Sustainable Abstraction of Bio-Polymer from Seafood Trash used for Soil Drenching and Analyzed for the Plant Growth Enhancer

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Abstract— *In worldwide distribution, chitin, which is present in crustacean shells, is the second most prevalent natural polymer. Chitosan is a natural amino-polysaccharide derived from chitin, with exceptional biocompatibility, biodegradability, and non-toxicity properties. Due to its characteristics and potential uses in sustainable agriculture, regenerative medicine, etc., chitosan has attracted much attention, resulting in an increasing number of publications and patents each year. This study's objective is to synthesize and characterize nanochitosan from waste seafood, especially blue crab (Callinectes sapidus). The prepared nanochitosan by high energy ball-milling method using sea species waste was synthesized and characterized for morphology, pore size, porosity, functional group, crystallinity, and thermal analysis determination by SEM, BET, FTIR, XRD, and DSC. All the characterization data were related to commercial chitosan and confirmed the structure and properties of chitosan. The results provide better in their nano-size and are further associated with the degree of crystallinity in physicochemical properties compared to the commercial chitosan. The prepared nanochitosan is more beneficial for environmental applications like agriculture due to its biodegradability. In comparison to commercial chitosan, the application of nanochitosan in soil yields excellent results in terms of moisture content, water holding capacity, total nitrogen, and carbon, which are 39%, 84%, 3.1g/kg, and 47.7g/kg, respectively.*

Keywords— Natural polymer, Nanochitosan, Commercial chitosan, Physical Characterization, Environmental application.

I. INTRODUCTION

Chitosan is a biopolymer formed from chitin, which is present in crustacean exoskeletons such as crabs and shrimp. Chitosan is a natural and linear polysaccharide derived from chitin by a chemical process that includes deproteinization, demineralization, and decolorization, and it is made by deacetylating chitin, which involves removing the acetyl groups from the polymer chain. (Figure 1). Chitosan has become beneficial and highly appreciated as a natural biodegradable high molecular polymer chemical that is a non-toxic and bioactive agent due to its fungicidal activities and elicitation of defensive systems in plant tissue. Chitin and chitosan are both polysaccharides that are chemically identical to cellulose, with the only difference being the presence or absence of nitrogen, which is absent in cellulose (Bautista-Baños et al., 2006). Chitin, a linear polysaccharide comprised of (1-4)-linked 2-acetamido-2-deoxy-b-D-glucopyranose units (Hu et al., 2007) (Dutta et al., 2002), is nature's second most common type of polymerized carbon. Even though it is not present in organisms that produce cellulose, it is classified as a cellulose derivative. It has a similar structure to cellulose, but it has an acetamide group (-$NHCOCH_3$) at the C_2 position. Molluscs, crustaceans, insects, fungi, algae, and other creatures make around 10 billion tons of chitin each year.

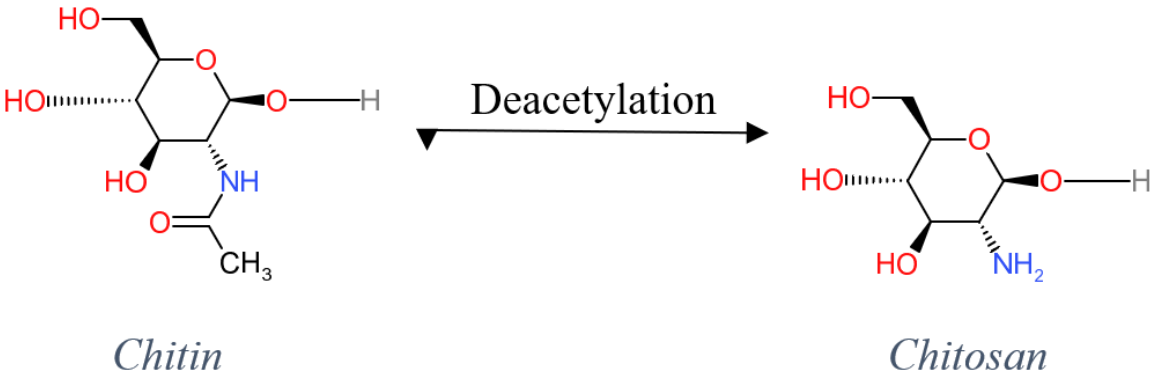

FIGURE 1: Structure of Chitosan after deacetylation from Chitin

The blue crab (*Callinectes sapidus*) (Figure 2) has a natural range that extends from Nova Scotia to northern Argentina, including Bermuda and the Antilles. The life cycle of blue crabs is similar to that of other estuarine-dependent species in the Gulf of Mexico. The blue crab sustains one of the Gulf of Mexico's most important commercial and leisure fisheries. Blue crabs were traditionally exploited by locals for immediate consumption; nevertheless, the first commercial mud crabs eventually reached local markets and formed a major component of the local crab fishery (Ikhwanuddin et al., 2011). Crab flesh is used in a variety of ways in the food business, including as an ingredient in culinary items. However, the crab shell is also beneficial in terms of cancer prevention and as a natural weight loss supplement. Chitosan, a polysaccharide, is also found in crab shells.

