International Journal of Earth Sciences Knowledge and Applications
journal homepage: http://www.ijeska.com/index.php/ijeska
e-ISSN: 2687-5993

Research Article https://doi.org/10.5281/zenodo.15342696

Biostimulatory Effects of Chitosan from Freshwater Prawn (*Macrobrachium rosenbergii*, De Man 1879) on Seed Germination and Seedling Growth

Win Lai Lai Htay[1,*], Khin Khin Gyi[2*]
[1]Department of Marine Science, Pathein University, 10014, Pathein, Ayeyarwady State, Myanmar
[2]Department of Marine Science, Mawlamyine University, 12012, Mawlamyine, Mon State, Myanmar

Article history
Received 29 January 2025
Accepted 15 March 2025
Published 30 April 2025

Contact
*Win Lai Lai Htay
Winlailaihtay8050@gmail.com (WLLH)
*Khin Khin Gyi
khinkhinmarinescience@gmail.com (KKG)

How cite
Htay, W.L.L., Gyi, K.K., 2025. Biostimulatory Effects of Chitosan from Freshwater Prawn (Macrobrachium rosenbergii, De Man 1879) on Seed Germination and Seedling Growth. International Journal of Earth Sciences Knowledge and Applications 7 (1), 102-109. https://doi.org/10.5281/zenodo.15342696.

Abstract
The present study investigates the extraction and characterization of chitosan from *Macrobrachium rosenbergii* shell waste and evaluates its potential as a biostimulant in seed germination. Chitin and chitosan were extracted using a sequential chemical process involving deproteinization, demineralization, decolorization, and deacetylation, employing sodium hydroxide, hydrochloric acid, and sodium hypochlorite. From 100 g of shell waste, the yields of chitin and chitosan were 29.0 ± 2.71% and 24.37 ± 3.38%, respectively. The extracted chitosan exhibited an off-white appearance with a particle size of 809.33 ± 49.66 µm, solubility in 1% acetic acid of 94.33 ± 2.19%, and a water-binding capacity of 775.17 ± 76.50%. The ash content (0.20 ± 0.10%) and moisture content (9.22 ± 3.89%) confirmed high purity and effective demineralization. Structural analysis via Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) validated the molecular and morphological integrity of the extracted chitosan. Seed priming with chitosan (1–2 mg mL^{-1}) significantly enhanced *Oryza sativa* germination by reducing the mean germination time and promoting shoot and root elongation. Treated seeds exhibited superior seedling vigor, demonstrating chitosan's efficacy as a natural agricultural biostimulant. These findings suggest that *M. rosenbergii*-derived chitosan holds promise for sustainable crop enhancement strategies.

Keywords
Chitin, chitosan, FTIR, SEM, seed priming, biostimulant, seedling vigor

1. Introduction

Chitin, a naturally occurring polysaccharide, is a major structural component of crustacean shells, comprising 15–40% of their composition, along with calcium carbonate (20–50%), proteins (20–40%), lipids (0–14%), omega-3 fatty acids, and pigments (Šimat et al., 2022). As the second most abundant biopolymer after cellulose, chitin consists of N-acetyl glucosamine (GlcNAc) units, which can be deacetylated into chitosan, a derivative with exposed amino ($-NH_2$) groups that significantly enhance its physicochemical and functional properties.

Chitosan is widely recognized for its biodegradability, biocompatibility, low toxicity, and high nitrogen content (6.89%), making it a promising alternative to synthetic cellulose derivatives (Gupta et al., 2000). Due to these attributes, chitosan has gained attention in agriculture, particularly as a biostimulant, a class of natural-origin compounds that enhance plant growth, improve stress resilience, and promote soil health without acting as conventional fertilizers or pesticides (Boehme et al., 2008).

Extensive research has demonstrated the agronomic benefits of chitosan in seed treatments. Chitosan-based coatings accelerate seed germination and improve stress tolerance in hybrid rice (Ruan et al., 2002), inhibit Sclerotinia rot in carrots (Cheah et al., 1997), and enhance maize seedling vigor (Shao et al., 2005).

Additionally, seed priming with chitosan has been reported

to ameliorate osmotic stress in wheat, further highlighting its role in improving plant resilience (Hameed et al., 2014).

Given its potential applications in sustainable agriculture, this study aims to extract and characterize chitosan from *Macrobrachium rosenbergii* shell waste and assess its efficacy as a biostimulant for seed priming. Specifically, the effects of chitosan-treated seeds on the germination performance and early seedling growth of *Oryza sativa* are examined, providing insights into its potential as an eco-friendly alternative for improving crop productivity.

2. Materials and methods

2.1. Sample Collection and Preparation

The shell waste of freshwater prawn (*Macrobrachium rosenbergii*) was sourced from local markets and seafood restaurants in Mawlamyine. The collected shell waste was transported to the laboratory, where residual meat and soft tissues were manually removed. The shells were thoroughly washed with tap water to eliminate impurities and residual proteins. Cleaned shell fragments were sun-dried, crushed into smaller particles, and stored in airtight plastic bags for subsequent chitin and chitosan extraction.

