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Larvicidal studies of *Avicennia marina* extracts against the dengue fever mosquito *Aedes aegypti* (Culicidae: Diptera)

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Abstract
Aedes aegypti is one of the most important medical insect pest found in tropical and non-tropical areas of the world, and its relevance as a model system. This study was undertaken to evaluate the impact of different doses of extracted *Avicennia marina* and its silver nanoparticles against 4th instar of *Ae. Aegypti* as a source of green nano insecticides. *A. marina* were extracted and Ag nanoparticles (AgNPs) were prepared fabricated with *A. marina*. Our results showed that *A. marina* synthesized AgNPs showed high larvicidal toxicity against 4th instar larvae of mosquitoes than the extracts alone at 17.53 times. These results suggest that the synthesized AgNPs of *A. marina* have the potential to be used as a perfect non-harmful synthesis compound for the control of the *Ae. aegypti* larvae.

Keywords: *Aedes aegypti*, *Avicennia marina*, nano insecticides, nanoparticles, mortality, sensitivity

1. Introduction
Aedes aegypti (Diptera: Culicidae) is an annoying creature to the human being and cause more diseases than any other organisms such as Dengue, Zika, Chikungunya and yellow fever [1]. Yellow fever and Dengue which transmitted by mean *Ae. aegypti* mosquito is responsible for thousands of deaths annually [2, 3]. Another example of *Ae. aegypti* disease is Zika fever, which has been re-infected in various parts of the word and became a serious problem as a human pathogen [4]. There is an integrated approach to reduce densities of *Ae. aegypti* and its lethal effects under experimentally controlled field conditions [5]. One of the most important technique to control the insect vector is to prevent mosquitoes breeding by using insecticides which can affect different stages [4] and involved using classical chemical insecticides such as DDT, chlordane, benzene hexachloride and hexamethyl tetraphosphate [6]. However, with due respect to their effective control in the elimination of mosquitoes population, these classical chemical cause serious undesirable effects on human health and to the environment. Many researchers are developing new strategies to control and reduce the use of toxic products, and one of the alternatives is to use a botanical insecticide which is easy to isolate chemical compounds from the extracts besides, they are sustainable and less toxic than chemical insecticides to combat *Ae. aegypti* mosquitoes [6- 8], and some of them were selected to produce commercial pesticides [9].
Mangroves (*Avicennia marina*) are marine woody plants [10], and it can produce lots of significant natural chemicals [11]. *A. marina* belong to the family Verbenaceae, and they contain abundant of chemical components [12], the barks, leaves, and fruits have been used as traditional medicine in Egypt to treat skin diseases [13, 14]. *A. marina* is known to be toxic to several organisms [15], but, their toxicity against mosquito is not completely understood.
Based on the foregoing, the main objective of this study was to scientifically evaluate the mosquito larvicidal activity of *A. marina* extracts and its silver nanoparticles against *Ae. Aegypti*, the vector responsible for the spread of dengue fever in Saudi Arabia.

2. Materials and Methods
2.1 Mosquito larval Culture
Ae. aegypti eggs were obtained in coordination with the Dengue Mosquito Experimental

Station (DMES), Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, where a colony was established under laboratory conditions [16], In order to obtain a sufficient number of larvae to carry out experiments.

2.2 Preparation of extracts

A. marina leaves were collected from Salman Gulf (21.5218.3 N, 38.5825.5 E) (Fig. 1) located on the Saudi Red Sea coast in Jeddah, Western Region, Kingdom of Saudi Arabia and classified by the Department of Marine Biology, King Abdulaziz University. Leaves were washed well and left at room temperature until completely dry, then they were ground with an electric grinder. The extraction was carried out using the Soxhlet Apparatus and the absolute ethyl alcohol was used as a solvent at 40 ° C. The extract was then concentrated using a rotary vacuum evaporator and kept in dark glass containers inside the refrigerator until the experiments were carried out.

