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Synthesis of silver nanoparticle using *Juniperus procera* and it's larvicidal activity against dengue and zika viruses vector *Aedes aegypt i*mosquito

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Abstract: The present study was planned to use the ethanolic leaves extract of *Juniperusprocera* as a bio-factor to the reduction of silver nitrate to silver nanoparticles and evaluate the larvicidal activity of plant extracts only and plant extracts with silver nanoparticles against *Aedes aegypti*. UV-Vis spectrum, Scanning electron microscope (SEM) and Fourier Transform Infrared Spectroscopy were used to characterized the silver nanoparticles. According to LC_{50} values (concentration which to kill 50% of larvae), The results showed that the plant extracts with silver nanoparticles (30.49 ppm) proved to be more effective against the mosquito *Ae. aegypti* than the plant extracts alone (111.17 ppm) by about 3.65 times. It is evident that from our results that the extract of the *J. procera* and the silver nanoparticles could be used on a safety method of control on the larvae of the dengue fever and Zika viruses vector.

Keywords: Dengue and zika virus, *Juniperus procera*. silver nanoparticle, *Ae. aegypti*

1. Introduction

DENV and ZIKV are single –stranded, positive-sense RNA viruses that are transmitted to human by Aedes mosquitoes [1, 2]. The 1st February 2016 the WHO indicate that the observed increase of congenital micro cephaly and other neurological disorders associated with Zika outbreaks constituted apublic health emergency of international concern [3]. The only way to avoid the development of severe forms of the disease and subsequent deaths is to maintain dengue at the lowest possible level of emergence by reducing the *Aedes aegypti* population [4]. The development of the process for the green synthesis of silver nanoparticles is an important form of nanotechnology research. Nanoparticles play an essential role in drug transfer, syndrome, imaging, sensing, gene transmission, and tissue engineering [5]. The biosynthesis of nanoparticles is advantageous over chemical and physical methods as it is a cost-effective and environment friendly method, where it is not necessary to use energy, high pressure, toxic chemicals, and temperature [6,7]. Silver nanoparticles (AgNPs) are reported to possess anti-viral activity [8], anti-inflammatory [9], and anti-fungal [10]. Green AgNPs have been synthesized using various natural products like Camellia sinensis [11], Glycine max [12], and *Azadirachta indica* [13]. Such studies could prove to have an enormous impact in immediate future if plant tissue culture and downstream processing procedures applied in order to synthesize metallic as well as oxide nanoparticles on industrial scale.

In the present study, we report the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the ethanolic leaf extract of *Juniperus procera*on the 4th instar larva of *Ae. aegypti*that we found a high larvicidal effects. This plant was used as essential oil against *Anophelesarabiensis* and record a strong repellent activity. [14]. Also, they found that the ethnomedicinal plant *J. procera* is highlyefficacious for the control of mosquitoes under bothlaboratory and semi-field conditions. It was also evident thatthe percent of larval mortality rate was dose dependent [15]. This study as the same as the result of the crude methanol and aqueous extrat of *Nelumbo nucifera* leaf extract with the biological synthesized nanoparticles [16].

2. Material and method

Collect the plant

Fresh leaves of *J. procera* were collected from Al Baha area Located in the southwestern part of Saudi Arabia (20°20′0″N,41°20′0″E). and identified by an expert taxonomist at the Biology Department, King Abdul-Aziz University, Saudi Arabia. The *J.procera* samples were washed with tap water and shade-dried at room temperature. Dried plant material was powdered using an electrical blender. 300 g of the powder was macerated in 1.5 L of methanol for 72 h. The crude plant extract was concentrated at reduced

temperature using a rotary evaporator, and stored at 22 _C. One gram of the plant residue was dissolved in 100 mL of acetone (fixative agent to separate the aqueous impurities altering the chemical composition of plant crude extract) and considered as 1% stock solution. From this stock solution, experimental concentrations were prepared [17].

