Optimization Of the Composition of The Nutrient Medium and Selection of Growth Conditions to Increase the Production of Phytohormones in The T. Harzianum Uzcf-55 Strain

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Abstract

It was found that Mandels medium was optimal for T. harzianum 55, and Capek medium was optimal for T. harzianum 54, T. asperellum 12. However, in order to increase the production of phytohormones by these strains, the composition of the medium was changed to enrich and reduce the cost of the medium. That is, Mandels and Capek mediums were prepared with 2% sucrose and 2% other carbon sources (molasses, cottonseed meal, crushed cotton leaves and corn extract) added, the strains were grown in them, and the production of GC and Indole complexes was studied. It was found that all three strains produced the most active GC and Indole complexes in mediums enriched with corn extract and molasses. In the control variant, it was found that in these nutrient media, the amount of indole compounds synthesized by T. harzianum 55 was 0.559 mg/ml and the amount of GC was 0.172 mg/ml, the amount of indole compounds synthesized by P. Canescens 54 was 0.418 mg/ml, the amount of GC was 0.189 mg/ml, and the amount of indole compounds synthesized by F. moniliforme 12 was 0.518 mg/ml and the amount of GC was 0.194 mg/ml.

Keywords: Auxin (AU), gibberellin (GK), indole acetic acid (ISK), indolyl-3-acetic acid (IAA), T. harzianum 55, strain, Mandels nutrient medium, Chapek nutrient medium, T. harzianum 54, T. asperellum 12.

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1. Introduction

Today, both in the world and in our republic, one of the main pressing issues is the cultivation of agricultural crops, increasing their productivity and thereby satisfying the population's demand for agricultural products. A number of measures are being taken in our country in this regard, including the selection of high-quality seeds, increasing soil fertility, developing agrotechnology and using biopreparations based on environmentally friendly biologically active compounds, etc.

Rhizosphere microorganisms play an important role in the growth and development of plants. They help plants to a certain extent meet their need for nutrients, hormones, vitamins and other physiologically active compounds. Micromycetes also have a positive effect on the development of plants, increase their productivity and protect them from diseases spread by various phytopathogens.

Mycelial fungi synthesize auxin (AU), gibberellin (GK) and indole acetic acid (ISA) during development. These biologically active substances are the main components of environmentally friendly biological preparations that accelerate seed germination, plant development and increase productivity.

In particular, species of micromycetes belonging to the Trichoderma genus synthesize phytohormones that stimulate the germination, development and yield of plant seeds. The creation of an environmentally friendly biopreparation with a complex effect based on the association of these active fungal strains allows for high and effective yields of agricultural crops. Therefore, the selection of promising local fungal strains that synthesize active phytohormones, the creation of an optimal nutrient environment for the selected strains (the influence of various carbon and nitrogen sources) and the optimization of growth conditions in a liquid nutrient medium for the production of phytohormones in large quantities, the study of the dynamics of their production of Gibberellin and Indole complexes, and the creation of environmentally friendly biopreparations with a stimulating effect on the yield of agricultural crops and protection against phytopathogenic fungi based on their association are of scientific and practical importance.

2. Materials And Methods Of Research

Currently, many studies are being conducted all over the world to search for and study fungi-producers to obtain high and effective yields from agricultural crops and protect them from various diseases. Indole-3-acetic acid (IAA) is a natural auxin, which belongs to the most widespread class of phytohormones that plays an important role in plant growth and development. It is noted that the multiphase activity of cytokinins is characteristic of all their forms. The effect of multiphase concentrations of cytokinins on seed germination, seedling development and root growth of rapeseed and tomato plants was studied [1].

Gibberellins are synthesized mainly in green leaves, and partial synthesis in roots has been found. Their most important and widespread property is that they have a stimulating effect on the growth and development of plants and have an active growth-promoting property. Like auxins, gibberellins also increase the yield of all types of crops. The main difference between gibberellins and auxins is that they participate in the processes of division and reproduction of meristem cells, awaken seeds from dormancy, ensure the development of the shoot, soften the endosperm layers and activate the synthesis of substances contained in them. [2].

