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ECO-FRIENDLY METHOD FOR CONTROLLING LAND SNAILS USING CHEMICALLY SYNTHESIZED Ag NANOPARTICLES IN NORTH SINAI, EGYPT

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ABSTRACT

Snails are among the dangerous organisms that affect plant life in North Sinai, Egypt, thus an effective chemical route was developed to destroy the land snails using a chemical solution containing sliver nanoparticles with/without sodium citrate ($Na_3C_6H_5O_7$) in addition to white sugar which act as a reducing agent. An economic system was implemented in order to ensure the formation of silver nanoparticles (AgNPs) due to the color change by Uv-Vis spectroscopy additionally, FTIR, and transmission electron microscopy (TEM) for further characterization which confirm the spherical morphology of AgNPs in the 10–25 nm range. The prepared AgNPs solution was used to be attached to the cell wall of *E. coli* and *Salmonella* sp. and successfully showed a great antibacterial and antifungal activity against the microorganisms. Moreover, the AgNPs solution exhibited excellent performance against land snails which have been felt and crashed in an impressive manner, without affecting the plant to be protected.

INTRODUCTION

The second-largest phylum of animals and the majority of the gastropods in the world are mollusks. It is one of the most varied animal species in terms of appearance and behaviour. Due to abrupt climatic change, which has caused continuing invasion of snails and then increased infestation in orchards, terrestrial snails (subclass: Pulmonata), one of the most widespread kinds of snails, have over 35,000 identified species worldwide. Many snails live in freshwater environments, where helminth larvae also spend some of their lives. Numerous water snails serve as nematode larvae carriers, spreading several diseases. Fasciolosis and schistosomiasis, two illnesses spread by aquatic snails, have a serious negative impact on people and their pets. Snails solely target insects, which is harmful for humans. Many parasite diseases that affect people, animals, and birds can be spread *via* a variety of terrestrial sail species (Mollusca, Gastropoda) **(Godan, 1983; Eshra, 2004)**. In Egypt, pests that impact various crops have been discovered over the past thirty to forty years. In recent years, wild gastropods have emerged as one of the most significant pests in the governorates of Ismaelia and Sharkia **El-Okda (1984) and Ismail (1997)**, respectively. In particular, in the places where they are located. Due to the pollution, the temperature is generally reasonable throughout the year. Fruits, particularly citrus fruits, pomegranates, guavas, and ornamentals, are grown there.

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Land snails and slugs are significant economic pests that infest and seriously harm some economically and important crops, including decorative plants, orchard trees, and vegetable crops. They are common in many governorates **El-Okda (1980) and Azzam (1998)**. To prevent the risks of chemical molluscicides, research on biological control agents is now required. A biological control agent against snails and slugs was Phasmarhabditis hermaphrodita, which was isolated from slugs. There aren't many studies on gastropod parasites.

Common types of Land Snail

Theba pisana (Müller) white snail, and the *Eobania vermiculata* brown garden snail, (Müller), are the most hazardous land snails in Egypt. These snails graze on the leaves of numerous gardens and ornamentals in addition to the citrus species that they harm. Therefore, it is crucial to keep these snails under control. Chemical pesticides are still one of the most efficient ways to control pests today **(El-Shahat *et al*., 2005; El-Shahat *et al.,* 2009).** Nanoparticle preparation is absolutely essential in the modern era; they are made from various elements and have a variety of applications. Environmentally friendly copper nanoparticle synthesis from waste printed circuit boards **Seif El-Nasr *et al.* (2020).** Synthesis of nanocrystalline Cu0.5Zn 0.5 Fe_2O_4 with catalytic activity **(Hassan *et al*., 2016).** The electrocatalytic performance of a Pd/Ce_2O_3 nanocomposite prepared has been improved by **Elkholy *et al.* (2019).**

Silica nanoparticles are synthesised from semi-burned rice straw ash **(Zakya *et al.*, 2008).** Synthesis of Co_3O_4 nanoparticles from amino acids mixed ligands can be used to remove dyes from wastewater (**El-Dossoki *et al.,* 2020).** AgNPs silver nano have been used in potential scientific and research sectors like biotechnical applications in biomedicine **(Xu *et al.,* 2020).** Due to their distinct and varied physical, chemical, and electrical properties, they are used in the environmental, optical, and electronic domains as well as in medication delivery and bio-imaging **(Bilal *et al.,* 2017).** The food and beverage due to its extensive ability to inhibit bacterial and fungal activities as well as its capacity to interact with diverse ligands and big molecules in the microbial cell, silver is one of the most commonly employed metals in the manufacture of nanoparticles. Due to its anti-inflammatory effects, silver has been frequently utilised to treat wounds and reduce microbial growth. AgNPs are used in the pharmaceutical industry because they are stable at a range of temperatures and have little toxicity to human cells.