2.2. Extraction of Chitosan by Chemical Method

Chitin and chitosan were extracted from 100 g (dry weight) of *M. rosenbergii* shells using a four-stage chemical process: deproteinization, demineralization, decolorization, and deacetylation. Deproteinization was carried out by soaking shell fragments in 5% sodium hydroxide (NaOH) at room temperature for 20 hours, followed by heating for 2 hours to remove proteins. The shells were then rinsed with tap water until a neutral pH (7) was achieved, and the alkaline supernatants were discarded. Demineralization was performed by immersing the deproteinized shells in 5% hydrochloric acid (HCl) at room temperature for 20 hours, followed by heating for 2 hours to eliminate minerals. The resulting chitin was thoroughly washed with tap water until neutrality was attained. Decolorization involved treating the chitin with 5% sodium hypochlorite (NaOCl) for 1 hour at room temperature, followed by repeated washing with tap water. The decolorized chitin was then air-dried at room temperature. Finally, deacetylation was conducted by immersing the dried chitin in 60% NaOH at room temperature for 20 hours, followed by heating for 2 hours, leading to chitosan formation. The extracted chitosan was washed with distilled water until neutral pH, dried overnight at room temperature, finely ground using an electric blender, and stored in airtight plastic bags for further analysis.

2.3. Yield and Characterization of Chitosan Biopolymer

2.3.1. Analysis of Chitin and Chitosan Yields

The yield of chitin and chitosan was calculated as the dry weight (g) of the extracted flakes relative to the dry weight (g) of the shrimp shell waste, expressed as a percentage:

$$\text{Yield (\%)} = (\text{Dried chitin/chitosan weight})/(\text{Dry weight of shrimp shell wastes}) \times 100 \quad (1)$$

2.3.2. Ash Content

To determine the ash content, 1.0 g of chitosan was placed in a pre-weighed crucible and heated at 575°C for 6 hours in a muffle furnace. The sample was then cooled in a desiccator for 15 minutes before weighing. Ash content was calculated as:

$$\text{Ash (\%)} = (\text{Ash weight})/(\text{Initial chitosan weight}) \times 100 \quad (2)$$

2.3.3. Moisture Content

Moisture content was assessed by drying 1.0 g of chitosan powder in a pre-weighed crucible at 105°C for 1 hour. The percentage of moisture was determined by the weight loss before and after drying:

$$\text{Moisture content (\%)} = (\text{Initial chitosan weight} - \text{Weight after drying})/(\text{Initial chitosan weight}) \times 100 \quad (3)$$

2.3.4. Solubility in 1% Acetic Acid

To evaluate solubility, 0.1 g of chitosan was dissolved in 10 mL of 1% acetic acid solution and continuously stirred until a homogeneous solution was obtained. The solution was filtered using Whatman Grade 1 filter paper (pore size: 11 µm) to separate insoluble particles. Solubility was calculated using:

$$\text{Insoluble (g)} = \text{final dry weight of filter paper (g)} - \text{initial weight of filter paper (g)} \quad (4)$$

$$\text{Insoluble (\%)} = (\text{Insoluble (g)})/(\text{Sample weight (g)}) \times 100 \quad (5)$$

$$\text{Solubility (\%)} = 100 - \text{insoluble (\%)} \quad (6)$$

2.3.5. Water-Binding Capacity (WBC)

The water-binding capacity of chitosan was determined by weighing 0.1 g of chitosan in a centrifuge tube, adding 10 mL of water, and mixing for 5 minutes. The sample was left at ambient temperature for 30 minutes, centrifuged at 4000 rpm for 45 minutes, and left overnight. The supernatant was decanted, and the tube was reweighed. WBC was calculated as:

$$\text{WBC (\%)} = (\text{Water bound chitosan weight (g)})/(\text{Initial chitosan weight (g)}) \times 100 \quad (7)$$

2.3.6. Scanning Electron Microscopy (SEM) Analysis

The microstructure of the extracted chitosan was analyzed using a JEOL NeoScope JCM-6000Plus Versatile Benchtop SEM. Prior to imaging, the dried chitosan sample was ground, mounted on a sample holder, and examined under an acceleration voltage of 15 kV to capture surface morphology.

2.3.7. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was employed to analyze the structural characteristics of chitosan. Spectra were recorded over a frequency range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using a PerkinElmer Spectrum Two FTIR Spectrometer.

2.4. Seed Germination Test with Chitosan Biopolymer

Preparation of Chitosan Solution

A 0.1% (w/v) chitosan solution was prepared by dissolving 0.1 g of extracted chitosan powder in 100 mL of 1% (v/v) aqueous acetic acid and allowing it to stand overnight. The

pH of the solution was adjusted to 6.0 by the dropwise addition of 1 M NaOH under continuous stirring for 30 minutes at room temperature (Reglinski and Taylor, 2004).

2.4.1. Seed Materials, Sterilization and Seed Priming

Seeds of *Oryza sativa* (local variety: Shwebo Pawsan) were obtained directly from a rice farm in Bonet village, Bilu Island, Mon State, Myanmar. Prior to germination testing, seeds were surface-sterilized by immersion in 1% sodium hypochlorite solution for 3 minutes, followed by three washes with distilled water (Abdel-Aziz, 2019).

Ten priming treatments were prepared, including a control (no chitosan application), three different concentrations of chitosan solutions (1.0, 2.0, and 3.0 mg mL^{-1}), three different concentrations of NPK solutions (0.5, 1.0, and 1.5 mg mL^{-1}), and three admixtures of chitosan and NPK solutions (CS: NPK at 1.0:0.5, 2.0:1.0, and 3.0:1.5 mg mL^{-1}).

