J. Trop. Plant Pests Dis.
Vol. 26, No. 2, September 2026
Pages: 342–352

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.226342-352

RESEARCH PAPER

Chitosan derived from crab shell waste: A natural coating against anthracnose disease in *Capsicum annuum* L. (Chilli)

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Manuscript received: 5 July 2025. Revision accepted: 24 November 2025. Available online: 12 Jnue 2026.

ABSTRACT

Chitosan, a polysaccharide derived from crustacean shells, serves as a promising natural coating for horticultural products such as chili (*Capsicum annuum* L.) to control postharvest diseases like anthracnose, caused by *Colletotrichum* species. This research aimed to extract chitosan from crab shell waste and evaluate its physicochemical properties and efficacy against anthracnose in chili fruit. Chitosan was produced from crab shell waste through demineralization, deproteinization, and deacetylation. The process yielded 32.29% chitosan. Key characteristics of the extracted chitosan included a violet result in the biuret test (indicating protein presence), partial solubility in acetic acid, 0.02% ash content, a melting point of 193.2 °C, 6.87% moisture content, and a low viscosity of 192.9 centipoise (cP). In vitro assays demonstrated that chitosan at a concentration of 2 mg/mL significantly inhibited the growth of *Colletotrichum gloeosporioides*. In in vivo trials, pure chitosan (2 mg/mL) and shell-derived chitosan (6 mg/mL) slightly prolonged the disease incubation period to 3.67 days and reduced disease severity by 33.33% compared to the control. However, the chitosan coating did not significantly affect fruit shrinkage. These results suggest that while crab shell-derived chitosan has strong antifungal properties, its formulation may need modification to improve physical barrier properties such as moisture retention.

Keywords: Anthracnose, *Capsicum annuum*, chitosan, crab shell, edible coating

INTRODUCTION

Chili (*Capsicum annuum* L.) is a high-value horticultural commodity in Indonesia and is widely consumed as a primary condiment (Muflikh & Kiloes, 2024). However, as a postharvest product, chili is susceptible to various diseases that reduce quality and quantity and pose health risks to consumers (Ahmad et al., 2024). Among the most important postharvest diseases of chili is anthracnose, caused by several *Colletotrichum* spp., including *C. fioriniae, C. fructicola, C. scovillei, C. siamense* and *C. truncatum,* which can potentially lead to yield losses ranging from 10% to 80%, depending on the chili cultivar, pathogen strain, and environmental conditions (Noor & Zakaria, 2024).

Therefore, effective, efficient, and safe postharvest management strategies are crucial, such as the use of biotic and abiotic agents, including natural resource materials (Megahed et al., 2023). Previous studies have demonstrated the effectiveness of natural compounds, such as plant extracts and microbial metabolites, in suppressing anthracnose in chili (Akin et al., 2024; Efri, 2011; Soesanto et al., 2020). One promising approach to protect chilies from postharvest pathogens is the use of coating technology with safe ingredients, known as edible coatings (Ma et al., 2024). Edible coatings can help maintain phytonutrient content and control physicochemical changes, thereby extending storage life (Pham et al., 2023).

Chitosan is one of the most widely utilized biopolymers for this purpose (Salgado-Cruz et al., 2021). It can be derived from various sources such as the shells of crustaceans, mollusks, algae, insects, and fungal cell walls through sequential processes of demineralization, deproteinization, and deacetylation (Pellis et al., 2022). Chitosan, as a natural coating agent for controlling postharvest pathogens, has been demonstrated on various horticultural crops, including strawberries (Petriccione et al., 2015), tomatoes (Paul et al., 2018), guava fruits (Silva et al., 2017), sweet peppers (Kehila et al., 2021), and chilies (Hu et al.,

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2020).

