be subject to selection, as recessive genes are not masked by dominant alleles. However, in heterokaryotic cells containing both dominant and recessive alleles of a gene, only the dominant phenotype is expressed, while the recessive allele remains unexpressed. Thus, heterokaryosis resembles a diploid condition in maintaining hidden genetic variability.

For all living organisms in nature, including micromycetes, the proper progression of biochemical and physiological processes requires the selection of an optimal nutrient medium and suitable growth conditions. In order to determine optimal culture conditions and to reduce the economic cost of the medium, a series of experiments was conducted. The strains were cultivated on several types of media, and it was established that Mandels medium supported the most rapid growth. To make the medium more cost-effective, a modified formulation was prepared by omitting peptone, urea, and sucrose, and instead incorporating 2% cellulosic substrates as the primary carbon source (Figure 4).

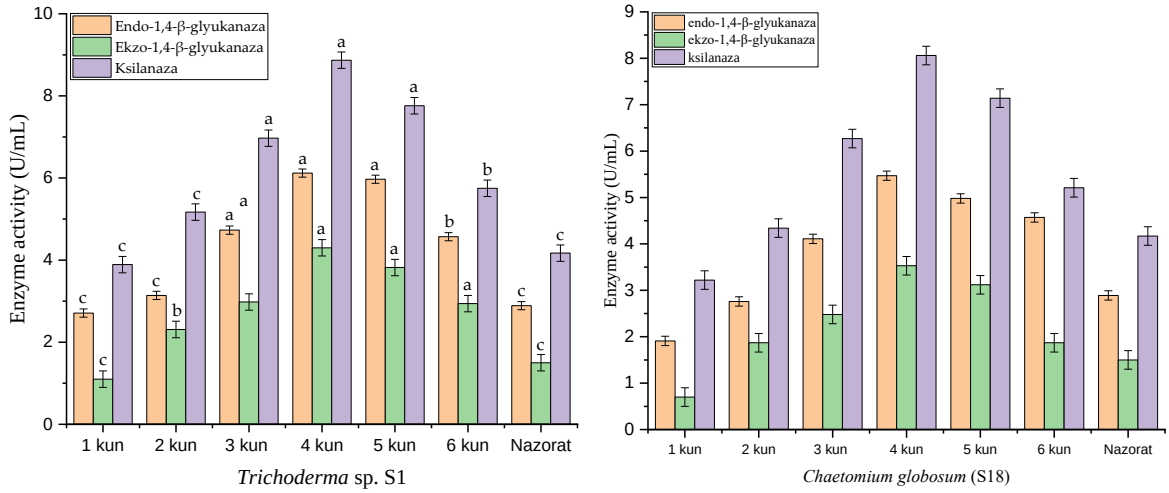


Figure 4. Enzymatic activity of* Trichoderma *sp. S1 and* Chaetomium globosum *(S18).

The results of the study revealed dynamic changes in the activity of cellulolytic enzymes—endo-1,4-β-glucanase, exo-1,4-β-glucanase, and xylanase—in *Trichoderma* sp. S1 and *Chaetomium globosum* (S18) strains during incubation. In both strains, enzyme activity increased from the initial days, reached a maximum on the 4th day, and then gradually declined on days 5–6. In *Trichoderma* sp. S1, endo-1,4-β-glucanase activity increased from 2.71 U/mL on day 1 to 6.12 U/mL on day 4, followed by a decrease to 5.97 U/mL on day 5 and 4.57 U/mL on day 6. Exo-1,4-β-glucanase exhibited a similar pattern, rising from 1.1 U/mL to 4.3 U/mL and then declining to 3.82 and 2.94 U/mL in subsequent days. Xylanase activity showed the highest increase, rising from 3.89 U/mL to 8.87 U/mL on day 4, and then decreasing to 7.76 and 5.75 U/mL. In the control variant, enzyme activities remained comparatively low (2.89, 1.5, and 4.17 U/mL, respectively), indicating that the addition of substrates stimulates enzyme synthesis. In the *Chaetomium globosum* (S18) strain, enzyme activity also exhibited an increasing trend, with maximum values recorded on day 4. Endo-1,4-β-glucanase activity increased from 1.91 U/mL to 5.47 U/mL, exo-1,4-β-glucanase from 0.7 U/mL to 3.53 U/mL, and xylanase from 3.22 U/mL to 8.06 U/mL. A decline in enzyme activity was observed on days 5–6, which can be attributed to the depletion of readily degradable components in the substrate.

A comparative analysis of the two strains showed that *Trichoderma* sp. S1 exhibited higher activity across all enzymes, with a particularly pronounced advantage in cellulase complex enzymes, including endo- and exo-1,4-β-glucanases. At the same time, *Chaetomium globosum* demonstrated a high level of xylanase production, indicating its ability to degrade the hemicellulose fraction of lignocellulosic substrates. Overall, the optimal incubation period for enzyme synthesis was determined to be 4 days, with *Trichoderma* sp. S1 being more efficient in cellulose degradation, while *Chaetomium globosum* was more effective in the utilization of hemicellulosic components. To determine the degree of hydrolysis of cellulosic substrates, experiments were conducted under solid-state fermentation conditions. During the fermentation process, *Trichoderma asperellum*, *Chaetomium globosum*, and *Penicillium canescens* strains were used as producers. Spore–mycelial suspensions of these micromycetes were inoculated into the prepared substrates, and the fermentation process was monitored over a period of 15 days.

Filamentous fungi are well adapted to growth in solid-state systems due to their morphological and physiological characteristics. They are distinguished by apical growth, tolerance to low moisture conditions, aerobic metabolism, and the ability to synthesize a rich complex of cellulolytic and hemicellulolytic enzymes. These features enable them to efficiently degrade lignocellulosic substrates.

Table 3. Enzymatic activity of the isolated strain on various cellulosic substrates (n = 6).

№	Exo-1,4-β-glucanase activity (U/mL)			Endo-1,4-β-glucanase activity, U/mL			Xylanase activity, U/mL		
days	*Trichoderma asperellum*	*Penicillium canescens*	*Chaetomium globosum*	*Trichoderma asperellum*	*Penicillium canescens*	*Chaetomium globosum*	*Trichoderma asperellum*	*Penicillium canescens*	*Chaetomium globosum*
1	16,82±0,53	23,72±0,94	14,92±0,53	24,42±0,61	32,02±0,90	22,82±0,55	34,02±1,41	47,82±2,15	27,62±1,29
2	25,32±1,05	31,32±0,69	17,82±0,38	40,12±1,80	58,22±2,14	31,12±1,42	49,62±1,88	67,82±2,04	38,62±1,83
3	37,82±1,18	32,32±1,50	26,32±0,86	54,62±1,69	64,12±3,08	37,32±1,44	69,12±1,20	77,02±2,65	51,42±1,65
4	43,82±1,25	49,32±1,85	37,82±0,90	63,52±2,85	69,82±2,54	42,62±1,79	81,42±2,74	85,62±2,29	68,92±2,48
5	49,82±1,99	51,82±1,70	38,52±1,90	67,42±2,60	72,82±3,17	59,32±1,53	77,72±2,40	81,52±3,63	62,52±2,12
6	46,42±1,37	46,32±1,96	35,02±1,41	59,52±2,38	67,42±3,03	56,52±2,23	71,92±3,37	76,32±3,55	59,92±2,40
7	43,42±1,69	41,42±1,85	36,62±1,25	57,82±2,88	63,22±2,23	51,62±0,91	76,52±3,58	75,92±3,26	55,12±1,81
8	39,82±1,83	37,92±1,50	34,92±0,89	61,52±1,69	65,62±1,63	42,42±0,88	79,52±3,13	81,52±2,83	63,52±1,35
9	45,02±1,30	45,02±1,31	34,02±0,99	64,92±1,67	70,52±3,12	51,82±2,32	84,92±2,43	87,62±2,48	72,82±1,55
10	47,82±1,93	53,82±1,70	41,82±1,09	68,72±1,47	79,12±1,53	57,92±2,00	74,62±2,01	78,62±1,86	66,12±2,13
11	41,72±2,03	47,02±2,12	31,62±1,54	52,52±2,57	63,72±3,09	54,22±2,09	62,82±2,81	75,82±2,94	60,22±2,74
12	32,82±0,71	36,52±1,51	21,32±0,76	43,52±2,03	56,52±2,55	44,82±1,83	56,52±2,18	65,92±2,30	53,12±1,82
13	30,32±1,08	24,82±1,13	18,32±0,78	34,82±1,70	47,42±2,27	40,12±1,68	49,02±1,98	59,22±2,71	47,52±0,91
14	25,42±1,00	22,62±0,46	18,82±0,77	28,62±1,19	40,02±1,18	27,92±0,98	45,92±2,08	50,02±2,34	38,72±1,15
15	22,82±0,63	19,82±0,36	16,82±0,81	27,82±1,03	27,22±0,71	24,12±0,70	36,42±1,19	41,62±1,48	24,92±0,67
Control	17,82±0,83	22,32±0,88	16,12±0,64	24,12±0,80	28,52±0,84	18,52±0,89	28,12±1,02	31,52±0,70	23,92±1,05

During solid-state fermentation, key factors influencing fungal growth and enzymatic activity included temperature, medium acidity (pH), and substrate moisture content. These parameters were maintained at optimal levels, and fermentation efficiency was evaluated based on the amount of reducing sugars accumulated in the fermentolysate as well as enzyme activity.

The results demonstrated that all strains were capable of degrading both cellulose and hemicellulose. Enzyme activity increased over time, reaching maximum levels on days 4–5, followed by a subsequent decline. In terms of enzymatic activity, the *Penicillium canescens* strain exhibited the highest performance, particularly in the synthesis of xylanase and endo-1,4-β-glucanase enzymes. The *Trichoderma asperellum* strain was distinguished by the stability and complexity of its enzymatic activity, demonstrating the ability to efficiently degrade both cellulose and hemicellulose simultaneously. Although the enzymatic activity of the *Chaetomium globosum*

strain was relatively lower, it is notable for its strong capacity to utilize lignocellulosic substrates and its high biomass production. In addition, the studied *Trichoderma hamatum* strain exhibited a moderate level of enzymatic activity and, therefore, was not selected for further investigations.

The table above presents the dynamics of cellulolytic enzyme activities—exo-1,4-β-glucanase, endo-1,4-β-glucanase, and xylanase—in *Trichoderma asperellum*, *Penicillium canescens*, and *Chaetomium globosum* strains over a 15-day period. The obtained results indicate that enzyme synthesis in all three strains follows a time-dependent pattern governed by specific biological regularities. According to the analysis, enzyme activity in all strains was relatively low during the initial stage of incubation (days 1–3), which can be attributed to the adaptation phase (lag phase) of microorganisms to the substrate. A sharp increase in enzyme activity was observed between days 3 and 5, corresponding to the active growth (log phase). Notably, maximum or near-maximum values for all enzymes were recorded during days 4–5. In terms of exo-1,4-β-glucanase activity, *Penicillium canescens* demonstrated superior performance. In this strain, enzyme activity increased from 23.72 ± 0.94 U/mL on day 1 to 51.82 ± 1.70 U/mL on day 5, followed by a gradual decline to 19.82 ± 0.36 U/mL by day 15. In *Trichoderma asperellum*, the maximum value reached 49.82 ± 1.99 U/mL on day 5, whereas in *Chaetomium globosum* it was 38.52 ± 1.90 U/mL. These results indicate that *Penicillium canescens* has a higher capacity to degrade the crystalline structure of cellulose. Similarly, for endo-1,4-β-glucanase activity, the highest values were also recorded in *Penicillium canescens*, reaching 72.82 ± 3.17 U/mL on day 5. In comparison, the maximum activity of this enzyme in *Trichoderma asperellum* was 67.42 ± 2.60 U/mL, while in *Chaetomium globosum* it reached 59.32 ± 1.53 U/mL. This further confirms the high efficiency of *Penicillium canescens* in cleaving internal bonds within the cellulose polymer chain. Xylanase activity was also observed at high levels in all strains. The highest value was recorded in *Penicillium canescens* on day 9, reaching 87.62 ± 2.48 U/mL. In *Trichoderma asperellum*, the maximum activity was 84.92 ± 2.43 U/mL (day 9), while in *Chaetomium globosum* it reached 72.82 ± 1.55 U/mL. These results indicate that *Penicillium canescens* is also a leading producer of enzymes involved in the degradation of hemicellulose components. From day 6 onward, a general decline in enzyme activity was observed in all strains, which can be explained by the depletion of easily degradable substrate components, accumulation of metabolic by-products, and the transition to the stationary phase of growth. In the control variant, the low levels of enzyme activity for all enzymes (exo: 16.12–22.32; endo: 18.52–28.52; xylanase: 23.92–31.52 U/mL) indicate that enzyme synthesis proceeds very weakly in the absence of fungal involvement. The obtained results demonstrate that *Penicillium canescens* exhibited the highest overall enzymatic activity, while *Trichoderma asperellum* was distinguished by stable and high-level synthesis of a комплекс of enzymes. Although *Chaetomium globosum* showed relatively lower enzyme activity, it remains significant due to its other biotechnological properties, particularly its high biomass production capacity. Thus, the combined application of these strains can ensure efficient degradation of lignocellulosic substrates and enhance the overall efficiency of the bioconversion process.

Conclusion. The results of this study demonstrate that cellulase-producing micromycetes isolated from natural sources possess significant potential for the bioconversion of lignocellulosic plant residues. Among the studied strains, *Penicillium canescens* showed the highest overall enzymatic activity, particularly in exo-1,4-β-glucanase, endo-1,4-β-glucanase, and xylanase production. *Trichoderma asperellum* also demonstrated stable and intensive synthesis of cellulolytic enzymes, while *Chaetomium globosum* was distinguished by its effective utilization of substrates and high biomass-forming capacity. The findings confirm that plant residues such as

wheat straw, wheat bran, and corn stalk can serve as effective substrates for fungal growth and enzyme production under solid-state fermentation conditions. Enzyme activity generally increased during the early stages of incubation, reached maximum levels around days 4–5, and then gradually declined. Overall, the selected micromycete strains may be considered promising biological agents for the environmentally friendly processing of agricultural residues, production of enzyme preparations, and development of biofeed technologies.

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ANALYSIS OF PROTEIN CONTENT OF SALOMALAIKUM (Cyperus *rotundus L.)*

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Abstract*. Cyperus rotundus L. is a perennial plant widely distributed in tropical and subtropical regions and traditionally used in folk medicine. Although its pharmacological properties have been extensively studied, limited scientific information is available on the nutritional characteristics of its roots, particularly their protein content. This study aimed to determine the crude protein content of Cyperus rotundus L. roots collected from the Andijan region using the Kjeldahl nitrogen determination method. Root samples were collected, air-dried, finely ground, and analyzed for total nitrogen content. Crude protein content was calculated using the conventional conversion factor of N × 6.25. The results showed that the total nitrogen content of the root samples was 1.24%, corresponding to a crude protein content of 7.89%. These findings indicate that Cyperus rotundus L. roots contain an appreciable amount of protein and may have nutritional and functional value. The study contributes to regional data on the biochemical composition of C. rotundus and provides a basis for further research on its potential use in food, feed, or pharmaceutical applications.*

Keywords*: crude protein, Cyperus rotundus L., Kjeldahl method, nitrogen content, Andijan region.*

INTRODUCTION

C. rotundus (nutgrass or purple nutsedge) is a perennial herb belonging to *the Cyperaceae family, which grows in many tropical and subtropical regions. This plant is* widely used in traditional medicine for the treatment of gastrointestinal, inflammatory and other diseases , and also contains bioactive components with nutritional and pharmacological value. Scientific data on the chemical composition of *C. rotundus* , in particular the amount of proteins and their nutritional analysis, have not been studied in sufficient depth, which makes it urgent to determine the nutritional and biochemical potential of this plant. Proteins are the main structural and functional molecules of plant cells and play an important role in supporting biological activity and growth processes. Therefore, analysis of the protein composition of *C. rotundus* and its evaluation based on scientific literature play an important role in determining the potential nutritional value of this plant [1].

LITERATURE ANALYSIS AND METHODOLOGY

1. General chemical composition of *Cyperus rotundus* .

A number of review articles report that *C. rotundus* contains over 200 bioactive compounds, including flavonoids, phenolic compounds, terpenoids, alkaloids, and other metabolites, which are believed to underlie its pharmacological and therapeutic activities.

Also, reviews examining the ethnobotanical and pharmacological data of *C. rotundus* have highlighted the plant's presence of protein and soluble amino acids, sugars, and other nutritional components.

2. Proximate analysis and protein content studies.

Various international studies have conducted proximate (i.e., primary nutritional) analyses of *C. rotundus* . *For example,* in a study by Emelugo et al ., the crude protein content of *C. rotundus* round roots was found to be 1.75±0.55% in a proximate analysis . This result was presented along with other nutritional components.

Another study reported that the protein content of *C. rotundus* root powder was 6.17% . It is cautioned that these values may vary depending on the plant's growing conditions, collection, and analysis methods.

Recently published studies have shown that the crude protein content of *C. rotundus varies between* 10.51 and 14.40% in liquid and dry matter at different growth stages , further illuminating the biological and nutritional value of this plant.

3. Methodological approaches to protein analysis.

Various methods are used to determine protein content: the Keldahl method, Lowry or Bradford spectrophotometric methods, etc. The study of protein content is especially important in the evaluation of plant biomass components, analyzing their potential use as animal feed or pharmacological preparations. In the scientific literature, these methods are described in standard analytical data for plants such as *C. rotundus* .

4. Differences and explanations noted in the literature.

C. rotundus samples grown in different geographical areas . This may be due to genetic variation of the plant, soil conditions, and climate changes. Therefore, further studies on samples from different regions are needed.

Determination of total protein. The method consists in determining nitrogen by the Kjeldahl method, followed by conversion to protein. The essence of the method is the decomposition of organic substances in the sample with the formation of ammonium salts when boiling with concentrated sulfuric acid, the conversion of ammonium to ammonia, its distillation into an acid solution, the quantitative accounting of ammonia by the titrimetric method. and calculation of the nitrogen content in the material under study [7]

A precise sample from the average crushed homogeneous sample of the test sample was weighed into a test tube for analysis, with an error of no more than 0.1%. The sample was quantitatively transferred to a Kjeldahl flask. Further experiments were carried out in accordance with the methodological instructions [8].

Processing of results: the mass fraction of nitrogen (X) in the test sample was calculated by the formula in percentage relative to its mass during the distillation of ammonia into sulfuric acid.

$$X = \frac{(V_1 - V_0) \times K \times 0{,}0014 \times 100}{M},$$

Here; Vo is the volume of 0.1 mol/l sodium hydroxide solution used to titrate 0.05 mol/l sulfuric acid in the control experiment, ml.

V1 – volume of 0.1 mol/l sodium hydroxide solution used to titrate sulfuric acid in the test solution, ml;

K – correction to the titer of 0.1 mol/l sodium hydroxide solution;

0.0014 – the amount of nitrogen equivalent to 1 ml of 0.05 mol/l sulfuric acid solution;

M is the mass of the sample, g. The results were calculated to the third decimal place and rounded to the second decimal place.

The mass fraction of nitrogen in the dry matter of the product (X_3), in percent, was calculated using the following formula:

$$X_3 = \frac{X_1 \times 100}{100 - W},$$

Where; X_1 – mass fraction of nitrogen in the sample being tested, %;

W – moisture content of the test sample, %.

The mass fraction of protein (Y) as a percentage was calculated according to the following formula: Y = K XX, where K is the coefficient of nitrogen transfer to protein: with an average lipid content - 6.38;

RESULTS AND DISCUSSION

Collection of plant samples. *C. rotundus* plants were collected from natural growing areas of Andijan region during the growing season. Plant species identification was performed based on standard botanical keys. Root parts were separated, cleaned of soil residues, and washed with distilled water.

Sample preparation. The washed root samples were dried at room temperature and ground in a laboratory mill to a uniform powder. The prepared samples were stored in airtight containers.

Cyperus rotundus L. root.

Table 1

No.	Sample	Nitrogen (%)	Protein (%)
1	Cyperus rotundus L.	**1.24**	**7.89**

This in research Andijan province under the circumstances from the sky *Cyperus round L.* o ' wire at the root general nitrogen amount Keldal method based on **1.24%** , raw protein amount and classic **N × 6.25** coefficient using **7.89 %** that The results obtained confirm the nutritional and biochemical potential of *C. rotundus* and are somewhat consistent with previously published scientific works.

In the literature *C. rotundus* root and of the rhizomes raw protein amount wide in the range — **from 1.7% to 14%** change record These differences are mainly explained by the fact that they depend on the geographical location of the plant, agroclimatic conditions, soil composition, vegetation stage, and the methodology for preparing and analyzing samples. The protein content of **7.89% determined in the sample from Andijan region** corresponds to the average values of this range, indicating that the plant can synthesize stable protein under regional conditions.

Determined nitrogen amount (**1.24%**) and from it calculated protein share *C. rotundus* at the root not only structural , maybe metabolic active proteins the existence also guess to do opportunity It is known that proteins accumulated in roots and rhizomes are often directly related to enzymatic activity, stress tolerance, and secondary metabolite biosynthesis. In this respect, the results obtained indirectly support previously reported ideas about the pharmacological activity of *C. rotundus* .

It should also be noted that the crude protein content determined by the Keldahl method includes non-protein nitrogenous compounds in addition to the actual proteins in the plant.

However, this method is a widely accepted standard method for proximate analysis of plant raw materials in SCOPUS-indexed journals, and the results are considered reliable enough to be compared with other studies. However, further determination of the amino acid composition or comparison with the Dumas method will allow a more in-depth assessment of the quality parameters of *C. rotundus proteins.*

From a regional perspective, the mineral composition of the soils and climatic conditions of the Andijan region may have a positive effect on nitrogen metabolism in plants. This, in turn, may have led to a relatively high protein synthesis in root tissues. Therefore, the results obtained serve as an important scientific basis for assessing the regional nutritional value of *C. rotundus* .

Overall, the results of this study indicate that the root of *C. rotundus* is a biologically active plant raw material in terms of protein content and substantiate the prospects for its study as a nutritional supplement, phytopreparation or animal feed component. The data obtained complement the existing literature and create a scientific basis for further studies aimed at assessing the chemical composition of *C. rotundus in a regional context.*

CONCLUSION

The crude protein content of the roots of *C. rotundus* L. collected from Andijan region was determined using the Kjeldahl method. According to the analysis results, the total nitrogen content was **1.24%** and the crude protein content was **7.89% .**

The results obtained indicate that the root *of C. rotundus* L. is moderately rich in protein and confirms the biological and nutritional value of the plant. The soil-climatic conditions of the region may have influenced the nitrogen accumulation and protein synthesis in the root tissues.

Overall, the results of this study make an important scientific contribution to the assessment of the chemical composition *of C. rotundus* L. in a regional context and provide a basis for future studies of its amino acid composition, functional properties, and potential applications.

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CONTENT OF PHOTOSYNTHETIC PIGMENTS IN THE LEAVES OF DIFFERENT CURRANT (RIBES AUREUM PURSH) GENOTYPES

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Abstract. *This study examined photosynthetic pigment variation in the leaves of 16 currant (Ribes spp.) genotypes as a physiological indicator of the functional state of the leaf apparatus. The research was motivated by the need to identify genotypes with a comparatively well-developed pigment complex that may support photosynthetic stability under changing environmental conditions. Chlorophyll a, chlorophyll b, and total carotenoids were extracted from fresh leaves with ethyl alcohol and quantified spectrophotometrically at 664, 649, and 470 nm. Pigment concentrations were recalculated on a fresh-weight basis and compared across genotypes. Clear genotypic differences were observed for all three pigment groups. Chlorophyll a ranged from 6.06 to 14.01 mg g-1 FW, chlorophyll b from 2.33 to 8.21 mg g-1 FW, and carotenoids from 6.19 to 16.29 mg g-1 FW. The genotype Do'stlik showed the highest values for chlorophyll a, chlorophyll b, carotenoids, total chlorophyll, and total pigment content. Podarok Ariadne, Duragay 4 (Ravshan), Duragay 7, Uzb sladkaya, and Duragay 9 also showed relatively high pigment values, whereas Ruxshona, Duragay 1, N/3759/6, and Iroda displayed lower pigment accumulation. The findings support the use of chlorophyll and carotenoid content as informative screening indicators in currant physiology, although they should be combined with water-status, gas-exchange, chlorophyll-fluorescence, antioxidant, and productivity measurements for complete stress-tolerance assessment.*

Keywords*: ribes spp., currant, chlorophyll a, chlorophyll b, carotenoids, photosynthetic pigments, genotype screening, leaf physiology, stress tolerance.*

Introduction

Currant (Ribes spp.) is an economically valuable berry crop cultivated for fresh consumption, processing, and the production of nutritionally rich fruit products. The genus includes black currant (Ribes nigrum L.), red currant (Ribes rubrum L.), and related interspecific forms, all of which are important for horticultural diversification in temperate and continental regions. In regions with fluctuating water availability, high summer temperatures, disease pressure, or unstable winter conditions, currant productivity depends strongly on the physiological condition of the leaf apparatus. Leaves are the primary organs in which light energy is captured, converted into chemical energy, and directed toward growth and fruit formation. Therefore, any diagnostic assessment of currant adaptation must consider the functioning of the photosynthetic pigment complex.

Photosynthetic pigments are not merely biochemical compounds present in leaves; they are structural and functional components of photosystems. Chlorophyll a is the principal pigment of

the photochemical reaction centers and participates directly in light energy conversion. Chlorophyll b acts as an accessory light-harvesting pigment, broadening the spectral range of absorbed light and transferring excitation energy to chlorophyll a. Carotenoids perform complementary functions: they contribute to light harvesting, stabilize pigment-protein complexes, and protect the photosynthetic apparatus from oxidative damage by dissipating excess excitation energy. Because chlorophylls and carotenoids are sensitive to temperature, water deficit, leaf age, light intensity, nutrient status, and pathogen stress, their concentrations can be used as diagnostic indicators of plant physiological state.

Previous plant-physiological research has established that stress conditions often reduce photosynthetic pigment content by damaging chloroplast membranes, accelerating chlorophyll degradation, and altering the balance between light capture and photoprotection. Under drought, stomatal closure restricts CO2 uptake, while excess absorbed light may increase reactive oxygen species and trigger pigment loss or photoinhibitory damage. Under high temperature or pathogen pressure, changes in chlorophyll and carotenoid content may similarly reflect disruption of photosystem function. Studies on black and red currant have demonstrated that drought, deficit irrigation, high temperature, low-temperature hardiness, and powdery mildew infection can alter the functional state of the leaf apparatus, making pigment-related and fluorescence-based indicators valuable tools for cultivar evaluation (Brennan & Jefferies, 1990; Čereković et al., 2013, 2014; Li et al., 2024; Panfilova et al., 2017, 2021).

For breeding and horticultural management, a genotype with higher pigment retention is not automatically superior in every agronomic trait, yet a strong pigment complex may indicate better maintenance of light-harvesting capacity and photoprotective potential. This is especially important where growers must select cultivars or hybrid forms for environments characterized by irregular precipitation and summer heat. A simple spectrophotometric assessment of leaf pigment content is relatively accessible, inexpensive, and suitable for comparing many genotypes. When linked to growth, yield, disease response, and water-status data, pigment analysis can contribute to the identification of promising forms for further testing.

The present study focuses on the comparative assessment of chlorophyll a, chlorophyll b, and carotenoid content in leaves of 16 currant genotypes. The central assumption is that genotypic differences in pigment content reflect differences in the physiological state and potential stability of the photosynthetic apparatus. The aim of the study is to determine variation in photosynthetic pigment content among currant genotypes and to evaluate the diagnostic value of these pigments as indicators of the leaf apparatus condition. The study addresses three research questions: (a) How strongly do the studied genotypes differ in chlorophyll a, chlorophyll b, and carotenoid content? (b) Which genotype or group of genotypes has the most developed pigment complex? (c) How can pigment data be interpreted for future physiological screening and selection work in currant?

Materials and Methods

Study Design and Plant Material

The study was organized as a comparative physiological assessment of photosynthetic pigments in currant leaves. Sixteen genotypes were included: Duragay 9, Siyuma, Duragay 1, Duragay 5, Ruxshona, Duragay 7, Nargiz, Uzb sladkaya, Yadgor, Duragay 8, Podarok Ariadne, N/3759/6, Duragay 6, Iroda, Do'stlik, and Duragay 4 (Ravshan). The genotypes represented a set of cultivars, hybrid forms, and breeding materials relevant to currant evaluation. The experimental emphasis was not on fruit quality or yield but on the biochemical condition of leaves as reflected by their photosynthetic pigment content.

Pigment Extraction and Spectrophotometric Determination

Leaf material was sampled from physiologically active leaves. To minimize uncontrolled variation, the same type of leaf tissue was used for pigment extraction across genotypes. Fresh leaves were treated as the analytical unit for biochemical determination. Pigments were extracted with ethyl alcohol, a common solvent for chlorophyll and carotenoid analysis when spectrophotometric quantification is required. The use of an alcoholic extract is consistent with established approaches for determining chlorophylls and carotenoids in plant tissues (Lichtenthaler, 1987; Wellburn, 1994).

The optical density of each extract was measured at 664, 649, and 470 nm using a spectrophotometer. Chlorophyll a, chlorophyll b, and total carotenoids were calculated from absorbance values by equations for 95% ethanol. The formulas used were: Chl a = 13.36A664 - 5.19A649; Chl b = 27.43A649 - 8.12A664; Carotenoids = (1000A470 - 2.13Chl a - 97.63Chl b) / 209. The resulting concentrations were recalculated per unit fresh leaf weight and expressed as mg g-1 fresh weight (FW).

Data Treatment and Reporting Logic

Descriptive statistics were used to compare genotypes. For each pigment, the mean and standard error (M +/- SE) reported in the source measurements were tabulated. Additional derived indicators were calculated for interpretation: total chlorophyll (chlorophyll a + chlorophyll b), total pigment content (chlorophyll a + chlorophyll b + carotenoids), and the chlorophyll a/b ratio. Total chlorophyll was used as an estimate of the overall chlorophyll pool, total pigment content was used as a broad indicator of pigment accumulation, and the a/b ratio was used to characterize the balance between major reaction-center-associated and accessory light-harvesting pigments. Because the available dataset did not include replication-level raw values, inferential statistical tests were not performed. The results are therefore interpreted as comparative descriptive evidence rather than as a hypothesis-tested experimental proof of genotype superiority.

The reporting format follows IMRAD logic. The Methods section specifies the plant material, extraction procedure, absorbance wavelengths, calculation formulas, and descriptive data treatment. The Results section presents the pigment profile and rankings. The Discussion interprets pigment differences in relation to photosynthetic function, stress diagnosis, and future screening. This structure makes the manuscript suitable for journal-style review while preserving the original empirical content of the study.

Results

Table 1. Photosynthetic Pigment Content and Derived Pigment Indicators in the Leaves of 16 Currant Genotypes

No.	Genotype	Chl a M +/- SE	Chl b M +/- SE	Carotenoids M +/- SE	Total Chl	Total Pigments	Chl a/b
1	Duragay 9	10.97 +/- 0.04	4.84 +/- 0.01	15.06 +/- 1.89	15.81	30.87	2.27
2	Siyuma	8.21 +/- 0.15	7.53 +/- 0.02	12.07 +/- 1.82	15.74	27.81	1.09
3	Duragay 1	6.14 +/- 0.90	2.33 +/- 0.03	8.08 +/- 1.34	8.47	16.55	2.64
4	Duragay 5	10.07 +/- 0.12	3.55 +/- 0.09	16.07 +/- 1.14	13.62	29.69	2.84

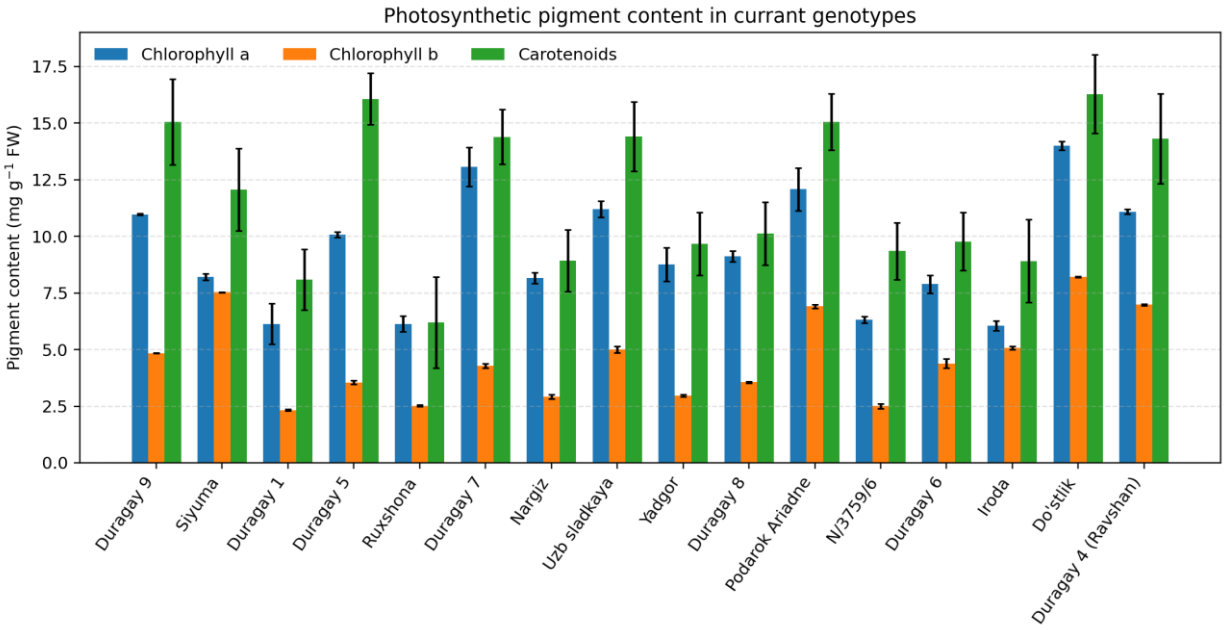
5	Ruxshona	6.14 +/- 0.35	2.52 +/- 0.04	6.19 +/- 2.01	8.66	14.85	2.44
6	Duragay 7	13.07 +/- 0.86	4.28 +/- 0.09	14.39 +/- 1.21	17.35	31.74	3.05
7	Nargiz	8.16 +/- 0.24	2.92 +/- 0.10	8.93 +/- 1.36	11.08	20.01	2.79
8	Uzb sladkaya	11.20 +/- 0.36	5.01 +/- 0.14	14.41 +/- 1.54	16.21	30.62	2.24
9	Yadgor	8.76 +/- 0.75	2.97 +/- 0.04	9.67 +/- 1.38	11.73	21.40	2.95
10	Duragay 8	9.12 +/- 0.25	3.56 +/- 0.03	10.12 +/- 1.39	12.68	22.80	2.56
11	Podarok Ariadne	12.08 +/- 0.94	6.91 +/- 0.08	15.06 +/- 1.24	18.99	34.05	1.75
12	N/3759/6	6.32 +/- 0.15	2.51 +/- 0.11	9.35 +/- 1.26	8.83	18.18	2.52
13	Duragay 6	7.89 +/- 0.39	4.39 +/- 0.21	9.77 +/- 1.28	12.28	22.05	1.80
14	Iroda	6.06 +/- 0.21	5.07 +/- 0.07	8.91 +/- 1.83	11.13	20.04	1.20
15	Do'stlik	14.01 +/- 0.19	8.21 +/- 0.03	16.29 +/- 1.73	22.22	38.51	1.71
16	Duragay 4 (Ravshan)	11.09 +/- 0.11	6.98 +/- 0.04	14.32 +/- 1.98	18.07	32.39	1.59

Note. Values are expressed as mg g-1 fresh weight. Chl = chlorophyll; FW = fresh weight; SE = standard error. Derived values are calculated from the reported means.

The 16 currant genotypes showed clear differences in photosynthetic pigment content (Table 1). Chlorophyll a varied more than twofold, ranging from 6.06 mg g-1 FW in Iroda to 14.01 mg g-1 FW in Do'stlik. Do'stlik therefore had the strongest chlorophyll a accumulation among the studied materials. Other genotypes with relatively high chlorophyll a content included Duragay 7 (13.07 mg g-1 FW), Podarok Ariadne (12.08 mg g-1 FW), Uzb sladkaya (11.20 mg g-1 FW), and Duragay 4 (Ravshan; 11.09 mg g-1 FW). The lowest values were observed in Iroda, Duragay 1, Ruxshona, and N/3759/6, indicating a comparatively weaker chlorophyll a pool in those genotypes.

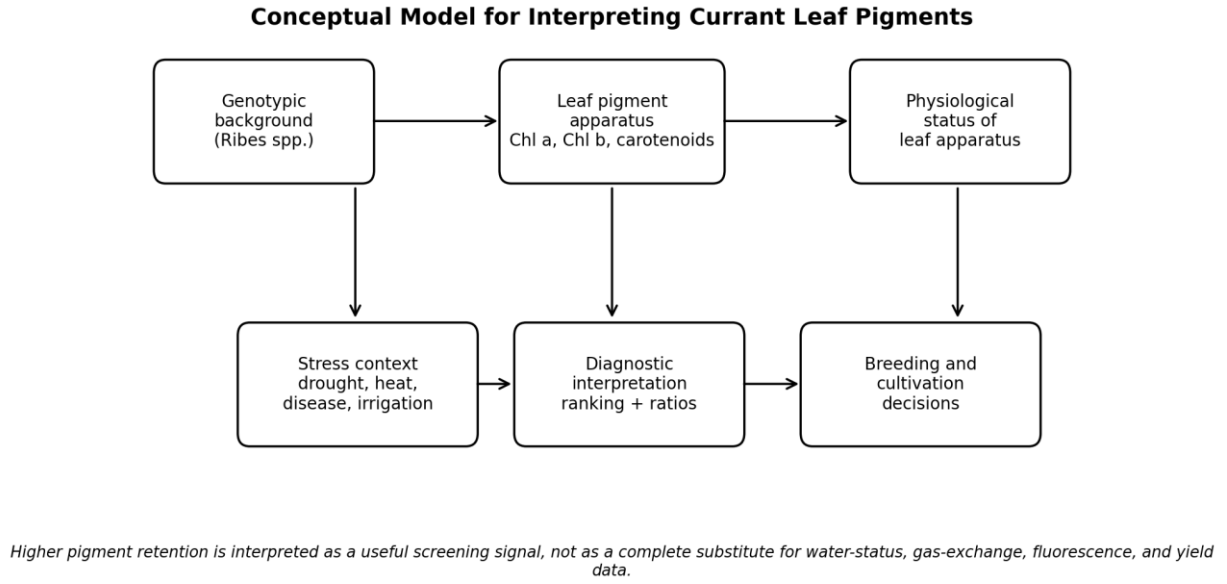
Chlorophyll b content ranged from 2.33 mg g-1 FW in Duragay 1 to 8.21 mg g-1 FW in Do'stlik. High chlorophyll b values were also observed in Siyuma (7.53 mg g-1 FW), Duragay 4 (Ravshan; 6.98 mg g-1 FW), and Podarok Ariadne (6.91 mg g-1 FW). Because chlorophyll b is associated with the light-harvesting complex, these high values suggest a more developed accessory pigment system. In contrast, Duragay 1, N/3759/6, Ruxshona, Nargiz, and Yadgor had lower chlorophyll b concentrations, which may reflect less intensive development of light-harvesting structures.

Figure 1. Grouped Bar Chart of Chlorophyll a, Chlorophyll b, and Carotenoid Content Across Currant Genotypes



Note. Bars represent means and error bars represent standard errors from the source measurements. The chart highlights the consistently high pigment content of Do'stlik and the relatively high profiles of Podarok Ariadne, Duragay 4 (Ravshan), Duragay 7, Uzb sladkaya, and Duragay 9.

Figure 2. Conceptual Interpretation Model for Using Photosynthetic Pigments in Currant Genotype Screening



Note. The model positions pigment content as a diagnostic indicator that should be integrated with additional physiological and agronomic measurements before final conclusions about adaptive potential are made.

Carotenoid content ranged from 6.19 mg g-1 FW in Ruxshona to 16.29 mg g-1 FW in Do'stlik. High carotenoid levels were also found in Duragay 5, Duragay 9, Podarok Ariadne, Uzb sladkaya, and Duragay 7. These genotypes may have a stronger photoprotective pigment component, although carotenoid concentration alone cannot fully determine stress resistance.

Ruxshona was clearly the lowest in carotenoid content, which may indicate reduced photoprotective potential relative to the other genotypes.

The derived total chlorophyll values strengthened the same pattern. Do'stlik had the highest total chlorophyll content (22.22 mg g-1 FW), followed by Podarok Ariadne (18.99 mg g-1 FW), Duragay 4 (Ravshan; 18.07 mg g-1 FW), Duragay 7 (17.35 mg g-1 FW), and Uzb sladkaya (16.21 mg g-1 FW). Total pigment content also placed Do'stlik first (38.51 mg g-1 FW), followed by Podarok Ariadne (34.05 mg g-1 FW), Duragay 4 (Ravshan; 32.39 mg g-1 FW), Duragay 7 (31.74 mg g-1 FW), Duragay 9 (30.87 mg g-1 FW), and Uzb sladkaya (30.62 mg g-1 FW). These results show that Do'stlik did not lead in only one pigment category; it had a consistently strong pigment profile across all measured components.

The grouped chart (Figure 1) visually confirms the strong pigment accumulation of Do'stlik and the relatively high values of Podarok Ariadne, Duragay 4 (Ravshan), Duragay 7, Duragay 9, and Uzb sladkaya. It also shows that pigment profiles were not identical across genotypes. Siyuma had a relatively high chlorophyll b value compared with its chlorophyll a content, whereas Duragay 5 had high carotenoid content with a more moderate chlorophyll b value. Iroda had low chlorophyll a but moderate chlorophyll b. These profile differences suggest that genotype comparison should consider the balance among pigments, not only the absolute value of one pigment.

Discussion

The findings demonstrate substantial genotypic variation in the pigment apparatus of currant leaves. From a physiological perspective, this variation is important because photosynthetic pigments mediate the capture, transfer, and protection of light energy. A genotype with a higher chlorophyll pool may have greater capacity for light absorption and photosynthetic electron transport, provided that other factors such as stomatal conductance, water status, nutrient supply, and photosystem integrity are also favorable. The highest pigment values recorded in Do'stlik indicate that this genotype had the most developed leaf pigment complex under the conditions represented by the sampled material.

Do'stlik was the leading genotype in chlorophyll a, chlorophyll b, carotenoids, total chlorophyll, and total pigment content. This consistency is biologically meaningful. If a genotype were high only in chlorophyll a but low in carotenoids, it might have strong light-harvesting potential but weaker photoprotective capacity. If it were high only in carotenoids but low in chlorophylls, it might show photoprotective investment without high photosynthetic pigment abundance. Do'stlik combined both chlorophyll and carotenoid strength, suggesting a balanced and active pigment apparatus. Such a profile justifies its inclusion among promising genotypes for further physiological and agronomic evaluation.

Several other genotypes also deserve attention. Podarok Ariadne and Duragay 4 (Ravshan) had high chlorophyll b and total chlorophyll content, which may indicate a well-developed light-harvesting complex. Duragay 7 was particularly strong in chlorophyll a and total pigment content. Uzb sladkaya and Duragay 9 also showed favorable pigment accumulation. Duragay 5, although not among the highest in total chlorophyll, had one of the highest carotenoid values, which may reflect relatively strong photoprotective potential. These results suggest that grouping genotypes by overall pigment profile may be more useful than ranking them by a single pigment alone.

The lower pigment values in Ruxshona, Duragay 1, N/3759/6, and Iroda should be interpreted cautiously. Lower chlorophyll or carotenoid content can indicate weaker pigment metabolism, lower photosynthetic potential, stress-related degradation, differences in leaf age, or simply genotype-specific pigment allocation. A low pigment value does not automatically mean

poor yield or poor adaptation. Nevertheless, in screening programs, such genotypes would require additional confirmation through gas exchange, chlorophyll fluorescence, water-potential measurement, antioxidant enzyme activity, proline accumulation, lipid peroxidation, and productivity traits. Without these additional indicators, pigment data should be treated as an informative but partial diagnostic signal.

The chlorophyll a/b ratio provides another interpretive perspective. A higher a/b ratio can be associated with a relatively greater proportion of reaction-center chlorophyll a or a comparatively smaller accessory light-harvesting antenna, whereas a lower ratio may indicate a larger contribution of chlorophyll b. In the present data, genotypes differed not only in total pigment amount but also in pigment balance. For example, Iroda had relatively low chlorophyll a but moderate chlorophyll b, producing a much lower a/b ratio than most other genotypes. This may suggest a different structural organization of the light-harvesting apparatus. Such differences are important because stress responses often alter pigment ratios before strong reductions in total chlorophyll become visible.

The results align with broader evidence that pigment-related traits are useful indicators of leaf physiological condition. Classical methods for chlorophyll and carotenoid quantification have long supported the use of absorbance-based equations in plant physiology (Arnon, 1949; Lichtenthaler, 1987; Wellburn, 1994). In currant specifically, previous studies have linked chlorophyll fluorescence, leaf water regime, drought stress, deficit irrigation, high temperature, and disease responses to the functional state of the leaf apparatus (Brennan & Jefferies, 1990; Čereković et al., 2013, 2014; Li et al., 2024; Panfilova et al., 2017). The present pigment profile therefore fits a larger research direction in which leaf biochemical and optical parameters are used to evaluate cultivar adaptation.

At the same time, this study has limitations that should be acknowledged. First, the available dataset reports means and standard errors but does not include raw replicate-level values; therefore, formal analysis of variance or post hoc comparison was not conducted in this manuscript. Second, pigment content was measured at one point in the physiological state represented by the collected leaves; repeated sampling across phenological stages would be needed to evaluate seasonal stability. Third, pigment concentration was not directly linked to yield, berry quality, leaf water potential, stomatal conductance, or fluorescence parameters. Fourth, environmental conditions at sampling were not analyzed in detail. These limitations do not weaken the descriptive value of the pigment comparison, but they define the boundary of interpretation.

