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Assessment of larvicidal activity of nanoemulsion from *Citrus sinensis* essential oil on *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Synthetic insecticides cause pollution to the environment. In addition, insects develop resistance toward them. So, there is an urgent need for effective safe alternatives. In the current study, nanoemulsion was prepared from essential oil of *Cirtus sinensis* by the ultrasonic method. The efficacy of the nanoemulsion was evaluated against larvae of *Culex pipiens* and compared with that of the bulk emulsion. The mean droplet size of the nanoemulsion was 78.8±14.2 nm with poly dispersity index (PDI) value 0.28. The LC_{50} for the nanoemulsion and the bulk emulsion were 27.4 and 86.3 ppm, respectively related to *Citrus sinensis* essential oil. The Larvicidal activity of the formulated nanoemulsion was more toxic than that of bulk emulsion. The results showed that nanoemulsion of *Cirtus sinensis* EO can be used for control of vector-borne disease *Culex pipiens* larvae.

INTRODUCTION

Culex mosquitoes transmit a number of arboviruses including West Nile, Rift Valley fever, and encephalitis viruses (Murugan *et al.*, 2012). The Use of chemical insecticides to control them is now difficult due to the increase of mosquito resistant (Bigoga *et al.*, 2013). In addition, such chemicals pollute the environment causing water pollution, decrease in fertility of the soil. Essential oils (EOs) were reported as effective Larvicidal against larvae of mosquito (Cheng *et al.***,** 2009 and Ghosh *et al.*, 2012). *Citrus sinensis* belongs to family Rutaceae, its EO is categorized under category of generally regarded as safe by the Authority of Food and Drug of USA, the oil contains many constituents such as limonene, monoterpenes, α-pinene, β-pinene, terpinolene, and Octanal (Akono *et al.*, 2015).

EO should be formulated to prevent vaporization of volatile compounds to reserve biological activity of the EO (Bakkali *et al.*, 2008 and Osanloo *et al.*, 2017). Nanoemulsions of EO are submicron emulsions with droplet size lower than 100 nm (McClements, 2011); such formulation enhances solubility of poorly water soluble oils (Magdassi *et al.*, 2013). Many nanoemulsions of EOs were reported as larvicides such as nanemulasions of eucalyptus and rosemary (Sugumar *et al.,* 2014 and Duarte *et al.*, 2015). The aim of the present study is to assess the larvicidal efficiency of a formulated nanoemulsion of *Citrus sinensis* EO by ultrasonic emulsification method against third instar larvae of *Culex pipiens.*

MATERIALS AND METHODS

Mosquito larvae:

Mosquito larvae were collected from a water body in Abu Rwash region, Giza governorate. Species identification was done in Faculty of Science Ain Shams University. The larvae were reared under optimum humidity (75 ± 5%) temperature (25 ± 2◦C), and a photoperiod of 16:8 light/dark hours, the bioassay was performed on the third generation.

Oil extraction:

Fresh fruits of *Citrus sinensis* were collected in March, 2018 from a market in Cairo, Egypt. Essential oil extraction from peels of *Citrus sinensis* (Rutaceae) was done through Hydro- distillation for 3 hours using a Clevenger-type apparatus according to Angioni *et al.* (2006).

Nanoemulsion formulation:

Bulk emulsion was prepared using EO, Tween 20 (Polyoxyethylene 20 sorbitan monolaurate) and distilled water according to Duarte *et al*. (2015). Then the bulk emulsion was subjected to a Sonicator (Ultrasonics, USA/ digital ultrasonic cleaner cd 4830) at frequency of 30 kHz and power output 750 W for 30 minutes according to Anjali *et al.* (2010) at the Electron Microscope unit in faculty of Science, Ain Shams University, energy input was given through sonitcator probe with a probe diameter of 13 mm.

Characterization of nanoemulsion

Droplet Size Distribution & PDI:

Droplet size was measured by dynamic light scattering (DLS) technique according to Sugumar *et al.* (2014) using particle size analyzer (Malvern-UK, 4700) at Egyptian Petroleum Research Institute (EPRI).

Physicochemical characterization:

The pH value of the nanoemulsion was measured using a pH meter (model HI 8417, Hanna Instruments Inc., Woonsocket, USA) at room temperature according to Shafiq *et al.* (2007).

The viscosity:

The viscosity of the nanoemulsion was measured by Ostwald viscometer performed at a temperature of 25 ± 0.5 ºC, experiments were performed in triplicate according to Abbas *et al.* (2010).

Stability:

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

Bioassay tests:

Bioassay was performed using third instar larvae of *Culex pipiens* kept at temperature 25 ± 2◦C and relative humidity of 75 ± 5%, The larvae were treated with different concentrations of nanoemulsion according to the standard WHO (2005) protocol.

