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Optimal Technological Parameters for Cultivating Trichoderma Consortia Isolated from Northern Kazakhstan Soils

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ABSTRACT

This study determined optimal technological parameters (pH of the medium, cultivation temperature, substrate) for the production of the Trichodermin-KZ biofungicide based on consortia of native strains isolated from the soils of Northern Kazakhstan. Three substrates were tested: Czapek-Dox medium, barley seeds, and wheat bran, in liquid and solid forms. Cultural and morphological features of consortia, such as the colour of the aerial and substrate mycelium, the edges of colonies, and the features of fungal sporulation were investigated on agar media. The surface of colonies, the colour of the aerial and substrate mycelium, and the weight of biomass before and after drying were studied on liquid media. Cultivation of the test consortia at different temperatures showed that the optimal temperature is +25°C, at which the colonies reach their maximum diameters: 45.0±0.8; 45.0±0.2; 55.0±0.7; intensive accumulation of biomass, sporulation, and conidia growth. To identify the optimum pH, the fungi were grown at a constant temperature (+25°C), but with variable pH (4.0; 5.0; 6.0; 7.0; 8.0; 9.0); pH 6-7 was found to be optimal for the accumulation of fungal biomass on liquid media, while pH 5-7 was optimal for agar media. Thus, the study established the technological parameters for the production of biofungicide. The materials of this paper are of practical value for producers of organic products.

1. INTRODUCTION

Biological control agents based on microorganisms, or their molecules play the predominant role in the fight against the excessive use of pesticides in agriculture, as proven by many in vitro and in planta studies [1]. The positive effect of Trichoderma on plant growth and development has proved that these fungi can be widely used to develop successful inoculation systems and effective delivery methods capable of stimulating plant growth and biocontrol against plant diseases [2]. The use of microorganisms in consortia outweighs their individual application. Thus, the combination of two compatible microorganisms, Serendipita indica and Trichoderma simmonsii, can serve as an essential supplement to the nutrition of crops and increase plant resistance against Phytopthtora 1apsica [3].

Several studies have reported on the use of Trichoderma species to combat phytopathogens in various agricultural crops. For instance, Trichoderma has been employed for the control of fusarium blight in beans, vascular wilting and early rot in tomatoes, and corn stem rot [4-7]. These studies highlight the effectiveness of Trichoderma in managing diseases across a range of crops, demonstrating its versatility as a biocontrol agent. Trichoderma species are recognized for their ability to thrive in various pathosystems without harming beneficial organisms. This minimal impact on soil balance makes Trichoderma a safe and environmentally friendly option for biocontrol across different crops [8]. For example, the development of a *Trichoderma asperellum* strain 6S-2 based biofertilizer has been effective in preventing apple tree transplant disease and promoting the growth of young apple tree seedlings [9].

The use of Trichoderma fungi as mycofungicides and biofertilizers offers significant economic benefits by reducing production costs and environmental impact. Trichoderma-based products dominate the biofungicide market, accounting for approximately 60% of the market share. These products are especially commercialized in Asia, particularly in India, and are also widely used in South and Central America [10]. Despite the rather considerable number of biological products on the market, scientists note that the effectiveness in experimental and field conditions may vary, even between strains within the same genus, depending on the growth conditions. Thus, the study of the effect of temperature on the fungi growth demonstrated that native strains of Trichoderma are better adapted to survive in changing conditions, providing better protection for vine plants upon joint evolution with each particular vineyard [11].

Agriculture in Northern Kazakhstan heavily relies on synthetic pesticides to protect crops, but these chemicals pose significant environmental and health risks, such as soil degradation, water contamination, and pesticide resistance. Introducing biofungicides based on native Trichoderma strains offers a sustainable alternative, providing environmental safety, reduced human health risks, and effective pathogen control without harmful residues. Utilizing these biofungicides aligns with sustainable agriculture principles, promoting soil health and ecosystem stability while enhancing crop yields. The study on native Trichoderma strains is crucial for advancing sustainable pest management practices, offering practical solutions to improve agricultural sustainability and economic resilience in the region.

The main objectives of the present study are:

1. To identify and isolate native strains of Trichoderma from soils in Northern Kazakhstan;
2. To determine the optimal technological parameters for cultivating these Trichoderma consortia;
3. To establish the technological parameters for the production of biofungicide.

