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Chitosan as A Plant Pathogen Control Agent: A Review

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Abstract. Pathogen attacks can cause decreased yields, even crop failure. Proper control can suppress pathogens' development and maintain yields. The concept of integrated pest management emphasizes the application of several techniques, one of which is the use of organic products as vegetable pesticides. Chitosan is a natural product obtained from crustacean shells or skins which can be used in various fields, including agriculture. Indonesia is one of the countries producing raw products from crustacean animals (shrimp, crab, lobster, and squid). This also has an impact on the amount of shell or skin waste produced. The processing of crustacean animal skin waste into chitosan can go through several stages, namely deproteinized, demineralization, and deacetylation. Chitosan application can be done by seed treatment, foliar spraying, soil application, and post-harvest products. The mode of action of chitosan against pathogenic fungi is binding to phospholipids in the plasma membrane of fungal cells, causing changes in hyphal morphology, degrading fungal enzymes, increasing levels of phenolics, sugar, and proline, and activating antimicrobial compounds and defense-related enzymes. The modes of action against pathogenic bacteria are interfering with gene expression, causing cell lysis, destroying bacterial biofilms, increasing defense enzyme activity, inducing systemic resistance, damaging and changing cell membranes, and causing cell wall permeability. The mode of action against pathogenic viruses is to increase the expression of genes related to defense, inhibit systemic viral multiplication and hypersensitivity response. The utilization of chitosan products is very important to study, especially in suppressing the use of chemical products and maintaining ecosystem sustainability.

Keywords: chitosan; pathogen; pathogen control

INTRODUCTION

Chitosan is a modified natural carbohydrate polymer that is processed through partial N-deacetylation of chitin, and can be obtained from the spinal structures of crustaceans and insects, and also in the cell walls of fungi (Xing *et al*., 2015). Indonesia is a country with high chitin-producing potential, this is due to the abundant availability of chitin raw materials from various crustacean animals such as shrimp, crabs, lobsters, and squid. Food processing of crabs and shrimp generally only utilizes the meat, while the shell or skin will become waste. (Hastuti *et al*., 2012) reported that around 57% of crab shell and shell waste from food processing becomes crab waste, which can pollute the environment. Overcoming this problem, there have been many studies on the utilization of chitin waste to become useful products, one of which is chitosan.

Chitosan has specific properties and promoting wound healing and is used in various applications (Kou *et al*., 2022). Several studies have proven that chitosan can be used as useful products such as fishery product preservatives and food product color stabilizers, flocculants and assisting the reverse osmosis process in water purification, additives for agrochemical products and seed preservatives (Ali *et al*., 2017; Tanasale *et al*., 2012; Trisnawati *et al*., 2013). Some research results show that, in agriculture, waste of marine organisms such as fish, seaweed, and crustaceans has been studied as nutrients supporting plant growth and controlling disease pathogens (Uge & Cahyaningrum, 2019; Ali *et al*., 2020; Pangaribuan *et al*., 2023). Chitosan is widely used as a pathogen control agent both in the field and post-harvest.

Several studies have reported the ability of chitosan to control the development of pathogenic viruses, namely the *Bean common mosaic virus* (BCMV), *Tobacco mosaic virus* (TMV), *Squash mosaic virus*, *Cucumber mosaic virus* (CMV), and *Tomato leaf curl virus* (ToLCV) (Damayanti *et al*., 2013; Firmansyah *et al*., 2017; Jia *et al*.,

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2016;Mishra *et al*., 2014; Nagorskaya *et al*., 2014; Uge *et al*., 2018). It is easy to decompose, so it is important to pay attention, especially in terms of application time and method of application. Based on the potential benefits of chitosan described above then it is important to conduct a literature study to determine the role of chitosan from crustacean animal waste in controlling plant pathogens.

METHODS

This type of research is a literature study that analyzes and synthesizes knowledge according to the problem raised. References were obtained from scientific publications available in the google scholar database with publication years 2012-2022. The literature review in this research focuses on the concept of integrated pest control which emphasizes the use of organic products as botanical pesticides, namely using chitosan obtained from crustacean shells or skin which can be used in agriculture.