Five concentrations of the nanoemulsion 5, 15, 25, 35, and 40ppm were used. While for bulk emulsion 50, 100, 150, 250, and 300 ppm were used. For each treatment, three replicates of twenty five larvae were used for each one.

For control, the same concentrations with surfactant only were used. Mortality was recorded after 24 hours post treatment. Mortality was analyzed using Minitab statistical software for dose and time mortality regression lines. 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 42.75 nm as shown in fig. 2; mean droplet diameter of the nanoemulsion was calculated to be 78.8±14.2 nm. The value of the PDI equal to 0.28.

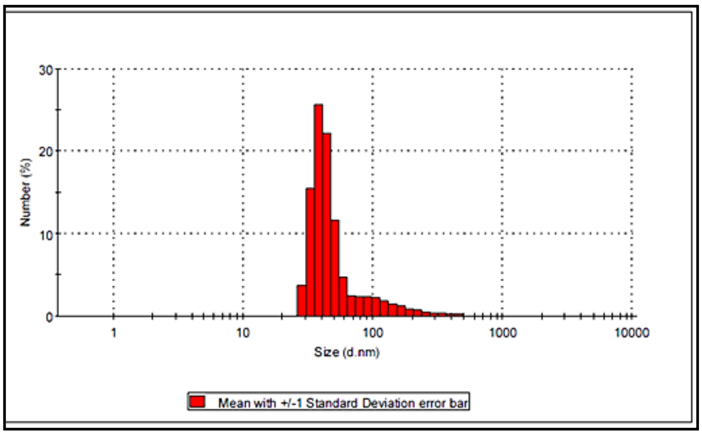

Fig. 1: Droplet size distribution of the nanoemulsion droplets by DLS.

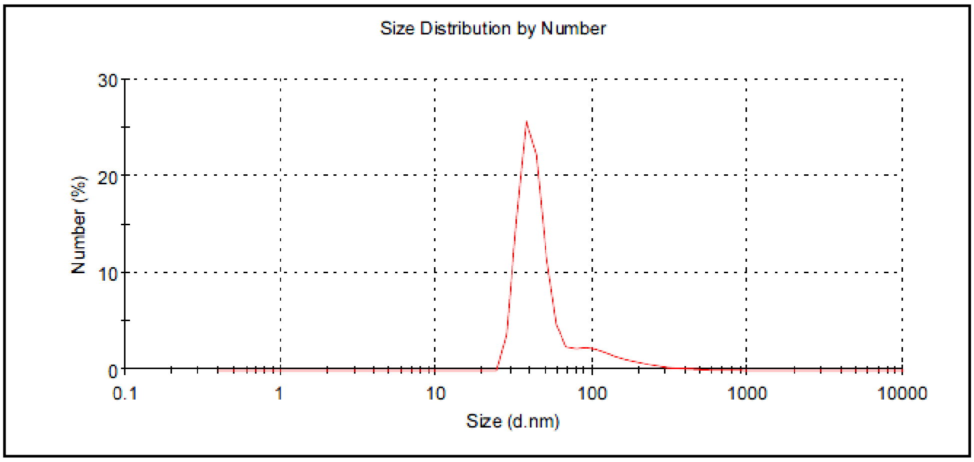

Fig. 2: Droplet size distribution with the peak at 42.75 nm.

Physicochemical characterization

The pH value of the nanoemulsion was 6.4 and the viscosity was 1.23 mpascal/sec.

Stability of nanoemulsion

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

Bioassay

The toxic effect of the nanoemulsion on the third instar larvae was significantly increased (P<0.05) with the increase of concentration. Regression analysis showed a concentration-dependent significant correlation of the nanoemulsion with larval mortality. The Mortalities percentage of the treated larvae caused by nanoemulsion and bulk emulsion (Table 1).

Table 1: Mortality percentages of nanoemulsion and bulk emulsion of *Cirtus sinensis* EO against 3rd instar *Culex pipiens* larvae after 24 hrs. Means bearing different letters within column are significantly different (*P*<0.05) ANOVA, LSD test.

Nanoemulsiom		**Bulk emulsion**	
Concentration ppm	Mortalities % (Mean ±SE)	Concentration Ppm	Mortalities % (Mean ±SE)
5	6.5±0.5 a	50	23.1±2.8 a
15	11.4± 1.95 b	100	54.7±0.7 b
25	37.4± 1.5 c	150	81.6±1.36 c
35	57.1±1.5 d	250	92.8±0.29 d
40	95.5±0.14 e	300	99±1 e

The LC_{50} value of nanoemulsion was estimated to be 27.4 ppm and for the bulk emulsion was 86.3 ppm. While the LC_{25} were 15.6 and 53.7, respectively as shown in Fig. 3. The nanoemulsion is more effective than the bulk emulsion regarding the LC_{50} and LC_{25} values (Fig. 4).