Its antimicrobial action is multifaceted, involving direct mode of action such as disrupting the cell membrane/wall of *Alternaria alternata* in apricots (Jieying et al., 2025), blocking nutrient exchange in tomato plants infected by *Fusarium oxysporum* (Carmona et al., 2021), affecting DNA/RNA and protein biogenesis of *F. oxysporum* in tomato plant (Carmona et al., 2021; Zhang & Shen, 2025), and chelating divalent metal cations that destabilize cell walls or membranes. Chitosan may also act indirectly, such as by inhibiting biofilm formation of *Xanthomonas oryzae* in rice (Yan et al., 2021; Ahmed et al., 2022).

In Indonesia, crab shell waste produced by a single fishery company can reach approximately 270 kg per day, representing more than 60% of the crab's total weight (Fachry & Alpiani, 2021; Amalia et al., 2021). This waste is used for multiple purposes, including animal feed, food products, fertilizers, and industrial raw materials such as chitosan (Amalia et al., 2021). However, shell waste is not optimally utilized and is mostly discarded into the environment or landfills. Consequently, it can cause environmental problems, including odor pollution, disease transmission, and aesthetic disturbances (Popović et al., 2023).

Therefore, this study aimed to produce chitosan from crab shell waste and evaluate its characteristics and potential to protect chili fruit and control anthracnose.

MATERIALS AND METHODS

Research Site. This study was conducted at two research facilities in the University of Jember, Indonesia. Chitosan production was carried out at the Laboratory of Plant Diseases, Faculty of Agriculture, University of Jember. The experimental work was performed at the Plant–Microbe Interaction Laboratory, Center for Development of Advanced Sciences and Technology (CDAST), University of Jember.

Extraction of Chitosan from Crab Shells. The crab shells used for the experiments were obtained from local fish processing industries in the Regency of Jember, East Java, Indonesia. The shells were washed, cleaned, and dried in an oven at 110–120 ºC for 30 min. The dried shells were cut into approximately 2 × 2 cm pieces and mixed with 1.5 M HCl solution at a ratio of 1:6 (weight per volume; w/v). Chitosan extraction included demineralization, deproteinization, and deacetylation (Pellis et al., 2022).

For demineralization, the mixture was heated at 60–70 ºC with stirring at 50 rpm for 4 hours, then filtered using Whatman No. 42 filter paper. The shell residue was washed with distilled water (dH_2O) to remove excess HCl until neutral pH was achieved. The obtained samples (rough powder) were dried in an oven at 85 ºC for 16 hours and cooled in a desiccator.

The shell powder was then deproteinized by adding 3.5% NaOH at a ratio of 1:16 (w/v) and homogenized at 60–70 ºC for 4 hours, followed by washing with dH_2O to remove residual NaOH until neutral pH was achieved. The sample was dried at 85 ºC for 16 hours, cooled in a desiccator, and weighed until constant weight.

For deacetylation, the samples were mixed with 60% NaOH at 1:18 (w/v) and heated at 110 ºC for 4 hours. The samples were then washed with dH_2O to remove residual NaOH solution until pH was achieved. The sample was dried at 80 ºC for 24 hours, cooled in a desiccator, and weighed to constant weight.

Characterization of Chitosan from Crab Shells. Chitosan extracted from crab shells was characterized and compared with pure and commercial food-grade chitosan. Water content was determined using a moisture determination balance by comparing the weight of the sample before and after heating the powder at 120 ºC for 20 min. Chitosan yield was calculated as the percentage of chitosan obtained from 100 g of crab shells.

Solubility was determined by dissolving 100 mg of chitosan in 10 mL of 1% acetic acid and observing solubility after standing for 60 min at room temperature (Du & Vuong, 2019). Ash content was calculated by comparing the weight of the sample before and after dry ashing in a furnace. Protein content was detected using Biuret reagent (Cong et al., 2023).

Particle shape was observed using a DN2500 microscope at 100× magnification. The melting point of chitosan was determined using differential scanning calorimetry, and viscosity was measured using the falling ball method (Song et al., 2022).

