



Synthesis, Phytochemical Screening and Toxicity Measuring Against *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae) of Silver Nano Particles from *Origanum marjorana* Extract in the Field

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ABSTRACT

Herein, we declare on the biosynthesis of synthesised silver nanoparticles utilizing the aqueous extract of *Origanum Marjorana* by reducing 1 mM silver nitrate at room temperature. AgNPs were characterized by using some common techniques such as an ultra violet spectrophotometer, Fourier transforms infrared spectroscopy, X-ray diffraction, and transmission electron microscopy. Morphologically, AgNPs demonstrated a spherical shape with an average particle size distribution of 63– 85 nm. FT-IR studies showed the presence of bioactive useful bunches such as phenolic compounds, amines, and aromatic rings are found to be the capping, stabilizing specialists and required for the reduction of silver ions of AgNPs. The measurement of toxicity of AgNPs and the plant extract of *O. marjorana* against *Earias insulana* was also discussed. After the treatment of synthesized nanoparticles for two successive seasons, general reduction percentages on the infestation of the green boll caused by *E. insulana* larvae in the second season was more by 60.4% than the first season which was 44.73%. Synthesized AgNPs treatment was considered more effective than plant extract for controlling *E. insulana*. General reduction percentages in the infestation of the green boll caused by *E. insulana* larvae in the first season associated with the treatments with AgNPs and plant extract were 44.73 and 37.53% while the second season record 60.4 and 51.82%; respectively.

INTRODUCTION

Cotton plant attacked by a number of insect pests that results in a reduction in cotton production. Bollworms are the major insect pests of cotton crops and among these spiny bollworms is particularly one of the most destructive pests that attached by infest squares, flowers and bolls causing dry and drop prematurely to them then cause reduce in the quantity through direct feeding of crops causing damage the lint quality of cotton

(Waleed 2013). Moustafa Hemat *et al.* (2015) use of less susceptible cotton cultivars infested with *E. insulana* can be considered as a part of IPM control program. Spiny bollworm *Earias insulana* (Boisd.) is dynamically nearly all throughout the year on its different host plants and is effective almost throughout the year on its diverse host plants. As a consequence of the attack, the quality and quantity of cotton are decreased. A single larva can ruin distinct buds and bolls in its life. To avoid the problems of insecticides, one of the best management methods is applied to plants products, since of their biodegradability and less venomous to non-target organisms. About 200 plants with insecticidal activities are known Singh *et al.* (2001). Moustafa Hemat (2016) found that plant extracts of *Conyza dioscoridis* and *Melia azedarach* have toxicity effects on *E. insulana*. Nanoscience is known as a topic counts on the prime properties of nano-size goals (Firdhouse and Lalitha 2015; Abou El-Nour *et al.* 2010; Mohanpuria *et al.* 2008). Nanoparticles possess optical, electronic, magnetic, and catalytic properties owing to their high surface area to volume ratio, so they drew attention to them (Poulose *et al.* 2014; Vijayakumar *et al.* 2013). Synthesis of nanoparticles has been applied in many cross-disciplinary fields, from agriculture to medicine. Silver metal nanoparticles show different colours owing to their Surface Plasmon Resonance (SPR) phenomenon. Synthesis of silver nanoparticles is most common due to their numerous applications in the medical field, health, and environmental importance (Bipin *et al.* 2020; Prabhu and Poulose 2012; Kumar and Yadav 2009). Silver is a delicate, white, glistening transition metal which has high electrical and thermal conductivity and it is applied in numerous shapes such as coins, vessels, solutions, foils, sutures, and colloids as lotions, ointments. Furthermore, silver compounds are non-toxic to the human (Sankara *et al.* 2013; Lara *et al.* 2011). *O. marjorana* is a traditional herb used worldwide recognized by its therapeutic property respiratory disarranges, indigestion, dental caries, rheumatoid joint pain, and urinary tract disarranges (Liang *et al.* 2012). It has anti-hyperglycaemic, antioxidant, anti-mutagenic, anti-fungal, anti-inflammatory, anti-viral, potent anti-bacterial, antioxidants, and antimicrobial properties, (Chou *et al.* 2010). They are employed in the nation's medication to treat several illnesses (Dorman and Deans 2000; Novak *et al.* 2003).

