Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2023.36.2.13
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 36(2), 175-184, 2023 E-ISSN: 2520-0860

Efficiency of Silicon and Silver Nanoparticles against the Infestation of *Tribolium castanium* (Herbst) (Coleoptera: Tenebrionidae) on Wheat Grains under Laboratory Conditions

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Received 2nd July 2023; Accepted 18th September 2023; Available online 29th December 2023

Abstract: In recent years, nanotechnology has emerged as one of the promising new technologies for pest control. In this study, a screening program was conducted to evaluate the impact of silicon nanoparticles (SiNPs) and silver nanoparticles (AgNPs) on the mortality of the adult stage of *Tribolium castanium* on wheat grains. The nanoparticles were synthesized using a laser ablation process, and their characterization was performed using Zeta-sizer, UV-visible spectroscopy, X-ray Diffraction, and Transmission Electron Microscopy. Different concentration levels of SiNPs and AgNPs (100 ppm, 200 ppm, 300 ppm, and 400 ppm) were tested to assess their effects on *T. castaneum* mortality. The LC_{50} value for SiNPs and AgNPs were calculated at 438.3 and 657.4 ppm NPs, respectively. The feeding method revealed that AgNPs resulted in 40% mortality, while SiNPs resulted in 70% mortality. These findings suggest that the synthesized SiNPs have a greater potential as an alternative option in managing pests in stored products compared to AgNPs at the same exposure time (four days). The results showed that both SiNPs and AgNPs were having a potential effect on adult stage of *T. castaneum*. This study demonstrated the utility of AgNPs and SiNPs in *T.castaneum* pest management programs.

Keywords: Nanotechnology, Pest control, *Tribolium castaneum*, Silicon nanoparticles, Silver nanoparticles.

Introduction

Grain crops are vulnerable to insect damage after harvesting, leading to significant losses in quantity and quality. In the case of Iraq, the exact extent of grain loss during storage remains unknown. However, according to the FAO's report, it is estimated to range of 10-20% (Rajendran & Sriranjini, 2008; Liu *et al.*, 2021; Papanikolaou *et al.*, 2022; FAO, 2023).

Wheat (*Triticum aestivum* L.) holds significant importance as a vital food source in various countries, including Iraq (FAO, 2023). During the 2019/2020 growing season, wheat cultivation in Iraq expanded across a substantial land area, as reported by the Central Statistical Organization (CSO) (Al-Hussine & Alyousuf, 2021; FAO, 2023). However, the grain production was severely affected by drought and water shortages; hence, Iraqi Ministry of Agriculture reduced agricultural cropping in irrigated areas by 50 percent in

2022. Consequently, wheat production experienced a significant decline (Al-Hussine & Alyousuf, 2021). According to post forecasts for MY 2022/23, wheat production is expected to reach only 3.25 million metric tons, a significant decrease from previous seasons (USDA, 2023). The Central Statistical Organization (CSO) estimated that the total amount of cereal harvested in 2022 was around 3.6 million tonnes, which is 45 percent reduction compared to the previous year's harvest, with wheat accounting for 3.1 million tonnes (FAO, 2023).

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a prevalent and significant insect pest infests stored grain products worldwide (Padín *et al.*, 2002; Padín *et al.*, 2013; Papanikolaou *et al.*, 2022). Cereal grains and their derivatives, including dry fruits, pulses, bran, coat, germ, grain dust, and processed cereal foods, are attacked by both adult beetles and larvae (Huang *et al.*, 2020; Li *et al.*, 2020). The mandible chews of adults and larvae can cause severe damage to the stored products, directly or indirectly. Additionally, the adult beetles, being strong fliers, have the capability to move easily between storage facilities and fields (Rajendran & Sriranjini, 2008; Liu *et al.*, 2021).

Ensuring the protection of stored agricultural grains and their products after harvest is crucial for global food security (Papanikolaou *et al.*, 2022). However, with increasing consumer awareness, research has shifted towards alternative methods of controlling postharvest pests, moving away from reliance solely on pesticides. While chemical insecticides are effective against many insects, they have several negative impacts on the environment and human health (Oliveira *et al.*, 2017; Campolo *et al.*, 2018). Furthermore, the effectiveness of major insecticide classes, such as organophosphates and pyrethroids, has been diminished due to the development of pesticide resistance by stored-product insects (Al-Jabr, 2006; Mossa, 2016). Consequently, alternative approaches have emerged, including gas treatments involving Carbon dioxide, Nitrogen, and ozone. These methods have proven to be effective in controlling postharvest pests with minimal impact on the nutritional, physical, and chemical properties of the grains (Jiang *et al.*, 2016; Al-Emara *et al.*, 2021).

