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LARVICIDAL EFFICACY OF *NIGELLA SATIVA* SEEDS OIL AND IT'S NANOPARTICLES AGAINST *CULEX PIPIENS* AND *MUSCA DOMESTICA*

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Abstract

Extensive and continuous use of synthetic chemical insecticides led to environmental hazards to non-target organisms, and development of insect's resistance. The present study evaluated essential oil of *Nigella sativa* seeds as a friendly larvicidal measure for *Culex pipiens* and *Musca domestica* vectors of infectious diseases. The *N. sativa* components essential oil was analyzed by GC-MS. Sixteen components were identified. The selenium nanoparticles (SeNPs) with *N. sativa* essential oil were prepared to form *N. sativa* SeNPs and proved by UV-Spectrophotometer, FT-IR, and SEM. The droplet size of nanoemulsion was 65±11.4 nm. The bioassay showed that *N. sativa* essential oil and its nanoparticles were more effective against larvae of *Cx. pipiens* than those of *M. domestica*.

Key words: *Nigella sativa* oil, Selenium nanoparticles, *Culex pipiens*, *Musca domestica*.

Introduction

No doubt the great majority of infectious arthropod-borne diseases, whether blood pa-rasites or gastrointestinal parasitic diseases (Morsy *et al,* 2019). But, the great majority of insect-borne diseases developed resistance to the chemical insecticides whether chlorinated or of phosphorous origins (Elnakib *et al*, 2018), apart from being risky to both man and animals (El-Bahnasawy *et al*, 2015), by causing environmental pollutions (Hijosa-Valsero *et al,* 2016). So, many authors have shafted to phytochemical plants and herbal extracts (Wolfin *et al,* 2019; Isman, 2020).

In Egypt, many plants and herbal extract were used as treatment for gastrointestinal diseases (Abouel-Nour *et al,* 2015), vectors control (Massoud and Labib, 2000; El gendy *et al*, 2019) and snail intermediate host control (Shoukry, 2006).

Nigella sativa seeds contained thymoquinone, p-cymene, carvacrol, terpineol, longifolene, α-pinene & thymol was recommended as phytochemical insecticides (Isman, 2006; Chaubey, 2007; Raj *et al,* 2015; Yimer *et al,* 2019). The chemical constituents were more specific to the target pests than insecticides against oviposition, larvae and adults anti-feeding against many insect vectors (Aboelhadid *et al*, 2015; Campolo *et al,* 2017).

Therapeutic agents such as antibody-based therapeutics, metal-based nanoparticles, etc. have been developed for treatment of infectious diseases and control of vectors (Anjali *et al,* 2010; Aderibigbe, 2017). Metal-based nanoparticles are characterized by small sizes between 10-100 nm accounted for good interaction with biomolecules within the cell and on cell surface, the high surface area promoted cell permeability (Kah *et al.* 2012; Mody *et al,* 2015).

Selenium (Se) is an essential trace element that is crucial for many cellular functions by the incorporation of selenoproteins (Skalickova *et al.* (2017). Selenium nanoparticles (SeNPs) were used in antimicrobial coatings, nutritional suppleme- nts, nanotherapeutics, diagnostics, medical devices, and other applications such as rectifiers (Vrček, 2018). SeNPs applications included drugs and targeted gene delivery, anti-cancer, anti-bacteria, anti-inflammatory activities, and biosensors (Chaudhary *et al*, 2014), and It possessed antiviral, antifungal activity (Nguyen *et al,* 2017). Many reports explain synthesis of SeNPs by various methods such as laser ablation meth-

od, microwave-assisted method, chemical reduction, electro-deposition method, salvo-thermal and green synthesis (Zhu *et al*, 2017: Shah and Zheng, 2019).

The present study aimed to assess the larvicidal efficiency of *Nigella sativa* seed oil and *N. sativa* SeNPs against *Culex pipiens* & *Musca domestica* 3rd stage larvae.

Materials and Methods

The *N. sativa* essential oils (black seed) were extracted by steam distillation at the National Research Center, Dokki. The seeds (25gm) were air dried in dark, mixed with 500ml of distilled water in one liter flask and subjected for hydro-distillation for 3 hrs. The volatile oils were dried with anhydrous sodium sulphate put in dark glass bottles in the refrigerator until needed.