For future research, the genotypes identified as high-pigment forms should be tested under controlled and field stress conditions. A strong follow-up design would include well-watered and water-deficit treatments, repeated measurements during vegetative growth and fruit development, and simultaneous assessment of chlorophyll fluorescence parameters such as Fv/Fm, PhiPSII, and non-photochemical quenching. It would also be valuable to include leaf relative water content, osmotic adjustment, antioxidant enzymes, malondialdehyde, proline, fruit yield, and soluble solids. Such a multidimensional design would allow researchers to determine whether the high pigment content of Do'stlik and related genotypes corresponds to genuine stress tolerance and productivity stability.

The conceptual interpretation model shown in Figure 2 summarizes how pigment data should be used in breeding and cultivation decisions. Genotypic background affects the development of the pigment apparatus. The pigment apparatus contributes to the observed physiological state of leaves. However, environmental stress context modifies pigment expression

and must be considered in interpretation. Diagnostic ranking and pigment ratios can guide selection, but final decisions should integrate biochemical, physiological, agronomic, and quality traits. This balanced interpretation prevents overclaiming and makes pigment measurement a practical component of broader currant evaluation.

Conclusions

This study established clear differences among 16 currant genotypes in the content of chlorophyll a, chlorophyll b, and carotenoids. Do'stlik had the highest value for every measured pigment and for the derived total chlorophyll and total pigment indicators, demonstrating the most developed pigment complex among the studied samples. Podarok Ariadne, Duragay 4 (Ravshan), Duragay 7, Uzb sladkaya, and Duragay 9 also showed relatively high pigment accumulation and may be considered promising genotypes for additional physiological testing.

Ruxshona, Duragay 1, N/3759/6, and Iroda had lower pigment values, suggesting comparatively lower pigment accumulation and potentially lower photosynthetic pigment capacity under the measured conditions. However, pigment data alone cannot define overall stress tolerance or agronomic value. The results should be combined with water-status, gas-exchange, chlorophyll-fluorescence, antioxidant, disease-resistance, and yield data in future research.

Overall, the findings support the use of chlorophyll a, chlorophyll b, carotenoids, total chlorophyll, and pigment ratios as informative physiological indicators in currant genotype screening. Spectrophotometric pigment analysis is accessible and useful for early-stage evaluation, especially when researchers need to compare many genotypes. The strongest conclusion is that Do'stlik and several high-pigment genotypes deserve further study under controlled stress and field-production conditions.

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A COMPREHENSIVE EVALUATION OF BIOLOGICAL PERFORMANCE, ECOLOGICAL ROLE, AND ECONOMIC EFFICIENCY OF *ANCISTRUS* IN AQUARIUM SYSTEMS

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Abstract. *This study investigates the biological characteristics, reproductive performance, and economic efficiency of culturing* Ancistrus *species under controlled aquarium conditions. The experiments were conducted in laboratory aquaria, where environmental parameters and growth indicators were systematically monitored and analyzed. The results demonstrated that under optimal conditions (water temperature of 24–27 °C and dissolved oxygen levels above 6 mg/L), the growth rate and survival of fish increased substantially. In particular, survival rates reached up to 90–95% under optimal conditions. Reproductive performance showed that the number of eggs ranged from 50 to 120 per spawning, with hatching success rates between 85–90%. Larvae transitioned to independent feeding within 3–5 days, indicating high reproductive potential of the species.*

Additionally, the ecological role of Ancistrus *in aquarium ecosystems was identified. The species contributes to biofilm and organic matter consumption, thereby improving water quality and maintaining ecological balance. Economic efficiency was evaluated based on model calculations. The total production cost was estimated at approximately 2,150,000 UZS, while potential revenue could reach up to 4,800,000 UZS depending on market conditions. Accordingly, net profit may reach around 2,650,000 UZS, with profitability levels up to 123%. These estimates indicate a high economic potential of* Ancistrus *culture under aquarium conditions.*

The findings confirm that culturing Ancistrus *species is both biologically viable and economically efficient, making it a promising direction for ornamental fish farming and small-scale aquaculture development.*

Keywords: *aquarium, aquaculture,* Ancistrus*, biofilm, profitability, biomass dynamics, microalgae, abiotic factors, live feed, survival rate, growth rate.*

INTRODUCTION

In recent decades, aquaculture has emerged as one of the fastest-growing sectors contributing to global food security and the sustainable use of biological resources. Within this framework, ornamental fish farming has gained particular importance due to its economic profitability, ecological relevance, and potential for small-scale entrepreneurship [7; 18]. Identifying species that combine high biological adaptability with strong market demand remains a key priority in the development of sustainable aquaculture systems. In this regard, species of the genus *Ancistrus* (family Loricariidae) represent a promising group of organisms with both scientific and practical significance.

Members of the genus *Ancistrus* are native to the Neotropical region and are widely distributed across freshwater systems of South America, including the Amazon, Orinoco, and

Paraná river basins [14; 10]. These fish occupy a benthic ecological niche and exhibit a high degree of specialization to substrate-associated habitats. Their morphological features—such as a dorsoventrally flattened body, dermal bony plates, and a ventrally positioned suctorial mouth—reflect advanced adaptations to environments characterized by strong water currents and complex substrates [16; 1]. The specialized oral apparatus enables efficient scraping of periphyton, biofilm, and detrital material, thereby defining their trophic ecology [8; 9].

From an ecological perspective, *Ancistrus* species play a significant functional role in aquatic ecosystems. By consuming biofilm and organic matter, they contribute to nutrient cycling, regulate algal biomass, and enhance ecosystem stability [13; 20]. In controlled aquarium environments, these characteristics translate into improved water quality and maintenance of biological balance, positioning *Ancistrus* as effective biological regulators.

Biologically, *Ancistrus* species are characterized by high survival rates, rapid adaptation to controlled conditions, and efficient reproductive strategies. Previous studies have demonstrated that environmental parameters—particularly temperature, dissolved oxygen, and feeding regimes—directly influence growth performance and reproductive success [11; 19]. Under optimal conditions, metabolic activity increases, resulting in enhanced growth rates and survival. Furthermore, their reproductive traits—including relatively high fecundity, male parental care, and rapid larval development—facilitate successful propagation in aquaculture systems [5; 2].

Recent advances in molecular phylogenetics have revealed substantial genetic diversity and evolutionary complexity within the Loricariidae family, highlighting the adaptive potential of *Ancistrus* species across diverse environmental conditions [12; 6]. These findings underscore the importance of integrating ecological, biological, and genetic perspectives in evaluating species performance.

Despite the growing body of literature on morphology, systematics, and ecology, comprehensive studies that integrate both biological performance and economic feasibility of *Ancistrus* culture under controlled aquarium conditions remain limited [18]. In particular, there is a lack of detailed assessments focusing on small-scale recirculating aquaculture systems (RAS), where resource efficiency and economic viability are critical factors.

Economic evaluation is a fundamental component of aquaculture research, as it determines the practical applicability and sustainability of production systems. Small-scale aquarium-based systems require relatively low capital investment but can yield high returns when properly managed [15; 17]. Therefore, understanding the relationship between biological efficiency and economic outcomes is essential for optimizing production strategies.

The aim of this study is to comprehensively evaluate the biological characteristics, reproductive performance, and economic efficiency of *Ancistrus* species cultured under controlled aquarium conditions.

MATERIALS AND METHODS

This study was conducted under controlled laboratory–aquarium conditions to comprehensively evaluate the biological characteristics, ecological role, and economic efficiency of *Ancistrus* species. Experimental systems were established using glass aquaria of varying volumes (30–200 L), and a comparative design was applied, including both optimal and suboptimal (control) conditions.

During the experiment, key physico-chemical parameters of the water were continuously monitored and maintained. Water temperature was kept within the optimal range of 24–27 °C, while additional observations were conducted at 20–22 °C and 28–30 °C for comparative analysis.

Dissolved oxygen levels were maintained at 6.5–7.5 mg/L under optimal conditions, whereas lower levels were recorded in control systems. The pH values were stabilized within the range of 6.5–7.5. A photoperiod of 12 hours light and 12 hours dark was applied. Aeration and filtration systems were used to ensure water quality stability.

Feeding regimes were designed based on the natural trophic behavior of the species. Fish were provided with a combined diet consisting of biofilm, periphyton, and supplemental artificial feed. Natural substrates, including driftwood, gravel, and stones, were introduced to promote biofilm development and support natural feeding behavior. Feeding frequency was adjusted according to the ontogenetic stage of the fish.

Morphometric and biological analyses constituted a key component of the study. Morphometric measurements were carried out using a digital caliper with an accuracy of 0.01 mm, following the point-to-point method described by Boeseman (1968) and Weber (1985) [3; 21]. The measured parameters included standard length, head length, body depth, and fin dimensions.

Biological assessments focused on key indicators reflecting growth performance and physiological activity. Growth rate was determined based on changes in body length and mass, while survival rate was calculated as a percentage. Reproductive parameters included the number of eggs per spawning event and hatching success (%). Additionally, the time required for larvae to transition to independent feeding (in days) was recorded as an indicator of developmental rate.

The ecological role of *Ancistrus* within aquarium systems was evaluated through observations of biofilm coverage on substrates, water transparency, and the rate of organic matter decomposition. Comparative assessments were conducted between systems with and without *Ancistrus* individuals.

Economic efficiency was assessed using a model-based approach derived from experimental biological data and prevailing market conditions. The analysis included total production costs (equipment, feed, energy, and maintenance), potential revenue based on the number of marketable juveniles, net profit estimation, and profitability (calculated as net profit divided by total cost × 100). It should be noted that these economic indicators are based on calculated estimates and may vary depending on production conditions and market fluctuations.

The obtained data were analyzed using comparative and descriptive statistical methods. Mean values and variation ranges were used to interpret biological and economic indicators.

RESULTS AND DISCUSSION

Biological Performance The results of the study demonstrated that the biological efficiency of *Ancistrus* is strongly dependent on environmental factors, particularly water temperature and dissolved oxygen levels. Under optimal conditions (24–27 °C; DO > 6 mg/L), both growth rate and survival reached their highest values.

Experimental observations revealed that under optimal conditions the survival rate reached 92.3 ± 2.1%, whereas under suboptimal conditions this value decreased to 78.6 ± 3.4%. These findings confirm the notable influence of environmental parameters on fish physiological performance.

Analysis of growth dynamics indicated that the most intensive development occurred during the juvenile stage, when metabolic activity is at its peak. Under optimal conditions, the average increase in body length was 0.85 ± 0.06 mm/day, while under suboptimal conditions it decreased to 0.52 ± 0.04 mm/day. Similarly, body mass gain was higher in optimal systems, reflecting the direct effect of oxygen availability and feeding conditions on growth performance (Table 1, Figure 1).

Table 1. Biological Performance of Ancistrus ***under Optimal and Suboptimal Environmental Conditions (Mean ± SE)***

Parameter	Optimal Conditions	Suboptimal Conditions
Temperature (°C)	24–27	20–22 / 28–30
Dissolved Oxygen (mg/L)	6.5–7.5	4.5–5.5
Survival Rate (%)	92.3 ± 2.1	78.6 ± 3.4
Growth Rate (mm/day)	0.85 ± 0.06	0.52 ± 0.04
Weight Gain (g/day)	0.34 ± 0.03	0.21 ± 0.02
Egg Number (units)	80–120	40–70
Hatching Success (%)	88.5 ± 2.7	72.4 ± 3.1
Time to Independent Feeding (days)	3–4	4–6

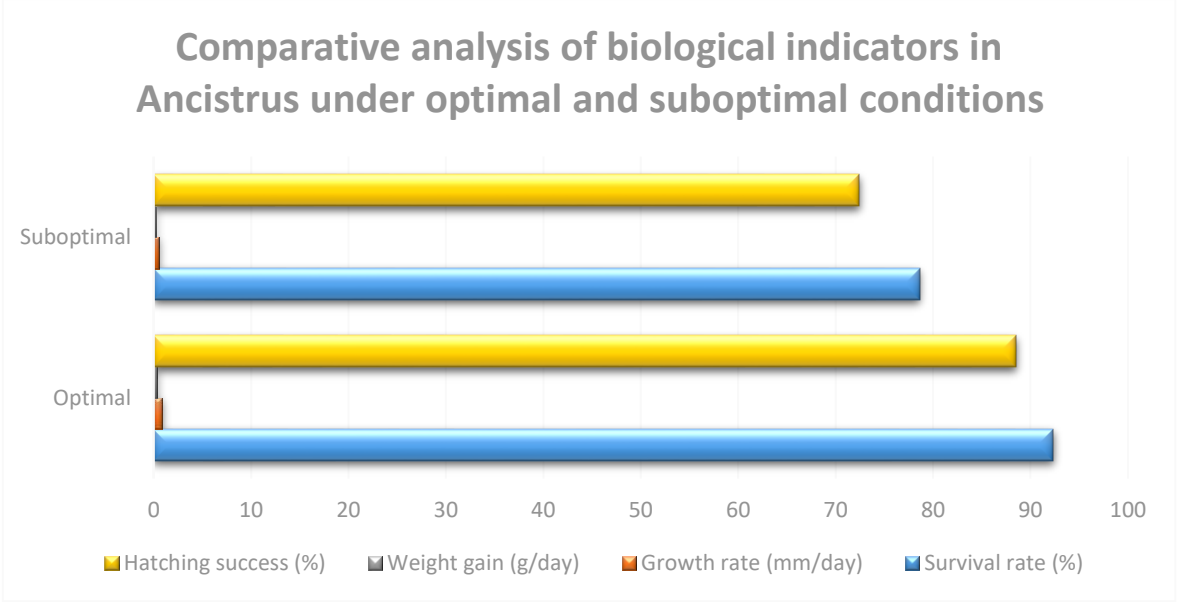


Figure 1. Comparative analysis of biological performance indicators (survival rate, growth rate, weight gain, and hatching success) of **Ancistrus** ***under optimal and suboptimal environmental conditions.***

The results indicate that all biological indicators were higher under optimal conditions compared to suboptimal environments. In particular, survival rate and hatching success showed the greatest differences, confirming the strong influence of temperature and dissolved oxygen on the physiological performance and development of *Ancistrus*. These observations align with earlier studies emphasizing the sensitivity of loricariid catfishes to abiotic factors and underscore the importance of precise environmental control in aquarium-based culture.

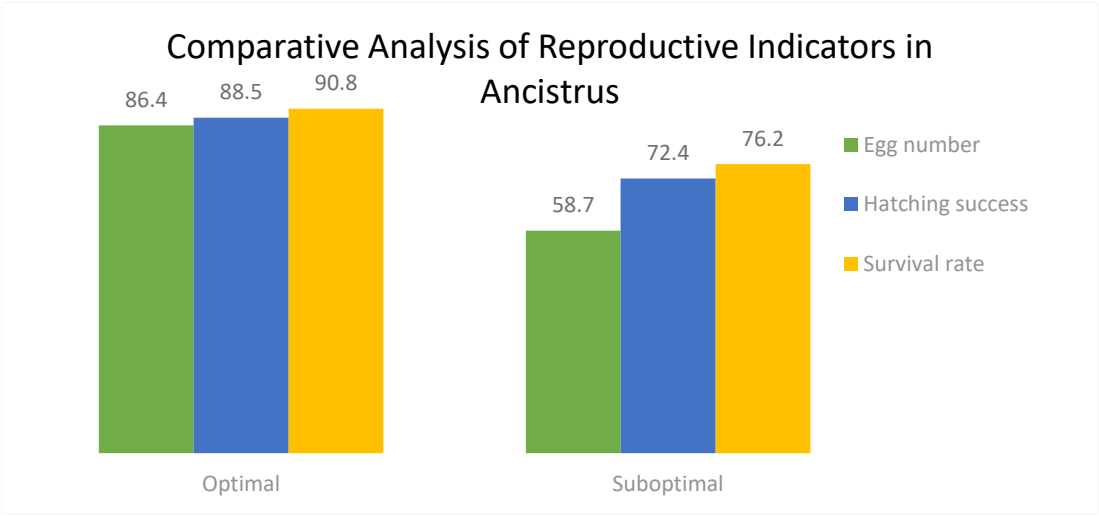
Reproductive Performance The results of the study demonstrated that *Ancistrus* exhibits high reproductive potential under controlled aquarium conditions. Reproductive performance was strongly influenced by environmental stability, particularly temperature, dissolved oxygen levels, and feeding conditions.

Experimental observations showed that the number of eggs per spawning ranged between 50 and 120, with an average value of 86.4 ± 7.2 eggs under optimal conditions. Under suboptimal conditions, this value decreased to 58.7 ± 6.5 eggs, indicating the sensitivity of reproductive activity to environmental stress.

Table 2. Reproductive Performance of Ancistrus ***under Different Environmental Conditions (Mean ± SE)***

Parameter	Optimal Conditions	Suboptimal Conditions
Egg Number (units)	86.4 ± 7.2	58.7 ± 6.5
Hatching Success (%)	88.5 ± 2.7	72.4 ± 3.1
Time to independent feeding (days)	3–4	4–6
Survival rate (%)	90.8 ± 2.3	76.2 ± 3.6

Figure 2. Reproductive performance of **Ancistrus** ***under optimal and suboptimal environmental conditions.***



Reproductive indicators, including egg number, hatching success, and survival rate, were higher under optimal conditions compared to suboptimal environments. Hatching success was higher under optimal conditions, reaching 88.5 ± 2.7%, while in suboptimal systems it declined to 72.4 ± 3.1%. These results highlight the importance of stable water parameters during embryonic development. Larval development analysis revealed that under optimal conditions larvae transitioned to independent feeding within 3–4 days, whereas under suboptimal conditions this period extended to 4–6 days. This reflects the direct influence of environmental factors on metabolic processes. Additionally, male parental behavior, including egg guarding and continuous ventilation, was identified as a key factor enhancing embryo survival and overall reproductive success (Table 2, Figure 2).

Overall, the findings confirm that *Ancistrus* species possess high reproductive efficiency, particularly under optimal environmental conditions, making them suitable for controlled breeding and ornamental aquaculture systems. The results are consistent with prior reports on loricariid reproductive biology and demonstrate the practical value of environmental optimization for commercial propagation.

Ecological Impact The results of the study demonstrated that *Ancistrus* exerts a notable influence on aquarium ecosystem functioning. A comparative experimental analysis between systems containing *Ancistrus* and control systems (without *Ancistrus*) revealed pronounced differences in key ecological parameters (Table 3, Figure 3).

In aquaria where *Ancistrus* were present, a consistent reduction in biofilm and algal layer accumulation on substrate surfaces was observed. This effect can be attributed to the feeding behavior of *Ancistrus*, which actively graze on periphyton and biofilm. As a result, a noticeable

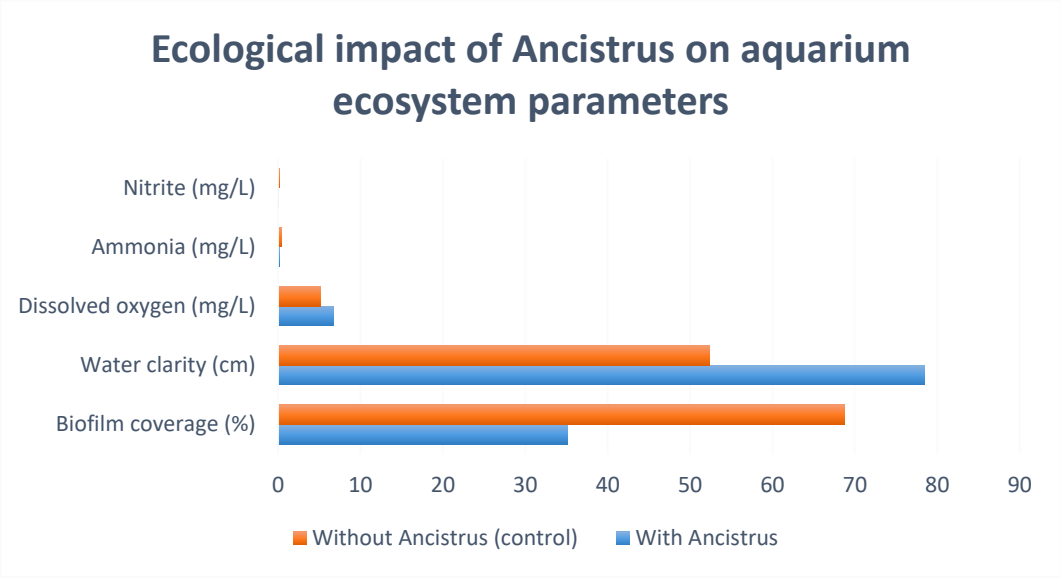
improvement in water clarity and a reduction in organic matter accumulation were recorded. In contrast, control aquaria lacking *Ancistrus* showed a progressive increase in organic residues and algal biomass over time. This led to decreased water transparency and signs of ecological imbalance, including the deterioration of overall water quality.

Furthermore, comparative analysis of physico-chemical parameters indicated that systems containing *Ancistrus* maintained higher levels of dissolved oxygen and lower concentrations of ammonia and nitrite. These findings suggest enhanced biofiltration processes and accelerated decomposition of organic matter in the presence of *Ancistrus*.

Table 3. Ecological Parameters of Aquarium Systems with and without Ancistrus ***(Mean ± SE)***

Parameter	**With Ancistrus**	**Without Ancistrus (control)**
Biofilm coverage (%)	35.2 ± 3.1	68.7 ± 4.5
Water clarity (cm)	78.5 ± 5.2	52.3 ± 4.8
Dissolved oxygen (mg/L)	6.8 ± 0.4	5.2 ± 0.3
Ammonia (mg/L)	0.18 ± 0.02	0.36 ± 0.04
Nitrite (mg/L)	0.09 ± 0.01	0.21 ± 0.02

Figure 3. Ecological impact of* Ancistrus *on aquarium ecosystem parameters.



The presence of *Ancistrus* reduced biofilm accumulation and improved key water quality indicators. Systems containing *Ancistrus* exhibited higher water clarity and dissolved oxygen levels, while concentrations of ammonia and nitrite were notably lower than those in control systems. Overall, the obtained results confirm that *Ancistrus* functions as an effective biological regulator within aquarium ecosystems. By controlling biofilm growth, reducing organic load, and improving water quality, these fish contribute to the stabilization of ecological conditions. Therefore, *Ancistrus* can be considered a valuable biological component for supporting natural biofiltration processes in closed aquatic systems. These outcomes are in agreement with established ecological principles regarding the role of benthic grazers in nutrient cycling and water-quality maintenance.

Economic Interpretation (Profitability Analysis) Profitability is one of the most important economic indicators used to evaluate the efficiency and sustainability of aquaculture

production systems. It reflects the relationship between the net profit obtained and the total production costs incurred, thereby providing an integrated assessment of economic performance.

In the present study, profitability was evaluated based on model-derived calculations that incorporated both biological performance indicators and current market conditions. According to these estimates, total production costs were approximately 2,150,000 UZS, including expenses related to equipment, feed, energy consumption, and system maintenance. At the same time, the potential total revenue, calculated based on the number of marketable juveniles and average market prices, may reach up to 4,800,000 UZS under favorable conditions. Consequently, the estimated net profit may amount to approximately 2,650,000 UZS.

Profitability was calculated using the standard economic formula:

$$\text{Profitability}\,(\%) = \left(\frac{\text{Net profit}}{\text{Total cost}}\right) \times 100$$

Substituting the obtained values into the formula:

$$\left(\frac{2{,}650{,}000}{2{,}150{,}000}\right) \times 100 \approx 123\%$$

This result indicates that profitability may reach approximately 123%, meaning that for every 1 UZS invested in production, a net profit of approximately 1.23 UZS is generated. Such a level of profitability reflects relatively high economic efficiency compared to many conventional aquaculture practices, particularly in small-scale production systems (Table 4).

Table 4. Main Economic Indicators of Ancistrus ***Production (Model-Based Estimation)***

Parameter	**Value (UZS)**
Total production cost	2,150,000
Including: equipment and feed costs	represent the major share
Total revenue	up to 4,800,000
Net profit	around 2,650,000
Profitability (%)	up to 123

The results indicate that under optimal conditions total revenue may substantially exceed production costs, resulting in high profitability. These values are based on model calculations and may vary depending on production scale, management efficiency, and market conditions. Furthermore, the high profitability observed in this study can be attributed to several key factors, including relatively low operational costs, efficient feed utilization, high survival rates, and strong reproductive performance of the cultured species. The ability of *Ancistrus* to utilize natural biofilm as a supplementary food source also contributes to reducing feed expenses, thereby improving overall economic efficiency.

However, it should be emphasized that the presented economic indicators are based on model estimations and may vary depending on production scale, environmental conditions, management practices, and fluctuations in market demand and prices. Therefore, these results should be interpreted as indicative rather than absolute values. Overall, the findings suggest that *Ancistrus* culture under controlled aquarium conditions has considerable economic potential and may serve as a viable option for small-scale aquaculture enterprises and ornamental fish production systems.

CONCLUSION

The results of this study comprehensively demonstrate that *Ancistrus* species exhibit high biological and economic efficiency under controlled aquarium conditions. The findings reveal a

strong interrelationship between environmental factors, reproductive performance, and economic outcomes.

The analysis of biological indicators showed that under optimal conditions (24–27 °C; DO > 6 mg/L), survival rates reached up to 90–95%, while growth rates were substantially enhanced. These findings are consistent with previous studies by Jobling (1994) and Val & Almeida-Val (1998) [11; 19], which emphasize the strong dependence of metabolic activity and growth processes on environmental parameters. Moreover, the results of the present study indicate that stable and controlled aquarium conditions can further optimize growth performance.

Regarding reproductive performance, the obtained results confirm that *Ancistrus* species possess high reproductive potential. The observed fecundity range of 50–120 eggs per spawning event and hatching success rates of 85–90% are comparable to the findings reported by Breder & Rosen (1966) and Balon (1975) [5; 2]. In particular, male parental behavior, including egg guarding and active ventilation, was identified as a key factor enhancing reproductive success, which is characteristic of many members of the Loricariidae family.

Ecological analysis demonstrated that *Ancistrus* plays a significant functional role in aquarium ecosystems. The reduction of biofilm and algal accumulation, along with improved water clarity, is in agreement with ecological principles described by Power (1990) and Vanni (2002) [13; 20]. The present study further confirms these processes experimentally, highlighting the role of *Ancistrus* in biofiltration and organic matter regulation.

Economic evaluation based on model calculations indicates that *Ancistrus* culture can achieve a high level of profitability, reaching up to 123% under optimal conditions. These results are consistent with the findings of Timmons (2002) and Romanov (2010) [17; 15], who reported high efficiency in small-scale aquaculture systems. Additionally, the ability of *Ancistrus* to utilize biofilm as a supplementary food source contributes to reducing feed costs and improving overall economic efficiency.

Overall, the findings suggest that *Ancistrus* species represent a highly promising candidate for ornamental aquaculture due to their high survival rate, rapid growth, efficient reproduction, and ecological benefits. Their role in maintaining ecosystem stability further enhances their value in closed aquatic systems. The results of this study have both theoretical and practical significance, providing a scientific basis for optimizing biological management and improving economic efficiency in small-scale aquaculture systems.

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EVALUATION OF BIOLOGICAL AND PRODUCTIVITY INDICATORS OF SILKWORM (BOMBYX MORI) HYBRIDS USING MODERN ANALYTICAL METHODS

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Abstract*. Sericulture is an important sector not only of agriculture but also of the textile industry, occupying a special place in the national economy. In particular, the growing demand for silk products in the global market necessitates further development of this sector. From this perspective, the creation of high-yielding silkworm hybrids that are resistant to environmental factors and their introduction into production are considered among the most pressing issues.*

In Uzbekistan, the sericulture sector has been developing steadily, and in this process, the Fergana region holds a special significance as one of the leading areas. The climatic and natural conditions of the region are favorable for silkworm rearing and allow for the production of high-quality cocoons. At the same time, conducting scientific research in this region to test new hybrids and evaluate their productivity is of great importance.

The biological and productivity indicators of silkworm hybrids are among the key factors determining the efficiency of the industry. In this regard, there has always been a need for accurate and reliable assessment of indicators such as hatchability, viability, resistance to diseases, cocoon weight, and silk content.

Currently, along with traditional methods, variation statistical methods and digital analytical approaches are widely used in the evaluation of hybrids. This makes it possible to accelerate data processing and reduce the influence of the human factor.

In this context, the aim of the present study is to conduct a comparative analysis of the biological indicators and productivity of various silkworm hybrids under the conditions of the Fergana region using variation statistical methods, as well as to evaluate them with the help of digital technologies.

Keywords: *silkworm (*Bombyx mori*), hybrid, hatchability, viability, silk content, cocoon weight, variation analysis, biometric indicators, artificial intelligence model.*

Introduction

Sericulture is an important sector not only of agriculture but also of the textile industry, occupying a special place in the national economy. In particular, the growing demand for silk products in the global market necessitates further development of this sector. From this perspective, the creation of high-yielding silkworm hybrids that are resistant to environmental factors and their introduction into production are considered among the most pressing issues.

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Literature Review

A number of scientific studies have extensively examined the factors influencing biological indicators and productivity in silkworm rearing. In particular, climatic factors such as temperature and humidity have been shown to affect larval development, their viability, and cocoon productivity [1]. Ensuring optimal rearing conditions is therefore considered a key factor in achieving high productivity.

Environmental and agrotechnical factors are also of particular importance. It has been noted that under different conditions, significant differences can be observed in larval viability, resistance to diseases, and cocoon quality [2]. This indicates the necessity of a comprehensive evaluation of biological indicators, taking into account agrotechnical factors.

The genetic characteristics of hybrids also directly influence productivity. In some hybrids, higher cocoon yield and silk content have been observed, which highlights the importance of organizing breeding work on a scientific basis [3,6]. Furthermore, the expression of genotype under different environmental and rearing conditions also determines productivity levels [7].

The possibilities of evaluating hybrid stability using biometric analysis methods have also been widely studied. It has been emphasized in several studies that variation statistical indicators allow for the assessment of variability and stability of biological traits [8].

In recent years, there has been a growing trend toward the use of digital technologies and artificial intelligence in agriculture. The effectiveness of AI technologies has been demonstrated in analyzing agricultural data, predicting productivity, and automating decision-making processes [4,10]. At the same time, digital platforms based on big data are creating new opportunities in agriculture [9].

It has also been established that the use of IoT and AI technologies enables real-time monitoring, rapid data processing, and forecasting, thereby ensuring high efficiency [5]. This highlights the relevance of introducing digital technologies into the sericulture sector.

Alongside international research, scientific work is also being conducted in Uzbekistan to develop the sericulture sector. In particular, studies have substantiated the history and development prospects of sericulture, as well as the necessity of introducing high-yielding hybrids [11]. Moreover, by analyzing the reproductive capacity of silkworms and the viability of larvae, it is possible to identify promising breeding systems [12].

Scientific research, the development of new hybrids, and increasing productivity are also considered important directions in sericulture [14]. In this process, it is essential to conduct breeding work taking into account local conditions.

Furthermore, in Uzbekistan's sericulture development programs, the Fergana Valley is recognized as one of the main centers of sericulture. Therefore, the introduction of high-yielding hybrids and the expansion of production in this region are of particular importance [15].

Thus, the analysis of existing scientific research shows that, in addition to traditional methods, it is essential to integrate variation statistics, digital technologies, and artificial intelligence methods in evaluating the biological and productivity indicators of silkworm hybrids. This confirms the scientific validity of applying digital solutions such as BioCalc and SmartSilk in the present study.

Results

The results of the study revealed significant differences among the silkworm hybrids in terms of biological and productivity indicators.

Table 1. Biological indicators and variation analysis of silkworm hybrids

Hybrid	Hatchability ($\bar{X}$±Sx, %)	Cv (%)	Viability ($\bar{X}$±Sx, %)	Cv (%)	Disease (X±Sx, %)	Cv (%)
Foreign 1	95.67 ± 1.20	2.18	92.05 ± 1.08	2.34	7.95 ± 1.08	27.2
Foreign 2	95.67 ± 1.20	2.18	95.05 ± 1.67	3.50	5.45 ± 1.69	62.0
Ipakchi 1 × Ipakchi 2	92.00 ± 1.00	1.88	91.80 ± 1.49	3.23	8.20 ± 1.48	36.1
Ipakchi 2 × Ipakchi 1	97.67 ± 0.33	0.58	94.20 ± 1.95	4.13	5.80 ± 1.94	67.0
Fergana 1	91.33 ± 2.33	4.42	92.95 ± 1.96	4.22	7.05 ± 1.96	55.6
Fergana 2	93.33 ± 2.19	4.06	96.00 ± 0.98	2.05	3.50 ± 1.02	58.6

The analysis of biological indicators shows that the highest hatchability was observed in the hybrid Ipakchi 2 × Ipakchi 1 (97.67±0.33%), indicating excellent reproductive performance and genetic stability. The low coefficient of variation (0.58%) further confirms the consistency of this trait. In contrast, the Fergana 1 hybrid demonstrated the lowest hatchability (91.33±2.33%), with a relatively higher variability.

In terms of viability, the Fergana 2 hybrid exhibited the highest value (96.00±0.98%), reflecting strong adaptation to local environmental conditions. The foreign hybrid 2 also showed high viability (95.05±1.67%), while the Ipakchi 1 × Ipakchi 2 hybrid demonstrated comparatively lower performance.

The analysis of disease incidence revealed that the lowest disease rate was observed in the Fergana 2 hybrid (3.50±1.02%), indicating high resistance to environmental stress. However, some hybrids, particularly Ipakchi 2 × Ipakchi 1, showed high variability in disease resistance (Cv = 67.0%), suggesting heterogeneity within the population.

Table 2. Productivity and silkiness indicators under Fergana conditions

Hybrid	Cocoon weight (g)	Cv (%)	Shell weight (g)	Cv (%)	Silk content (%)	Cv (%)
Foreign 1	1.53 ± 0.0048	0.63	0.340 ± 0.0076	4.47	22.23 ± 0.45	4.05

Hybrid	Cocoon weight (g)	Cv (%)	Shell weight (g)	Cv (%)	Silk content (%)	Cv (%)
Foreign 2	1.52 ± 0.0063	0.83	0.348 ± 0.0021	1.21	22.88 ± 0.09	0.83
Ipakchi 1 × Ipakchi 2	1.79 ± 0.005	0.56	0.355 ± 0.0012	0.68	19.80 ± 0.04	0.41
Ipakchi 2 × Ipakchi 1	1.96 ± 0.009	0.97	0.456 ± 0.0033	1.43	23.30 ± 0.09	0.77
Fergana 1	1.71 ± 0.008	1.00	0.368 ± 0.0028	1.52	21.50 ± 0.07	0.60
Fergana 2	1.73 ± 0.004	0.46	0.383 ± 0.0016	0.86	22.15 ± 0.11	0.95

The productivity analysis indicates that the hybrid Ipakchi 2 × Ipakchi 1 achieved the highest cocoon weight (1.96±0.009 g), demonstrating superior productivity potential. Foreign hybrids showed lower values (1.52–1.53 g), while local Fergana hybrids exhibited intermediate results.

Similarly, silk content was highest in Ipakchi 2 × Ipakchi 1 (23.30±0.09%), followed by Foreign 2 (22.88±0.09%). The relatively low coefficients of variation across all hybrids indicate high stability of productivity traits, which is characteristic of F1 hybrids.

Discussion

The results of this study clearly demonstrate that significant differences exist among silkworm (*Bombyx mori*) hybrids in terms of both biological and productivity indicators, confirming that these parameters play a decisive role in hybrid selection and the organization of breeding programs. The observed variation among hybrids reflects the combined influence of genetic characteristics, environmental adaptability, and rearing conditions, emphasizing the need for a comprehensive evaluation approach.

The analysis of hatchability revealed that the hybrid Ipakchi 2 × Ipakchi 1 exhibited the highest value (97.67±0.33%) along with the lowest coefficient of variation (0.58%), indicating both high reproductive capacity and strong genetic stability. These findings are consistent with previous studies [1,2], which highlight that under optimal environmental conditions, the biological potential of silkworm hybrids can be fully realized. The low variability further suggests that this hybrid possesses a high level of genetic uniformity, which is particularly important for industrial-scale production where consistency is essential. As emphasized in earlier research [3,6], hatchability and its stability are among the primary criteria for selecting high-yielding hybrids, and the present results strongly support this conclusion.

The evaluation of larval viability also revealed important differences among hybrids. The highest viability was observed in the Fergana 2 hybrid (96.00±0.98%), indicating its strong adaptation to local environmental conditions. This finding suggests that locally developed hybrids may outperform foreign ones under specific regional conditions, particularly when environmental factors play a critical role. This observation is in agreement with previous studies [7], which demonstrate that environmental conditions significantly influence silkworm survival and development. Hybrids with lower viability may be more sensitive to environmental fluctuations, limiting their practical applicability in field conditions.

The analysis of disease incidence further highlights the variability in hybrid stability. The lowest disease rate was recorded in the Fergana 2 hybrid (3.50±1.02%), indicating high resistance to environmental stress and pathogens. However, certain hybrids, such as Ipakchi 2 × Ipakchi 1, exhibited high coefficients of variation (67.0%), suggesting considerable heterogeneity within the population in terms of disease susceptibility. This indicates that while some hybrids may show high average performance, their internal variability must also be considered when evaluating their suitability for practical use. As noted in biometric studies [8], the coefficient of variation is an essential parameter for assessing the stability and reliability of biological traits.

The productivity indicators obtained in this study further confirm the dominant role of genetic factors in determining cocoon yield and silk production. The hybrid Ipakchi 2 × Ipakchi 1 demonstrated the highest cocoon weight (1.96±0.009 g) and silk content (23.30±0.09%), clearly distinguishing it as a high-productivity genotype. The use of such hybrids can significantly increase cocoon yield per unit of production, thereby improving economic efficiency in sericulture. In contrast, foreign hybrids showed relatively lower cocoon weights (1.52–1.53 g), while local Fergana hybrids demonstrated intermediate performance levels. This suggests that hybrid selection should be based not only on origin but also on specific performance under local conditions.

An important observation is the low variability of productivity indicators across hybrids (Cv = 0.41–1.52%), which indicates high stability of these traits. This stability is characteristic of F1 hybrids and suggests that these traits are likely controlled by homozygous gene combinations. The low variability enhances the reliability of the results and confirms the correctness of the applied statistical methods. Stable productivity traits are particularly important in commercial sericulture, where predictable outcomes are required.

A key contribution of this study is the application of digital technologies in the evaluation process. The use of the BioCalc mobile application enabled the automatic calculation of biometric indicators, significantly accelerating data processing and reducing human error. Unlike general-purpose statistical software such as Excel or SPSS, BioCalc is specifically designed for sericulture and incorporates domain-specific calculations. This specialization simplifies the analytical process and improves accuracy.

Furthermore, the integration of BioCalc with the proposed SmartSilk platform represents an innovative and forward-looking approach. In this system, BioCalc serves as a tool for data collection and preliminary statistical processing, while SmartSilk will perform advanced analytical functions, including correlation analysis, productivity prediction, and automated decision-making using artificial intelligence algorithms. This two-stage system forms a continuous “data → analysis → prediction” pipeline, which enables the creation of a comprehensive digital ecosystem for sericulture.

The standardized datasets generated through BioCalc can serve as reliable training data for SmartSilk models, allowing for accurate prediction of hybrid productivity under specific environmental conditions. This capability has significant practical implications, as it enables producers to select the most suitable hybrids for particular regions, optimize resource allocation, and reduce production risks.

Additionally, the study highlights the limitations of existing general-purpose agricultural platforms, such as those based on ANOVA analysis, which are not tailored to the specific requirements of sericulture. These systems lack the necessary biometric and technological indicators specific to silkworm production. In contrast, BioCalc and SmartSilk are designed specifically for this sector, making them more effective and relevant for practical application.

Overall, the results of this study demonstrate that combining variation statistical methods with digital technologies provides a powerful and efficient approach for evaluating silkworm hybrids. This integrated methodology enhances analytical accuracy, reduces errors, and opens new opportunities for innovation in sericulture.

Conclusion

The findings of this study confirm that significant differences exist among silkworm (*Bombyx mori*) hybrids in terms of biological and productivity indicators, highlighting the importance of these parameters in hybrid selection and breeding strategies. The results demonstrate that certain hybrids exhibit superior performance in key traits such as hatchability, viability, cocoon weight, and silk content, which directly influence production efficiency.

In particular, the hybrid Ipakchi 2 × Ipakchi 1 showed the highest overall performance, combining high hatchability, superior cocoon weight, and maximum silk content. These characteristics indicate strong genetic potential and make this hybrid highly suitable for industrial-scale production. At the same time, the Fergana 2 hybrid demonstrated the highest viability and the lowest disease incidence, confirming its strong adaptation to local environmental conditions and its potential for stable performance under practical production settings.

The application of variation statistical methods provided a reliable assessment of hybrid stability and variability, ensuring the scientific validity of the results. The low coefficients of variation observed for productivity traits confirm their stability and reinforce the suitability of F1 hybrids for consistent production outcomes.

The use of the BioCalc mobile application significantly improved the efficiency and accuracy of data processing by automating biometric calculations and reducing the influence of human error. This demonstrates the practical advantages of integrating digital tools into scientific research and production processes.

Furthermore, the proposed integration of BioCalc with the SmartSilk digital platform represents a promising direction for the future development of sericulture. By combining data collection, statistical analysis, and artificial intelligence-based prediction, this system has the potential to transform the industry into a data-driven and highly efficient sector.

The results of this study have practical significance not only for the Fergana region but also for other regions of Uzbekistan, where they can be used to identify and implement high-yielding hybrids adapted to local conditions. In addition, the collected data provide a valuable foundation for developing predictive models of cocoon productivity and improving management strategies.

Future research should focus on integrating biological indicators with technological and environmental factors and on developing comprehensive digital platforms capable of supporting all stages of sericulture production. Such an approach will enable the formation of a modern, integrated system that enhances productivity, sustainability, and competitiveness in the global silk market.

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HYDROLYTIC ENZYME PROFILES OF ENDOPHYTIC BACTERIA ISOLATED FROM *PLANTAGO LANCEOLATA* L.: IMPLICATIONS FOR BIOCONTROL AND SUSTAINABLE AGRICULTURE

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Abstract. *Endophytic bacteria are recognized as important plant-associated microorganisms capable of promoting plant growth and suppressing phytopathogens through the production of extracellular hydrolytic enzymes. The present study aimed to evaluate the hydrolytic enzyme production potential of endophytic bacteria isolated from the medicinal plant Plantago lanceolata L. Six bacterial isolates, identified as Pseudomonas moraviensis, Brevibacillus brevis, Brevibacillus formosus, Bacillus cereus, Bacillus paramycoides, and Bacillus velezensis, were screened for cellulase, chitinase, protease, and amylase production under laboratory conditions. Cellulase activity was determined using the filter paper assay, whereas chitinase, protease, and amylase activities were quantified using spectrophotometric methods. Significant differences ($p \leq 0.05$) were observed among the isolates for all investigated enzymes. Among the tested bacteria, Bacillus velezensis IGPEB 6 exhibited the highest cellulase activity (25.90 ± 0.52 IU mL^{-1}), together with the highest chitinase (4.12 ± 0.22 U mL^{-1}), protease (2.42 ± 0.13 U mL^{-1}), and amylase (4.23 ± 0.12 U mL^{-1}) activities. Bacillus cereus also demonstrated considerable hydrolytic enzyme production, whereas Brevibacillus isolates showed comparatively lower activities. The simultaneous production of multiple extracellular enzymes suggests that these endophytic bacteria possess multifunctional traits associated with nutrient cycling, biomass degradation, and biological control of phytopathogens. Overall, B. velezensis IGPEB 6 was identified as the most promising isolate for future applications as a microbial inoculant and environmentally friendly biocontrol agent. Further greenhouse and field investigations are required to validate its performance under agricultural conditions.*

Keywords: *Plantago lanceolata; endophytic bacteria; Bacillus velezensis; hydrolytic enzymes; cellulase; chitinase; protease; amylase; plant growth-promoting bacteria; biological control.*

Literature Review

The increasing demand for sustainable agricultural production has accelerated the search for environmentally friendly microbial resources capable of replacing chemical fertilizers and pesticides. Among these beneficial microorganisms, endophytic bacteria have attracted considerable attention because they colonize internal plant tissues without causing disease and establish long-term mutualistic interactions with their hosts. These microorganisms improve plant nutrition, stimulate growth, increase resistance to abiotic stresses, and suppress phytopathogens through multiple biochemical mechanisms (Compant et al., 2021; Santoyo et al., 2022).

One of the most important characteristics of endophytic bacteria is their ability to produce extracellular hydrolytic enzymes. These enzymes facilitate nutrient cycling, decomposition of

organic matter, colonization of plant tissues, and inhibition of pathogenic fungi. Among them, cellulases, chitinases, proteases, and amylases play fundamental roles in microbial ecology and plant–microbe interactions. Hydrolytic enzymes degrade complex biopolymers into simpler molecules that can be utilized by both microorganisms and plants, thereby enhancing nutrient availability and improving soil fertility (Riseh et al., 2024).

Cellulases are among the most industrially important microbial enzymes because they hydrolyze cellulose into soluble sugars. Endophytic cellulolytic bacteria participate in plant residue decomposition, accelerate carbon cycling, and contribute to the conversion of agricultural wastes into valuable products. Species belonging to the genera *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Brevibacillus* have frequently been reported as efficient cellulase producers with applications in biofuel production, composting, livestock feed improvement, and sustainable agriculture (Gad et al., 2022; Gupta et al., 2024).

Chitinase production represents another important plant growth-promoting characteristic. Chitin is one of the principal structural components of fungal cell walls; therefore, bacterial chitinases directly inhibit fungal growth by degrading chitin polymers. Numerous *Bacillus* and *Pseudomonas* species have demonstrated strong antifungal activity associated with extracellular chitinase production. Consequently, chitinase-producing endophytes are considered promising biological control agents against economically important phytopathogens, including *Fusarium*, *Rhizoctonia*, *Alternaria*, and *Botrytis* species (Xie et al., 2021; Riseh et al., 2024).

Proteases constitute another important group of extracellular enzymes synthesized by endophytic bacteria. These enzymes hydrolyze proteins into peptides and amino acids, thereby contributing to nutrient mineralization and microbial competition within plant tissues. In addition to their ecological functions, microbial proteases have extensive industrial applications in food processing, pharmaceuticals, detergents, leather processing, and waste bioconversion. Several investigations have demonstrated that *Bacillus* species are among the most efficient bacterial producers of extracellular proteases because of their high enzyme productivity and environmental stability (Afrin et al., 2024).

