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ARTICLE

Comparative Toxicological Effects of Silica and Nanosilica against *Trogoderma granarium* under Variable Temperature Conditions

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ABSTRACT

According the importance of the stored grains and other products, it is an essential to keep them from khapra beetle, *Trogoderma granarium* infestation. This study determined the mortality percentage of 5th instar larvae of *T. granarium* fed on wheat seeds (25 gm) treated with different weights of silica as well as silica nanoparticles (20, 40, 60 and 80 mg) at different temperature (9 °C, 25 °C, and 35 °C). Study showed that using silica nanoparticles in cold temperature (9 °C) was the most efficient treatment with the lowest LC_{50} (lethal concentration required to kill 50% of the population) value and caused the highest toxicity index. In contrast, the least efficient treatment (25 °C) with the highest LC_{50} value and showed lowest toxicity index was using silica in normal temperature, when using silica nanoparticles, the cold temperature was the best condition followed by hot temperature (35 °C) and finally the normal temperature. On the other hand, using silica in hot temperature was most effective followed by silica with cold temperature and finally silica with normal temperature. The biochemical assays revealed that the change in the experimental temperature had a nonsignificant effect on the total protein content of the larvae. The total lipids and total carbohydrates exhibited a significant increase due to hot treating. 5th instar larvae of *T. granarium* treated with LC_{50} of silica at high temperature led to a nonsignificant ($p \leq 0.05$) decrease in Acetylcholinesterase (AchE) activity compared to treatment at normal temperature. In contrast, Glutathione S-transferase (GST) and Peroxidase

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activities were significantly ($p \leq 0.05$) raised due to the treatment conducted at high temperature. Additionally, treating larvae with LC_{50} of silica nanoparticles at low temperature caused a significant increase in both GST and peroxidase activities, while the increase in AChE was nonsignificantly ($p \leq 0.05$) compared to treatment at normal temperature. Using silica at low temperature could be used as an alternative to chemical insecticides to control *T. granarium* larvae.

Keywords: Biochemical Studies; Control; Khapra Beetle; Nanosilica; Silica; Temperature

1. Introduction

One of the most significant crops that provides a consistent source of sustenance to a sizable portion of the world's population is wheat (*Triticum aestivum*); it is the second-largest grain in the world. Almost 785 million metric tons of wheat were produced worldwide in the 2023–2024 marketing year[1]. Wheat accounts for approximately 20% of all agricultural imports and 10% of the total value of agricultural production. Therefore, it is important to work hard to produce wheat in a sustainable manner and to improve its quality by using fewer chemical pesticides[2, 3]. A large percentage of the population's access to food is impacted by insects, which are among the primary problems associated with stored grains globally[4].

Stored grains and other products are thought to be extremely susceptible to pests such as the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), in many parts of the world[5–7]. Because of its rapid consumption of stored grains, the khapra beetle (*Trogoderma granarium*) is classified as an A2 quarantine global insect pest. It is one of the most damaging arthropods for stored goods because of its development of insecticidal resistance and its capacity to endure famine for extended periods of time. *T. granarium* is mostly recognized as a pest of stored wheat, where it can result in postharvest losses of up to 30%[8]. The larval stage is the most detrimental[9, 10]. It infests a variety of raw grains and processed grain products and can drastically lower the quality and quantity of stored commodities by consuming the seed embryo before the full kernel or seed, leaving only the husk. *T. granarium* is one of the most hazardous and widespread pests in the tropical and subtropical regions of Asia and Africa. The development rates and survival of the khapra beetle are significantly influenced by temperature, light, moisture, and season[11]. Additionally, Rizwan et al.[12] reported that the developmental period of the khapra beetle was very short at 30 °C and 35 °C, which led to greater population growth. Therefore, temperatures below 30 °C or above 35 °C were unsuitable for development. As a result, temperature and relative humidity can significantly affect the life stages of internal pests such as Trogoderma granarium. These factors can be used in conjunction with any chemical or physical measures because they can increase their effectiveness and decrease pest resistance.

Synthetic chemical pesticides have been used for many years to control stored grain pests[13]. However, because of their widespread abuse, groundwater, sediments, plants, soil, and animals and insects that have become tolerant of them are all contaminated[14]. Therefore, we must look for new control measures that could help manage the khapra beetle. NPs constitute a new generation of environmental remediation technology that can reasonably address some of the most challenging environmental cleanup problems, as claimed by Cinnamuthu and Murugesa[15]. Additionally, they can be utilized to develop novel insecticides, pesticides, and insect repellents[16]. Furthermore, Zahran and Sayed[17] found that O. surinamensis adults' mortality percentages increased as the exposure time and nanosilica increased.

