

The effectiveness of the biological preparation "Ivan Ovsinsky-fulvohumate" on the cotton variety "Bukhara-6" in the conditions of irrigated meadow-alluvial soils of the Bukhara region

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Abstract. Growing high quality crops in the country depends on the fertility of the soil, the amount of humus in it and beneficial microflora. In recent years, Fusarium wilt disease caused by the soil fungus *Fusarium oxysporum* has been observed in some crop growing areas to manifest as a "flat" disease. Fusarium wilt has been known abroad and in Uzbekistan for many years. Fusarium wilt has been associated with 1-2 % damage to fine-grained cotton varieties (*Gossypium barbadense*) in previous years, but medium-fiber cotton varieties (*Gossypium hirsutum*) have also become affected in recent decades.

1 Introduction

There are reports of Fusarium wilt causing significant economic damage to the US states of California, Louisiana and Australia. Fusarium wilt affects cotton from germination to maturity. In affected cotton, the supply of water and nutrients is disrupted. When the diseased stem of the plant is cut away, the conductive tissue is greatly darkened. The fungus releases toxins from itself, which leads to poisoning of plants. During the growing season, a pathogenic fungus can spread from one field to another through irrigation water, soil adhering to tillage equipment and machinery. Pathogenic fungus develops in the soil for a long time (10 years or more), is highly resistant to changes in weather temperatures, humidity and the effects of antagonistic microorganisms in the soil. Fusarium wilt fungus can accumulate in seeds and thus enter the field where the soil is not damaged. Fusarium wilt (wilt) of cotton caused by a soil fungus, a facultative parasite *Fusarium oxysporum* Schlecht.: Fr. f. sp. *vasinfectum* W.C. Snyder et H.N. Hansen, is one of the most common and very harmful diseases of fine and medium staple cotton in the world, including in Uzbekistan. Due to the way of life of the pathogen - the ability to remain on the roots of many vegetative plants, on their remains and in the soil at rest for a long time - the fight against Fusarium wilt is a very difficult task. When cotton seedlings are affected by Fusarium wilt in the development phase

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of 4–6 leaves, the plant dries quickly in 3–5 days. In the future, in affected plants, growth slows down and cases of decay are not uncommon.

In Uzbekistan, varieties of medium staple cotton (*Gossypium hirsutum* L.) are mainly cultivated, among which there are no wilt-resistant varieties. Other control measures, including agrotechnical (crop rotation, etc.), chemical (seed treatment with fungicides, spraying of vegetative plants) and others, are ineffective against cotton Fusarium wilt or have not been studied enough. Therefore, the testing of various methods of control, including the search for effective fungicides-treaters of cotton seeds against this disease, is a very topical and urgent task. The purpose of this study was to test and evaluate the effectiveness of fulvohumate with trichodermin Ivan Ovsinsky, a liquid low molecular weight humic biofertilizer-chelator (humate, potassium). Developer and manufacturer: Research and Production Association "Alfa-Group" LLC Novosibirsk region, Novosibirsk district., r. Krasnoobsk village, SIBNIIZIKH FANO, Russia.

Literature review: Information about the disease and measures to combat cotton wilt. Cotton wilt disease is present in all cotton growing countries of the world and occurs in two different forms. Verticillium wilt - *Verticillium dahliae* Kleb. and Fusarium wilt - *Fusarium oxysporum* f. sp. *vasinfectum* which stimulate fungi. In recent years, Fusarium wilt in cotton has caused enormous economic damage to all cotton-growing countries of the world (the United States, China, India, Pakistan, Australia, and others). In particular, the 4th race of the Fusarium wilt fungus, which originated in Australia, has done a lot of damage, spreading through seeds in the United States and other countries.

Pathogenic fungi live in the soil and infect more than 880 cultivated and wild plants (Filippov, Andreev, Bazilinskaya, 1978). Mushrooms are stored in soil, plant debris and seeds, and can persist in the soil for more than 25 years, even if the plant is not planted. In Uzbekistan, wilt disease affects medium-staple and fine-staple varieties of cotton. Under our conditions, 35-40 years ago, medium-staple varieties of cotton were infected with verticella wilt, and fine-staple varieties were infected with fusarium, but now medium-staple varieties of cotton are also susceptible to infection with fuarirose wilt. In Uzbekistan, wilt disease affects medium-staple and fine-staple varieties of cotton. Under our conditions, 35-40 years ago, medium-staple varieties of cotton were infected with verticella wilt, and fine-staple varieties were infected with fusarium, but now medium-staple varieties of cotton are also susceptible to infection with fuarirose wilt.

