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BIOLOGICAL CONTROL OF COTTON DISEASE BY BACTERIAL AGENTS

БИОЛОГИЧЕСКАЯ КОНТРОЛЬ С БОЛЕЗНЬЮ ХЛОПКА С ПОМОЩЬЮ БАКТЕРИАЛЬНЫХ СРЕДСТВ

PAXTA KASALLIGINI BAKTERIAL AGENTLAR BILAN BIOLOGIK NAZORAT QILISH

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Аннотация

В этом исследовании изучается эффективность восьми бактериальных штаммов в борьбе с корневой гнилью хлопчатника, вызванной Fusarium oxysporum f, в условиях засоленной почвы. Саженцы хлопчатника очень восприимчивы к почвенным грибковым патогенам, что приводит к значительным потерям урожая, несмотря на использование фунгицидов. Бактериальные изоляты были получены из почв ризосферы хлопчатника и оценены их потенциал биоконтроля. Эксперимент заключается в инокуляции семян хлопчатника бактериальными суспензиями и выращивании их в зараженной Fusarium засоленной почве. Результаты показали, что четыре бактериальных изолята: Pseudomonas chromraphis TSAU13, Pseudomonas putida TSAU1, Pseudomonas putida 1T1 и Pseudomonas extremorientalis TSAU20 значительно снизили заболеваемость до 75% по сравнению с контролем. Эти результаты подчеркивают потенциал конкретных бактериальных штаммов в качестве эффективных средств биоконтроля против корневой гнили хлопчатника, устойчивой альтернативы химическим фунгицидам. Необходимы дальнейшие исследования для изучения генетического разнообразия микроорганизмов биоконтроля и их адаптации к конкретным сельскохозяйственным условиям.

Annotatsiya

Ushbu tadqiqot sho‘rlangan tuproq sharoitida Fusarium oxysporum f keltirib chiqaradigan g‘o‘za ildizlarini chirishga qarshi kurashda sakkiz bakteriya shtammlarining samaradorligini o‘rganadi. G‘o‘za ko‘chatlari tuproqdagi qo‘ziqorin qo‘zg‘atuvchilariga juda sezgir bo‘lib, fungitsidlar qo‘llanilishiga qaramay, hosilning sezilarli darajada yo‘qotilishiga olib keladi. Bakterial izolatlar paxta rizosferasi tuproqlaridan olingan va ularning bionazorat salohiyati uchun baholangan. Tajriba davomida g‘o‘za chigitlarini bakterial suspenziyalar bilan emlash va ularni Fusarium bilan zararlangan sho‘rlangan tuproqda o‘stirishdan iborat. Natijalar shuni ko‘rsatdiki, to‘rtta bakterial izolat, Pseudomonas chlororaphis TSAU13, Pseudomonas putida TSAU1, Pseudomonas putida 1T1 va Pseudomonas extremorientalis TSAU20, nazorat bilan solishtirganda kasallik bilan kasallanishni sezilarli darajada 75% gacha kamaytirdi. Ushbu topilmalar kimyoviy fungitsidlarga barqaror muqobil bo‘lgan paxta ildizi chirishiga qarshi samarali bionazorat agenti sifatida o‘ziga xos bakterial shtammlarning salohiyatini ta’kidlaydi. Bionazorat mikroorganizmlarining genetik xilma-xilligini va ularning muayyan qishloq xo‘jaligi muhitiga moslashuvini o‘rganish uchun keyingi tadqiqotlar kafolatlanadi.

Abstract

This study investigates the efficacy of eight bacterial strains in controlling root rot of cotton caused by Fusarium oxysporum f in salinated soil conditions. Cotton seedlings are highly susceptible to soil-borne fungal pathogens, leading to significant crop losses despite the use of fungicides. The bacterial isolates were sourced from cotton rhizosphere soils and evaluated for their biocontrol potential. The experiment involved inoculating cotton seeds with bacterial suspensions and growing them in Fusarium-infested salinated soil. Results showed that four bacterial isolates, Pseudomonas *chlororaphis TSAU13, Pseudomonas putida TSAU1, Pseudomonas putida 1T1, and Pseudomonas extremorientalis TSAU20, significantly reduced disease incidence by up to 75% compared to the control. These findings highlight the potential of specific bacterial strains as effective biocontrol agents against cotton root rot, offering a sustainable alternative to chemical fungicides. Further research is warranted to explore the genetic diversity of biocontrol microorganisms and their adaptation to specific agricultural environments.*

