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Evaluation of growth regulatory effect of chitin and chitin based derivatives extracted from fresh water crustaceans

Junaid Alam*[1], Akhilesh Kushwaha[3] and Abhishek Mathur[2, 3]

[1]CMJ University, Shillong, Meghalaya, India
[2]Department of Research & Development (R&D), Sheetal Life Sciences, Dehradun (U.K), India.
[3]Institute of Transgene Life Sciences, Lucknow (U.P), India.

Abstract

The present investigation was carried out to investigate the natural growth supplement in fields as an alternative source instead of synthetic and traditional fertilizers. Since the traditional and synthetic fertilizers used in fields causes toxicity in the grains etc. and thus are lethal to the lives of flora and fauna that resides within the soil and also to humans. The study thus focused to explore some natural products/molecules which can be utilized as growth regulator and supplement in the fields which shows no toxicity. In the present investigation, natural polysaccharide viz. chitin was extracted from fresh water crustaceans and its derivatives (chitosan) was prepared by the process of deacetylation by the treatment of chitin with 40% (w/v) NaOH at 120^{o}C for 3h. The growth regulating effect of crustacean waste, extracted chitin and chitin based derivatives viz. chitosan on mung bean seeds was observed by the treatment of sterilized mung bean seeds with these products for 12 h to 96 h. Non-treated seeds with these products was used as negative control while seeds treated with standard chitin (as procured from Hi-Media Pvt. Ltd., Mumbai, India) was used as the reference positive control. The results were found to be very surprising as the seeds treated with chitin and chitin based derivatives showed significant germination. It was found that seeds treated with crustacean waste and standard chitin promotes prominent growth regulating activities with the passage of time. It was found that 50% germination in mung bean seeds (treated with crustacean waste and standard chitin) occurred after 96h in comparison to standard; extracted chitin and chitosan treated seeds (33%).

Keywords: Chitin, chitosan, natural products/molecules, growth regulation/promotion activity.

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. Chitin hold great economic value due to their versatile biological activities and chemical applications, mainly in medical [1, 2] and pharmaceutical areas [3, 4]. Chito-oligosaccharides and their N-acetylated analogues are useful for applications in various fields because they have specific biological activities such as antimicrobial activity, antitumor activity and immune-enhancing effects [5]. Some chito-oligosaccharides such as (GlcNAc) and (GlcNAc) have been reported to possess antitumor activity [6, 7]. Chitinolytic enzymes have been widely used in various processes including the agricultural, biological and environmental fields [8, 9]. Several chitinolytic enzymes have been identified in various *Streptomyces* sp., including, *Streptomyces plicatus* [10], *S. lividans* [11], *S. virdificans* [12] and *S. halstedii* [13]. The chitinases were purified and characterized from marine bacterium [14]. The potent chitinolytic activity of marine actinomycetes species and enzymatic production of chito-oligosaccharides was investigated [15]. Chitin and chitin based derivatives have beneficial effects in agriculture [16]. As few reports are available on the growth stimulating effects of chitin and it's based derivatives, our study is thus based on the assessment of growth regulating/promoting activities of chitin and chitin based derivatives.

*Corresponding Author

Junaid Alam
CMJ University, Shillong, Meghalaya, India

Email: junaidbiotechnology@hotmail.com

MATERIALS AND METHODS

All the chemicals and reagents used were of Analytical grade and were procured from Ranchem and Hi-Media, India.

Collection of Chitinous wastes

The chitinous wastes of fresh water crustaceans were collected from the fresh water areas of Dehradun and Rishikesh (U.K), India and were washed properly in order to remove the sand debris present on the surfaces. The chitinous wastes were then after air dried and powdered material obtained was used as chitin.

