استخدام بعض المواد النانوية كمبيدات حشرية ضد خنفساء الحبوب المنشارية
Oryzaephilus surinamensis L. (Coleoptera: Silvanidae)

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الخلاصة

جمعت عينات من رز العنبر المصاب بحشرة خنفساء الحبوب المنشارية من الأسواق المحلية في مدينة كركوك خلال شهر كانون الأول 2021 ، وأجريت التجارب في مختبرات كليتي التربية للعلوم الصرفة و الطب البيطري بجامعة كركوك للمدة من شباط ولغاية أيار 2022 .

استخدم ثلاث مواد نانوية في هذه الدراسة شملت (الذهب النانوي ، ثنائي أوكسيد التيتانيوم النانوي و أوكسيد الحديد النانوي) درست التأثيرات السمية لهذه الجسيمات النانوية بأربعة تراكيز مختلفة (0 ، 500 ، 1000 ، 1500) ppm على حياتية خنفساء الحبوب المنشارية كمعدل وضع البيض ، مدة طوري اليرقي و العذري ونسبة القتل لبالغاتها .

أظهرت هذه المواد تأثيرا معنويا على الجوانب الحياتية للحشرة ،حيث بلغت مدة حضانة البيض 11.08 يوم في معاملة ثنائي أوكسيد التيتانيوم بينما بلغت مدة حضانة البيض 10.62 و 9.99 يوما في معاملتي الذهب النانوي و أوكسيد الحديد النانوي على التوالي ، بالمقارنة مع معاملة المقارنة والتي بلغت 8.59 يوما. أظهر ثنائي أوكسيد التيتانيوم تأثيرا معنويا على مدة الطور اليرقي حيث بلغ 18.84 يوم بينما بلغ في معاملتي الذهب النانوي و أوكسيد الحديد النانوي بلغت 18.03 و 17.32 يوما على التوالي ، بالمقارنة مع معاملة المقارنة والتي بلغت 15.5يوما . أما في طور العذراء فقد بلغت المدة 5.93 يوما في معاملة ثنائي أوكسيد التيتانيوم ولم يكن هناك فروق معنوية بين كل من الذهب النانوي و أوكسيد الحديد النانوي فقد بلغت 5.42 و 5.38 يوما على التوالي ، بالمقارنة مع معاملة المقارنة والتي بلغت 4.00 يوما. وأظهرت المواد النانوية تأثيرا واضحا في نسب القتل فقد بلغت في بالغات حشرة خنفساء الحبوب المنشارية 100 % بعد 16 يوم من بدء التجربة ، بالمقارنة مع معاملة المقارنة والتي بلغت 45.55 % . وبناءا على نتائجنا يمكن استخدام المواد النانوية كمبيدات فعالة للسيطرة على الحشرات المخزنية وخاصة خنفساء الحبوب المنشارية .

الكلمات المفتاحية : خنفساء الحبوب المنشارية ، تقنية النانو ، AuNPs ، IONPs ، TiO_2NPs ، الرز

use some nanomaterials as insecticides against the saw-toothed grain beetle *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae)

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Abstract

Samples of rice amber infected with the saw-toothed grain beetle were collected from local markets in the city of Kirkuk during the month of December 2021, and incubator the experiments were conducted in the laboratories of the Colleges of Education for Pure Sciences and Veterinary Medicine at the University of Kirkuk for the period from February to May 2022, Three nanomaterials were used in this study, which included (nano gold, nano titanium dioxide and nano iron oxide). The toxic effects of these nanoparticles were studied at four different concentrations (0, 500, 1000, 1500) ppm on the life of the saw-toothed grain beetle as the rate of egg laying duration Larval and pupa phases and the rate of killing of its adults. The incubation period for the eggs reached 11.08 days in the titanium dioxide treatment while the incubation period for the eggs reached 10.62 and 9.99 days in the nano gold and nano iron oxide treatments respectively, Compared to the comparison treatment, which was 8.59 days . titanium dioxide showed a significant effect on the duration of the larval stage which amounted to 18.84 days while it reached 18.03 and 17.32 days in the nano gold and iron oxide treatments respectively, Compared with the comparison treatment, which amounted to 15.5 days . while in the pupa stage the period reached 5.93 days in the titanium dioxide treatment and there were no differences Significant differences between gold nanoparticles and iron oxide nanoparticles were 5.42 and 5.38 days respectively, compared to the comparison treatment, which amounted to 4.00 days. and the nanomaterials showed a clear effect on the rates of Dead as it reached 100 % in adults of the sawn grain beetle 16 days after the start of the experiment, Compared to the

comparison treatment, which amounted to 45.55 % . Based on our results nanomaterials can be used as effective pesticides to control insects especially saw-toothed grain beetle .

