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Review Article

Green Nano-Biotechnology: A New Sustainable Paradigm to Control Dengue Infection

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Dengue is a growing mosquito-borne viral disease prevalent in 128 countries, while 3.9 billion people are at high risk of acquiring the infection. With no specific treatment available, the only way to mitigate the risk of dengue infection is through controlling of vector, i.e., *Aedes aegypti*. Nanotechnology-based prevention strategies like biopesticides with nanoformulation are now getting popular for preventing dengue fever. Metal nanoparticles (NPs) synthesized by an eco-friendly process, through extracts of medicinal plants have indicated potential anti-dengue applications. Green synthesis of metal NPs is simple, cost-effective, and devoid of hazardous wastes. The recent progress in the phyto-synthesized multifunctional metal NPs for anti-dengue applications has encouraged us to review the available literature and mechanistic aspects of the dengue control using green-synthesized NPs. Furthermore, the molecular bases of the viral inhibition through NPs and the nontarget impacts or hazards with reference to the environmental integrity are discussed in depth. Till date, major focus has been on green synthesis of silver and gold NPs, which need further extension to other innovative composite nanomaterials. Further detailed mechanistic studies are required to critically evaluate the mechanistic insights during the synthesis of the biogenic NPs. Likewise, detailed analysis of the toxicological aspects of NPs and their long-term impact in the environment should be critically assessed.

1. Introduction

In the age of emerging and reemerging pathogens, resistant bugs, deadly cancers, and neglected tropical diseases like dengue necessitate the need of holistic approaches to foster health and well-being [1–4]. In this regard, the mosquito-borne diseases got immense significance as mosquitoes serve as a vector for various deadly infections like yellow fever, malaria, filariasis, dengue, etc. [5]. Among the mosquito-borne viral diseases, dengue fever has attracted attention of researchers, epidemiologists, health, and social workers [6], because of their life threatening nature, massive disease burden, climatic conditions, vector expansion, urbanization, and other socio-demographic factors [7]. The dengue virus is transmitted by the *Aedes aegypti*, and *Aedes albopictus* has put billions of people at risk of the dengue infection, especially threatening the tropical and subtropical regions [8, 9]. The annual reported cases of the infection are estimated to be between 50 to 100 million. It is further estimated that the actual number of the dengue incidence are around 390 million with 96 million of symptomatic cases and 25,000 estimated annual mortalities" [10]. Dengue has now an endemic status in 128 countries. The situation is further aggravated by the resistant strains of dengue which are proposed to be the primary cause of the transmission on a large scale. The origination of resistant strains of dengue virus is the main cause of dissemination of dengue infections and its influence on human health. Dengue virus has four different serotypes, referred as DENV 1–4, that have substantial genotypic variations within each serotype. Recently, the fifth serotype of the dengue virus (DENV-5) was also identified [11]. Infection caused by all serotypes reveals similar symptoms [12]. Lifelong immunity is achieved upon recovery of the patient from one particular serotype, while the recovered patient is not protected from a secondary infection from other serotypes. The secondary infection may lead to more severe cases like dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) [13]. DSS and DHF results through the antibody mediated disease enhancement (ADM), resulting in either from the previous infection or induced by the vaccine [14]. Dengue infection has no specific treatment, while the only option is supportive care and symptomatic treatment. Therefore, an early diagnosis and vector management is a key to controlling dengue fever.

As of now, despite tremendous research for antiviral drugs or moieties, there has been no significant development to combat the DENV, and usually, symptomatic treatment is provided to the affected patients. At present, the WHO recommends only one dengue vaccine for all serotypes in children >9 years [15, 16]. The vaccine is only implemented in countries with greater than 70% sero-prevalence of the dengue virus; however, the vaccinations are only recommended for dengue sero-positive cases [17]. Extensive research is required to develop synthesize chemical entities that enable to inhibit the virus. E-gene, NS-1 gene, and NS-3 genes are considered as potential pharmaceutical targets for drugs. Previous studies revealed that bromocriptine exhibit antiviral potentials by inhibiting its replication. Other drugs like balapiravir, chloroquine, prednisolone, and celgosivir have not revealed any significant results during trials. Clinical trials with other drugs like ribavirin, ketotifen, and ivermectin are currently underway. Other researchers have been tirelessly working to search anti-dengue phytochemicals that can be useful in the control of dengue. The prevalence of dengue fever has prompted scientists to look for novel therapies, antiviral drugs, and nanotechnology based innovations. This study aims to update researchers' knowledge about the use of natural products-mediated synthesis of biogenic NPs and their possible role in the management of dengue infection and anti-dengue mechanisms of biogenic NPs.

2. Mitigating the Dengue Infection

Dengue virus represents Flaviviridae having a spherical shape and size of ~50 nm [18]. Dengue virus comprises ten proteins, in which 3 are structural proteins and 7 nonstructural proteins (NS). These nonstructural proteins play an important part in immune evasion, replication, and assembly of the virus. Nonstructural proteins like NS-1, NS-3, and NS-5 are absolutely vital for formation of viral particles and, therefore, also present an opportunity to design effective antiviral drugs. Dengue prevalence is a pressing problem for the developing world that signifies a dire need of innovative approaches for curing the disease or limiting their prevalence. There is a need for novel anti-dengue agents apart from transcription or protease activity that works on viral stages. Entry inhibitors alongside fusion are viable options that limit dengue entry into the target cell, repressing its replication and rendering the virus ineffective [19, 20].

Currently available vector control strategies are grouped into including physical control via GIS mapping for locating dengue foci, effective surveillance, determination of oviposition sites, and community-driven control programs. Next strategy is through biological control including paratransgenesis, vectors genetic modifications, sterile insects techniques, and use of crustacean and larvivorous fish, whereas chemical control strategies include the use of insecticides, plant derived compounds, use of insects growth regulators, and the "attract and kill" approach using pheromones. Others include immunotherapy strategies via the use of vaccines. Such approaches encompass biological, chemical, and environmental methods to curtail breeding and proliferation of the vector for dengue virus, i.e., *Aedes aegypti.* Due to the lack of awareness, poor sanitation hygiene, and other socio-economic motives, vector control becomes more challenging in developing countries [21, 22]. Effective and efficient vector control strategies through chemical or biological products are used worldwide [23]. However, chemicals such as synthetic lead have powerful impacts on public health that bring about resistance in different species of mosquitoes [24, 25]. Eco-friendly ways to control mosquito vectors with ultra-efficiency are needed. The mosquito is generally targeted by organophosphates and other growth regulators. Indoor spraying and bed nets are used to decrease the transmission. Phytochemicals with strong mosquitocidal and insecticidal potential are considered an alternative to synthetic insecticides in vector control programs. These plant-derived bioactive entities are characterized by their larvicidal, pupicidal, and adulticidal properties. Furthermore, both naturally occurring and synthetic chemicals are revealed to alter the oviposition behavior in mosquitoes or possess the ovicidal properties or may act as mosquito repellant [19, 26–29].