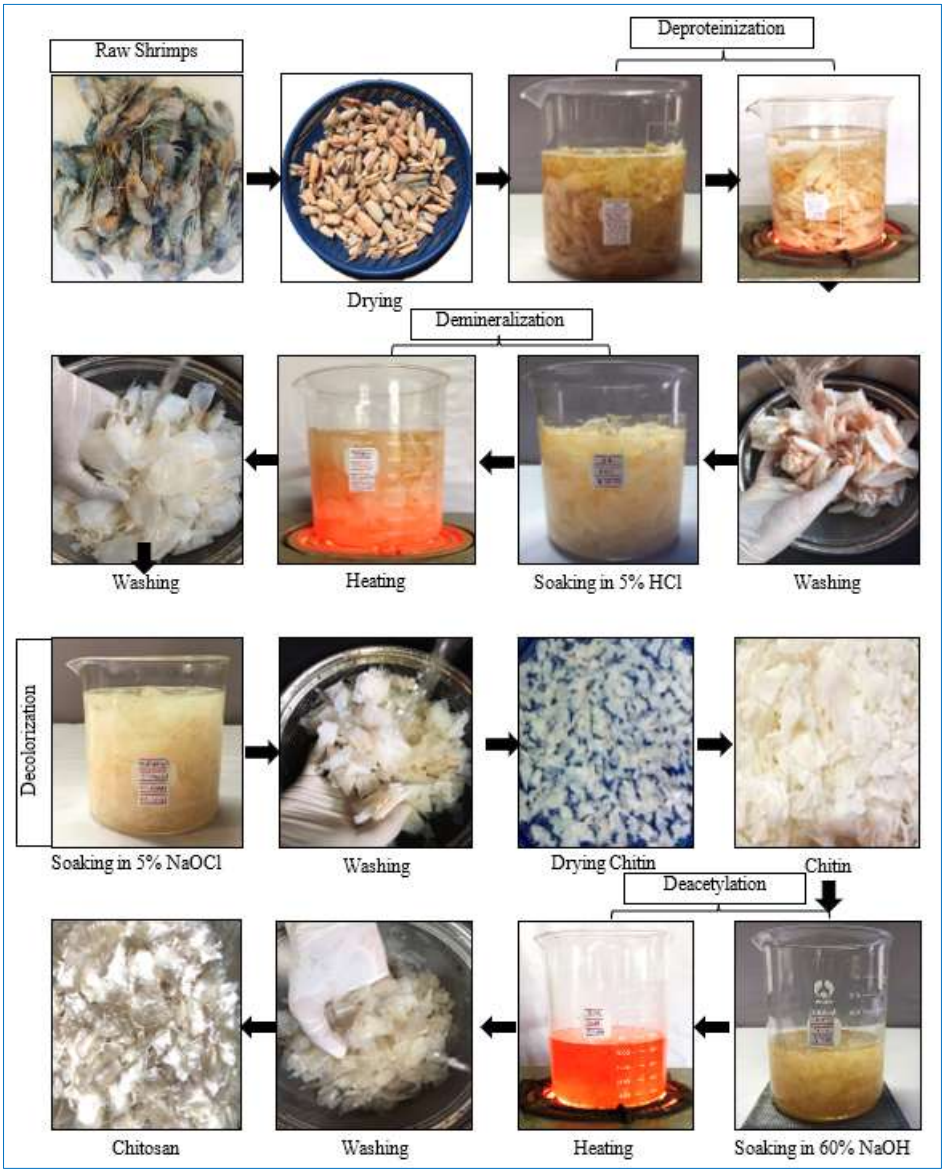

Fig. 1 The extraction procedures of chitin and chitosan from *Macrobrachium rosenbergii*

Fifteen seeds were immersed in each priming solution and germinated in PYREX® crystallizing dishes (120 × 120 × 60 mm) in triplicate. Each dish contained five seeds placed on Whatman Grade 1 filter paper, which was moistened with 20 mL of the respective priming solution. Distilled water was added daily to maintain moisture levels.

2.4.2. Germination Percentage

The germination percentage (GP) was recorded daily from the first day of germination up to 14 days after sowing. Seeds were considered germinated when radicle emergence of at least 1 mm was observed. The GP was calculated using the following equation:

$$GP = (Number\ of\ germinated\ seeds)/(Total\ number\ of\ seeds)\times 100 \quad (8)$$

2.4.3. Mean Germination Time

Mean germination time (MGT) represents the average duration required for seed emergence or germination. MGT

was calculated following the equation proposed by Ling et al. (2022):

$$MGT = (\sum (D \times n)) / (\sum n) \quad (9)$$

where; D denotes the number of days, and n represents the number of seeds germinated on the corresponding days.

2.4.4. Germination Energy

Germination energy (GE) is defined as the percentage of seeds that germinate within a short, predefined period, typically within the early phase of the germination test, when most viable seeds are expected to germinate. In this study, GE was determined at the point when 80% of the total planted seeds had germinated:

$$GE = \frac{\text{Number of seeds obtained when 80\% of the planted seeds germinated}}{\text{Total number of seeds tested}} \times 100 \quad (10)$$

2.4.5. Seedling Length

Seedling length, defined as the sum of root and shoot lengths, was measured in millimeters using a ruler after 14 days of germination.

2.4.6. Seedling Vigor Index

The seedling vigor index (SVI) was calculated according to the method described by Islam et al. (2013) using the following equation:

$$SVI = \text{Seedling length (mm)} \times \text{Germination percentage} \quad (10)$$

2.4.7. Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Significant differences among treatment means were determined using one-way analysis of variance (ANOVA), followed by Tukey's LSD test at $p < 0.05$. Statistical analysis was performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA).

3. Result and Discussion

3.1. Yield and Characterization of Chitosan

The yields of chitin and chitosan extracted from *Macrobrachium rosenbergii* shell waste were 29.0 ± 2.71 g and 24.37 ± 3.38 g, respectively. These values are consistent with previous reports, such as the 23% chitosan yield reported by No and Meyers (1989). Variations in yield are attributed to differences in processing conditions, particularly reaction time, which significantly affects extraction efficiency.