Therefore, the current work aimed to evaluate the insecticidal efficacy of silica and nanosilica against *T. granarium* larvae at different temperatures condition (9 °C, 25 °C, and 35 °C), as well as comparing the effect of silica and nanosilica on certain biochemical features.

2. Materials and Methods

2.1. Rearing Technique of Insect Culture

Khapra beetle (*T. granarium*) cultures of various ages were acquired from the Plant Protection Research Institute in Giza, Egypt. They were reared on wheat grains that were bought from a neighborhood store. Each cylindrical glass jar containing the *T. granarium* cultures was covered with

muslin fabric, secured with a rubber band, and maintained in a low-light environment with a temperature of 25–32 °C and a relative humidity of 60–70%. Periodically, the cultures were cleansed. Khapra beetle adults were permitted to deposit their eggs, and the fifth larval instars of the cultures were removed for the bioassay [18].

2.2. Source of Silica and Silica Nanoparticle Particles

Silica and silica nanoparticle particles were purchased from Naqaa Company, Cairo, Egypt, and were prepared and previously characterized in El-Didamonya et al. [19].

2.3. Toxicological Study

Effects of various concentrations of silica and silica nanoparticles (20, 40, 60, and 80 mg) at various temperatures (9 °C, 25 °C, and 35 °C) were assessed. A total of 25 g of clean uninfested wheat was weighed into small jars, and each concentration was added separately. Ten 5th instar larvae were placed in each jar. The jars were covered with muslin cloth for sufficient ventilation. The negative control group was prepared with clean wheat without silica or silica nanoparticles. Three replicates were carried out for each concentration. and incubated at the tested temperature and 70 ± 2% relative humidity. The daily mortality counts were recorded until the 6th day and were corrected via the Abbott formula [20]. The cumulative corrected mortality percentages after 3 days of exposure to silica and silica nanoparticles were plotted against the corresponding concentrations via the probit LdP line software program to obtain the LC_{50} (lethal concentration required to kill 50% of the population) values.

2.4. Biochemical Assays

2.4.1. Chemicals Purchasing

All required chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4.2. Sample Preparation

1 gm larvae treated with the LC_{50} of silica or silica nanoparticles were homogenized in 5 ml. distilled water after 3 days of treatment for biochemical analysis.

2.4.3. Total Protein Concentration Estimation

The total protein concentration was determined via the Bradford method [21]. One hundred milligrams of Coomassie Brilliant Blue G-250 was dissolved in 50 milliliters of 95% ethanol to create the protein mixture. One hundred milliliters of 85% (Weight/Volume) phosphoric acid was added to this mixture. A final volume of one liter was achieved by diluting the resulting solution. Fifty microliters of sample solution or standard curve test tubes were pipetted with 50 µl of serial quantities of bovine serum albumin ranging from 10 to 100 µg. Phosphate buffer (0.1 M, pH 6.6) was used to reduce the capacity of the test tube to 1 ml. After adding five milliliters of protein reagent to the test tube, the contents were combined by vortexing or inversion. After two minutes and before one hour, the absorbance at 595 nm was measured against a blank made from one milliliter of phosphate buffer and 5 ml of the "protein" reagent.

2.4.4. Total Lipid Content Estimation

The total lipid content was estimated via the method of Knight, Anderson and Rawle [22]. The phosphovanillin reagent, which is made by dissolving 0.6 grams of pure vanillin in 10 milliliters of ethanol and then adding 100 milliliters of distilled water, was used. Then, 400 cc of concentrated phosphoric acid was added. In a test tube, 250 µl of the sample was combined with 5 ml of concentrated sulfuric acid, and the mixture was heated in a boiling water bath for 10 minutes. The digest was added to 6 milliliters of phosphovanillin reagent after it had cooled to room temperature. The color produced was measured at 525 nm against the reagent blank after 45 minutes. The optical density of the reference standard was compared, and the results are reported as mg lipids per ml hemolymph.

