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Antifungal Activity of Chitin, Chitosan and their Derivatives from *Artemia Parthenogenetica* Cystes of the Aral Sea against Phytopathogenic Fungi

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A B S T R A C T

In the present study, the antimicrobial activity of chitin, chitosan, and their carboxymethylated and sulfated derivatives obtained from the cysts of *Artemia partenogenetica* from the Aral Sea was evaluated against phytopathogenic fungi. The antifungal activity was studied using the disk diffusion method on PDA medium, followed by measuring the inhibition zones of fungal growth. The results showed that low-molecular-weight chitosan (XT3-1) exhibited the highest antifungal activity, with growth inhibition zones up to 30 mm against *F. vasinfectum* and *V. dahliae*. Carboxymethyl derivatives (KMXT and KMX3) demonstrated significantly lower activity (6–24 mm), which can be attributed to the reduction of the macromolecule's cationic charge. Sulfated forms (CX3-1 and CX3-2) retained high activity comparable to native chitosan, with inhibition zones up to 27–30 mm against *F. culmorum* and *F. vasinfectum*, confirming the effectiveness of sulfation in enhancing antifungal properties. The obtained data emphasize the practical significance of chemically modified chitosan derivatives for plant protection against phytopathogenic fungi and highlight their potential for developing safe and efficient biofungicides.

Introduction

Plant-pathogenic fungi are considered economically significant worldwide, as they cause damage to a wide range of agricultural crops during both the growing season and post-harvest storage. Controlling these diseases is of great importance and has traditionally been achieved through the use of synthetic pesticides. However, the application of fungicides, particularly those of synthetic origin, is associated with increasing environmental problems (1).

In recent years, there has been a growing interest in finding alternatives to chemical fungicides that are safe and pose minimal risks to human health and the environment. Among these approaches, promising results

have been obtained through the use of natural compounds such as chitosan (2). The biopolymer chitosan is one of the most abundant natural aminopolysaccharides, composed of glucosamine and N-acetylglucosamine units, and has attracted considerable attention due to its unique physicochemical properties and biological activity (3–4).

Among the various biologically active properties of chitosan, its antimicrobial activity is of particular interest because of the challenges posed by the use of traditional fungicidal agents (3,5). Moreover, numerous studies have focused on the antimicrobial properties of chitosan and its derivatives against phytopathogens (6) and have been summarized in several review articles (7). It has been established that the inhibitory effect of chitosan manifests at various stages of fungal development, including mycelial growth, sporulation, spore viability and germination, as well as the synthesis of virulence factors (8–9).

The natural antifungal properties of chitosan and its derivatives (10–12) have led to their use in commercial disinfectant formulations. Chitosan offers several advantages over other types of disinfectants, including higher antimicrobial activity, a broader spectrum of action, and low toxicity to mammalian cells (13–14).

The aim of the present study was to compare the antimicrobial activity of chitin, chitosan, and their carboxymethylated and sulfated derivatives obtained from the cysts of *Artemia partenogenetica* from the Aral Sea against phytopathogenic fungi — the causative agents of alternariosis (*Alternaria* sp.), root rot (*F. oxysporum*), verticillium wilt (*V. dahliae*), vascular wilt of cotton (*F. vasinfectum*), helminthosporiosis (*Fusarium culmorum*), and other diseases caused by *Fusarium* spp.

Materials and Methods

The activity of chitin/chitosan and its carboxymethyl and sulfate derivatives against phytopathogenic fungi (*F. oxysporum*, *Fusarium sp.*, *Fusarium culmorum*, *F. vasinfectum*, *V. dahliae*, and *Alternaria sp.*) was evaluated using the disk diffusion method.

Cultures of phytopathogenic fungi were grown in Potato Dextrose Broth (PDB). Three-day-old fungal cultures were suspended in sterile 0.9% NaCl solution, and the cell density was adjusted to 5×10^5 CFU/mL using the McFarland standard (0.5). The prepared fungal suspensions (100 µL) were spread evenly on the surface of Potato Dextrose Agar (PDA) plates using a sterile glass rod to form a uniform lawn.

