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Synergistic effect of silica or nanosilica and different temperatures conditions to control *Trogoderma granarium* (Coleoptera: Dermestidae) adults

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Abstract
This study assessed the mortality rate of the newly emerged adult of *Trogoderma granarium*. Adults were fed on wheat seeds treated with varying amounts of silica or nano silica (20, 40, 60, and 80 mg per 25 grams of wheat) under different temperature conditions (Cold, normal, and hot). The findings indicated that silica was most effective at cold temperatures, yielding the lowest LC_{50} value and highest toxicity index. Conversely, the least effective treatment, with the highest LC_{50} value and lowest toxicity index, was using silica at normal temperatures. Among the conditions tested, nano silica at hot temperatures proved the most effective, followed by cold and then normal temperatures. For silica, normal temperatures were most effective, followed by hot and cold temperatures. Biochemical analyses showed alterations in total protein, total lipid, carbohydrates, acetylcholinesterase (AchE), glutathione S-transferase (GST) and peroxidase in adults fed on wheat treated with LC_{50} silica or nano silica, which was clearer when using nano silica.

Keywords: Khapra beetle, nano silica, silica, temperature, control, biochemical studies

Introduction
Wheat (*Triticum aestivum*) is a crucial crop that provides essential nutrition for a significant portion of the global population, including in Egypt [1]. It constitutes approximately 20% of all agricultural imports and 10% of the total value of agricultural production. Therefore, it is important to focus on sustainable wheat production and quality improvement while minimizing the use of chemical pesticides [2, 3]. Insects pose a major challenge to stored grains worldwide, impacting food availability for many people [4].
Stored grains and other products are highly susceptible to pests like the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), particularly in tropical and subtropical regions [5-7]. The Khapra beetle is recognized as a global quarantine pest due to its aggressive feeding habits on stored grains. Its ability to develop insecticidal resistance and endure long periods without food makes it one of the most destructive pests for stored products, particularly wheat, where it can cause up to 30% postharvest losses [8].
Historically, synthetic chemical insecticides have been used to manage stored grain pests [9]. However, their excessive use has led to groundwater contamination, as well as the development of tolerance in insects[10]. Thus, there is a need for new control strategies. Nanoparticles are emerging as a promising solution for environmental remediation and could be used to develop new pesticides, insecticides, and repellents at a lower cost [11, 12].
The aim of this study was to evaluate the insecticidal effectiveness of silica and nano silica against *Trogoderma granarium* adults under various temperature conditions and to examine their impact on certain biochemical aspects.

Materials and Methods
Source of Strain
Mixed-age cultures of *Trogoderma granarium* (Khapra beetles) were obtained from Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt. These cultures were maintained on healthy wheat grains purchased from a local market. The beetles were reared in cylindrical glass jars covered with muslin cloth and secured with rubber bands. The rearing conditions included low light intensity, a temperature range of 25-32 °C, and a

relative humidity of 60-70%. The cultures were cleaned periodically [13]. To prepare adults for the bioassay, fifth instar larvae were collected from these cultures and kept in separate jars filled with wheat till mature for adults.

Silica and Nano Silica Particles Source

Silica and nano silica particles were procured from Naqaa Company, Cairo, Egypt. The particles were prepared following the protocol outlined by El-Didamonya *et al.* [14] and characterized using a Transmission Electron Microscope (TEM). The nanoparticles were observed at 180 kV using an AMT camera unit at the Electron Microscopy Unit, Al-Azhar University, Egypt.

Toxicological Study

The study evaluated the effects of various concentrations of silica and nano silica (20, 40, 60, and 80 mg) at different temperatures (7 °C, 25 °C, and 45 °C). Clean, un infested wheat (25 grams) was placed in small jars, to which each weight was added separately. Ten newly adults were introduced into each jar, which was then covered with muslin cloth to ensure ventilation. A control group was maintained with wheat but without any silica or nano silica. Each concentration was tested in triplicate, and jars were incubated at 28±2 °C with 70±2% relative humidity. Daily mortality counts were recorded for six days and adjusted using Abbott's formula [15]. The cumulative corrected mortality percentages after three days were plotted against the corresponding concentrations using probit software (LPD line) to determine the LC_{50} values.