RESULTS AND DISCUSSION

Chitin Waste Potential in Indonesia

Indonesia is the world's third-largest shrimp-producing country (Ministry of Maritime Affairs and Fisheries, 2012). As a shrimp exporting country, Indonesia certainly has the opportunity to produce chitin or chitosan (Ministry of Maritime Affairs and Fisheries, 2012). Exported shrimp are generally peeled and frozen so that the skin, legs, tail, and head weighing about 60-70% will only become waste (Azhar *et al.*, 2010). It is reported that shrimp waste contains protein, fat, calcium carbonate, chitin, pigments, and other ashes which have many benefits (Amalia & Arga, 2018; Rinaudo, 2012). Other than from shrimp, chitosan can also be obtained from other crustaceans such as crabs, squid, and lobster. (Arafat *et al*., 2015) Shrimp shells contain 18,1% chitosan, while crab shells contain 28,19% chitosan (Agusta, 2021; Nerdy *et al*., 2022). Data on the average production volume of crustaceans (crabs, shrimp, and lobster) from 2020 to 2021 tends to increase (Figure 1), with the highest production is shrimp followed by crabs, while lobsters is the lowest.

The Ministry of Maritime Affairs and Fisheries, (2015) states that the export volume of shrimp (peeled and without heads) is around 90 thousand tons annually, so it is predicted that 12 thousand tons of (dried) shrimp shells will be available, while crab exports (generally canned) are estimated to be around 4000 tons per year. produce crab shell waste of as much as 1000 tons.year^{-1}. Both of these wastes have the potential to be processed into chitin, with an estimated production of 1,700 tons.year^{-1}. In Indonesia, it was reported that the distribution of shrimp processing facilities covered several areas including the islands of Java, North Sumatra,

Lampung, South Sulawesi, Southeast Sulawesi, Central Sulawesi, and East Kalimantan, while for crab shells, it covered North Sumatra, the east coast of Sumatra, the coast of Java, Kalimantan and South Sulawesi. The abundant amount of crustacean animal skin waste, if processed into chitin and chitosan, is expected to increase the country's foreign exchange (Ministry of Maritime Affairs and Fisheries, 2015).

It is known that the number of provinces in Indonesia that cultivate crabs, shrimp, and lobsters is quite a lot. Data from the Ministry of Marine Affairs and Fisheries show that interest in shrimp farming in Indonesia is higher than that of in crabs and lobsters (Figure 2). From the data below (**Table 1**), it is known that approximately in 2021 and 2022 the percentage of provinces in Indonesia cultivated crabs were 50% (in 2021) and 50% (in 2022); shrimp were 100% (in 2021) and 89,4% (in 2022); while lobsters were 35,2% (in 2021) and 34,2% (in 2022). Thus, it is known that the potential for the most widely cultivated crustacean animal in Indonesia is shrimp, with the percentage of provinces in Indonesia that cultivate it reaching 89,4%. From this information, more or less shrimp, crab, and lobster shell waste in Indonesia will

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certainly continue to be produced every year. Therefore a proper management is becoming an important concern.

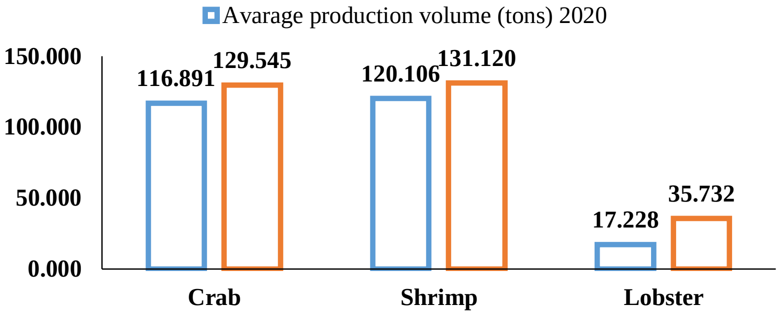
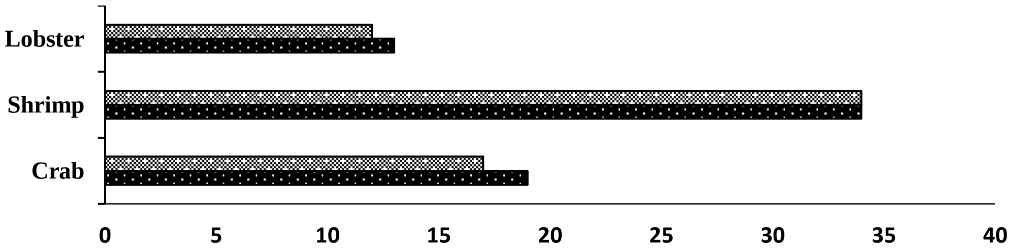