The test samples, whose antifungal activity was to be determined, were dissolved in 100 mL of 1% acetic acid (CH_3COOH) at a concentration of 30 µg. Sterile Whatman No.1 filter paper discs (6 mm in diameter) were impregnated with the prepared solutions and dried under aseptic conditions in a laminar flow cabinet. The dried discs were then placed on the surface of PDA plates inoculated with the fungal cultures.

Petri dishes were incubated at 28°C for 96 hours. The antifungal activity of the samples was evaluated by measuring the inhibition zone around each disc.

The larger the diameter of the inhibition zone, the higher the antifungal activity of the sample against the phytopathogenic fungi.

As a negative control, the reagents used for sample preparation were applied. A fluconazole solution was used as the positive control.

Results and Discussion

The study of the antifungal (antiphytopathogenic) activity of chitosan and its chemically modified forms is of significant interest, as this characteristic determines their practical value for protecting plants against fungal diseases.

In this study, the tested samples were designated with the following codes: XT3-1 – low molecular weight chitosan (16 kDa), XT3-2 – chitosan (147 kDa), KMXT – carboxymethyl chitin, KMX3 – carboxymethyl chitosan, CX3-1 – sulfated chitosan obtained using chlorosulfonic acid, and CX3-2 – sulfated chitosan synthesized using a pyridine–sulfur trioxide complex. Figure 1 shows the antifungal activity of the samples XT3-1, KMXT, CX3-2, XT3-2, KMX3 and CX3-1 against phytopathogenic fungi (*F. oxysporum*, *Fusarium sp.*, *Fusarium culmorum*, *F. vasinfectum*, *V. dahliae*, and *Alternaria sp.*).

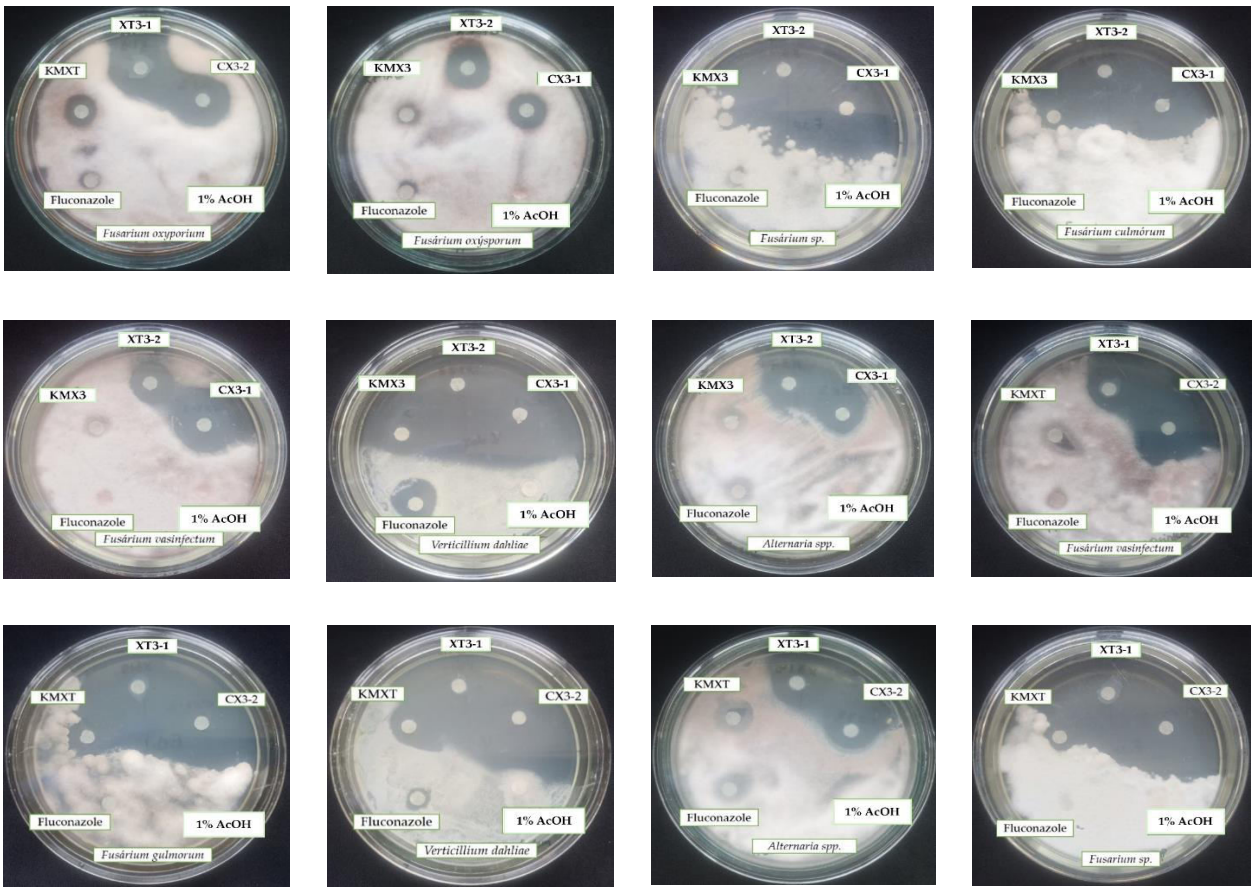
The results showed that the tested samples exhibited varying degrees of antifitopathogenic activity (Table 1). The most pronounced inhibitory effect was observed for the low–molecular-weight chitosan (XT3-1), with growth inhibition zones ranging from 22 mm (*F. oxysporum*) to 30 mm (*F. vasinfectum, V. dahliae*). The high efficacy of

