

Vector borne diseases and their control strategies on *Anopheles sp*, *Aedes sp*, and *Chironomus sp*: An overview focused on nanoparticles and its mode of action

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Abstract

Vector-borne diseases (VBDs) exist in intricate socio-ecological systems, and their impacts extend far beyond human health, affecting various aspects of our world. It is clear that vector-borne illnesses are a part of a complex web of interactions, even if prior research has concentrated on the direct effects of these diseases on human health and death. Vector-borne diseases are intricately linked to environment, ecology, diseases, and societal responses, creating feedback loops in fuel disease transmission. Consequently, they have left an indelible mark on human history. There has been significant interest in utilization of nanoparticles, synthesized through different methods, as innovative pesticides. Numerous studies have explored the toxic effect against a wide range of pests and insect vectors, particular attention is given to mosquitoes. A few prominent exceptions are silica, alumina, silver, and graphene oxide nanoparticles in respect to insects, but overall, our knowledge of the specific processes by which nanoparticles operate against insects and mites is still rather restricted. Metal nanoparticles have been found to interact with phosphorus (P) and sulfur (S) in proteins and its nucleic acids, leading to reduced membrane permeability, denaturation of enzyme, and cell death. Additionally, silver nanoparticles have been observed to regulate key insect genes, resulting in decreased synthesis of proteins and release of gonadotrophin that in turn leads to abnormalities and reproductive failures. In the final section, we critically examine the research trends in insect nanotoxicology, highlighting the significant challenges in predicting the ecotoxicity implications stemming from the present world application of nanoparticles as pesticides.

Keywords: Vector borne disease, silver nanoparticles, mode of action, glutathione S-transferases (GST)

Introduction

Malaria, dengue fever, Zika, Chikungunya, and Japanese encephalitis are all vector-borne diseases that have long been a major global public health problem. These diseases do not directly transmit between humans but depend on the convergence of hosts, pathogens and environmental conditions. Changes in social and environmental variables, such as international commerce and global climate changes, have led to a rise in the proliferation and transmission of vector-borne illnesses (Athni *et al.*, 2021) [2]. Vector control

stands as the primary approach for managing VBDs. In their 2017 publication "Global Vector Control Response: An Integrated Approach for the Control of VBDs," the World Health Organization (WHO) emphasized the importance of vector control in preventing an estimated 663 million cases of malaria in Africa, with more than half of those cases being attributed to the widespread implementation of vector control measures. The expenses of treating diseases can be greatly decreased by using effective vector control measures (Ma *et al.*, 2022) [14].

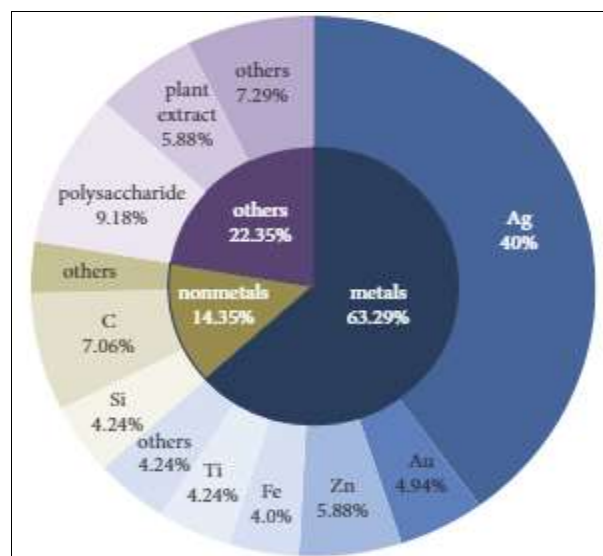


Fig 1: The classification of nanoparticles (NPs) employed for insect resistance, they can be broadly categorized based on their distinct properties. Metal-based NPs constitute the largest share at 63.29%, while nonmetal-based NPs, including those composed of silicon (Si) and carbon (C), represent a smaller portion at 14.35% (Nie *et al.*, 2023) [19].

