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Research

The Physiological Effect Of Zinc Oxide (ZnO) Nanopesticide On *Aedes aegypti* Larvae

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ABSTRACT

Aedes aegypti is responsible for transmitting various mosquito-borne diseases. Recently, there have been concerns about the negative impacts of the insecticides used in vector control including insecticide resistance development in the mosquito population. These circumstances lead to efforts to develop other strategies for controlling mosquito vectors. As technology in nanoparticles advances, zinc oxide (ZnO) nanoparticles have the potential as the alternative for chemical pesticides for mosquito larvicides due to their optical properties and widespread usage in different industries. The purpose of this study was to determine the toxicity of ZnO nanoparticles towards *Ae. aegypti* larvae and to examine the physiologies of *Ae. aegypti* mosquito larvae treated with ZnO nanoparticles at LC_{50} level. Toxicity bioassays were carried out to determine LC_{50} and LC_{90} values. The larvae surface and midgut treated with LC_{50} ZnO were examined using the Scanning Electron Microscope (FESEM) and Dispersive X-ray spectroscopy (EDX). The LC_{50} and LC_{90} concentrations of ZnO nanoparticles after 4 hr of direct UV exposure against *Ae. aegypti* larvae were 49.141 mg/L and 64.195 mg/L, respectively. After exposure to ZnO nanoparticles, *Ae. aegypti* larvae showed morphological abnormalities, including distorted and shrunk body parts as well as midgut rupture. Overall, the findings suggest that ZnO nanoparticles have the potential to replace chemical pesticides as a means of reducing the populations of *Ae. aegypti* mosquitoes.

Key words: Mosquito, nanoparticles, pesticide, zinc oxide

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INTRODUCTION

Aedes aegypti is the primary vector of the chikungunya, Zika, dengue, and yellow fever viruses (Eisen *et al*., 2014). These diseases are expected to spread globally in the next few decades as the population of *Aedes* mosquitoes continues to grow due to rapid urbanization, improved connections between continents as well as climate change (Kraemer *et al*., 2019). As of the year 2023, over 6.5 million cases were reported in over 80 countries with more than 7300 deaths related to dengue fever. In Malaysia alone, 111400 dengue cases were reported in 2023 (WHO, 2024).

Dengue control and reduction of vector-host contacts are the main control strategies for mosquitoes in Malaysia (Hamdan & Kilicman, 2019) as vaccines for dengue are relatively new (Vethasalam, 2024), and current medications only treat the symptoms of other mosquito-borne diseases. Several mosquito control strategies have been implemented, including environment management, source reduction, larvicide application, fogging, and house inspection in Malaysia (Ong, 2016) Primarily, organophosphates and pyrethroid insecticides were used in mosquito-borne disease control programs (Yusof *et al*., 2022) and these were available for common households in the form of aerosol spray and mosquito coil. However, recent studies show that *Ae. Aegypti* mosquitoes were resistant to either one or both types of chemical groups across different regions in Malaysia

(Rasli *et al*., 2021; Akhir *et al*., 2022). In the aspect of human health, occupational pesticide exposure by mosquito control workers is associated with reduced neurobehavioral performances (Yusof *et al*., 2022). There are reports of adverse effects of commercial insecticides on humans and non-targeted organisms (Rezende-Teixeira *et al*., 2022). Thus, there is a need to find an alternative chemical to control mosquito populations.

Metal oxide nanoparticles, particularly, have been proven to cause toxicity to organisms in small amounts and these include zinc oxide nanoparticles. Due to their distinctive physical and chemical characteristics, they were widely used in several industrial sectors and commonly applied as photocatalysts, UV absorbers, fillers, and more (Kołodziejczak-Radzimska & Jesionowski, 2014). Zinc oxide nanostructures have high catalytic activity and a large surface area, making them ideal for use in catalytic reaction processes (Thirumavalavan *et al*., 2013). Similar to other metal oxide nanoparticles, ZnO nanoparticles can also be characterized via particle size distribution, particle morphology, particle reactivity, and surface chemistry (Kołodziejczak-Radzimska & Jesionowski, 2014), and all these aspects influence its effectiveness in toxicology application.

