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Development and Study of Mosquitocidal and Antibacterial Properties of *Typhonium trilobatum*-Synthesized ZnO NPS and their Impact on the Predation Efficiency of Guppy *Poecilia reticulata* against the Dengue vector, *Aedes aegypti*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Dengue fever is an arbovirus spread mostly by Aedes mosquitos. Every year, there is an increase in the incidence of dengue infection and transmission. As a result, the need for an effective measure remains a top concern. The current work aims to assess the effectiveness of *Typhonium trilobatum* leaf extract synthesized zinc oxide nanoparticles (ZnO NPs) against the Dengue vector, *Aedes aegypti*. We synthesized zinc nanoparticles by reducing and stabilizing it using *T. trilobatum* leaf extract. UV-vis spectrophotometry, Fourier transform infrared spectroscopy, X-ray diffraction, energy dispersive X-ray analysis, field emission scanning electron microscopy, transmission electron microscopy and EDAX analysis were used for analyzing nanoparticles. *T. trilobatum* leaf extract and biosynthesized ZnONPs were extremely efficient against *Ae. aegypti* young instars, with LC_{50} values ranging from 36.633 ppm (larva I) to 102.436 ppm (pupa) and 1.555 ppm (larva I) to 6.906 ppm (pupa). The predation effectiveness of *Poecillia reticulata* guppy fish against *A. aegypti* I - IV instar larvae were 49.8 and 25.9 %, respectively. Predation was 78.3 and 39.8 % in ZnO-contaminated environments, respectively. The results of *T. trilobatum* leaf extract synthesized ZnONPs showed considerable larvicidal action with an increase in predatory potential of the guppy fish *P. reticulata*. To screen the antibacterial activities of *T. trilobatum* leaf extract and Biosynthesized ZnO NPs against *Bacillus subtilis, Klebsiella pneumoniae,* and *Salmonella typhi,* the agar disk diffusion and minimum inhibitory concentration procedures were used. The biosynthesized ZnO NPs showed excellent antibacterial effects against bacterial pathogens. Overall, our study suggests that *T. trilobatu*m leaf extract synthesized ZnO NPs could be used in mosquito control as well as the creation of novel chemotherapeutic medicines with low systemic toxicity.

Keywords: Aedes aegypti; antibacterial activity; mosquitocidal activity; predation efficiency; ZnO Nanoparticles.

1. INTRODUCTION

Aedes aegypti mosquito is well known for transmitting viruses and causes viral borne diseases. Therefore, mosquito control management is the foremost remedy to control the viral break-through. Usually the utilization of toxic substances as prominent insecticidal agents and targeting life stages of mosquito is a more prominent and sustainable method to stop the diseases transmission through insects as mediator [1]. Although the chemical mediated control is now widely adopted as primary controlling remedy; but still it is well observed of leading to environmental toxicity and contamination for the aquatic system [2,3] and this adverse impact highly encouraged the eco-friendly approach for such interventions.

Now the research is focused on phytochemical assay of leaf extract from plants as potential alternative to conventional insecticides. The vital role of leaf extract in crop plant protection such as antifungal, antiviral, antibacterial, insecticidal properties and also against the herbivore attacks [4]. "*Typhonium trilobatum* (L.) (*T. trilobatu*m) Schott belongs to the family Araceae, is a small to moderate sized perennial herb, commonly known as Bengal arum, Ghatkanchu or Ghatkol in Bangladesh. It is widely grown in India, Bangladesh, China, Thailand, Vietnam, Malaysia, and Srilanka for its rhizomes, leaves and petioles. It contains thiamine, niacin, carotene, folic acid, sterols and β-sitosterol" [5,6]. The uses of this plant as traditional medicine confirms that it may possess some important biological activities. Previous scientific investigations have reported that different parts of this *T. trilobatum* plant possess antimicrobial, nematocidal [7] and larvicidal activity [8]. "It also has antibacterial activities against pathogenic bacteria" [9].