The ash content of the extracted chitosan, determined through anaerobic combustion in a muffle furnace (William and Wid, 2019), was 0.20 ± 0.10%, indicating effective demineralization. This result aligns with findings by Pădureţu et al. (2018), who noted that lower ash content reflects successful demineralization.

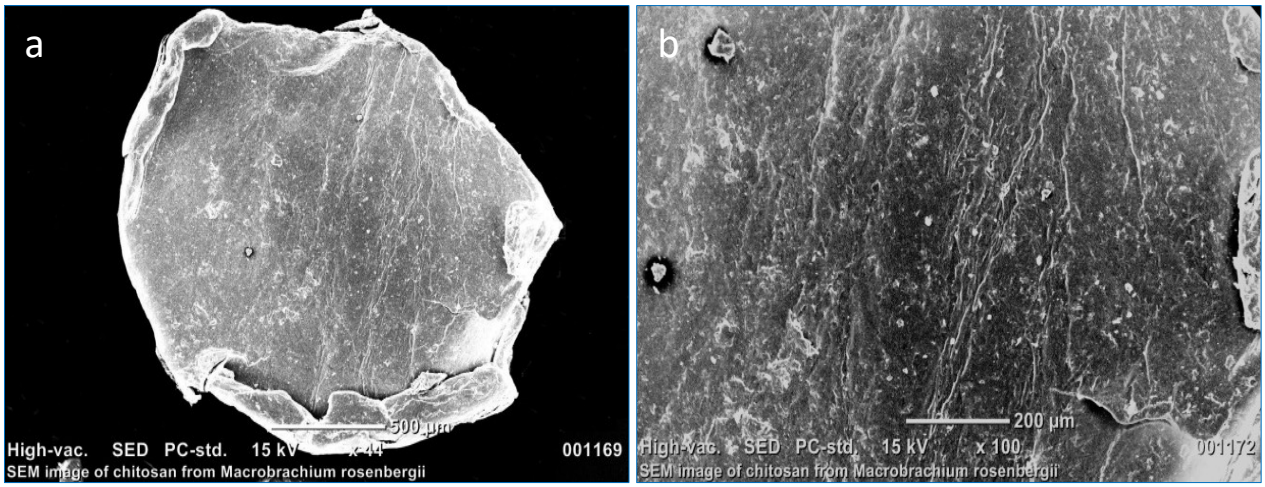

Fig. 2. SEM images of chitosan: a) Chitosan flake (44×) and b) Rough surface with porous and fibril structures (100×)

The moisture content of the chitosan was 9.22 ± 3.89%, within the typical commercial range of 7–11% (Rege et al., 1999). Moisture content is influenced by the degree of deacetylation, as the alkaline treatment during deacetylation increases the number of free amino groups ($-NH_2$) that interact with water (Ahing and Wid, 2016).

Chitosan solubility in 1% acetic acid was 94.33 ± 2.19%, significantly higher than the 66.43 ± 2.61% reported by Iber et al. (2023) for *M. rosenbergii* chitosan. High solubility enhances chitosan's functionality by improving its dispersion and incorporation into formulations (Ahing and Wid, 2016).

The water-binding capacity (WBC) of the chitosan was 775.17 ± 76.50%, consistent with commercial chitosan ranges of 458–805% (Cho et al., 1998) and 812.67 ± 7.64% (Gadgey and Bahekar, 2017). The WBC is attributed to chitosan's polycationic nature, which facilitates hydrogen bonding with water molecules.

The extracted chitosan was off-white in color, reflecting variations in purity and residual pigments due to the source material and extraction methods (Kurita, 2001; Prashanth and Tharanathan, 2007). The particle size ranged from 809.3 to 859.0 µm, indicating a relatively coarse material. Larger particle sizes are advantageous in agricultural applications, as they enable slower release of bioactive compounds, improving soil conditioning, water retention, and sustained

nutrient or pesticide release, thereby enhancing plant growth and disease resistance (Mourya and Inamdar, 2008; Ibrahim et al., 2019).

3.1.1. SEM and FTIR Analyses of Chitosan

SEM revealed that the chitosan exhibited a fibril and porous surface morphology with a notably rough texture, consistent with findings by Iber et al. (2023) for commercial chitosan.

The FTIR spectrum of the extracted chitosan sample exhibited characteristic absorption bands at 3358.5, 3287.4, 2925.5, 2870.6, 1645.8, 1571.5, 1347.3, 1151.3, 1067.3, 1022, and 896.1 cm^{-1} (Fig. 3). The absorption bands at 3358.5 and 3287.4 cm^{-1} correspond to O-H and N-H stretching vibrations, respectively. The peak at 2925.5 cm^{-1} is attributed to the stretching vibration of $-CH_3$, while the band at 2870.6 cm^{-1} corresponds to $-CH_2$ stretching. The presence of a peak at 1645.8 cm^{-1} indicates carbonyl stretching (amide I), whereas the band at 1571.5 cm^{-1} is associated with N-H bending (amide II), both of which are characteristic of chitosan. The peak observed at 1347.3 cm^{-1} corresponds to C-N stretching, while those at 1151.3, 1067.3, and 1022 cm^{-1} are indicative of C-O-C bonds. Additionally, the absorption band at 896.1 cm^{-1} is attributed to the β-1,4 glycosidic bond. These spectral features align with previous findings (Vino et al., 2015), confirming the structural integrity of the extracted chitosan. Chitosan-derived amides have been recognized for their role as natural growth promoters, contributing to enhanced seed germination, improved seedling vigor, and increased stress tolerance. These properties collectively support plant development, leading to healthier and more resilient crops (Orzali et al., 2017).