Historically, insect growth regulators and insecticides have been used to target vector larvae. Additionally, tactics like bed nets treated with pesticide and indoor residual spraying have been used. Nonetheless, it is impossible to ignore the problems with resistance, residue, and the effects chemical treatments have on the environment. Vector control operations are complicated by insecticide resistance, as different vectors exhibit resistance in different geographic areas. Furthermore, research indicates that exposure to pesticides raises the risk of cancer in people (Nie *et al.*, 2023) ^[19]. To manage insecticide-resistant vectors that are transmitting outdoors, different therapies with a variety of active components are desperately needed. In this context, nanotechnology has emerged as a promising option for vector control. Gold nanoparticles (Au NPs), according to a literature review, have shown effectiveness against insect vectors transmitting diseases such as encephalitis, Zika, leishmaniasis, and trypanosomiasis (Onen *et al.*, 2023) ^[20]. There are other nanoparticles that work well against insect vectors than gold ones; various nanoparticles, including TiO₂, Ag, CuO, and Pd, have demonstrated insecticidal activity against vectors. However, the mechanisms underlying the enhanced toxicity of nanoparticles at low concentrations remain insufficiently understood (Slavin *et al.*, 2017) ^[24].

Recent research has shifted the focus towards controlled-release nano-sized pesticide systems based on nanoparticles.

Biodegradable and light-responsive polymers have been synthesized, enabling controlled pesticide release that gradually increases upon exposure to UV light. Nanoparticles have also demonstrated their potential to reduce pesticide consumption by protecting active ingredients from hydrolysis and photolysis, which is environmentally beneficial (Zhang *et al.*, 2023) ^[27]. Furthermore, greener synthesis methods for nanoparticles, using biological resources such as plants, bacteria, fungi, yeast, and algae as precursors, offer low toxicity and high biocompatibility. Despite potential applications of nanoparticles in insect control for public health, questions remain about the safety of nanoparticles and their impact on non-target organisms (Dikshit *et al.*, 2021) ^[5]. Some studies in nanoecotoxicology suggest no environmental or human health risks in the potential applications of polymeric nanoparticles. However, conflicting findings exist, with some studies indicating that nanoparticles can have both stimulatory and inhibitory effects on plant growth, depending on their concentration and exposure levels. Currently, there is no clear standard for defining high and low concentrations of different nanoparticles (Martínez *et al.*, 2020) ^[15]. In this review, we focus on the development and application of nanoparticles (NPs) for vector control, delving into the mechanisms behind NPs' lethality to vectors and assessing the environmental safety of nanoparticles.

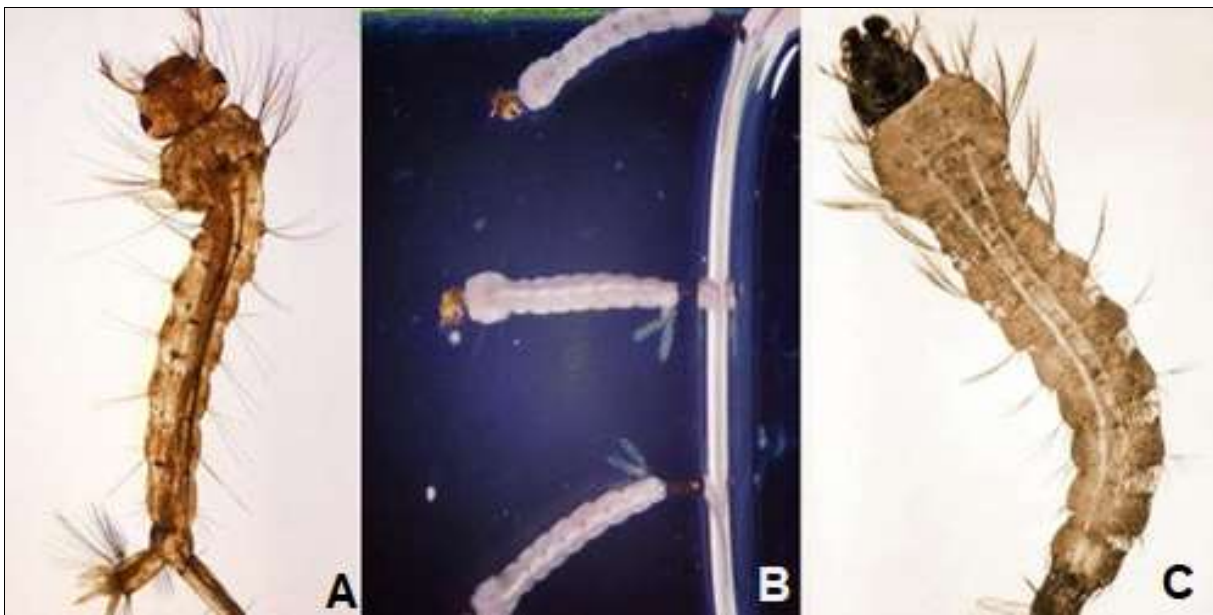


Fig 2: Research on toxicity of green-synthesized nanoparticles against arthropod vectors mostly focused on three major mosquito vectors: (A) *A. stephensi*, (B) *A. aegypti*, and (C) *C. quinquefasciatus* (Benelli, 2018) ^[3]

