CHITOSAN FOR FUNGAL DISEASES CONTROL

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Abstract
The use of chitosan is a promising alternative; it can be applied in many fields based on its biological activity and easy-to-obtain procedures. Such it was used in agriculture, environmental protection, pharmaceutical and biomedical applications. The fungicidal activity of the chitosan against *Fusarium graminearum*, *Penicillium chrysogenum* and *Aspergillus orizae* were done by using the plate growth rate method.
Key words: chitosan, fungicidal activity

Introduction

Chitosan; a linear polysaccharide is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible and was reported by several researchers to have strong antimicrobial and antifungal activities [1, 2].
Chitosan possesses three types of reactive functional groups: an amino group at the C-2 position of each deacetylated unit, as well as primary and secondary hydroxyl groups at the C-6 and C-3 positions, respectively, of each repeat unit (Figure 1) [3].

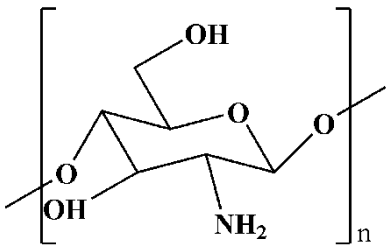

Figure 1. The structure of chitosan

Fresh vegetables and fruits with good quality have benefits for the health of human body due to their good flavor, and the contents in nutrients [4]. The safety of vegetables and fruits is related to its high decay rate and short shelf life period. The rot of vegetables and fruits could be observed because the loss of nutrients and the infection of microorganism during storage. Chitosan can inhibit several plant diseases because it has a fungicidal effect against various fungi. The article demonstrations that the aqueous acid solutions of chitosan presented fungicidal activity against different fungi species as follow: *Fusarium graminearum* - ATCC 46779m, *Penicillium chrysogenum* ATCC 9179 and *Aspergillus orizae* ATCC 10124.

Material and Methods

The chitosan solution was prepared in 2 % (v/v) acetic solution acid. This solution has been preserved at 37°C to maintain its fluidity. The solution of chitosan was prepared by weighing 0.5 grams of low molecular weight chitosan and adding to 100 ml of 2% acetic acid solution (2 ml of glacial acetic acid was added to 98 ml of distilled water).

The Erlenmayer containing the solution of chitosan acetic acid was sonicated for 2 hours for the complete dissolution of chitosan.
The antifungal activity of this chitosan acid aqueous solution was tested on strains of different fungi species as follow: *Fusarium graminearum* - ATCC 46779m, *Penicillium chrysogenum* ATCC 9179 and *Aspergillus orizae* ATCC 10124.
These standard fungi cultures were maintained in laboratory conditions, at 4°C, in tubes with yeast glucose agar (CYGA). From these species were obtained active cultures by inoculation on Petri dishes with melted chloramphenicol yeast glucose agar (CYGA). Antifungal tests against *Fusarium graminearum* (ATCC 46779m), *Penicillium chrysogenum* (ATCC 9179) and *Aspergillus orizae* (ATCC 10124) were performed by using the plate growth rate method [5-7].
Into each Petri plate was aseptically introduced 1 ml from the chitosan acid aqueous solution and after was added 15 ml of chloramphenicol yeast glucose agar (CYGA). The content of Petri plates was gently swirled to achieve uniform mixing of the content. The final concentration of the chitosan in each Petri plates was 0.33%. In the control plates was poured only 15 ml of chloramphenicol yeast glucose agar. After the solidification of the media discs with 5 mm diameter were extracted. Also inoculum discs of the test fungus species were obtained from the original cultures and these were inoculated on the solidified media.
In each plate were inoculated three inoculums discs. The plates were incubated at 25 + 2°C and after seven days the radial growth of mycelium was measured. The results were compared with negative control. The mean of three readings was taken for calculations using equation:
Antifungal activity (%) = (Dc − Ds) / Dc) × 100 ; [5]
Where: Dc is the diameter of growth in control plate and Ds is the diameter of growth in the plate containing tested antifungal agent

Results and discussion
The FTIR spectra of chitosan is presented in Figure 1.