Zinc oxide is unique as it can absorb UV light (Kołodziejczak-Radzimska & Jesionowski, 2014). Ultraviolet (UV) illumination can improve photoconductivity by reducing the surface of the electron depletion region of ZnO (Bao *et al*., 2011), and induce an oxidation process that can damage and be fatal to organisms (Baruah *et al*., 2010). Under UV radiation, ZnO nanoparticles in an aqueous solution have a phototoxic effect, producing Reactive Oxygen Species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide ions (O_2) (Zhang *et al*., 2011; Kołodziejczak-Radzimska & Jesionowski, 2014). These ROS caused by ZnO nanoparticles are proven to be cytotoxic to most organisms but are still considered safe for humans (Nagar *et al*., 2022). As nanotechnology advances, ZnO nanoparticles are characterized as target-specific while remaining non-toxic for humans and other non-target organisms with the influence of the environment's natural UV supply. Zinc oxide nanoparticles have the potential of becoming an alternative chemical pesticide based on their properties. Thus, the study aims to determine the lethal concentrations of the ZnO nanoparticles on *Aedes aegypti* mosquito larvae and to investigate the post-mortem physiological and morphological changes of larvae after being treated with ZnO nanoparticles.

MATERIALS AND METHODS

Rearing of mosquitoes

Eggs of *Aedes aegypti* were obtained from the Vector Research Control Unit, School of Biological Sciences, Penang, Malaysia. Eggs were hatched and the larvae were reared in enamel plates (30 cm diameter and 5 cm depth) with dechlorinated tap water at a temperature of 24°C. A powdered mixture of beef liver, cat food, milk powder, and yeast in the ratio of 2:1:1:1 per total weight was supplied twice a day as food for mosquito larvae. Late third and early fourth instar larvae were used for bioassay tests.

Stock zinc oxide nanoparticle preparations.

Factory-produced ZnO nanoparticles with 99.9% purity were purchased from Approfit ZnO Manufacturing Sdn Bhd. The size of ZnO was 20 nm. 10 g/L stock solution of pure ZnO nanoparticles was prepared by diluting 0.5 g ZnO powder in 50 mL distilled water. The stock solutions were sonicated by using an ultrasonic bath for 40 to 50 min to agitate the particles in a solution. For each bioassay test, the stock solution was shaken thoroughly for an even mix and distribution of ZnO nanoparticles.

Toxicity test of ZnO nanoparticles on *Aedes aegypti* larvae

The toxicity test was modified based on the larvicide susceptibility test by the World Health Organization (2016). In each toxicity assay, 20 mosquito larvae were placed in a disposable test cup containing ZnO nanoparticles and tap water, with a total volume of 100 mL. Test concentrations (30, 35, 40, 45, 50, 55, 60 mg/L) of nanoparticles were obtained by diluting matching volumes of ZnO stock solution with tap water to make 100 mL volume. Each test included one control group (tap water only) with three replicates for each concentration. UV light was applied on all test replicates for four hr to activate ZnO nanoparticles in the solution. The UV lamp used was at UVA wavelength ranging between 315-400 nm, with the intensity of 0.9-1 mW/cm^2 on the top surface. Black cloth and an additional opaque box were used to keep the UV light within. The mortality was measured after 24 hr of UV light exposure. After needle probing, larvae with no movement were counted as dead. If the mortality percentage of the control group was between 5% to 20%, Abbott's formula (Abbott, 1925) was applied to the ZnO-treated group, whereby:

$$\text{Corrected mortality (\%)} = \frac{\text{\% Test mortality - \% Control mortality}}{\text{100 - \% Control mortality}} \times 100$$

Statistical analysis

Mortality data were used to determine the LC_{50} and 90%-LC_{90} values (The lethal concentration that kills 50% & 90% of tested mosquito larvae). LC values and confidence limit interval were calculated using log dosage–probit analysis via SPSS version 25 software program. The LC values were calculated by transforming the sigmoid dose-response curve to a linear straight line. The detailed mathematical steps used to derive the regression line equation were based on studies by Vincent (2008) and Finney (1952).

Midgut extraction and stereo microscopy

LC_{50} of ZnO nanoparticles-treated larvae and untreated larvae were dissected on a petri dish and then monitored using a dissecting microscope. The anterior end of the larvae was removed, and the midgut of the larvae was pulled out from the abdomen. The external bodies and the midguts of both treated and non-treated larvae were observed under a stereo microscope to inspect for any morphological changes.

Scanning electron microscopy

The external bodies and the midguts of both treated and untreated larvae were then fixed using prepared McDowell and Trump's fixatives overnight before Scanning Electron Microscope (SEM) viewing. The samples were washed using 0.1 M phosphate buffer and postfixed in 1% Osmium tetroxide. The samples were later washed in distilled water and then dehydrated in ethanol with increasing purity (50% to 100%). Finally, the samples were immersed in hexamethyldisilazane (HMDS), and taken out to be air dried. Once completed, the samples were mounted onto a stub with a double-sided tape, gold coated, and examined using a scanning electron microscope (Carl Zeiss Leo Supra 50 VP).