Key Words : *Oryzaephilus surinamensis* , Nanotechnology **,** AuNPs **,** IONPs **,** TiO_2NPs , Rice .

Introduction

Rice (*Oryza Sativa* L.) is the second most important cereal crop in economic terms after wheat as it is one of the main foods for more than two and a half billion people in the world [1] Asia is the largest producer and consumer of it [2] and , [3] Insects generally destroy stored products by eating them directly [4] and store insects cause an increase in temperature and humidity in commodities and fungi encourage growth and seed germination [5] and . Thus cause pollution and economic loss by reducing the quality and quantity of stored commodities [6] , So thus is one of the well-known pests in granaries that is widespread in the world as it has the ability to cause damage to different types of grains [7] . Severe infections cause great losses in the percentage of weight loss, as in addition to grains it attacks dry fruits, nuts, sugar and sweets [8] . Tobacco, dried meat, and many plant products consumed by humans [9]. The insect goes through four stages: the egg, the larva, the pupa stage, and the adult. The egg phase is characterized by white color and oval shape, and with the passage of time it becomes dark brown and the larva is white in color and contains from the front with brown pigmentation and a pointed body in the back that later turns to yellow and then brown. . The Virgin is inside a cocoon[10] . The adult insect is small, flat, dark brown in color, about 3 mm long. It is called the serrated grain beetle because it has six serrated teeth on each side of its pectoral rings [11] . The importance of using nanotechnology is the use of tiny particles ranging in size from 1 to 100 nanometers, as these particles possess new physical, chemical and biological properties and herein lies the advantages of these unique particles [12].Nano-pesticides are one of the alternatives to overcoming conventional groups of pesticides to reduce the environmental risks of the active substances in chemical pesticides[13] .

Our study aims to evaluate the effect of some Nanomaterials (gold nanoparticles (AuNPs , iron oxide nanoparticles IONPs and titanium dioxide nanoparticles TiO2NPs) on the life of the sawfly beetle because of this insect's great impact on stored grains especially rice grains . we decided to study the effect of these materials against this insect to find out the possibility of benefiting from these materials as future insecticides due to their low cost ease of use and friendliness with the environment compared to chemical pesticides.

Materials and working methods

Breeding of the sawtooth grain beetle *Oryzaephilus surinamensis*

Sawtooth grain beetle was collected from infected rice seeds from some local market stores in the city of Kirkuk for the period from the twenty-sixth of December 2021 to the twenty-ninth of it good ventilation tied with rubber belts, and placed in the incubator at 2±25 C° and relative humidity 5±65 % the insect was allowed to reproduce and lay eggs for 10 days then removed and obtained new generation individuals [14].

Nanoparticles used in the study

Table 1 showing the Nanoparticles used in the study

Nanomaterials	Size (nm)	Purity %	Color
Gold nanoparticles	28	99.97+	Pink
Iron oxide nanoparticles	20 - 40	98+	Red
Titanium dioxide nanoparticles	20	99+	White

Prepare a base solution of ppm1500 for each of the above-mentioned nanomaterials by dissolving 1.5 g of it in 1 liter of distilled water in order to obtain a homogeneous solution and reduce the agglomeration of the sample a device (Silent Crusher Homogenizer _ Heidolph 10rmp×1000) was used for 10 minutes for each solution and a series of concentrations was prepared (0 , 500 , 1000 , 1500) ppm of diluting the base solution for each of the materials used in the study using distilled water.

Calculation of the incubation period of eggs, the period of the larval stage and the pupa stage of the sawtooth grain beetle

10 gm of crushed rice seeds were placed in Petri dishes and for each repeater as the crushed rice seeds of the aforementioned size is the best food medium for obtaining insect larva and pupa and 5 pairs (5 females + 5 males) newly hatched were placed inside each plate and the dishes were closed with openings For the purpose of ventilation by boring cloth and placed inside the incubator at a temperature of 2±25 C° and relative humidity of 5±70 % with daily monitoring. And when observing the

change in the color of the eggs the time period for hatching eggs the duration of the larval stage and the pupa stage were studied by taking a number of seeds and carefully breaking them under the microscope.