Isolation of the Pathogen from Chili and Pathogenicity Assay. Chili fruit exhibiting typical anthracnose symptoms were surface sterilized with 70% ethanol, followed by washing with sterile distilled water and drying on sterile tissue paper. The fruits were placed on sterile filter paper (Whatman) in Petri plates and incubated at 25 ºC under a 12-hour light/dark cycle for two days until spore layers appeared on the chili fruit surface (Rungjindamai, 2016).

Using an autoclaved toothpick, spore layers were transferred onto potato dextrose agar (PDA) plates to obtain pure cultures for morphological characterization using a DN2500 microscope and identification (Than et al., 2008; Oo et al., 2018).

For the pathogenicity assay, a 7-day-old pure culture was inoculated onto surface-sterilized red chilies that had been pierced two to three times with a sterile needle. The samples were incubated for 7 days at room temperature. Pathogenicity was confirmed by observing lesion formation at the inoculation points and fungal growth development (Hodiyah et al., 2024).

Preparation of Chitosan Coating Agent. The stock solution was prepared by dissolving 0.5 g of chitosan in 50 mL of 0.25 N HCl with agitation on a hotplate magnetic stirrer at 40 ºC for 60 min. The solution was adjusted to pH 5.6 with 1 N NaOH and autoclaved for 20 min. The sterile solution was diluted to 50 mL at various concentrations using sterile dH_2O (Morachis-Valdez et al., 2017).

Bioassay in Vitro. The inhibitory effect of chitosan on pathogen growth in vitro was evaluated based on radial mycelial growth, hyphal diameter, and septal distance. A 5-mm plug of 7-day-old pathogen mycelia was placed in the center of PDA medium containing chitosan at final concentrations of 2, 4, 6, or 8 mg/mL in 9-cm Petri plates.

Radial growth was measured 7 days after incubation at 28 ºC. Hyphal diameter and septal distance were measured at the second hyphal segment from the tip under microscopic observation using a DN2500 microscope at 400× magnification. Colony diameter was determined as the average of two perpendicular measurements (Hendricks et al., 2017).

Relative inhibition (%) was calculated as the difference between fungal diameter in the control and treatment, divided by the control diameter and multiplied by 100 (Tovar et al., 2019). The experiment was conducted in triplicate.

Anthracnose Control Assay on the Chili Fruit. Freshly collected fruits were washed with water and surface sterilized with 70% ethanol. Fruits were wounded by piercing five times with a sterile needle and immersed in chitosan solutions (2, 4, 6, or 8 mg/mL) for 60 s. Sterile water and pure chitosan at 2 mg/mL served as controls (Hodiyah et al., 2024).

For pathogen inoculation, 5 µL of conidial suspension (1.25×10^6 conidia/mL) was dropped onto the wounded area. Fruits were placed in containers and incubated in a growth chamber at 28 ºC with a 12-hour light/dark cycle. At least four fruits with three inoculation sites each were used.

Disease assessment. Weight loss, incubation period, and disease severity were evaluated. Weight loss (WL) was calculated as the percentage reduction from initial weight using the formula (Martínez-Blay et al., 2020):

$$\mathrm{WL}\,(\%) = \frac{(\mathrm{X} - \mathrm{Y})}{\mathrm{X}}\%$$

WL = Percentage of weight loss;
X = Initial weight before storage;
Y = Weight after storage.

The incubation period was defined as the time until first visible symptoms appeared. Disease severity was assessed at 7 days after inoculation using the formula described by Hodiyah et al. (2024):

$$\%\mathrm{DS} = \left[\frac{\sum (n_i \times v_i)}{\mathrm{Z} \times \mathrm{N}}\right] \times 100\%$$

n_i = The number of fruits in each category;
v_i = The score for each category;
N = The highest score of the category;
Z = The total number of fruits observed.

The score for the category was determined as:
0 = No lesion or symptoms;
1 = Lesion area of 0–20%;
2 = Lesion area of 21–40%;
3 = Lesion area of 41–60%;
4 = Lesion area of 61–80%;
5 = Lesion area of 81–100%.