Scientists have also proposed certain genetic strategies to prevent the transmission of DENV to human beings. This is done by the introduction of the genes responsible for the disease resistance in the vector. Among them, one of the endosymbiotic bacteria (*Wolbachia*) is frequently used to spread disease resistant genes into mosquitoes. A transfected line of the *Aedes aegypti* with *Wolbachia* revealed suppression of the DENV by increasing the basal immunity in the insect that led to the reduced transmission. These *Wolbachia* transfected *A. aegypti* female mosquitoes possess an additional reproduction advantage over the uninfected ones [30]. Other researchers have tried to use the life span shortening strain of *Wolbachia,* to reduce the lifespan of the mosquito, which can decrease the burden of the vector borne diseases spread by *A. aegypti* [31]. Such genetic strategies are, however, primitive and mostly successful at the lab scale, while their implementation on ground would require a deeper understanding of the underlying mechanisms and further research.

3. Nano-Biotechnology, an Emerging Interface

The successful apprehension and manipulation of nanomaterials using the environmentally benign resources like plant extracts or their derived chemical entities have paved a way for using nanotechnology in an economical, sustainable, and compatible way [32–34]. The process is characterized by treating plant extracts with metal salts in different combinations that lead to the reduction of metal salt and subsequent capping and stabilization of NPs [35, 36]. The convergence of nanotechnology and biotechnology has revealed exciting results for different health-hygiene, nanomedicinal, environmental, and industrial applications [37–39]. These applications have paved a way for the crystallization of nano-biotechnology or nanobiology. Metal NPs like silver, gold, zinc, etc., are known to possess multifunctional properties owing to their unique surface area to a volume ratio. These NPs can be assembled by a variety of physical, chemical, or biological processes [40, 41]. The physical means are often characterized by high energy inputs making the overall process expensive while chemical means can generate hazardous wastes [42].

Recently, medicinal plants have been reported to exhibit efficacy in various diseases including cancer, infectious diseases, diabetes, and neurological disorders [43–50]. They inhibit the dengue virus by blocking the replication of virus particles through interacting with the genome, or by blocking their entry. The anti-dengue effect is manifested through destabilization of NS proteins. Natural products obtained from plants are reported to stop the viral replication either by interfering with the enzymes like inhibiting polymerases, interacting with glycoproteins, or inhibiting the replication by interfering with the RNA synthesis pathway. Despite the advances in screening potential inhibitors, no such therapies have been approved due to the heterotypic dengue infections [51–55].

A significant volume of research is now focused on the biological methods that include extracts from the medicinal plants as an eco-friendly, simple, and economical process for assembling nanomaterials or composite nanomaterials [56–61]. Other biological forms like microorganisms can also be utilized for the synthesis of metal NPs [33] but possess additional requirements like culture maintenance and sterile conditions. On the contrary, plants do not possess any expensive requirements, and are easy to handle. Phytochemicals can reduce and stabilize NPs [62]. Apart from the industrial applications, these biogenic NPs have revealed excellent biomedical potential [63, 64]. Converging experimental evidence suggests that the biogenic NPs can be used against the dengue virus and controlling their vectors [19]. The phyto-fabricated NPs present an excellent opportunity to control the dengue virus. A detailed review of the literature is presented in Table 1, summarizing the plant used, type of the metal NPs, their size, and application in vector control.

4. Anti-Dengue Properties of Biogenic Nanoparticles; Molecular Aspects

Few studies have documented the anti-dengue effect of the phytogenic silver NPs against DENV-2. The likelihood utilizing green-synthesized NPs in the fight against dengue (serotype DEN-2) has been acknowledged lately. One of the research articles encompasses the biosynthesis of silver NPs from *Bruguiera cylindrica* (L.) Blume and evaluated their effects on the dengue virus as well as their toxicity was evaluated against the vector [65]. Interestingly, the silver NPs treatment revealed decreased expression of dengue viral E-gene that codes for structural envelope (*E*) protein. These results were confirmed through the western blot and RT-PCR. The viral E-gene was found to be down-regulated in a dose dependent manner leading to significant reduction in envelope proteins as compared to the control. Significant downregulation at $30\,\mu g \cdot mL^{-1}$ was observed. The synthesized silver NPs were found to be toxic to the *A. aegypti* larvae and pupae. Similar results are concluded for the *Moringa oleifera* synthesized silver NPs for anti-dengue applications [19]. Sonneratia alba Sm. derived silver NPs tested in the concentration range of $5\,\mu g/mL$ to $15\,\mu g \cdot mL^{-1}$ also revealed significant reduction in the Viral E-protein, indicating a potential anti-dengue effect [66]. The aforementioned findings put forth the hypothesis that the reduction in the formation of *E* protein may occur due to silver NPs inhibiting the *E* gene and reducing the number of units that are ineffective [65]. Subsequently, *Centroceras clavulatum* (C.Agardh) Montagne synthesized silver nanoparticles (AgNPs) that were tested at 50 mg/ml showed no toxicity which is relevant against Vero cells, while the inhibition of growth of DEN-2 viral occurred for more than 80 percent [67]. The importance of screening different biosynthetic methods has been felt by these studies that can explore ways for the production of novel and safer nano drugs producing NPs having different features. Available studies show the important role of screening different plants which act as a source of reducing molecules of nanosynthesis because different paths frequently guide us to manifold various aspects of NPs and characteristics of biological toxicity [66] (Figure 1).

Conclusively, these studies show strong and tangible potential of screening substantial species of plants for biosynthesis of NPs with anti-dengue applications. The scarce literature further necessitates conducting assemble NPs other than silver, using medicinal plants for investigating their anti-dengue properties.

4.1. Phyto-Nano-Interface for Vector Control. The use of synthetic insecticides for potential vector control is undesired because of environmental hazards and the elimination of the nontarget organisms [68, 69]. Besides, environmental issues, health concerns, and emerging insect resistance to