2. MATERIALS AND METHODS

2.1 Consortia selection and preparation

Consortia of strains *Tr. Lignorum* and *Tr. Album* were used as the basis for the development of the Trichodermin-KZ biofungicide (Table 1) [12]. The strains were obtained from a reputable fungal culture collection and were selected based on their known antagonistic, hyperparasitic, growth-stimulating, and cellulose-decomposing properties. The rationale behind their selection was to leverage these beneficial characteristics to enhance the biofungicide's effectiveness. Technological parameters (an effective nutrient substrate, optimal pH values of the medium and temperature for cultivation, and the titre of the consortium) have been investigated in previous studies.

Table 1. Consortia composition based on the collection of strains-antagonists of Trichoderma fungi

Consortia	Strains of Trichoderma Fungi	Properties
K-1	T134, T340, T100	Antagonistic, hyperparasitic, growth-stimulating, cellulose-decomposing
K-2	T134, T115, T200	
K-3	T17, T90, T350	

2.2 Experimental design

The experiment was arranged systematically to cover different aspects of fungal growth and viability. Replicates were included for each treatment to ensure consistency and reproducibility of the results. Specifically, each pH treatment and medium type was tested in triplicate to provide robust data for analysis. Randomization was applied where possible to minimize bias and ensure the reliability of the experimental outcomes.

2.3 Nutrient media preparation

This study investigated both liquid and agar forms of wheat bran, barley, and Czapek-Dox media to identify the most effective nutrient medium. Substrates weighing 30 g were placed in glass jars, moistened with 5ml of distilled water, and then sterilised for 20 minutes (101kPa, 121℃). After sterilisation, 2ml of suspension culture of various consortia of Trichoderma fungi were introduced into each flask.

2.4 Fungal growth and pH experiments

The effect of the acidity of the medium on the viability of consortia of Trichoderma fungi was determined on two nutrient media: on wheat bran broth and Czapek-Dox agar medium, at +25℃, with variable pH values of the medium (4.0, 5.0, 6.0, 7.0, 8.0, and 9.0). The pH values were adjusted using appropriate buffer solutions to maintain the desired acidity levels. The fungi were grown according to the surface culture method for 15 days. The diameter of the consortium colonies was measured every day of cultivation on a solid medium.

The growth of mycelium on a liquid medium was assessed using a qualitative scale that ranges from 0 to 3. This scale provides a standardized way to describe the extent and quality of mycelium formation:

0 - no growth or seed block fouling (this score indicates that the conditions were not conducive to fungal growth);

1 - mycelium growth by individual colonies (mycelium appears in isolated patches or colonies; these colonies are small and scattered, indicating limited or initial growth);

2 - mycelial mat is solid and thin (a continuous layer of mycelium forms across the surface of the medium; this score indicates moderate growth and spread of the fungal mycelium);

3 - mycelial mat is solid and thick (this score indicates robust growth and extensive colonization by the fungus).

2.5 Spore titre determination

The consortia of Trichoderma fungi were first cultured on suitable growth media (Czapek-Dox, wheat bran, or barley) for 20 days, after which spores were harvested by adding sterile distilled water and gently scraping the surface. The resulting spore suspension was filtered to remove mycelial fragments and then subjected to serial dilution. Each dilution (100μl) was pipetted onto Petri dishes containing solidified agar medium and spread evenly using a sterile spreader. The inoculated dishes were incubated at 25℃ for 5-7 days to allow spore germination and colony formation. Post incubation, colonies were counted, and the spore titre was calculated using the Eq. (1). This calculation ensured accuracy by averaging results from dilutions.

$$\text{Spore Titre(CFU/ml)} = \frac{(\text{Number of Colonies} \times \text{Dilution Factor})}{\text{Volume of Inoculum (ml)}} \quad (1)$$

2.6 Statistical analysis

To compare the growth rates, biomass accumulation, and spore titers among different conditions and consortia, statistical analyses were conducted. Results indicated significant differences ($p<0.05$) in growth rates, biomass accumulation, and spore titers across various nutrient media, temperatures, and pH levels. The data were presented as mean ± standard deviation, and statistical significance was determined at the 95% confidence level.