Biosynthesis of Silver Nanoparticles

Prepare the nanoparticle particles by adding 1 ml of plant extracts to 1 mM AgNO3 solution adding addition to 0.5 triton x-100 and 97.5 distilled water in an Erlenmeyer flask and incubated at room temperature for 24 hour until the color change. A brown-yellow solution indicated the formation of AgNPs (Fig.1).

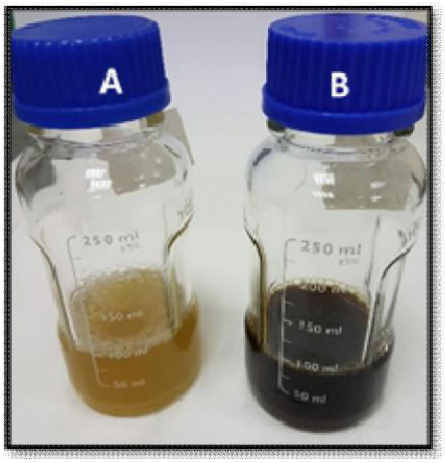

Fig 1: shwing the extracts liquid of *Juniperus procera*

A) Without $AgNo_3$ B

B)) After adding $AgNo_3$ **(H-5)**

Characterizations

UV-visible spectrophotometer (PG instrument) was used to analyse and monitor the synthesis of AgNPs. The surface morphology of prepared AgNPs was studied by using scanning electron microscopy (SEM; Hitachi S4800). Fourier transform infrared spectroscopy (FTIR) was recorded at room temperature to identified the functional groups and bioreductant on a Fourier transform infrared spectrometer (JASCO 460 plus) using the KBr pellet technique.

Rearing the mosquitoes

Ae. aegypti was the a mosquito colony that established under laboratory conditions in the premises of the mosquito dengue fever research station that belong to the Department of Biological Sciences at King Abdul-Aziz University, Jeddah. It was maintained at 27 ± 2 °C, 75–85% RH, under 14 L:10 D photoperiod cycles. The larvae were reared until pupation and adult emergence took place for maintaining the stock culture.

Larval bioassay

The larval susceptibility test was conducted according the method of [18]. Treatments were carried out by exposing early 4^{th} instar larvae of *Ae.aegypti* to various concentrations of the *J.procera* and AgNPs for 24 hr, in groups of glass beakers containing 100 ml of tap water. Five replicates of 20 larvae each per concentration, and so for control trials were set up. The larvae were given the usual larval food during these experiments. Larval mortalities were recorded at 24 hr post-treatment for the *J.procera* as well as the AgNPs The dead larvae were identified when they failed to move after being probed by a needle in siphon or cervical region.

Statistical analysis

LC_{50} and LC_{90} regression equations, were estimated by a computerized log- probit analysis software program, and by using LDP Line program.

3. Results and Discussion

Characterization of the silver nanoparticles

Fig. 2 displays the absorption spectra of synthesized silver nanoparticles (H-5) by using extract and triton x-100 along with pure extract. Two narrow absorption peaks were obtained at 230 and 275 nm for pure extract that would be responsible for the reduction of silver ion to silver metal due to the presence of polyphenol and other functional groups [19]. It is clear from the pure extract absorption peak that there is no silver nanoparticle. The characteristic absorption spectra obtained at 417 nm due to Surface Plasmon Resonance (SPR) which proves the synthesis of silver nanoparticles at room temperature by using extract.

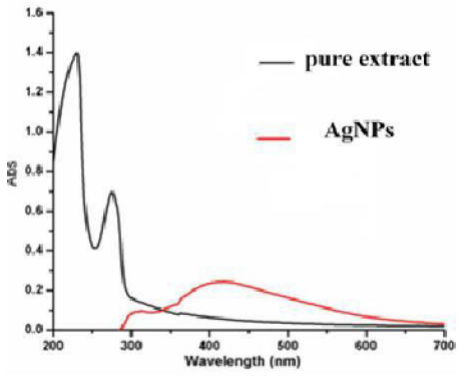

Fig 2. UV-visible spectrum of pure extract and AgNPs

Fig. 3 illustrated the FT-IR spectrum of prepared silver nanoparticles at room temperature. The broad

