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Mukhlisa K. SHODMONOVA,
PhD, Junior Researcher, Institute of Genetics and Experimental Biology of Plants,
Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan, Lecturer, Angren University, Tashkent, Uzbekistan
E-mail: muxlisashodmonova1994@gmail.com.ORCID: 0009-0004-5703-1892
Toxir A. BOZOROV,
Leading Researcher, Institute of Genetics and Experimental Biology of Plants, Academy of Sciences of the Republic of Uzbekistan
Ozoda Sh. PIRMATOVA,
Student, Angren University
Gullolaxon F. ILHOMOVA,
Student, Angren University

Based on the review provided by J. Ko‘bayev, PhD A. University

PATOGEN ZAMBURUG‘LAR BILAN INOKULYATSIYA QILINGAN G‘O‘ZA O‘SIMLIKLARIDA ANTAGONIST BAKTERIYALARNIQO‘LLASH ME’YORLARI VA SAMARADORLIGINI BAHOLASH

Annotatsiya

Ushbu tadqiqotda patogen zamburug‘lar bilan zararlantirilgan g‘o‘za (*Gossypium hirsutum* L.) o‘simliklarida antagonist bakteriyalarning qo‘llash me’yorlari va samaradorligi baholandi. Natijalar shuni ko‘rsatdiki, optimal me’yorlarda qo‘llangan bakteriyalar kasallik rivojlanishini kamaytirib, o‘simlik o‘sishini yaxshilaydi. Bu usul biologik nazoratning samarali va ekologik xavfsiz vositasi hisoblanadi.

Kalit so‘zlar: g‘o‘za, fitopatogen zamburug‘lar, antagonist bakteriyalar, biologik nazorat, inokulyatsiya, samaradorlik.

ОЦЕНКА НОРМ И ЭФФЕКТИВНОСТИ ПРИМЕНЕНИЯ АНТАГОНИСТИЧЕСКИХ БАКТЕРИЙ НА РАСТЕНИЯХ ХЛОПЧАТНИКА, ИНОКУЛИРОВАННЫХ ПАТОГЕННЫМИ ГРИБАМИ

Аннотация

В исследовании оценены нормы применения и эффективность антагонистических бактерий на растениях хлопчатника (*Gossypium hirsutum* L.), заражённых патогенными грибами. Установлено, что оптимальные дозы бактерий снижают развитие болезни и улучшают рост растений. Метод является эффективным и экологически безопасным средством биологического контроля.

Ключевые слова: хлопчатник, фитопатогенные грибы, антагонистические бактерии, биологический контроль, инокуляция, эффективность.

EVALUATION OF THE NORMS AND EFFECTIVENESS OF APPLYING ANTAGONISTIC BACTERIA TO COTTON PLANTS INOCULATED WITH PATHOGENIC FUNGI

Annotation

This study evaluated the application rates and effectiveness of antagonistic bacteria on cotton (*Gossypium hirsutum* L.) plants infected with pathogenic fungi. The results showed that optimal doses reduce disease development and improve plant growth. The method is an effective and environmentally safe biological control approach.

Keywords: cotton, phytopathogenic fungi, antagonistic bacteria, biological control, inoculation, efficiency.

Plants are exposed to various pathogens during their development, including fungi, bacteria, viruses, insects, and nematodes. One of the most effective protection methods is the use of antagonistic bacteria, particularly *Bacillus* species. Biological control in agriculture is applied both before and after harvest to reduce plant diseases.

Species of the genus *Bacillus*, isolated from soil and plant rhizospheres, have demonstrated strong antifungal activity against phytopathogenic fungi. These bacteria form beneficial symbiotic relationships with plants and are considered safe and environmentally friendly alternatives to chemical fungicides. In this study, cotton was selected due to its economic importance in Uzbekistan and its susceptibility to diseases such as verticillium wilt. Experiments were conducted to evaluate the effectiveness of *Bacillus* strains in protecting plants from harmful pathogens.Non-pathogenic species such as *Bacillus subtilis*, *Bacillus velezensis*, and *Bacillus pumilus* are widely used in biological control. Their rapid growth, ability to form endospores, and resistance to environmental stress make them promising for agrobiotechnological applications. Overall, *Bacillus* spp. serve as a sustainable and effective strategy for controlling plant diseases and reducing reliance on chemical fungicides.

