

Research Article

Extraction and evaluation of chitosan as an insecticide against saw-toothed grain beetle, *Oryzaephilus surinamensis* **L. (Coleoptera: Silvanidae)**

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ABSTRACT: Rice samples infested with saw-toothed grain beetles have been collected from a local market in the city of Kirkuk/Iraq. The study was conducted in Kirkuk during the period 15 December 2023 to 25 April 2024, at College of Pure Sciences at the University of Kirkuk, Iraq. Chitosan was utilised in the study to examine its toxic effects at three different concentrations (0.5, 1.0, 1.5 ppm) on the life cycle of the saw-toothed grain beetle, focusing on oviposition rate, duration of larval and pupal stages, and mortality rate of its adults. The chemical demonstrated a substantial effect in lowering the number of eggs laid as the concentration increased, with the control treatment registering the highest oviposition rate of 314.14 eggs compared to 209.31, 117.03, and 61.12 eggs for chitosan concentrations of 0.5, 1.0, and 1.5 ppm, respectively. The shortest egg incubation period recorded was 8.17 days, Incubation times for chitosan treatments increased significantly to 9.66, 11.69, and 14.00 days for concentrations of 0.5, 1.0, and 1.5 ppm, respectively, demonstrating an inverse association with concentration levels. Furthermore, the emergence rate of beetles decreased as concentration increased. The emergence counts for the chitosan treatments at concentrations of 0.5, 1.0, and 1.5 ppm dropped to 133.11, 55.69, and 30.12 beetles, respectively, with the control treatment having the highest average emergence of 289.43 beetles. The larval stage duration showed a direct proportionality with chitosan concentrations, with the 1.5 ppm concentration marking the longest larval duration at 22.00 days, significantly surpassing all other concentrations. The control treatment recorded the shortest duration at 13.56 days, whereas 0.5 and 1.0 ppm concentrations resulted in duration of 17.19 and 19.66 days, respectively. For the pupal stage, significant differences were observed with increasing chitosan concentration; the control treatment displayed the shortest pupal duration at 4.33 days. A direct relationship was found between the concentrations and pupal stage duration, reducing the period to 6.00, 7.59, and 9.07 days for concentrations of 0.5, 1.0, and 1.5 ppm, respectively. Chitosan exhibited significant differences from the second day of the experiment, as the mortality rate increased with concentration and over time. The concentration of 1.5 ppm showed the highest mortality rate at 98.62% after 16 days, whereas the control treatment recorded the lowest rate at 33.17%. Based on the results of the current study, chitosan can be utilized as an effective pesticide for controlling stored-product pests, particularly the saw-toothed grain beetle.

KEYWORDS: Chitosan, concentrations, life cycle, rice, saw-toothed grain beetle

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INTRODUCTION

In tropical and subtropical regions, rice (*Oryza sativa*) is the staple food consumed by over half of the world's population (Al-Yunis *et al*., 1987). Due to its nutritional composition of proteins, vitamins, carbohydrates, and other elements including phenolic compounds, rice is not only necessary for existence but also offers several health benefits. These elements greatly reduce the incidence of cancer and chronic disorders and are essential for preserving good health (Halul, 2018). The saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae), poses a major risk to stored grain. This is a widespread insect that affects several grains, especially wheat, and often results in substantial weight loss (Pricket, 1990). Pest damage to stored grains and products derived from them affects between 10-40% of the world's grain stocks, with major economic consequences (Al-Hadidi *et al*., 2014). Chitin is the second most prevalent natural carbohydrate polymer after cellulose, derived from crustacean exoskeletons, as well as fungal and insect cell walls (Xia *et al*., 2011). This biopolymer is deacetylated, resulting in chitosan, chitin's most notable derivative, which dissolves in mildly acidic solutions (Jia & Xu, 2001). This adaptable material is used in a variety of research areas due to properties such as biodegradability, biocompatibility, and non-toxicity, as well as biological capabilities such as antibacterial, antioxidant, and cholesterol-lowering effects (Panith *et al*., 2016). Chitosan is largely derived from shrimp exoskeletons and crab shells, both of which are byproducts of the fishing and food industries (Younes & Rinaudo, 2015). The study by Sabbour & Abdel-Hakim (2018) highlighted the