Energy Dispersive X-Ray analysis (EDX)

The presence of Zinc (Zn) elements and Oxygen (O) elements was determined using the Energy Dispersive X-ray analysis (EDX) on samples of control and LC_{50} of ZnO nanoparticles-treated *Ae. aegypti* samples.

RESULTS

The toxicity study of larvae *Aedes aegypti* under ZnO nanoparticles treatment

The LC_{50} and LC_{90} of ZnO nanoparticles-*Ae. aegypti* larvae with UV exposure after 24 hr were 49.141 mg/L and 64.195 mg/L, respectively (Table 1). The results confirmed that ZnO nanoparticles caused toxicity and were fatal to *Ae. aegypti* larvae.

Table 1. LC_{50} and LC_{90} of concentration of zinc oxide nanoparticles under 4 hr of UV exposure against the larvae *of Ae. aegypti*

ZnO (mg/L)	Mortality (%)	LC_{50} (mg/L)	95% confidence limit (mg/L)		LC_{90} (mg/L)	95% Confidence limit (mg/L)		X^2 value	df
			LCL	UCL		LCL	UCL		
30	0.0								
35	5.0								
40	16.7								
45	33.3	49.14	47.03	52.69	64.20	59.20	77.33	0.017	12
50	53.7								
55	70.0								
60	100.0								

#LC_{50}: Lethal concentration that causes 50% mortality; LC_{90}: Lethal concentration that causes 90% mortality; LCL: Lower confidence limit; UCL: Upper confidence limit; X^2: Chi square value; df: Degrees of freedom.

Larval morphological changes and zinc distribution

After exposure to ZnO nanoparticles at LC_{50} level, the larvae showed several morphological changes. Some noticeable damage included distortion and shrinking on the thorax, abdomen, and the siphon (Figure 1b). The segments in the thorax and abdomen regions became indistinguishable (Figure 2b). Dead larvae turned dark after ZnO treatment. The midgut of the LC_{50} ZnO nanoparticles-treated sample under FESEM was severely damaged i.e. it was ruptured and broken down into several parts (Figures

3b and 4b). White particles found in the midguts of treated larvae are most likely the accumulation of ZnO nanoparticles, as EDX spectra showed the presence of Zinc and Oxygen elements in the midgut of LC_{50} ZnO nanoparticles-treated sample (Figure 5), but not in the untreated sample (Figure 6).

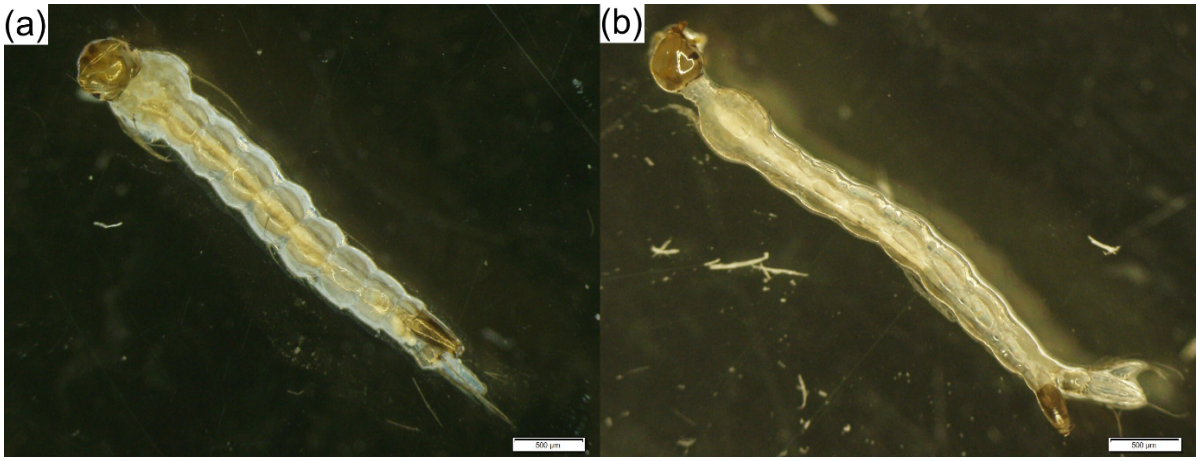

Fig. 1. Images of *Ae. aegypti* larvae under a stereo microscope. (a). Untreated *Ae. aegypti* larvae with normal morphological structure. (b). LC_{50} ZnO nanoparticles-treated *Ae. Aegypti* larvae with shrinkage abdomen and thorax region.

