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Original Research Article

Isolation and Application of Chitin and Chitosan from Crab shell

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A B S T R A C T

Chitin and chitosan are an important family of linear polysaccharides consisting of varying amounts of β-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2- amino-2-deoxy-β-D-glucopyranose (GlcN) units. The specific objectives for this study were to extract the chitosan from crab exoskeletons and evaluate its growth enhancing properties for plants. The soil sample and crab shells are collected in around Thanjavur for chitosan estimation and its efficacy as a biofertilizer. Low nutrient soil samples were enriched with chitosan and also used for growth rate growth of Black gram plant (*Vigna mungo).*

Introduction

Crab Shell is an excellent dry organic source of NPK, Calcium (23%) and Magnesium (1.33%). It will also help with nematode and fungus problems. Crab Shell is high in Chitin (Kite-en), which promotes the growth of Chitin eating bacteria in the soil. The exoskeletons of fungus and nematode eggs are high in chitin. Crab Shell when added to the soil helps to create a hostile environment for the fungus and nematodes by feeding the biological life that eats chitin and chitin based organisms. To reduce the impact of excess chemical fertilizers in the field of agriculture the Biofertilizer is being considered as a potential tool; biologically fixed nitrogen is such a source which can supply an adequate amount of Nitrogen to plants and other nutrients to some extent.

Biofertilizer material as a substitute for Nitrogen fertilizer. In general two types of Biofertilizer are used. When chitin is mixed with soil, the most common polysaccharide, stimulates the micro-organisms and this chemically stable compound is mineralized in a short period (Alexander, 1977). Soil amendment with chitin has also resulted in significant control of root knot nematode and root-infecting fungi by changing the soil micro-flora resulting in an increase in microorganisms around the roots, antagonistic to root pathogens (Godoy *et al.*, 1983).

Chitin and chitosan are an important family of linear polysaccharides consisting of

varying amounts of β-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN) units (Muzzarelli, 1973).

Chitin was first found in mushrooms in 1811 by Professor Henri Braconnot, Director of the Botanical Gardens at the Academy of Sciences in Nancy, France. The English word "chitin" comes from the French word "chitine," which first appeared in 1836. These words were derived from the Latin word "chitōn," meaning mollusk.

Chitin is a poly - B - (1-4) - N - acetyl -D-glucosamine. It is a nitrogen containing polysaccharide, related chemically to the cellulose; hence, they are insoluble in water and common organic solvents. On the other hand, they dissolve only in solvents such as N,N dimethylacetamide, hexafluoroacetone or hexafluoro-2-propanol . When the degree of N-acetylation (defined as the average number of N-acetyl-D-glucosamine units per 100 monomers expressed as a percentage) is less than 50%, chitin becomes soluble in aqueous acidic solutions (pH < 6.0) and is called chitosan. This means that the term “chitosan” represents a group of fully and partially deacetylated chitins, but a rigid nomenclature with respect to the degree of N deacetylation between chitin and chitosan has not been established.

Some authors consider that chitosan is a polysaccharide containing at least 60% GlcN residues. According to the nomenclature proposed by the European Chitin Society (EUCHIS) (Pillai *et al.*, 2009), chitin and chitosan should be classified on the basis of their insolubility and solubility in 0.1 M acetic acid; the insoluble material is called chitin, whereas the soluble one is chitosan. The structures of “ideal” chitin and “ideal” chitosan,and the “real“ structures of these compounds are resented in Figure 1.

Application

Various applications of chitin are of great industrial importance. The proper utilization of these shell wastes not only solves the problem of their disposal but also forms the basis for many potential products used in the fields such as textiles, photography, medicine, agriculture, food processing etc.

Crab is a decapod crustacean There are about 4500 species crabs that come in many different size and colors. About 30-40% of crustacean shell waste consists of protein, 30-50% calcium carbonate and 20-30% chitin (Johnston and Peniston, 1982). These proportions vary with species and season.

Chitin and its derivative, chitosan, are biocompatible, biodegradable, nontoxic, anti-microbial and hydrating agents.

The aim of the study was to enhance the utilization of crab waste and help to minimize the environmental pollution. The specific objectives for this study were to extract the chitosan from crab exoskeletons and evaluate its growth enhancing properties for plants.