role of nanochitosan in affecting and controlling the tortoise beetle *Cassida vittata* on sugar beet crops. Concentrations of 110 and 150 ppm resulted in a decrease in the oviposition rate to 22 and 3 eggs/female, respectively, as compared to the control, which had a rate of 266 eggs/female. Meanwhile, the study by Keshmar (2023) found that the addition of chitosan at levels of 1.0 and 2.0 mL/L recorded a mortality rate of *Callosobruchus maculatus* (Southern cowpea weevil) larvae of 61.66% and 72.5%, respectively. While, Hadi (2021) utilized chitosan-nanocoated ethanol extract at a concentration of 3430 ppm, which reduced the hatching rate of eggs of the khapra beetle (*Trogoderma granarium*) to 0%, whereas a concentration of 2401 ppm resulted in a hatching rate of 38.96%, compared with the control treatment that had a hatching rate of 86.66%. Our current study aims to extract chitin from local carp fish and convert it to chitosan using established chemical methods, evaluating its impact as an insecticide against the saw-toothed grain beetle. This is due to its cost-effectiveness, ease of use, and environmental friendliness, as an alternative to using carcinogenic and mutagenic chemical pesticides.

MATERIALS AND METHODS

Breeding of saw-toothed grain beetle (*O. surinamensis***)**

Saw-toothed grain beetles were collected from local amber rice seeds that were infested in some local market stores in the city of Kirkuk from 3 February 2024 to 6 February 2024. The infested seeds were placed in 60 ml Petri dishes, each containing 30 gm of seeds, and small holes were made in the lids of the Petri dishes. These were then placed in an incubator at a temperature of 30°C and a relative humidity of 70% to allow the insects to reproduce and lay eggs for two weeks. Adults were isolated to obtain first-generation insects (Kalaf *et al*., 2022).

Chitosan extraction

Carp fish scales were collected and dried at room temperature for seven days. They were washed with 99.9% absolute ethanol at a temperature of 50-60°C to sterilize and remove any strong odours and then dried for 24 hr at room temperature. The dried scales were ground using an Arzum brand blender made in Turkey. The powder was treated with 5% Hydrochloric Acid (HCl) at 25°C for 24 hr to remove Calcium Carbonate (CaCO₃). The powder was then filtered using filter paper and washed with distilled water. A deproteinization step was performed by treating the sample with 10% Sodium Hydroxide (NaOH) to remove protein components at 60°C for 24 hr. The samples underwent deacetylation through treatment with 50% sodium hydroxide (Haj *et al*., 2020).

PREPARATION OF CONCENTRATIONS

The base solution was prepared with a concentration of 1500 ppm of the chitosan used in the study by dissolving 0, 0.5, 1, and 1.5 ppm in a litre of distilled water. To achieve a homogenous solution and reduce sample agglomeration, a Silent Crusher Homogenizer–Heidolph at 10000 rpm was used for 10 min for each solution. A series of concentrations, 0, 0.5, 1, and 1.5 ppm, were prepared by diluting the base solution according to the equation $C_1V_1 = C_2V_2$.

Calculating the incubation period of eggs and the larval and pupal stages for the saw-toothed grain beetle

Broken rice seeds were chosen as the optimal medium for obtaining larvae and pupae. 30 gm of these seeds were put in Petri plates for each replicate, along with five pairs (5 males/5 females) of newly hatched beetles. The dishes were sealed with ventilation holes and put in an incubator at a temperature of 25 ± 2 °C and relative humidity of 70 ± 5 %. Daily observations included removing the beetles from the duplicates, discarding the eggs, and keeping just ten eggs in each dish. The eggs were returned to the incubator to examine the change in egg colour to determine the hatching time and the duration of the larval and pupal phases.

Calculating the mortality percentage of adult saw-toothed grain beetles

In the described study, chitosan-treated rice seeds were allocated into 300 ml glass bottles, measuring 30 gm per bottle, across three experimental replications, with an additional untreated control group for comparative analysis. Into each bottle, we introduced quintets of sexually dimorphic newly emerged beetles (comprising 5 males and 5 females). These containers were then sealed with muslin cloth, and fastened securely with rubber bands to allow for aeration while preventing insect escape. The bottles were placed into an incubator, maintaining a regulated climate of 25±2°C and a relative humidity threshold of 70±5%. Biannually, over 16 days, we meticulously sorted and enumerated the expired specimens in each replicate to assess their survival rates. The percentage of mortality was systematically calculated using a predefined equation (Hill, 2002).