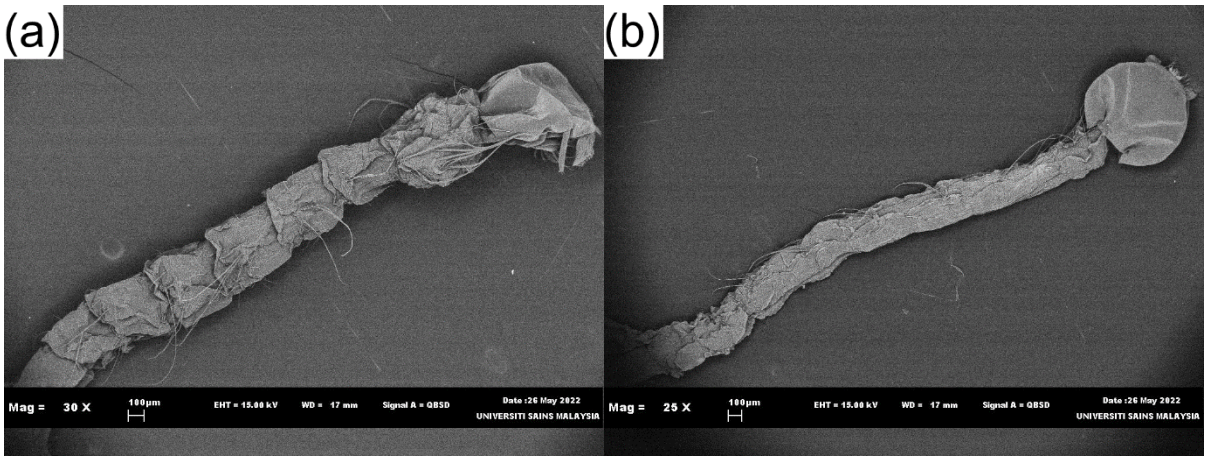

Fig. 2. SEM Images of *Ae. Aegypti* larvae. (a). Untreated *Ae. aegypti* larvae with normal morphological structure. (b). LC_{50} ZnO nanoparticles-treated *Ae. aegypti* larvae with shrinkage abdomen and thorax region as well as segments on both abdomen and thorax became indistinguishable.

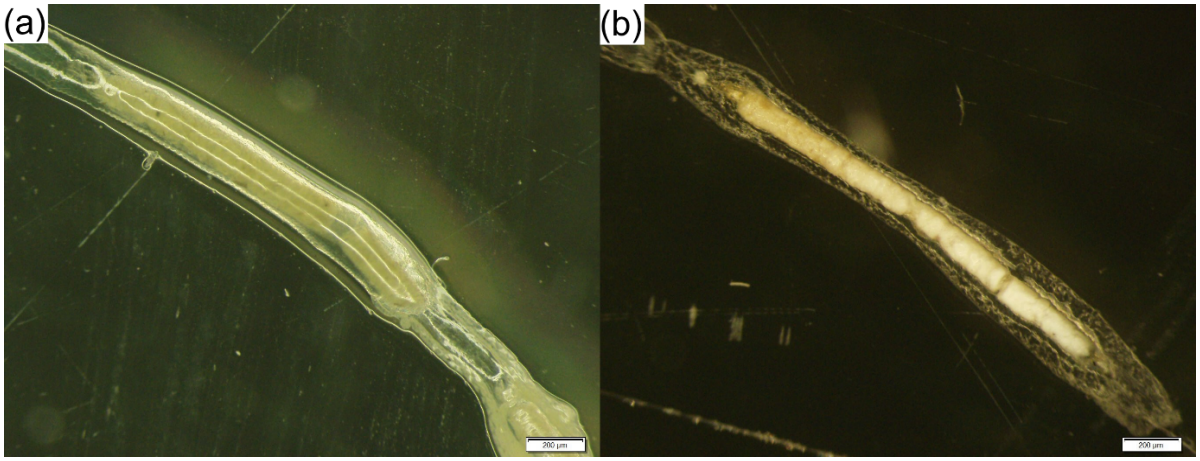

Fig. 3. Images of *Ae. Aegypti* larvae midgut under a stereo microscope. (a). Untreated *Ae. aegypti* larvae midgut with normal morphology structure. (b). LC_{50} ZnO nanoparticles-treated *Ae. aegypti* larvae midgut with the accumulation of whitish ZnO content.