Synthesis of Silver NPs against VBD

The nanoparticles are made using the so-called green synthesis process, in which extracts from fungi, bacteria, plants their purified metabolites, and even dead insects have been used to reduce and stabilize nanoparticles in aqueous suspensions. When compared to traditional physical and chemical synthesis methods, the green manufacturing process has significant benefits since it does not require the use of extremely hazardous chemicals or large energy inputs (Hano *et al.*, 2021) ^[8]. The total process is inexpensive and simple, yielding a diverse range of nanoparticles such as gold, zinc oxide, iron, silver, titania, palladium, and carbon. These nanoparticles have been successfully tested against a

wide range of noxious arthropods, including agricultural pests and vectors of public health and cattle science (Salem, 2023) ^[23]. Although some interesting work on lice and mites has been done, the great majority of researchers focused on mosquito vectors and ticks. Furthermore, research has been conducted to investigate the stabilization and increase of the efficacy of *B.thuringiensis* and deltamethrin treatments against vector populations utilizing particular nanomaterials such as gold nanoparticles (Benelli, 2018) ^[3]. The research on nanoparticles as new insecticides and acaricides is accompanied by a growing body of evidences demonstrating the limited impact of green-capped nanoparticles in the aquatic environment, because at the concentrations used to

combat mosquito young instars, scarce acute and chronic toxicity has been detected in most cases, along with minimal genotoxicity and lack of detrimental behavioural changes in non-target species such as fishes, dragonfly nymphs, tadpoles. Being exposed to silver nanoparticles has been shown to cause morphological deformities and DNA damage in invertebrate and vertebrate creatures, as well as a negative influence on the enzymatic activities of several non-target species.

Recent research has given insight on the repercussions of mosquito early instars being subjected to green-synthesized nanoparticles. Kalimuthu *et al.* (2017) ^[11] showed that exposing *A. aegypti* (L.) fourth instar larvae for 24 h to *H. coronarium* J. Koenig rhizome-synthesized Ag nanoparticles (LC₅₀ = 72 ppm) led to partial lyses of the midgut epithelial cells; vesicles and damaged membranes at the apical side of epithelial cells were observed. It is difficult to determine whether the toxicity of nanoparticles, particularly metal ones, is due to the nanomaterial itself or to ions generated by it in a number of cases, and it has been discussed that several types of nanoparticles can act as toxic agents by passing through cellular barriers and then generating toxic ions that damage cell machinery (Huang *et al.*, 2017) ^[10]. In response to this challenge, Nair and Choi (2011) investigated the effect of commercial silver nanoparticles (0.2, 0.5, and 1 mg/l) on the aquatic midge *C. riparius* (Meigen), focusing on the effect of the nanomaterial on the expression of glutathione S-transferase (GST) genes, which are associated with the occurrence of oxidative stress (Park *et al.*, 2015) ^[21]. The scientists discovered that, depending on the measured dose and length of exposure to the contaminant, all GST genes investigated in *C. riparius* displayed up- or down regulation to varied degrees, with the greatest mRNA expression in the Delta3, Sigma4, and Epsilon1 GST classes. These findings highlighted the critical function of GST genes in shielding against oxidative stress after nanosilver exposure (Nair and Choi 2011) ^[17]. Nair *et al.* (2011) ^[17] found that silver nanoparticles up to 4 mg/l did not cause acute toxicity in *C. riparius*, but they did cause chronic toxicity (pupation, emergence, and reproductive failure) when tested at doses ranging from 0.2 to 1 mg/l. To shed insight on the toxicity mechanisms, the scientists examined changed gene expression in midges exposed to nanosilver, revealing down regulation of the ribosomal protein gene (CrL15), which regulates ribosomal assembly and therefore protein synthesis.