Micromycetes Phoma glomerata LWL2 and Penicillium sp. LWL3 were isolated from the rhizosphere. It was found that endophytic fungi of this species synthesize high amounts of GA (GA1, GA3, GA4 and GA7) and indolyl acetic acid (IAA). It was found that the synthesized phytohormones had a positive effect on the growth and development of cucumber plants and increased the tolerance of plants to some abiotic stresses, such as salinity and drought, compared to the control. [3].

It has been found that the use of indolyl acetic acid, synthesized by the rhizosphere fungus Gibberella fujikuroi, increases olive yield, improves the quality of the resulting crop, and reduces tree disease. [4].

Among micromycetes, the most widespread auxin-synthesizing species are Fusarium, Rhizoctonia, Rhizopus, Absidia, Aspergillus, and Penicillium. Cytokinins have also been found to be actively synthesized by several other fungi, including Dictiostellium, Fusarium, Paxillus, Phoma, Rhizopogon, Schizophyllum, Suillus, Taphrina, Trichoderma, and Uromyces. [5].

Abiotic stress conditions present in nature can cause problems in the cultivation of agricultural products and obtaining high yields from them. It was found that the fungal strain Penicillium spadiceum AGH786 has a stimulating effect on the development of soybean plants and increases their tolerance to salinity. It was also found that the culture fluid of the fungal strain contains biologically active phytohormones (GAs, ABA, JA and SA). After inoculation of plant seeds, their germination was accelerated, and at the initial stages of development, the strength of the stem, the area of the leaf blade, and the ability to withstand salinity were found. [6]

In nature, microbial producers of cellulose and hemocellulose play a significant role in the production of various fungal strains of the genus Trichoderma, which is associated with their high secretion capacity, however, the Trichoderma enzyme complex has shortcomings, in which they have low cellulose activity and a low content of β-glucosidases. In contrast to Trichoderma, fungi of the genus Penicillium have been found to efficiently degrade cellulose and cellulosic waste, and to synthesize large amounts of cellulose and hemocellulose by enzyme complexes. Individual enzymes synthesized by micromycetes have a high degree of stability in performing their activity [7].

The growth potential of Trichoderma strains on plant substrates - wood processing and hydrolysis industry wastes - was assessed. It was found that the morphological parameters of growth and the intensity of spore formation of Trichoderma strains are closely related to natural substrates. A biotechnology for the mass production of a biopreparation for plant protection using wood processing industry wastes has been developed [8].

Auxins act as a signal that warns plants of biotic and abiotic factors. They are phytohormones necessary for the life of plants, regulating their growth and development. There are several forms of auxins in nature, the most widespread active form is indole acetic acid (ICA). Even small amounts of indole acetic acid are important for plant development. In agriculture, the use of rhizosphere micromycetes to increase the yield of grain crops in the cultivation of barley, wheat or rice is advisable. The use of the UV-vis method is effective in determining phytohormones synthesized by fungi [9].

When plants were inoculated with a culture medium of T. harzianum and fungal strains grown for 7 days, root development and nutrient uptake significantly increased (+76 and +61%). The content of indolyl-3-acetic acid, trans-zeatin riboside, dihydrozeatin riboside, GC and abscisic acid was analyzed 10 days after inoculation. After inoculation with culture fluids of micromycete strains, the content of ISK and GC increased by 49+71% in plant leaves and by 40+143% in roots. Trans-zeatin riboside decreased by 51% in leaves and by 37% in roots. The results of the study showed that micromycete strains synthesize several types of phytohormones, and these phytohormones ensure the strong development of the plant root system and activate its growth. [10].

