The potentiality of Fenugreek nanoemulsion for control of mosquito-Borne Disease (Culex quinquefasciatus; Diptera: Culicidae)

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ABSTRACT

In the present study fenugreek nanoemulsion (FN) was tested against mosquitoes vector; so; characterization of this nanoemulsion using a transmission and scanning electron microscope (TEM and SEM). The used molecules appear to be spherical with a mean diameter of 80 nm. The larvae in the fourth stage were exposed to higher concentrations of FN from 10 to 320 μl/ml each for 1 and 2 hours of exposure time. The results proved that LC50 (lethal concentration) against mosquitoes 4th larval stage was 200 μl/ml/ for 2h and LC90 was 65 μl/ml / for 2 h while the positive control material (temephos) was 0.02 mg/L. As the two previous doses increased to 160 l/ml/2 h and 320 l/ml/2 h, Culex quinquefasciatus pupae showed a slightly high tolerance to the influence of these nano-emulsions. These findings suggest a new field for eradicating this vector (Culex quinquefasciatus) which can avoid the resistance that has been observed in previous chemical applications in this field. Nanotechnology rises up to give a wide scale for pest control and medicine.

INTRODUCTION

Arthropods are extremely dangerous vectors of illnesses and parasites that could cause epidemics or pandemics in the world's growing human and animal populations. Mosquitoes (Diptera: Culicidae) are among them, posing a serious threat to millions of people around the world by transmitting diseases such as malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis, and Zika virus (Jensen and Mehlhorn, 2009; Benelli and Mehlhorn, 2016; Pastula et al., 2016; Saxena et al., 2016).

Dog heartworm, West Nile virus, and Eastern equine encephalitis are among the microbial illnesses transmitted by Culicidae to dogs and horses (WHO, 2012; 2014; Mehlhorn, 2015). Unfortunately, there is no treatment for most arboviruses transmitted by mosquitoes, particularly dengue fever. Furthermore, even for other mosquito-borne
diseases, such as malaria, significant hurdles still exist that prevent effective control (Benelli and Mehlhorn, 2016).

*Culex quinquefasciatus* (*Cx. quinquefasciatus*), one of deadly mosquito, transmits the disease. Adult nematodes impede lymphatic system flow, resulting in lymphatic channel inflammation and elephantiasis. Filariasis is primarily common in Africa and India, although it is also found in China, Japan, Sri Lanka, and several Pacific islands.

On the other hand, lymphatic filariasis, also, known as elephantiasis, threatens over 1.4 billion people in 73 nations throughout the world. Over 120 million individuals are currently afflicted, with the illness disfiguring and incapacitating about 40 million people. Controlling or eradicating the mosquito population could reduce the disease transmission.

In the year 2000, the World Health Organization began the “Global Programme to Eliminate Lymphatic Filariasis. WHO, 2012 recommended the eradication of filariasis vector using a disease map for the parasites and its vector. In this context, vector control in specific areas, in addition to preventive treatment and morbidity management, contributed to the elimination of lymphatic filariasis (WHO, 2014).

Particular attention should be paid to the emergence of mosquito resistance strains, as well as environmental considerations, while using synthetic pesticides (Hemingway and Ranson, 2000; Naqqash et al., 2016). Indeed, in the past, organophosphates, carbamates, and pyrethroids were used extensively to target Culicidae juvenile instars, with serious consequences for human health and the environment (Naqqash et al., 2016).

Synthetic insecticides were widely utilised in modern agriculture production only a few years ago, resulting in widespread environmental contamination, food toxicity, resistance development, and health hazards. As a result, insect control now necessitates contemporary technology that poses less health risks, leaves no residue in their meal, and reduces organism resistance (Norris and Norris, 2011).

The specific fields of nanobiotechnology, which include liposomes nanoparticles for medication delivery, emulsions, imaging, biomaterials, food, optical, electronics, pathogens, biosensors, and in vitro diagnostics have been widely applied. Nanomaterials have unique physiological and chemical properties. Nanomaterials are similar in size to the organelles found in cells and have the potential to interfere with essential cell functioning, perhaps causing toxicity (Zhang et al., 2012). Attachment of extraordinary biomolecules such as bacteria, parasites, proteins as well as nucleic acids (Crean et al. 2011). beside the creation of environmentally acceptable nano-formulations with an efficient delivery method and minimal amounts of material are encouraging criteria for nanotechnique.