Materials and Methods

Sample Collection

The Crabs were collected from Thanjavur market. The Crab Exoskeletons were placed in Ziploc bags and refrigerated overnight. The samples were dried under room temperature for 10days and ground well to make it into a powder. The ground sample was used as powder for the preparation of Biofertilizer. and D-Glucosamine.

Soil Collection

The soil was collected from four different areas and their parameters were analyzed

before and after application of chitosan. (Table: 1)

Extraction of Chitin and Chitosan (Kim *et al.*, 1999)

1. The crabs' exoskeletons were placed in 250 ml beakers and treated in boiling sodium hydroxide (4% v/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin.

2. After the samples were boiled in the sodium hydroxide, the beakers containing the crab shell samples were removed from the hot plate, placed in the hood and allowed to cool for 30 minutes at room temperature.

Demineralization

The exoskeletons were weighed approxitely 25 g

Sample was demineralized with 100 ml of HCl using concentrations 0.5% or 1.0%. The samples were allowed to soak for 24 hours to remove minerals (mainly calcium carbonate)

The demineralized crab shell samples were then treated for one hour with 50ml of a 20% NAOH solution to decompose the albumen into water soluble amino-acids.

The remaining chitin was washed with deionized water, which was then drained off. The chitin was further converted into chitosan by the process of deacetylation.

Deacetylation

The deacetylation process was carried out by adding 100 ml of 50% NaOH to each sample and then boiled at 100°C for 2 h on a hot plate.

The samples were then placed under the hood and cooled for 30 min at room temperature.

Afterwards the samples were washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan.

The prepared chitosan was then placed in 250 ml beakers and labeled according to the treatment used.

The samples were then left uncovered and oven dried at 120°C for 24 h. The chitosan was then in a creamy-white form.

Field Study as a Biofertilizer

The seeds of *Vigna mungo* were collected and it was soaked in various concentrations at overnight. The standard chitosan concentration was prepared i.e. 10g of chitosan is dissolved in 10ml of distilled water.

Materials Used

Boiling tubes, Cotton, Distilled water.

Seed Germination Technology

The above mentioned materials were sterilized in Autoclave.

By using cotton make a layer of bud in boiling tubes under sterilized condition.

The seeds soak in different concentration of chitosan were taken (10%, 30%, 70%, Control).

Then the seeds were inoculated in boiling tubes, and kept in to incubation for 24 hours. After the germination of the seed, is transferred in to the pot. The same

concentration is used for to soak the seeds is mixed in the soil.

6. Every 15 days the Calcium, Magnesium, Organic matter, Nitrogen, Potassium was analyzed.

Chitosan Estimation (george *et al.*, 1983)

The chitosan solution (1 ml) was added to 0.25 ml of 4% acetyl acetone (4% acetyl acetone in 1.25 N sodium carbonate [vol/vol]) and heated at 90°C for 1 h in a test tube covered with a Teflon-lined screw cap.

After cooling, 2 ml of ethanol was added, with shaking to dissolve precipitates.

Add 0.25 ml of Ehrlich reagent (1.6 g of N-N-dimethyl-p-aminobenzaldehyde in a 30:30 ml mixture of ethanol and concentrated HCI) was added.

The color formed in the solution was measured at 530 nm spectrophotometer.

Duplicate determinations were performed for each hydrolysate.

Results and Discussion

Soil Analysis

The Soils were collected from four different areas and their quality was analyzed. The Mathakottai sample shows the lowest nutrient content compare with other selected samples. Table: 2 shows the nutrient values of selected samples. After the application of chitosan the plants were grow well in mathakottai soil and their nutrients such as organic matter, N, P, K , Iron, Calcium, Magnesium and Heavy metals were analyzed. (Table: 8).

After 2 months the results showed that seeds exposed to chitosan treatment at 70% achieved a better germination percentage when compared to those exposed to chitosan treatment at 10% and 30%. The seeds exposed to 70% chitosan treatments were taller than the untreated control. However, the germination ratio (%) for plants exposed to chitosan 10% and 30% was homogenous with the one of the untreated control.