2.4.5. Total Carbohydrates Assessment

The phenol–sulfuric acid reaction of Dubois et al. [23] was used to quantify the total carbohydrates in the acid extract of the sample. One gram of the sample was homogenized in five milliliters of 0.3 N $HClO_4$ at 0 °C for one minute. For an additional ten minutes, the homogenate was held on ice. After the insoluble material was centrifuged for three minutes at 2000 r.p.m., it was rinsed twice in five milliliters of ice-cold $HClO_4$ by centrifugation and redispersion. Acid

extract was created by combining the three supernatants. To 0.5 ml of phenol (20% w v^{-1}), 100 microliters of the acid extract was added to a colorimetric tube. Then, 5 millilitres of sulfuric acid were added quickly while shaking. Before the readings were taken, the tubes were shaken and left in a water bath at 25 to 30 °C for 10 to 20 minutes after being allowed to stand for 10 minutes. The sugar solution was replaced with distilled water to create blanks. At 490 nm, the absorbance of the distinctive yellow–orange hue was measured and compared with that of a blank. The formula for total carbohydrates is µg glucose per g.b.wt.

2.4.6. Acetylcholinesterase Activity Estimation

AchE (acetylcholinesterase) activity was measured according to the method described by Simpson, Bulland and Linquist[24], utilizing the substrate acetylcholine bromide (AchBr). Two hundred microlitres of enzyme solution, 0.5 ml of 0.067 M phosphate buffer (pH 7), and 0.5 ml of AchBr (3 mM) were present in the reaction mixture. For thirty minutes, the test tubes were incubated at 37 °C. The test tubes were filled with 1 milliliter of alkaline hydroxylamine, which is equivalent to 2 milliliters of hydroxylamine chloride and 3.5 milliliters of NaOH. Next, 0.5 ml of HCl was added, which was composed of two parts ΔH_2O and one part concentrated HCl. After the mixture was shaken well, it was left to stand for two minutes. After 0.5 ml of ferric chloride solution (0.9 M $FeCl_3$ in 0.1 M HCl) was added, the mixture was thoroughly mixed. At 515 nm, the decrease in AchBr caused by AchE hydrolysis was measured.

2.4.7. Glutathione S-Transferase Assessment

Glutathione S-transferase (GST) was detected as described by Habig, Pabst and Jakoby[25] by detecting S-(2,4-dinitro-phenyl)-L-glutathione formed by the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB). One milliliter of the potassium salt of phosphate buffer (pH 6.5), one hundred microliters of GSH, and two hundred microliters of larval homogenate made up the reaction mixture. A total of 25 µl of the substrate CDNB solution was added to initiate the reaction. Both the GSH and CDNB concentrations were set to 5 mM and 1 mM, respectively. The reagents and enzymes were incubated for five minutes at 30 °C. To find the nanomolar substrate conjugated per min per larva via a molar.

2.4.8. Peroxidase Activity Evaluation

Peroxidase activity was determined according to Vetter, Steinberg and Nelson[26]. One milliliter of 0.3% hydrogen peroxide (in distilled water) and one milliliter of 1% o-phenylenediamine (in 95% ethyl alcohol; fresh every four hours) were used. After five minutes, the process was stopped by adding two milliliters of saturated sodium bisulfite to each 200 µl sample. Each sample's reagent blank is made by adding dye, sulfite, and hydrogen peroxide in that order. When hydrogen peroxide is added, sulfite inhibits the enzyme, rendering it inactive. Twenty-five milliliters of 95% ethyl alcohol was added to the sample and blank to flocculate the starch. To ensure adequate flocculation, the starch suspension needs to be continually spun while alcohol is added. After that, the samples were centrifuged for five minutes at approximately 3000 r.p.m. After being decanted into a colorimeter tube, the absorbance of the clear supernatant was measured at 430 nm. For every sample, the colorimeter was adjusted to 100% transmittance using the matching blank. The change in absorbance at 430 nm was used to express the enzyme activity (ΔOD430) per minute per g fresh weight).

2.5. Statistical Analysis of Data

The experimental design permitted identifying the variance among groups with an SD of 9% of the mean. Power (β) needed to detect alteration of the same degree to that detected was calculated by post hoc power calculations with the smallest sample range N = 3 per group has β = 1 with an error = α–0.05 using MiniTab 18 software.

Version 20.0 of the IBM SPSS software program was used to input and analyze the data (Armonk, NY: IBM Corp). The means and standard deviations were used to characterize the quantitative data. The results were deemed significant at the 5% level. To compare the two groups under study, Student's t test for normally distributed quantitative variables was employed.

