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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

CHITOSAN AND CHITO-OLIGOSACCHARIDES EXTRACTED AND PURIFIED FROM EXOSKELETONS OF CRUSTACEANS: POTENT GROWTH PROMOTIONAL AND PESTICIDAL ACTIVITIES ON COTTON CROP

Manjit Kaur*[1], Sanjeev Kalia[1], Abhishek Mathur[2]

[1]*Dept. of Zoology, RIMT University, Mandi-Gobindgarh, Punjab, India*

[2]*Dept. of Research &Development, Prathista Industries Limited, Telangana State, India*

**Corresponding author: manjitkaur380@gmail.com*

ABSTRACT

Chitosan and chito-oligosaccharides (COS) are the natural molecules having significant pharmacological action and promising agricultural activities. Such preparations were obtained from exoskeletons of crustaceans after series of processing steps. These molecules are responsible for inhibiting microbial growth attacking the crops, inducing natural defensive mechanisms in plants and enhancing the growth of the crops. In the present study, the antifungal activities of chito-oligosaccharides (COS), growth promoting and pesticidal activities of chitosan were determined on cotton crop and the reduction in percent population of cotton boll worm was studied. The results of the study showed promising activities of Chitosan and chito-oligosaccharides (COS) as illustrated in the study.

Keywords: Chitosan, Chito-oligosaccharides (COS), Crustaceans exoskeleton, Growth promotion activities, Pesticidal activities, Cotton crop.

1. INTRODUCTION

Chitin, a polysaccharide of animal origin, is obtained from waste material of seafood industries. It occurs in the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish. Chitin is also the important component of exoskeleton of Arthropods. Chitin is also forming the important composition of fungus. Chitosan is the deacetylated product formed by treatment of chitin with concentrated (50%) caustic alkali. The regulatory and toxicological status of Chitosan has already been established. Chitin and Chitosan are described as a family of linear polysaccharides consisting of varying amounts of linked residues of β (1, 4) linked N-acetyl-2 amino-2-deoxy - D - glucose and 2 - amino - 2 - deoxy-Dglucose residues (fig.1). Chitin samples have a low amount of D units and hence the polymer is insoluble in acidic aqueous media. Chitin is a versatile molecule having wide pharmacological and agricultural activities [1-6].