Demineralization of chitinous wastes

The demineralization of chitinous wastes was performed [17]. The chitinous wastes were treated with 1.75 N acetic acid at room temperature for about 12-15 hours. The ratio of waste to solvent were maintained (1:15 w/v). The demineralized material obtained

were recovered by filteration and rinsing with de-ionized water and will be dried in forced hot air oven at 65^{0}C.

Deproteinization and removal of lipids

The new and advanced methodology for deproteinization of proteins from demineralized chitinous wastes was designed by using deproteinization agents. This process can be 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 a ratio 1:20 (w/v) in flasks at various temperatures in incubator-shaker for about 72 h and adding mixture of solvents (phenol: chloroform, 1:1 ratio) again and again to the residue obtained and centrifuging the mixture until the residue gives no test for the presence of protein content. After repeating the procedure for 3-4 times, finally the residue was treated with 2N sodium hydroxide (1:25 w/v) at 70^{0}C for 1 hour. The lipid content gets dissolved in phenol: chloroform mixture and was removed from the chitinous wastes. Grease spot test can be performed in order to determine qualitatively the presence of lipid content if any present in the residual material. The residual materials left were dried in hot air oven at 60^0 C.

Preparation of Chitosan

The demineralized and deproteinized chitin material was subjected to concentrated sodium hydroxide at 40% w/v [18]. The deacetylated forms of chitosan obtained were solubilized in 2 M dilute acetic acid.

Growth Regulating Assay of Chitin and Chitin based derivatives

The growth regulating/promoting assay was performed as per our experimental procedure. Mung bean seeds (about equal in no.) were sterilized initially in Tween-20 and further in N-saline. The seeds were then soaked separately in crustacean waste solution (20 mg/ml in 0.1 N NaOH), extracted chitin (20 mg/ml in 0.1 N NaOH), standard chitin (20 mg/ml in 0.1 N NaOH), extracted chitosan (20 mg/ml in 0.1 N NaOH) and standard chitosan (20 mg/ml in 0.1 N NaOH). The petri plates having untreated seeds/seeds soaked in N-saline were used as Negative control. Further the treated and untreated seeds were transferred to petri plates having sterilized cotton bedding. The germination of seeds was recorded from 12 h-96 h duration.

RESULTS

The assessments of growth regulation/promotion activities of chitin and chitin based derivatives were performed. The results were found to be very surprising as the seeds treated with chitin and chitin based derivatives showed significant germination. It was found that seeds treated with crustacean waste and standard chitin promotes prominent growth regulating activities with the passage of time. It was found that 50% germination in mung bean seeds (treated with crustacean waste and standard chitin) occurred after 96h in comparison to standard; extracted chitin and chitosan treated seeds (33%). The results are shown in Table 1 and Figure 1.

Table 1. Data Table for Growth Regulation activities of Chitin and its Derivatives

	Control	Exoskeleton Powder	Standard Chitin	Extracted Chitin	Standard Chitosan	Extracted Chitosan
18 hours	17	33	33	17	17	No Growth
24 hours	17	33	50	17	17	No Growth
43 hours	17	33	50	33	33	17
48 hours	17	50	50	33	33	17
67 hours	17	50	50	33	33	17
72 hours	17	50	50	33	33	17
96 hours	17	50	50	33	33	17