3. Results

Table 1 shows the mean daily mortality percentage of 5th instar larvae of *T. granarium* fed wheat seeds treated with different weights of silica or silica nanoparticles (20,

40, 60 and 80 mg per 25 gm wheat) at a normal temperature (25 ± 2 °C). The data revealed that larval mortality increased with increasing silica concentration and exposure time. The mortalities on the 1st day were 0, 13.3, 20, and 33.3%, respectively, and increased to 13.3, 46.6, 46.6 and 60%, respectively, on the 2nd day of treatment with 20, 40, 60 and 80 mg per 25 gm wheat. The results revealed 100% larval mortality after 4 days of treatment with 80 mg. The other tested concentrations, 20, 40, and 60 mg, caused 100% mortality after 6 days of treatment. When silica nanoparticles were used, the mortality percentages of larvae after one day increased from 0 to 26.6%, 33.3%, and 26.6%, respectively, whereas on the 2nd day, the mortality percentages were 26.6%, 33.3%, 66.6% and 53.3%, respectively. On the 3rd day, the mortality percentages were 40%, 53.3%, 73.3%, and 86.6%, respectively, whereas on the 4th day, the percentages were 46.6%, 53.3%, 93.3%, and 93.3%, respectively, when 0, 20%, 40%, 60%, and 80 mg silica nanoparticles per 25 gm wheat were used, respectively. On the 5th day, larval mortality reached 100% at a concentration of 80 mg per 25 gm, whereas mortality rates of 66.6%, 73.3% and 93.3% were recorded at concentrations of 20, 40 and 60 mg silica nanoparticles, respectively. The data revealed that the use of 60 mg silica nanoparticles per 25 gm caused 100% mortality on the 6th day of treatment; however, at concentrations of 20 and 40 mg per 25 gm, 86.6% and 93.3% mortality, respectively, increased to 100% on the 7th day.

Table 1. Mean mortality percentage of 5th instar larvae of *Trogoderma granaria* fed wheat seeds treated with different concentrations of silica or silica nanoparticles at a normal temperature (25 ± 2 °C).

Concentrations (mg per 25 gm Wheat)	Silica						Silica Nanoparticles					
	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	20	40	53.3	100	0	26.6	40	46.6	66.6	86.6
40	13.3	46.6	66.6	73.3	86.6	100	26.6	33.3	53.3	53.3	73.3	93.3
60	20	46.6	66.6	80	93.3	100	33.3	66.6	73.33	93.3	93.3	100
80	33.3	60	86.6	100	100	100	26.6	53.33	86.6	93.3	100	100

Note: The values represent the means of 3 replicates.

The data in **Table 2** show that when the 5th instar larvae of *T. granarium* fed on wheat were treated with different weights of silica or silica nanoparticles, larval mortality increased with increasing weight of silica (20, 40, 60 and 80 mg per 25 gm wheat), as did the exposure time of the experiment *(*1–6 days). The mortality percentage of larvae exposed to 20, 40, 60, or 80 mg of silica per 25 gm wheat at a hot temperature **(**35 ± 2 °C) after one day increased from 13.3% to 20%, 40%, and 40%, respectively, whereas on the 2nd day, the mortality percentages were 33.3%, 60%, 53.3%, and 66.6%, respectively. The results revealed that 60 and 80 mg of silica per 25 gm caused 100% mortality after 4 days of treatment. Moreover, at concentrations of 40 and 20 mg per 25 gm, 100% mortality was recorded on the 6th day of treatment. When silica nanoparticles were used, the mean mortality percentages of *T. granarium* larvae after 1 day of feeding on wheat seeds treated with 20, 40, 60 and 80 mg of silica nanoparticles per 25 gm of wheat at a hot temperature **(**35 ± 2 °C) were 13.3%, 13.3%, 13.3%, and 33.3% mortality, respectively. On the 2nd day, 33.3% mortality was detected at concentrations of 20, 40 and 60 mg of silica nanoparticles per 25 gm wheat, and 53.3% mortality was detected at concentrations of 80 mg of silica nanoparticles per 25 g. On the 3rd day, the mean mortality percentages were 53.3, 60, 60 and 86.6%, and on the 4th day, the mean mortality percentages were 60, 73.3, 93.3 and 100% for 20, 40, 60 and 80 mg of silica nanoparticles per 25 gm of wheat, respectively. On the 5th day, the mean mortality percentages were 66.6, 86.6, 100 and 100, respectively. On the 6th day, the mean mortality rate was 80% at the 20 mg concentration and 100% at the other concentrations.