Fusarium wilt can affect cotton from seedlings to the end of the growing season. In most cases, cotton seedlings become very sick during weeding, asymptomatic and suddenly dry up.

Plant yields and fiber quality are reduced. The quality and fat content of seeds deteriorate sharply, and all this causes great material damage. As a result of alternate sowing of cotton with other crops, the activity of the fungus that causes cotton disease in the soil is somewhat reduced, but does not completely disappear.

The lack of organic matter in the upper soil layer as a result of the lack of crop rotation leads to a sharp decrease in the number of beneficial microorganisms. In recent years, it has been noted that Fusarium wilt affects varieties grown in all regions of the country.

Table 1. Information on the state of prevalence of wilt in cotton crops (2016-2020) in the Bukhara region.

№	Name of districts	Cotton planting area, ha	2016 year			2017 year			2018 year			Cotton planting area, ha	2019 year			Cotton	2020 year		
			Projected area	Actual spreading area	Total cultivated area	Projected area	Actual spreading area	Total cultivated area	Projected area	Actual spreading area	Total cultivated area		Projected area	Actual spreading area	Total cultivated area				
1	Bukhara	108	172	880	880	124	118	118	110	107	156	900	129	115	168	802	129	124	347
2	Vobkent	100	158	880	100	111	106	106	164	124	380	103	149	133	297	104	150	144	325
3	Jondor	142	609	307	307	424	404	404	374	370	584	133	444	396	562	116	444	427	361
4	Kogon	7120	2214	990	990	1341	1277	1277	683	385	1844	7400	462	412	1992	7006	460	445	2303
5	Qorakul	1028	2993	1434	1434	1288	1227	1227	2636	2460	3570	9950	2952	2632	3856	8956	2950	2843	2893
6	Karavulbozor	5715	2161	660	660	2567	2445	2445	2765	2478	1660	5515	2974	2651	1793	5905	2970	2864	5251
7	Olot	8580	2156	2317	2317	3703	3527	3527	3745	3527	4200	8620	4232	3774	4536	7914	4230	4076	3513
8	Peshko	9855	3011	660	660	4080	3886	3886	1884	1391	2594	9200	1669	1488	2818	8511	1670	1607	3592
9	Romit'an	121	379	880	120	395	376	376	394	376	402	118	432	403	434	108	432	435	413
10	Shafirkon	102	290	880	150	519	494	494	312	248	461	102	298	265	497	904	298	287	556
11	Gizhduwon	1030	1827	980	1510	2224	2118	2118	2318	2119	2354	1037	2543	2267	2542	9364	2540	2449	3265
12	Bukharacity	300	0	0	0	0	0	0	0	0	150	150	0	0	130	0	0	0	0
Total		1096	3046	1363	1523	3096	2949	2949	2758	2464	3605	1060	2956	2636	3713	9790	2955	2847	4085

In 2019, 26366 ha of cotton fields were infected with Fusarium wilt in the Bukhara region. (Table-1) In particular, 1,153 ha were registered in the Bukhara district, 3,962 ha in the Jondor district, 2,632 ha in the Karakul district, 2,651 ha in the Karaulbozor district, 3,774 ha in Alat district, 4033 ha in Romitan district and 2657 ha in Shafirkon district. This problem is extremely urgent, and research in this area should be carried out on a regular basis in cooperation with phytopathologists, breeders, agronomists and other scientists.

The following measures are recommended to prevent damage to cotton by Fusarium wilt disease.