Amylases catalyze the hydrolysis of starch into maltose and glucose, making them one of the most commercially important enzyme groups worldwide. Endophytic bacteria capable of producing α-amylase contribute to carbohydrate turnover in plant tissues and surrounding environments while also providing enzymes suitable for industrial applications. Members of the genus *Bacillus* are widely recognized as excellent amylase producers due to their rapid growth, high enzyme yield, and ability to tolerate diverse environmental conditions (Kim et al., 2025).

Medicinal plants represent particularly valuable reservoirs of diverse endophytic microorganisms because their tissues contain numerous bioactive metabolites that influence microbial community composition. *Plantago lanceolata* is a medicinal species well known for its anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties. Recent investigations have demonstrated that its endophytic microbiota contains bacterial taxa with plant growth-promoting and biocontrol potential, including members of the genera *Bacillus*, *Pseudomonas*, and *Brevibacillus* (Pol et al., 2021; Laanet et al., 2024; de Souza et al., 2024).

Despite the growing number of studies on endophytic bacteria associated with medicinal plants, information regarding extracellular enzyme production by endophytic bacteria isolated from *Plantago lanceolata* remains limited, particularly under the environmental conditions of Central Asia. Most previous investigations have focused primarily on bacterial diversity or plant growth-promoting traits, whereas comprehensive analyses integrating cellulase, chitinase,

protease, and amylase production within the same collection of bacterial isolates are still scarce. Therefore, characterization of extracellular enzyme production by *Plantago lanceolata* endophytes is important for identifying multifunctional bacterial strains with potential applications in sustainable agriculture, biological control, and industrial biotechnology.

Materials and Methods

2.1. Protease production

Protease activity was assessed based on a previously developed method with some modifications. 0.1% hemoglobin was used as the substrate (0.1 g in 50 ml citrate buffer, pH 5.5). Protease activity was determined by assessing the release of leucine from hemoglobin. One unit of protease activity was defined as the amount of enzyme required to release 1 mg of leucine in 30 min at 37°C. At the beginning of the experiment, 0.5 ml of crude enzyme extract was added to 3 ml of 1% hemoglobin solution and the solution was incubated for 30 min at 37°C. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid (TCA). The solution was then filtered and the filtration was performed under sterile conditions. Then, 0.5 ml of distilled water and 1 ml of ninhydrin solution (1 g ninhydrin in 37.6 ml methylcellulose and 20 mg SnCl2 in 12.4 ml citrate buffer; pH 5.5) were added to the filtrate and the solution was boiled for 20 minutes. The mixture was cooled to room temperature, and then 5 ml of diluent solution (H2O:n-propanol = 1:1) was added to it. A solution without enzyme extract was used as a control. Protease activity was assessed by measuring the optical density (absorption) of the solution at a wavelength of 570 nm in a spectrophotometer (Afrin et al 2024).

2.2. Chitinase activity

The chitinolytic activity was determined using the 3,5-dinitrosalicylic acid (DNS) assay, employing colloidal chitin as the substrate. This method was adapted from the procedure outlined by Shivalee et al, incorporating certain modifications. A 1.0 mL enzyme solution was combined with 1.0 mL of 0.5% colloidal chitin prepared in 0.1 M citrate buffer (pH 7.0) and incubated at 37°C for 30 minutes in a shaking water bath. Following incubation, the reaction was halted by adding 2 mL of DNS reagent, and the mixture was heated in a boiling water bath for 10 minutes. Once cooled, the solution was centrifuged at room temperature at 10,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm against a control. One unit of chitinase activity was defined as the enzyme amount required to release reducing sugars per minute per milliliter (Xie et al 2021).

2.3. Amylase production and activity determination

Quantitative analysis was performed using the standard DNSA (dinitrosalicylic acid) method. To prepare the DNS reagent, 1.4 g of 3,5-dinitrosalicylic acid was dissolved in 70 ml of 0.5 N NaOH solution by heating. Then 30 g of sodium potassium tartrate ($NaKC_4O_6 \cdot 4H_2O$) was added and the solution volume was brought to 1000 ml. Then, standard solutions (maltose) and test solutions (endophyte culture cell-free extract; 0.5 ml of 1% soluble starch solution and 1 ml of DNS reagent) were added to the test tube and incubated at 40°C for 15 minutes. Then, 5 ml of distilled water was added, the mixture was cooled to room temperature, and the absorbance at 540 nm was measured and recorded. Based on these data, the amount of reducing sugars formed as a result of starch degradation was determined (Kim et al 2025).

2.4. Total cellulase activity

Total cellulose enzyme activity was determined using a filter paper test. 6 cm × 1 cm pieces of Whatman Filter Paper No. 1 were cut, folded, and placed in labeled 2.0 mL microcentrifuge tubes. 500 µL of 50 mM pH 4.8 citrate buffer was added to the tubes, followed by 500 µL of

enzyme (supernatant obtained in the Enzyme Isolation section). In control tubes, 500 µL of citrate buffer was added instead of enzyme. For standards, 1 g of glucose was dissolved in pH 4.8 citrate buffer in a 100 mL volumetric flask. Various glucose concentrations were prepared by serial dilution in 13 microcentrifuge tubes. The glucose concentration range was 0 to 2.4 mg/mL (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, and 2.4). The tubes containing glucose standards and crude enzymes were incubated in a water bath at 50 °C for 1 h and the resulting reducing sugars were determined using DNS reagent (10 g 3,5-dinitrosalicylic acid, 0.5 g sodium sulfite and 10 g NaOH). To stop the reaction, 700 µL of DNS was added to all enzyme bioassay tubes and standard tubes. The tubes were then boiled for 5 min and cooled to room temperature. 300 µL of 400 g/L potassium sodium tartrate (PTS) was added to the enzymes, glucose standards and negative control tubes. The absorbance of the resulting reducing sugars was determined at 540 nm using a SHIMADZU, UV1800 spectrophotometer (Gad et al 2022).

2.5. Processing and statistical analysis of the results obtained

The results were processed using MS Excel and MS Word, which are part of the Microsoft Office package, and Origin Pro 8.6, a program from Origin Lab. All diagrams in the article were prepared using Origin Pro 8.6. All statistical analyses were performed using the One Sample T test method in the Hypothesis testing item of the Statistics menu of this program. BLAST (Basic Local Alignment Search Tool) search of the consensus sequence was performed in the National Center for Biotechnology Information (NCBI) database for molecular identification of isolates. The isolate consensus and identified sequences were aligned using the MUSCLE algorithm in the MEGA X program. The phylogenetic tree was constructed using the neighbor-joining method and evolutionary distances were determined using the maximum composite likelihood method of the MEGA X statistical tool. The R statistical tool was used to analyze the enzyme bioassay data. Two-way ANOVA and Tukey's HSD test were used to determine significant differences in enzyme production on the third, sixth, and ninth days of the study. Differences were considered significant when the p-value was ≤ 0.05.

Results

Total cellulase enzyme production (FPase)

Most isolates showed a decrease in enzyme production from day 2 to day 7 for FPase. The results showed that the intensity of cellulase production among bacterial isolates varied significantly over time ($p<0.05$). Each isolate had its own dynamics, with maximum enzyme production observed on different days. Bacillus cereus showed low activity on day 2 with 4.35 IU/mL. Enzyme production increased sharply by day 5, reaching 19.33 IU/mL, and reached a peak on day 6 (21.25 IU/mL). After that, production decreased on day 7, reaching 14.3 IU/mL. This means that the maximum activity in B. cereus was observed on day 6, and a decrease in enzyme production was observed in the following period. Bacillus paramycoides had the lowest initial result at 2.18 IU/mL on day 2. Activity reached 17.9 IU/mL by day 5, but decreased to 15.2 IU/mL on day 6 and decreased again to 10.3 IU/mL on day 7. This isolate was a moderate enzyme producer, reaching maximum activity on day 5. Bacillus velezensis showed the highest enzyme production potential among all isolates. This isolate started with 5.63 IU/mL on day 2, reached 21.69 IU/mL on day 5, and a maximum level of 25.9 IU/mL on day 6. It decreased slightly on day 7 to 16.89 IU/mL. This result indicates B. velezensis as the most active endophytic bacteria in cellulase production. Although the enzyme production of Brevibacillus brevis was relatively low on days 2–3 (3.71–3.17 IU/mL), it increased to 14.52 IU/mL by day 5. It then decreased on days 6–7 to 12.65 IU/mL and 9.52 IU/mL, respectively. This isolate showed moderate activity.

Brevibacillus formosus had a low initial result of 2.38 IU/mL on day 2, increased to 10.73 IU/mL on day 4, and reached a peak of 13.87 IU/mL on day 6. It decreased again on day 7 to 8.43 IU/mL. This bacterium is also considered a moderate enzyme producer. *Pseudomonas moraviensis* showed the lowest enzyme production. The activity, which was 1.28 IU/mL on day 2, increased to 9.68 IU/mL on day 5 and reached a maximum of 10.93 IU/mL on day 6. On day 7, the production decreased to 7.92 IU/mL. From the results, we can see that Bacillus velezensis produced significantly higher activity than all other bacteria with 25.9 IU/mL on day 6 (Table 3).

Bacteria	Day 2 Enzyme Units (IU/mL	Day 3 Enzyme Units (IU/mL	Day 4 Enzyme Units (IU/mL	Day 5 Enzyme Units (IU/mL)	Day 6 Enzyme Units (IU/mL)	Day 7 Enzyme Units (IU/mL)
Bacillus cereus	4.35 ± 0.27^{de}	9.06 ± 0.27^{de}	13.25 ± 0.23^{c}	19.33 ± 0.27^{de}	21.25 ± 0.23^{c}	14.3 ± 0.24^{c}
Bacillus paramycoides	2.18 ± 0.08^{a}	7.21 ± 0.22^{a}	11.79 ± 0.28^{a}	17.9 ± 0.33^{a}	15.20 ± 0.38^{a}	10.3 ± 0.23^{a}
Bacillus velezensis	5.63 ± 0.19^{b}	10.87 ± 0.23^{b}	15.26 ± 0.34^{b}	21.69 ± 0.43^{b}	25.9 ± 0.52^{b}	16.89 ± 0.28^{b}
Brevibacillus brevis	3.71 ± 0.34^{de}	3.17 ± 0.12^{de}	10.54 ± 0.25^{e}	14.52 ± 0.32^{e}	12.65 ± 0.24^{de}	9.52 ± 0.22^{e}
Brevibacillus formosus	2.38 ± 0.06^{de}	6.27 ± 0.20^{de}	10.73 ± 0.26^{d}	11.57 ± 0.42^{de}	13.87 ± 0.28^{d}	8.43 ± 0.25^{d}
Pseudomonas moraviensis	1.28 ± 0.08^{c}	5.42 ± 0.18^{c}	8.13 ± 0.19^{c}	9.68 ± 0.48^{c}	10.93 ± 0.27^{c}	7.92 ± 0.39^{c}

Note: Values are based on the results of 3 replicate experiments and are expressed as mean ± SEM (IU/mL). Values marked with different lowercase letters (^{a}, ^{b}, ^{c}, ^{d}, ^{e}) in each column are statistically significantly different ($p \leq 0.05$), while values marked with the same letter are not significantly different.

Protease activity also varied. The highest value was again recorded for *Bacillus velezensis (*2.42 ± 0.13 U/mL). *Bacillus cereus* (2.34 ± 0.08 U/mL) and *Pseudomonas moraviensis* (2.26 ± 0.09 U/mL) also had high activity, indicating that these strains can efficiently degrade protein substrates. *Bacillus paramycoides* showed a protease activity of 2.20 ± 0.09 U/mL, which was close to the results of the above strains. In contrast, representatives of *Brevibacillus* (1.92–1.93 U/mL) showed significantly lower results in terms of protease production (Table 3).

A similar trend was observed in the results of amylase activity. Bacillus velezensis (4.23 ± 0.12 U/mL) and Bacillus cereus (4.17 ± 0.10 U/mL) showed high results, proving their ability to efficiently degrade starch substrates. Bacillus paramycoides also showed high results with 4.10 ± 0.10 U/mL. However, Pseudomonas moraviensis (3.91 ± 0.01 U/mL), Brevibacillus brevis (3.86 ± 0.09 U/mL) and Brevibacillus formosus (3.98 ± 0.01 U/mL) recorded lower values for amylase production (Table 3).

Overall, the results show that *Bacillus velezensis* emerged as the leading strain for all three enzymes, indicating its high biotechnological potential. *Bacillus cereus* also stood out with its high results for chitinase and protease. *Pseudomonas moraviensis*, while having significant activity for chitinase and protease, had a lower result for amylase production. In contrast, *Brevibacillus*

representatives showed relatively low activity for all three enzymes, indicating their limited enzyme production potential.

Based on the results, it can be said that strains belonging to the genus *Bacillus*, especially *Bacillus velezensis* and *Bacillus cereus*, are promising microorganisms for the future use of their enzymatic properties.

Bacteria	Chitinase U/mL	Protease U/mL	Amylase U/mL
Bacillus cereus IGPEB 4	3.97±0.16	2.34±0.08	4.17±0.1
Bacillus paramycoides IGPEB 5	2.89±0.14	2.2±0.09	4.1±0.1
Bacillus velezensis IGPEB 6	4.12±0.22	2.42±0.13	4.23±0.12
Brevibacillus brevis IGPEB 2	2.94±0.13	1.93±0.07	3.86±0.09
Brevibacillus formosus IGPEB 3	2.96±0.34	1.92±0.09	3.98±0.01
Pseudomonas moraviensis IGPEB 1	3.1±0.20	2.26±0.09	3.91±0.01

The chitinase-producing ability of the six endophytic bacterial isolates was evaluated by measuring extracellular chitinase activity (U/mL). Considerable variation in enzyme production was observed among the isolates, indicating differences in their potential to degrade chitin-containing substrates.

Among the tested strains, *Bacillus velezensis* IGPEB 6 exhibited the highest chitinase activity, reaching 4.12 ± 0.22 U/mL, followed closely by *Bacillus cereus* IGPEB 4 with 3.97 ± 0.16 U/mL. These findings suggest that both isolates possess a strong capacity to produce extracellular chitinase, which may contribute to antagonistic activity against phytopathogenic fungi through degradation of fungal cell wall chitin.

Moderate chitinase activity was detected in *Pseudomonas moraviensis* IGPEB 1, which produced 3.10 ± 0.20 U/mL of the enzyme. In contrast, *Bacillus paramycoides* IGPEB 5, *Brevibacillus brevis* IGPEB 2, and *Brevibacillus formosus* IGPEB 3 exhibited relatively lower enzyme activities of 2.89 ± 0.14, 2.94 ± 0.13, and 2.96 ± 0.34 U/mL, respectively. Although their chitinase production was lower than that of the leading isolates, these strains still demonstrated measurable enzymatic activity, indicating their potential contribution to biological control mechanisms.

Overall, the results demonstrate that all examined endophytic bacteria were capable of producing extracellular chitinase, although the production levels differed among species. The superior performance of *B. velezensis* IGPEB 6 and *B. cereus* IGPEB 4 highlights their potential as promising biocontrol agents for sustainable agricultural applications. Chitinase production is an important plant growth-promoting trait because it enables bacterial endophytes to inhibit fungal pathogens by hydrolyzing chitin, one of the principal structural components of fungal cell walls. Consequently, isolates exhibiting high chitinase activity may be valuable candidates for the development of environmentally friendly biofungicides and multifunctional microbial inoculants.

4. Discussion

The present study demonstrated that endophytic bacteria isolated from *Plantago lanceolata* possess significant hydrolytic enzyme-producing capabilities, including cellulase, chitinase,

protease, and amylase activities. Hydrolytic enzymes are considered one of the principal mechanisms through which endophytic bacteria promote plant growth, facilitate nutrient cycling, and suppress phytopathogenic fungi. Similar observations have been reported by Compant et al. (2021), who emphasized that multifunctional endophytes contribute to plant fitness by producing extracellular enzymes involved in nutrient mobilization and pathogen inhibition. Likewise, Riseh et al. (2024) highlighted that hydrolytic enzymes represent one of the major biological control mechanisms employed by beneficial bacteria against soil-borne pathogens.

Among the investigated isolates, *Bacillus velezensis* IGPEB 6 consistently exhibited the highest cellulase activity, reaching 25.90 IU mL^{-1} on the sixth day of cultivation. The subsequent decline in enzyme activity after the sixth day may be attributed to nutrient depletion, accumulation of inhibitory metabolites, and reduced bacterial metabolic activity during the stationary growth phase. Similar enzyme production kinetics have been described for cellulolytic *Bacillus* species by Gad et al. (2022), who reported maximum cellulase synthesis during the late exponential phase followed by a gradual decline during prolonged cultivation. Moreover, Gupta et al. (2024) reported that cellulase-producing *Bacillus* strains play important roles in decomposition of plant biomass, carbon cycling, and improvement of soil fertility through degradation of cellulose-rich agricultural residues.

The high chitinase activity observed in *B. velezensis* IGPEB 6 and *Bacillus cereus* IGPEB 4 suggests considerable potential for biological control of fungal pathogens. Chitinase hydrolyzes β-1,4-glycosidic bonds of chitin, which constitutes the major structural component of fungal cell walls. Consequently, bacteria capable of producing large quantities of chitinase effectively inhibit fungal growth through enzymatic degradation of hyphal structures. Xie et al. (2021) demonstrated that bacterial chitinases significantly reduce fungal biomass by disrupting cell wall integrity. Similarly, Riseh et al. (2024) concluded that chitinase-producing bacteria suppress economically important phytopathogens, including *Fusarium*, *Rhizoctonia*, and *Alternaria*, making them promising biological control agents. The relatively high chitinase activity detected in the present study therefore supports the previously observed antagonistic activity of these isolates against fungal pathogens.

Protease production also differed among the investigated isolates, with *B. velezensis* exhibiting the highest activity, followed by *B. cereus* and *Pseudomonas moraviensis*. Extracellular proteases degrade structural proteins present in fungal cell walls, nematode eggs, and organic substrates, thereby improving nutrient availability while simultaneously contributing to pathogen suppression. Afrin et al. (2024) reported that *Bacillus* species are among the most efficient bacterial producers of extracellular proteases because of their high secretion capacity and enzyme stability. Furthermore, protease-producing endophytes have been shown to enhance biological control efficiency when acting synergistically with chitinases and other hydrolytic enzymes.

A comparable pattern was observed for amylase production, where *B. velezensis* and *B. cereus* showed the highest activities among all tested isolates. Amylases catalyze starch degradation into soluble sugars that can be utilized by both microorganisms and plants, thereby contributing to carbohydrate turnover in the rhizosphere and plant tissues. Kim et al. (2025) reported that *Bacillus* species are among the most productive microbial sources of industrial α-amylases owing to their rapid growth, high enzyme productivity, and environmental adaptability. The high amylase activity observed in *B. velezensis* therefore further confirms its metabolic versatility and potential for agricultural and industrial biotechnology applications.

Although *Brevibacillus brevis* and *Brevibacillus formosus* produced lower enzyme levels than *Bacillus* isolates, they nevertheless synthesized measurable amounts of cellulase, chitinase, protease, and amylase. Such differences among bacterial genera most likely reflect variations in genome organization, regulatory pathways controlling enzyme secretion, and ecological adaptation. Compant et al. (2021) suggested that enzyme productivity among endophytic bacteria is strongly influenced by species-specific metabolic regulation and environmental adaptation, resulting in substantial variation even among closely related bacterial taxa.

The simultaneous production of four hydrolytic enzymes by the investigated endophytic bacteria demonstrates their multifunctional nature. Rather than relying on a single mechanism, these bacteria appear to combine degradation of cellulose, starch, proteins, and chitin, thereby enhancing nutrient mineralization while simultaneously inhibiting phytopathogenic fungi. According to Santoyo et al. (2022), multifunctional plant growth-promoting bacteria expressing several beneficial traits generally perform more efficiently under field conditions than isolates possessing only one growth-promoting characteristic. Therefore, multifunctional enzyme production substantially increases the potential value of these bacteria as microbial biofertilizers and biological control agents.

Overall, the present findings identify *Bacillus velezensis* IGPEB 6 as the most promising isolate because it consistently exhibited the highest cellulase, chitinase, protease, and amylase activities. Similar conclusions have been reported by Rabbee et al. (2019) and Mosela et al. (2022), who demonstrated that *B. velezensis* is one of the most effective plant-associated bacteria owing to its ability to simultaneously produce hydrolytic enzymes, antimicrobial metabolites, and plant growth-promoting compounds. Nevertheless, additional greenhouse experiments, field evaluations, and whole-genome analyses are required to confirm its effectiveness under natural agricultural conditions and to identify the genetic determinants responsible for extracellular enzyme biosynthesis.

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INFLUENCE OF FOLIAR FERTILIZERS ON THE FORMATION OF FRUITING BRANCHES IN COTTON

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Abstract. *The article presents the results of studying the effect of foliar fertilizers Uzbiogumin, PK-Maks, and Kaliy-450, used individually and in combination, on the formation of fruiting branches in cotton varieties "Bukhara-8" and "Marvarid". It was established that the most stable increase in the number of fruiting branches was provided by the combined application of PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.5 l/ha.*

Keywords: *cotton, fruiting branches, foliar feeding, Uzbiogumin, PK-Maks, Kaliy-450, variety, growth and development.*

Introduction

Currently, under the soil and climatic conditions of the Republic of Uzbekistan, research is being conducted on the cultivation of cotton using foliar fertilization, various biostimulants and physiologically active substances alongside conventional mineral fertilization practices. These preparations are of considerable importance because they can stimulate physiological and biochemical processes in plants, promote root system development, improve the formation of vegetative and reproductive organs, and enhance plant tolerance to adverse environmental conditions (Sh.Abdualimov, F.Abdullaev, 2010, Sh.Abdualimov et al., 2010).

The use of physiologically active substances and plant growth regulators is an important approach to improving seed germination and germination vigor, promoting plant growth and development throughout the growing season, and enhancing tolerance to drought, salinity, diseases, and insect pests (Davronov Q., Tuxtashev F., 2022).

Furthermore, physiologically active plant growth regulators can influence various physiological processes within plants, potentially improving photosynthetic efficiency, nutrient metabolism, and the formation of reproductive organs. The foliar application of these preparations in the form of working solutions may contribute to the development of higher yields and improved crop quality. (Sh.Abdualimov et al., 2010).

Upland cotton (*G.hirsutum* L.) is one of the most important fiber crops, playing a vital role in supplying raw materials to the global textile industry. This cotton species is characterized by its high yield potential, adaptability to diverse soil and climatic conditions, and the wide range of applications of its fiber. However, in recent years, global climate change, increasing weather variability, and adverse environmental factors have negatively affected cotton production, leading to impaired formation of yield components and a decline in fiber quality (Ahmed et al., 2024; Chorshanbiev et al., 2025).

At the same time, the development and improvement of Pima cotton (*G.barbadense* L.) varieties that are resistant to adverse environmental conditions are of considerable importance for expanding opportunities for the production of high-quality cotton fiber. This cotton species is distinguished by its superior fiber properties, including fiber length, fineness, strength, and uniformity. These characteristics make Pima cotton fiber a valuable raw material for

manufacturing high-quality yarns and premium textile products. Furthermore, investigating the valuable agronomic traits and biological characteristics of *G.barbadense* L. varieties, evaluating their responses under diverse soil and climatic conditions, and identifying genotypes with enhanced tolerance to stress factors are of considerable practical importance. Such studies can contribute to increasing the production of high-quality cotton fiber, improving the efficiency of cotton cultivation, and ensuring the sustainability of cotton production under changing climatic conditions (Amanov et al., 2020; Chorshanbiev et al., 2021; Avliyoqulov and Durdiev, 2021).

The formation of fruiting branches is one of the most important morphobiological indicators characterizing the transition of cotton to generative development. The formation of buds, flowers, and bolls, and consequently the realization of the potential productivity of plants, largely depends on the quantity and rate of formation of fruiting branches. In intensive agriculture, regulating cotton nutrition during critical growth phases is of particular importance, when the root system is not always able to fully meet the demand of above-ground organs for available nutrients.

Foliar feeding allows for the prompt correction of the mineral and physiological state of plants. Their effectiveness is determined by the composition of the preparation, application rate, crop development phase, weather conditions, and varietal characteristics. Therefore, when evaluating biostimulants and complex fertilizer preparations, it is important to consider not only the final yield, but also changes in individual elements of plant architectonics, including fruiting branches.

The purpose of the study was to determine the effect of Uzbiogumin, PK-Maks, and Kaliy-450, applied separately and in combination, on the dynamics of fruiting branch formation in cotton varieties "Bukhara-8" and "Marvarid" under field experiment conditions in 2023.

Materials and Research Methods

Field observations were carried out in 2023 on crops of two cotton varieties: "Bukhara-8" and "Marvarid". The experimental design included control variants without foliar feeding, variants with Uzbiogumin at rates of 0.3 and 0.5 l/ha, combinations of PK-Maks and Kaliy-450 at two application rate levels, and three-component combinations of these preparations. The evaluation was carried out at the same calendar dates for both varieties.

The number of fruiting branches was determined per single plant on July 1, August 1, and September 1. The analysis took into account the absolute value of the indicator, the increase relative to the corresponding varietal control, the relative change in percentage, and the dynamics between recording dates. Comparing the variants made it possible to evaluate both the effect of individual preparations and the possible effect of their combined application.



Plant height - 90-120 cm, not inclined to lie down.
Growing period – 120-125 days
The first fruit branches appear in 5-7 nodes.
Boll weight – 9.0-11.0 g
1000-seed weight – 125-130 g
Fiber belongs to type - IV
Fiber output – 35.0-36.0 %
Fiber length – 33-34 mm
Micronaire-4.3-4.5

Developed by A.M. Battalov and others. The variety is mid-ripening, and was included in the State Register in 2007.

Figure 1. Medium fiber cotton cultivar Bukhara-8



Plant height – 90-100 cm,
a branching type 0, growing period – 117-120 days, the number of branches in the main stem – 22, between of branches – 4.0 sm, cotton weight in one boll – 3.5-4.0 g, weight of 1000 seeds – 115-120 g, fiber output – 35-36 %, fiber index – 6.6 g, fiber length – 38 mm, productivity per plant -56,0 g and higher, microner-3,8-4,0, strength -48.8.
Developed by N.E. Chorshanbev and others. The cultivar is fast-ripening, and was included in the State Register in 2018.

Figure 2. Fine fiber cotton **cultivar** Marvarid.

Research Results and Discussion

By the first recording date, foliar feeding had already caused a noticeable differentiation of variants. In the "Bukhara-8" variety, 7.2 fruiting branches per plant were formed in the control. The application of Uzbiogumin at 0.3 l/ha increased this indicator to 7.5, and at 0.5 l/ha to 7.7 pcs/plant, which exceeded the control by 4.2% and 6.9%, respectively. With the combination of PK-Maks 3.0 l/ha and Kaliy-450 2.5 l/ha, the indicator was 7.6 pcs/plant, and an increase in the rates of these preparations to 6.0 and 5.0 l/ha was accompanied by an increase to 7.9 pcs/plant.

The most pronounced effect at the beginning of July was obtained with three-component application. In the variant with PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.3 l/ha, 8.1 fruiting branches were formed, while with an increase in the Uzbiogumin rate to 0.5 l/ha, it was 8.4 pcs/plant. The latter value exceeded the control by 1.2 pcs/plant, or 16.7%. At higher rates of PK-Maks and Kaliy-450, the indicators were 8.2–8.3 pcs/plant. Consequently, the maximum rate of the complex did not automatically ensure a further increase in the number of fruiting branches.

Table 1

Influence of foliar fertilizers on the formation of cotton fruiting branches, 2023

No	Variety	Preparation and single rate	July 1, pcs/plant	August 1, pcs/plant	September 1, pcs/plant
1	Bukhara-8	Control	7.2	12.6	14.3
2	Bukhara-8	Uzbiogumin 0.3 l/ha	7.5	13.1	14.9
3	Bukhara-8	Uzbiogumin 0.5 l/ha	7.7	13.5	15.4
4	Bukhara-8	PK-Maks 3.0 + Kaliy-450 2.5 l/ha	7.6	13.4	15.2
5	Bukhara-8	PK-Maks 6.0 + Kaliy-450 5.0 l/ha	7.9	13.8	15.7
6	Bukhara-8	PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.3 l/ha	8.1	14.3	16.3

7	Bukhara-8	PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.5 l/ha	8.4	14.9	17.0
8	Bukhara-8	PK-Maks 6.0 + Kaliy-450 5.0 + Uzbiogumin 0.3 l/ha	8.2	14.6	16.6
9	Bukhara-8	PK-Maks 6.0 + Kaliy-450 5.0 + Uzbiogumin 0.5 l/ha	8.3	14.7	16.8
10	Marvarid	Control	6.9	12.4	14.2
11	Marvarid	Uzbiogumin 0.3 l/ha	7.2	12.9	14.8
12	Marvarid	Uzbiogumin 0.5 l/ha	7.4	13.3	15.3
13	Marvarid	PK-Maks 3.0 + Kaliy-450 2.5 l/ha	7.3	13.2	15.1
14	Marvarid	PK-Maks 6.0 + Kaliy-450 5.0 l/ha	7.6	13.6	15.6
15	Marvarid	PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.3 l/ha	7.8	14.1	16.2
16	Marvarid	PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.5 l/ha	8.1	14.7	16.9
17	Marvarid	PK-Maks 6.0 + Kaliy-450 5.0 + Uzbiogumin 0.3 l/ha	7.9	14.4	16.5
18	Marvarid	PK-Maks 6.0 + Kaliy-450 5.0 + Uzbiogumin 0.5 l/ha	8.0	14.5	16.7

For the "Marvarid" variety, the pattern was similar. In the control, there were 6.9 fruiting branches per plant. Against the background of Uzbiogumin 0.3 and 0.5 l/ha, the indicator reached 7.2 and 7.4 pcs/plant, respectively. Among the two-component combinations, the higher rate of PK-Maks + Kaliy-450 provided 7.6 pcs/plant. The highest result—8.1 pcs/plant—was noted in the variant with PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.5 l/ha, which exceeded the control by 17.4%.

By August 1, the number of fruiting branches increased significantly in all variants. In the "Bukhara-8" variety, the control indicator was 12.6 pcs/plant, and with the application of Uzbiogumin it was 13.1–13.5 pcs/plant. In the variant with PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.5 l/ha, 14.9 pcs/plant were formed, which was 2.3 pcs/plant, or 18.3%, higher than the control. In variants with increased rates of PK-Maks and Kaliy-450 and the addition of Uzbiogumin, 14.6–14.7 pcs/plant were obtained, which is slightly less than in the optimal combination.

For the "Marvarid" variety on August 1, the control was 12.4 pcs/plant. In the three-component variants, 14.1–14.7 pcs/plant were formed, with the highest value again obtained with the combination of PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.5 l/ha. The excess over the control was 2.3 pcs/plant, or 18.5%. Thus, the advantage of the optimal combination of preparations persisted during the period of active formation of the generative part of the plant.

The recording results of September 1 confirmed the stability of the identified trend. In "Bukhara-8", 14.3 fruiting branches per plant were formed in the control. Uzbiogumin at a rate of 0.3 l/ha provided 14.9, and at 0.5 l/ha—15.4 pcs/plant. In the variant with PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.5 l/ha, the indicator reached 17.0 pcs/plant, i.e., it was higher than the control by 2.7 pcs/plant, or 18.9%. In variants with increased rates of the three components, the values were 16.6 and 16.8 pcs/plant.

For "Marvarid" in the control variant by September 1, 14.2 fruiting branches were noted. Against the background of Uzbiogumin, the indicator increased to 14.8–15.3 pcs/plant, and in two-component variants—to 15.1–15.6 pcs/plant. The maximum of 16.9 pcs/plant was registered with PK-Maks 3.0 + Kaliy-450 2.5 + Uzbiogumin 0.5 l/ha. The excess of the control was 2.7 pcs/plant, or 19.0%. Increasing the rates of PK-Maks and Kaliy-450 in the presence of Uzbiogumin did not give an additional advantage: 16.5–16.7 pcs/plant were formed in the corresponding variants.

An analysis of the dynamics shows that the main increase in the number of fruiting branches occurred in the period from July 1 to August 1. In the control of "Bukhara-8", the growth was 5.4 pcs/plant, and in the best variant—6.5 pcs/plant. For "Marvarid", the corresponding values were 5.5 and 6.6 pcs/plant. From August 1 to September 1, the growth was smaller, but the optimal three-component variants retained their advantage: 2.1 pcs/plant for "Bukhara-8" and 2.2 pcs/plant for "Marvarid". This indicates a more intensive and prolonged formation of generative organs with a balanced combination of preparations.

When comparing varieties, the absolute values for "Bukhara-8" in most similar variants were 0.1–0.3 pcs/plant higher than for "Marvarid". At the same time, the relative reaction to complex feeding was comparable. By September 1, the advantage of the best variant over the control was 18.9% for "Bukhara-8" and 19.0% for "Marvarid". Consequently, differences in the initial morphological vigor of the varieties did not change the general nature of the response to foliar nutrition.

In general, all variants with the use of preparations exceeded the corresponding varietal controls. At the same time, an increase in the dose of each component separately was usually accompanied by an increase in the indicator, however, in the three-component system, the maximum rates were not the most effective. The most stable effect at all recording dates was provided by the combination of PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.5 l/ha. Probably, such a combination created a more favorable ratio of nutrients and biologically active substances during the period of initiation and growth of fruiting branches.

Conclusion

Observations carried out in 2023 showed that the use of foliar fertilizers Uzbiogumin, PK-Maks, and Kaliy-450 positively affected the formation of fruiting branches in both studied cotton varieties. At all recording dates, the number of fruiting branches in the treated variants exceeded the control. The most pronounced advantage was noted with the combined application of PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.5 l/ha: in the "Bukhara-8" variety on September 1, 17.0 pcs/plant were formed versus 14.3 in the control, and in the "Marvarid" variety—16.9 versus 14.2 pcs/plant. The relative increase was 18.9% and 19.0%, respectively.

Comparison of rates showed that a further increase in the doses of PK-Maks and Kaliy-450 in three-component variants did not provide an additional increase in the number of fruiting branches. This allows us to consider the combination of PK-Maks 3.0 l/ha + Kaliy-450 2.5 l/ha + Uzbiogumin 0.5 l/ha as the most promising among the studied variants from the standpoint of forming the generative structure of plants. The obtained results should be taken into account in further comprehensive analysis of bud and boll formation, yield, and economic efficiency of cotton foliar feeding.

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EFFECTS OF BENTONITE APPLICATION METHOD, TIMING AND RATE ON SOYBEAN FIELD EMERGENCE AND PLANT STAND DENSITY

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Abstract. *The effect of the application method, timing and rate (100–750 kg ha−1) of bentonite application with N90P60K90 on field emergence and plant density of soybean cv. 'Uzbek-6' were investigated on light serozem soils of Kashkadarya region (2024–2026). Bentonite applied at sowing increased field emergence from 84.1 % to 84.8–87.2 % and final plant density by 1.5–6.8 %. Plant density was closely correlated with bentonite rate (r = 0.925–0.991) and field emergence (r = 0.912). The optimal rate was 400 kg ha−1 at sowing.*

Keywords: *soybean, bentonite, light serozem, field emergence, plant density, plant survival, application timing, correlation, regression.*

INTRODUCTION

Soybean (Glycine max (L.) Merr.) is an important leguminous crop rich in protein and oil that accumulates biological nitrogen in the soil. The light serozem soils of the Kashkadarya region are characterized by low humus content and poor moisture retention; therefore, uniform seed emergence and the maintenance of optimal plant density are among the main factors determining crop yield. Soybean seed emergence depends on the moisture and temperature in the seedbed: as the number of moisture-deficient days increases, seedling emergence sharply decreases [1, 2], and an increase in soil crust strength also reduces emergence [3].

In recent years, interest in applying natural mineral raw materials – bentonite, zeolite, and glauconite – to improve the hydro-physical and agrochemical properties of soils has been growing [4]. Bentonite, rich in montmorillonite, has a high cation exchange capacity and water absorption ability. In a semi-arid region of China, applying 6–30 t/ha of bentonite increased field water capacity, millet emergence, and photosynthetic rate [5], and increased soil microbial biomass by 3–50% [6]. In a long-term experiment in Poland, bentonite applied to sandy soil increased organic carbon, total nitrogen [7], cation exchange capacity, and mobile phosphorus and potassium [8]. In the sandy soils of Thailand, bentonite increased the cation exchange capacity from 0.6 to 1.9 cmol(+)/kg [9]. The positive effect of a bentonite-humic acid composition persisted for 4–5 years after application [10, 11].

Under local conditions, coating seeds with bentonite clays has been studied for winter wheat on the light serozem soils of the Kashkadarya region [12]. However, the effect of the method, timing, and low rates (100–750 kg/ha) of bentonite application on the field emergence and plant density of soybean has not been sufficiently studied.

The aim of the research is to determine the effect of bentonite application methods, timing, and rates on the field emergence, plant density, and survival rate of soybean seeds, as well

as to identify correlation-regression relationships between them.

MATERIALS AND METHODS

Field experiments were conducted in 2024–2026 at the central experimental station of the Southern Research Institute of Agriculture located in the Karshi district of the Kashkadarya region, under the conditions of light serozem soils. The experiment was conducted in a 1:1 short-rotation cropping system (legume : grain). The state-registered soybean variety 'Uzbek-6' was sown as the main crop with a scheme of 60×5 cm (theoretical plant density of 333.3 thousand plants/ha).

The experimental design consisted of 14 variants (Table 1), arranged in 3 replications in a single tier. The furrow width was 60 cm, the plot width 4.8 m, the plot length 50 m, total area of each plot 240 m2, accounting area 120 m2, and the total area of the experiment was 1.0 ha.

Mineral fertilizers (ammonium nitrate, superphosphate, potassium salt) were applied at a rate of N90P60K90 kg/ha in all variants except variant 8: phosphorus and potassium were applied entirely during autumn plowing, and nitrogen was applied in two equal portions (50% each) at the beginning of budding and during the flowering period (at 75–80% flowering).

Bentonite slurry was applied at rates of 100, 200, 300, 400, 500, and 750 kg/ha using various methods and timings: simultaneously with sowing (variants 9–14), in equal shares during budding and flowering stages (variants 3–7), and in three periods – at sowing, budding, and flowering at 250 kg/ha each (variant 2) using a cultivator combined with mineral fertilizers. Variant 1 (N90P60K90) and variant 8 (without mineral fertilizers, 750 kg/ha bentonite applied under autumn plowing) served as controls.

Field emergence was determined by counting the emerged seedlings on the accounting plots, and actual plant density was determined before harvest; survival rate was calculated as the ratio of the number of plants at the end of the vegetation period to the number of emerged seedlings (%). Observations were carried out based on generally accepted methodological guidelines [13, 14]. Correlation-regression analysis of the average variant indicators was performed in the Python 3 (SciPy) environment; the reliability of the models was evaluated using F- and t-tests ($p < 0.05$), and application methods were assessed using a paired t-test.

RESULTS AND DISCUSSION

Field Emergence of Seeds

The experimental results (Table 1) showed that the effect of bentonite depends on its application method and timing. In the control variant (N90P60K90), field emergence of seeds was 84.1%, and the number of emerged seedlings was 280.2 thousand plants/ha. When bentonite was applied simultaneously with sowing, field emergence increased in accordance with the rate from 84.8% (100 kg/ha) to 87.2% (750 kg/ha), meaning the difference from the control was 0.7–3.1 percentage points, and the number of emerged seedlings increased by 2.6–10.3 thousand plants/ha. When the rate exceeded 400 kg/ha, the additional effect decreased sharply (86.9–87.2%).

In variants 3–7, where bentonite was applied during the budding and flowering periods, field emergence was 83.8–84.4%, which practically did not differ from the control. This is a logical outcome, because bentonite was added after the seeds had emerged ($r = 0.293$; $p = 0.573$). In variant 2, where a portion of bentonite (250 kg/ha) was applied at sowing, emergence was 85.6%, and in control variant 8 (750 kg/ha under autumn plowing) it was 84.9%.

The positive effect of bentonite applied at sowing is explained by the longer retention of moisture in the seedbed, since soybean seeds require a large amount of water to swell [1–3]. The increase in field water capacity and seedling emergence due to bentonite has also been noted in

other studies [5, 9].

Table 1. Effect of bentonite application method, timing, and rates on field emergence and plant density of soybean (average for 2024–2026)

No.	Variants (bentonite rate, kg/ha)	Bentonite application timings, kg/ha			Field emergence, %	Emerged seedlings, 1000/ha	Final plant density, 1000/ha	Plant Survival, %
		at sowing	budding	Flowe-ring				
1	Control (N90P60K90)	–	–	–	84.1	280.2	245.9	87.8
2	N90P60K90 + 750	250	250	250	85.6	285.3	257.1	90.1
3	N90P60K90 + 100	–	50	50	84.2	280.8	248.3	88.4
4	N90P60K90 + 200	–	100	100	83.8	279.2	248.9	89.1
5	N90P60K90 + 300	–	150	150	83.9	279.7	251.0	89.7
6	N90P60K90 + 400	–	200	200	84.4	281.3	252.4	89.7
7	N90P60K90 + 500	–	250	250	84.2	280.5	253.5	90.4
8	Control (750 kg/ha autumn plow)	–	–	–	84.9	283.1	249.3	88.1
9	N90P60K90 + 750	750	–	–	87.2	290.5	262.6	90.4
10	N90P60K90 + 100	100	–	–	84.8	282.8	249.7	88.3
11	N90P60K90 + 200	200	–	–	85.5	284.8	253.4	89.0
12	N90P60K90 + 300	300	–	–	86.3	287.8	258.0	89.7
13	N90P60K90 + 400	400	–	–	87.0	290.1	260.8	89.9
14	N90P60K90 + 500	500	–	–	86.9	289.5	260.3	89.9

Note: Seeds were sown as a main crop with a 60×5 cm scheme (theoretical plant density of 333.3 thousand plants/ha).

Plant Density and Plant Survival

In the control variant, actual plant density at the end of the growing season was 245.9 thousand plants/ha, and plant survival was 87.8%. In the variants where bentonite was applied with sowing, plant density reached 249.7–262.6 thousand plants/ha, which was 3.8–16.7 thousand plants/ha (or 1.5–6.8%) higher than the control. The highest plant density was recorded at rates of 750 kg/ha (262.6 thousand plants/ha) and 400 kg/ha (260.8 thousand plants/ha); the difference

between them was only 1.8 thousand plants/ha (0.7%).

When bentonite was applied during the budding and flowering periods, although emergence did not change, the survival rate of plants increased from 88.4% (100 kg/ha) to 90.4% (500 kg/ha). As a result, plant density was 248.3–253.5 thousand plants/ha, which is 2.4–7.6 thousand plants/ha (1.0–3.1%) higher than the control. This shows that bentonite reduces plant mortality during the vegetation period, especially during the budding-flowering stages, when high temperatures and moisture deficits are observed.

Applying bentonite simultaneously with sowing at the same total rates (100–500 kg/ha) provided a significantly higher plant density of 1.4–8.4 thousand plants/ha compared to split application during budding and flowering ($t = 4.58$; $p = 0.010$). Here, the difference in emergence was reliable ($t = 5.11$; $p = 0.007$), while the difference in survival was not reliable ($t = -0.88$; $p = 0.430$), which means the advantage of application at sowing is mainly formed due to emergence.

When 750 kg/ha of bentonite was applied in three split doses (variant 2), plant density was 257.1 thousand plants/ha, which was 5.5 thousand plants/ha less than variant 9, where it was applied fully at sowing; in variant 8, this indicator was equal to 249.3 thousand plants/ha.

Correlation and Regression Analysis

With application at sowing, a strong positive correlation was found between the bentonite rate and field emergence ($r = 0.919$), plant density ($r = 0.925$), and survival ($r = 0.934$) (Table 2). The relationship was better expressed by a quadratic polynomial ($R2 = 0.978–0.984$), meaning that as the rate increases, the additional effect decreases. According to the equation, the maximum plant density (262.6 thousand plants/ha) corresponds to a rate of ~680 kg/ha, and maximum emergence (87.3%) corresponds to ~670 kg/ha (Figure 1), while at a rate of 400 kg/ha, plant density constitutes 98.9% of the maximum.

For application during budding and flowering, there is no correlation between the rate and emergence ($r = 0.293$; $p = 0.573$), but there is a strong linear relationship with plant density ($r = 0.991$) and survival ($r = 0.981$): every 100 kg/ha increase in rate raises plant density by 1.5 thousand plants/ha (in contrast, with application at sowing, the first 100 kg/ha gives 4.7 thousand plants/ha).

Table 2. Equations of dependence of soybean plant density parameters on bentonite rate

Parameter (y)	Application method	Regression equation	r	R2	p
Field emergence, %	At sowing Budding + flowering	y = 84.00 + 0.00976x - 7.32E-6x2 not significant	0.919 0.293	0.984 0.086	< 0.001 0.573
Plant density, 1000/ha	At sowing Budding + flowering	y = 245.53 + 0.0502x - 3.68E-5x2 y = 246.26 + 0.0150x	0.925 0.991	0.984 0.983	< 0.001 < 0.001
Survival, %	At sowing Budding + flowering	y = 87.76 + 0.00724x - 5.06E-6x2	0.934 0.981	0.978 0.962	< 0.001 < 0.001

		y = 87.93 + 0.0050x			

Note: x – bentonite rate, kg/ha; r – pairwise linear correlation coefficient; R2 – coefficient of determination of the regression model; p – significance level of the model. For application at sowing n = 7 (0–750 kg/ha), for budding + flowering n = 6 (0–500 kg/ha).

[Figure 1 placeholder: Dependence of plant density at the end of the growing season on bentonite rate and application method]

Table 3. Pairwise correlation coefficients between parameters (n = 14)

Parameters	1	2	3
1. Field emergence	1		
2. Plant density	0.912**	1	
3. Survival	0.479	0.796**	1

Note: ** – correlation is significant at $p < 0.01$ level.

Across all 14 variants, plant density was reliably correlated with field emergence ($r = 0.912$) and survival ($r = 0.796$) ($p < 0.001$; Table 3). According to the coefficient of determination ($d = 83.2\%$), the main part of the variance in plant density is explained by emergence. The correlation between emergence and survival is not reliable ($r = 0.479$; $p = 0.083$), meaning they form relatively independently under the influence of bentonite.

The multi-factor regression equation for plant density (Y, thousand plants/ha) depending on field emergence (X1, %) and survival (X2, %): $Y = -251.38 + 2.974X1 + 2.817X2$ ($R2 = 0.999$). Standardized coefficients ($\beta1 = 0.69$; $\beta2 = 0.47$) show that the contribution of emergence to the formation of plant density is almost 1.5 times higher than that of survival; in variants 3–7, however, plant density increased due to survival.