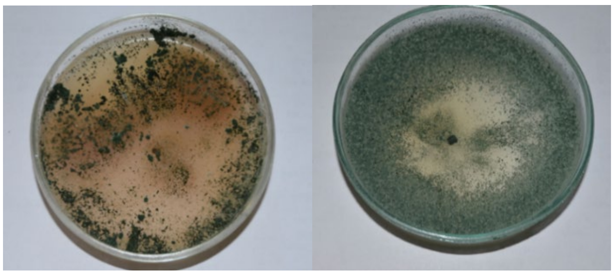
3. RESULTS

3.1 Selection of optimal nutrient media for the growth of consortia of Trichoderma fungi

Table 2 shows the cultural and morphological features of the Trichoderma fungi-based consortia, including the colour of the aerial and substrate mycelium, the edges of colonies, and the features of the fungal sporulation.

Table 2. Characteristics of consortium colonies on bran and barley agar, Czapek-Dox medium on the 7th day of growth

No.	Nutrient Medium		
	Bran Agar	**Barley Agar**	**Czapek-Dox agar**
K-1	The surface of the colonies is velvety, the edges are fleecy. The aerial mycelium is dark green, with white speckles. The substrate mycelium is dark green. The growth is intense.	The surface of the colonies is velvety, the edges are irregular. The aerial mycelium is green. The substrate mycelium is dark green. Mycelium is scattered.	The surface of the colonies is velvety, the edges of the colonies are irregular. The aerial mycelium is dark green with white speckles. The substrate mycelium is dark green. Moderate growth.
K-2	The surface of the colonies is velvety, the edges are irregular. The aerial mycelium is dark green with white speckles. The substrate mycelium is dark green. Mycelium is dense.	The surface of the colonies is velvety, the edges are fleecy. The aerial mycelium is yellow, the substrate mycelium is dark green. Moderate growth.	The surface of the colonies is powdery, the edges of the colonies are not outlined. The aerial mycelium is yellow, the substrate is yellow green. Moderate growth.
K-3	The surface of the colonies is velvety, the edges are irregular. The aerial mycelium is green. The substrate mycelium is green. The growth is moderate.	The surface of the colonies is velvety, the edges of the colonies are irregular. The aerial mycelium is green with white speckles. The substrate mycelium is dark green. Moderate growth.	The surface of the colonies is velvety, the edges of the colonies are not outlined. The aerial mycelium is light yellow. The substrate mycelium is light green. Mycelium is scattered.



A) **B)**

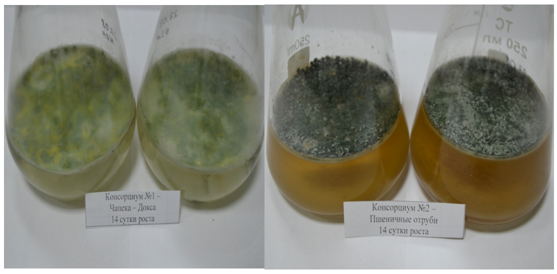
Figure 1. Cultural and morphological features of the K-3 consortium on different solid nutrient media. A - barley, B - wheat bran

Two types of Trichoderma fungi-based consortia were identified upon investigating the colony structure on barley broth. The first type (including K-1 and K-3) had a poorly developed aerial mycelium from light green to green, and dark green stroma. The edges of the colonies were irregular. The sporulation was poor (Figure 1). The second type had yellow aerial mycelium and a dark green substrate mycelium (K-2). The aerial mycelium was well-developed, it expanded completely and covered the Petri dish on the seventh day of cultivation. The edges of the colonies were fleecy. The sporulation was moderate.

To a certain extent, the cultural and morphological features of the Trichoderma fungi-based consortia changed when grown on the Czapek-Dox medium. On this medium, the aerial mycelium was cobwebby, poorly developed, the stroma had a pale colour.

Properties such as colony surface, colour of aerial and substrate mycelium, and biomass weight before and after drying were studied on the following liquid media: agar-free Czapek-Dox medium, wheat bran and barley broths (Table 3).