Figure 1. The average production volume of crab, shrimp, and lobster in 2020 and 2021 (Ministry of Maritime Affairs and Fisheries, 2022b)



	Crab	Shrimp	Lobster
2021	17	34	12
2020	19	34	13

Figure 2. Data on provinces in Indonesia that cultivate crabs, shrimp, and lobsters (Ministry of Maritime Affairs and Fisheries, 2022)

Table 1. Percentage of Indonesian's provinces cultivated crabs, lobster and shrimps in 2021-2022

Commodity	Total of Indonesian's Provinces	Periode (Year)	Total of Provinces cultivated crab, Losbter and shrimp	Percentage of Indonesian's provinces cultivated crab, lobster and shrimp (%)
Crab	34	2021	17	50,0
	38	2022	19	50,0
Shrimp	34	2021	34	100
	38	2022	34	89,4
Lobster	34	2021	12	35,2
	38	2022	13	34,2

Chitin Extraction Method for Chitosan

Crab shells, shrimp shells, lobsters, and squid bones are washed and then dried, then the waste is crushed and sifted. Furthermore, the waste flour was extracted using the method (Bastaman, 1989; Ningtyas *et al*., 2021).

De-proteinase: the deproteinization process is carried out by mixing the waste powder into 3% NaOH with a ratio of 1:6 (w/v) and heating it in a water bath at 80–85 ^{0}C for 30 minutes. The mixture was cooled and separated between solid and liquid using a 90-mesh sieve. The solids were then rinsed with distilled water by spraying 1 liter of

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distilled water.

Demineralization: this process is carried out by mixing the deproteinized waste powder into 1.25 N HCl with a ratio of 1:10 (w/v) and heating it in a water bath at 70–75 ^{0}C for 1 hour. After cooling, solids and liquids were separated using a 90- mesh sieve. The solids were rinsed with distilled water in the same way as above. From this process chitin is obtained, then the chitin is deacetylated.

Deacetylation: This is the process of mixing chitin with 50% NaOH in a ratio of 1:10 (w/v) and heating it in a water bath at 95–100 ^{0}C for 30 minutes. After the solution has cooled, the solids are separated using a 90-mesh sieve and rinsed with distilled water. Furthermore, the solids were dried in an oven at 80 ^{0}C for 24 hours.

