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Original Article

Evaluation of the Efficacy of Chitosan Extracted from Shrimp Against the Moth *Ephestia cautella*

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Abstract

To control the date moth on a domestic scale in 2025-2026, this study was conducted. It analyzed the biological efficacy and physiological symptoms of chitosan (from shrimp shells), applying two methods: immersion and through the nutritional food, and at three concentrations (0.1%, 0.25%, 5.0%).

Efficacy was demonstrated in a concentration-dependent manner in the results. was caused by damage to their cuticle and loss of moisture to the body in direct contact application, whereas feeding inhibition and chronic enterotoxemia occurred in a strong response after application through the food chain. Larvae in the early instar (second instar) were much more sensitive than larvae in the later instar (fourth instar) and were more resistant and stronger. Chitosan was effective as a growth regulator in the insects over 23 days; although the pupae were not immediately killed, growth arrest was seen, with a 40% emergence rate at higher concentrations. The 5.0% concentration completely eliminated the insect and showed 100% mortality of the adults within 72 h, which disrupted the reproductive cycle by preventing adult mating and oviposition. On the other hand, lower concentrations (0.25%) were chronic inhibitors through sterilization of the surviving insects. The inclusion of chitosan formulation during the post-harvest phase in the following stages of the management process is recommended by the study – the mixing of this compound directly into the stored commodities, and the application of chitosan at early larval phases – as an environmentally safe and eco-friendly solution for the sustainable protection of the crops during the post-harvest stage.

Keywords: *Ephestia cautella*, shrimp shells, dates, chitosan.

1-Introduction

Iraq is a major date producer, yielding approximately 450,000 metric tons annually, of which around 300,000 metric tons are exported. One of the key factors influencing the economics of the date industry in Iraq (1).

It is infested by stored-product insect pests (2), such as the moth (Ephestia cautella (Walker)) and the saw-toothed grain beetle (Oryzaephilus surinamensis).

1-1. Classification and Morphology

The insect belongs to the kingdom Animalia, phylum Arthropoda, class Insecta, order Lepidoptera, family Pyralidae, genus Cadra (or Ephestia), and species Ephestia cautella.

The insect is a small moth, approximately 6–8 mm in length with a wingspan of 10–15 mm. It is commonly referred to as the moth (3), Indian flour moth (4), or rice grain moth.

Adult Morph: The body and forewings are pale, clay-yellow to straw-colored, marked with small

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black dots. The gray hindwings are aggressively fringed with long hairs and sharply taper to a point (Picture 1).

Larval Morph: The larva is a minute, creamy-white caterpillar (about 8 mm long) with a heavily sclerotized brown head capsule. Its true thoracic and abdominal legs are drastically reduced, which serves as an evolutionary adaptation for an internal life cycle within grain kernels (Picture 2) (5).

Picture (1): Represents the adult stage of the moth (*Ephestia cautella*).

Picture (2): Represents the larval stage of the moth (*Ephestia cautella*).

Pupa: 7–8 mm long, yellow; the cocoon is 10–12 mm long and approximately 3.5 mm wide, dirty white in color.

Picture (3): Represents the pupa stage of the moth (*Ephestia cautella*).

1-2. Ecological and Economic Impacts

A. Advantages (Laboratory Context Only)

- **Biological Host Substitute:** is mass-reared in industrial entomological laboratories as a factitious host. Its mass-produced eggs serve as a cost-effective nutrition source for breeding beneficial parasitoids, such as *Trichogramma chilonis*, used in biological control programs (6).

B. Disadvantages and Damage Parameters

- **Agricultural Impact:** It functions as a primary pest capable of destroying 30% to 50% of total stored grain weight. Consuming the grain germ obliterates seed viability and germination capacity (7).
- **Livestock Impact:** Infested grains emit a repulsive, pungent odor due to accumulated larval frass, silk webbing, and cast pupal skins. This feed tends to be rejected by livestock, and forced intake can result in very severe gastrointestinal distress.
- **Human Impact:** It poses a threat to global food security and causes huge economic losses (8). Grinding grain contaminated with insects or bugs will not make flour. In addition, moisture, identified as a result of larval hyperventilation, may create hot spots that favor the development of carcinogenic and mycosis-producing molds. (such as *Aspergillus*).