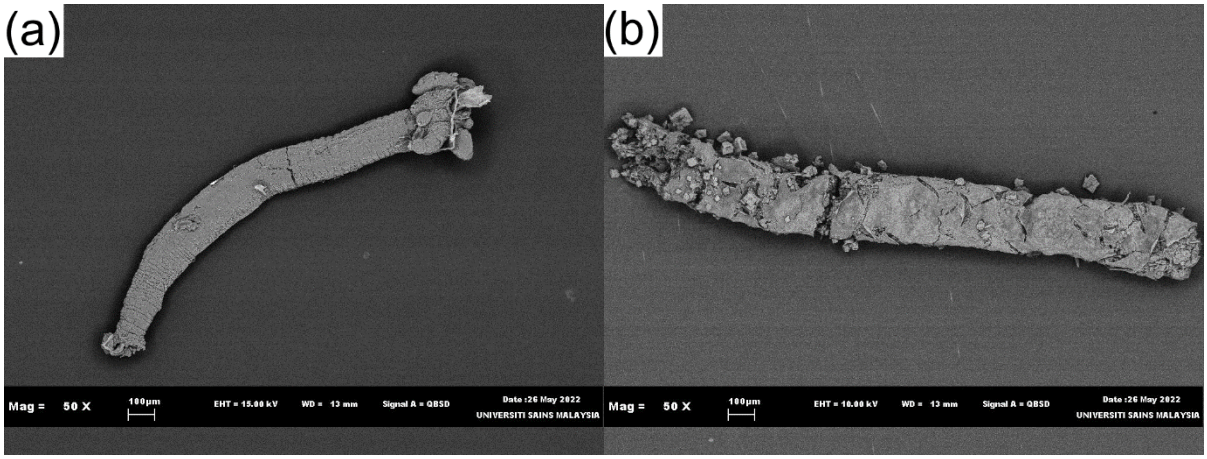

Fig. 4. SEM Images of *Ae. aegypti* larvae midgut. (a). Untreated *Ae. aegypti larvae* with normal morphology structure. (b). LC_{50} ZnO nanoparticles-treated *Ae. aegypti* larvae midgut with the ruptured parts on the midgut structure.

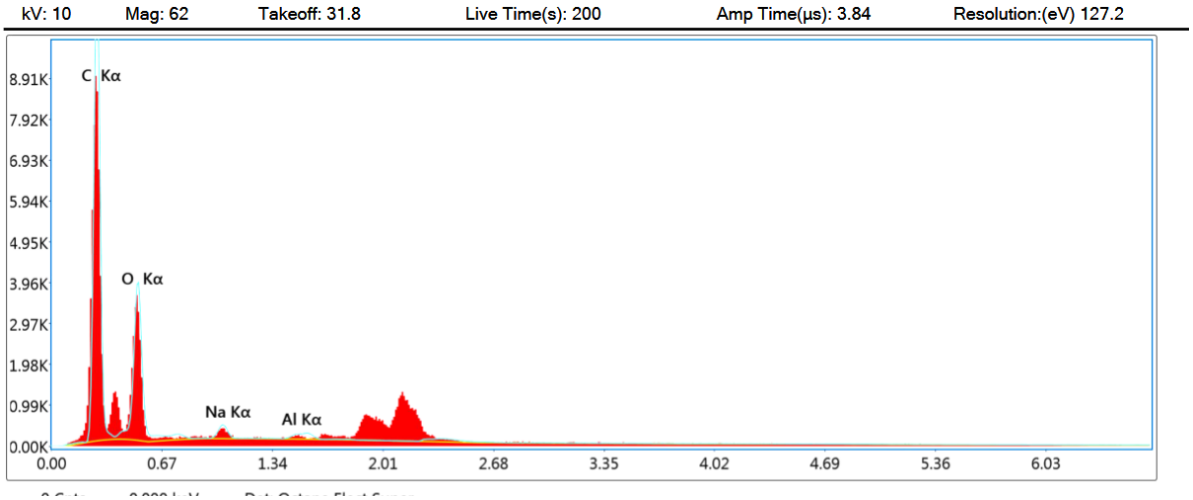

Fig. 5. EDX spectra for elements present in the midgut of untreated samples with the absence of Zn element.

