



Comparing the effect of nano-neem extract with nano-zinc oxide in controlling the whitefly *Bemisia tabaci*

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Abstract:

The study was conducted in the Graduate Studies Laboratory - Department of Life Sciences - College of Education - Samarra University in the period from 8/1/2024 to 4/22/2025, Integrated Pest Management (IPM) and molecular diagnosis in controlling the whitefly (Hemiptera: Aleyrdidae) *Bemisia tabaci* in Samarra. The results of this study reached the following: Nanotechnology was used for two compounds, namely zinc oxide and nano neem, and it gave good results in killing the whitefly. The control factor in the experiment was distilled water only. The experiment showed that the killing rate was good compared to other control methods, as it was higher than the rest of the treatments. The results of Table 5 showed significant differences in the killing rates due to the interaction between concentration and exposure duration, as the highest rate was 96.7% after 72 hours of treatment, while the lowest killing rate was 33.3% after 24 hours of treatment, while the average killing rate with the effect of concentration showed that the highest killing rate was at a concentration of 0.625, which amounted to 76.67%, while the lowest average killing rate was at a concentration of 0.250, which amounted to 31.10%. As for the average killing rate with the effect of killing duration, the highest killing rate was after 72 hours, which amounted to 74.47%, and the lowest killing rate was after 24 hours, which amounted to 33.30%. The results showed that the whitefly killing rate increased with increasing concentration and treatment duration.

key words: *Bemisia tabaci*, zinc oxide, nano neem, , whitefly, neem

مقارنة تأثير مستخلص النيم النانوي مع أكسيد الزنك النانوي في مكافحة الذبابة البيضاء *Bemisia tabaci*

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الملخص:



أُجريت الدراسة في مختبر الدراسات العليا - قسم علوم الحياة - كلية التربية - جامعة سامراء، في الفترة من 2024/8/1 إلى 2025/4/22، باستخدام الإدارة المتكاملة للآفات والتشخيص الجزيئي في مكافحة الذبابة البيضاء (نصفيات الأجنحة: *Bemisia tabaci* Aleyrodidae) في سامراء. النتائج توصلت هذه الدراسة إلى النتائج التالية: استُخدمت تقنية النانو لمركبين، هما أكسيد الزنك والنيم النانوي، وأعطت نتائج جيدة في القضاء على الذبابة البيضاء. وكان عامل الضبط في التجربة هو الماء المقطر فقط. وأظهرت التجربة أن معدل القتل كان جيدًا مقارنةً بطرق الضبط الأخرى، إذ كان أعلى من باقي المعاملات. أظهرت نتائج الجدول 5 فروقًا معنوية في معدلات القتل نتيجةً للتفاعل بين التركيز ومدة التعرض، حيث بلغ أعلى معدل قتل 96.7% بعد 72 ساعة من المعالجة، بينما بلغ أدنى معدل قتل 33.3% بعد 24 ساعة من المعالجة. أما متوسط معدل القتل بتأثير التركيز، فقد أظهر أن أعلى معدل قتل كان عند تركيز 0.625، والذي بلغ 76.67%، بينما بلغ أدنى معدل قتل عند تركيز 0.250، والذي بلغ 31.10%. أما بالنسبة لمعدل القتل المتوسط بتأثير مدة القتل، فقد سُجِّل أعلى معدل قتل بعد 72 ساعة، حيث بلغ 74.47%، وأدنى معدل قتل بعد 24 ساعة، حيث بلغ 33.30%. وأظهرت النتائج ارتفاع معدل قتل الذبابة البيضاء بزيادة التركيز ومدة المعاملة.

الكلمات المفتاحية: *Bemisia tabaci*، أكسيد الزنك، نيم نانوي، ذبابة بيضاء، نيم

Introduction:

The whitefly species of the Aleyrodidae family, belonging to the order Hemiptera, are among the most dangerous pests that infect many agricultural crops around the world, as they are capable of feeding on a wide range of crops inside greenhouses and cause material losses to agricultural production in the world, the Arab world, and Iraq. The percentage of losses due to this pest between 1980 and 2000 was approximately \$10 billion (1). *Bemisia tabaci* is a deadly pest that causes significant damage to greenhouse products (2).