Statistical Analysis. Differences among treatments were analyzed using one-way ANOVA with SAS software (version 9.1; SAS Institute, Cary, NC, USA). Duncan's multiple range test was applied at $p \leq 0.05$. At least three replicates were performed, and statistical analysis was conducted on the collected data.

RESULTS AND DISCUSSION

Chitosan from Crab Shells. This study found that crab shells contained approximately 32.29% chitosan. During chitosan extraction (Figure 1), the shape and physical weight changed depending on the specific stage. During the demineralization stage, the crab shells (Figure 1A) lost roughly 55.99% of their weight (Table 1) without noticeable alteration in shape (Figure 1B).

During the deproteinization stage, the demineralized product resulted in chitin, indicating a weight loss of approximately 13.17%, from 43.11 g to 38.30 g (Table 1). Additionally, significant physical changes were observed (Figure 1C). Chitin was subsequently deacetylated in the final step to produce chitosan, which had a yellowish-white color (Figure 1D) and weighed 32.29 g, indicating a chitosan yield of 32.29% (Table 1).

In general, chitosan produced from crab shells can vary considerably depending on the extraction method, source, and raw materials used (Ibram et al., 2019; Eddya et al., 2020). Abirami et al. (2021) reported a chitosan yield of approximately 37.5% from crab shells, whereas Musmade & Mahatma (2021) obtained only 19.34% from the same material. Chemical deproteinization and demineralization methods have been reported to be highly effective, with differences in chitosan color depending on the raw material and source (Trung et al., 2020).

This study used international standards to describe the quality of chitosan extracted from crab shells (Table 2). The Biuret test showed that all chitosan solutions turned purple, except for food-grade chitosan (Table 2), indicating the presence of residual protein

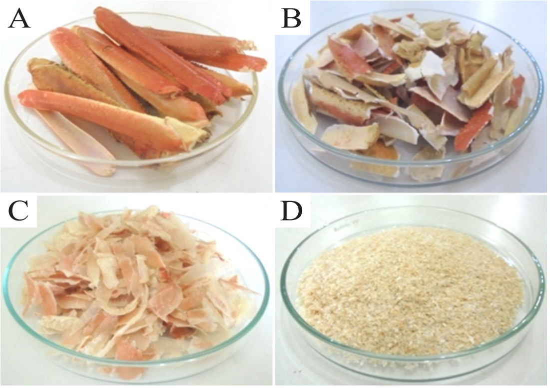

Figure 1. Changes in physical appearances during chitosan extraction. A. Crab shell waste before treatment; B. After demineralization; C. After deproteinization; D. After deacetylation.

Table 1. Analysis of products during chitosan extraction from crab shells

Sample	Value
Initial weight of crab shell (g)	100.00
Weight after demineralization (g)	44.11
weight after deproteination (g)	38.30
Yield (%)	38.30
Weight after deacetylation (g)	32.29

Table 2. Physicochemical characteristics of chitosan based on international standards

Properties	Character of			
	Crab shells chitosan	Commercial food-grade chitosan	Pure chitosan	International standard
Yield (%)	32.29	NA	NA	NA
Biuret test	Violet	Greenish yellow	Dark violet	Violet
Solubility	Partially dissolved	Dissolved	Dissolved	Dissolved
Ash (%)	0.02	0.013	0.01	< 1
Melting temperature (°C)	193.2	179.3	176.2	NA
Water content (%)	6.87	9.4	8.83	2–10
Particle's shape	Crystal form	Crystal form	Crystal form	Crystal form
Viscosity (cP)	192.9	199.2	207.8	< 200 (low) 200–799 (medium) 800–2000 (high) > 2000 (very high)

(Table 2). Indicating that chitosan still contains protein (Koirala et al., 2024). Unlike the others, chitosan extracted from crab shells was only partially soluble in 1% acetic acid, likely due to its ash content and melting point. According to Kurniasih et al. (2016), residual ash in chitosan can influence its solubility and melting point.

