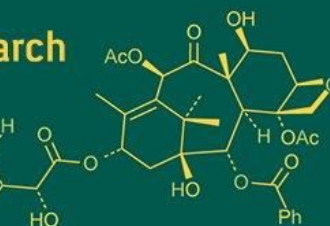
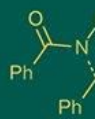


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## Chitosan: A natural ally in pest management

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**Abstract**

The adverse effects of synthetic pesticides have spurred a search for alternative pest management options, leading to a focus on biopolymers like chitosan. Chitosan, derived from chitin, offers biodegradability, biocompatibility and human non-toxicity. Traditionally sourced from shrimps and crabs, recent studies explore insects as alternative chitin sources due to efficient extraction methods. The process involves demineralization, deproteinization, and deacetylation, with extraction conditions influencing biopolymer properties. Insects, comprising 95 percent of animal biodiversity, offer substantial potential for chitin and chitosan production. Chitosan's versatility finds applications in various industries, including agriculture for pest management, disease control and enhancing plant defense. Its insecticidal properties make it an attractive alternative to synthetic pesticides, aligning with organic agriculture principles. Research indicates chitosan's efficacy in disease control, with factors such as polymerization degree and environmental conditions affecting performance. Furthermore, chitosan induces plant responses and exhibits antimicrobial activity against phytopathogens. Embracing chitosan and its derivatives represents a promising step towards sustainable pest management, reducing reliance on synthetic pesticides while fostering agricultural and environmental health.

**Keywords:** Chitin, chitosan, biopolymer, insects as chitin source, pest management, insecticidal activity, antimicrobial properties

**Introduction**

Adverse effects of synthetic pesticides lead to the quest of alternative options for pest management. Biopolymers such as cellulose, starch, gelatines and chitosan have gained much attention due to their valuable characteristics, including biodegradability, biocompatibility and non-toxicity for humans (Ravi, 2000) <sup>[24]</sup>. Chitosan is the most important derivative of chitin. It was discovered in mushrooms by Henri Braconnot back in 1811, who named it "fungine". Later, in 1821, Auguste Odier isolated the same compound from beetle cuticles and named it "chitin". Chitin was, the first polysaccharide identified by man, preceding cellulose by about 30 years, and the most abundant polymer after the latter. Indeed, chitin is the most abundant component in the exoskeleton of arthropods and in the structural membranes and spores of fungi (Kou *et al.*, 2021) <sup>[16]</sup>. Shrimps and crabs, characterized by a high chitin content (around 20-30%), are the most common commercial sources of this polysaccharide, although their use as a source of chitin has been much debated recently (Gillett, 2008) <sup>[11]</sup>. Chitosan's origins can be traced back to Charles Rouget's study in 1859, when he produced the material by heating chitin in an alkaline medium. However, Felix Hoppe-Seyler coined the word "chitosan" in 1894 and the chemical structure was discovered only in 1950 (Ruiz *et al.*, 2017) <sup>[26]</sup>. As the global demand for chitosan is increasing, with an annual growth rate of 15.4 percent, there is a need for alternative sources to meet the market demand. In recent years, research has also focused on the study of insects, characterized by 10-15 percent of chitin, as an alternative source of the latter. The extraction of chitin from insects is also advantageous in terms of the rearing method, substance consumption, and time of production. The most investigated insect species for chitosan production are *Hermetia illucens* (Linnaeus, 1758) (Diptera Stratiomyidae), *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera Tenebrionidae) and *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera Tenebrionidae). Chitosan production from chitin, regardless of the source, is mainly characterized by three steps of extraction, as follows: demineralization, deproteinization and deacetylation (Mohan *et al.* 2020) <sup>[21]</sup>.