Biochemical Assays

After three days of treatment with the LC_{50} concentrations of silica and nano silica, the adult were homogenized for biochemical analysis. Total proteins were measured using the Bradford method [16]. Total lipids were estimated according to Knight *et al.* [17]. Total carbohydrates were determined in the acid extract of the samples using the method by Dubois *et al*. [18]. Acetylcholinesterase (AchE) activity was assessed using the method described by Simpson *et al.* [19]. Glutathione S-transferase (GST) activity was detected as per the method outlined by Habig *et al.* [20]. Peroxidase activity was determined using the method by Vetter *et al.* [21].

Statistical Analysis

Data were analyzed using IBM SPSS software version 20.0 (Armonk, NY: IBM Corp). Descriptive statistics including mean and standard deviation were calculated. Significance was set at the 5% level. The student's t-test was employed to compare normally distributed quantitative variables between groups.

Results

Table (1) showed the means mortalities' percentage of the newly emerged adult of *T. granarium* for the 1st day fed on wheat seeds treated with 20, 40,60 and 80 concentrations of silica/25 gm wheat in normal temperature **(**25±2 °C) were 0, 33.3, 33.3 and 46.6% respectively. While for the 2nd day were 13.3, 73.3, 73.3 and 100%. On the 3rd day, the mortalities' percentages were 26.6, 73.3, 93.3 and 100 respectively. While on the 4th day they were 53.3,80, 100 and 100%, respectively with 20, 40, 60 and 80 mg nano silica/25 gm wheat. While on the 5th day the averages mortalities were 66.6, 93.3, 100 and 100% respectively. On 6th day and 7th days the mortalities were 100% at all applied silica concentration.

While when using nano silica, the mortality percentage the newly emerged adults of *T. granarium* for the 1st day fed on wheat seeds treated with 20, 40,60 and 80 concentrations of nano silica in normal temperature **(**25±2 °C) were 6.66, 33.3, 33.3 and 60% respectively. While for the 2nd day were 40, 60, 73.3 and 100% respectively. On the 3rd day the mean mortality percentages were 60, 86.6, 100 and 100%. While on the 4th day the averages were 60, 86.6, 100 and 100% and increased to 86.6, 93.3 100 and 100% at the 5th day with 20, 40, 60 and 80mg/25 gm wheat, respectively. On the 6th day the mortalities' percentages recorded 93.3, 100, 100 and 100% for 20, 40, 60 and 80 mg/25 gm wheat, respectively. Moreover, at the 7th days of the treatment the adult mortality with the treatment 20mg nano silica became100%.

Table 1: The mean mortality percentage of the newly emerged adults of *Trogoderma granaruim* fed on wheat seeds treated with different concentrations of silica or nano silica in normal temperature (25±2°C).

Weight (mg/25 gm wheat)	Silica						Nano silica					
	Corrected % mortality											
	1 day	2 days	3 days	4 days	5 days	6 days	1 day	2 days	3 days	4 days	5 days	6 days
0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	13.3	26.6	53.3	66.6	100	6.66	40	60	60	86.6	93.3
40	33.3	73.3	73.3	80	93.3	100	33.3	60	86.6	86.6	93.3	100
60	33.3	73.3	93.3	100	100	100	33.3	73.3	100	100	100	100
80	46.6	100	100	100	100	100	60	100	100	100	100	100

Values represent the mean of 3 replicates.

Data in Table (2) displayed that when the newly emerged adults of *T. granarium* feed on wheat treated with different weights of silica or nano silica, the data revealed that average mortalities increased with increasing the concentration of silica within 1 – 7 days of the experiment. The mortalities' percentages of adults exposed to 20, 40, 60, 80 mg of silica in hot temperature **(**35±2 °C) for one day were significantly increased from 13.3 to 33.3, 40 and 40, respectively. While, on the 2nd day the mortalities' percentages were 46.6, 80, 80 and 80 respectively. On the 3rd day the mortality percentages were 66.6, 86.6, 93.3 and 100 respectively, while on the 4th day the percentage were 80, 100, 100 and 100, respectively. On 5th day, 6th day and 7th days the mortalities were 100% at all applied silica concentration.