Micromycetes isolated from the root rhizosphere of gray soils and agricultural crops (maize, wheat, cotton) of the Tashkent region and available in the collection of the Laboratory of Biological Processing of Plant Raw Materials were screened for their plant growth properties using the hypocotyl method. For this purpose, 8 micromycetes belonging to the Trichoderma genus were grown in liquid Mandels [11] and Chapek [12] nutrient media for 10 days and the culture fluids were filtered. The effect of the filtered culture fluids on wheat and maize coleoptiles was studied. For this purpose, micromycetes were grown in mineral nutrient media Mandels (pH 5.5-5.8) and Capek (pH 6.5-6.8) at a temperature of 28-30°C and a rotation speed of 200-220 times per minute for 10 days, and their culture liquid was separated from the biomass by filtration. The culture liquid was exposed to 1 cm long coleoptiles of wheat and maize. After 24 hours, the length of the coleoptiles was measured. Also, the effect of the culture liquid of the selected Trichoderma sp-55 fungi on the germination, growth and development of cotton, wheat and maize seeds was studied in microvegetation and vegetative experiments. The fungi were grown in liquid nutrient media for 6 days as described above. The extracted culture fluids and culture fluids diluted in the ratios of 1:50, 1:100, 1:300, 1:400, 1:500 were applied to plant seeds, that is, the seeds were inoculated for 18 hours. In the control variant, water and Capek nutrient medium were used to treat the seeds. 50 plant seeds were taken for each experimental variant. The experiment was carried out in three replicates. The seeds were germinated in a Petri dish in a thermostat at a temperature of 250C for 48 hours and the germination percentage was determined [13].

As is known, in order to achieve efficiency in the use of microorganisms, it is necessary to correctly select the nutrient medium and growth conditions. It is especially important to pay attention to economic efficiency in the development of commercial preparations. Therefore, it is advisable to use industrial waste to make the nutrient medium cheaper.

3. Results And Discussion

Many authors have found that Mandels medium is optimal for the growth of fungi of the T. harzianum 55 genus, and Capek medium for T. harzianum 54 and T. asperellum genera. Therefore, in our studies, Mandels medium was used for the growth of T. harzianum 55 strain, Capek medium for T. harzianum 54 and T.

asperellum 12 strains. However, in order to increase the production of phytohormones by these strains, the composition of the medium was changed in order to enrich and reduce the cost of the medium. That is, Mandels and Capek nutrient media were prepared with 2% sucrose and 2% other carbon sources (molasses, cottonseed meal powder, crushed cotton leaves, and corn extract), and strains were grown in them and their production of GC and indole compounds was studied. The results of the study showed that the carbon sources added to the nutrient media significantly affected the growth of fungi and the activity and amount of phytohormones synthesized. It was found that all three strains produced the most active GC and indole compounds in nutrient media enriched with corn extract and molasses. In the control variant, the amount of Indole bricks synthesized by T. harzianum 55 was 0.559 mg/ml and the amount of GC was 0.172 mg/ml in these feed media, the amount of Indole bricks synthesized by T. harzianum 54 was 0.418 mg/ml, the amount of GC was 0.189 mg/ml, and the amount of Indole bricks synthesized by T. asperellum 12 was 0.518 mg/ml and the amount of GC was 0.194 mg/ml (see Figure 1). When molasses was used as a carbon source in the enrichment of the feed media, although GC and Indole bricks were produced in slightly smaller amounts than in the corn extract, the use of industrial waste molasses was relatively economical, so its use in the enrichment of the feed media was determined in subsequent studies.