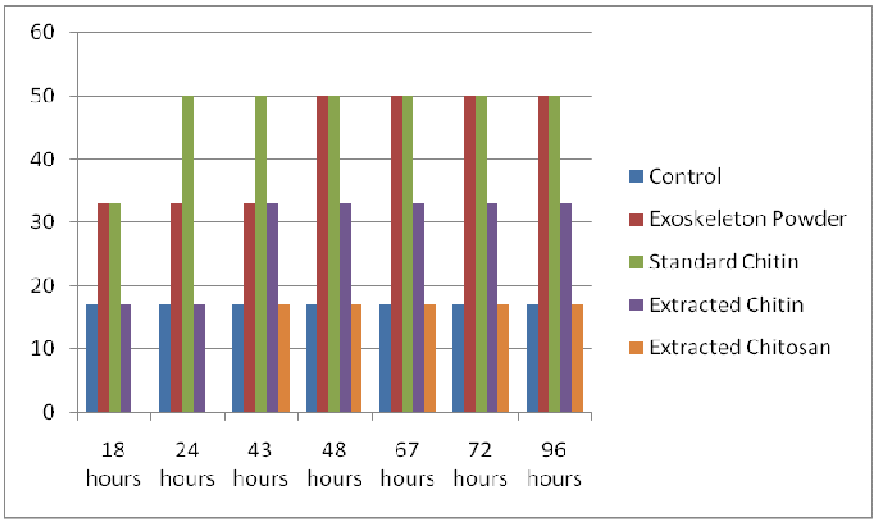

Fig 1. Growth Regulation activities of Chitin and its Derivatives

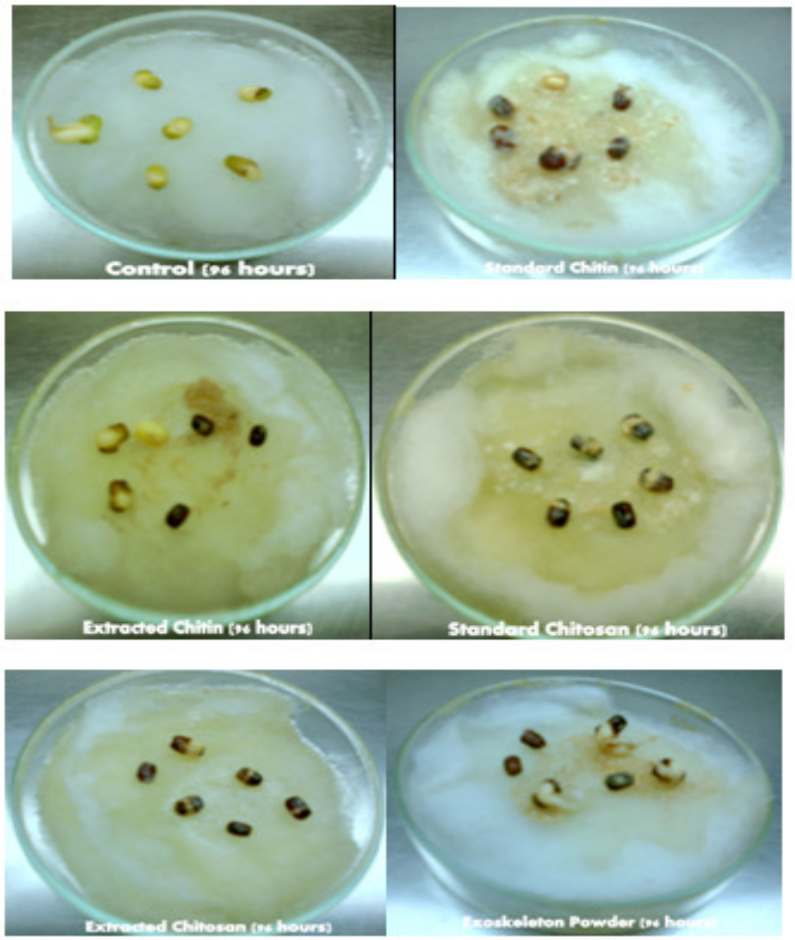

Fig 1. Growth Regulation activities of Chitin and its Derivatives

DISCUSSION AND CONCLUSION

The results confirmed that chitin and chitin based derivatives can acts as growth accelerators in fields. Our studies thus provide the approach for assessing natural molecules and products that can acts as growth regulators and promoters of plants/dormant seeds. The study thus illustrates that natural molecules and products can be utilized in growth promoting activity. Our results thus correlate the previous findings [15]. Further studies are however needed to refine the technique and interrogating the growth activities of chitin and chitin based derivatives using different experimental procedures.

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