In order to avoid microbial infections linked to medical tools (catheters, wound dressings, orthopedics and cardiovascular implants), various silver salt coatings are typically used. Researchers from all around the world are paying close attention to the synthesis of AgNPs because of these wide-ranging applications. Because they can combat antibiotic resistance, AgNPs are frequently utilised as antimicrobial agents. Both Gram-positive and Gram-negative bacteria are successfully combatted by them. According to literature, for example, AgNPs attaches to the cell wall of *B. subtilis* and *S. Typhimurium* then creates holes on the membrane that ultimately cause cell's death. AgNPs also interacts with and penetrates the bacterial cell wall, substantially disturbing cellular activity (**Yan *et al.*, 2018; Burdușel *et al.,* 2018).**

Aim of the Work

In this study, it has become more beneficial to create an integrated strategy to get rid of this pest to control and to eliminate land snails in the region of North Sinai, Egypt. While protecting the environment from contamination. A new chemical route for synthesis of AgNPs were implemented then the prepared is well characterized then we apply sliding nanoparticles (AgNPs) using the green approach.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical grade and were used as received without any further purification and were obtained from Sigma-Aldrich, Germany. The deionized water was obtained from Milli-Q system (Direct 8, Millipore SAS, Molshiem, France).

Sample synthesis

Two methods of chemical synthesis of AgNPs were used in the first one (solution A) 0.17 g of tri-sodium citrate was added in 100.0 mL of deionized water was added then it stirred and heated at 60°C for 10 min, second solution was prepared by adding 0.1 g of $AgNO_3$ in 10 mL of deionized water which was added dropwise to first solution and the whole mixture was stirred for 10 min. Then after, 25.0 g of sucrose was added slowly and keep stirred to ensure that the reduction process begins which could be achieved by the color change from colorless to amber yellow which confirms the formation of Ag-NPs then stirring was continued for 10 min, the final solution was stored (**Suriati *et al.*, 2014)**.

The second method (solution B) which is tri-sodium citrate– free was prepared by adding 0.1 g of $AgNO_3$ in 10.0 mL of deionized water and then heated at 40°C and stirred for 5 min. After that, 25.0 g of sucrose was added slowly and keep stirred while the temperature raised to 60°C for 10 min to the color change as abovementioned and then stirring was continued for 15 min then the final solution was stored **(Meshram *et al.*, 2013)**.

Materials characterization (instruments)

UV/VIS spectroscopy (UV-2505, Spectro UV-Vis RS, labomed inc., Los Angeles, USA) and FTIR (Nicolet Avatar 660, Nicolet, USA) were used to confirm the structure of the obtained samples. Moreover, Transmission Electron Microscopy (TEM) (JEM-100 CX 11, Japan) was used to confirm the morphology and the size of the particles where a drop of the prepared solution was applied on the carbon-coated copper grates (CCG) then it allowed to be dried at room temperature prior to the TEM analysis. AgNPs dried samples were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

Microbial tests and land snail's control

Diffusion agar antibacterial activities were carried out before assay plates using 100 ml (5 mg/ml concentration) of the examined samples were used to determine the sensitivity of clinically pathogenic microorganisms against antibiotics and the prepared AgNPs. The plates were incubated for three days at 28°C (for fungus) and one day at 37 °C (for bacteria).

Trees and plants which infected by snails were sprayed with the prepared solution of AgNPs where 50 ml of the solution was sprayed the olive and palm tree from the root to the branches in the field.