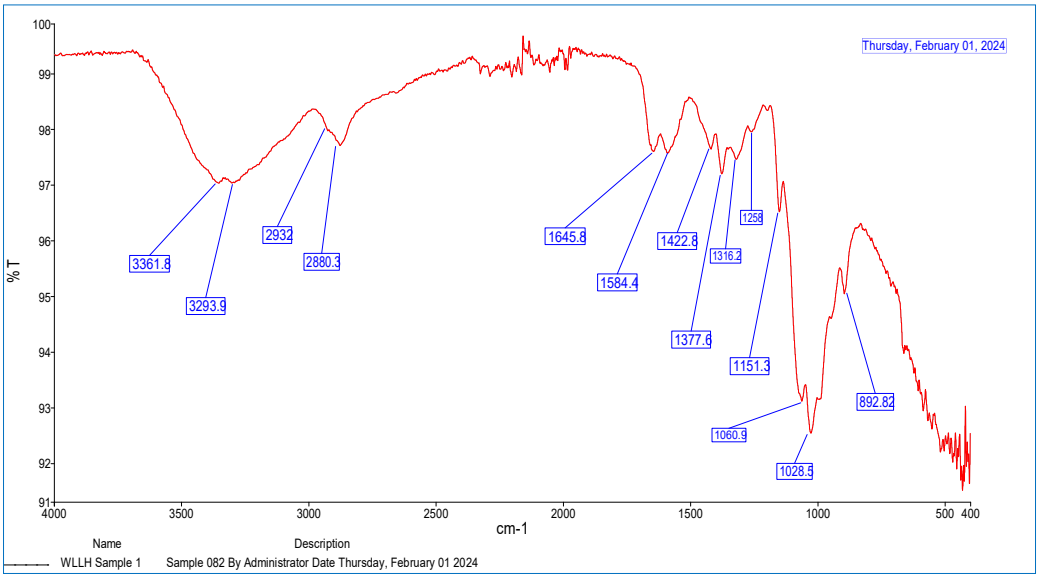

Fig. 3. FTIR analysis of chitosan extracted from *Macrobrachium rosenbergii*

3.2. Seed germination and growth response of Oryza sativa to different concentrations of chitosan extracted from Macrobrachium rosenbergii, NPK, and chitosan-NPK

The GP of seeds treated with chitosan did not exhibit significant differences compared to other treatments (Fig. 4A, Table 1). However, chitosan priming effectively reduced the MGT. Seeds treated with chitosan at concentrations of 1.0, 2.0, and 3.0 mg mL^{-1}, as well as the control group, demonstrated a reduced MGT of 3.2–3.3 days.

In contrast, seeds treated with NPK (0.5, 1.0, and 1.5 mg mL^{-1}) exhibited a longer MGT of 3.6–3.9 days, while those subjected to chitosan-NPK treatments had the highest MGT, ranging from 4.1 to 4.5 days (Fig. 4B, Table 1). The ability of *M. rosenbergii*-derived chitosan to significantly decrease MGT aligns with previous studies by Suchada et al. (2007) and Hameed et al. (2013), which reported reduced MGT in rice and wheat following chitosan priming.

GE was highest (100%) in seeds treated with 1.0 mg mL^{-1} chitosan and in the control group, followed by 93.3% for 3.0 mg mL^{-1} chitosan, 86.7% for 2.0 mg mL^{-1} chitosan and 0.5 mg mL^{-1} NPK, and 80% for chitosan-NPK (1.0:0.5 mg mL^{-1}) treatments. A decline in GE was observed with increasing NPK (1.5 mg mL^{-1}) and chitosan-NPK (3.0:1.5 mg mL^{-1}) concentrations (Fig. 4C, Table 1). These findings are consistent with the work of Hameed et al. (2014), who also reported enhanced germination energy following chitosan priming.

Chitosan application significantly increased shoot length, with the maximum shoot length (125 mm) observed at 2.0 mg mL^{-1} chitosan. However, higher concentrations of chitosan-NPK (3.0:1.5 mg mL^{-1}) did not confer additional benefits to shoot elongation (Fig. 4D, Table 1). Root length was similarly enhanced by chitosan, particularly at 1.0 and 2.0 mg mL^{-1}, while chitosan-NPK treatments demonstrated a less pronounced effect. Notably, root length was significantly lower in control and NPK-treated seeds compared to those treated with chitosan alone (Fig. 4E, Table 1). These results corroborate previous studies by Boonlertnirun et al. (2008) and Li et al. (2016), which reported improved shoot and root development following chitosan application in various plant species.

SVI was significantly lower in the control and NPK-treated seeds, whereas chitosan priming, with the exception of high-dose chitosan-NPK (3.0:1.5 mg mL^{-1}), markedly improved seedling vigor (Fig. 4G, Table 1).