Gas chromatography/mass spectrometry (GC-MS) analysis of *N. sativa* essential oil: GCMS was analyzed, Faculty of Pharmacy, Ain Shams University, using a Shimadzu 2010 Plus GC-MS (Germany) equipped with a Quadrupole (QP-5050) detector. Capillary column was CP-Wax 52 CB (50mx 0.32mm, film thickness 0.25µm). Injector temperature was 240°C & detector temperature, 250°C. The oven temperature program was from 60°C (10min. hold) to 220°C, raised to 2°C/min. and increased to 220°C (11.5min. hold) raised to 20°C/min. flow speed, 10pound/square inch. Detector: 70electorn volt; ionization type, helium as carrier gas (20ml/minute).

The injected sample was 1µl. constituents, and identification was determined by comparing the standard substances retention times with WILEY, NIST, & TUTOR libraries data.

Synthesis of *N. sativa* extract *in-situ* selenium nanoparticles (SeNPs): Selenious acid (H_2SeO_3, 0.013gm, 0.01mmol) was dissolved in 85ml deionized water in conical flask. Tween 80 (8ml) was added to selenious acid heated to 60°C. The oil (2.5ml.) was added to the mixture with continuous stirring at 60°C for 1hr and then 200µL of 40mM ascorbic acid was added as a catalyst the ruby red SeNPs were suspended. Nanoparticles were evaluated by UV-Spectrophotometer, particle size and TEM.

Ultraviolet-Visible (UV-VIS) Spectra (Shimadzu spectrophotometer): It followed selenium nanoparticles formation in the oil aqueous solution. UV-VIS spectra were recorded between 400-700nm.

Infra-Red (IR) spectroscopy: IR spectroscopy was done by using Bruker IR Spectrometer.

TEM: Shape and size of SeNPs were obtained by using High Resolution Transmission Electron Microscopy (HRTEM) JEOL (JEM -2100 TEM). TEM colloidal nanocrystals were prepared by placing a drop of colloidal solution on 400mesh carbon coated copper grid and dried at room temperature.

Larvicidal Assay: 1- of *Cx. pipiens* were egg rafts obtained from the mosquito-culture at RTC, Ain Shams University. The larvae were reared in dishes containing 1000ml distilled water. Newly hatched larvae were fed on the Tetra-min (Germany). Adults were reared in (35x35x35cm) wooden cages and provided with 10% sucrose solution daily as well as a pigeon for female blood feeding.

2- *Musca domestica*: A laboratory bred pupae were obtained from the Research Institute for Medical Entomology in Dokki, Giza, and colonized in the Insectary, Department of Entomology. Adults were given powdered milk mixed with distilled water (1:1) in cotton pads in separate Petri dishes for feeding and breeding. The larval diet was a mixture of wheat bran, yeast, sugar and powder milk (40:10:3:3), respectively and maintained under controlled conditions of 14:10 light: dark photoperiod, 27±2°C, & 60-70% RH (Abdel-Haleem *et al,* 2018).

Larvicidal bioassay (WHO 2005): 1-*Cx. pipiens*: *N. sativa* oil and its nanoparticles were used against 3rd larval instar under the laboratory conditions. Batches of 25 3rd instar larvae were transferred by to six small

disposable test cups, and treated with *N. sativa* SeNPs different concentrations (5, 10, 20, 30, 40 & 50 ppm) and (50, 100, 200, 300 & 400 ppm for oil). Experiments were repeated 3 times**.** Same concentrations with 1% surfactant only were used as control. 2- *M. domestica* 20 3rd instar larvae were used. *N. sativa* SeNPs & oil were prepared as 10ml. concentration series added to 10gm. bran media in 120ml. plastic cups and gently mixed. Dilutions (10, 20, 30, 40 & 50 ppm. for *N. sativa* SeNPs) and (60, 120, 240, 480 & 960 ppm. for *N. sativa*) were used side by side with controls and repeated 3 times as well. Mortality recorded after 48 hrs.

Statistical analysis: Data were analyzed by statistics package (LDP-line) for goodness of fit (*Chi* square test) and to detect LC_{50} and LC_{95} values with corresponding 95% confidence limits (C.L.), slope, correlation coefficient and standard error.

Results

The *N. sativa* GC-MS analysis showed sixteen compounds (Tab. 1).

Table 1: Chemical components of *N. sativa* essential oil as identified Gas Chromatography Mass Spectrometry (GC-MS).