This finding is consistent with the ash content data, where chitosan from crab shells had the highest ash content compared with food-grade and pure chitosan (Table 2). In addition, chitosan from crab shells had the highest melting point (193.2 ºC), whereas food-grade and pure chitosan had melting points of 179.3 ºC and 176.2 ºC, respectively. The results also showed that chitosan from crab shells had the lowest moisture content (6.87%) and belonged to the lowest viscosity category (192.9 cPa). No differences in particle shape were observed among all chitosan samples (Table 2; Figure S1). Similarly, chitosan extracted from crustacean shells is typically produced in irregular crystal shapes (Aberoumand & Hoseinian, 2025) is produced in irregular crystal shapes.

Characteristics of the Pathogen of Anthracnose. The disease and its pathogen were identified through macroscopic and microscopic observations. The anthracnose pathogen was isolated from chili fruit exhibiting concentric and wrinkled lesions (Figure 2A). On PDA, the colony appeared white macroscopically (Figure 2B), while the reverse side showed an orange coloration at the colony base (Figure 2C).

Microscopically, the isolate produced cylindrical conidia with rounded ends, with an average width of 3.5–6.0 µm and length of 10–16 µm, along with septate hyphae (Figure 2D). The fungal colony also produced dark-colored setae (Figure 2E). Based on these morphological characteristics (Hodiyah et al., 2024; Than et al., 2008; Oo et al., 2018), the fungus was identified as belonging to the *Colletotrichum gloeosporioides* species complex.

Chitosan Inhibits *C. gloeosporioides* In Vitro. The in vitro results showed that chitosan at concentrations starting from 2 mg/mL significantly reduced the growth and development of *C. gloeosporioides* compared with the control. The assessment was based on colony diameter (Figure 3, Table 3), hyphal diameter, and septal distances (Table 3).

Macroscopically, fungal growth was significantly slower on PDA media containing chitosan than on control media. These results indicate that chitosan affected pathogen growth and development. Several studies have reported that chitosan inhibits growth and causes structural and molecular disorganization in *C. gloeosporioides* cells on papaya (Braga et al., 2019).

In addition, chitosan has been shown to inhibit conidial germination, induce hyphal lysis, and shorten hyphal segments in *Fusarium pseudograminearum* (Zhang et al., 2022), *Sclerotium sclerotiorum*, and *Rhizoctonia solani* (De Bona et al., 2021; Ismail et al., 2024), resulting in reduced disease severity. In general, chitosan inhibits hyphal growth through several mechanisms, including disruption of cell membranes and walls, formation of a dense polymer film on the

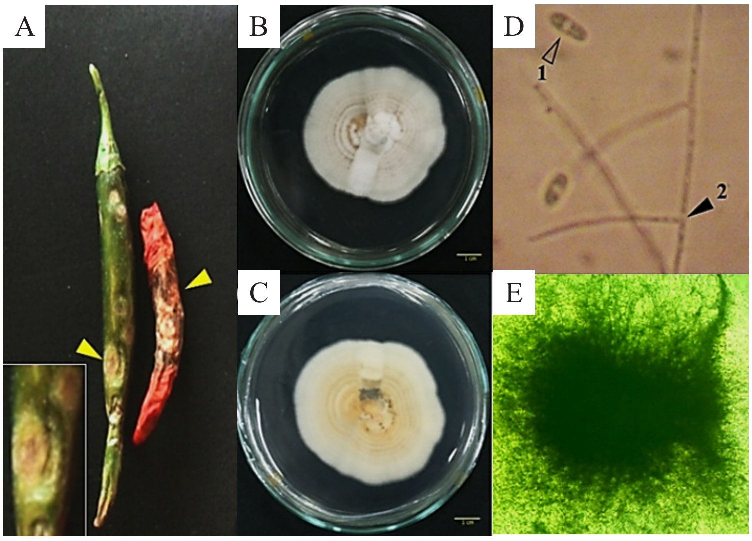

Figure 2. Isolation of the anthracnose pathogen from symptomatic chili fruits. A. Lesions on chili fruits (yellow arrowheads); B. Lesions on chili fruits (yellow arrowheads); C. Colony morphology viewed from the bottom after 7 days on PDA medium; D. Conidia (empty arrowhead) and hyphae (filled arrowhead) of at 400× magnification; E. Setae (Scale bar = 1 cm).

