EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 16 No. 1 (2024)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F. Toxicology& Pest control) *Vol.16(1) pp51-61 (2024)*
DOI: 10.21608/EAJBSF.2024.345753

Egypt. Acad. J. Biology. Sci., 16(1):51-61(2024)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Efficacy of Nano-Encapsulated Mandarin Oil using (Polyethylene Oxide/Polyacrylamide) and Gamma Irradiation against *Culex pipiens* Larvae

Reda S. Hassan[1]; Thanaa M. Sileem[1]; Waheed A. A. Sayed[1] and Mohamed M. Ghobashy [2]

[1]Biological Applications Department, Nuclear Research Centre- Egyptian Atomic Energy Authority. Cairo, Egypt.

[2]Radiation Research of Polymer Chemistry Department, National Center of Radiation Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.

***E. Mail :** redahassan28812@yahoo.com ; thanaasileem@yahoo.com ; waheed.sayed@eaea.org.eg ; Mohamed.ghobashy@eaea.org.eg

ARTICLE INFO
Article History
Received:8/2/2024
Accepted:14/3/2024
Available:18/3/2024

***Keywords*:**
Nano-gel, mosquito, Larvicide, mortality-responses, physical characteristics.

ABSTRACT

Nano-encapsulation of essential plant oils may prove to be the most effective approach to overcome their application challenges against insect pests. Larvicidal properties of polyethylene oxide/polyacrylamide (PEO/PAAM) nanogels of mandarin essential oil (MEO) against Culexpipiens larvae were investigated. The nano-gel was prepared using an intramolecular crosslinking method initiated by gamma irradiation. The resulting mandarin essential oil (PEO/PAAm) nanogel (MEON) exhibited monodispersity, a relatively small size distribution, and a low negative surface charge of -1.1 mV, suggesting enhanced stability and reduced electrostatic interactions. The 2nd instar larvae were more sensitive to MEON than the 3rd and 4th instar larvae at the lower doses of 10 and 15 ppm. The LC_{50} and LC_{99} for the MEON were (11.5 and 18.4 ppm) for 2nd instar larvae; (14.5 and 23.6 ppm) for 3rd instar larvae; and (15.3 and 33.8 ppm) for the 4th instar larvae, respectively, at 48 hours after treatment. These results shed light on MEON's physical attributes, surface qualities, and biological assay and suggest that it might be a useful substance for *Culex pipiens* management.

INTRODUCTION

Culex mosquitoes are known to spread important diseases to humans, for instance, elephantiasis by the transmission of Lymphatic filariasis and West Nile, Rift Valley fever, and encephalitis by the transmission of arboviruses (Madeira *et al.*, 2024). *Culex pipiens* is the most abundant mosquito worldwide and is widely distributed in urban and suburban regions (Villena *et al.*, 2024). Excessive use of chemical insecticides has caused environmental pollution and vector resistance (Hillary *et al.*, 2024). Essential oils (EOs) were reported as effective toxic materials against mosquito larvae (Vivekanandhan *et al.*, 2023). The United States Authority for Food and Drugs has classified orange oils as safe. The oil contains many constituents such as limonene, monoterpenes, α-pinene, βpinene, terpinolene, and octanal (de Jesus Oliveira *et al.*, 2024). *Citrus reticulata*, a mandarin EO that is a member of the Rutaceae family, has been the subject of numerous attempts to

Citation: *Egypt. Acad. J. Biolog. Sci.* (F. Toxicology& Pest control) *Vol.16(1) pp51-61 (2024)*
DOI: 10.21608/EAJBSF.2024.345753

demonstrate the insecticidal action of orange essential oils (EOs) against various insect pest species (Sayed *et al.,* 2020; Marouf and Harras, 2022). (Brah *et al.,* 2023).

Although EOs have a toxic action against insect pests, they should be formulated to avoid the vaporization of volatile chemicals to preserve their actions (Osanloo *et al.,* 2022). The submicron emulsion of nano-encapsulated essential oils (EOs) has been suggested as a potentially effective pesticide formulation; these formulations improve the solubility of poorly water-soluble oils (Ibrahim, 2020). Many nanoencapsulations of EOs were reported as pesticide formulations against mosquitoes (Esmaili *et al.,* 2021). Nano-gels, as a class of nanoscale hydrogels, have attracted significant attention in recent years due to their unique properties and promising applications in various fields, including biomedicine and drug delivery. One of the key challenges in nano-gel synthesis is achieving a controlled and uniform size distribution, which is crucial for their performance and effectiveness in specific applications. Various methods have been explored for nano-gel synthesis, including chemical crosslinking, self-assembly, and radiation-induced crosslinking (Matusiak *et al.,* 2020a). The ideal intramolecular crosslinking method for synthesizing nanogels has been highlighted (Rosiak *et al.,* 2005). Because gamma irradiation can both start and control crosslinking reactions in aqueous polymer systems, it has become a potential process for creating nano encapsulation. This technique offers advantages such as simplicity, scalability, and the absence of the need for initiators or additional chemicals. Specifically, the use of gamma irradiation in the intramolecular crosslinking process has demonstrated potential in the production of nano-gels with a restricted size distribution, a high crosslinking density, and a semi-permeable membrane (Alshangiti *et al.,* 2019). For crosslinked hydrogel (Ghobashy *et al.,* 2021b) to be used as a template (Ghobashy *et al.,* 2021a) and blend polymer (Ghobashy *et al.,* 2017) hydrogel points to utilize as green renewable assets to protect the environment from negative effects (Madani *et al.,* 2022). Previous studies from our laboratory have indicated that irradiation treatment is a useful method for these hydrogel points. In order to boost the larvicidal efficacy of mandarin essential oil and expand its application against *Culex pipiens*, the current trial intends to create a unique formulation of the oil.