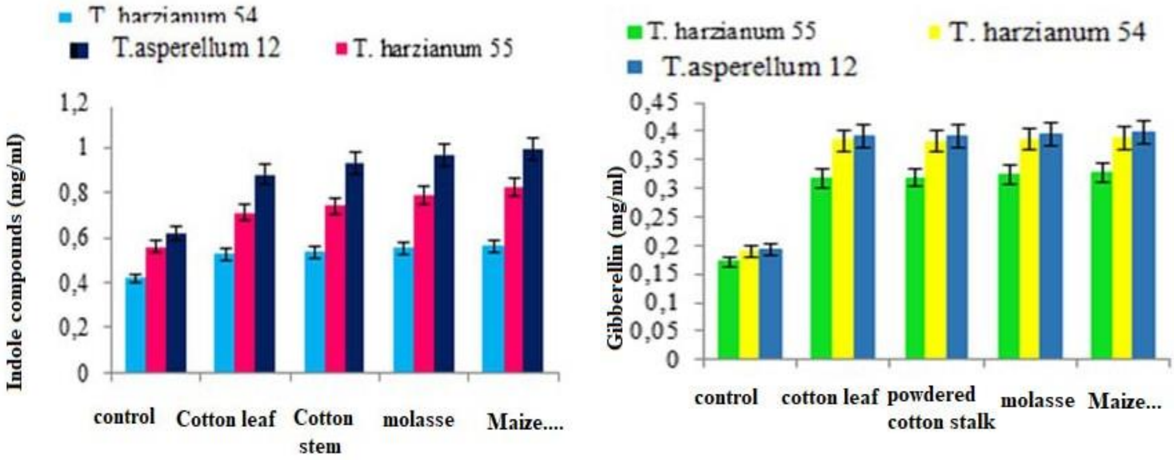

Figure 1. Effect of different carbon sources on the production of ISC and GC of T. harzianum UzCF-55, T. harzianum UzCF-54, T. asperellum 12 strains

The effect of pH and temperature on the production of ISC and GC of T. harzianum UzCF-55, T. harzianum UzCF-54, T. asperellum strains was studied. For this purpose, the strains were grown in nutrient media with a pH value of 4.0 to 7.5. It was found that the cultures grew well from pH 4.5 to 7.0 and synthesized phytohormones to a certain extent. In media with an initial pH value of 4.0, T. harzianum 55 fungus grew weakly, produced very small amounts of phytohormones, and did not produce phytohormones, although it grew weakly at pH 7.5. At a pH value of 4.0, the growth of T. harzianum UzCF-54 and T. asperellum strains was not observed. In the nutrient medium with an initial pH value of 7.5, the phytohormones produced by the strains were low, but had a certain value (see Table 1). It was found that for good growth of T. harzianum 55 and the activity of the synthesized phytohormones, the initial pH value of the nutrient medium was 5.0-5.5, and for T. harzianum UzCF-54 and T. asperellum, the initial pH value of the nutrient medium was 6.5 and 6.8 (see Figure 2).

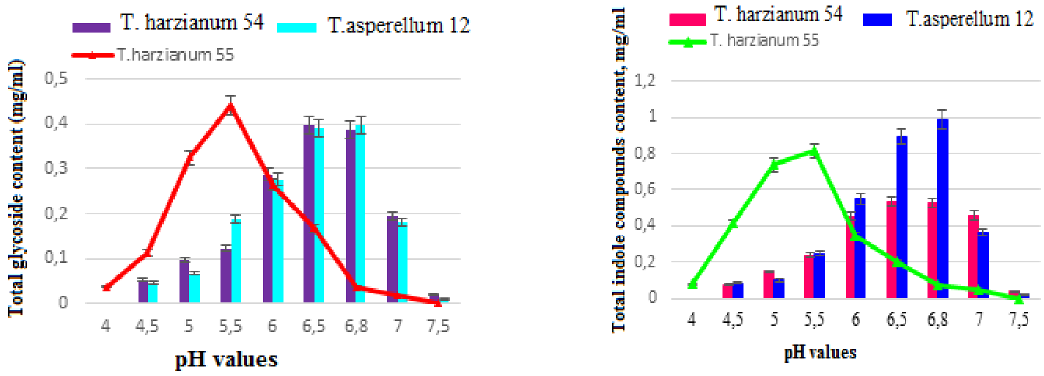

Figure 2. The effect of the initial pH value of the nutrient medium on the formation of ISK and GK of the studied strains

The effect of temperature on the production of phytohormones by micromycetes was studied. The fungi were grown at temperatures ranging from 20°C to 45°C. As a result, the highest value of phytohormone activity was detected in the range of 28-30°C, and a decrease in the activity of the studied phytohormones was observed with a decrease in temperature to 20°C and an increase in temperature to 45°C (see Table 1).