Predation is a sustainable life force, for the predator interaction and it's limed the ecosystems level [10-12]. For the past several years, chemicals are being used for the control of dengue vectors [13]. On the contrary, fishes play an important role as natural enemy for the sustainable management of (I to IV instar) of mosquitoes [14]. The species *Poecilia reticulata* fish predator is well active in food chain [15], and actively involved in the management of the larval vectors [16]. Moreover, the larvivorous fishes are more efficient to control mosquitoes at their larval stages [17].

Presently, nanoscience technology is bridging the interdisciplinary aspects of sciences by acting

on the molecular and physical level, which exhibited considerable variation in the field of medical science areas as well as in the parasitology and vector control program [18,19]. In the realm of nanotechnology based inventions for different disease control activities, the plant based synthesized nanoparticles of potential therapeutic compounds or molecules has been developed as most prominent and effective biological activities as non-toxic, bio-compatibility, biodegradability, and delivery of drugs, biologics and vaccines which create a noble platform in the utilization of the natural resources apart from the molecular capability. Besides these natural system of mosquito control, the use of nanobiomaterials one of the great options in efficient control of target mosquito vectors and safer to the coexisting natural complex in the aquatic system [20].

So we switch on to green synthesized insecticide. In this study, we proposed the *T. trilobatum* leaf extract -synthesis of zinc oxide nanoparticles as a novel and effective tool against dengue vector *Ae. aegypti*. ZnONPs were characterized using a variety of biophysical methods including UV–vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and sorted for size categories. Further, *T. trilobatum* leaf extract and green-synthesized ZnONPs were evaluated for larvicidal, pupicidal toxicity against the primary dengue vector *Ae. aegypti*. Besides this evaluation, for more confirmation and practical implementation, the efficiency on predation of larvivorous fish, *P. reticulata* targeting the all larval stages at the normal condition is measured by treating with lower dosage of *T. trilobatum* leaf extract -synthesis of ZnO NPs. In addition, antibacterial properties of biosynthesized nanoparticles were evaluated against *Bacillus subtilis, Klebsiella pneumoniae,* and *Salmonella typhi* using the agar disk diffusion and minimum inhibitory concentration protocol.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The *Typhonium trilobatum* plant was obtained from Bharathiar University campus, Coimbatore, Tamil Nadu, India is 11.01 Latitude and 76.91 Longitude. The plant was identified properly and voucher specimens were deposited in the Botanical Survey of India, TNAU, Coimbatore, Tamilnadu, India (BSI/SRC/5/23/2023-24/Tech-445). The collected samples were washed, shade dried, ground to fine powder and stored in a sterile container for further studies. The 6 g of *T. trilobatum* leaf extract powder was incubated in 100 mL of deionized water for 24 h. The sample was filtered and the extract was used for nanoparticles synthesis as a stabilizing agent.

2.2 Preparation of n-ZnO

ZnO NPs was synthesized using zinc acetate, sodium hydroxide and *Typhonium trilobatum* leaf extract by co-precipitation method. Briefly, 0.1 mol/L zinc acetate (Zn $(CH_3COO)_2$ $2H_2O$) and plant extract was stirred for 30 min at room temperature. Simultaneously, 0.2 M sodium hydroxide (NaOH) was prepared under constant stirring for 30 min at room temperature. The formation of white precipitate was obtained by adding NaOH aqueous solution drop wise into zinc acetate solution and stirred for 4h. Then the solution was kept overnight for nanoparticles to settle. Further, the precipitated nanomaterials particles were repeatedly washed by using de-ionized water to discard the residual compounds. The plant synthesized ZnO NPs were dried and calcined at 600°C for 4 h.

2.3 Characterization of the Biosynthesized n-ZnO

The morphological and physio-chemical features of the bio-synthesized ZnO NPs were confirmed by sampling the reaction mixture by UV–visible spectra analysis and characterized by different techniques like FESEM, TEM, EDAX. The phase purity of the biosynthesised ZnO NPs was studied by XRD analysis. Particle size and its relative functional groups were observed using FTIR spectroscopy [21].