Key words: *Biocontrol, plant beneficial microorganisms, phytopathogens, cotton.*
Ключевые слова: *Биоконтроль, полезные для растений микроорганизмы, фитопатогены, хлопок.*
Kalit so'zlar: *Bionazorat, o'simlik uchun foydali mikroorganizmlar, fitopatogenlar, paxta.*

INTRODUCTION

Biological control has been described as the reduction of the amount of pathogen inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man [1,2]. According to this definition, organisms and procedures involved in biological control include: (1) avirulent or hypo-virulent individual or population within the pathogenic species, (2) antagonistic microorganisms and (3) effective resistance of the pathogen by the host plant through manipulation. Plant pathogens and insect pests, among others, are the populations within the pathogenic species that are targeted by biological control.

Over the past three decades studies on the use of beneficial microorganisms as biocontrol agents for plant protection have increased greatly. Several strains have been reported to show good performance *in vitro* and in specific trials, nonetheless, only few have demonstrated consistent and effective biocontrol in different field situations [3]. As a result only very few get to the market. Fravel [4] estimated the number of biocontrol products in the market as 1% of agricultural chemical sales.

Microbial antagonists include naturally occurring microorganisms that are antagonistic to crop pathogens, and have the potential to protect crop against the harmful effect of the pathogen, consequently providing an alternative to chemical fungicides [5]. Representatives of a range of bacteria and fungi and in a few cases nematodes have been identified as biocontrol agents (BCAs) against soil-borne plant pathogens; the most abundant soil and plant-associated bacterial genera among such groups are *Bacillus, Pseudomonas, Serratia* and *Streptomyces* [6].

Most strategies were based on pre-screening of microbes for their ability to produce anti-fungal metabolites [7]. This screenings resulted in the appearance of over one hundred bacterial biocontrol agents (BCAs) on the market. Very recently a method was developed to enrich for strains which efficiently colonize the root system [8]. This strategy appeared to enrich for biocontrol strains which do not produce antibiotics. This is an advantage since production of antibiotics is a disadvantage in the registration process. Some naturally occurring soil bacteria and fungi have demonstrated great potential to antagonize crop pathogens, hence, biological control involving the use of such plant beneficial microorganisms for plant protection is being considered as a viable substitute to reduce the use of chemical pesticides [9]. Biological control, based on microorganisms to suppress plant disease, offers a powerful alternative to synthetic chemicals [10].

Representatives of a range of bacteria and fungi and in a few cases nematodes have been identified as biocontrol agents (BCAs) against soil-borne plant pathogens; the most abundant soil and plant-associated bacterial genera among such groups are *Agrobacterium, Burkholderia, Bacillus, Pseudomonas, Serratia* and *Streptomyces* [11]. After application of microbiological control agents to the seed, these micro organisms must inhibit growth of the target pathogen to reduce infection by the pathogens and proliferate on the appropriate plant root surface [12]. Also, inoculation with some bacterial endophytes has been demonstrated to reduce disease incidence and symptoms of *F. oxysporum* in cotton [13] and *V. dahliae* in oilseed rape [14]. Seed coating with biocontrol bacteria in potato, radish, sugar beet and fruits has been shown to increase crop yield [15].

These plant beneficial microorganisms are known to antagonize phytopathogens through competition for niches or nutrients (e.g. iron through siderophores synthesis); parasitism that may involve production of hydrolytic enzymes, for example, chitinase, glucanase, protease and cellulase that can lyse pathogen cell walls; inhibition of the pathogens by anti-microbial compounds (antibiosis); induction of systemic resistance in host plants [16].

The chance of selecting effective biocontrol agents may be improved by isolating biocontrol strains from the same environment in which they are to be used. Hence, two widely used approaches to select for potential biocontrol agents focus first on isolation of antagonists from soils that are naturally suppressive to a particular pathogen (suppressive soils); the second approach comprises isolation from intended environment of use, such as soils, seeds or roots [17]. In many disease suppressive soils fluorescent *Pseudomonas* spp. were found to be plant disease control agents. They have a high growth rate relative to many other rhizosphere bacteria, possess various and diverse mechanisms for controlling plant growth and diseases, are easy growth under laboratory conditions and in batch cultures and can subsequently be reintroduced into the rhizosphere by seed bacterization [18].