FIGURE 2: Blue crab *(Callinectes sapidus)*

Chitosan is the resultant material, and it has several unique features that make it valuable in a range of applications. When chitosan comes into contact with water, it forms a gel-like material, which is one of its most remarkable features. Because of its features, it is beneficial in a variety of sectors, including food packaging and wastewater treatment. Chitosan also possesses antibacterial characteristics, which make it valuable in medicinal applications such as wound healing and drug delivery. Furthermore, chitosan has been demonstrated to have potential use in agriculture, where it may be utilized as a natural pesticide or fertilizer. Because of its biodegradability, cationic nature, film-forming capacity, antibacterial characteristics, chelating capabilities, water-holding capacity, pH sensitivity, biocompatibility, and adhesion qualities, chitosan is a flexible and important agent in agriculture. It may be used to enhance soil, control disease, regulate nutrients, and promote sustainable agricultural methods. Its features are pH-adjustable, making it appropriate for organic and sustainable activities. Researchers are always looking for new methods to exploit its benefits for better agricultural yield and environmental sustainability. The degree of N-acetylation (DA) of chitosan determines its characterization, which affects not only its physicochemical properties but also its immunological activities (Mahlous et al., 2007). The functional qualities of chitin and chitosan are influenced by

physicochemical parameters, which vary depending on the crustacean species and method of manufacture. The physicochemical characteristics of various preparations will differ, particularly the degree of deacetylation, solubility, viscosity, and molecular weight. To efficiently use chitinous products for specific applications, the functional characteristics of chitin and chitosan products should be carefully evaluated (Cho et al., 1998). The presence of free amine groups throughout the chitosan chain affects its solubility, allowing it to dissolve in diluted aqueous acidic solutions.

Chitosan derived from crab shells has been shown to have several agricultural advantages. One significant advantage is its ability to improve soil structure by boosting porosity and water-holding capacity. This promotes root growth and nutrient absorption in plants. Chitosan has been found to improve plant development by promoting seed germination and boosting plant biomass, in addition to improving soil structure. It can also assist in minimizing the demand for artificial fertilizers by increasing the availability of nutrients in the soil.

Chitosan stimulates several defensive mechanisms to increase plant tolerance to a wide range of biotic and abiotic stresses, including drought, cold, salt, and water-related difficulties.(Ali et al., 2021) It has been proven that chitosan treatment increased chlorophyll content, hence increasing tomato growth under salt-induced stress conditions. (Wang et al., 2021) Under salt stress, maize height, and main root length were dramatically reduced, whereas shoot and root dry weights were both reduced, and sodium absorption increased. Salt stress dramatically reduced maize seedling photosynthesis, including photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate. (Jiao et al., 2024)

The use of chemical fertilizers to improve soil nutrition has increased agricultural production in recent decades (Mącik et al., 2020). However, there are numerous known disadvantages to continuously applying chemical fertilizers to the soil, such as increasing irrigation requirements, suppressing phyto-beneficial microbes in the soil, and negatively impacting soil ecology, despite some additional benefits, such as ease of handling and predictable results (Bisht and Chauhan, 2020). Simultaneously, the physical, chemical, and biological health of arable land has deteriorated due to excessive chemical use and changes to traditional agricultural methods(Chaudhary et al., 2020). As a result, with dwindling land resources and soil biological potential, the health of diverse agricultural production systems, as well as total biological resources, require proper attention. Under these circumstances, there is a compelling case for using microorganisms in integrated plant management systems to improve plant performance (Saberi-Riseh and Moradi-Pour, 2021). Chitosan, being a polysaccharide, works as a bioremediation molecule, stimulating the activity of beneficial soil microbes such as *Bacillus spp*., fluorescent *Pseudomonas spp*., *Actinomycetes*, *Mycorrhiza*, and *Rhizobacteria*. This affects the rhizosphere's microbial balance, favoring beneficial bacteria. Bioremediation of soil disturbed with a variety of heavy metals was helped by chitosan treatment in conjunction with mycorrhizal inoculation (Angelim et al., 2013). It encapsulates a consortium of various PGPR within chitosan aided in delivery while also stimulating the development and activity of the bacteria for bioaugmentation and biostimulation of hydrocarbon-polluted soils. *Bacillus subtilis* is a fungal pathogen and one of the most extensively used biopesticides in agriculture. *B. subtilis* produces chitinases in its growth media (Chen et al., 2010). The addition of chitosan to the carrier material increased *B. subtilis* multiplication and fungicidal effect, as well as the control of Fusarium wilt in pigeon pea and crown rot in peanut induced by *Aspergillus niger*. The addition of chitosan increased *B. subtilis* effectiveness against powdery mildew in strawberries (Lowe et al., 2012). It also improves soil water retention behavior by indirectly conditioning the soil (Pandey and De, 2017).

The microorganisms included in biofertilizers maintain the earth's natural nutrition cycle while increasing soil organic matter. Using biofertilizers leads to cultivating healthy plants while also enhancing soil health and sustainability. Chitosan-encapsulated microbial biofertilizer benefits tomato crops by improving nutrient absorption, disease resistance, and root growth. (Isabel et al., 2024). Overall, the usage of chitosan in agriculture has the potential to raise crop yields while also improving soil health over time. The current study aimed to develop value-added products from blue crab waste in the extraction and processing of chitosan, as well as their influence on soil drenching with plant growth enhancement.

II. MATERIALS AND METHODS

2.1 Materials

Blue crab shells (*Callinectes sapidus*) were obtained from Central de Pescados y Mariscos, La Nueva Viga market, in Mexico City. The crab shells were completely cleaned, washed, and dried under sunlight. Commercial chitosan with medium molecular weight, catalog number 448877 was from the Sigma-Aldrich company ltd. All the other chemicals used were analytical grade. Soil used for drenching is procured from the garden of CINVESTAV without any addition of fertilizer. The tomato seeds and the germination tray were purchased from the hydro environment shop.

2.2 Methods

2.2.1 Extraction of chitosan

Mechanical pulverizing method: The dried crab shells and commercial chitosan were made into coarse powder and kept in a high-speed shimmy ball mill (model number LB60G-2S0007BER) separately for 8 hours at 550rpm/m and resting for 5 hours, again 3 hours at 550rpm/m to make it as a nanopowder.

Demineralization: The crab shell powder from the pulverizing method was treated with 1N HCl at 1000rpm/ 80°C for 24 hours to remove all the mineral content. The sample was then filtered and washed with distilled water for 30 minutes until it reached a neutral pH (=7). The demineralized crab shell powder was dried for 24 hours (Shimahara and Takiguchi, 1988).