MATERIALS AND METHODS

Insects and Chemicals:

Mosquito larvae were reared in the insectary of the nuclear reach center, Inshas, Egypt. The larvae were reared under optimum humidity (75 ± 5%), temperature (25 ± 2◦C), and a photoperiod of 12:12 light/dark hours. Mandarin essential oil was purchased from the "Binsib Company for Agriculture Development" in Cairo, Egypt. PEO was obtained from Loba-chemieindoaustranal Co., Mumbai, India. Acrylamide monomer (AAm) was obtained from Sigma-Aldrich Co. Hydrochloric acid (35.5 wt.%) and sodium hydroxide flakes were supplied from the market.

Gamma-Irradiation-Induced Polymerization of (PEO/PAAm) Nanogel:

The optimum intramolecular crosslinking technique is used in the particular instance of the synthesis of polyethylene oxide/polyacrylamide (PEO/PAAm) nanogel, with gamma irradiation catalyzing radical polymerization processes. The experimental procedure involves preparing a 0.5 wt% solution of the AAm monomer and a 0.1 wt% solution of PEO by dissolving 0.05 g and 0.01 g of PEO in 10 ml of distilled water. The resulting mixture is then subjected to sonication for 5 minutes, which promotes the mixing and dispersion of the components within the solution. The pH of the solution is adjusted to 1 using a 35.5 wt% hydrochloric acid (HCl) solution. The pH adjustment is crucial for the subsequent polymerization reaction. Following the pH adjustment, the solution is exposed to gamma irradiation, which serves as the initiator of radical polymerization reactions. The irradiation dose employed in this synthesis is 5 kGy. The synthesis is conducted using a 60Co source established at NCRRT, EAEA, in Cairo, Egypt. The gamma irradiation triggers the crosslinking of the polymers, leading to the formation of the PEO/PAAm nanogel.

Encapsulation of Mandarin Oil Inside (PEO/PAAm) Nanogel:

The pH of the resulting suspension solution of PEO/PAAm) nanogel was adjusted to 6 by adding a concentrated solution of NaOH (added slowly while stirring at 150 rpm, allowing the nanogel particles to agitate). The resulting clear solution (A) was taken to the ultrasonic bath (70 w) for 15 minutes at room temperature. For the encapsulation of mandarin oil inside the PEO/PAAm) nano-gel, the emulsion solution of oil (B) was prepared by dissolving 5 ml of oil with 1 ml of tween 80 in 10 ml of distilled water, stirring at 350 rpm for 10 minutes, and keeping at 4 ºC for 24 hours. Then, both solutions (A) and (B) were mixed gradually with stirring for 10 minutes, and the pH of the solution was adjusted to 4 using HCl solution (35.5 wt. %). The pH adjustment is crucial for maintaining the stability and properties of the encapsulated mandarin oil within the nanogel. The resulting mixture now contains the encapsulated mandarin (MEO) essential oil within the PEO/PAAm) nanogel (MEON).

Characterization of (PEO/PAAm)/MEO Nano-gel (MEON):

Transmission Electron Microscope (TEM):

The structure of the MEO nanogel was examined by transmission electron microscopy (H-7650, Hitachi, Japan); the prepared formulation was already diluted with distilled water before the examination. One drop of the MEON formulation was put on a film-coated copper grid before staining with a (2% w/v) phosphotungstic acid solution. The grid was allowed to dry for 10 minutes at ambient temperature before visualization under a transmission electron microscope.

Dynamic Light Scattering (DLS) and Zeta Potential:

The particle size (PS) and polydispersity index (PDI) were investigated using Zetasizer Nano ZS (Ver.6.12, Malvern Instruments Ltd., Worcestershire, England) using the dynamic light scattering technique at room temperature. MEON was previously diluted with distilled water (100-fold) before any measurements. The structure of the MEON was examined by transmission electron microscopy (H-7650, Hitachi, Japan); the prepared formulation was already diluted with distilled water before the examination. One drop of the EO-loaded formulation was put on a film-coated copper grid before staining with a (2% w/v) phosphotungstic acid solution. The grid was allowed to dry for 10 minutes at ambient temperature before visualization under a transmission electron microscope.

Bioassay Tests:

A bioassay was performed using second, third, and fourth instar larvae of *Culex pipiens* kept at a temperature of 25 ± 2 °C and a relative humidity of 75 ± 5%. The larvae were treated with different concentrations of PEO, PAAM, and MEO nanogel according to the standard protocol. Four concentrations of nanogel (0, 10, 15, 20, 25, and 30 ppm) were used. For each treatment, five replicates of twenty-five larvae were used. Mortality was recorded after 24- and 48-hours post-treatment.

Statistical Analysis:

Lethal concentrations were determined at the 95% confidence level using a probit regression line. LC_{50} and LC_{99} were calculated (Finney, 1971). The percentages of larval mortality were calculated for each concentration of the tested samples. Correction for control mortality was conducted using Abbott's formula. A one-way ANOVA followed by Tukey's multiple comparison tests ($P < 0.05$) was carried out to illustrate the significance of the tested samples from the control groups. All statistical analysis was conducted using the statistical package for social science (SPSS) software version 14.

RESULTS AND DISCUSSION

Characterization of (PEO/PAAm)/MEO Nano-gel:

Figure 1, shows a transmission electron microscopy (TEM) image of MEON particles. The nano-gel particles appear monodisperse, meaning they have a uniform size distribution as seen in the TEM image. The diameters of the nano-gel particles are reported to be in the range of 130–160 nm based on the scale bar in the TEM micrograph.