Our present study is focused on the synthesis of silver nanoparticles by reducing the silver nitrate solution using an aqueous leaf extract of *O. marjorana*. The nanoparticles were characterized by techniques like visible ultraviolet spectroscopy, Fourier infrared spectroscopy (FT-IR), X-ray diffraction (XRD), transmission electron microscopy (TEM), The toxicity of both the plant extract and the nanoparticles of *O. marjorana* extract against *E. insulana* is also measured.

MATERIALS AND METHODS

Chemicals:

Silver nitrate (AgNO_3 , Merck) and ethanol (HPLC, S d fine-chem limited) were used in this study without further purification.

Plant Material:

The plant (*O. marjorana*) was collected from Saudi Arabia. It was distinguished at the office of Botany, Faculty of Science, El-Menoufia University, Shebin El-Kom, Egypt.

Preparation of Plant Extract:

The plant leaves clear out (20 g) were washed a few times with running tap water, taken after by refined water to evacuate the clean, and extricated by maceration with 70% ethanol/water (100 ml) at room temperature for 7 days. The extracts were shifted with filter paper, collected, put away at 4°C, and used for asset tests Zayed *et al.* (2012).

Synthesis of Silver Nanoparticles:

Silver nanoparticles were arranged by including 0.4 ml of the extract to 10 ml of fluid silver nitrate solution (1 mM) at room temperature. The blend was hand-shaken and permitted to stand within the dim at room temperature. The experiment was observed via colour change with the naked eye as well as UV–vis spectrophotometer. The gotten nanoparticle arrangement was filtered by rehashed centrifugation at 12,000 rpm for 20 min taken after by redispersion of the pellet in deionised water.

Characterization of Silver Nanoparticles:

The analysis was carried out in the research centre lab of king Abdelaziz University in Jeddah in Saudi Arabia. The UV–vis spectroscopy estimations were recorded on a Jasco dual-beam spectrophotometer (model UV–VIS–NIR 570) operated at a resolution of 2 nm. FTIR measurements were recorded on Perkin Elmer Inc ranging from 4000 nm to 650 nm. XRD analysis was recorded on D8 DISCOVER Family (BRUKER) at the wavelength of 1.5406 Å. Morphological ponders were employed utilizing transmission electron microscopy (TEM). The TEM images were gotten by a JEOL-JEM-1011 version of transmission electron microscopy.

Field Experimental:

Field trials were done during two successive seasons 2018 and 2019 at Qaha experimental station, Qalyoubia governorate, cultivated with Giza 86 cotton variety by area of 350 m² to assess the effect of plant extract and AgNPs of against infestation of *E insulana* on cotton plants. The design of this experiment was randomized complete blocks with three replicates, divided into three plots, the plot was treated with plant extract and the other one with AgNPs, and a plot was left as a control. Cotton seeds were sown at a 20cm distance between hills. Spraying of the tested treatments took place on cotton plants three times on June 30th, July 23th, and 6th August in the first season and on July 1st, 15th, and 30th respectively in the second season. Each treatment was applied individually. The experiments were done under local meteorological conditions of 33°C average temperatures, 55% R.H., and 4.6 m/sec. as an average wind velocity during the experiment. Spraying of treatments was done by a knapsack sprayer Cifarili. To evaluate the effect of the two treatments against spiny bollworm, samples of 100 bolls/plot were randomly picked before and a week after the application of each treatment. Sampling continued weekly until harvest and bolls collected were carefully dissected and recorded the percentage of larval infestation in green boll was determined according to Henderson and Tilton (1955).

RESULTS AND DISCUSSION

Ultra Violet Spectral Analysis (UV–Vis):

AgNPs was confirmed by UV–vis spectra as obvious in Figure (1). to observe the progress of the reaction during the reduction of Ag⁺ ions. The diminishment of Ag⁺ ions to Ag particles during the exposure of the plant extract was followed by a change in colour from green to dark yellowish-brown in a fluid arrangement. From Figure (1) a band centred at 418 nm characteristic for colloidal silver and this affirmed the arrangement of AgNPs. We thought that arrangement of the assimilation top with a diminished in bandwidth is a sign of smaller, spherical shaped particles with some agglomeration Jaidev and Narasimha (2010). The width of the surface plasmon retention of the dielectric job of silver almost disappeared Mukherjee *et al.* (2002).