When using nano silica, the mean mortality percentage of. The mortality of the newly emerged adult of *T. granarium* adults for the 1st day fed on wheat seeds treated with 20, 40, 60 and 80 nano silica/25gm wheat in hot temperature (35±2 °C) were 13.3, 13.3, 26.6 and 26.6% respectively. While in

the 2nd day, the adult's mortalities recorded 40, 66.6, 73.3 and 73.3% respectively. The mortality was 73.3, 86.6, 86.6 and 93.3% in the 3rd day, while on the 4th day the mortality percentages were 86.6, 93.3, 100 and 100 respectively with 20, 40, 60 and 80 mg nano silica/25 gm wheat. Also, the 5th day showed increasing in the adults' mortalities that reached 93.3, 100, 100 and 100% for 20, 40, 60 and 80mg nano silica, respectively. The average mortality reached 100% the 6th day of application with 20 mg.

Table 2: The mean mortality percentage of newly emerged adults of *Trogoderma granaruim* fed on wheat seeds treated with different concentrations of silica or nano silica in hot temperature (35±2 °C).

Weight (mg/25 gm wheat)	Silica						Nano silica					
	Corrected % mortality											
	1 day	2 days	3 days	4 days	5 days	6 days	1 day	2 days	3 days	4 days	5 days	6 days
0	0	0	0	0	0	0	0	0	0	0	0	0
20	13.3	46.6	66.6	80	100	100	13.3	40	73.3	86.6	93.3	100
40	33.3	80	86.6	100	100	100	13.3	66.6	86.6	93.3	100	100
60	40	80	93.3	100	100	100	26.6	73.3	86.6	100	100	100
80	40	80	100	100	100	100	26.6	73.3	93.3	100	100	100

Values represent the mean of 3 replicates.

The obtained results in Table (3) clearly show that the effect of silica or nano silica on newly emerged adults' mortality at cold temperature was in parallel correlation with both silica weights (20, 40, 60 and 80 mg/25 gm wheat) and time of exposure. The results show that there was a significant effect of silica on adults' mortality at cold temperature. The mortalities of *T. granarium* adults at the 1st day fed on 25gm wheat seeds treated with 20, 40, 60 and 80 mg of silica in cold temperature (9±2 °C) were 13.3, 20, 33.3 and 33.3% respectively. While in the 2nd day, the adults' mortalities recorded 60, 60, 73.3 and 73.3% respectively. The mortalities were 73.3, 80, 80 and 93.3% in the 3rd day, while on the 4th day the mortality percentages were 80, 80, 80 and 100 respectively with 20, 40, 60 and 80mg silica/25gm wheat. Also, the 5th day showed an increasing in the adults' mortalities that being 86.6, 100, 100 and 100% respectively. The average mortality reached 100% in both 6th and 7th day of application at all concentrations.

The mortality percentages of adults exposed to 20, 40, 60, 80 mg of nano silica in cold temperature **(**9±2°C) for one day were increased from 0 to 6.6, 33.3, 33.3 and 53.3% respectively. While on the 2nd day the mortality percentages were 46.6, 66.6, 73.3 and 80% respectively. On the 3rd day the mortality percentages recorded 73.3, 93.3,100 and 100 respectively, while on the 4th day the percentage were 86.6,100,100 and 100 with 20, 40, 60, 80 mg of nano silica, respectively. On 5th day the mortality percentages reached 66.6 for 20 mg nano silica and 100 for the rest, while at the 6th it increased to 100%.

Table 3: The mean mortality percentage of the newly emerged adults of *Trogoderma granaruim* fed on wheat seeds treated with different concentrations of silica or nano silica in cold temperature (9±2°C).