2.4 *Ae. aegypti* Rearing

Eggs of *Ae. aegypti* were provided by the National Centre for Disease Control (NCDC) field station of Mettupalayam (Tamil Nadu, India). The eggs were cultured in the standard size of plastic containers and 1 L distilled water was given. The larval food was given, and sucrose with honey solutions is the adult food material was given [22].

2.5 Larvicidal and Pupicidal Toxicity on *Ae. aegypti*

Ae. aegypti were cultured and maintained was done by following the method [23]. In the toxicity

experiments, 25 numbers of *Ae. aegypti* larva (1st, 2nd, 3rd& 4th) and pupa were exposed for 24 h in a conical flask occupied with 250 ml of distilled H_2O with desired concentration of *T. trilobatum* leaf extract (20, 40, 60, 80, and 100 ppm) or green-synthesized ZnO nanoparticles (2, 4, 6, 8, and 10 ppm). In each treatment, 5 replications were carried out, beside with negative controls. Death rated (%) was determined by the formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead individuals}}{\text{Number of treated individuals}} \times 100\%$$

2.6 Predatory Potential of *P. reticulata*

P. reticulata was maintained at the laboratory in a definite size of fish tank and cultured fishes were used to test the predatory potential of guppy fish. Different larval stages (I-IV) of *Ae. aegypti* have been used for the predatory bioassay. A feeding potential of fish was recorded with decrease in doses for the 1st to 4th larvae of *Ae. aegypti* i.e., 1/3 of the LC_{50} values of *T. trilobatum* leaf extract and green-synthesized ZnO NPs. Experiments were replicated for five times and larvae were replaced daily and the experiments were monitored periodically (predatory/prey potential) the predatory efficiency were calculated by using formula originally defined by [24,14].

2.7 Antimicrobial Effect of ZnO NPs

The agar well-diffusion method was used to test the *T. trilobatum* leaf extract synthesized ZnO NPs against strains of *Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae.* An axenic culture of each bacterium, cultured in nutrient broth medium, was removed and dispersed using a sterile spreader over separate agar plates for the antibacterial assay. Using a borer, 6 mm-diameter wells were punched into nutritional agar plates. *T. trilobatum* leaf extract and biosynthesized ZnO NPs (10, 20 and 30 µg mL^{-1}) solutions were added to the wells of all the media plates. The plates were incubated at 28±2°C for 24 h, and on the second day of incubation, the amount of the zone of bacterial growth inhibition was determined [25].

2.8 Statistical Analysis

All analyses were used in SPSS software package 16.0 version. Mosquitocidal experiments were analyzed data by Probit analysis [26]. Generalized linear model JMP 7 used to analyze Fish predation data. A probability level of $P<0.05$ was used to test significance of differences among values.

3. RESULTS AND DISCUSSION

3.1 Biosynthesized ZnO Nanoparticles Characterization- Visual Observation

The first important evidence that ZnO NPs have been biosynthesised is visual observation. The ZnONPs (Zn $(CH_3COO)_2$ $2H_2O$) were added to the *T. trilobatum* leaf extract, resulting in a cream-colored precipitate rather than the original light red color (Fig. 1). similarly, Abdelbaky [27] proved the biosynthesis of ZnO NPs by displaying similar color fluctuations of produced ZnO NPs utilizing leaf extract of *Pelargonium odoratissimum (L.)*, from light red to cream-colored precipitate.

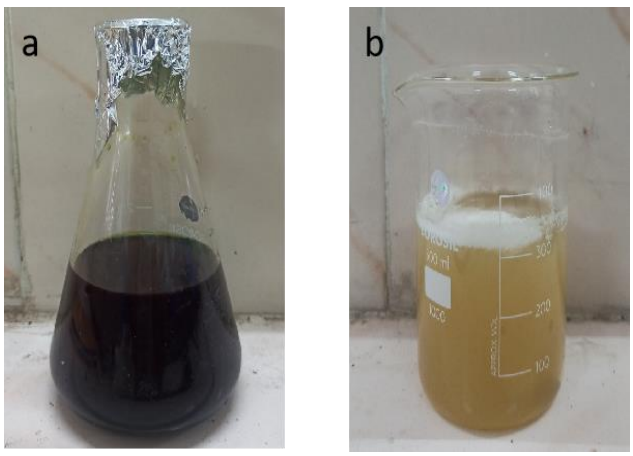

Fig. 1. The visual observation of colour changes (a) *T. trilobatum* leaf after 30 min (b) *T. trilobatum* leaf and (Zn(CH3COO)2·2H2O)