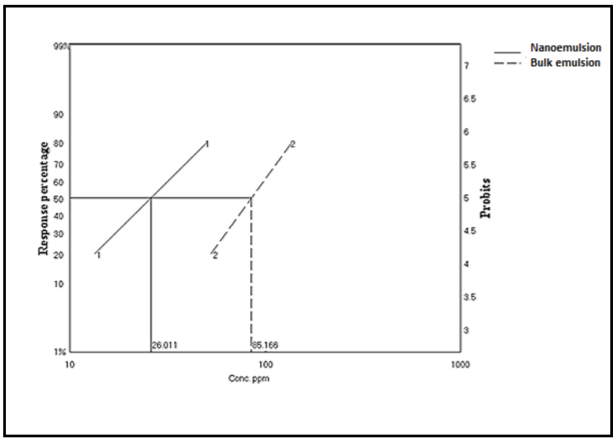

Fig. 3: Regression line of probit mortality of *Culex pipiens* larvae against the log concentrations of the nanoemulsion and bulk emulsion of *Citrus sinesis* EO.

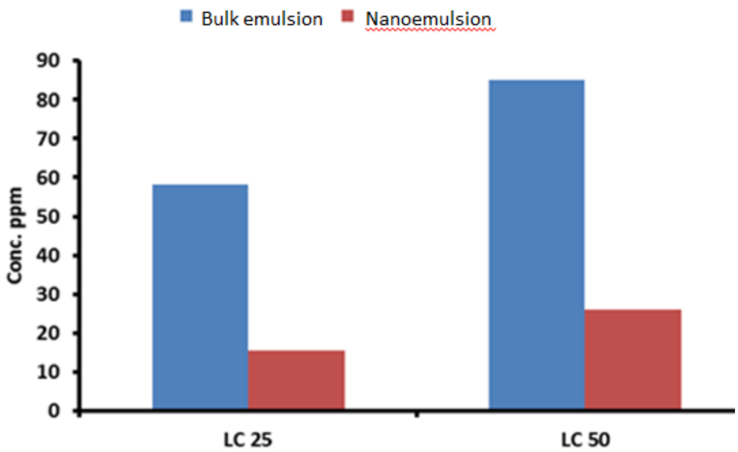

Fig. 4: LC_{25}, LC_{50} of nanoemulsion and bulk emulsion of *Cirtus sinensis* EO against 3rd instar *Culex pipiens* larvae after 24 hrs.

DISCUSSION

Control of mosquitoes is facing economic and ecological challenge because of the hazards caused by majority of the synthetic insecticides. In contrast, bio insecticides have long been described as safe alternatives to synthetic insecticides (Sugumar *et al.*, 2014). *Culex pipiens* larvae are aquatic so the larvicide used to control them has to be soluble in water, while EO has poor water solubility. Therefore, EO should be formulated as Nano emulsion to overcome this obstacle (Duarte *et al*., 2015). Characterization of the prepared Nano emulsion in this study proved that the mean droplet size was less than 100 nm, which means that was within the nano-range according to McClements (2011). PDI is a measure of the stability and uniformity of the droplet size in the nanoemulsion (Anjali *et al.,* 2010). The low PDI of the formulated Nano emulsion results in high uniformity of droplet size and provide long-term stability. The stabilization of the nan emulsion is due to the surfactant, as it provides a mechanical barrier to prevent accumulation. The viscosity measurement was low which confirms that the Nano emulsion is of oil-in-water type according to . *Citrus sinensis* EO components were analyzed by GC-MS in a previous study, (Akono *et al*., 2015) and the major component was found to be limonene with 94.92% of total peak area. Larvicidal activity of the oil based nano emulsion may be due to the major component (limonene) which reported to have insecticidal property according to Saad (2013). 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 and Duarte *et al.,* 2015).

The higher efficiency of the nanofomulation than the bulk emulsion may be due to the smaller size of the nanoemulsion droplets, which increases the surface area and facilitates the penetration of nanoemulsion into the larvae according to Anjali *et al*. (2010).The botanical molecules are able to interact with body hormones, enzymes and bind to membranes and cellular components and thus interfere with biochemical pathways of the mosquito. (Powell, 2009 and Fallatah, 2010). Nanoemulsions based on EO have various advantages including stability, long shelf life (Mishra *et al*., 2017). To our knowledge, this is the first time that a nanoemulsion of *Citrus sinensis* EO, reported as a potent Larvicide against *Culex pipiens.*

CONCLUSION

It can be concluded that the formulation of nanoemulsion of *Citrus sinensis* EO can be used as low-cost safe Larvicide to the environment for controlling the disease-vector *Culex pipiens*.

