

Effectiveness of *Rhazya stricta* Extracts and Silver Nanoparticles Against *Aedes aegypti*, the Vector of Dengue Fever Makkah Region

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Abstract

Currently there are 90 arboviruses that have been isolated from the natural mosquito population. The most globally and widespread viruses are the dengue. In the recent years, dengue fevers cases have increased significantly in Makkah Region. To the present day there is no vaccine for protection against these viruses. Therefore, the best way to control these disease is to control the mosquito vector (*Aedes aegypti*). The objectives of this research are to use new potential alternatives control measures for the vector control through the evaluation of the efficiency of the extract of the *Rhazya stricta* in pregnated with the synthetic silver nanoparticles. On a safely method of curbing its population these plant were collected from the local environment of Makkah Region and the preparation of the extract and the experiment was conducted according to the WHO protocols. Our results showed a high efficiency of the *Rhazya* extract where the fatal concentration was 50 % of the population (LC50) mm (323,447 ppm). Moreover, with the addition of the silver nanoparti the (LC50) was 91,342 ppm.

The present study was planned to evaluate the biological activity of *Rhazya stricta* extracts and silver Nanoparticles against mosquito larvae of *Aedes aegypti*, the vector of dengue fever and Zika virus.

The extract *R. stricta* has a fatal effect against the different stages of inequities and the extract mixture with **silver nanoparticles** could yield a greater synergistic effect for the extract and the increase with efficiency

1. The independent variable included a series of the extract concentration alone and in a mixture by using 5 replicates for each concentration.
2. The mosquito larvae group composed of 20 experimented larvae for each replicate five replicate for each concentration
3. Control group larvae + water only

Importance of study and back ground

1. The mosquitoes vector is known to transmit- almost 90 arboviruses to animals and specifically to human as far as dengue fever and contemporary them on slaughters of zika infection^[1].
2. Currently there is no vaccine for controlling any of these viruses^[2].

3. The best approach for all evocative the infection in the control of the mesquites insect – vector (*A. aegypti*)
4. Most methods for controlling the vector population are directed to the use of chemicals (insecticides) but with no complete control and in addition there are many side effects from these chemicals^[3].

Currently scientist is in a great search for best alternatives which is one of the objective of this study.

Material and methods

This study was conducted at dengue mosquito research station, Biology Department, Faculty of Science, King Abdulaziz Univeristy, Jeddah, Saudi Arabia.

Preparation of plant extracts

The Rhayza leaves were recovered from the field (Hada AlSham locally) washed carefully then dried at room temperature, then extracted thing in soxhlet apparatus (Fig.1.) and the concentrated extract thing in the rotary vacuum evaporator (Fig.2.) According to ^[4].

Biosynthesis of silver nanoparticles

The silver nanoparticles were prepared by using 1 ml of the extract and 1ml AgNO₃ in addition to 0.5 triton x-100 and 97.5 distilled water then all the extract was left at room temperate until the color changes (Fig.3.)

The preparation of standard solutions

The stock solution was prepared by adding 0.1 gm of it to 100 ml of distilled water containing 0.5% triton X- 100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations were prepared in distilled water. The standard WHO larval susceptibility test method ^[5] was used (Fig4).

Statistical analysis

LC₅₀ and regression equations were estimated by a computerized log-probit analysis software program.