3.2 UV–Visible Spectroscopy

In the UV-vis spectrophotometer of *T. trilobatum* leaf extract synthesized ZnO NPs, a band about 354 nm was detected. The band's shape was symmetrical, demonstrating particle the existence and stability and indicating similar diffusion (Fig. 2). In agreement with our findings, Ashokan [28] reported the *M. fragrans* production of ZnO NPs as a top with the highest retention at 350 nm. Recently, Lakshmi [29] discovered a high absorption band at 378 nm, confirming an efficient and successful green production of ZnO NPs. The UV analysis produced in this study is comparable with additional Reports [30].

3.3 FTIR Studies

The FTIR transmittance spectra of *T. trilobatum* leaf extract synthesized ZnO NPs are shown in Fig. 3. The characteristic FT-IR bands of plants and ZnO NPs revealed a substantial peak in the range of (500-4500 cm^{-1}) 395, 871, 1043 and 1419 cm-1. We discovered an FTIR spectrum value indicating stretching at 1410 cm^{-1}, with a focus on C-C stretching. The C=N stretch of aliphatic amines causes the peak at 1043. 871 cm^{-1} is associated with stronger alkene groups with C-H bending. A peak at 1043 cm^{-1} is assigned to the C=N stretching modes of aliphatic amines [31].

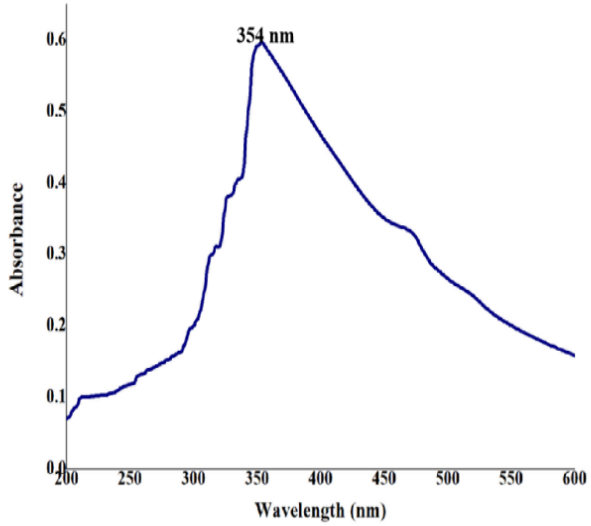

Fig. 2. UV–Vis spectra for *T. trilobatum* leaf extract synthesized ZnO NPs

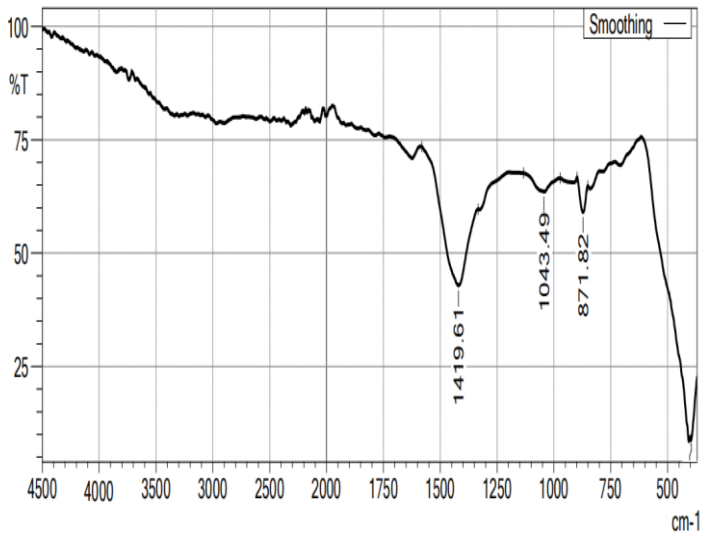

Fig. 3. FTIR spectrum of ZnO NPs of leaf extracts of *T. trilobatum*

Furthermore, this result was discovered to be consistent with an earlier report on the production of n-ZnO utilizing *O. europa* and *Raphanus sativus var. Longipinnatus* leaf extract [32,33]. The FTIR results revealed that there are band shifts that are either increased or decreased from their initial values; this change in the wave number could be caused by the presence of distinct functional groups in the biosynthesized nanoparticles.