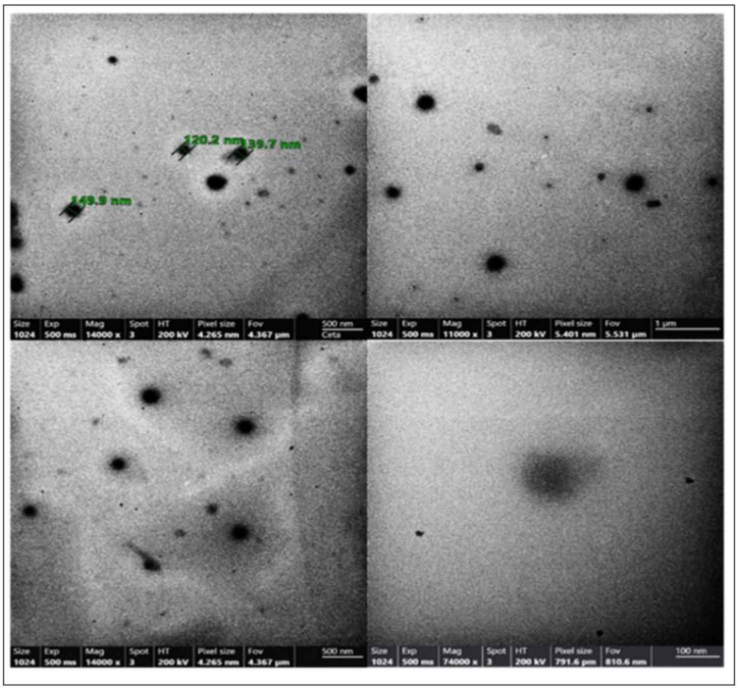


Fig. 1: Transmission electron microscopy (TEM) of MEON.

The monodispersity and relatively small nano-gel particle diameters suggest the nano-gel may be suitable for various biomedical applications. The hydrodynamic diameter and distribution of encapsulated mandarin oil were determined by the dynamic light scattering (DLS) technique (Fig. 2.a). To deliberate the size distribution profile and polydispersity of the synthesized nanogel of mandarin oil, the particle size distribution of the MEON particles, as measured using a particle size analyzer, is reported to be 205 nm. This indicates that the nanogel has a relatively uniform size distribution centered on this value.

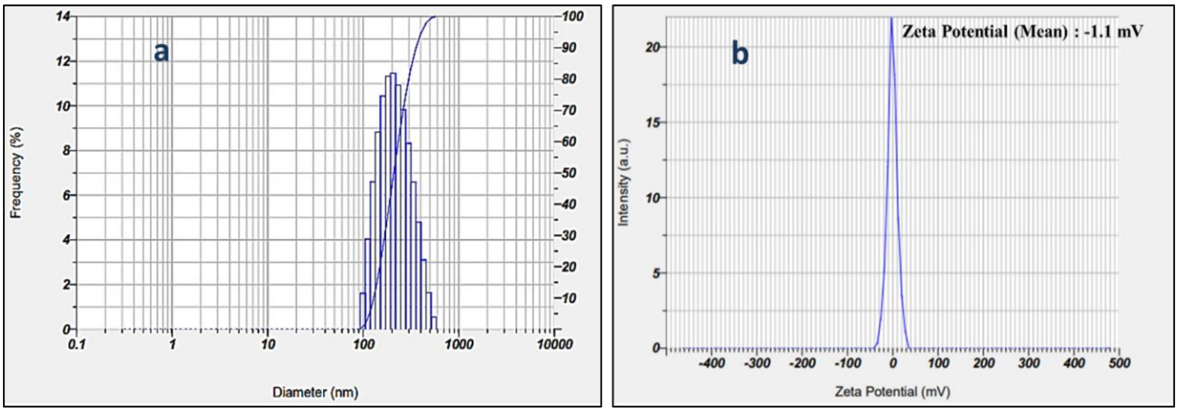


Fig. 2: particle size analyzer (a) particle size distribution = 205 nm and Zeta potential = -1.1 mV (b) of MEON.

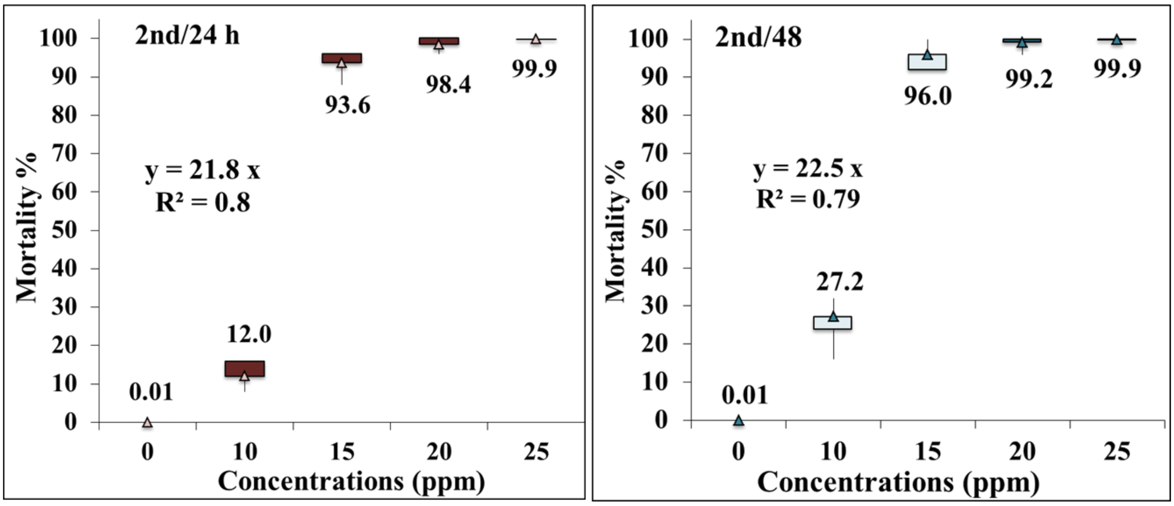
The zeta potential of the nanogel indicates that all of the samples were negatively charged. The absolute value of the zeta potential reached a maximum when the amount of nanogel was 4.2 × 10−2% w/v. The zeta potential value of encapsulated mandarin oil was 23 mV (Fig. 2.b), which indicates the strong repulsion between particles and the increasing stability of the encapsulated oil.