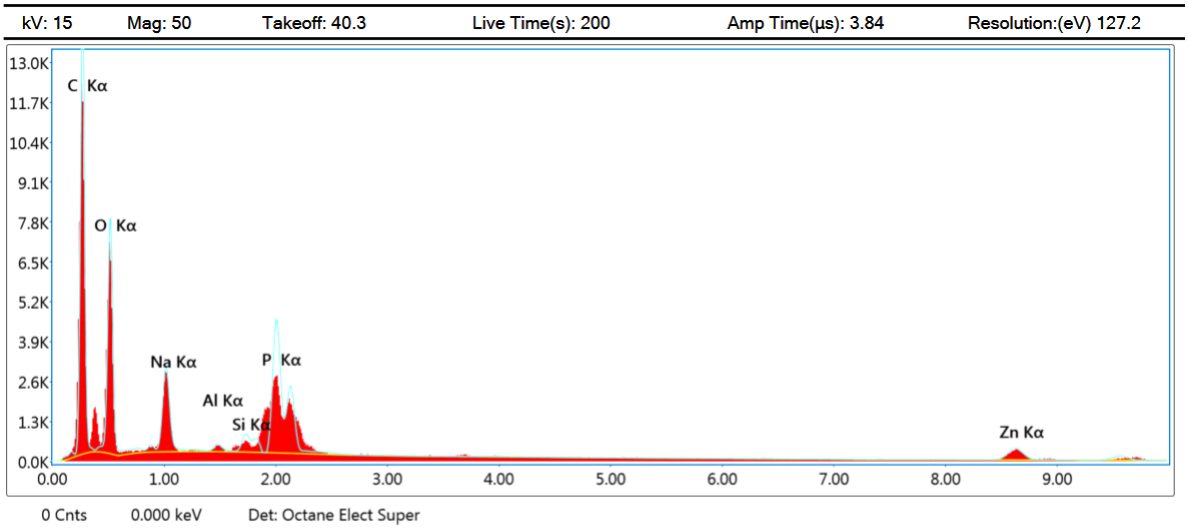

Fig. 6. EDX spectra for elements present in the midgut of LC_{50} ZnO nanoparticles-treated samples with the presence of zinc element.

DISCUSSION

The mortality of mosquito larvae is proposed as the result of the accumulation of reactive oxygen species (ROS), whereby cytotoxic Zn^{2+} induces the oxidative organelles to produce ROS to induce cell apoptosis that overtakes the detoxification process resulting in larvae death (Nagar *et al*., 2022).

The morphological changes after LC_{50} treatment in both the cuticle and midgut of mosquito larvae may be due to the toxic effect of ZnO nanoparticles, as these changes are also described similarly by several other studies using the same or other mosquito species but with ZnO nanoparticles of different characterizations and modifications (Banumathi *et al*., 2017; Abinaya *et al*., 2018; Yazhiniprabha *et al*., 2019; Dhavan & Jadhav, 2020; Vinotha & Vaseeharan, 2023). The presence of Zn elements in LC_{50} ZnO nanoparticles-treated larvae could indicate accumulation and direct intake of ZnO nanoparticles into the midgut cells. The midgut tissue layer is thin as it consists of simple epithelial tissues surrounded by an outer muscle layer (Fernandes *et al*., 2014), which allows ZnO nanoparticles to enter the cell easily.

As summarized by Shahzad & Manzoor (2021), ZnO nanoparticles may be inhaled, consumed, or physically in contact with insect cuticles. From these exposure routes, the nanoparticles were able to reach and accumulate in different parts of the body, including the midgut. These nanoparticles either penetrate or get internalized by endocytic vesicles in the cells or tissues. As endocytosis approaches its end in lysosomes, Zn^{2+} is released quickly into cells and potentially produces many free radicals as a byproduct (Nagar *et al*., 2022). The release of ROS is further accelerated by UV light as it excites ZnO nanoparticles present in both aqueous solution and in larvae tissues (Sirelkhatim *et al*., 2015). At the same time, Zn^{2+} within the larvae tissue binds to multiple types of intracellular proteins including nucleic acid and enzymes, causing damage to protein and inhibiting enzymatic activity. These occasions then successfully overtake the detoxification process of ZnO, cause cell apoptosis, and finally induce mortality in mosquito larvae. Some harmful impacts of nanoparticles in insect midgut include oxidative stress, de-nucleation of cells, destruction of brush borders, and loss of organization in cell layers (Shahzad & Manzoor, 2021). The midgut rupture of treated larvae could be the aftermath of these impacts combined.