Studying the mechanism of toxic effect of different types of nanoparticles on different organisms such as bacteria, fungi, and fungi (3) found that sublethal doses of silver nanoparticles (AgNPS) affect the process of hardening in the body wall of adults such as fruit flies, as it was observed that the wall becomes white with a pale color, The researcher explained that the silver nanoparticles caused a decrease in dopamine levels directly by targeting the genes and enzymes responsible for the dopamine pathway.

Nanoparticles are divided into two main groups: organic nanoparticles, which include carbon nanoparticles (alkali), and the second group is inorganic nanoparticles, which include magnetic nanoparticles, nanoparticles of noble metals (gold and silver), as well as semiconductors (titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles because of their distinctive properties and many uses and functions in various medical, agricultural, and industrial fields (4).

The aim of the study:

1- Bio-manufacture of silver nanoparticles, zinc oxide nanoparticles, neem nanoparticles and their effectiveness on the whitefly *Bemisia tabaci*.



2 - Due to the lack of research studies on this insect, many researchers have worked in this field and discovered modern methods and techniques for an integrated program to control the whitefly insect *Bemisia tabaci* on plants and crops and reduce the number of whiteflies on the crop by controlling the immature and adult stages to reduce the numerical density of the whitefly.

Materials and Methods:

The current study was conducted in the Graduate Studies Laboratory / Samarra University / College of Education / Department of Life Sciences / from August 2024 to March 2025 AD. 2-3- 2 Laboratory rearing of the whitefly *Bemisia tabaci*:

Sites were selected from Salah al-Din Governorate / Samarra District / Al-Huwaish, where large quantities of the whitefly *Bemisia tabaci* are present, due to the availability of crops infested by the fly during the summer. The insect is active in large numbers, and these farms were not treated with pesticides to ensure accurate results in the experiments conducted in our study during the months of June, July, and August. *Bemisia tabaci* was classified at the University of Baghdad / Research Center / Museum of Natural History in book number (10) dated (February 13, 2024), issue 240. *Bemisia tabaci* were collected in 1-liter plastic containers prepared for this purpose. The leaves of the plants infected with the insect were transferred to the incubator under laboratory conditions at a temperature of $26 \pm 2^\circ\text{C}$, a relative humidity of $5 \pm 65\%$, and a light period of 10-14 hours. They were then placed in the rearing cage. The whitefly *Bemisia tabaci* had dimensions of 85 cm length x 50 cm width x 85 cm height.

Preparation of *V. lecanii* biomass:

To obtain biomass, the fungal isolate was grown in a sterile Petri dish containing sterile potato dextrose agar (PDA) medium. The culture medium was incubated at $2 \pm 26^\circ\text{C}$ and $5 \pm 85\%$ relative humidity for 7 days. Four 5 mm diameter discs were then taken from the colonies grown on the PDA solid medium and seeded onto the sterile liquid medium placed in a 1000 ml glass container with the addition of 125 mg of tetracycline to prevent bacterial growth. The glass containers were continuously stirred and incubated at $2 \pm 26^\circ\text{C}$ for 21 days with daily manual shaking. The biomass was harvested after 21 days of incubation using a glass funnel and filter paper. The biomass was then washed with distilled water three times, followed by washing with deionized water twice to remove all residual media. Weigh 10 g of fungal biomass. Using a sensitive balance, it was transferred to 1000 ml glass containers containing 250 ml of deionized water and also incubated under the same conditions above with daily shaking using a shaker for 120 hours. After the period, the mushroom biomass was filtered using filters to obtain the mushroom biomass filtrate. The filtrate was collected and incubated at a temperature of $2 \pm 26^\circ\text{C}$ and a relative



humidity of $5 \pm 75\%$ until use (5). The biomass is then dried to obtain a powder for experiments on whiteflies.