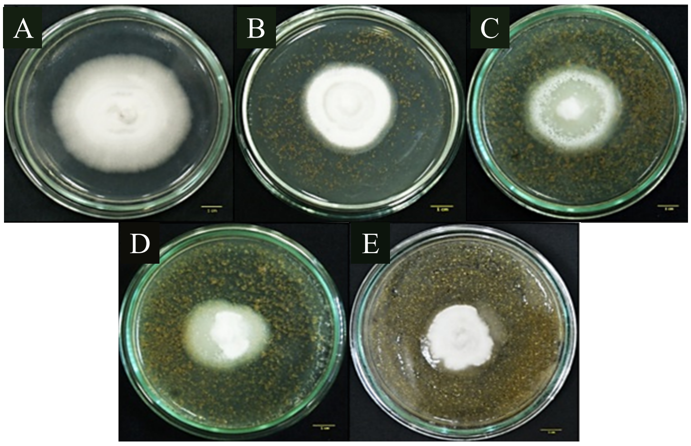

Figure 3. *Colletotrichum gloeosporioides* cultures grown on PDA medium containing different concentrations of chitosan derived from crab shells at 7 days after incubation. A. 0 mg/mL; B. 2 mg/mL; C. 4 mg/mL; D. 6 mg/mL; and E. 8 mg/mL (bar scale = 1 cm).

Table 3. Effect of different concentrations of chitosan on the in vitro growth of *C. gloeosporioides*

Chitosan concentration	Growth parameters		
	Colony diameter (cm)	Hypae diameter (µm)	Segment distances (µm)
Untreated control (0 mg/mL)	6.26 a	6.70 a	321.97 a
2 mg/mL	4.96 b	4.59 b	135.28 b
4 mg/mL	4.23 c	4.30 b	123.61 b
6 mg/mL	3.66 d	3.89 c	84.03 c
8 mg/mL	3.65 d	3.35 d	83.42 c

Means within the column followed by the same letter(s) are not significantly differed at $p \leq 0.05$.

cell surface, interaction with microbial DNA, nutrient chelation, and antifungal activity (Yan et al., 2021).

Effect of Chitosan on *C. gloeosporioides* In Vivo. Chitosan coating applied to chilies delayed the incubation period. Chilies coated with chitosan from crab shells, starting at 2 mg/mL, showed a longer incubation period and significantly reduced disease severity compared with the control (Table 4). However, although the delay in incubation time was statistically significant, similar delays were also observed in the positive control, suggesting that the effect was comparable.

The results indicated that chitosan derived from crab shells at 6 mg/mL was as effective in preventing disease development in chili fruits as pure chitosan at 2 mg/mL (Table 4). The lowest disease severity was observed in fruits coated with both concentrations, which showed slower symptom development, including sunken necrotic tissue and black concentric ring spots.

These results suggest that the most likely mechanism is direct antimicrobial activity, since the responses were observed in postharvest fruits. This mechanism may involve pathogen membrane disruption, interference with nucleic acids, chelation of essential nutrients, and formation of a physical barrier restricting pathogen growth and spread (Riseh et al., 2022). Consequently, disease development was reduced, resulting in lower disease severity (Zhang & Shen, 2025).

In contrast, symptoms in the control treatment appeared more severe (Table 4; Figure 4). The reduced disease severity in coated fruits likely resulted from inhibition of *C. gloeosporioides* growth (Table 3).