RESULTS AND DISCUSSION

UV-Vis Analysis

The analysis was performed by combining 5.0 mL of the prepared solutions of the two methods with 100 mL of binary distilled water to be diluted, resulting in concent-rations of 2.6×10^{-3} M, then placing the samples in radio-spectroscopy ultraviolet / visible at wavelengths between 200 and 800 nm, in order to determine the concentration of the two samples. (The relationship between absorbance and wavelength is plotted using Origin software ver.2018 as can be seen in Fig. 1 as the spectrum revealed a strong peak at 420 nm which corresponds to Plasmon resonance and is consistent with the optical properties of silver nanoparticles **Prakash (2013).**

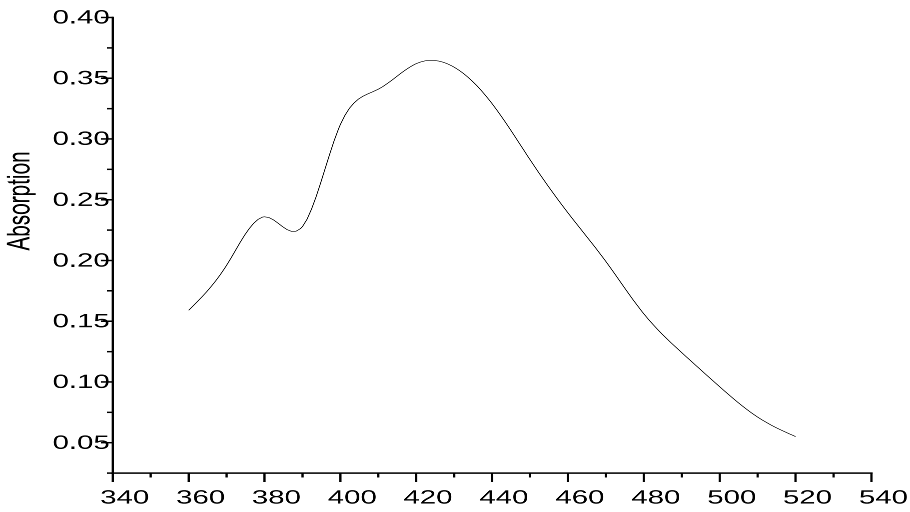

Fig. 1. Uv-vis absorbance spectrum of AgNPs

TEM Analysis

It can be seen in Fig. 2 that the structure of AgNPs is spherical, monodispersed and its size distribution is ranged from 10.0 to 25.0 nm., emphasis the nano size of the prepared AgNPs particles.

FTIR Analysis

The capping factor of AgNPs was confirmed by FTIR analysis. The FTIR spectrum was performed in the range 400 to 3000 cm^{-1}, some strong peaks appeared at 2353, 1649, 1532, 1019, and 664 cm^{-1} as can be shown in Fig. 3. The gluconic acid formed after the released glucose is oxidized then encapsulates the AgNPs. The transformation of gluconic acid into metallic silver begins with the encapsulating of the acid in AgNPs. A reducing agent reduces (Ag^+) to produce metallic silver (Ag^0), which is then aggregated into oligomeric groups. Finally, these groups result in the emergence of metallic colloidal Ag^0 particles **(Iravani *et al.*, 2014).** The first carbon of glucose is oxidized to generate a gluconic acid and a carboxylic acid with chelating and antibacterial effects. The cyclic glucono-delta-lactone ester structure of gluconic acid's aqueous solution chelates metal ions and creates extraordinarily stable complexes. This substance exhibits potent chelating properties in an alkaline solution toward anions like calcium, iron, silver, aluminum, copper, and other heavy metals. C-H, C-O bending vibrations are responsible for the peak at 664 cm^{-1}, 1532 cm^{-1} and 1019cm^{-1} **(Khalil *et al.*, 2012).**

Antimicrobial Activities

A reliable agar diffusion technique was used to assess the antibacterial effectiveness of chemically produced AgNPs against four pathogenic bacterial strains (*Salmonella* sp., *E. coli*, *Bacillus* and *Staphylococcus*) (**Valgas *et al.*, 2007).** The investigated bacterial strains were cultured on a nutritious broth medium consisting of cultivars of pathogenic bacteria that were purchased from the International Microbial Centers (American Type Culture Collection (ATCC)) (peptone, beef extract, yeast extract and sodium chloride). Cultivated for 24 hr at 37°C. The following day aids the nutritional agar medium for cultivating the bacterial strains, which includes peptone, beef extract, yeast extract, sodium chloride, and agar. Five wells with a 5mm diameter were created. Colloidal solutions containing various quantities of AgNPs ($2.6x10^{-3}$M) were prepared. Deionized water was used as a negative control for antibacterial activity. After the 24 hr., as incubation period, all plates were inspected to determine the region