Chitosan stimulates soil biodiversity and restores beneficial organisms that attack, repel, or otherwise antagonize disease-causing plant pathogens. It's like a vaccination render a soil disease-suppressive. Plants growing in disease-suppressive soil resist diseases much better than in soils low in biological diversity. The chitosan were estimated in various parts of the plant, like leaf, stem, and root. In that chitosan is greatly absorbed in roots of the plant compared to stem.

Field Study

The results of the field studies shown in Table 6 and Table 7. The average leaf count height and stem diameter of the plants that were under investigation for the three month study.The results for average leaf count of plants whose seeds were treated with 70% chitosan and 30% chitosan treatments, revealed better leaf production. The average leaf count for the untreated control was less than the plants whose seeds were treated with chitosan treatments. The average height of the plants treated with chitosan was greater than the untreated control. The average height for 70% chitosan and 30% chitosan treatments were 27.8 and 21.7cm respectively. The overall results for the untreated control in regard to the height of the plant were significantly lower than the experimental plants; therefore the height growth process was much slower for the controls. There were also some pest and

predator activities during the study which might have affected the growth success of the plants.

Grain Yield

The maximum yield was achieved by applying chitosan by seed soaking and soil application; the average yield was 135g / plant, whereas the control was 75g /plant (Table:9). This result could explain that application Of Chitosan by incorporation in soil showed the maximum grain yield. Moreover, chitosan had a positive ionic charge which chemically binds with plant nutrients that showed a negative ionic charge resulting in a slowly released action in plants which closely contributed to yield increasing.

Table.1 Sample Collection Areas

S.No	Soil Collection
1.	Mathakottai
2.	E.B colony
3.	Mariamman Kovil
4.	Ammapet

Table.2 Seed Growth Enhancement

Si.No	Concentration
Control	Soak in 10ml Distilled water
10%	9ml of Distilled water in 1ml of chitosan
30%	7ml of Distilled water in 3ml of chitosan
70%	3ml of Distilled water in 7ml of chitosan

Table.3 The Nutrient Analysis of Soil

Soil collection	Calcium (mg)	Magnesium (mg)	Organic Matter (%)	Nitrogen (%)	Potassium (%)
Mathakottai	25.4	11	3.9	0.048	0.069
E.B colony	27.8	11.4	7.7	0.050	0.072
Mariamman Kovil	31	13.5	12.7	0.650	0.076
Ammapet	35.4	14.4	13.2	0.117	0.075

Table.4 Chitosan Estimation in Soil

Concentration	Chitosan Estimation
Control	0.27
10%	1.014
30%	1.325
70%	1.340

Table.5 Nutrient Analyses in Soil Using Different Concentration of Chitosan for Plant Growth

Chitosan Concentration	Calcium	Magnesium	Organic Matter	Nitrogen	Potassium
70%	35.4	24.35	7.0	0.73	0.141
30%	27.1	36.52	4.2	0.670	0.136
10%	25.5	14.61	6.4	0.62	0.118
Control	23.4	12.2	3.9	0.056	0.09

Table.6 Chitosan Estimation in Different Parts of Plant

Concentration Of Chitosan	Chitosan analysis %		
	Root	Stem	Leafs
Control	1.27	0.20	1.086
10%	1.453	1.21	1.09
30%	2.204	1.124	1.40
70%	2.124	1.762	1.394