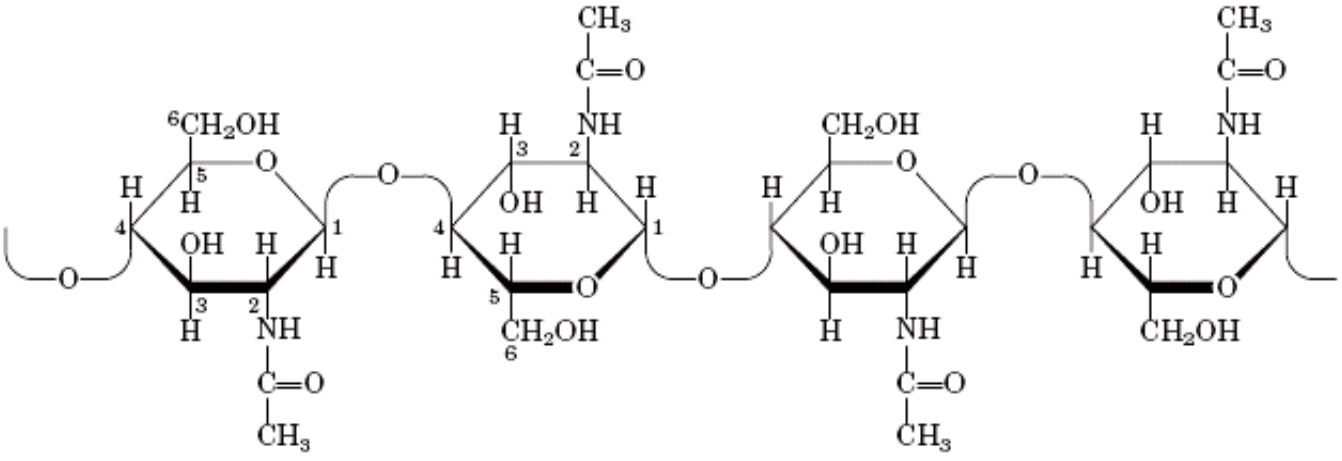


Fig. 1: Chitin structure: polymer of N- acetyl β- D glucosamine

In the present study, Chitosan and chito-oligosaccharides were prepared from Chitin obtained from fresh water crustaceans. The molecules were utilized to assess the growth regulatory activity, fungicidal activity and pesticidal activity on the cotton crop. The scope of present study can also be utilized to study the mechanism and mode of action of chitin and its derivatives on plant growth promoting rhizobacteria responsible for overall growth of the crops

2. MATERIAL AND METHODS

2.1. Collection of chitinous waste and preparation of chitosan solution

The chitinous wastes of crustaceans (exoskeleton waste of crabs and prawns) were procured from coastal areas of sea located. The chitinous wastes were washed properly to remove the sand debris and salts present on the surfaces.

2.2. Demineralization of chitinous wastes

The demineralization of chitinous wastes was performed according to the method adopted. The chitinous wastes were treated with 1.75 N acetic acids at room temperature for about 12-15 hours. The ratio of waste to solvent was maintained (1:15 w/v). The demineralized material obtained was recovered by filtration and rinsing with normal saline followed with drying in forced hot air oven at 65°C [7].

2.3. Deproteinization and removal of lipids

The methodology for deproteinization of proteins from demineralized chitinous wastes was designed by using deproteinization reagents/enzymes. The process was performed either by using proteolytic enzymes such as proteinase-k dissolved in buffer containing 0.05 M Tris base (pH 6.5-9.1) in the ratio 1:20 (w/v) in flasks at various temperatures in incubator shaker for about 72 hours and added mixture of solvents (Phenol: chloroform, 1:1 ratio) repeatedly to the residue obtained followed by centrifugation until the residue gave no test for the presence of protein content. Protein content left in the residue was treated with 2 N sodium hydroxide in water (1: 25 w/v) at 70°C for 1 hour [7].

2.4. Preparation of colloidal chitin and degradation of chitin by bacterial chitinases

The colloidal chitin was prepared by using 1g of standard chitin, in 1N HCl for 2 h at room temperature. The colloidal chitin prepared of each of the samples was washed several times with large volume of distilled water to adjust the pH to 7.0 [8].

2.5. Isolation of microbes for production of chitinase

The soil samples were collected from agricultural field by performing the serial dilution method and maintained on glycerol yeast medium plates at 37°C.

2.6. Screening and culture conditions

For the screening purpose, strain was inoculated in 100 ml of medium (3% w/v chitin, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 50 mM sodium phosphate buffer, pH 6.0) in a 250 ml Erlenmeyer flask at 30°C.

2.7. Chitinase Production

For the production of chitinase, the strain was grown in 100 ml of fresh medium (3% w/v chitin; 0.1% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; 50mM Sodium Phosphate buffer, pH 6.0) in a 250 ml Erlenmeyer flask at 30°C. For reflecting the growth of the culture in this medium OD at 660 nm was taken using blank as medium in which no inoculum was added. The supernatant (enzyme) was collected from 3-day old cultures by centrifuging the mixture at 12,000 g for 20 minutes. The enzyme was concentrated by condensing the solution in order to reduce its volume. The enzyme was further utilized to determine the degradation of chitin. The degraded chitin derived products (Chito-oligosaccharides/COS) were further purified by HPLC technique.

