

Original Research Article

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Stimulation of Plant Growth by Microbes Synthesizing Melanin

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ABSTRACT

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In this work, the effect of a new generation biopreparation (btmstr-biopreparation) based on the metabolites of the fungus *Trichoderma harzianum* and the bacterium *Bacillus thuringiensis* on the yield of mungbean plant was studied. According to the results of btmstr-biopreparation, prepared from the culture fluid of the *Trichoderma harzianum* sp.76 strain synthesizing ISC, as well as from a mutant strain of the bacterium *Bacillus thuringiensis* var. *thuringiensis* synthesizing melanin, showed the possibility of obtaining a harvest 77.4% more than the control variant (Btnms biopreparation), or 61.4% more compared to the template an option. During the research, it was found that under production conditions, various aphids, mustache, candela, grass moth, leaf beetles and fungi causing fusarium, as well as microbial objects causing bacterial spotting occur on the mungbean plant. Therefore, this biopreparation is recommended for use to increase the yield of agricultural plants and combat various diseases and pests.

Introduction

When growing agricultural products in our country, 20-30% of yields are lost as a result of various insect pests and microbiological diseases. Practical experiments have shown that from 10% to 50% of the crop of agricultural plants is lost as a result of the development of some extremely harmful microorganisms. Practical studies show that crop yields are achieved by increasing the

amount of mineral fertilizers and chemicals, which, in turn, leads to a sharp increase in environmental pollution. Currently, the main attention of scientists is focused on the creation of environmentally friendly, growth-controlling plant stimulants by biotechnological means and their widespread introduction into practice (Avetisyan *et al.*, 2013; Abdel-Razek *et al.*, 2013).

The main purpose of this research work is to study the effect of a new generation biopreparation (biopreparation

BtmsTr), created on the basis of the Scientific Laboratory of the Department of "Biotechnology" of TICHT using metabolites of the fungus *Trichoderma harzianum* and the bacterium *Bacillus thuringiensis*, on the yield of mungbean plants.

The *Trichoderma fungus* is widely distributed in nature and recorded as a microbial object, easily isolated in pure form, with very rapid biomass formation, and with a high level of biological activity compared to phytopathogenic fungi without damage to the plant (Grondona *et al.*, 1997). This fungus has been particularly recognized by scientists as an ideal biological target for controlling the growth and development of phytopathogenic fungi. Hence, it is hypothesized that -btmstr biopreparation developed as a new generation biopreparation exhibits biological activity against phytopathogenic microorganisms found in mungbean plant as it contains the culture fluid of *Trichoderma fungus*.

Furthermore, since 50% of the biopreparation consists of the culture fluid of a mutant strain of the melanin-synthesizing bacterium *Bacillus thuringiensis*, the fact that this biopreparation, in addition to its property of positively affecting plant growth and development, also stores Spore-crystalline toxins that have insecticidal activity against some of the insect pests found in them indicates that they have at least two scopes of action (Korobov and Kamenek, 2010; Reyes-Ramírez *et al.*, 2004). Also, as is often noted in scientific sources, the presence of strains of entomopathogenic bacteria *Bacillus thuringiensis* activity against various fungal and bacterial diseases of plants with antifungal and antibacterial effects may also be the reason for recognizing this bacterial fluid as one of the promising agents (Barboza-Corona *et al.*, 1999; Raymond *et al.*, 2010; Armada *et al.*, 2015; Kassogué *et al.*, 2015; Adounigna Kassoguél *et al.*, 2016).

Materials and Methods

Bacillus thuringiensis var, *thuringiensis*-M_{1st} (Khujamshukurov *et al.*, 2006) and *Bacillus thuringiensis* var. *thuringiensis* mutant strains -btms, as well as strain sp.76 *Trichoderma harzianum* stored in the collection of the Tashkent Institute of Chemical Technology, scientific laboratory of the Department of Biotechnology were used *Trichoderma harzianum* sp. Strain 76 was grown on a modified Mendels' nutrient medium, and the strain of the bacterium *Bacillus thuringiensis* was grown on a non-standard nutrient medium recommended for production (Khujamshukurov *et al.*, 2001).