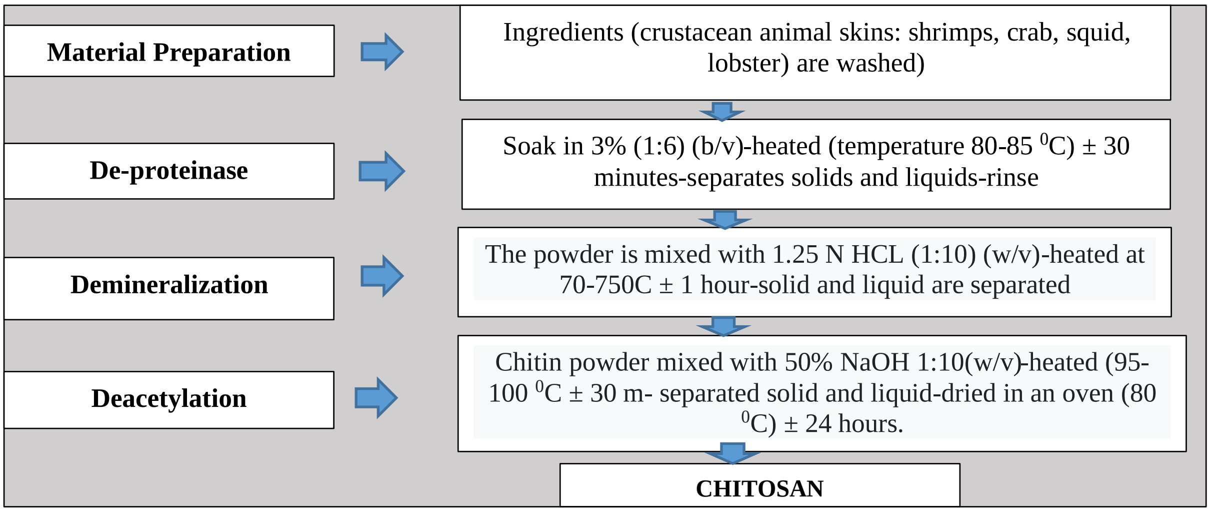

Figure 3. Process flow for making chitosan

Methods of Application of Chitosan in Agriculture

The application of chitosan to plants can be done in various ways including through seed treatment, spraying on leaves, spraying on the soil, and a combination of treatments with biological agents. Each treatment showed different doses according to the recommendations of the research results.

Seed treatment; This technique is generally carried out by soaking the seeds in a chitosan solution according to the concentration for one hour. Other recommended doses for the application of fenugreek seeds to suppress *Fusarium solani* are 2 g.l^{-1} and 4 g.l^{-1} of acetic acid for treating potato seeds to suppress *F. oxysporum* (Xue *et al*., 2021). The recommended concentration to suppress the development of seed-borne pathogenic viruses is 1% (Firmansyah et al., 2017). Another benefit provided by chitosan is seed coating, which can improve the quality and germination ability of seeds and increase tolerance to stress conditions (Hameed *et al*., 2014).

Treatment of the leaves is a simple technique. Application to the leaf surface allows direct contact with symptoms and pathogens on the leaf surface. In addition, treatment in this way can cause a physical barrier between the pathogen and the host plant. Treatment on leaves can be carried out when the plants are 5 HST, by spraying evenly over the entire surface of the plant leaves (Firmansyah *et al*., 2017). Application on cucumber plants as much as 0.05 -0.1% can suppress *Colletotrichum* sp and *Exobasidium vexans* infections in tea plants (Chandra *et al*., 2017; Dodgson & Dodgson, 2017). This was also supported by Uge *et al*., (2018) where a concentration of 1% was able

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to suppress *Cucumber mosaic virus* infection in pepper plants.

Table 2. Mode of action of the treatment using chitosan on fungi, bacteria, and viruses

Pathogen group	Species	*Mode of Action*	Reference
Fungus	*Sclerospora graminicola*	Chitosan with a low molecular weight causes inhibition of mycelium growth, while chitosan with a high molecular weight affects spore form, sporulation, and germination.	(Oliveira *et al*., 2012)
		Chitosan initiated of plant defense boosters in treated plants may be attributable to the ability of chitosan to modulate salicylic acid phytohormone pathways which include the synthesis of phenylalanine ammonia lyase and pathogenesis-related proteins	(Siddaiah *et al*., 2018)
	Penicillium citrinum, Penicillium mallochii	Chitosan inhibits the growth of two Penicillium species by compacting the fungal spores and reducing the activity of plant cell walls by degrading the enzymes produced by the fungi. In addition, chitosan enhances the wound healing process in citrus fruits.	(Coutinho *et al*., 2020)
	Colletotrichum capsici	Chitosan completely inhibits the growth of the *C. capsici* mycelium by forming a physical barrier around the penetration sites of the pathogen, preventing it from spreading to healthy tissues. In addition, chitosan activates antimicrobial compounds related to defense, induces a decrease in malondialdehyde content, and increases the concentration of soluble sugar and proline, as well as peroxidase and catalase activity.	(Akter *et al*., 2018)
	Colletotrichum camelliac	Chitosan increases the activity of H2O2, defense-related enzymes, such as polyphenol oxidase, peroxidase, catalase, phenylalanine ammonia-lyase, and dissolved protein concentrations in camellia plants.	(Li & Zhu, 2013)
Bacterial	*Xanthomonas oryzae* pv. *oryzae, Xanthomonas oryzae* pv. *oryzicola*	Chitosan causes membrane lysis and destruction of bacterial biofilms and increases the activity of phenylalanine ammonia-lyse, peroxidase and polyphenol oxidase in rice seedlings.	(Li *et al*., 2013b)
	Xanthomonas vesicatoria	Chitosan induces systemic resistance mechanisms in tomatoes by activating several defense enzymes and elicits the expression of the PIN II and ETR-1 genes from several molecular pathways involved in pathogen defense.	(Ramkissoon *et al*., 2016)
	Pseudomonas fluorescens	Chitosan reduces disease incidence, lesion diameter and induces systemic resistance mechanisms in broccoli.	(Li *et al*., 2010)
	Acidovorax citrulli	Chitosan significantly inhibits the growth of *A. citrulli* by damaging and altering the cell membrane, separating the cytoplasmic membrane from the cell envelope, agglomerating cytosolic components, forming vacuole-like structures, and breaking down the cell wall, which causes	(Li *et al*., 2013)

