Int.J.Curr.Microbiol.App.Sci (2018) *7*(7): 61-66

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 07 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.707.007

Antifungal Effect of Chitosans and Chito-oligosaccharides against Early Blight of Tomato

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Keywords

Antifungal effect, Chitosans, Chito-oligosaccharides Tomato

Article Info

Accepted:
04 June 2018
Available Online:
10 July 2018

A B S T R A C T

The present investigation was to determine the antifungal ativity of different chitosans and chito-oligosaccharides against *Alternaria solani.* Out of four chitosans tested, chitosan DA10 and 134 was recorded as 53.44 and 51.47% at 1000ppm, respectively where as at 1500 ppm conc, the inhibition percent was 67.54 and 59.01%, respectively. Among the treatments, chitosans exhibited maximum growth inhibition of pathogen at 1500ppmconcentration.Chito-oligosaccharides obtained by enzymatic degradation of the chitosans using promising biocontrol agents have exhibited a positive effect on the growth of the test pathogen showing that they were not effective in reducing the disease.

Introduction

Tomato (*Lycopersicon esculentum* Mill) belongs to the family Solanaceae and is one of the most remunerable and widely grown vegetable in the world.Tomato (*Lycopersicon esculentum*.L) is one of the most important economic vegetable crops cultivated in India in an area of 7.60 Mha with a production of 18.38Mt and productivity of 24.2 Mt/ha (Indiastat, 2015- 2016).Tomato crop is usually susceptible to many diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors. Among the fungal diseases, early blight also known as target spot disease caused by *Alternaria solani.* Chitosan is a natural non-toxic biopolymer derived as a major component of the shells of crustacean such as crab, shrimp, and crawfish.

Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident, are chitin and its main derivative, chitosan. Both are the simplest linear polysaccharides composed of α 1-4 linked D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) monomers in various linkage patterns and compositions. Chitin and its derivatives have become a promising alternative treatment due to its

natural character, antifungal activity and elicitation of defense responses in plant tissue. Chitosan and its derivatives have been commercially formulated for use in agriculture.

Interestingly, many biocontrol agents produce a battery of hydrolytic enzymes as part of their biocontrol mechanism. Among these hydrolytic enzymes, chitinases form one of the major enzymes. Such chitinolytic activity helps chitin and its derivatives to be broken down into smaller fragments called chitooligosaccharides. This break down is a natural process occurring in the rhizosphere due to which, there is a possibility of presence of such molecules in the vicinity of plant roots. Reports show that chito-oligosaccharides act either as antimicrobials or induce host plant resistance.

Materials and Methods

Preparation of chitosan solution

The stock solutions, 10mg/ml of four different chitosans were prepared by dissolving each purified chitosan in 0.25 N HCl under continuous stirring. The pH was adjusted to 5.5-6.0 using 2N sodium hydroxide, dialysed for 12h against cold distilled water and autoclaved. From the stock solutions, various concentrations (1000ppm and 1500ppm) of chitosans were prepared with distilled water.

Effect of chitosans and chito-oligosaccharides on test pathogen

The antimicrobial activity of chitosans and chito-oligosaccharides against *A. solani* was assessed using microtitre plates. Malt extract Dextrose broth supplemented with chitosans of different concentrations was inoculated with 10µl of spore suspension of test pathogen. MDA with only test pathogen served as control. After 48 h of incubation, optical density (O.D) was measured in a microtiterplate reader at 595nm.Percent inhibition will be calculated according to the formula;

$$I\ \% = C - T / C \times 100$$

where,
I = Inhibition of pathogen growth
C= Pathogen growth in control
T= Pathogen growth in treatment

The efficient chitosans and the selected potential bacterial and fungal bioagents were inoculated in their respective media and incubated at 28°C for 72h to obtain chito-oligosaccharides. The culture broth was filtered through membrane filter followed by heat inactivation at 60°C for 1hour to arrest any further enzymatic activity. Thus obtained culture filtrate containing chito-oligosaccharides was co-inoculated with test pathogen into fresh medium and media with test pathogen served as control. After 48 h of incubation, optical density (O.D) was measured in a microtiterplate reader at 595nm. Percent inhibition was calculated according to the formula

$$I\ \% = C - T / C \times 100$$

where,
I = Inhibition of pathogen growth
C= Pathogen growth in control
T= Pathogen growth in treatment

Compatibility of chitosans and biocontrol agents

The cell/spore suspension of biocontrol agents were co-inoculated with chitosans into MD broth and incubated at 28±2°C for 24h. Optical density was recorded at 600nm and595nmandfor bacterial and fungal growth, respectively.