band appeared at ~3442 cm^{-1} is due to the presence of O–H stretching vibration [20,21,1]. The strong band appeared at 1026 cm^{-1} is ascribed to the C-O stretching vibration of OH group, which also confirms the presence of phenolic group in the extract and interacted with silver ion. Scissoring vibration of the NH_2 group in amines and the stretching mode of the C=C bonds in aromatic rings seem to be responsible for the band at 1616 cm-1. Beside these modes, the bending vibration of the N-H bonds in carboxylic acids & derivatives could also contribute to the neighboring band at 1620 cm-1. A stretching vibration band appears at ~452 cm^{-1} may be due to the adsorption or interaction of O-H on the surface of silver nanoparticles. The FTIR results revealed that the biomolecules of extract present on the surface of silver nanoparticles [22,17]. The FTIR results demonstrated that synthesized AgNPs might be stabilized by the existence of polyphenols bioactive materials in the extract, which act as bio-reducing and capping agent, therefore they help in the formation of stabilized silver nanoparticles.

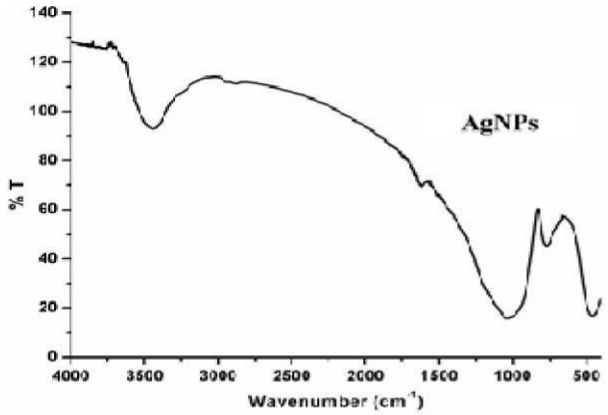

Fig 3. FTIR spectrum of AgNPs

Fig. 4 displays the image of silver nanoparticle by using Scanning electron microscopy (SEM). The SEM images of silver nanoparticle show spherical and cubic shape with uniform size distribution. The small degree of agglomeration could be seen in SEM investigations that may be due to the effect of bioextract and surfactant during synthesis. The SEM studies clearly depicts that the bioorganic extract and surfactant play a momentous role for producing the spherical AgNPs materials.

The larvicidal activity of *J.procera* and AgNPs

Table 1 shows the toxicity of *J.procera* and AgNPs against mosquito larvae of *Ae.aegypti*. The effective concentrations of the above test compound against 4th instar larvae were 50 - 250 ppm, and 10-100 ppm, respectively. The corresponding larval mortalities were in respect 18 – 92.78, and 17.5 - 9175 %. Taking LC_{50} values obtained from toxicity lines into Consideration (Fig.5), the records showed that AgNPs (30.49 ppm) proved to be the more effective compound than *J. procera* (111.17ppm) by about 3.65 folds.

However, it has been suggested that the variation in susceptibility status of the present mosquito larvae to the test compound is a dynamic process depending on the frequency of use, type of compound and its concentration [23,24]. The fluctuations in the percentage mortalities obtained for the different concentrations of the test compound against the present *Ae.aegypti* larvae support this conclusion [25]. Generally, when comparing the lethal concentrations to 50% of the treated larvae with the coastal plant before and post addition of the silver nitrate, hence the efficacy of the extract has increased after the formation of the silver nanoparticles (AgNo3) Moreover the increased efficacy might be attributed to the small size of the particles that facilitate its passage through the body wall to inside the cells which might contradict negatively the ecdysis and molting in addition to other physiological processes.