Calculation of the death percentage of adults of the sawtooth grain beetle

Rice seeds treated with nanomaterials were placed in glass bottles with a capacity of 300 ml at a rate of 20 g/bottle and for three replications in addition to the comparative treatment. Five pairs of the newly hatched insect (5 females + 5 males) were introduced to each bottle with its nozzle covered tightly by the boring cloth and tied Rubber belts were placed in the incubator at a temperature of 2±25° C and the relative humidity was set at 5±65 % the number of dead insects was counted every two days for 16 days, and in each replicate a reading of the percentage of Dead was taken according to the equation [15].

$$\text{Percentage of Dead Ratio} = \frac{\text{Dead Insects Number}}{\text{Total Insects Number}} \times 100\%$$

Statistical analysis

Our experiments were designed using a Completely Randomized Design (C.R.D the arithmetic averages of the coefficients were compared using Duncan's Multiple Range test with a probability level of 0.05 and the results were analyzed using SAS version 6 . [16]

Characterization of Nanomaterials

The phenotypic characteristics of nanomaterials were studied using Transmission Electron Microscopy (TEM) and Zeta potential .

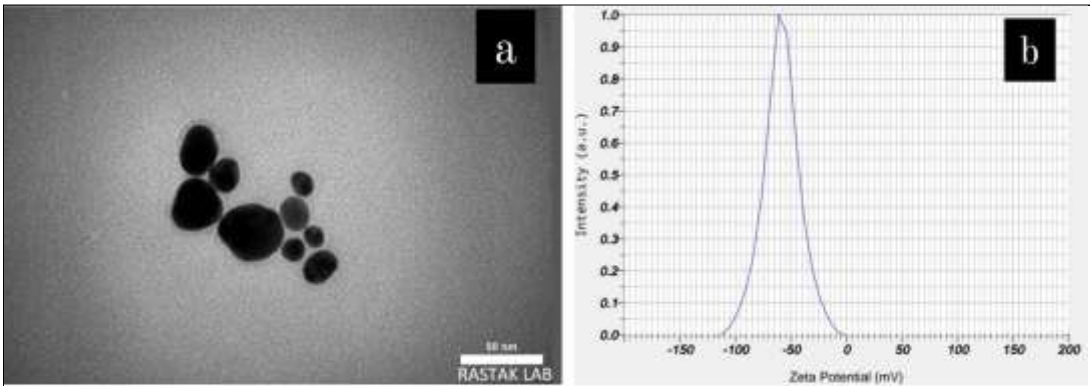

Figure 1 : Characterization of AuNPs (a) TEM image showing monodispersed AuNPs (b) Zeta potential

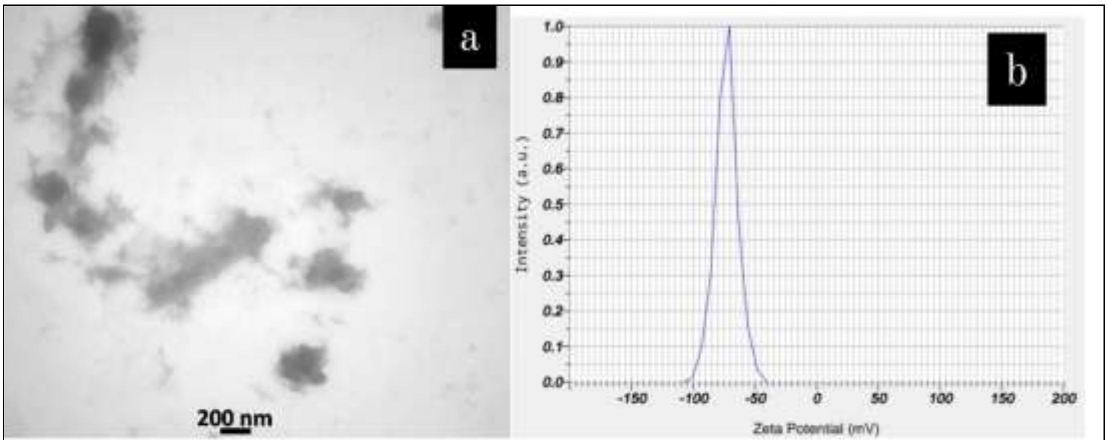

Figure 2 : Characterization of IONPs (a) TEM image showing monodispersed IONPs (b) Zeta potential