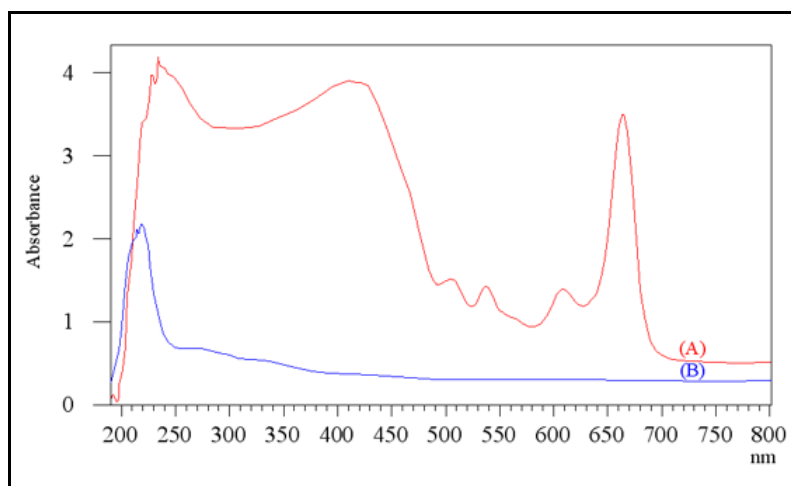


Fig. (1). (A) UV of AgNPs, (B) UV of *O. marjorana* leaf extract

Fournier Infrared analysis (FTIR):

FTIR estimations were carried out to distinguish the biomolecules for capping and productive stabilization of AgNPs. From Figure (2), there are peaks at 3400 to 3200 cm^{-1} and 3000 to 2850 cm^{-1} were allotted to O-H stretching of alcohol and phenol compounds and aldehydic -C-H- stretching of alkanes, separately. The bands in the region of 1640 to 1550 cm^{-1} and 1450 to 1375 cm^{-1} corresponded to N-H (bend) of primary and secondary amides and C-H (-CH₃ - bend) of alkanes, respectively. The peaks in the region of 1350 to 1000 cm^{-1} assigned to -C-N- stretching vibration of the amine or -C-O- stretching of alcohols, ethers, carboxylic acids, esters, and anhydrides Kumar *et al.* (2012). A few reports proposed that the arrangement of AgNPs might be due to the presence of proteins, free amine, carbonyl, and phenolic groups (Basavaraja *et al.* 2008; Kalimuthu *et al.* 2008; Mandal *et al.* 2005). We promoted that the carbonyl group from the amino acid build-ups and proteins has the more grounded capacity to bind metal which demonstrates that the proteins seem conceivably from the metal nanoparticles (i.e., capping of silver nanoparticles). This recommended that the natural atoms seem to be the result of double capacities of the arrangement and the stabilization of AgNPs Sathyavathi *et al.* (2010). The peaks were relegated to fragrant rings, geminal methyl, and ether linkages appeared the nearness of flavones and terpenoids mindful for the stabilization of AgNPs Nabikhan *et al.* (2010).

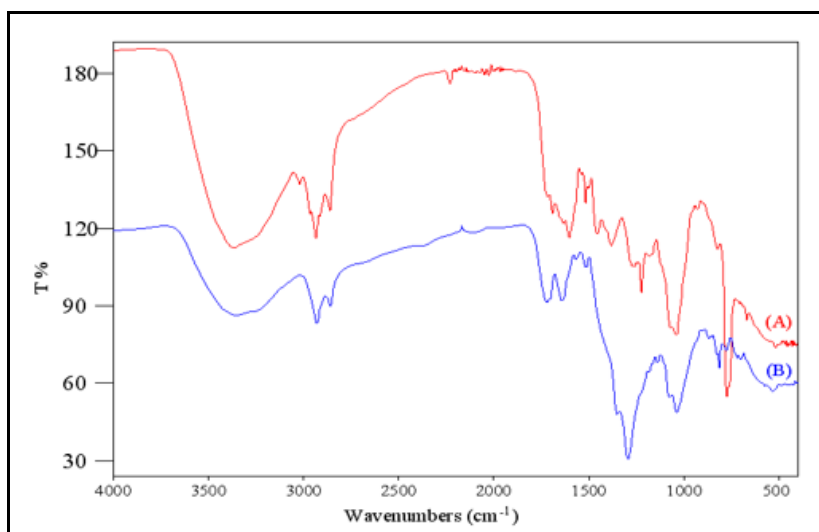


Fig. (2). (A) FTIR of *O. marjorana* leaf extract, (B) FTIR of AgNPs

X-ray Diffraction Analysis (XRD):