Deproteinization: The demineralized shell powder was treated with 1M NaOH with constant stirring for 15 hours at 100°C to remove all the proteins. The sample was then filtered and washed for 30 minutes with distilled water until it reached a neutral pH (=7). The deproteinized nano crab shell powder was dried for 24 hours to obtain chitin (Abdou et al., 2008).

Deacetylation of chitin: The deacetylation of chitin was then conducted according to the method by (Yen et al., 2009). The chitin obtained was treated with 40 % (w/w) aqueous sodium hydroxide (NaOH) at 110°C for 3 hours. Then, the chitin was filtered and washed with deionized water until neutral pH to obtain the chitosan. The obtained chitosan was then dried for 24 hours for further studies and analysis.

2.2.2 Characterization of chitosan

Yield of chitin and chitosan: Chitin yield was calculated by dividing the weight of extracted chitin by the initial dry crab shell weight, and chitosan yield was calculated by dividing the weight of produced chitosan by the dry chitin weight before deacetylation (Demir et al., 2017). The following yields were calculated:

$$\text{Yield of chitin (\%)} = [\text{Extracted chitin (g)}/\text{Crab shells (g)}] \times 100 \quad (1)$$

$$\text{Yield of chitosan (\%)} = [\text{Produced chitosan (g)}/\text{Chitin (g)}] \times 100 \quad (2)$$

2.2.3 Determination of degree of deacetylation

The direct titration method was used to determine the degree of deacetylation of chitosan extracted from blue crabs, which was conducted according to the method by(Kjartansson, 2008) with some modifications. Chitosan samples (0.1 g) were dissolved in 25 ml of 0.06 M HCl for 1 h at room temperature. The solutions were diluted to 50 ml before being titrated with 0.1 N NaOH to pH 3.75 under constant stirring. The volume of NaOH at pH 3.75 was acquired and recorded. Titration was continued to pH 8 and the total volume of NaOH (0.1 M) was recorded. The degree of deacetylation was then calculated using the following equation.

$$DD = \frac{161.16 \times (v_2 - v_1)N}{w_1} \quad (3)$$

Where, 161.16 is the mass of the chitosan monomer, V_1 and V_2 are the volumes of NaOH solution used, N is the strength of the NaOH solution (0.1 N) and W_1 is the mass of the sample after correction for moisture. The degree of deacetylation (DD) of the samples was determined in triplicate.

2.2.4 Instrumentation

A Varian 640-IR spectrophotometer detected FTIR spectra of materials. Spectral scanning was acquired in a wave number ranging from 4000 to 400 cm^{-1}. XRD patterns were obtained with the Bruker D8 Advance eco. X-ray diffractometer operating at 40kV and 30mA producing CuKα with $\lambda_{1/2}$ = 1.5416 Å. Micrographs were obtained with JOEL JSM-7401F. SEM images were obtained at 100, 500, and 30,000x magnifications. DSC analysis was done by using a TA Instruments Q2000 calorimeter under a high-purity N_2 atmosphere. The heating rate was 5 $Kmin^{-1}$, and the sample mass was weighed in Mettler-Toledo UMX2 microbalance. The specific surface area, pore size, and pore volume of the extracted chitosan were investigated using TriStar 3000 Plus, Surface Area and Pore Size Analyzer (Micromeritics Instrument Co., Ltd, America) with an N_2 adsorption-desorption method under 0.08 MPa.

2.2.5 Soil Drenching

The soil drenching process was carried out by adding chitosan solution with a 60mg/ml concentration in the soil without any addition of commercial fertilizer. The extracted chitosan was mixed with 1% glacial acetic acid in 99ml of distilled water and the pH was adjusted to 6.5 using NaOH. Control is used without the addition of chitosan. (In patent process)

2.2.6 Soil analysis

2.2.6.1 pH

In a beaker, 10 g of soil is weighed and 25 ml of distilled water is added. Approximately for 10 minutes it was shaken with a magnetic stirrer and allowed for a 10-minute resting period. Using a pH meter, it was calibrated and readings were taken.

2.2.6.2 Moisture content

Weighed a dry and clean empty container, such as a petri dish lid (weight A). The container is later filled with soil and the combined weight of the container and soil (weight B). Keep the Petri dish and soil in a 105 °C oven for 24 hours. Allow the sample to cool to room temperature. Fill the container halfway with dry soil (weight C).

(B - A) = initial wet sample weight (4)

(B - C) = denotes the initial water content (5)

$$\text{Water content percentage} = \frac{(B-C)}{(B-A)} \times 100 \quad (6)$$

2.2.6.3 Water holding capacity

Place a defined amount of soil (20 g), of known moisture, on a piece of paper previously weighed filter on a funnel, containing glasses in the lower part to receive the drained water. Saturate both the soil and the filter paper with distilled water. Add a little excess distilled water (approx. 10 ml). Simultaneously run blanks with only filter paper, previously weighed, added with enough distilled water to saturate, and a little more. Cover both the sample and the filter papers with aluminum foil to avoid water evaporation. Let the excess water drain for 24 hours. Weigh the soil together with the filter paper and the retained water. Also, weigh the water-saturated filter papers and calculate the water retention factor. water per filter paper (weight of wet filter paper divided by the weight of dry filter paper). It is usually 3.2 or higher. Calculate the Water Retention Capacity, subtracting the weight of the filter paper by its factor and the dry weight of the soil (weight of the soil sample x (1- moisture fraction)) at the total weight drained (soil + water retained by the soil + filter paper + water retained by the filter paper). Divide this result by the dry weight of the soil and multiply by 100 if expressed as a percentage, or per 1000, if it is expressed per 1000 g (kg).

2.2.7 Soil type

Weigh 50 g of soil. Add approx. 600 mL of distilled water and place in the glass of the blender 10 mL of the dispersant (Sodium hexametaphosphate solution 50 g/L). Shake for 2 min. Transfer to a 1 L measuring cylinder and dilute with distilled water. Shake by inversion for 1 min to homogenize, rest for 40 sec, and take the first reading of Temperature (T_1) and hydrometer. Let stand for 2 hours and take the second reading of the hydrometer and Temperature (T_2).