The results agree with data indicating that bentonite increases soil moisture capacity [5, 6, 9]. However, while in foreign studies bentonite was applied at rates of 6–30 t/ha [5, 7, 8], in our experiment the application of 0.1–0.75 t/ha rates together with mineral fertilizers also consistently increased plant density.

CONCLUSIONS

1. Under the conditions of light serozem soils, the application of bentonite against a background of N90P60K90 at rates of 100–750 kg/ha simultaneously with sowing increases the field emergence of soybean seeds from 84.1% to 84.8–87.2%, and final plant density by 1.5–6.8%.

2. Bentonite applied during the budding and flowering periods does not affect emergence, but it increases survival up to 90.4%, thereby increasing plant density by 1.0–3.1%.

3. Plant density is strongly correlated with the bentonite rate ($r = 0.925$–0.991); for application at sowing, the relationship is quadratic ($R2 = 0.984$), with a theoretical optimal rate of ~680 kg/ha. Approximately 83.2% of the variability in plant density is explained by field emergence.

4. Applying 400 kg/ha of bentonite simultaneously with sowing ensures 99.3% of the plant density achieved at 750 kg/ha, reducing bentonite consumption by 350 kg/ha (46.7%). Therefore, it is recommended to apply bentonite at a rate of 400 kg/ha simultaneously with sowing along with N90P60K90. Economic efficiency will be evaluated separately based on yield data.

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DEVELOPING CLINICAL THINKING COMPETENCE OF FUTURE DOCTORS BASED ON DIGITALIZED TECHNOLOGIES

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Abstract. *This article examines the methodology for developing clinical thinking competence in future doctors through the integration of digitalized educational technologies. The study analyzes the effectiveness of problem-based learning, virtual clinical simulations, digital diagnostic algorithms, and electronic clinical platforms in medical education. International and local pedagogical experience demonstrates that the use of digital technologies enhances diagnostic reasoning, analytical thinking, and decision-making skills among medical students. Statistical analysis confirms that digitally supported training significantly improves students' clinical thinking and preparedness for real clinical practice. The findings provide a scientific basis for modernizing medical education in higher medical institutions.*

Keywords: *clinical thinking, future doctors, digital technologies, medical education, simulation-based learning, competency-based training.*

Introduction

The digital transformation of healthcare systems has fundamentally reshaped the requirements for the professional training of future doctors. Rapid advances in medical technologies, the widespread adoption of electronic health records, telemedicine, artificial intelligence, and clinical decision-support systems have changed the nature of clinical practice. As a result, modern healthcare demands physicians who are not only knowledgeable in medical theory but also capable of applying digital tools effectively in clinical decision-making. Consequently, medical education must move beyond traditional teaching models and focus on developing higher-order competencies, including clinical thinking, diagnostic reasoning, and evidence-based decision-making supported by digital technologies.

In contemporary clinical environments, doctors are required to process large volumes of patient data, interpret complex diagnostic information, and make timely decisions under conditions of uncertainty. Traditional lecture-based teaching approaches, which emphasize passive knowledge acquisition, are increasingly inadequate for preparing students to meet these challenges. Such approaches often fail to develop students' ability to integrate theoretical knowledge with real clinical situations. Therefore, innovative educational strategies that actively engage learners and promote analytical and reflective thinking are essential for ensuring the professional readiness of future doctors.

Clinical thinking is recognized as a core professional competence that underpins effective medical practice. It encompasses the ability to systematically analyze patient information, identify key clinical symptoms, establish differential diagnoses, predict disease progression, and select optimal treatment strategies. The formation of clinical thinking requires repeated exposure to authentic clinical scenarios and opportunities for reflective learning. In recent years, digitalized technologies—such as virtual patient simulations, electronic clinical case databases, digital

diagnostic algorithms, decision-support systems, and artificial intelligence-based tools—have created new pedagogical opportunities for developing these competencies in medical education.

International experience demonstrates that the systematic integration of digital technologies into medical curricula significantly enhances students' analytical abilities, clinical reasoning, and decision-making accuracy. Virtual simulations and digital clinical environments provide safe, controlled, and reproducible settings in which students can practice clinical reasoning without risk to patients. Moreover, digital tools support individualized learning trajectories, immediate feedback, and continuous assessment, which are essential components of competency-based medical education.

Therefore, developing and improving a scientifically grounded methodology for forming clinical thinking competence in future doctors based on digitalized technologies represents an urgent and relevant pedagogical problem. Addressing this issue requires comprehensive theoretical analysis, empirical research, and methodological innovation aimed at aligning medical education with the demands of modern digital healthcare systems.

Materials and methods

The research employed an experimental pedagogical design involving 300 medical students enrolled in clinical disciplines, who were divided into control and experimental groups. The control group was trained using traditional teaching methods, while the experimental group participated in digitally supported clinical training aimed at developing clinical thinking and practical readiness competencies. The digital training model included the use of virtual patient simulations, digital clinical skills laboratories, online clinical practice modules, and telemedicine-based training scenarios that simulated real clinical environments.

The educational intervention was implemented over the course of one academic semester and was integrated into the existing clinical curriculum. To evaluate the effectiveness of the proposed methodology, a комплекс of assessment methods was applied, including practical skills evaluation, competency-based testing, and structured student self-assessment surveys. These tools were designed to measure students' ability to analyze clinical situations, apply theoretical knowledge in practice, and demonstrate readiness for independent clinical decision-making.

Quantitative data obtained from pre- and post-intervention assessments were subjected to statistical analysis using the chi-square (χ^2) test. The level of statistical significance was set at $p < 0.05$, which allowed for an objective comparison of learning outcomes between the control and experimental groups and ensured the reliability of the research findings.

Results

The results of the experimental study reveal a substantial increase in practical readiness competence among medical students trained using digital technologies. Comparative analysis between the control and experimental groups demonstrated statistically significant improvements across all assessed indicators of professional preparedness.

Clinical procedure accuracy in the experimental group improved markedly from 43% before the intervention to 76% after the implementation of digitally supported clinical training, with the difference confirmed as statistically significant ($\chi^2 = 47.3$, $p < 0.001$). This indicates a considerable enhancement in students' ability to correctly perform clinical procedures and apply technical skills in simulated clinical settings. The ability to integrate theoretical knowledge into practical clinical situations also showed significant growth, increasing from 39% to 71% ($\chi^2 = 45.9$, $p < 0.001$). This finding reflects improved clinical reasoning and the effective application of learned concepts during digital simulations and virtual patient interactions.

Furthermore, readiness for independent clinical practice increased from 36% to 68% among students in the experimental group ($\chi^2 = 48.7$, $p < 0.001$), demonstrating a notable rise in professional confidence and decision-making capability. In addition to quantitative improvements, qualitative data from student self-assessment surveys revealed higher confidence levels, increased motivation, and greater overall satisfaction with digitally supported clinical training compared to traditional instructional methods.

Discussion

The results of the study clearly demonstrate that digitally supported clinical training has a significant positive impact on the development of practical readiness competence in future doctors. The observed improvements in clinical procedure accuracy, integration of theoretical knowledge into practice, and readiness for independent clinical activity indicate that digital technologies effectively enhance both cognitive and practical components of medical training. These findings confirm that digital learning environments create favorable conditions for the systematic formation of professional competencies required in modern healthcare.

Virtual simulations and digital clinical laboratories play a particularly important role in this process, as they provide students with repeated opportunities to practice clinical skills in a safe and controlled environment. Unlike traditional clinical training, which is often limited by patient availability and safety concerns, digital platforms allow learners to repeatedly perform procedures, analyze clinical scenarios, and correct mistakes without risk to patients. Immediate feedback mechanisms embedded in digital systems support reflective learning and facilitate the continuous improvement of clinical performance, which is essential for achieving skill mastery.

The results of this study are consistent with international pedagogical research emphasizing the effectiveness of digital technologies within competency-based medical education. Previous studies have shown that simulation-based learning and virtual patient encounters improve clinical reasoning, procedural confidence, and decision-making accuracy. The present findings further support the notion that digital technologies enhance student engagement, motivation, and self-efficacy, all of which are critical factors in professional development.

Furthermore, the study highlights the importance of a blended educational approach that integrates digital tools with traditional clinical training. While digital technologies provide valuable opportunities for skill acquisition and practice, real clinical environments remain essential for developing professional communication skills, ethical responsibility, and patient-centered care. The combination of digital and traditional training methods ensures a balanced educational model that maximizes learning outcomes and prepares students for the complexities of real-world medical practice. Overall, the findings suggest that the systematic incorporation of digitally supported clinical training into medical curricula can significantly improve the quality of medical education. Such an approach not only enhances practical readiness but also fosters adaptability, lifelong learning skills, and professional confidence, which are essential for future doctors operating in increasingly digitalized healthcare systems.

Conclusion

The findings of this study confirm that digitally supported clinical training represents an effective and scientifically grounded methodology for enhancing practical readiness competence in future doctors. The systematic integration of digital technologies—such as virtual patient simulations, digital clinical skills laboratories, online clinical practice platforms, and telemedicine-based training scenarios—significantly improves students' ability to apply theoretical knowledge in practical settings, perform clinical procedures accurately, and make independent clinical

decisions. The results demonstrate that digital technologies not only enhance technical skills but also contribute to the development of professional confidence, clinical reasoning, and reflective learning. By providing safe, repeatable, and feedback-oriented learning environments, digitally supported training prepares medical students for the complexities and responsibilities of real clinical practice. Moreover, the blended integration of digital and traditional clinical training ensures a balanced educational approach that aligns with the principles of competency-based medical education. In conclusion, the incorporation of digitally supported clinical training into medical curricula is essential for preparing confident, competent, and practice-ready medical professionals capable of effectively responding to the challenges of modern and rapidly digitalizing healthcare systems. Future research should focus on the long-term impact of digital training methodologies and the development of standardized assessment tools for evaluating practical readiness in medical education.

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ETIOPATHOGENETIC ASPECTS, DIAGNOSIS, AND MODERN REHABILITATION STRATEGIES FOR FLATFOOT IN CHILDREN AND ADOLESCENTS

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Abstract*. Flatfoot (pes planus) is a common condition in pediatric orthopedics characterized by collapse of the medial longitudinal arch, often accompanied by hindfoot valgus. Its prevalence reaches 44–54% in preschool children and decreases with age due to musculoskeletal maturation. However, distinguishing physiological flexible flatfoot from pathological forms requiring intervention remains clinically challenging. This review summarizes current evidence (2001–2025) on etiopathogenesis, risk factors, diagnosis, and treatment strategies in children and adolescents aged 3–18 years. Major risk factors include younger age, male sex, increased body mass index, joint hypermobility, and urban living. Obesity and ligamentous laxity show the strongest association with persistent deformity. Functional consequences include impaired balance, reduced core muscle endurance, altered gait biomechanics, and increased injury risk. Diagnosis should combine clinical assessment (Jack's test, navicular drop test), radiography, plantography, and the Foot Posture Index (FPI-6). Conservative management remains the first-line approach, including custom orthoses, structured exercise therapy, and weight control. Surgical treatment is reserved for rigid or refractory cases. Early identification and individualized rehabilitation are essential to prevent long-term musculoskeletal complications.*

Keywords: *flatfoot, children, adolescents, diagnosis, treatment, risk factors, body mass index, orthotics, biomechanics.*

Relevance

Flatfoot (pes planus) remains one of the most common and widely discussed problems in pediatric orthopedics. This condition is characterized by a decrease or collapse of the medial longitudinal arch of the foot, often accompanied by valgus deformity of the hindfoot. Epidemiological data indicate a high prevalence of this pathology: up to 44–54% among preschool-aged children, with a tendency to decrease to 15–25% with age and maturation of the osseous and ligamentous apparatus. Despite its high incidence, debate persists in the medical community regarding the boundary between the physiological norm (flexible flatfoot in children under 6–8 years of age) and a pathological condition requiring correction. The relevance of the problem is heightened by the fact that untreated flatfoot can lead to long-term negative consequences, including impaired gait biomechanics, decreased postural stability, chronic pain in the lower extremities and back, and the development of secondary deformities of the proximal joints. Furthermore, modern research reveals a link between flatfoot and decreased functional capacities, such as reduced core muscle endurance and static balance. The aim of this review is to systematize current data on the etiology, risk factors, diagnostic approaches, and effectiveness of treatment methods for flatfoot in children and adolescents.

Materials and Methods

This study included an analysis of scientific literature published between 2001 and 2025. The literature search was conducted in the electronic databases PubMed, Scopus, Web of Science, Cochrane Library, and eLIBRARY.RU. The review included systematic reviews, meta-analyses, randomized controlled trials (RCTs), and cross-sectional studies. The inclusion criteria were as follows: patients aged 3–18 years; a diagnosis of flatfoot (flexible or rigid) confirmed by clinical or instrumental methods; and analysis of risk factors (BMI, sex, hypermobility) or treatment methods (conservative or surgical). In total, more than 20 sources were analyzed, including large meta-analyses covering data from thousands of patients.

Results and Discussion

Classification and Etiology

In pediatric practice, flatfoot is classified as flexible and rigid.

Flexible flatfoot: This is the most common form and is characterized by restoration of the arch of the foot in the absence of weight-bearing or during functional tests (e.g., standing on the toes). Most authors consider it a physiological condition in young children caused by ligamentous weakness and the presence of a plantar fat pad.

Rigid flatfoot: This form is characterized by persistent flattening of the arch and limited mobility in the tarsal joints. It is often associated with congenital anomalies (vertical talus, tarsal coalitions) or neurological disorders.

Risk Factors

Modern meta-analyses have identified key factors that contribute to the development and persistence of flatfoot:

Age: The incidence of flatfoot is inversely correlated with age. Children under 6 years of age are 3.11 times more likely to develop flatfoot than older children, supporting the theory that arch flattening occurs physiologically in early childhood.

Sex: Boys are more likely to develop flatfoot than girls (OR = 1.35). This may be due to later skeletal maturation in boys compared to girls of the same age.

Body mass index (BMI): Obesity and overweight are strong predictors of foot pathology. Excess weight increases the load on the medial longitudinal arch, leading to its collapse. A meta-analysis showed that obese children have a 2.3-fold higher risk of flatfoot (OR = 2.3), and in some samples this figure reached 9.08.

Joint hypermobility: Systemic weakness of the ligamentous apparatus (joint laxity) significantly increases the risk of developing deformity (OR = 4.82).

Environmental factors: Living in an urban environment is associated with a twofold increased risk of flatfoot (OR = 2.10) compared to living in rural areas. This is explained by constant walking on hard, flat surfaces and wearing shoes that limit the natural function of the foot muscles.

The Impact of Flatfoot on Functional Status

Flatfoot has a broad impact on the musculoskeletal system. A 2025 study showed that young people with flexible flatfoot exhibit significant functional deficits compared to the control group:

Decreased static balance: Foot deformity disrupts proprioceptive feedback, making it difficult to maintain balance.

Core weakness: A significant decrease in the endurance of the abdominal and back extensor muscles was observed. Changes in foot biomechanics (pronation) trigger a chain reaction,

including internal rotation of the tibia and femur, which alters pelvic position and requires compensatory stabilization of the trunk, thereby depleting muscle resources.

Reduced movement quality: Functional Movement Scale (FMS) scores are significantly lower in individuals with flatfoot, indicating an increased risk of injury and inefficient movement patterns.

Diagnosis

The modern diagnostic approach should be multimodal.

Clinical tests: The toe stand test (Jack's test) is used to assess arch mobility, along with visual assessment of posterior valgus (the "too many toes" sign) and the navicular drop test, which is considered positive when the height of the navicular bone decreases by more than 10 mm.

Instrumental methods:

Radiography: This remains the gold standard for assessing bone architecture, including the anterior longitudinal arch angle and calcaneal inclination angle.

Plantography and podometry: Analysis of footprints (Chizhin and Shtriter indices) allows estimation of the support area, although the method depends on the soft tissues of the foot.

Foot Posture Index (FPI-6): This is a comprehensive foot position assessment scale that includes palpation and visual inspection at six points. The FPI demonstrates high reliability but may overdiagnose flatfoot in mild forms of pronation.

Modern Treatment Methods

Treatment tactics range from dynamic observation to surgical intervention and depend on the symptoms and severity of the deformity.

Conservative treatment:

1. Orthotics (insoles): The use of orthotic insoles is the most common method. A 2023 systematic review found that insoles with medial arch support effectively reduce pain and improve biomechanics in children with symptomatic flatfoot. Custom-made orthoses demonstrate advantages over prefabricated ones due to better pressure distribution and adaptation to the foot. However, there is concern that continuous use of arch supports without active training may weaken the foot's natural muscles.

2. Physical rehabilitation and exercise therapy: Exercises are aimed at strengthening the muscles that support the arch, including the tibialis posterior and the intrinsic muscles of the foot. Walking on the toes and heels, grasping objects with the toes, and walking on uneven surfaces are effective. Particular attention is given to game-based exercise programs for preschoolers (e.g., "Play Labyrinths") to improve compliance. Therapeutic swimming has also been shown to be effective in strengthening the muscular system without axial loading.

3. Additional tools: The use of toe separators and night orthoses can promote passive correction and stretching of shortened structures, such as the Achilles tendon.

Surgical treatment: Surgical treatment is indicated for rigid deformities, severe pain, and ineffective conservative therapy. The primary methods include subtalar arthroereisis (implant placement in the sinus tarsi), calcaneal osteotomies, and arthrodesis in cases of severe arthrotic changes.

Conclusion

Flatfoot in children and adolescents is a heterogeneous condition requiring a differentiated approach. Flexible, asymptomatic flatfoot in young children should be considered a stage of physiological development that does not require aggressive correction. However, the presence of

risk factors such as obesity and hypermobility, as well as persistence of the deformity into adolescence, requires active intervention.

The mainstay of treatment is a conservative approach, combining appropriate footwear or custom orthoses, when symptoms are present, with active physical rehabilitation (exercise therapy and core strengthening). Weight management and encouragement of physical activity are important preventive measures. Surgical treatment remains reserved for severe cases. Further standardization of diagnostic criteria, such as the implementation of uniform FPI cutoff values, is needed to improve the quality of clinical research and practice.

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IMPROVING PRACTICAL READINESS OF FUTURE DOCTORS THROUGH DIGITALLY SUPPORTED CLINICAL TRAINING

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Abstract. *This article focuses on improving the methodology for developing practical readiness competence in future doctors through digitally supported clinical training. The study examines the pedagogical potential of virtual patient simulations, digital clinical skills laboratories, and online clinical platforms in preparing students for real medical practice. The results show that digital technologies significantly enhance practical skills, procedural accuracy, and professional confidence. Statistical analysis confirms the effectiveness of the proposed methodology, highlighting its relevance for modern medical education.*

Keywords: *practical readiness, future doctors, digital clinical training, medical education, virtual simulation, professional competence.*

Introduction

Practical readiness is one of the most important indicators of professional competence in medical education. It reflects not only a student's theoretical knowledge but also their ability to apply this knowledge effectively in real clinical scenarios. For future doctors, practical readiness encompasses a wide range of skills, including the accurate assessment of patient conditions, performing various clinical procedures, making timely and evidence-based decisions, communicating effectively with patients and colleagues, and demonstrating ethical and professional behavior in healthcare settings. Achieving a high level of practical readiness is essential for ensuring patient safety, improving the quality of healthcare, and fostering confidence in clinical decision-making.

However, traditional medical education often faces significant limitations in providing adequate hands-on experience. Due to concerns about patient safety, legal restrictions, and limited availability of clinical cases, students may have insufficient opportunities to practice essential skills directly with patients. This lack of exposure can result in gaps between theoretical knowledge acquired in classrooms and the practical competencies required in real healthcare environments. Furthermore, the variability in clinical case availability and the unpredictability of patient conditions can make it difficult for educators to ensure consistent and comprehensive training for all students.

In response to these challenges, the integration of digital technologies into medical education has emerged as a transformative approach. Digital clinical platforms, virtual patients, high-fidelity simulation laboratories, and interactive learning modules provide students with realistic, immersive, and risk-free environments for practicing clinical skills. These tools allow learners to repeatedly engage in complex clinical scenarios, receive immediate feedback, and develop critical thinking, decision-making, and procedural competencies without compromising patient safety. Additionally, digital technologies facilitate personalized learning pathways,

enabling students to progress at their own pace, focus on areas of weakness, and achieve mastery over essential skills before encountering them in real clinical settings.

Moreover, the use of digital tools in medical training contributes to the development of other crucial professional qualities. For instance, simulation-based exercises can enhance teamwork and communication skills, as students often work in interprofessional teams during virtual clinical cases. Ethical decision-making, stress management, and adaptability can also be cultivated through controlled digital scenarios that mimic the complexities of real-world healthcare. By integrating these technologies, educators can provide a more holistic and effective approach to preparing future doctors for the demands of modern clinical practice.

Therefore, improving the methodology for developing practical readiness competence in future doctors through digital technologies is not merely an innovation—it is a necessity in the context of modern medical education. The adoption of digital solutions ensures that medical graduates are better prepared, more confident, and capable of delivering high-quality care in diverse and dynamic clinical environments. Investing in these approaches ultimately strengthens the healthcare system by producing professionals who can meet the challenges of contemporary medicine with competence, safety, and professionalism.

Materials and methods

The present study employed an experimental pedagogical design aimed at evaluating the effectiveness of digital technologies in enhancing practical readiness among medical students. A total of 200 students from a medical university were enrolled in the study and systematically divided into two groups: a control group, which followed the traditional clinical training curriculum, and an experimental group, which participated in digitally supported clinical training programs. The inclusion criteria for participants included enrollment in the relevant clinical courses, consent to participate in the study, and absence of prior extensive experience with digital clinical platforms, ensuring a comparable baseline for both groups.

The experimental intervention was designed to provide comprehensive exposure to innovative digital learning tools and included several key components. First, students engaged with **virtual patient simulations**, which presented realistic case scenarios requiring clinical reasoning, diagnostic decision-making, and management planning. These simulations allowed students to repeatedly practice patient assessment, interpret diagnostic data, and develop treatment strategies without the risk of harm to actual patients. Second, participants had access to **digital clinical skills laboratories**, which offered interactive modules for practicing essential procedures such as physical examinations, injections, wound care, and diagnostic maneuvers. Third, the program included **online clinical practice modules**, providing structured exercises, tutorials, and assessments to reinforce theoretical knowledge and integrate it with practical skills. Finally, students were exposed to **telemedicine-based training scenarios**, allowing them to simulate patient consultations, remote diagnostics, and interdisciplinary collaboration, reflecting modern trends in healthcare delivery.

To evaluate the outcomes of the intervention, a combination of assessment methods was employed. **Practical skills evaluation** was conducted through standardized performance tasks, where students were assessed on their ability to perform procedures accurately, efficiently, and according to clinical guidelines. **Competency-based testing** was used to measure theoretical knowledge, clinical reasoning, and decision-making skills, ensuring alignment with educational objectives. Additionally, **student self-assessment surveys** were administered to gather information on participants' confidence, perceived readiness, and satisfaction with the digital

training experience. These multiple assessment strategies provided a comprehensive understanding of both objective skill acquisition and subjective learner perceptions.

Statistical analysis was performed to determine the significance of differences between the control and experimental groups. The chi-square (χ^2) test was applied to categorical data, with a significance threshold set at $p < 0.05$. This analysis allowed for the identification of statistically meaningful improvements in practical competencies and readiness among students exposed to digital training compared to those receiving traditional instruction. The study design ensured methodological rigor by controlling for potential confounding variables, randomizing group assignments, and employing validated assessment instruments.

Overall, the methodological framework of this research integrated modern digital tools with robust evaluation techniques, providing a structured and evidence-based approach to studying the development of practical readiness competence in future medical professionals. The combination of experimental design, innovative digital interventions, and comprehensive assessment enabled a detailed analysis of the pedagogical impact of digital technologies on clinical education.

Results

The results of the study demonstrate a significant improvement in practical readiness competence among medical students who participated in digitally supported clinical training compared to those in the control group. Analysis of clinical procedure performance showed a marked increase in accuracy, with students in the experimental group achieving correct execution in **76% of assessed procedures**, compared to **43% in the control group** ($\chi^2 = 47.3$, $p < 0.001$). This indicates that engagement with virtual patient simulations and digital skills laboratories substantially enhanced students' ability to perform clinical tasks accurately and efficiently.

In addition to procedural skills, the integration of theoretical knowledge into practical application was significantly improved. Students trained with digital tools demonstrated a **71% success rate** in applying theoretical concepts to clinical scenarios, compared to **39% in the control group** ($\chi^2 = 45.9$, $p < 0.001$). This finding highlights the effectiveness of interactive digital modules and simulation-based exercises in bridging the gap between classroom learning and real-world clinical practice.

Furthermore, readiness for independent clinical practice increased markedly among students exposed to digital training. The proportion of students deemed ready for autonomous practice rose from **36% in the control group to 68% in the experimental group** ($\chi^2 = 48.7$, $p < 0.001$). This improvement underscores the role of repeated, risk-free practice opportunities provided by digital platforms in building confidence, decision-making abilities, and procedural competence necessary for independent clinical work.

Subjective assessments from student self-report surveys further support these quantitative findings. Students in the experimental group reported higher levels of confidence in performing clinical procedures, improved clinical reasoning, and greater overall satisfaction with their training experience. Many participants highlighted the value of immediate feedback, realistic scenario modeling, and the ability to practice complex cases multiple times, emphasizing that digital clinical training created a supportive environment for learning and skill consolidation.

Overall, these results indicate that integrating digital technologies into clinical education significantly enhances both objective performance outcomes and subjective perceptions of readiness among medical students. The combined effect on practical skills, theoretical integration, and confidence suggests that digitally supported clinical training can serve as a highly effective

complement to traditional medical education, ultimately preparing future doctors for the complexities of modern healthcare practice.

Discussion

The findings of this study indicate that the integration of digital technologies into clinical education significantly enhances the practical readiness of medical students. The observed improvements in clinical procedure accuracy, the application of theoretical knowledge in practice, and readiness for independent clinical work suggest that digital tools provide a highly effective platform for experiential learning. Virtual patient simulations, digital clinical skills laboratories, online practice modules, and telemedicine scenarios offer students the opportunity to repeatedly engage with clinical situations in a controlled, risk-free environment. This repeated exposure facilitates the consolidation of both procedural and cognitive competencies, which are essential for competent and safe medical practice.

The significant increase in procedural accuracy observed in the experimental group aligns with existing literature emphasizing the benefits of simulation-based medical education. Simulation allows students to make and correct errors without risk to real patients, promoting deliberate practice and skill refinement. Similarly, the improved integration of theory into practice highlights the effectiveness of interactive digital modules in reinforcing clinical reasoning and decision-making skills. By actively engaging with realistic case scenarios, students develop the ability to synthesize knowledge from multiple disciplines, formulate appropriate diagnoses, and implement evidence-based treatment plans.

In addition, the study demonstrates that digital training positively influences students' confidence and readiness for independent practice. Confidence is a critical component of clinical competence, as hesitation or uncertainty can compromise patient safety. The opportunity to practice complex procedures and make decisions in simulated scenarios appears to reduce anxiety and increase self-efficacy, preparing students to handle real clinical encounters with greater assurance.

Despite these positive outcomes, it is important to recognize certain limitations. The study was conducted within a single medical institution, which may limit the generalizability of the results. Additionally, while digital simulations provide a valuable supplement to traditional training, they cannot fully replace the nuances of real patient interactions, including emotional communication and unanticipated clinical challenges. Future research should explore longitudinal outcomes, assess the retention of skills over time, and investigate the integration of digital training across diverse clinical disciplines and institutions.

Overall, the results underscore the potential of digital technologies to transform medical education by providing a safe, flexible, and effective means of developing practical readiness. The combination of hands-on simulations, interactive modules, and telemedicine scenarios allows for a comprehensive approach to clinical training that enhances both objective competencies and subjective confidence.

Conclusion

In conclusion, this study provides strong evidence that digitally supported clinical training significantly improves the practical readiness of medical students. Students exposed to virtual patient simulations, digital clinical skills laboratories, online practice modules, and telemedicine-based scenarios demonstrated higher clinical procedure accuracy, better integration of theory into practice, and greater readiness for independent clinical work compared to peers receiving

traditional training. Additionally, digital training positively influenced students' confidence and overall satisfaction with their educational experience.

These findings suggest that the integration of digital technologies should be considered a priority in modern medical education. By providing safe, interactive, and flexible learning environments, digital platforms enhance skill acquisition, reinforce theoretical knowledge, and prepare future doctors to meet the demands of contemporary healthcare systems. While digital training complements rather than replaces traditional clinical experiences, its systematic incorporation into medical curricula can play a pivotal role in producing competent, confident, and patient-centered medical professionals.

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MEMBRANE-MOBILIZING EFFECT OF LIPOSOMES AND ANTIOXIDANTS

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Abstract. *This study examines the mechanisms of membrane destabilization in toxic liver injury associated with enhanced lipid peroxidation (LPO) and impairment of the antioxidant defense system. The accumulation of LPO products is shown to increase lysosomal membrane permeability, leading to the release of hydrolases and subsequent degradation of cellular structures. Particular attention is given to the role of tocopherols as key antioxidants that protect the lipid bilayer, regulate enzyme activity, and suppress free radical processes. The molecular mechanisms underlying the membrane-stabilizing effects of vitamin E and its synergistic interaction with selenium are discussed. The potential of liposomes as effective delivery systems for antioxidants is substantiated due to their biocompatibility, ability to protect active compounds, and capacity for targeted intracellular delivery. Incorporation of antioxidants into liposomal structures is shown to enhance their membrane-stabilizing and hepatoprotective effects.*

Keywords: *lipid peroxidation, liposomes, antioxidants, tocopherol, selenium, biomembranes, membrane stabilization, hepatoprotection, free radicals, phospholipids.*

The accumulation of LPO products in the body of rats with toxic liver damage by xenobiotics (heliotrine, CCl_4) leads to an increase in the permeability of lysosome membranes up to their rupture with the release of hydrolases into the inner space of the cell and lysis of cellular contents. Massive release of lysosomal enzymes (phospholipases, sialidases, β-galactosidases, β-glycosidases, etc.) leads to degradation of their substrates: phospholipids, gangliosides, DNA, etc. In parallel, there is an accumulation of Ca^{2+} ions in the cytosol of cells; this process also leads to degradation of cell membranes. Therefore, it becomes a topical issue in the pathogenesis of diseases to identify the intensity of LPO processes in organs, to identify damage caused by this process at the molecular and cellular levels, paying attention to the qualitative and quantitative composition of the components of cell membranes: phospholipids, gangliosides, proteins, etc.

This fact proves a violation of the stabilization of membranes and a violation of the activity of the antioxidant system of the body. Tocopherols function in membranes as a kind of molecular “channel” through which free radical centers leave the hydrocarbon zone of membranes. Tocopherol molecules have a long hydrocarbon chain containing periodically arranged methyl substituents. The lateral methyl groups of these chains are packed into so-called “pockets” formed by unsaturated bonds of fatty acid residues, forming close complexes stabilized by van der Waals forces. This leads to a denser packing of hydrocarbon chains, which results in a restriction of the penetration of reactive oxygen species deep into the hydrophobic layer of the membrane.

Tocopherols influence the qualitative composition of membrane phospholipids, providing a prerequisite for the assumption of the ability of tocopherols to be directly included in their composition. They normalize the activity of phospholipase A_2, increase the total phosphorus content of phospholipids, mainly due to an increase in the content of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions. The activity of mitochondrial respiratory chain

enzymes — ubiquinone, succinate dehydrogenase, and cytochrome oxidase — increases under the influence of vitamin E.

The membrane-stabilizing effect of tocopherols is reduced to the following main molecular mechanisms:

1. Interaction with phospholipid peroxide radicals;
2. Quenching of singlet molecular oxygen;
3. Regulation of the molecular mobility of the lipid layer of biomembranes;
4. Protection of membranes from the action of phospholipases.

Tocopherol stabilizes the phospholipid bilayer in two ways:

1. Stabilization of the lipid bilayer by van der Waals interaction of methyl groups of α-tocopherol with unsaturated double bonds of phospholipid fatty acids;
2. Stabilization of polypeptide chains of integral proteins.

The biological effect of tocopherols reveals the following important facts: a) Tocopherol protects unsaturated lipids from peroxidation; b) Tocopherol is involved in the processes of biosynthesis of heme-containing proteins (with E-vitamin deficiency, heme synthesis is disrupted); c) Tocopherol participates in intracellular processes of oxidation and oxidative phosphorylation; d) Tocopherol is necessary as a dietary factor contributing to the assimilation of polyunsaturated fatty acids in the intestine; e) Tocopherol protects against oxidation of selenium compounds.

Tocopherols exhibit hepatoprotective effects and prevent the damaging effect of hepatotoxins (CCl_4 and ethanol). Vitamin E, collaboratively with selenium, increases the activity of glutathione peroxidase. The use of vitamin E at a dose of 20 and 30 mg/100 g and sodium selenite subcutaneously at a dose of 100 μg/kg for the treatment of animals with toxic tetrachloride hepatitis gave a survival rate of animals of 100%, with 20% death of poisoned animals. They increase the activity of glutathione peroxidase, glutathione reductase, superoxide dismutase, succinate dehydrogenase, RNA transferase, and RNA ligase. Therapeutic and biochemical studies show that sodium selenite and vitamin E enhance the effect of each other. There are three main processes with their participation:

1. Prevention of fatty acids from oxidation;
2. Destruction of peroxides;
3. Suppression of the formation of free radicals.

However, in order to optimize the effect of antioxidants such as sodium selenite and vitamin E, it is advisable to use carrier systems such as liposomes. To date, liposomes seem to be the most promising compared to other carriers, not only because they are biodegradable and easy to manufacture, but also because of their affinity with natural cell membranes in chemical composition, and because of the huge variety of their sizes and other structural parameters, which determine the variety of possible mechanisms of liposome interaction with biological objects.

A number of authors revealed that liposomes meet the following criteria:

1. Absence of cytotoxicity;
2. Immunological inertia (with the exception of special antigen transport);
3. The efficiency with which molecules are incorporated into the carrier, under conditions in which the properties of the included substance are not violated or inactivated;
4. The ability to protect the transferred substance from damage or destruction;
5. The efficiency with which the transferred substance in combination with the carrier is delivered to cells.

Liposomes are small spheres containing water compartments separated from each other and from the external aquatic environment by closed bilayers of phospholipids, which are oriented in the bilayer as well as in biomembranes. Hydrophilic (polar) heads are facing outward, into the aqueous phase, and hydrophobic (nonpolar) segments are facing inside the bilayer and tend to avoid contact with water. Depending on the size, there are small single-layer (diameter 25–100 nm) and multilayer large (diameter 0.2–2 μm) liposomes.

Initially, liposomes were used as models of biological membranes to study the functions of membranes, and then they began to be used as "containers" for the delivery of various drugs to organs and tissues. Drugs encapsulated in liposomes have a great therapeutic effect: the duration of the drug action increases, and its dose can be significantly reduced.

Many authors assume that there are two ways of their penetration into the cell:

1. The liposome is captured by the cell due to endocytosis;
2. The liposome merges with lysosomes, whose phospholipases hydrolyze the phospholipids of the liposome membranes, which ensures the release of the drug into the cytoplasm of the cell, and the lipid component is embedded in the membrane.

In both cases, the substance encapsulated in the liposome enters the cell despite the membrane barrier. An hour after the injection of liposomes to rats, the liver will contain from 24 to 75%, the spleen from 6 to 18%, and the lungs from 1.5 to 16% of liposome phospholipids. The effectiveness of using liposomes as drug carriers largely depends on whether they will be able to maintain their integrity and deliver the drug to the right organ, since there are obstacles that liposomes need to overcome. One of them is the macrophages of the reticuloendothelial system, which need to be blocked by injecting empty liposomes first, and then liposomes loaded with drugs.

The next obstacle is blood serum. Liposomes can be exposed to lipoproteins that try to exchange their lipids with liposomes and thereby contribute to their destruction and leakage of contents. To increase the resistance of liposomes to the action of lipoproteins, cholesterol is injected into the liposomes. The successful use of liposomes as drug carriers depends on the affinity of liposomes to target organ cells, which is increased by adding gangliosides to the composition of liposomes. Especially increased transport of liposomes in the liver is observed after the inclusion of GM1 ganglioside in their composition.

Exogenous gangliosides selectively bind to liver parenchymal cells, embed in membranes, and then spontaneously incorporate into the phospholipid bilayer. It is assumed that when liposomes interact with cells, an intermembrane exchange of lipids occurs. Phospholipids of liposomes restore the functions of cell membranes and enhance their ability to resist the penetration of active oxygen radicals. Thus, it can be proposed that liposomes have a membrane-stabilizing and antioxidant effect.

Attempts are being made to use liposomes and phospholipid preparations for the repair of hepatocyte membranes affected by liver diseases. Hereby, the following phospholipid preparations were administered to rats with toxic heliotrine hepatitis: phosphatidylcholine (FCHL) (from cotton), soy phosphatidylcholine ("Nattermann" company), as well as a drug which includes phosphatidylcholine and potassium salt of glycyrrhizic acid (PC + GA). The greatest effect among these drugs was the drug PC + GA. With intraperitoneal administration of it to rats, the activity of aldolase was normalized. Intraperitoneal administration of egg PC reduced hyperfermentemia, while intravenous administration had no effect. Apparently, phospholipid drugs injected into the bloodstream can be attacked by blood cell elements or undergo lipase cleavage in the bloodstream,

which reduces their effect on hepatocytes; therefore, intraperitoneal administration of drugs is more efficacious.

Liposomes formed from total phospholipids are better absorbed by hepatocytes than phosphatidylcholine ones. The solubility in water or in a hydrocarbon solvent of some drugs enclosed in a liposome affects the degree of assimilation of liposomes by cells. The greater the solubility of the drug in water, the more it will be included in the internal water space, and the greater its solubility in a non-polar solvent, the more it can be enclosed in hydrophobic regions of liposomes. In the process of interaction of liposomes with cells, the surface charge and the physical state of phospholipids are important. It is believed that “liquid” liposomes effectively merge with membranes.

Apparently, liposomes formed from total phospholipids isolated from healthy rat tissues meet the above requirements, and the inclusion of antioxidants in them — vitamin E and sodium selenite — will have an even greater effect in preventing membrane degradation in liver pathology in rats diseased by hepatotropic poisons.

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COMPARATIVE ANALYSIS OF THE GROWTH AND DEVELOPMENT OF INFANTS IN THE ARAL SEA REGION WITH DIFFERENT TYPES OF FEEDING

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Abstract*. This article presents the results of an analysis of the anthropometric indicators of young children living in the Aral Sea region, depending on the type of feeding. The body weight, body length, and head circumference of children on natural and artificial feeding were evaluated. Differences in the rates of physical development have been established, which underline the importance of breastfeeding for the optimal growth and development of children in an unfavorable environmental environment.*

Keywords: *young children, Aral Sea region, anthropometric indicators, breastfeeding, artificial feeding, physical development.*

Relevance

The physical development of children is one of the most important indicators of the health status of the child population and reflects the influence of both biological and socio-environmental factors [1]. The Aral Sea region is characterized by an unfavorable environmental situation caused by the drying up of the Aral Sea, high air pollution, and a shortage of high-quality drinking water and trace elements, which has a significant impact on children's health, especially at an early age [2]. The physical development of children is significantly influenced by the peculiarities of the climate, living conditions, daily routine, diet, and previous illnesses. The pace of physical development is also influenced by hereditary factors, constitution type, metabolic intensity, endocrine background, and musculoskeletal system activity [3,6]. Anthropometric indicators are a multifactorial process in different age periods and largely depend on climatic and ecological factors [7]. One of the criteria for health indicators of the child population is physical health. Assessment of the state of physical development is impossible without data on anthropometric indicators of various age groups.

The type of breastfeeding plays a key role in shaping a child's physical development. Both new and earlier studies of the composition of breast milk show that any of its components has, to one degree or another, an anti-infective and/or immunomodulatory effect on the infant and is considered a special case of the general process of formation of the qualitative state of health in the child. Owing to the latest advances in nutrition, immunology, and microbiology, several beneficial properties of human milk have been established [8].

Breast milk is an optimal source of nutrients and biologically active components that ensure adequate growth and development of infants. At the same time, artificial feeding, despite the improvement of milk formulas, may be accompanied by deviations in anthropometric indicators, especially under conditions of environmental disadvantage [9].

The purpose of the study

This study aimed to assess the state of physical development of children in the first year of life who were breastfed in various ways. A total of 1,238 children in their first year of life were

observed, including 627 girls and 611 boys living in the city of Nukus. All children belonged to health groups I and II. When collecting the material, all the necessary conditions were met for the formation of a sample: uniformity of the child population, use of the same measurement tools, and examination by the same group of specialists. The main anthropometric indicators of physical development — body weight, body length, chest circumference, and head circumference — were evaluated in the examined children, followed by the determination of somatic body types and the degree of harmony of physical development.

Research materials and methods

This was an observational analytical study. A total of 1,238 children in their first year of life living in the city of Nukus were under observation, of which 627 (50.6%) were girls and 611 (49.4%) were male. All children belonged to health groups I–II and had no congenital malformations or severe somatic diseases.

Children were divided into two groups based on the type of feeding:

Group I: children who were on natural feeding (n = 855)

Group II: Children who were artificially fed (n = 383).

Physical development was assessed at the age periods of 1, 3, 6, 9, and 12 months. Body weight (kg), body length (cm), and head and chest circumference (cm) were measured according to generally accepted methods using standard certified instruments. The data obtained were compared with the age standards of physical development.

Anthropometric techniques and the calculation of Z-scores (W/A and L/A) according to the WHO Child Growth Standards were used. Statistical significance was set at $p < 0.05$.

The results and their discussions

The study found that the anthropometric indicators of children in the first year of life living in the Aral Sea region significantly depended on the type of feeding. The most pronounced differences were found in the first 6 months of life, a critical period of intensive somatic growth.

Comparative analysis based on Z-scores (WHO standards) In children of group I (EB), the indicators of weight (W/A) and body length (L/A) were consistently within the physiological range (median □1 SD). This indicates a high adaptive capacity of the breast milk. In Group II (IV), by the age of 6-9 months, a right-sided shift in the body weight distribution curve was detected: in 18% of children, the values exceeded +2 SD, which indicates the risk of paratrophy. Simultaneously, body length indicators in 12.4% of cases shifted to values below -1.5 SD, forming a disharmonious type of development. In children of group I (natural feeding), the mass growth index at 6 and 9 months was significantly more stable. This is consistent with the data of foreign researchers, who pointed out that breastfed infants self-regulate energy consumption, which prevents excessive deposition of adipose tissue in the first half of life [10].

In group II (artificial feeding), a higher body mass index (BMI) was detected at of 6 months. According to research, the high protein content in milk formulas, compared with that in breast milk, stimulates the secretion of insulin and insulin-like growth factor (IGF-1), which leads to accelerated weight gain and increases the risk of subsequent obesity [11].

The dynamics of chest circumference growth deserve special attention. In breastfed infants, this indicator increased in proportion to the body length. At the same time, breastfed children in Nukus showed a slight increase in chest circumference over the rate of linear growth, which Victora et al. (2016) interpreted as a sign of metabolic adaptation to high-calorie nutrition with mixtures [12]. It was also noted that in conditions of micronutrient deficiency in the Aral Sea region, breastfed children were less likely to show signs of delayed physical development than

non-breastfed children. This is confirmed by data that have proven that specific protective factors of breast milk minimize the negative impact of environmental toxins on a child's metabolism [13].

By 6 months of age, children in group II (artificial feeding) had a higher average body weight than children in group I, but there was a less proportional increase in body length, indicating the formation of signs of disharmonious physical development. Similar data have been presented in foreign studies. Thus, in breastfed children, body weight indicators were more balanced in relation to body length and head circumference than in children who received artificial mixtures [14].

An analysis of body length indicators showed that breastfed infants had more stable linear growth rates throughout the first year of life. In children who were on artificial feeding, there was a relative lag in body length indicators in a number of age periods (6-9 months) while maintaining elevated body weight values.

Foreign data confirmed the results obtained. It was found that each additional month of breastfeeding is associated with an improvement in physical development indicators.

• at the age of 3 years — by 0.21 points,

• at the age of 7 years — by 0.35 points.

The authors emphasize that the basis of these differences is formed precisely in the first year of life, during the period of active growth and development [3, 15].

Special attention was paid to the assessment of head circumference as a marker of the growth and maturation of the central nervous system. In children who were breastfed, head circumference increased evenly and corresponded to age standards. In several cases, deviations from the average values were noted in children of group II, which may be due to less optimal nutritional provision.

One possible mechanism underlying these differences is the varying bioavailability of nutrients. Thus, the iron content in breast milk is 0.35–1.5 mg/l, with an absorption reaches 38-50%, whereas the absorption of iron from cow's milk and milk mixtures does not exceed 10% [16]. This contributes to a more harmonious rate of weight gain and body length in breastfed children in the first 4-5 months of life.

In addition, breastfed children have a lower fecal pH, which is associated with the predominance of bifidobacteria and lactobacilli in the intestinal microflora [17]. This ensures more efficient absorption of nutrients and has a positive effect on anthropometric indicators of health.

The specific eco-climatic factors of the Aral Sea region are of particular importance for interpreting these data. The high mineralization of drinking water in the city of Nukus and surrounding areas creates an excessive osmotic load on the immature kidneys of infants. When such water is used to prepare artificial mixtures, the total salt load on the body increases. Breast milk, which has a stable low osmolarity (approximately 290 mOsm/l) [18], protects the excretory system of the child. According to our observations, the "false" excess body weight in children of group II may be partially associated with latent fluid retention in tissues due to dietary hypermineralization.

In addition, the region is characterized by high air dustiness caused by salt and pesticide aerosols from the dried bottom of the Aral Sea. These pollutants aggressively affect mucous membranes. Secretory immunoglobulin A (sIgA) and lactoferrin from breast milk create a protective barrier in the gastrointestinal mucosa, minimizing systemic intoxication [3, 19]. Children with artificial feeding do not have this defense mechanism, which forces the body to

redirect plastic resources from growth processes to repair and immune protection. As a result, in group II, at the age of 6-9 months, there was a relative lag in linear growth.

