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# Larvicidal efficiency of zinc oxide nanoparticles against mosquito vectors

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**Abstract**
Nanotechnology has enhanced the management in controlling pest, due to its ability to produce nanoparticles with its unique physio-chemical properties. The zinc oxide nanoparticles show high efficacy in larvicidal activity against various mosquito vector. The species *Aedes aegypti* transmits various important diseases such as dengue, chikungunya, and zika. The species *Aedes aegypti* is also known as cosmopolitan. The present review summarizes about the nanoparticles based insecticides against the mosquito larvae. By the vast usage of chemical insecticides increases the resistances in insects. So, the alternative to chemical insecticides is botanical based nanoparticles helps to reduces or prevent the mosquito *Aedes aegypti* has increased its attention in previous years. This present review is about the traditional methods to control mosquitoes and disadvantages of conventional mosquito control, the effectives of ZnO nanoparticles against the larvicidal mosquito, involvement of various other metallic nanoparticles against larvicidal, the effect of plant based nanoparticles is used against the species against *Aedes aegypti* and its mechanism of nanoparticles used to control the species. Additionally, the carboxypeptidase enzyme is inhibited by the zinc ions, preventing mosquito proliferation and development.

**Keywords:** Zinc oxide nanoparticles, insecticides, resistances, dengue, *Aedes aegypti* and larvicidal activity

## Introduction

Mosquitoes are important carriers of infectious diseases, and they belong to the Family Culicidae and Order Diptera. The major carriers of infections that cause diseases like chikungunya, dengue, filariasis, zika fever, yellow fever, Japanese encephalitis, malaria, West Nile fever, and Rift Valley fever (Abinaya *et al.*, 2018; Agarwal *et al.*, 2017) [1, 2]. There are over 35,000 species of Mosquitoes in the world. Among them, 53 species are from the genus *Anopheles*, and nine of them are known to carry the Malarial infection. Interestingly, *Anopheles stephensi* seems to be the predominant species that infects people with the Malarial disease (Al-Dhabi *et al*., 2018; Ahmad *et al*., 2022) [3, 4]. Filariasis is spread by *Culex quinquefasciatus*, one of the *Culex* species (Alphey *et al*., 2010; Ali *et al*., 2019) [5, 6]. *Aedes* species, specifically both *Aedes aegypti* and *Aedes albopictus*, have also been a part of human societies for centuries and are responsible for the transmission of dengue. Tropical and subtropical nations are the areas where dengue outbreaks are most common (World Statistics and Indian Statistics for the year 2023, taken from NVCB). An estimated 1121 dengue cases were recorded in Tamil Nadu, India (Amuthavalli *et al*., 2021) [7].

The traditional mosquito control techniques have drawbacks such resistance development, environmental issues, and challenges in extracting strong bioactive chemicals from natural sources. The application of nanomaterials for mosquito control is an inventive strategy that has gained popularity recently. Because of their unique chemical and physical characteristics, nanoparticles provide a flexible platform for long-term, focused mosquito control. The environmentally benign and sustainable character of phytomediated nanoparticle production has also drawn attention. Zinc oxide nanoparticles (ZnO NPs) have shown great promise in the management of mosquito larvae. Numerous plant sources have been used in studies; each provides unique phytochemicals to the nanomaterials. This review examines the complex interaction between synthesis techniques, particle properties, and larvicidal performance, and it compiles current knowledge on the use of phyto-mediated zinc oxide (ZnO) nanoparticles as repellent agents.

## Managing of Mosquitoes using Traditional methods

Traditionally, chemical pesticides and the microbial toxic substances (Endotoxin from *Bacillus thuringiensis*) have been used to control mosquitoes. These chemical pesticides, however, have detrimental impacts on the ecosystem and present threats to both humans and non-target species. In addition to these drawbacks, one major issue with limiting vector transmissions is mosquito resistance to chemical pesticides. Alternative approaches are therefore necessary to address these issues in managing pathogenic pathogens and mosquito populations (Ananth and Thangamathi, 2018; Ananthi *et al*., 2022) [8, 9]. For a number of reasons, using phytochemicals to reduce mosquito populations has shown to be very successful. It is easy to use and eco-friendly. In comparison with certain commercial insecticides, it achieves an excellent efficiency at modest dosages. It doesn't need artificial surfactants. It has several mechanisms of action, which prevents mosquito populations from becoming resistant to it (Anitha *et al*., 2018) [10].

