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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 mosquitos, 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 elephantasis. 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 CO_2 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^{o}C) to develop into first larvae stag (**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 ~~of~~ the prior trials were used to determine LC_{50} and LC_{90}. 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-cholrinated 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 tape 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**).

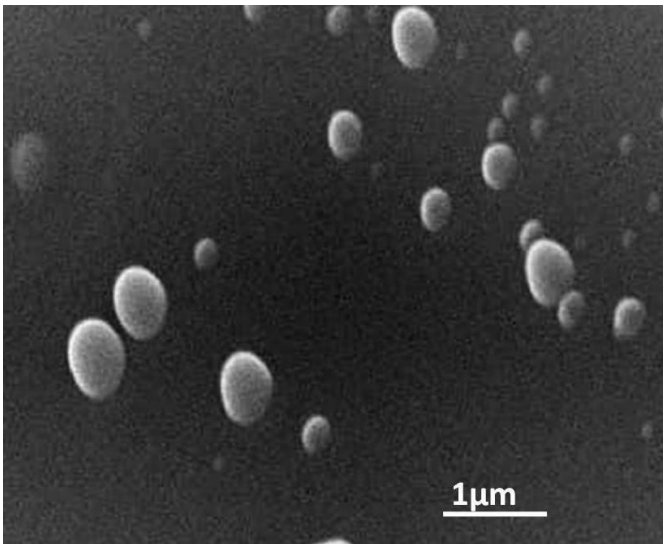

Fig.1: Scanning electron microscopic characterization of FN which appear as spherical in shape.

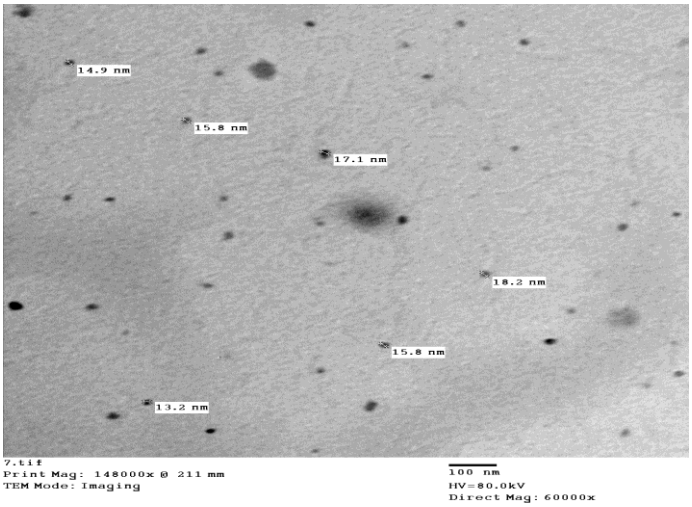

Fig.2: Transmission electron microscopic characterization of FN which appear the size of the synthesized FN.

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.

Conc µl/ml	Larval mortality(%)		Pupa(%)		Pupae mortality (%)		Adult emergence (%)		Adult mortality (%)	
	1	2	1	2	1	2	1	2	1	2
320	100	100	-	-	-	-	-	-	-	-
160	82	87	-	-	-	-	-	-	-	-
80	63	69	67.5	64.5	60	60	50	37.5	60	100
40	35	40	84.6	72.7	27.27	25	50	73.3	50	26.6
20	15	22	85	78	17.6	25	82.3	74.35	28.5	25.8
10	5	9	95	91	5.49	10.5	90	81	-	-
Control p	100	100	0	0	0	0	0	0	0	0
Control N	0	0	100	100	0	0	100	100	0	0

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-cholrinated 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 (**Table1**).

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 (**Table1**).

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 (**Table1**).

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 LC_{50} and LC_{90} of larvicidal activity of Fenugreek nanoemulsion on *Cx. quinquefasciatus* were 65 µl/ml / 2 h for LC_{50} and 200 µl/ml/ 2h for LC_{90} 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 (**Moemenbellah-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-Ahmadi *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 (LC_{50}) 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

Abu-Elala, N.M.; Attia, M.M. and Abd-Elsalam, R. M. (2018). Chitosan-silver nanocomposites in goldfish aquaria: A new perspective in *Lernaea cyprinacea* control, Int. J. Biol. Macromol., 111: 614-622.

Abdel-Daim, M.M.; Abd Eldaim, M.A. and Mahmoud, M.M.. (2014). *Trigonella foenum-graecum* protection against deltamethrin-induced toxic effects on haematological, biochemical, and oxidative stress parameters in rats. Can. J. Physiol. Pharmacol. 92:679–685.

AbdElKader, N.A.; Sheta; E.; AbuBakr; H.O. and others (2021). Effects of chitosan nanoparticles, ivermectin and their combination in the treatment of *Gasterophilus intestinalis* (Diptera: Gasterophilidae) larvae in donkeys (*Equus asinus*). Int. J. Trop. Insect. Sci. http://dx.doi.org/10.1007/s42690-020-00171-2.