Picture (4): Mating between adult insects is observed following treatment with chitosan.

1-3. Modern Integrated Pest Management (IPM) Strategies

1. Ozone Fumigation Technology: Recent trials with ozone under gaseous application at 2500 ppm (parts per 1000 ppm of air) in a completely enclosed silo show a 100% mortality rate in every insect egg and larval stage of the insects without affecting the nutritional or baking quality of the grain (9,10).

2. Botanical Biopesticides and Plant Oils: Adult knockdown and prevention of egg laying of rice are excellent when stored rice is treated with Neem oil (Azadirachta indica) or Pongamia oil at 5 mL/kg. Also, VOCs from botanical dusts, such as Dysphania ambrosioides, show high larvicidal activity (11).

3. Genetic Host Resistance: Wheat cultivars that are highly resistant to breaking the fruit wall with their hard pericarp (such as certain wheat varieties like PBW 373) fail to allow penetration of neonate larvae, increasing the grain's incubation time and hence reducing the possibility of population explosions.

4. Physical and Digital Trapping: New silos make the use of light traps based on the unique optical sensitivity of Gelechiidae moths possible by using energy-efficient LED lights, automatically providing early detection and monitoring.

5. Chemical Fumigation: Emergency used in severe outbreaks in industry, with strict hermetic sealing (12).

2- Materials and Methods

2-1. Insect Rearing and Sample Preparation

A-Stock Maintenance: The experiment studied in Controlled household conditions. A bulk quantity of host commodity (dates) was initially stored in a large plastic container in an airtight condition to ensure the container does not contaminate the host commodity.

B-Experimental Units: A smaller sub-population of the commodity was taken out and evenly spread across small plastic bioassay cups for the bioassay. Every cup was fitted with a specially designed lid with a pattern of small holes for proper ventilation and to prevent the larvae from being suffocated.

C-Insect Introduction: Five uniform-sized larvae (V1) of the target pest were carefully introduced into each bioassay cup, and there were multiple replicates (n>4) were prepared for each experimental group to achieve statistical accuracy.

2-2. Chitosan Treatment Application Methods

Two different approaches with the shrimp-produced chitosan solution were tested in the bioassay under different concentrations:

A- Dietary Medium Treatment: The following is the procedure used to ensure thorough treatment of the host diet (dates) in the cups before larvae were introduced; the highest amount of chitosan solution applied each time is shown. It was designed this way to assess the stomach toxicity and feeding deterrence of this compound.

B- Direct Larval Treatment (Dipping Method): Five larvae were carefully picked out of each of the replicated cups and dipped for a few seconds directly into the chitosan solution employing a fine mesh strainer. Larvae were transferred directly from filter paper to the fully untreated food in cups and isolated to enable and assess the contact toxicity of the biopolymer via the larval cuticle.

C- Control Group: Replicated cups were not treated and/or dipped only in distilled water in order to observe the usual mortality and normal development of the larvae.

3- Results and Discussion

- **Immediate Toxicity Patterns:** During the initial 24 hours, the control group maintained 0% mortality. Contact treatment induced a faster immediate knockdown effect than dietary treatment, particularly at the 5.0% concentration, killing 80% of 2nd instars and 60% of 4th instars. This fast contact response suggests initial integument stress (13).
- **Delayed Stomach Toxicity:** Conversely, dietary treatments acted more slowly within the first 24–48 hours because they required an incubation and ingestion period. However, as shown in later stages, this dietary path acts as a chronic physiological disruptor rather than an immediate contact toxin, aligning with (14).

Table 1: Acute cumulative mortality percentage (%) of 2nd and 4th instar larvae under contact (direct) versus dietary application across time.