When preparing a new composition of a biopreparation, work was carried out according to the following scheme: 1- Btms - *Bacillus thuringiensis* var. *thuringiensis* - M_{1st} - strain that does not synthesize melanin (cultivated for 72 hours, untreated, accepted as a control variant); 2- Btms-*Bacillus thuringiensis* var. *thuringiensis*-culture fluid of a mutant strain synthesizing melanin btms (cultured for 72 hours, accepted as a template version compared to the btmstr biopreparation); 3- Btmstr is a culture liquid of the *Trichoderma harzianum* sp.76 strain synthesizing ISC as well as a mutant strain of the bacterium *Bacillus thuringiensis* var. *thuringiensis* synthesizing melanin 76 (cultured from 72 hours, 1:1 ratio).

Experiments on the introduction into production were carried out using the example of a Mash plant planted as a second crop on the territory of the farm "Shabboda Shamoli" Bakht SIU, Zarbdor district, Jizzakh region. Plots with a total area of 6 hectares were selected for experiments.

Culture fluids were introduced by soaking the seeds for 4 hours before sowing the Mash. Also, after germination of the seeds, they were treated with leaf spraying using an OVH-600 sprinkler tractor at the rate of 200 liters per hectare in the presence of two leaves. When sowing, Masha used a tractor-seeder, which was used for sowing wheat.

Results and Discussion

The results obtained on the basis of the btms biopreparation (which does not synthesize melanin) are shown in Table 1. Under the action of the biopreparation btms (culture liquid), the average number of leaves of the Mash plant is 52. It can be seen that these leaves are on average 5.0 cm wide and on average 6.0 cm long. The length of the stem averaged 11.1 cm, and the length of the resulting thorns averaged 7.9 cm. It was found that the number of ears per plant corresponded to 33.7, and the number of immature ears was 9.8, that is, 29.0%.

It was noticed that the weight of the obtained grains is 11.6 grams. The effect of btms biopreparation (synthesizing melanin) - the development of the Masha plant is shown in Table 2. According to the results, it was noted that the average number of leaves is 117.3, which is 65 (55.52%) more than that of the biopreparation btms (which does not synthesize melanin). It was also found that the average width of the leaves is 6.54 cm, and

the height is 7.55 cm. This indicates that the leaves were 1.54 cm wide and 1.55 cm high compared to the results obtained on the basis of the biopreparation btnms (which does not synthesize melanin).

In addition, it was found that the number of spikes in Btms biopreparation is 70.38, which is 36.6 more than Btnms biopreparation, and the length of spikes is 2.05 cm. These indicators of the biopreparation -Btms have a huge impact on the yield of midges. In experiments, it was found that the grain weight under the action of the Btms biopreparation is 8.2 g higher than that of the Btnms biopreparation.

However, this small difference can be explained by the number of immature spikes in both variants. For example, in the btnms biopreparation, the average number of immature thorns was 9.8, and in the btms biopreparation – 18.25. Based on this indicator, it is possible to give a high assessment of the biological effectiveness of the Btms biopreparation. *There is Bacillus thuringiensis var. thuringiensis* is a culture fluid of a mutant strain synthesizing melanin btms and sp of the fungus *Trichoderma* synthesizing ISK.

The results obtained from the btmstr biopreparation prepared from the culture fluids of strain 76 at a 1:1 ratio are shown in Table 3. From the results obtained, it was observed that the number of leaves of Mash plant treated with BtmsTr biopreparation averaged 151.5, that is, 65.6% (99.4 pieces) more than Btnms biopreparation and 22.5% (34.2 pieces) more than Btms biopreparation. It was also found that the average width of the leaves is 8.0 cm, and the height is 9.0 cm.

This showed that, compared with the results obtained on the basis of the Btnms biopreparation, the leaves were 2.1 cm wider and the leaf height was 2.5 cm higher. According to the same indicators, it can be seen that the width of the sheet is 0.86 cm, and the height of the sheet is 0.95 cm higher than that of the Btms biopreparation.