3.4 X-ray Diffraction Studies

X-ray diffraction was used to examine the phase and crystalline structure of the biosynthesized ZnO NPs utilizing *T. trilobatum* leaf extract, and the resulting diffraction pattern is shown in Fig. 4. The X-ray diffraction peaks of biosynthesized ZnO NPs sample exhibited various peaks of 2θ = 31.91, 34.55, 36.38, 47.67, 56.72, 62.9, 66.40, 68.0o, 69.1, 72.65, and 77.07 which are in well consistent with the corresponding (hkl) diffraction planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) respectively. These peaks match those of (JCPDS card No: 36-1451), showing that the hexagonal wurtzite structure of ZnO NPs production has been confirmed [34]. Sharp diffraction line peaks in the XRD pattern supported the biosynthesized ZnO NPs crystallinity. The obtained result is consistent with the findings of previous studies using leaf extracts from *C. gigantean, Indigofera tinctoria , and Cayratia pedata* to synthesize n-ZnO [35-37].

3.5 Morphology and Elemental Studies - Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

The morphological structure of *T. trilobatum* leaf extract synthesized ZnO NPs was investigated using scanning electron microscopy (FESEM) in different magnification ranges to determine the shape of the ZnO NPs. SEM analysis clearly depicts the hexagonal shape of the synthesized nanoparticles. The result obtained is shown below as (Fig. 5). “Various research reports the effect of surface morphology and its relationship in the synergistic activity of ZnO” [37]. Our results result was found to coincide with an earlier report, which too showed different ZnO morphologies were formed depending on the pH of the synthesis conditions [38].

The surface structure and particle size of the *T. trilobatum* leaf extract synthesized ZnO NPs were further examined using transmission electron microscopy (TEM), as shown in Fig. 6 (a-c). “TEM image of synthesized ZnO NPs shows different sizes and shapes of the NPs and also outline the aggregation of nanoparticles. We confirm the formation of ZnO-NPs by comparing the particle size obtained from X-ray diffraction and transmission electron microscopy. Our results are in harmony with previous reports” [39].