Table 2. Mean mortality percentage of 5th instar larvae of *Trogoderma granaria* fed wheat seeds treated with different concentrations of silica or silica nanoparticles at hot temperatures (35 ± 2 °C).

Concentrations (mg per 25 gm) Wheat)	Silica						Silica Nanoparticles					
	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	33.3	46.6	60	86.6	93.3	13.3	33.3	53.3	60	66.6	80
40	20	60	73.3	86.6	100	100	13.3	33.3	60	73.3	86.6	100
60	40	53.3	73.3	100	100	100	13.3	33.3	60	93.3	100	100
80	40	66.6	93.3	100	100	100	33.3	53.3	86.6	100	100	100

Note: The values represent the means of 3 replicates.

The results in **Table 3** clearly show that the effects of silica or silica nanoparticles on larval mortality at cold temperatures were correlated in parallel with both the silica concentration (20, 40, 60 and 80 mg per 25 gm wheat) and the duration of exposure. On the 1st day, the mortalities were 6.6, 6.6, 13.3 and 33.3%, whereas on the 2nd day, the mortalities increased to 20, 26.6, 26.6 and 40% at concentrations of 20, 40, 60 and 80 mg per 25 gm wheat, respectively. The mortality rates were 40, 46.6, 66.6 and 66.6% on the 3rd day, whereas on the 4th day, the mortality percentages were 66.6, 66.6, 86.6 and 86.6% for 20, 40, 60 and 80 mg per 25 gm wheat, respectively. On the 5th day, the use of 80 mg of silica caused 100% larval mortality, whereas 40 and 60 mg of silica caused 86.6% mortality. The average mortality reached 100% on the 6th day after the application of 20, 40 and 60 mg of silica per 25 gm of wheat. The results revealed that the mortalities were 6.66, 6.66, 6.66.3 and 46.6% with concentrations of 20, 40, 60 and 80 mg silica nanoparticles per 25 gm wheat, respectively. For the 2nd day, the values were 26.6, 26.6, 33.3 and 80. On the 3rd day, the mean mortalities were 60, 60.6, 60.6 and 93.3%, respectively. On both the 4th and the 5th days, the percentages of mortality caused by silica nanoparticles were 86.6, 86.6, 93.3 and 100%, respectively, with 20, 40, 60 and 80 mg of silica nanoparticles per 25 gm of wheat. The average mortality reached 100% on the 6th day of application at all concentrations.

Table 3. Mean mortality percentage of 5th instar larvae of *Trogoderma granaria* fed wheat seeds treated with different concentrations of silica or silica nanoparticles at a cold temperature (9 ± 2 °C).

Concentrations (mg per 25 gm) Wheat)	Silica						Silica Nanoparticles					
	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	6.66	20	40	66.6	73.3	100	6.66	26.6	60	86.6	86.6	100
40	6.66	26.6	46.6	66.6	86.6	100	6.66	26.6	66.6	86.6	86.6	100
60	13.3	26.6	66.6	86.6	86.6	100	6.66	33.3	66.6	93.3	93.3	100
80	33.3	40	66.6	86.6	100	100	46.6	80	93.3	100	100	100

Note: The values represent the means of 3 replicates.

Table 4 and **Figure 1** show that the use of silica nanoparticles at cold temperature was the most efficient treatment, with the lowest LC_{50} value of 15.839 mg per 25 gm wheat and the highest toxicity index of 100. In contrast, the least efficient treatment with the highest LC_{50} value and lowest toxicity index was the use of silica at normal temperatures. The use of silica nanoparticles at a cold temperature was the best environment, followed by a hot temperature, and finally, the use of silica at a hot temperature was the most effective, followed by the use of silica at a cold temperature and, finally, silica at a normal temperature.

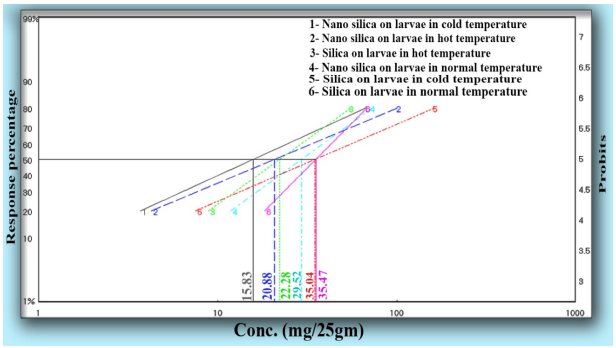

Figure 1. LC_{50} of silica or silica nanoparticles on *Trogoderma granria* larvae at different temperatures after 3 days of treatment.