The worldwide species *Aedes (Stegomyia) aegypti* is responsible for spreading important arboviruses like dengue, Zika, and chikungunya. The use of larvicidal chemicals is the primary method used to control this insect. However, a surge in resistant insects has resulted from the abuse of synthetic chemical larvicides, making management challenging. The significant benefits of nanostructured delivery mechanisms, such as controlled release; large surface area; enhancement of biological activity; defense of natural bioactive agents from the environment and thus obtaining stability; and lipophilic drugs are easier to disperse even in aqueous vehicles, have led to a greater focus on the use of botanical insecticide–based nanotechnology as a potential alternative to the use of chemical agents for the control of *Ae. Aegypti*. The information currently available regarding botanical insecticide-based nanosystems that is larvicidal against *Ae. aegypti* larvae is compiled in this review. The bulk of papers employed metallic nanoparticles (NPs) as larvicidal agents; silver nanoparticles (AgNPs) in particular showed promise as an alternative. Next came nanoemulsions that contained vegetable oils, the majority of essential oils, and nanosystems that permit the dispersion of this highly hydrophobic product in water, the larval development environment. The last section closes the knowledge gap in the literature by summarizing scientific research on these nanoparticles mode of action.

**Disadvantages of Conventional Mosquito Control**

The discovery of DDT, attempts to control mosquitoes were very successful with the use of numerous synthetic pesticides (Duarte *et al*., 2020) [21]. Insecticides have been used extensively ever since to keep mosquitoes under control. However, over use of these pesticides resulted in the development of resistant in vector species, raising questions about their detrimental effects on the environment and human health (Baruah *et al*., 2021) [11]. Hence, when looking for a solution, the top priorities should be insecticide substitutes that are reasonably priced, efficient, and safe for both people and the environment. Since mosquito species are increasingly developing resistance, which makes it difficult to control mosquito populations and stop the spread of vector-borne infections, alternative pesticides should have the ability to be resistant to mosquito populations (Basnet *et al*., 2018) [12]. The mosquito species can even become resistant to a variety of chemical combinations (Bekkari *et al*., 2017) [13]. When chemical pesticides are sprayed on the surface, residual chemicals are left behind that are harmful to people if they are breathed (Benelli, 2015) [16]. The unpleasant smell of organic pesticides and the challenges encountered in observing the effects of space spraying made chemical agents unacceptable. Further, they are quite expensive and needs technical abilities.

The emergence of tolerance by mosquito vectors posed a threat to the biological insecticides derived from plants and microbial sources (Basnet *et al.*, 2018) [12]. Because biocontrol agents alter the food chain and reduce biodiversity, they may pose a threat to non-target organisms (Benelli and Mehlhorn, 2016; Benelli *et al.*, 2018) [14, 15]. As part of a mosquito control effort, the establishment of transgenic mosquitoes must overcome challenges include maintaining mutant stains, interacting with non-target organisms, and ethical concerns (Brintha and Ajitha, 2015; Chandrasekaran *et al*., 2016) [18, 19].

**The activity of ZnO-NPs against Mosquito Vectors**

ZnO-NP toxicity is dependent on a number of variables. ZnO-NPs' cytotoxicity is influenced by their form. Furthermore, the size of nanoparticles has a big impact on how harmful they are to insects and mosquito larvae. ZnO-NPs are more hazardous at the nanoscale than they are at the macroscale (Chinnathambi *et al*., 2023) [20]. The first mechanism that accounts for ZnO-NPs' toxicity to mosquito larvae is the breakdown of cell membranes and their accumulation in the cytoplasm. Which cause apoptosis by generating ions that are harmful to zinc ($Zn_2^+$) and induce cell damage through intracellular ROS cytotoxicity.