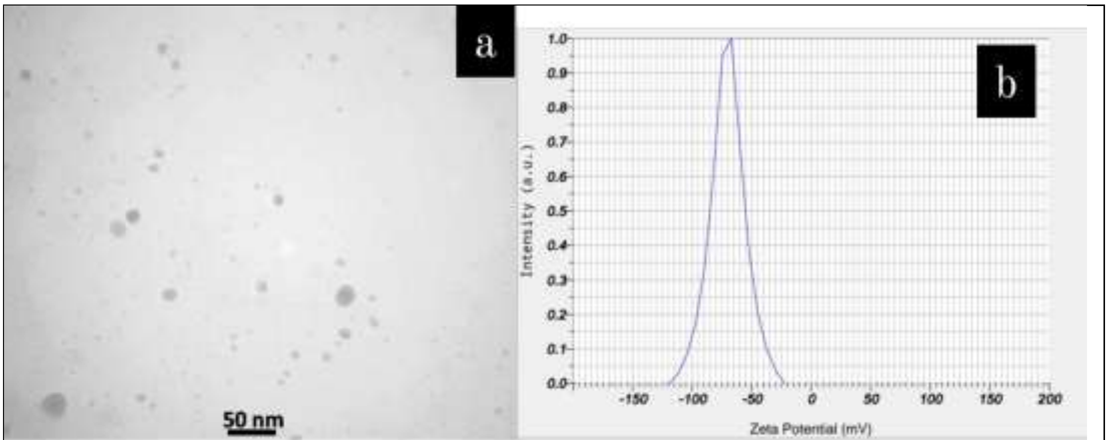

Figure 3 : Characterization of TiO_2NPs (a) TEM image showing monodispersed TiO_2NPs (b) Zeta potential

Effect of nanoparticles on the incubation period of egg stage

In the serrated grain beetle The results in Table 2 show the effect of nanomaterials on the incubation period of eggs, as we note that nano-titanium dioxide recorded the highest effect in prolonging the incubation period, which amounted to 13.34 days while there were no significant differences between the effect of iron oxide and nano-gold as their average effect was 11.48 and 12.68 days respectively and the interaction between the materials and their concentrations showed that there were no significant differences between iron oxide and gold nanoparticles at the concentration of 500 ppm as the average effect of iron oxide at a concentration of 500 ppm was 9.33 days and the rate of nano-gold was 9.50 days at the same concentration while there were significant differences at the concentrations of 500 , 1000 and 1500 ppm which reached 9.50 , 11.69 and 12.68 days respectively in gold nanoparticles and reached 9.33, 10.53 and 11.48 days respectively in iron oxide nanoparticles and reached 10.17, 12.22 and 13.34 days respectively in Nano titanium dioxide The results also show that the incubation period is directly affected by the concentrations as it showed that the incubation period increases the higher the concentration so the 1500 ppm concentration exceeded all concentrations as it reached 12.50 days Through the air hole the small size of these particles leads to embryo poisoning and this means that the nanomaterials affect the life aspects of the sawn grain beetle and thus it can act as insecticides. These results are consistent with what was found by [17].

Table 2 Effect of nanoparticles on the incubation period of the eggs of the saw toothed grain beetle

Con. / N.M.	**Egg incubation period\day**				**Nano material impact rate**
	0	**500**	**1000**	**1500**	
Nano gold	8.59 g	9.50 f	11.69 d	12.68 b	10.62 b
Nano iron oxide	8.59 g	9.33 f	10.53 e	11.48 d	9.99 c
Nano titanium dioxide	8.59 g	10.17 e	12.22 c	13.34 a	11.08 a
LSD 5 %	0.25				0.21
Effect rate of nanoparticales	8.59 d	9.67 c	11.48 b	12.50 a	
LSD 5 %	0.43				

Numbers in the same field and bearing different letters differ from each other statistically at the 5% probability level.