In both pot and field studies, the administration of B. velezensis strain ELS2 and B. amyloliquefaciens strain ETL2 as foliar and seed treatments proved to be effective in preventing bacterial blight on cotton. When compared to chemical controls and chemical plant defense inducers in pot and field trials, plants treated with B. amyloliquefaciens strain ETL2 and B. velezensis strain ELS2 showed maximum plant growth and seed cotton output, and these treatments also improved disease control efficiency [19].

Biological control agents include antagonistic bacteria or metabolic products. studies investigated the inhibitory effects of two plant growth-promoting rhizobacteria (PGPR) strains, namely Bacillus tequilensis C-9 and *Sphingobacterium* A1, against *V. dahliae* in vitro and in the field. Antimicrobial assays using a mixed fermentation of two bacteria to inhibit *V. dahliae* were performed and showed that spore formation and germination and virulence protein of *V. dahliae* were reduced after treatment with the cell-free mixed fermentation broth. In field studies, a reduction in disease severity, incidence and index was observed after treatment at the bud and bell stages [20,21].

The complexity of the interactions involved in biocontrol and the wide range of environmental conditions found globally in agriculture make it unlikely that any one strain will suppress even a single disease in all settings. The genetic diversity of microorganisms with disease-suppressive potential remains a powerful yet largely untapped resource for biocontrol of plant disease [22]. There is a need to seek new biocontrol strains, particularly strains adapted to the site where they will be used [23].

Plant-growth-promoting microbes (PGPMs) help with efficient root colonization, compete with other soil microorganisms, stimulate host defense systems against pathogens, and promote plant growth through different mechanisms [24]. PGPMs have biocontrol agents (BCAs) called plant-growth-promoting rhizobacteria (PGPR) and plant-growth-promoting rhizofungi (PGPF) that fight crop diseases [25]. Earlier studies reported that *Bacillus*, *Pseudomonas*, *Actinobacteria*, and *Lactobacillus* have been used in different crop protection strategies. The biocontrol strains *B. pumilis* and *B. amyloliquefaciens* displayed many important characteristics, such as siderophore production, phosphate solubilization, IAA production, and antagonistic activity toward fungal pathogens, which could improve plant growth in terms of leaf number, biomass, and shoot length under field conditions [26]. However, at this moment, more research into the effective management of cotton bacterial blight by plant extracts in terms of concentration determination and development of biopesticides will provide future avenues to avoid environmental pollution [27].

The aim of this article is to evaluate the effectiveness of various bacterial strains in suppressing root rot of cotton caused by *Fusarium oxysporum* in salinated soil conditions. This involves isolating and screening beneficial microorganisms from cotton rhizospheres, particularly those adapted to suppressive soils, to identify potential biocontrol agents that can provide an alternative to chemical fungicides for managing cotton seedling diseases.

MATERIAL AND METHODS

Approximately one third of a seven day old PDA Petri dish culture of fungal pathogens was homogenised and used to inoculate 200 ml of Chapek-Dox medium in a 1 L Erlenmeyer flask. After growth for 3 days at 28^{o}C under aeration (110 rpm), the fungal material was poured over sterile glass wool to remove the mycelium and the filtrate, containing the spores, was adjusted to a concentration of 5 x 10^6 spores/ml. For soil infestation, spores were mixed thoroughly with salinated soil to 3.0 x 10^7 spores/kg soil. The cotton seeds were sterilised by immersion in 70% ethanol for 5 minutes and subsequently in 0.1% $HgCl_2$ for 1 min, washed several times with sterile water, and allowed to germinate for 4 days at room temperature. Subsequently, they were coated with bacteria by soaking them in a suspension of 1x10^8 CFU/ml bacteria in sterile PBS buffer whereas control seeds were soaked in sterile PBS buffer, both for 15 minutes. Seeds were dried in a sterile air stream. The soil used for pot experiments was selected from irrigated agricultural field affected by salinity from the Sayhunobod district. One seed was sown per plastic pot (9 cm diameter; 15 cm deep), each containing 300 g of salinated soil, at a depth of approximately 1.5 cm. Each treatment contained four groups of twelve plants. The plants were grown under open natural conditions at 21-24°C and were watered when necessary. The number of diseased plants was determined when 50 to 70% of the plants in the control without bacteria were diseased, usually four weeks after sowing. Plants were removed from the soil, washed and the plant roots were examined for foot and root rot symptoms as indicated by browning and lesions. Roots without any disease symptoms were classified as healthy.