An alternative approach involves the use of DEs (diatomaceous earths) are primarily silica-based and are obtained from fossilized phosphatlankton. Dietomaceous earths are used to control stored product insect populations. Subramanyam *et al.* (2000), Roesli *et al.* (2001) and Mewis *et al.* (2003) proposed that DEs are more effective against insect pests if they are high in silica, have a uniform size distribution and possess certain physical characteristics. To achieve these characteristics, altering their size to the nanoscale has been proposed by Debnath *et al.* (2011).

Nanomaterials have emerged as a promising alternative to traditional pesticides for the control of stored product insect pests, such as the red flour beetle *T. castaneum*. In recent studies, the efficacy of various types of nanomaterials, including metal nanoparticles, for controlling *T. castaneum* has been highlighted. For instance, silver nanoparticles have exhibited remarkable insecticidal properties against *T. castaneum* at low concentrations (Jafari *et al.*, 2021). Similarly, copper oxide nanoparticles were effective in reducing the development and survival of *T. castaneum* larvae (Hossein *et al.*, 2021). However, the Silicon nanoparticles (SiNPs) can also affect the insect respiratory system, leading to suffocation and subsequent mortality. SiNPs can enter the insect tracheal system, leading to the obstruction of airflow and subsequent asphyxiation (Hossein *et al*., 2021). The objective of this study was to evaluate the insecticidal activity of AgNPs and SiNPs against red flour beetle *T. castaneum*.

Materials & Methods

Synthesis of AgNPs and SiNPs

In this study, the laser ablation in water technique (LAW) was employed for the synthesis of silver nanoparticles (AgNPs) and silicon nanoparticles (SiNPs). LAW was conducted at the Nanotechnology lab, Physics Department, Collage of Sciences, University of Basrah. Silicon /silver target (purity > 99.99%) is placed in a glass container filled with deionized water (DI). The container is irradiated with a high-power laser beam, Q-switched Nd: YAG laser (1064nm wavelength), which vaporizes the surface of the silver target. The laser beam is focused on the silver with a pulse duration of 6 ns and a repetition rate of 10 Hz. The laser energy is 500 mJ to obtain AgNPs and SiNPs the laser ablation is performed for 5 minutes. The resulting vapor rapidly condenses to form AgNPs in the water. The AgNPs /SiNPs suspension is collected for further characterization and applications. The LAW method does not require any reducing agents or stabilizers, making it an eco-friendly approach for the production of SiNPs and AgNPs. The size and morphology of the samples were determined by scanning electron microscope (SEM) and zeta sizer (Aslani *et al*., 2003; Rai, *et al*., 2009; Mathew & Narayanankutty, 2010; Zhang *et al*., 2017; Vahid & Zangeneh, 2019; Abbas & Abbas, 2019; Shah *et al*., 2020; Dongshi Zhang, 2021; Jasrotia *et al*., 2022). The size and shape of the AgNPs and SiNPs produced by the LAW method can be controlled by adjusting various parameters, such as laser power, irradiation time, and distance between the target and the water surface.

Nanoparticles characterization

Samples of AgNPs and SiNPs were exposed to X-ray diffraction (XRD) at a wavelength of 1.54 A with a scanning time of 0.52 per second. EDA was carried out by x-ray Oxford (Model 6647, England) at 5.9 keV. Preparations were scanned using scanning electron microscope (SEM) at the Faculty of Science, University of Basrah (Jasrotia *et al*., 2022). To determine the sizes and zeta potentials of the silicon and silver nanoparticles, advanced instrumentation was employed. The measurements were carried out using a state-of-the-art device, the Malvern Zetazizer ZEN 36W (United Kingdom), which provided particle size distribution data in the range of 2nm to 3nm (Jakinala *et al*., 2021).

Wheat grains

The wheat grains, *Triticum aestivum* (var. Adana), were commercially acquired from the local market that widely used and stored for sowing purposes. However, the red flour beetle is a secondary pest of stored grain products, the insects found to be attacking the stored grains. The grains were cleaned to remove any impurities by sieving, sterilized and stored at 25°C until to use for the experiments.