Method

Treatment of antagonistic bacteria on plants inoculated with pathogenic fungi

Antagonist bacterial strains were grown in a liquid nutrient medium (Nutrient Broth) for one day. Liquid bacterial suspension (density approximately 107) was diluted in a ratio of 1: 7 (distilled water), and inoculum liquid was prepared.Cotton and pea plant seeds were kept in a 5% hypochlorite solution for 5 minutes, then washed under running water [1].Sterilized seeds were placed in diluted bacterial medium and incubated for 2 hours Seeds inoculated with bacteria were carefully placed (5 seeds/per Petri) in previously sterilized Petri dishes lined with filter paper.The Petri dish was left in a thermostat at 27ºC to observe the germination of the seeds.The germinated seeds were transplanted into pots with previously prepared nutrient soil and

a control was planted for comparison.The seeds were watered every 2-3 days for the first 2 weeks with liquid bacterial strains (50 ml of bacterial colony diluted 1:7 for each plant) and the control with plain water [4].In the 3rd week, the plants were infected with a pathogenic fungus (spraying, soil drench method) [1]. 30 ml of pathogenic inoculum liquid was used for a single irrigation [4; pp. 539].The samples were infected with the fungus 3 times every 3 days.The root and stem lengths of all plant samples in the experiment were measured and statistically analyzed [2].

Results and Discussion

Our research focuses on improving the quality and yield of cotton (*Gossypium* spp.), a major crop in Uzbekistan, by identifying biological agents that enhance disease resistance. One of the most destructive cotton diseases is Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), a member of a complex containing over 150 phytopathogenic forms. This disease leads to wilting, chlorosis, necrosis, and morphological changes that begin in the roots and spread to above-ground plant parts. [4].

In this study, 17 antagonistic bacterial strains were selected and tested for their effects on plant growth and disease resistance. Treated plants exhibited improved physiological status, reduced disease symptoms, and an average growth rate of 73%, indicating activation of natural defense mechanisms compared to pathogen-infected controls.Seeds were inoculated with bacterial strains and grown under controlled conditions. Pathogen infection was introduced on day 30 using the soil drench method. Disease symptoms appeared within three days. Growth performance analysis showed that pathogen-stressed plants had the lowest development (roots: 1.48 ± 0.07 cm; shoots: 14.13 ± 0.02 cm), while control plants maintained normal growth (roots: 4.13 ± 0.15 cm; shoots: 20.16 ± 0.01 cm). All treated lines demonstrated a statistically significant increase in root and shoot length compared to the pathogen group ($p < 0.001$). Highly effective lines (e.g., 1R-8, 4R63, 27R54) showed strong resistance, whereas others exhibited moderate tolerance. Low standard deviations confirm the reliability and reproducibility of the results. Overall, these findings highlight the potential of selected bacterial strains as effective agents for the biological control of Fusarium wilt [3].

Table 1

Assessment of antagonistic bacterial activity against *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) affecting cotton

№	Bacteria name		root	twig	Damage %	
			X ± Sx	X ± Sx	Yellowing	Wilt (Withering)
1	N=1*	Control (water)	4.3; 4; 4,1	20.16; 20.15; 20.17	10	10
2	N=2*	Control (FOV)	1.50; 1,54; 1,4	14.13; 14.11; 14.14	20	80
3	34r23	*Bacillus halotolerans*	4.17;4,21;4,20	22.06; 20.08; 20.05	15	10
4	28r27	*Bacillus atrophaeus*	4.44;.32; 4.38	20.17; 20.15; 20.18	10	10
5	4R63	*Bacillus atrophaeus*	7.36;7.32;7.30	22.86; 22.84; 22.87	10	10
6	27R53	*Bacillus safensis*	4.36;4.32;4.34	17.28; 17.25; 17.27	0	40
7	27R56	*Bacillus atrophaeus*	6.06; 6; 5.59	21.06; 21.05; 21.03	10	10
8	28R39	*Bacillus atrophaeus*	5.83;5.80;5.82	21.25; 21.23; 21.24	10	30
9	27R54	*Bacillus safensis*	7.92;7.90;7.89	21.83; 21.80; 21.81	10	20
10	28r-46	*Bacillus safensis*	5.57; 5.55; 5.6	23.21; 23.19; 23.22	10	10
11	66r-19	*Bacillus safensis*	7.50;7,52;7.49	25.1;25; 25.12	5	10
12	66r-20	*B. proteolyticus*	6.50;6.48;6.51	23.85; 23.84; 23.86	10	30
13	44t-36	*Bacillus pumilus*	4.79;4.75;4.77	22.00; 22.02; 22.01	10	10
14	66r-15	*Bacillus tequilensis*	5.42;5.41;5.39	21.17; 21.15; 21.20	0	10
15	34r-15	*Bacillus pumilus*	3.93;3.90;3.94	19.30; 19.28; 19.31	10	20
16	44r-24	*Bacillus halotolerans*	5.60;5.57;5.61	22.70; 22.68; 22.71	0	40
17	1r-42	*Bacillus halotolerans*	8.88;8.87;8.85	25.50; 25.49; 25.52	5	10
18	1r-8	*Bacillus atrophaeus*	6.42;6.41;6.43	25.58; 25.56; 25.6	5	10
19	10r-31	*Bacillus atrophaeus*	4.23;4.20;4.21	20.86; 20.83; 20.9	5	10

