Research Paper

Extraction and evaluation of the effectiveness of chitosan as insecticides on the immature stages of the saw-toothed grain beetle oryzaephilus surinamensis l. (coleoptera: silvanidae)

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Article Info

Article History:
Received: 23 July 2024
Revised: 01 October 2024
Accepted: 08 October 2024
Published: 25 November 2024

Keywords:
Oryzaephilus Surinamensis
Chitosan
Insecticides
Rice
Grain
Saw-Toothed

ABSTRACT

This study aimed to collect samples of amber rice infected with the saw-toothed grain beetle from local markets in Kirkuk city during 13 December 2023 to 25 April 2024, and the experiments were conducted in the laboratories of the College of Education for Pure Sciences at Kirkuk University from February to April 2024. The toxic effects of chitosan were studied at three different concentrations: 0.5, 1.0, and 1.5 ppm on the immature stages of the saw-toothed grain beetle. The substance exhibited significant effects on the immature stages of the insect, recording the shortest egg incubation period with an average of 8.17 days, while chitosan treatment showed a significantly longer periods of 9.66, 11.69, and 14.00 days for concentrations of 0.5, 1.0, and 1.5 ppm respectively. The larval stage duration had a direct proportionality with the chitosan concentrations, where the 1.5 ppm concentration registered the longest larval period of 22.00 days, as opposed to the control which recorded the shortest period of 13.56 days, and the 0.5 and 1.0 ppm concentrations recorded durations of 17.19 and 19.66 days respectively. In the pupal stage, significant differences were found when the chitosan concentration was increased, with the control treatment having the shortest pupal stage duration of 4.33 days; a direct correlation was found between the concentrations and the pupal stage duration, which decreased to 6.00, 7.59, and 9.07 days for the 0.5, 1.0, and 1.5 ppm concentrations, respectively.

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1. INTRODUCTION

Rice, scientifically known as Oryza sativa, is a staple grain for the people of tropical and subtropical countries, serving as the basic food for more than half of the world's population [1]. Its nutritional composition, rich in proteins, vitamins, carbohydrates, essential nutrients, and phenolic compounds, plays an essential role in maintaining health and reducing the risks of chronic and cancerous diseases [2]. This crop is susceptible to a variety of damages and for a variety of causes, including contamination with Insect excrement, becausee the damage produced by insects as a consequence of their contamination outweighs the harms caused by direct feeding and Infestation destruction. As a result, its commercial worth and consumer appeal drop. This occurs through several primary methods, including the mixing of complete insect bodies or parts thereof, or of their various stages such as eggs, larvae, nymphs, and pupae with grains or their products, or exuviae, eggshells, cocoons, and the mingling of their feces with the grains, resulting in foul odors [3] Among the most widespread pests in the grain storage sector, the saw-toothed grain beetle (Oryzaephilus surinamensis), which belongs to the Silvanidae family of flat bark beetles and the Coleoptera order, poses a global threat, causing significant damage to a variety of grain crops, including notable weight loss due to infestation [4]. Insect pest infestations in stored grains lead to considerable economic consequences, with losses estimated to range from 10-40% of global grain reserves [5]. Furthermore, flour and stored pharmaceuticals are afflicted, and adult insects and larvae can be found in all types of grains that were previously impacted by other pests and were stored poorly. It has also been observed that this insect likes packaged items for direct eating [6]. Chitin is the second-most plentiful polysaccharide in nature after cellulose, sourced from the exoskeletons of crustaceans, and the cell walls of fungi and insects [7]. Chitosan, the main derivative of chitin, is produced by deacetylating chitin. As a biopolymer in the form of a dilute acidic solution, it readily dissolves in various substances [8]. Its properties include biodegradability, biocompatibility, and non-toxicity, allowing chitosan to have broad applications in various fields of scientific research. Additionally, it possesses useful biological characteristics, such as antimicrobial, antioxidative, and cholesterol-lowering properties [9]. Notably, the chitosan is primarily obtained from the exoskeletons of shrimp and crabs [10]. Every year, 8 x 10% tons of waste are generated, with exoskeletons containing 15-40% chitin accounting for 40% of that. However, its limited supply owing to seasonal changes, along with high demand due to its various applications, has prompted the quest for alternative sustainable sources. Insects, for example, are seen to be a potential and sustainable supply of chitin and chitosan [11]. Our present research intends to assess the impact of chitosan as an insecticide on the juvenile stages of the saw-toothed grain beetle, as an alternative to chemical pesticides that pollute the environment.