Results and Discussion

Antifungal activity of chitosans and chito-oligosaccharides

Chitosans

The efficacy of four chitosans was tested *in vitro* against *A. solani*. The results indicated that the chitosans were effective with significant difference in inhibiting growth of *A. solani* in varying degrees. In the present study, growth inhibition of *A. solani* with chitosans DA10 and 134 was recorded as 53.44 and 51.47% at 1000ppm, respectively whereas at 1500 ppm conc, the inhibition percent was 67.54 and 59.01%, respectively. Among the treatments, chitosans exhibited maximum growth inhibition of pathogen at 1500ppmconcentration. The data on percent inhibition was recorded and is presented in Table 1. These two effective chitosans were used for further studies (Fig. 1).

The inhibition of growth of *A. solani* under *in vitro* conditions may be due to the antifungal properties of chitosan. Roller and Covill (1999) showed that chitosan at a concentration of 1mg/ml reduced the growth rate of *Mucor racemosus*. Prapagdee *et al.,* (2007) reported that a chitosan bearing broth at 1mg/ml concentration completely inhibited the growth of *F. solani* f.sp. *glycine.* In the *in vitro* experiments, growth of the test fungus was successfully inhibited by chitosan treatment, varying from partial inhibition at low concentration to complete inhibition at high concentrations, which is in agreement with the findings of Reglinski *et al.,* (2010), Abd-Alla and Haggag (2010), El Hassni *et al.,* (2004) and Munoz and Moret (2010).

Among natural elicitor compounds, chitosan offers a great potential as a biodegradable substance that has antimicrobial and eliciting activities (Benhamou, 1996). The results of the present experiments showed that different chitosans at all concentrations effectively reduced the growth of *A. solani* and the maximum inhibition was observed at1500ppm. These results are in confirmation with the findings of Pongphen *et al.,* (2007) who found the effect of chitosans on mycelial growth and spore germination of *Colletotrichum gloeosporioides* at 0.5%, 1.0%, 1.5%, and 2.0% (w/v) chitosan. The higher concentration of chitosan (1.5% and 2.0%) has more inhibitory effect on fungal growth than the lower concentrations (0.5% and 1.0%).

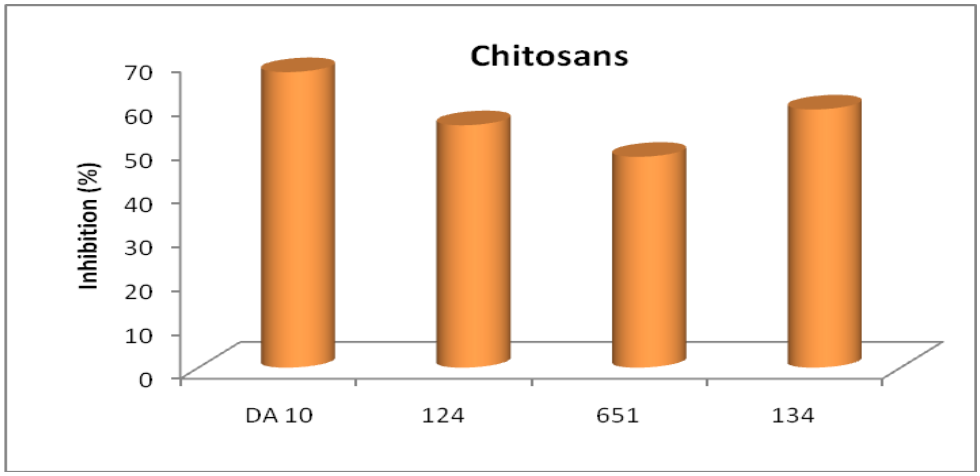
Table.1 Antimicrobial activity of different chitosans against *A. solani*

Chitosans	Inhibition of *Alternaria solani* (%) at	
	1000 ppm	**1500 ppm**
DA 10	53.44	67.54
124	35.40	55.40
651	29.01	48.19
134	51.47	59.01
SE (m)	0.013	0.02
SE (d)	0.018	0.02
CD	0.039	0.06
CV (%)	6.523	12.33