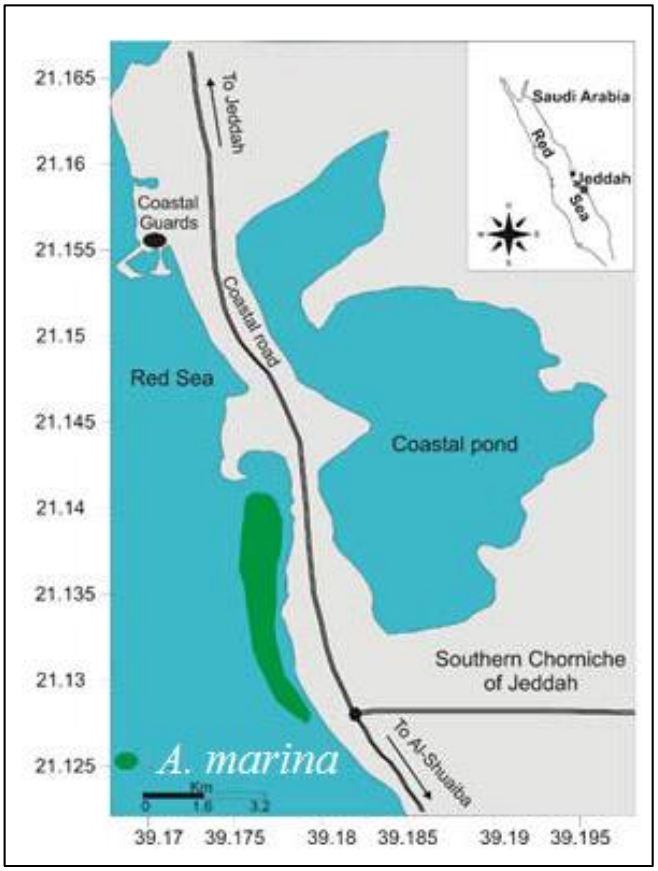

Fig 1: Sample collecting sites

2.3 Preparation of standard solutions

The standard solutions for testing were prepared by adding 1 ml of *A. marina* extracts to 99 ml of deionized water and 0.5 ml of Triton-X100 as an emulsifier to help mix the extract with water.

2.4 Biosynthesis of silver nanoparticles (AgNPs)

The silver nanoparticles were prepared by adding 1 ml of silver nitrate solution concentration of 100 micromolar, to 99 ml of the standard solution *A. marina* extracts, which was prepared and left at room temperature until the color change to brown which indicates the formation of nanoparticles.

2.4 Characterization of silver nanoparticles

The absorbance spectra were measured for each silver nitrate solution extracted before and after the addition of silver nitrate and color appearance using the UV / Vis / NIR Spectrophotometer at a wavelength of 300-800 nanometers and one-nanometer brightness. The samples were then prepared for the tests by subjecting the silver nanoparticle solution to centrifugation at a rate of 7800 rpm for 30 min., the precipitate was then discarded. In order to determine the shape and size of particles, a quantity of dried precipitation was dissolved in ethanol alcohol and the suspension was placed for the ultrasonic bath (BRANSON 1510) for 30 min. A drop of suspension was placed on a carbon-covered copper grid and left to dry thoroughly and then scanned by scanning electron microscope (SEM) at an accelerating voltage of 90 KV.

In order to identify the effective functional groups of the extract, which is responsible for the reduction of silver nitrate into nanoparticles, the infrared spectroscopy Fourier transform infrared (FTIR) was used to conduct a scan of the extract before and after adding of silver nitrate within the range 600-4000 cm^{-1} at 16 times, and a clarity of 4 cm^{-1} by placing a small portion of the raw extract in the sample area, and followed the same method with the powdered silver particles already dried.

2.5 Larval bioassay

The sensitivity of *Ae. aegypti* larvae to *A. marina* extracts was estimated, as well as for prepared nanoparticles in laboratory conditions at 27 ° ± 2 ° C, and relative humidity of 60-70% following the WHO standard method of immersion method (WHO, 2005). The 4th larval stage was treated to a series of concentrations for five replicates, containing 20 larvae in each. For control, five groups were used. Experiments were carried out and the results recorded daily until the complete insects were released. The dead stages of the treatments (larvae -pupa-adults) were isolated and examined to identify abnormalities that may occur in their visual form with an anatomical microscope equipped with a digital camera connected to a computer.

2.6 Statistical analysis

The larvae mortality percentages were calculated for each concentration. The results were analyzed using [17], using LDP line software to derive statistical values and constants at 95% confidence intervals and a significant level of 0.05.

3. Results

3.1 Characterization of AgNPs and changing color

In this study, the color change was a sign as evidence of the formation of nanoparticle. After adding the silver nitrate to the extracts, the color of the extracts gradually changed over time. This indicates that the ionic silver nitrate was reduced to unsaturated silver particles. While, no change in the color for extracts without adding silver nitrate as well as for silver nitrates without the extract (Fig. 2 a, b, c).