No.	RT	Area%	Name	Formula	Weight
1	4.64	1.98	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}O_2$	136.12
2	5.98	5.62	Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)-	$C_{10}H_{16}$	136.12
3	6.56	5.30	Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)-	$C_{10}H_{16}$	136.12
4	7.57	20.73	p-Cymene	$C_{10}H_{14}$	134.11
5	9.25	1.31	Longifolene	$C_{15}H_{24}$	204.18
6	9.58	10.31	Thymoquinone	$C_{10}H_{12}O_2$	164.08
7	10.66	2.71	Phenol, 2,3,5,6-tetramethyl-	$C_{10}H_{14}O$	150.10
8	11.94	8.91	p-Cymene-2,5-diol	$C_{10}H_{14}O_2$	166.09
9	12.43	1.64	Naphthalene, decahydro-1,1-dimethyl-	$C_{12}H_{22}$	166.17
10	12.99	8.05	trans-Farnesol	$C_{15}H_{26}O$	222.19
11	13.15	5.60	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.24
12	13.65	13.51	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294.25
13	14.1	5.56	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.24
14	16.30	2.42	9,12-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	266.22
15	18.13	4.76	Morphinan-6-ol, 4,5-epoxy-N-methyl-2-[(4-trifluoromethyl) phenoxy]-,	$C_{18}H_{21}NO_3$	431.17
16	18.61	1.70	2-Hydroxy-N'-(1-(2 thienyl)ethylidene) benzohydrazide, 2TBDMS derivative	$C_{18}H_{14}N_2O_2$	488.23

Nanoemulsion by droplet size was characterized by Gaussian distribution analysis (Tab. 2).

Table 2: Intensity-weighted Gaussian distribution analysis (Solid Particle).

Parameter	Value	Parameter Value
Mean Diameter	55-75 nm	Variance (P.I.)= 0.138
Stnd. Deviation	78.3 nm (37.2%)	Chi Squared=161.391
Norm. Stnd. Dev	0.372(Coeff. of Var'n)	Baseline Adj.= 0.531 %
Z-Avg. Diff. Coeff.	2.07E-008 cm^2/s	

Physicochemical characterization: Nanoemulsion pH value was 6.4 & viscosity was 2.2m pascal/sec. Stability of nanoemulsion was not the sign of nanoemulsion included phase separation or creaming, and stable after centrifugation at 10,000rpm for 30minutes and when stored for a month at 4˚C.

Synthesis: *N. sativa* oil was rich in unsaturated fatty acids, mainly linoleic acid (50-60%), protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%) and total ash (4.8%), vitamins and minerals as Cu, P, Zn & F…etc, oleic acid (20%), eicodadienoic acid (3%) & dihomolinoleic acid (10%), Saturated fatty acids (palmitic, stearic acid) amount of 30% or less. α-Sitosterol was a major sterol accounted to 44% & 54% of total sterols in Tunisian stigmasterol (6.57-20.92%). Others were ellone, avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, lophenol, obtusifoliol, stigmastanol, & stigm-asterol-7-ene. Oil had relatively low reducing activities, but high stabilizing characteristic during SeNPs preparation. So, ascorbic acid was used as a catalyst. Se+ cation reduction into Seo was done by sugar with the ascorbic acid catalyst that acted as an aldehyde to form SeNPs and stabilized nanostructure of SeNPs, but glucosinolates were oxidized to Gluconic acid.

The preparation of SeNPs from Se^+ using *N. sativa* oil caused the reaction change in

color. Fresh oil was characterized by yellow coloration. But, after adding H_2SeO_3 and stirring for 1hr. at 60ºC; solution turned red (Fig. 1). Formation of SeNPs was monitored by UV-VIS spectroscopy & UV-VIS absorption spectrum of selenium nanoparticles (Fig. 2). Selenium colloidal solution according to plasmon resonance surface in absorption spectra showed the peak at wave length 530nm indicating that the nanoparticles were formed and dispersed in aqueous solution without aggregation formation. Selenium colloidal nanoparticles characters were proved by TEM. Synthesized selenium nanoparticles using this green method have spherical shape morphology with an average size of 55-75nm; with some clusters.

SEM particle size: *N. sativa* SeNPs synthesized by *N. sative* oil was scanned (Fig. 3). *N. sativa* SeNPs were spherical and particles form cluster with a few number of micrometers sized particles.

N. sativa oil and *N. sativa* SeNPs were bioassayed against 3rd instars larvae of *Cx. pipiens* (Tab. 3, Fig. 4). Confidential limits of *N. sativa* & *N. sativa* SeNPs were significantly calculated for LC_{50} & LC_{95} (P=0.05), 133.49 & 17.39, with slope function of 2.48 & 1.72 respectively. Toxicity of *N. sativa* SeNPs for *Cx. pipiens* larvae was about 8 folds more than of *N. sativa* oil with relative potency 7.676.