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		leaching out of the mass of nutrients and nucleic matter.	
Viral	*TMV*	Chitosan-induced TMV resistance in wild-type Arabidopsis plants and deficiency of the jasmonic acid pathway (jar1) increased expression of the defense-related PR1 gene.	(Jia *et al.*, 2016)
	AMV	Chitosan induces systemically acquired resistance, increases total carbohydrate and total phenolic content, and triggers transcriptional levels of peroxidase, pathogen-associated protein-1, and phenylalanine ammonia-lyase.	(Abdelkhalek *et al.*, 2021)
	BYMV	chitosan-based nanomaterials have a superior effect in controlling plant viruses as an antiviral curing agent. ChiNPs promote the plant defense mechanism against virus invasion by promoting expression of the PR-1 gene and some defense-related enzymes (phenylalanine ammonia lyase, polyphenol oxidase) and increasing the total phenolic content.	(El Gamal *et al.*, 2022)

Source: (Abdellatef *et al.*, 2022)

Soil treatment can be done by watering soil amendments at the soil surface, on plants aged 4-6 WAP. This treatment is generally accompanied by spraying on the leaf surface and the application of PGPR (Firmansyah *et al.*, 2017). The application of chitosan into the soil can help increase the population and abundance of beneficial microorganisms such as *Pseudomonas fluorescens*, actinomycetes, mycorrhiza, and rhizobia.

Treatment of post-harvest products. The application of chitosan on the surface of fruit and vegetable products can help suppress pathogen infection, induce resistance responses in plant tissues, and suppress respiration so that products do not ripen easily. The study by (Munhuweyi *et al.*, 2017) showed that the application of 0.1-10 $g.l^{-1}$ on Pomegranate fruit can reduce the incidence of rot disease caused by *Botrytis* sp, *Penicillium* sp., and *Pilidiella granati*, while (Zheng *et al.*, 2017) the 5 $mg.l^{-1}$ treatment was shown to be able to suppress diseases caused by *B. cinerea* and *P. expansum*. In the treatment of peaches with chitosan and oligochitosan 5 $g.l^{-1}$, the incidence of disease caused by *Monilinia fruiticola* was drastically reduced (Ma *et al.*, 2013). Chitosan nanoparticles can inhibit *Colletotrichum gloeosporioides* activity by up to 85.7%, and inhibit spore germination by up to 61% (Suryadi *et al.*, 2017). The application of chitosan in increasing the shelf life of fruit has also been studied by Hesami *et al.*, (2021) which showed that chitosan 1% with low temperature storage (5 ^{0}C) is the most effective for improving the physiological quality and prolong the shelf life of ber fruits.

Mechanism of Action of Chitosan in Plant Pathogen Control

Chitosan as a plant pathogen control agent has several roles or properties as antifungal, antifungal, and antibacterial, while in other fields it also has anti nematode and abiotic antistress properties. This characteristic is of course related to the mode of action against pathogen infection. The following is a grouping of modes of action of chitosan in controlling pathogenic fungi, pathogenic bacteria, and pathogenic viruses (**Table 2**).