Exposure Period	Concentration	2nd Instar - Contact	2nd Instar - Dietary	4th Instar - Contact	4th Instar - Dietary
24 Hours	Control (Water + Acetic)	0%	0%	0%	0%
	Chitosan 0.1%	20%	20%	0%	0%
	Chitosan 0.25%	60%	0%	20%	0%
	Chitosan 5.0%	80%	20%	60%	20%
48 Hours	Control (Water + Acetic)	0%	0%	0%	0%
	Chitosan 0.1%	40%	20%	20%	20%
	Chitosan 0.25%	60%	20%	20%	0%
	Chitosan 5.0%	80%	40%	60%	20%
72 Hours	Control (Water + Acetic)	0%	0%	20%	20%
	Chitosan 0.1%	60%	40%	20%	20%
	Chitosan 0.25%	60%	20%	20%	0%
	Chitosan 5.0%	80%	40%	80%	20%

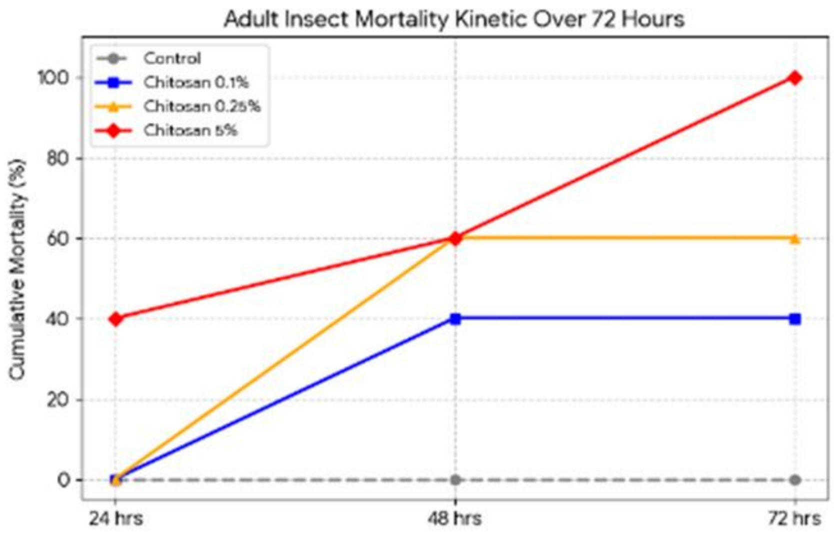

Figure 1: Comparison of treatment methods and the effect of concentrations over short-term exposure)

Table 2: Chronological development and long-term phenotypic mapping after 23 days of initial exposure.

Initial Form	Concentration	Status After 23 Days: Contact Treatment	Status After 23 Days: Dietary Treatment
2nd Instar	Control	40% Pupae, 40% Active Larvae, 20% Dead	60% Pupae, 40% Active Larvae
	Chitosan 0.1%	20% Pupae, 20% Larvae, 60% Dead	60% Dead (Delayed Cumulative Toxicity)
	Chitosan 0.25%	40% Pupae, 20% Larvae, 40% Dead	60% Pupae, 40% Dead
	Chitosan 5.0%	**100% Absolute Mortality**	60% Pupae, 40% Dead
4th Instar	Control	20% Pupae, 60% Copulating Adults, 20% Dead	20% Pupae, 60% Copulating Adults, 20% Dead
	Chitosan 0.1%	80% Emerged Adults (Deformed), 20% Dead	80% Emerged Adults, 20% Dead
	Chitosan 0.25%	80% Dormant Pupae (Developmental Arrest), 20% Dead	20% Larvae, 60% Pupae, 20% Adults
	Chitosan 5.0%	20% Emerged Adults, 80% Retarded Development/Dead	40% Pupae, 40% Adults, 20% Dead