Therefore, this study is planned to use the scanning and transmission electron microscopy to characterize the selected FN, then test its larvicidal efficacy against 4th larval instar of *Cx. quinquefasciatus* under controlled laboratory conditions and measure its effect on the life cycle of mosquitoes.
MATERIALS AND METHODS

Nanoemulsion analysis
The Fenugreek nanoemulsion was purchased from Nanotech (Egypt); this nanoemulsion was analyzed using TEM (Abu-Elala et al., 2018) and SEM. The particles were sonicated in ethanol and put on a copper-coated carbon grid, where it was allowed to evaporate. The nanoparticles was imaged using a Jeol-JEM Japan 2100 running at 80 KV. Furthermore, after drying the particles in a CO₂ critical point drier (Autosamdri-815, Germany), the specimens were glued over stubs and coated with 20 nm gold in a sputter coater (Spi-Module sputter Coater, UK), then, the specimens were examined and photographed using a scanning electron microscope at a magnification ranging from 35X to 500X (JSM 5200, Electron prob) (Attia et al., 2021; 2022a).

Mosquito rearing
*Culex quinquefasciatus* eggs were collected from the Giza Medical Research Institute of Insects. The eggs were reared in de-chlorinated clean water in suitable aquaria (40X60X10 cm) at room temperature (26 ±2°C) to develop into first larvae stage (WHO, 2005; Attia and Salaeh, 2020).

Testing of Mortality rate caused by Fenugreek nanoemulsion
The larvicidal activity was determined using an approach of Attia et al. (2017); the studies took place in a room with a temperature of 26 ±2 °C and RH of 70 ±10% with a photoperiod of 14 hrs light/ 10 hrs dark. On *Cx. quinquefasciatus*, the efficacy of the tested FN was assessed using 4th instar larvae. In a 100 mL plastic jar, each stage was exposed independently in five replicate (each with 20 stage). *Cx. quinquefasciatus* larvae were exposed to different concentrations of FN as; 320; 160; 80; 40; 20 and 10 μl/ml of distilled water for different times 1 and 2 hours in de-chlorinated tape water (each time in a separate group with 20 larvae each); to study the effects of FN. After that, all the prior trials were used to determine LC₅₀ and LC₉₀. The Temephose (0.02 mg/L) was chosen as a reference larvicidal agent (control positive; Rai et al., 2020). Their concentration and exposure time were measured in accordance with the manufacturer's instructions. Twenty larvae in de-chlorinated tape water were used as control non treated negative control.

Evaluation of mortalities
At the end of each exposure period, the nanoemulsion was removed, and each stage was rinsed multiple times with de-chlorinated tape-water, transferred to clean beakers, and observed for 14 days after treatment. The average mortality of the five replicates was used to calculate the efficacy. The intended association between mortality percent and measured FN concentrations and exposure time was used to establish the LC₅₀ and
LC90. The control negative non-treated tests were run in parallel, with de-chlorinated tap water used in each experiment (Attia et al., 2017).

**Statistical analysis**

SPSS version 21 was used to analyse the data, and probit analysis was used to establish 95 percent confidence intervals. The LC50 and LC90 values were determined using either the regression equation (\(Y=\) Mortality percent; \(X=\) Log concentration) or by drawing a transverse line from probit 0.5 on the y-axis to the x-axis and computing the log concentration (Finney, 1971; Attia et al., 2022b).

**RESULTS**

The synthesized FN were found to be spherical in shape using scanning electron microscope and, in the size, ranged from 13.2- 18.2 nm in diameter using transmission electron microscopy (Figs.1&2).

![Fig.1: Scanning electron microscopic characterization of FN which appear as spherical in shape.](image1.png)

![Fig.2: Transmission electron microscopic characterization of FN which appear the size of the synthesized FN.](image2.png)
The potentiality of Fenugreek nanoemulsion for control of *Culex quinquefasciatus*

The degree of the larvicidal action is proportionally related to the increase in concentration and exposure duration for FN. Concentrations less than 10 μl/ml in the exposed stage, there was no mortality. (Table 1).