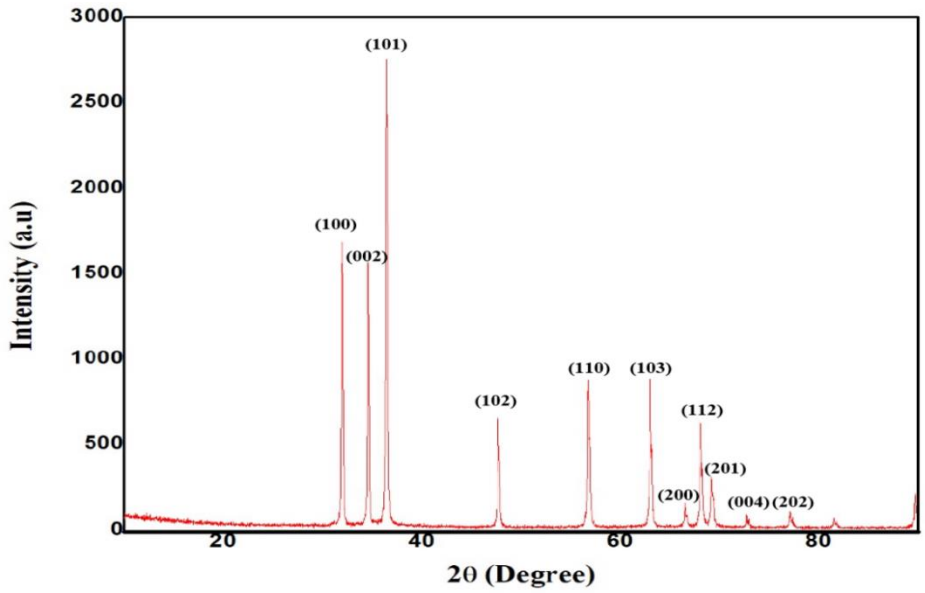

Fig. 4. XRD pattern of biosynthesized ZnO NPs via *T. trilobatum* leaf extract

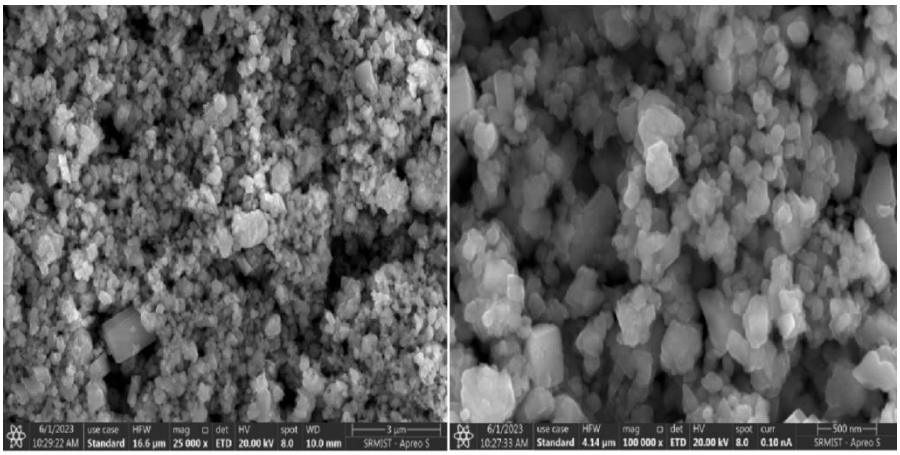

Fig. 5. FE-SEM image of biosynthesized ZnO NPs

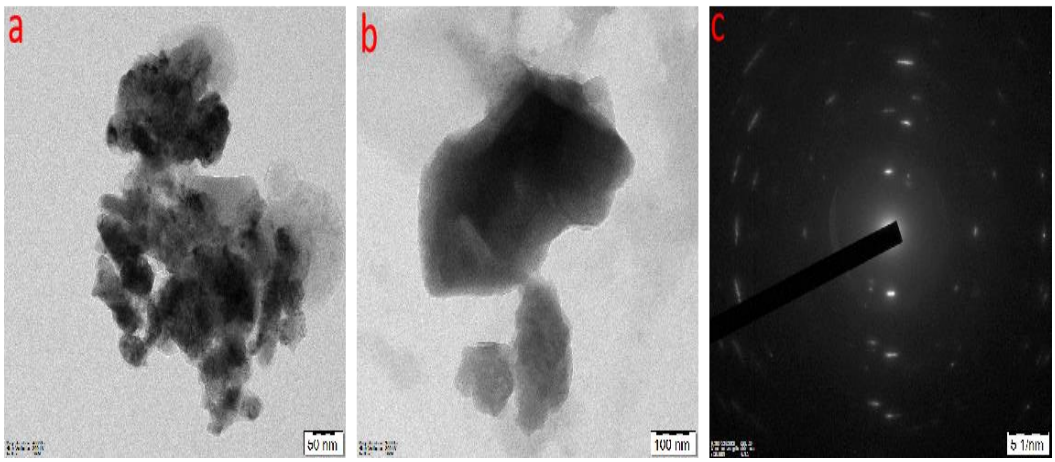

Fig. 6. (a-c) TEM analysis of biosynthesized ZnO NPs

3.5.1 Energy dispersive X-ray Analysis (EDX) spectrum of ZnO NPs

Fig. 7 and Table 1 depicts the elemental composition of *T. trilobatum* leaf extract synthesized ZnO NPs acquired by EDS. The EDS data clearly demonstrated that ZnO was formed up of only Zn and O atoms.