Table 1: Plant based nanoparticles used against the dengue vector.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Characterization			MIC	References
					Size	Shape	Technique used		
1.	*Leucas aspera* (willd.) link	Larvae	Not reported	AgNPs	25–80 nm	Clustered and irregular shapes, and mostly aggregated	UV-vis, XRD, FTIR, SEM	0.01–5 $mg{\cdot}L^{-1}$	[86]
2.	*Feronia elephantum* L.	Larvae	Not reported	AgNPs	20 to 60 nm	Triangular, pentagonal, and hexagonal structures	UV-vis, FTIR, SEM, EDX,TEM	37.534 $\mu g{\cdot}mL^{-1}$	[87]
3.	*Annona muricata*	Larvae	Not reported	AgNPs	20 to 53 nm	Spherical	UV-vis, FTIR, XRD, SEM, EDX, TEM	—	[88]
4.	*Phyllanthus niruri* L.	Larvae	Larvae is perforated through the breathing tube, eradicating them by contamination and suffocation	AgNPs	30–60 nm	Spherical, mostly aggregated	UV-vis, SEM, EDS, FTIR, XRD, EDX	—	[89]
5.	*Holarrhena antidysenterica*	Larvae	Not reported	AgNPs	20 to 80 nm	Dispersed, crystalline, and mostly spherical	UV-vis, XRD, SEM, TEM, FTIR	—	[90]
6.	*Coleus aromaticus* Lour.	Larvae	Not reported	AgNPs	262.7 to 553.9 nm	Spherical and aggregate	UV-vis, EDX, FTIR, XRD, SEM	—	[91]
7.	*Artemisia vulgaris*	Larvae	Interfere with molting and other physiological processes	AgNPs	30–70 nm	Polydispersed, irregularly shaped	UV-vis, FTIR, XRD, EDX, SEM	—	[92]
8.	*Gracilaria firma*	Larvae	Not reported	AgNPs	12–200 nm	Spherical	UV-vis, FTIR, XRD, EDX, TEM	—	[93]
9.	*Myristica fragrans*	Larvae	Not reported	ZnO NPs	100 to 200 nm	Rod-like	UV-vis, DLS, FTIR, Zeta Potential, XRD.EDX, SEM, TEM	—	[94]
10.	*Beauveria bassiana*	Larvae	Not reported	AgNPs	36.88 to 60.93 nm	Spherical	UV-vis, SEM, EDX	—	[95]
11.	*Aganosma cymose*	Larvae	Not reported	AgNPs	1 to 16.5 nm	Polydispersed, spherical	UV-vis, FTIR, XRD, AFM, SEM, TEM, XRD, AFM	—	[96]
12.	*Cocos nucifera*	Larvae	Inhibition of major detoxifying proteins glutathione-S-transferase and cytochromeP450	AgNPs	5–65 nm	Spherical, pseudo spherical and rectangle	UV-vis, TEM, XRD	—	[97]
13.	*Carissa carandas*	Larvae	Route through the exoskeleton of insect into cells of individual and intervention with sloughing	AgNPs	1.6 to 7.4 nm	Spherical poly-dispersed	UV-vis, FTIR, XRD, AFM, SEM, TEM	—	[98]
14.	*Zeuxine gracilis*	Larvae	Not reported	AgNPs	20–40 nm	Orbicular, cubic	UV-vis, EDX, FTIR, XRD, DLS, SEM,TEM	—	[99]

TABLE 1: Continued.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Characterization Size	Characterization Shape	Characterization Technique used	MIC	References
15.	*Halodule uninervis*	Deformed adults	Inhibit neurosecretory cells, shrink internal cuticle, and/or can act directly on epidermal cells causing cuticular oxidation	AgNPs	25–40 nm	Spherical or with cubic	UV-vis, FTIR, SEM, EDX, XRD, Raman analysis	—	[100]
16.	*Chomelia asiatica*	Larvae	Not reported	AgNPs	15–31 nm	Triangular, pentagonal, and hexagonal	UV-vis, FTIR, SEM, EDX	—	[21]
17.	*Parthenium hysterophorus*	Larvae	Not reported	TiO_2 NPs	20–50 nm	Spherical	UV-vis, FTIR,SEM, EDX, XRD	—	[101]
18.	*Sida acuta*	Larvae	Not reported	AgNPs	20 to 60 nm	Spherical, triangular, pentagona l, and hexagonal	UV-vis, FTIR, SEM.TEM, EDX	—	[102]
19.	*Arachis hypogaea*	Anal papillae region and cuticle layer.	Reduce membrane permeability, deactivate enzymes in midgut, liberate peroxides leading to cell death	AgNPs	20 to 50 nm	Spherical and polyhedral	FTIR, XRD, TEM, SEM, EDX	—	[103]
20.	*Azadirachta indica*	Larvae and pupae	Penetration through the membrane	AgNP	30 to 50 nm	Spherical	UV-vis, FTIR, SEM, EDX, XRD	3.969 (larva I) to 8.308 ppm (pupa)	[104]
21.	*Heliotropium indicum*	Larvae	Not reported	AgNP	18 to 45 nm	Spherical, triangle, truncated triangles, and decahedral	UV-vis, FTIR, TEM, SEM, EDX, XRD	35.97 μg/mL	[105]
22.	*Feronia elephantum*	Larvae III	Bind to sulfur-containing proteins or phosphorus-containing compounds like DNA, causes denaturation of some enzymes and organelles	AgNP	20 to 60 nm	Triangular, pentagonal, and hexagonal	UV-vis, FTIR, SEM, EDX, XRD	23.12 $\mu g\ mL^{-1}$	[106]
23.	*Carmona retusa*	Larvae	Not reported	AgNP	20 to 40 nm	Cubic	UV-vis, XRD, FTIR, TEM, SAED	198.766 ppm	[107]
24.	*Plumeria rubra*	Larvae II, IV	Not reported	AgNP	32–200 nm	Spherical	UV-vis, TEM, PSA and zeta potential	500 ppm	[108]
25.	*Catharanthus roseus*	Larvae	Altered physiological processes	AgNP	35 to 55 nm	Spherical	UV-vis, H1NMR, FTIR, and mass spectroscopy	40 ppm	[109]
26.	*Anisomeles indica*	Larvae III	Nor reported	AgNP	18 and 35 nm	Spherical	UV-vis, FTIR, SEM, EDX	35.21 mg/mL	[110]
27.	*Ulva lactuca*	Larvae IV	Gastric caeca, muscles, nerve cord ganglia appeared damaged and disorganized, spoiled epithelium	ZnO NPs	10–50 nm	Sponge-like asymmetrical	XRD, UV-vis, FTIR, SAED, TEM	50 μg/ml	[111]

Table 1: Continued.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Characterization Size	Shape	Technique used	MIC	References
28.	*Sargassum muticum*	Larvae and pupae	Binds to sulfur from proteins or to phosphorus from DNA, causes swift denaturation of organelles and enzymes	AgNP	43–79 nm	Spherical	FTIR, SEM, EDX, and XRD analyses	10 ppm	[112]
29.	*Cymbopogon citratus*	Larvae and pupae	Interfere with molting and other physiological processes	AuNPs	20–50 nm	Orbicular, trigonal, hexagonal, and rod-like	UV-vis, FTIR, TEM, EDX, XRD	41.5 ppm	[113]
30.	*Pedilanthus tithymaloides*	Larvae and pupae	Denature ribosome, suppress the expression of enzymes and proteins crucial to ATP production causing disruption of the cell	AgNPs	15–30 nm	Spherical	UV-vis, FTIR, XRD, EDX, AFM		[114]
31.	*Pongamia pinnata*	Larvae	Not reported	AgNPs	10 to 80 nm	Spherical	UV-vis, XRD, FTIR, TEM	0.25–1 ppm	[115]
32.	*Delphinium denudatum*	Larvae II	DNA loses its replication ability and cellular proteins become inactivated on	AgNPs	85 nm	Spherical	UV-vis, XRD, SEM, FTIR	9.6 ppm	[116]
33.	*Bauhinia variegata*	Larvae III	Penetration through membrane to midgut epithelial membrane, the enzymes gets inactivated, and produce peroxide causing cell death	AgNPs	38 to 65 nm	Spherical, triangle, truncated triangles, and decahedral	UV-vis, XRD, SEM, FTIR,TEM, EDX	89.42 μg/m L	[117]
34.	*Zornia diphylla*	Larvae III	Not reported	AgNPs	28 to 61 nm	Spheres, triangle, truncated triangles, and decahedral	UV-vis, XRD, SEM, FTIR,TEM, EDX	13.42 μg/ml	[118]
35.	*Melia azedarach*	Larvae	Not reported	AgNPs	3 to 31 nm	Spherical	UV-vis, XRD,TEM,	23.82 ppm	[119]
36.	*Suaeda maritima*	Larvae I and pupae	Inhibit neurosecretory cells, causing shrinkage of internal cuticle, and/or can act directly on Epidermal cells responsible for the production of enzymes leading tanning and/or cuticular oxidation process	AgNPs	20 to 60 nm	Spherical	UV-vis, XRD, SEM, FTIR, EDX	8.668 to 17.975 ppm	[120]
37.	*Hedychium coronarium*	Larvae and pupae	Damaged midgut epithelium	AgNPs	9.54 nm to 49.0 nm	Spherical and oval	UV-vis, XRD, FTIR,TEM, EDX	24.2 ppm(I), 39.7 ppm(II), 52.7 ppm(III) 72.6 ppm(IV), 348.6 ppm	
38.	*Achyranthes aspera*	Larvae IV	Not reported	AgNPs	7 to 14 nm	Cuboidal and spherical	UV-vis, SEM, TEM, FTIR and XRD	8.92 mg/ml	[121]