ZnO-NP treatment was shown to cause disruption to the head, thorax, abdomen, and siphon regions in mosquito larvae. The zinc oxide nanoparticles were able to attach to the larvae's surface and cause harm while also effortlessly piercing the cuticle due to their nano size. After piercing the cuticle, they target particular cells and interfere with the physiological processes of mosquito larvae. ZnO-NPs efficiently killed *Aedes aegypti, Anopheles stephensi* IV instar larvae, and *Culex quinquefasciatus* larvae when mediated by *Pterolobium hexapetalum* the aqueous leaf extract (Agarwal *et al*., 2017) [2]. The larval epithelial cells were successfully disrupted by ZnO-NPs made from the *Elettaria cardamomum* extract, and it was also demonstrated that these particles accumulated in the mid-gut region (Chinnathambi *et al*., 2023) [20]. When larval cells are subjected to ZnO-NPs for a prolonged length of time, they result in abnormal cell shape, changes to the cell cycle, and a reduction in mitochondrial activity (Toshima and Yonezawa, 1998) [26].

Different structural characteristics influence the toxicity of ZnO-NPs. For example, compared to spherical-shaped structures, rod-shaped ZnO-NPs are substantially more dangerous (Agarwal *et al.*, 2017) [2]. ZnO-NPs shaped like stars were very poisonous to the larvae of *Aedes albopictus* and *Anopheles vagus.* The increased toxicity of star-shaped ZnO-NPs was attributed to several factors. The larvae's membranes are abused and damaged when a star-shaped particles come into direct interaction with cells due to their spatial layout and randomly aligned spikes (Sahai and Goswami, 2015) [25]. It may also absorb a large number of oxygen molecules due to its large surface to volume ratio and its abundance of polar surfaces. ZnO-NPs have a higher larvicidal ability because of the abundance of oxygen molecules, which contribute to the production of intracellular ROS (Tso *et al*., 2010; Turek and Stintzing, 2013) [27, 28].

ZnO-NPs exhibits ovicidal, larvicidal, and adulticidal activities against a wide variety of mosquito species. The toxicity of these compounds has been associated with the presence of zinc and oxygen ions. All phases of the mosquito life cycle are prevented from growing due to the production of reactive species from singlet oxygen ions (Vellore Nagarajan and Vijayarangan, 2019) [29]. The buildup of zinc ions prevents mosquito larvae from developing and causes metal ion poisoning in them. Additionally, the carboxypeptidase enzyme is inhibited by the zinc ions, preventing mosquito proliferation and development. Zinc oxide nanoparticles have an impact on the alimentary canal and Malpighian tubules. The cytoplasm can vacuolate and cluster and the peritrophic membrane can become injured. ZnO-NPs induce the gastric caeca to shrink and cause the basement membrane to develop before the epithelial cells, some of which still had nuclei form. Degradation and disconnection occur among the tall cuboidal and regenerative cells of the posterior midgut. Cell-shaped adoration was seen in Malpighian tubules. Exposure of hemoglobin-producing cells to ZnO-NPs causes structural distortion that ultimately lowers their viability (Velsankar *et al*., 2019) [30]. ZnO-NPs caused *Aedes aegypti* to exhibit morphological and histological alterations, which were mediated by the aqueous extract of

*Solanum lycopersicum*. Death was the consequence of syphon tube suffocation brought on by the ZnO-NPs (Vijayakumar *et al*., 2016) [31]. Zn ions reduced by *Syzygium cumini* had an $LC_{50}$ value of 49.22 ppm, making them effective against IV-instar *Aedes aegypti* (Vinotha *et al*., 2020) [32]. ZnO-NPs generated by *Lumnitzera racemosa* aqueous have been observed to accumulate in the gastrointestinal tract and interfere with physiological processes including moulting. This interference impeded the mosquito larvae's ability to develop. The larvae's body shrank and took on a charred look due to ZnO-NP toxicity. Furthermore, the nanoparticles had a detrimental effect on the syphon, which resulted in larval death (Benelli and Mehlhorn, 2016) [15].

**Involvement of Metal ions nanoparticles**

The usage of insecticides is extremely hazardous to humans, necessitating the use of more sophisticated pesticides, insects remain a major cause for human diseases and agricultural loss. For example, because metallic nanoparticles may be produced sustainably from natural resources, they have garnered interest in studies as potential insecticides. In this paper, we examine the use of metal nanoparticles as insecticides against crop pests, blood-feeding parasites, ticks, and mosquitoes. Gold, copper, iron, nickel, palladium, and oxides of zinc, titanium, aluminum, iron, copper, magnesium, and cadmium are the elements that make up metal nanoparticles. The intrinsic characteristics of nanoparticles, metal ion release, redox disequilibrium, enzyme inactivation, and genetic damage all contribute to toxicity. Additionally, we contrast conventional pesticides with metal Nano-pesticides. Additionally, we contrast conventional pesticides with metal nano-pesticides. The larvicidal and pupicidal properties of metallic nanoparticles against different mosquito vectors of dengue fever, malaria, and other illnesses were discovered. Pesticidal effects could potentially be produced by the phytochemicals in the unprocessed natural materials used to make metallic nanoparticles. Nanoparticle sizes, shapes, and charges determine insecticidal efficiency (Li *et al*., 2023) [22].