Furthermore, upregulation of the gonadotrophin releasing hormone gene (CrGnRH1) and the Balbiani ring protein gene (CrBR2.2) can indicate the activation of gonadotrophin releasing hormone mediated signal transduction pathways, as well as reproductive failure and the organism's defences (Flanagan and Manilall., 2017) ^[6]. Furthermore, Nair *et al.* (2013) ^[18] investigated how stress response transcription of antioxidant and detoxifying genes is affected by exposing *C. riparius* to three different concentrations of commercial silver nanoparticles and free ionic silver for 24 hours. The scientists demonstrated that Cu and Mn superoxide dismutase were upregulated after exposure to Ag⁺ ions and

silver nanoparticles, respectively. Catalase, phospholipid hydroperoxide glutathione peroxidase 1, and thioredoxin reductase 1 transcript levels were increased following exposure to silver nanoparticles, while no alterations were found after exposure to free ionic silver. The expression levels of glutathione S-transferase classes delta-3, sigma-4, and epsilon-1 were higher following exposure to Ag nanoparticles compared to free ionic silver. Overall, the results showed that when compared to free silver ions, exposure to silver nanoparticles had a significant influence on the activation of oxidative stress and detoxification genes.

Mode of action of NPs against VBR

Despite the significant potential of nanoparticles as insecticides and acaricides, concerns persist regarding their practical use in both terrestrial and aquatic ecosystems. The limited understanding of their ecotoxicological implications makes it challenging to predict their fate in the environment and their potential effects on human health (Zaheer *et al.*, 2022) ^[26]. Surprisingly, despite a plethora of data on their toxicity against certain pests and vectors, thorough insights into the exact mechanisms of action of nanoparticles against insects and mites are lacking. This knowledge is critical to foreseeing the toxicological effects of nanoparticle pesticide applications in the actual world. Mechanisms underlying cytotoxicity and genotoxicity have been studied particularly for silver nanoparticles, as their toxicity in model organisms is substantially impacted by parameters such as dimensions, form, and charge (Benelli, 2018) ^[3].

One method includes the nanoparticle scale effect, in which pesticide adherence in the environment is strengthened, increasing the possibility of insect exposure to nanoparticles in the environment. Following that, nanoparticles can dehydrate cells by adsorption on the stratum corneum, causing morphological and histological problems in insects (Chaud *et al.*, 2021) ^[4]. Among the many nanoparticles tested, silica and aluminium were shown to connect with the cuticle layer of ticks, resulting in the physical absorption of lipids and waxes, cell dehydration, and final cell death (Fig 1). Experiments conducted by Sultana found that carbon-dotted silver nanoparticles exhibited high toxicity to *A. stutzeri* and *C. quinquefasciatus*. Scanning electron microscopy (SEM) analysis in this experiment revealed that these carbon-dotted silver nanoparticles caused deformities in larvae, while X-ray analysis confirmed the presence of nano-hybrids in the treated specimens. The finding of silver in mosquito tissues shows that the cellular-level toxicity of nano-silver may be to blame for their extinction. Furthermore, damage to the stratum corneum and cellular tissue was seen by high-resolution transmission electron microscopy (HR-TEM). Third-instar *A. aegypti* larvae exposed to zinc oxide nanoparticles (1.57 mg/mL, 24 hrs) derived from *Lobelia* displayed abdominal contractions, altered thorax shape, mid gut lesions, and the loss of lateral hairs, anal gills, and brushes, while the accumulation of zinc oxide nanoparticles was observed in the chest and abdomen (Nie *et al.*, 2023) ^[19].

