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 bromoquiprine exhibit antiviral potentials by inhibiting its replication. Other drugs like balapiravir, chloroquine, prednisolone, and celsogivir 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 non-structural 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 para-transgenesis, 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 repellent [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,
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 phytoogenic 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 µg·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 µg/mL to 15 µg·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, Centrotceras 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
| Sr. No | Plant used                        | Target Stage | Mechanism                                                                 | Type of NPs | Size        | Characterization                                                                 | Technique used          | MIC            | References |
|-------|-----------------------------------|--------------|---------------------------------------------------------------------------|-------------|-------------|--------------------------------------------------------------------------------|--------------------------|----------------|------------|
| 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·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 μg·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, 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.    | *Coles aromatica* 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   | Polydisperse, irregularly shaped                                              | UV-vis, FTIR, XRD, EDX, SEM | —             | [92]       |
| 8.    | *Gracilaria firma*                | Larvae       | Not reported                                                              | AgNPs       | 12–200 nm  | Spherical                                                                   | UV-vis, DLS, FTIR, Zeta Potential, XRD, EDX, SEM, TEM | —             | [93]       |
| 9.    | *Myristica fragrans*              | Larvae       | Not reported                                                              | ZnO NPs     | 100 to 200 nm | Rod-like                                                                   | UV-vis, SEM, EDX, TEM, XRD | —             | [94]       |
| 10.   | *Beauveria bassiana*              | Larvae       | Not reported                                                              | AgNPs       | 36.88 to 60.93 nm | Spherical                                                                 | UV-vis, FTIR, XRD, AFM, SEM, TEM, XRD, AFM | —             | [95]       |
| 11.   | *Aganosma cymose*                 | Larvae       | Not reported                                                              | AgNPs       | 1 to 16.5 nm | Polydisperse, spherical                                                       | UV-vis, FTIR, XRD, AFM, SEM, TEM, XRD, AFM | —             | [96]       |
| 12.   | *Cocos nucifera*                  | Larvae       | Inhibition of major detoxifying proteins glutathione-S-transferase and cytochrome P450 Route through the exoskeleton of insect into cells of individual and intervention with sloughing | AgNPs       | 5–65 nm    | Spherical, pseudo spherical and rectangle                                  | UV-vis, TEM, XRD         | —             | [97]       |
| 13.   | *Carissa carandas*                | Larvae       | Inhibition of major detoxifying proteins glutathione-S-transferase and cytochrome P450 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, FTIR, XRD, DLS, SEM, TEM | —             | [99]       |
| Sr. No | Plant used       | Target Stage | Mechanism                                                                 | Type of NPs | Size         | Characterization Shape       | 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                                                              | TiO₂ 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 papilla 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 μg 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    | Not 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    | 50μg/ml                                     | [111]      |
| Sr. No | Plant used               | Target Stage       | Mechanism                                                                 | Type of NPs | Size       | Characterization 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 penetration through membrane to midgut epithelial membrane, the enzymes gets inactivated, and produce peroxide causing cell death | AgNPs       | 85 nm      | Spherical             | UV-vis, XRD, SEM, FTIR, TEM, EDX | 9.6 ppm  | [116]      |
| 33.   | Bauhinia variegata      | Larvae III         | Not reported                                                               | AgNPs       | 38 to 65 nm | Spherical, triangle, truncated triangles, and decahedral | UV-vis, XRD, SEM, FTIR, TEM, EDX | 89.42 μg/m L |           |
| 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 |           |
| 35.   | Melia azedarach         | Larvae             | 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       | 3 to 31 nm  | Spherical             | UV-vis, XRD, SEM, FTIR, TEM, EDX | 23.82 ppm |           |
| 36.   | Suaeda maritima         | Larvae I and pupae | Inhibit neurosecretory cells, 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 | [117]    |
| 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]      |
| Sr. No | Plant used                     | Target Stage | Mechanism                                                                 | Type of NPs | Size       | Characterization | Technique used                  | MIC            | References |
|-------|--------------------------------|--------------|----------------------------------------------------------------------------|-------------|------------|------------------|---------------------------------|----------------|------------|