**Table(1): Different concentrations of FN on *Culex quinquefasciatus* as larvicidal.**

<table>
<thead>
<tr>
<th>Conc μl/ml</th>
<th>Larval mortality(%)</th>
<th>Pupa(%)</th>
<th>Pupae mortality (%)</th>
<th>Adult emergence (%)</th>
<th>Adult mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
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<tr>
<td>320</td>
<td>100</td>
<td>100</td>
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<td>160</td>
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<td>80</td>
<td>63</td>
<td>69</td>
<td>67.5</td>
<td>64.5</td>
<td>60</td>
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<td>40</td>
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<td>40</td>
<td>84.6</td>
<td>72.7</td>
<td>27.27</td>
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<td>15</td>
<td>22</td>
<td>85</td>
<td>78</td>
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<td>10</td>
<td>5</td>
<td>9</td>
<td>95</td>
<td>91</td>
<td>5.49</td>
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<tr>
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<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control N</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

1: group treated after 1 hour.
2: group treated after 2 hour.

**Control P:** Larvae treated with temephose (0.02 mg/L).
**Control N:** larvae present in de-chlorinated tape water used as control non treated negative control.

In concentration of 10 μl/ml; 5 and 9% of the total exposed larvae were died after 1 and 2 hour exposure periods respectively. Then, the survived larvae (95% and 91%) were molting into pupae. Those pupae were active and express low mortality rate; 5.49% (5) and 10.5% (10). All emerged adult were active normal not deformed adult (90% and 81% were emerged adult).

While, by regarding the larvae exposed to concentration 20 μl/ ml; 15% and 22% larvae were found dead after 1 and 2 hours exposure time respectively. While the remaining 85 and 78 larvae were pupate, then 15 pupa out of 85 and 20 pupa of 78 were died. Finally, 50 out of 70 survived pupae and 43 out of 58 pupae were emerged to adult (Table1).

At concentration 40 μl/ml; 35 % and 40% of exposed larvae were found dead after 1 and 2 hours of exposure. Then 10 and 20 larvae were died after 36 and 38 hours of exposure. The remaining 55 and 40 larvae were started to pupate. Only 40 and 30 pupa were succeed to complete their pupation, while the other 15 and 10 pupa were failed to
emerge to adult. Twenty and 22 pupa were finally developed to form adult while the other pupae were failed to emerge to adult (Table 1).

At concentration of 80 μl/ml; 63% and 69% larvae were dead at 1 and 2 hours of exposure period; the 1st group in 1h exposure; 12 larvae were dead after 36 hours and 11 larvae were dead after 24 hours in the 2nd group of 2 hours exposure time. Ten pupae were still alive from 25 and 15 pupa were dead in the 1st group; while in group of 2 hours exposure period; 12 pupae were dead and 8 pupae were alive. From the 1st group; 5 adult emerged and 3 deformed adult; while in the 2nd group 3 adult emerged and 5 were died; All 3 adult were deformed (Table 1).

At the concentration 160 μl/ml; 82% and 87% were died from the total exposed larvae in the two groups (1 and 2 hours); but no larvae were pupate; in the 1st group 18 larva dead after 18 hours while 13 larvae dead after 12 hours of exposure period (Table 1).

At the high concentration 320 μl/ml all exposed 4th stage larvae were died in the exposed time 1 and 2 hours exposure period.

The calculated LC50 and LC90 of larvicidal activity of Fenugreek nanoemulsion on Cx. quinquefasciatus were 65 μl/ml / 2 h for LC50 and 200 μl/ml/ 2h for LC90 while the positive control material was temephose was 0.02 mg/L.

DISCUSSION

Nanotechnology is a new-changing technology which used in a variety of fields, including medicine, biology, and agriculture (Salem et al., 2022). Nanomaterials as active pesticide agents or nanocarriers for their transport are used in the formulation of nano-emulsions for smart nano-pesticides. It can also be determined that it is more valuable than chemical insecticides for targeting pesticide delivery for controlling of mosquito larvae and adults (Ahmed et al., 2019; AbdElKader et al., 2021).

The emergence of resistance from recurrent application of the same synthetic chemicals for vector control had been necessitating for the development of another new technology to disrupt the life cycle of these vectors.