- cleaning the area covered with cotton stalks;

- after harvesting the grain, plow the fields and turn them upside down rest in the soil for 2–3 months (July, August) without sowing;

- apply nitrogen, phosphorus, potash fertilizers only for the specified period and in moderation (exceeding the nitrogen norm of plants increases the risk of Fusarium wilt, the use of potash fertilizers reduces the disease);

- after harvesting on the planting areas, sowing green manure crops (rapeseed, mustard, winter rye), in the fall, grind the crop of these plants with KIR -1.5 and plow the soil to a depth of 35–40 cm, (the measure leads to an increase in the amount of organic matter in the soil);

- organize root removal of weeds in the fields (in this case, the fungus causing wilt on the roots of weeds will be removed);

- widespread introduction of cotton-alfalfa crop rotation in the affected areas;

- sowing grain in cotton fields affected by Fusarium wilt. (but in this case the soil is not cleared of infestation by wilting);

- in order to enrich the soil with organic matter, organize the application of decomposed organic fertilizers (undecomposed manure, the introduction of feces into the soil contributes to the spread of the disease);

- to increase the immunity of plants, before sowing, the seeds are treated with a solution of biologically active immunoregulatory substances (Humimax, Edagum (Russia), Uzgumi (Uzbekistan), Sofgard (China). Application of a suspension in the phase of 2-3 true leaves in cotton, mixed with a solution of microbiological drugs and 1.5% urea;

- apply nitrogen fertilizers before the cotton flowering period, phosphorus and potash fertilizers should preferably be applied before sowing based on the recommendations;

- breeding cotton varieties resistant to Fusarium wilt disease;

- it is necessary to carry out disinfection and deodorizing measures for equipment working in areas affected by Fusarium wilt and other working tools, pieces of soil on shoes preventing the entry of spores of pathogenic fungi into an undamaged field;

- prohibit the receipt of seeds from areas affected by wilt by more than 5%;

- Introduce the use of microbiological preparations against Fusarium wilt of cotton of domestic production BIST (National University of Uzbekistan named after Mirzo Ulugbek), Trichodermin (Institute of Plant Protection of Uzbekistan) and Sporagin (Anguzal Agroservice), Baktofit (Russia) based on the organization of bacteria *Bacillus subtilis*. pre-sowing treatment of seeds with biological preparations and additional treatment of soil and plants with these preparations during the growing season;

Experts predict the spread of Fusarium wilt in the Bukhara region in 2020 on 29,550 hectares of cotton fields. The implementation of the proposed organizational, agrotechnical measures in areas where the spread of the disease is predicted and the experimental use of the microbiological preparations listed above can have a positive effect.

To instruct the Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan, the National University of Uzbekistan named after Mirzo Ulugbek, the Research and Production Center under the Ministry of Agriculture and Water Resources, the Research Institute of Plant Protection to create effective, biologically and environmentally friendly biofertilizers against Fusarium wilt and it would be advisable to allocate the necessary means to carry out this work.

In order to prevent the spread of this disease in the fields of our country, local authorities, officials and farm managers need to control the spread of Fusarium wilt, as well as develop and implement measures to combat it based on the above recommendations.

When cotton seedlings are affected by Fusarium wilt in the development phase of 4–6 leaves, the plant dries quickly in 3–5 days. In the future, in affected plants, growth slows down and cases of decay are not uncommon.

- Regulation of the use of microbiological preparations against Fusarium wilt in cotton:

Bacillus subtilis produced by the Sibbiopharm plant (Russia) has fungicidal properties against verticillium and fusarium wilt, root rot. This drug is widely used on grain and vegetable crops in Russia and other countries.

Laboratory tests have shown that the drug, developed jointly with the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan and Anguzal Agroservis (Uzbekistan) based on a local strain (*Bac. Subtilis*), also has antagonistic properties against homozygous, verticillium and fusarium wilt and root rot.

It has been shown that the drug developed by the Uzbek Research Institute of Plant Protection on the basis of local strains of the *Trichoderma* fungus has an effect on Fusarium wilt.

The above preparations are being tested in the laboratories of research institutes using soil samples from the Bukhara region infected with Fusarium wilt.

Local authorities, officials, farmers will organize the implementation of the above-mentioned comprehensive measures and ensure the timely full use of microbiological preparations to prevent the spread and damage of cotton by Fusarium wilt over large areas.

2 Research methodology

Testing of Ivan Ovsinsky - fulvohumate against Fusarium wilt cotton on a natural infectious background in the Bukhara region.