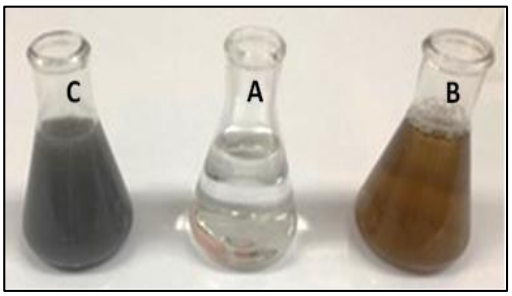

Fig 2: Biosynthesis silver nanoparticles for *A. marina* extract A) silver nitrates B) *A. marina* extract C) silver nanoparticles

3.2 Spectrophotometer UV/Vis/NIR

From studying the absorption spectra in the visible light area and ultraviolet radiation of the wavelength at which the strongest absorption and formation of nanoparticle particles occurred, the results showed that the mangrove extract obtained the absorption strength at the wavelength 316 nm (Fig. 3).

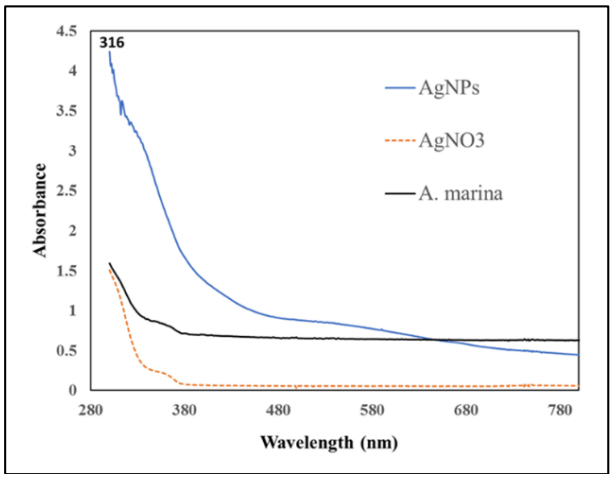

Fig 3: The ultraviolet radiation spectrometer for silver nitrate ($AgNO_3$), *A. marina* extract and silver nanoparticles (AgNPs).

3.3 Particles examinations using an electron microscope

To determine the size and shape of the formed particles, the scanning electron microscope was used. Results showed that the shape of the particles varied from spherical to rectangular, depending on the extracts used in their preparation. The size of the silver particles prepared from the mangrove extract ranged from 10-40 mm (Fig. 4).

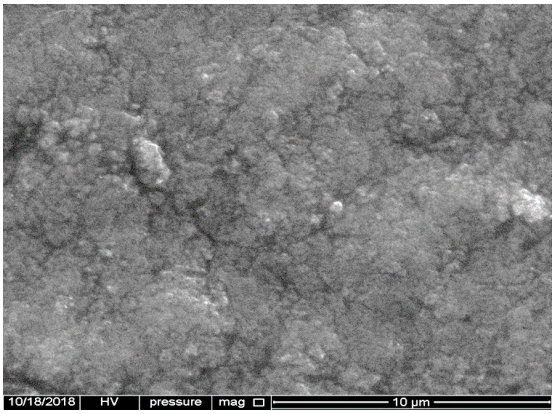

Fig 4: Analysis of the scanning electron microscopy device for the silver particles prepared from the *A. marina* extract.

3.4 Infrared spectrometer (FTIR) analysis

The analysis of the infrared spectrometer (FTIR) was compared before and after adding silver nitrate and the composition of nanoparticles (nano) through a displacement of values from 122.46 to 1566.75 with changes at absorption peaks 144648 and 1525.79 and decay of the absorption peak 225.445 (Fig. 5).

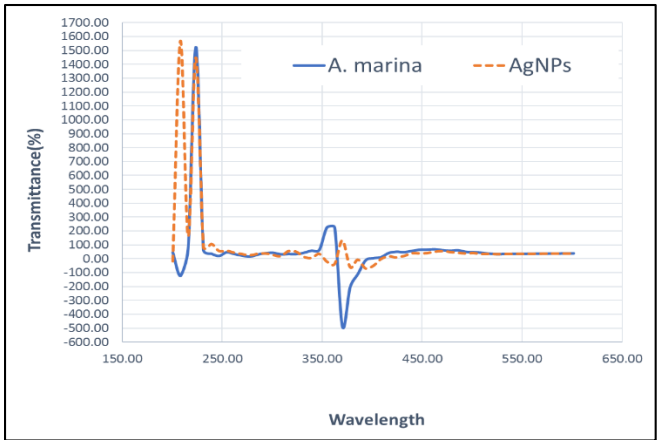

Fig 5: Analysis of the FTIR for *A. marina* extract before and after adding silver nitrates.