Table 4. LC_{50}, LC_{90}, toxicity index and resistance ratio slope of the effects of silica and silica nanoparticles on *Trogoderma granaria* larvae at different temperatures after 3 days.

Treatment	LC_{50} (mg per 25 gm Wheat)	Toxicity Index	Resistance Ratio	Slope	LC_{90} (mg per 25 gm Wheat)
Silica nanoparticles on larvae in cold temperature	15.839	100	1	1.339	143.61
Silica nanoparticles on larvae in hot temperature	20.888	75.828	1.319	1.223	233.379
Silica on larvae in hot temperature	22.281	71.087	1.407	2.122	89.502
Silica nanoparticles on larvae in normal temperature	29.527	53.642	1.864	2.133	117.799
Silica on larvae in cold temperature	35.044	45.197	2.213	1.261	364.183
Silica on larvae in normal temperature	35.47	44.655	2.239	2.94	96.764

Note:
- Indexes of the effects of silica nanoparticles on larvae at low temperatures.
- Resistance ratio (RR) of larvae in cold-temperature media compared with that of silica nanoparticles.

The data in **Figure 2** show the concentrations of total protein, total lipids and total carbohydrates in the 5th instar larvae of *T. granarium* fed wheat treated with the LC_{50} of silica at normal and hot temperatures. The results revealed that the change in the experimental temperature had a non-significant effect on the total protein content of larvae (68.23 and 67.10 mg per g.b.wt for normal and hot temperatures, respectively). The total lipid and total carbohydrate contents were 27.33, 35.07, 26.87 and 39.07 mg per g.b.wt. for normal and hot samples, respectively. Statistical analysis revealed a significant increase in total lipids and total carbohydrates due to heat treatment.

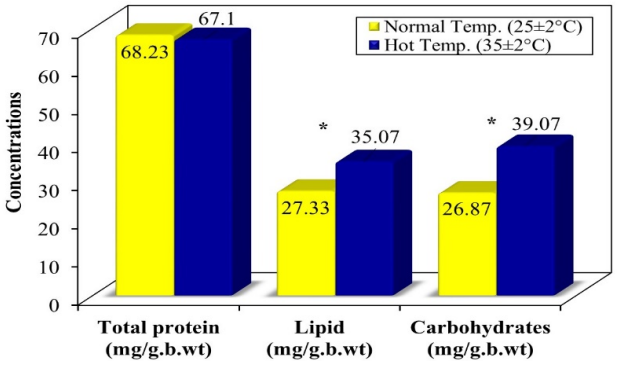

Figure 2. Effects of the LC_{50} of silica on total protein, total lipid and total carbohydrate concentrations in 5th instar larvae of *Trogoderma granarium* at normal and hot temperatures.

Note:
- The data are expressed as the means of 3 replicates.
- *: Statistically significant at $p \leq 0.05$ (t test).

The results in **Figure 3** show the comparison between the total protein, total lipid and total carbohydrate contents of the 5th instar larvae of *T. granarium* with the LC_{50} of silica nanoparticles at normal and cold temperatures. The total protein content was not significantly ($p \leq 0.05$) elevated at normal and cold temperatures, with values of 68.23 and 73.63 mg per g.b.wt., respectively. In contrast, the total lipids and total carbohydrates significantly increased ($p \leq 0.05$) from 27.33 to 30.77 mg per g.b.wt for total lipids and from 26.87 to 37.30 mg per g.b.wt for the Lc_{50} of silica nanoparticles at normal and cold temperatures, respectively.

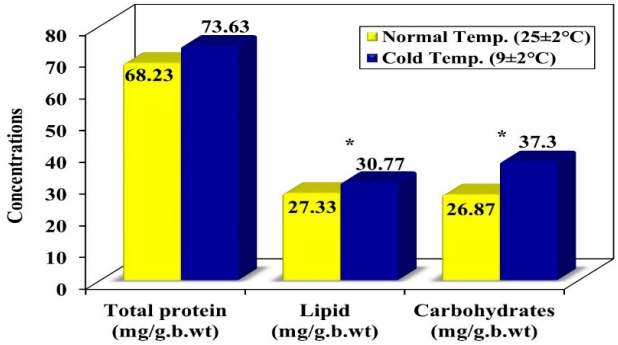

Figure 3. Effects of the LC_{50} of silica nanoparticles on the total protein, total lipid and total carbohydrate contents of 5th instar larvae of *T. granarium* at normal and cold temperatures.