chitosan against phytopathogenic fungi is consistent with the literature data, which describe a significant inhibitory effect of both native chitosan and its nanostructured forms against *Fusarium solani* and *F. culmorum* (15–16).

Carboxymethyl chitosan and carboxymethyl chitin exhibited significantly lower activity (6–24 mm), which is associated with a decrease in the cationic charge of the macromolecule due to the introduction of –COOH groups. A reduction in the antimicrobial potential of carboxymethyl derivatives has also been reported by other researchers (17). The sulfated samples (CX3-1 and CX3-2) demonstrated relatively high activity, comparable to that of unmodified chitosan: inhibition zones for *F. gulmorum* and *F. vasinfectum* reached 27–30 mm. The obtained results are consistent with literature data indicating a pronounced antifungal activity of sulfated chitosan derivatives against *Fusarium graminearum* (18).

The reference drug fluconazole showed low inhibition zone values (6–7 mm) and no activity against several strains, highlighting the potential of chitosan and its modified forms as biopolymeric fungicides with a broader spectrum of activity. For clarity, the obtained data are also presented as statistical charts in Fig. 2.

Fig.1 Activity of the samples XT3-1, KMXT, CX3-2, XT3-2, KMX3 and CX3-1 against phytopathogenic fungi (*F. oxysporum*, *Fusarium* sp., *Fusarium culmorum*, *F. vasinfectum*, *V. dahliae*, *Alternaria* sp.).



The obtained data on the activity of samples XT3-1, KMXT, CX3-2, XT3-2, KMX3 and CX3-1 against phytopathogenic fungi are presented in Table 1.

Fig.2 Antifungal activity of chitosan/chitosan and its derivatives against phytopathogenic fungi