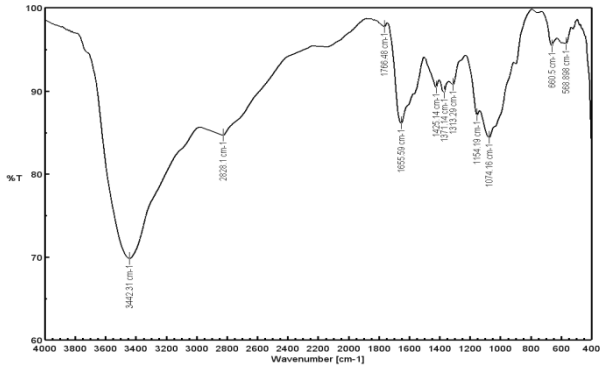

Figure 2. FTIR spectrum of Chitosan

The FTIR spectra shows characteristic absorption bands at approximately 1666 has been assigned as the –NH bending of NH_2 group and at 3442 cm^{-1} has been assigned as the hydroxyl group. At 1371 cm^{-1} has been assigned as amide band. The skeletal vibrations involving the C-O-C stretching band was observed at 1074 cm^{-1}.
In the prezent article the elemental composition of chitosan was confirmed by energy dispersive analysis of X-rays (EDAX), see Figure 3.

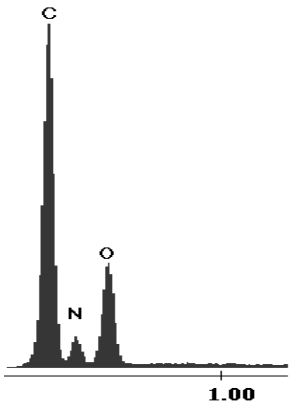

Figure 3. EDAX image for Chitosan

The results of the chitosan acid aqueous solutions on the fungi speies tested are presented in Table 1.

Table 1. Antifungal activity of chitosan acid aqueous solution

Fungi specie	Diameter of grow (mm)								Antifungal activity (%)
	Control				Chitosan				
	1	2	3	M	1	2	3	M	
Fusarium graminearum	48	40	35	41	0	0	0	0	100
Penicillium chrysogenum	18	20	20	19.3	0	0	0	0	100
Aspergillus orizae	20	20	20	20	0	0	0	0	100

The efficacy of chitosan acid aqueous solution was 100% against all the fungi species tested. In Figure 4 are presented results with *Fusarium graminearum* of *control* sample (M) and sample with chitosan.

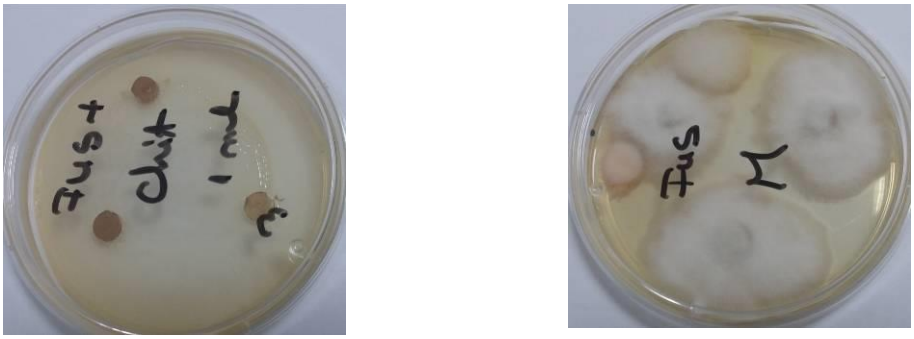

Figure 4. Images of the Fusarium graminearum (sample M and sample with chitosan)

Our results could be compared with others studies but there are not uniformity between these results due to different types of chitosan tested [8].

The effect of chitosan on fungal cell walls is dependanted on the concentration, degree of acetylation and the pH. In a study on cultures of *Rizhopus solani* was observed that the fungus germination decreased with increasing the chitosan concentration in the medium. When the inhibition process occurs, the medium became alkalin and this reduces the effectiveness of the chitosan [9].

Inhibition rate in order of 80% against plant fungus such as *Phomopsis asparagi* and as high as 95% against *Fusarium oxysporum, Cucumernum owen, Rhizoctonia solani* and *Fusarium oxysporum* have been, however, known to occur with low chitosan concentration (20-150 $mg \cdot L^{-1}$) [10].

The inhibitory effect of chitosan depends also on the type of solvent. The best effect had chitosan dissolved in lactic acid compared to dissolve in formic acid and acetic acid [11].

Conclusion

The article shows the results of fungicidal activity of aqueous acid solutions of chitosan. This acid aqueous solution of chitosan had a good fungicidal activity against all the fungi species tested. Procedure of using of the aqueous acid solutions of chitosan as biopesticides is very effective and eco-friendly for the reason that, it is no-toxic, biodegradable and biocompatible. Much of study is still to be done in the field of biochemical mode of action of chitosan against fungi.

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