2.8. Separation and purification of Chito-oligosaccharides by HPLC

The purified compound obtained through gradient column chromatography and TLC was subjected to HPLC analysis. HPLC of the Chito-oligosaccharides were performed in NIMR, Haridwar (Uttarakhand), India using a Shimadzu LC-2010 HPLC system (Kyoto, Japan), equipped with a Shimadzu LC 2010 UV-VIS detector with a thermostated flow cell. The detector signal was recorded on a Shimadzu LC2010 integrator. The column used was a C-18 block heating-type Shim-pack VP-ODS (4.6 mm interior diameter × 150 mm long) with a particle size of 5 μm. Then, a 20-μl of sample was chromatographed using linear gradients of CH_3CN-H_2O from 70% to 55% in 30 minutes at a flow rate of 1 ml/minute. The oligosaccharides were monitored at 205 nm with a spectrophotometric detector.

2.9. Preparation of Chito-oligosaccharides (COS) solution and determination of anti-microbial activity

The chito-oligosaccharides were solubilized in 2 M dilute acetic acid. The prepared solution was processed for determination of antifungal activity by well diffusion method [9-13].

2.10. Determination of pesticidal activity of Chitosan on cotton boll worm population

The chitosan solution was utilized to determine the pesticidal activity on cotton boll worm as foliar spray (5 ml/Liter) in comparison to control set (Cotton crops with no treatment) [14-20].

3. RESULTS AND DISCUSSION

The Chito-oligosaccharides were purified via HPLC. The chromatogram is shown in fig. 2. In the present study, the antifungal activity of chito-oligosaccharides was determined against phyto-pathogens. The results are shown in table 1, fig. 3. The results showed promising activities against phyto-pathogens. The growth promotion and pesticidal activities on cotton crop was determined.

The results were found to have significant growth promotion activities on cotton crop in terms of Height (cm), leaves number and boll formation appearance (table 2; fig. 4). The pesticidal effect of Chitosan solution (5 ml/Liter) on percent population reduction of boll worms was determined. The results showed significant reduction in population of cotton boll worm (table 2; fig. 4). The results of the antimicrobial study were found to be corelated with the previous findings of researchers [21-23]. The results of the growth regulatory activities correlate with the previous findings [24-27].

Table 1: Antifungal activity of Chito-oligosaccharides (COS) against fungal phyto-pathogens

Diameter of zoned of inhibition of Chito-oligosaccharides (COS) via well diffusion method			
Sample/Positive Control	***Fusarium oxysporum***	***Sclerotium rolfsii***	***Colletotrichum sp.***
Chito-oligosaccharide (1 mg/ml)	18.0±0.43	23.0±0.45	35.0±0.018
Fluconazole/ Positive Control (1 mg/ml)	42.0±0.28	43.0±0.32	42.0±0.020