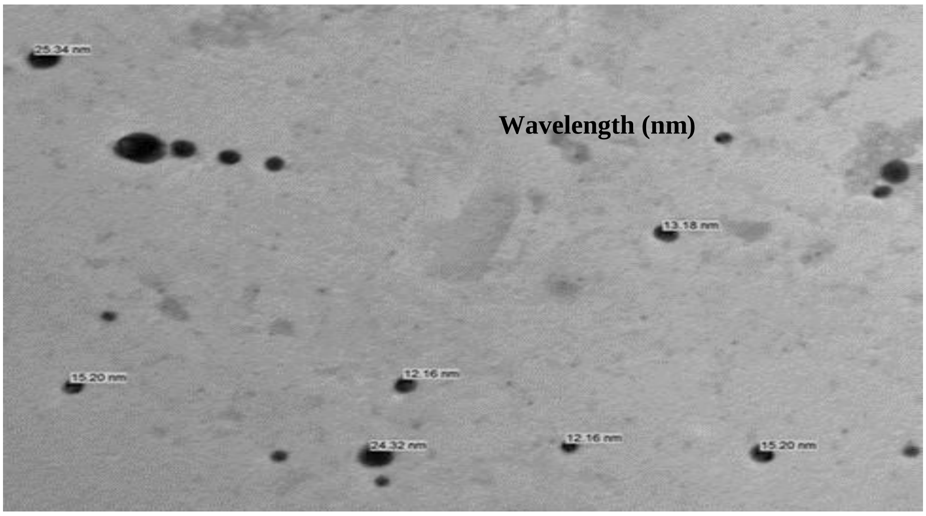

Fig. 2. TEM micrographs of AgNPs

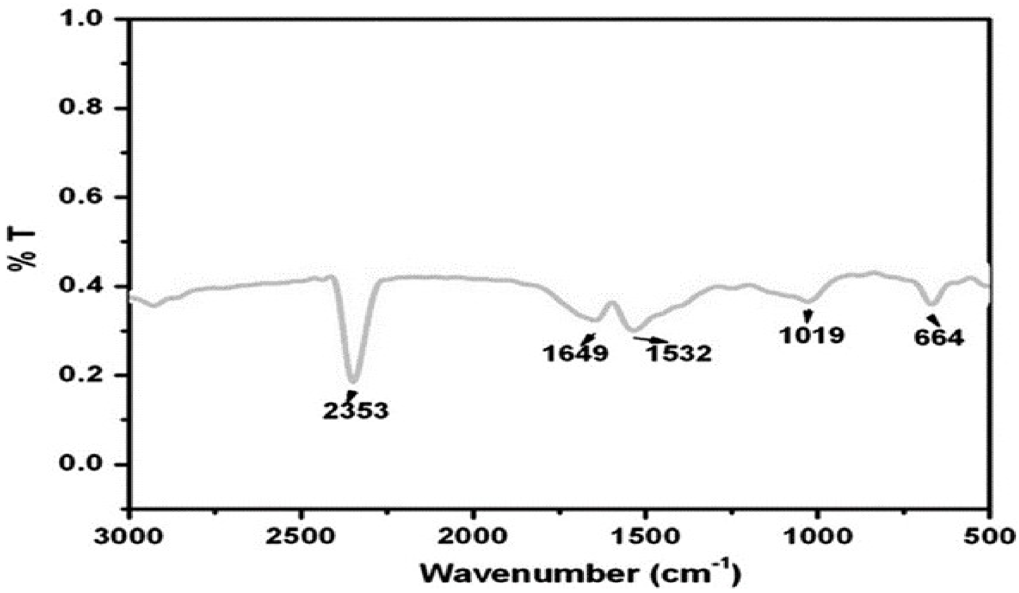

Fig. 3. FTIR spectra of AgNPs

of inhibition around the wells. All dishes were then incubated at 37°C for a further 24 hr. During the test which demonstrated that AgNPs attach to the *Escherichia coli* cell wall, determined the lowest inhibitory concentration (MIC) of silver nitrogen that resulted in visual suppression of bacterial growth in *Salmonella* sp. and *E. coli* with IC 30 and IC 40 values at 2 mM impacting antibacterial action, it creates holes on the membrane that ultimately cause cell death and limit growth. Smaller particles were more effective than larger ones. AgNPs exhibit antibacterial and bactericidal activity against the microorganisms that generate biofilms. On the other hand, *Bacillus* and *Staphylococcus* bacteria remain unaffected as can be seen in Fig. 4.