Table 1: Continued.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Size	Characterization Shape	Technique used	MIC	References
39.	*Azadirachta indica*	Larvae III	Interfere with moulting and other physiological processes	AgNPs	41–60 nm	Spherical	UV-vis, XRD, SEM, FTIR,EDX	0.04 mg/l	[122]
40.	*Morinda citrifolia*	Larvae	Not reported	TiO_2NPS	20.46–39.20 nm	Spherical, oval and triangle	UV- vis, XRD, SEM, FTIR,EDX	31.685 mg/L	[123]
41.	*Clausena dentata*	larvae	Denaturation of the sulfur-containing proteins or phosphorous- containing compound like DNA	SeNPs	46.32 nm to 78.88 nm	spherical	UV-vis, XRD, SEM, FTIR,EDX	104.13 mg/L	[124]
42.	*Hyptis suaveolens*	Larvae	Not reported	AgNPs	5–25 nm	Spherical, hexagonal, triangular and polyhedral	UV-vis, XRD, SEM, FTIR,TEM	10 mg/L	[125]
43.	*Chloroxylon swietenia*	Larvae	Not reported	AuNPs	18–37 nm	Spherical	UV-vis, XRD, FTIR, TEM, EDX, Zeta potential analyses	0.340 ppm	[126]
44.	*Ambrosia arborescens*	Larvae III	Bind macromolecules such as proteins and DNA, altering their structure	AgNPs	10–14 nm	Spherical	UV-vis,FTIR, TEM, SEM, EDX, AFM	0.43 ppm	[127]
45.	*Lobelia leschenaulti ana*	Larvae III	The disappearance of antenna and mouth brush, shrinkage in ventral area, loss of lateral hair, changes in structure of thorax, breakage of minutes of midgut, disappearance of anal gills, and brushes	ZnONps	15–46 nm	Spherical	UV-vis, XRD, FTIR, SEM, TEM	10 mg/L	[128]
46.	*Acacia caesia*	Larvae III, ova, adults	Midgut epithelial membrane damaged, enzymes were inactivated and generated peroxides leading to cell death	AgNPs	20 to 46 nm	Spherical	UV-vis, XRD, FTIR, EDX, SEM, TEM, AFM	11.32 μg/ml for larvae, 75 μg/m for ova, 20.94 μg/ml for adults	[129]
47.	*Melia azedarach*	Larvae III	Interfere with intracellular cell signaling, bounds with sulfur contain proteins	Pd NPs	10 to 20 nm	Spherical	UV-vis, FTIR, XRD, TEM		[111]
48.	*Azadirachta indica*	Larvae III and IV	Increase ROS and other radicals production causing apoptosis via phosphatidyl serine externalization, DNA, nuclear fragmentation, activation of meta- caspases, mitochondrial dysfunction	AgNPs	35–60 nm	Spherical	UV-vis,SEM, EDX, TEM, FTIR, XRD, DLS	10.92 mg/L (III) 11.88 mg/L(IV)	[130]
49.	*Artocarpus heterophyllu s*	Larvae	Not reported	CuNPs	132 nm	Asymmetrical dispersed	UV-vis, XRD, FTIR,SEM	3.85, 4.24, 4.66 and 5.08 mg/ml	[131]

Table 1: Continued.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Characterization Size	Shape	Technique used	MIC	References
50.	*Morinda tinctoria*	Larvae III	Denature sulfur- containing proteins or phosphorous containing compound like DNA, causing in denaturation of organelles and enzymes	AgNPs	60–95 nm	Spherical	UV-vis, AFM, FTIR	3.631 ppm	[132]
51.	*Euphorbia milii*	Larvae II, IV	Not reported	AgNPs	208 nm	Spherical	UV-vis, SEM, EDX, XRD,FTIR, particle size, and zeta potential analysis	281.28 ± 23.30 and 178.97 ± 37.82 ppm	[133]
52.	*Mukia maderaspatana*	Larvae	Denature sulfur- containing proteins or phosphorous containing compound like DNA	AgNPs	13–34 nm	Spherical	UV-vis, XRD, FTIR, ART, SEM,	0.506; 1.082, 0.392; 0.870 ppm	[134]
53.	*Cassia fistula*	Larvae and pupae	Disturbed protein mechanism	AgNPs	148–938 nm	Spherical	FTIR, TEM, SEM, UV- vis, XRD	51.3, 47.1, 56.0, 78.0 and 519.3 mg/L	[135]
54.	*Chrysanthe mum* sp.	Larvae	Interference with the process of dissociation and other physiological processes	AgNPs	40–100 nm	Clustered and irregular shapes	UV-vis, FTIR, SEM	12.754 ppm	[136]
55.	*Carissa spinarum*	Larvae III	Not reported	AgNPs	40–100 nm	Cubic and spherical	FTIR, SEM, UV-vis, XRD, TEM	9.01 μg/ml	[137]
56.	*Nicandra physalodes*	Larvae III	Interfere with molting and other physiological processes	AgNPs	5–35 nm	Cubic and spherical	UV-vis, XRD, FTIR, SEM, TEM	13.61 μg/ml	[138]
57.	*Clerodendrum chinense*	Larvae III	Not reported	AgNPs	25–30 nm	Irregular, Spherical or with Cubic structures	UV-vis, SEM, TEM,EDX, FTIR	11.10 μg/ml	[139]
58.	*Calotropis gigantea*	Larvae and pupae	Not reported	AgNPs	20–35 nm	Clustered and irregular	UV-vis, SEM, EDX, FTIR	24.33 ppm, 34.01 ppm, 51.92 ppm, 63.38 ppm and 83.88 ppm	[140]
59.	*Tagetes* sp.	Larvae IV	Not reported	CdNps		Roughly spherical	UV-vis, SEM, FTIR and fluorescence	10 ppm	[141]
60.	*Cleistanthus collinus*	Larvae	Inhibit neurosecretory cells and gut enzyme of larvae, toxic effect on epidermal cells	AgNPs	66.27 to 75.09 nm	Triangular and pentagonal	UV-vis, FTIR, XRD, SEM, EDX	20 mg/l	[142]
61.	*Strychnos nux-vomica*	Larvae	Inhibitory influence on neurosecretory cells and gut enzyme of larvae, toxic efficacy on epidermal cells	AgNPs	54.45 to 60.84 nm	Irregular, spherical and round	UV-vis, FTIR, XRD, SEM, EDX	25 mg/l	[142]
62.	*Tridax procumbens*	Larvae	Not reported	CuONps	16 nm		XRD, FTIR, SEM, EDX, UV-vis, and fluorescence spectroscopy	4.209 mg/L	[143]