After the fungal mat has formed, the fungal mass is filtered using sterile Whatman filter paper (PaperNO 1) and washed thoroughly with sterile distilled water to remove any residual medium. The fungal mass is then placed in a conical flask containing 100 ml of sterile distilled water and left in an incubator for 72 hours. The fungal mass is filtered again using sterile filter paper to ensure all medium components are removed. Then, 10 g of the fungal mass is weighed and placed in 100 ml of a 1 mM AgNO_3 solution and placed in an incubator in complete darkness. After the period has elapsed, we filtered the biomass again using Whatman filter No. 1 filter paper using centrifugation at 6,000 rpm for 10 minutes. We took 100 ml of the fungal biomass filtrate and added 100 ml of the previously prepared 1 mM zinc nitrate solution (ZnNO_3). We left 100 ml of the filtrate for comparison and further testing. All flasks were placed in a shaking incubator at 100 rpm at $25-26^\circ\text{C}$ under dark conditions for 96 hours (6).

Bio-prepared nanocomposite from the fungus *V. lecanii*

The zinc oxide nanoparticles (ZnONPs) used in this study were obtained from the Ministry of Science and Technology in Baghdad, Iraq. The compound was in the form of a yellowish-white powder with a particle size of less than 5 micrometers and a purity of 99%. The ministry prepared the compound in a plastic container containing 7 grams, and it was received in the form of a nanopowder with a particle size of less than 100 nanometers. Preparation of the nanocomposite:

Zinc nanoparticles were synthesized by crushing the mushroom extract using an ultrasonic device for five minutes. The previously prepared zinc oxide solution was then placed on a hot plate with a magnetic stirrer for 30 minutes. The mushroom extract was then added to the zinc oxide solution in droplets, then placed in an ultrasonic device for 30 minutes. The mixture was then mixed with a magnetic mixer without heat for 30 minutes (7). We weighed 0.5 grams of nano zinc oxide powder and placed a drop of concentrated nitric acid on it and mixed it with the powder, noting the rise of vapors from the powder after mixing it with nitric acid. Then, the homogeneous material was placed in a glass flask containing 1000 ml of distilled water with continuous stirring for 10 minutes to ensure the dissolution of the nano compound with water. Thus, we have the main stock concentration. After that, we conduct several dilutions to reach the concentrations required in the experiment, which are a concentration of 0.250 (0.125, 0.062) for a time period of 24, 48, 72, 96)) with three replicates for each concentration. Only distilled water was used as the control factor in this experiment. Monitoring and recording of killing was carried out after 24, 48, and 72 hours.

Statistical Analysis:

The results analyzed statistically by applying the statistical program (MINITAB VER.17) according to the Anova analysis test (Anova), the mathematical



averages were compared according to the Duncuns Multiple Range test and at a possibility of $0.05 \geq p$ (8).

Results:

Whitefly sensitivity to the bio-prepared nano-neem extract:

The results in Table 1 showed significant differences in the kill percentages due to the interaction between concentration and exposure duration. The highest kill percentage was 96.7% after 72 hours of treatment, while the lowest kill percentage was 33.3% after 24 hours of treatment. The average kill percentages, depending on the concentration, showed that the highest kill percentage was at a concentration of 0.625, reaching 76.67%, while the lowest kill percentage was at a concentration of 0.250, reaching 31.10%. The average kill percentages, depending on the duration of the exposure, showed that the highest kill percentage was at 72 hours, reaching 74.47%, and the lowest kill percentage was at 24 hours, reaching 33.30%. The results showed that the whitefly kill percentage increased with increasing concentration and treatment duration. The results of Table (1) demonstrate the effectiveness of nano-neem concentrations on whiteflies, demonstrating that nano-neem was superior to the other nano-treatments used in the experiment within 72 hours of using concentrations (0.250, 0.125, and 0.625). The study results demonstrated that the use of nano-neem at low concentrations of each resulted in the whitefly being unable to fly, unable to feed, and reduced movement and activity, paralyzing it after 24, 48, and 72 hours due to the targeting of its nervous system.