According to the results, wheat bran and barley broth both provided the maximum growth of the mycelial mat with the sporulation of the fungus. A continuous thin mycelial mat with sporulation was observed on these media after three days of cultivation, while within the Czapek-Dox medium, the mycelium had only overgrown the seed block.



A B

Figure 2. Cultural and morphological features of consortia on liquid nutrient media. A - Czapek-Dox, B - wheat bran

Czapek-Dox broth had powdery, poorly developed aerial mycelium of light green to green colour, the substrate mycelium was pale. Biomass weight ranged within 5.15-5.33g per 100ml, dry biomass weight was within 0.25-0.29g.

A solid thick mycelial mat with sporulation was formed after 10 days of growing on wheat bran and barley broth, while Czapek-Dox broth yielded individual colonies and a solid mycelial mat did not form (Figure 2). The maximum mass of dry mycelium after 10 days of cultivation was determined only on barley broth and was 0-1.0g. This time point was selected for assessing biomass accumulation and sporulation on liquid media. By this time, the fungi have typically reached a significant stage of growth, allowing for the measurement of biomass before any potential nutrient depletion or overcrowding impacts growth.

Table 3. Description of Trichoderma fungi-based consortia on the 10th day of growth on various liquid nutrient media (per 100 ml of the liquid medium)

No.	Nutrient Medium		
	Bran broth	Barley broth	Czapek-Dox broth
K-1	The surface of the colonies is velvety, the aerial mycelium is dark green with white speckles. The substrate mycelium is white. The sporulation is intensive. Quick gain of biomass - 5.76g. The weight of biomass after drying - 0.3g	The surface of the colonies is velvety, the aerial mycelium is dark green with white speckles. The substrate mycelium is white. The sporulation is average. Biomass weight - 6.51g, after drying - 0.6g	The surface of the colonies is powdery, the aerial mycelium is light green. The substrate mycelium is white. Sporulation is intensive. Biomass weight - 5.33g, after drying - 0.29g
K-2	The surface of the colonies is tomentose, the aerial mycelium is dark green with white speckles. The substrate mycelium is white. Sporulation is intensive. Quick gain of biomass - 6.24g, after drying - 0.7g	The surface of the colonies is tomentose, the aerial mycelium is dark green with white speckles. The substrate mycelium is white. Sporulation is intensive. Average growth. Biomass weight - 6.94g, after drying - 0.8g	The surface of the colonies is powdery, the aerial mycelium is light green, the substrate mycelium is white. Sporulation is intensive. Average growth. Biomass weight - 5.28g, after drying - 0.28g
K-3	The surface of the colonies is tomentose, the aerial mycelium is dark green with white speckles. The substrate mycelium is white. Sporulation is intensive. Quick gain of biomass - 6.31g, after drying - 0.58g	The surface of the colonies is tomentose, the aerial mycelium is dark green with white speckles. The substrate mycelium is white. Sporulation is above average. The growth is scattered. Biomass weight - 7.75g, after drying - 1.0g	The surface of the colonies is powdery, the aerial mycelium is green. The substrate mycelium is white. Sporulation is above average. Biomass weight - 5.15 g, after drying - 0.25g

Wheat bran media demonstrated the highest rates of biomass gain and sporulation, as well as less time required for the development of dense mycelium. Furthermore, wheat bran is an easily accessible and cost-effective nutrient substrate, since it is used in agriculture as animal feed.

3.2 Determination of the optimal ambient temperature for the growth of Trichoderma fungi-based consortia

Table 4 presents the results of cultivation of the strains under study on the Czapek-Dox medium at different temperatures (+6°C; +15°C; +25°C; +30°C; +35°C).

Table 4. Influence of temperature on the growth rate of the Trichoderma fungi-based consortia

Consortium	Diameter of Colonies, Mm				
	+6°C	+15°C	+25°C	+30°C	+35°C
K-1	-	28.0±0.2	45.0±0.8	25.0±0.5	2.0±0.1
K-2	-	32.0±1.5	45.0±0.2	25.0±0.7	7.0±0.9
K-3	-	35.0±0.8	55.0±0.7	40.0±0.6	10.0±0.1

The diameter of the consortium colonies grown on agar medium was measured every day of cultivation. An analysis of the data in Table 4 shows that all consortia had no vegetation at +6°C. At+35°C, the fungi started growing on day 6; on day 8, the colonies reached a diameter of 2mm, while the conidia did not form. At +15°C, the fungi started growing on day 5; on day 10, the colony diameter reached 28-30mm and active sporulation was observed. At +25°C, the growth began as early as day 3; on day 7, active sporulation and mass formation of conidia were observed; on days 13-14 - a set of dense green biomass formed. At +30°C, the colony diameter reached 20 mm on day 7; the sporulation started on day 5, full coverage of the culture dish and intensive sporulation occurred after 15 days.

