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Biocontrol of Cotton Pathogens Using Soil Antagonists

Rustam N. Mannanov* • **Rano K. Sattarova**

Department of Agricultural Biotechnology and Phytopathology, Tashkent State Agrarian University 100140 Tashkent, Republic of Uzbekistan

Corresponding author: * **rustam.mannanov@mail.ru, sattarova-rano@mail.ru**

ABSTRACT

In field trials, the biological efficacy of pre-sowing cotton seed treatment with the antagonist bacteria *Bacillus subtilis* 23 was studied on upland variety of cotton *Gossypium hirsutum* C-6524. The strain showed significant ($P < 0.05$) repression of bacterial blight and damping-off of cotton whereas infection of cotton seedlings by *R. solani* and *X. malvacearum* was reduced from 98 to 35% and from 92 to 37%, respectively. Use of *B. subtilis* 23 resulted in an increase of cotton yield by 26 and 27% in the presence of bacterial blight and root rot pathogens, respectively. The highest control efficacy (64%) was recorded for *Bacillus subtilis* 23 against *Rhizoctonia solani*.

Keywords: *Bacillus subtilis*, biocontrol, cotton diseases

INTRODUCTION

Cotton pathogens may dramatically decrease yield, so disease control plays a very important role in cotton cultivation (Hillocks 1992; Ismailov 1996; Gnanamanickam 2002). Uzbekistan is the world's fifth largest cotton grower, and second after the USA in terms of cotton exports, growing cotton on 1.4 million ha. In this respect, cotton disease management is of high economic significance. Chemical pesticides have been a traditional method used to protect the crop from diseases. However, growing public and scientific concern about the presence of synthetic pesticides in the food-chain and in the environment has led to great interest in biological control as a means of plant protection (Hanson 2000; Vinale *et al*. 2008). Many studies reported that a number of microbial isolates has proven to be an effective biocontrol agent against cotton root diseases caused by *Fusarium*, *Rhizoctonia*, *Verticillium* and *Pythium* (Safiyazov *et al*. 1995; Brunner *et al*. 2005; Demin 2006; Shumilina *et al*. 2006; Erdogan and Benlioglu 2010). Selecting agents based on prescreens for antibiosis in *vitro* has led to the discovery of many biocontrol agents, some of which had been shown through mutational analysis to provide control through antibiosis *in vivo*. There are various working hypotheses for effective biocontrol of fungal cotton pathogens: from necessary endophytic colonization of the cotton plant to evaluation of epiphytic colonization of plant surfaces (Griffin *et al*. 2005).

A number of bacterial isolates collected from the cotton rhizosphere were as effective as commercial fungicides in suppressing seedling disease pathogens *R. solani* and *P. ultimum* on cotton in the field (Hagedorn *et al*. 1993). Analysis of laboratory and small-plot trials on biocontrol of cotton pathogens indicates the effective biocontrol of early cotton phytopathogens *Xanthomonas malvacearum* and *Rhizoctonia solani* using soil bacterial antagonist *Bacillus subtilis* 23 by means of pre-sowing treatment (Safiyazov *et al*. 1995). In this article, we report the results from our field trials aiming to evaluate the biocontrol activity of tested bacterial antagonist *B. subtilis* 23 against two serious cotton pathogens *X. malvacearum* and *R. solani*, with the hope of developing a complex biological product to control cotton pathogens.

MATERIALS AND METHODS

Strains

Antagonistic bacteria *Bacillus subtilis* 23 was kindly provided by the Institute of Microbiology of Academy of Sciences of the Republic Uzbekistan and were grown on glucose-peptone agar (GPA) containing 10 g peptone, 10 ml glycerol, 5 g NaCl and 20 g agar in 1000 ml of water. Liquid cultures of the antagonists were grown on glucose-peptone broth (GPB) which is GPA without agar. Phytopathogenic microorganisms (*Xanthomonas malvacearum* and *Rhizoctonia solani*) were isolated from infected cotton plants collected in plantations of the Tashkent region. To confirm their pathogenicity, a preliminary infection of cotton seeds under laboratory conditions was made. Pathogenic microorganisms were grown on potato agar (PA) prepared from 400 g of potatoes boiled in 1 l of water for 20 min, then passed through cheesecloth. To the filtrate, 30 g sucrose and 20 g agar were added.

Seed treatment

Seeds of widely used Uzbekistan industrial upland cotton cultivar 'C-6524' were surface sterilized in concentrated H_2SO_4 for 3 min, thoroughly rinsed in sterile water and soaked in an antagonist cell suspension for 18 h. The cell suspension was prepared by growing the antagonist on GPB at 25-28°C for 3 days and by centrifuging the liquid culture (8000 rpm, 10 min). The pellet obtained was re-suspended in tap water with a cell titer of 1-1.5 × 10^9 cells/ml. The dosage was equal to 4 l of suspension/ton of cotton seeds.