Table 1: Continued.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Characterization			MIC	References
					Size	Shape	Technique used		
63.	*Rhizophora mucronata*	Larvae III	Denaturation of the sulfur-containing proteins or phosphorous containing compound like DNA	AgNPs	60–95 nm	Spherical	UV-vis, XRD, FTIR, and AFM analysis	0.585 mg/L	[144]
64.	*Belosynapsis kewensis*	Larvae IV	Not reported	AgNPs	10 to 28 nm	Spherical	UV-vis, FTIR,TEM, and XRD	84.2 ppm	[145]
65.	*Cynodon dactylon*	Larvae	Bio uptake and toxicity	AgNPs	14 nm	Spherical	UV-vis, XRD, TEM	2.50, 2.78, 3.02, 3.05 μg/mL	[146]
66.	*Sida acuta*	Adults	Interfere with molting and other physiological processes.	AgNPs	5–35 nm	Spherical	UV-vis, SEM, TEM, FTIR, EDX	35.12 μg/mL	[147]
67.	*Mussaenda glabra*	Larvae	Not reported	AgNPs	15 to 25 nm	Spheres, Triangle, truncated Triangles and decahedral	UV-vis, XRD, FTIR, SEM, TEM	17–19 μg/mL	[147]
68.	*Psychotria nilgiriensis*	Ova, larvae, pupae, adults	Not reported	AgNPs	40–60 nm	Spherical and cubic	UV-vis, SEM, FTIR, EDX	20.26, 24.08, 29.37, 35.33 and 43.12 μg/ml	[148]
69.	*Berberis tinctoria*	Larvae and pupae	Interfere with molting and other physiological processes	AgNPs	65–70 nm	Spherical	UV-vis, XRD, SEM	4.97 ppm (I instar), 5.97 ppm (II), 7.60 ppm (III), 9.65 ppm (IV), and 14.87 ppm (pupa)	[149]
70.	*Derris trifoliata*	Larvae III and IV	Binding to DNA and enzymes and impairs cellular metabolism	AgNPs	18–50 nm	Spherical and cubic	UV-vis, FTIR, SEM, EDX, XRD, TEM	12.11 mg/l (III), 17.76 mg/l (IV)	[150]
71.	*Cassia roxburghii*	Larvae III	Not reported	Ag NPs	57 to 95 nm	Orbicular, trigonal, truncated triangles, and decahedral morphologies	UV-vis, FTIR, SEM, EDX, XRD.	31.27 and 48.81 μg/mL	[151]
72.	*Artemisia nilagirica*	Larvae and pupae	Damage midgut epithelial membrane, inactivate enzymes and generate peroxide leading to cell death	AgNPs	6.723 nm	Spherical to irregular	UV-vis, FTIR, SEM, XRD		[152]
73.	*Scadoxus multiflorus*	Larvae and ova	Affect the epithelial cell/midgut or cortex, lateral hair loss, deformation in gills as well as brushes	ZnO NPs	31 ± 2 nm	Irregular spherical	UV-vis, FTIR, SEM, EDX, XRD	34.04 ppm and 32.73 ppm	[153]
74.	*Pergularia daemia*	Larvae	Not reported	AgNPs	44 to 255 nm	Spherical	UV-vis, TEM, particle size and zeta potential analysis	9.90, 11.13, 12.40, 12.95 ppm	[154]
75.	*Ipomoea batatas*	Larvae	DNA structure deformation, and generation of excessive reactive oxygen species.	AgNPs	20–50 nm	Orbicular	UV-vis, FTIR, SEM, EDX, XRD	15.657 μg/mL	[155]

Table 1: Continued.

Sr. No	Plant used	Target Stage	Mechanism	Type of NPs	Characterization Size	Shape	Technique used	MIC	References
76.	*Annona squamosa*	Larvae	Not reported	AgNPs		Spherical and cluster shaped	UV-vis, XRD, FTIR, SEM	7.52, 8.34, 9.06, 9.15 μg/mL	[156]
77.	*Achyranthes aspera*	Larvae IV	Reduce ATP synthesis, ion exchange, reduce membrane permeability causing cell death	AgNPs	1–30 nm	Three dimensional cuboid	UV-vis, FTIR, SEM, TEM, EDX, XRD	26.693 μg/mL	[157]
78.	*Habenaria plantaginea*	Larvae	Not reported	AgNPs	0.1 to 29 nm	Polydispersed and spherical	UV-vis, AFM, FTIR, SEM, TEM, XRD	13.38 μg/ml	[158]
79.	*Rubus ellipticus*	Larvae	Decrease membrane permeability, disturb proton motive process, Cellular function is disrupted	AgNPs	<30 nm	Spherical	UV-vis, XRD, FTIR, SEM, TEM, EDX	13.83 μg/mL	[159]
80.	*Menyanthes trifoliata*	Adults	Detiriorated midgut	AgNPs	10 to 50 nm	Orbicular, Trigonal, pentagonal, hexagonal	UV-vis, XRD, FTIR, SEM, EDX	14.99 μg/mL	[160]
81.	*Manihot esculenta*	Larvae III	Not reported	AgNPs		Spherical and aggregates	UV-vis, XRD, FESEM, and HRTEM	4.53 mg/mL	[161]
82.	*Couroupita guianensis*	Larvae IV	Not reported	AgNPs	10–45 nm	Spherical	UV-vis, XRD, FTIR, TEM	2.1 ppm	[162]
83.	*Couroupita guianensis*	Larvae IV	Not reported	AgNPs	5–15 nm	Orbicular	UV-vis, XRD, FTIR, TEM	2.09 ppm	[162]
84.	*Trichoderma atroviride*	Larvae	Not reported	AgNPs	14.01–21.02 nm	Hexagonal (diamond shape)	UV-vis, confocal laser microscopy (CLSM),	1 ppm, 2 pp m, 3.12 ppm, 6.30 ppm	[163]
85.	*Hedyotis puberula*	Larvae and ova	Not reported	AgNPs	10–16 nm	Mostly spherical, a few nanorods, hexagonal and polygonal nanoprisms	UV-vis, FTIR, XRD, AFM, SEM, TEM, EDX and DLS analysis	18.05 μg/ml (larvae) 100 μg/ml (ova)	[164]
86.	*Carica papaya*	Larvae II and III	Inhibit AChE, GABA- gated chloride ion channel, disruptna, K ion exchange, cyt-P450, hormones, osmotic pressure and ionic balance. cause mitotic poisoning, inhibit cholinergic system, neuromuscular coordination	AgNPs	12 ± 6 nm	Spherical	FTIR, GCMS	1.46 (II) 1.76 ppm (III)	[73]
87.	*Syzygium cumini*	Larvae	Not reported	AgNPs	50 nm	Spherical, round, triangular, and Hexagonal	UV-vis, FTIR, EDX, XRD, SEM	16.45 μg/ml	[165]