Antifungal Activities

Excellent antifungal efficacy was demonstrated by the produced AgNPs against pathogenic fungus such *Aspergillus fumigatus*, *Candida albicans*, and *Fusarium* sp

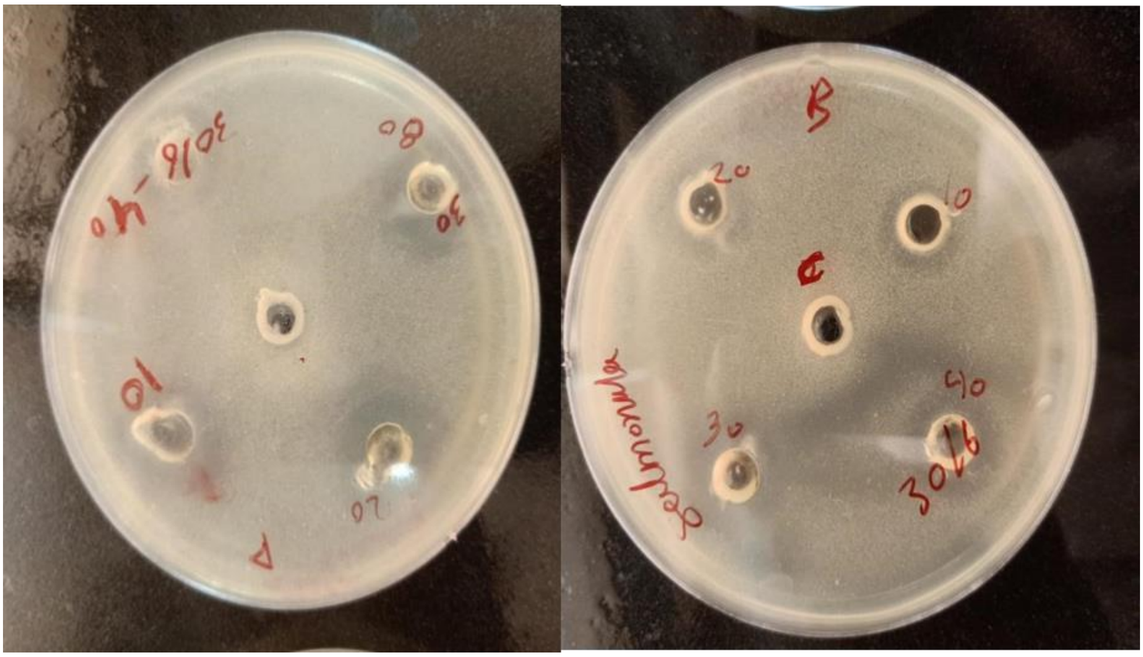

Fig. 4. Effects of AgNPs on *salmonella* and *E. coli*

(Pantidos and Horsfall, 2014). The size and form of the AgNPs affect their antifungal action as can be shown in Fig. 5.

The high surface area of these tiny nanoparticles ensures that microbial growth is inhibited and could demonstrate how AgNPs nanoparticles prevent fungus from producing conidia. Last but not least, AgNPs offer enormous potential for controlling fungus that produce spores.

Land Snails Control

Snail repellents such as AgNPs are used to force snails and slugs to lose a large proportion of the water when ingested or if contacted the animal as a result of continuous secretions, which results in the animal's dehydration and death. Two methods were developed to chemically control the snails: firstly, by collected snails as can be seen in fig. 6 and adding AgNps solutions A (with trisodium citrate) and solution B (without trisodium citrate) for 60 min at room temperature after that, it is observed that most of snails were controlled as can be seen in Fig. 6. In the second method snails were sprayed by the prepared solution on an olive tree and palm show Fig.7. On the following day it was noticed that snails fell from the tree and charred snails from inside show Fig. 8.

Conclusion

The spread of land snails in North Sinai Governorate is a major concern due to their impact on food security in particular the agricultural crops. The deficiency of current control methods to control their rapid spread. In our study, we were able to get rid of the snail by dissolving the inner part just as the substance was able to dissolve the snail's outer shell, and thus the snail is completely dissolved which will not cause any harmful effects to the environment.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the reported work.