Date of processing of seeds and sowing: April 18, 2020

Type of experiment: field small-plot on a natural infectious background.

Place of testing: Bukhara province, Bukhara dist, Podshokhi MFY, Bafo Mardon Sharif farm, farmer Sharipov Otabek's field.

Repetitions: 4.

Plot location: randomized.

Number of plots: 3 variants x 4 replications = 12 plots.

The size of one plot: 24 m², 4 plots (one variant in 4 repetitions) - 96 m².

Total area under experiment: 12 plots x 24 m² = 288 m².

Repetitions: 4.

Plot location: randomized.

Number of plots: 3 variants x 4 replications = 12 plots.

The size of one plot: 24 m², 4 plots (one variant in 4 repetitions) - 96 m².

Total area under experiment: 12 plots x 24 m² = 288 m².

Seed consumption for 1 plot: 84 g, for 4 plots (for option 1) - 336 g, for 12 plots - 1.008 kg.

Total number of seeds required: 1 kg 008. Sowing: manual.

Row spacing: 60 cm, protection between lanes - 2x0.6 m = 1.2 m.

Pre-sowing treatment of seeds, spraying of crops with Ivan Ovsinsky-fulvohumate preparations and reference preparations was carried out at the appropriate periods during the growing season, in accordance with the instructions of manufacturers (Table 2). Agrotechnical measures on the field were carried out in accordance with the practice adopted on the farm, including plants on May 17, 2020 (in the phase of 2-3 true leaves).

Table 2. Experience options in Bukhara region

Experience options, drug, manufacturer (active ingredient)	Processing type	Consumption rate
Ivan Ovsinsky - water 4-6% (40-60 g/l)	Presowing treatment of seeds	0.5 l + 40 l water/t
	Spraying crops in the phase of 2-3 true leaves	0.5 l/ha
	Spraying crops in the budding phase	0.5 l/ha

Standard "P-4 65%"	Presowing treatment of seeds	2 l + 20-30 l water/t
	Spraying crops in the phase of 2-3 true leaves	2 l + 100-300 l water/ha
	Spraying crops in the budding phase	2 l + 100-300 l water/ha
Control	No processing	-

Before sowing, soil sampling was carried out in the experimental field in order to determine the amount of *Fusarium oxysporum* propagules in the soil: 1) date of sampling: April 19, 2020. 2) the number and weight of samples taken: from three points along the diagonal of the experimental plot, at each point from three tiers (from layers 0-10, 10-20 and 20-30 cm deep), 9 samples in total.

The analysis showed that *Fusarium* spp. propagules were contained in the soil. in the following quantities: in the horizon 0-10 cm 66.7 propagules/g, 10-20 cm - 88.9 propagules/g and 20-30 cm - 44.5 propagules/g of soil. In addition to this fungus, propagules of the fungus *Verticillium dahliae* were found in the soil horizon of 10-20 cm in the amount of 22.2 propagules/g.

From the beginning of the manifestation of wilt on crops, samples of wilted or fading cotton plants were taken from the experimental and control plots at each count. Mycological analysis showed that all dead and diseased plants were affected only by the causative agent of *Fusarium* wilt.

The first count was carried out by the number of sprouted seedlings, in subsequent counts the number of living, diseased (obviously withering) and dead (withered) plants was recorded. Records were made for the following phases of plant development:

- 1) April 30/2020 - for full shoots;
- 2) May 15/2020 - 15 days after the 1st count (in the phase of 2-3 true leaves);
- 3) 06/05/2020 - 35 days after the 1st count (in the budding phase);
- 4) June 25/2020 - 55 days after the 1st count (in the flowering-boll formation phase);
- 5) 07/15/2020 - 75 days after the 1st count (in the phase of the beginning of the opening of the boxes).

3 Results and discussion

In an experiment conducted in the Bukhara region. pre-sowing treatment of cotton seeds with preparations Ivan Ovsinsky-fulvohumate and spraying in the phase of 2-3 true leaves and the budding phase had a certain positive effect on the incidence of plants with *Fusarium* wilt. At the same time, 28.4% of plants died due to *Fusarium* wilt during the 75 days elapsed from the time of emergence of full seedlings, and the death of a large number of plants (22.0%) was observed during the first 15 days - in the seedling phase. The development of the disease continued after this period, however, during the following periods, significantly fewer plants died. The biological effectiveness of the preparation Ivan Ovsinsky - fulvohumate, calculated on the basis of the difference in the number of plants killed from the disease in the experimental and control variants, by the phase of the beginning of opening of the boxes was 60.2%. The biological efficiency of the reference preparation was close to that of the Ivan Ovsinsky preparation and amounted to 66%.