In addition, the number of adhesions in BtmsTr biopreparation was found to be 115.8, i.e. 82.1 more than Btnms biopreparation and 45.42 more than Btms biopreparation. It was observed that plants treated with BtmsTr biopreparation had an average spike length of 11.8 cm, which was 3.9 cm longer than Btnms biopreparation and 1.85 cm longer than Btms biopreparation. These indicators, such as the ones above, are sure to have their effect on the yield of the plant.

Analyzing the data presented in Tables 1-3, it can be seen that in the plant variants treated with BtmsTr biopreparation, the average grain weight was 51.4 grams, which was 39.8 grams more than that of Btnms biopreparation and 31.6 grams more than that of Btms biopreparation. If we analyze the number of immature spikes, we can see that plant variants treated with BtmsTr biopreparation had 29.5 immature spikes, while they were 9.8 in Btnms biopreparation and 18.25 in Btms biopreparation. Since the number and weight of immature ears were not measured during the research, theoretically, if the grain of immature ears is counted, it can be recognized that even by this indicator the biopreparation BtmsTr has a positive effect on the yield of mungbean.

Based on these experiments, btmstr-a biopreparation prepared from the culture fluid of the *Trichoderma harzianum* sp.76 strain synthesizing ISC, as well as from a mutant strain of the bacterium *Bacillus thuringiensis var. thuringiensis* synthesizing melanin, showed the possibility of obtaining a harvest 77.4% more than the control variant (Btnms biopreparation), or 61.4% more compared to with a template version.

When comparing these data obtained on the basis of production experiments with data from scientific sources published internationally, it can be seen that they correspond to the results recognized by several scientists (Coyne and Al-Harathi, 1992; Hoti and Balaraman, 1993; van de Sande *et al.*, 2007; Liu *et al.*, 2013; Sansinenea and Ortiz, 2015).

These results showed that the results were significantly different from the results obtained at the farm “Jurabek”, located in the territory of Bakht SIU, Zarbdor district. In particular, in the studies conducted at the Jurabek farm according to the above scheme, the following results were noted: *Bacillus thuringiensis var. a mutant strain of the bacterium thuringiensis* synthesizing melanin, as well as SP of the fungus *Trichoderma* synthesizing ISK.

It was noted that the btmstr biopreparation prepared from culture fluids of strain 76 in a 1:1 ratio yields 82.0% more yield than the Mosh plant yield control variant (btnms biopreparation), or 60.4% more than the template variant. It was noted that studies using the same scheme conducted in two regions showed differences in the yield of the BtmsTr biopreparation compared with the control variant (Btnms biopreparation) by 4.6% or 1.0% compared with the template variant.

Table.1 The effect of the btnms biologics on the growth and development of the Mash plant

Measurement sources	Experimental options (for example, 10 seedlings taken every 10 meters in a staggered manner)										Average
	Btnms-1	Btnms-2	Btnms-3	Btnms-4	Btnms-5	Btnms-6	Btnms-7	Btnms-8	Btnms-9	Btnms-10	
Quantity of leaves, pieces	42	54	30	81	54	59	69	39	37	46	52,1
Width of the sheet, cm	4.7	5,8	4,7	5,1	4,28	5,6	6,1	5,2	4	4,55	5,0
Leaf height, cm	5.6	6,6	6,1	6,5	5,8	6,03	7,05	7,1	3,9	5,52	6,0
Root length, cm	10.1	8,5	17	8,7	10,8	10,5	10,5	12,3	8,8	13,7	11,1
Spike length, cm	6.9	8,8	7,39	7,6	9,6	8,7	6,8	7,8	7,57	7,7	7,9
Number of ears, pcs.	16	28	38	55	44	39	22	38	19	38	33,7
Quantity of unripe ears	0	9	16	10,8	19	13	9	21			9,8
Grain weight, g	42	54	30	81	54	59	69	39	37	46	52,1