Weight (mg/25 gm wheat)	Silica						Nano silica					
	Corrected % mortality											
	1 day	2 days	3 days	4 days	5 days	6 days	1 day	2 days	3 days	4 days	5 days	6 days
0	0	0	0	0	0	0	0	0	0	0	0	0
20	13.3	60	73.3	80	86.6	100	6.66	46.6	73.3	86.6	86.6	100
40	20	60	80	80	100	100	33.3	66.6	93.3	100	100	100
60	33.3	73.3	80	80	100	100	33.3	73.3	100	100	100	100
80	33.3	73.3	93.3	100	100	100	53.3	80	100	100	100	100

Values represent the mean of 3 replicates.

Table (4) and Fig. (1) exposed that LC_{50} values of silica against the new emerged adults of *T. granarium* in cold, hot and normal temperature were 5.786, 12.929 and 28.4mg, respectively. The results revealed that the adults were more resistant in normal temperature that showed the highest ratio 4.908. In contrast, the treatment in cold temperature caused the adults be more sensitive to the silica that showed toxicity index 100%.

While in nano silica against the newly emerged adult of *T. granarium* in hot, cold and normal temperature were 6.444, 12.836 and 17.89 mg, respectively. The treatment in hot temperature caused the adults be more sensitive to the nano silica that showed toxicity index 100%. Otherwise, the results revealed that the adults were more resistant in normal temperature that showed the highest ratio 2.776.

Table 4: LC50, LC90, toxicity index and resistance ratio slope of silica and nano silica on the newly emerged adult of *Trogoderma granaruim* in different temperature after 3 days.

Treatment	LC_{50} (mg/25 gm wheat)	Toxicity Index	Resistance Ratio	Slope	LC_{90} (mg/25m wheat)
Silica on adults in cold temperature	5.786	100	1	1.049	96.466
Nano silica on adults in hot temperature	6.444	89.789	1.114	1.287	63.828
Nano silica on adults in cold temperature	12.836	45.076	2.218	3.125	32.998
Silica on adults in hot temperature	12.929	44.752	2.235	2.118	52.085
Nano silica on adults in normal temperature	17.89	32.342	3.092	3.923	37.955
Silica on adults in normal temperature	28.4	20.373	4.908	4.347	55.996

Index and Resistance Ratio (RR) compared with silica on adult in cold temperature.