Percentage of mortality = (Number of dead insects/Total number of insects) x %100

Calculating the percentage loss in weight of seeds infested by the saw-toothed grain beetle

Healthy rice seeds treated with chitosan were placed in 300 ml glass bottles, at 30 gm per bottle for three replicates, along with a control treatment. Five pairs of newly hatched beetles (5 males/5 females) were added to the bottles. The

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bottles were incubated at 25±2°C and 70±5% relative humidity after being covered with muslin fabric and fastened with rubber bands. The glass bottles for the treatments were compared against the control bottles, and the percentage loss in their weight was calculated at intervals of 7, 14, 21, 28, 35, 42, and 49 days using the following equations (Keita *et al*., 2001):

Percentage loss in seed weight $=$ (Final weight - Initial weight)/Initial weight) x %100

Testing the attractant and repellent effect of chitosan on adult saw-toothed grain beetles

A locally manufactured chemotaxis device, based on the design of a Chemotropometer (Folsum, 1931), was used. The device consists of a wooden box measuring 48 cm in length and 20 cm in width with a movable cover that contains two opposing openings on the sides of the box to allow a tube to pass through. The tube is a glass cylinder, 100 cm in length and 2.5 cm in diameter, featuring an opening in the centre for the introduction of insects. The tube is graduated in centimetres. At the end of the tube, ground rice seeds treated with chitosan in previously selected concentrations were placed, while at the other end, rice seeds intended for control, soaked in distilled water, were placed. Ten insects (5 males/5 females) were introduced for each replicate, with three replicates in total. The number of insects that moved inside the tube and covered a distance of 25 cm towards the openings was recorded after 20 minutes of their introduction, and the percentage of attraction and repulsion was calculated using the following equations (Shaaban & Mallah, 1993):

Percentage of attraction $=$ (Number of insects that moved toward the tested material and crossed a distance of 25 cm from the centre/Total number of insects) x %100

Percentage of Repellency $=$ (Number of insects that moved away from the tested material and crossed a distance of 25 cm from the centre/Total number of insects) x %100

Equilibrium Percentage = Attraction Percentage - Repellency Percentage

* The positive value indicates that the material is attractant to the insects.

* The negative value indicates that the material is repellent to the insects.

STATISTICAL ANALYSIS

Using SAS software version 9, the findings were statistically evaluated as a factorial experiment by a Completely Randomized Design (CRD). The Least Significant Difference test (LSD) was used to compare means between multiple groups. It calculates the minimum detectable difference between means to determine the extent of difference between them.

RESULTS AND DISCUSSION

The effect of chitosan on the productivity of the sawtoothed grain beetle (*O. surinamensis* **L.)**

From the results presented in Table 1, it is observed that chitosan significantly affected the oviposition rate, as there was a decrease in the number of eggs laid with an increase in chitosan concentration. The control treatment recorded the highest egg-laying rate with an average of 314.14 eggs, compared with the chitosan treatments at 0.5, 1.0, and 1.5 ppm, which showed averages of 209.31, 117.03, and 61.12 eggs, respectively. A direct correlation is noted between the levels of chitosan application and the oviposition rate. This could be due to chemical alterations in the enzyme acetylcholinesterase, leading to its inhibition, which negatively impacts the overall activity of the insect and consequently reduces its egg-laying capacity (Rajkumar *et al*., 2020). Alternatively, it could be attributed to the chitosan possibly containing sterilizing agents such as metepa-p32, c4-tepa, Apholate, and Fluorouracil, which reflected in the decreased egg numbers or negatively influenced the feeding of the adults by affecting the primary food reserve necessary for egg formation, resulting in a reduction. These findings align with those found by Sabbour & Abdel-Hakim (2018), who indicated the role of nanochitosan in affecting and controlling the tortoise beetle, *C. vittata*, on sugar beet crops. Here, concentrations of 110 and 150 ppm resulted in a decrease in the oviposition rate, with averages of 22 and 3 eggs per female, respectively, for the aforementioned concentrations, compared to the control which recorded an average of 266 eggs per female. Table 1, shows that the percentage of emerged insects decreased with increasing concentrations of chitosan, with the control treatment yielding the highest average of 289.43 insects, while the number of emerged insects decreased to 133.11, 55.69, and 30.12 for the chitosan treatments at concentrations of 0.5, 1.0, and 1.5 ppm, respectively. This decline upon increasing its concentration could be due to its effect on the egglaying process, which affects the number of emerged insects. Furthermore, a thin layer may form on the eggshell, preventing respiration, or an increase in the entry of toxic compounds into it, impeding embryo formation. These findings corroborate those of Hadi (2021), who observed that the khapra beetle (*T. granarium*) hatched at a rate of 0% when ethanol extract coated with nanochitosan was used at a concentration of 3430 ppm, and a concentration of 2401 ppm, the rate was 38.96%, as opposed to 86.66% for the