No significant differences in weight loss were observed, indicating that coating did not significantly affect water loss from chilies (Table 4). This may be due to the limited ability of chitosan coatings to prevent moisture loss during incubation. The inability of chitosan to effectively coat the fruit surface may be related to its viscosity. All chitosan samples in this study showed low viscosity (Table 2).

Water loss through transpiration is one of the main factors contributing to weight reduction. High-viscosity coatings are generally more effective in maintaining fruit freshness and flavor (Pham et al., 2023; Chen et al., 2024). Therefore, further formulation using

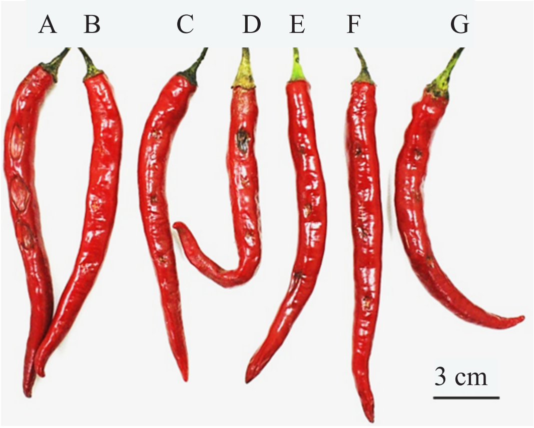

Figure 4. Anthracnose symptoms on chili fruits under different chitosan treatment. A. Non-treated healty control; B. Chili fruits coated with pure chitosan at 2 mg/mL; C. Chili fruits coated with crab shell chitosan at 2 mg/mL; D. 4 mg/mL; E. 6 mg/mL; F. 8 mg/mL; G. Symptoms observed 7 days after pathogen inoculation.

Table 4. Disease severity of anthracnose caused by *C. gloeosporioides* on chili fruits

Treatments	Parameter		
	Incubation time (days)	Disease severity (%)	Weight loss (%)
Positive control	3.25 b	51.67 a	20.13 a
Non-treated healty control	ND a	0.00 e	20.44 a
2 mg/mL (pure chitosan)	3.67 e	33.33 de	18.86 a
2 mg/mL (crab shells chitosan)	3.42 c	41.67 b	20.53 a
4 mg/mL B (crab shells chitosan)	3.50 cd	38.33 bc	20.40 a
6 mg/mL B (crab shells chitosan)	3.67 e	33.33 de	20.04 a
8 mg/mL B (crab shells)	3.67 e	35.00 cd	20.26 a

Value within a column followed by the same letter(s) are not significantly different at $P \leq 0.05$. ND = No disease.

more viscous materials may improve the effectiveness of chitosan coatings for both disease protection and maintenance of chili fruit physical quality.

CONCLUSION

This study demonstrated that crab shell waste, a significant environmental byproduct in Indonesia, can be effectively converted into chitosan with a yield of 32.29%. The extracted chitosan exhibited potent antifungal activity against *C. gloeosporioides* in both in vitro and in vivo assays. A coating containing 6 mg/mL of crab shell–derived chitosan was as effective as 2 mg/mL of pure chitosan in reducing disease severity on chili fruits. However, the coating did not prevent fruit weight loss, which may be attributed to the low viscosity of the extracted chitosan.

ACKNOWLEDGMENTS

The authors thank the University of Jember and the Center for Development of Advanced Science and Technology (CDAST), University of Jember, for providing research facilities.

FUNDING

This research did not receive financial support from any funding agency.

AUTHORS' CONTRIBUTIONS

HSA and MAB conceived and designed the experiment. MAB carried out the experiments and collected and analyzed the data under the supervision of HSA. HSA performed in-depth data analysis and interpretation and prepared the manuscript. Both authors contributed to the research design, data analysis, interpretation, and manuscript preparation. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no conflicts of interest related to the publication of this manuscript.

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