Insect colony

A colony of *T. castaneum* was maintained in the Entomology laboratory of the Plant Protection Department at the University of Basrah, College of Agricultural. The insects were reared on wheat grains in sterilized glass jars (1000 ml). The wheat grains as a food source for reared insects (Sial *et al*., 2017; Al-Emara *et al*., 2021). The rearing conditions were set at 28 ± 2° C and 65 ± 5% relative humidity in the incubator (Binder, Germany)

(Al-Emara *et al*., 2021). The adult beetles used for all bioassays were selected from a mixed-sex population with less than two weeks old (Padín *et al*., 2013; Bhavaniramya *et al*., 2019; Al-Emara *et al*., 2021).

Bioassay

Contact toxicity assay was used to determine the effects of AgNPs and SiNPs on adults of *T. castanium* at four doses of 100, 200, 300, and 400 ppm nanoparticles prepared according Austin *et al*. (2020). The experiments were conducted in 500 ml glass Jars with a tight glass cap, and 20 g of sterilized wheat grains was well mixed to get homogenious with each NPs. The experiment was a Completely Randomized Design (CRD) with five replications, each containing 20 adults. Nanoparticles were applied to each container and then manually shaken for about 1min to ensure uniform nanoparticle distribution. The treated containers were allowed to settle for 10 min. *T. castanium* adults were added to each vessel and incubated for 28 min at 28°C±1 (RH 70±5). Each concentration was repeated three times. Mortality was observed at 24-, 48-, 72- and 96-hours post-treatment.

Statistical analysis

The corrected mortality data were analyzed using SPSS 16 software. An analysis of variance (ANOVA) according to randomized complete design using factorial arrangement followed by a Tukey post-hoc analysis was conducted. The results were presented as means (±SE) of the untransformed data; statistically significant differences were determined using a significance level of $p < 0.05$. Probit analysis software was utilized to estimate the LC_{50} of the corrected values with fiduciary limits (SPSS Inc., 2006).

Results & Discussion

Structural study of nanoparticles

In this study, the nanoparticles were prepared and characterized using scanning electron microscope (SEM). The SEM images (Fig. 1) revealed the original morphology of the silicon and silver nanoparticles. Both types of nanoparticles are approximately spherical shape, with diameters ranging from 20 nm to 70 nm.

Fig. (2) showed the particle size distribution and Zeta Potential of AgNPs. The average particle size of the synthesized AgNPs in this study was determined to be 40 nm using dynamic light scattering spectrometry. The measurements were conducted on an aqueous solution of AgNPs with an approximate size of ~45 nm and a zeta potential of -19 mV. These findings regarding hydrodynamic size and zeta potential of AgNPs are consistent with the recent reports by Badoei-Dalfard *et al*. (2019) and Jakinala *et al*. (2021). Hydrodynamic size falls within the range of 20-70 nm and a zeta potential of -4.6 mV for AgNPs (Jakinala *et al*., 2021).

Figure 3 illustrated the particle size distribution and zeta potential of SiNPs studied in this research. The average particle size of synthesized SiNPs was 35 nm. The size of the SiNPs were calculated by dynamic light scattering spectroscopy using an aqueous solution, revealing a size of approximately 45 nm and a zeta potential of -19 mV (Fig. 3). The hydrodynamic size and zeta potential of AgNPs observed in this study are in accordance with the findings reported by Badoei-Dalfard *et al*. (2019) and Jakintala *et al*. (2021). Specifically, the AgNPs exhibit a hydrodynamic size ranging from 20 to 70 nm and a zeta potential of -4.6 mV (Jakinala *et al*., 2021). These consistent results from different studies validate the accuracy and reliability of our measurements and add to the growing body of knowledge on AgNPs properties.

