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Effect of green synthesized silver nanoparticles using *Sargassum natans* extract as a pesticidal against the dengue fever mosquito *Aedes aegypti* (Culicidae: Diptera)

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Abstract
This study aims to use Brown Algae extract, Sargassum natans as a biological agent for reducing silver nitrate to silver nanoparticles and evaluate their effectiveness in controlling of Aedes aegypti that transfer dengue fever in Jeddah province. The fourth-year larvae were exposed to different concentrations of both the extract alone and the silver nanoparticles prepared from it according to the WHO standard method. The silver nanoparticles were characterized using UV-visible spectroscopy, scanning electron microscope and IR spectroscopy. The larvae sensitivity to the extract was ecaluated before and after the addition of the nitrate. The results showed that the silver nanoparticles were nearly 1.46 times superior against larvae compared to the extract alone. The values of the lethal concentrations for 50% (LC_{50}) of the treated larvae were 282.55 ppm for the extract and 194.5 ppm for silver nanoparticles. The results of this study revealed the presence of deformations in the larvae treated with both the extract and nanoparticles and these effects were observed all stages of growth, which caused in the destruction of the insect without completing its life cycle.

Keywords: *Aedes aegypti*, dengue fever, biocidal, *Sargassum natans*, synthesized silver nanoparticles

1. Introduction
In recent years, Dengue Fever has become one of the most important viral diseases transmitted by mosquitoes to humans, seeing global distribution similar to Malaria. In some countries, Dengue Fever has reached as much as 5% of the population. The disease is classified as emerging or re-emerging. It is endemic to most countries outside the European Region. Its incidence increased fourfold between 1970 and 1995; in the American Territory there were 1.3 million cases in 1998, of which 3,600 were deaths [1]. In Saudi Arabian, the first case of Dengue Fever (caused by the virus type 2-DEN) was confirmed in October 1993, according to the World Health Organization (WHO). In 1994, Dengue Fever broke out in Jeddah, where 289 confirmed cases of the virus were recorded, and the virus was first isolated from a case that resulted in death in Jeddah due to Dengue Hemorrhagic Fever [2]. Most control methods in the past have focused on using conventional chemical pesticides. Frequent and intensive use came to be associated with harmful effects on non-target organisms, such as mosquito predators or through the food chain, known as biomagnification, causing serious damage to the environment and chronic health problems [3]. This has led scientific research centers to limit manufacture and use of these chemical pesticides, at the same time generating increasing interest in finding new and unconventional means of controlling the vectors, such as insect growth regulators (IGRs), biological insecticides and plant extracts [4].
Algae and Seagrasses are aquatic angiosperms, confined to the marine environment. Marine biodiversity is a key source of chemical compounds, which have many biomedical [5]. In recent years, marine bio resources have been extensively investigated in the field of nano science and nanotechnology to develop novel, greener and affordable methods for the metal nanoparticle synthesis [6]. This study aims to use the ethanolic of Sargassum natans extract as a bio-factor to the reduction of silver nitrate to silver nanoparticles and evaluate its activity against larvae of dengue fever vector Aedes aegypti (L.) by exposing larvae to different concentration for each of the extract and its silver nanoparticles using the standard WHO method.

2. Materials and Methods

2.1 Mosquito rearing

The eggs of *Aedes aegypti* were provided by the Research unit of Dengue Fever and Vector Control at King Abdualaziz University, Jeddah, Saudi Arabia. This help to establish a colony of flies under lab conditions according to Hanan *et al*.(2018) [7] method to get abundant number from larvae and carry out the bioassay experiments.

2.2 Preparation of Extracts

Sargassum natans brown algae were collected from Salman Gulf (21.5218.3 N, 38.5825.5 E) (Figure 1) located on the Saudi Red Sea coast in Jeddah province, Western Region, Saudi Arabia. It was classified by the Department of Marine Biology at King Abdulaziz University. The leaves were washed properly and left at room temperature until completely dried and then grinded using an electric grinder. The extraction process was carried out using the Soxhlet Apparatus and absolute ethyl alcohol as solvent at 40 °C. The extract was then concentrated using a Rotary Vacuum Evaporator and stored in dark glass containers inside the refrigerator until testing.

2.3 Preparation of Standard Solutions

The required standard solutions for experimentation were prepared by adding 1 mL of *S. natans* extract to 99 mL deionized water with 0.5 mL Triton-x100 as an emulsifier to help in mixing the extract with water.

3.4 Bioprocessing of Silver Nanoparticles

The silver nanoparticles were prepared by adding 1 mL of 100 mmol silver nitrate solution to 99 mL of the standard solution of *S. natans* brown algae extract, which was prepared earlier and left at room temperature until the color changes to brown indicating the formation of silver nanoparticles.