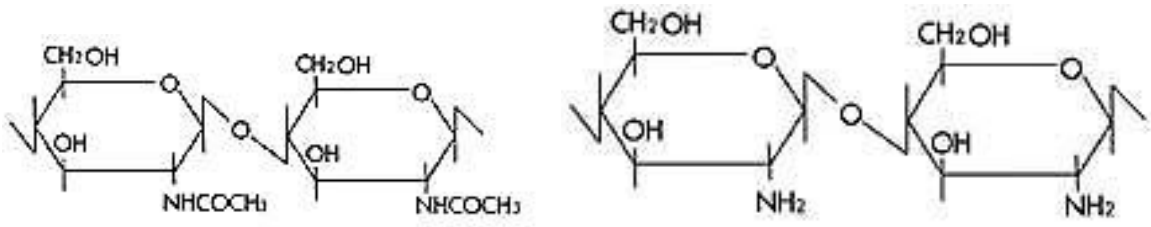
Table.7 Heavy Metal Analyses in Soil

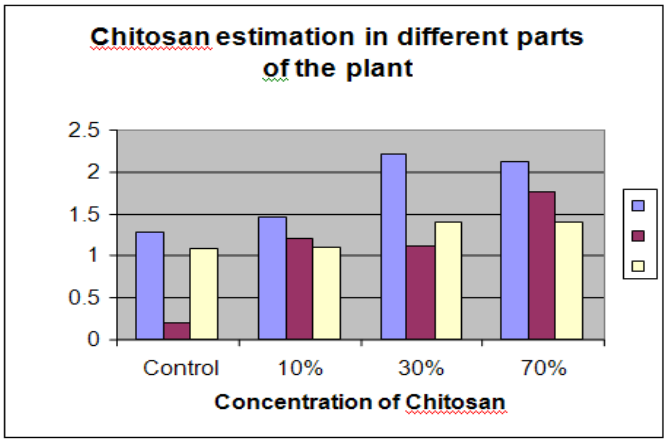
Chitosan Concentration	Cadmium	Lead	Zinc	Mercury
Control	Nil	2.507	0.5	0.65
10%	Nil	0.8	0.1	0.32
30%	Nil	1.1	0.1	0.33
70%	Nil	1.05	0.2	0.52

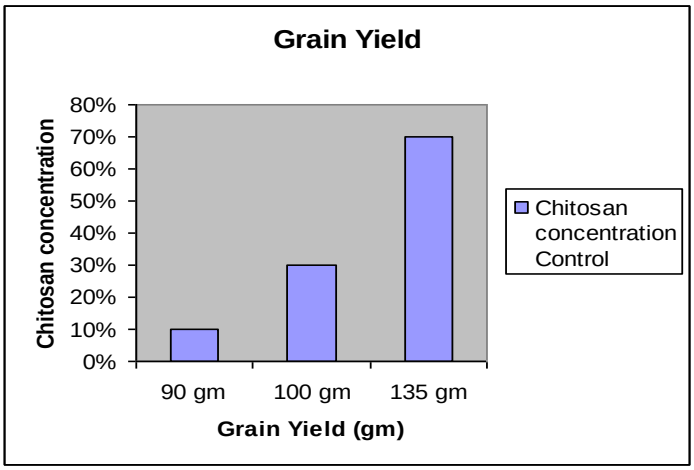
Table.8 Grain Yield

Chitosan concentration	Grain yield
Control	75 gm
10%	90 gm
30%	100 gm
70%	135 gm

Fig.1 The Chemical Structures of Chitin and Chitosan







Chitin, a major component of discarded shellfish is reported from Pakistan to contain 16.1% chitin in shrimp, 19.5% in crab and 14.5% in prawn (Sultana *et al*., 2000). There are reports that chitin and chitosan amendments to soil effectively reduced soilborne diseases (Benhamou & Theriault, 1992; Bell *et al.*, 1998).

Chitin to soil resulted in a marked suppression of the severity of root rot of bean caused by *Fusarium solani* f.sp. *phaseoli* and vascular wilt of radish caused by *F.oxysporum, F. conglutinans* (Mitchell & Alexander, 1961). Bell *et al.*, (2000) reported a reduction in the abundance of the plant parasitic nematodes *Heterodera trifolii* in white clover and *Paratylenchus* sp., in ryegrass roots by the application of chitin to soil. In the present study, use of crustacean chitin at 0.1% or crustacean waste powder (having 0.1% chitin) alone or with *Pseudomonas aeruginosa* or *Paecilomyces lilacinus* was efficacious in the control of root-infecting fungi attacking chilli roots. Rodriguez-Kabana *et al*., (1984) reported that the addition of chitin (1% or more) to soil control root knot nematode. Others reported that the use of chitin alone or with organic materials reduced fungal infection (Bade & Wick, 1988; Sultana *et al*., 2000) and root knot nematode establishment (Ehteshamul-Haque *et al*., 1997). Similarly Manjula & Podile (2001) reported that a chitin-supplemented formulation improved biocontrol activity and plant growth promoting potential of *Bacillus subtilis*.

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