The maximum growth rate of mycelium was observed at 15-30°C. Within 4-5 days, the entire surface of the medium in Petri dishes was covered with a colony of fungus. The sporulation began on days 5-6 and reached almost 100% on day 8.

At +35°C, the growth of fungi slowed down. On day 15, the diameter of the colony was a maximum of 10mm. This time point was used to study the effects of different pH levels and temperatures on fungal growth. It represents a period where the fungi have had adequate time to respond to the environmental conditions, showing both initial growth responses and any longer-term adaptations or inhibitions. The sporulation started on day 7, but after sporulation, conidia did not actively form. This time point was chosen to evaluate early-stage growth and morphological features on solid nutrient media. It allows sufficient time for initial colony formation and sporulation without reaching full maturity, providing insights into the early development of fungal colonies.

3.3 Selection of the optimum pH of the nutrient medium for the growth of Trichoderma fungi-based consortia

During the experiment, the most active growth of colonies was recorded within the pH values of 5-7. Fungi growth also occurred in alkaline and highly alkaline media with pH 8-9, but the colonies were 1.5-2 times smaller. The growth slowed down after 5 days, with no active sporulation and conidia development, leading to scattered and grey mycelium. Strongly acidic medium at pH 4 inhibited the growth of consortia. On day 4, only growth remnants were observed - a slight pubescence of the seed agar block; in the following days, the growth of the fungus was also poor, the mycelium was scattered and grey (Table 5).

After three days of cultivation, a continuous thin mycelial mat with sporulation appeared on media with pH of 6-7. While on media with an alkaline and acidic reaction (pH 8 and 5), the mycelium has only overgrown the seed block in the depth of the medium. After 7 days of cultivation, solid thick mycelial mats with sporulation and a thick dark green colour were formed on liquid bran broth at pH 6-7 (Table 6).

On media with pH 5, consortia K-1 and K-2 formed a solid thin mycelial mat with weak sporulation, while K-3 formed a solid thick dark green mat. On the medium with pH 8-9, consortia K-1 and K-3 developed only individual colonies, while K-2 formed a continuous thin mycelial mat with sporulation. Strongly acidic media (pH 4) resulted in no vegetation for K-1, while K-2 and K-3 grew in individual colonies.

Table 5. Influence of various pH values of the medium on the consortia growth on day 15

pH Values	Diameter of Colonies, Mm		
	K-1	K-2	K-3
pH 4	49.5±3.96	64.5±0.28	49.5±3.96
pH 5	61.2±2.16	69.2±1.87	61.2±2.16
pH 6	69.0±2.02	65.3±0.69	69.0±2.02
pH 7	65.0±1.15	61.6±2.20	65.0±1.15
pH 8	43.0±0.57	28.3±1.66	43.0±0.57
pH 9	41.2±1.01	40.5±2.02	41.2±1.01
LSD	7.64	7.70	7.64

Table 6. Influence of various pH levels of the medium on the accumulation of biomass of consortia on day 15

Strain	Mycelium Growth, Point/Weight of Dry Mycelium, g					
	pH of the Medium, Biomass Weight					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
K-1	0/0	2/3.1	3/6.9	3/7.3	1/1.9	1/2.0
K-2	1/1.4	2/4.9	3/8.7	3/9.0	2/4.8	2/4.8
K-3	1/1.2	3/6.7	3/10.2	3/9.9	1/3.4	1/3.6
Strain	pH of the Medium, Biomass Weight after Drying					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
K-1	0	0.22±0.04	0.41±0.05	0.42±0.04	0.2±0.03	0.17±0.02
K-2	0.2±0.03	0.29±0.04	0.54±0.06	0.52±0.05	0.36±0.04	0.38±0.03
K-3	0.2±0.03	0.4±0.05	0.6±0.07	0.58±0.06	0.28±0.04	0.3±0.03

The mass of dry mycelium K-1 after 15 days of cultivation was maximal at pH 7. The largest biomass of K-2 and K-3 was at a pH of 6.