However, different conditions during the extraction process, such as concentration of reagents, time and temperature, pH, presence, or absence of metal cations, pKa, molecular weight and degree of deacetylation, may affect the properties of the biopolymer Kong *et al.* 2010) [15]. Chitin is naturally present as a structural element in the exoskeletons of a wide range of eukaryotic organisms like crustaceans, insects and cell walls of fungi and the second most abundant biopolymer next to cellulose on the planet. The biocompatibility, non-toxicity and versatility of chitin favour its usage in several industrial sectors (Antoniraj *et al.* 2020) [2]. Crab and shrimp exoskeletons are considered the main source for commercially produced chitin (Arbia *et al.* 2013) [3]. Although, chitin is the main structural polysaccharide of insect exoskeleton only limited numbers of insect species have been documented as sources for chitin extraction (Liu *et al.*, 2012) [19]. Crab, shrimp and crayfish have been preferred for commercial production of chitin but new alternative chitin sources like corals, fungus and insects could be exploited (Ifuku *et al.*, 2011) [12]. Chitin is also a primary component in insect cuticles. Therefore, insects are an alternative source of chitin and consequently, of chitosan. Recently, the production of chitin and chitosan from insect sources has drawn increased attention. First, insects possess enormous biodiversity and represent 95 percent of the animal kingdom (Labandeira *et al.*, 1998) [17]. Therefore, they offer a tremendous potential as a natural resource for chitin and chitosan production. Chitin and chitosan as a biopolymer have received more momentum in last two decades as basic and applied research, industrial uses due to its macromolecular structure, physicochemical properties, bioactivities and versatility over synthetic version. There is a wide practical application of chitosan and its oligosaccharides in food, beverage, pharmaceutical, cosmetics, agriculture and veterinary, aquaculture, textile, pulp-paper industry, biotechnological applications (Bio-sensor & bio-devices) and green chemistry (Morin *et al.*, 2019) [22]. They have been utilized to control disease or reduce their spread; to chelate nutrient and minerals, preventing pathogens from accessing them; or to enhance plant innate defense (Singh *et al.*, 2017) [28]. The

heterogeneity of chitin caused by differences in source materials is a hindrance for the application of chitin and its derivatives. The insecticidal properties of chitin and chitosan make them a novel tool in pest management strategy. Perusal of literature revealed that products based on these may serve as good alternatives for broad-spectrum and highly persistent pesticides. Their insecticidal properties on homopteran insects were studied by (Casal *et al.*, 2002) [7] and their activity on some lepidopteran insects was demonstrated by Zhang *et al.* (2003) [35]. The pesticides application and their related effects constitute a topic of major concern, nowadays the government promotes organic agriculture as an alternative approach that maximizes the performance of renewable resources and optimizes nutrient and energy flows in agro-ecosystems. For this reason, many scientific works and researches have been focused in developing alternative approaches to the use of pesticides for managing crop diseases, through experimentations that have followed different paths including physical methods integrated pest management and biological control (Tinivella *et al.*, 2009) [29]. A promising approach consists in the use of natural compounds such as plant extracts and their active principles (alkaloids, phenols, monoterpenes and sesquiterpenes, isoprenoids), which have been studied for their various antifungal, antibacterial and antioxidant properties (Riccioni and Orzali, 2011) [25] and animal derived compounds like chitosan. The efficiency of chitosan on disease control depends on the dose and virulence of the pathogen. However, the degree of polymerization, molecular weight, chemical composition, and environmental conditions are the critical factors. It has proved to be very interesting for controlling plant diseases (El Hadrami *et al.*, 2008) [8]. In fact, it has been shown both to possess a broad-spectrum antimicrobial activity against several phytopathogenic organisms and to induce numerous biological responses in plants (Malerba and Cerana 2016) [20]. In order to avoid using synthetic pesticides, chitosan and their derivatives are new tools in the arsenal of pest management techniques because of their insecticidal and microbial activity (Figure 1).