control treatment. Productivity likewise declined noticeably as chitosan concentration increased; the control treatment showed the maximum productivity rate of 92.13%, which dropped to 63.59, 47.59, and 49.28% at 0.5, 1.0, and 1.5 ppm, respectively. The reason for this decrease might be due to the effect of this material on the egg-laying process and the number of emerged insects, which subsequently reflected in productivity as it is the result of the difference between the number of eggs and the number of emerged insects. On the other hand, it is an organic polymer known for its efficiency in delivering toxic chemical compounds, which work on penetrating the eggshell and thereby paralyzing and eventually killing the embryo (Athanassiou *et al*., 2017).

The effect of chitosan on the egg-laying period of the sawtoothed grain beetle (*O. surinamensis)*

Table 2, demonstrates the effect of chitosan on the incubation period of eggs. It is observed that the control treatment recorded the shortest incubation period with an average of 8.17 days, while the chitosan treatment showed a significant superiority with incubation periods of 9.66, 11.69, and 14.00 days for concentrations of 0.5, 1.0, and 1.5 ppm, respectively. A direct relationship between the incubation period and the concentrations was noted, which might be attributed to the elongation of the incubation period due to its role in the biological aspects of the insect. The polymeric materials form a thin layer on the eggshells, preventing them from breathing or increasing the penetration of toxic compounds inside the egg and hindering embryo formation.

The impact of chitosan on the larval stage duration of the saw-toothed grain beetle (*O. surinamensis)*

Table 3 illustrates that the larval stage duration is directly proportional to chitosan concentrations, with the concentration of 1.5 ppm recording the longest period of 22.00 days, significantly surpassing all other concentrations. Conversely, the control treatment registered the shortest duration of 13.56 days, while the concentrations of 0.5 and 1.0 ppm recorded periods of

17.19 and 19.66 days, respectively. The prolonged duration could be attributed to the systemic nature of chitosan and its high efficacy in combating the saw-toothed grain beetle, negatively affecting their development and the growth rate of the larvae within (Zhang *et al*., 2013). Chitosan possesses chemical compounds that cause hormonal disruption leading to an extended larval stage duration by inhibiting the formation of the molting hormone (Ecdyson Hormone) (Campos *et al*., 2018). In addition, it plays a role in extending the egg incubation period subsequently elongating the duration. These results are in agreement with Keshmar (2023), which found that the addition of chitosan at levels of 1.0 and 2.0 ml/l resulted in mortality rates of the larvae of the southern cowpea weevil *C. maculatus* of 61.66% and 72.50%, respectively.

The effect of chitosan on the pupal stage duration of the saw-toothed grain beetle (*O. surinamensis)*

Table 4 shows significant differences when increasing the concentration of chitosan, as the control treatment gave the shortest pupal stage period of 4.33 days. A direct relationship was found between the concentrations and the duration of the mentioned stage, as the periods decreased to 6.00, 7.59,

Table 1. The effect of chitosan on the productivity of the saw-toothed grain beetle(*O. surinamensis*)

Table 2. The effect of chitosan on the egg incubation period of the saw-toothed grain beetle (*O. surinamensis*)

Chitosan Concentration ppm	Egg incubation period/days				
	8.17 d				
0.5	9.66c				
	11.69 _b				
1.5	14.00a				
L.S.D 5%	0.3533				
Similar letters in the same row or column indicate no significant differences at the probability level $p < 0.05$					