RESULTS AND DISCUSSION

Cotton seedlings are vulnerable to attack by a number of soil-borne fungal pathogens, including *R. solani*, *Colletotrichum gossypii*, *F. oxysporum* f sp. *vasinfectum* and *Verticillium dahliae*. The resulting damping-off disease is widespread in most cotton-growing areas of the world, causing extensive crop losses [28] despite the widespread use of fungicides. A number of bacterial isolates collected from the cotton rhizosphere have been reported to be effective alternatives to commercial fungicides in suppressing disease caused by *R. solani, P.ultimum* and *F. oxysporum* f sp. *vasinfectum* [29].

In this work eight bacterial strains, were screened for their ability to suppress root rot of cotton caused by *F. oxysporum* f sp. *vasinfectum.* Fourty one percent of the plants which had grown in soil infested with *Fusarium* spores were diseased (Figure 3.1).

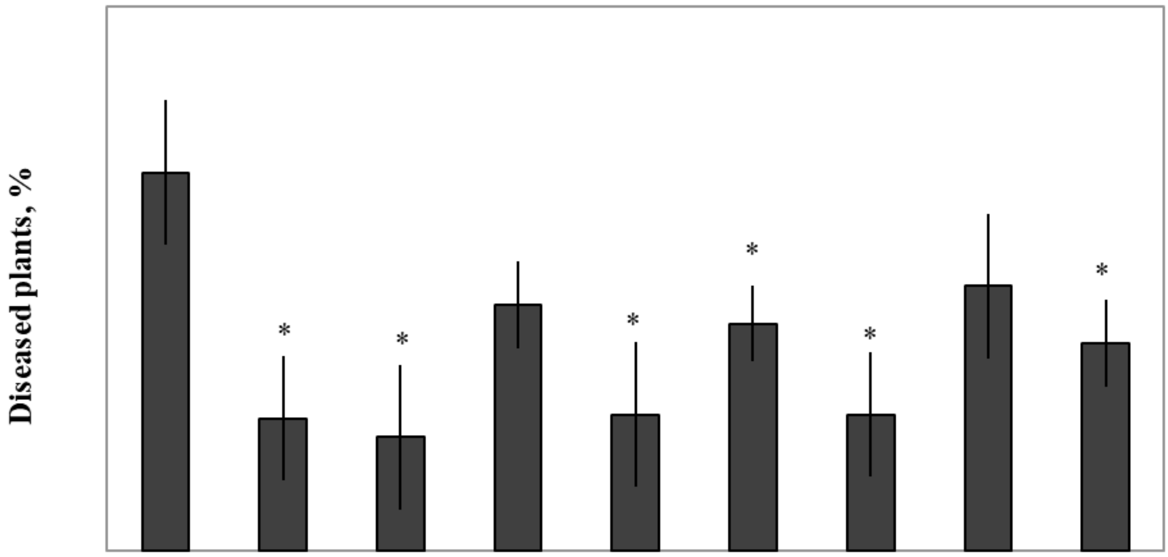

Figure 3.1 Control of cotton root rot in salinated soil by selected bacterial isolates (Treatments were: control in soil infested with *F. oxysporum* f sp. *vasinfectum* and experiments in infested soil using seeds treated with bacterial inoculants, *Significantly different from the control at P<0.05)

From the eight bacterial strains only four bacterial isolates *P. chlororaphis* TSAU13, *P. putida* TSAU1, *P. putida* 1T1 and *P. extremorientalis* TSAU20 showed statistically significant disease control (75%) in comparison to the *Fusarium*-infected control plants (Figure 3.1; 3.2). Other authors also reported that *P. fluorescens* CS85 isolated from the rhizosphere of cotton seedlings grown in suppressive soil in China has proven to be an effective biocontrol agent against cotton seedling diseases, both in the greenhouse and in the field**.** This strain, which does not inhibit the growth of the fungal pathogens *R. solani*, *C. gossypii*, *F. oxysporum* f sp. *vasinfectum*, or *V. dahliae* on agar plates [30], is thought to compete with these pathogens for binding to plant roots**.**

Figure 3.2 The biological control of cotton root rot caused by *F. oxysporum* by biological control agent *P. extremorientalis* TSAU20

CONCLUSIONS

In conclusion, the results of this study highlight the promising potential of using specific bacterial strains as biocontrol agents against cotton root rot in salinated soils. The significant disease suppression exhibited by certain strains underscores the importance of exploring microbial diversity for sustainable agricultural practices. By reducing reliance on chemical fungicides, these biocontrol agents offer a more environmentally friendly solution to managing crop diseases. Overall, this study provides valuable insights into harnessing the power of beneficial microorganisms for enhancing crop production and minimizing losses due to disease.

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