X-ray diffraction analysis (XRD) analysis uncovered three diffraction peaks at 38.080° , 64.560° and 77.640° that recorded the planes 1 1 1, 1 1 3 and 0 8 0 of face centred cubic structure of silver Shameli *et al.* (2011).

The XRD results showed the biosynthesized AgNPs are composed of crystals. Scherrer's formula revealed the estimation of the size of the nanoparticles is 65 nm substantiating electron microscopic ponders. the diffractogram also showed several additional intense peaks characteristic for fcc Ag, which remained Unassigned It is clear that the escalated of crests reflected the tall degree of crystallinity of the silver nanoparticles. Be that as it may, the diffraction crests are wide which shows that the crystallite measure is exceptionally little Wani *et al.* (2011).

Transmission Electron Microscope (TEM) Analysis:

The size and morphology of AgNPs were assessed by TEM analysis as shown in Figures (3A & 3B). Fig. (3A) shows a layout of the arranged AgNPs, whereas Fig. (3B) illustrates the global shape of the AgNPs. The nanoparticles are very polydisperse and extended in an estimate from 2 to 25 nm with an average size of ca. 12 nm. Generally, The AgNPs are well scattered, in spite of the fact that a few of them were famous to be agglomerated. The TEM pictures clearly uncover the nearness of a few natural layers encompassing the surface of the AgNPs. Strikingly, the larger part of the particles within the TEM pictures are not in physical contact with each other but showed up isolated by the natural layer. Hence, high-resolution TEM pictures clearly demonstrate the coating of AgNPs with a natural layer.

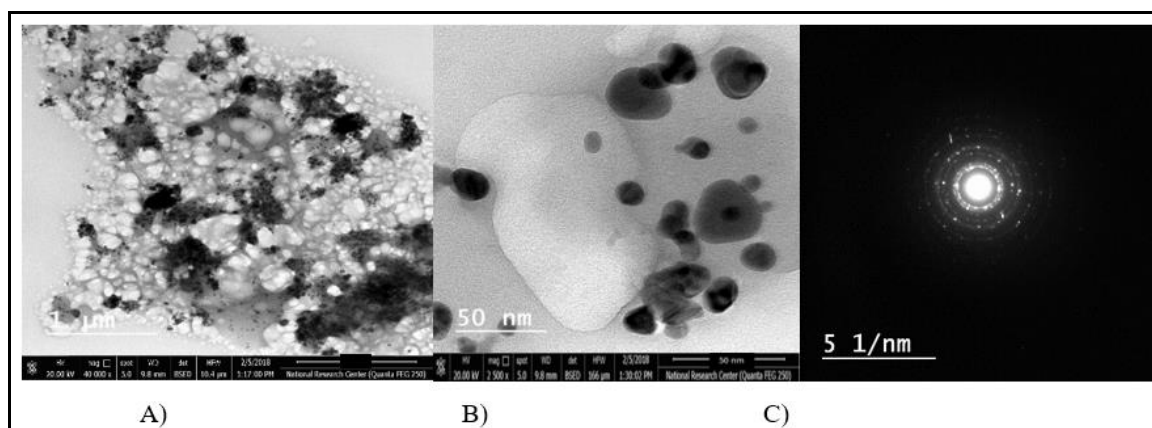


Fig. (3). TEM images of AgNPs (A,B) and the corresponding diffraction pattern (C).