Silt % + clay % = 1st reading + (T_1-20) x 0.36 x100 g of soil (7)

% Sand = 100 – (% Silt + % Clay) (8)

$$\%\ Clay = \frac{\text{2nd reading + (T2}-20) \text{ x 0.36 x 100}}{Gram\ of\ soil} \quad (9)$$

2.2.8 Total nitrogen

Weigh 2 grams of soil and place it in the tube of the Kjendal. Weigh 3 blanks (reactive only). Add 1 gr. potassium sulfate powder, 1 pinch (1/3 g) of copper sulfate, and 10 ml of the H_2SO_4 to each tube including blanks. Place the tubes in the digester. This is ignited and brought to 120°C, and the vacuum is opened (watch for large bubbles, the valve is opened approximately ¾). Then every 30 minutes the temperature is raised in blocks of 80°C up to 320 °C. Turn off the appliance to stop the digester. Wait for the temperature to drop to 90°C and raise the tubes so that the grill cools faster, it takes about 1 hour. close the void. The samples are placed for 3 days (8 h day^{-1}) to digest. Distilled H_2O is added to detach the material from the tube, if it does not detach, heat the bottom of the tube with a lighter. The contents of the tube are placed in a 500 ml flask, brought to 200 ml with distilled H_2O, distilled, and titrated with 0.01 N HCl at the first color change.

2.2.9 Total Carbon

The soil was milled and homogenized using a mortar. A sample of 200 mg of dry soil was weighed.

The Total Organic Carbon was determined by a TOC-VCSN carbon analyzer (Shimadzu, Canby, USA). This method is based on the complete combustion of Organic Carbon to CO_2 and then measured by an Infrared Analyzer.

2.2.10 Preparation of seeds for sowing

The seeds were surface sterilized with sodium hypo chloride and then washed continuously with distilled water in a beaker. After that, it was kept at room temperature overnight and sown in the soil.

2.2.11 Planting of seeds

The germination trays were watered and 3 seeds per each cavity were sown with a spacing of 1cm apart and observed for germination.

III. RESULTS AND DISCUSSION

3.1 Extraction:

The experiment procedures were done in triplicates and all the results were reported with average in standard deviation. Blue crab (*Callinectus sapidus*) shells were used as the starting material for extraction in this study (Figure 3). The key components of the crab shells were chitin, protein, and calcium carbonate, which were around 30%, 16%, and 55% as w/w, respectively, indicating that calcium carbonate made up a large portion of the crab shells (Yihun et al., 2020). The (International, n.d.) AOAC (1990) procedures were used to determine the chitin and chitosan yield, which ranged from 43.34±0.69% and 45.01±1.06% in this study. According to earlier research, the yield of chitosan produced from *Matuta lunaris* shells ranged from 24.04 to 34.5% (Haziman Abdullah et al., 2019). *Pachygrapsus mamoratus* yielded 17% and *Sesarma plicatum* yielded 41.37%, respectively (Hossain and Iqbal, 2014). The yield of chitosan extracted from shrimp and crab shells (deacetylated using 65% (w/v) sodium hydroxide, NaOH, at 30 °C for 3 days) was 46% (Rajendran et al., 2015). The yield of chitosan produced in this study is comparable to or less than that achieved in earlier investigations, which may be influenced by the high mineral content of *Callinectus sapidus* shells.

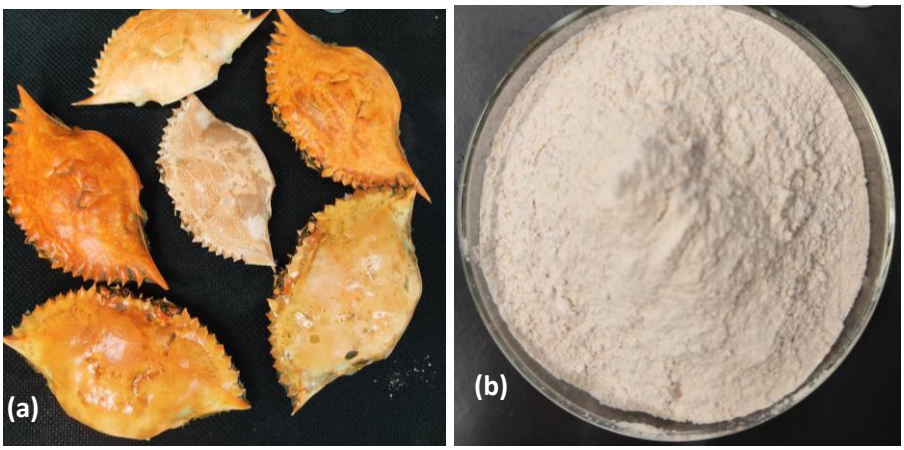

FIGURE 3: (a) Dried crab shell (*Callinectus sapidus*) (b) Extracted chitosan

3.2 Degree of deacetylation (DD)

The chitosan degree of deacetylation, DD, is a measure that reflects the molar proportion of glucosamine monomeric units and ranges from 0 (chitin) to 100 (completely deacetylated chitin). The DD of chitin/chitosan is the most critical characteristic that impacts their biological, physicochemical, and mechanical properties, and hence the efficacy of chitosan and its derivatives (Pérez-Álvarez et al., 2018). Deacetylation removes acetyl groups from the molecular chain of chitin, leaving behind a molecule (chitosan) with a high degree of chemically reactive amino group ($-NH_2$) (Baskar and Sampath Kumar, 2009). In any event, the degree of deacetylation (DDA) may be used to distinguish chitin from chitosan since it determines the concentration of free amino groups in the polysaccharides (Sarbon et al., 2015). DDA is a characteristic that influences chitosan properties such as chemical reactivity, covalent bonding ability, solubility, viscosity, and biodegradability (Lamarque et al., 2005) (LERTSUTTHIWONG et al., 2002). In this study, the degree of deacetylation of commercial chitosan was 78.69±0.0045 % which is less than the Chitosan extracted from blue crabs at 90.12±0.0014 %. Depending on the source and preparation method,

the degree of deacetylation (DD) might range from 30 to 95% (Di Martino et al., 2005). The degree of deacetylation values is highly dependent on the source and technique of purification, as well as the kind of analytical methods utilized, sample preparation and equipment type used, and a variety of other circumstances that may impact the degree of deacetylation analysis (No et al., 2002).