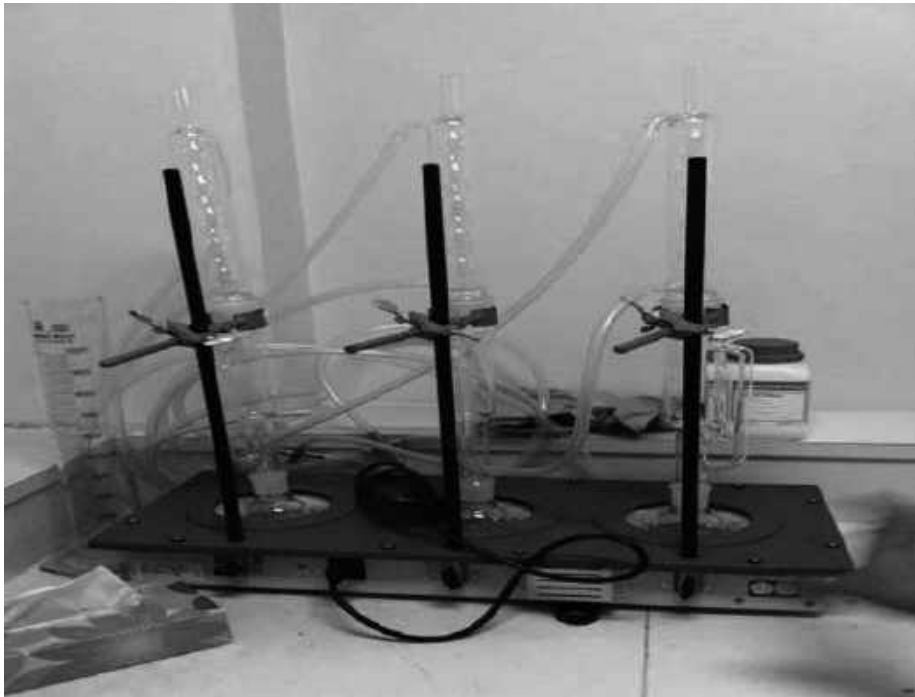


Fig. (1): Soxhlet apparatus



Fig. (2): Rotary Vacuum Evaporator

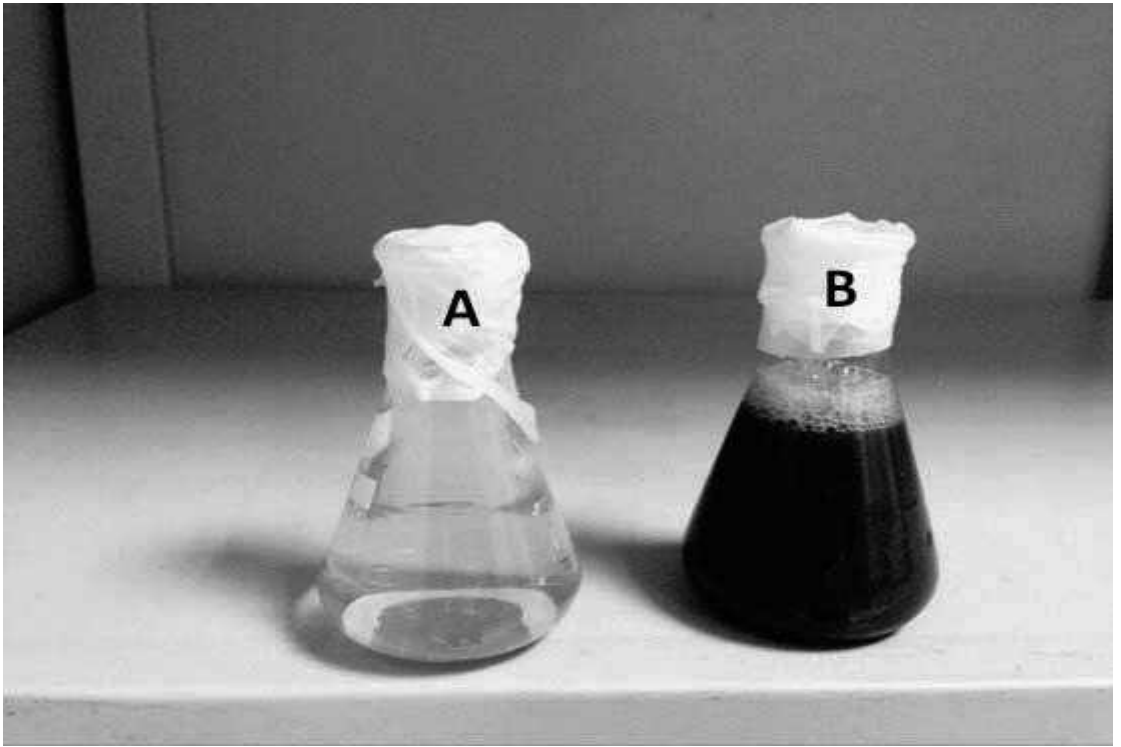


Fig. (3): Biosynthesis and silver nanoparticles
(A) - Before Biosynthesis (B) - After Biosynthesis