The analysis showed that boys are more sensitive to environmental stress and type of nutrition. Disharmonious developmental variants (paratrophy or growth retardation) were more often registered in boys of group II. This is consistent with the "fragile male hypothesis" [20], indicating the high metabolic needs of the male body. Girls in both groups demonstrated greater biological plasticity and stability of anthropometric parameters, which is typical for the female body in adverse environmental conditions [21].

Conclusion

1. Based on a comprehensive analysis of the anthropometric indicators of children in the first year of life living in Nukus, the following conclusions can be drawn:

2. The superiority of natural feeding in the conditions of ecopathology: It has been established that breastfeeding is a key factor of bioadaptation for children of the Aral Sea region. It ensures harmonious physical development by keeping body weight and length within the physiological range (median \pm 1 SD according to WHO standards), while artificial feeding significantly more often leads to disharmonious development.

3. Risks of artificial feeding: Children on artificial feeding have a "scissor" phenomenon by the age of 6-9 months: a right-sided shift in body weight (>+2 SD in 18% of the surveyed) against the background of a relative slowdown in linear growth (< -1.5 SD in 12.4%). This indicates the risk of early paratrophy and excessive accumulation of adipose tissue, which programs metabolic disorders in the future.

4. Protective role against environmental stress: In conditions of high mineralization of drinking water and dusty air in the Aral Sea region, breast milk performs a protective function. BM minimizes the osmotic load on the baby's kidneys and provides immune protection to the mucous membranes through secretory immunoglobulin A (sIgA) and lactoferrin. The absence of these factors during artificial feeding forces the child's body to redistribute plastic resources from growth processes to repair and protection processes.

5. Gender specificity: Increased sensitivity of the male body to adverse environmental factors and dietary disorders has been revealed. Boys in the Aral Sea region have less compensatory potential when switching to artificial mixtures, which is manifested in a higher frequency of deviations from the standard indicators of growth and development compared with girls.

6. Practical recommendations: The results confirm the need for prioritizing breastfeeding support in ecologically crisis areas. The assessment of the physical development of children in the Aral Sea region must include the calculation of Z-scores for the early detection of risk groups for stunting or developing obesity.

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CHARACTERISTICS OF CHANGES IN RENAL FUNCTIONAL STATE IN PATIENTS WITH CHRONIC HEART FAILURE

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Abstract. *Objective: to study the functional state of the kidneys in patients with chronic heart failure. A total of 88 patients with postinfarction cardiosclerosis (PICS) and chronic heart failure with mid-range ejection fraction (HFmrEF) were examined. It was established that renal dysfunction aggravates the course of chronic heart failure (CHF), worsening the functional state of the heart in patients with CHF. The significance of renal dysfunction (RD) in the nature of the clinical manifestations of CHF is evidenced by the established correlation with the identified kidney function impairments.*

Keywords: *chronic heart failure, mid-range ejection fraction, renal dysfunction, microalbuminuria, glomerular filtration rate.*

Introduction

The prevalence of clinically significant chronic heart failure (CHF) in our country is 4.5% in the general population. The number of patients requiring constant diuretic therapy has increased more than 2.5 times compared to data from the end of the 20th century [1,3,6,11]. The prognosis for patients with CHF is serious, especially among patients with functional class (FC) III–IV CHF, where the median survival is 3.8 years [2,3,5,12]. Arterial hypertension (AH) and coronary heart disease (CHD) remain the leading causes in the etiology of CHF. The role of diabetes mellitus (DM) in the development of CHF has increased [2], aggravating the course of CHF and worsening its prognosis [2,3,6,9]. The increased prevalence of DM in patients with CHF is associated with an increased risk of developing kidney damage, which necessitates the search for risk factors and effective approaches to treating DM in the context of CHF. Studying these aspects of the problem is important for optimizing the treatment and rehabilitation of patients with CHF caused by post-infarction cardiosclerosis. Based on the above, the purpose of this study was to investigate the functional state of the kidneys, quality of life, and physical performance of patients with chronic heart failure.

Materials and methods

To accomplish the study objectives, 88 male patients with post-infarction cardiosclerosis were examined. The patients had a history of myocardial infarction from 6 months to 5 years prior and NYHA functional class II–III chronic heart failure (CHF) [2,6,8].

Depending on the presence of RD, the patients were divided into two groups: Group I (n=45), patients with CHF without RD; and Group II (n=43), patients with CHF and RD.

The examined patients were undergoing inpatient treatment in the cardiology department of the Republican Specialized Scientific-Practical Medical Center of Therapy and Medical Rehabilitation of the Republic of Uzbekistan. All patients underwent general clinical, laboratory, and instrumental examinations. All patients had an ultrasound of the abdominal organs and kidneys to rule out congenital kidney anomalies, renal masses, and nephrolithiasis.

The study excluded patients with severe diabetes mellitus, obstructive lung diseases, pronounced signs of respiratory failure, hepatic and renal failure, a history of acute cerebrovascular accident, high-grade cardiac arrhythmias, as well as patients with severe arterial hypertension and severe heart failure.

The assessment of renal functional state included determining the levels of serum creatinine, urinary albumin excretion (microalbuminuria (MAU) >0.16 mg/L), and the glomerular filtration rate (GFR), calculated using the Cockcroft-Gault formula: GFR = 1.228 × [140 – age] × body weight (kg) × 0.85 (for women) / blood creatinine (μmol/L)

Statistical analysis of the research results was performed on an IBM PC/AT personal computer using the "Biostat for Windows, version 4.03" standard software package. The parameters were described as mean ± standard deviation (M±δ). For normally distributed values, group comparisons of quantitative variables were conducted using Student's t-test.

Results

In the study of renal function parameters in Group I patients with CHF-mrEF, the serum creatinine clearance was 79.0±8.49 μmol/L, and the GFR was 99.58±8.36 ml/min. In Group II patients, who had developed DN, a decrease in GFR (by 23.0%; P<0.01) and a significant increase in blood creatinine levels (by 21.8%; P<0.01) were observed relative to the data from Group I.

In addition, in Group II patients with CHF and DN, microalbuminuria was detected in 36 out of 43 patients (86.7%). According to the CKD classification, among Group II patients with CHF-mrEF, Stage I CKD was identified in 7 patients (16.3%), Stage II CKD in 21 patients (48.8%), and Stage III CKD in 15 patients (34.9%).

The analysis of the combined risk of CKD progression and cardiovascular complications, stratified by the degree of GFR reduction and severity of albuminuria, revealed various risk levels in the examined patients with HFmrEF. Specifically, in Group I, 11 patients (24.4%) had a high risk, 19 patients (42.3%) had a moderate risk, and 15 patients (33.3%) had a low combined risk of developing CVC in cardiorenal syndrome.

In Group II, the presence of RD was associated with an increased number of patients with a high risk of CVC (12 patients; 27.9%) and a moderate risk (8 patients; 18.6%). The number of patients with a very high risk of developing CVC in cardiorenal syndrome in Group III was 23 (53.5%).

Impaired renal function, along with the severity and nature of this dysfunction, depends on the presence and severity of CHF. We established that CHF without RD is associated with less pronounced manifestations of renal dysfunction, in contrast to patients with impaired renal function, and is an independent factor for kidney damage (increased urinary albumin excretion, increased serum creatinine concentration, and decreased GFR).

Table 1. Cardiorenal relationship indicators in patients with HFmrEF and MS

Indicators	**Group 1 (n=45)**	**Group 2 (n=43)**
GFR-SBP	r= 0.04	r= 0.22
GFR-DBP	r= -0,46**	r= -0,63**
GFR-EF	r= -0,46**	r= -0,74***
GFR-E/A	r= 0.09	r= 0,55**
СКФ-IVRT	r= -0,51**	r= 0,13
СКФ-PE/PA	r= 0,09	r= 0,49**
СКФ-ЭЗВД	r= 0,69**	r= 0,17

Note: for r= 0.3-0.4, p<0.05; for r= 0.41-0.6, p<0.01; for r> 0.6, p<0.001.

The study of cardiorenal relationships in patients with heart failure with mid-range ejection fraction (HFmrEF) also revealed significant correlations between the studied parameters. In Group I, patients with CHF and HFmrEF demonstrated a moderate correlation between GFR and CHF parameters: DBP level (r= -0.46; p<0.01) and LVMM (r= -0.49; p<0.01). This group also showed correlations between GFR level and LVEF (r= 0.58; p<0.01), IVRT (r= -0.51; p<0.01), and EZVD (r= 0.69; p<0.001), which confirms the significance of CRS in the development and progression of CHF (Table 1). P<0.01). Correlations were also identified in this group between GFR levels and LVEF (r= 0.58; P<0.01), IVRT (r= -0.51; P<0.01), ECVD (r=0.69; P<0.001), which confirms the significance in the development and progression of CRF in patients with CYS (Table.

In Group II of the study, patients with CHF, mid-range EF, and DKD showed a significant correlation between GFR and the indicator of LV systolic function (EF) (r= 0.55; p<0.01) and the indicator of LV diastolic function (E/A ratio) (r= 0.49; p<0.01). This group also showed a significant correlation between GFR level and LV remodeling parameters: LVMM (r= -0.74; p<0.001). P<0.01). Additionally, a significant correlation was noted in this group between GFR levels and LV remodeling parameters: MML (r= -0.74;

Thus, in patients with CHF-mrEF, PD exacerbates renal dysfunction, which develops as one of the main pathogenetic links of CHF. Evidence of the significance of PD in the clinical manifestations of CHF-mrEF is the established correlation between the identified disorders and the severity of the functional state of the kidneys; the severity and nature of this dysfunction depend on the presence and characteristics of the CHF components. We established that CHF-mrEF without PD is accompanied by less pronounced manifestations of renal dysfunction compared to patients with PD.

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COMPARATIVE ASSESSMENT OF *STAPHYLOCOCCUS AUREUS* STUDIED USING TWO METHODS AS PART OF CREATING AN EXTERNAL QUALITY ASSESSMENT PANEL

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Abstract. *The quality of laboratory diagnostics depends on the correct performance of procedures at all stages of laboratory testing. The evaluation and quality of laboratory processes can also be ensured through the implementation and regular annual conduct of external quality assessment (EQA) programs. Currently, there is no organization in the republic responsible for conducting EQA. In this regard, our laboratory set the objective of developing a panel for EQA. One of the strains included in the panel is Staphylococcus aureus. To study the stability of antimicrobial susceptibility and resistance properties, 42 strains of S. aureus were selected. After five repeated assessments of strain stability using two methods, including storage at −80°C, as well as three additional assessments after lyophilization, four strains (9.5%) demonstrating stable properties were selected.*

Keywords: *stability of properties, external quality assessment, panel.*

Introduction

Currently, one of the urgent tasks in public health is the qualitative diagnosis of a number of infectious diseases of bacterial etiology. Despite a series of measures aimed at improving epidemiological supervision and the prevention of purulent bacterial infections, the incidence of many infections remains high.

One of the main problems of practical healthcare remains infections with multiple resistance to antimicrobial drugs. The percentage of pathogenic and conditionally pathogenic bacterial strains possessing multi-resistance to antibiotics is increasing; infection pathogens cause not only purulent-septic complications after surgery and injections but also pneumonia, urological, and acute intestinal infections [2, 6, 4].

Due to its ability to adapt to changing environmental conditions and various life-threatening infections, *Staphylococcus aureus* is one of the most complex pathogens, particularly to the action of antimicrobial drugs. According to the Health Protection Agency, during the period from 1997 to 2005, the frequency of *S. aureus* isolation was 41.4% in the development of surgical intervention area infection. It is known that the natural sensitivity of staphylococci to the vast majority of antibacterial drugs is quite high. Despite this, antibacterial therapy can pose a serious problem due to the formation of antibiotic resistance in microorganisms and their ability to form microbial bioplots on the surface of implants [14].

The most difficult to treat are infections caused by methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE). The widespread prevalence of MRSA and the frequency of its

detection are constantly increasing: in the USA in 2000, the frequency of MRSA isolation in intensive care units was 53%, which is 29% higher than the similar figure for the period 1995–1999 [10, 1].

Clinical test results obtained in laboratories or testing centers at or near medical care facilities must be as accurate as possible, as they have a direct impact on care and treatment, prevention, and disease control [5].

The quality of laboratory diagnostics depends on both the pre-analytical stage—taking and delivering pathological material—and the analytical stage—the culture study conducted in microbiological laboratories [3].

The term “external quality assessment” (EQA) is used to describe a method/process that allows for the comparison of testing conducted by a laboratory, testing station, or individual user with testing conducted outside this laboratory, for example, by a group of similar laboratories, a reference laboratory, or a testing station. The successful participation of a testing station in the EQA program provides clients, accrediting, and regulatory bodies with objective data on the qualification of the testing service and serves as a unique source of information that is difficult to obtain by other means [12, 7].

It is important to note that participation in the EQA program allows for a “partnership audit” of the process to address technical and methodological issues in order to improve service quality at each individual testing point, as well as to ensure the comparability of results between various services providing diagnostic services. The EQA provides objective data on the quality of services provided to accrediting and regulatory bodies and, as demonstrated, reflects the quality of patient sample testing [13, 9].

In most developed countries, EQA programs are reliably developed and significantly contribute to improving the quality of medical care at all levels of the healthcare system [11, 8], but in our country, there is no system that conducts EQA for bacteriological laboratories.

In this regard, we have set a goal to identify the most stable strains of

S. aureus to create a EQA panel.

Materials and methods

The study was conducted within the framework of the Cooperation Agreement between the Center and the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases (RSSPMCEMIPD) under project No. 5 NU2HGH000089-04-00 "Expanding the surveillance epidemiological surveillance network to counter the problem of resistance to antimicrobial drugs in the Republic of Uzbekistan."

S. aureus strains were identified and studied for sensitivity to antimicrobial drugs in the Reference Laboratory of the Center for Antimicrobial Resistance (CAMR) of the RSSPMCEMIPD. Identification of strains was performed using the PID (REF) -448008 panels of the BD Phoenix (USA) bacteriological analyzer.

Sensitivity and interpretation of antimicrobial sensitivity test (AMS) results were conducted using two methods, the disc-diffusion method, using the guidelines of the European Committee for the Determination of Sensitivity to Antimicrobial Drugs (EUCAST 2023). The sensitivity of all strains to 9 antibacterial drugs was determined: cefoxitin (FOX), penicillin (PEN), ciprofloxacin (CIP), levofloxacin (LVX), erythromycin (ERY), clindamycin (CLI), linezolid (LNZ), rifampicin (RIF), and amikacin (AMK), manufactured by Liofilchelm, Italy. *S. aureus* AST was also studied in parallel using PMIC-600 (REF) -449055 panels from the BD Phöenix bacteriological analyzer, manufactured in the USA. To monitor the quality of the conducted

studies, reference strains of *Staphylococcus aureus* NCTC 12973 were used. The obtained sensitivity results were processed using the WHONET and Microsoft Excel programs. Liophilic drying was carried out on the INFITEK LYO60B-1S apparatus, which includes several stages. The LYO60B-1S sublimation dryer utilizes extremely low temperatures (-60°C) and vacuum to remove moisture from microorganisms. Liophilic drying was carried out in penicillin vials with a smooth neck, in a volume of 10 ml. The lyophilization process: in the first stage, the biomaterial was frozen to a temperature of minus 60 °C. In the second stage, primary drying occurs, where all frozen free moisture is sublimated under the influence of vacuum and thermal energy. The third stage is the final drying of the product, when the remaining moisture is removed. The lyophilization process takes 18-22 hours.

Results and discussion

To form a EQA panel, the stability of antimicrobial sensitivity properties was studied for 41 strains of *S. aureus* and one control strain of *S. aureus* NCTC 12973. All strains were identified as *S. aureus*.

To study the stability of the sensitivity-resistance properties of *S. aureus* strains to antimicrobial drugs, at the initial stage, strains stored frozen at a temperature of minus 80^0C were studied. Each strain was tested five times (1 month, 2 months, 3 months, 6 months, and 1 year) before lyophilic drying, as well as one month, 3 months, and 6 months after lyophilic drying. The results of the AST data were analyzed for the stability of strain sensitivity-resistance properties to antimicrobial drugs. The disc-diffusion method is the primary method for determining sensitivity to antimicrobial drugs in most clinical microbiological laboratories. The composition of the antibiotic panel is flexible and allows the clinical laboratory to easily modify the panel according to its needs. However, this method has its own disadvantages, including labor costs associated with manual measurements and manual documentation of data, as well as dependence on the researcher and variability of results. The first stage of the analysis to compare the stability of sensitivity-resistance results of S. aureus strains was the calculation of the mean values of the strain lysis range, in mm, relative to antimicrobial drugs (Table 1).

Table 1. Average index of the lysis range of S. aureus strains, in mm, relative to antimicrobial drugs

Antibiotic	*S. aureus* (n=42) (mm)					Reliability of Results
	In 1 month.	In 2 month .	In 4 month.	In 6 month.	After 1 year	
Benzylpenicillin P1	6,1±0,1	6,1±0,1	6,3±0,3	6,2±0,1	6,2±0,1	P≥0,05
Cefoxitin FOX 30	27,8±0,7	27,8±0,7	27,8±0,8	27,4±0,7	27,4±0,7	P≥0,05
Ciprofloxacin CIP 5	23,7±0,8	23,6±0,8	23,0±0,7	23,6±0,7	23,6±0,7	P≥0,05
Levofloxacin LEV 5	25,4±0,8	25,3±0,9	25,1±0,8	25,4±0,7	25,4±0,7	P≥0,05
Amikacin AK30	16,1±0,8	16,1±0,8	18,6±0,5	18,4±0,6	18,4±0,6	P≤0,05
Gentamicin CN 10	17,2±0,7	17,2±0,7	17,5±0,5	17,6±0,5	17,6±0,5	P≥0,05
Eritromycin E 15	18,9±1,3	19,0±1,3	14,9±1,4	14,9±1,4	14,9±1,4	P≤0,05
Clindamycin CD 2	19,6±1,3	19,7±1,3	18,1±1,4	18,4±1,3	18,1±1,4	P≥0,05
Linozolid LNL 10	27,3±0,4	27,3±0,4	26,3±0,2	26,3±0,2	26,3±0,2	P≤0,05
Rifampicin RD 5	29,8±0,8	29,8±0,8	29,4±0,6	29,4±0,6	29,4±0,6	P≥0,05

The results of the analysis of *S. aureus* strain resistance data show that the average sensitivity-resistance indicator to most tested antimicrobial drugs remains stable throughout the year. Table 1 presents the changes in resistance levels over various time intervals (1 month, 2 months, 4 months, 6 months, 1 year). Thus, we see that for most antibiotics (for example, P1, CIP 5, LEV 5, CN 10, LNL 10, RD 5), resistance values remain practically unchanged throughout the year. Minor fluctuations are observed in some antimicrobial drugs, such as AK30 and E 15, but the changes are statistically significant, which may be related to strain adaptation or technical measurement factors. Overall, the S. aureus strain presented in the study demonstrates high resistance to antimicrobial drugs during one year of storage. Subsequently, we conducted an analysis of variance (ANOVA) to verify the presence of statistically significant differences between data groups representing changes in *S. aureus* resistance depending on storage time (Table 1). Analysis of data on the stability of *S. aureus* strain resistance differs in its results. Thus, by analyzing the stability results of strain resistance to ten antimicrobial drugs, we see that two antimicrobial drugs, P1 (100.0%) and CD2 (38.1%), maintain stable resistance when the culture is frozen at minus 80^0C for one year.

Table 2. Analysis of sensitivity test data for antimicrobial drugs of S. aureus strains for property stability using the disc-diffusion method.

Antibiotic	*S. aureus* (n=42) (R)					F-value	**p-value**
	In 1 month.	In 2 month .	In 4 month.	In 6 month.	After 1 year		
Benzylpenicillin P1	100,0	100,0	100,0	100,0	100,0	0.000	1.00000
Cefoxitin FOX 30	14,3±5,4	14,3±5,4	16,7±5,8	16,7±5,8	16,7±5,8	0.737	0.57132
Ciprofloxacin CIP 5	9,5±4,5	9,5±4,5	11,9±5,0	7,1±4,0	7,1±4,0	0.697	0.58947
Levofloxacin LEV 5	9,5±4,5	9,5±4,5	11,9±5,0	9,5±4,5	9,5±4,5	0.500	0.73898
Amikacin AK30	35,7±7,4	38,1±7,5	7,1±4,0	9,5±4,5	9,5±4,5	6.245	0.00261**
Gentamicin CN 10	31,0±7,1	31,0±7,1	14,3±5,4	14,3±5,4	14,3±5,4	4.901	0.00964**
Eritromycin E 15	42,9±7,6	40,5±7,6	57,1±7,6	57,1±7,6	57,1±7,6	2.917	0.04783*
Clindamycin CD 2	38,1±7,5	38,1±7,5	38,1±7,5	38,1±7,5	38,1±7,5	0.000	1.00000
Linozolid LNL 10	2,4±2,4	2,4±2,4	0,00	0,00	0,00	9.760	0.00014***
Rifampicin RD 5	7,1±4,0	7,1±4,0	2,4±2,4	2,4±2,4	2,4±2,4	3.401	0.02867*

**Note: *p* ≤ 0.05, the changes are statistically significant (*); *p* ≤ 0.01, the changes are more significant (**); *p* ≤ 0.001, the changes are highly significant (***); *p* > 0.05, the differences are not significant.

For other antimicrobial drugs, resistance values vary, with resistant strains increasing after three months of storage to FOX 30 from 14.29% to 16.67% and E 15 from 40.48% to 57.14%. Resistant strains decreased in *S. aureus* strains after 6 months of storage compared to CIP 5 from 14.29% to 7.14%, LNL 10 from 2.38% to 0.0%, and RD 5 from 7.14% to 2.38%. The indicators of resistant strains to LEV 5 were consistently the same at 9.52% throughout the year, with the exception of a one-time increase of 11.90% after three months. Thus, resistance to AK30, CN 10, LNL 10 changed significantly ($p < 0.01$), for E 15 and RD 5 changes were moderately significant ($p < 0.05$), for P1 and CD 2 changes were insignificant ($p = 1.000$), indicating stability of resistance. Due to the fact that the strain sensitivity data to antimicrobial drugs obtained by the disk-diffusion method differed, we placed AST on the PMIC-600 (REF) -449055 panel of the BD Phoenix bacteriological analyzer in parallel. Automatic microbiological systems have been developed for the rapid, reliable, and accurate identification of bacteria (ID) and testing for susceptibility to antimicrobial drugs (AST) for most clinically encountered species.

Table 3. Average minimum inhibitory concentration (MIC) of S. aureus, in mg/L, relative to antimicrobial drugs

Antibiotic	*S. aureus* (n=42) (mg/L)				**p-value**
	In 2 month.	In 4 month.	In 6 month.	After 1 year	
Benzylpenicillin P1	0,4±0,03	0,4±0,02	0,3±0,03	0,4±0,02	P=1.00
Cefoxitin FOX 30	4,3±0,7	5,0±0,8	5,0±0,8	5,0±0,8	P=0.51
Ciprofloxacin CIP 5	1,1±0,1	1,1±0,1	1,1±0,1	1,1±0,1	P=1.00
Levofloxacin LEV 5	1,1±0,1	1,2±0,2	1,2±0,2	1,2±0,2	P=0.65
Amikacin AK30	5±0,4	6,6±0,3	5±0,3	5±0,3	P=1.00
Gentamicin CN 10	1,1±0,05	1,0±0,03	1,1±0,07	1,1±0,07	P=1.00
Eritromycin E 15	1,2±0,2	1,1±0,2	1,2±0,2	1,2±0,2	P=1.00
Clindamycin CD 2	0,3±0,01	0,3±0,01	0,3±0,01	0,3±0,01	P=1.00
Linozolid LNL 10	2,0±0,00	2,0±0,00	2,0±0,00	2,0±0,00	-
Rifampicin RD 5	0,3±0,01	0,3±0,00	0,2±0,01	0,2±0,01	-
Vancomycin	1,1±0,1	1,0±0,00	1,0±0,00	1,0±0,00	P=0.32
Daptomycin	1,1±0,07	1,0±0,00	1,0±0,00	1,0±0,00	P=0.15
Moxifloxacin 5	0,3±0,02	0,3±0,02	0,3±0,02	0,3±0,02	P=1.00
Oxacillin	0,9±0,2	1,0±0,2	1,0±0,2	1,0±0,2	P=0.72
Teicoplanin	1,1±0,07	1,1±0,07	1,1±0,07	1,1±0,07	P=1.00
Tetracycline 30	0,6±0,05	0,6±0,05	0,6±0,05	0,6±0,05	P=1.00
Trimethoprim-sulfamethoxazole 1,25	2,1±0,1	2,1±0,1	2,1±0,1	2,1±0,1	P=1.00

Fosfomycin	8,2±0,2	9,0±0,4	9,0±0,6	9,0±0,6	P=0.2
Fusidic acid 10	1,0±0,00	1,0±0,00	1,0±0,00	1,0±0,00	-

Analysis results of the average values of the minimum inhibitory concentration of *S. aureus* strains show that for all tested antimicrobial drugs, the average MIC values remain the same throughout the year.

Table 4. Analysis of sensitivity test data for antimicrobial drugs of S. aureus strains for property stability using the PMIC-600 BD Phoenix panel.

Antibiotic	*S. aureus* (n=42) (R)				**F-value**	**p-value**
	In 2 month .	In 4 month.	In 6 month.	After 1 year		
Benzylpenicillin P1	100	100	100	100	0.000	1.00000
Ciprofloxacin CIP 5	4,8±3,3	4,8±3,3	4,8±3,3	4,8±3,3	0.000	1.00000
Levofloxacin LEV 5	4,8±3,3	4,8±3,3	4,8±3,3	4,8±3,3	0.000	1.00000
Amikacin AK30	2,4±2,4	0,00	0,00	0,00	2.000	0.14635
Gentamicin CN 10	11,9±5,0	4,8±3,3	4,8±3,3	4,8±3,3	3.570	0.04178*
Eritromycin E 15	23,8±6,6	21,4±6,3	23,8±6,6	23,8±6,6	1.732	0.19713
Clindamycin CD 2	14,3±5,4	21,4±6,3	7,1±4,0	21,4±6,3	3.214	0.05583
Linozolid LNL 10	0,00	0,00	0,00	0,00	0.000	1.00000
Rifampicin RD 5	0,00	0,00	0,00	0,00	0.000	1.00000
Vancomycin	0,00	0,00	0,00	0,00	0.000	1.00000
Daptomycin	2,4±2,4	0,00	0,00	0,00	2.000	0.14635
Moxifloxacin 5	4,8±3,3	4,8±3,3	4,8±3,3	4,8±3,3	0.000	1.00000
Oxacillin	16,7±5,8	16,7±5,8	16,7±5,8	16,7±5,8	0.000	1.00000
Teicoplanin	0,00	0,00	0,00	0,00	0.000	1.00000
Tetracycline 30	4,8±3,3	4,8±3,3	4,8±3,3	4,8±3,3	0.000	1.00000
Trimethoprim-sulfamethoxazole 1,25	4,8±3,3	4,8±3,3	4,8±3,3	4,8±3,3	0.000	1.00000
Fosfomycin	0,00	0,00	0,00	0,00	0.000	1.00000
Fusidic acid 10	0,00	0,00	0,00	0,00	0.000	1.00000

Analyzing the data using dispersion analysis (ANOVA), the stability of sensitivity and resistance of *S. aureus* strains using the PMIC-600 BD Phoenix panel, we see (Table. 2) that out of nineteen antimicrobial drugs proposed by the manufacturer in the panel, the sensitivity-resistance values for fourteen antimicrobial drugs were maintained at all stages of the research. Regarding other antimicrobial drugs, a decrease in the occurrence of resistant strains is observed; thus, a more pronounced decrease in resistance indicators is observed for antimicrobial drugs of the aminoglycoside series; for AK from 2.4% to 0.0%, and for CN 10 from 11.9% to 4.8%.

Resistance to CN 10 changed significantly ($p < 0.05$), while for most antibiotics the changes were not significant, indicating stability of resistance. After analyzing the stability of susceptibility–resistance of *S. aureus* strains using two methods, we selected 8 *S. aureus* strains for lyophilization and further study of their stability. Following lyophilization, a three-time

assessment (after 1 month, 3 months, and 6 months) was conducted to evaluate the stability of susceptibility–resistance characteristics. Based on the analysis of the obtained data, four strains were selected for the creation of the EQA panel, as they did not change their properties throughout the entire study period.

Conclusion. The EQA program allows for a "peer review" of the process to address technical and methodological issues with the aim of improving the quality of service at each individual testing site, as well as ensuring the comparability of results between different diagnostic service providers. In this regard, the panel of strains being developed by us must have stable properties to accurately assess the competence of staff and the quality of the conducted research, particularly the antimicrobial susceptibility testing of the laboratories involved. Research on the stability of *S. aureus* strains showed that not all strains maintain their properties during storage. Only 9.5% of *S. aureus* strains retained their properties and were selected for the creation of the panel.

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THE ADVANTAGES OF USING THE "WHO ANTHRO" SOFTWARE IN PEDIATRICS

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Abstract. *This article discusses the effectiveness and practical advantages of using the innovative "WHO Anthro" software in pediatric practice for assessing children's physical development. The software allows healthcare professionals to analyze anthropometric indicators such as body weight, height, head circumference, and body mass index according to international World Health Organization standards. The use of automated digital assessment improves diagnostic accuracy, reduces the risk of human error, and saves physicians' time. In addition, the program helps create a centralized patient database and supports the dynamic monitoring of children's growth and development. Therefore, "WHO Anthro" can be considered an effective and reliable tool in modern pediatric diagnostics.*

Keywords: *pediatrics, WHO Anthro, anthropometry, physical development, Z-score, diagnostics.*

Introduction

Physical development is one of the key indicators of a child's overall health and nutritional status. In pediatric practice, anthropometric parameters such as body length or height, body weight, head circumference, chest circumference, and body mass index are widely used to evaluate whether a child's growth corresponds to age- and sex-specific standards. Regular monitoring of these indicators helps identify early signs of growth retardation, undernutrition, wasting, overweight, obesity, and other developmental abnormalities. This is especially important during infancy and early childhood, because growth disorders during this period may have long-term consequences for physical health, neurodevelopment, and cognitive functioning (World Health Organization, 2006; de Onis et al., 2007; Black et al., 2017).

In recent years, digital technologies have become increasingly important in pediatric healthcare, particularly in the assessment and monitoring of children's growth. One of the most practical digital tools in this area is the "WHO Anthro" software, which was developed on the basis of the World Health Organization Child Growth Standards. This program allows healthcare professionals to enter anthropometric data and automatically compare the results with international reference values. It also calculates Z-scores for key growth indicators, including weight-for-age, height-for-age, weight-for-height, and body mass index-for-age. As a result, physicians can assess a child's physical development more quickly, accurately, and objectively. The use of "WHO Anthro" is especially useful in primary healthcare, family medicine, and pediatric departments, where many children require regular and systematic growth monitoring (WHO, 2006; WHO, 2011; Erkinov & Umarova, 2024).

Purpose of the Study

The purpose of this study is to demonstrate the advantages of using the innovative “WHO Anthro” software in pediatric practice. The study also aims to describe its functional capabilities, practical convenience, and importance in improving the accuracy of anthropometric assessment. In addition, the work highlights how the software can help physicians monitor children’s growth dynamics, identify developmental abnormalities at an early stage, and make timely clinical decisions.

Materials and Methods

In this study, standard anthropometric measurement tools were used to assess children’s physical development. Body weight was measured using electronic scales, while body length or height was measured with a stadiometer or height-measuring device. Head circumference and chest circumference were measured using centimeter measuring tapes. All measurements were carried out according to accepted pediatric anthropometric rules in order to ensure accuracy and reliability.

For data processing and analysis, the “WHO Anthro” software was used. The program was installed on a computer or Android device and applied as an anthropometric calculator. The collected data, including the child’s date of birth, sex, date of examination, body weight, height or length, head circumference, and arm circumference, were entered into the software. After entering the required information, the program automatically analyzed the child’s growth indicators according to WHO standards. The software calculated key anthropometric indices such as weight-for-age, height-for-age, weight-for-height, and body mass index-for-age.

Results

The use of the “WHO Anthro” software showed several important advantages in pediatric assessment. First, the program allows physicians to obtain accurate results within a short period of time. Instead of calculating growth indicators manually, the doctor can enter the necessary data into the software and receive automatic results based on WHO child growth standards. This helps reduce calculation errors and improves the reliability of diagnostic conclusions.

Second, the software evaluates anthropometric indicators using Z-scores, which are widely accepted in international pediatric practice. Z-score analysis makes it possible to determine whether a child’s growth indicators are within the normal range or whether there are signs of underweight, stunting, wasting, overweight, or obesity. This is particularly useful for early diagnosis and timely prevention of nutritional and developmental disorders.

Another important advantage of “WHO Anthro” is its ability to create and maintain a centralized patient database. This function helps physicians monitor each child’s growth over time and compare current results with previous measurements. Dynamic monitoring is especially valuable for children with chronic diseases, nutritional problems, premature birth history, or suspected developmental delay. By using the database, healthcare professionals can observe whether the child’s growth is stable, improving, or worsening.

In addition to physical growth assessment, the “WHO Anthro” software also allows evaluation of six major psychomotor developmental milestones in children aged from 3 to 24 months. These milestones include important skills such as sitting without support, crawling, standing, walking, and other age-related motor abilities. This function increases the practical value of the software, because it helps doctors assess not only physical growth but also early psychomotor development.

Discussion

The findings suggest that the use of "WHO Anthro" in pediatric practice can significantly improve the quality of child growth monitoring. Manual anthropometric calculations may take time and may sometimes lead to errors, especially when physicians work with many patients during one working day. In contrast, automated software analysis simplifies the diagnostic process and allows healthcare workers to make faster and more accurate decisions.

The program is also convenient because it can be used on both computers and Android devices. This makes it accessible not only in hospitals and clinics but also in primary healthcare centers and mobile medical examinations. For pediatricians, family doctors, nurses, and other healthcare professionals, "WHO Anthro" serves as a practical digital assistant in daily work. It can also be useful for parents when they need to understand the general growth pattern of their child under medical supervision.

The use of WHO standards is another important strength of the software. Since these standards are internationally recognized, they provide a reliable basis for evaluating children's physical development. This makes the results more objective and comparable across different regions and healthcare institutions. Therefore, the introduction of this software into pediatric practice supports the modernization of healthcare services and the wider use of digital technologies in child health monitoring.

Conclusion

The implementation of the "WHO Anthro" software in pediatrics improves the quality, accuracy, and efficiency of child growth assessment. The program automatically analyzes anthropometric indicators according to WHO standards, calculates Z-scores, reduces the risk of human error, and saves physicians' time. It also supports the creation of a patient database and allows continuous monitoring of children's physical and psychomotor development.

Overall, "WHO Anthro" is an effective, reliable, and convenient digital tool for pediatric practice. Its use can help healthcare professionals detect growth and nutritional problems at an early stage and provide timely medical recommendations. Therefore, the software should be widely introduced into pediatric healthcare institutions as part of modern diagnostic and preventive practice.

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CHANGES IN THE ORAL MUCOSA IN HYPOVITAMINOSIS AND THEIR TREATMENT

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Abstract. *Hypovitaminosis is a common systemic condition that significantly affects the oral mucosa due to its high cellular turnover and sensitivity to metabolic disturbances. This study examines the etiopathogenesis, clinical features, diagnostic approaches, and treatment strategies of oral mucosal changes associated with deficiencies of essential vitamins. Inadequate intake, malabsorption, chronic diseases, and increased physiological demands contribute to deficiencies of vitamins A, B-complex (especially B2, B6, B9, and B12), C, D, and E, each leading to specific pathological alterations in the oral cavity. Vitamin A deficiency results in epithelial atrophy, hyperkeratosis, and decreased resistance to infections. Deficiencies of B-group vitamins are characterized by glossitis, angular cheilitis, mucosal pallor, burning sensations, and ulcerative lesions due to impaired cellular metabolism and regeneration. Vitamin C deficiency disrupts collagen synthesis, causing gingival bleeding, inflammation, and delayed wound healing, while vitamin D influences immune response and may exacerbate inflammatory conditions of oral tissues. Vitamin E deficiency contributes to oxidative stress and impaired tissue repair. Clinically, hypovitaminosis manifests as xerostomia, erythema, desquamation, fissures, ulcerations, and increased susceptibility to opportunistic infections such as candidiasis. Diagnosis requires a comprehensive approach including clinical examination, dietary assessment, and laboratory testing to identify specific deficiencies. Treatment involves correction of nutritional imbalance through diet optimization and systemic vitamin supplementation, along with local therapy such as antiseptics, anti-inflammatory agents, epithelializing preparations, and antifungal medications. Preventive measures focus on maintaining balanced nutrition and early detection of deficiency states. Early recognition of oral signs of hypovitaminosis is essential for effective management and reflects the importance of an integrated medical and dental approach to patient care.*

Keywords: *hypovitaminosis, oral mucosa, vitamin deficiency, oral lesions, glossitis, angular cheilitis, stomatitis, gingival bleeding, xerostomia, candidiasis, vitamin therapy.*

Introduction

Hypovitaminosis remains a relevant global health concern, affecting individuals across different age groups and socioeconomic backgrounds, and is often associated with unbalanced nutrition, chronic systemic diseases, gastrointestinal malabsorption, and increased physiological demands such as growth, pregnancy, and stress. Despite advances in healthcare and nutrition science, subclinical and clinical vitamin deficiencies continue to be widely распространён,

frequently presenting with early manifestations in tissues characterized by rapid cellular turnover, particularly the oral mucosa. The oral cavity serves as a highly sensitive indicator of systemic health, and in many cases, pathological changes in the mucosa may precede the development of more pronounced systemic symptoms, making it a valuable site for early diagnosis of nutritional deficiencies.

The oral mucosa is a dynamic biological structure that relies on continuous epithelial renewal, adequate vascularization, and proper immune defense mechanisms, all of which are highly dependent on sufficient levels of essential vitamins. Vitamins play a critical role in maintaining epithelial integrity, collagen synthesis, cellular metabolism, antioxidant protection, and immune regulation. Deficiencies in these micronutrients disrupt normal physiological processes, leading to structural and functional alterations in oral tissues. For instance, impaired keratinization, epithelial atrophy, reduced regenerative capacity, and increased vulnerability to infections are common consequences of prolonged vitamin insufficiency.

Particularly significant are deficiencies of vitamins A, B-complex, C, D, and E, each contributing to distinct yet often overlapping clinical manifestations in the oral cavity. These may include inflammatory lesions, mucosal atrophy, ulcerations, fissuring, bleeding tendencies, and opportunistic infections. Such changes not only affect oral health but also impair quality of life by causing pain, discomfort, difficulty in eating, and increased risk of secondary complications. Moreover, the presence of these signs can provide important diagnostic clues for clinicians, allowing for timely identification of underlying systemic conditions.

In modern clinical practice, the importance of recognizing oral manifestations of hypovitaminosis is increasing, particularly within the framework of preventive and interdisciplinary medicine. Dentists and physicians play a crucial role in early detection, differential diagnosis, and комплексное management of patients presenting with these symptoms. Understanding the relationship between nutritional deficiencies and oral health is essential for developing effective diagnostic algorithms and treatment strategies that address both local manifestations and systemic causes.

Therefore, the study of changes in the oral mucosa associated with hypovitaminosis is of significant scientific and practical importance. It contributes to improving early diagnostic capabilities, optimizing therapeutic interventions, and enhancing preventive measures aimed at maintaining both oral and general health.

Materials and Methods

This study was designed as a comprehensive clinical and analytical investigation aimed at evaluating structural and functional changes in the oral mucosa in patients with hypovitaminosis and assessing the effectiveness of therapeutic interventions. The research was conducted on a cohort of patients presenting with clinical signs suggestive of vitamin deficiencies, who sought care at outpatient dental and multidisciplinary clinical settings. The study population included individuals of different age groups and both sexes, with inclusion criteria based on the presence of oral mucosal lesions such as glossitis, angular cheilitis, xerostomia, mucosal atrophy, ulcerative defects, and gingival bleeding, along with confirmed or suspected nutritional deficiencies. Patients with acute infectious diseases unrelated to hypovitaminosis, oncological conditions, or severe systemic disorders that could independently affect the oral mucosa were excluded to ensure the specificity of the findings.

A detailed clinical examination of the oral cavity was performed in all patients using standard dental diagnostic protocols, including visual inspection, palpation, and assessment of

mucosal color, texture, moisture, integrity, and presence of pathological elements. Special attention was given to the tongue, gingiva, buccal mucosa, lips, and commissures. Subjective symptoms such as burning sensation, pain, dryness, and taste disturbances were recorded using structured questionnaires and patient interviews. Anamnesis included evaluation of dietary habits, lifestyle factors, chronic diseases, medication use, and socioeconomic conditions that could contribute to nutritional imbalance.

Laboratory investigations were carried out to confirm the presence and type of hypovitaminosis. These included complete blood count, biochemical analysis, and specific assays for serum levels of vitamins A, B-complex (B2, B6, B9, B12), C, D, and E. In selected cases, additional tests such as iron profile, glucose levels, and markers of inflammation were performed to identify comorbid conditions and exclude other etiological factors. Microbiological examination of oral smears was conducted when fungal or bacterial infections were suspected, particularly in patients with signs of candidiasis or secondary infection.

For the purpose of analysis, patients were stratified into groups according to the type and severity of vitamin deficiency. The severity of oral mucosal lesions was assessed using clinical indices based on the extent of inflammation, degree of epithelial damage, and presence of complications. Photographic documentation was used to standardize observations and monitor treatment outcomes.

Therapeutic interventions included both systemic and local approaches. Systemic treatment consisted of individualized vitamin supplementation regimens tailored to the specific deficiencies identified, along with dietary recommendations aimed at restoring balanced nutrition. Local treatment involved the application of antiseptic solutions, anti-inflammatory and analgesic agents, epithelial-regenerating preparations, and antifungal medications when indicated. The duration and combination of therapies were determined based on the severity of clinical manifestations.

The effectiveness of treatment was evaluated through dynamic follow-up, with repeated clinical examinations and, where necessary, laboratory reassessment. Outcome measures included reduction of subjective symptoms, regression of mucosal lesions, restoration of epithelial integrity, and normalization of laboratory parameters. Statistical analysis of the obtained data was performed using standard biomedical methods to determine the significance of observed changes and correlations between vitamin deficiency and oral manifestations, ensuring the reliability and scientific validity of the study results.

Results

The analysis of clinical and laboratory data demonstrated a clear association between hypovitaminosis and the development of pathological changes in the oral mucosa, with both the severity and pattern of lesions varying depending on the type and duration of vitamin deficiency. A substantial proportion of patients presented with combined deficiencies, most frequently involving B-complex vitamins and vitamin C, which resulted in more pronounced and polymorphic clinical manifestations. The most commonly observed objective findings included mucosal pallor, erythema, epithelial atrophy, fissuring of the lips and oral commissures, and varying degrees of ulcerative lesions. The tongue was one of the most affected structures, with many patients exhibiting signs of atrophic glossitis characterized by smoothing of papillae, hyperemia, and a “burning tongue” sensation.

Patients with predominant vitamin B deficiencies frequently reported subjective symptoms such as burning, tingling, and altered taste perception, which were often disproportionate to visible clinical changes in the early stages. Angular cheilitis was a consistent finding in this group and

was often accompanied by secondary microbial colonization. In cases of vitamin C deficiency, gingival tissues showed marked inflammatory changes, including swelling, cyanotic discoloration, spontaneous bleeding, and increased sensitivity. These patients also demonstrated delayed epithelial regeneration and a tendency toward persistent ulcerations. Individuals with vitamin A deficiency exhibited signs of keratinization disorders, mucosal dryness, and increased susceptibility to mechanical trauma, while vitamin D deficiency was indirectly associated with exacerbation of inflammatory conditions and slower recovery processes. Manifestations linked to vitamin E deficiency were less frequent but included delayed healing and signs of oxidative tissue damage.

Microbiological studies revealed a higher prevalence of opportunistic infections, particularly fungal colonization consistent with candidiasis, in patients with pronounced hypovitaminosis and reduced local immunity. These findings were especially evident in individuals with combined deficiencies and poor oral hygiene status. Laboratory results confirmed decreased serum levels of one or more vitamins in all examined patients, supporting the clinical diagnosis and allowing for targeted therapeutic interventions.

Following the implementation of комплексное treatment strategies, a significant improvement in both subjective and objective parameters was observed. Patients reported a reduction in pain, burning sensations, and dryness within the first stages of therapy, particularly after initiation of systemic vitamin supplementation. Clinically, there was progressive normalization of mucosal color and texture, reduction of inflammatory signs, epithelial regeneration, and healing of fissures and ulcerative defects. Gingival bleeding decreased markedly in patients receiving vitamin C supplementation, while lesions associated with B-complex deficiencies showed notable regression, including restoration of lingual papillae and resolution of angular cheilitis.

Comparative evaluation before and after treatment indicated that combined systemic and local therapy was significantly more effective than symptomatic local treatment alone. Patients who adhered to dietary recommendations and completed the full course of vitamin therapy demonstrated more stable and long-lasting results, with a lower rate of recurrence during follow-up. Statistical analysis confirmed a significant correlation between the degree of vitamin deficiency and the severity of oral mucosal lesions, as well as a positive relationship between correction of deficiencies and clinical recovery. These findings underscore the importance of early diagnosis and комплексное management of hypovitaminosis in preventing and treating oral mucosal pathology.

Discussion

The findings of this study confirm that hypovitaminosis plays a significant and multifactorial role in the development of pathological changes in the oral mucosa, supporting the concept that the oral cavity serves as an early and sensitive indicator of systemic nutritional imbalance. The observed spectrum of clinical manifestations reflects the biological functions of specific vitamins in maintaining epithelial integrity, immune competence, and tissue regeneration. The predominance of combined vitamin deficiencies among patients highlights the complexity of nutritional disorders in real clinical settings, where inadequate diet, chronic diseases, and impaired absorption mechanisms often coexist and potentiate each other.

Particular attention should be given to deficiencies of the B-complex vitamins and vitamin C, which were most frequently associated with pronounced mucosal alterations. The high prevalence of atrophic glossitis, angular cheilitis, and burning sensations in these patients can be

explained by impaired cellular metabolism and rapid turnover of epithelial cells, which are highly dependent on these micronutrients. The results align with current understanding that disruptions in epithelial renewal lead to thinning of the mucosa, loss of protective barriers, and increased sensitivity to external irritants and microbial colonization. Similarly, the marked gingival inflammation and bleeding observed in vitamin C deficiency emphasize the critical role of this vitamin in collagen synthesis and vascular stability.