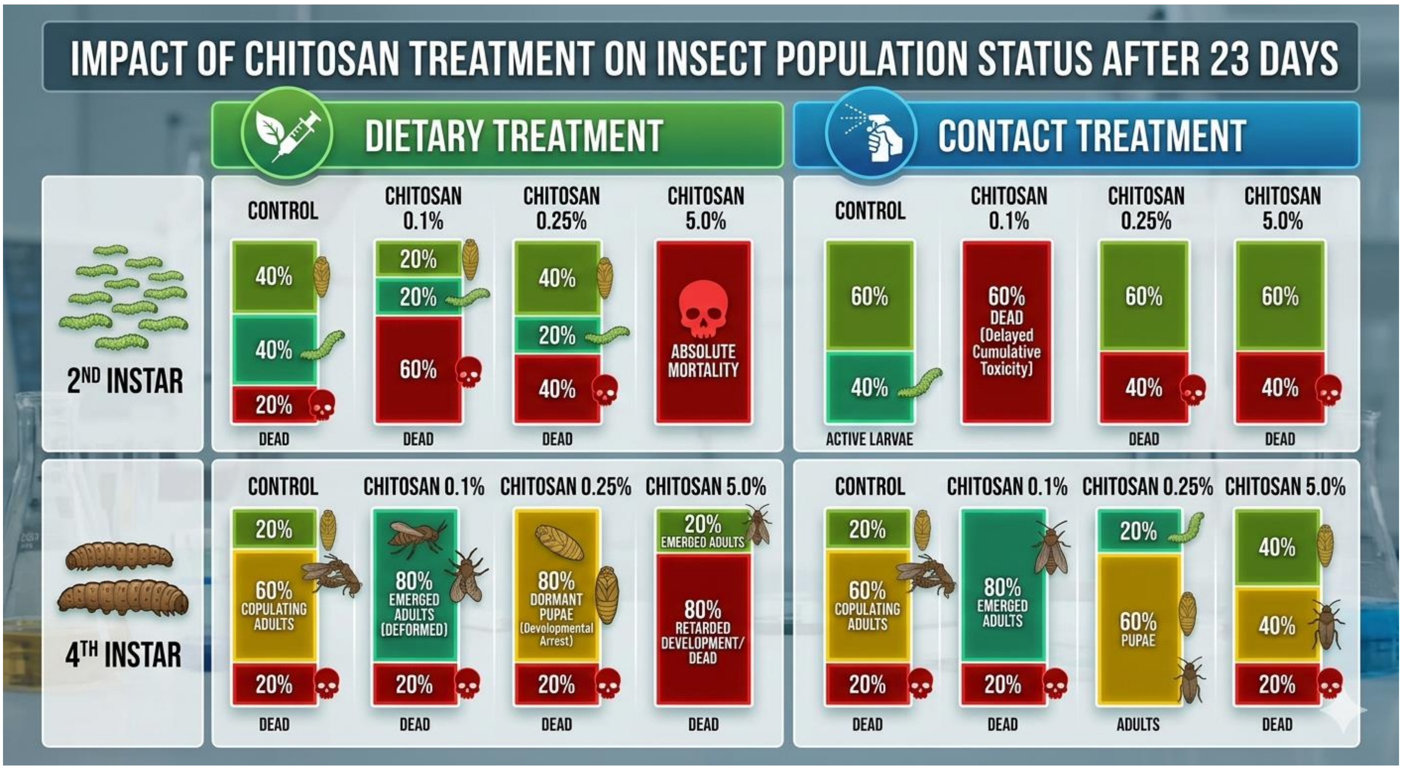

Figure 2: Observations after 23 days of exposure demonstrated that early developmental exposure significantly alters long-term larval phenotypic status and metamorphosis success.