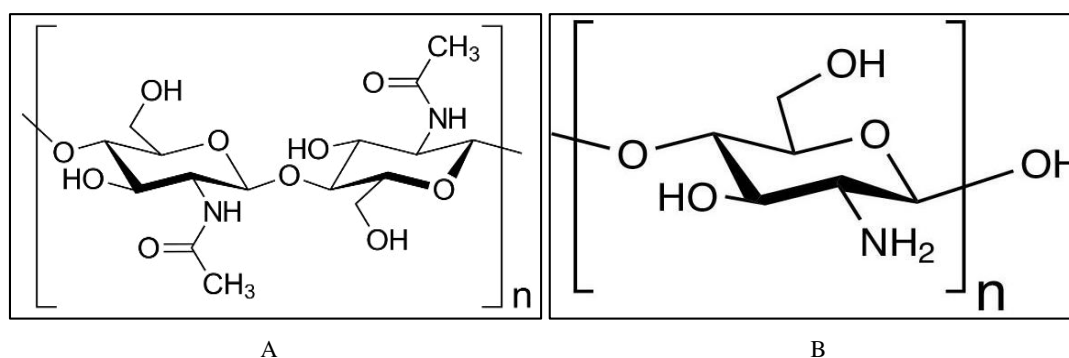


**Fig 1:** The represented structures of A chitin and B chitosan (de-acetylated form of chitin).

#### Extraction of chitosan from crustaceans/insects/fungus:

Chitosan can be extracted from chitin, a polysaccharide found in the exoskeletons of crustaceans, using two main methods: chemical and biological. Chemical methods involve treating the chitin with alkaline and acidic solutions, while biological methods utilize microorganisms and enzymes to extract chitin (Figure 2). Chitosan production from chitin, regardless of the source, is mainly characterized

by four steps of extraction, as follows: deproteinization, demineralization, decolourization and deacetylation. However, different conditions during the extraction process, such as concentration of reagents, time and temperature, pH, presence, or absence of metal cations, pKa, molecular weight and degree of deacetylation, may affect the properties of the biopolymer.

**Deproteinization:** The fine powder weighing 30g of each respective insect specimen were subjected to deproteinized by treating dried powder samples with a 4.0 percent NaOH solution at 90°C for 2 hrs in glass flask (250 ml capacity) with constant stirring through glass rod to remove the protein and was filtered. Then, the filtrate was washed with tap water for 30 minutes until the pH becomes neutral (7). The deproteinized filtrate was dried in the oven at 60°C for 24 hrs.

**Demineralization:** The remaining deproteinized powder were demineralized with 2.5 percent (w/v) of hydrochloric acid (HCl) at room temperature (28 °C) for 6 hrs to remove the mineral content with a ratio of 1:20 (w/v). After that, the samples were filtered and washed with tap water for 30 minutes, or until the pH reaches neutral (7). The demineralized samples were dried in the oven at 60°C for 24 hrs.

**Decolourization and Dewatering:** The ddecolourization process was accomplished by soaking the samples in acetone for 10 minutes, drying them for 2 hrs at room temperature (28 °C) and then removing the acetone residues. The decolourized sample particles was washed in running tap water, rinsed, filtered and dried at 60°C for 24 hrs in the oven to obtain chitin from respective powder samples of the moths.

**Deacetylation of chitin:** The chitin so obtained after decolorization was treated with 50 percent (w/v) aqueous NaOH with a ratio of chitin to the solution of 1:15 (w/v) at 105 °C for 2 hrs. Then, the chitin was filtered and washed with deionized water until the pH becomes neutral (7) to obtain the chitosan. The obtained chitosan were oven dried for 24 hrs at 60 °C. Finally, the product from each specimen so obtained were pulverized and packed as chitosan as dry basis.

