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EXPERIMENTAL ARTICLES

Fungicide Features of the Nanosystems of Silkworm (*Bombyx mori*) Chitosan with Copper Ions

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Abstract—The fungicidal activity of some chitosan samples and their nanostructured systems with copper was studied. The samples of *Bombyx mori* chitosan and its nanostructured systems with copper were found to inhibit growth and development of phytopathogenic fungi *Fusarium solani* 169 and *Verticillium dahlie* 57. The growth inhibition zone was of considerable size (22 to 60 mm).

Keywords: Bombyx mori chitosan, chitosan nanosystems with copper ions, *Fusarium solani* 169, *Verticillium dahlie* 57, fungicidal activity

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It is known that chitosan and its modifications exhibit biological activity against viral infections, bacteria, and phytopathogenic fungi [1–5].

Development of metal-containing preparations based on polymers (including chitosan) is a major area of nanochemistry. It is known that Ag-chitosan composites have bactericidal and bacteriostatic effects against *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* [6].

Chitosans with low-molecular masses were found to have more pronounced antimicrobial properties than high-molecular chitosans. We also researched antibacterial activity of metal complexes of chitosan with copper against *Salmonella enteritidis*. Our results showed that the complex with the chitosan : copper ratio of 1 : 1 caused 100% inhibition of growth of phytopathogens [7].

Manufacturing of chitosan preparations from silkworm pupae, as well as synthesis and application of the chitosan-based nanostructured systems developed previously [8, 9], which contain both copper ions and nanoparticles has both basic and applied importance. Some aspects of it are described in this study.

MATERIALS AND METHODS

Extraction of *Bombyx mori* chitin and production of chitosan were carried out according to the method described in the study [8].

Nanostructured systems were constructed from *Bombyx mori* chitosan (Chs) and Cu^{2+} in the presence of reducing agents. For synthesis of copper nanoparticles, aqueous 0.5 M water solution of $CuCl_2$ was heated at 70°C in the presence of ethanol for 1 h, as it was described [10]. Calculated amounts of metal salts were added to Chs solutions. The samples were identified by UV spectroscopy and under an AFM5500 atomic force microscope. This method made it possible to obtain nanostructural systems based on chitosan and copper ions within the size range of 2.5–25 nm.

Content of nitrogen in the samples was determined according to the Dumas method [11], by incineration of a weighted sample in a quartz tube in CO_2 atmosphere by the oxygen of solid oxidizers. The amount of metal in the samples was determined by chelatometric titration [12]. The copper content in the Chs : Cu nanosystem thus obtained was 24%.

Fungicidal features of Chs solutions and of Chs : Cu nanostructured systems were determined by their effect on growth and development on *Fusarium solani* 169 and *Verticillium dahliae* 57 phytopathogenic fungi.

Pure cultures of phytopathogenic strains *F. solani* 169 and *V. dahliae* 57 isolated from plant stems and roots were obtained from collections of cultures of Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan.

For detection of fungicidal features of chitosan and its nanostructural systems with copper ions, the Czapek-Dox agar medium was used, which contained the following (g/L): glucose, 30.0; $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; agar, 25; pH 6–6.5. The medium was sterilized at 0.5 atm and dispensed into sterile petri dishes. Phytopathogenic fungi were plated on lawn. Spores of phytopathogenic fungi were transferred to the agar plate

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Effect of molecular mass characteristics of chitosan and copper-bearing nanosystems on growth and development of phytopathogenic fungi *F. solani* 169 and *V. dahliae* 57

No.	Samples	Molecular mass of chitosan, 10^3	Deacetylation degree, %	Mass of active compounds in solution, mg	Growth inhibition zone, mm	
					F. solani 169	*V. dahliae* 57
1	CH_3COOH	–	–	4.0	10	14
2	Chs-1	48	86	5.0	23	28
3	Chs-2	50	81	5.0	22	24
4	Chs-3	35	70	2.5	23	17
5	Chs : Cu-1	35	70	1.5	25	27
6	Chs : Cu-1	35	70	3.0	45	60

by a loop and spread on the surface by a sterile spatula. The agar blocks were excised with a sterile Forstner bit (diameter 6–8 mm); then, chitosan solution or its nanostructural systems with copper ions (0.2 mL) were applied to the holes, and the plates were incubated at 28–30°C for 8–10 days, as described in [13]. Acetic acid solution (2%) was used as the control.

RESULTS AND DISCUSSION

Research of the biological activity of Chs solution and of the Chs : Cu nanostructural systems against *F. solani* 169 and *V. dahliae* 57 phytopathogenic fungi showed that solutions of the studied samples effectively inhibited growth and development of these fungi. Considerable zones of growth inhibition were formed, from 22 to 60 mm, depending on the amount of Chs : Cu specimens. The results are presented in the table.

Phytopathogenic fungi under study develop in roots and stems of plants, and cause growth retardation and such diseases as root rot and wilt. These pathogenic fungi are usually present in soil and may cause decreased harvests of industrial crops.

Our results showed that Chs solutions had fungicide properties, i.e. zones of fungal growth inhibition, 22–28 mm in diameter developed between Chs solutions and the lawns. Retardation of mycelium development around the zone was also observed. Chitosan solutions probably had a destructive effect on fungal hyphae. It should be noted that the samples of chitosan with the highest rate of deacetylation showed stronger fungicidal properties. Generally, our results showed that the studied samples of chitosan exhibited moderate fungicidal activity against the tested strains of phytopathogenic fungi.

A number of studies dealt with the antibacterial activity of chitosan depending on its molecular mass and degree of deacetylation (DDA). The authors determined that aminoglycans with high degrees of polymerization, when applied as 1% solution in 0.2% of hydrochloric acid, showed antimicrobial activity against many microorganisms [14]. Examination of chitosans with molecular mass of 4000 Da and DDA values of 55, 77, 78, and 86% revealed a tendency to increased cell death levels with increasing DDA values. This was due to the fact that chitosan with high DDA had a higher concentration of positive charges along its main chain, causing formation of more stable bonds with the microbial cell wall surface [15]. Our results confirmed these data. We found that introduction of copper nanoparticles into chitosan molecules increased their fungicidal activity against phytopathogenic fungi. Increased content of Chs : Cu-1 in the system resulted in an increase of fungicidal activity by 2.2 times (table; nos. 5 and 6). Efficient inhibition of growth and development of phytopathogenic fungi *V. dahliae* 57 resulted in lysis of mycelial hyphae and development of a growth inhibition zone 60 mm in diameter between the Chs : Cu system and the pathogens.

Thus, we showed fungicidal activity of several samples of chitosan and chitosan nanosystems with copper ions against phytopathogenic fungi *F. solani* 169 and *V. dahliae* 57. We determined that *Bombyx mori* samples of chitosan and its nanostructural systems with copper effectively inhibited growth and development of phytopathogenic fungi. The samples obtained may be of use for prevention and treatment of some fungal plant diseases.

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