The results obtained in our trial are consistent with numerous literature reports that the main damage of *Fusarium* wilt in cotton occurs in the early phases of plant development. Symptoms of the disease on seedlings included the appearance of chlorosis and necrosis on the leaves, followed by wilting and death of plants.

The data in Table 2.1 show that the use of Ivan Ovsinsky - fulvohumate for seed dressing and spraying plants in the phase of 2-3 true leaves and the budding phase with Ivan Ovsinsky

- had a certain positive effect on the incidence of plants with Fusarium wilt - the number of plants dying from the disease was 12.2% less than in the control variant,

In the Bukhara region, in the period from April 30 to May 15, 2020, about 30 samples of diseased seedlings were collected, and then 3-5 samples of wilted and withering plants were collected on June 5 and 25 and July 15, 2020. All collected samples were subjected to mycological analysis - the causative agent of the disease was isolated from them on nutrient media.

To do this, the roots, root neck and lower part of the stem of the specimens were cut with sterile scissors into segments 0.4–0.5 cm long, washed under running tap water for 2 hours, surface sterilized, washed three times with sterile distilled water, and dried between the leaves. sterile filter paper and laid out on the surface of 2% starvation agar (with streptomycin, 0.5 g/l) in Petri dishes (5-8 segments per dish). The plates were incubated in a thermostat at 24°C for 5-7 days.

The fungal hyphae emerging from the segments were subcultured onto potato sucrose agar (PSA) in Petri dishes and incubated in a thermostat at 25±2°C for 7-10 days. The resulting cultures of the fungus were used to identify the pathogen and the culture from withered seedlings, as well as to propagate the inoculum during the experiment on an artificial infectious background of Fusarium wilt in IGEBR lysimeters.

Colonies of Fusarium spp. on CCA, they were mostly white with a slightly noticeable light pink tint or white-light pink (Fig. 3 and 4). Based on cultural, morphological and microscopic features, the fungus was presumably identified as *F. oxysporum*, f. sp. *vasinfectum*.

In both (field and lysimetric) experiments, samples of withered or fading cotton plants were taken from the experimental and control plots. Mycological analysis showed that all dead and diseased plants were affected only by the causative agent of Fusarium wilt.

In the lysimetric experiment, the first count was carried out according to the number of sprouted seedlings; in subsequent counts, the number of living, diseased (obviously withering) and dead (withered) plants was recorded. Records were made for the following phases of plant development:

- 1) 06/20/2020 - for full shoots;
- 2) 07/10/2020 - 20 days after the 1st count (in the phase from 2-3 leaves - the beginning of budding);
- 3) 07/30/2020 - 40 days after the 1st count (in the budding phase - opening the first boxes).

The biological effectiveness of the tested preparations was calculated by comparing the number of dead plants in the experimental and control variants (at the last count) according to the following modified VIZR formula:

$$Be = \frac{a - b}{a} \times 100$$

where: a - percentage of dead plants in control; b - percentage of dead plants in the experiment.

Pre-sowing treatment of seeds, spraying of crops during the corresponding periods of the growing season were carried out in accordance with the instructions of manufacturers (Table 3.2). In the soil under the plots in the phase of 2-3 true leaves, 33% sodium nitrate was introduced at a rate of 90 kg/ha. Plants in the phase of 2-3 leaves and budding were treated with a suspension at a rate of 3 and 5 kg/ha, respectively.

In particular, in the control variant, the average height of cotton was 88.1 cm, and in the 2nd, 3rd and 4th variants of the experiment, the value of this indicator was 5.4–7.3 cm higher than in the control variant.

A similar picture was observed in terms of the number of fruit elements in the plant. At the end of the growing season, the average number of fruit elements in the control variant was 9.1, and in experimental variants 2, 3 and 4, the number of fruit elements was 2.0–3.4 more than in the control variant. In addition, data recording actual plant density levels at the

end of the growing season show that no significant differences were observed between the variants in this parameter.