Field trials

Field trial for evaluating the biocontrol ability of *Bacillus subtilis* 23 against *X. malvacearum* and *R. solani* were performed in the BO'Z-SUV BIOZERNO farm of Zangiata district, in the Tashkent region. The plot size was 20 m^2 for each of the 4 replications. Seeds were sown in mid-April, and yield of harvested cotton fiber was recorded mid- to late-September.

For cultivation of the bacteria, 750-ml flasks containing 100 ml of Corn-molasses medium, were inoculated with 5 ml of a bacterial suspension and grown in a shaker (220 rpm) at 28°C for 48 h. These flasks were used for inoculation of 5-L bottles, each containing 1 L of growth medium. After cultivation in a shaker (220 rpm) at 28°C for 48 h, the cell suspensions were aseptically transferred into sterile 10-L canisters and stored at + 5°C. Coated and

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Short Communication

Table 1 Effect of pre-sowing treatment with cell suspension of *Bacillus subtilis* 23 on cotton fiber yield.

Treatment	Infection (%)	Biocontrol efficacy (%)	Yield (t/ha)
Control (*X. malvacearum)*	92.5	--	0.22
X. malvacearum + *B. subtilis* 23	37.5	59.4	2.62
X. malvacearum + *B. subtilis* N	42.6	53.9	2.24
Control (*R. solani*)	97.6	--	0.14
R. solani + *B. subtilis* 23	35.2	63.9	2.74
R. solani + *B. subtilis* N	41.6	57.4	2.34
LSD between means ($P = 0.05$)	12.6	17.4	0.7

uncoated seeds were sown in field plots. The soil is a calcareous serozem with 2.4% organic matter, N 0.1%, P 1.34%, K 7.1%. The pH is 7.8. Weeds were removed by hand and plots were irrigated after visual inspection of plants. Yield was calculated after five months as t/ha. *B. subtilis* N, isolated from the bio product Khlopkosporin (Karimov 1993), was used as the standard. Biological efficacy was calculated by the method of Dement'eva (1977): (Rk - Ro) × 100/Rk (%), where Rk = disease development in the control and Ro = disease development in trial variant. The plants in true leaf stage were examined for foot and root rot symptoms as indicated by browning and lesions. Angular leaf spot with red to brown borders in cotton indicated bacterial blight infection.

Statistical analyses

All experiments were carried out in 4 replications. Standard deviations and LSD between means were conducted according to Dospekhov (1985).

RESULTS AND DISCUSSION

This research is a follow-up of laboratory investigations initiated in 1994 (Safiyazov *et al.* 1995). Subsequent to the results of previous study, we continued research with field trials using the most effective antagonist *B. subtilis* 23 in the form of a cell suspension, in water, as described above, to control *R. solani* and *X. malvacearum*, showing significant biocontrol of this antagonist (**Table 1**). The strain showed significant ($P < 0.05$) repression of bacterial blight and damping-off of cotton whereas infection of cotton seedlings by *R. solani* and *X. malvacearum* was reduced from 98 to 35% and from 92 to 37%, respectively. Use of *B. subtilis* 23 resulted in an increase of cotton yield by 26 and 27% in the presence of bacterial blight and root rot pathogens, respectively. The *B. subtilis* N strain, used as standard, positively influenced the reduction of infection; however, the biological efficacy of *B. subtilis* 23 was higher. The highest control efficacy (64%) was recorded by *B. subtilis* 23 against *R. solani*.

Several authors reported that microbial bacterial inoculants such as *Pseudomonas fluorescens*, *Bacillus* spp., *Burkholderia* cepacia isolates can effectively control *R. solani*-induced damping off of cotton seedlings both in the laboratory and field conditions (Wather and Gindrat 1988; Zaki *et al.* 1998; Wang *et al*. 2004). In other studies, Salah Eddin *et al*. (2007) reported that seed treatment followed by foliar application of *P. fluorescens* Pf1 significantly reduced the incidence of bacterial blight and recorded the percent disease index of 14.5 as against 43.8 in control.

Overall, our laboratory and field experiments proved the biocontrol activity of *B. subtilis* 23 against four cotton pathogens and allow us to recommend this antagonist for developing a bioproduct for industrial application as a means of pre-sowing seed treatment. Further studies of the antagonist's activity on other agricultural crops and in both directions – stimulation of growth and pathogen inhibition – could lead to the development of products, either synthetic or prepared from microbiological cultures, for use in the field.

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