2. RELATED WORK

A study [12] reported that the use of nanochitosan had an effect on pest control, particularly in targeting the tortoise beetle (Cassida vittata), where its impact was observed in reducing the egg-laying rate on sugar beet crops. Concentrations of 110 and 150 parts per million resulted in reduced egg-laying rates to 22 and 3 eggs/female, respectively, compared to the control sample rate of 266 eggs/female. In a related context, another study [13] revealed the high efficacy of chitosan on the larvae of the southern cowpea weevil (Callosobruchus maculatus), where the addition of chitosan at levels of 1.0 and 2.0 ml per liter resulted in mortality rates of 61.66% and 72.50%, respectively. Meanwhile, [14] indicated the effectiveness of ethanol extract coated with nanochitosan, explaining that its use at a concentration of 3430 parts per million completely eradicated the hatching of eggs of the khapra beetle (Trogoderma granarium), with a hatching rate of 0%. In contrast, the lower concentration of 2401 parts per million achieved a hatching rate of 38.96%, compared to the control treatment, which resulted in an egg hatching rate of 86.66%. [15] observed that raising the concentration of chitosan lengthened the pupal stage of the common housefly, Musca domestica.[16] discovered that injecting it at a concentration of 2% resulted in the lowest rate of decrease (0.63 - 0.64) % for date fruits infected by the date palm beetle, phoenix dactylifera, in the agricultural seasons of 2014 and 2015, respectively. In comparison, the control treatment showed a decrease rate of 1.15 and 1.10 for the two seasons, respectively. [17] Discovered that chitosan killed both the rice weevil, Sitophilus oryzae, and the red flour beetle, Tribolium castaneum. [18] In their study evaluating the effectiveness of chitosan against the diamondback moth, Plutella xylostella, and the tomato fruit worm, Helicoverpa armigera, observed mortality rates of 72 and 40% within 24 and 72 hours, respectively.

3. METHODOLOGY

3.1 Breeding the Saw-Toothed Grain Beetle

Saw-toothed grain beetles were collected from locally infected amber rice seeds from some local market stores in the city of Kirkuk during the period between 3 February 2024, and 6 February 2024. The infected seeds were then placed in 90 ml Petri dishes, each containing 60 g, and made small holes in the Petri dish lids. Then placed them in an incubator at a temperature of 30°C and a relative humidity of 70% to allow the insects to reproduce, lay eggs, for a period of two weeks. After that, the adults were isolated to obtain individuals of the first generation [19].

3.2 Chitosan Extraction

Carp fish scales were collected and left to dry at ambient temperature for 7 days, and then the scales were washed using 99.9% absolute ethanol at a temperature ranging between 50 °C and 60 °C to sterilize them and eliminate any foul odors. Subsequently, the scales were dried for 24 hours at ambient temperature and then it was ground using a Turkish - made Arzum blender. The resulting powder was treated with 5% hydrochloric acid (HCl) at 25 °C for 24 hours to remove calcium carbonate ($CaCO_3$), after which the powder was filtered using filter paper and washed with distilled water. The samples then underwent a 10% sodium hydroxide (NaOH) bath to eliminate the protein component at 60 degrees Celsius for 24 hours, and the process was concluded by treating the samples with 50% sodium hydroxide for de-acetylation (NaOH) [20].

3.3 Preparation of Chitosan Concentrations

The preparation of the basic solution involved a chitosan concentration of 1500 ppm for the substance under investigation. This was achieved by dissolving precise quantities of 0, 0.5, 1, and 1.5 ppm in one liter of distilled water. In order to ensure a uniform solution and to reduce the potential for sample clumping, each solution was subjected to a 10 - minute homogenization process utilizing a Silent Crusher Homogenizer - Heidolph at 10000 rpm. A gradient of concentrations at 0, 0.5, 1, and 1.5 ppm was meticulously prepared from the parent solution by employing the dilution formula $C_1V_1=C_2V_2$, to achieve the required dilutions.

3.4 Calculating the Incubation Period of the Eggs and the Larval and Pupal Stages for the Oryzaephilus Surinamensis Beetle

Crushed rice seeds were used as the preferred medium to obtain larvae and pupae. 30 grams of these seeds were placed in Petri dishes for each replicate. Into each dish, five freshly hatched pairs (5 males / 5 females) were introduced, and then the dishes were sealed with ventilation holes made in their lids. The dishes were placed in an incubator at a temperature of 25±2 °C with a humidity level of 70±5%, and monitored daily. Dead insects were removed from the replicates, and the eggs were also removed, leaving only 10 eggs per dish. The dishes were then returned to the incubator to observe changes in the color of the eggs and to study the time period for hatching, as well as the duration of the larval and pupal development.

