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Assessment of larvicidal efficiency of nanoemulsion from *Pimpinella anisum* L. essential oil on *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

In the current study, the nanoemulsion prepared from the essential oil of *Pimpinella anisum* was evaluated as an efficient larvicide against the insect disease vector *Culex pipiens*. The essential oils were distilled from seeds of *P. anisum* by steam distillation, then the nanoemulsion was prepared using the ultrasonication method. The mean droplet size of the nanoemulsion was 116 nm with a polydispersity index of 0.21. The efficacy of nanoemulsion was assessed against the third instar larvae through several concentrations of 30, 35, 40, 45 and 50 ppm. The mortality of larvae was recorded 24h after exposure. The LC_{50} and LC_{90} for the nanoemulsion were 40.1 and 55.1 ppm while the LC_{50} and LC_{90} for the bulk emulsion were 44.9 and 69.2 ppm, respectively related to *P.anisum* essential oil. The Larvicidal activity of the formulated nanoemulsion was more toxic than that of the bulk emulsion. The findings of the present study revealed that the nanoemulsion of *P. anisum* EO can be used as a candidate in the integrated management programs to control *C. pipiens*.

INTRODUCTION

Culex mosquitoes are significant vectors of many diseases as they transmit a number of arboviruses including encephalitis, Rift Valley fever and West Nile viruses (Murugan *et al.*, 2012). *C. pipiens* L. is the most abundant mosquito in Egypt, it is found in urban and suburban regions. Chemical insecticides are not recommended nowadays due to several reasons such as the development of insect resistant and the resulted health problems (Bigoga *et al.*, 2013). In addition, these synthetic compounds leave residues that accumulate in the environment causing pollution such as water and soil pollution (Scott *et al.* 2013; Shah *et al.*, 2015). Insecticides with botanical origin may provide efficient and suitable alternatives (Pavela & Govindarajan, 2017) Most botanicals are safer than synthetic insecticides and less harmful to non-target organisms. Essential oils (EOs), also named volatile oils, are natural volatile compounds obtained from parts of aromatic plants such as roots, stems, leaves, flowers, fruits and seeds according to the type of plant (Aktar *et al.*, 2009). EOs are widely used in several industries including food applications, pharmaceutical, perfumery, medicine, sanitary, and cosmetic products (Azmy, 2021b). EOs were proved to be efficient Larvicides against mosquito (Cheng *et al.*, 2009 and Ghosh *et al.*, 2012). To prevent vaporization of the volatile compounds and to reserve biological activity of the EO, the EO should be formulated as nanoemulsion (Bakkali *et al.*, 2008; Osanlo *et al.*, 2017).

EO Nanoemulsions are emulsions with droplet size in the nano-range (McClements, 2011); this formulation improves solubility of oils (Magdassi *et al.*, 2013). Many EOs nanoemulsions were reported as effective larvicides such as nanemulasions of eucalyptus, rosemary, orange (Sugumar *et al.*, 2014; Duarte *et al.*, 2015, Azmy *et al.*, 2019; Azmy *et al.*, 2021 a). *P. anisum* belongs to family Apiaceae, the EO of this plant can be extracted from the seeds, the major component of *P. anisum* EO is trans-anethole (Orav *et al.*, 2008), the insecticidal activity of this compound was reported against *C. pipiens* (Zoubiri *et al.*, 2014;El Zayyat *et al.*, 2017). From this prospective, the present study intended to evaluate the larvicidal efficiency of nanoemulsion of *P. anisum* EO against third instar larvae of *C. pipiens* under laboratory conditions.

MATERIALS AND METHODS

Mosquito larvae:

Mosquito larvae were obtained from the Egyptian Research Institute of Medical Entomology. Mass rearing of larvae was under optimum humidity (75 ± 3%) temperature (25 ± 2◦C), and 16 L:8 D photoperiod.

Oil extraction:

Essential oil extraction from seeds of *P. anisum* (Apiaceae) was done through Hydro- distillation using a Clevenger-type apparatus according to Angioni *et al.* (2006).