Effect of nanoparticles on the larval stage of the saw toothed grain beetle

The results of Table 3 show that the period of the larval stage is directly affected by the concentrations as it turns out that the period of the larval stage increases with the increase in the concentration as the concentration of 1500 ppm exceeded all concentrations and the period of the larval stage at this concentration reached 21.6 days. Between nano titanium dioxide on the one hand and gold and nano iron oxide on the other hand nano titanium dioxide had the most effect in prolonging the period of the larval stage reaching 18.84 days While the period of the larval stage reached 18.03 and 17.32 days in each of the gold and iron oxide nanoparticles respectively the interaction between the materials and their concentrations shows that there are significant differences between the nanomaterials so the period of the larval stage increases with the increase in concentrations. These results are consistent with the evidence of [18] that the materials The nanoparticles prolonged the time of the larval stage of the southern cowpea beetle, possibly because the nanomaterials act as trypsin inhibitors and disrupt the growth and reproduction processes.

Table 3 Effect of nanoparticles on the period of the larval stage of the saw toothed grain beetle

Con. / N.M.	Larval phase period\day				Nano material impact rate
	0	**500**	**1000**	**1500**	
Nano gold	15.50 f	16.67 e	18.40 d	21.53 b	18.03 b
Nano iron oxide	15.50 f	16.33 fe	17.26 e	20.17 c	17.32 c
Nano titanium dioxide	15.50 f	17.23 e	19.53 c	23.10 a	18.84 a
LSD 5 %	0.54				0.47
Effect rate of nanoparticales	15.50 d	16.74 c	18.39 b	21.60 a	
LSD 5 %	0.93				

Numbers in the same field and bearing different letters differ from each other statistically at the 5% probability level.

Effect of nanoparticles on the Pupal phase period of the saw toothed grain beetle

The results from Table 4 show that there are significant differences in the rate of effect of each of the nanomaterials used in the study as the results showed the superiority of nanomaterials as the nano-titanium dioxide reached when the average period of the pupal phase reached 5.93 days while there are no significant differences between each of the nanomaterials. Iron nanoparticles at a rate of 5.38 days and nano-gold at a rate of 5.42 days respectively the rate of effect of the concentration of nanomaterials also shows that the period of the pupal phase is directly proportional to the concentrations the higher the incubation period as we notice that the concentration exceeds 1500 ppm at a rate of 7.89 days. The interaction between the nanomaterials and their concentrations showed that there were no significant differences at the concentration of 500 ppm between the nanomaterials used in the current study as the average period of the virgin phase reached 4.67 , 4.6 and 4.57 days in each of gold , iron oxide and titanium dioxide nanoparticles, respectively, and the superiority of two Nano titanium oxide at concentrations 1000 and 1500 ppm on each of gold and nano iron oxide at the same concentrations where the period of the pupal phase reached 6.43 days in titanium dioxide at a concentration of 1000 ppm while there were no significant differences between gold and iron oxide which amounted to 5.4 and 5.58 day in a row The period of the pupal phase was 8.7 days in titanium dioxide at a concentration of 1500 ppm while there were no significant differences between gold and iron oxide, as it reached 7.63 and 7.33 days respectively meaning that nanomaterials have an effect in prolonging the pupal phase period. The reason may be that Nanomaterials affect the growth and division of insect cells and thus affect the time required for the insect to transform from a virgin to an adult insect and these results are in line with what [19] said that nanomaterials have a significant effect in increasing the period of the pupal stage.

Table 4 Effect of nanoparticles on the pupal period of the serrated grain beetle

Con. / N.M.	Pupa phase period \day				Nano material impact rate
	0	**500**	**1000**	**1500**	
Nano gold	4 f	4.67 e	5.40 d	7.63 b	5.42 d
Nano iron oxide	4 f	4.60 e	5.58 d	7.33 b	5.38 b
Nano titanium dioxide	4 f	4.57 e	6.43 c	8.70 a	5.93 a
LSD 5 %	0.21				0.18
Effect rate of nanoparticales	4 d	4.61 c	5.80 b	7.89 a	
LSD 5 %	0.36				

Numbers in the same field and bearing different letters differ from each other statistically at the 5% probability level.