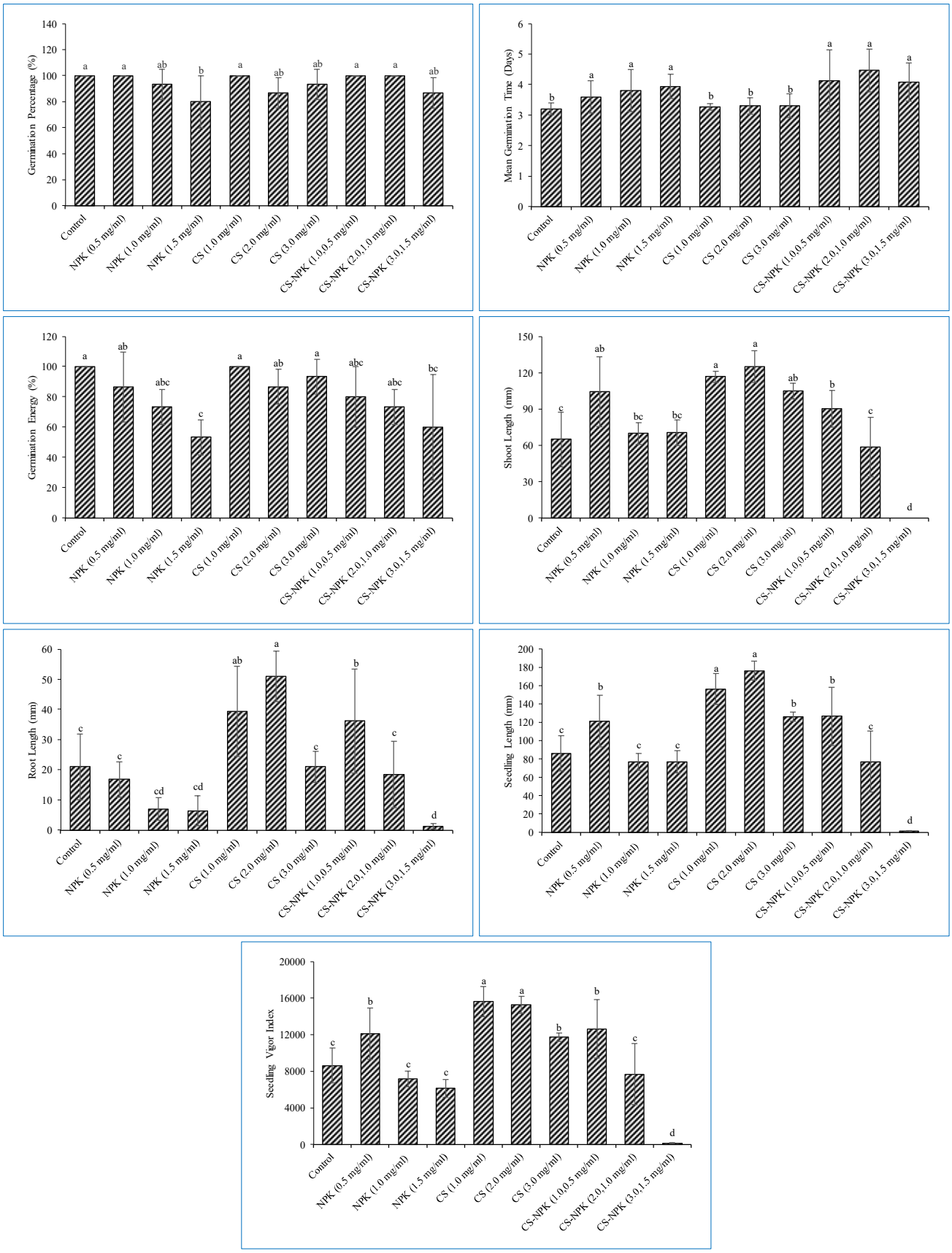

Fig. 4 (a-g). The effects of control, NPK, chitosan, and admixture of chitosan-NPK on seedling vigor index of *Oryza sativa*. Each data point represents the mean of triplicate analyses ± SD. Bars with different letters are significantly different at $p<0.05$ using the Tukey LSD test

Chitosan at 1.0 and 2.0 mg mL^{-1}, as well as chitosan-NPK (1.0:0.5 mg mL^{-1}), significantly increased seedling length compared to the control and NPK treatments (Fig. 4F, Table 1). Given that SVI is a critical indicator of seedling growth potential, the observed enhancement with chitosan priming is in agreement with previous findings by Liu et al. (2015) and Abdel-Aziz et al. (2019).

Overall, chitosan priming, particularly at 1.0 and 2.0 mg mL^{-1}, demonstrated superior efficacy in enhancing seedling growth and vigor compared to NPK and control treatments. However, excessive chitosan concentrations negatively affected seedling development, a trend consistent with the observations of Kanawi et al. (2021) in cucumber, basil, and cowpea. These findings underscore the potential of *M. rosenbergii*-derived chitosan as a natural seed priming agent for improving germination performance and early seedling establishment.

Table.1. Effects of control, NPK, Chitosan (CS), and CS-NPK on germination parameters and seedling growth of *Oryza sativa*