Data presented in Table (1), showed that applied of the synthesized AgNPs and the plant extract of *Origanum marjorana* during two successive seasons 2018/2019 caused reduction in infestation percentages of cotton bolls caused by the spiny bollworm *E. insulana*, the reduction percentages in the infestation of the green boll during July and August were 58.09 and 69.71%, respectively after application of synthesized AgNPs compared with 54.78 and 63.94% of the plant extract treatment of 2018 season and 59.04 and 61.76%; respectively after application of AgNPs treatment compared with 56.57 and 47.07% after application of plant extract treatment at 2019 season during the two months that mentioned previously. As shown in Table (1), general reduction percentages on the green bolls infestation caused by *E. insulana* larvae in season 2019 were more by 60.4 and 51.82% respectively after applied the synthesized AgNPs and plant extract treatments than season 2018 which was 44.73 and 37.53% for the same treatments. As shown in Table (1), synthesized AgNPs treatment is considered more effective than plant extract

for controlling *E. insulana*. rates of infestation with *E. insulana* in the treated plot with synthesized AgNPs were lower during the months of July and August of the seasons 2018 and 2019, respectively than the plot applied with plant extract during the same seasons.

Table 1: Effect of spraying synthesized AgNPs and plant extract on the rate of the infestation of the green boll of cotton plant in Qalyoubia governorate during seasons 2018 and 2019.

Date of inspection	(2018) Season			Date of inspection	(2019) Season		
	Untreated (check)	Synthesized AgNPs	Plant extract		Untreated (check)	Synthesized AgNPs	Plant extract
18/6/2018	1	1	1	18/6/2019	2	2	2
25/6/2018	4	4	4	25/6/2019	4	4	3
30/6 1 st spray	12	12	12	1/7 1 st spray	18	10	8
7/7/2018	40	10	8	8/7/2019	20	8	8
15/7/2018	52	22	25	15/7 2 nd spray	30	10	12
23/7 2 nd spray	64	32	35	22/7/2019	32	8	10
30/7	68	20	24	30/7 3 rd spray	16	8	10
July mean	34.43	14.43	15.57	July mean	17.43	7.14	7.57
% Reduction	-	58.09	54.78	% Reduction	-	59.04	56.57
6/8 3 rd spray	68	30	35	6/8/2019	24	4	10
13/8	68	15	19	19/8/2019	20	10	12
27/8	72	18	21	28/8/2019	24	12	14
August mean	69.33	21	25	August mean	22.67	8.67	12
% Reduction	-	69.71	63.94	% Reduction	-	61.76	47.07
General mean	51.88 ^a	17.72 ^c	20.29 ^b	General mean	20.05 ^a	7.91 ^b	9.79 ^b
% General reduction	-	44.73 ^b	37.53 ^b	% General reduction	-	60.4 ^a	51.82 ^b
LSD:	2.54			LSD:	1.96		
P-value	0.0000***			P-value	0.0001***		

Values within the same season and the same row having the same letter are not significantly different (ANOVA, by Duncan's multiple range tests, $P < 0.05$)

In this respect, (Rajput *et al.* 2017) observed the effect of botanical extracts versus pink bollworm through two successive cotton growing seasons (2015 and 2016). Tobacco, neem, and datura were used in the classical method. In general, results showed that the sprays at different periods indicated the upmost pest population reduction at tobacco followed by neem and datura, and the same way was also suggested in the second year of the study. Also, many experiments were done by laboratory treatments with plant extract like (Yousef Heba *et al.* 2016, and Moustafa Hemat *et al.* 2018) estimated the insecticidal efficacy of *Calotropis procera*, *Ocimum sanctum* and *N. oleander* extracts on *P. gossypiella*. Treatment of different plant extracts helped to remove the bollworm larvae from cotton, also the same was found by (Bardin *et al.* 2008; Rafiq *et al.* 2012) that plant extract application on cowpea field caused increasing in flower production per plant. Field infestation of insects has been identified as the major hitch to crop production. The effectiveness of plant-based insecticidal applications may be promoted if it is sprayed either in the early morning or late evening Oparaeke *et al.* (2003). Also, Moustafa Hemat *et al.* (2015) estimate the formulation and characterization of bio-based oil in water nanoemulsion and its insecticidal activity versus newly-hatched larvae of *Pectinophora gossypiella* and *Earias insulana* under laboratory conditions. This study gives a share in natural products nanobiotechnology, presenting a probable insecticidal nanoemulsion prepared with eucalyptus essential oil. And, Kiruba and Thirunavukkarasu (2017) investigated the effects of methanolic extracts of *Andrographis paniculata* on *Earias vittella* under laboratory conditions. Treatments included with different concentrations of extract to compare with untreated found that there were poor hatching and poor egg-laying. Also, Sharaby *et al.* (2019) evaluated the toxicity, antifeedant activity and biological effects of ethanolic leaves extract of Eucalyptus on *H.*

armigera larvae. All leave extracts cause mortality to larvae and can be used in combination with other methods in the integrated management program.