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and 9.07 days for concentrations of 0.5, 1.0, and 1.5 ppm, respectively. This could be attributed to the fact that these polymers possess effective conduction advantages, causing an increase in the surface area in contact and enabling them to deliver a greater quantity of toxic chemicals in the required amounts and release them in small quantities over long periods, leading to the demise of the targeted pest (Ghormade *et al*., 2011; Hayles *et al*., 2017). Alternatively, it may be due to their effects on the growth and cell divisions of the insect, and consequently on the time required for transformation from the pupal stage to the adult insect. These results are consistent with Hamid (2023), who found that increasing the addition of chitosan prolonged the period of the pupal stage for the housefly *Musca domestica*.

The effect of chitosan on mortality of the adult sawtoothed grain beetles (*O. surinamensis)*

The mortality percentage of the saw-toothed grain beetle adults was calculated for different concentrations of chitosan starting from the second day of the experiment. It is observed from Table 5, that there were significant differences from that day, as the mortality rate increased with the increase in chitosan concentration and further increased over the days. The concentration of 1.5 ppm gave the highest mortality rate, reaching 22.10%, 34.00%, 51.41%, 69.56%, 78.02%, 87.54%, 94.12%, and 98.62% after 2, 4, 6, 8, 10, 12, 14, and 16 days, respectively. Meanwhile, the control treatment recorded the lowest mortality rate, reaching 0.00%, 2.76%, 5.98%, 9.08%, 16.00%, 22.66%, 27.75%, and 33.17% for the aforementioned days, respectively. This might be due to its ability to penetrate the hard outer cuticle layer and the flexible areas through the respiratory openings (Stigmata) to reach cell tissues, as well as its effect on the nervous and digestive systems of the hairy grain beetle, causing its death. On the other hand, the effect in killing adults might be explained by its possession of functional chemical groups, most importantly organic acids and phosphorus compounds, which are distinguished by their high capacity to kill insects (Saharan *et al*., 2015). These results are in agreement with Rajkumar *et al*. (2020), who found that chitosan had a lethal effect on the rice weevil *Sitophilus oryzae* and the red flour beetle *Tribolium castaneum*. Similarly, these results align with Zhang *et al*. (2013) in their study assessing the efficacy of chitosan on the diamondback moth *Plutella xylostella* and the tomato fruitworm *Helicoverpa armigera*, achieving mortality rates of 72% and 40% within 24 and 72 hr, respectively.

The effect of chitosan on the percentage of weight loss in grains infested by saw-toothed grain beetle (*O. surinamensis)*

The results presented in Table 6, indicate that chitosan treatment significantly affected the percentage of weight loss in grains, where an inverse relationship was found between the concentrations and percentage loss; as the percentage increased over time. The control treatment recorded the highest rates of 4.01, 4.55, 5.02, 5.41, 5.75, and 6.09%, after 7, 14, 21, 28, 35, 42, and 49 days, respectively. These values decreased with increasing concentrations until they reached the lowest percentage at 1.5 ppm, registering 0.53, 0.69, 0.87, 1.08, 1.20, 1.37 and 1.53% for the aforementioned days,

Table 3. The effect of chitosan on the larval stage period of the saw-toothed grain beetle (*O. surinamensis*)

Table 4. The effect of chitosan on the pupal stage duration of the saw-toothed grain beetle (*O. surinamensis*)

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Chitosan Concentration ppm	Mortality percentage / day							
	2	4	6	8	10	12	14	16
Ω	0.00 d	2.76d	5.98 d	9.08d	16.00 d	22.66 d	27.75d	33.17d
0.5	8.07c	21.12c	35.19c	46.01 c	54.37 c	66.91c	72.42 c	81.17c
	15.22 b	29.03 b	44.12 _b	62.00 b	72.77 _b	81.09 b	87.35 b	92.88 b
1.5	22.10a	34.00 a	51.41 a	69.56a	78.02 a	87.54 a	94.12 a	98.62 a
L.S.D 5%	1.2662	1.9105	0.8445	2.0109	1.4328	2.1467	2.0304	2.328
Similar letters in the same row or column indicate no significant differences at the probability level $p < 0.05$.								