3.6 Larvicidal and Pupicidal Toxicity

Mosquitocidal potential of *T. trilobatum* leaf extract at various as treatment such as twenty, fourty, sixty, eighty and hundred ppm larval young instars and pupae stages of dengue vector, *Ae. aegypti* is given in Table 2. The acquired LC_{50} and LC_{90} values were calculated using the observed mortality and LC_{50}values for *T. trilobatum* leaf extract are 36.633 ppm, 48.733 ppm, 65.580 ppm, 85.159 ppm and 102.436 ppm and LC_{90} values are 119.212 ppm,149.732 ppm, 172.404 ppm, 193.372 ppm and 205.984 ppm for also first, second, third, fourth in stars and pupal population. The control treatment, on the other hand, had no mortality. There was considerable larval pupal toxicity in four larval instars (I, II, III, and IV) and pupae of *Ae. aegypti* after exposure to *T. trilobatum* leaf extract synthesized ZnO NPs. When exposed with low doses of *T. trilobatum* leaf extract synthesized ZnO NPs, all larval instars and pupae died. The LC_{50} *T. trilobatum* leaf extract synthesized ZnO NPs on instar larval stages (I,II,III,IV) and pupae of *Ae. aegypti* was 1.555 ppm, 1.639 ppm, 3.667 ppm,5.022 ppm and 6.906 ppm while LC_{90} values of 6.338 ppm, 9.134 ppm,11.575 ppm, 14.273 ppm and 16.561 ppm, (Table 3), respectively. Supporting our data, Amuthavalli [40] reported high activity of extract of *Lawsonia inermis* LC50 73.439 (I), 95.204 (II), 110.731 (III), 123.173 (IV), and 131.816 ppm (pupae). Similarly, the Zinc oxide nanoparticles using plant *Lawsonia inermis* showed good larvicidal activity after 24 h 5.494 (I), 6.801 (II), 9.336 (III),

10.736 (IV), and 12.710 ppm (pupae) respectively.

Dose dependent mortality was recorded after the exposure of n-ZnO in the larval and pupal populations [28], and in the present study also similar toxicity effect against the mosquito. Chinnathambi [41] have experimented by using phyto-synthesized zinc oxide nanoparticles showed promising larvicidal and pupicidal activities against mosquito. Moreover, it is relevant to correlate our results with an earlier research by 100% larval mortality of *Ae. aegypti* were reported by n-ZnO with LC_{50} values of 4.030 ppm in first instar and 7.213 ppm in pupae, respectively [36]. The results clearly indicated that, *T. trilobatum* leaf extract synthesized ZnO NPs showed larvicidal and pupicidal toxicity and it can be used as environmentally sound larvicidal and pupicidal control agent at stagnant water ecosystem for the control of dengue vector, *Ae. aegypti.*

3.7 Impact of Nanoparticles on *P. reticulata* Predation

Mosquito fish performed good predatory efficacy against *Ae. aegypti* After 24 hours, predation towards instar larvae of *Ae. aegypti* were 49.80 % (I), 44.4% (II), 34.3% (III) and 25.9% (IV instar) (Table. 4). Mosquito fish are good predation of mosquito larvae is quite a normal phenomenon at its breeding sites [42-45]. Predatory fish can successfully decrease larval mosquito populations in the standing water ecosystem [46]. The post treatment of *T. trilobatum* leaf extract and biosynthesized ZnO NPs against *Ae. aegypti* after 24 hours, predation towards instar larvae of *Ae. aegypti* were 57.4% (I instar), 50.7% (II instar), 39.9% (III instar) and 30.7% (IV instar) (Table. 5) respectively and 78.3% (I instar), 67.5% (II instar), 54.8% (III instar) and 39.8% (IV instar) (Table. 6) respectively. Fishes have good feed potential and can be used as a natural enemy agent of mosquito breeding sites. It was found that *P. reticulata* fish had the highest predation efficacy after the treatment of *T. trilobatum* leaf extract synthesized ZnO NPs against *Ae. aegypti.* Similarly, it was also reported that *S. alba* synthesized AgNPs, did not negatively influence the mosquito fish against *Ae. Aegypti* [24]. In agreement with our results, *Pergularia daemia*-synthesized Ag NP have been reported as non-toxic against the non-target fish P. reticulata, while they are able to evoke good mortality rates against mosquito vectors *An. stephensi* and *Ae. aegypti* [47].