NPs: nanoparticles; X-ray diffraction (XRD); Fourier transform infrared (FTIR); scanning electron microscope (SEM); energy dispersive X-ray analysis (EDX); UV-visible spectroscopy (UV-vis); field emission scanning electron microscope (FESEM); high resolution transmission electron microscopy (HRTEM); transmission electron microscopy (TEM); dynamic light scattering (DLS).

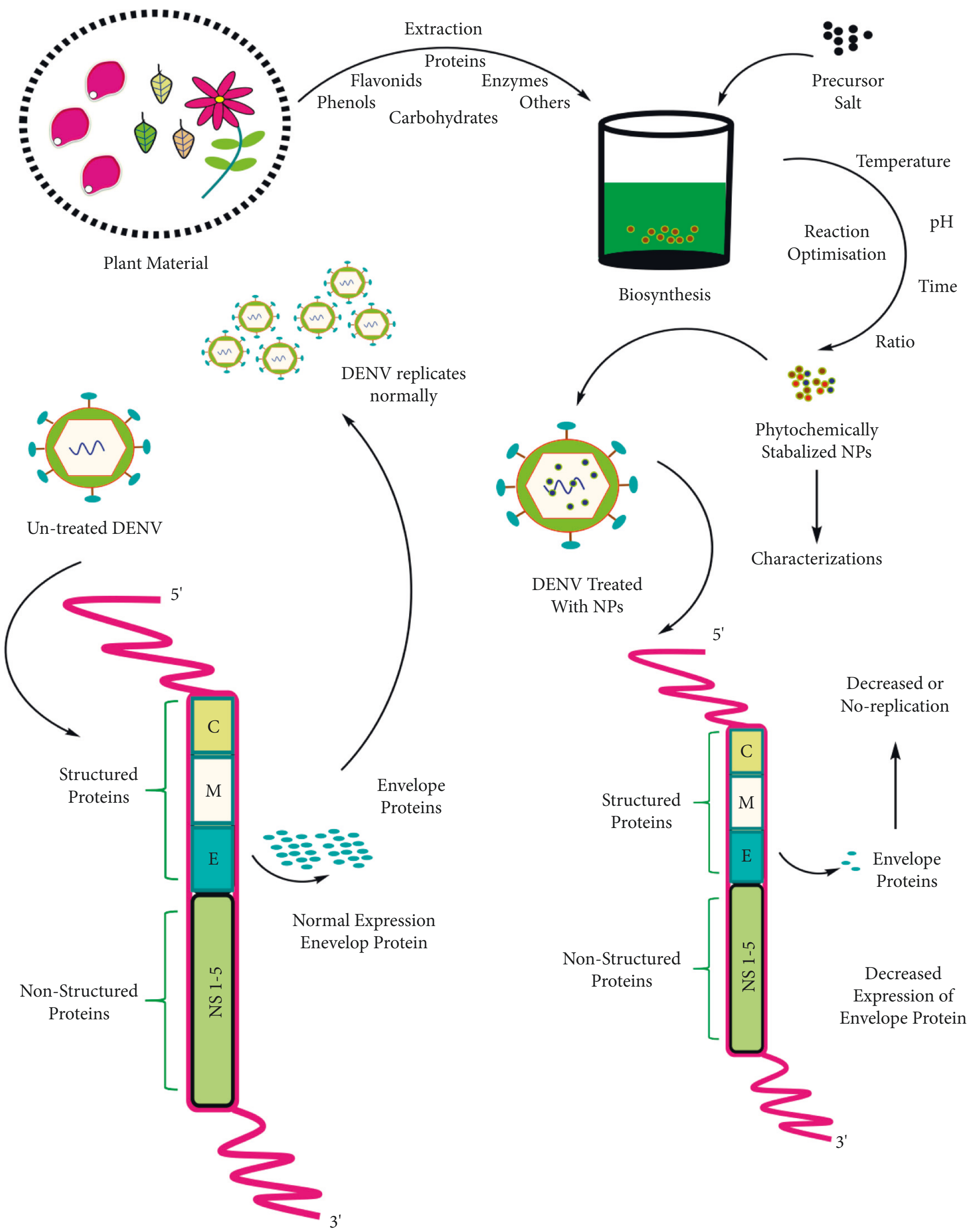

Figure 1: Molecular interaction of biogenic NPs with the DENV genome causing decreased expression of viral E-gene.

insecticides have led to the realization that these synthetic chemicals may not be reliable in the long-term [70]. Such pesticides are an instant danger to human health if used in a nonjudicious manner. According to estimates, the synthetic pesticides lead to around 3 million cases of poisoning and 222,000 deaths annually. Similarly, escaping of the pesticides residues and their accumulation in the food chain represents an unforeseen danger [71]. Thankfully, nanotechnology-based interventions have emerged has a promising and alternative source of insecticides due to their potent insecticidal nature, mobility, solubility, and stability [70]. The promising potential of green-synthesized NPs has paved a way for novel vector control strategies. Their toxicity against some arthropod pests and vectors, especially mosquitoes has been well documented. There is a significant volume of literature on the toxicity of biogenic NPs on mosquitoes; however, the information on the precise mechanistic aspects is scarce. The underlying mechanism is pivotal to investigate the toxicological consequences arising from the use of NPs as pesticides.

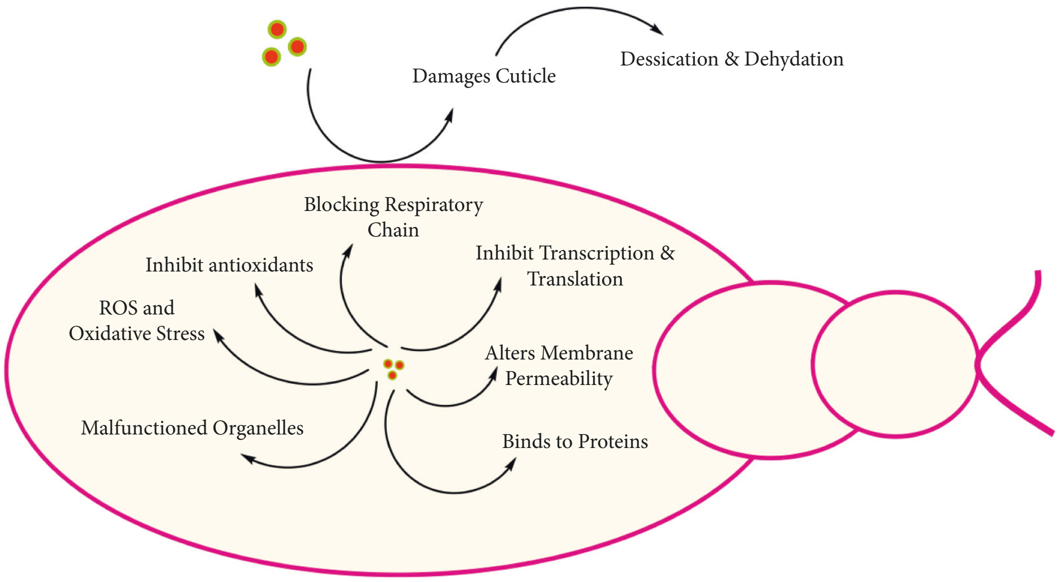

Figure 2: Mechanism of nanoparticles toxicity against insects.