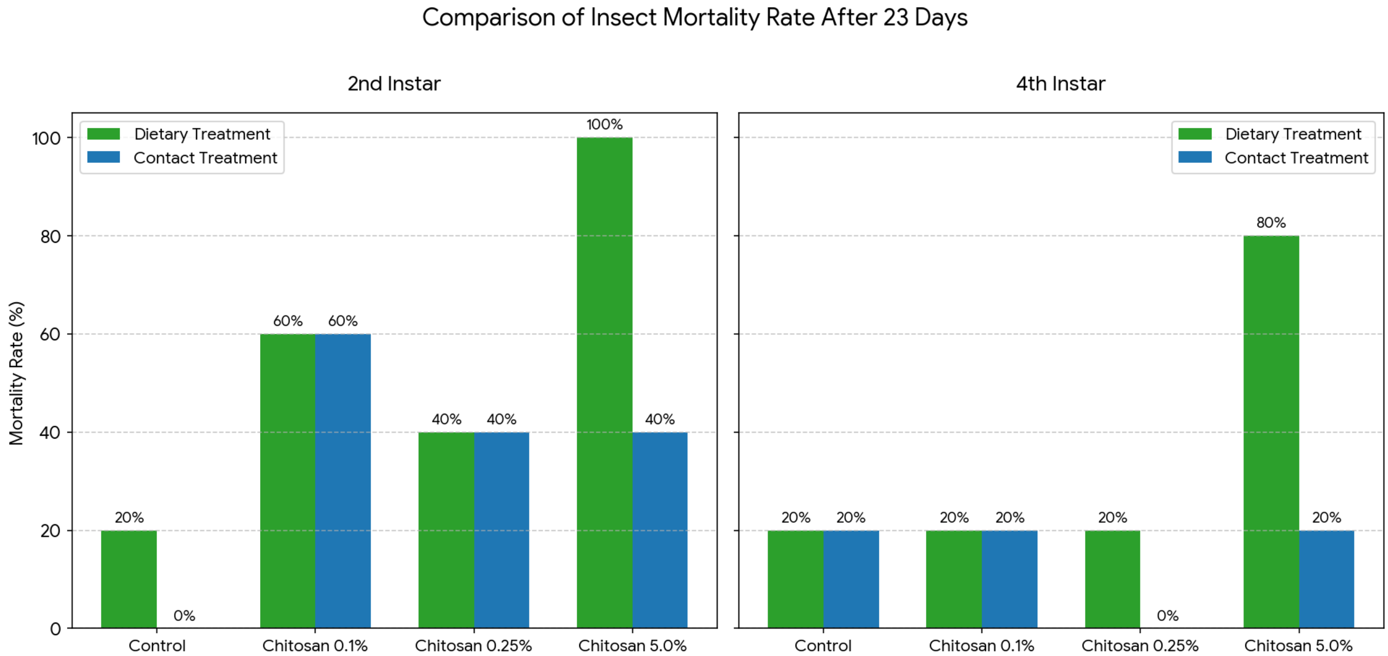

Figure 3: Comparison of insect mortality rate after 23 days

Figure 2 and **Figure 3** here: Long-term phenotypic distribution and developmental arrest mapping

- **Larval Instar Susceptibility:** 2nd instar larvae exhibited severe vulnerability compared to 4th instars. Contact treatment at 5.0% achieved 100% absolute mortality against 2nd instars. This sensitivity is linked to their thinner cuticle and underdeveloped digestive tract. In contrast, 4th instars showed high tolerance due to a thicker, sclerotized chitinous exoskeleton capable of restricting biopolymer penetration (15).
- **Developmental Arrest and Deformities:** Surviving 4th instars exposed to 0.25% and 5.0% chitosan exhibited significant developmental arrest (e.g., 80% dormant pupae) or emerged as deformed adults. This chronic disruption highlights the biopolymer's efficacy as an Insect Growth Regulator (IGR) that compromises subsequent generations (16).

Table 3: Susceptibility of the Pupal stage and adult emergence dynamics.

Concentration	Mortality (24–48 Hours)	Ultimate Biological Status After Several Days	Successful Emergence Rate (%)	Total Pupal Failure (%)
Control	0%	3 Normal Adults Emerged, 2 Dead Pupae	60%	40%
Chitosan 0.1%	0%	3 Normal Adults Emerged, 2 Dead Pupae	60%	40%
Chitosan 0.25%	0%	2 Normal Adults Emerged, 3 Dead Pupae	40%	60%
Chitosan 5.0%	0%	2 Normal Adults Emerged, 3 Dead Pupae	40%	60%

To evaluate the bio-delayed effect during metamorphosis, the transitional pupal stage was isolated and monitored.