Fig. 5. Antifungal activity of AgNPs

Fig.6. Snails after treatment

Fig. 7. Snails befor the treatment in the farm

Fig. 8. Snails after the treatment in the farm

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الملخص العربي

طريقة صديقة للبيئة لمقاومة القواقع الأرضية بإستخدام جزيئات نانو الفضة المُصنعة كيميائيًا في شمال سيناء، مصر

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تعتبر القواقع الأرضية من أخطر المشكلات التي تواجه المزارعين في شمال سيناء، مصر. لإنتشارها بطريقة كبيرة في سيناء وإتلافهاالعديد من المحاصيل الزراعية. تكمُن خطورة هذه القواقع في تغذيتها على العصارة النباتية للنبات. وللأسف فإن مكافحتها باستخدام المبيدات الحشرية والطرق الأخرى غير مجدية لعدم قدرة جزيئات المبيد على إختراق الجسم الصدفي للقواقع كذلك فإن عملية تجميع القواقع وحرقها يمثل خطورة كبيرة لزيادة نسبة الإنبعاثات الكربونية. كما إن دفنها يساعدها على تكاثرها وعودتها مرة أخري بأعداد كبيرة. لذا بات من الضروري البحث عن طريقة خضراء ذكية تقاوم هذه القواقع وتمنع وصولها للنباتات فجاءت فكرة تحضير جزيئات نانو الفضة بطريقة خضراء. بطريقة غير مكلفة وموفرة للجهد والطاقة. يتم استخدام السكر الأبيض في عملية الإختزال بديلًا عن مادة سترات الصوديوم الثلاثية. وتم تحضير جزيئات النانو في وجود السكر الأبيض وسترات الصوديوم الثلاثية وتارة أخري بوجود السكر الأبيض فقط للمقارنة. وتم دراسة حجم هذه الجزيئات وشكلها عن طريق الميكروسكوب الإلكتروني لإثبات أن جزيئات محلول الفضة بحجم النانو، كما تم عمل تحليل الأشعة تحت الحمراء لمعرفة المجموعات الفعالة وكذلك دراسة التحليل الطيفي للعينات التي أثبتت وجود حزمة امتصاص عند طول موجي 420 نانومتر وهي الدالة علي تكون هذه الجزيئات. قبل البدء في عملية المكافحة تم دراسة تأثير هذه الجزيئات على أربعة أنواع من البكتريا: *Salmonella sp., E. coli, Bacillus* and *Staphylococcus* وقد تبين فاعليتها الكبيرة في القضاء على نوعين وهم *Salmonella* sp and *E. coli*، ويرجع تفسير ذلك الي القوة المزدوجة لجزيئات نانو الفضة ومجموعة الكربوكسيل المتكونة من عملية أكسدة السكر ما يجعل لها تأثير مضاعف. أوضحت التحاليل أن حجم الجزيئات 10 - 25 نانومتر ولها شكل كروي. تم استخدام السكر الأبيض كعامل مختزل قوي لإختزال جزيئات الفضة بشكل سريع يوفر الوقت والمجهود والطاقة مع قدرته على جذب القواقع. معمليًا تم وضع مجموعة من القواقع متوسطة الحجم داخل كأس يحتوي على محلول به معلق جزيئات نانو الفضة لمدة ٤٠ دقيقة وظهر الجزء الداخلي وقد تآكل تمامًا ثم الجزء الخارجي للقواقع وعند تطبيق هذا بمزرعة حقيقية من خلال شجرة زيتون ونخلة فتم رش محلول معلق جزيئات نانو الفضة لكل منهما وشملت عملية الرش كلا من الساق والأرواق والجذور ثم لوحظ تساقط القواقع مع احتراق الجزء الداخلي لها. نستهدف في المستقبل حساب نسبة تركيز جزيئات النانو الموجودة بالنبات في الثمار والأوراق لمعرفة الحدود الآمنة بواسطة حساسات خاصة وسيتم عمل دراسة تحليل احصائي مع التوصية بمراقبة المناطق الموبوءة عن طريق تقنيات الإستشعار عن بُعد لسرعة معالجتها قبل تفاقم المشكلة.

الكلمات الإسترشادية: القواقع الأرضية، نانو الفضة، حمض الجلوكونيك، المجهر الإلكتروني.

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