Table 1. The effect of temperature on the formation of ISK and GK of the studied strains

№	Temperature,°C	*T. harzianumUz CF-55*		**T. harzianum UzCF-54**		**T. asperellum 12**	
		ИСК, мг/мл	ГК, мг/мл	ИСК, мг/мл	ГК, мг/мл	ИСК, мг/мл	ГК, мг/мл
1	20	0,133	0,092	0,143	0,086	0,153	0,091
2	25	0,291	0,297	0,246	0,241	0,544	0,257
3	28	0,813	0,441	0,533	0,396	0,896	0,390
4	30	0,738	0,435	0,528	0,386	0,987	0,395
5	35	0,154	0,111	0,457	0,195	0,365	0,182
6	40	0,126	0,097	0,097	0,093	0,099	0,085
7	45	0,078	0,086	0,041	0,037	0,059	0,044

To determine the maximum amount of ISC and GC production of the studied strains on which day, the cultures were grown in liquid nutrient medium for up to 10 days. The study used nutrient media with sucrose and molasses added in amounts from 1%+1% to 5%+5% as carbon sources. According to the results, in all nutrient media, the maximum amount of ISC synthesis was found on days 6 and 7, and GC synthesis on days 3, 6 and 9. Below is information on the dynamics of ISC and GC production of T. harzianum UzCF-55, T. harzianum UzCF-54, T. Asperellum 12 strains grown in nutrient media with the addition of 2%+2% sucrose and molasses.

The highest amounts of ISK were synthesized in the T. harzianum UzCF-55 strain on days 7 and 6, amounting to 1.011 and 1.167 mg/ml, respectively. The same correlation was observed in the control, with ISK produced on days 6 – 0.749, on days 7 – 0.559 mg/ml. In the following days of the experiment, the amount of ISK decreased in both variants, and was slightly higher on days 4 and 9 (see Figure 2). Also, the highest amounts of GC were synthesized on days 3, 6 and 9, amounting to 0.271; 0.292 and 0.318 mg/ml, respectively. The same correlation was observed in the control, with GC produced on days 3 – 0.076, on days 6 – 0.087 and on days 9 – 0.172 mg/ml. In the following days of the experiment, it became clear that the amount of GK in both options decreases (Fig. 2).

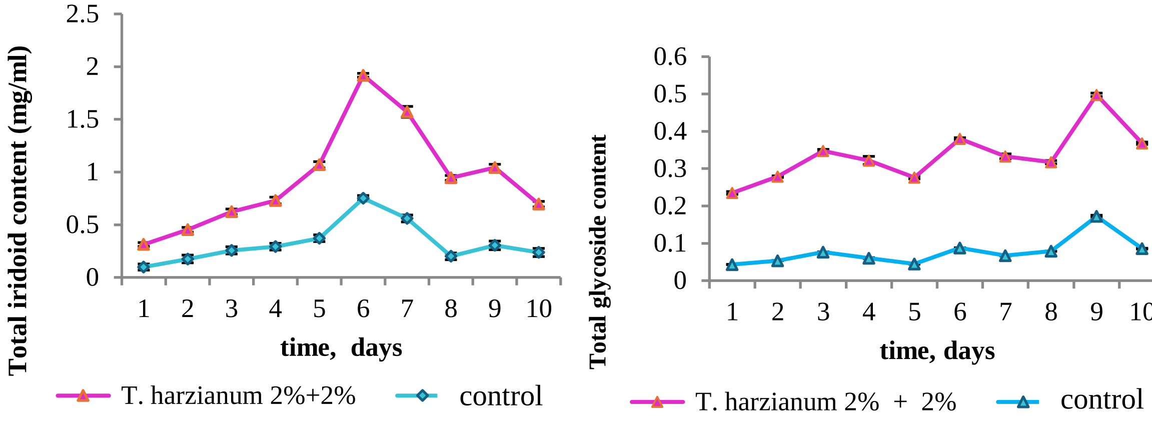

Figure 2. Amount of ISC and GC synthesized by T. Harzianum UzCF-55 strain on 2% sucrose + 2% molasses nutrient medium (day 10).