Trigonella foenum-graecum L., generally known as fenugreek, is a member of the Fabaceae family. It is the most promising therapeutic herb that has been discovered since ancient times. Fenugreek seed oil has antibacterial, anti-diabetic, antioxidant, and wound-healing effects (Srinivasan, 2006; Abdel-Daim et al., 2014; Dharajiya et al., 2016; Sharma et al., 2017). Fenugreek oil has been shown to have antifungal and anticancer activities in recent investigations (Aqil and Ahmad, 2003; Nandagopal et al., 2012; Palambo and Semple 2001). The worldwide analysis based on fenugreek investigations demonstrates that the seeds and oil of this plant have substantial antibacterial action in concentrations ranging from mg/mL to g/mL (Dash, 2011; Liu et al., 2017).
The mosquitocidal effectiveness of the tested FN was found to start at 10 μl/ml. For these FN emulsion, there is a clear relationship between the severity of the effects and the increase in concentration and exposure duration. With higher concentrations and exposure time, the effectiveness improves. Pupation rates and adult emergences decreased as the concentration increase. When used in large amounts, it can be dangerous. After 2 hours, the larvicidal effects of raising particle concentration from 80 μl/ml to 160 l/ml rose from 69 to 87 percent for both concentrations, respectively, and reached 100 percent by increasing exposure period to 12 hours. After 2 hours of exposure, the impact rose to 100% after raising the concentration to 320 μl/ml.

The capacity of the tested FN to kill larvae may be linked to the method of action reported by Mansuri et al. (2022), in which it may be absorbed by phospholipid in the cuticle of larval instars via physiosorption and lysis, resulting in insect mortality. Because the exposed tegument is thin, the effect was strong.

Essential oils (EOs) are naturally oily liquids recovered by hydro distillation from various sections of aromatic plants (bark, stem, flower, and rhizome) using the Clevenger type apparatus (Osanloo et al., 2017). They have a wide range of biological properties, including antibacterial activity (Osanloo et al., 2020), leishmanicidal activity (Noorpisheh Ghadimi et al., 2020), larvicidal activity (Osanloo et al., 2018), and repellent activity (Moemenbollah-Fard et al., 2020). Because of their selective action on target and negligible side effects on non-target organisms, EO-based insecticides have recently been proposed as alternatives to synthetic insecticides for mosquito control (Soleimani-Ahmad et al., 2017). Many research on the use of EOs against mosquitoes have been published (Khanavi et al., 2013). However, because some of the chemicals in EOs are volatile, their effectiveness as an insecticide and repellant is restricted.

In another investigation involving non-formulated EO, the perfect larvicidal impact (100 percent mortality) was attained at 4 hours instead of 24 hours (Sugumar et al., 2014). Nanoemulsions' reported lethal concentration 50 (LC₅₀) was substantially higher than that of non-formulated EOs in ten studies. The two most recent reports looked into the persistence of larvicidal activity. At two distinct doses, tarragon essential oil was entrapped in chitosan nanoparticles. The larvicidal action of tarragon EO at concentrations of 1.6 and 6% was sustained for 2 and 4 days, respectively, whereas these durations were greatly improved in nanoformulated forms.

The physical stability of nanoemulsions is normally good; however, when they are diluted (100-200 times) during larvicidal testing, their stability plummets. Instability eventually leads to the larvicidal effects lasting just a short time (Osanloo et al., 2017). As a result, the long-term larvicidal activity of nanoemulsions has not been documented; only the efficiency of EOs has been enhanced.

Herbal extracts are utilized as reducing agents in the manufacture of silver nanoparticles from their salts, such as AgNO₃ (Siddiqi et al., 2018). Toxic reducing chemicals are not required in this approach (Amini, 2019). However, a crucial issue has
been missed in these reports: silver nanoparticles interact efficiently with chemical functional groups. As a result, the final characteristics of silver nanoparticles are greatly influenced by the reducing agent, such as herbal extracts (Amiri et al., 2018). The LC$_{50}$ of plant-derived silver nanoparticles against An. stephensi has been found to range from 2 to 12470 ppm (Subarani et al., 2013). Chemically produced nanoparticles with a particle size of 30 nm had only a 20% larvicidal efficacy at 100 ppm (Osanloo et al., 2018).

**CONCLUSION**

The current investigation showed that FN may be utilised as an effective larvicidal against Cx. quinquefasciatus larvae when administered at concentrations of 160 l/ml and 320 l/ml following exposure times of 12 and 2 hours, respectively. As a result, preventing the transmission of dangerous viral and parasite infections by such vectors. The successful use of FN in the treatment of aquatic stages of vector-borne parasitic illness opens up a new avenue for battling these hazardous parasites and avoids the resistance that has been observed in this sector due to repeated chemical applications.

**REFERENCES**


The potentiality of Fenugreek nanoemulsion for control of *Culex quinquefasciatus*


The potentiality of Fenugreek nanoemulsion for control of *Culex quinquefasciatus* 623