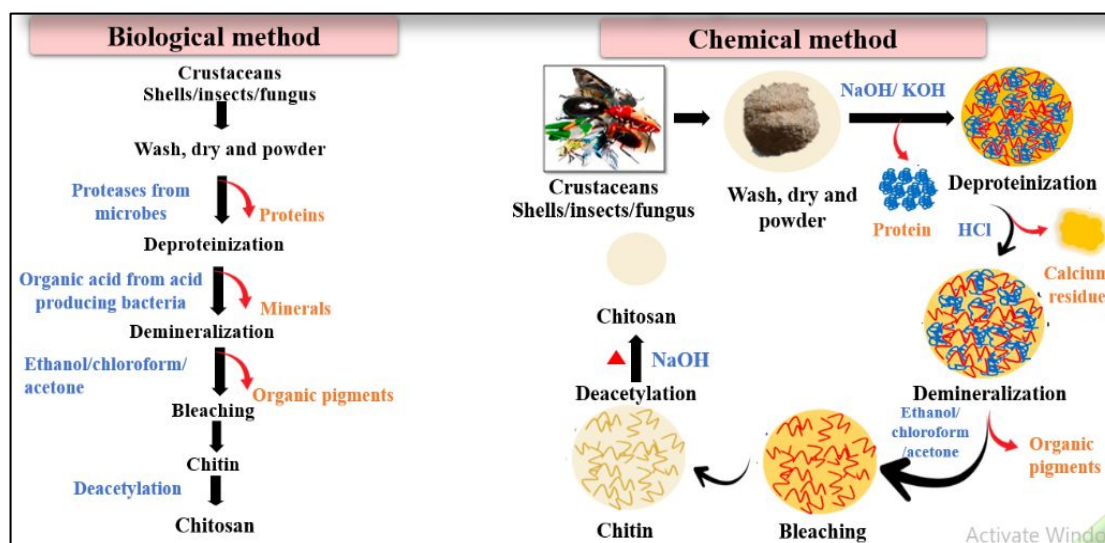


Fig 3: Extraction process of chitin by biological and chemical method

### Mode of action of chitin, chitosan and its derivatives

#### Insecticidal activity:

While studying the insecticidal and fungicidal activities of chitosan and oligo-chitosan, Zhang *et al.* (2003) [35] presumed that the chitosan forms a double layered film (hydrophobic and hydrophilic) on the surface of insects and the film can block air from the insects and the insects died

due to asphyxiation or the application of chitosan may induce chitosanase activity and that lead to insecticidal activity (Figure 3). The different methods of extraction of chitin and preparation of chitosan played significant role in histopathology and enzymatic activity in the midgut and their insecticidal efficacy (Said *et al.*, 2011) [27].

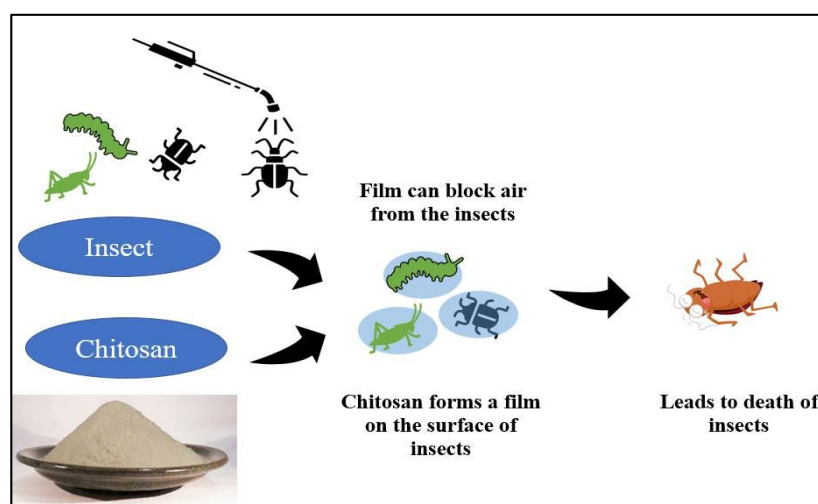


Fig 3: Potential insecticidal actions of chitosan against insects

Zhang *et al.* (2003) <sup>[35]</sup> found 40, 72 and 93 percent mortality of *Helicoverpa armigera* (Hubner), *Plutella xylostella* (Linnaeus) and *Rhopalosiphum padi* (Linnaeus), respectively when tested through leaf dip bioassay at chitosan solution of 3.0 g/L after 72 hours of exposure. Chitosan with different molecular weights and its metal complex also showed insecticidal activity against cotton leaf worm and aphid (Badawy and Aswad, 2012) <sup>[6]</sup>. Chitosan has antifeedant activity as soybean seed coating against soybean pod borer and soybean aphid (Zeng *et al.*, 2012) <sup>[33]</sup>. Chitosan also showed termiticidal activity against *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Raji *et al.*, 2018) <sup>[23]</sup>. Ascrizzi *et al.* (2021) <sup>[5]</sup> reported that the seed coating of common bean (*Phaseolus vulgaris* L.) with chitosan-essential oil (CH-EO) (*Ferulago campestris* Besser) solutions at 20 percent were found best in protective treatment against bean weevil, *Acanthoscelides obtectus* (Say) as it recorded lowest eggs, larva and entrance holes in the seeds. Uddin *et al.* (2021) <sup>[30]</sup> observed highest larval mortality of *Spodoptera litura* Fab. (62.72%) in chitosan @ 3.0 g/L through combined bioassay technique followed by topical (52.51%) and leaf dip (46.14%) method at 7 days after treatment. Highest growth inhibition of larva (27.61%) and reduction in pupal weight (37% reduction over control) was also noticed in combined bioassay technique. Wang *et al.* (2021) <sup>[31]</sup> found that the spinosad/chitosan controlled-release suspension (SCCS) at concentration higher than 30

mg/L showed >70 percent mortality of the third-instar larvae of *P. xylostella*.