3.3 Fourier transform infrared (FTIR)

Extracted chitosan had some similar functional groups as commercial chitosan. The O-H stretching band in extracted chitosan was exhibited at 3514.91cm $^{-1}$, indicating the alcohol group in the chitosan. According to ("Organic Chemistry: A Lab Manual - PAVIA ET.AL: 9788131512432 - AbeBooks," n.d.)Pavia et al. (2009), the alcohol group (O-H band) was found to be between 3,650 cm^{-1} and 3,200 cm^{-1}. The C-O stretching band measured 1017.23cm^{-1}, while the commercial chitosan stretching band measured 1023.80cm^{-1}. Furthermore, the stretching band of N-H in the extracted chitosan was in the range of 3111.91cm^{-1}, whereas the stretching band for N-H in commercial chitosan was in the range of 3260.46cm^{-1}. According to ("Organic Chemistry: A Lab Manual - PAVIA ET.AL: 9788131512432 - AbeBooks," n.d.)Pavia et al. (2009), the amine group (N-H stretching bands) absorbs infrared between 3,500 cm^{-1} and 3,100 cm^{-1}. The bending band of N-H in the extracted chitosan was in the range of 1562.24cm^{-1}, whereas the bending band for N-H in commercial chitosan was in the range of 1565.52cm^{-1}. The amine group (N-H bending bands) absorbs infrared between 1640 cm^{-1} and 1550 cm^{-1} ("Organic Chemistry: A Lab Manual - PAVIA ET.AL: 9788131512432 - AbeBooks," n.d.). The peak at 2718.74 cm^{-1} and 2934.61 cm-1 reveals the C-H for commercial and extracted chitosan, according to Li, Weng, Wu, and Zhou (1998). When the spectra in Figure 4 are examined, the little peak at 1465.92 cm^{-1} and 1375.99 cm^{-1} is assigned as -CH^2, 1375.12 cm^{-1}, and 1450.64 cm^{-1} are the peak that shows -CH^3 groups for commercial and extracted chitosan ("Organic Chemistry: A Lab Manual - PAVIA ET.AL: 9788131512432 - AbeBooks," n.d.). The extracted and commercial chitosan had some distinct peaks and similar peaks on the spectrum, which might be due to the fact that they were produced from different sources, with the extracted chitosan being created from blue crab shells.

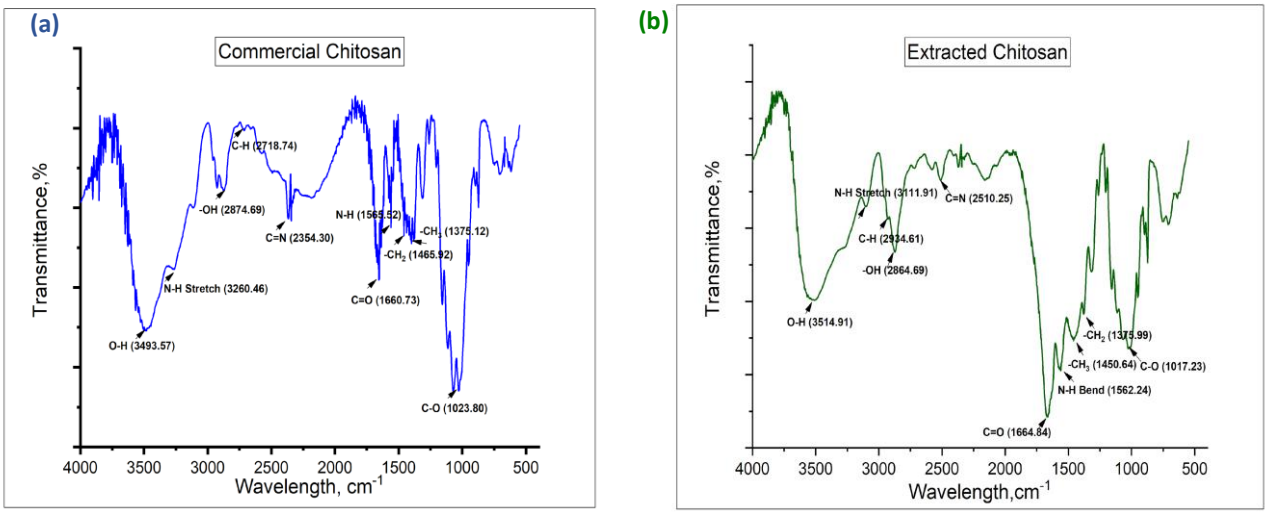

FIGURE 4: Fourier transform infrared (FT-IR) spectra of (a) commercial chitosan (b) Extracted chitosan

3.4 X-ray Diffraction (XRD)

The XRD of commercial chitosan, and nanochitosan extracted are shown in Figure 5. Sharp peaks with a diffraction angle of 2Θ 10.116°, 20.034°, 25.458°, 26.758°, 35.294°, 43.451°, and 57.559° are found in the X-ray diffractogram of commercial chitosan, and 10.901°, 12.593°, 19.084°, 26.516°, 35.252°, and 43.512° are the diffractogram of extracted chitosan, indicating that the medication is present as a crystalline substance. The commercial chitosan contains two peaks at 2Θ-10.12° and 2Θ-20.03° owing to 100 and 020 with the latter peak being weaker than the first, and the extracted chitosan contains diffraction peaks around 2Θ-10.9° and 2Θ-19.08° owing to Miller indices of 111 and 201 lattice planes, although many XRD patterns of chitosan in the literature have two typical peaks that are usually around 2Θ-10° and 2Θ-20° (Yen et al., 2009). According to