The toxic effect of NPs may be linked to some stress stimuli caused by NPs (Figure 2). The exact mechanism is not understood completely but scientific findings have revealed that NPs may cause morphological alterations like loss of lateral hair and damaged gills and brushes [72]. This may affect the respiratory activity of larvae, since the larval stages rely solely on gills for breathing. At the cellular level, severe membrane degradation is observed, as NPs penetrate easily through the membrane. NPs may get accumulated in midgut causing shrinkage of abdomen and damaged epithelium or cortex. Blocking of the trypsin enzyme activity is also considered as one of the causes of NPs mediated insecticidal activity [73]. Activity of this digestive protease is linked with the signal transduction system as it regulates the expression of a second gene, i.e., the late trypsin gene. The presence of two trypsin allows the mosquito to assess the quality of the meal and adjust the levels of late trypsin for a particular meal with remarkable flexibility. Feeding activity is disturbed when trypsin activation is halted and the quality of meal cannot be assessed [74]. Another factor contributing to the toxicity of NPs is directly related to their small size due to which they can pass easily into the cuticle and act directly on epidermal cells and interfere with enzyme production necessary for tanning and cuticle oxidation, ultimately affecting the whole molting process. Alternatively, they may inhibit neurosecretory cells resulting in cuticular shrinkage. Some NPs are also associated with the disturbing of muscular layers causing loss of distinction in endocuticle and exocuticle leading to insect inactivity. NPs may bind to the cuticle, sorbing the cuticular lipids and waxes resulting in body wall desiccation, de-pigmentation, abrasion, spiracle blockage, and insect dehydration, to which the insect ultimately succumbs [72, 74]. This factor contributes to the utilization of NPs against the early instars and pupae and prevents their development to adult stage rendering them as a powerful larvicidal agent [75]. Authors have reported interruption of acetylcholinesterase activity by NPs. Acetylcholine is a compound involved with nerve impulse transmission from nerve to nerve cell or involuntary muscles, and this activity is regulated by acetylcholinesterase (AChE) [63, 76]. It is reported the NPs interfere with AChE resulting in disturbance of nerve impulse transmission across cholinergic synapses [77]. Therefore, this could be useful to assess the potential neurotoxic capacity of some NPs [74]. Hormonal imbalances are also reported in insects which are manifested by NPs. Further, NPs are reported to interfere with the cytochrome P450, involved in the molting of insects [73, 78]. A critical impact on reproduction and development is also reported [74], where Gonadotropin production is downregulated resulting in reduced fitness and reproductive failure. Reduced female fertility is observed as NPs disrupt the oogenesis process and ovaries become defective, having a negative effect on egg laying capabilities [72]. Moreover, NPs damage the organism by penetrating through the exoskeleton [79], enter in the intracellular space, and then the nanoscale material binds to sulfur from proteins or to phosphorus from DNA which leads to the rapid denaturation of organelles and enzymes. Due to the decrease in membrane permeability and disturbance in proton motive force, loss of cellular function, and cell death occur [80, 81]. At the cellular level, NPs can penetrate the cytosol and interrupt the cellular signaling pathways, causing disruption in ion exchange and neuromuscular coordination [73].

Even though several evidences exist on the toxicity of NPs, different experimental designs with diverse NPs sizes, coatings, concentrations, times of exposure, measured endpoints, and cell types make it difficult to compare results and determine the mode of action by which these particles inflict damage to organisms [82–84]. Generation of reactive oxygen species (ROS) and free radicals have been observed and implicated in the cause of oxidative stress, namely, in the form of antioxidant defense system activation/inhibition such as depletion of glutathione, lipid peroxidation and