3.5 Characterization of silver nanoparticles

The absorbance spectra of each silver nitrate solution and the extract before and after the addition of silver nitrate and color appearance were measured using the UV - Vis / NIR Spectrophotometer at a wavelength range of 300-800 nm and one nm brightness. The samples were then prepared for the rest of the tests by subjecting the silver nanoparticle solution to centrifugation at a rate of 7800 round/minute for half an hour and then disposing of the filtrate and drying the precipitate. In order to determine the shape and size of the particles, a quantity of dried precipitate was dissolved in ethyl alcohol and the suspension was placed for ultrasonic bath (BRANSON 1510) for half an hour. A drop of suspension was placed on a copper grid covered with carbon and left to dry completely and then scanned by scanning electron microscope (SEM) at an accelerating voltage of 90 kilovolt.

In order to identify the effective functional groups that present in the extract, which is responsible for the reduction of silver nitrate into silver nanoparticles, the Fourier Transform Infrared Spectrometer (FTIR) was used. The survey was conductedfor the extract before and after the addition of silver nitrate within the range of 600-4000 cm^{-1} at a rate of 16 times and a clarity of 4 cm^{-1} by placing a small portion of the raw extract in the sample place. The same method was followed with the powdered silver particles that were already dried.

3.6 Larval Susceptibility Tests

The sensitivity of the 4th instar larvae of *Ae. aegypti* to the brown algae extract *S. natans* and its synthesized silver nanoparticles was tested in laboratory conditions at 27 °C ± 2 °C and relative humidity of 60-70% using dipping method according to WHO methods [8]. The larvae were exposed to a series of concentrations of five replicates using 20 larvae in each time and as well as control trials were set up. Experiments were carried out and results were recorded daily until the adult emergence. The dead stages that caused by treatments (larvae - pupa - adult) were isolated and examined to identify abnormalities that may occur in their morphological using dissecting microscope that equipped with a digital camera connected to a computer.

3.7 Statistical analysis

The percentages of larvae mortality for each concentration were calculated and the outcome results was analyzed according to Finney (1971) [9] method using LDP line software to obtain the different statistical values with the lower and upper confident limits at 95% with significance level of 0.05. The ratio of activation and relative effectiveness were calculated based on the lethal concentration of 50% (LC_{50}) of larvae that treated with brown algae extract and silver nanoparticles according to Lee *et al*. (1974) [10].

3. Results and Discussion

3.1 Characterization of Silver Nanoparticles

This study included the use of brown algae extract as a as a bio-factor for reducing silver nitrate to silver nanoparticles. The color of the extract was gradually changed over time after the addition of silver nitrate until it reached brown color after 24 hours (Fig. 2). The results of the UV-visible spectrum analysis shown in Fig. 3 indicated the appearance of two absorbance peaks at wavelengths of 280 nm and 425 nm for the extract. After the addition of nitrates, the broad peak disappeared and instead a small peak at a wavelength of 340 nm was appeared. This indicates that the reaction took place and the silver nanoparticles formed. The nanoparticles were shown by the scanning electron microscope (Figure 4).

The Infrared (IR) spectral analysis for the brown algae extract before and after the addition of silver nitrate is shown in figure (5). A broad band at 2000 cm^{-1}appeared for the algae extract. Other peaks at 1500, 1000 and 500 cm^{-1} were also appeared which can be attributed to the presence of alcoholic, phenolic and aliphatic compounds and carbonyl groups.

On comparing the FTIR spectrum of the extract before and after the addition of silver nitrate and the formation of silver nanoparticles, it was noticed a shift for the peaks which indicating the role of the phenolic and aliphatic compounds in the stability of the silver nanoparticles [11-13]. Several studies have suggested that plant extracts can be used to produce metal nanoparticles because they contain many secondary metabolites that play a major role in altering the charge of the metal ions and converting them into nanoparticles [14]. The ability of the Apiin compound that was extracted from Henna plant leaves on the reduction and the production of gold and silver nanoparticles is due to its containment of carbonyl and secondary hydroxyl groups [15]. It was reported that the proteins and some amino acids found in the soya bean extract (Glycine extract) act on the reduction of palladium ions into palladium nanoparticles and inhibit their aggregation [16].