Ahmed, T.; Hyder, M. Z.; Liaqat, I. and Scholz, M. (2019). Climatic conditions: conventional and canotechnology-based methods for the control of mosquito vectors causing human health issues Int. J. Environ. Res. Public Health, 16(17):3165; https://doi.org/10.3390/ijerph16173165.

Amini, S.M. (2019). Preparation of antimicrobial metallic nanoparticles with bioactive compounds. Mater. Sci. Eng. C, 103, https://doi.org/10.1016/j.msec.2019.109809.

Amiri, S.; Yousefi-Ahmadipour, A.; Hosseini, M.J.; Haj-Mirzaian, A.; Momeny, M.; Hosseini-Chegeni, H.; Mokhtari, T.; Kharrazi, S.; Hassanzadeh, G.; Amini, S.M.; Jafarinejad, S. and Ghazi-Khansari, M. (2018). Maternal exposure to silver nanoparticles are associated with behavioral abnormalities in adulthood: Role of mitochondria and innate immunity in developmental toxicity. Neurotoxicology, 66 **:** 66-77, https://doi.org/10.1016/j.neuro.2018.03.006.

Aqil, F. and Ahmad, I. (2003). Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World J. Microbiol. Biotechnol., 19: 653–657.

Attia, M.M.; Soliman, S. M. and Khalf, M.A. (2017). Hydrophilic nanosilica as a new larvicidal and molluscicidal agent for controlling of major infectious diseases in Egypt. Vet World. 10(9): 1046–1051.

Attia, M.M. and Salaeh, N.M.K. (2020). Ultrastructure of adult *Gasterophilus intestinalis* (Diptera: Gasterophilidae) and its puparium. Int. J. Trop. Insect Sci. 40(4):1-9.

Attia, M. M.; Yehia, N.; Soliman, M. M.; Shukry, M.; El-Saadony, M. T. and Salem H M. (2022a). Evaluation of the antiparasitic activity of the chitosan-silver nanocomposites in the treatment of experimentally infested pigeons with *Pseudolynchia canariensis*. Saudi J. Biol. Sci. 29: 1644–1652.

Attia, M.M.; Abou-Okada, M.; Shamseldean, M.S.M.; et al. (2022b). Insecticidal effects of Curcumin (*Curcuma longa*) against the horse stomach bot fly, *Gasterophilus intestinalis* (Diptera: Oestridae). Int. J Trop. Insect. Sci. https://doi.org/10.1007/s42690-021-00618-0.

Benelli, G. and Mehlhorn, H. (2016). Declining malaria, rising dengue and Zika virus: insights for mosquito vector control. Parasitol. Res.115: 1747–1754.

Crean, C. et al (2011). Polyaniline nanofibres as templates for the covalent immobilisation of biomolecules. Synth Met. Elsevier, 161(3–4):285–292.

Dash, B. (2011). Antibacterial activities of methanol and acetone extracts of fenugreek (*Trigonella foenum*) and coriander (*Coriandrum sativum*). Life Sci. Med. Res. 27: 1-8.

Dharajiya, D.; Jasani, H.; Khatrani, T.; Kapuria, M.; Pachchigar, K. and Patel, P. (2016). Evaluation of antibacterial and antifungal activity of fenugreek (*Trigonella foenum-graecum*) extracts. Int. J. Pharm. Pharm. Sci., 8: 212–217.

Finney, D. J. (1971). Probit Analysis. Cambridge University Press, Cambridge. pp-333.

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. Annu. Rev. Entomol. 45:371–391.

Jensen, M. and Mehlhorn, H. (2009). Seventy-five years of Resochin_ in the fight against malaria. Parasitol. Res. 105: 609–627.

Khanavi, M.; Vatandoost, H.; Dehaghi, N.K.; Dehkordi, A.S.; Sedaghat, M.M.; Hadjiakhoondi, A. and Hadjiakhoondi, F. (2013). Larvicidal activities of some Iranian native plants against the main malaria vector, *Anopheles stephensi*. Acta. Med. Iran., 51: 141-147.

Liu, Q.; Meng, X.; Li, Y.; Zhao, C. N.; Tang, G.Y. and Li, H.B. (2017). Antibacterial and antifungal activities of spices. Int. J. Mol. Sci., 18: 1283**.**

Mehlhorn, H. (Ed.) (2015). Encyclopedia of Parasitology, fourth ed. Springer, New York, p. 893.

Moemenbellah-Fard, M.D.; Shahriari-Namadi, M.; Kelidari, H.R.; Nejad, Z.B.; Ghasemi, H. and Osanloo, M. (2020). Chemical composition and repellent activity of nine medicinal essential oils against *Anopheles stephensi*, the main malaria vector. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-020-00325-2.

Mansuri, A.; Chaudhari, R.; Nasra, S.; Meghani, N.; Ranjan, S. and Kumar, A. (2022). Development of food-grade antimicrobials of fenugreek oil nanoemulsion-bioactivity and toxicity analysis. Environ. Sci. Pollut. Res. Int. doi: 10.1007/s11356-022-19116-y. Epub ahead of print. PMID: 35150423.

Nandagopal, S.; Dhanalakshmi, D.; Kumar, A.G. and Sujitha, D. (2012) Phytochemical and antibacterial studies of fenugreek *Trigonella foenum- graecum* L.-a multipurpose medicinal plant. J. Pharm. Res. 5: 413–415.