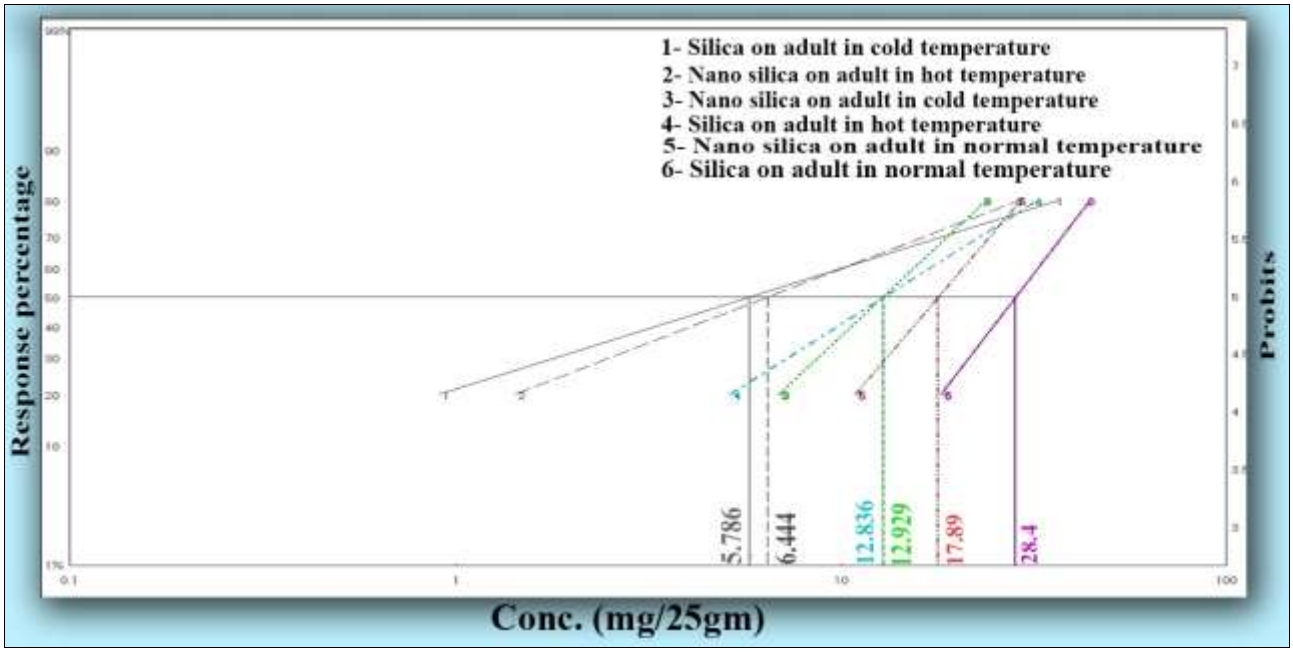
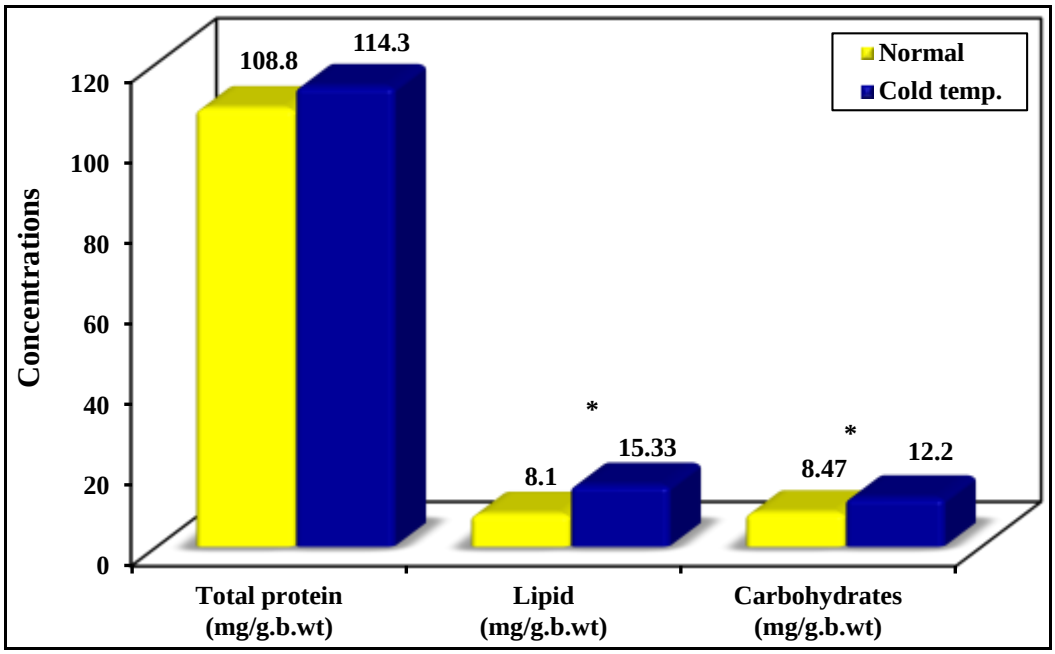


Fig 1: LC_{50} of silica or nano silica on *Trogoderma granaruim* adults in different temperature after 3 days of the treatments.