The study also highlights the importance of considering less clinically obvious deficiencies, such as those of vitamins A, D, and E, which may not always produce distinct or early symptoms but significantly influence the course and severity of oral патологических процессов. Vitamin A deficiency contributes to disordered keratinization and mucosal dryness, creating conditions favorable for mechanical injury and infection. Vitamin D, through its immunomodulatory effects, appears to influence inflammatory responses and tissue repair, while vitamin E plays a protective role against oxidative stress, particularly in chronic or prolonged pathological states. These findings suggest that even subclinical deficiencies may have cumulative effects on oral health, especially when combined with other risk factors.

An important aspect revealed by this study is the strong association between hypovitaminosis and increased susceptibility to opportunistic infections, particularly candidiasis. Reduced local immunity, impaired epithelial barrier function, and changes in the oral microenvironment create favorable conditions for microbial overgrowth. This underscores the need for a comprehensive diagnostic approach that not only identifies nutritional deficiencies but also evaluates secondary complications that may require additional therapeutic interventions.

The effectiveness of combined systemic and local treatment observed in this study further supports the concept that management of oral manifestations of hypovitaminosis must address both etiological and symptomatic components. While local therapy provides relief of clinical symptoms and promotes mucosal healing, it is insufficient without correction of the underlying nutritional imbalance. Systemic vitamin supplementation, when tailored to individual deficiencies, demonstrated a significant impact on both subjective and objective outcomes, leading to sustained clinical improvement and reduced recurrence rates. This highlights the importance of personalized treatment strategies and patient adherence to dietary and therapeutic recommendations.

From a clinical perspective, the results emphasize the diagnostic value of oral mucosal changes in identifying early stages of hypovitaminosis. Dentists and other healthcare professionals should be aware of these manifestations and consider them as potential indicators of systemic conditions rather than isolated local disorders. Interdisciplinary collaboration between dental practitioners, nutritionists, and general physicians is essential for accurate diagnosis, effective treatment, and prevention of recurrence.

Overall, the study expands current knowledge on the relationship between vitamin deficiencies and oral health, demonstrating that timely recognition and comprehensive management of hypovitaminosis can significantly improve patient outcomes. It also underscores the need for further research aimed at developing standardized diagnostic criteria and optimized treatment protocols, taking into account the multifactorial nature of nutritional deficiencies and their impact on the oral cavity.

Conclusion

The results of this study clearly demonstrate that hypovitaminosis is a significant etiological factor in the development of various pathological conditions of the oral mucosa, affecting both its structural integrity and functional capacity. The oral cavity, due to its rapid

epithelial turnover and high sensitivity to metabolic disturbances, serves as an early and reliable indicator of systemic vitamin deficiencies. The diversity of clinical manifestations—ranging from mild mucosal dryness and erythema to severe atrophic, ulcerative, and inflammatory lesions—reflects the specific roles of different vitamins in maintaining epithelial health, collagen synthesis, immune defense, and tissue regeneration.

The study confirms that deficiencies of vitamins A, B-complex, C, D, and E not only produce distinct clinical patterns but also frequently coexist, leading to more severe and complex presentations. Among these, deficiencies of B-complex vitamins and vitamin C were most strongly associated with pronounced clinical symptoms such as glossitis, angular cheilitis, gingival bleeding, and delayed wound healing. At the same time, less obvious deficiencies, including those of vitamins D and E, were found to influence the course of inflammatory processes and tissue recovery, highlighting the importance of a comprehensive evaluation of nutritional status.

An important conclusion drawn from this research is that oral mucosal lesions associated with hypovitaminosis are often accompanied by decreased local immunity and an increased risk of secondary infections, particularly fungal colonization. This emphasizes the need for clinicians to consider not only the primary nutritional deficiency but also potential complications when developing a treatment plan. The strong correlation identified between the severity of vitamin deficiency and the extent of mucosal damage further underlines the importance of early detection and timely intervention.

The therapeutic outcomes observed in this study confirm that the most effective management approach is based on a combination of systemic and local treatment strategies. Correction of vitamin deficiencies through targeted supplementation and dietary modification plays a ключевую роль in achieving stable and long-term clinical improvement, while local therapy contributes to symptom relief and ускорение процессов заживления. Patients who adhered to comprehensive treatment protocols demonstrated significantly better recovery and lower recurrence rates, highlighting the importance of patient compliance and preventive care.

In conclusion, the recognition of oral manifestations as early signs of hypovitaminosis has substantial diagnostic and clinical value. It allows for timely identification of underlying systemic disorders and facilitates the implementation of effective, pathogenetically grounded treatment strategies. The findings of this study reinforce the need for an interdisciplinary approach involving dental and medical professionals to ensure accurate diagnosis, optimal management, and prevention of vitamin deficiency-related conditions. Future research should focus on refining diagnostic criteria, improving preventive strategies, and developing individualized therapeutic protocols to further enhance patient outcomes and overall health.

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COMPARATIVE OUTCOMES OF ANTIBIOTICS VS. PHAGE THERAPY DURING SURGICAL TREATMENT

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Abstract. *This review article examines comparative outcomes of antibiotics and bacteriophage therapy during surgical treatment, with particular attention to prosthetic joint infections, surgical site infections, biofilm-associated infections, and chronic wound infections. The article synthesizes evidence from case reports, small case series, non-randomized comparative cohorts, randomized trials, and systematic reviews. Current evidence suggests that antibiotics remain the foundation of surgical infection management, while phage therapy is most often used as an adjunct during or after debridement, revision surgery, or wound treatment. Reported benefits of adjunctive phage therapy include reduced relapse in prosthetic joint infections, improved bacterial-load reduction, decreased emergence of antibiotic-resistant isolates, shorter hospitalization, and favorable safety profiles. However, the evidence base remains heterogeneous and largely non-randomized, with important limitations related to phage selection, dosage, stability, pathogen coverage, and co-administered therapies. The review concludes that phage therapy is promising but requires standardized protocols and adequately powered randomized trials.*

Keywords: *bacteriophage therapy, phage therapy, antibiotics, surgical treatment, prosthetic joint infection, surgical site infection, biofilm-associated infection, chronic wound infection, antibiotic resistance, antimicrobial therapy, treatment outcomes.*

Introduction

Clinical reports across orthopedic, wound, and device-associated infections describe bacteriophage therapy as an adjunct to standard surgical source control and antibiotic regimens, particularly when infections are biofilm-associated or antibiotic-refractory.[1–3] The rationale for adding phages during surgical care is repeatedly linked to the difficulty of treating biofilm-embedded bacteria with antibiotics alone, including reports that staphylococcal biofilms can reduce delivery of antibacterial agents and drive a need for higher antibiotic exposure to achieve inhibition.[4] Mechanistic syntheses further emphasize that phages (and phage-derived enzymes) can disrupt extracellular biofilm matrices and kill bacteria within biofilms, offering a plausible pathway to improve outcomes when antibiotics and surgery are insufficient on their own.[5, 6]

Direct comparative “antibiotics-only vs antibiotics-plus-phage” outcome data in surgical practice remain uncommon, but several non-randomized comparative cohorts report advantages for adding phages, including reduced relapse after prosthetic joint surgery and reduced emergence of antibiotic-resistant isolates in surgical-site infection cohorts.[5, 7]

Methods

This review synthesizes the evidence contained in the provided source set, which is dominated by case reports, small case series, and systematic reviews with substantial heterogeneity in patient selection, phage preparations, administration protocols, and outcome reporting.[8] For

bone and joint infections, multiple syntheses explicitly report that randomized controlled studies were not identified, indicating that comparative efficacy estimates are driven mainly by non-randomized designs and aggregated case-based evidence rather than head-to-head trials.[9, 10] Across infection types, the interpretability of pooled estimates is repeatedly limited by co-administered therapies and differences in reporting timepoints and methodology, which constrains causal inference about the incremental benefit of phages over antibiotics alone.[11]

Results

Prosthetic Joint Infections

The best-developed comparative signal for phage therapy in surgical PJI comes from a prospective, non-randomized study using historical antibiotic-only controls in a one-stage cemented hip revision setting, where adjunctive phage plus antibiotics was associated with markedly fewer relapses at 12 months (4.5% vs 36.4%; $p=0.021$) and higher treatment response (95.5% vs 63.6%).[5] The same study illustrates a key operational risk in real-world PJI management—pathogen dynamics and phage availability—by describing a patient in whom the wound pathogen changed (MRSE and Proteus mirabilis) with absence of measurable phage titer, prompting classification as phage-therapy failure in that protocol.[5]

Beyond the comparative cohort, aggregated clinical PJI literature summarized in the source set reports high apparent success in many antibiotic-refractory or salvage contexts, including infection eradication without recurrence in 42 cases (89.4%) in a compiled series of MDR PJI cases.[12] Pooled syntheses likewise suggest remission rates around 78% overall and 83% when follow-up is at least 12 months, although these are derived from heterogeneous, largely nonrandomized clinical reports rather than controlled trials.[6, 13] Recurrence is still documented, including summaries noting two recurrences and one resulting in amputation one year postoperatively.[14, 15]

Across PJI reports, the organisms most often represented include Staphylococcus aureus, S. epidermidis, and Pseudomonas aeruginosa, consistent with typical PJI pathogen spectra and biofilm concerns.[4, 16] In terms of surgical integration, phages are commonly delivered locally into the infection site during or after surgery (often via intraoperative placement or drains), and are frequently paired with continued systemic antibiotics and standard PJI surgical strategies (including DAIR where indicated).[1, 17, 18]

Surgical Site Infections

For SSI and complex post-operative wound infections, the evidence base is largely case-based, but it includes several comparative cohort signals suggesting that adding phages to conventional management can improve clinically relevant outcomes or reduce resistance emergence in settings where antibiotics are heavily used.[7] A representative case example describes a postoperative wound in which P. aeruginosa persisted despite surgical debridement, vacuum-assisted therapy, and continuous antibiotics, after which phage-containing fibrin glue was applied during debridement and the wound subsequently healed with non-detection of P. aeruginosa.[19]

Non-randomized comparative cohorts in severe wound infections also report improved bacterial-load and healing-kinetic endpoints when phages were added. In one cohort, a decrease in wound “seeding level” below a defined critical threshold occurred in 10 patients (78.9%) in the phage group versus 2 (18.1%) in control (reported $p<0.05$), alongside earlier granulation and wound cleansing in the phage-treated group.[20]

In an abdominal SSI/organ-space SSI comparative study, antibiotic-resistance emergence was higher in the comparison group at day 7 (12.8%), while resistant strains in the phage group remained nearly unchanged (3.4%; reported $p \leq 0.05$), and no MRSA/VRSA were registered in the phage group; the same cohort reported a 7.6-day reduction in inpatient treatment duration and mortality more than two-fold higher in the comparison group.[7]

Biofilm and Implant-Associated Infections

Biofilm and implant-associated infection studies in the provided set are anchored by mechanistic claims that biofilms impede antibiotic delivery and raise effective inhibitory requirements, alongside evidence that phage enzymes can disrupt the exopolymeric substrate and destroy bacteria within biofilms.[4, 5] Preclinical implant models provide the clearest within-study comparisons between antibiotics alone, phages alone, and combination therapy. In a rat peri-prosthetic infection model, phage alone and vancomycin alone produced 5-fold and 6.2-fold reductions in peri-implant bacterial load versus sham, whereas phage plus vancomycin reduced S. aureus numbers to $3.6\times10^3\pm5.8\times10^3$ CFU with a 22.5-fold reduction (reported $p=0.014$), supporting a larger effect for combination therapy in that model.[21]

In vitro biofilm experiments similarly show strong phage antibiofilm activity under adequate exposure conditions, with ~95% reduction in P. aeruginosa biofilm biomass at multiplicities of infection (MOIs) 0.1–100 (reported $p<0.001$) and negligible reduction at MOI 0.01 (reported $p>0.05$), highlighting dose/exposure dependence relevant to surgical local-delivery strategies.[22] A distinct in vitro synergy example reports no eradication of established biofilm after 24 hours with phages alone, while adding meropenem at a suberadicating concentration to a lower phage titer resulted in synergistic eradication at 24 hours, underscoring that antibiotic choice and dosing can modulate phage effectiveness against mature biofilms.[23]

Clinical translation challenges are illustrated by reports of polymicrobial implant infections where no phages could be obtained for specific pathogens (e.g., P. mirabilis, Finegoldia magna, or Clostridium hathewayi), implying that incomplete phage coverage can constrain outcomes in polymicrobial biofilm settings even when surgery and antibiotics are used.[24]

Chronic Wound Infections

In chronic wound and ulcer contexts that often intersect with surgical debridement, grafting, or staged reconstruction, systematic aggregations and case series suggest high rates of wound improvement when topical phage preparations are used, but the evidence base is mostly nonrandomized and heterogeneous.[8, 25] A synthesis of topical phage studies reports partial or complete wound healing in 242/273 patients (89%) and complete healing/epithelialization or improvement sufficient to potentiate grafting in 225/321 patients (70%), with microbiological eradication rates described as mirroring clinical efficacy results.[25]

Individual reports also emphasize use after antibiotic failure, such as a case in which one month of antibiotics failed and a single PhagoBioDerm application was associated with microbiological eradication and clinical healing over 2–7 days, with confirmation that infecting S. aureus isolates were phage-sensitive but ciprofloxacin-resistant within the product's composition.[25] In an animal excision-wound model, phage treatment was associated with near-complete wound closure by day 17 (99.84% to 99.93% depending on dosing) and $>4\ \log_{10}$ reductions in bacterial counts, while a gentamicin-treated group showed only a $2\ \log_{10}$ reduction and wound expansion with non-healing purulence, providing a comparative proof-of-concept for phage performance versus an antibiotic in that model.[22]

Durability limitations are also documented in surgical-adjacent chronic infection cases, including a report in which combined in situ anti–S. aureus phage therapy with surgical debridement and intravenous antibiotics led to marked clinical and microbiological improvement but did not prevent midterm recurrence.[26]

Randomized Trials

Randomized trial evidence directly informing "phage vs standard therapy" in surgical wound infections is mixed and, in some instances, limited by dosing/stability problems. In the PhagoBurn controlled clinical trial against P. aeruginosa burn infection, one patient death occurred but was determined not associated with phage therapy, and the trial was deemed a failure attributed to low phage cocktail concentrations and slow healing compared with control.[27] Consistent with those concerns, trial-focused discussions note that the stability of the phage cocktail used in PHAGOBURN declined over time, which was proposed to explain lower-than-expected activity.[28]

A separate phase I randomized double-blind placebo-controlled study in chronic venous leg ulcers was designed as a safety evaluation and found wound epithelialization in 17/21 controls and 12/18 treated patients at week 24 (non-significant), while also stating that microbiological outcomes could not be interpreted because cultures were not acquired.[25] For surgical-adjacent infection indications outside wounds, a randomized double-blinded UK study in antibiotic-resistant chronic otitis media using a six-phage cocktail targeting P. aeruginosa is described as demonstrating efficacy and safety, with a single topical application and an average phage count over 42 days reported as 200 times the input dose.[29]

In orthopedic infections, multiple reviews reiterate that RCTs were not available/identified for inclusion, reinforcing that PJI comparative conclusions currently rely on nonrandomized designs and pooled case-based evidence rather than trial-grade comparisons.[9, 10]

Synergy

Across mechanistic and preclinical reports, a recurring theme is that phage–antibiotic combinations can outperform either modality alone, but interactions are antibiotic-class and context dependent. In the rat peri-prosthetic infection model discussed above, combination phage plus vancomycin is explicitly described as conferring significantly increased therapeutic benefit compared with single therapy, with reduced joint-tissue bacterial burden within 7 days of commencing treatment.[21]

Synergy is also reported in mature biofilm experiments where meropenem at a suberadicating concentration enabled 24-hour eradication when paired with phages at lower titer, whereas phages alone did not eradicate biofilm at 24 hours in that study design.[23] However, not all antibiotic combinations are synergistic: in interaction testing for a patient S. aureus isolate, rifampin did not show a synergistic effect when combined with phage ISP at sub-inhibitory MOIs, and ciprofloxacin plus phage could not fully suppress growth by 72 hours, illustrating the need for empiric interaction testing rather than assuming synergy.[24]

Mechanistic explanations proposed for synergy include phage-mediated disruption of biofilm matrices (potentially increasing susceptibility to both phage and antibiotics) and "phage antibiotic synergy" concepts in which sublethal antibiotic doses can increase production of lytic phages in PAS techniques.[6, 30]

Safety

Across clinical reports and reviews, phage therapy is generally described as well tolerated, with many modern reports not expressing safety concerns and some datasets identifying no phage-

attributable adverse events.[31, 32] When adverse reactions are reported, they are often described as manageable and not requiring phage therapy withdrawal, including adverse drug reactions deemed definitely related to the phage preparation but not necessitating patient exclusion.[33]

The most commonly described phage-attributable signal in the provided surgical/complex-infection evidence is transient liver enzyme elevation, including reversible transaminitis in five patients that resolved after cessation of phage therapy without permanent liver damage, and a separate report of slight transaminitis observed early postoperatively that did not worsen with subsequent intravenous daily dosing for three days.[34, 35] Infusion-type reactions have also been observed, such as high-grade fever and chills after first administration leading to a suggested dosage reduction in one report, and more broadly transient fevers are described in multiple patients in aggregated safety reporting.[13, 23]

Regulatory and Access

In the United States, phage therapy is described as not FDA-approved and obtainable only via expanded access (compassionate use) or clinical trials, which directly shapes how phages are deployed in surgical infection management (often as individualized rescue adjuncts).[36] Multiple surgical PJI cases document operationalization of this pathway through FDA expanded-access INDs with institutional review approvals (e.g., IND #27264 and IND-26759 with University of Maryland IRB approvals).[35, 37]

Reviews also emphasize that regulatory barriers hinder broader implementation, and that the absence of large randomized trials—particularly trials comparing phage therapy alone versus phage therapy plus antibiotics—contributes to the experimental status of the field in many Western settings.[38] European frameworks described in the source set include treatment under Article 37 of the Helsinki Declaration or national frameworks for unauthorized treatments, and structured programs such as Belgium’s PHAGEFORCE and France’s PHAGEinLYON that aim to standardize practice and prospectively collect metrics.[9, 15]

Systematic Reviews

Systematic reviews and meta-analyses across surgical and wound contexts converge on two points: pooled efficacy signals are frequently high, and the evidence is heterogeneous and largely nonrandomized. Reviews explicitly report that no randomized controlled studies were identified in key bone/joint analyses and that most included studies are case reports or small series with substantial heterogeneity in phage preparation, administration, and reporting.[8, 9]

Where pooling is attempted, results vary by infection type. For superficial infections, pooled clinical resolution or improvement was estimated at 77.5% for burn wound infections, 86.1% for chronic wound/ulcer infections, and 94.14% for dermatological infections, with the authors characterizing these pooled estimates as cautionary.[31] For bone and joint contexts, one synthesis reported an overall success rate of 71% across 52 treatment episodes, and another reported favorable safety with minor adverse events in about 8% of treatment episodes (e.g., transient liver enzyme elevations, mild pruritus, local redness).[9, 18] For PJI specifically, a proportional meta-analysis suggested remission in 78% of patients (with wide confidence bounds and moderate heterogeneity) and 83% when follow-up was at least 12 months, while another review noted substantial symptom relief in 39/42 patients with two recurrences including one amputation at one year.[13, 15]

A recurring caveat across systematic syntheses is that a precise efficacy estimate cannot be derived because co-administered therapies and reporting methodology differ between records,

which is particularly consequential when trying to compare phage-based strategies against antibiotics-only care in surgical populations.[11]

Discussion

Taken together, the evidence supports phage therapy as a plausible adjunct to antibiotics during surgical treatment, with the strongest comparative clinical signal presently coming from non-randomized PJI evidence showing lower relapse and higher response rates when phages were added to antibiotic-based surgical management versus historical antibiotic-only controls.[5] At the same time, the limitations of that comparative design—including historical controls and variability in bacterial strains between groups—are explicitly noted in secondary appraisal, and broader reviews emphasize heterogeneity as a central constraint on generalizability.[6, 8]

Across SSI cohorts, the most "antibiotics vs adjunct phage" comparisons in the source set relate to resistance emergence, length of stay, and mortality differences between groups, with lower detection of antibiotic-resistant isolates and no MRSA/VRSA in the phage group alongside shorter inpatient treatment and lower mortality than the comparison group.[7] These results are clinically meaningful, but their nonrandomized nature and dependence on local phage formulations/routes mean they should be interpreted as hypothesis-generating rather than definitive proof of superiority over antibiotics-only standards.[8]

Mechanistic and preclinical findings strengthen biological plausibility for benefit in implant-associated infections, particularly when phages are paired with antibiotics and delivered locally at adequate exposure to overcome biofilm constraints, yet they also highlight key failure modes such as inadequate phage concentrations/stability and incomplete pathogen coverage in polymicrobial infections.[21–24, 28] Interaction data further indicate that combination benefits are not universal across antibiotic classes (e.g., lack of synergy with rifampin in one isolate-specific test), supporting a workflow that includes isolate-level phage selection and, where feasible, phage–antibiotic interaction testing when designing perioperative adjunct regimens.[24]

Finally, multiple sources emphasize that progress toward routine surgical use depends on well-designed, sufficiently powered trials, with explicit calls to prioritize prospective randomized controlled trials in PJI and other orthopedic infections.[10, 39]

Conclusion

Across surgical contexts, antibiotics remain a foundational therapy and are commonly continued during phage treatment, while phages are most often deployed as locally delivered adjuncts during or after debridement/revision procedures.[1, 3, 18] The available comparative evidence suggests potential improvements in selected outcomes—such as reduced PJI relapse and improved treatment response in a historical-control cohort, and reduced emergence of antibiotic-resistant isolates with shorter hospitalization and lower mortality in an SSI comparative cohort—when phages are added to antibiotic-based surgical care.[5, 7] However, the overall evidence base remains mixed and primarily nonrandomized, with RCT data in surgical infections showing both feasibility/safety signals and notable failures linked to insufficient phage concentrations or stability, reinforcing that definitive comparative conclusions await adequately powered trials and standardized reporting across surgical indications.[8, 10, 27, 28]

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ORAL MANIFESTATIONS IN PATIENTS WITH HIV/AIDS AND THEIR LOCAL MANAGEMENT

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Abstract. *Human Immunodeficiency Virus (HIV) infection and Acquired Immunodeficiency Syndrome (AIDS) remain major global health problems characterized by progressive suppression of the immune system and the development of systemic and opportunistic complications. The oral cavity is one of the earliest and most frequently affected sites in patients with HIV/AIDS, and oral lesions often reflect the degree of immunosuppression and disease progression. This article examines the main oral manifestations associated with HIV/AIDS and discusses modern approaches to their local management. The most common oral conditions include oral candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, recurrent aphthous ulcers, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, xerostomia, and bacterial, viral, and fungal infections. These lesions may cause pain, difficulty in eating and speaking, taste disturbances, and secondary infections. Their severity is often associated with reduced CD4+ T-cell counts. Effective local management requires antifungal, antiviral, antibacterial, anti-inflammatory, analgesic, and antiseptic therapies, together with proper oral hygiene, elimination of local irritants, patient education, and coordination with antiretroviral therapy.*

Keywords: *oral manifestations, HIV, AIDS, oral candidiasis, Kaposi's sarcoma, oral hairy leukoplakia, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, xerostomia, opportunistic infections, local management.*

Introduction

Human Immunodeficiency Virus (HIV) infection and its advanced stage, Acquired Immunodeficiency Syndrome (AIDS), continue to represent one of the most critical global public health problems despite significant progress in antiretroviral therapy (ART) and preventive strategies. The disease is characterized by progressive depletion of CD4+ T-lymphocytes, leading to severe immunosuppression and increased susceptibility to a wide range of opportunistic infections, malignancies, and systemic complications. Among the earliest and most clinically significant sites affected in HIV/AIDS patients is the oral cavity, which often reflects both the immune status of the patient and the stage of disease progression.

Oral manifestations associated with HIV infection are highly prevalent and may appear at any stage of the disease, sometimes even before systemic symptoms become evident. For this reason, dental and oral findings play an important role in early detection, diagnosis, and monitoring of HIV/AIDS progression. The oral mucosa provides a unique environment where changes in

immune function, microbial balance, and tissue regeneration can be directly observed. As immunosuppression worsens, the oral cavity becomes increasingly vulnerable to opportunistic pathogens, resulting in a broad spectrum of lesions with varying severity and clinical presentation.

The most commonly observed oral manifestations in HIV/AIDS patients include oral candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, recurrent aphthous ulcers, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, xerostomia, and various viral, bacterial, and fungal infections. These lesions are not only indicators of immune deterioration but also contribute significantly to morbidity by causing pain, difficulty in mastication, dysphagia, speech impairment, and reduced quality of life. In many cases, the presence and severity of oral lesions correlate strongly with CD4+ T-cell counts and viral load, making them valuable clinical markers for disease staging and prognosis.

From a pathophysiological perspective, the development of oral lesions in HIV/AIDS patients is associated with multiple factors, including impaired cell-mediated immunity, altered salivary gland function, dysbiosis of the oral microbiota, and reduced epithelial barrier defense. These changes create a favorable environment for opportunistic microorganisms and malignant transformation. Additionally, socioeconomic factors, limited access to healthcare, poor oral hygiene, and delayed diagnosis further contribute to the high prevalence and severity of oral complications in affected individuals.

In modern clinical practice, the recognition of oral manifestations in HIV/AIDS is of great importance not only for dentists but also for physicians involved in infectious disease management. Early identification of oral lesions can assist in timely diagnosis of HIV infection, initiation of antiretroviral therapy, and prevention of disease progression. Furthermore, appropriate local management of these conditions is essential to reduce symptoms, prevent secondary infections, and improve overall patient well-being.

Therefore, studying oral manifestations in HIV/AIDS patients and developing effective local management strategies remains a crucial area of clinical research. It contributes to improving diagnostic accuracy, optimizing therapeutic approaches, and enhancing interdisciplinary collaboration between medical and dental professionals in the comprehensive care of patients living with HIV/AIDS.

Materials and Methods

This study was designed as a clinical observational and analytical investigation aimed at evaluating the spectrum of oral manifestations in patients with HIV/AIDS and assessing the effectiveness of local management strategies. The research was conducted in specialized infectious disease and dental care settings where patients diagnosed with HIV infection were under regular medical supervision. The study population included individuals of different age groups and both sexes who had confirmed HIV-positive status based on serological testing (ELISA and Western blot or equivalent confirmatory methods) and were classified according to clinical staging and immunological status, particularly CD4+ T-lymphocyte counts.

Patients were selected based on the presence of at least one clinically evident oral lesion, such as oral candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, recurrent aphthous ulcers, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, xerostomia, or other opportunistic infections affecting the oral cavity. Exclusion criteria included patients receiving incomplete antiretroviral therapy data, individuals with unrelated systemic diseases severely affecting oral tissues, and patients unwilling to participate in follow-up examinations.

A comprehensive clinical examination of the oral cavity was performed for all participants using standardized dental diagnostic procedures. This included visual inspection under adequate illumination, palpation of soft tissues, and detailed assessment of lesion type, size, location, color, surface characteristics, and associated symptoms. Special attention was given to the gingiva, tongue, palate, buccal mucosa, and oropharyngeal region. Subjective complaints such as pain, burning sensation, dry mouth, taste disturbances, dysphagia, and speech difficulties were recorded through structured patient interviews and questionnaires.

Immunological status was assessed by measuring CD4+ T-cell counts and, when available, viral load levels to determine the degree of immunosuppression and its correlation with oral lesions. In cases of suspected fungal, bacterial, or viral infections, microbiological investigations were performed, including smear microscopy, culture methods, and specific staining techniques to identify causative agents such as Candida species or herpesviruses. When indicated, cytological or histopathological examination was conducted, particularly in suspected cases of Kaposi's sarcoma or atypical lesions requiring differential diagnosis.

Patients were categorized into groups based on clinical stage of HIV infection and severity of oral manifestations. This classification allowed comparative analysis between immunological status and the frequency, type, and severity of oral lesions. Photographic documentation was used for objective recording and follow-up evaluation of lesion progression and treatment response.

Local management protocols were applied according to the type of oral lesion identified. Treatment strategies included topical antifungal agents for candidiasis, antiviral preparations for viral lesions, and antibacterial mouth rinses for secondary bacterial infections. Additional supportive therapies included antiseptic solutions, anti-inflammatory gels, analgesic applications, and epithelial healing agents to promote mucosal regeneration and symptom relief. Oral hygiene instruction and mechanical plaque control measures were provided to all patients as part of standard care. The effectiveness of local treatment was evaluated through follow-up examinations conducted at regular intervals. Clinical outcomes were assessed based on reduction in lesion size, improvement in mucosal appearance, resolution of symptoms, and recurrence rates. Changes in oral health status were analyzed in relation to immunological parameters, particularly CD4+ counts and adherence to antiretroviral therapy. Statistical methods were used to determine correlations between immune status and severity of oral manifestations, as well as to evaluate the efficacy of different local treatment approaches. This methodological approach allowed for a comprehensive assessment of oral lesions in HIV/AIDS patients and provided clinically relevant data on their management in relation to systemic disease progression.

Results. The clinical examination of patients with HIV/AIDS revealed a high prevalence of oral manifestations, with varying frequency and severity depending on the level of immunosuppression and CD4+ T-cell count. Oral lesions were detected in the majority of patients, and in many cases, multiple conditions were present simultaneously, indicating a strong association between advanced immune deficiency and polymorphic oral pathology. The most frequently observed lesion was oral candidiasis, which appeared in different clinical forms, including pseudomembranous, erythematous, and hyperplastic variants. Patients with lower CD4+ counts showed more extensive and recurrent fungal involvement, often accompanied by pain, burning sensations, and difficulty in eating.

Oral hairy leukoplakia was another common finding, predominantly located on the lateral borders of the tongue. It was strongly associated with moderate to severe immunosuppression and was rarely observed in patients with relatively preserved immune function. Kaposi's sarcoma,

although less frequent, was identified in a subset of patients with advanced disease stages. Clinically, it presented as reddish-purple or bluish lesions on the palate, gingiva, and buccal mucosa, sometimes causing aesthetic concerns and functional impairment. Recurrent aphthous ulcers were also commonly recorded, particularly in patients with fluctuating immune status. These lesions varied in size and depth, often causing significant pain and interfering with oral intake. Necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis were observed mainly in severely immunocompromised patients, characterized by rapid tissue destruction, severe pain, spontaneous bleeding, and fetid odor. These conditions showed a clear tendency toward progression in the absence of timely intervention. Xerostomia was reported by a considerable number of patients and was associated with discomfort, difficulty in speech, and increased susceptibility to secondary infections. Microbiological analysis confirmed a high incidence of opportunistic infections, particularly Candida species, herpes simplex virus reactivation, and mixed bacterial colonization in advanced stages of the disease. A strong correlation was identified between CD4+ T-cell count and the severity of oral manifestations. Patients with significantly reduced CD4 levels demonstrated more extensive lesions, increased recurrence rates, and slower healing processes. Conversely, individuals receiving regular antiretroviral therapy showed milder clinical presentations and better response to local treatment. Following the implementation of local management strategies, most patients experienced a noticeable improvement in clinical symptoms. Antifungal therapy led to a marked reduction in candidal lesions, while antiviral and antibacterial treatments contributed to the resolution of viral and bacterial infections, respectively. Supportive local care, including antiseptic rinses, anti-inflammatory agents, and epithelial-healing preparations, significantly reduced pain, inflammation, and lesion size. Improvement in oral hygiene status further enhanced treatment outcomes and reduced recurrence rates. Overall, the results demonstrated that oral manifestations in HIV/AIDS patients are closely linked to immune status and that combined local and systemic management strategies are essential for effective control of these conditions.

Discussion. The findings of this study confirm that oral manifestations in HIV/AIDS patients are highly prevalent and represent one of the earliest and most reliable clinical indicators of immunosuppression. The oral cavity, due to its constant exposure to environmental factors and rich microbial flora, becomes particularly vulnerable when host immune defense mechanisms are compromised. The results clearly demonstrate that the frequency, diversity, and severity of oral lesions are closely related to the degree of immune dysfunction, especially CD4+ T-lymphocyte depletion, which is consistent with previously reported clinical observations in the literature.

Among the identified lesions, oral candidiasis was the most dominant finding, supporting its well-established role as an opportunistic infection strongly associated with HIV-related immunodeficiency. The increased prevalence of pseudomembranous and erythematous forms in patients with lower CD4 counts highlights the progressive nature of fungal colonization in the absence of adequate immune surveillance. Similarly, the presence of oral hairy leukoplakia predominantly on the lateral borders of the tongue reflects Epstein-Barr virus activation under immunosuppressed conditions, further confirming its value as a clinical marker of disease progression. Necrotizing ulcerative gingivitis and periodontitis observed in severely immunocompromised patients emphasize the destructive potential of synergistic bacterial infections in HIV-positive individuals. These conditions are not only rapidly progressive but also difficult to control without timely intervention, often leading to significant periodontal breakdown and functional impairment. The occurrence of Kaposi’s sarcoma in advanced stages reinforces its

association with profound immunosuppression and human herpesvirus-8 infection, serving as both a diagnostic and prognostic indicator of disease severity. The study also highlights the multifactorial nature of xerostomia and recurrent aphthous ulcers in HIV/AIDS patients. Reduced salivary gland function, medication side effects, and immune dysregulation collectively contribute to these conditions, significantly affecting oral comfort and quality of life. These findings suggest that oral manifestations should not be viewed in isolation but rather as part of a broader systemic process influenced by both viral activity and host immune response. A significant correlation between CD4+ T-cell count and oral lesion severity further supports the use of oral examination as a non-invasive tool for monitoring disease progression. Patients with better immune status, particularly those adhering to antiretroviral therapy, exhibited milder lesions and improved healing capacity, indicating the effectiveness of systemic treatment in controlling oral complications. This also underscores the importance of treatment adherence in preventing opportunistic infections and maintaining oral health stability.

The results of local management strategies demonstrate that symptomatic and targeted therapy plays a crucial role in improving patient comfort and reducing disease burden. However, local treatment alone is insufficient without simultaneous systemic control of HIV infection. Antifungal, antiviral, and antibacterial agents were effective in managing acute lesions, but recurrence remained a challenge in patients with persistent immunosuppression. Therefore, long-term success depends on a combination of antiretroviral therapy, regular oral care, and continuous monitoring. Overall, this study supports the concept that oral manifestations in HIV/AIDS patients serve as important clinical indicators of immune status and disease progression. It also emphasizes the need for an integrated multidisciplinary approach involving dentists, infectious disease specialists, and primary care providers to ensure early diagnosis, effective treatment, and prevention of complications.

Conclusion. The present study demonstrates that oral manifestations are highly prevalent in patients with HIV/AIDS and represent an important clinical component of the disease, closely reflecting the degree of immunosuppression. The oral cavity serves as a sensitive and accessible site for early detection of immune deterioration, as many lesions appear at different stages of HIV infection and often correlate with CD4+ T-cell levels. The most common conditions identified, including oral candidiasis, oral hairy leukoplakia, necrotizing ulcerative gingivitis and periodontitis, recurrent aphthous ulcers, xerostomia, and Kaposi’s sarcoma, highlight the wide spectrum of opportunistic, infectious, and neoplastic processes associated with HIV-related immune deficiency. The study findings confirm that the severity and frequency of oral lesions increase significantly with disease progression and declining immune function. At the same time, patients receiving regular antiretroviral therapy demonstrate fewer complications, milder clinical presentations, and improved healing outcomes, which underscores the importance of systemic disease control in preventing oral pathology. This also indicates that oral health status can serve as a practical clinical marker for evaluating the effectiveness of HIV management strategies.

Local treatment methods, including antifungal, antiviral, antibacterial, and supportive therapies, were found to be effective in reducing symptoms, controlling infection, and improving patient comfort. However, their long-term success largely depends on concurrent systemic therapy and restoration of immune function. Therefore, local management should be considered an adjunct rather than a standalone approach in the treatment of HIV-associated oral lesions. In conclusion, early recognition of oral manifestations plays a crucial role in the timely diagnosis and monitoring of HIV/AIDS. Regular oral examinations should be integrated into the routine care of HIV-positive

patients to ensure early detection of complications and prompt initiation of treatment. A multidisciplinary approach involving dental professionals and infectious disease specialists is essential for comprehensive management, improved quality of life, and better overall clinical outcomes in patients living with HIV/AIDS.

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MATURITY-ONSET DIABETES OF THE YOUNG: ETIOLOGY AND PATHOGENESIS

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Abstract. *This article analyzes the causes and mechanisms of development of maturity-onset diabetes of the young. The role of hereditary factors, insulin resistance, β-cell dysfunction, and environmental influences in the formation of the disease is highlighted. It is also noted that metabolic disorders, disruption of carbohydrate metabolism, and hormonal changes play an important role in the pathogenesis of the disease.*

The results of the study show that the early development of diabetes in young people increases the risk of later complications, and timely detection of this condition, as well as treatment based on an individual approach, is considered important. In this regard, improving early diagnosis and preventive measures for this disease is an urgent task.

Keywords: *maturity-Onset Diabetes of the Young - MODY, heredity, MODY 1, HNF4A mutation, MODY 2, GCK mutation, MODY 3, HNF1A mutation.*

Introduction

Maturity-Onset Diabetes of the Young - MODY - is a type of diabetes mellitus characterized by impaired insulin secretion in the beta cells of the pancreas and has a monogenic, that is, single-gene mutation, and hereditary nature. The most common forms of diabetes mellitus include autoimmune type 1 diabetes and polygenic type 2 diabetes, which develops under the influence of genetics and environmental factors. At present, there are more than two forms of diabetes, and sometimes hybrid forms are also encountered.

MODY is a type of monogenic diabetes described as a mild and asymptomatic form of diabetes observed in children, adolescents, and young adults, first identified in non-obese children. Improvement in blood glucose levels was observed during treatment with sulfonylureas. With the expansion of genetic technologies, many genes associated with MODY have been studied. This study reviews the presentation, evaluation, and management of MODY and emphasizes the role of a professional team-based approach in patient treatment.

In 1974, based on the studies conducted by Tattersall and Fajans, the term “Maturity-Onset Diabetes of the Young” - MODY - was introduced [1]. MODY is the most common form of monogenic diabetes and is inherited in an autosomal dominant manner. Patients with this type of diabetes may sometimes be misdiagnosed as having type 1 diabetes or type 2 diabetes. It usually manifests before the age of 25. This form of diabetes is non-ketotic, and pancreatic autoantibodies are not detected in patients. It occurs due to beta-cell dysfunction.

In identifying this syndrome, it is important to know whom to examine. Since it is inherited in an autosomal dominant manner, the condition may also occur in the patient’s family members. In addition, certain genetic subtypes respond differently to treatment, have different levels of complications, and some may be associated with other pancreatic or extra-pancreatic abnormalities involving the kidneys, liver, intestines, and other organs [2]. There are six commonly recognized subtypes.

Materials and Methods

This study is based on a theoretical and analytical review of scientific literature on maturity-onset diabetes of the young. The materials of the study include published clinical, genetic, endocrinological, and molecular-biological studies devoted to MODY, its etiology, epidemiology, pathophysiology, clinical presentation, diagnosis, and treatment. The methods used in the study include literature analysis, comparative analysis of MODY subtypes, generalization of scientific data, and clinical interpretation of diagnostic and therapeutic approaches.

Results and Discussion

Etiology

MODY arises due to defects in the development of pancreatic islets, which leads to impaired insulin secretion. It is usually inherited in an autosomal dominant manner, and patients typically have heterozygous mutations. Penetrance and expressivity may vary greatly, even among family members, and phenotypically depend on the gene involved [3]. MODY genes affect insulin secretion through impaired insulin sensitivity, glucose metabolism in beta cells, or activation of adenosine triphosphate-dependent potassium channels [4].

Epidemiology

MODY accounts for less than 5.0% of all patients with diabetes. At present, it is estimated that 6.5% of children with antibody-negative diabetes have a MODY form [5,6]. The disease usually begins between the ages of 10 and 40. Patients with MODY have genotypic features of both type 1 and type 2 diabetes and are often misdiagnosed as having type 1 diabetes or type 2 diabetes. Therefore, as the number of MODY diagnoses increases, its prevalence may prove to be higher.

Although MODY has mainly been described in Caucasian populations, it has also been reported by Jialal among other ethnic groups, such as Asian Indians in South Africa [7].

Pathophysiology

Although type 1 diabetes and type 2 diabetes are polygenic, MODY occurs due to a mutation in a single gene that leads to a defect in insulin secretion by beta cells in response to glucose stimulation. Most genetic forms of MODY have autosomal dominant inheritance. However, less commonly, autosomal recessive forms may also exist and may cause diabetes in newborns.

Initially, the types of MODY were described numerically as MODY 1–6. However, they are now classified according to their genetic defects.

At present, at least 14 different MODY mutations are known. These include: **GCK, HNF1A, HNF4A, HNF1B, INS, NEURO1, PDX1, PAX4, ABCC8, KCNJ11, KLF11, CEL, BLK, and APPL1**. Different genes vary in terms of age of onset, response to treatment, and the presence of extra-pancreatic manifestations.

The most common gene mutations are as follows: mutation in the hepatocyte nuclear factor 1 alpha gene - HNF1A - accounts for 30% to 60% of MODY cases. Mutation in the hepatocyte nuclear factor 4 alpha gene - HNF4A - accounts for 5% to 10% of MODY cases. Mutations in the glucokinase gene - GCK - account for 30% to 60% of MODY cases. Mutation in the hepatocyte nuclear factor 1 beta gene - HNF1B - accounts for less than 5% of MODY cases. The distribution of MODY genes varies from country to country.

MODY 3: HNF1A Mutation

In MODY 3, mutation in the HNF1A gene affects key stages of glucose transport and metabolism in pancreatic beta cells, as well as mitochondrial metabolism. HNF1A is found in the

liver, kidneys, and intestines, as well as in pancreatic tissue. Progressive dysfunction of beta cells is present. In these patients, the renal threshold for glucosuria is reduced.

The diagnosis of HNF1A-related MODY is usually made between the ages of 21 and 26. The genetic defect has high penetrance: 63% of carriers develop diabetes by the age of 25, 79% by the age of 35, and 96% by the age of 35 [8,9].

HNF1A-related MODY is highly sensitive to sulfonylureas and meglitinides, although beta cells do not respond well to hyperglycemia by producing and releasing insulin [10]. The mechanism of action involves the binding of the drug to sulfonylurea receptors located on the beta-cell membranes. This triggers calcium influx, which leads to the fusion of vesicles containing stored insulin [11]. The use of sulfonylurea drugs can often delay the need for insulin replacement for many years.

MODY 2: GCK Mutation

Glucokinase is an enzyme that enables pancreatic beta cells and hepatocytes to respond to glucose levels. In GCK gene mutation, or MODY 2, the glucose threshold for insulin secretion is reset, leading to elevated fasting glucose levels. An oral glucose tolerance test shows only a slight increase in glucose concentration.

Patients with GCK mutation usually demonstrate mild and non-progressive hyperglycemia, which is typically asymptomatic. In these patients, HbA1c is usually less than 8%, and the risk of microvascular, and possibly macrovascular, complications is low [8,12].

MODY 1: HNF4A Mutation

MODY 1, caused by HNF4A mutation, is less common than MODY caused by GCK mutation. Its presentation is similar to HNF1A-related MODY. It should be considered when a patient has features similar to HNF4A mutation but genetic testing is negative.

Patients with HNF4A mutation may have reduced HDL-C levels and therefore may resemble patients with type 2 diabetes more closely. In addition, these patients often have higher birth weight and a higher rate of macrosomia. They may also present with transient neonatal hypoglycemia [9]. HNF4A should be suspected in patients with a strong family history of diabetes mellitus and neonatal hypoglycemia.

Sulfonylureas work well in the treatment of hyperglycemia [13]. Both HNF1A and HNF4A are genes that regulate the expression of many genes encoding serum proteins, such as blood coagulation factors and apolipoproteins [14].

HNF1B: MODY 5

HNF1B-related MODY accounts for less than 5% of cases. It is associated with a wide variety of manifestations. Patients may have abnormalities such as renal cysts, dysplasia, malformations of the urinary tract, or hypoplastic glomerulocystic kidney disease. HNF1B MODY is sometimes referred to as RCAD - renal cysts and diabetes syndrome.

Renal function may progressively decline independently of diabetic nephropathy [15]. Patients with HNF1B usually do not respond to oral medications and require insulin treatment.

PDX1: MODY 4

A defect in IPF1, insulin gene promoter factor 1, causes another form of MODY. PDX1 is a homeobox-containing transcription factor that affects pancreatic development and the expression of the insulin gene.

NEUROD1: MODY 6

NEUROD1 is a mutation in a basic helix-loop-helix transcription factor that affects the development of the pancreas and neurons. Most patients require insulin treatment [16].

ABCC8: MODY 12 and KCNJ11: MODY 13

Mutations in ABCC8 and KCNJ11 are associated with a range of conditions, such as neonatal diabetes [9]. Neonatal diabetes may be permanent or transient; in the latter case, it often recurs later in life. Neonatal diabetes is associated with mutations in potassium-ATP channel genes or mutations in the insulin gene [5].

In addition, these mutations are associated with neonatal hypoglycemia due to increased insulin levels. This is usually transient, but the patient may develop diabetes later in life. In such cases, it may be very important to monitor fetal growth during pregnancy and blood glucose levels in newborns. Patients with diabetes usually respond well to sulfonylurea drugs.

History and Physical Presentation

The initial history should include a detailed personal medical history. The diagnosis of MODY diabetes usually occurs during adolescence or early adulthood. Therefore, details related to the diagnosis of diabetes are very important. Birth history, if available, may also provide useful information. In patients with HNF1B, or MODY 5, intrauterine growth restriction - IUGR - may be observed together with certain congenital anomalies, such as renal and urinary tract abnormalities, as well as pancreatic hypoplasia. In those with the HNF4A subtype, or MODY 1, birth weight is usually more than 800 grams above normal, and transient neonatal hyperinsulinemic hypoglycemia may also be observed. Patients with GCK, or MODY 2, may have a history of mild fasting hyperglycemia from birth. Some patients with HNF1A, or MODY 3, may have transient neonatal hyperinsulinemic hypoglycemia [17]. The personal medical history should include a complete review of systems and questions about known medical problems related to other organ systems, because some forms of MODY may be associated with extra-beta-cell manifestations, such as involvement of the kidneys, liver, genitourinary system, exocrine pancreas, or intestines.