(Yen et al., 2009), the explanation for the various characteristic diffraction peaks might be due to the source of chitin. (Okuyama et al., 2000) demonstrate that chitosan chains crystallize in an orthorhombic unit cell. The wide peak at 10.12° validates the amorphous phase, whereas the sharp peak at 20.03° and 19.08° indicates the crystalline phase created by the commercial and extracted chitosan intramolecular hydrogen bonding. The intensity and diffraction angles of nano-sized extracted and commercial chitosan XRD patterns varied. The degree of crystallinity for commercial chitosan is 61.35%, and extracted chitosan is 29.66% respectively. The results show that the crystallites are substantially clustered and reasonably distributed in the milled powders.

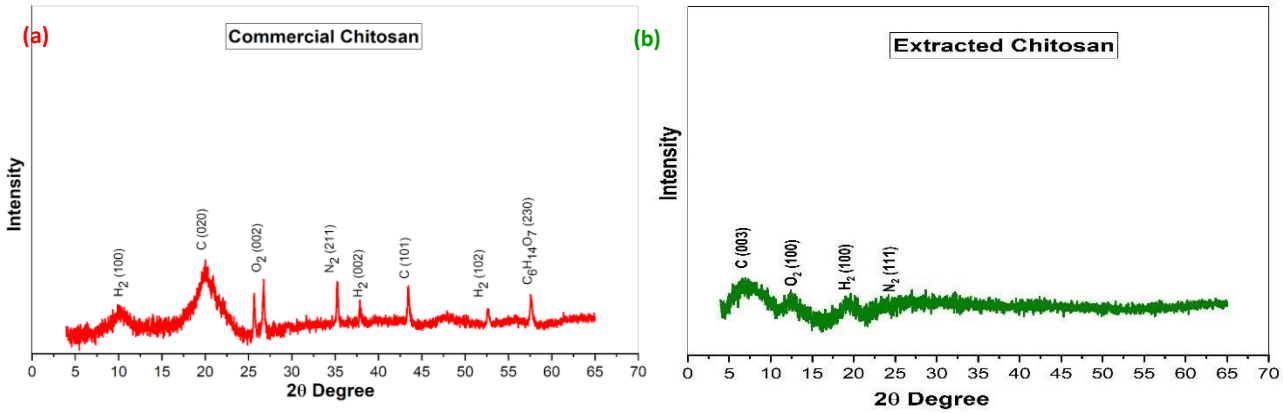

FIGURE 5: XRD Spectra of (a) commercial Chitosan (b) Extracted Chitosan

3.5 Scanning Electron Microscopy (SEM)

The morphology of the surface chitosan was examined by SEM. Figure 6 shows SEM images of commercial chitosan (Figures 6 a,b,c) and extracted chitosan (Figures 6 d,e,f). Commercial chitosan SEM scans revealed a nonporous, smooth membranous phase with dome-shaped orifices, microfibrils, layers of flakes, and crystallites. The determined pore size is 288±98.6µm and the particle size is 79.29±11.08nm. Figure 6 d,e,f shows electron micrographs of chitosan-extracted with a porous and chain-like structure. The SEM picture further revealed that the extracted chitosan had a near-spherical shape with a smooth surface. The pore size is approximately a standard deviation of 42.43±28.92µm and the particle size is 33.37±3.79nm. Chitosan nanoparticles were discovered to be spherical and have a smooth surface, regardless of size.

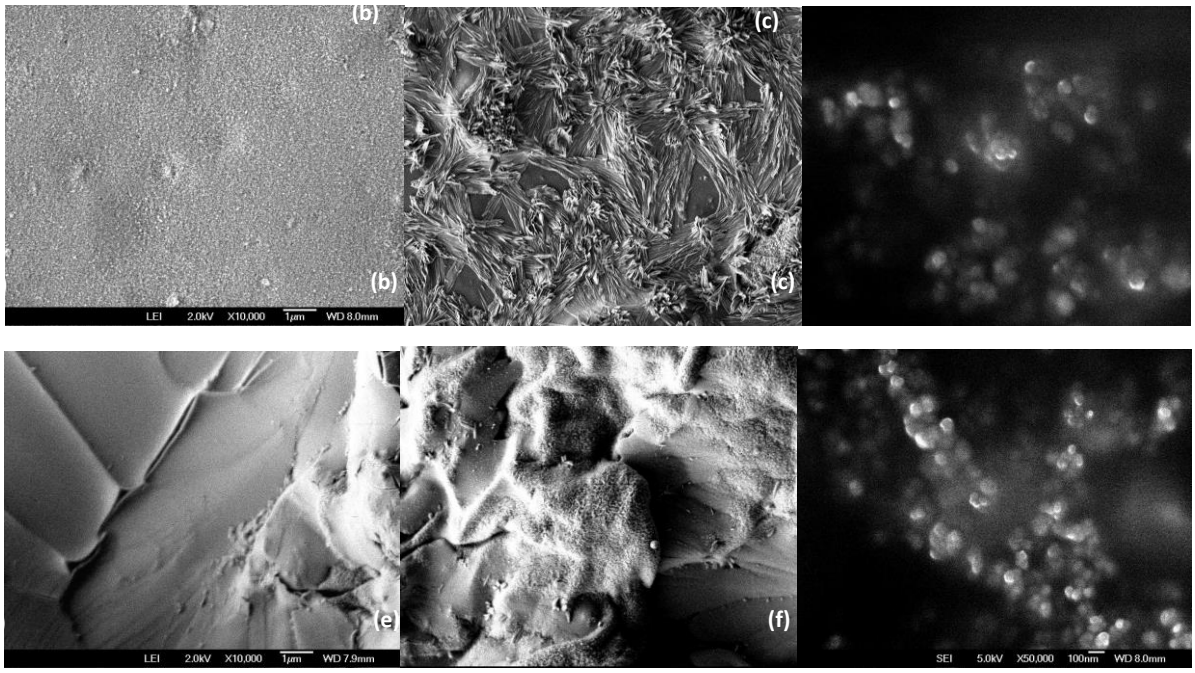

FIGURE 6: (a) Commercial Chitosan in X 10,000 (b) X 30,000 (c) Commercial Chitosan nanoparticles (d) Extracted Chitosan in X 10,000 (e) X 30,000 (f) Extracted Chitosan nanoparticles