Nanoemulsion formulation:

The emulsion was prepared using EO, Tween 20 and distilled water according to Duarte *et al*. (2015). To get the nanoemulsion, the emulsion was subjected for 30 minutes according to Anjali *et al.* (2010) to Sonicator 30 kHz frequency and 750 W power output (Ultrasonics, USA/ digital ultrasonic cleaner cd 4830) at the Electron Microscope unit in faculty of Science, Ain Shams University. The part of emulsion which was not subjected to soncication was considered to be the bulk emulsion.

Characterization of nanoemulsion

Droplet Size Distribution & poly dispersity index PDI:

Measurement of the Droplet size was done using dynamic light scattering technique according to Sugumar *et al.* (2014) at Egyptian Petroleum Research Institute using particle size analyzer (Malvern-UK, 4700).

Stability:

Thermodynamic stability of the Nanoemulstion was evaluated via storing it at 4 ºC and 25 ºC for a month. In addition, it was centrifuged at 10,000 rpm for 30 min, and then was observed for any cracking, phase separation or creaming according to Ghosh *et al.* (2013).

Bioassay tests:

Bioassay was performed on the third instar larvae of *C. pipiens*, the larvae were treated with different concentrations of nanoemulsion according to the standard WHO (2005) protocol.

Five concentrations of the nanoemulsion and bulk emulsion 30, 35, 40, 45, and 50 ppm were used. Three replicates of twenty larvae were used for each treatment. For the control, the same concentrations were used but with the surfactant only. Mortality was recorded after 24 hours of treatment. Lethal concentration were determined at the 95% confidence level using probit analysis, the percentages of larval mortality were calculated for each concentration of the nanoemulsion.

RESULTS

Charazterization of nanoemulsion

Droplet size:

The droplet size distribution of the nanoemulsion is shown in Fig. 1, with the peak at 116 nm as shown in Fig. 2, the value of the PDI equal to 0.21.

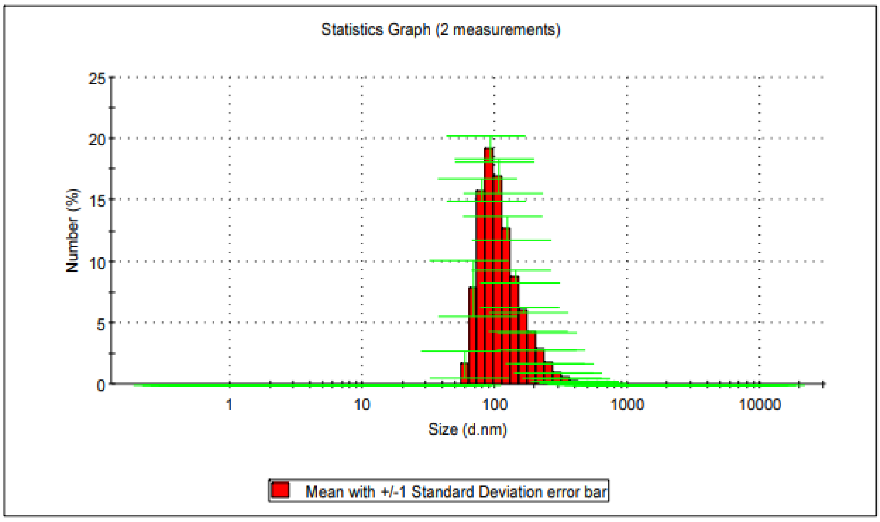

Fig. 1: Droplet size distribution of the nanoemulsion droplets by dynamic light scattering.

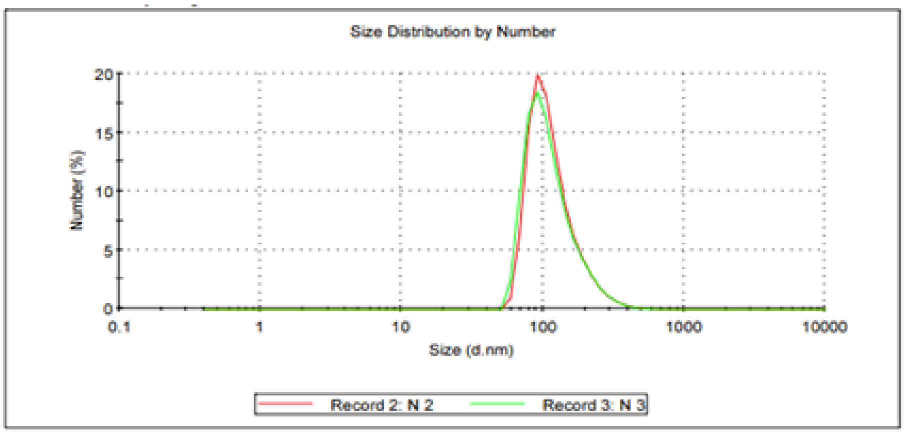

Fig. 2: Droplet size distribution.