Parameters	Control	NPK (0.5 mg/ml)	NPK (1.0 mg/ml)	NPK (1.5 mg/ml)	CS (1.0 mg/ml)	CS (2.0 mg/ml)	CS (3.0 mg/ml)	CS-NPK (1.0, 0.5 mg/ml)	CS-NPK (2.0, 1.0 mg/ml)	CS-NPK (3.0, 1.5 mg/ml)
Germination percent (%)	100 ± 0^{a}	100 ± 0^{a}	93.3 ± 11.6^{ab}	80 ± 20^{b}	100 ± 0^{a}	86.7 ± 11.6^{ab}	93.3 ± 11.6^{ab}	100 ± 0^{a}	100 ± 0^{a}	86.7 ± 11.6^{ab}
Mean germination time (days)	3.2 ± 0.2^{b}	3.6 ± 0.5^{ab}	3.8 ± 0.7^{ab}	3.9 ± 0.4^{ab}	3.3 ± 0.1^{b}	3.3 ± 0.3^{b}	3.3 ± 0.4^{b}	4.1 ± 1.0^{ab}	4.5 ± 0.7^{a}	4.1 ± 0.6^{ab}
Germination energy (%)	100 ± 0^{a}	86.7 ± 23.1^{ab}	73.3 ± 11.6^{abc}	53.3 ± 11.6^{c}	100 ± 0^{a}	86.7 ± 11.6^{ab}	93.3 ± 11.6^{a}	80 ± 20^{abc}	73.3 ± 11.6^{abc}	60 ± 34.6^{bc}
Shoot length (mm)	65 ± 22.6^{c}	104.4 ± 28.9^{ab}	69.8 ± 9.1^{bc}	70.4 ± 10.7^{bc}	116.8 ± 4.6^{a}	125 ± 12.9^{a}	104.8 ± 6.4^{ab}	90.2 ± 15.2^{b}	58.6 ± 24.7^{c}	0^{d}
Root length (mm)	21 ± 10.8^{c}	16.8 ± 5.8^{c}	7 ± 3.7^{cd}	6.4 ± 5.0^{cd}	39.4 ± 14.9^{ab}	51 ± 8.4^{a}	21 ± 5.2^{c}	36.2 ± 17.3^{b}	18.4 ± 11.1^{c}	1.3 ± 1.0^{d}
Seedlings length (mm)	86 ± 19.0^{c}	121.2 ± 27.9^{b}	76.8 ± 9.3^{c}	76.8 ± 12.3^{c}	156.2 ± 16.8^{a}	176 ± 10.5^{a}	125.8 ± 5.1^{b}	126.4 ± 31.8^{b}	77 ± 33.1^{c}	1.3 ± 1.0^{d}
Vigor index	8600 ± 1902.6^{c}	12120 ± 2791.4^{b}	7168 ± 864^{c}	6144 ± 982^{c}	15620 ± 1675.4^{a}	15253.3 ± 911^{a}	11741.3 ± 473.2^{b}	12640 ± 3175.4^{b}	7700 ± 3312.1^{c}	112.7 ± 84.5^{d}

Values are means ± SD of three replicates. Values followed by different superscript letters within each row differ significantly at $p < 0.05$ using one-way analysis of variance (ANOVA), Tukey's LSD comparison test

4. Conclusion and Recommendations

This study successfully isolated chitin and chitosan from the shells of the freshwater prawn *Macrobrachium rosenbergii* using a chemical extraction method. The extracted chitosan exhibited a low ash content (<1%), indicating efficient demineralization and high purity. Its high solubility and water-binding capacity further highlight its potential for diverse applications. FTIR analysis confirmed the characteristic functional groups of chitosan, including Amide I (C=O stretching), Amide II (N-H bending and C-N stretching), and Amide III (a combination of N-H bending, C-N stretching, and C-H bending).

The application of *M. rosenbergii*-derived chitosan in seed priming was evaluated using rice (*Oryza sativa*) seeds treated with different chitosan concentrations, compared to NPK fertilizers and a control. Chitosan concentrations of 1.0 and 2.0 mg mL^{-1} significantly enhanced root length, shoot length, and seedling vigor. While chitosan-treated seeds exhibited similar germination percentages to other treatments, chitosan proved more effective in promoting root and shoot elongation during later growth stages, ultimately improving the seedling vigor index. In contrast, although NPK fertilizer provided essential nutrients, it did not enhance seedling growth to the same extent as chitosan.

These findings underscore the importance of chitosan's source and physiochemical properties in determining its effectiveness as a seed-priming agent. The results highlight the potential of chitosan priming as a sustainable agricultural technique for improving crop performance and resilience. Future research should focus on optimizing chitosan extraction methods, exploring its effects on different crops, and evaluating its interaction with other bio-stimulants to maximize its agricultural benefits.

Acknowledgement

I would like to express my gratitude to my science teacher, Dr. Khin Khin Gyi, Lecturer, Department of Marine Science, Mawlamyine University, Myanmar, provided me with valuable advice and suggestions to improve my experiment. This study is part of the MSc thesis of the corresponding author, titled *"Extraction and Characterization of Chitosan Biopolymer from Shrimp Shell Wastes and Its Application in Biostimulation of Seed Germinative Capacity and Seedling Growth."* The authors acknowledge all contributors who provided technical assistance and valuable insights that enriched this research.

Reference

Abdel-Aziz, H.M.M., 2019. Effect of priming with chitosan nanoparticles on germination, seedling growth and antioxidant enzymes of broad beans. The Egyptian Society for Environmental Sciences. 18 (1), 81-86.

Ahing, F.A., Wid, N., 2016. Extraction and characterization of chitosan from shrimp shell waste in Sabah. Transactions on Science and Technology 3 (1-2), 227-237.

Boehme, M., Schevschenko, Y., Pinker, I., 2008. Use of biostimulators to reduce abiotics stress in cucumber plants (*Cucumis ativus* L.). Horticultural Sciences 75, 339-344.

Boonlertnirun, S., Boonraung, C., Suvanasara, R.Y., 2008. Application of chitosan in rice production. Journal of Metals, Materials and Minerals 18 (2), 47-52.

Cheah, L. H., Page, B. B. C. and Shepherd, R. 1997. Chitosan coating for inhibition of sclerotinia rot of carrots. New Zealand Journal of Crop Horticultural Science 25 (1), 89-92.

Cho, Y.I., No, H.K., Meyers, S.P., 1998. Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. Journal of Agricultural and Food Chemistry 46, 3839-3843.

De Man, J.G., 1879. On some species of the genus *Palaemon* Fabr. with descriptions of two new forms. Notes from the Leyden

Museum 1 (3), 165-184.

Gadgey, K.K., Bahekar, A., 2017. Studies on extraction methods of chitin from crab shell and investigation of its mechanical properties. International Journal of Mechanical Engineering and Technology 8 (2), 220-231.