Application of Chitosan on Plant Pathogens

Fungal

Chitosan has the ability as an antifungal. Chitosan showed an antibiofilm activity and

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a potential to inhibit fungal (Vijayan *et al*., 2020), reducing the colony diameter (Lipsa et al., 2020) and improve the antifungal properties (Hussein *et al*., 2020). Amino acid groups in the form of acetylamino (HCOCH3) and glucosamine (C6H9NH2) in chitosan which are positively charged can bind to negatively charged macromolecules on the surface of fungal cells such as the peptidoglycan layer and lipopolysaccharide which are fats and proteins (Trisnawati *et al*., 2013). The presence of this group can interfere with the formation of appressorium and the growth of the fungus (Sitorus *et al*., 2014). Several research results related to the role of chitosan in controlling plant pathogenic fungi are shown in **Table 3**.

Table 3. Results of research on the role of chitosan in controlling fungal pathogens

Fungi Isolate	Treatment Dose	Treatment effect	Reference
Colletotrichum capsici	Concentration 32%	Prevented the mycelial development	(Long *et al*., 2018)
Phytophthora megakarya L (caused of cocoa pod rot)	concentration 0,4%, 0,6% dan 0,8% on acetic acid solution	Inhibits colony development up to 48-64%	(Septiana *et al*., 2018)
Rigidoporus lignosus (caused oof clove white root fungus disease)	8 $g.l^{-1}$ dan 10 $g.l^{-1}$	Inhibits mycelial development up to 100% and cause lysis	(Widiantini et al., 2016)
Colletorichum gloeosporioides Penz (antracnose on papaya fruit)	concentration 0,75%	Inhibits fungi development 72-85%	(Hamdayanty *et al*., 2016)
Colletotrichum spp.) cause of anthracnose in chilies	0,5-1 $ml.l^{-1}$	Suppressing the rate of anthracnose infection in chilies to > 50%	(Suryadi *et al*., 2019)
Sclerotinia sclerotirum and *Fusarium oxysporum* f.sp. *cucumerinum* (FOc) cause of stem rot and wilting	1 $mg.ml^{-1}$ and 2 $mg.ml^{-1}$	Suppresses the development of pathogenic fungi up to 70-90%	(Onaran *et al*., 2021)

Bacterial

Chitosan application affects the activity of bacteria. This can be seen from several parameters including cell lisis, inhibits of biofilm formation and bacterial growth. The direct antibacterial activity of chitosan is related to the lysis of the bacterial membrane which can be observed through an electron microscope. Chitosan treatment is known to be able to increase seed germination. This can also be seen from the fresh and dry weight parameters of watermelon seeds in the application of chitosan to soil (Li *et al*., 2013). Yang *et al*., (2014) suspected that the mechanism of inhibition by chitosan that occurred could be through disruption and lysis of the bacterial membrane, decreased formation of bacterial biofilms, and changes in gene expression. Several research results related to the role of chitosan in controlling plant pathogenic bacteria are shown in **Table 4**.

Viral

Chitosan and its derivatives are known to act as inducers or inducers of resistance, increasing plant resistance responses both locally around the infected area, to signal healthy plant parts. This mechanism occurs by involving early signals, and the

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accumulation of metabolites and proteins related to defense mechanisms such as phytoalexins and PR-proteins (Siddaiah *et al*., 2018).

Table 4. Results of research on the role of chitosan in controlling pathogenic bacteria

Bacterial isolates	Treatment Dose	Treatment effect	Reference
Bulkholderia seminalis causes of apricot fruit rot	1 $mg.ml^{-1}$ and 2,0 $mg.ml^{-1}$ on an acid solvent	Treatment for 3 and 12 hours suppressed the infection	(Lou *et al*., 2011)
Xanthomonas sp. cause of rot disease in *Euphorbia pulcherrima*	10 $mg.ml^{-1}$	Inhibit bacterial development	(Li *et al*., 2008)
Xanthomonas campestris Pv campestris (Xcc) cause of black rot	300 $\mu g.ml^{-1}$	Inhibits growth and viability of bacteria up to 50 %	(Sreelatha *et al*., 2022)
Psudomonas fluorescens cause of stem rot disease in broccoli	0,10 $mg.ml^{-1}$	Treatment with an incubation time of 12 and 24 hours can reduce the incidence of the disease	(Li *et al*., 2010)
Acidovorax citrulli causes bacterial fruit spot on watermelon	0.40 $mg.ml^{-1}$	Inhibit the growth of *A.citrulli*	(B. Li *et al*., 2013)
Acidovorax avenae subsp. avenae cause of brown bacterial disease in rice plants	0.10, 0.20, 0.40 $mg.ml^{-1}$	Inhibits bacterial growth and formation of biofilms	(Yang *et al*., 2014)