The T. harzianum UzCF-54 fungal strain synthesized the highest amount of ISC on days 7 and 6, which amounted to 0.601 and 0.635 mg/ml, respectively. The same correlation was observed in the control, with ISC produced in the amount of 0.475 and 0.418 mg/ml on days 6 and 7, respectively. In the following days of the experiment, the amount of ISC in both variants decreased, and was slightly higher on days 4 and 9 (Fig. 3). Also, GC was synthesized in the highest amount on days 3, 6 and 9, amounting to 0.266; 0.372 and 0.386 mg/ml, respectively. The same correlation was observed in the control, 0.143, 0.189 and 0.225 mg/ml were produced on 3 days, 6 days and 9 days, respectively. In the following days of the experiment, it became clear that the amount of GK in both variants decreases (Fig. 3).

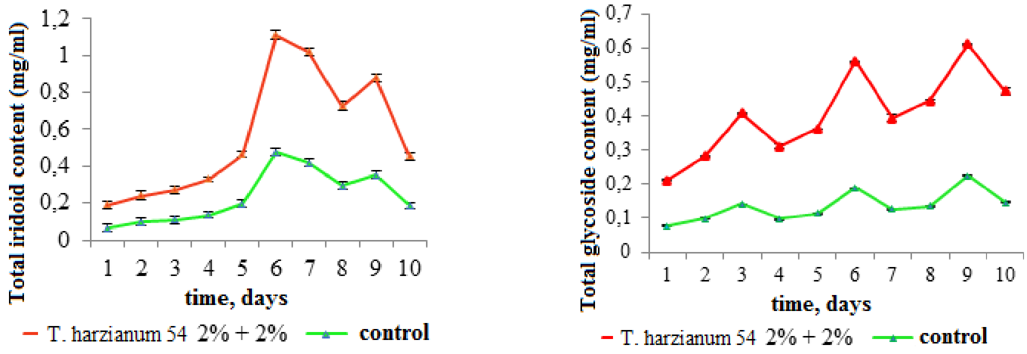

Figure 3. Amount of ISC and GC synthesized by T. harzianum UzCF-54 strain on 2% sucrose + 2% molasses nutrient medium (day 10).

The T. asperellum 12 fungal strain produced the highest amount of ISK on days 7 and 6, which amounted to 1.257 and 0.954 mg/ml, respectively. The same correlation was observed in the control, with ISK produced in the amount of 0.876 on days 6 and 0.518 mg/ml on days 7. In the following days of the experiment, the amount of ISK decreased in both variants, and was slightly higher on days 4 and 9 (Fig. 4). The highest amount of GC was synthesized on days 3, 6 and 9, amounting to 0.172; 0.202 and 0.396 mg/ml, respectively. The same correlation was observed in the control, with GC produced in the amount of 0.107 on days 3, 0.126 on days 6 and 0.194 mg/ml on days 9. In the following days of the experiment, it became clear that the amount of GK in both variants decreases (Fig. 4).

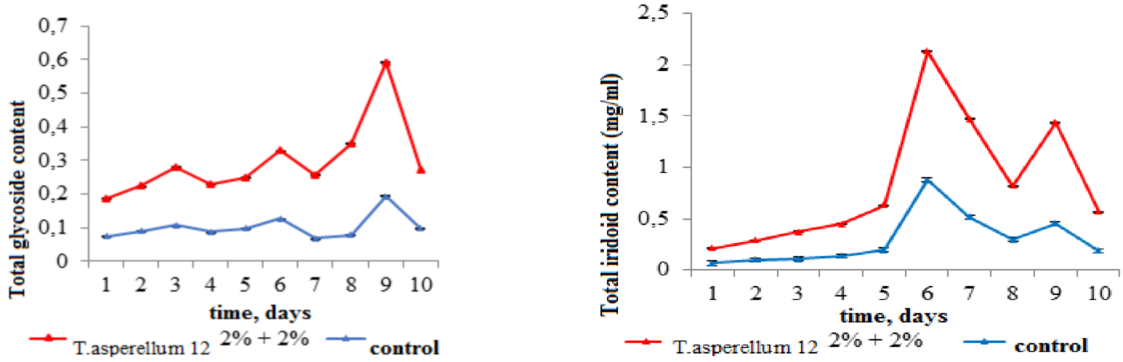

Figure 4. Amount of ISC and GC synthesized by T. asperellum strain 12 in a nutrient medium with 2% sucrose + 2% molasses (day 10).