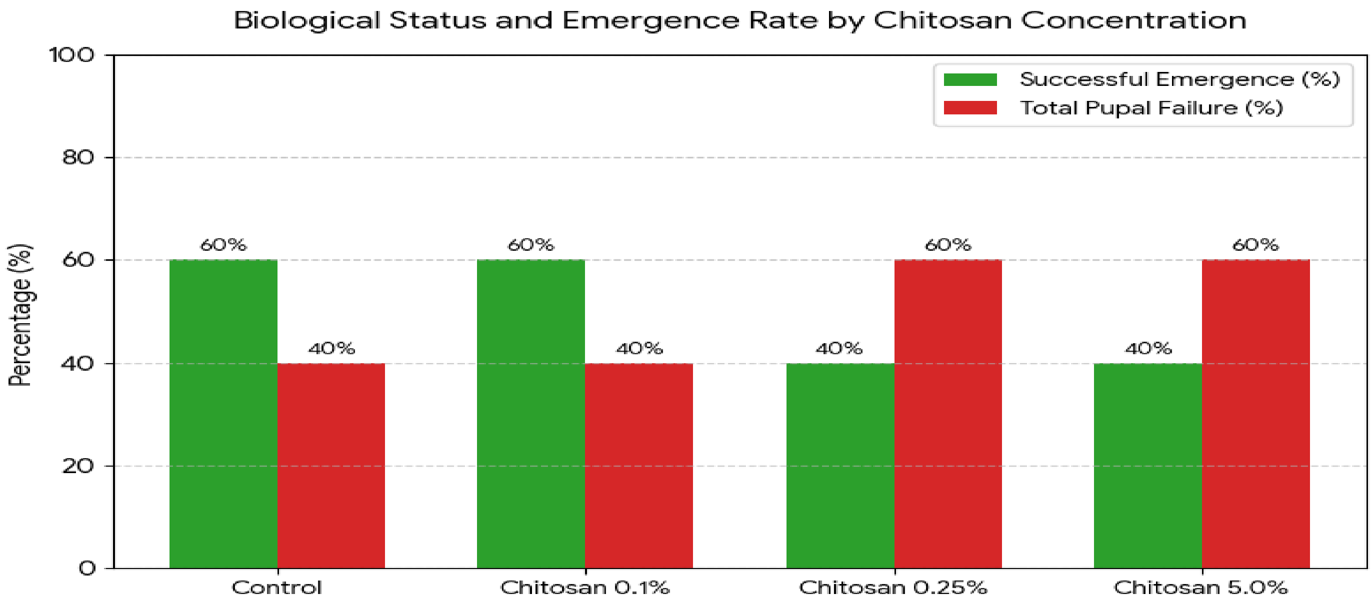

Figure 4: Correlation between Chitosan concentrations and adult emergence success

- **Absence of Acute Pupal Toxicity:** The results showed 0% mortality in all concentrations for the first 24-48 hours, indicating that chitosan does not have immediate chemical shock or knockdown effect on the protective pupal stage (17).
- **Metamorphosis Disruption:** There was a latent effect of the true insecticidal activity after some days. The use rates of 0.25% and 5.0% resulted in 40% and 60% adult emergence, respectively, and none at all emerged when no use was made. The results corroborate the study conducted (18), which reported that the higher the concentration of Chitosan, the lower the adult emergence of stored product pests.
- **Physiological Mechanism:** The molting enzymes (chitinases) are one of the natural factors that the insect needs for its exoskeleton renewal, and exogenous chitosan disrupts the equilibrium of molting enzymes. The resulting disruption causes severe morphological defects at ecdysis, physically fixing the insect in the cocoon, which results in pupal failure within the cocoon (19).

Table 4: Kinetic cumulative mortality percentage (%) of adult insects and reproductive behavior.