#### Antimicrobial activity:

The antimicrobial properties of chitosan are highly associated with its structure, physicochemical properties and environmental conditions (Ke *et al.*, 2021) <sup>[13]</sup>. High molecular weight chitosan is generally unable to penetrate the cell membrane; its potential antimicrobial effects involve acting as a chelator of essential metals, preventing nutrients from being taken up from cells extracellular and altering cell permeability. However, low molecular weight chitosan not only has extracellular antimicrobial activity but also intracellular antimicrobial activity, thereby affecting RNA, protein synthesis and mitochondrial function. Antibacterial activity of chitosan against gram-negative bacteria is due to more negative charge because lipopolysaccharides are often attached to phosphorylated groups and more negatively charged surface allows the binding of cationic chitosan to phospholipids when the environmental pH is below 6.5 whereas in gram-positive bacteria, teichoic acids are also negatively charged due to the presence of phosphate groups in their structure. However, deletion of the teichoic acid biosynthesis pathway in *Staphylococcus aureus* (Rosenbach) resulted in increased resistance to chitosan.

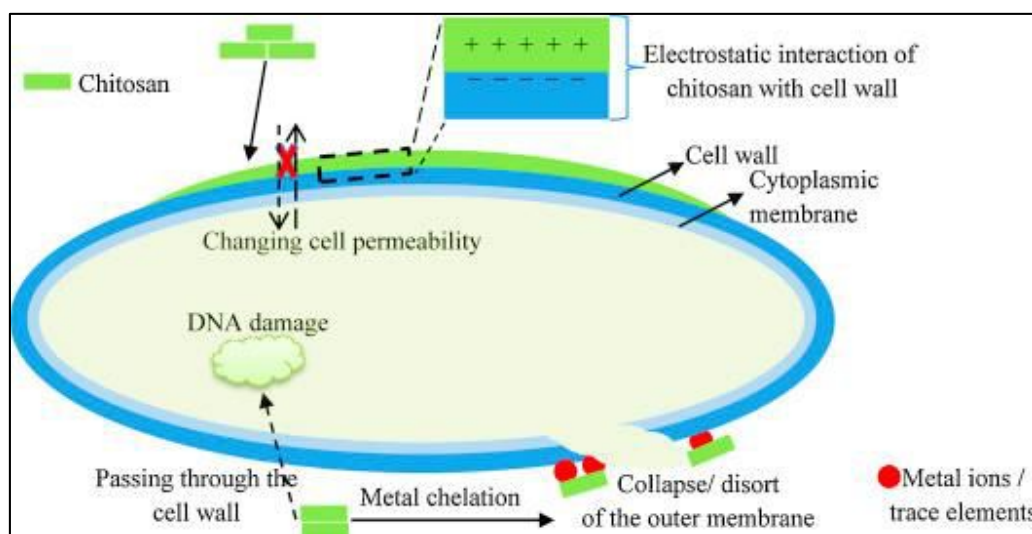


**Fig 4:** Potential antimicrobial actions of chitosan against fungus and bacteria

Li *et al.* (2012) <sup>[18]</sup> reported the antibacterial activity of chitosan A (1129kDa) and chitosan B (607kDa) against bacterial leaf blight and leaf streak of rice, which significantly reduced disease incidence and severity by comparison with the control. Similar result was finding by Wang *et al.* (2012) <sup>[32]</sup> against twelve *Xanthomonas* strains isolated from *Euphorbia pulcherima* (Willdenow) of different countries. Algam and Elwagia (2015) <sup>[1]</sup> found that chitosan at 3 and 5 mg/mL showed strong antifungal activity against the early blight, *Alternaria alternata* in in-vitro experiment and chitosan @ 5 mg/mL applied as foliar spray alone, or in combination with seed treatment or seed treatment alone, reduced disease severity by 46 percent and were found comparable to fungicide spraying. Gaikwad (2016) <sup>[10]</sup> tested *in-vitro* effect of chitosan on growth inhibition of *Alternaria porri* by poison food technique and observed >80 percent growth inhibition in treatments of

chitosan at 2.4 percent and Mancozeb @ 2 percent. According to Zhang *et al.* (2024) <sup>[34]</sup>, chitosan significantly inhibited the mycelia growth and spore germination of *Fusarium solani* causing post-harvest soft rot of ginger. *In-vitro* study indicates chitosan treatment effectively reduced disease rate and severity of post-harvest soft rot through modulating the expression of phytopathogenic response LysM genes.