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REFERENCES

Abbas, K.; Abdulkarim, S.; Saleh, A. and Ebrahimian, M. (2010). Suitability of viscosity measurement methods for liquid food variety and applicability in food industry - A review Journal of Food, Agriculture & Environment, 8(3&4): 100-107.

Akono, P.; Dongmo, P.; Tonga, C.; Kouotou, S. ; Kekeunou, S.; Magne, G.; Lehman, L.G. and Menut, C. (2015). Larvicidal activity of essential oils from pericarps of ripe Citrus fruits cultivated in Cameroon on pyrethroids sensitive and resistant strains of *Anopheles gambiae*. Journal of Entomology and Zoology Studies, 3(4): 334-339.

Anjali, C.; Khan, S.; Goshen, K. and Magdassi, S. (2010). Formulation of water-dispersible nanopermethrin for larvicidal applications. Ecotoxicology and Environmental Safety., 73: 1932-1936.

Anjali, C. ; Sharma, Y., Mukherjee A. and Chandrasekaran N. (2011). Neem oil (*Azadirachta indica*) nanoemulsion - a potent larvicidal agent against *Culex quinquefasciatus*. Wiley Online Library.

Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. (2008) Biological effects of essential oils - a review. Food Chem Toxicol, 46(2):446–475.

Bigoga, J.; Saahkem, P.; dindeng, S.; Ngondi, J. and Negue, M. (2013). Larvicidal and Repellent Potential of *Chenopodium ambrosiodes* Linn Essential oil against *Anopheles gambiae* Giles (Diptera: Culicidae). The open Entomolgy journal, 7:16-22.

Cheng, S.; Huang, C.; Chen, Y.; Yu, J.; Chen, W. and Chang, S. (2009). Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. Bioresource Technology, 48: 4868 - 4873.

Duarte, J.; Amado, J.; Oliveira, A.; Cruz, R.; Ferreira, A.; Souto, R.; Falcão, D.; Carvalh,o J. and Fernandes, C. (2015). Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. Rev. Bras., 25(2):189–192.

Fallatah, S. (2010). Histopathological Effects of Fenugreek (*Trigonella foenumgraceum*) extracts on the larvae of the Mosquito *Culex Quinquefasciatus* Agricultural Technology and Biological Sciences, 5(2): 123-130.

Ghosh, A.; Choudhary, N.; and Chandra, G. (2012). Plant extracts as potential mosquito larvicides. The Indian Journal of Medical Research,135(5):581-598.

Ghosh, V.; Mukherjee, A.; and Chandrasekaran, N. (2013). Formulation and characterization of plant essential oil based Nanoemulsion: evaluation of its Larvicidal activity against *Aedes egypti*. Asian J. Chem., 25:S321- S323.

Magdassi, S.; Mukherjee, A. and Chandrasekaran, N. (2013). Distinctive effects of nano-sized permethrin in the environment. Environmental Science and Pollution Research, 20: 2593-2602.

McClements, D. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter, 7(6): 2297-316.

Mishra, P.; Tyagi, B.; Chandrasekaran, N. and Mukherjee, A. (2017). Biological nanopesticides: a greener approach towards the mosquito vector control Environ Sci. Pollut, Res., 1–13.

Murugan, K.; Kumar, P.; Kovendan, D.; Subrmaniam, J. and Hwang, J. (2012). Larvicidal, pupicidal, repellent and adulticidal of Citrus sinensis orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol, Res., 111(4):1757-1769.

Osanlo, M.; Amani, A.; Sereshti, H.; Abai, M.; Esmaeili, F. and Sedaghat, M. (2017). Preparation and optimization nanoemulsion of Tarragon (*Artemisia dracunculus*) essential oil as effective herbal larvicide against *Anopheles stephensi*. Ind Crop. Prod., 109: 214–219.

Powell, R. (2009). Plant seeds as sources of potential industrial chemicals, pharmaceuticals, and pest control agents. J. Nat. Prod., 72(3):516-23.

Saad, M. (2013). Chemical Composition and Biological Activities of Four *Citrus* Essential Oils. J. Plant Prot. and Path., 4 (9): 767 – 780.

Shafiq, S.; Faiyaz S.; Sushma, T.; Farhan, J.; Khar, R.; and Ali, M. (2007). Design and development of ramipril nanoemulsion formulation: in vitro and in vivo assessment. J. Biomed Nanotechnol, 3:28–44.

Sugumar, S.; Clarke, S.; Nirmala, M.; Tyagi, B.; Mukherjee, A.; and Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. Bull Entomol Res.,104(03): 393–402.

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