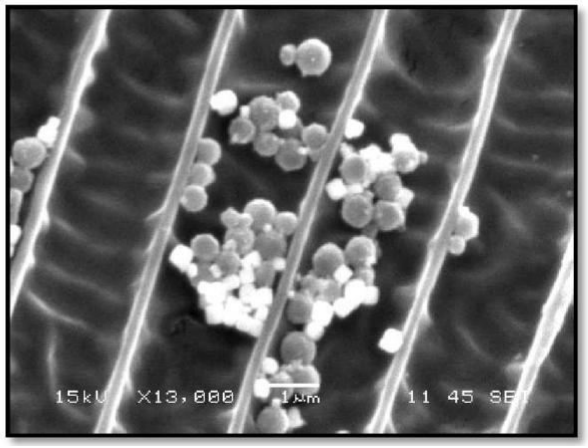

Fig 4. SEM micrograph of AgNPs

These results are in line with what was reported by [26]. Hence the efficacy of the silver particles prepared from the extract of the plant *Sterculia foetida* seeds as a larval insecticide on two species of mosquitoes was due to the small size which might enhance the quick. As well as there was another similar observation reported by [27] where he mentioned that the silver nanoparticles that were synthesized form the leaves of the plant Melia dubia has high efficacy than the crude extract against the *Culex quinquefasciatus* mosquito larvae. Also, [28] has reported that the silver particles produced from the extract of the plant *Nelumbo nucifera* has strong efficacy in inducing the mortality of two species of mosquito larvae followed by the methalonic extract then followed by the aqueous extract.

Table 1: Effect of 24 hrs exposure to aqueous extract of *J. procera* and its synthesized silver nanoparticles (AgNps H-5) against 4th instar larvae of *Ae. aegypti*

Compound tested	Conce. (ppm)	Larval Mortality (%) Mean* ± SD	LC	Con. (ppm)	Confidence limit Lower -Upper	Slope	Chi**
J.procera	50	18.00*± 0.3	50	111.17	100.7 - 121.5	3.22	6.99
	100	37.11*± 1.2					
	150	63.92*± 1.4					
	200	77.32*± 0.8	90	278.16	242.4 - 333.8		
	250	92.78*± 1.2					
AgNPs	10	17.53*± 0.6	50	30.49	26.3 - 34.7	2.26	5.06
	30	42.27*± 1.8					
	50	65.98*± 1.1					
	80	83.51*± 1.1	90	112.51	93.1 - 144.2		
	100	91.75*± 0.4					

*. The mean difference is significant at the 0.05 level.

** Chi square tabulated = 7.8 (When tabulated (Chi)2 larger than calculated at 0.05 level of significance indicates the homogeneity of results).

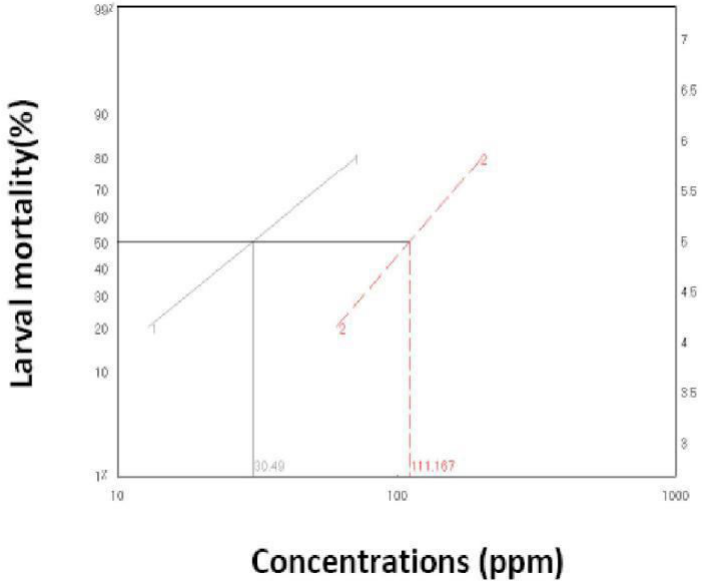

Fig 5: LC-P lines of *J. procera* against fourth instar larvae of *Ae. Aegypti*

1-After adding AgNo3 for 24h 2- Without $AgNo_3$(H-5)

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