3.5 Calculating the Percentage of Weight Loss in Seeds Infested by the Oryzaephilus Surinamensis Beetle

Healthy rice seeds treated with chitosan were placed inside glass bottles, each with a capacity of 300 ml, at a rate of 30 g per bottle for three replicates, in addition to the control treatment which involved introducing five pairs of insects (5 males / 5 females) freshly hatched into the bottles. The bottles were covered with muslin cloth and tied with rubber bands, then incubated at a temperature of 25±2 °C and a relative humidity of 70±5%. The glass bottles for the controls were placed alongside those for the test group, and the percentage of weight loss was calculated at the intervals (7, 14, 21, 28, 35, 42, 49) days using the following equations [21]:

The percentage of seed weight loss = (Final Weight-Initial weight) / (Initial weight) x 100%.

4. RESULTS AND DISCUSSION

4.1 The Effect of Chitosan on the Egg-Laying Period of the Oryzaephilus Surinamensis Beetle

Table 1 elucidates the effects of chitosan on the incubation period of eggs. It is observed that the control treatment exhibited the shortest incubation duration, averaging 8.17 days. In contrast, the chitosan treatments demonstrated a statistically significant prolongation of incubation periods, recording values of 9.66, 11.69, and 14.00 days for concentrations of 0.5, 1.0, and 1.5 ppm, respectively. There is a direct correlation between the incubation duration and the concentrations utilized. This prolongation in incubation time can be attributed to the role of chitosan in the biological aspects of the insect's lifecycle. The polymer material forms a thin layer over the eggshell, which inhibits respiration, potentially enhances the penetration of toxic substances into the eggs, and prevents embryogenesis, consequently leading to an extended incubation period for the eggs.

Table 1. Shows the Effect of Chitosan during the Egg-Laying Period of the Oryzaephilus Surinamensis Beetle

Chitosan Concentrations Ppm	Incubation Period of Eggs / Days
0	8.17 d
0.5	9.66 c
1	11.69 b
1.5	14.00 a
L.S.D %5	0.3533
Similar letters in the same row or column indicate no significant differences at the probability level of p<0.05	

4.2 The Effect of Chitosan on the Larval Stage of the Oryzaephilus Surinamensis Beetle

Table 2 demonstrates that the larval stage duration of the saw-toothed grain beetle is directly proportional to the concentrations of chitosan applied. The concentration of 1.5 ppm registered the longest larval stage, totaling 22.00 days, and exhibited significant superiority over the other concentrations. Meanwhile, the control treatment recorded the lowest duration of 13.56 days, while concentrations of 0.5 and 1.0 ppm noted larval durations of 17.19 and 19.66 days, respectively. The extension of this stage may be attributed to chitosan's systemic properties and its high efficacy in combating the saw-toothed grain beetle, which has had a detrimental effect on their development and growth rate of the larvae [18]. Chitosan also possesses chemical compounds that induce hormonal disturbances, leading to an increase in the larval phase duration such as its capability to inhibit the synthesis of the ecdysone hormone [22]. Furthermore, its role in extending the egg incubation period subsequently results in a protracted larval stage. These findings are consistent with [13], which reported that the addition of chitosan at levels of 1.0 and 2.0 ml per liter recorded mortality rates of larvae of the southern cowpea beetle, Callosobruchus maculatus, at 61.66% and 72.50% respectively.

Table 2. The Effect of Chitosan during the Larval Stage of the Oryzaephilus Surinamensis Beetle

Chitosan Concentration Ppm	Metamorphosis Stage/Days
0	13.56 d
0.5	17.19 c
0	19.66 b
1.5	22.00 a
L.S.D %5	0.748
Similar letters in the same row or column indicate no significant difference at a probability level of p<0.05	

4.3 The Effect of Chitosan on the Pupal Stage of the Oryzaephilus Surinamensis Beetle

Table 3 exhibits significant differences when increasing the concentration of chitosan, with the control treatment presenting the shortest pupal stage at 4.33 days. A positive correlation was detected between the concentrations and the pupal stage duration, with the duration extending to 6.00, 7.59, and 9.07 days for concentrations of 0.5, 1.0, and 1.5% respectively. This lengthening may be due to the fact that these polymers possess efficient conduction properties, causing an increase in surface area contact which enables them to deliver a larger amount of toxic chemical substances in the required quantities, releasing them in small amounts over extended periods, thus leading to the eradication of the targeted pest [23], [24]. Alternatively, it could be related to their impact on the growth and cellular divisions of

The insect, thereby affecting the required time for transformation from the pupal to the adult stage. These results are congruent with the findings from [15], which found that increased addition of chitosan prolonged the pupal stage duration of the housefly, Musca domestica.