Effect of nanoparticles on the killing rate of the sawtooth grain beetle The killing percentage was calculated from the second day of the experiment, and we note from the results in Table 5 that there were significant differences from the second day between the nanomaterials and the superiority of nano-titanium dioxide from the second day over the rest of the materials in the percentage of killing while there was no significant difference between any of the materials. On the sixteenth day of the experiment as for the average effect of the concentrations of nanomaterials used in the study the results showed that the concentration exceeded 1500 ppm since the second day of the experiment as the killing rate was 34.62 % while the killing rate on the same day was 0% In the control treatment and this superiority continues until the fourteenth day of the experiment we notice the superiority of the concentration 1500 ppm at a rate of 99.43 % followed by the concentration 1000 ppm at a rate of 98.83 and then the concentration 500 ppm at a rate of 97.08 % and we note on the sixteenth day that there is no significant difference in the rate of killing rate for all concentrations All insects were killed in the treatments while the rate of killing in the Table 5 Effect of nanomaterials on the percentage of killing (%) in the saw toothed grain beetle

Nano material impact rate\days		2	4	6	8	10	12	14	16
Nano gold		11.94 b	56.45 a	62.38 a	68.11 a	71.81 a	78.61 b	84.39 a	86.39 a
Nano iron oxide		3.31 c	13.20 c	29.29 b	43.99 b	56.33 b	72.94 c	80.86 b	86.39 a
Nano titanium dioxide		26.27 a	51.21 b	61.79 a	68.50 a	73.12 a	80.23 a	84.54 a	86.39 a
LSD 5 %		1.73	2.37	1.52	1.66	1.92	1.45	0.85	0.19
rate of effect of concentration of nanomaterials									
0		0.00 c	2.97 d	8.55 d	11.08 d	13.22 d	25.00 c	38.15 d	45.55 b
500		9.42 b	41.88 c	55.94 c	65.91 c	75.98 c	91.13 b	97.08 c	100 a
1000		11.33 b	52.54 b	64.67 b	76.87 b	86.85 b	96.21 a	98.38 b	100 a
1500		34.62 a	63.75 a	75.47 a	86.94 a	92.27 a	96.69 a	99.43 a	100 a
%5 LSD		2.00	2.73	1.75	1.92	2.21	1.67	0.99	0.22
Interaction between nanomaterials and their concentrations									
Nano gold	**0**	0.00 d	2.97 g	8.55 i	11.08 h	13.22 f	25.00 d	38.15 d	45.55 b
	500	13.57 c	66.91 c	73.99 d	75.57 d	78.53 d	89.43 c	99.40 a	100 a
	1000	15.83 cb	75.97 b	80.66 c	91.36 b	96.37 ba	100 a	100 a	100 a
	1500	18.38 b	79.96 b	86.33 b	94.42 b	99.00 ba	100 a	100 a	100 a
Nano iron oxide	**0**	0.00 d	2.97 g	8.55 i	11.08 h	13.22 f	25.00 d	38.15 d	45.55 b
	500	0.00 d	13.97 f	29.59 h	46.41 g	66.13 e	88.07 c	91.85 c	100 a
	1000	0.00 d	15.40 f	36.59 g	52.07 f	68.17 e	88.63 c	95.15 b	100 a
	1500	13.25 c	20.47 e	42.44 f	66.40 e	77.82 d	90.07 c	98.30 a	100 a
Nano titanium dioxide	**0**	0.00 d	2.97 g	8.55 i	11.08 h	13.22 f	25.00 d	38.15 d	45.55 b
	500	14.70 c	44.77 d	64.23 e	75.77 d	83.27 c	95.90 b	100 a	100 a
	1000	18.17 b	66.27 c	76.77 d	87.17 c	96.00 b	100 a	100 a	100 a
	1500	72.22 a	90.83 a	97.63 a	100 a	100 a	100 a	100 a	100 a
LSD 5 %		3.46	4.74	3.03	3.33	3.83	2.89	1.71	0.38

Numbers in the same column with different letters differ from each other statistically at the 5% probability level.