Table 5. Results of research on the role of chitosan in controlling viral pathogens

Viral Isolate	Treatment Dose	Treatment Effect	Reference
Tobacco mosaic virus (TMV) causes tobacco mosaic disease	1 $mg.ml^{-1}$	Infectivity and low of viral coat protein content	(Nagorskaya *et al*., 2014)
Bean common mosaic virus (BCMV) causes of mottled mosaic inlong bean plants	Concentration 1%	Extend incubation time, reduces disease severity, viral titer, and peroxidase activity	(Damayanti *et al*., 2013)
Squash mosaic virus cause of disease in cucumber plants	concentration 5% + PGPR 1%	Promotes plant growth and inhibits the development of symptoms reducing the incidence and intensity of the disease as well as stimulate plant growth	(Firmansyah *et al*., 2017)
Cucumber mosaic virus (CMV) cause of mottled disease in pepper	concentration 1%	Reducing the incidence and intensity of the disease as well as stimulate plant growth	(Uge *et al*., 2018)

Chitosan is recognized by plant PRRS (pattern recognition receptors) and can trigger plant defense responses and can behave like PAMPs/MAMPs (Pathogen/Microbe-associated molecular patterns). PAMPs/MAMPs, which are recognized by the plant PRRS system and trigger a defense response, by inducing non-host plant resistance and especially promoting systemic resistance. Enhanced defense responses after chitosan application include increasing H+ and Ca2+ for entry into the cytosol, activation of MAP-kinase, callus apposition, oxidative burst, hypersensitive response, synthesis of abscisic acid, jasmonic, phytoalexin, and PR-protein

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(Laflamme *et al*., 2000), accumulation of phytoalexins, PR (pathogenesis-related) proteins (glucanase, proteinase, peroxidase, ribonuclease like protein) and proteinase inhibitors, lignin synthesis and callus formation (El Hadrami *et al*., 2010). Several studies related to the role of chitosan in controlling plant pathogenic bacteria are shown in **Table 5**.

CONCLUSION

Chitosan is a natural product of crustaceans which is abundant in Indonesia. Crustacean waste processing in Indonesia is still very limited. The utilization of crustacean waste in chitosan will greatly assist farmers in managing plant diseases in environmentally friendly cultivation. Crustacean shells (shrimp, crab, lobster, and squid) can be processed into chitosan through deproteinized, demineralization, and deacetylation processes. Chitosan has been studied and has been known to control fungal, bacterial, and viral pathogens. Applications to plants can be carried out in seed treatment, soil treatment, leaf treatment, and post-harvest products. The application dose of chitosan in suppressing pathogens varies greatly depending on each species. Modes of action of chitosan in controlling fungi include remembering the cell plasma membrane, changing the morphology of hyphae, inhibiting mycelium development, affecting spore shape, causing abnormal growth in cells, increasing peroxidase activity and enzymes related to inhibition of pathogens. The modes of action of chitosan in controlling bacteria are interfering with bacterial cell gene expression, causing cell lysis and destruction of bacterial biofilms, inducing resistance mechanisms, damaging and changing cell membranes, impairing cell membrane permeability, reducing disease severity, and causing bacterial death. The mode of action of chitosan in controlling viruses is increasing gene expression, increasing phenolic content and enzymes related to defense reactions, inhibiting viral multiplication, and increasing hypersensitivity responses. Biological properties of chitosan are different between product of degree deacetalizations. Therefore, studies related to differences in the degree of deacetalization and biological properties for plant pathogen control need to be researched further.

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