Table 3. The Effect of Chitosan on the Pre-Pupal Stage of the Oryzaephilus Surinamensis Beetle

Chitosan Concentrations Ppm	A Period of Probation/Recruitment Day.
0	4.33 d
0.5	6.00 c
1	7.59 b
1.5	9.07 a
L.S.D %5	0.2568
Similar letters in the same row or column indicate no significant difference at a probability level of p<0.05.	

4.4 The Effect of Chitosan on the Percentage of Weight Loss in Grains Infested with Oryzaephilus Surinamensis Beetle

The results of Table 4 indicate that the chitosan treatment significantly affected the percentage of weight loss in grains, with an inverse relationship found between the concentrations and the percentage of loss. The percentage increased over time, with the control treatment recording the highest rates at 4.01, 4.55, 5.02, 5.41, 5.75, 6.09, and 6.31% after 7, 14, 21, 28, 35, 42, and 49 days, respectively. Conversely, these values decreased with the increase in concentrations, reaching the lowest rate at 1.5 ppm, which was 0.53, 0.69, 0.87, 1.08, 1.20, 1.37, and 1.53 for the aforementioned days, respectively.

Table 4. Effect of Chitosan on the Percentage of Weight Loss (%) In Seeds Infested With the, Oryzaephilus Surinamensis Beetle

Chitosan Concentration Ppm	Percentage of Weight Loss (%) in Seeds/ Day						
	7	14	21	28	35	42	49
0	4.01 a	4.55 a	5.02 a	5.41 a	5.75 a	6.09 a	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	1.09 c	1.26 c	1.39 c	1.57 c	1.78 c	1.96 c	2.15 c
1.5	0.53 d	0.69 d	0.87 d	1.08 d	1.20 d	1.37 d	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 difference at a probability level of $p<0.05$.							

The potential reason for their reduction may be due to its effect on the larval stages, which are one of the harmful stages of the insect. The impact on the seeds is not limited to the adults only but also their larvae, and their feeding leads to their poisoning and death, hence the reduction in their numbers and the decrease in the loss rates in their weights. This aligns with what was illustrated by [13], stating that chitosan is very effective against the larvae of the southern pea beetle. Observing the results from this table, there is an inverse relationship between the killing rates in adults and the loss in seed weight, which can be attributed to the direct proportionality between the concentrations and the killing rates. The higher the concentration, the higher the killing rate, which in turn leads to a decrease in the number of emerging insects and the loss rate. On the other hand, although the seeds are dry, they contain a moisture level of 15-20% [25], and the reason for the decrease in loss rates in their weights could be that spraying chitosan on their shells reduces the loss in weights by reducing the evaporation process from their surfaces or it may also be due to water loss due to evaporation, in addition to the consumption of food storage through the respiration process. It serves as a barrier that reduces water loss and provides protection for the outer surfaces of the seeds from mechanical injuries, as well as sealing minor scratches in them, thereby maintaining their moisture [26]. These results agree with those of [16] , who found that its addition at a concentration of 2% resulted in the lowest decrease rate of (0.63 – 0.64)% for the agricultural seasons of 2014 and 2015, respectively, for dates infested with the scale beetle phoenix dactylifera, while the control treatment recorded a decrease rate of 1.15 and 1.10 for the seasons, respectively.

5. CONCLUSIONS

Based on the findings of the current study, we infer that chitosan has a substantial effect on eggs, by forming a thin layer on the eggshells that prevents them from breathing and hinders embryo development. In terms of the larval stage, chitosan shown excellent efficacy in repelling the saw-toothed grain beetle, which hampered larval development and growth. Meanwhile, the effect of chitosan on the pupal stage was detected in its impact on the insect's development and cell divisions, changing the time necessary to transition from the pupal stage to an adult insect.

Acknowledgments

The authors have no specific acknowledgments to make for this research.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Asmaa Mahmoud Jamal	✓	✓	✓	✓		✓		✓	✓	✓	✓			✓
Adel Ali Haidar	✓	✓		✓		✓		✓	✓	✓	✓			
Mohsen Omar Mohammed	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓		

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to Cite: Asmaa Mahmoud Jamal, Adel Ali Haidar, Mohsen Omar Mohammed. (2024). Extraction and evaluation of the effectiveness of chitosan as insecticides on the immature stages of the saw-toothed grain beetle oryzaephilus surinamensis l. (coleoptera: silvanidae). International Journal of Agriculture and Animal Production (IJAAP), 4(2), 126–134. https://doi.org/10.55529/ijaap.46.16.24

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