The effect of nanoparticles against the *T. castaneum*

The mortality rate of adult *T. castaneum* treated with the nanoparticles was observed within 24 hours under laboratory conditions. Significant interaction effects were observed between dates. These results agreed with Jafari *et al*. (2021) which mentioned that the silver nanoparticles have been shown to have significant insecticidal activity against *T. castaneum* adults. Based on this observation, day-by-day treatment effects were reported. Analysis of variance indicated that AgNPs had no significant effect on the treated adults at the first day across all concentrations. However, there was a significant difference at the second day (F= 7.733, P =0.009), the third day (F = 13.33, P = 0.002), and the fourth day (F = 20.79, *P* = <0.001) at a 1% level of significance (Table 1). Similarly, the analysis of variance revealed that the effect of silicon nanoparticles on the treated adults was non-significant on the first and second days across all concentrations. However, significant differences were observed on the third day (F = 7.33, P = 0.011) and the fourth day (F = 22.07, P < 0.001) at a 1% level of significance (Table 2). These results partially agreed with Rouhani *et al*. (2012) and Jafari *et al*. (2021). At the highest concentration of 400 ppm, the presence of nanosilicon and nanosilver resulted in 70% and 40% mortality of *T. castaneum* adults, respectively, on the fourth day (Tables 1 and 2). It was observed that the percentage of adult mortality increased with an increase in nanoparticle concentrations, which is consistent with previous studies (Rouhani *et al*., 2012; Padín *et al*., 2013; Bhavaniramya *et al*., 2019; Al-Emara *et al*., 2021).

Table (1) showed that the highest percentage of mortality achieved with silver nanoparticles (40%) on the fourth day at a concentration of 400 ppm. In comparison, SiNPs resulted in the mortality of 70% of *T. castaneum* adults at a similar concentration.

The LC_{50} value for SiNPs and AgNPson adults of *T. castaneum* were revealed in Table 3. Based on these values, adults exhibited higher susceptibility to SiNPs compared to AgNPs. There was no significant difference between LC_{50} of SiNPs and AgNPs. The LC_{50} values decreased with increasing nanoparticle concentrations (Table 3). Hence, these results found to be in consistency with Raduw & Mohammed (2020) when they mentioned that the Silicon oxide and aluminium oxide NPs were more effective than zinc oxide NPs, where the mortality of the second instars on wheat treated with 200 mg. kg^{-1} concentrations.

Concurrently, the mortality rate increased with the rise in nanoparticle concentrations. These results were compatible previous studies (Elchiguerra *et al*., 2005; Reddy *et al*., 2007; Meng *et al*., 2009; Donaldson *et al*., 2009; Rouhani *et al*., 2012; Padín *et al*., 2013; Bhavaniramya *et al*., 2019). The LC_{50} values for SiNPs and AgNPson adults after four days of exposure were estimated (Table 3). The LC50 value comparison using the LC_{50} and their lower and upper 95% confidence limits showed that there was a significant difference between LC_{50} value for SiNPs and this value for AgNPs (582- 751.7) in adults. Statistic t ratio >1.96 for all nanoparticles, g factor <0.5, and heterogeneity factor <1 for all nanoparticles. Due to the significant LC_{50} difference between SiNPs and AgNPs in adults, the use of silicon nanoparticles may be more suitable for control of *T. castaneum*.

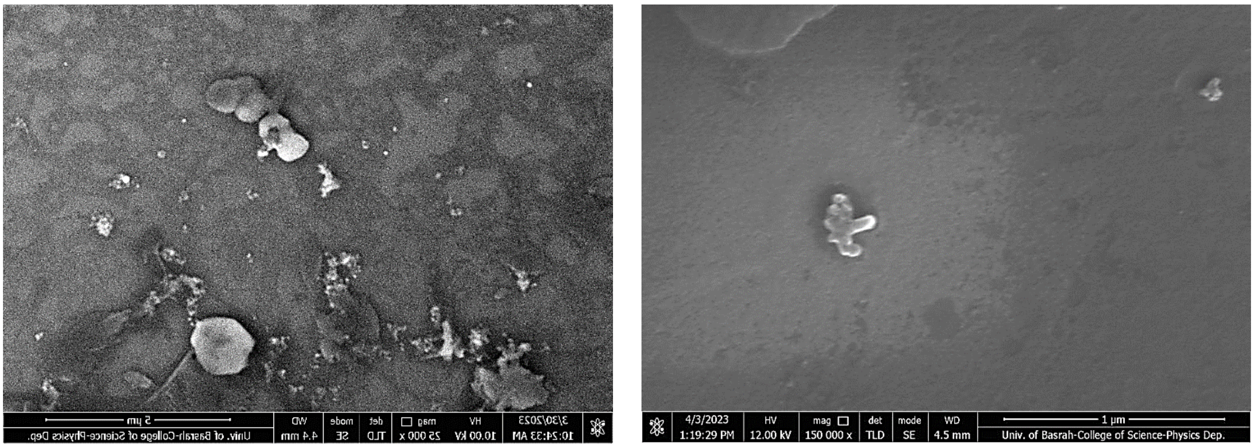

Fig. (1): The SEM images of the synthesized Si (left) and Ag (right) nanoparticles.