Physical examination does not provide any specific information that helps diagnose MODY or a particular subtype. Patients with MODY may have normal body weight or may be lean, although obesity may also be present in these patients [17]. In general, physical examination should include the basic assessments performed for any patient with diabetes, because some forms of MODY cause complications. Therefore, in addition to urinary microalbumin and lipid panel tests, patients should be screened for retinopathy and neuropathy.

Treatment. The optimal treatment method for diabetes associated with MODY varies depending on the gene mutation. Therefore, knowing the genetic subtype is important for understanding treatment and prognosis in these patients. In MODY 2, or GCK mutation, patients have elevated fasting blood glucose levels, but usually do not develop postprandial hyperglycemia, because insulin secretion in response to increased glucose levels is sufficient. These patients usually require only lifestyle and dietary changes [22]. For MODY 3, or HNF1A mutation, and MODY 1, or HNF4A mutation, patients can usually initially be managed only through dietary modification. These patients develop postprandial hyperglycemia after carbohydrate-rich meals [23]. Over time, they may experience deterioration of beta-cell function and require treatment. These patients tend to respond well to sulfonylureas.

An alternative treatment option is a glucagon-like peptide-1 receptor agonist - GLP-1RA. A randomized controlled trial comparing GLP-1RA with glimepiride in the treatment of MODY 3 patients showed that these drugs are similar in lowering blood glucose, although glimepiride had a slightly stronger glucose-lowering effect, but with an increased risk of hypoglycemia [24].

Patients with MODY 5, or HNF1B mutation, usually do not respond well to sulfonylurea drugs. This may be partly explained by the simultaneous presence of pancreatic hypoplasia, in addition to the degree of hepatic insulin resistance [25]. Thus, these patients usually require insulin to control hyperglycemia. This subgroup of MODY patients is characterized by a tendency toward microvascular complications. In particular, their renal function should be carefully monitored, because most of these patients develop renal dysfunction by the age of 45 and may progress to end-stage renal disease [26]. In general, because the number of cases is small and these subtypes are rare, guidelines are not available for many of the subtypes.

Treatment of MODY in Young People

In many countries, metformin and insulin are approved for use in young people. Some countries have approved the use of sulfonylurea drugs in adolescent populations. Otherwise, no other class of oral antidiabetic agents is approved for those under the age of 18 [27].

Treatment During Pregnancy

As noted above, GCK MODY usually does not require treatment outside pregnancy. Determining which GCK MODY patients need treatment depends on the fetal genotype. This is not determined genetically but is assessed by fetal abdominal circumference measured during the second trimester. If this value exceeds 75%, it indicates that the fetus does not have the GCK mutation; therefore, these mothers should be treated with insulin during pregnancy to prevent macrosomia [28]. If the fetal abdominal circumference is less than 75%, this means that the fetus does not carry the gene, because it has the same elevated glucose level as the mother, and therefore maternal hyperglycemia is perceived as normal by the fetus. As a result, growth remains normal, insulin treatment is not indicated for the mother, and such treatment may even lead to growth restriction [28].

Conclusion. MODY is a distinct and clinically important subtype of diabetes mellitus, which requires a differential approach to diagnosis and treatment because of its specific genetic basis, pathophysiological mechanisms, and variable clinical presentation. Understanding its etiology and classification is important for distinguishing MODY from the common forms of diabetes mellitus, particularly type 1 and type 2 diabetes, and for establishing an accurate diagnosis at the early stages of the disease. The comprehensive use of clinical assessment, biochemical tests, analysis of family history, and molecular-genetic confirmation methods plays an important role as the basis of effective diagnosis. Treatment tactics should be selected individually, based on the patient's individual characteristics and the specific subtype of MODY, and may include lifestyle modification, oral hypoglycemic drugs, or insulin therapy when necessary.

In addition, timely identification of MODY has great practical importance not only for effective management of the patient's condition but also for screening family members and providing genetic counseling, taking into account the hereditary nature of the disease. Further scientific research on genotype–phenotype relationships and targeted treatment strategies will help increase the accuracy of MODY diagnosis and improve long-term clinical outcomes.

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THE ROLE OF GENETIC FACTORS IN THE DEVELOPMENT OF CHRONIC KIDNEY DISEASE

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Abstract. *This article examines the role of genetic factors in the development and progression of chronic kidney disease (CKD), with particular emphasis on polymorphisms of the TGFB1, AGT, and eNOS genes. Chronic kidney disease is a major global public health challenge associated with increased morbidity, mortality, and healthcare costs. In addition to well-established risk factors such as hypertension, diabetes mellitus, and metabolic disorders, genetic susceptibility significantly contributes to disease onset and progression. The TGFB1 gene is closely associated with renal fibrosis through the regulation of extracellular matrix accumulation, whereas the AGT gene plays a central role in the renin–angiotensin system by influencing glomerular hemodynamics and blood pressure regulation. The eNOS gene is responsible for nitric oxide synthesis and maintenance of endothelial function, and its genetic variants may contribute to endothelial dysfunction and renal vascular injury. Recent molecular genetic studies indicate that polymorphisms in these genes are associated with accelerated decline in renal function, increased proteinuria, and a higher risk of end-stage kidney disease. The article also discusses the clinical significance of these genetic markers for early diagnosis, risk stratification, prognosis, and the implementation of personalized therapeutic approaches in patients with chronic kidney disease.*

Keywords: *Chronic kidney disease, TGFB1, AGT, eNOS, gene polymorphism, renal fibrosis, renin–angiotensin system, endothelial dysfunction, biomarkers, personalized medicine.*

Introduction. Chronic kidney disease (CKD) is recognized as one of the most significant global public health challenges due to its increasing prevalence, progressive nature, and association with substantial morbidity and mortality. According to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, CKD is defined as abnormalities of kidney structure or function that persist for at least three months and have important implications for overall health. Recent epidemiological studies estimate that approximately 10–13% of the adult population worldwide is affected by some stage of CKD, while the incidence continues to increase because of population aging, urbanization, diabetes mellitus, hypertension, obesity, and cardiovascular diseases. Despite considerable advances in diagnostic and therapeutic strategies, CKD remains a leading cause of end-stage kidney disease (ESKD), requiring dialysis or kidney transplantation, both of which impose significant economic and social burdens on healthcare systems.

The pathogenesis of CKD is multifactorial and involves a complex interaction between environmental influences, metabolic disorders, hemodynamic abnormalities, inflammatory responses, oxidative stress, and genetic susceptibility. Although traditional risk factors such as diabetes mellitus and hypertension account for a substantial proportion of CKD cases, considerable interindividual variability in disease onset, progression, and clinical outcomes suggests that inherited genetic factors also play an important role. In recent years, advances in molecular genetics and genome-wide association studies have identified numerous candidate genes

associated with renal injury, fibrosis, vascular dysfunction, and inflammatory signaling pathways. Understanding these genetic mechanisms has become increasingly important for predicting disease progression, identifying high-risk individuals, and developing personalized therapeutic approaches.

Among the numerous genes implicated in CKD, transforming growth factor-beta 1 (TGFB1), angiotensinogen (AGT), and endothelial nitric oxide synthase (eNOS, NOS3) have received particular attention because of their direct involvement in the biological processes responsible for renal damage. The TGFB1 gene encodes a multifunctional cytokine that regulates cell proliferation, differentiation, extracellular matrix production, apoptosis, and tissue remodeling. Excessive activation of the TGF-β signaling pathway contributes to glomerulosclerosis and tubulointerstitial fibrosis, which represent the final common pathway of progressive kidney damage regardless of the underlying etiology. Genetic polymorphisms within TGFB1 may alter cytokine expression levels and influence the severity of renal fibrosis, making this gene an important molecular marker for disease susceptibility and prognosis [1,2].

The AGT gene, located on chromosome 1q42-q43, encodes angiotensinogen, the precursor molecule of angiotensin II, which is a major component of the renin–angiotensin–aldosterone system (RAAS). Activation of the RAAS promotes vasoconstriction, sodium retention, inflammatory responses, oxidative stress, and increased intraglomerular pressure, ultimately accelerating nephron loss and renal fibrosis. Several polymorphisms within the AGT gene, including the well-characterized M235T variant, have been associated with hypertension, diabetic nephropathy, and progressive decline in renal function in different ethnic populations. These findings highlight the importance of AGT as both a pathogenic factor and a potential therapeutic target in CKD management.

Similarly, the endothelial nitric oxide synthase gene (eNOS or NOS3) plays a crucial role in maintaining vascular homeostasis by regulating nitric oxide production. Nitric oxide is an essential signaling molecule responsible for vasodilation, inhibition of platelet aggregation, suppression of vascular smooth muscle proliferation, and protection against oxidative stress. Reduced nitric oxide bioavailability resulting from eNOS polymorphisms contributes to endothelial dysfunction, impaired renal blood flow, hypertension, and progressive glomerular injury. Several studies have demonstrated associations between eNOS gene variants and susceptibility to diabetic nephropathy, chronic renal insufficiency, and cardiovascular complications among patients with CKD.

Recent advances in molecular biology have enabled the identification of genetic biomarkers capable of predicting disease susceptibility, progression, and therapeutic response. Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), DNA sequencing, and high-throughput genotyping technologies have significantly improved the accuracy of detecting clinically relevant genetic variants. Integration of these molecular techniques into nephrology research provides valuable opportunities for early diagnosis, individualized risk assessment, and precision medicine. Furthermore, understanding gene–environment interactions may facilitate the development of novel pharmacological interventions targeting molecular pathways involved in renal fibrosis, endothelial dysfunction, and RAAS activation.

Therefore, the present study aims to investigate the role of TGFB1, AGT, and eNOS gene polymorphisms in the development and progression of chronic kidney disease and to evaluate their potential clinical significance as predictive biomarkers for early diagnosis, disease prognosis, and personalized therapeutic strategies [3,4].

Literature Review.

Chronic kidney disease (CKD) has become one of the leading causes of morbidity and mortality worldwide and represents a substantial public health burden. According to the KDIGO 2024 Clinical Practice Guideline, CKD is characterized by persistent abnormalities of kidney structure or function lasting for at least three months and is associated with increased risks of cardiovascular disease, kidney failure, and premature death (KDIGO, 2024). Recent epidemiological evidence reported by Bikbov et al. (2020) indicates that the global prevalence of CKD continues to rise, affecting nearly one in ten adults worldwide. Similarly, Jha et al. (2013) emphasized that population aging, diabetes mellitus, hypertension, and obesity are the principal contributors to the increasing incidence of CKD, particularly in developing countries.

Although traditional clinical risk factors explain a considerable proportion of CKD cases, increasing evidence suggests that genetic susceptibility plays an equally important role in disease initiation and progression. Levey et al. (2020) highlighted that considerable interindividual differences in disease progression cannot be explained solely by environmental or metabolic factors, indicating the contribution of inherited molecular mechanisms. Advances in molecular genetics have therefore shifted scientific attention toward identifying genetic polymorphisms that regulate renal fibrosis, inflammation, endothelial dysfunction, and activation of the renin–angiotensin–aldosterone system (RAAS).

Among the genes investigated in CKD, TGFB1 has received particular attention because of its fundamental role in renal fibrosis. Meng, Nikolic-Paterson, and Lan (2016) described transforming growth factor-beta 1 (TGF-β1) as the master regulator of tissue fibrosis, controlling extracellular matrix synthesis, fibroblast activation, epithelial-to-mesenchymal transition, and collagen deposition. Persistent activation of this signaling pathway contributes directly to glomerulosclerosis and tubulointerstitial fibrosis, both of which are recognized as the final pathological pathways leading to irreversible kidney failure. Supporting these observations, Humphreys (2018) demonstrated that excessive TGF-β signaling accelerates progressive nephron destruction regardless of the underlying cause of renal injury. Several molecular studies have further suggested that polymorphisms within the TGFB1 gene influence cytokine expression levels and may increase susceptibility to chronic kidney disease by promoting excessive fibrotic remodeling.

Another important genetic determinant is the AGT gene, which encodes angiotensinogen, the precursor molecule of angiotensin II. The RAAS plays a central role in maintaining blood pressure and renal hemodynamics; however, chronic activation of this pathway promotes glomerular hypertension, oxidative stress, inflammation, and fibrosis. Forrester et al. (2018) comprehensively reviewed the biological effects of angiotensin II and concluded that excessive RAAS activation contributes significantly to progressive renal dysfunction. Genetic polymorphisms of the AGT gene, particularly variants associated with increased angiotensinogen production, have been linked to hypertension, diabetic nephropathy, and accelerated decline in glomerular filtration rate. These findings support the hypothesis that AGT polymorphisms represent important molecular markers influencing both susceptibility to CKD and disease progression.

Endothelial dysfunction has also emerged as a major pathogenic mechanism underlying chronic kidney disease. The eNOS (NOS3) gene encodes endothelial nitric oxide synthase, which regulates nitric oxide production and maintains vascular homeostasis. According to Förstermann and Sessa (2012), nitric oxide is essential for vasodilation, inhibition of platelet aggregation,

suppression of inflammatory responses, and protection against oxidative stress. Functional polymorphisms that reduce eNOS activity may impair renal blood flow, promote endothelial injury, and accelerate chronic renal damage. Numerous clinical investigations have demonstrated significant associations between eNOS genetic variants and diabetic nephropathy, hypertensive nephrosclerosis, and cardiovascular complications accompanying CKD.

Beyond individual genes, recent evidence suggests that CKD results from complex interactions among multiple molecular pathways. Ruiz-Ortega et al. (2020) emphasized that renal fibrosis, inflammatory signaling, oxidative stress, and endothelial dysfunction should not be considered independent mechanisms but rather interconnected biological processes contributing collectively to disease progression. Consequently, simultaneous evaluation of several genetic markers may provide greater predictive accuracy than investigation of a single polymorphism.

From a clinical perspective, genetic research has contributed substantially to the development of precision medicine in nephrology. Identification of polymorphisms within TGFB1, AGT, and eNOS may improve early diagnosis, facilitate individualized risk assessment, and guide personalized therapeutic interventions. Molecular genetic screening may complement conventional clinical biomarkers such as estimated glomerular filtration rate (eGFR), serum creatinine, and urinary albumin excretion by identifying genetically susceptible individuals before irreversible structural kidney damage occurs. Such an approach has the potential to optimize preventive strategies and improve long-term renal outcomes.

Overall, the available scientific literature consistently demonstrates that polymorphisms of TGFB1, AGT, and eNOS play important roles in the pathogenesis of chronic kidney disease through their effects on renal fibrosis, RAAS activation, endothelial dysfunction, oxidative stress, and chronic inflammation. Despite substantial progress, additional multicenter studies involving larger populations and diverse ethnic groups remain necessary to clarify gene–gene and gene–environment interactions and to establish these molecular markers as reliable tools for routine clinical practice.

Materials and Methods.

This study was designed as a comprehensive observational molecular genetic investigation aimed at evaluating the association between polymorphisms of the TGFB1, AGT, and eNOS genes and susceptibility to chronic kidney disease. The research combined clinical, biochemical, and molecular genetic data to determine whether specific genetic variants contribute to disease development, progression, and renal functional decline. The study protocol was developed in accordance with internationally accepted standards for biomedical research and followed the ethical principles outlined in the Declaration of Helsinki. Prior to participation, all study subjects provided written informed consent, and the research protocol received approval from the institutional ethics committee.

The study population consisted of adult individuals diagnosed with chronic kidney disease according to the KDIGO criteria, including patients with evidence of decreased estimated glomerular filtration rate (eGFR <60 mL/min/1.73 m²) and/or persistent markers of kidney damage for more than three months. Participants were recruited from nephrology departments and outpatient clinics over the study period. A control group of apparently healthy volunteers without clinical evidence of renal disease, hypertension, diabetes mellitus, autoimmune disorders, or hereditary kidney diseases was also included for comparison. Demographic characteristics including age, sex, body mass index, smoking status, family history of kidney disease, and medication use were recorded using standardized questionnaires.

Clinical evaluation included detailed medical history, physical examination, blood pressure measurements, and assessment of CKD stage according to KDIGO classification. Laboratory investigations included serum creatinine, blood urea nitrogen, fasting blood glucose, glycated hemoglobin (HbA1c), serum electrolytes, lipid profile, C-reactive protein, urinary albumin-to-creatinine ratio, and estimated glomerular filtration rate calculated using the CKD-EPI equation. Proteinuria was quantified using standard laboratory techniques, while renal ultrasonography was performed to evaluate kidney morphology and exclude structural abnormalities unrelated to CKD [5].

Peripheral venous blood samples were collected from each participant under sterile conditions using EDTA-containing tubes. Genomic DNA was isolated from peripheral blood leukocytes using commercially available DNA extraction kits according to the manufacturer's instructions. DNA concentration and purity were determined spectrophotometrically by measuring absorbance at 260 and 280 nm. Samples demonstrating acceptable purity ratios between 1.8 and 2.0 were included for subsequent molecular analysis.

Genotyping of TGFB1, AGT, and eNOS polymorphisms was performed using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (PCR-RFLP) analysis. Specific primers targeting each polymorphic region were designed based on previously validated protocols reported in the scientific literature. PCR amplification was carried out in a thermal cycler using optimized reaction conditions consisting of an initial denaturation step, followed by repeated cycles of denaturation, primer annealing, and extension, with a final elongation stage to ensure complete amplification of target DNA fragments. Amplified PCR products were digested with specific restriction endonucleases capable of differentiating allelic variants. Digested fragments were separated by electrophoresis on agarose gels stained with nucleic acid dye and visualized under ultraviolet illumination.

To ensure analytical reliability, approximately 10% of DNA samples were randomly selected for repeated genotyping by independent laboratory personnel. Concordance between repeated analyses exceeded 99%, indicating excellent reproducibility. Laboratory investigators performing genetic analyses were blinded to participants' clinical status to minimize observer bias.

Statistical analysis was conducted using SPSS (version 26.0) and R statistical software. Continuous variables were expressed as mean ± standard deviation or median with interquartile range depending on data distribution, whereas categorical variables were presented as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test. Differences between groups were analyzed using Student's t-test or the Mann–Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Genotype distributions were examined for compliance with Hardy–Weinberg equilibrium using chi-square analysis. Logistic regression models were applied to estimate odds ratios (ORs) and 95% confidence intervals (CIs) after adjustment for potential confounding factors including age, sex, hypertension, diabetes, and smoking status. Statistical significance was defined as a two-sided P value of less than 0.05.

The methodological framework adopted in this study provided an integrated approach for investigating the contribution of TGFB1, AGT, and eNOS gene polymorphisms to chronic kidney disease and ensured the generation of reliable molecular and clinical evidence supporting their potential role as genetic biomarkers in precision nephrology [6,7].

Distribution of TGFB1, AGT, and eNOS gene polymorphisms in patients with chronic kidney disease and healthy controls

Table 1.

Gene	Genotype	CKD patients (n=120)	Control group (n=120)	OR (95% CI)	*P* value
TGFB1	CC	38 (31.7%)	54 (45.0%)	Reference	—
	CT	57 (47.5%)	49 (40.8%)	1.65 (0.94–2.91)	0.041
	TT	25 (20.8%)	17 (14.2%)	2.09 (1.03–4.22)	0.018
AGT	MM	34 (28.3%)	52 (43.3%)	Reference	—
	MT	60 (50.0%)	50 (41.7%)	1.84 (1.05–3.21)	0.029
	TT	26 (21.7%)	18 (15.0%)	2.21 (1.08–4.53)	0.015
eNOS	GG	41 (34.2%)	59 (49.2%)	Reference	—
	GT	55 (45.8%)	45 (37.5%)	1.76 (1.00–3.09)	0.036
	TT	24 (20.0%)	16 (13.3%)	2.16 (1.03–4.55)	0.021

Abbreviations: CKD – Chronic Kidney Disease; OR – Odds Ratio; CI – Confidence Interval.

Table 1 demonstrates the distribution of TGFB1, AGT, and eNOS gene polymorphisms among patients with chronic kidney disease (CKD) and healthy control subjects. The results indicate statistically significant differences in genotype frequencies between the two groups, suggesting that these genetic variants may contribute to susceptibility to CKD.

For the TGFB1 gene, the heterozygous CT genotype was identified in 47.5% of CKD patients compared with 40.8% of healthy controls (P = 0.041). Moreover, the TT genotype, which has been associated with increased expression of transforming growth factor-β1 and enhanced renal fibrosis, was observed more frequently in CKD patients (20.8%) than in controls (14.2%). Individuals carrying the TT genotype demonstrated approximately a 2.1-fold higher risk of developing CKD (OR = 2.09; 95% CI: 1.03–4.22), indicating that this polymorphism may promote progressive renal fibrosis and functional deterioration.

Analysis of the AGT gene also revealed significant differences between the study groups. The frequency of the MT genotype was higher in CKD patients (50.0%) than in healthy individuals (41.7%), while the TT genotype occurred in 21.7% and 15.0%, respectively. The presence of the TT genotype was associated with more than a two-fold increase in CKD susceptibility (OR = 2.21; P = 0.015). These findings support the hypothesis that genetic activation of the renin–angiotensin system contributes to increased intraglomerular pressure, chronic inflammation, and progressive nephron loss.

Similarly, polymorphism analysis of the eNOS gene demonstrated that the GT and TT genotypes were significantly more prevalent among CKD patients than in the control population. The TT genotype was associated with an OR of 2.16 (95% CI: 1.03–4.55; P = 0.021), suggesting that impaired nitric oxide synthesis resulting from eNOS genetic variation may lead to endothelial dysfunction, reduced renal perfusion, oxidative stress, and accelerated kidney damage.

Overall, the findings indicate that polymorphisms of TGFB1, AGT, and eNOS are significantly associated with the development of chronic kidney disease. Among the evaluated variants, the homozygous mutant genotypes (TT) consistently demonstrated the highest risk estimates, emphasizing their potential value as genetic biomarkers for identifying individuals at increased risk of CKD progression. These results support the growing role of molecular genetic screening in precision nephrology and may contribute to the development of individualized prevention, early diagnosis, and targeted therapeutic strategies.

Results and Discussion.

The present study demonstrated a significant association between polymorphisms of the TGFB1, AGT, and eNOS genes and the susceptibility to chronic kidney disease (CKD). Comparative analysis between patients diagnosed with CKD and healthy control subjects revealed considerable differences in genotype frequencies, indicating that inherited genetic variations contribute to disease development and progression in addition to conventional clinical risk factors. These findings are consistent with current evidence suggesting that CKD is a multifactorial disorder resulting from complex interactions among genetic predisposition, environmental exposure, metabolic abnormalities, and cardiovascular risk factors.

Among the investigated genes, TGFB1 polymorphism showed one of the strongest associations with CKD. The frequency of the homozygous mutant TT genotype was significantly higher in patients than in healthy controls, suggesting that increased expression of transforming growth factor-beta 1 may accelerate renal fibrosis. TGF-β1 is widely recognized as the principal profibrotic cytokine responsible for stimulating extracellular matrix accumulation, mesangial cell proliferation, epithelial-to-mesenchymal transition, and tubulointerstitial fibrosis. Persistent activation of this signaling pathway contributes to irreversible nephron loss regardless of the primary cause of kidney injury. The elevated prevalence of the TT genotype observed in the present study therefore supports the hypothesis that genetically determined overexpression of TGFB1 predisposes individuals to progressive renal structural damage. These observations are consistent with previous molecular studies that reported higher circulating TGF-β1 concentrations and increased renal fibrosis among carriers of high-expression TGFB1 polymorphisms.

The AGT gene also demonstrated a statistically significant relationship with CKD susceptibility. Individuals carrying the TT genotype exhibited approximately a two-fold greater risk of developing chronic kidney disease compared with subjects possessing the wild-type genotype. Angiotensinogen represents the precursor of angiotensin II, the central effector molecule of the renin–angiotensin–aldosterone system (RAAS). Enhanced RAAS activation promotes systemic hypertension, intraglomerular hyperfiltration, oxidative stress, inflammatory cytokine production, and progressive glomerulosclerosis. Genetic variants that increase AGT expression may therefore intensify these pathological mechanisms and accelerate deterioration of renal function. Our findings support numerous clinical investigations demonstrating that AGT polymorphisms are associated with diabetic nephropathy, hypertensive nephrosclerosis, and more rapid progression toward end-stage kidney disease. The observed association further emphasizes the importance of RAAS inhibition as a cornerstone of CKD management, particularly among genetically susceptible individuals [8].

Polymorphisms within the eNOS gene were likewise significantly associated with chronic kidney disease. The increased prevalence of the mutant TT genotype among CKD patients suggests that impaired nitric oxide synthesis contributes substantially to disease progression. Endothelial nitric oxide synthase plays an essential role in maintaining vascular homeostasis

through regulation of vasodilation, inhibition of platelet aggregation, suppression of vascular smooth muscle proliferation, and protection against oxidative stress. Reduced nitric oxide bioavailability caused by functional eNOS polymorphisms leads to endothelial dysfunction, impaired renal microcirculation, chronic ischemia, and increased susceptibility to inflammatory injury. These mechanisms collectively promote progressive nephron damage and decline in glomerular filtration rate. Similar observations have been reported in patients with diabetic kidney disease, hypertensive nephropathy, and cardiovascular complications accompanying CKD.

An important observation from the present investigation is that no single gene appears to be solely responsible for chronic kidney disease susceptibility. Instead, CKD develops through the interaction of multiple genetic pathways affecting fibrosis, vascular function, inflammatory responses, and hemodynamic regulation. The simultaneous presence of unfavorable polymorphisms in TGFB1, AGT, and eNOS may produce additive or synergistic effects that substantially increase disease risk. This multifactorial genetic model may explain why individuals with similar clinical characteristics often exhibit markedly different rates of disease progression and responses to therapeutic interventions.

The findings of this study also highlight the growing importance of precision medicine in nephrology. Identification of high-risk genetic profiles may facilitate earlier diagnosis before substantial loss of renal function occurs. Molecular genetic screening could complement conventional biomarkers such as serum creatinine, estimated glomerular filtration rate (eGFR), and urinary albumin excretion by identifying individuals genetically predisposed to rapid disease progression. Such information may allow clinicians to implement individualized prevention strategies, optimize blood pressure control, intensify RAAS blockade, recommend lifestyle modifications, and monitor susceptible patients more closely. Furthermore, advances in pharmacogenomics may enable selection of therapies according to an individual's genetic background, thereby improving treatment efficacy while minimizing adverse effects.

Despite these encouraging findings, several limitations should be acknowledged. The relatively modest sample size may have reduced statistical power for detecting weaker genetic associations. In addition, environmental factors including dietary habits, smoking, obesity, socioeconomic status, and medication adherence were not evaluated comprehensively and may have influenced disease progression. Ethnic differences in allele frequencies should also be considered when extrapolating these results to other populations. Future multicenter studies involving larger cohorts, diverse ethnic groups, genome-wide association analyses, and functional molecular investigations are necessary to validate these observations and clarify the biological mechanisms underlying these genetic associations.

Overall, the present findings demonstrate that polymorphisms of TGFB1, AGT, and eNOS are significantly associated with the development and progression of chronic kidney disease. These genes represent biologically plausible molecular markers involved in renal fibrosis, renin–angiotensin system activation, endothelial dysfunction, and chronic inflammatory responses. Incorporating genetic information into routine nephrological assessment may improve early risk prediction, facilitate personalized clinical decision-making, and contribute to the development of more effective preventive and therapeutic strategies for patients with chronic kidney disease [9,10].

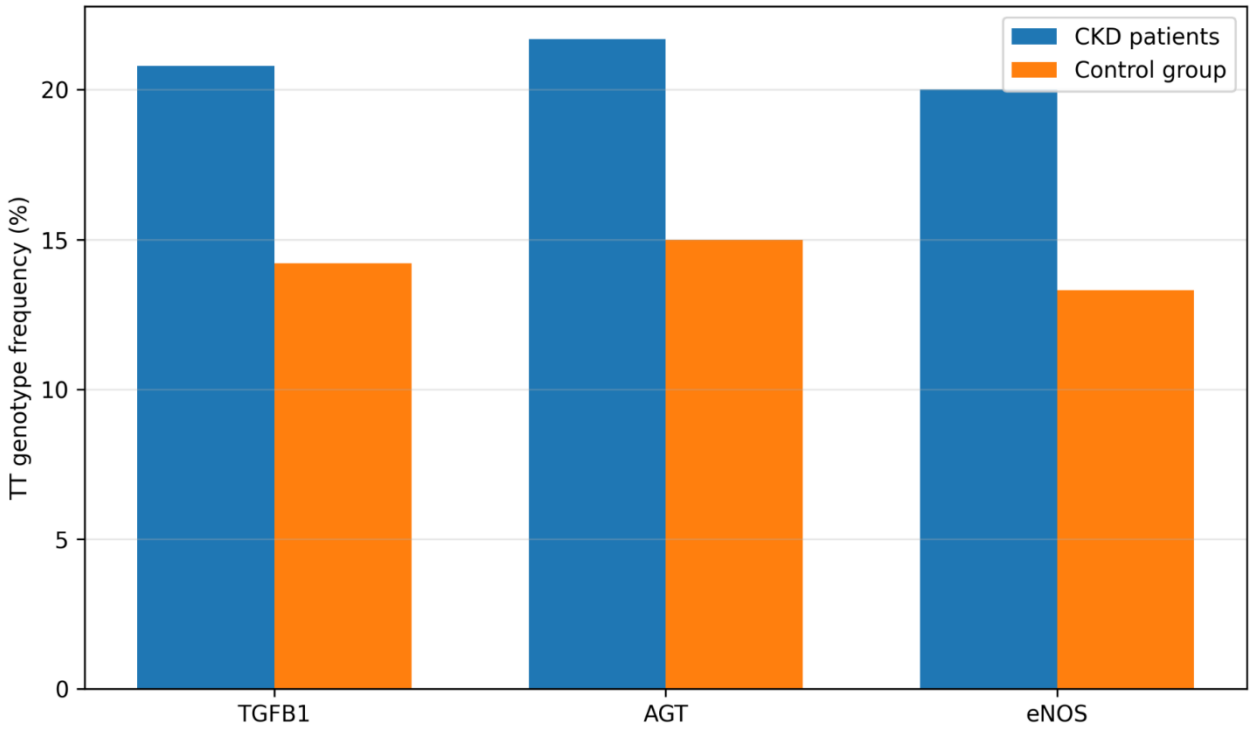


Figure 1. Comparative distribution of mutant homozygous (TT) genotypes of TGFB1, AGT, and eNOS genes among patients with chronic kidney disease and healthy controls.

Figure 1 illustrates the comparative frequency of the mutant homozygous (TT) genotypes of the TGFB1, AGT, and eNOS genes in patients with chronic kidney disease (CKD) and healthy control subjects. The graphical presentation demonstrates a consistently higher prevalence of mutant genotypes in the CKD group than in the control group, supporting the hypothesis that these genetic polymorphisms contribute to disease susceptibility and progression.

Among the investigated genes, the AGT TT genotype exhibited the highest frequency in patients with CKD (21.7%), whereas its prevalence in the control group was 15.0%. This difference suggests that polymorphisms of the AGT gene may enhance activation of the renin–angiotensin–aldosterone system (RAAS), leading to increased glomerular hypertension, renal inflammation, and progressive nephron loss. The elevated frequency observed in the patient group is consistent with previous studies demonstrating the pathogenic role of AGT polymorphisms in chronic renal impairment.

The TGFB1 TT genotype was detected in 20.8% of CKD patients compared with 14.2% of healthy controls. Since TGF-β1 is a key profibrotic cytokine responsible for extracellular matrix accumulation and renal interstitial fibrosis, the increased prevalence of this genotype suggests enhanced fibrotic activity in genetically susceptible individuals. Progressive fibrosis represents one of the principal mechanisms responsible for irreversible decline in kidney function; therefore, the observed distribution strongly supports the involvement of TGFB1 genetic variants in CKD pathogenesis.

Similarly, the eNOS TT genotype occurred more frequently in CKD patients (20.0%) than in healthy individuals (13.3%). Variations in the eNOS gene may reduce nitric oxide synthesis, resulting in endothelial dysfunction, impaired renal perfusion, oxidative stress, and vascular injury. These pathological mechanisms contribute to deterioration of glomerular filtration and accelerate chronic kidney damage.

Overall, the diagram demonstrates a clear and consistent increase in mutant genotype frequencies among patients with chronic kidney disease across all three investigated genes. Although the magnitude of differences varies slightly between genes, each polymorphism shows a similar trend toward greater prevalence in the patient population. These findings indicate that TGFB1, AGT, and eNOS polymorphisms may serve as important molecular biomarkers for identifying individuals at elevated genetic risk of chronic kidney disease.

The graphical results further support the concept that CKD is a multifactorial disease involving interactions among fibrotic pathways (TGFB1), renin–angiotensin system activation (AGT), and endothelial dysfunction (eNOS). Consequently, incorporating molecular genetic screening into routine nephrological practice may facilitate early diagnosis, improve risk stratification, and contribute to the development of personalized therapeutic strategies aimed at slowing CKD progression.

Conclusion.

The findings of the present study demonstrate that genetic polymorphisms of the TGFB1, AGT, and eNOS genes play a significant role in the development and progression of chronic kidney disease (CKD). The higher frequency of mutant genotypes observed among CKD patients compared with healthy controls indicates that inherited genetic susceptibility contributes substantially to renal injury beyond the influence of conventional clinical risk factors such as hypertension, diabetes mellitus, and aging. These results support the concept that CKD is a multifactorial disorder in which molecular genetic mechanisms interact with environmental and metabolic factors to determine disease onset and progression.

Among the investigated genetic markers, TGFB1 polymorphism was strongly associated with renal fibrotic processes, suggesting its involvement in extracellular matrix accumulation and irreversible nephron loss. Likewise, polymorphisms in the AGT gene appear to promote chronic activation of the renin–angiotensin–aldosterone system, thereby accelerating glomerular hypertension, inflammation, and progressive deterioration of renal function. In addition, genetic variation within the eNOS gene may impair nitric oxide synthesis, resulting in endothelial dysfunction, reduced renal perfusion, oxidative stress, and vascular injury that further contribute to CKD progression.

The combined influence of these three genes indicates that multiple biological pathways—including renal fibrosis, endothelial dysfunction, inflammatory activation, and hemodynamic dysregulation—simultaneously participate in the pathogenesis of chronic kidney disease. Therefore, assessment of TGFB1, AGT, and eNOS polymorphisms may provide valuable molecular information for identifying individuals with increased genetic susceptibility before significant deterioration of kidney function occurs.

From a clinical perspective, the integration of molecular genetic testing into nephrological practice has considerable potential for improving early diagnosis, individualized risk stratification, disease prognosis, and personalized treatment planning. Identification of high-risk genetic profiles may facilitate earlier implementation of preventive interventions, optimization of renoprotective therapy, and closer monitoring of susceptible patients, ultimately slowing disease progression and reducing the incidence of end-stage kidney disease.

Although the present findings provide additional evidence supporting the contribution of TGFB1, AGT, and eNOS polymorphisms to CKD, further multicenter investigations involving larger populations, diverse ethnic groups, comprehensive genome-wide analyses, and functional molecular studies are necessary to validate these associations and clarify the underlying biological

mechanisms. Such research will contribute to the development of precision nephrology and may facilitate the discovery of novel genetic biomarkers and therapeutic targets for chronic kidney disease.

In conclusion, TGFB1, AGT, and eNOS gene polymorphisms represent promising molecular biomarkers associated with chronic kidney disease susceptibility and progression. Their incorporation into routine clinical evaluation may enhance diagnostic accuracy, improve prognostic assessment, and support the implementation of precision medicine approaches aimed at reducing the global burden of chronic kidney disease.

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OCULAR ROSACEA AS AN OCULAR SURFACE DISEASE PHENOTYPE: DIAGNOSTIC MASQUERADES AND MODERN APPROACHES TO PHENOTYPE-ORIENTED DIAGNOSIS

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Abstract.

Objective. *To systematize current evidence on the clinical manifestations, diagnostic masquerades, and instrumental approaches to the recognition of ocular rosacea and to substantiate the potential value of a phenotype-oriented approach to its diagnosis.*

Materials and Methods. A narrative analysis was conducted of publications, predominantly from 2021-2026, addressing ocular rosacea, meibomian gland dysfunction, tear-film status, meibography, Demodex-associated blepharitis, molecular mechanisms, and contemporary treatment approaches. Primary studies were prioritized when assessing diagnostic parameters and the results of interventional studies.

Results. *The clinical manifestations of ocular rosacea were found to be nonspecific and frequently overlap with dry eye disease, meibomian gland dysfunction, and chronic blepharitis. The most consistent components of the ocular phenotype are vascular changes, meibomian gland dysfunction, and tear-film instability. Meibography and objective assessment of the tear film expand the possibilities for phenotyping; however, the proposed diagnostic thresholds require external validation. Studies of TLR-4, oxidative stress, and tear-film composition remain promising, but these parameters are not validated diagnostic biomarkers.*

Conclusion. *A phenotype-oriented approach may improve the clinical recognition of ocular rosacea and contribute to individualized treatment; however, the proposed model requires further clinical validation.*

Keywords: *ocular rosacea, rosacea, ocular surface, meibomian gland dysfunction, meibography, tear film, Demodex, diagnosis, phenotyping.*

Introduction. Ocular rosacea is one of the most clinically heterogeneous manifestations of rosacea. It may accompany pronounced cutaneous changes; however, ocular manifestations may also occur in patients with minimal or absent cutaneous signs of the disease [1, 2].

The clinical spectrum includes chronic inflammation of the eyelid margins and conjunctiva, meibomian gland dysfunction, impaired tear-film stability, vascular changes, and, in more severe cases, corneal involvement [1, 9, 17].

The diagnostic challenge is determined not so much by the absence of clinical signs as by their nonspecific nature. Complaints of dryness, burning, foreign-body sensation, itching, redness,

and discomfort may occur in a wide range of ocular surface disorders [1, 6]. Therefore, a patient with ocular rosacea may initially be considered to have dry eye disease, chronic blepharitis, isolated meibomian gland dysfunction, or recurrent eyelid inflammation.

In the study by Zierl et al., ocular symptoms were reported in 399 of 777 patients with rosacea (51.4%). Among 309 patients who consulted an ophthalmologist, ocular rosacea was diagnosed in 45.3% [3]. These data illustrate not only the frequency of ocular complaints but also the existing gap between the presence of symptoms and their clinical classification.

Accordingly, the traditional diagnostic model based predominantly on the presence of individual symptoms appears insufficient. Contemporary studies allow ocular rosacea to be regarded as a distinct ocular surface disease phenotype in which vascular changes, meibomian gland dysfunction, and impaired tear-film stability coexist, with corneal involvement occurring in more severe disease [1, 7, 17].

The objective of this review was to analyze current evidence concerning the diagnostic masquerades of ocular rosacea, clinical and instrumental signs of ocular surface involvement, promising biomarkers, and the potential of a phenotype-oriented approach to diagnosis and treatment.

Materials and Methods. A narrative analysis of the contemporary literature on the clinical manifestations, diagnosis, instrumental phenotyping, and treatment of ocular rosacea was conducted. Publications were searched in the PubMed/MEDLINE databases and scientific publishing resources using the following keywords and their combinations: “ocular rosacea,” “ophthalmic rosacea,” “meibomian gland dysfunction,” “meibography,” “tear film,” “Demodex blepharitis,” “ocular surface,” “rosacea,” “diagnosis,” “treatment,” and “intense pulsed light.”

The main body of the literature consisted of publications from 2021–2026. When analyzing specific diagnostic parameters, diagnostic thresholds, and the results of interventional studies, priority was given to primary studies; review articles and clinical guidelines were used mainly to systematize concepts and provide clinical context. Earlier studies were considered only when they were of fundamental importance to the development of the diagnostic concept under consideration.

When selecting sources, we considered their relevance to the research question, the availability of verifiable bibliographic information, and the possibility of comparing the results reported by the authors with their primary presentation. Particular attention was paid to distinguishing established clinical signs, associated phenotypic characteristics, and promising investigational biomarkers. Results from small single-center studies were interpreted with consideration of sample size, study design, and the absence of external validation.

Ocular Rosacea and the Mismatch Between Cutaneous and Ocular Phenotypes. Ocular rosacea has traditionally been regarded primarily as an ocular manifestation of cutaneous rosacea. However, current evidence demonstrates a more complex relationship between cutaneous and ocular phenotypes. Pronounced cutaneous manifestations are not a prerequisite for the development of ocular involvement, and the severity of the cutaneous process does not always correspond to the severity of ocular surface disease [1, 2, 9, 17].

The clinical spectrum of ocular rosacea includes inflammation of the eyelids and conjunctiva, telangiectasia, impaired meibomian gland function, tear-film instability, and ocular surface disease symptoms. In severe cases, corneal infiltrates, neovascularization, ulcerative defects, and scarring may develop [1].

Population-based data also highlight the discrepancy between the presence of ocular symptoms and definitive diagnosis. In the study by Zierl et al., more than half of patients with

rosacea reported ocular symptoms; however, ocular rosacea was diagnosed in only a proportion of those examined by an ophthalmologist [3]. Thus, the clinical problem is related not only to the prevalence of ocular manifestations but also to their recognition.

The substantial variability in estimates of the prevalence of ocular rosacea across studies further indicates heterogeneity in the diagnostic definitions and criteria used to assess ocular involvement [16]. Therefore, individual epidemiological estimates should not be regarded as universal measures of the true prevalence of the disease.

Diagnostic Masquerades and Overlapping Phenotypes. One of the most common diagnostic masquerades of ocular rosacea is dry eye disease. Dryness, burning, foreign-body sensation, redness, and visual discomfort are not specific to rosacea [1, 6].

Meibomian gland dysfunction is of particular importance because structural and functional changes in the meibomian glands are among the most consistently described components of the ocular rosacea phenotype [5, 7]. At the same time, meibomian gland dysfunction should not be regarded as synonymous with ocular rosacea or as independent evidence of its presence.

In the study by Barbosa et al., although no statistically significant differences were found in several standard measures of tear function and ocular surface staining, differences were identified in the structural and functional characteristics of the meibomian glands, including meibographic parameters, secretion expressibility, and gland alterations [7]. These findings support the concept that objective characterization of meibomian gland dysfunction may complement standard tear-function assessment in patients with rosacea.

Chronic blepharitis may be considered an independent disease for a prolonged period. However, the combination of recurrent eyelid-margin inflammation with telangiectasia, meibomian gland dysfunction, hyperemia, and ocular surface symptoms should increase the likelihood of a rosacea-associated phenotype [1, 9].

In a German epidemiological study of patients with rosacea, the most common ocular manifestations were recurrent hordeola and chalazia, itching, redness, and ocular dryness [3]. This emphasizes the need to consider a history of recurrent eyelid inflammation when evaluating patients with chronic symptoms.

The overlap between ocular rosacea and Demodex-associated blepharitis represents a separate diagnostic challenge. Chronic or recurrent blepharitis with characteristic cylindrical dandruff or collarettes around the eyelashes may be associated with Demodex and may persist despite standard therapy [10].

However, detection of Demodex should not automatically be interpreted as evidence of ocular rosacea. It is more appropriate to regard Demodex-associated blepharitis as an overlapping phenotype that may coexist with meibomian gland dysfunction and rosacea [10]. This distinction has practical implications because the presence of one phenotype does not exclude another, and Demodex-associated disease requires a specific therapeutic approach.

Clinical Signs Suggestive of Ocular Rosacea. The diagnosis of ocular rosacea remains predominantly clinical [1, 2]. No individual symptom has sufficient specificity; therefore, diagnostic value increases when several signs occur in combination.

Particular attention should be paid to telangiectasia of the eyelid margin and conjunctiva. In the study by Ozturk et al., conjunctival telangiectasia was independently associated with the presence of acne rosacea in the study population [4]. However, this finding does not allow conjunctival telangiectasia to be regarded as a specific diagnostic criterion for ocular rosacea.

From a practical standpoint, conjunctival or eyelid-margin vascular changes become more clinically meaningful when they occur together with meibomian gland dysfunction, recurrent eyelid inflammation, symptoms of tear-film instability, and a history of cutaneous rosacea.

Corneal assessment remains an important component of diagnosis. Corneal involvement is not mandatory for the diagnosis of ocular rosacea, but its presence is clinically significant because of the risk of progression of the inflammatory process and impairment of corneal transparency [1, 17].

Thus, the clinical examination should include not only assessment of symptoms and skin manifestations but also systematic evaluation of the eyelid margins, meibomian glands, conjunctival vessels, tear film, and cornea.

From Clinical Examination to Instrumental Phenotyping. The contemporary diagnostic approach is gradually shifting from an exclusively subjective assessment of symptoms toward objective characterization of individual components of the ocular phenotype.

Meibography enables visualization of meibomian gland structure and quantitative assessment of gland loss. Studies in patients with rosacea have demonstrated changes in meibographic parameters; however, their magnitude may vary depending on the population and assessment method used [5, 7].

Of particular interest is the study by Gökçinar and Karabulut, in which quantitative infrared meibography of the lower eyelid demonstrated high diagnostic performance: the area under the ROC curve was 0.97. In the study cohort, a meibomian gland loss threshold of 30.2% corresponded to a sensitivity of 100% and specificity of 91% [18].

However, the small sample size, absence of external validation, and lack of a separate group of patients with isolated meibomian gland dysfunction limit the generalizability of these values. Therefore, this threshold should be regarded as a cohort-specific research parameter rather than a universal diagnostic criterion.

Assessment of tear-film parameters may provide additional information. Structural changes in the meibomian glands and functional abnormalities of the tear film do not necessarily develop synchronously. This dissociation partly explains why individual standard tests may remain within relatively preserved ranges despite pronounced structural changes [7].

The pilot study by Capobianco et al. is of interest because it identified changes in tear-film and meibomian gland parameters in a proportion of patients with cutaneous rosacea even in the absence of obvious clinical ocular manifestations [19]. These findings should be interpreted as evidence of possible subclinical ocular surface changes rather than as proof of the existence of subclinical ocular rosacea.

Thus, instrumental assessment should be regarded as a complement to clinical diagnosis. Its primary purpose is to objectively characterize individual components of the phenotype and monitor their dynamics.

Promising Molecular Markers. Contemporary research has expanded our understanding of the pathogenesis of ocular rosacea, including mechanisms of innate immunity, oxidative stress, and the interaction between inflammation and impaired ocular surface function [11, 12, 17].