CONCLUSION

From this work, we recorded a basic, fast, and compelling green blend of AgNPs from the *O. marjorana* plant leaf extract. The characterization of AgNPs was determined well-utilizing UV-vis spectroscopy, FT-IR, TEM, and X- XRD analysis and this is evidence of the formation of nanoparticles. FT-IR demonstrated that bioactive compounds capable of silver bioreduction may be proteins and flavonoids assumed to act as lessening and capping operators for the silver nanoparticles avoiding the agglomeration of the particles and in this manner stabilizing the nanoparticles. at long last the estimate and shape of AgNPs were distinguished by TEM analysis. AgNPs showed promising toxicity activity against of cotton spiny bollworm *E. insulana*. The results showed that AgNPs treatment is considered more effective than plant extract only for controlling *E. insulana*. General reduction percentages in the green bolls infestation caused by *E. insulana* larvae in season 2018 was 44.73 and 37.53% after treatment with synthesized AgNPs and plant extract while season 2019 associated to the treatments with nano and plant extract was 60.4 and 51.82%; respectively.

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ARABIC SUMMARY

التركيب والفحص الكيميائي وقياس سمية جزيئات الفضة النانوية لمستخلص نبات البردقوش ضد دودة اللوز الشوكية في الحقل.

هبة الشاطر^١ - همت زكريا محمد مصطفى^٢ - هبة يوسف^٣

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٢- معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي

٣- قسم الكيمياء - كلية العلوم والاداب بخليص - جامعة جدة - المملكة العربية السعودية

نعلن عن التخليق الحيوي للجسيمات النانوية الفضية المركبة باستخدام المستخلص المائي لنبات البردقوش. باستخدام ١ ملم من نترات الفضة في درجة حرارة الغرفة. تم التعرف علي جزيئات الفضة النانوية باستخدام بعض التقنيات الشائعة مثل مقياس الطيف فوق البنفسجي، تحويلات فورييه الطيفية بالأشعة تحت الحمراء، حيود الأشعة السينية، المجهر الإلكتروني النافذ. من الناحية الشكلية تميزت جزيئات الفضة النانوية بالشكل الكروي متوسط توزيع لحجم الجسيمات يبلغ ٦٣-٨٥ نانومتر. وقد اظهرت الاشعة تحت الحمراء وجود باقات مفيدة نشطة بيولوجيًا مثل المركبات الفينولية والأمينات والحلقة العطرية لتكون مختومة ومتوازنة ومطلوبة لتقليل أيونات الفضة من جزيئات الفضة النانوية. كما تمت مناقشة قياس سمية جزيئات الفضة النانوية والمستخلص النباتي للبردقوش ضد دودة اللوز الشوكية، بعد معاملة جزيئات النانو المركبة لموسمين متتاليين، كانت النسبة المئوية للخفض العام في ww إصابة اللوز الأخضر التي تسببها يرقات دودة اللوز الشوكية في الموسم الثاني أكثر بنسبة ٦٠,٤٪ من الموسم الأول الذي كان ٤٤,٧٣٪. اعتبرت معاملة جزيئات الفضة النانوية المركبة أكثر فعالية من المستخلص النباتي مكافحة دودة اللوز الشوكية. وكانت النسب المئوية للخفض العام في إصابة اللوز الأخضر التي تسببها يرقات دودة اللوز الشوكية في الموسم الأول لمعاملات جزيئات الفضة النانوية والمستخلص النباتي ٤٤,٧٣ و ٣٧,٥٣٪ بينما سجل الموسم الثاني ٦٠,٤ و ٥١,٨٢٪ على التوالي.