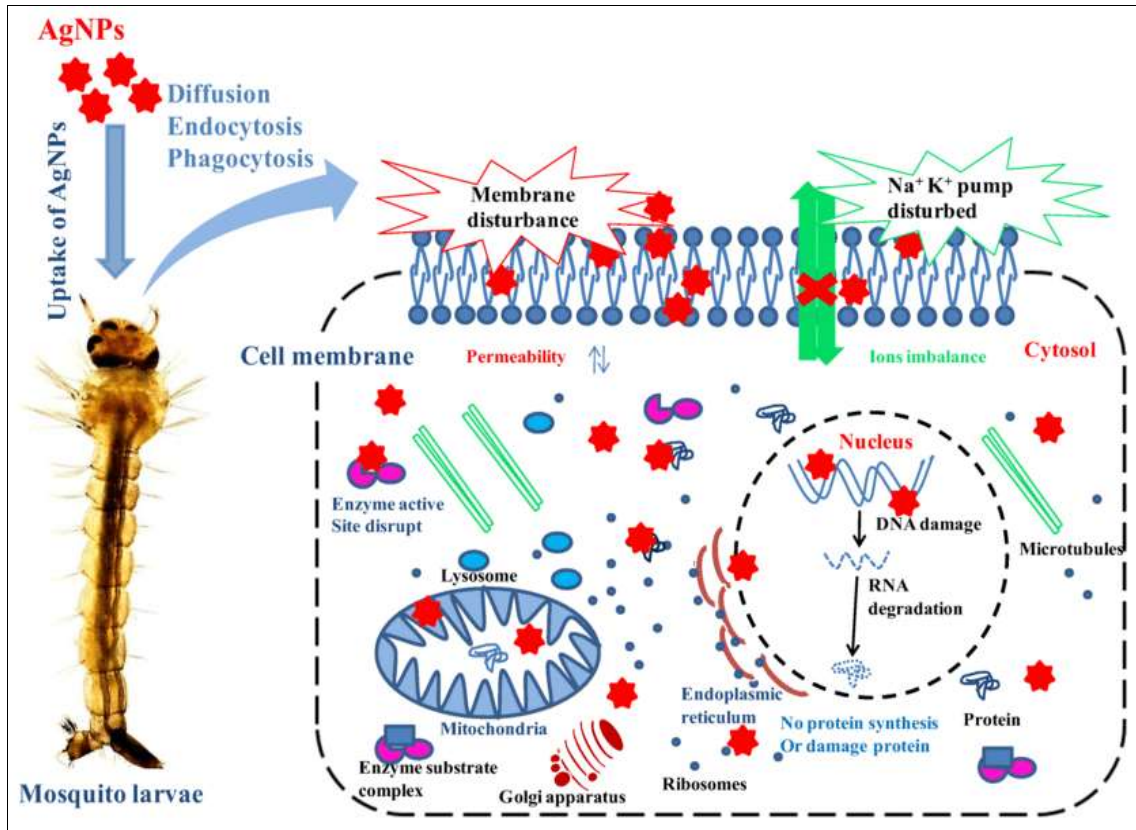


Fig 3: Mode of action of silver nanoparticles in mosquito larvae (Kumar *et al.*, 2020)^[12]

Another process includes nanoparticles entering the insect body and causing oxidative stress, protein damage, and disruption of the insect's normal physiological functioning. Whenever nanoparticles are picked up by cells, damage to DNA and oxidative stress are detected in in-vivo investigations. Nanoparticles have been proven in previous studies to have insecticidal action due to their ability to kill cells (Mir *et al.*, 2020)^[16]. Particular nanoparticles, such as TiO₂, may absorb UV rays from their surroundings. When electrons absorb energy, they become very active and form holes in the valence band. Oxygen radicals are formed when oxygen in the air reacts with these electrons. Water

undergoes an oxidation reaction with these generated holes, leading to the production of reactive oxygen species (ROS), such as H₂O₂ and O²⁻. These ROS cause cell death when they react with glycosides, unsaturated fatty acids, proteins, and other biological components (Li *et al.*, 2020)^[13]. Furthermore, metal ions in the substance might enter cells during the outward release phase and bind to amino acids in proteins such as sulphur and phosphorus, lowering cell membrane permeability. Nanoparticles may induce DNA damage and impede the function of intracellular enzymes once within cells (Wang *et al.*, 2017)^[25].

Table 1: Different types of nanoparticles and their action against various mosquito species (Nie *et al.*, 2023)^[19].