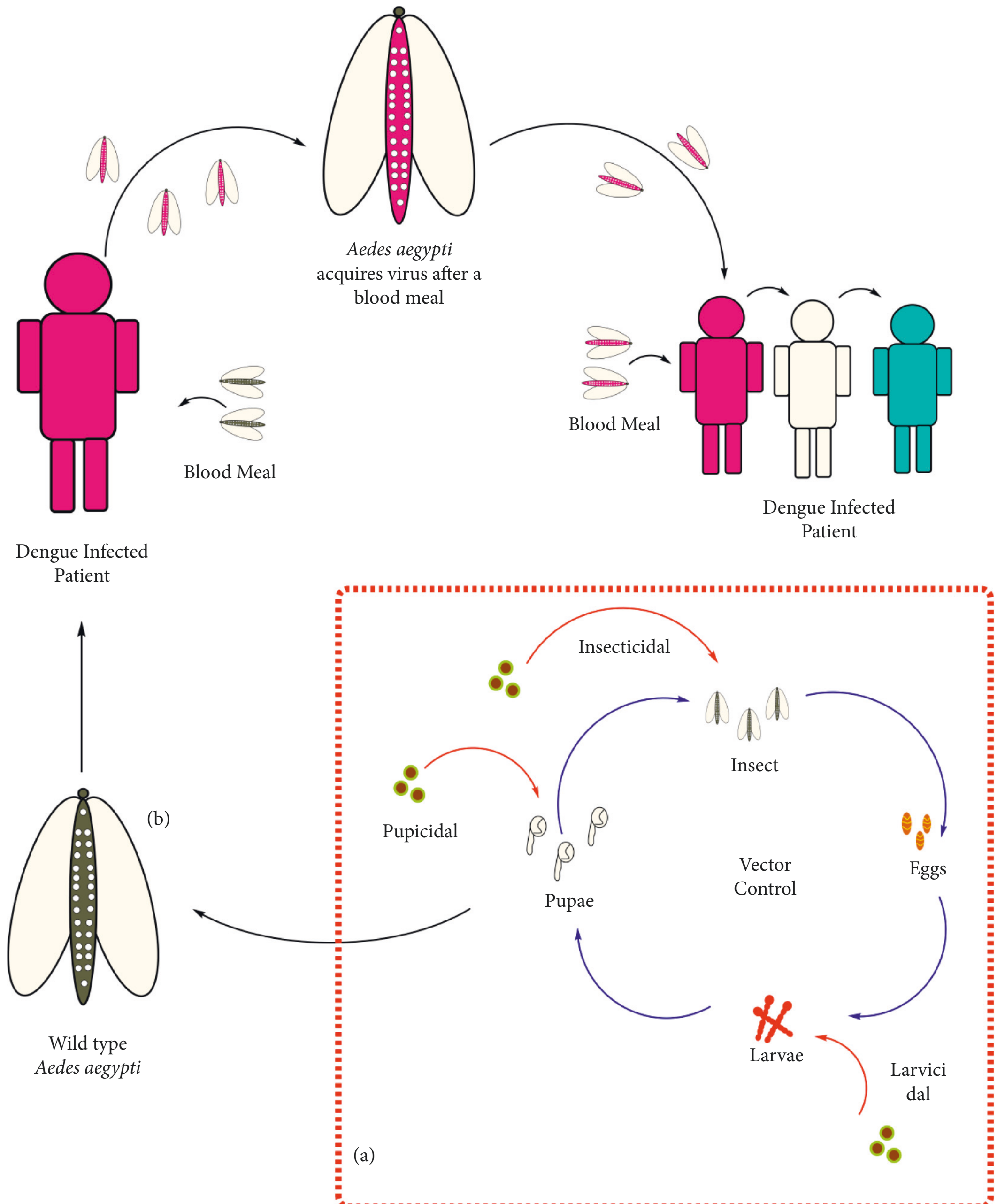

Figure 3: Vector control and dengue transmission.

DNA damage, decreased mitochondrial activity, inflammatory processes, and apoptosis in a wide variety of cell types [85] (Figure 3).

Converging evidence suggests an inverse correlation between the size of NP and their toxicity and penetration into the body of insects. Despite a number of pieces of evidences, there is a dire need to conduct extensive studies on the effects of the biogenic metal NPs on insects with reference to their physicochemical nature like size, shape, charge, etc. Moreover, the present body of literature only indicates silver and gold NPs for their anti-parasitic properties and applications in entomology. Research can be extended to other metal NPs of composite nonmaterial's biosynthesized from medicinal plants.

NPs: nanoparticles; X-ray diffraction (XRD); Fourier transform infrared (FTIR); scanning electron microscope (SEM); energy dispersive X-ray analysis (EDX); UV-visible spectroscopy (UV-vis); field emission scanning electron microscope (FESEM); high resolution transmission electron microscopy (HRTEM); transmission electron microscopy (TEM); dynamic light scattering (DLS).

5. Nanoparticles Enhances Predation Efficiency

Biological control of dengue vectors seems another probable solution. The prospective biological control of dengue vectors can be performed using natural predators like fish, young instar tadpoles, copepods, and water bugs. Fishes

Table 2: Effect of NPs on the efficiency of predators of dengue vector.

S.No	Predator	Plant used	Nanoparticles (NPs)	Salt used (as a precursor)	Efficiency		Reference
					Before	After	
1	*Mesocyclo ps aspericornis*	*Cymbopogon citratus*	AuNPs	$HAuCl_4$	56%	77.30%	[113]
2	*Megacyclo ps formosanus*	*Hedychium coronarium*	AgNPs	$AgNO_3$	7.22, 5.88, 1.28, and 0.28 larvae	8.11, 6.88, 1.95, 1.06 larva/day	[168]
3	*Poecilia sphenops*	*Psychotria nilgiriensis*	AgNPs	$AgNO_3$	65% (larva I), 49.62% (larva II)	92.25% (larva I), 76.50% (Larva II)	[148]
4	*Gambusia affinis*	*Mimusops elengi*	AgNPs	$AgNO_3$	81.7% (larvae III)	88.60%	[169]
5	*Poecilia reticulata*	*Sonneratia alba*	AgNPs	$AgNO_3$	6.5, 4.8, 3.8, 2.6 larvae/day	8.2, 6.4, 5.0, 3.9 larvae/day	[66]
6	*Oryzias melastigma*	*Chenopodium ambrosioides*	AgNPs	$AgNO_3$	65.5 (II) and 59.0% (III)	91.0 (II) and 85.5% (III)	[170]

were predominantly considered for biological control of mosquitoes. Places that have the possibility to breed mosquitoes such as dams, marshes, canals, ponds, etc., were inundated with numerous predatory fishes [148]. The cyclopoids are also reported to be among the efficient predators of the larvae of the mosquito involved in the spread of dengue [113]. Copepods represent another economical and cost-effective biological control of culicidae larvae in urban and semiurban areas [166, 167]. The most effective agents of copepods that control mosquitoes biologically are *Mesocyclops*, i.e., *Mesocyclops pericornis, Mesocyclops longisetus, Mesocyclops guangxiensis, and Mesocyclops thermocyclopoides* [113]. Recently, the effect of NPs on the predation behavior of these natural predators has been studied (Table 2). The striking findings are the increase in predation efficiency. It has been clearly demonstrated that the rate of predatory activity rises up administering NPs; however, the underlying exact mechanism is yet to be explored. The efforts, however, have been made to investigate the nontarget effects of NPs towards predatory copepods are somewhat limited.

6. Conclusion and Insights for Future Research

In the synthesis of the metal nanoparticles, the green synthesis method stands out due to its eco-friendly and sustainable nature. Based on the available research, it can be concluded that the biogenic nanoparticles have an enormous potential to answer the pressing healthcare challenges, such as the mitigation of the dengue infections. Dengue virus is now considered as global threat that requires innovative approaches for its control. Nano-biotechnology interventions can be helpful in reducing the disease burden in a cost-effective and sustainable manner. Biogenic nanoparticles can reduce the dengue infection with by direct interaction or indirect interaction with the vector. Numerous studies have supported the potential of biogenic NPs for manifesting the anti-dengue effect by interfering and downregulating the critical structural genes necessary for the viral assembly. Furthermore, these biogenic NPs have successfully demonstrated vector control potential which is manifested through their biocidal nature. From an application standpoint, the production of these biogenic NPs is free of any hazardous chemicals, with no special energy requirements and an easy scale up potential. The challenge is to implement these nano-biotechnology-based interventions on ground.

The major focus in the green synthesis is centered on the synthesis of silver and gold nanoparticles; however, these studies should be extended to other innovative composite nanomaterials. Literature of the mechanistic insights of green synthesis is scarce and further studies should be undertaken to critically evaluate the mechanistic insights during synthesis of the biogenic nanoparticles. Similarly, detailed studies should be conducted to evaluate the toxicity of the nanoparticles and their long-term impact in the environment should be critically assessed.

Abbreviations

NPs: Nanoparticles
DENV-5: Dengue virus fifth serotype
DSS: Dengue shock syndrome
DHF: Dengue hemorrhagic fever
ADM: Antibody mediated disease enhancement
NS: Non-structural proteins
AgNPs: silver nanoparticles
AChE: Acetylcholinesterase
XRD: X-ray diffraction
FTIR: Fourier transform infrared
SEM: Scanning electron microscope
EDX: Energy dispersive X-ray analysis
UV-vis: UV-visible spectroscopy
FESEM: Field emission scanning electron microscope
HRTEM: High resolution transmission electron microscopy
TEM: Transmission electron microscopy
DLS: Dynamic light scattering.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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