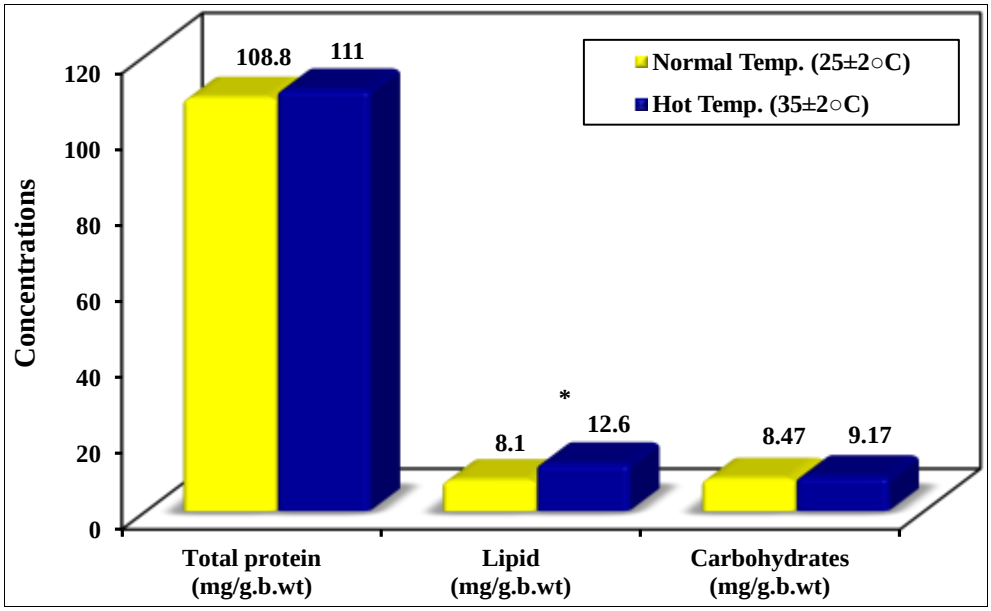
Data in Fig. (2) showed showed that comparison between treatment of the newly emerged adults of *T. granarium* with LC_{50} of silica in normal and cold temperature on total protein, total lipid and total carbohydrates respectively. The total protein exhibited a non-significant alteration in normal and cold temperature, where it recorded 108.8 and 114.3 mg/g.b.wt, respectively. While the total lipids were 8.10 mg/g.b.wt and 15.33 mg/g.b.wt and the total carbohydrates were 8.47 mg/g.b.wt and 12.20 mg/g.b.wt for Lc_{50} of silica in normal and cold temperature, respectively. Statistical analysis showed a significant raise in total lipids and carbohydrates for cold treatment.



Data was expressed using mean of 3 replicates.
*: Statistically significant at $p \leq 0.05$ (t-test).

Fig 2: Effect of LC_{50} silica on total proteins, total lipid and total carbohydrates concentrations in the newly emerged adults *Trogoderma granarium* in normal and cold temperature.

Results in fig. (3) demonstrated the comparison between treatment of the newly emerged adults of *T. granarium* with LC50 of nano silica in normal and hot temperature on total protein, total lipid and total carbohydrates. The total protein and total carbohydrates were non-significantly increase. The total protein was 108.8 mg/g.b.wt and 111.0 mg/g.b.wt and the total carbohydrates were 8.47 mg/g.b.wt and 9.17 mg/g.b.wt for Lc50 of nano silica in normal and hot temperature, respectively. Contrary, the total lipids revealed a significant elevation in hot temperature (8.10 mg/g.b.wt) than in normal temperature (12.60 mg/g.b.wt).



Data was expressed using Mean of 3 replicates.
*: Statistically significant at $p \leq 0.05$ (t-test).

Fig 3: Effect of LC_{50} nano silica on total proteins, total lipid and total carbohydrates of the newly emerged adults of *T. granarium* in normal and hot temperature.