Note:
- The data are expressed as the means of 3 replicates.
- *: Statistically significant at $p \leq 0.05$ (t test).

The enzymatic activities of acetylcholinesterase (AchE), glutathione S-transferase (GST) and peroxidase at the LC_{50} of silica at normal (25 ± 2 °C) and hot (35 ± 2 °C) temperatures are presented in **Figure 4**. The data revealed a non-significant decrease ($p \leq 0.05$) in AchE activity in the normal temperature treatment group compared with that in the cold temperature group. In contrast, a significant increase ($p \leq 0.05$) in GST and peroxidase activity was detected in the hot-temperature treatment group compared with the normal-temperature treatment group.

The data in **Figure 5** revealed a nonsignificant increase ($p \leq 0.05$) in AChE activity in the cold temperature treatment group compared with that in the normal temperature treatment group. However, a significant increase ($p \leq 0.05$) in GST and peroxidase activity was detected in the cold-treated samples (9 ± 2 °C) compared with the normal-temperature

samples. In general, compared with treatment at a normal temperature, the LC_{50} of silica nanoparticle treatment at a low temperature significantly increased ($p \leq 0.05$) peroxidase activity.

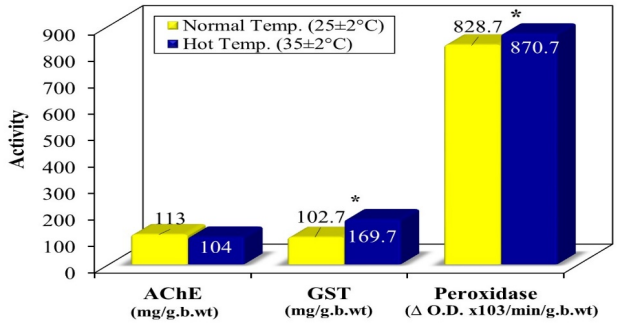

Figure 4. Effects of silica at the LC_{50} on acetylcholinesterase (AChE), glutathione S-transferase (GST) and peroxidase enzymes in 5th instar larvae of *Trogoderma granarium* at normal and hot temperatures.

Note:
- The data are expressed as the means of 3 replicates.
- *: Statistically significant at $p \leq 0.05$ (t test).

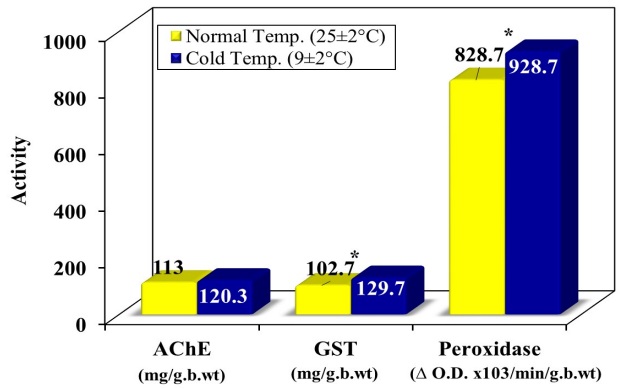

Figure 5. Effects of the LC_{50} of silica nanoparticles on acetylcholinesterase (AChE), glutathione S-transferase (GST) and peroxidase enzymes in 5th instar larvae of *Trogoderma granarium* at normal and hot temperatures.

Note:
- The data are expressed as the means of 3 replicates.
- *: Statistically significant at $p \leq 0.05$ (t test).

4. Discussion

This study demonstrated that silica and silica nanoparticles (SNPs) might be used to help with the control of stored-grain pests such as *T. granarium* pest control (IPM). The application of SNPs could greatly increase the mortality effect of NPs with increasing time after application, according to a comparison of our results with those of previous studies [27].