3.2 Larval Susceptibility Tests

The study included evaluating of the efficacy of the brown algae extract and its silver nanoparticles prepared against the

4th instar larvae of *Ae. aegypti*. The results shown in table 1 revealed that the silver nanoparticles were more effective against the mosquito larvae compared to the extract itself. The effective concentrations of the crude extract ranged from 100 to 900 ppm in which the percentages of the treated larval mortalities were in the range of 20.62 - 89.69%. While, the effective concentrations for the silver nanoparticles were between 100-300 ppm in which the percentage of the treated larval mortalities were 17.53 – 93.81%.

According to the results of the statistical analysis (Table 2 and Fig. 6), the LC_{50} value (concentration in which kill 50% of larvae) with brown algae extract was 298.72 ppm. While, the LC_{50} with silver nanoparticles was 167.46 ppm. This confirms that mixing the extract with the silver nitrate has increased its effectiveness after the formation of the silver nanoparticle particles (AgNPs) by about 1.784 times., where the combined effect value was (+43.94) which is an indication on the compatibility of mixing processes and the potentiation. This may be due to the synergy that occurs between the extract and the silver particles during the reduction process, where the compounds present in the extract are bonded to the particles' surface, increasing the strength of their impact [17]. Or, the strength of the effect may be due to their small size, which facilitates its pass through the wall of the body into the cells where it interferes with the process of moulting and other physiological processes. This is consistent with what was reported by Rajasekharreddy *et al*. (2014) [18] that the effectiveness of silver particles prepared from the extract of the seeds of *Sterculia foetida* plant as larvae pesticide for two types of mosquitoes is due to its small size that help in rapid penetration through the body wall and its removal rate from the attached sites in the insect's body is slow. Another similar note was reported by Karthikeyan, *et al* (2014) [19] which stated that the silver nanoparticles prepared from *Melia dubia* plant leaves extract was more effective than the crude extract against *Cx. quinquefasciatus* mosquito larvae. Also, what was reported by Santhoshkumar *et al* (2011) [20] that the silver particles produced from *Nelumbo nucifera* plant extract were having the strongest effect in the destruction of two types of Mosquitoes larvae followed by the methanolic extract and water extract. This study is also consistent with what was reported by Alyaha *et al*. (2018) [21].

The present study showed also some morphological deformations on the treated larvae with brown algae extract and silver nanoparticles; for instance body pigmentation (entire or spots black on the larvae body), shrinking body resulted from abdomen segments contraction and neck elongation. Others abnormal shape was recorded in the emerged pupa such as incomplete pupa attached with its exuvia by head or siphon area and emergence of intermediate stage between between pupa and adult. These deformations occurred in the treated larvae and pupa could be attribute to the chemical component of the brown algae extract which interfered with physiological processes during metamorphosis or could disrupt the secretion mechanical of endocrine system of insects [22]. Therefore, an abnormal growth stages and mortality cases had been observed. These observations are correspondent to other previous studies. For example, it was recorded in several observations such as growth inhibition, morphological deformities prolong eggs hatching and development when *A. indicum* extract used against three species of mosquito [23]. It was found also in similar observation when marine plants extracts were tested against larvae and pupa stages of *Anopheles d'thali* [24].