3.6 Differential Scanning Calorimetry (DSC)

DSC was used to investigate the glass transition (T_g) and thermal behavior of chitosan .Figure 7 depicts the DSC thermogram of a commercial chitosan and extracted chitosan. Chitosan's DSC thermogram reveals two large endothermic peaks at 87.84°C and 95.14°C. The first peak might be exhibited due to water vapor. At 301.93°C and 319.18°C, there is a large and sharp exothermic peak associated to the thermal breakdown of the chitosan chains. DSC peaks indicate that the breakdown of chitosan nanoparticles will occur well over 300°C. Reduced crystallinity implies a change in the solid state structure of chitosan as a result of crosslinking (Zhang et al., 2004).

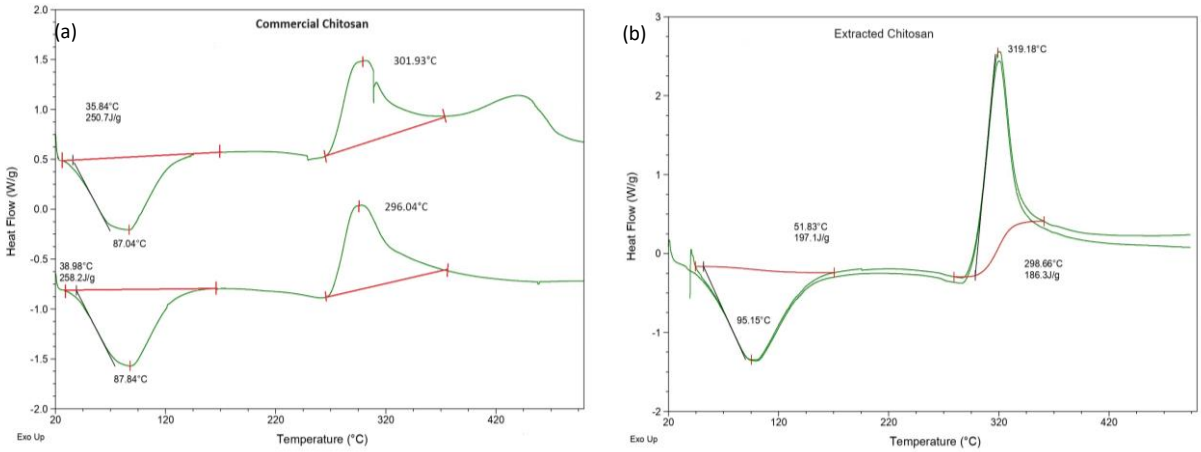

FIGURE 7: DSC Spectra of (a) Commercial Chitosan (b) Extracted Chitosan

3.7 Brunauer-Emmett-Teller (BET) analysis

To investigate the porosity nature of the chitosan, Brunauer-Emmett-Teller (BET) gas sorptometry measurements were performed. Using the BET (Brunauer, Emmett, and Teller) equation, the surface area may be estimated from the amount of gas necessary to produce an adsorbed monolayer. The gas pressure is gradually raised until all pores are filled with liquid to determine the pore volume and pore size distribution. The gas pressure is then gradually lowered, evaporating the condensed gas from the system. The analysis of the adsorption and desorption isotherms offers details on the pore volume and size distribution. The commercial and extracted chitosan BET surface area and pore volume were 11.3892 m^2/g, 15.9057 m^2/g, and 0.0102 cm^3/g, 0.197 cm^3/g respectively. Table 1 summarizes and compares the BET surface area of either natural or manufactured chitosan adsorbents reported in earlier research with that of the extracted chitosan from blue crab. The BET data clearly corroborate the porous nature of the chitosan (Luo et al., 2018a).

TABLE 1
CHITOSAN BET SURFACE AREA AND TOTAL POROSITY COMPARISON WITH OTHER LITERATURE STUDIES

S.No	Samples	BET surface area (m^2/g)	Total porosity volume (cm^3/g)	Reference
1	Chitosan-EGDE beads	0.62	-	(AZLAN et al., 2009)
2	Chitosan	1.22	-	(Zhang et al., 2021)
3	PVA/chitosan	1.95	-	(Rajeswari et al., 2020)
4	CMC beads	0.49	-	(Luo et al., 2018b)
5	Chitosan powder	11.85	0.010	(Ngamsurach et al., 2022)
6	Chitosan beads mixed ZnO	12.46	0.013	(Ngamsurach et al., 2022)
7	Commercial Chitosan	11.39	0.010	This work
8	Extracted Chitosan	15.90	0.197	This work

3.8 Soil Analysis

The most important practical application of Chitosan is in agriculture and horticulture, particularly for conserving water in arid and desert environments and accelerating plant development. As a result, research into the moisture content, soil type , pH,

Total nitrogen and carbon of chitosan in addition to the soil is essential. Table 2 describes the analysis between commercial and extracted chitosan drenched in the soil. Soil pH is a critical state that has a significant impact on soil biology, chemistry, and physical processes, all of which have direct effects on plant growth and development. It is obvious that pH affects soil and crop yield. Soil pH is classified as follows by the United States Department of Agriculture's National Resources Conservation Service: ultra-acidic (3.5), extremely acidic (3.5-4.4), very strongly acidic (4.5-5.0), strongly acidic (5.1-5.5), moderately acidic (5.6-6.0), slightly acidic (6.1-6.5), neutral (6.6-7.3), slightly alkaline (7.4-7.8), moderately alkaline (7.9-8.4), strongly alkaline (8.5-9.0), and very strongly alkaline (>9.0). (Burt 2014) Agricultural crop production is often undertaken in the slightly acidic to slightly alkaline range, a window linked with optimal soil nutrient availability. Though plant tolerance to high pH varies, most agricultural plants grow best at a pH close to neutral (Läuchli and Grattan, 2017). The impact of crop production and plant development is influenced by the moisture content of the soil. Water retention is essential and less uptake of water due to the addition of chitosan to the soil plays an important role in water saving.