Stability:

There was no observed sign of instability of the nanoemulsion, including creaming, phase separation or cracking, it was stable after centrifugation for 30 min at 10,000 rpm and also it was stable when stored for a month at 4 ºC and 25 ºC .

Bioassay

The toxic effect of the nanoemulsion on the 3rd larval instar of *C. pipiens* was significantly increased with the increase of concentration($P<0.05$). Regression analysis showed a concentration-dependent correlation of the nanoemulsion with mortality of larvae. Table 1 shows Mortalities percentage of the larvae caused by bulk emulsion and nanoemulsion after 24 h of treatment.

Table 1: Mortality percentages of nanoemulsion *P. anisum* EO against 3rd instar larvae of *C. pipiens* after 24 hrs.

Nanoemulsiom		**Bulk emulsion**	
Concentration ppm	Mortalities % (Mean ±SE)	Concentration Ppm	Mortalities % (Mean ±SE)
30	17±0.5 a	30	12±0.5 a
35	23± 1.9 b	35	20±1 b
40	52± 1.1 c	40	40± 1c
45	68.1±1. d	45	50± 0.2d
50	83±0.1 e	50	62±0.2e

Fig. 3 and 4 Show the LC_{50} of the nanoemulsion and bulk emulsion on the regression line of probit mortality . The nanoemulsion is more effective than the bulk emulsion regarding the LC_{50} and LC_{25} values.

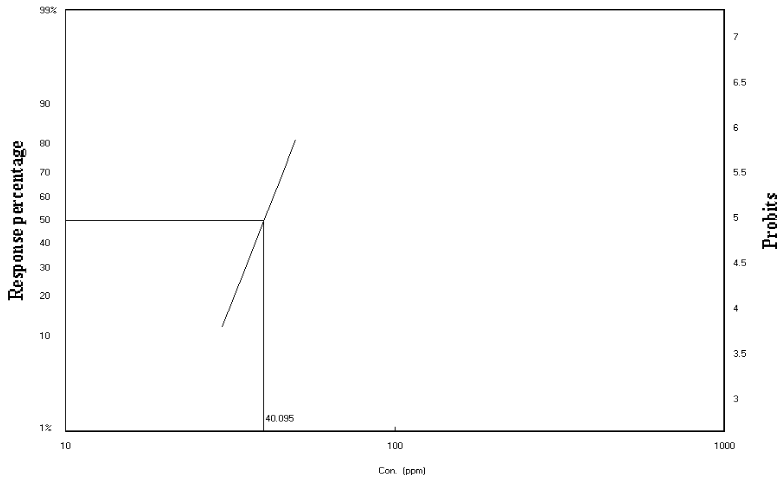

Fig. 3: Regression line of probit mortality of *C .pipiens* larvae against the log concentrations of the nanoemulsion of *P. anisum* EO.

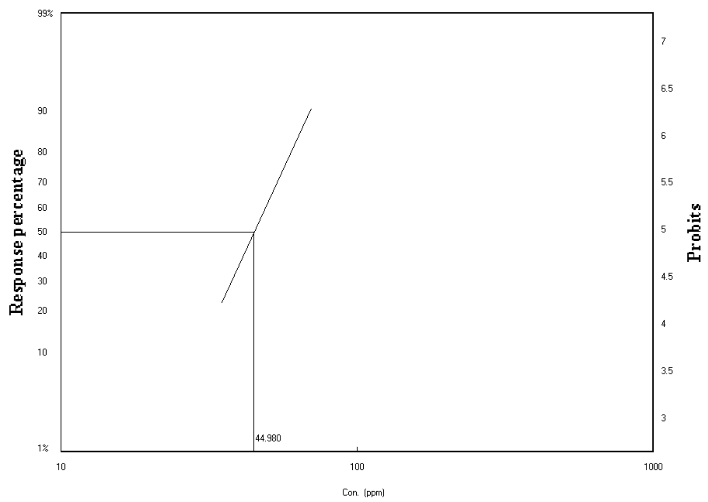

Fig. 4: Regression line of probit mortality of *C. pipiens* larvae against the log concentrations of the bulk emulsion of *P.anisum* EO.