Control treatment was 45.55 % . It is clear from the above that the killing rate is directly proportional to the concentration used . The results of the interaction between the nanomaterials and their concentrations on the second day showed that the nanoparticles of titanium dioxide with a concentration of 1500 ppm had a killing rate of 72.22 % followed by nano-gold with a killing rate of 18.38 % at a concentration of 1500 ppm and on the fourth day the results show that the treatment of nano-titanium dioxide particles at a concentration of 1500 ppm was 90.83% and then followed by nano-gold at a concentration of 1500 ppm with a killing rate of 79.96 % and then the concentration of 1000 ppm for gold nanoparticles with a killing rate of 75.97 % with no significant differences between the two concentrations. On the sixth day the superiority of the treatment of nano-titanium dioxide with a concentration of 1500 ppm was observed as the killing rate reached 97.63 % and then followed by nano-gold with a concentration of 1500 ppm With a killing rate of 86.33 % on the eighth day the killing rate was 100 % by treating nano-titanium dioxide with a concentration of 1500 ppm followed by nano-gold at a concentration of 1000 and 1500 ppm with a killing rate of 91.36 % and 94.42 % respectively. With no significant differences between the two concentrations and on the tenth day we note the superiority of the treatment of nanoparticles treated with titanium dioxide at a concentration of 1500 ppm over most of the other concentrations while on the twelfth and fourteenth days we notice the superiority of titanium dioxide and nano gold at concentrations 1000 and 1500 ppm with a killing rate. It reached 100 % while on the sixteenth day the killing rate was 100 % for all treatments which indicates the death of all insects on that day while the killing rate on the same day was 45.55 % in the control treatment. The reason for the high killing rate may be due to the toxic effect of nanoparticles inside the insect's cuticle as it affects the physiological processes as it destroys many vital molecules such as enzymes and protein decomposition and affects the permeability of the plasma membrane. Treatment with nanoparticles helps them to enter their digestive system and because of the high permeability of these materials, they reach their cells which leads to cell death and damage to its DNA and thus its death in addition nanomaterials have significant effects on insect antioxidants and detoxification enzymes which leads to oxidative stress and cell death and nanomaterials can affect membrane permeability and thus denaturation of the organelle and enzyme followed by cell death as [20] used titanium dioxide particles with a size of 179.6 nm against the diamond butterfly insect and the values of LC25, LC50, LC75, and LC95 were (20.81, 37.57, 67.83, 158.69) respectively and the results are consistent with what was indicated by [16] which showed when using silver nanoparticles against the sawfly grain beetle were The killing rate is 86.7 % after three days of treatment the study of [21] also showed the lethal effect of titanium oxide nanoparticles against adults of the sunn bug as the percentage of killing in the phase was 41.15 % at the concentration of 100 mg/l compared to the control treatment which amounted to 0%. Also the study of [22] showed the effect of using zinc oxide silver and nano selenium against the small grain borer bug a clear effect on the rate of killing in its adults the killing rate reached 100 % after 9 days from the start of the experiment and the results converge with what [23] showed Where it achieved the highest killing rate of 74 % at 8000 ppm concentration after 96 hours of treatment with silver nanoparticles. As for the reason for increasing concentration causing an increase in killing rates it may be due to the fact that increasing the number of minutes leads to its ability to penetrate the cuticle and reach important centers and influence faster [24] Nanoparticles penetrate the exoskeleton of the insect [25] and then bind with sulfur to proteins or phosphorous from DNA which leads to rapid deformation of organelles or enzymes, and this causes reduced membrane permeability protein movement disturbance loss of cellular function and then programmed cell death. Hence the insect's death [26] The toxic effect of nanoparticles may be attributed to the absorption of nanoparticles by the insect wall and this leads to the breakdown of the waxy barrier composed of fats such as fatty acids which serve as a protection to prevent water loss or evaporation because the loss of the wax barrier causes dehydration and then death of the insect [27] The genotoxicity of nanoparticles results from the formation of reactive oxygen species (ROS) either by the particles themselves by inducing cellular responses or stimulating target cells and ROS results in the formation of OH which is one of the species harmful to the DNA of the insect [28] . [29] showed that the insect brain is also vulnerable to oxidative stress through high concentrations of peroxidizase enzyme saturated fatty acids, high rate of oxygen consumption and lack of antioxidant enzymes, as well as various nanoparticles can cross the blood-brain barrier and reach the central nervous system as well as nanoparticles can inhibit the action of (ACHE) Acetylcholinesterase which is one of the important enzymes in the work of the nervous system which is responsible for transmitting nerve impulses through the hydrolysis of the neurotransmitter acetylcholine and acetic acid in the synapses of the insect As shown by [30] to the reason for the death of the different stages of insects is attributed to the fact that nanoparticles can cause abnormalities or abnormalities in chromosomes and damage the DNA, which leads to cell damage and death.

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