TABLE 2
ANALYSIS OF SOIL AFTER BEING DRENCHED WITH CHITOSAN AND COMPARED WITH CONTROL

Source	Moisture content %	Water holding capacity %	pH	Sand %	Clay %	Slit %	Texture Classification	Total Nitrogen (g/Kg)	Total Carbon (g/Kg)
Commercial chitosan	34	82	7.3	47	19	34	Loam	2.9	46.4
Extracted Chitosan	39	84	7.2	46	20	42	Loam	3.1	47.7
Control	20	63	7.8	40	17	30	Loam	2.0	22.1

The total nitrogen concentration of the soil demonstrated statistically significant changes between treatments in the study's interaction zone. According to (Man et al., 2021), the total N concentrations in soil (2.0-2.2 g/kg). A substantial outcome in soil was obtained with the treatment of commercial and extracted chitosan are 2.9g/kg ha^{-1} and 3.1 g/kg ha^{-1} of N, which had a 45% increase of extracted chitosan in N content when compared to the control without chitosan.

The amount of carbon inputs into the soil rises when crop production and crop litter reach the soil (Batlle-Bayer et al., 2010) (Khan et al., 2007) (Mahlous et al., 2007), and so soil carbon stocks may grow with N fertilization (Batlle-Bayer et al., 2010) (Buyanovsky and Wagner, 1998). Soil organic carbon contents varied from 21.7 to 23.1 g/kg and did not differ substantially. The soil organic carbon increased after the addition of chitosan to the soil ranging around 46.4g/Kg for commercial and 47.7g/Kg for the extracted chitosan. Soil organic carbons have an important role in soil physical, chemical, and biological qualities, as well as overall soil fertility.

3.9 Seed Germination

Seed germination is an important process that impacts crop output and quality. Understanding the molecular components of seed dormancy and germination is therefore critical for increasing agricultural output and quality (Tuan et al., 2019). Both chitosan were applied properly in order to examine the normal effect of chitosan in seed germination and seedling growth of Roma tomatoes (*Solanum lycopersicum*). Its use resulted in considerable improvements in many plant components. For healthy growth, plants rely on minerals found in the soil. Plant production suffers as a result of nutrient deficiency. These nutrients are provided by chitosan. The time of seed germination in experimental plots was closely monitored. Table 3 shows the total number of seeds germinated and their potential percentage.

TABLE 3
EFFECT OF CHITOSAN ON SEED GERMINATION AND GROWTH PARAMETER

Parameter	Time of germination (in Day)	Seed germination(%)	Plant Height(cm)	Wet weight (g)	Dry weight (g)
Commercial Chitosan	8	55.56	23.92±1.05	0.626933±0.078908	0.083867±0.003535
Extracted Chitosan	6	78	29.14±0.55	1.435167±0.0056	0.213±0.013018
Control	11	22	17.60±10.16	0.4105±0.237002	0.0665±0.038394

Different parts of the plant were measured using suitable methods to determine the effect of chitosan amendments. After 35 days, the shoot length was measured on a centimeter scale. Extracted chitosan-treated seedlings germinated in 6 days, whereas commercial chitosan-treated and controlled seeds sprouted in 8 and 11 days. The maximal length of the shoot seen with extracted chitosan and commercial chitosan treatment was 29.14±0.55cm and 23.92±1.05cm after 35 days respectively, whereas control exhibited 17.60±10.16cm after 35 days, respectively. Dry weight refers to all plant elements excluding water and is a more trustworthy weight analysis method. The dry weight and wet weight of the germinated plants are given in Table 3.

IV. CONCLUSION

This study investigated the physicochemical properties and characterization of chitosan derived from blue crab (*Callinectus sapidus*) shells, which are discarded and pollute the environment. The study's findings were also compared to commercial chitosan derived from crabs. Based on the findings, it is claimed that blue crab has a high potential for producing chitosan. With appropriate deacetylation conditions, this study shows that blue crab (*Callinectus sapidus*) may be employed as a possible source for chitosan extraction. The chemical composition of the extracted chitosan was determined using FTIR, and the crystalline diffraction peaks were examined using XRD. Chitosan has a porous structure and porosity, according to morphological studies. Chitosan, when added to the solution, functions as a plant growth booster in various crops such as bean plants, radishes, passion fruit, potatoes, cabbage, soybean, and others. It improves plant productivity and protects plants from diseases. Chitosan has a considerable influence on root, shoot, blooming, and flower number growth rates (Pandey and De, 2018). Chitosan application in soil reduced seed germination time and increased germination percentage. The foregoing explanation suggests that drenching chitosan in soil resulted in improved tomato plant growth and development.

AUTHORS CONTRIBUTION

Divya Shanmugavel: Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Validation, Methodology. **Omar Solorza – Feria :** Conceptualization, Methodology, Validation, Resources, Supervision, Project administration, Funding acquisition. **Sathish Kumar Kamaraj:** Conceptualization, Methodology, Validation, Resources, Supervision, Project administration, Funding acquisition.

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COMPETING INTEREST

There are no conflicts that we need to report.

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