Table 5. The effect of chitosan on the mortality percentage of adult saw-toothed grain beetles (*O. surinamensis*)

Table 6. The effect of chitosan on the loss percentage (%) in the weight of seeds infestedwith the saw-toothed grain beetle (*O. surinamensis*)

Chitosan Concentration ppm	Seed weight loss percentage/day							
	7	14	21	28	35	42	49	
θ	4.01a	4.55a	5.02a	5.41 a	5.75 a	6.09a	6.31 a	
0.5	2.11 _b	2.48 _b	2.67 _b	2.85 _b	3.01 _b	3.22 _b	3.54 b	
	1.09c	1.26c	1.39c	1.57c	1.78c	1.96c	2.15 c	
1.5	0.53d	0.69d	0.87d	1.08 _d	1.20d	1.37d	1.53 d	
L.S.D 5%	0.1044	0.1419	0.1354	0.186	0.1302	0.0888	0.0993	
Similar letters in the same row or column indicate no significant differences at the probability level $p < 0.05$.								

Table 7. The effect of chitosan on the attraction, repellent, and balancing for adult saw-toothed grain beetles (*Oryzaephilussurinamensis*)

respectively. The probable cause of this decrease could be due to its impact on the larval stages, which constitute one of the harmful phases of the insect's life cycle. Its effect on the seeds is not limited to the adults but also includes the larvae, and their feeding leads to poisoning and death, thereby reducing their numbers and consequently diminishing the weight loss percentages. This is to what was stated in Keshmar (2023) that chitosan is very effective against the larvae of the Southern cowpea weevil. Through our observation of the results from Tables 5 and 6, it appears that there is an inverse proportion between the adult mortality rate and the seed weight loss. This is attributed to the direct correlation between the concentrations and mortality rate; as the concentration increases, so does the mortality rate, which in turn leads to a decrease in the emerging insect population and the loss percentage. On the other hand, although the seeds are dry, they contain a moisture content ranging between 15-20% (Sahuki, 1990). The reason for the decreased weight loss percentage might be that spraying chitosan on seed husks reduces weight loss by minimizing the water evaporation process from their surfaces. The weight loss can also be attributed to water loss due to evaporation in addition to the consumption of food reserves through respiration. It acts as a barrier that reduces water loss and provides protection to the outer surface of the seeds from mechanical injuries, as well as sealing minor scratches, thereby maintaining their moisture (Ribeiro *et al.,* 2007).These results agreed with Taain *et al*. (2019) who found that adding it at a concentration of 2% resulted in the lowest percentage decrease at (0.63-0.64)% for the agricultural seasons of 2014 and 2015, respectively, for date palm fruits infested with the scale beetle (*Phoenix dactylifera*), while the control treatment recorded a decreasing percentage of 1.15 and 1.10 for the respective seasons.

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The effect of chitosan on the attraction, repellency, and balancing for adult saw-toothed grain beetles (*O. surinamensis***)**

The results of Table 7, show that the attraction rate increased with increasing chitosan concentration, with concentrations of 0.5, 1.0, and 1.5% recording attraction rates of 36.09, 43.27, and 51.00%, respectively. As for the balancing effect, positive results were recorded, and the attracting effect (Attractant) was higher than the repelling effect (Repellent), with the 1.5% concentration registering the highest balancing rate at 13.05%. Overall, from our results, we notice chitosan has an attractive effect on the saw-toothed grain beetle, which could be due to the positive effects of organic polymers on the insect's sensory receptors, making it beautiful. These results are in line with Abid (2012), which found that adding a 5% hexane extract of fenugreek seeds gave an attraction rate of 90% for the hairy grain beetle (Khapra beetle). This indicates that materials of natural organic origin can protect stored grains or those treated with them.

CONCLUSION

The effect of chitosan on eggs experienced, where a thin layer on the eggshell prevents it from breathing and hinders embryo formation. The development and growth rate of the larvae and the pupal stage, was affected by chitosan. It's impact on the time required to transform from pupa to adult insect was also evidenced. As for its effect on adults, chitosan demonstrated its ability to penetrate the hard outer cuticle and flexible areas through respiratory openings, reaching body tissues and affecting both the nervous and digestive systems. A direct relationship was found between the concentration of chitosan and the mortality rate of the saw toothed grain beetle chitosan can also be used as an attractant for the saw-toothed grain beetle.

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