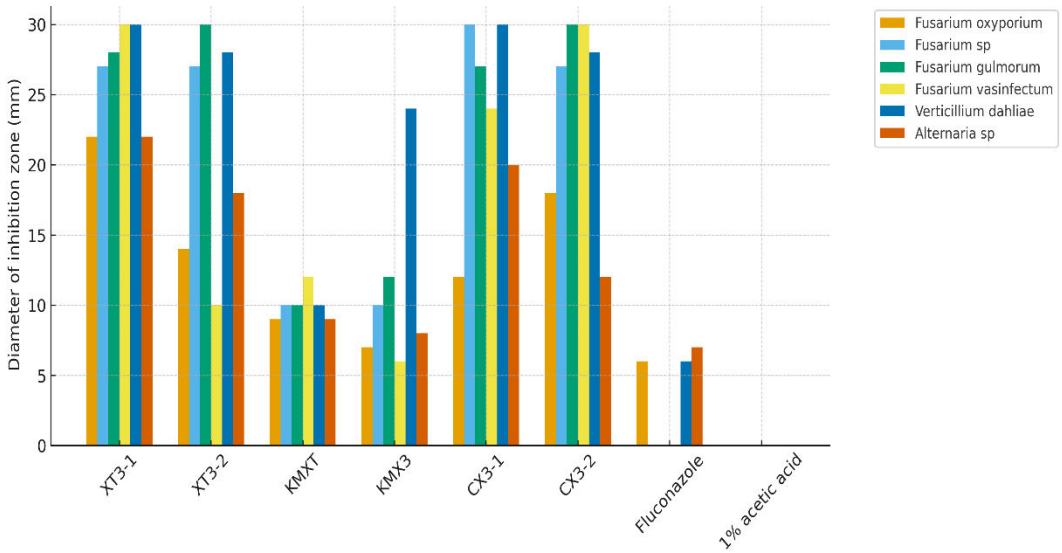


Table.1 Activity of samples XT3-1, KMXT, CX3-2, XT3-2, KMX3 and CX3-1 against phytopathogenic fungi

Test samples	*Fusarium oxyporium*	*Fusarium sp*	*Fusarium gulmorum*	*F. vasinfectum*	*V. dahliae*	*Alternaria sp*
	Diameter of inhibition zone (mm)					
XT3-1	22±1.2	27±1.4	28±1.3	30±1.0	30±1.2	22±1.3
XT3-2	14±1.0	27±1.0	30±1.1	10±1.2	28±1.2	18±1.2
KMXT	9±1.3	10±1.2	10±1.5	12±1.1	10±1.5	9±1.4
KMX3	7±1.7	10±1.2	12±1.1	6±1.0	24±1.1	8±1.0
CX3-1	12±1.0	30±1.0	27±1.2	24±1.0	30±1.1	20±1.1
CX3-2	18±1.4	27±1.1	30±1.4	30±1.1	28±1.3	12±1.5
Fluconazole	6.0±1.0	-	-	-	6±1.0	7±1.4
1% acetic acid	-	-	-	-	-	-

The charts illustrate the differences in the activity of the samples against fungi such as *F. oxysporum*, *Fusarium sp.*, *V. dahliae*, and others. They also show how changes in molecular weight and chitosan modification affect its antimicrobial activity against *F. oxysporum*, *V. dahliae*, and *Alternaria sp.* (Fig. 2).

Thus, the conducted studies demonstrated that chitosan and its carboxymethylated and sulfated derivatives possess the highest antifitopathogenic activity among the tested samples, which is consistent with the literature data. The carboxymethyl derivatives exhibited a significantly lower level of inhibition due to the reduction of the macromolecule's cationic charge. The sulfated forms of chitosan showed activity comparable to that of native chitosan, confirming the potential of this modification. The obtained results indicate the promise of using chitosan and its derivatives as biopolymeric fungicides for plant protection against phytopathogenic fungi.

Author Contributions

B.P. Karlybaeva: Conceived and designed the research, extracted and processed the samples, synthesized chitin/chitosan derivatives, performed data analysis, prepared the original manuscript draft, and participated in article administration and funding acquisition. I. Zh. Yerniyazova: Contributed to experimental design and data interpretation, assisted in methodology optimization,

and participated in funding and resource management. K.G. Khajibaev: Assisted in experimental procedures and data collection, contributed to laboratory analyses, and participated in article funding. G. E. Berdimbetova: Provided scientific supervision and critical review of the manuscript, contributed to conceptual guidance, editing and overall research coordination.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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