The gamma irradiation technique enables the synthesis of nano-gels in a one-step, simple, and scalable manner. Water-soluble polymers can be intramolecularly crosslinked by radiation to create nano-gels (Matusiak *et al.,* 2020). Based on the research of Rosiak *et al.* (2005), the optimal intermolecular crosslinking technique uses high-energy, low-LET (linear energy transfer) ionizing radiation to cause aqueous polymers to crosslink. This method results in nano-gels with a high degree of crosslinking and a narrow size distribution (Ashfaq et al., 2021). Radiation-induced crosslinking of polymers can be used to create hydrogels and their nanoscale counterparts, nano-gels (Dispenza *et al.*, 2017). To develop botanical biopesticides for use in plant protection, numerous studies have attempted to create EO-based nano-emulsions (Campolo *et al.,* 2020). Nano-emulsions are kinetically stable systems with droplets ranging in size from 50 to 200 nm (Lakshmayya *et al.,* 2023). Nano-encapsulation using polymers is a well-known method for the preservation of essential oils. It offers plenty of benefits, including improved water solvency, compelling assurance against degradation, the avoidance of volatile component evaporation, and tightly controlled and targeted release (Lammari *et al.,* 2020).

Bioassay

Figure 3, shows the mortality of 2nd instar larvae of C. pipines at 24 h after treatment with MEON concentrations. Significant mortalities were obtained in the cases of 15, 20, and 25 ppm of MEON compared to concentrations of 0.0 and 10 ppm ($F_{(4, 24)} = 2464.2$, $P < 0.05$).

Fig. 3: Mortality percentages of different concentrations (ppm) of MEON against 2nd instar larvae of *C. pipines* at 24 and 48 h post treatments.



While after 48 hours, mortality increased gradually by increasing the concentration levels ($F_{(4,24)} = 29.5$, $P < 0.05$). The higher mortalities (96.0, 99.2, and 99.9%) were observed at the tested concentrations (15, 20, and 25 ppm), respectively.

Similarly, higher mortality (99.7%) was obtained for 3rd larvae treated for 25 ppm concentration after 24 h treatments than the mortalities recorded for the other tested concentrations ($F_{(4,24)} = 461.6$, $P < 0.05$) (Fig. 4). While within 48 h after treatment, the mortality at 10 ppm concentration was still drastically low compared to 15, 20, and 25 ppm ($F_{(4,24)} = 682.5$, $P < 0.05$). Obtained data revealed that the mortalities of the 3rd larvae were lower than those of the 2nd larvae.

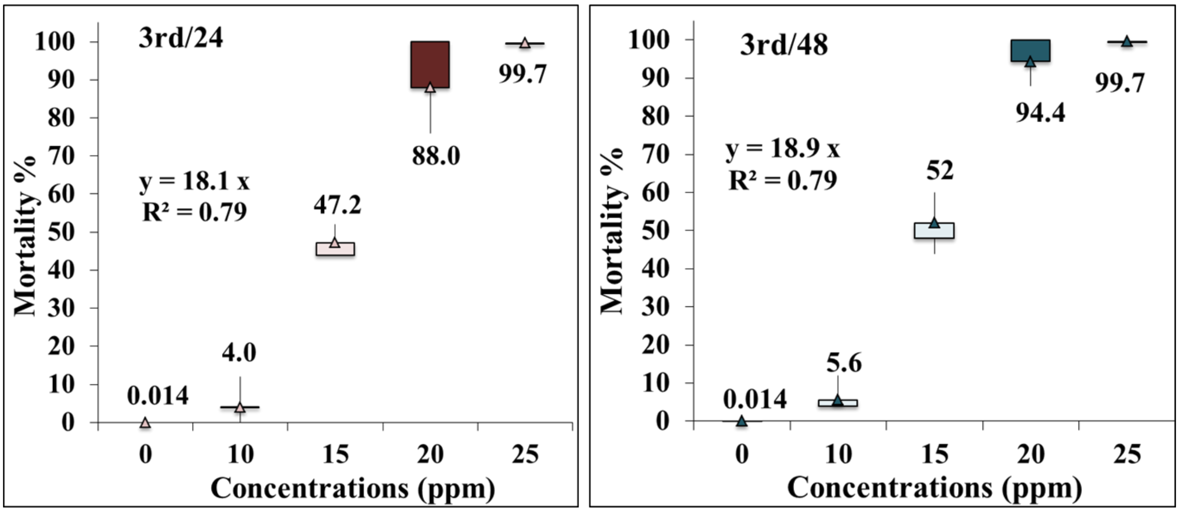


Fig. 4. Mortality percentages of different concentrations (ppm) of MEON against 3rd instar larvae of *C. pipines* at 24 and 48 h post treatments.