Cotton yields were usually determined by manual harvesting and the results showed that about 70% of the main crop was harvested at the first harvest. On the control variant, the yield of raw cotton is 36.6 centners / ha, on the 2nd experimental variant it is 38.9 centners / ha, 40.1 centners / ha, and on the 1st variant of the experiment (Table 3).

Table 3 The yield of cotton variety "Bukhara-6" in conditions of meadow-alluvial soils of the Bukhara region

№	Options	Number of boxes pcs / height	Average weight of boxes, gr	Productivity c/ha	Increase	
					u/ra	%
1.	Ivan Ovsinsky - water 4-6% (40-60 g/l)	11,9	6,4	40,1	+3,5	8,8
2.	Standard "P-4 65%"	10,8	5,6	38,9	+2,3	6,8
3.	Control without processing	9,2	4,9	36,6	-	-

Thus, it was noted that the yield of cotton under the influence of fulvohumate "Ivan Ovsinsky" in the experimental variants was on average 2.3-3.5 c/ha higher than in the control. It has been established that fulvohumate from the preparation "Ivan Ovsinsky" affects the rate of stimulation of cotton growth during the growing season. It was also found that the cotton yield increased by 3.5 c/ha (or 8.8%) compared to the control.

4 Discussion

Based on the test carried out, the following conclusions can be drawn:

Experience on a natural infectious background in the Bukhara region:

1. The natural conditions of the Bukhara oasis differ from the rest of the territory of Uzbekistan in their peculiar properties: arid climate, low content of plant residues, mineralization and proximity to the surface of groundwater, special geomorphological, lithological conditions of soil formation and its properties (morphogenetic, agrochemical, physicochemical, chemical and biological activity) soil formation under the influence of human activity.

2. The soil of the experimental field is heavily infested with propagules of the Fusarium wilt pathogen, as well as a small amount of propagules of the cotton verticillium wilt pathogen. During the growing season, the defeat and death of seedlings and adult cotton plants was caused only by the causative agent of Fusarium wilt.

3. High harmfulness of Fusarium wilt in cotton has been revealed. In the control variant, by the beginning of the bolls opening phase, 71.3% of the plants died in the full germination phase.

4. The main damage of Fusarium wilt in the field was manifested during the first 35 days after the emergence of cotton seedlings, i.e. until the budding phase. Before this phase, 21.1-24.2% of plants died in the experimental and reference variants and 56.3% of the plants in the control variant. After budding, there was a sharp decrease in the activity of the Fusarium wilt pathogen and the number of dead plants varied in the phases of flowering-boll formation

and the beginning of opening of bolls in the experimental and reference variants, within 1.3-2.4%, and 1.1-2.9% , and in the control - 12.0% and 3%, respectively.

5. Pre-sowing treatment of cotton seeds with Ivan Ovsinsky-fulvohumate preparations (and reference preparations) to a certain extent reduced the infestation of plants with Fusarium wilt, although it did not provide complete protection of crops from this disease. The biological effectiveness of Ivan Ovsinsky-fulvohumate was 60.2%.

5 Conclusion

Given that Fusarium wilt in cotton is a disease, the fight against which is extremely difficult, against which there are no effective and registered chemical or biological fungicides both in Uzbekistan and in foreign cotton-growing countries of the world, we recommend registering Ivan Ovsinsky - fulvohumate, for pre-sowing seed treatment cotton with a consumption rate of 0.5 l / t, followed by spraying of crops in the phase of 2-3 true leaves and the budding phase Ivan Ovsinsky - fulvohumate with a consumption rate of 0.5 l / ha, against Fusarium wilt of cotton.

On irrigated meadow-alluvial soils, due to the use of fulvohumate "Ivan Ovsinsky", the resistance of cotton to various diseases has increased and the agrochemical properties of the soil have improved, an additional yield of 2.3-3.5 c/ha of cotton has been obtained. The profitability of the farm increased from 15.9% to 21.4%, respectively. It is recommended to use this preparation on meadow-alluvial soils of the Bukhara oasis.

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