**Plant mediated Nanoparticles against Mosquitoes**

Mosquitoes (Diptera: Culicidae) pose a serious hazard to millions of people globally because they are carriers of harmful parasites and infections. Insect growth regulators, microbial control agents, and organophosphates are typically used to target young instar mosquitoes. Insecticide-treated bed nets and residual spraying indoors are also used. Nonetheless, the environment and human health are severely harmed by these substances. Recently, safer and more advanced tools have been used to improve mosquito control. Silver mosquitocidal nanoparticles make up the bulk of plant-fabricated mosquitocidal NPs. UV-visualization spectroscopy, scanning electron microscopy, transmission electron microscopy, energy-dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy, and X-ray diffraction studies are typically used to confirm the synthesis of mosquitocidal nanoparticles. It's interesting to note that certain mosquito species of medical and veterinary significance have been shown to be effectively targeted by metal nanoparticles synthesized from plants as ovicides, larvicides, pupicides, adulticides, and oviposition deterrents. The dengue vector *Aedes aegypti*, the Filariasis mosquito *Culex quiquefasciatus*, and the malaria vector *Anopheles stephensi* are all severely poisoned by a few parts per million of various MNP. However, despite the mounting evidence of MNP's effectiveness, very modest attempts have been made to elucidate any potential off-target effects on other aquatic creatures and the natural adversaries of mosquitoes. This topic received special attention in the last section. Highlighted are certain hotspots that require more investigation and collaboration between entomologists and parasitologists (Benelli, 2016) [18].

**Action of ZnO Nanoparticles on *plasmodium falciparum***

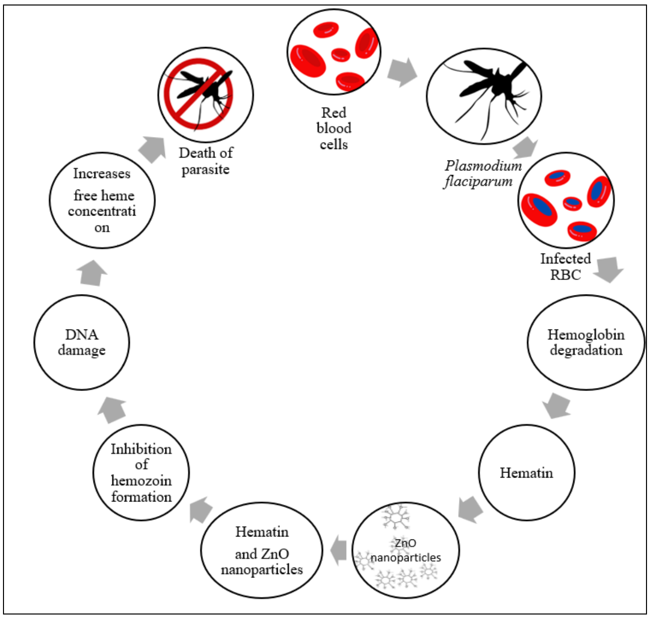


**Fig 1:** Action of ZnO nanoparticles on *Plasmodium falciparum*

**Conclusion**

Based on the nanoparticle characteristics and method of synthesis, the size and shape of the synthesized nanoparticles, and the functional groups present on the surface of the synthesized nanoparticles shows the efficacy of the ZnO-NPs on larvicidal activity. The use of natural products as pesticides is highly preferred than chemical pesticides, due to their rapid environmental degradation and low toxicity to other non-target organisms. The botanical insecticides are useful for mosquito control due to a wealth of secondary metabolites, which are applied given the evolution and adaptation suffered against predators. Among them, larvicides have the advantage of eliminating a large population of individuals in a small area, being a good alternative in eliminating vectors.

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