On the other hand, the results of the statistical analysis showed in Table 1 that there was a direct correlation between the concentrations of *A. marina* extract, larvae and pupa mortality, and inhibition of adults from treated larvae. Where the effective concentrations were 100, 300, 50, 800 and 1000 ppm. The percentage of inhibition of adults from larvae treated at previous concentrations ranged from 1791% with recorded larval mortality at 4%, whereas larvae mortality was 13.83% and pupa mortality was 87-17%. The pupa percentages were high in larvae treated with low concentrations as well as the rate of conversion from pupa to adults.

By comparing of calculated Chi square $(Chi)^2$ and tabulated one which obtained from the statistical at df = n-2 and α = 0.05, levels were higher than the values of the calculated Chi square, confirming that the difference is significant between the concentrations and mean of adults inhibitor from treated larvae.

Table 1: Sensitivity of *Ae. aegypti* larvae for the extract of *A. marina*

Conc. (ppm)	Larval Mortality (%)*	Pupae Produced (%)	Adults emergence	
			Total	Inhibition (%)
100	13	87	83	17
300	42	58	48	52
500	60	40	32	68
800	72	28	21	79
1000	83	17	9	91
0	4	96	96	4

* Five replicates; 20 mosquito larvae each

Chi-square calculated from the data = 1.9137

Tabulated Chi-square at 0.05 probability level = 7.81

The line is good fit and the data are significantly homogenous.

IC_{50} = 290.19 ppm

Fiducial limits of IC_{50}= 247.59– 333.23 ppm

IC_{90} = 1169.98 ppm

Fiducial limits of IC_{90} = 955.35– 1532.80 ppm

Slope= 2.1166

The results shown in Table 2 the laboratory toxicity data and the delayed effects of silver nanoparticles prepared from *A. marina* extract. Effective concentrations were ranged from 10-50 ppm. Larval mortality rates ranged from 18-88%, and the inhibition adult's rate were ranged from 29-97%.

The results of the statistical analysis showed in Fig. 6, that the values of the inhibitory concentrations were 50-90% of the adults produced from the treated larvae. These values were about 16.558 and 44.093 ppm, respectively.

According to the values from Fig. 7 of the concentration that inhibited of 50% of adults, the results showed that the silver particles prepared from the *A. marina* extract were higher than the extracts alone at 17.53 times.

Table 2: Sensitivity of *Ae. aegypti* larvae for silver particles prepared from mangrove extract *A. marina*

Conc. (ppm)	Larval Mortality (%)*	Pupae Produced (%)	Adults emergence	
			Total	Inhibition (%)
10	18	82	71	29
20	47	53	46	54
30	65	35	23	77
40	77	23	15	85
50	88	12	3	97
0	4	96	96	4

* Five replicates; 20 mosquito larvae each
Chi-square calculated from the data = 5.2184
Tabulated Chi-square at 0.05 probability level = 7.81
The line is good fit and the data are significantly homogenous.
IC_{50} = 16.5586 ppm
Fiducial limits of IC_{50}= 14.5607– 18.417 ppm
IC_{90} = 44.093 ppm
Fiducial limits of IC_{90} = 38.6233– 52.4572 ppm
Slope = 3.0131