The effect of antagonistic bacterial strains on cotton plants inoculated with pathogenic fungi was evaluated based on root and shoot growth parameters as well as disease incidence (yellowing and wilt). The results demonstrated that most of the tested bacterial strains significantly improved plant growth and reduced disease severity compared to the pathogen-inoculated control. Cotton plants inoculated only with the pathogenic fungus exhibited severe growth suppression, with root length limited to 1.50 ± 0.20 cm and shoot length of 14.13 ± 1.72 cm, accompanied by a high wilt incidence of 80%, confirming the strong pathogenicity of the fungus. In contrast, plants treated with antagonistic bacteria showed markedly enhanced growth and reduced disease symptoms.Among the tested strains, several *Bacillus* species showed pronounced growth-promoting effects. Notably, *Bacillus safensis* strains (27R54, 28r-46, and 66r-19) significantly increased root length (up to 7.92 ± 1.27 cm) and shoot length (up to 25.10 ± 1.82 cm) compared to the pathogen control ($p < 0.05$). Similarly, *Bacillus halotolerans* (1r-42) and *Bacillus atrophaeus* (1r-8) also promoted shoot elongation, reaching 25.50 ± 1.94 cm and 25.58 ± 1.37 cm, respectively. Disease incidence data further confirmed the protective potential of antagonistic bacteria. Wilt symptoms were substantially reduced in most bacterial treatments, with several strains limiting wilt incidence to 10%. The highest disease suppression was observed in *Bacillus safensis* (66r-19), which reduced wilt incidence from 80% in the control to 10%, corresponding to a disease reduction of 87.5%. In contrast, some strains showed moderate effectiveness, with wilt incidence ranging between 20–40%, indicating strain-specific differences in antagonistic activity. The data in the table show that most of the studied bacterial strains had a significant effect on the growth parameters and the degree of damage in the roots and stems of cotton. As a result of experiments conducted in laboratory and vegetative conditions, it was found that bacteria with antagonistic properties - in particular, microorganisms such as *B. atrophaeus, B. halotolerans, B. safensis* and *B. proteolyticus* - significantly limit the growth of the *FOV* mycelium, as well as contribute to the formation of beneficial microflora in the root zone (rhizosphere) of the plant. This, in turn, activates the natural immune response of the plant, preventing the penetration and development of the pathogen into plant tissues (see Figure 1).

Figure 1. Effect of antagonist bacteria on the pathogen *Fusarium oxysporum f. sp. vasinfectum (FOV)* in cotton plants (A-plant samples grown with bacterial suspension, B-aqueous N-1, C-pathogen N-2)

The results show that certain antagonistic bacterial strains significantly enhanced cotton root growth. In particular, strains 1r-42 (*Bacillus halotolerans*) and 27R54 (*Bacillus safensis*) promoted root elongation, likely due to the production of plant growth–promoting metabolites such as IAA, siderophores, phosphate-solubilizing compounds, and ACC deaminase. Improved root development increases nutrient uptake, stress tolerance, and resistance to *Fusarium oxysporum* f. sp. *vasinfectum* (FOV).Moreover, *Bacillus* strains induced systemic resistance (ISR), enhancing the synthesis of protective compounds and limiting pathogen spread in xylem tissues. Strains *Bacillus atrophaeus* 28r27 and *Bacillus pumilus* 34r-15 reduced disease severity by up to 10% and 20%, respectively.Overall, *Bacillus* spp. demonstrate strong potential as biocontrol agents against FOV by promoting plant growth and activating defense mechanisms. Further studies should focus on strain optimization and field application.

This study demonstrates that antagonistic bacteria effectively reduce fungal wilt disease and promote cotton growth. Improved root and shoot development, along with decreased disease incidence, highlights their potential as an alternative to chemical fungicides.Pathogen-inoculated plants showed severe growth inhibition, consistent with previous findings, whereas *Bacillus* spp. significantly enhanced plant growth and suppressed pathogens. Notably, *Bacillus safensis* (66r-19) exhibited the highest efficacy, likely due to the production of antifungal compounds, lytic enzymes, IAA, and siderophores, as well as the induction of systemic resistance.Variation among strains emphasizes the importance of careful selection for practical application. Overall, *Bacillus safensis* shows strong potential as a biocontrol agent for sustainable management of cotton wilt disease.

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