Naqqash, M.N.; Gokce, A.; Bakhsh, A. and Salim, M. (2016). Insecticide resistance and its molecular basis in urban insect pests. Parasitol. Res. 115: 1363–1373.

Noorpisheh Ghadimi, S.; Sharifi, N. and Osanloo, M. (2020). The leishmanicidal activity of essential oils: A systematic review. J. Herb. Med. Pharmacol., 9: 300-308, https://doi.org/10.34172/jhp.2020.38.

Norris, L.C. and Norris, D.E. (2011). Insecticide resistance in *Culex quinquefasciatus* mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. J. Vector Ecol. 36 (2): 411–420.

Osanloo, M.; Amani, A.; Sereshti, H.; Shayeghi, M. and Sedaghat, M.M. (2017). Extraction and chemical composition essential oil of *Kelussia odoratissima* and comparison its larvicidal activity with Z-ligustilide (major constituent) against *Anopheles stephensi.* J. Entomol. Zool. Stud., 5: 611-616.

Osanloo, M.; Sereshti, H.; Sedaghat, M.M. and Amani, A. (2018). Nanoemulsion of Dill essential oil as a green and potent larvicide against *Anopheles stephensi*. Environ. Sci. Pollut. Res., 25, 6466-6473, https://doi.org/10.1007/s11356-017-0822-4.

Osanloo, M.; Ghaznavi, G. and Abdollahi, A. (2020). Surveying the chemical composition and antibacterial activity of essential oils from selected medicinal plants against human pathogens. Iran. J. Microbiol., 12, 505-512, https://doi.org/ 10.18502/ ijm.v12i6.5032.

Palambo, A. and Semple, S. (2001). Antibacterial activity of traditional medicinal plants. J. Ethnopharmacol., 77: 151–157.

Pastula, D.M.; Smith, D.E.; Beckham, J.D. and Tyler, K.L. (2016). Four emerging arboviral diseases in North America: Jamestown Canyon, Powassan, chikungunya and Zika virus diseases. J. Neurovirol. http://dx.doi.org/10.1007/s13365-016-0428-5.

Rai, P.; Bharati, M. and Saha, D. (2020). Insecticide resistance to Temephos and synthetic Pyrethroids in *Culex quinquefasciatus* say from sub- Himalayan West Bengal, India. Int. J. Trop. Insect Sci.; 9: 1–8.

Salem H M, Salaeh NM.K., Ragni M, Swelum A. A., H. Alqhtani A, Abd El-Hack M E., El-Saadony M T., Attia M M.(2022). Incidence of gastrointestinal parasites in pigeons with an assessment of the nematocidal activity of chitosan nanoparticles against Ascaridia columbae, Poult. Sci., 101, 6, 101820. https://doi.org/10.1016/j.psj.2022. 101820.

Saxena, S.K.; Elahi, A.; Gadugu, S. and Prasad, A.K. (2016). Zika virus outbreak: an overview of the experimental therapeutics and treatment. Virus Dis. http://dx.doi.org/10.1007/s13337-016-0307-y.

Sharma, V.; Singh, P. and Rani, A. (2017). Antimicrobial activity of Trigonella foenum-graecum L.(Fenugreek). Eur J Exp Biol 7:4 Aqil F, Ahmad I (2003)

Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World J. Microbiol. Biotechnol. 19: 653–657.

Siddiqi, K.S.; Husen, A. and Rao, R.A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. J. Nanobiotechnology., 16, https://doi.org/10.1186/s12951-018-0334-5.

Soleimani-Ahmadi, M.; Gorouhi, M.A.; Azani, S.; Abadi, Y.S.; Paksa, A.; Rashid, G. and Sanei-Dehkordi, A. (2017). Larvicidial Effects of essential oil and methanol extract of *Achillea wilhelmsii* C. Koch (Asteraceae) against *Anopheles stephensi* Liston (Diptera: Culicidae), a malaria vector. J. Kerman. Univ. Med. Sci., 24: 58-67.

Srinivasan, K. (2006). Fenugreek (*Trigonella foenum-graecum*): a review of health beneficial physiological effects. Food Rev. Int. 122: 203–224.

Subarani, S.; Sabhanayakam, S. and Kamaraj, C. (2013). Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res., 112, 487-499, https://doi.org/10.1007/s00436-012-3158-5.

Sugumar, S.; Clarke, S.K.; Nirmala, M.J.; Tyagi, B.K.; Mukherjee, A. and Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. Bull. Entomol. Res. , 104 (3): 393-402.

WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides.

WHO (2012) Handbook for Integrated Vector Management. World Health Organization, Geneva.

WHO (2014) Lymphatic Filariasis. Fact Sheet N_102. World Health Organization, Geneva.

Zhang, X.; Xu, X.; Bertrand, N.; Pridgen, E., Swami, A. and Farokhzad, C.O. (2012). Interactions of nanomaterials and biological systems: Implications to personalized nanomedicine. Adv. Drug Deliv. Rev., 64 (13): 1363-1384.