Table.2 Antimicrobial activities of chito-oligosaccharides against *A. solani*

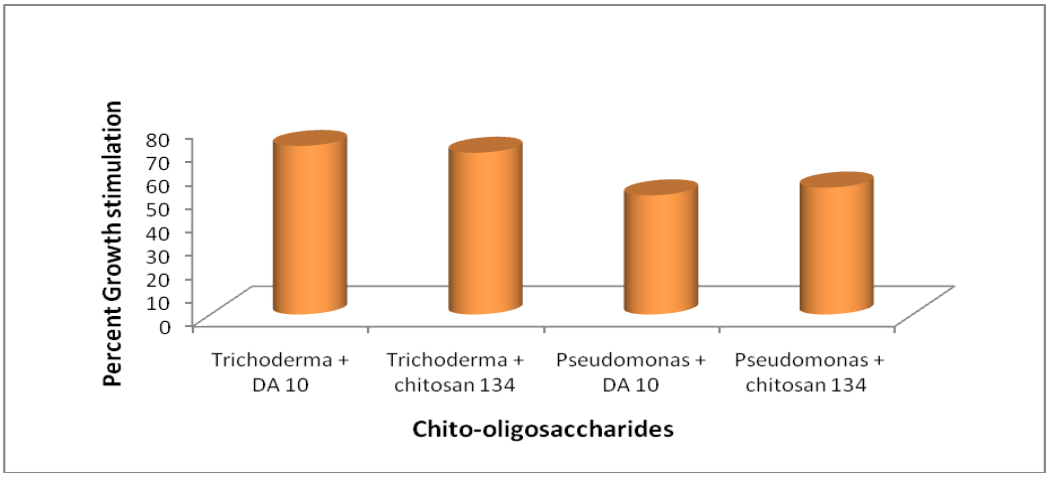
Treatments		Growth stimulation of *A.solani* %
T1	*Trichoderma* + DA 10	71.97
T2	*Trichoderma* + chitosan 134	69.08
T3	*Pseudomonas* + DA 10	50.88
T4	*Pseudomonas* + chitosan 134	54.19
T5	Control	0
	SE (m)	0.03
	SE (d)	0.04
	CD	0.09
	CV (%)	2.25

Fig.1 Evaluation of chitosans against *A. solani* on tomato i*n vitro*



DA = Degree of acetylation

Fig.2 Evaluation of chito-oligosaccharides against *A. solani* on tomato *in vitro*



T1= *Trichoderma* + DA 10, T2= *Trichoderma* + chitosan 134, T3= *Pseudomonas* + DA 10 and T4= *Pseudomonas* + chitosan134

Chito-oligosaccharides

Like chitosans, chito-oligosaccharides have also been shown to possess antimicrobial/defense inducing properties. Most of the biocontrol agents are known to possess very high chitinolytic activity. Hence, by treating DA10 and 134 chitosans with potential isolates of *Pseudomonas* (P28) and *Trichoderma* (T4), chito-oligosachhaides were produced as described in materials and methods and their efficacy against *A. solani* was studied.

Trichoderma with DA10 and 134 produced oligosaccharides, exhibited growth stimulation of test pathogen with 71.97% and 69.08% whereas oligosaccharides produced by *Pseudomonas* with DA10 and 134 exhibited growth stimulation of 50.88% and 54.19%respectively.Chito-oligosaccharides thus obtained have exhibited a positive effect on the growth of the test pathogen. The results were presented in Table 2 (Fig. 2).

Laokuldiloka *et al.,* (2017) reported antimicrobial properties of chito-oligosaccharides produced by three different enzyme treatments. However, in this study, there was no inhibitory effect of chito-oligosaccharides against test pathogen. On the other hand, Oliveira *et al.,* (2008) studied the growth rate of *A. alternata* and *Rhizopus stolonifer* in media containing different mixtures of oligosaccharides (Q1, Q2 and Q3). Q1 exhibited growth-stimulating effect whereas Q2 and Q3 fractions inhibited the growth the test fungi.

Hence concluded that the early blight of tomato is an economically important disease caused by *A. solani* (Ellis and Martin) Jones and Grout. Under *in vitro* conditions, the antifungal activity of chitosans against early blight of tomato pathogen may be attributed to direct antifungal activity such as destroying mycelium. None of the chito-oligosaccharides obtained by treating with effective biocontrol isolates identified in this study could inhibit growth of *A. solani*. On the contrary, growth promotion of *A. solani* was observed across treatments.

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How to cite this article:

Ramakrishna, A., S. Desai, G. Uma Devi and Uma Maheswari, T. 2018. Antifungal Effect of Chitosans and Chito-oligosaccharides against Early Blight of Tomato. *Int.J.Curr.Microbiol.App.Sci.* 7(07): 61-66. doi: https://doi.org/10.20546/ijcmas.2018.707.007