3.4 Study of various substrates to produce biofungicide based on Trichoderma fungi strains

Studies have shown that all Trichoderma fungi-based consortia can grow on various substrates. Notably, the K-1 fungi turned out to be the most fastidious, the titre of which was comparatively lower than that of other consortia in all three substrates (Table 7).

Table 7. Titre of spores of Trichoderma fungi-based consortia on various substrates

Substrate	Biofungicide Titre, Million Spores		
	K-1	K-2	K-3
Czapek-Dox	83±3.30	175±6.78	136±10.09
Wheat bran	179±2.30	250±7.65	240±6.31
Barley	124±7.64	210±3.67	270±9.50

Natural substrates such as wheat bran and barley seeds provided the greatest biomass gain and sporulation in K-2 and K-3. The biofungicide titre did not exceed 83 million spores/ml on the synthetic substrate, while this figure reached up to 270 million spores/ml in suspension of barley seeds. High rates of K-2 and K-3 sporulation were observed on all substrates. Moreover, K-3 intensively formed spores on barley and wheat bran, with 270 million spores/ml and 240 million spores/ml of suspensions, respectively. Consortia K-2 most actively developed spores on the synthetic Czapek-Dox medium, where the spores reached up to 175 million in 1 ml of suspension on day 20 of cultivation, compared to other consortia. This time point was chosen to assess the titre of spores on various substrates for biofungicide production. By this stage, the fungi have usually completed their growth cycle and reached maximum spore production, providing a clear comparison of the effectiveness of different substrates. Barley and wheat bran are also suitable as a substrate for this consortium.

One unexpected result was the differential response of the Trichoderma fungi consortia to various nutrient media and environmental conditions. While wheat bran and barley broth generally promoted robust growth and sporulation across all consortia, Czapek-Dox medium yielded significantly less biomass and poorer mycelial development, despite being a synthetic medium often used for fungal growth. Additionally, the fungi's response to temperature variations revealed that growth was optimal at a moderate range (15-30°C), but dramatically reduced at the extremes (6°C and 35°C). This highlights the sensitivity of Trichoderma fungi to temperature changes, which was particularly unexpected at higher temperatures where growth was minimal, contrary to typical fungal behavior that can often tolerate a broader range of temperatures. Furthermore, the varying pH levels demonstrated that while Trichoderma could adapt to slightly acidic to neutral conditions, growth in highly alkaline or acidic media was substantially inhibited, contrary to the more robust growth expected across a wider pH range. These findings underline the importance of optimizing both nutrient media and environmental conditions to maximize the growth and efficiency of Trichoderma fungi-based consortia for practical applications.

4. DISCUSSION

4.1 Importance of trichoderma strains in agriculture and medicine

The importance of using Trichoderma strains as biological control agents for agricultural and medicinal crops are confirmed by numerous studies in recent years. Thus, all strains of *Trichoderma virens*, *T. pseudokoningii* and *T. harzianum* demonstrated good potential as biocontrol agents against *A. alternata* and stimulated the growth of the medicinal plant *Ashwagandha* [13]. The antagonistic effect of *Trichoderma harzianum* strains against many pathogens, such as *Alternaria alternata*, *Bipolaris cynodontis*, *Fusarium* *culmorum*, and *F. oxysporum* was shown on the example of sunflower [14]. The potential of the Trichoderma fungi as the basis of environmentally friendly and harmless biofungicides is evidenced by many authors [15-17]. The ability of wheat plants to adapt the antioxidant mechanism and tolerate water stress is also associated with the use of Trichoderma cultures grown on potato broth [18-20].

4.2 Use of microbial consortia for enhanced biocontrol

Currently, the creation of microbial consortia is considered as one of the ways to improve the reliability of modern methods of biological control of plant pathogens of agricultural crops, as well as the influence of biotic and abiotic environmental factors. This will expand the spectrum of action of microbial consortia. This area is the main trend in biotechnology, which has already been commercialised in sustainable agriculture [21-23].