This result is consistent with the study by (9), which used three types of aromatic plant oils. The results showed significant differences between neem oil and the mortality rates for all stages of the tomato moth. The study attributed this to the effect of the active ingredient Azadirachtin (a tetranortriterpenoid) in neem oil, which has a high toxicity on the digestive enzymes of whitefly stages. The results also showed an increase in larval mortality rates with increasing exposure duration and with the nanopesticide application rate compared to conventional, non-encapsulated pesticides. This is consistent with (10), who found that the relative effectiveness of biopesticides increased with increasing concentrations used for all pesticides. Mortality rates were highest at higher concentrations in the experiment and increased with increasing exposure time. (11) found that nanoencapsulation of biopesticides represents an effective method for enhancing insecticide activity and the durability of active ingredients for sustainable pest management. The results of the nanoconcentration of neem oil (3 ml/L) are attributed to the synergy between the size of the nanocapsules and the insecticidal effect of neem oil. The nanocapsules help improve the absorption of neem oil by insects, increasing its effectiveness in killing adults. This technique enables the active compound to be delivered more efficiently to the target tissues (12).



Average kill by concentration	72 ساعة	48 ساعة	24 ساعة	time/hour Concentration/L
55.57 b	76.7	56.7	33.3	0.125
c031.1	50.0	30.0	13.3	0.250
				0.625
76.67 a	96.7	80.0	53.3	
	74.47 a	55.57 b	33.30 c	Average kills by time
d0.0	0.0 d	0.0 d	0.0 d	Control

Table (2) Whitefly sensitivity to bio-prepared nano-neem extract
Effectiveness of Zinc Oxide in Killing Whiteflies

The results in Table 2 showed significant differences in the kill rates due to the interaction between concentration and exposure duration. The highest kill rate was 73.3% after 72 hours of treatment, while the lowest kill rate was 16.7% after 24 hours of treatment. The average kill rate, depending on the concentration, showed that the highest kill rate was at a concentration of 0.250, reaching 74.43%, while the lowest kill rate was at a concentration of 0.6250, reaching 35.57%. The average kill rate, depending on the duration of the exposure, showed that the highest kill rate was at 72 hours, reaching 75.57%, and the lowest kill rate was at 24 hours, reaching 34.43%. The results showed that the whitefly kill rate increased with increasing concentration and treatment duration. The results of the study were consistent with those of (13), who indicated that the zinc oxide nanocomposite had an effective effect in controlling the red flour beetle, *T. castaneum*, compared to the pesticide malathion. The results showed a significant effect of the zinc oxide nanocomposite on the mortality rate, productivity, and weight loss of whole grains. These rates increased with increasing concentration and exposure duration. These particles cause deformities and dehydration of the insect and provide protection for the grains by reducing the rate of the first generation of *T. castaneum*, and subsequently reducing the percentage of weight loss in the grains. The results of this study were also similar to those of (14), who indicated the effect of nanocomposites, including zinc oxide, in protecting grains from infestation by the Khapra beetle for up to 40 days. The weight loss rate was 0.67%, 0.73%, and 3.44%, while the loss rate in the control treatment was 0.73%. 11.74%. Zinc oxide nanoparticles have been used to develop pesticides due to their antimicrobial, physical, and other properties (15).

Table (2) Effectiveness of zinc oxide to kill whiteflies



Average kill by concentration	72 ساعة	48 ساعة	24 ساعة	time/hour Concentration/L
35. 57 c	56. 7 c	33. 3 d	16. 7 e	0.625
74. 43 a	96. 7 a	73. 3 b	53. 3 c	0.250
b053. 3	73. 3 b	53. 3 c	33. 3 d	0.125
	75. 57 a	53. 30 b	34. 43 c	Average kills by time
d0.0	0.0 d	0.0 d	0.0 d	Control

References:

- 1 - **Horowitz** ,A. **Rami** ,et al. (2020) ,Insecticide resistance and itmanagement in *Bemisia tabaci* species. Journal of Pest Science ,93:893,910.
- 2- **Vyskočilova** ,S. ,Tak ,W. T. ,Van Brunschot ,S. ,Seal ,S. ,& Colvin , J.(2018). An integrative approach to discovering cryptic species within the *Bemisia tabaci* whitefly species complex. Scientific Reports ,8 ,10886.
- 3- **Catherine** ,S., D. Reaves , F. Turner & J.Bang. 2011.Impact of Silver Nanoparticles Ingestion on Pigmentation and Development Progression in *Drosophila* .Atlas.J.biology 1(3):52-61.
- 4- **Xu** , Z.P., Q.H.Zeng,G.Q. Lu, A.B.Yu .2006. Inorganic nanoparticles as carriers for efficient cellular delivery. Chemical Engineering Science 61: 1027- 1040.
- 5- **Al-Shammari**, Hazem Eidan Abdul-Hussein (2015). Effect of the predator *Dicrodiplosis manihoti* Harris Diptera: Cecidomyiidae and silver nanoparticles prepared by biological methods on some biological aspects of the citrus mealybug *Planococcus citri* (Risso) Hemiptera: Pseudococcidae, PhD thesis - College of Agriculture - University of Baghdad - Iraq
- 6- **Gupta** , S. and Dikshit, A.K. (2010) Biopesticides: An eco-friendly approach for pest Control. J. Biopesticides 3(1) : 186 – 188.
- 7- **Al-Naimi**, MT. Hamdan, NT. Abdul Rahim, E., and Al-Janabi, Z. (2019). Biological degradation of malathion pesticides by silver bioparticles from extracts of *Bacillus licheniformis*. Crop Research, 20 spl., (79-84).



- 8 - **Reddy**, S. J. (2015). Silver Nanoparticles - Synthesis, Applications and Toxic Effects on Humans: A Review. *International Journal of Bioassays* 4(11) Pp 4563-4573.
- 9 - **Ahmed**, D. M., Mohsen, A. E. A. M., El-Deeb, M. A., Alkhedaide, A., El-Tahan, A. M., & Metwally, E. S. M. (2022). The larvicidal effect of neemazal T/S, clove oil and ginger oil on tomato leafminer, *Tuta absoluta* compared to coragen. *Saudi Journal of Biological Sciences*, 29(3), 1447-1455.
- 10 - **Hanash**, A. A. R. (2023). Estimation insecticidal action of Abamectin and Neem oil against *Tuta Absoluta* insect (Lepidoptera: Glechiidae). *Wasit Journal for Pure Sciences*, 2(4). 131-135
- 11- **Emamjomeh**, L., Imani, S., Talebi Jahromi, K., & Moharramipour, S. (2023). Nanoencapsulation enhances the contact toxicity of Eucalyptus globulus Labill and *Zataria multiflora* Boiss essential oils against the third instar larvae of *Ephestia kuehniella* (Lepidoptera: Pyralidae). *International Journal of Pest Management*, 69(3), 207-214.
- 12- **Fahmi**, M. Z., Suwito, H., Susilo, A., Joeniarti, E., Jaswidi, A. M. R., & Indrasari, N. (2017). Chitosan-based neem seed extract nanocapsules: A new approach on enhancing its effectiveness as an insecticide delivery agent. *Journal of Chemical Technology and Metallurgy*, 52, 6, 1129-1134.
- 13 - **Abdel-Salam**, M. M. (2016). Effect of foliar application of salicylic acid and micronutrients on the berry quality of "Bez El Naka" local grape cultivar. *Middle East J. Appl. Sci.*, 6(1): 178–188.
- 14 - Radhiou, Ghadir Abdul Jabbar. (2020) Evaluation of the efficiency of some commercial nano-compounds and alcoholic extracts of some plants in controlling the hairy grain beetle (*Trogodema granarium* Evest 1898 (coleopteran): Dermestidae) under laboratory conditions. Master's Thesis. College of Agriculture. University of Kufa. Republic of Iraq. Issue 2 (1).
- 15- **Akbar**, S., Tauseef, I., Subhan, F., Sultana, N., Khan, I., Ahmed, U., & Haleem, K. S. (2020). An overview of the plant-mediated synthesis of zinc oxide nanoparticles and their antimicrobial potential. *Inorganic and Nano-Metal Chemistry*, 50(4), 257-271.