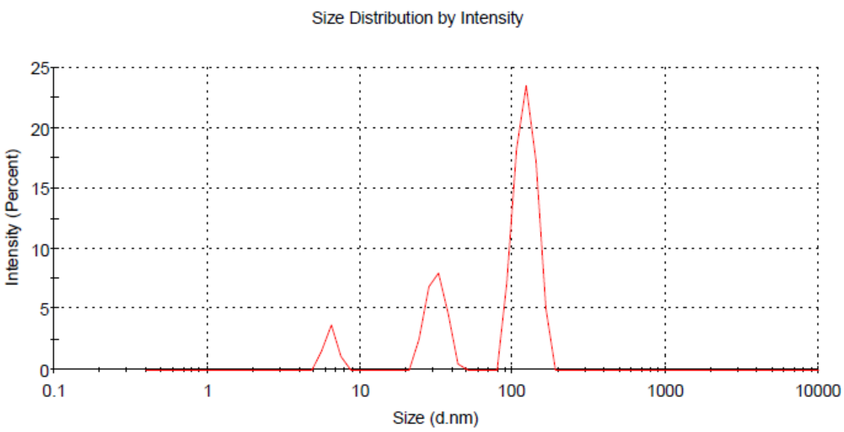

Fig. (2): Size distribution of synthesized AgNPs.

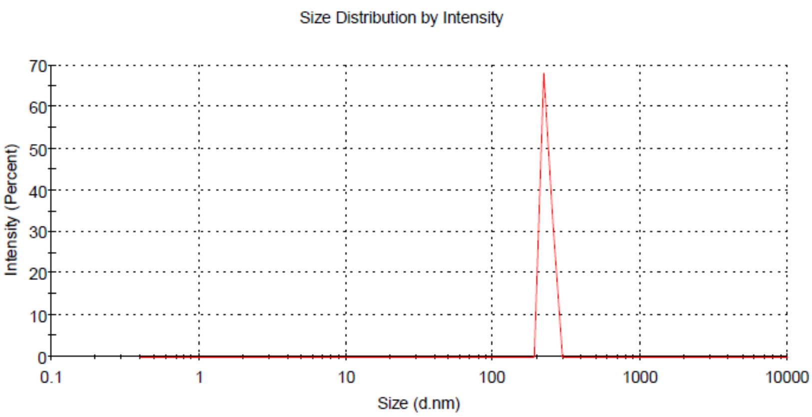

Fig. (3): Size distribution of synthesized SiNPs.

Table (1.): Effect of silver nanoparticles (AgNPs) on the mortality rate *T. castaneum* adult.

Time (hrs)	The mortality rate (%±SE) at concentration				Mean	F_1	p_1
	100 ppm	200 ppm	300 ppm	400 ppm			
24	0.0±0.00^{a}	0.0±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00	–	–
48	0.0±0.00^{A}	0.0±0.00^{aA}	0.00±0.00^{bA}	3.30±0.33^{bA}	0.825	1.000	0.441
72	0.0±0.00^{B}	0.0±0.00^{aB}	6.70±0.67^{abAB}	13.3±1.33^{bA}	5.00	7.333*	0.011*
96	0.0±0.00^{B}	6.7±0.67^{aB}	16.70±1.67^{aB}	40.00±4.00^{aA}	15.85	22.067*	<0.001*
Mean	0.00	1.68	5.85	14.15			
F_2	–	4.000	11.167*	23.667*			
p_2	–	0.052*	0.003*	<0.001*			

p_1: p-value for comparing the different concentrations each time
p_2: p-value for comparing the different times in each concentration
*: Statistically significant at $p \le 0.05$
Means in the same Column with lowercase letters are not statistically significant
Means in the same Row with uppercase letters are not statistically significant

Table (2): Effect of silicon nanoparticles (SiNPs) on on the mortality rate *T. castaneum* adult.