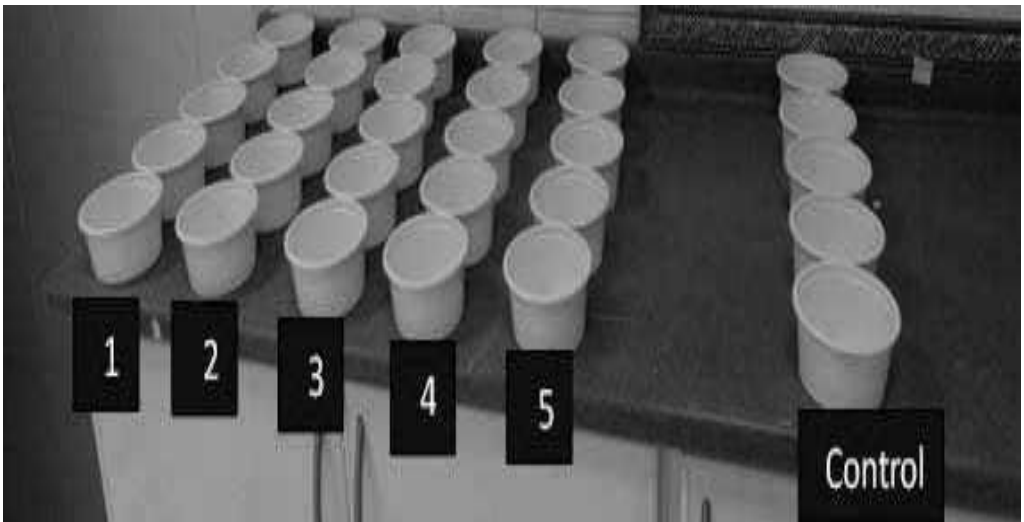


Fig. (4):The experimental design was conducted according the (WHO) methods

Results and Discussion

Susceptibility levels of *A. aegypti* larvae following treatments with different concentrations of *R. stricta* only & *R. stricta* with AgNo₃ against 4th larval instars of *A. aegypti* are shown in figure (5&6). In general, 30– 97% and 24 – 98 % larval mortalities were obtained when the 4rd instar larvae of *A.aegypti* were treated with the effective concentrations of *R. stricta* only (200 – 1000ppm) and *R. stricta* with silver nanoparticles (30 – 150ppm) extracts. Taking LC₅₀ values (concentration which to kill 50% of larvae) into consideration, the records showed that the *R. stricta* with silver nanoparticles (91.342 ppm) proved to be more effective extract than the *R. stricta* only (323.447 ppm). by about 3.46 folds figure (6).

In addition to the fatal direct effect of the extract and silver nanoparticles on the larvae dengue fever vector more morphology effects were evident figure (6) including:

1. Shrinks of the larva body parts.
2. Cell explosion and pigmentation.
3. Clear larva neck prolongation
4. Penetration of nanoparticles on the effected larvae body.

The results obtained from this study showed that the effect of the extract of *R. Stracta* and the nanoparticles against the larvae of the dengue fever vector (*A. aegypti*) one in accordance with what was repeated by other workers [6, 7, 8].

Conclusion

It is evident that from our results that the extract of the *R. stricta* and the silver nanoparticles could be used on a safety method of control on the larvae of the dengue fever and Zika viruses. As a potential candidate and an alternative for the use of chemical coater

Recommendations

We recommend that more active research should be develop to the use of natural product alone or strengthened with other sophisticated technology as the use of silver nanoparticles to reduce the population mosquito dengue fever (*A. aegypti*)

Commercial practical application

It is possible to prepare the extract in commitment formulations easy applicable in the abatement of the campaigns of the larvae of the dengue and Zika Viruses

Future work

More work should be directed towards the separation of the pure active ingredient so that it can be formulated different usable forms.