#### Nematicidal activity

Fan *et al.* (2023) <sup>[9]</sup> studied the activity of chitosan oligosaccharide (COPS) against plant pathogenic nematode (PPN) in soil and presumed that the application of chitosan partly stimulates the parasitism of nematophagous microorganisms, increasing nematicide substances release and enhancing resistance of host (Figure 5).

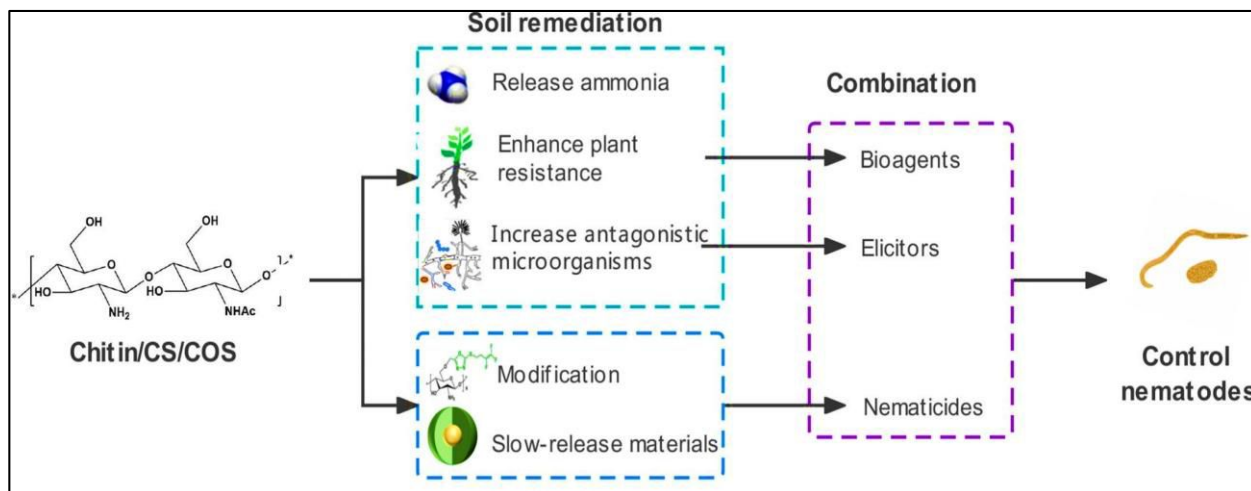


**Fig 5:** Potential nematocidal actions of chitosan against nematodes

Khalil and Badawy (2012) <sup>[14]</sup> reported nematicide activity of the chitosan ( $2.27 \times 10^5$  g/mol and  $3.60 \times 10^5$  g/mol) against root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) infesting roots of tomato plants. Amongst chitin, chitosan and bioproducts, chitin at 5 and 7 percent showed equal effectiveness against juveniles of *Rotylenchulus reniformis* after three days of treatment recording 74.16 and 78.54 percent mortality, respectively (Archana, 2017) <sup>[4]</sup>.

### Conclusion

Chitosan is the second most widely distributed abundant natural polymer obtained from the chitin. Chitosan derived from the exoskeleton of crustaceans, cuticles of insects, cell walls of fungi and some algae. *In-vitro* effectiveness of chitin, chitosan and its derivatives as insecticidal activity (*H. armigera*, *P. xylostella*, *S. litura*, *Agrotis ipsion* Hufnagel, Soybean pod borer and aphids), as termiticidal activity (*R. flavipes* and *R. virginicus*), as antifeedant activity (*S. littoralis*) as antimicrobial activity (early blight of tomato, soft rot of ginger, bacterial leaf blight and leaf streak of rice and purple blotch of onion) and as nematocidal activity (*M. incognita* and *R. reniformis*) showed promising results. Chitosan as carrier with insecticides (spinosad) or as chitosan-metal complex (CH-Cu, CH-Ni and CH-Hg) also showed promising results. Looking to the harmful effect of pesticides and adverse effect on the environment, chitosan as natural ally for pest management holds great promise for sustainable agriculture and ecofriendly pest management.

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