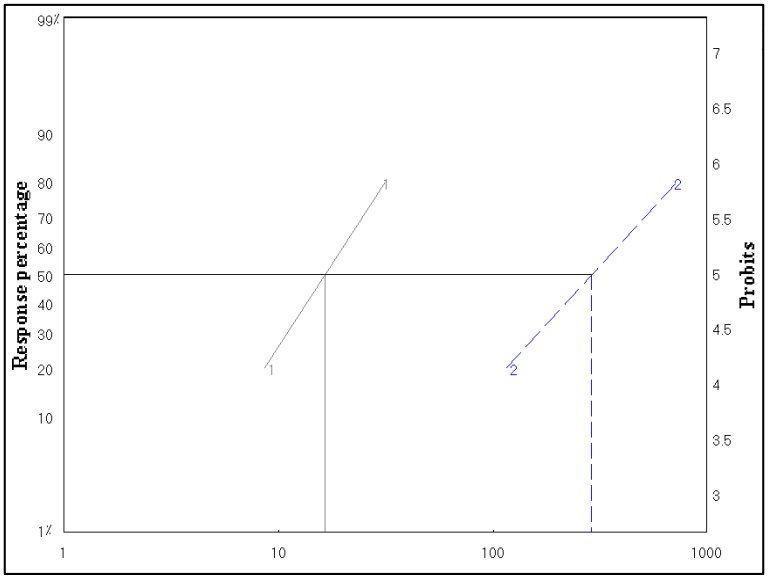

Fig 6: The LDP line of the total insects produced from the treated larvae

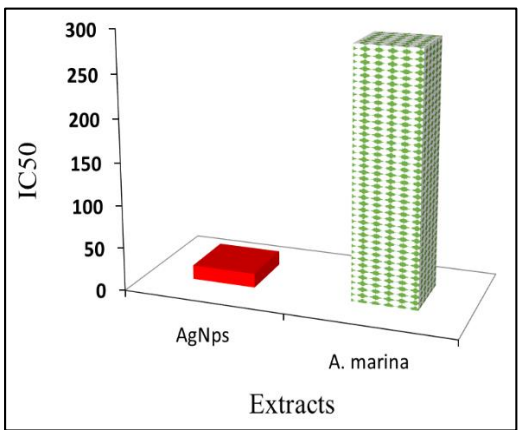

Fig 7: Comparison between the silver particles prepared from the *A. marina* extract alone.

4. Discussion

The development of non-harmful and effective insecticides is important for public health worldwide. In this study, we used the Mangroves (*A. marina*), a marine plant that was used as traditional medicine in Egypt, as a source of green nano insecticides against *Ae. aegypti*, as well as growth inhibitors. In our research, we found that the mortality of silver particles prepared from the *A. marina* extract against *Ae. aegypti* larvae were higher than the extracts alone as shown in Table 1&2 and illustrated by Fig. 1 &2. In general, 13%, 42%, 60%, 72% and 83% of larval mortality was obtained when the 4th instar larvae were treated with only A. marina, and 18%, 47%, 65%, 77% and 88% of larval mortality were obtained when the 4th instar larvae were treated with silver particles prepared from A. marina extract. Therefore, in the present work, cumulative mortalities during larval development to pupa and adults have

been taken as a sign for evaluating the tested plant against Ae. aegypti. The records concentrations of only A. marina extract caused 17%, 52%, 68%, 79%, and 91%, and the records when they treated with silver particles prepared from A. marina extract were 29%, 54%, 77%, 85% and 97% inhibition of adult emergence, respectively. The larvicidal activities of silver nanoparticles synthesized with *A. marina* leaf extract were effective against the larvae of *Ae. Aegypti* were also observed [18]. Ag nanoparticles (AgNPs) fabricated with the *A. herba-alba* extract on Indian and Saudi Arabian strains of *Anopheles*, *Aedes* and *Culex* mosquitoes were tested [19]. Another important finding in this study was that when we added silver nitrate to the extract it changed to brown color. The characteristic brown color is attributed to the excitation of Surface Plasmon Response (SPR) with the silver nanoparticles as suggested by [20]. $AgNO_3$ when it was mixed with *A. herba-alba* leaf aqueous extract it showed a yellowish-brown coloration which indicated the formation of *A. herba-alba* synthesized AgNPs [19] which corporate with our results. The most interesting finding was that the size of the silver particles prepared from *A. marina* extract ranged from 10-40 mm that provides an excellent larvicidal activity for the synthesized nanoparticles. The SEM analyses of the synthesized AgNPs were clearly clustered and irregular shapes with an average size of 40–100 nm which observed by [21]. One of the initial objectives of this study was to effect of the *A. marina* leaf extract before and after the addition of silver nitrate to the larvae of *Ae. aegypti*. Our results showed that the percentage of *A. marina* extract ranged from 100-1000 ppm where effective concentration which inhibited adults derived from treated larvae. These results agree with the finding of other studies [20], while the results recorded with different values for the lethal concentrations of 50% and 90% of *Ae. aegypti* larvae treated with silver particles prepared from the extract were effective concentrations ranged from 10-50 ppm. Larval mortality rates ranged from 18-88%. Inhibition rates for adults ranged from 29-97%. However, results are promising, since relevant toxicity potential on mosquitoes has been detected. As an example, *A. nilagirica* synthesized AgNPs have LC_{50} values ranging from 0.34 for first instar larvae, to 0.05 µg/ml for pupae on An. stephensi, and 0.46, for first instar, to 0.16 µg/ml for pupae, on Ae. aegypti were reported [22]. More generally**,** there are many reports available about the promising potential of AgNPs as nano insecticides [23, 24]. This conclusion might be due to the fact that this marine extract contains an effective component with larvicidal properties against mosquitoes [25-27].

5. Conclusion

Based on the results obtained against the targeted insect vectors, both extracts and the prepared AgNPs can be used as an insecticide against *Ae. aegypti* mosquito. Mixing of the extract and the nanoparticles at low concentrations lead to better and powerful insecticide effects, promising, since they are effective at low doses, and may constitute an advantageous alternative to build newer and safer control tools.

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