Time (hrs)	The mortality rate ((%±SE) at concentration				Mean	F_1	p_1
	100 ppm	200 ppm	300 ppm	400 ppm			
24	0.0±0.00^{bB}	3.3±0.33^{cAB}	3.3±0.33^{cAB}	13.3±1.33^{bA}	4.98	4.000	0.052
48	6.7±0.67^{abB}	10.0±1.0^{bcB}	13.3±1.33^{cAB}	30.0±3.0^{bA}	15.00	7.733*	0.009*
72	10.0±1.00^{abB}	20.0±2.0^{abB}	33.33±3.33^{bAB}	56.7±5.67^{aA}	30.00	13.333*	0.002*
96	23.3±2.33^{aB}	30±3.0^{aB}	53.3±5.33^{aA}	70±7.0^{aA}	44.15	20.792*	<0.001*
Mean	10.00	15.83	25.81	42.50			
F_2	6.933*	12.250*	25.286*	21.424*			
p_2	0.013*	0.002*	<0.001*	<0.001*			

p_1: p-value for comparing the different concentrations each time
p_2: p-value for comparing the different times in each concentration
*: Statistically significant at $p \le 0.05$
Means in the same Column with lowercase letters are not statistically significant
Means in the same Row with uppercase letters are not statistically significant

Table (3): LC_{50} (ppm) values of silicon and silver nanoparticles against *T. castaneum* adults after 4 days

Nanoparticles	Estimated LC_{50} (ppm)	Slope(±SE)	Limits 95%
AgNPs	657.4	2.91 ± 0.78	582.0-751.7
SiNPs	438.3	3.82 ± 0.98	381.4-508.3

Acknowledgements

The authors would like to express their sincere gratitude to the College of Agriculture, University of Basrah, for their support and funding of this study. A special acknowledgment goes to Professor Dr. Mazin Auny Mahdi from the Department of Physics, College of Sciences, University of Basrah, for his valuable assistance in the synthesis and characterization of the nanoparticles.

Contributions of authors

S.S.: Methodology, software, investigation resources data, writing original draft .

A.A. and **M.H.A.**: supervision. read and approved the draft and final manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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كفاءة مركبات السيليكون والفضة النانوية ضد الاصابة بحشرة *Tribolium castanium* (Herbst) (Coleoptera: Tenebrionidae) على حبوب الحنطة مختبرياً

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المستخلص: في السنوات الأخيرة، ظهرت التكنولوجيا النانوية كإحدى التقنيات الجديدة الواعدة لمكافحة الآفات. في هذه الدراسة، تم إجراء برنامج فحص لتقييم تأثير مركبات السيليكون (SiNPs) ومركبات الفضة (AgNPs) على معدل القتل لبالغات حشرة *Tribolium castanium* على حبوب الحنطة. تم تصنيع المركبات النانوية باستخدام الليزر، وتم إجراء توصيفها باستخدام تقنية قياس زيتا، وطيف فوق البنفسجي المرئي، وتشتت الأشعة السينية، والمجهر الإلكتروني النافذ. تم اختبار مستويات تركيز مختلفة من المركبات النانوية للسيليكون والفضة (100 جزء في المليون، 200 جزء في المليون، 300 جزء في المليون، و 400 جزء في المليون) لتقييم تأثيرها على قتل *T. castanium.* . تم حساب قيمة LC50 لمركبات السيليكون والفضة النالنونية عند 438.3 و 657.4 جزء في المليون على التوالي. أظهرت طريقة الاستجابة للتغذية أن مركبات الفضة النانوية أدت إلى قتل 40%، في حين أدت مركبات السيليكون النانوية إلى قتل 70%. تشير هذه النتائج إلى أن المركبات السيليكون النانوية المصنعة لها إمكانية أكبر كبديل في إدارة الآفات في المنتجات المخزنة مقارنة بمركبات الفضة النانوية في نفس فترة التعرض (أربعة أيام). أظهرت النتائج أن كل من مركبات السيليكون والفضة النانوية لهما كفاءة ضد بالغات *T. castanium.*

الكلمات المفتاحية: التكنولوجيا النانوية، مكافحة الآفات، *Tribolium castaneum*، مركبات السيليكون النانوية، مركبات الفضة النانوية.