CONCLUSION

This study discovers that ZnO nanoparticles are lethal for *Ae. aegypti* larvae at considerably low concentrations under UV radiation exposure. Several physiological and morphological changes found on the body surface of larvae as well as on the midgut may indicate some of the many physiological impacts of ZnO nanoparticles. Midgut was proposed as one of the accumulation sites of ZnO nanoparticles based on the presence of the Zn element detected. With future modification and refinement of ZnO nanoparticles, it has the potential to be a green alternative to commercial larvicide.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18(2): 265- 267.

Abinaya, M., Vaseeharan, B., Divya, M., Sharmili, A., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., & Benelli, G. 2018. Bacterial exopolysaccharide (EPS)-coated ZnO nanoparticles showed high antibiofilm activity and larvicidal toxicity against malaria and Zika virus vectors. Journal of Trace Elements in Medicine and Biology, 45: 93– 103. https://doi.org/10.1016/j.jtemb.2017.10.002

Akhir, M.A.M., Wajidi, M.F.F., Lavoué, S., Azzam, G., Jaafar, I.S., Awang Besar, N.A.U., & Ishak, I.H. 2022. Knockdown resistance (kdr) gene of *Aedes aegypti* in Malaysia with the discovery of a novel regional specific point mutation A1007G. Parasites & Vectors, 15: 1-15. https://doi.org/10.1186/s13071-022-05192-z

Banumathi, B., Vaseeharan, B., Ishwarya, R., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., & Benelli, G. 2017. Toxicity of herbal extracts used in ethno-veterinary medicine and green-encapsulated ZnO nanoparticles against *Aedes aegypti* and microbial pathogens. Parasitology Research, 116: 1637-1651. https://doi.org/10.1007/s00436-017-5438-6

Bao, J., Shalish, I., Su, Z., Gurwitz, R., Capasso, F., Wang, X., & Ren, Z. 2011. Photoinduced oxygen release and persistent photoconductivity in ZnO nanowires. Nanoscale Research Letters, 6: 1-7.

https://doi.org/10.1186/1556-276X-6-404

Baruah, S., Mahmood, M.A., Myint, M.T.Z., Bora, T., & Dutta, J. 2010. Enhanced visible light photocatalysis through fast crystallization of zinc oxide nanorods. Beilstein Journal of Nanotechnology, 1: 14-20. https://doi.org/10.3762/bjnano.1.3

Dhavan, P.P., & Jadhav, B.L. 2020. Eco-friendly approach to control dengue vector *Aedes aegypti* larvae with their enzyme modulation by *Lumnitzera racemosa* fabricated zinc oxide nanorods. SN Applied Sciences, 2: 1-15. https://doi.org/10.1007/s42452-020-2636-0

Eisen, L., Monaghan, A.J., Lozano-Fuentes, S., Steinhoff, D.F., Hayden, M.H., & Bieringer, P.E. 2014. The impact of temperature on the bionomics of *Aedes* (Stegomyia) *aegypti*, with special reference to the cool geographic range margins. Journal of Medical Entomology, 51(3): 496-516. https://doi.org/10.1603/ME13214

Fernandes, K.M., Neves, C.A., Serrão, J.E., & Martins, G.F. 2014. *Aedes aegypti* midgut remodeling during metamorphosis. Parasitology International, 63(3): 506-512. https://doi.org/10.1016/j.parint.2014.01.004

Finney, D.J. 1952. Probit analysis: a statistical treatment of the sigmoid response curve (2nd ed.). Cambridge University Press.

Hamdan, N.I., & Kilicman, A. 2019. Analysis of the fractional order dengue transmission model: A case study in Malaysia. Advances in Difference Equations, 2019: 1-13. https://doi.org/10.1186/s13662-019-1981-z

Kołodziejczak-Radzimska, A., & Jesionowski, T. 2014. Zinc Oxide—From Synthesis to Application: A Review. Materials, 7(4): 2833– 2881. https://doi.org/10.3390/ma7042833

Kraemer, M.U.G., Reiner, R.C., Brady, O.J., Messina, J.P., Gilbert, M., Pigott, D.M., Yi, D., Johnson, K., Earl, L., Marczak, L.B., Shirude, S., Davis Weaver, N., Bisanzio, D., Perkins, T.A., Lai, S., Lu, X., Jones, P., Coelho, G.E., Carvalho, R. G., Bortel, W.V., Marsboom, C., Hendrickx, G., Schaffner, F., Moore, C.G., Nax, H.H., Bengtsson, L., Wetter, E., Tatem, A.J., Brownstein, J.S., Smith, D.L., Lambrechts, L., Cauchemez, S., Linard, C., Faria,N.R., Pybus, O.G., Scott, T.W., Liu, Q., Yu, H., Wint, G.R.W., Hay, S.I..,Golding, N. 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nature Microbiology, 4: 854–863. https://doi.org/10.1038/s41564-019-0376-y