The results revealed that the death rate of *T. granarium* larvae depended on the concentration of silica or silica nanoparticles as well as the exposure duration at various temperatures. According to Rigaux, Haubruge and Fields [28], the effect of silica caused *T. castaneum* adults to become brittle, mostly as a result of desiccation and a subsequent decrease in the water content of their bodies following silica treatment, which ultimately resulted in their mortality. Additionally, Korunic [29] reported that the vulnerability of stored-product insects to diatomaceous earth varies by species and that silica dust containing amorphous silica shows outstanding efficacy at lower treatment rates than other forms do.

Our results were in accordance with El-Gendy et al.'s [17] results; who reported an increase in *T. castaneum* adults' mortality with increasing silica or nanosilica concentrations. Moreover, they revealed that using nanosilica at hot temperatures proved the most effective treatment.

Similarly, Saed et al. [30] stated that the adults' mortality of *T. confusum* and *R. dominica* increased with the increasing exposure time, and percent mortality was more than 86% and 95%, respectively, after 14 days of exposure to silica nanoparticles. Zayed, El-Sagheer and Hussain [31] proved that silica oxide nanoparticles at the concentration of 1.5 gm kg^{-1} caused accumulative mortality percentages of *T. castaneum*, and the percent mortality increased gradually with increased exposure time, with the percent of higher mortality reaching to 40.0% and 65.0% individuals after one and two weeks, respectively.

The mortality of insects by silica nanoparticles was explained by many authors [32, 33]. They declared that the toxicity of SiO_2 nanoparticles is due to their binding to the insect cuticle, followed by physisorption of waxes and lipids, leading to insect dehydration.

The alterations in the tested biochemical aspects (total protein, total lipids, total carbohydrate, GST, AChE, and peroxidase) in some treatments and increase with other treatments depend on many factors such as temperature. NPs have a significant impact on the insect's antioxidant and detoxifying enzymes, protein synthesis, gene regulation, thus leading to oxidative stress, disrupting development and reproduction, enzyme denaturation, and cell death [34].

The overexpression of some proteins as a result of the interaction of nanoparticles with cellular proteins, such as those involved in cell division, causes an increase in total protein [35]. The mechanism of AgNP cytotoxicity may also be related to the intracellular release of silver ions, which are

then bound to SH groups, most likely derived from proteins or amino acids, and impact protein functions as well as the antioxidant defense system of cells [36]. Additionally, Said, Hammam and Abd-El Kader [37] proposed that the conversion of lipids and carbohydrates to proteins or the increased synthesis of new proteins by the larvae's fat body, hemolymph, and other tissues could be the cause of the increase in total protein concentrations.

El-Gendy et al. [17] recorded the same observations of alterations in total protein, total lipid, carbohydrates, acetylcholinesterase (AChE), glutathione-S-transferase (GST), and peroxidase in *T. granarium* adults fed on wheat treated with LC_{50} silica or nanosilica, which was clearer when using nanosilica.

From our results, the decrease in the activity of enzymes can be explained by Benelli [32, 38], who reported that nanoparticles cause the degradation of enzymes and organelles by penetrating through the exoskeleton and then binding to sulfur or phosphorus from the DNA. This is the major pathway of nanoparticle exposure. These factors reduce the permeability of the membrane and affect cellular function, leading to cell death. In agreement with these results, nanoparticles affect insect species by penetrating the exoskeleton; they enter the intracellular space and lead to expeditious denaturation of enzymes and organelles, which affects the function of cells, and cell death can also take place.

5. Conclusions

The above-mentioned results clearly revealed that nanosilica was more effective than silica in controlling *Trogoderma. granarium* larvae, especially when applied in cold storage conditions. Therefore, this combination could be used as an eco-friendly control program.

5.1. Study Limitations

Owing to the variation of insect species, these treatments must be further studied to determine the required concentration and the best temperature condition for each pest.

5.2. Practical Applications

We could mix the required concentrations of the nanosilica with the wheat seed in the bags and control the store temperature.

5.3. Future Research Directions

Studying the effect of our treatments on the wheat seed quality to confirm the ability of using them as a pest control tool in stores.

Author Contributions

This paper is a part of the D.A.E.-G.'s Ph.D. thesis. D.A.E.-G., A.Z.E.S., D.S.F., S.A.R. and R.M.S. designed the study. D.A.E.-G. carried out all the experiments and recorded the data. D.A.E.-G., S.A.R. and R.M.S. analyzed the results. D.A.E.-G. wrote the first draft of the manuscript. A.Z.E.S., D.S.F., S.A.R. and R.M.S. read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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