As shown in Figure 5, the mortality percentages of treated 4th larvae at 20 ppm were relatively lower (50.4%) compared to 25 ppm (99.56%) at 24 h after treatment ($F_{(4,24)} = 433.1$, $P < 0.0005$), while they were highly increased to 68.0% at 20 ppm, and the same percentage of mortality (99.56%) was obtained at 25 ppm for 48 h after treatment ($F_{(4,24)} = 249.3$, $P < 0.0005$). Moreover, the percentage of mortality increased from 7.2 to 15.2 for 24 and 48 hours at 10 ppm, respectively. Indeed, many trials indicated that orange EOs have toxic effects on mosquito vector diseases, for instance, anopheles mosquitoes (Umar *et al.,* 2024), *Culex quinquefasciatus* (Suwansirisilp *et al.,* 2013), *Culex pipiens* (Michaelakis *et al.,* 2009), and Aedes albopictus (Li *et al.,* 2023). The obtained results indicated that the MEON concentration (25 ppm) exhibited more than 99 % mortality in *C. pipiens* instars larvae, which was drastically lower than those recorded for bulk orange EOs in the previous studies, which ranged from 300 to 1000 ppm (M. Azmy *et al.,* 2019a) (Manimaran *et al.,* 2012). It was demonstrated that mandarin EO inhabited the acetylcholine esterase, carboxyl esterase, acid phosphatase, and alkaline phosphatase of *C. pipiens* larvae (Badawy *et al.,* 2018). It was found that over 2000 secondary metabolites have been identified from orange essential oils, with the most important active ingredients being diterpenes, monoterpenes, terpenes, and sesquiterpenes, which may refer to the toxicological effects of the orange EOs against insect pests (Brah *et al.,* 2023). The current study is comparable with several studies on nanoemulsions based on EOs as effective insecticides (Sogan *et al.,* 2023; Abdel-Baki *et al.,* 2021). Because the EOs have destitute water solubility, larvicides utilized to manage mosquito larvae ought to be dissolvable in water since they are aquatic life forms. To bypass this natural barrier, EOs should be formed into nanoemulsions (Sharifiyan *et al.,* 2024). The obtained results in the concentration mortality bioassays were satisfactorily described by the probit model (Fig. 6).

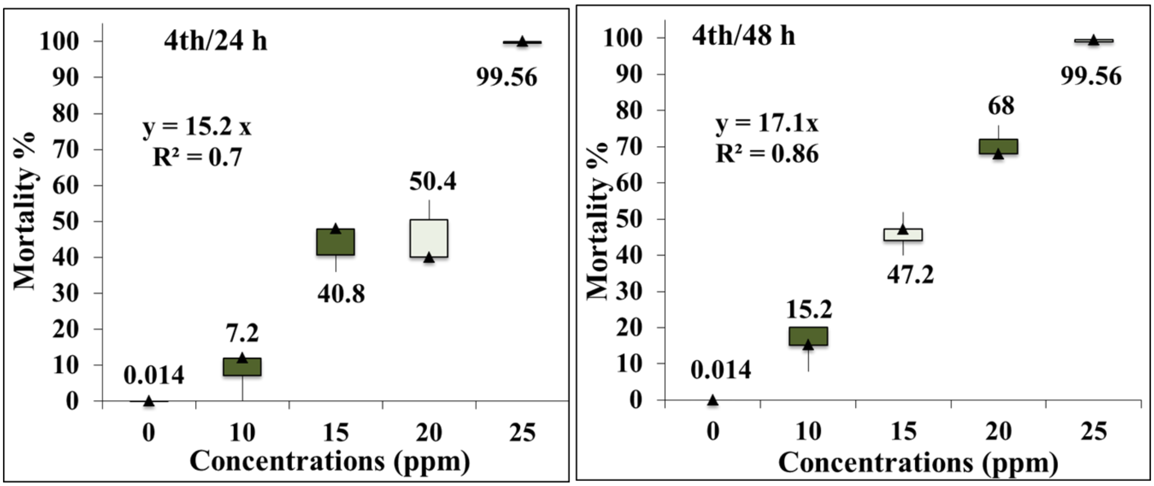


Fig. 5: Mortality percentages of different concentrations (ppm) of MEON against 4th instar larvae of *C. pipines* at 24 and 48 h post treatments**.**