Data in fig. (4) demonstrated that GST and peroxidase activities were significantly elevated in the newly emerged adults of *T. granarium* as a result of LC_{50} of silica treatment in cold temperature compared with normal temperature. The recorded activates of GST were 127.0 in normal temperature and 144.7 in cold temperature. In case of peroxidase activates, results exposed 660.3 and 912.3 (mg/g.b.wt) for both normal and hot temperature respectively. In contrast, LC_{50} of silica treatment in cold temperature compared led to a significant decline in AChE activity from 667.7 to 221.3 in normal temperature.
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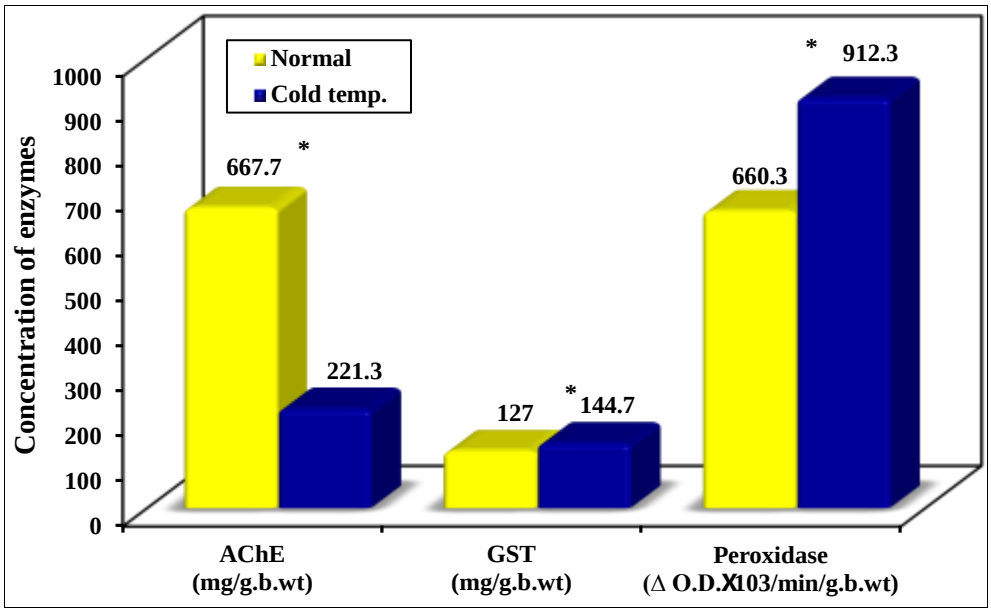
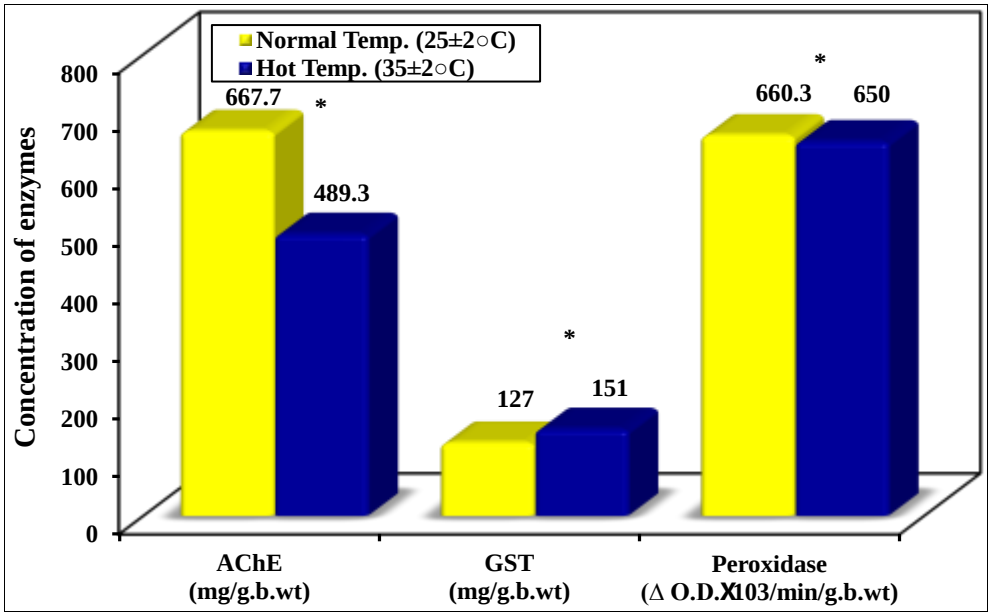


Data was expressed using Mean of 3 replicates.
*: Statistically significant at $p \leq 0.05$ (t-test).

Fig 4: Effect of LC_{50} silica on Acetylcholinesterase (AChE), Glutathione S-transferase (GST) and Peroxidase enzymes of the newly emerged adults of *Trogoderma granarium* in normal and cold temperature.