Concentration	24 Hours	48 Hours	72 Hours	Long-term Reproductive Impact
Control	0%	0%	0%	Normal copulation, egg laying, and successful progeny.
Chitosan 0.1%	0%	40%	40%	Copulation and delayed oviposition observed.
Chitosan 0.25%	0%	60%	60%	Severely restricted mating and minimal progeny.
Chitosan 5.0%	40%	60%	**100%**	**Total eradication. Copulation completely prevented.**

The post-emergence effect of chitosan showed that the biopolymer has a significant effect in decreasing the longevity of adults and making them incapable of reproduction in the long-term.

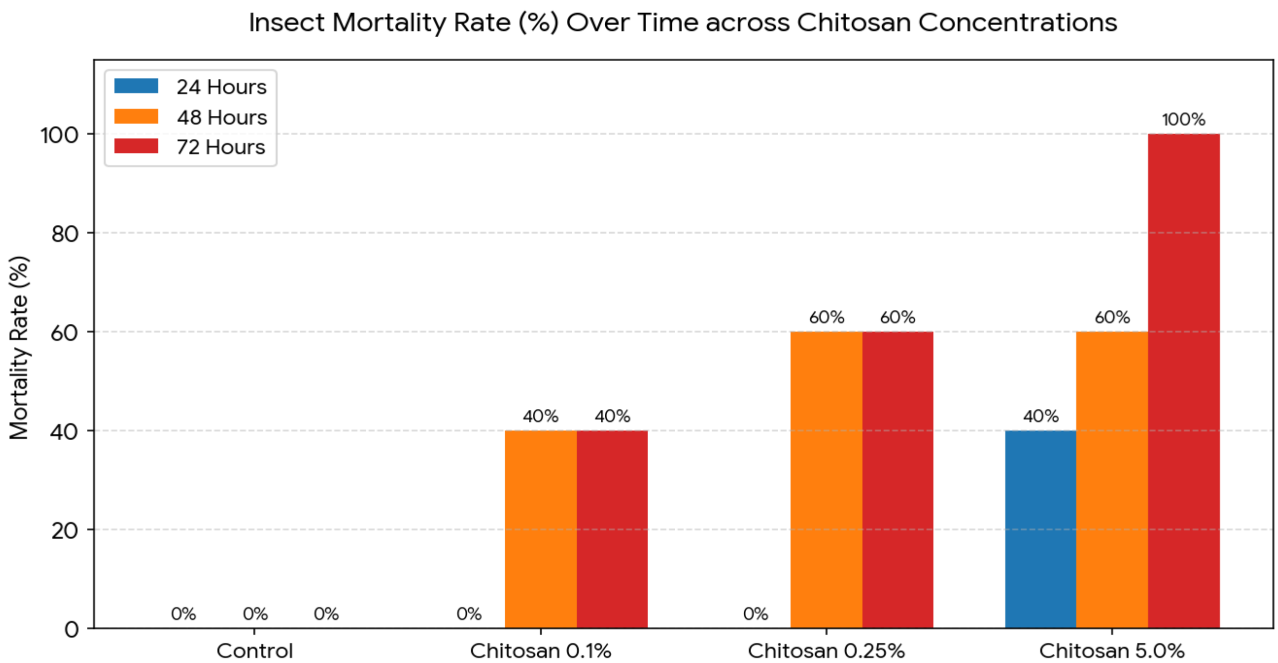

Figure 5: Insect mortality rate (%) over time across different chitosan concentrations compared to the control group

- **Adult Mortality Dynamics:** Overall, an adult response was observed over time that was evident in a clear concentration-dependency. The mortality rate of the control group was kept at 0% to validate the experimental setup. No immediate mortality was observed for 24 hours after exposure to lower concentrations (0.1% and 0.25%), but progressive mortality was observed at 48 hours, where 40% and 60% were obtained for the two lower concentrations, respectively. This is an internal physiological accumulation threshold to which the latency refers. The other concentration of 5.0% was acutely toxic, with 40% mortality at 24 hours and 100% absolute mortality at 72 hours.
- **Sublethal Reproductive Alterations:** In addition to death, sublethal exposures to chitosan caused significant damage to the reproductive organs of survivors. It caused delayed oviposition (egg-laying) and thereby delayed pest inv Jalal population increase in storage at 0.1%. Mating behavior was greatly reduced, if it occurred at all, at 0.25%, leading to low brood production. Total eradication of the population at 5.0% prevented any insects from mating, effectively destroying the insect's life cycle before the insects were able to be reproductive (20).