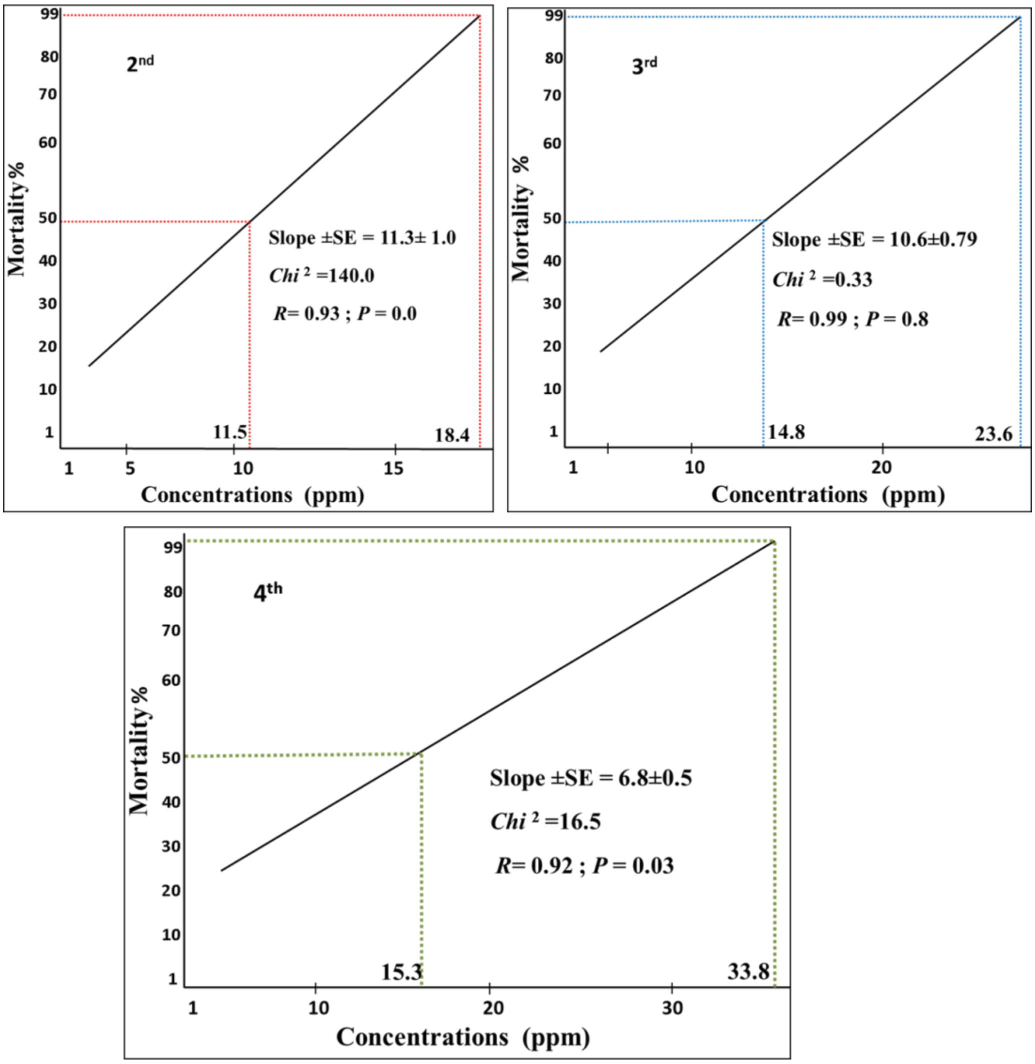


Fig. 6: Mortality-responses of *C. pipines* larvae (2nd, 3rd and 4th) for different concentrations (ppm) of MEON at 48 h post treatments.

The LC_{50} was (11.5, 14.8 and 15.3 ppm) for the 2nd instar, 3rd and 4th instar, respectively (Fig. 6). Also, the LC_{99} was (18.4, 23.6 and 33.8 ppm) for the 2nd instar, 3rd and 4th instar, respectively. The results indicated that LC_{50} the fourth instar was more resistant to treatment, but the second instar was much more vulnerable to MEON (no overlap in 95% fiducial limits), according to the LC_{50} and LC_{99} values. As shown in Figure 6, a concentration-dependent association between MEON and larval mortality was demonstrated via regression analysis. Insecticidal properties of orange oils were reported for larvicidal activity against *C. pipiens* in a previous study that suggested it might be because of trans-anethole, the main component of the EO (Kamaraj *et al.,* 2023). The orange EO nanoemulsion's larvicidal action could be attributed to limonene, the main ingredient, which Saad (2013) showed has insecticidal qualities. Our results are in line with several studies on nanoformulations that use essential oils (EOs) as effective pesticides (Duarte *et al.,* 2015; Barradas and de Holanda e Silva, 2021). Our results are consistent with those of Sanei-Dehkordi *et al.* (2022), who discovered that the LC_{50} and LC_{90} of nano orange EO for the third larvae of *Anopheles stephensi* and *Culex quinquefasciatus*, respectively, were the low concentrations (6.63 and 12.29 µg/mL) and (4.9 and 16.4 µg/mL), respectively. The results we obtained are in agreement with those reported by Theochari *et al.* (2020), who observed that a concentration of 27.4 ppm of nanoemulsion orange EO was reported as an LC_{50} against *C. pipines* larvae in their third instar. Based on our research, it appears that the MEON could be the most effective way to address the issues with using EO against *C. pipiens*. It does this by preventing rapid evaporation and degradation in the environment, offering a well-soluble formulation, and lowering the dosage required. To guarantee this novel material's safety for the environment and non-target creatures, more investigation is necessary for the risk evaluation of the material. Studying field applications and challenges related to economic feasibility is also necessary.

CONCLUSIONS

Orange essential oils have become one of the major bio-pesticides against mosquito vector diseases due to their known toxicity and their environmental benefits. However, it is essential to preserve the orange essential oils by maintaining their stability against environmental conditions since they are chemically unstable and can degrade when exposed to air, light, moisture, and mild temperatures. High doses and poor water solubility are also significant considerations when using essential oils. The designated emphasis in this trial is using nano-gel as a green encapsulation of mandarin essential oil in the hope of revolutionizing their application challenges. The aqueous polymer polyethylene oxide/polyacrylamide was crosslinked by gamma irradiation, producing polyethylene oxide/polyacrylamide nano-gels as a result. Mandarin essential oil (MEO) was used to dissolve the solution and create mandarin essential oil nano-gel (MEON), which has a restricted size distribution and a high degree of cross-linking. The resulting MEON presented spherical shapes with homogeneous surfaces. The average size of MEON particles ranged from 130 to 160 nm; a low negative zeta potential of −23.0 mV indicated high stability of the emulsion. The results demonstrated that the dose of 25 ppm of MENO exhibited 99.9% mortality in the three instar larvae of *C. pipiens*. Further research on the risk assessment of MENO is required for its safety properties.

Ethics Approval and Consent to Participate: Not applicable.

Competing interests: The authors declare no conflict of interest.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Funding: No funding was received.

Availability of Data and Materials: The authors declare that they have no objection to the availability of data and materials.

EFERENCES

Abdel-Baki, A. A. S.; Aboelhadid, S. M.; Sokmen, A.; Al-Quraishy, S.; Hassan, A. O. and Kamel, A. A. (2021). Larvicidal and pupicidal activities of Foeniculumvulgare essential oil, trans-anethole and fenchone against house fly Muscadomestica and their inhibitory effect on acetylcholinestrase. *Entomological Research,* 51(11): 568-577.

Alshangiti, D. M.; Ghobashy, M. M.; Alkhursani, S. A.; Shokr, F. S.; Al-Gahtany, S. A., and Madani, M. M. (2019). Semi-permeable membrane fabricated from organoclay/PS/EVA irradiated by ɣ-rays for water purification from dyes. *Journal of Materials Research and Technology,* 8(6), 6134-6145.