Data in figure (5) indicated displayed that AChE and peroxidase were decline in the newly emerged adults of *T. granarium* as a result of LC_{50} of nano silica treatment in hot temperature when compared to normal temperature. Furthermore, the decrease in Ach was significantly and in peroxidase was non-significantly. The activities of AChE were 667.7 and 489.3 for normal and hot temperature, respectively. While in peroxidase, activity results showed 660.3 and 650.0 for normal and cold temperature, respectively. In case of GST being 127.0 in normal temperature and 151.0 in hot temperature. On the other hand, Statistical analysis indicated a significant growing of GST being 151.0 in hot temperature when compared to 127.0 in normal temperature.



Data was expressed using Mean of 3 replicates.
*: Statistically significant at $p \leq 0.05$ (t-test).

Fig 5: Effect of LC_{50} nano silica on Acetylcholinesterase (AChE), Glutathione S-transferase (GST) and Peroxidase enzymes of the newly emerged adults of *Trogoderma granarium* in normal and hot temperature.

Discussion

This study demonstrated that both silica and nano silica (SNP) are effective tools for pest control management (IPM) of stored grain pests such as *Trogoderma granarium*. Our findings align with earlier research by Debnath *et al.* [22], which highlighted the significant increase in mortality effects with SNP as the time post-application extended.

The results indicate that the mortality rate of *T. granarium* larvae is dependent on both the concentration of silica or nano silica and the duration of exposure, across different temperatures. Rigaux *et al.* [23] explained that silica treatment caused brittleness in *T. castaneum* adults, primarily due to desiccation and reduced body water content, leading to mortality. Additionally, Korunic [24] found that amorphous silica dusts are highly effective even at lower application rates compared to other formulations, noting variability in insect susceptibility to diatomaceous earth among species.

Saed *et al.* [25] reported that the mortality rates of *T. confusum* and *Rhyzopertha dominica* increased with prolonged exposure to nano silica particles, reaching over 86% and 95%, respectively, after 14 days. Similarly, Zayed *et al.* [26] showed that silica oxide nanoparticles at a concentration of 1.5 g/kg led to cumulative mortality rates of *T. castaneum*, which increased progressively over time, reaching 40.0% and 65.0% after one and two weeks, respectively.

The mechanisms behind the mortality induced by nano silica have been discussed by several authors. Benelli [27] and Ayoub *et al.* [28] explained that SiO_2 nanoparticles adhere to the insect cuticle, causing physical disruption of waxes and lipids, which leads to dehydration and mortality.

The study also observed changes in biochemical aspects such as total protein, total lipids, total carbohydrates, GST, AchE, and peroxidase activity. The impact of nanoparticles on these factors varies depending on the treatment conditions, such as temperature. Nanoparticles significantly affect antioxidant and detoxifying enzymes, protein synthesis, and gene regulation, leading to oxidative stress, disrupted development and reproduction, enzyme denaturation, and cell death [29].

An increase in total protein observed in some treatments could be attributed to the overexpression of proteins as nanoparticles interact with cellular proteins involved in processes like cell division [30]. Alternatively, cytotoxic mechanisms of nanoparticles, such as those seen with silver nanoparticles, might involve the release of silver ions that bind to SH-groups in amino acids or proteins, disrupting protein functions and antioxidant defenses [31]. Said *et al.* [32] also suggested that increased total protein content could result from the enhanced synthesis of new proteins by the hemolymph, fat body, or conversion of lipids and carbohydrates to proteins.

The observed decrease in enzyme activity can be explained by Benelli *et al.* [27, 33] who found that nanoparticles degrade enzymes and organelles by penetrating the exoskeleton and binding to sulfur or phosphorus in DNA. This leads to reduced membrane permeability, altered cellular function, and potentially cell death. Consistent with these findings, nanoparticles can affect insects by penetrating the exoskeleton and causing rapid denaturation of enzymes and organelles, disrupting cellular function and leading to cell death.

Conclusion

The results indicate that nano silica is more effective than silica in controlling *Trogoderma granarium* adults, particularly when used in normal (room temperature) and cold storage conditions. This suggests that nano silica could be a valuable component of an eco-friendly pest control strategy.

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