**P<0.05, level of significance*

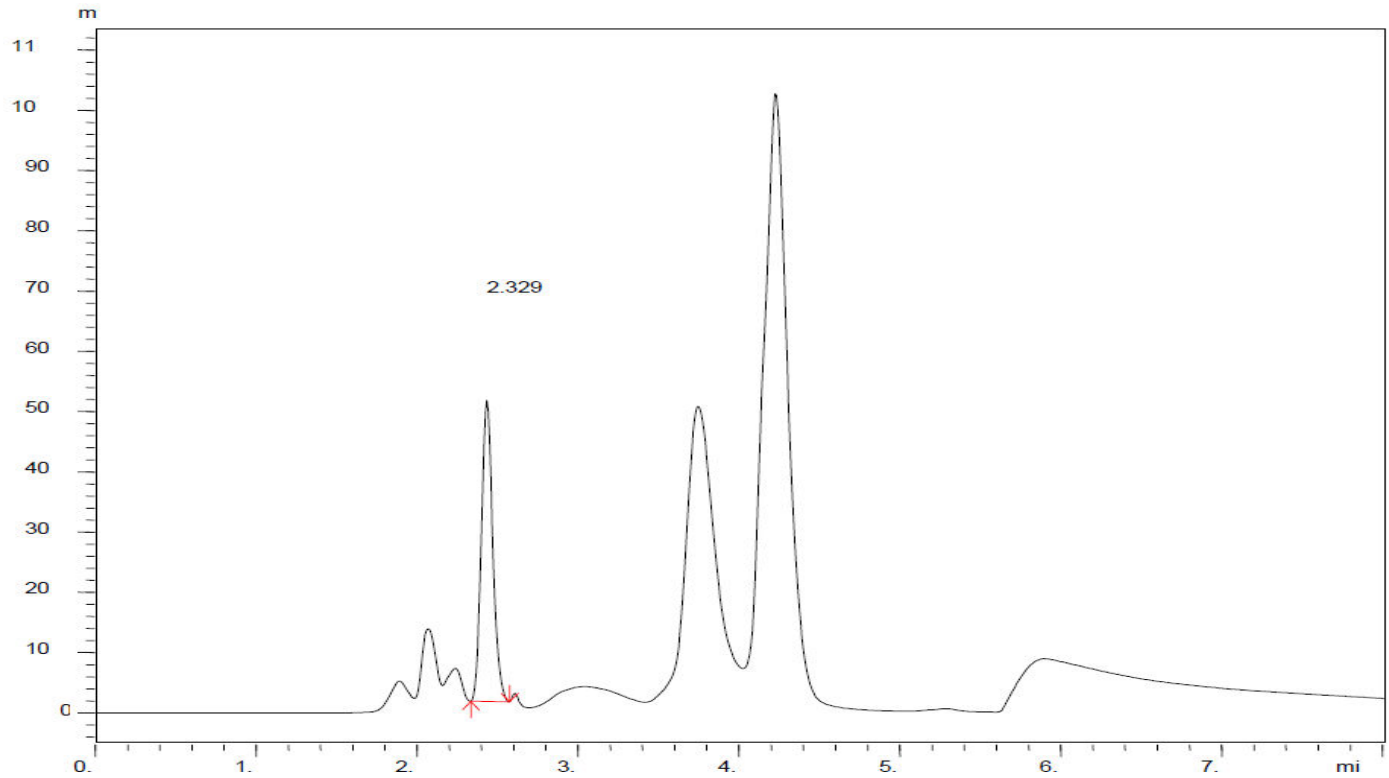


Fig. 2: HPLC chromatogram peaks showing the separation of Chito-oligosaccharides (COS)

Table 2: Effect of Chitosan on vegetative growth; reproductive growth and reduction in population of boll worm on cotton crops

Treatment Sets/Control	Average readings of best 5 plots (25 crops per plot) after 2 months				
	Crops number per plot	Height (cm)	Leaves number (cm)	Boll formation appearance	Percent reduction of population of Boll worm
Control plot (Without treatment)	25	15.0	22.0	70.0	0.0
Treatment with Chitosan solution only (5 ml per liter)	25	56.0	33.0	12.0	33.0

(Average readings of best 5 plots)- after 2 months' time period (Fertilizer - cow dung manure)