Ashfaq, A.; An, J.-C.; Ulański, P. and Al-Sheikhly, M. J. P. (2021). On the mechanism and kinetics of synthesizing polymer nanogels by ionizing radiation-induced intramolecular crosslinking of macromolecules. *Pharmaceutics,* 13(11): 1765.

Badawy, M. E.; Taktak, N. E. and El-Aswad, A. F. (2018). Chemical composition of the essential oils isolated from peel of three citrus species and their mosquitocidal activity against Culex pipiens. *Natural product research,* 32(23): 2829-2834.

Barradas, T. N. and de Holanda e Silva, K. G. J. E. C. L. (2021).Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. *Environmental Chemistry Letters,* 19(2): 1153-1171.

Brah, A. S.; Armah, F. A.; Obuah, C.; Akwetey, S. A. and Adokoh, C. K. (2023). Toxicity and therapeutic applications of citrus essential oils (CEOs): A review. *International Journal of Food Properties,* 26(1): 301-326.

Campolo, O.; Giunti, G.; Laigle, M.; Michel, T.; Palmeri, V. J. I. C. and Products (2020). Essential oil-based nano-emulsions: Effect of different surfactants, sonication and plant species on physicochemical characteristics. *Industrial Crops and Products,* 157: 112935.

de Jesus Oliveira, J.; dos Passos, E. M.; Alves, S. M.; Sarmento, V. H.; Bjerk, T. R.; Cardoso, J. C.; Blanco-Llamero, C.; Souto, E. B.; Severino, P. and da Costa Mendonça (2024). Microemulsion of essential oil of Citrus aurantium var. dulcis for control of *Aleurocanthus woglumi* and evaluation of selectivity against *Aschersonia aleyrodis* and *Ceraeochrysa cornuta. Crop Protection*, 106586.

Dispenza, C.; Spadaro, G.; and Jonsson M. J. (2017). Radiation engineering of multifunctional nanogels." Applications of Radiation Chemistry in the Fields of Industry, *Biotechnology and Environment*, 95-120.

Duarte, J. L.; Amado, J. R.; Oliveira, A. E.; Cruz, R. A.; Ferreira, A. M.; Souto, R. N.; Falcão, D. Q.; Carvalho, J. C. and Fernandes, C. P. (2015). Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil.*Revista Brasileira de Farmacognosia*, 25: 189-192.

Esmaili, F.; Sanei-Dehkordi, A.; Amoozegar, F. and Osanloo, M. J. (2021). A review on the use of essential oil-based nanoformulations in control of mosquitoes. *Research in Applied Chemistry,* 11(5): 12516-12529.

Finney, D. J. (1971). Probit analysis: 3d ed,Cambridge University Press. Cambridge University Press.

Ghobashy, M. M.; El-Sawy, N. M. and Kodous, A. S. (2021a). Nanocomposite of cosubstituted carbonated hydroxyapatite fabricated inside Poly (sodium hyaluronate-acrylamide) hydrogel template prepared by gamma radiation for osteoblast cell regeneration. *Radiation Physics and Chemistry,* 183: 109408.

Ghobashy, M. M.; El-Damhougy, B. K.; El-Wahab, H. A.; Madani, M.; Amin, M. A.; Naser, A. E. M.; Abdelhai, F.; Nady, N.; Meganid, A. S. and Alkhursani, S. A. (2021b). Controlling radiation degradation of a CMC solution to optimize the swelling of acrylic acid hydrogel as water and fertilizer carriers. *Polymers for Advanced Technologies,* 32(2): 514-524.

Ghobashy, M. M.; Reheem, A. M. A. and Mazied, N. A. (2017). Ion etching induced surface patterns of blend polymer (poly ethylene glycol–poly methyl methacrylate) irradiated with gamma rays. *International Polymer Processing,* 32(2): 174-182.

Hillary, V. E.; Ceasar, S. A. and Ignacimuthu, S. J. (2024). Efficacy of plant products in controlling disease vector mosquitoes, a review. *Entomologia Experimentaliset Applicata,*172 (3): 195-214.

Ibrahim, S. S. (2020). Essential oil nanoformulations as a novel method for insect pest control in horticulture. *Horticultural crops,* 195-209.

Kamaraj, C.; Satish Kumar, R. C.; Al-Ghanim, K. A.; Nicoletti, M.; Sathiyamoorthy, V.; Sarvesh, S.; ... and Govindarajan, M. (2023). Novel Essential Oils Blend as a Repellent and Toxic Agent against Disease-Transmitting Mosquitoes. *Toxics,* 11(6), 517.

Lakshmayya, N.; Mishra, A. K.; Mohanta, Y. K.; Panda, J.; Naik, B.; Mishra, B.; Varma, R. S. B. and Biotechnology, A. (2023). Essential oils-based nano-emulsion system for food safety and preservation: Current status and future prospects. *Biotechnology,* 102897.

Lammari, N.; Louaer, O.; Meniai, A. H. and Elaissari, A. J. (2020). Encapsulation of essential oils via nanoprecipitation process: Overview, progress, challenges and prospects. *Pharmaceutics,* 12(5): 431.

Li, Y.; Wu, W.; Jian, R.; Ren, X.; Chen, X.; Hong, W. D.; Wu, M.; Cai, J.; Lao, C. and Xu, X. (2023). Larvicidal, acetylcholinesterase inhibitory activities of four essential oils and their constituents against *Aedes albopictus,* and nanoemulsion preparation. *Journal of Pest Science,* 96(3): 961-971.