|    39 | *Azadirachta indica*<br>*Morinda citrifolia* | 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                                                               | TiO₂NPs     | 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 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 | AgNPs       | 10–14 nm    | Spherical        | UV-vis, FTIR, TEM, SEM, EDX, AFM | 0.43 ppm | [127]     |
|    45 | *Lobelia leschenaultiana*      | Larvae III   | Bind macromolecules such as proteins and DNA, altering their structure 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 Interfere with intracellular cell signaling, bounds with sulfur contain proteins Increase ROS and other radicals production causing apoptosis via phosphatidyl serine externalization, DNA, nuclear fragmentation, activation of meta-caspases, mitochondrial dysfunction | 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 Increase ROS and other radicals production causing apoptosis via phosphatidyl serine externalization, DNA, nuclear fragmentation, activation of meta-caspases, mitochondrial dysfunction | Pd NPs      | 10 to 20 nm | Spherical        | UV-vis, FTIR, XRD, TEM         |               | [111]     |
|    48 | *Azadirachta indica*          | Larvae III and IV | Interfere with intracellular cell signaling, bounds with sulfur contain proteins 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 heterophyllus*    | 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]     |
| Sr. No | Plant used            | Target Stage          | Mechanism                                                                 | Type of NPs | Size         | Characterization 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.   | *Chrysanthemum 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 and 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 Inhibitory influence on neurosecretory cells and gut enzyme of larvae, toxic efficacy 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 mnx-vomica* | Larvae               | Inhibit 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        | Roughly spherical | XRD, FTIR, SEM, EDX, UV-vis, and fluorescence spectroscopy | 4.209 mg/L          | [143]     |
| Sr. No | Plant used                     | Target Stage | Mechanism                                                                 | Type of NPs | Characterization | Technique used                                                                 | MIC         | References |
|--------|--------------------------------|--------------|---------------------------------------------------------------------------|-------------|------------------|--------------------------------------------------------------------------------|-------------|------------|
| 63.    | *Rhizophora mucronata*         | Larvae III   | Denaturation of the sulfur-containing proteins or phosphorous containing compound like DNA | AgNPs       | 60–95 nm         | Spherical, FTIR, XRD, AFM analysis                                            | 0.585 mg/L | [144]      |
| 64.    | *Belosynaps kewensis*          | Larvae IV    | Not reported                                                              | AgNPs       | 10 to 28 nm      | Spherical, FTIR, TEM, XRD                                                     | 84.2 ppm    | [145]      |
| 65.    | *Cynodon dactylon*             | Larvae       | Bio uptake and toxicity                                                   | AgNPs       | 14 nm            | Spherical, 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, XRD, 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 morphologies, UV-vis, XRD, FTIR, SEM, TEM | 17–19 µg/mL | [147]      |
| 68.    | *Psychotria nilghiriensis*     | 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, TEM, FTIR, EDX                                       | 12.11 mg/l (III), 17.76 mg/l (IV) | [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, SEM, FTIR, XRD, TEM                            | 3.27 and 48.81 µg/mL | [150]      |
| 71.    | *Cassia roxburghii*            | Larvae III   | Not reported                                                              | Ag NPs      | 57 to 95 nm      | Orbicular, trigonal, truncated triangles, and decahedral morphologies, UV-vis, XRD, FTIR, XRD, TEM | 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, SEM, XRD                                     | 34.04 ppm and 32.73 ppm | [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, XRD                                  | 9.90, 11.13, 12.40, 12.95 ppm | [153]      |
| 74.    | *Pergularia daemia*            | Larvae       | Not reported                                                              | AgNPs       | 44 to 255 nm     | Spherical, UV-vis, TEM, particle size and zeta potential analysis            | 15.657 µg/mL | [154]      |
| 75.    | *Ipomoea batatas*              | Larvae       | DNA structure deformation, and generation of excessive reactive oxygen species. | AgNPs       | 20–50 nm         | Orbicular, UV-vis, TEM, particle size and zeta potential analysis            | 15.657 µg/mL | [155]      |
| Sr. No | Plant used                     | Target Stage | Mechanism                                                                 | Type of NPs | Size                  | Characterization 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, FTIR, SEM, 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 ppm, 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 nanoparticles | 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, disrupts 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, 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).
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 as 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 1**: Molecular interaction of biogenic NPs with the DENV genome causing decreased expression of viral E-gene.
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 trypsins 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
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
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]. 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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