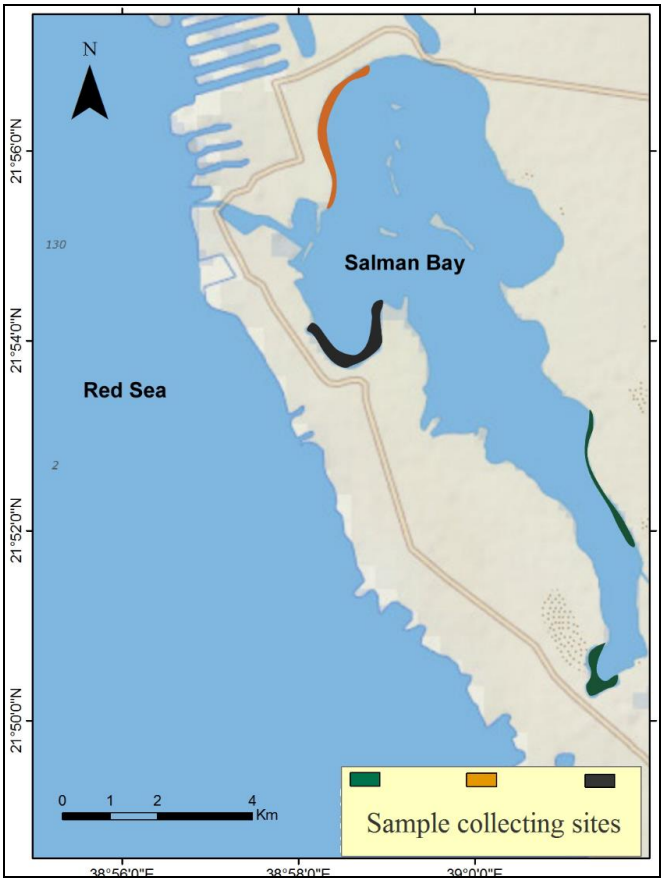

Fig 1: Sample collecting sites

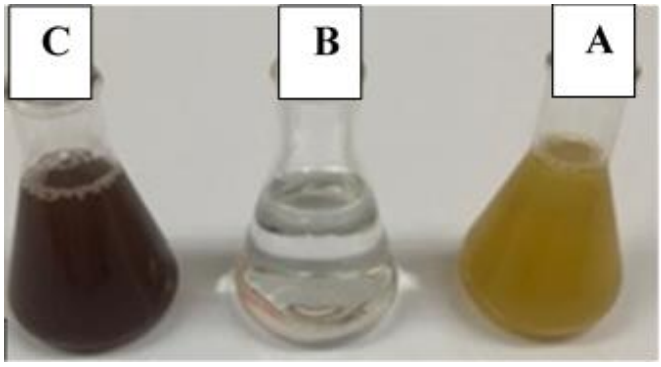

Fig 2: Biosynthesis silver nanoparticles for *A. marina* extracts **A)** silver nitrates **B)** *S. natans* extracts **C)** silver nanoparticles

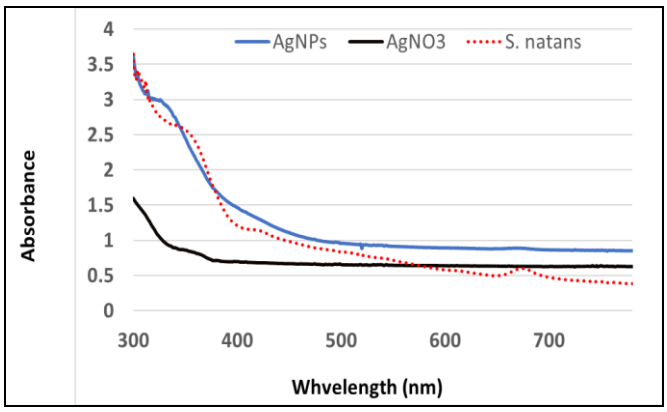

Fig 3: The UV-visible absorption spectrum of Brown Algae extract and the prepared AgNPs

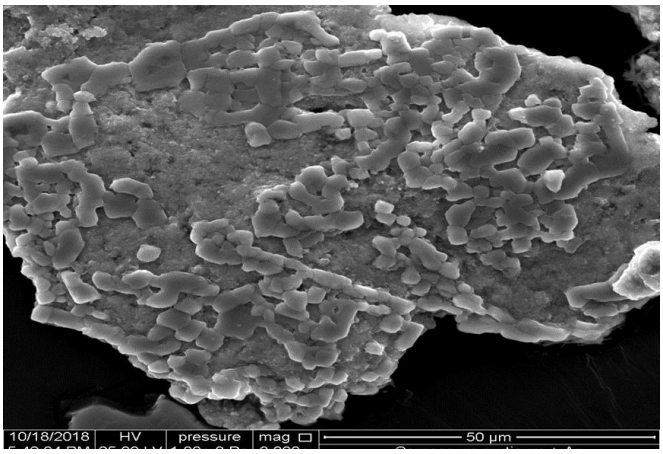

Fig 4: SEM micrograph of AgNP, papered from brown algae extract (*Sargassum natans*)

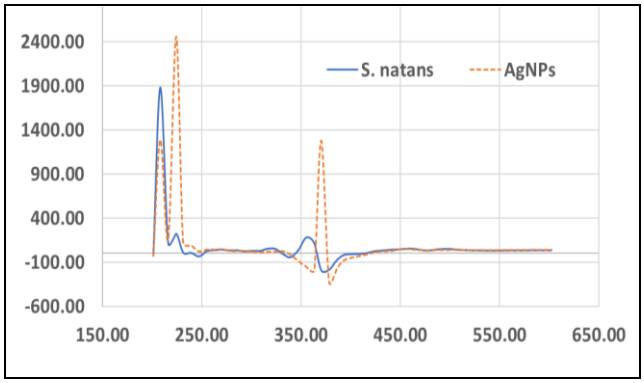

Fig 5: FT-IR spectra of brown algae extract *S. natans* and AgNPs

Table 1: The Susceptibility level of larvae of *Ae aegypti* against brown algae extract and its prepared AgNPs