M Azmy, R.; E El Gohary, E. G.; M Mahmoud, D.; AM Salem, D.; A Abdou, M. and Salama, M. (2019a). Assessment of larvicidal activity of nanoemulsion from Citrus sinensis essential oil on Culex pipiens L.(Diptera: Culicidae). *Egyptian Journal of Aquatic Biology Fisheries,* 23(3): 61-67.

Madani, M.; Hosny, S.; Alshangiti, D. M.; Nady, N.; Alkhursani, S. A.; Alkhaldi, H.; Al-Gahtany, S. A.; Ghobashy, M. M. and Gaber, G. A. (2022). Green synthesis of nanoparticles for varied applications: Green renewable resources and energy-efficient synthetic routes. *Nanotechnology Reviews,* 11(1): 731-759.

Madeira, S.; Bernardino, R.; Osório, H. C. and Boinas, F. J. (2024). Mosquito (Diptera: Culicidae) Fauna of a Zoological Park in an Urban Setting: Analysis of Culex pipiens sl and Their Biotypes. *Egyptian Journal of Aquatic Biology and Fisheries,* 15(1): 45.

Manimaran, A.; Cruz, M.; Muthu, C.; Vincent, S. and Ignacimuthu (2012). Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston). *Advances in Bioscience Biotechnology,*3(07): 855-862.

Marouf, A. E. and Harras, F. (2022). Efficacy of Three Plant Oil Extracts and their Nano-Emulsions on the Leaf Miner, *Tuta absoluta* Larvae (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Plant Protection Pathology,* 13(9): 207-213.

Matusiak, M.; Kadlubowski, S. and Rosiak, J. M. (2020). Nanogels synthesized by radiation-induced intramolecular crosslinking of water-soluble polymers. *Radiation Physics and Chemistry*, 169: 108099.

Michaelakis, A.; Papachristos, D.; Kimbaris, A.; Koliopoulos, G.; Giatropoulos, A. and Polissiou, M. G. (2009). Citrus essential oils and four enantiomeric pinenes against *Culex pipiens* (Diptera: Culicidae). *Parasitology research*, 105: 769-773.

Osanloo, M.; Firooziyan, S.; Abdollahi, A.; Hatami, S.; Nematollahi, A.; Elahi, N. and Zarenezhad, E. J. (2022).Nanoemulsion and nanogel containing Artemisia dracunculus essential oil; larvicidal effect and antibacterial activity." *BMC Research Notes "* 15(1): 276.

Rosiak, J. M.; Ulanski, P.; Kujawa, P.; Janik, I.; Stasica, P.; Kadlubowski, S. and Kozicki, M. (2005). Radiation-Induced Transformations of Polymers in Aqueous Solutions. *Eurasian Chemico-Technological Journal* ,7: 225-250.

Saad, M. M. (2013). Chemical composition and biological activities of four citrus essential oils. *Journal of Plant Protection and Pathology*, 4(9): 767-780.

Sanei-Dehkordi, A.; Moemenbellah-Fard, M. D.; Saffari, M.; Zarenezhad, E. and Osanloo, M. (2022).Nanoliposomes containing limonene and limonene-rich essential oils as novel larvicides against malaria and filariasis mosquito vectors. *BMC Complementary Medicine and Therapies*, 22(1): 140.

Sayed, W. A. A.; El-Bendary, H. and El-Helaly, A. (2020). Increasing the efficacy of the cotton leaf worm *Spodoptera littoralis* nucleopolyhedrosis virus using certain essential oils. *Egyptian Journal of Biological Pest Control*, 30(1): 1-7.

Sharifiyan, M.; Mehrkhou, F. and Negahban, M. (2024). Lethal and sublethal effects of Menthapiperita L. and its nanoformulation form on the biological and population growth parameters of *Trialeurodes vaporariorum* (Westwood) under laboratory conditions. *Journal of Entomological Society of Iran,* 44.1 (2024): 20-30.

Sogan, N.; Kala, S.; Kapoor, N.; Singh, H.; Verma, P.; Nautiyal, A. and Nagpal, B. (2023). Utilization and re-use of orange peel derived oil by formulating nanoemulsion for efficient vector control application. *Waste Biomass Valorization,* 1-13.

Suwansirisilp, K.; Visetson, S.; Prabaripai, A.; Tanasinchayakul, S.; Grieco, J. P.; Bangs, M. J. and Chareonviriyaphap, T. (2013). Behavioral responses of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) to four essential oils in Thailand. *Journal of pest science,* 86: 309-320.

Theochari, I.; Giatropoulos, A.; Papadimitriou, V.; Karras, V.; Balatsos, G.; Papachristos, D. and Michaelakis, A. (2020).Physicochemical characteristics of four limonene-based nanoemulsions and their larvicidal properties against two mosquito species, *Aedes albopictus* and *Culex pipiens* molestus. *Insects,* 11(11): 740.

Umar, S.; Kokori, B.; Bashir, A. and Umaru, W. J. (2024). Larvicidal Efficacy of Citrus sinensi (Sweet Orange) Peel Extract against Anopheles Mosquito Larva from Nagazi and Adavi Local Government Area, Kogi State, Nigeria. *Journal of Applied Sciences and Environmental Management*, *28*(1): 123-128.

Villena, O. C.; McClure, K. M.; Camp, R. J.; LaPointe, D. A.; Atkinson, C. T.; Sofaer, H. R. and BerioFortini, L. (2024). Environmental and geographical factors influence the occurrence and abundance of the southern house mosquito, *Culex quinquefasciatus*, in Hawai ‘i. *Scientific Reports,* 14(1): 604.

Vivekanandhan, P.; Panikar, S.; Sethuraman, V.; Usha-Raja-Nanthini, A. and Shivakumar, M. S.R. (2023). Toxic and synergetic effect of plant essential oils along with nano-emulsion for control of three mosquito species. *Journal of Natural Pesticide Research,* 100045.