Nagar, V., Singh, T., Tiwari, Y., Aseri, V., Pandit, P.P., Chopade, R.L., Pandey, K., Lodha, P., & Awasthi, G. 2022. ZnO Nanoparticles: Exposure, toxicity mechanism and assessment. Materials Today: Proceedings, 69: 56–63. https://doi.org/10.1016/j.matpr.2022.09.001

Ong, S.Q. 2016. Dengue vector control in Malaysia: A review for current and alternative strategies. Sains Malaysiana, 45(5): 777-785.

Rasli, R., Cheong, Y.L., Che Ibrahim, M.K., Farahininajua Fikri, S.F., Norzali, R.N., Nazarudin, N.A., Hamdan, N.F., Muhamed, K.A., Hafisool, A.A., Azmi, R.A., Ismail, H.A., Ali, R., Ab Hamid, N., Taib, M.Z., Omar, T., Wasi Ahmad, N., & Lee, H.L. 2021. Insecticide resistance in dengue vectors from hotspots in Selangor, Malaysia. PLOS Neglected Tropical Diseases, 15(3): 1-15. https://doi.org/10.1371/journal.pntd.0009205

Rezende-Teixeira, P., Dusi, R.G., Jimenez, P.C., Espindola, L.S., & Costa-Lotufo, L.V. 2022. What can we learn from commercial insecticides? Efficacy, toxicity, environmental impacts, and future developments. Environmental Pollution, 300: 1- 20. https://doi.org/10.1016/j.envpol.2022.118983

Shahzad, K., & Manzoor, F. 2021. Nanoformulations and their mode of action in insects: A review of biological interactions. Drug and Chemical Toxicology, 44(1): 1–11. https://doi.org/10.1080/01480545.2018.1525393

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., & Mohamad, D. 2015. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Letters, 7(3): 219-242. https://doi.org/10.1007/s40820-015-0040-x

Thirumavalavan, M., Huang, K.L., & Lee, J.F. 2013. Preparation and morphology studies of nano zinc oxide obtained using native and modified chitosans. Materials, 6(9): 4198- 4212. https://doi.org/10.3390/ma6094198

Vincent, K. 2008. Probit analysis. San Francisco State University.

Vethasalam, R. 2024. First dengue vaccine launched in Malaysia [WWW Document]. The star. URL https://www.thestar.com.my/news/nation/2024/06/11/first-dengue-vaccine-launched-in-malaysia (accessed 19.6.24).

Vinotha, V., & Vaseeharan, B. 2023. Bio-fabricated zinc oxide and cry protein nanocomposites: Synthesis, characterization, potentiality against Zika, malaria and West Nile virus vector's larvae and their impact on non-target organisms. International Journal of Biological Macromolecules, 224:

699–712. https://doi.org/10.1016/j.ijbiomac.2022.10.158
WHO. 2016. Monitoring and managing insecticide resistance in *Aedes* mosquito populations: interim guidance for entomologists. World Health Organization, Geneva. 11 pp.
WHO. 2024. Dengue and severe dengue [WWW Document]. World Health Organization. URL https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (accessed 19.6.24).
Yazhiniprabha, M., Vaseeharan, B., Sonawane, A., & Behera, A. 2019. *In vitro* and *in vivo* toxicity assessment of phytofabricated ZnO nanoparticles showing bacteriostatic effect and larvicidal efficacy against *Culex quinquefasciatus*. Journal of Photochemistry and Photobiology B: Biology, 192: 158-169. https://doi.org/10.1016/j.jphotobiol.2019.01.014
Yusof, M.Z., Cherrie, J.W., Samsuddin, N., & Semple, S. 2022. Mosquito Control Workers in Malaysia: Is Lifetime Occupational Pesticide Exposure Associated with Poorer Neurobehavioral Performance? Annals of Work Exposures and Health, 66(8): 1044-1055. https://doi.org/10.1093/annweh/wxac038
Zhang, H., Chen, B., Jiang, H., Wang, C., Wang, H., & Wang, X. 2011. A strategy for ZnO nanorod mediated multi-mode cancer treatment. Biomaterials, 32(7): 1906-1914. https://doi.org/10.1016/j.biomaterials.2010.11.027