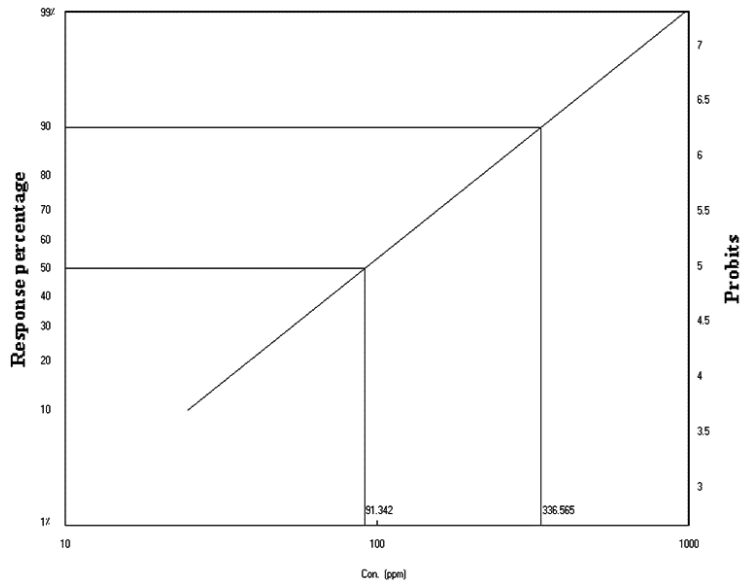


Fig. 5. : Regression lines for Rhazya stricta extracts and silver Nanoparticles Bioassay of larvae of *A. aegypti* using dipping technique.

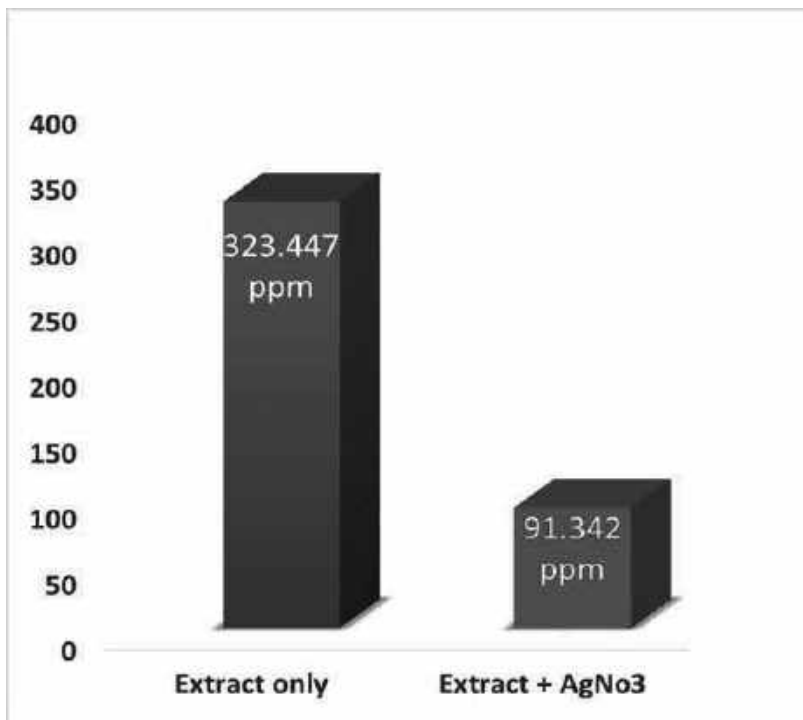


Fig. (6): Toxicity values of Rhazya stricta only & Rhazya stricta with AgNo3 against 4th larval instars of *Aedes aegypti*.

Comparison on basis of LC₅₀ values.



Fig (7)The Biological effects of Harmal extracts and silver Nanoparticles on larvae *A. aegypti* :

- | | |
|--------------------------|-----------------------------|
| A) Control | B) Segment Body Contraction |
| C) Pigmentation | D) Cells Explosion |
| E) Prolongation the neck | F) penetration |