Table 3: Larvicidal activity of *N. sativa* & *N. sativa* SeNPs against 3rd instar larvae of *Cx. pipiens* 48hrs post treatment.

Compound (ppm)	*N. sativa* oil	*N. sativa* SeNPs
LC_{50} (*Co*-limits)	133.49 (117.74-146.45)	17.39 (14.83-20.40)
LC_{95}(*Co*-limits)	613.02 (493.43-819.69)	157.07 (103.44-296.2)
Slope± *SE*	2.48± 0.45	1.72± 0.83
Relative potency	1.00	7.676
Toxicity index	13.027	100

Larvicidal activities of *N. sativa* oil and *N. sativa* SeNPs against *M. domestica* 3rd larvae instar (Tab. 4, Fig. 5) showed confidential limits for LC_{50} & LC_{95} at (P=0.05). LC_{50} values were 232.87 and 24.94, respectively. *N. sativa* SeNPs showed high toxicity as larvicide against *M. domestica* larvae by 9 folds more than *N. sativa* oil. Toxicity index of *N. sativa* oil was (10.7) compared with *N. sativa* SeNPs (100).

Table 4: *N. sativa* & *N. sativa* SeNPs efficacy against *M. domestica* 3rd instar larvae 48hrs post-treatment.

Compound (ppm)	*N. sativa* oil	*N. sativa* SeNPs
LC_{50} (*Co*-limits)	232.87 (200.88-269.71)	24.94(21.96-27.40)
LC_{95} (*Co*-limits)	1608.22 (1190.1-21413.8)	72.44 (60.14-98.03)
Slope± *SE*	1.96± 0.37	3.55± 0.11
Relative potency	1.00	9.33
Toxicity index	10.7	100

Larvicidal activity caused high toxicity to *M. domestica* and *Cx. pipiens* larvae with LC_{50} 24.94 & 17.3, respectively.

Discussion

Generally speaking, some plants and herbs have aromatic volatile components with characteristic flavor, and odor (El-Zaeddi *et al*, 2016). South Mediterranean Region, including Egypt, is a rich source of medicinal plants with several uses as alternative medicine across history (El-Demerdash, 2001).

Hasaballah (2015) evaluated the toxic effects of both ethanolic and petroleum ether plant extracts under laboratory conditions to co-ntrol 3rd instar larvae of *C. pipiens*, found that *Azadirachta indica* followed by *Rhizophora. australis*, and *N. sativa* were the most effective ones among ten plants extracts. Al-Seeni *et al.* (2016) reported that *N. sativa* was effective in liver protection against the tetracholorocarbon toxicity Elgohary *et al.* (2018) found that ultrasound, topical application of N. *sativa* oil, phonophoresis, and MEBO ointment potentially accelerated the wound healing induced by chemical burns, and modalities may be used to treat wounds. Aborehab and Waly (2019) studied possible hepato-protective effects of N. *sativa*, *P. ginseng*, and *C. sempervirens* in Aflatoxin B1 (AFB-1) induced hepatocellular carcinoma rat model, found histopathological grades of the liver healing.

Conclusion

This study proved that *N. sativa* oil in nanoparticles form was more effective than its oil against larvae of *Cx. pipiens* and *M. domestica*. High larvicidal activity was against *Cx. pipiens* than *M. domestica*. *N. sativa* oil and its nanoparticles proved to be safe, eco-friendly, commercially available and promising in controlling insect-vectors of diseases. Besides, *N. sativa* is safe treatment for many human communicable diseases.

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Explanation of figures

Fig. 1: Nanoparticles formation, Fig. 2: UV-V=absorption spectra of seleneim nanoparticles synthesized by *N. sativa* essential oil with 0.1mM selenious acid.
Fig. 3: SEM micrographs of *N.s* SeNPs synthesized by *N. sativa* oil.
Fig. 4: Susceptibility of *Cx. pipiens* larvae to *N. sativa* oil and *N.s* SeNPs: 1=*N.s* SeNPs. 2= *N. sativa*
Fig. 5: Susceptibility of *M. domestica* larvae to *N. sativa* oil and *N.s*SeNPs:1-*N.s* SeNPs on and 2= *N. sativa*.
Fig. 6: Larvicidal activity (LC_{50}) of *N.sativa* oil and *N.s* SeNPs against *Cx. pipiens* and *M. domestica.*