Sl no	Nanoparticles	Source	Target's species	Lethal indices (LC ₅₀)
1	Silver	<i>B. marisfavi</i>	<i>A. aegypti</i>	13.96 ppm
			<i>C. quinquefasciatus</i>	24.54 ppm
			<i>A. stephensi</i>	29.14 ppm
2	Silver	<i>I. batatas</i>	<i>A. aegypti</i>	17.578 µg/mL
			<i>C. quinquefasciatus</i>	10.069 µg/mL
			<i>A. stephensi</i>	12.568 µg/mL
3	Silver	<i>C. roxburghii</i>	<i>A. Aegypti</i>	26.35 µg/mL
			<i>C. quinquefasciatus</i>	28.67 µg/mL
			<i>A. stephensi</i>	31.27 µg/mL
4	Gold	<i>P. sulcata</i>	<i>A. aegypti</i>	70.16 ppm
5	Zinc	<i>Cucurbita</i>	<i>C. tritaeniorhynchus</i>	39.007 ppm
6	Zinc	Chemical method	<i>C. quinquefasciatus</i>	291.0 mg/L
7	Zinc	<i>P. aeruginosa</i>	<i>C. pipiens</i>	75 ppm
8	Zinc	<i>Cucurbita</i>	<i>C. tritaeniorhynchus</i>	44.68 ppm
9	Magnesium	<i>P. chrysogenum</i>	<i>A. stephensi</i>	12.5–15.5 ppm
10	Magnesium	Chemical method	<i>C. quinquefasciatus</i>	83.4 mg/L
11	Copper	Chemical method	<i>C. quinquefasciatus</i>	100.8 mg/L
12	Copper	<i>T. procumbens</i>	<i>A. aegypti</i>	4.209 mg/L
13	Silicon	Chemical method	<i>C. quinquefasciatus</i>	27.81 mg/L

Future perspectives and research challenges

The current study reveals a substantial gap in the number of publications on the toxicity of nanoparticles against agricultural, medicinal, and veterinary arthropods. The potential of environmentally friendly nanoparticle production technologies holds considerable promise and has sparked broad interest among research organisations throughout the world, potentially opening up new pathways for arthropod pest and vector control (Amarasinghe *et al.*, 2020) ^[1]. Nonetheless, despite the meticulous efforts of certain researchers to clarify the processes by which silica, alumina, silver, gold, titania, and graphene nanoparticles cause toxicity in arthropods, our knowledge in this study domain remains restricted. Furthermore, the processes underlying the observed toxicity of copper, iron, and chitosan nanoparticles, which are important nanomaterial classes used in entomological and parasitological research, need to be investigated further (Gambardella and Pinsino, 2022) ^[7].

Furthermore, the impact of nanoparticle size, shape, and charge on the many possible modes of action remains unknown. To avoid the insecticidal and acaricidal effects of botanicals and microbial compounds employed as reducing and capping agents, green manufacturing procedures using particular chemicals such as -caryophyllene, stearic acid, and zein are desirable. This method avoids errors in findings caused by the variety of the green reducing agents examined (Patil and Chandrasekaran, 2020) ^[22]. Finally, more work is needed to evaluate the suggested nanopesticides in real-world field circumstances, while additionally tracking their stability, fate in the environment, and sublethal impacts on non-target species. This involves a particular emphasis on genotoxicity as well as subtle physiological and behavioural changes. A comprehensive understanding of the various pathways leading to chronic nanoparticle toxicity in vertebrates, with a primary emphasis on human health, is of utmost importance and urgently needed.

Conclusion

In conclusion, the study of vector-borne diseases (VBDs) has evolved significantly over time, expanding beyond the narrow focus on their direct impact on human health. We now recognize the intricate interplay between VBDs, environmental conditions, disease burdens, vector ecology and societal responses. This complexity has shed light on the profound historical influence of VBDs on human societies. One promising avenue of research in the fight against VBDs is the utilization of nanoparticles as innovative pesticides. While numerous studies have explored their effectiveness against arthropod pests and vectors, our understanding of the underlying mechanisms remains somewhat limited. Notable exceptions include nanoparticles like silica, alumina, silver, and graphene oxide, which have demonstrated their potential in insect control. Metal nanoparticles, in particular, have shown the ability to interact with crucial elements in proteins and nucleic acids, disrupting cellular processes and ultimately leading to cell death. The regulation of key insect genes by silver nanoparticles presents a fascinating aspect of their mode of action, resulting in developmental abnormalities and reproductive failures in targeted pests. As we look ahead, it is clear that the application of nanoparticles as pesticides holds great promise for managing vector-borne diseases and agricultural pest control. However, the real-

world implications of these applications present significant challenges in terms of ecotoxicological consequences. It is essential to conduct further research and assessment to better understand the broader environmental impacts, ensuring that the benefits of this innovative approach do not come at the cost of unintended harm to ecosystems. In the quest to combat VBDs and enhance agricultural sustainability, the integration of nanotechnology must be approached with caution and care, considering its potential risks alongside its benefits. Only through a comprehensive understanding of the ecological ramifications can we make informed decisions and develop responsible practices to harness the potential of nanoparticles in our ongoing battle against vector-borne diseases and arthropod pests.

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