Thus, the use of the Trichoderma consortium (BHU51 and BHU105) to evaluate growth stimulation and induction of protection against *Sclerotium rolfsii* was more effective than upon using microorganisms separately [24, 25]. The authors also used consortiums of native strains of Trichoderma to develop a biofungicide, since such strains are more adapted to survive in changing conditions and can provide better plant protection, which is consistent with the studies of many authors [11].

4.3 Optimization of growth conditions for trichoderma strains

The main components of the technological regulations are the cultivation temperature, the pH of the medium, and the substrate [26-28]. According to the experimental data, native Trichoderma strains demonstrated satisfactory growth between +15°C and +30°C, of which the most optimal temperature was +25°C. Optimal culture growth on a solid medium was in the pH range from 5 to 7. In a liquid medium, pH 6-7 ensured maximum growth of the mycelial mat with sporulation of the fungus. The results obtained are consistent with the data in the scientific literature. Thus, during the study of mycoparasitism of *T. asperellum* and *T. asperelloides* isolates grown at temperatures from 7°C to 42°C, Trichoderma isolates showed growth at temperatures from +12°C to +37°C, with a maximum growth reached at 27°C [29]. Study of biomass production by Trichoderma fungi at +20°C, +25°C, +30°C, and +35°C and pH 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5, and 8.0 showed active growth at +25-30°C and pH 5.5-7.5 [30-32].

In the context of optimizing growth conditions for Trichoderma strains, the presented study revealed that the optimal temperature range for growth was between +15°C and +30°C, with the most favorable temperature being +25°C. This temperature range aligns with findings in the scientific literature, where Trichoderma isolates showed maximum growth at 27°C. Furthermore, the optimal pH for solid medium growth was found to be between 5 and 7, while for liquid medium, pH 6-7 supported maximum mycelial mat growth and sporulation. This is consistent with other studies that have indicated active growth within pH 5.5-7.5. The substrates used also played a critical role, with wheat bran and barley seeds yielding the highest biomass gains, sporulation rates, and dense mycelium formation. These natural substrates provided superior results compared to the synthetic Czapek-Dox medium.

4.4 Substrate selection for large-scale production

For large-scale production of microbial biomass, the main factor is the substrate as the main condition for maximum accumulation of the target product [33-35]. In this regard, the organic substrate is of particular interest for modern science, as evidenced by recent studies [36-38]. A sufficient variety of organic substrates should be noted, e.g., cotton waste, manure, poultry manure, rice bran, spent corn cobs, vermicompost, barley straw, wheat grain, spent fungus substrate, sorghum, sugar beet pulp, etc. [39-41]. Therewith, both the use of native strains and a local organic product as a substrate is important [42, 43].

As a result of this study, the highest rates of biomass gains, sporulation, and formation of dense mycelium were achieved upon using wheat bran and barley seeds as a substrate. The use of such substrates is also practical and cost-effective for large-scale production, highlighting their potential for commercial biofungicide applications. This detailed analysis underscores the importance of optimizing both environmental conditions and substrate selection to maximize the efficacy of Trichoderma fungi-based consortia.

5. CONCLUSIONS

The study described cultural and morphological features of the Trichoderma fungi, such as the colour of the aerial and substrate mycelium, the edges of colonies, the features of the sporulation of the fungus depending on nutrient media. This study identified the optimal growth conditions for Trichoderma fungi-based consortia, finding that +25°C and a pH of 6-7 are ideal for maximum biomass and sporulation. Temperatures below +15°C inhibit growth, and temperatures of +30°C and above block conidia formation. Barley seed and wheat bran proved to be the most effective substrates, while the Czapek-Dox medium was ineffective for spore production. These findings are significant for developing effective biofungicides, contributing to more reliable and sustainable agricultural biocontrol methods.

While this study provides valuable insights into the optimal growth conditions for Trichoderma fungi-based consortia, it is limited by its laboratory setting, which may not fully replicate field conditions. Future research should explore the effectiveness of these consortia under diverse environmental conditions, test the biofungicides in field trials to assess their real-world applicability.

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