In the study, when the effect of sucrose and molasses concentrations on the synthesis of phytohormones was studied, an increase in the production of phytohormones was observed depending on their concentration. The highest synthesis of phytohormones by all three strains was observed in nutrient media with the addition of sucrose and molasses in an amount of 1% + 5%, while the amount of indole compounds synthesized by the T. harzianum UzCF-55 strain was 3 times higher and the amount of GC was 4 times higher than the control, respectively (see Figure 5). It was found that the amount of ISK synthesized by the T. harzianum 54 strain was 2.3 times higher than the control, and the amount of GC was 3 times higher (see Figure 5), and the amount of ISK synthesized by the T. asperellum 12 strain was 3.4 times higher and the amount of GC was 4 times higher than the control (see Figure 5)

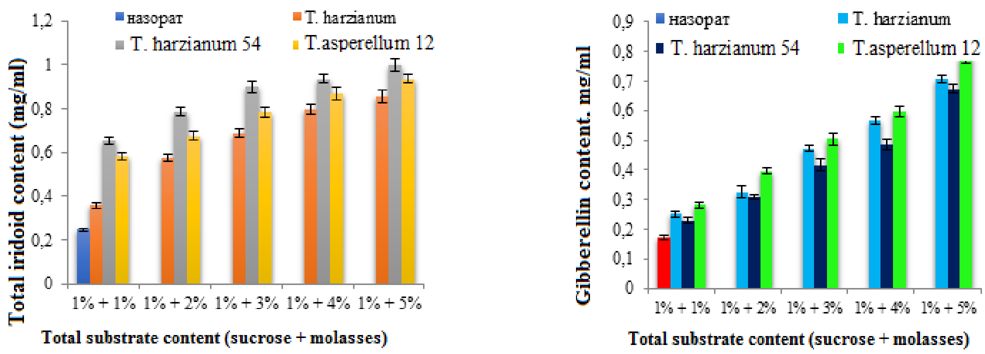

Figure 5. Effect of carbon source amount on indole and GC production of fungal strains T. harzianum 55, T. harzianum 54, T. asperellum 12

Thus, in molasses-enriched feed medium, the fungal strains T. harzianum 55, T. harzianum 54, and T. asperellum 12 produced significantly higher amounts of ISC and GC and were significantly different from the control on all days studied [15-16].

4. Conclusion

From the laboratory collection of micromycetes isolated from the rhizosphere of gray soils and root crops of the Fergana region, 8 T. harzianum UzCF-55, T. harzianum UzCF-54, T. asperellum fungi were selected and the growth properties of the plants were studied. It was found that T. harzianum UzCF-55, T. harzianum UzCF-54, T. asperellum strains have a higher growth effect than other strains of fungi.

For the production of biologically active substances phytohormones, indole compounds and GC in large quantities, Mandels and Chapek nutrient media enriched with molasses, which is an industrial waste, as a carbon source, are recommended for growing producers in liquid nutrient media. It is convenient to use Mandels (rN 5.5) and Chapek (rN 6.8) nutrient mediums at a concentration of 106-7 spores/ml for 6 days in the amount of 2%, and the cultivation temperature should be 28-30°C.

Word count:

The article was 3200 words long. This section includes the introduction, main body, and conclusions. Abstract, keywords, bibliographies, and graphics were not included.

Data Access Statement:

All the data used in this study are open and available. Key data supporting this study are included in the appendix of this article.

We have made the data, code, and methods fully available for the replication or extension of the analyses reported in this manuscript. For further information, please contact the author and send a request.

Ethical statement

This study did not include human subjects, animals, or tissues. Therefore, ethical approval was not required for this study.

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Declaration: This article is the result of independent scientific research by the author(s), and references are made to the work of others.

Conflict of intrest statement: The author(s) declare that there are no conflicts of interest in the conduct of this research.

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