Gupta, K.C., Ravi Kumar, M.N.V., 2000. An overview on chitin and chitosan applications with an emphasis on controlled drug release formulations. Journal of Macromolecular Science, Part C: Polymer Reviews 40 (4), 273-308.

Hameed, A., Sheikh, A., Hameed, A., Farooq, T., Basra, S. M. A., and Jamil, A., 2013. Chitosan priming enhances the seed germination, antioxidants, hydrolytic enzymes, soluble proteins and sugars in wheat seeds. Agrochimica 57 (1), 31-46.

Hameed, A., Sheikh, A., Hameed, A., Farooq, T., Basra, S.M.A., Jamil, A., 2014. Chitosan seed priming improves seed germination and seedling growth in wheat (*Triticum aestivum* L.) under osmotic stress induced by polyethylene glycol. The Philippine Agricultural Scientist. 97(3), 294-299.

Iber, B.T., Torsabo, D., Chik, C.E.N.C.E., Wahab, F., Sheikh Abdullah, S.R., Abu Hassan, H., Kasan, N. A., 2023. A study on the effects of interfering with the conventional sequential protocol for chemical isolation and characterization of chitosan from biowaste of giant freshwater prawn *Macrobrachium rosenbergii*. Heliyon 9 (e13970), 1-19.

Ibrahim, A.I., Ebeid, H.M., Kishk, Y.F.M., Abdel Fattah, A.A., Mahmoud, K.F., 2019. Effect of grinding and particle size on some physical and rheological properties of chitosan. Arab Universities Journal of Agricultural Sciences 27 (2), 1513-1527.

Islam, M.R., Rahman, M.A., Rashid, M.M., Shahin-Uz-Zaman, M., 2013. Effect of moisture level and storage container on the quality of chickpea seed (*Cicer arietinum*). Bulletin Institute of Tropical Agriculture, Kyushu University 36, 61-69.

Kanawi, M.A., Haydar, M.A., Radhi, W.N., 2021. Effect of chitin and chitosan in improvement of plant growth and anti-fungal activity. Egyptian Journal of Botany 61 (2), 513-519.

Kurita, K., 2001. Controlled functionalization of the polysaccharide chitin. Progress in Polymer Science 26 (9), 1921-1971.

Li, H., Zhao, X., Wang, W., Li, L., 2016. Effect of chitosan on growth and dry matter accumulation of rice seedlings. Ecology and Environmental Sciences 25 (4), 691-696.

Ling, Y., Zhao, Y., Cheng, B., Tan, M., Zhang, Y., Li, Z., 2022. Seed priming with chitosan improves germination characteristics associated with alterations in antioxidant defense and dehydration-responsive pathway in white clover under water stress. Plants 11 (15), 1-13.

Liu, S., Remley, M., Bourland, F.M., Nichols, R.L., Stevens, W.E., Phillips, J.A., Fritschi, F.B., 2015. Early vigor of advanced breeding lines and modern cotton cultivars. Crop Science 55, 1729-1740.

Mourya, V. K., and Inamdar, N. N., 2008. Chitosan-modifications and applications: opportunities galore. Reactive and Functional Polymers 68 (6), 1013-1051.

No, H.K., Meyers, S.P., 1995. Preparation and characterization of chitin and chitosan-a review. Journal of Aquatic Food Product Technology 4 (2), 27-52.

Orzali, L., Corsi, B., Forni, C., Riccioni, L., 2017. Chitosan in agriculture: A new challenge for managing plant disease. *In*: E. A. Shalaby (Ed.), Biological activities and application of marine polysaccharides. *IntechOpen*: pp. 87-96.

Pădureţu, C.C., Apetroaei, M.R., Rău, I., Schroder, V., 2018. Characterization of chitosan extracted from different Romanian black sea crustaceans. Chemistry and Materials Science 80 (3), 13-24.

Prashanth, K.V.H., Tharanathan, R.N., 2007. Chitin/chitosan: modifications and their unlimited application potential-an overview. Trends in Food Science & Technology 18 (3), 117-131.

Rege, P.R., Shukla, D.J., Block, L.H., 1999. Chitinosans as tableting excipients for modified release delivery systems. International Journal of Pharmaceutics 181 (1), 49-60.

Reglinski, T., Taylor, J.T., 2004. Chitosan induces resistance to pitch canker in *Pinus radiata*. New Zealand Journal of Forestry Science 34 (1), 49-58.

Ruan, S., Xue, Q., Tylkowska, K., 2002. Effect of priming on germination and health of rice (*Oryza sativa* L.) seeds. Seed Science and Technology 30 (2), 451-458.

Shao, C.X., Hu, J., Song, W.J., Hu, W.M., 2005. Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. Journal of Zhejiang University (Agriculture and Life Science) 31 (6), 705-708.

Šimat, V., Rathod, N.B., Čagalj, M., Hamed, I., Mekinić, I.G., 2022. Astaxanthin from crustaceans and their byproducts: a bioactive metabolite candidate for therapeutic application. Marine Drugs 20 (3), 1-22.

Suchada, B., Sarobol, S.E., Meechoui, S., Sooksathan, I., 2007. Drought recovery and grain yield potential of rice after chitosan application. Kasetsart Journal- Natural Science 41, 1-6.

Vino, A.B., Ramasamy, P., Shanmugam, V., Shanmugam, A., 2012. Extraction, characterization and in vitro antioxidative potential of chitosan and sulfated chitosan from cuttlebone of *Sepia aculeata* Orbigny, 1848. Asian Pacific Journal of Tropical Biomedicine 2, 334-341.

William, W., Wid, N., 2019. Comparison of extraction sequence on yield and physico-chemical characteristic of chitosan from shrimp shell waste. Journal of Physics. Conference Series 1358, 1-7.