4. Statistical Interpretation and Comprehensive Discussion

The overall results show that the chitosan derived from shrimp skins is highly biopesticidal in nature and can be considered an environment-friendly agent for suppressing the stored-product pest (21).

. **Contact Toxicity vs. Dietary Stomach Toxicity**

One of the central conclusions of this research is that the two application techniques exhibited distinct behavioral and physiological differences:

- **Contact Action (Direct Larval Dipping):** Immediate knockdown effects were achieved by direct contact. The number of 2nd instar larvae killed, for example, by 5.0% of a contact treatment was 80% in 72 hours. From a physiological point of view, chitosan is a cationic poly glucosamine which can directly bind to the negatively charged epicuticle of insects. This physical interaction leads to rapid cellular dehydration, closure of the respiratory spiracles, and impedes mechanical mobility.
- **Dietary Treatment (Stomach Toxicity):** Application to the diet resulted in greater feeding deterrence activity (antifeedant response). Chronic failure increased during long-term metamorphosis, while larvae actively avoided consumption of treated medium, which extended their lives in short-term assays. When ingested late, it is a slow toxin working by interfering with the peritrophic membrane in the insect gut, and syncing up with (22).

Instar Susceptibility and Long-Term IGR Effects

- **Vulnerability Gradients:** The younger larvae2nd were much more vulnerable than the older 4th. This gap is related to the thickness of the integument: the early instars have thin, soft integuments that allow chemical penetration quickly, while the thick and heavily sclerotized integuments of 4th instars are a good physical barrier.
- **Insect Growth Regulator (IGR) Properties:** In our extended observation period of 23 days, chitosan was effective and acted as an IGR. In an 80% pupal arrest, sublethal exposures (0.25% contact) resulted in complete eradication of 2nd instars and blocked pupation completely (0%). This indicates that a sub-lethal dose level is sufficient to completely inhibit chitin synthesis, ecdysis, and the insect life cycle (23).

5. Conclusion

1. **Promising Biopesticide Candidate:** Chitosan, as excreted by crustaceans, is a highly interesting, environmentally friendly, and non-toxic biopesticide ideal for achieving all the requirements of Green Integrated Pest Management (IPM) in storage protection systems for stored grains and date commodities without

leaving harmful chemical residues in the stored grains.

2. **Dual Mechanism of Action:** There is a dual mode of action through direct contact with the external surface of the cuticle, leading to degradation and dehydration of the cuticle, plus chronic internal (dietary) starvation and strong feeding repellence upon ingestion of chitosan, which dominates.
3. **Insect Growth Regulator (IGR) Effects:** The results from this study agree with the action of chitosan as an Insect Growth Regulator (IGR). The protective cocoon protects the pupal stage from actual death, but sublethal exposure leads to a chronic disruption of metamorphosis, resulting in mortality as high as 40% of overall adults, and sterilizing the survivors so that they are unable to launch another generation of explosions.
4. **Concentration-Dependent Deployment:**

1. **High Concentration (5.0%):** Applies the best operating point for rapid elimination in 72-hours and provides adulticidal action, eradicates early larvae, and completely prohibits mating and oviposition.
2. **Low Concentrations (0.25%):** May be strategically used to establish long-term suppression of a population through reproductive arrest and/or delay of development.

5. **Strategic Recommendations:** In order for post-harvest control of this stored-product pest to be optimised, applications of chitosan should be made as a dietary medium treatment (dietary medium admixed to the stored commodity) rather than on the surfaces of the commodity. Also, early larval stages must be targeted for control to ensure the best opportunity for eradication before the pest enters more difficult life stages.

Conflict of interest: NIL

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