The lethal concentration values of nanoemulsion and bulk emulsion calculated from the regression line of probit mortality of *C. pipiens* larvae against the log concentrations are shown in Table 2.

Table 2: The LC25, LC50 and LC90 values of nanoemulsion and bulk emulsion of *P. anisum* EO against 3rd instar larvae of *C. pipiens* after 24 hrs.

Lethal concentration	**Nanoemulsiom Concentration Ppm**	**Bulk emulsion Concentration Ppm**
LC_{25}	33.9	35.8
LC_{50}	40.1	44.9
LC_{75}	47.3	56.4
LC_{90}	55.1	69.2
LC_{99}	71.3	98.4

DISCUSSION

Insecticides from botanical sources such as EOs have been reported as safe effective substitutes to synthetic insecticides as they are recognized being environmentally friendly (Sugumar *et al.*, 2014; Azmy *et al.*, 2021c). On the other hand, EOs have poor water solubility, while larvicides used to control mosquito larvae should be soluble in water because they are aquatic organisms. Therefore, EOs should be formulated as nanoemulsions to overcome this natural obstacle (Duarte *et al.*, 2015).

The larvicidal activity of the nanoemulsion of *P. anisum* against the *C. pipiens* larvae in this study may be due to trans-anethole which is the major component of the EO, it was reported to have insecticidal property (Abdel- Baki *et al.*, 2021) . Our results agree with several studies on the nanoemulsions based on EOs as effective insecticides (Anjali *et al.*, 2011; Ghosh *et al.*, 2013; Sugumar *et al.*, 2014; Duarte *et* *al.*, 2015, Azmy *et al*, 2019; Azmy *et al.*, 2021a). In addition, the results of this research come in contact with the research that studied the toxic effects of *P. anisum* EO on the different life stages of *Cx. quinquafasciatus* (Pavela & Govindarajan, 2017), Knio *et al*, 2008 reported that *P. anisum* seed EO had toxic effect on larvae of *Ochlerotatus caspius* . Furthermore, It was reported that *P. anisum* Eo resulted in complete mortality to *Tribolium castaneum* and *Ephestia kuehniella* adults after 24 h after treatment with 64 µl/L air concentration (Mikhaiel, 2001). Our findings could be attributed to the penetration of EO to the body of the larvae causing neural toxicity to their nervous system and inhibition of the normal biological processes (Chantawee & Soonwera, 2018). Nanoemulsions based on EO have various advantages including stability, long shelf life (Mishra *et al*., 2017). The uniformity and stability of the droplet size in the nanoemulsion can be indicated by PDI value is an indication of (Anjali *et al.*, 2010), the low value of PDI of the prepared nanoemulsion in this study indicates the high uniformity of droplet size and the long-term stability. Stabilization of the nanoemulsion is because of the presence of the surfactant as it provides a mechanical barrier to prevent the droplets from accumulation. The high efficiency of the nanemulsion may be due to the small size of the droplets, which increases the surface area and facilitates the penetration of the effective component into the larvae according to Anjali *et al*. (2010). The botanical molecules are able to interact with enzymes, hormones and bind to membranes and cellular components and thus interfere with biochemical pathways of the mosquito (Powell, 2009 and Fallatah, 2010). Further investigation is needed to reveal the mode of action of this larvicide and its effect on the different biological aspects of the treated larvae.

CONCLUSION

The present research reported that natural products of botanical origin such as the nanoemulsion of *P. anisum* EO can be used as low-cost safe alternative to humans and the environment for controlling the disease-vector *C. pipiens*.

ETHICAL APPROVAL:

This research paper was approved by the research ethics committee from Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2023/3/1).

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