Table.2 Effect of btms biopreparation on growth and development of mungbean plant

Measurement sources	Experimental options (for example, 10 seedlings taken every 10 meters in a staggered manner)								Average
	Btms-1-1	Btms-1-2	Btms-1-3	Btms-1-4	Btms-2-1	Btms-2-2	Btms-2-3	Btms-2-4	
Quantity of leaves, pieces	124	78	93	81	129	156	135	142,0	117.3
Width of the sheet, cm	5,6	6,6	6,8	6,5	6	6,8	6,8	7,20	6.54
Leaf height, cm	7,5	7,6	8,1	8,3	7,4	7,5	6,8	7,20	7.55
Root length, cm	11,1	8.2	12,5	13,2	21,7	33,6	19,82	22,45	17.82
Spike length, cm	8,3	10,5	9,1	10,5	10,2	7,7	12,5	10,80	9.95
Number of ears, pcs.	51	46	70	55	63	106	76	96,00	70.38
Quantity of unripe ears	8	33	17	17	27	12	14	18	18.25
Grain weight, g	18,35	8,02	20,26	19,21	10,12	35,76	18,9	27,8	19.80

Table.3 The effect of BtmsTr biologics on the growth and development of the Mash plant

Measurement sources	Experimental options (for example, 10 seedlings taken every 10 meters in a staggered manner)				Average
	BtmsTr-1	BtmsTr-2	BtmsTr-3	BtmsTr-4	
Quantity of leaves, pieces	152	168	132	154	151.5
Width of the sheet, cm	7,6	7,1	7,8	7	7.4
Leaf height, cm	8,1	7,5	8,8	9,7	8.5
Root length, cm	26,3	20,1	22,3	19,8	22.1
Spike length, cm	12,9	11,1	12,9	10,3	11.8
Number of ears, pcs.	164	97	133	69	115.8
Quantity of unripe ears	28	17	39	34	29.5
Grain weight, g	80,1	45,1	50,3	30,25	51.4

Considering that the results obtained both theoretically and practically repeat each other, BtmsTr-a biopreparation prepared from culture fluids of strain 76 of the *Trichoderma* fungus synthesizing ISC and melanin-synthesizing mutant strain *Bacillus thuringiensis* var. *thuringiensis*, in a ratio of 1:1, is environmentally friendly, exhibits plant growth control properties, allows to recognize it as a biopreparation with low cost and ease of use in practice.

Btmstr-a biopreparation prepared from the culture fluid of the *Trichoderma harzianum* sp.76 strain synthesizing ISC, as well as from the mutant Btms strain of the bacterium *Bacillus thuringiensis* var. *thuringiensis* synthesizing melanin, makes it possible to obtain a harvest 77.4% more than the control variant (Btms biopreparation), or 61.4% more compared to the template variant (Btms biopreparation).

Therefore, we recommend using this biopreparation to increase the yield of agricultural plants and to control various diseases and pests. During the research, it was found that under production conditions, various aphids, mustache, candela, grass moth, leaf beetles and fungi causing fusarium, as well as microbial objects causing bacterial spotting occur on the mungbean plant.

Treatment with the microbiological biopreparation BtmsTr should also be carried out at the moment when insect pests start attacking the plants. We believe that this will further increase the availability of BtmsTr, a microbiological biopreparation in agricultural practice.

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Institutional Review Board Statement

Not applicable.

Transparency

The authors confirm that the manuscript is an honest, accurate and transparent account of the study that no vital features of the study have been omitted and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Authors' Contributions

All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript. Razikova Malikaxon F.: Formal Analysis, Investigation, Data Curation, Writing –Original Draft, Writing – Review & Editing. Sayidxonov Toyirxon M.: Formal Analysis, Investigation, Resources. Dmitry V. Voitka.: Conceptualization, Formal Analysis, Investigation. Kuchkarova Dilafuz K. H.: Writing – Review, Investigation & Editing. Nortozi A. Khujamshukurov.: Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration, Funding Acquisition, Supervision.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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