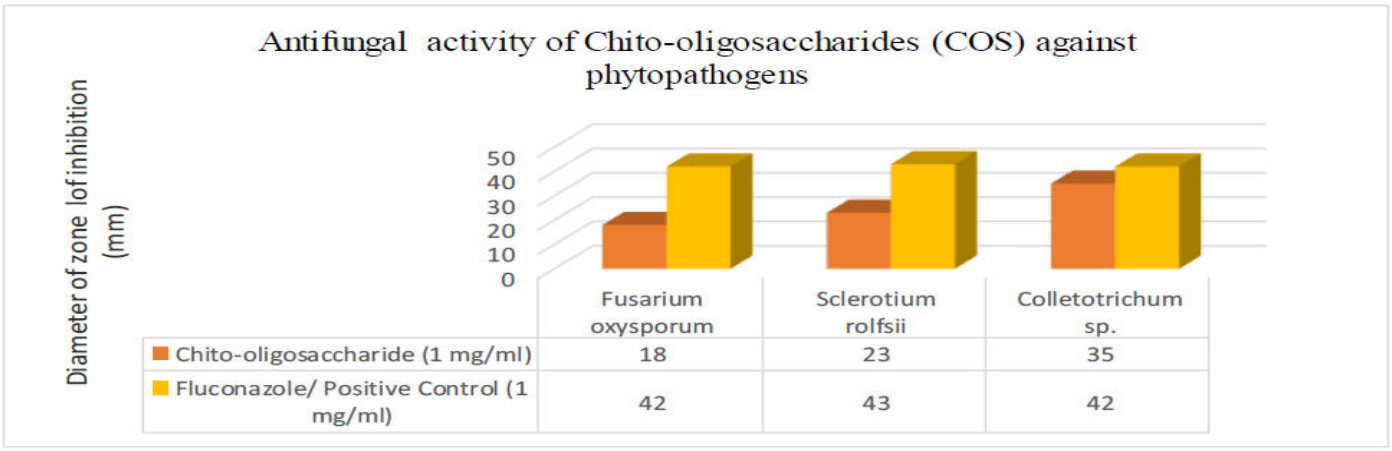
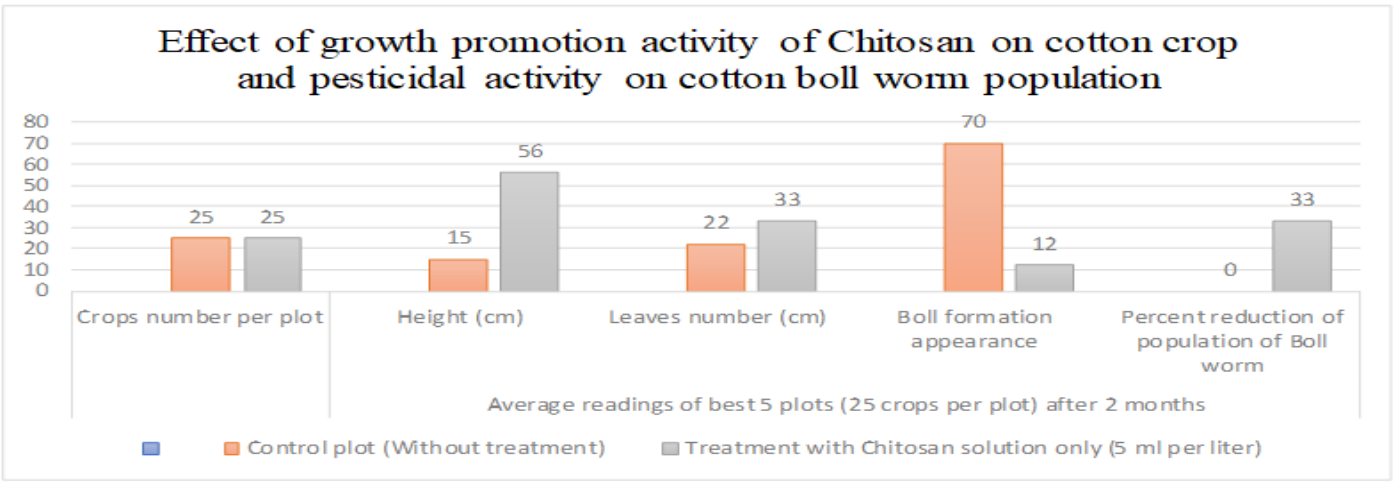


Fig. 3: Graphical representation of antifungal activity of Chito-oligosaccharides (COS) against fungal phyto-pathogens



(Average readings of best 5 plots)- after 2 months' time period (Fertilizer - cow dung manure)

Fig. 4: Graphical representation of Effect of Chitosan on vegetative growth; reproductive growth and reduction in population of boll worm on cotton crops

4. CONCLUSION

The studies thus suggest that, Chitosan and chito-oligosaccharides (COS) can be utilized as promising growth promoter, fungicidal and pesticidal agent in cotton crop. The results of the study thus validate the concept as determined in the study. Future studies must be done to determine the mechanism and mode of action of Chitosan and chito-oligosaccharides for growth

promotion, fungicidal and pesticidal action in crops. The present study can also be utilized to study the mechanism and mode of action of chitin and its derivatives on plant growth promoting rhizobacteria responsible for overall growth of the crops.

Conflict of interest

None declared

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