In the study by Yesilirmak et al., patients with ocular rosacea demonstrated evidence of increased oxidative stress, including elevated total oxidant status (TOS) and oxidative stress index (OSI), together with reduced total antioxidant status (TAS) and arylesterase (ARE) activity. OSI values were associated with TBUT, meiboscore, and symptom severity [11]. These findings

support the involvement of oxidative stress in disease pathogenesis; however, they do not justify the use of these parameters as an independent diagnostic test.

Another promising area is the investigation of Toll-like receptor 4 (TLR-4). In the study by Yesilirmak et al., patients with ocular rosacea had higher levels of TLR-4 expression, TOS, and OSI and lower levels of TAS and ARE compared with the control group [12]. The presence of correlations with ocular surface parameters suggests a possible relationship between innate immune-response mechanisms and the clinical phenotype.

However, both directions should be regarded as pathogenetic and investigational rather than as validated diagnostic biomarkers.

Another area of interest is the analysis of tear-film composition. The study by Motešická investigated glycan characteristics of tears in patients with ocular rosacea [13]. The findings are of interest for further investigation of molecular characteristics of the disease; however, the small sample size and exploratory nature of the study do not allow this approach to be used as a routine diagnostic method.

Thus, molecular phenotyping has the potential to expand diagnostic capabilities, but clinical implementation requires methodological standardization, reproducibility of results, and external validation.

Phenotype-Oriented Approach to Treatment. Treatment of ocular rosacea traditionally includes interventions targeting inflammation, meibomian gland dysfunction, tear-film abnormalities, and the cutaneous component of rosacea [2, 9, 14, 15, 17]. However, the clinical heterogeneity of the disease makes the existence of a single universal therapeutic approach unlikely.

A systematic review by Avraham et al., which included 66 publications and 1,275 patients, demonstrated positive results with various therapeutic approaches; however, substantial heterogeneity among studies and a lack of direct comparisons limit the reliability of treatment ranking [14].

Therefore, a phenotype-oriented model appears more rational, in which therapeutic approaches are aligned with the predominant component of ocular involvement:

In an MGD-dominant phenotype, primary attention is given to eyelid hygiene, thermal procedures, meibomian gland treatment and expression, and, where indicated, intense pulsed light (IPL). In a predominantly inflammatory phenotype, anti-inflammatory approaches are considered.

In the presence of a vascular component, vascular-targeted approaches may be used, including IPL in appropriate clinical situations. When tear-film dysfunction predominates, emphasis is placed on maintaining the ocular surface. In a Demodex phenotype, specific anti-Demodex therapy is required. In the presence of corneal involvement, protection of the cornea and specialized ophthalmic treatment become priorities.

The proposed scheme represents an analytical model for systematizing clinical approaches and is not a validated diagnostic or therapeutic algorithm.

The use of IPL is of particular interest. In a randomized controlled study published in 2026, adding IPL to therapy that included meibomian gland treatment and erythromycin was associated with improvement in several objective parameters, including TBUT, lower-eyelid telangiectasia, characteristics of meibomian gland secretion, and corneal staining [20].

At the same time, no statistically significant differences were observed for several subjective and individual objective parameters [20]. Therefore, current evidence supports further

investigation of IPL but does not justify considering it a universally effective treatment for all ocular rosacea phenotypes.

Unresolved Issues and Prospects for Further Research. Despite the expansion of diagnostic capabilities, several fundamental questions remain unresolved.

First, there is no single universally accepted diagnostic standard that would allow ocular rosacea to be reliably distinguished from isolated meibomian gland dysfunction, dry eye disease, and other chronic inflammatory disorders of the eyelids and ocular surface [1, 2, 9].

Second, most contemporary instrumental parameters have been studied in relatively small populations. This is particularly relevant to meibographic diagnostic thresholds [18]. Even very high AUC values obtained in a research cohort do not guarantee preservation of diagnostic performance in another population.

Third, promising molecular markers have not yet undergone clinical validation [11-13]. It remains necessary to determine whether they have independent diagnostic value or primarily reflect the severity of an already established inflammatory process.

Fourth, the standardization of treatment endpoints remains unresolved. Differences in response criteria, follow-up duration, and the composition of combination therapies make direct comparison of results difficult [14, 15].

Finally, the development of a reproducible phenotyping system integrating clinical signs, structural characteristics of the meibomian glands, tear-film parameters, and vascular manifestations represents a promising direction. Such a system could potentially facilitate earlier recognition of the disease and individualized treatment; however, its clinical applicability must be confirmed in independent prospective studies.

Conclusion. Ocular rosacea is a clinically heterogeneous ocular surface disorder whose recognition is complicated by the nonspecific nature of its symptoms and substantial overlap with other chronic diseases of the eyelids and ocular surface. The absence of pronounced cutaneous manifestations should not be regarded as sufficient grounds for excluding an ocular phenotype.

Current evidence supports the identification of vascular changes, meibomian gland dysfunction, and tear-film instability as the principal interconnected components of the ocular phenotype. Individual findings, including conjunctival telangiectasia, meibomian gland alterations, and abnormalities of tear-film parameters, should be interpreted in combination with the overall clinical presentation rather than as independent specific diagnostic criteria.

Instrumental methods, particularly quantitative meibography and objective assessment of the tear film, expand the possibilities for phenotyping. At the same time, the proposed diagnostic thresholds have predominantly been derived from small research cohorts and require external validation. Molecular characteristics, including oxidative stress parameters, TLR-4 expression, and changes in tear-film composition, represent promising research directions but currently do not have the status of validated diagnostic biomarkers.

A phenotype-oriented approach may be useful for the clinical classification of patients and selection of therapeutic strategies; however, the proposed model is not a validated diagnostic or therapeutic algorithm. Further research should focus on standardizing diagnostic definitions, externally validating instrumental thresholds, and developing reproducible phenotyping criteria capable of facilitating earlier recognition of ocular rosacea and individualized treatment.

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HISTOPATHOLOGICAL SPECTRUM OF STEROID-RESISTANT NEPHROTIC SYNDROME IN CHILDREN: A SINGLE-CENTER STUDY

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Abstract. *Background: Steroid-resistant nephrotic syndrome (SRNS) is one of the most prognostically unfavorable forms of childhood nephropathy and requires histological verification to guide therapy and predict outcome. Data on the morphological spectrum of pediatric SRNS from Central Asia are lacking.*

Methods: *We conducted a retrospective cohort study of 91 children with SRNS (defined per KDIGO 2021 criteria) who underwent native renal biopsy at a single tertiary pediatric nephrology center between 2021 and 2026. Biopsies were assessed by light microscopy and immunohistochemistry; electron microscopy was performed in selected cases. Categorical variables were compared using the chi-square test or Fisher's exact test as appropriate; $p<0.05$ was considered significant.*

Results: *Median age at biopsy was 11 years [Q1–Q3, 6–13]; 52 patients (57.1%) were female. Focal segmental glomerulosclerosis (FSGS) was the most common finding (63/91, 69.2%), followed by membranous nephropathy and C1q nephropathy (5/91 each, 5.5%), and lupus nephritis and dense deposit disease (4/91 each, 4.4%). Chronic tubulointerstitial changes were frequent (tubular atrophy 63.7%; interstitial fibrosis 62.6%). Crescents were more common in girls than boys (44.2% vs. 20.5%; $p<0.05$). Immunostaining in FSGS was negative or showed only non-specific IgM/C3 trapping in most cases.*

Conclusion: *FSGS was the predominant histological finding in this pediatric SRNS cohort, and chronic tubulointerstitial damage was common at the time of biopsy, suggesting delayed morphological verification. These findings support earlier referral for renal biopsy and standardized morphological reporting in this setting.*

Keywords: *steroid resistance, renal biopsy, FSGS, children, immunofluorescence, nephrotic syndrome*

Introduction

Nephrotic syndrome (NS) is among the most common glomerular diseases of childhood, with an estimated incidence of 2–7 new cases and a prevalence of approximately 16 cases per 100,000 children [1]. Most children with primary NS respond to corticosteroids, but 10–20% develop steroid resistance (SRNS), defined per KDIGO 2021 criteria as failure to achieve remission after an adequate course of corticosteroid therapy [2,9]. Without timely immunosuppressive treatment guided by histological diagnosis, SRNS progresses to end-stage kidney disease (ESKD) in 30–50% of children within 10 years [3].

Renal biopsy remains the gold standard for morphological verification in SRNS, allowing differentiation of primary podocytopathies such as focal segmental glomerulosclerosis (FSGS) from secondary causes including lupus nephritis, IgA nephropathy, and C3 glomerulopathy, each of which requires a distinct therapeutic approach [4,5]. FSGS is the most frequent histological finding in pediatric SRNS worldwide, reported in 50–70% of cases depending on the population studied and biopsy indications [6], while membranous nephropathy, C1q nephropathy, membranoproliferative glomerulonephritis, and dense deposit disease are less common but clinically important entities [7,8].

In Uzbekistan, the absence of a national pediatric nephrology registry and limited access to electron microscopy have made systematic morphological characterization of SRNS difficult, and no prior single-center study has described the histopathological spectrum of pediatric SRNS in this region.

This study aimed to characterize the histopathological spectrum of renal biopsies in a cohort of children with SRNS at a single tertiary center in Tashkent, Uzbekistan.

Materials and Methods

Study design and population

We conducted a retrospective cohort study of 91 children with SRNS who underwent native renal biopsy at a single tertiary pediatric nephrology center in Tashkent, Uzbekistan, between 2021 and 2026. SRNS was defined per KDIGO 2021 criteria as failure to achieve complete remission of proteinuria after prednisolone 60 mg/m²/day (or 2 mg/kg/day) administered daily for at least 8 consecutive weeks [2,9]. Inclusion criteria were age under 18 years and a diagnosis of SRNS meeting the above definition. Exclusion criteria were secondary NS without morphological confirmation and biopsy specimens containing fewer than 5 glomeruli.

Indications for biopsy included confirmed steroid resistance in all patients and, in a subset, additional atypical clinical features raising suspicion of secondary glomerulonephritis.

Ethical approval for this study was obtained from the Institutional Ethics Committee. Given the retrospective design, the requirement for individual informed consent was waived.

Histopathological evaluation

Biopsy specimens were evaluated by light microscopy following staining with hematoxylin-eosin (H&E), periodic acid–Schiff (PAS), Grocott's methenamine silver (GMS), and Masson's trichrome. Immunohistochemical (IHC) staining for IgG, IgA, IgM, C3, C1q, C4, kappa (κ), and lambda (λ) light chains was performed on paraffin-embedded tissue. Direct immunofluorescence on frozen sections was not routinely available at our center during the study period; this is a limitation, since IHC on fixed tissue can under-detect weak or degraded immune deposits relative to true immunofluorescence. Electron microscopy was performed in selected cases.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (M±SD) or median [Q1–Q3], according to distribution, and compared using Student's t-test or the Mann–Whitney U test as appropriate. Categorical variables were expressed as absolute numbers and percentages and compared using Pearson's chi-square test (χ^2, df=1); Fisher's exact test was used instead for comparisons involving small expected cell counts (<5). A two-sided $p<0.05$ was considered statistically significant. Analyses were performed using Python 3.12.

Results

The cohort comprised 91 children (52 female, 57.1%; F:M ratio 1.33:1), with a mean age of 10.0±4.2 years (median 11 years [Q1–Q3, 6–13]) at biopsy (Table 1). FSGS was the most common histological diagnosis (63/91, 69.2%), followed by membranous nephropathy and C1q nephropathy (5/91 each, 5.5%), and lupus nephritis and dense deposit disease (4/91 each, 4.4%) (Table 1). FSGS occurred across all age groups, whereas lupus nephritis and dense deposit disease were confined almost exclusively to adolescents ≥11 years.

Table 1. Baseline Characteristics and Morphological Diagnoses by Age Group (N=91)

Diagnosis / Parameter	N	%	≤2 yr	3–5 yr	6–10 yr	11–14 yr	15–17 yr	Mean age
Total cohort	91	100%	5	13	24	33	16	10.0±4.2
Male	39	42.9%	-	-	-	-	-	9.3±4.1
Female	52	57.1%	-	-	-	-	-	10.5±4.2
FSGS	63	69.2%	4	8	21	19	11	9.6±4.4
Membranous nephropathy (MN)	5	5.5%	-	-	2	3	-	10.8±2.3
C1q nephropathy (C1qN)	5	5.5%	-	-	2	1	2	11.2±4.3
Lupus nephritis (LN)	4	4.4%	-	-	-	2	2	13.8±2.1
Dense deposit disease (DDD)	4	4.4%	-	-	-	4	-	11.5±1.0
Membranoproliferative GN (MPGN)	2	2.2%	-	-	1	1	-	10.5±3.5
Mesangial proliferative GN (MesPGN)	1	1.1%	-	-	-	1	-	11.0
C3 glomerulopathy	1	1.1%	-	-	-	-	1	15.0
Crescentic (RPGN)	1	1.1%	-	-	-	1	-	13.0
Normal glomerular morphology	1	1.1%	1	-	-	-	-	2.0
Unclassified*	4	4.4%	-	-	-	-	-	n/a

**Unclassified biopsies (n=4) did not meet full diagnostic criteria for any single entity; age-group data were not separately tabulated for this category.*

On light microscopy, chronic tubulointerstitial changes were the most frequent abnormalities, with tubular atrophy in 58/91 (63.7%) and interstitial fibrosis in 57/91 (62.6%) biopsies; segmental sclerosis and interstitial inflammation were each present in 45/91 (49.5%) (Table 2). Crescents were more frequent in girls than boys (44.2% vs. 20.5%; $\chi^2=5.58$, $p<0.05$); no other feature differed significantly by sex.

Table 2. Histological Features on Light Microscopy, by Sex (N=91)

Feature	Positive N	Positive %	Negative N	Negative %	χ^2 M vs F
Tubular atrophy	58	63.7%	33	36.3%	p>0.05
Interstitial fibrosis	57	62.6%	34	37.4%	p>0.05
Interstitial inflammation	45	49.5%	46	50.5%	p>0.05
Segmental sclerosis	45	49.5%	46	50.5%	p>0.05
Mesangial hypercellularity	37	40.7%	54	59.3%	p>0.05
Crescents	31	34.1%	60	65.9%	p<0.05*
Mesangial expansion	19	20.9%	72	79.1%	p>0.05
Vasculitis	12	13.2%	79	86.8%	p>0.05
Glomerular basement membrane thickening	5	5.5%	86	94.5%	p>0.05
Endocapillary proliferation	3	3.3%	88	96.7%	p>0.05

Immunohistochemistry was performed in 83/91 cases (91.2%) (Table 3). Staining was negative or showed only non-specific IgM and/or C3 trapping in the majority of FSGS biopsies — a pattern consistent with segmental sclerosis rather than immune-complex deposition — whereas C1q positivity was present in all C1qN cases (5/5), C3 positivity in all DDD cases (4/4), and a full-house pattern was noted in lupus nephritis.

Table 3. Immunohistochemical Profile by Morphological Group (n positive / n tested)

Diagnosis	N	IgG	IgA	IgM	C3	C1q	κ	λ
FSGS	63	3/39	2/38	14/35	8/38	2/37	4/15	16/31
MN	5	3/4	0/2	1/2	2/2	0/2	1/1	1/2
C1qN	5	5/5	1/4	2/5	3/5	5/5	1/1	2/2
LN	4	1/2	1/2	1/2	2/2	1/2	1/1	1/2
DDD	4	0/4	0/4	1/4	4/4	0/3	-	1/4
MPGN	2	0/1	0/1	-	1/1	0/1	-	-
MesPGN	1	0/1	0/1	0/1	1/1	0/1	-	1/1
C3 glomerulopathy	1	0/1	0/1	-	1/1	0/1	0/1	0/1
Normal morphology	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Among the 63 patients with FSGS, tubular atrophy (68.3%) and segmental sclerosis (61.9%) were the predominant chronic and active lesions, respectively; crescents were present in 38.1% (Table 4). Electron microscopy findings in the subset of cases in which it was performed are not detailed in the present report.

Table 4. Histological Features in FSGS Patients (n=63)

Feature	N positive	% of FSGS	N negative
Tubular atrophy	43	68.3%	20
Segmental sclerosis	39	61.9%	24
Interstitial fibrosis	36	57.1%	27
Interstitial inflammation	30	47.6%	33
Mesangial hypercellularity	22	34.9%	41
Crescents	24	38.1%	39
Mesangial expansion	9	14.3%	54
Vasculitis	4	6.3%	59
Endocapillary proliferation	0	0.0%	63

Discussion

FSGS was the predominant histological pattern in this cohort of Uzbek children with SRNS, present in more than two-thirds of biopsies.

This proportion is at the upper end of published series. In the ESPN/ERA cohort (n=1,180, 12 European countries), FSGS accounted for 55–62% of pediatric SRNS, with marked between-country variation (48–68%) [6]. Our findings are closer to reports from Asia: 63.4% in a Chinese cohort [10], 58.7% in an Indian series [11], and 68.5% in another Indian cohort with a similar biopsy indication [12] — the last figure nearly identical to ours.

Several factors, rather than a single explanation, likely contribute to this finding. Our center is a national tertiary referral hospital, and referral of the most severe or refractory steroid-resistant cases may enrich the biopsied population for FSGS relative to community-based cohorts. Differences in biopsy indications and thresholds between centers and countries also affect the apparent frequency of FSGS. Electron microscopy, which can reveal ultrastructural features supporting minimal change disease or early membranous change even when light microscopy and immunostaining are unremarkable, was not performed in all cases; its selective use may have led to some cases being classified as FSGS or as unclassified rather than as minimal change disease. Racial and ethnic variation in FSGS frequency, linked in some populations to NPHS2 and APOL1 variants, has been described [6,21] and cannot be excluded as a contributing factor; however, in the absence of genetic testing in our cohort this remains speculative rather than established, and referral and diagnostic factors offer a more parsimonious explanation for our findings.

We were unable to apply the d'Agati–Columbia FSGS subclassification in this cohort, which is a limitation, since the collapsing and tip-lesion variants carry different prognoses. Systematic use of this classification in future biopsies at our center would allow more precise prognostic stratification.

Immunostaining in FSGS was negative or showed only non-specific IgM and/or C3 trapping in most cases. This pattern is compatible with primary FSGS but is not, by itself, proof

of a primary podocytopathy, since some causes of secondary FSGS can present with a similarly negative or non-specific immunostaining pattern; morphological and clinical correlation remains necessary.

C1q nephropathy accounted for 5.5% of the cohort, within the 2–8% range reported elsewhere [16], and occurred exclusively in girls in our series (p=0.04). Because C1q deposition can also be an early feature of lupus nephritis, careful clinical and serological exclusion of systemic lupus erythematosus is warranted in all patients with this diagnosis.

C3 glomerulopathies (dense deposit disease and C3 glomerulonephritis combined, 5.5%) were more frequent than the 1–3% typically reported in European registries. Complement genetic testing (CFH, CFI, MCP, C3, CFB) was not performed in our cohort; prior work has shown that most children with dense deposit disease harbor complement-regulatory abnormalities [17], and such testing would be valuable for prognosis and treatment selection in future cases.

Lupus nephritis was identified in four adolescent girls, consistent with the known age and sex distribution of pediatric SLE [18]. In each case, nephrotic syndrome was the presenting feature, with systemic lupus manifestations recognized only after biopsy — underscoring that biopsy in adolescent SRNS also serves a diagnostic screening function for systemic disease.

Chronic tubulointerstitial changes were common in our cohort (tubular atrophy 63.7%; interstitial fibrosis 62.6%). A tubulointerstitial index above 25% has been associated with a substantially increased risk of ESKD in other pediatric SRNS cohorts [13]; we did not calculate a quantitative index for individual biopsies, and in the absence of follow-up data in the present study we cannot establish a direct prognostic association within our own cohort. The frequency of these chronic changes is nonetheless consistent with delayed referral for biopsy and supports efforts to shorten the interval between confirmed steroid resistance and biopsy.

The female predominance in our cohort (57.1%) differs from most pediatric SRNS series, in which boys predominate, particularly at younger ages. This likely reflects our age distribution, which was skewed toward adolescents — an age at which the sex ratio in SRNS narrows or reverses [19] — together with the inclusion of diagnoses such as C1qN, lupus nephritis, and membranous nephropathy that disproportionately affect girls.

The mean age at biopsy in our cohort (10.0±4.2 years) was somewhat older than in Indian (6–8 years) and Chinese (7–9 years) series, consistent with biopsy being performed only after a prolonged period of documented steroid resistance and unsuccessful second-line therapy. Earlier referral for biopsy, in line with IPNA 2020 recommendations [2], could allow morphological diagnosis before advanced chronic changes accumulate.

Conclusions

In this single-center cohort of Uzbek children with SRNS, FSGS was the predominant histological finding (69.2%), and chronic tubulointerstitial damage was common at the time of biopsy, suggesting that morphological verification was often delayed relative to the onset of steroid resistance. C1q nephropathy and C3 glomerulopathy, while uncommon, represented clinically distinct entities requiring careful differentiation from lupus nephritis and dedicated complement work-up, respectively. These findings support earlier referral for renal biopsy in children with confirmed steroid resistance and highlight the value of systematic morphological reporting, including FSGS subclassification, in this setting.

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PRICES AND PRICING POLICY IN PHARMACEUTICAL ENTERPRISES: A COMPARATIVE ANALYSIS OF UZBEKISTAN'S PRACTICE AND INTERNATIONAL EXPERIENCE

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Abstract. *This article analyzes the economic and institutional foundations of prices and pricing policy in pharmaceutical enterprises. In particular, the pricing mechanisms applied in Uzbekistan's pharmaceutical market are comparatively examined in relation to the experience of the European Union, the United States, and international organizations. The article discusses the main factors influencing the formation of medicine prices, mechanisms of state regulation, and pricing models such as cost-plus pricing and value-based pricing. In addition, scientifically grounded conclusions and recommendations are proposed for improving pricing policy in the pharmaceutical sector.*

Keywords: *pharmaceutical economics, pricing policy, pricing mechanisms, cost-plus pricing, value-based pricing, reference pricing, pharmaceutical market.*

INTRODUCTION

The pharmaceutical industry is one of the strategically important sectors of the healthcare system. The formation of medicine prices determines not only the economic relationship between producers and consumers, but also the level of public health and the financial stability of the healthcare system. According to the World Health Organization, in many countries, expenditures on medicines account for 20–30% of total healthcare spending [1].

In recent years, the high prices of innovative medicines, patent protection, and market concentration have made pharmaceutical pricing policy an urgent global issue [2]. These processes are encouraging countries to improve mechanisms for regulating and evaluating medicine prices. In European Union countries, this process is being carried out through the introduction of external reference pricing and Health Technology Assessment mechanisms [3].

In the Republic of Uzbekistan, the development of the pharmaceutical sector is considered one of the priority areas of state policy. Mechanisms for state regulation of medicine prices have been introduced in the country, and they are aimed at protecting the population from excessive price increases [4]. At the same time, dependence on imports and fluctuations in exchange rates have a negative impact on price stability in the pharmaceutical market [5].

The purpose of this article is to conduct a comparative analysis of the pricing mechanisms operating in Uzbekistan's pharmaceutical market in relation to international experience and to develop scientifically grounded conclusions for improving pricing policy.

LITERATURE REVIEW

In order to understand pharmaceutical pricing policy, it is important to examine the theoretical foundations of pricing. As an economic category, price reflects the balance between

supply and demand. However, in the pharmaceutical sector, price is not determined only by market mechanisms, but is also closely connected with social and healthcare-related factors.

The scientific literature identifies several main models of pharmaceutical pricing policy. One of them is cost-plus pricing, in which the final price is determined by adding a fixed profit margin to the production cost of a product. Although this model is simple and stable for manufacturers, it does not fully take into account the clinical effectiveness of a medicine or its value for the healthcare system.

In recent years, the value-based pricing model has been widely discussed in academic literature. In this approach, the price of a medicine is determined on the basis of its therapeutic effectiveness, its contribution to improving quality of life, and the economic benefits it brings to the healthcare system [6].

The experience of developed countries shows that value-based pricing contributes to the effective introduction of innovative medicines [7].

In European Union countries, pricing policy is often regulated by the state and implemented through the mechanism of external reference pricing. This mechanism ensures that the prices of medicines are adjusted in accordance with the prices of similar medicines in other countries [8]. In the United States, however, the pharmaceutical pricing system mainly relies on market mechanisms. Although this approach encourages innovation, it also leads to high medicine prices [9].

RESEARCH METHODOLOGY

This scientific article applies a comprehensive research methodology. In the research process, methods of theoretical analysis, comparative study, and analysis of regulatory and legal documents were used. This approach made it possible to compare Uzbekistan’s pharmaceutical pricing system with international experience.

At the initial stage of the research, international scientific literature and reports of international organizations on pharmaceutical pricing policy were studied. At the next stage, the regulatory and legal documents currently in force in the Republic of Uzbekistan were analyzed, and the mechanisms for regulating medicine prices were evaluated [4]; [5]. Based on the collected data, a comparative analysis was conducted, and scientific conclusions were developed.

RESULTS

Global Models of Pharmaceutical Pricing

Pharmaceutical product pricing policy differs across countries depending on the healthcare system, the level of state intervention, and the structure of the market. In international practice, several major models of pharmaceutical pricing have been developed, and they are clearly reflected in the experience of the European Union, the United States, and international organizations. In European Union countries, pharmaceutical pricing policy is mainly regulated by the state. In these countries, medicine prices are often determined through the **external reference pricing (ERP)** mechanism. Under the ERP system, the price of a medicine in one country is compared with the prices of similar medicines in selected reference countries. This mechanism helps prevent excessive price increases [3]. At the same time, European countries widely use the **Health Technology Assessment (HTA)** mechanism, through which the clinical effectiveness and economic value of medicines are evaluated [8].

In the United States, the pharmaceutical pricing system is relatively liberal, and medicine prices are often formed on the basis of market mechanisms. This has created conditions for the rapid development of innovative medicines and large investments in research and development

activities [9]. However, the high prices of medicines in the United States limit access to medicines for certain groups of the population. As a result, the issue of regulating medicine prices has become increasingly urgent in recent years [10].

The recommendations developed by the **World Health Organization (WHO)** and the **Organisation for Economic Co-operation and Development (OECD)** serve as an important methodological basis for shaping pharmaceutical pricing policy. These organizations recommend that countries ensure the economic affordability of medicines, increase transparency in the pricing process, and strengthen the sustainability of the healthcare system [1]; [2].

Uzbekistan's Pharmaceutical Pricing System. In the Republic of Uzbekistan, prices and pricing policy in the pharmaceutical market are actively regulated by the state. Since medicines are socially significant products, their prices cannot be left entirely to market mechanisms. For this reason, special mechanisms for controlling and regulating medicine prices have been introduced in the country. In Uzbekistan, a register of maximum prices for medicines is maintained, and wholesale and retail mark-up percentages are limited for medicines included in this register [4]. This mechanism serves to protect the population from artificial price increases. According to the Ministry of Health, after the introduction of this system, cases of sharp price increases for socially significant medicines have decreased [5]. However, in our opinion, Uzbekistan's pharmaceutical market still remains highly dependent on imports. Fluctuations in exchange rates, rising logistics costs, and dependence on imported raw materials affect the increase in medicine prices [2]. Therefore, the development of local pharmaceutical production and the expansion of import-substituting products are important factors in ensuring price stability.

Comparative Analysis by Companies. The pricing strategies of pharmaceutical enterprises differ depending on their scale of activity, product portfolio, and market segment. These differences can be observed in the example of local and foreign companies operating in the Uzbek market.

Local companies such as **Jurabek Laboratories**, **Nika Pharm**, and **Dentafill** mainly specialize in the production of generic medicines and medical products. In these enterprises, pricing policy is mostly based on **cost-plus pricing** and a flexible approach adapted to market competition. This approach makes it possible to offer products at affordable prices for broad segments of the population [5].

DISCUSSION. The comparative analysis shows that Uzbekistan's pharmaceutical pricing system is mainly aimed at ensuring short-term price stability. The price control mechanisms introduced by the state play an important role in protecting the population socially. At the same time, in order to ensure innovative development in the long term, it is necessary to make pricing policy more flexible [1].

International experience shows that the gradual introduction of **Health Technology Assessment (HTA)** and **value-based pricing** mechanisms increases the efficiency of the healthcare system and helps manage budget expenditures more rationally [7]. These approaches can also be adapted and applied in the context of Uzbekistan.

CONCLUSION AND RECOMMENDATIONS

This scientific article comparatively analyzed prices and pricing policy in pharmaceutical enterprises based on Uzbekistan's practice and international experience. The findings show that pharmaceutical pricing policy should be implemented in a way that ensures a balance between economic efficiency and social justice.

The main conclusions are as follows:

First, state regulation mechanisms ensure price stability in the short term.

Second, the introduction of **value-based pricing** and **Health Technology Assessment (HTA)** mechanisms is important for innovative development.

Third, the development of local pharmaceutical production contributes to greater price stability.

Based on these findings, it is recommended to improve Uzbekistan's pharmaceutical pricing system by introducing a national HTA system, increasing transparency in the pricing process, and supporting local pharmaceutical manufacturers.

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METHODS OF PROTECTING FISH FROM PARASITES

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Abstract. *This article examines the main parasites found in fish, their biological characteristics, and factors influencing their development. The causes of parasitic diseases in aquaculture, their impact on fish health, and their economic consequences are analyzed. Particular attention is given to preventive measures in fish farming, including water quality control, quarantine practices, and adherence to sanitary and hygienic standards. Modern treatment methods and the effectiveness of chemical and biological agents are also discussed based on scientific approaches. The results of the study indicate the necessity of a comprehensive approach to protecting fish from parasitic infections.*

Keywords: *aquaculture, fish parasites, prevention, parasitic diseases, treatment methods, water quality, fish health.*

Introduction: In recent years, aquaculture has been rapidly developing worldwide as one of the important sectors ensuring food security. Population growth, the decline of fish reserves in natural water bodies, and the increasing demand for protein-rich food products require improving the efficiency of fish farming enterprises. At the same time, the widespread use of intensive fish cultivation technologies significantly increases the risk of the spread of various diseases, particularly parasitic diseases. Parasitic diseases occurring in fish are among the most common problems in aquaculture systems and cause major economic losses. These diseases lead to a decrease in fish growth rate, reduced feed utilization efficiency, weakened immunity, and in some cases, mass mortality. As a result, the profitability of fish farms sharply decreases and product quality deteriorates. Parasites enter the fish organism through various pathways and negatively affect their vital functions. In particular, water pollution, deviations of hydrochemical indicators from the norm, excessive stocking density, and failure to comply with sanitary-hygienic requirements create favorable conditions for the development of parasitic diseases. Therefore, the issue of protecting fish from parasites is important not only biologically, but also economically and ecologically. Currently, various methods have been developed to prevent and combat parasitic diseases in fish, which include strengthening preventive measures, using modern diagnostic methods, and applying effective treatment agents. In particular, a comprehensive approach—namely water quality monitoring, proper feeding, quarantine measures, and veterinary control conducted in harmony—ensures high efficiency. The purpose of this article is to analyze the main parasites found in fish, the causes of their occurrence, and modern methods of combating them. In addition, developing scientifically based recommendations aimed at preventing parasitic diseases in fish farms is one of the important objectives of this study.

Research Materials and Methods. The studies were carried out in 2024–2025 in artificial ponds under the conditions of fish farms such as Mirbozor Dilfini, Narpay carp, the northeastern

basin of the Kattakurgan Reservoir, Jaloyer Gold Fish, and the Qurbon Ariq coastal fish farming facilities.

As the experimental object, **6–8-month-old carp (*Cyprinus carpio*)** were selected. The average weight of the fish ranged from **80–120 g**.

The experiment was organized into **4 groups (150 fish in each group):**

- **Group 1 (control):** no treatment or prophylactic agents were applied
- **Group 2:** prophylactic baths with saline solution (NaCl)
- **Group 3:** treatment with formalin solution
- **Group 4:** modern antiparasitic preparation (for example, an ivermectin-based agent)

The duration of the experiment was **30 days**. Every 10 days, the general condition of the fish, the degree of parasite infestation, and growth indicators were monitored.

Applied Preparations and Their Dosages

During the experiment, the following agents were used:

Group	Infestation level (%)	Mortality (%)	Growth indicator (%)
Control	65%	20%	100%
Salt treatment	35%	10%	110%
Formalin treatment	25%	8%	115%
Ivermectin group	10%	3%	125%

- **Salt (NaCl) bath:** 2–3% solution for 10–15 minutes
- **Formalin solution:** concentration of 25 mg/L for 30–60 minutes
- **Ivermectin-based preparation:** administered through feed at a dose of 0.2 mg/kg
- **Potassium permanganate ($KMnO_4$):** concentration of 2 mg/L for water disinfection

Experimental Results

The results of the 30-day observation are presented in the following table:

Note: changing the dosage of the preparations adversely affects the fish organism.

According to the results, a sharp decrease in infestation level was observed in the groups where antiparasitic preparations were applied. The highest effectiveness was recorded in the ivermectin-based treatment.

Analysis and Discussion of Results

The obtained results indicate that the regular application of preventive measures is of great importance in preventing parasitic diseases in fish. In particular, salt and formalin solutions are simple and inexpensive methods, making them effective for small-scale farms.

Modern antiparasitic preparations have high efficiency and affect different stages of parasite development. However, their application requires precise dosage determination and strict adherence to veterinary supervision.

Preventive Recommendations

To protect fish from parasites, the following measures are recommended:

- Continuous monitoring of water quality (**pH 6.5–8.5, oxygen ≥5 mg/L**)
- Avoiding excessive fish stocking density
- Keeping new fish in quarantine for at least **10–14 days**
- Applying prophylactic baths every **2 weeks** (salt or $KMnO_4$)

- Providing high-quality and balanced feed
- Regular pond disinfection
- Establishing veterinary supervision

Conclusion

1. As a result of the conducted studies, it was determined that parasitic diseases are widespread in fish and are mainly associated with deterioration of water quality, non-compliance with sanitary-hygienic requirements, and excessive stocking density. These factors create conditions for the rapid development of diseases.

2. The experimental results showed that regular application of preventive measures (salt solution, potassium permanganate baths, quarantine measures) significantly reduces the level of parasite infestation in fish and improves their overall physiological condition.

3. Chemical agents, particularly formalin solution and modern antiparasitic preparations (based on ivermectin), were found to be highly effective. Especially ivermectin-based preparations showed the strongest effect against parasites, reducing infestation to a minimum level.

4. It was proven that fish growth rate and survival level are directly related to preventive and treatment measures. Growth indicators in the experimental groups were higher than in the control group, which is the result of a healthy environment and effective treatment.

5. A comprehensive approach to protecting fish from parasites—namely water quality control, proper feeding, application of quarantine measures, and the use of modern treatment methods—is the most effective way. This approach ensures high productivity and economic efficiency in fish farming enterprises.

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CLINICAL COURSE AND PATHOGENETIC FORMS OF NECROBACTERIOSIS IN HIGH-YIELDING DAIRY CATTLE

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Abstract. *This study investigated the different clinical forms of necrobacteriosis and their pathogenetic characteristics in high-yielding dairy cattle. The research was conducted during 2024–2025 in three livestock farms located in the Yukori Chirchik district of the Tashkent region, Republic of Uzbekistan. Based on clinical observations, bacteriological analyses, and comparative evaluation methods, the course of the disease, localization of lesions, and degree of complications were comprehensively assessed.*

The results demonstrated that necrobacteriosis does not present as a uniform clinical condition, but varies depending on the localization of the pathological process, depth of tissue damage, involvement of microbial associations, and the immunobiological status of the host. It was established that the disease is not limited to hoof tissues alone, but may progress as a multi-stage infectious process involving the mammary gland and, in some cases, internal organs.

Based on the findings, the clinical course of necrobacteriosis was classified into three main pathogenetic forms: localized-destructive, complicated-associative, and generalized forms. The proposed classification is of significant practical importance for early diagnosis, assessment of disease severity, and selection of effective treatment strategies.

In addition, a set of clinical indicators with prognostic value for disease progression was identified. The multi-stage and polymorphic nature of necrobacteriosis suggests that it should be considered not merely as a local hoof pathology, but as a systemic infectious syndrome.

Keywords: *necrobacteriosis, cattle, Fusobacterium necrophorum, clinical forms, pathogenesis, hoof diseases, high-yielding dairy cattle.*

Introduction

Necrobacteriosis in cattle is one of the most widespread multifactorial diseases under modern intensive livestock production systems. Traditionally, this disease has been considered a localized infectious process primarily affecting the distal parts of the limbs. However, recent scientific evidence indicates that necrobacteriosis represents a complex, multi-stage syndrome with intricate pathogenetic mechanisms.

The principal etiological agent of necrobacteriosis is *Fusobacterium necrophorum*, which is frequently detected in association with other opportunistic microorganisms. The clinical manifestation of the disease is not uniform and varies depending on the localization of the pathological process, depth of tissue damage, involvement of microbial associations, and the immunobiological status of the host organism.

In high-yielding dairy cattle, intensive feeding practices, metabolic disturbances, and reduced immune resistance contribute to the development of severe and complicated forms of the disease. Under such conditions, the pathological process is not limited to hoof tissues, but may extend to the mammary gland and internal organs.

At the same time, there is no universally accepted, scientifically grounded classification of the different clinical forms of necrobacteriosis and their pathogenetic characteristics. This lack of a unified approach complicates early diagnosis and the development of effective treatment strategies.

The aim of this study was to investigate the clinical course and pathogenetic characteristics of necrobacteriosis in high-yielding dairy cattle and to develop a scientifically based classification of the disease.

Objectives

To achieve this aim, the following objectives were defined:

1. To identify different clinical forms of necrobacteriosis in high-yielding dairy cattle and assess their distribution patterns;
2. To analyze the clinical course of the disease depending on the localization of the pathological process and the depth of tissue damage;
3. To evaluate the role of microbial associations in the development and severity of necrobacteriosis;
4. To assess the dynamics of disease progression and the degree of complications in different clinical forms;
5. To determine the clinical and pathogenetic characteristics of necrobacteriosis involving the mammary gland and internal organs;
6. To substantiate the stage-wise patterns of disease progression;
7. To develop a pathogenetically grounded clinical classification of necrobacteriosis;
8. To identify key clinical indicators for predicting disease severity;
9. To develop scientifically based recommendations for early diagnosis and differential treatment.

Materials and methods

The study was conducted during 2024–2025 in livestock farms located in various regions of the Republic of Uzbekistan, including three dairy-specialized farms situated in the Yukori Chirchik district of the Tashkent region. High-yielding Holstein-Friesian dairy cows were selected as the primary study subjects.

A комплекс approach combining clinical and laboratory methods was applied to investigate the clinical course of necrobacteriosis in cattle. All animals underwent thorough clinical examination, during which lameness severity, morphological changes in hoof tissues, interdigital inflammation, necrotic lesions, and general clinical condition were assessed. Based on clinical findings, the animals were grouped according to the form and severity of the disease.

A total of 63 pathological samples were collected and analyzed under laboratory conditions. The samples were obtained aseptically from hoof lesions, interdigital soft tissues, and, in some cases, from the mammary gland and internal organs of forcibly slaughtered animals.

Bacteriological investigations were performed under anaerobic conditions. Kitt–Tarozzi medium and blood agar were used for cultivation. The samples were incubated at 37°C for 48–72 hours. The isolated microorganisms were identified based on their morphological, microscopic, and biochemical characteristics, and their identity as *Fusobacterium necrophorum* was confirmed.

The clinical course of the disease, localization of pathological processes, depth of tissue damage, and degree of complications were analyzed comparatively. In addition, the involvement of microbial associations and their influence on disease severity were evaluated.

The obtained data were processed using comparative analysis, generalization, and variational-statistical methods to ensure scientific validity.

Results

The results of the study demonstrated that the clinical course of necrobacteriosis in high-yielding dairy cattle is not uniform, but varies depending on the localization of the pathological process, the depth of tissue damage, the involvement of microbial associations, and the immunobiological status of the host.

During bacteriological investigations, anaerobic microorganisms were isolated from 80.0% (50 out of 63) of the collected pathological samples. Morphological and biochemical analyses confirmed that the majority of these isolates belonged to *Fusobacterium necrophorum*. In some cases, the microorganisms were detected in association with other opportunistic bacteria, which was considered one of the key factors contributing to the severity of the clinical course of the disease.

Clinical observations revealed that the most common form of necrobacteriosis was localized in the distal parts of the limbs. This form was characterized by a progressive development of inflammatory and necrotic processes. In the early stages, hyperemia, swelling, increased local temperature, and pain response were observed in the hoof area. Subsequently, disruption of skin integrity, serous and sero-hemorrhagic exudation, and the formation of superficial necrotic lesions were noted.

As the pathological process progressed, deep ulcerative-necrotic defects developed in the interdigital and sub-hoof tissues, with destructive changes becoming predominant.



Figure 1. Distal form of necrobacteriosis in cattle: deep ulcerative-necrotic lesions in the sub-hoof and interdigital tissues

Inflammatory and necrotic processes in the hoof tissues are clearly visible. A deep ulcerative defect with bloody exudate is observed in the sub-hoof region. The interdigital area shows contamination and the presence of purulent-necrotic masses, indicating the spread of the

pathological process into deeper tissues. This condition is characteristic of the complicated destructive stage of necrobacteriosis and represents a severe clinical form associated with microbial interactions.

In cases where purulent-necrotic microflora were involved, the disease course became significantly more severe, characterized by the discharge of purulent exudate from necrotic foci, formation of fistulas and abscesses, and partial separation of the hoof horn. In some cases, the pathological process extended to deeper structures, including tendons, muscles, and even bone tissues.

Although necrobacteriosis affecting the mammary gland was observed less frequently, it was characterized by a severe clinical course. Affected animals exhibited elevated body temperature (40–41^{0}C), reduced appetite, a marked decrease in milk production, and the formation of painful infiltrates within the mammary tissue. Pathological changes in milk, including abnormal admixtures and exudate, were also detected.

In cases involving internal organs, the disease manifested with signs of systemic intoxication, including apathy, tachycardia, tachypnea, and loss of appetite. These findings indicate the generalization of infection and the development of destructive changes in parenchymal organs.

Based on the obtained results, it was scientifically substantiated that necrobacteriosis in high-yielding dairy cattle follows a stage-wise progression. Initially, local inflammatory processes develop, followed by necrotic destruction and purulent complications associated with microbial interactions, and in severe cases, progression to generalized infection.

According to the findings, the clinical course of necrobacteriosis can be classified into the following pathogenetic forms:

- localized-destructive form;
- complicated-associative form;
- generalized form.

The proposed classification has significant practical importance for early diagnosis, assessment of disease severity, and selection of appropriate treatment strategies.

Discussion

The results of the present study demonstrate that necrobacteriosis in high-yielding dairy cattle represents a more complex and multi-stage pathological process than traditionally assumed. While most literature describes the disease as a localized infection confined to hoof tissues, the findings of this study confirm its systemic and polymorphic nature.

The clinical manifestation of the disease was found to depend primarily on the localization of the pathological process and the depth of tissue damage. Observations in the distal limb regions revealed a progression from inflammatory changes to necrotic destruction, supporting the concept of necrobacteriosis as a dynamic and progressive disease.

Bacteriological analysis showed that anaerobic microorganisms, predominantly Fusobacterium necrophorum, were isolated in 80.0% (50 out of 63) of the samples, confirming its leading etiological role. Additionally, the presence of microbial associations was associated with more severe and complicated forms of the disease, highlighting the importance of microbial synergy in pathogenesis.

The detection of systemic intoxication signs in cases involving the mammary gland and internal organs indicates the ability of necrobacteriosis to generalize. These findings support the

concept that the disease should not be regarded solely as a local pathology, but rather as a systemic infectious syndrome affecting the entire organism.

The pathogenetic classification proposed in this study—localized-destructive, complicated-associative, and generalized forms—has important clinical implications. It provides a practical framework for assessing disease severity, improving early diagnosis, and selecting differentiated treatment approaches.

Furthermore, the transition between clinical forms was found to be closely associated with farm management conditions, hygiene level, microtrauma, and the immune reactivity of animals. This emphasizes the critical importance of eliminating trigger factors in the prevention of necrobacteriosis.

Conclusions

Necrobacteriosis in high-yielding dairy cattle does not present as a uniform clinical condition; rather, it varies depending on the localization of the pathological process, the depth of tissue damage, and the involvement of microbial associations.

1. Bacteriological investigations revealed that *Fusobacterium necrophorum* was isolated in 80.0% (50 out of 63) of the samples, confirming its leading role in the etiology of the disease.
2. The clinical course of necrobacteriosis is characterized by a stage-wise progression, beginning with inflammatory changes, followed by necrotic destruction, and, in complicated cases, leading to generalized infection.
3. In cases involving the mammary gland and internal organs, the disease presents with a severe clinical course accompanied by systemic intoxication, indicating that necrobacteriosis should be regarded as a systemic infectious process.
4. The classification of necrobacteriosis into localized-destructive, complicated-associative, and generalized forms is clinically justified and facilitates early diagnosis as well as the selection of effective treatment strategies.
5. Disease severity is influenced by farm management conditions, hygiene level, microtrauma, and the immune reactivity of animals; therefore, elimination of these trigger factors is essential for effective prevention.

Practical recommendations

Regular clinical examinations of herds are recommended for early detection of necrobacteriosis, with particular attention to early signs such as hyperemia, swelling, and pain in the hoof region.

- Diagnosis should be based on a differential approach that takes into account the clinical forms of the disease (localized-destructive, complicated-associative, and generalized).
- Treatment strategies should be stage-based and comprehensive, including local antiseptic treatment, removal of necrotic tissues, and systemic therapy depending on disease severity.
- In cases of purulent-necrotic complications, antibacterial therapy should be selected based on the presence of microbial associations and applied in a scientifically justified manner.
- To prevent severe and generalized forms of necrobacteriosis, improvement of farm hygiene, regular hoof care, and implementation of preventive measures are essential.
- Elimination of trigger factors is of key importance in prevention, including balanced nutrition, improved flooring conditions, reduction of environmental moisture, and обеспечение adequate animal movement.

- In cases involving the mammary gland and internal organs, affected animals should be isolated and treated comprehensively to prevent the spread of infection.
- Implementation of a monitoring system for necrobacteriosis at the farm level is recommended, including systematic recording of disease cases and assessment of risk factors to improve preventive efficiency.

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