Extract	Conc. (ppm)	Larval Mortality %
S. natans	100	20.6
	300	45.4
	500	60.8
	700	77.3
	900	89.7
	Control	3
AgNPs	100	17.5
	150	40.2
	200	55.7
	250	79.4
	300	93.8
	Control	3

Table 2: The statistical analysis of Susceptibility level of larvae of *Ae aegypti* against brown algae extract and its prepared AgNPs

Statistical Values	*silver nanoparticles* (AgNPs)	Brown Algae ***S. natans***
LC_{50} Confidence limets (Lower -Upper)	167.46 (156.85-177.81)	298.72 (254.23-343.729)
LC_{90} Confidence limets (Lower -Upper)	310.76 (282.99-351.73)	1293..72 (1031.04-1771.56)
Slop	4.77	2.013
$(Chi)^2$ (*df=3*)*	6.86	6.73
Activation Ratio	1.784	
Relative Effectiveness **	43.94%	

**The $(Chi)^2$ calculated lower than the tabulated one that equal 8.7. This indicates the homogeneity of results as well as the LDP line is representative of these results

*Relative Effectiveness = $\frac{LC50\ (S.natans) - LC50\ (AgNps)}{LC50\ (S.natans)} x\ 100$

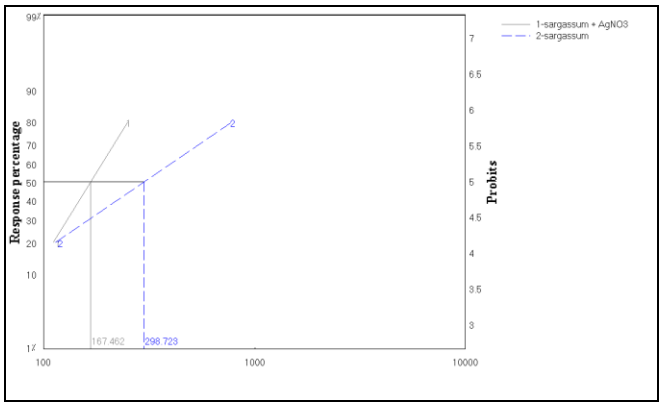

Fig 6: The LC-P lines toxicity of the tested compounds 1- *silver nanoparticles* (AgNPs) 2- Brown Algae extract

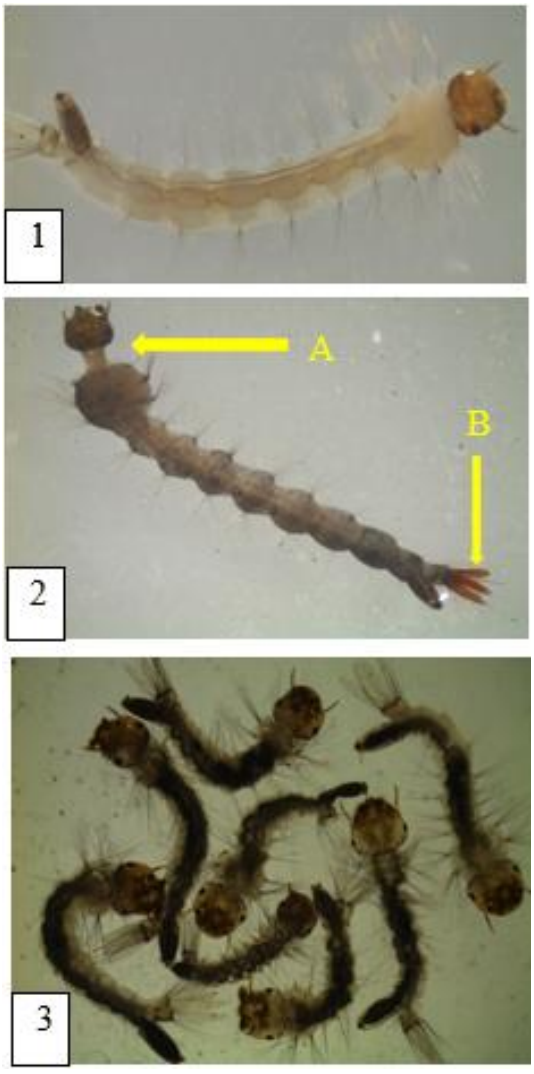

Fig 7: Some morphological deformations occurred on *Ae. Aegypti* satges after exposing to Brown Algae extract **1.** Control. **2.** Prolongation the neck (A) and Pigmentation (B). **3.** Segment Body (b)

4. Conclusion

The results of this study revealed the possibility of using Sargassum natans extract and its silver nanoparticles tested against the 4th instar larva of Ae. aegypti mosquito in control program as a safety compound and friendly to humans and the environment.

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