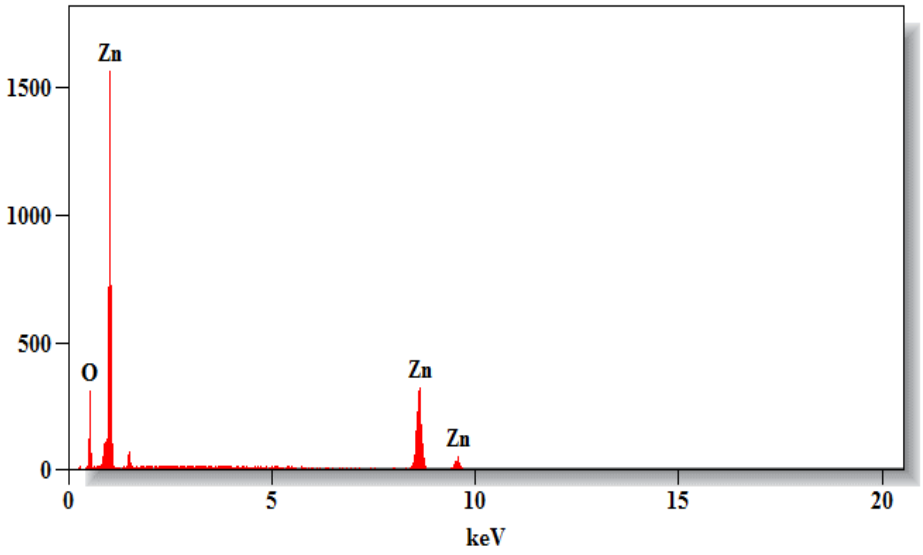

Fig. 7. Energy dispersive X-ray analysis of *T. trilobatum* leaf extract synthesized ZnO NPs

Table 1. Elemental constituents of *T. trilobatum* leaf extract synthesized ZnO NPs

Element	Net Counts	Weight %	Atom %
O	1586	17.13	45.79
Zn	5368	82.87	54.21
Total		100.00	100.00

Table 2. Larvicidal and pupicidal effect of *T. trilobatum* leaf extract against the dengue vector, *Ae. Aegypti*

Larval and pupal stage	Larval and pupal mortality (%) (Mean±S.D) Concentration (ppm)					LC_{50} (LC_{90})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	χ^2 (*d.f.*=4)
	20	40	60	80	100		Lower	Upper		
Larva I	39.56±1.9	51.32±1.8	66.14±2.0	74.28±1.3	83.48±1.8	36.633 (119.212)	25.215 (104.280)	44.806 (144.141)	*x*= 0.016 *y*= -0.569	0.226 n.s
Larva II	35.16±1.0	43.64±1.6	59.02±1.9	67.04±1.8	71.94±2.1	48.733 (149.732)	37.487 (126.450)	57.636 (193.597)	*x*= 0.013 *y*= -0.618	1.011 n.s
Larva III	26.96±2.2	36.0±1.5	45.8±1.0	57.16±2.5	66.04±1.9	66.580 (172.404)	57.241 (143.328)	77.430 (229.341)	*x*=0.012 *y*= -0.806	0.234 n.s
Larva IV	21.76±2.4	29.9±0.9	37.84±1.5	48.76±1.6	56.24±0.9	85.159 (193.372)	74.613 (158.648)	101.969 (263.674)	*x*= 0.012 *y*= -1.009	0.095 n.s
Pupae	15.4±1.9	22.1±1.2	29.36±1.4	39.86±1.1	48.48±1.9	102.436 (205.984)	89.452 (168.681)	125.788 (281.398)	*x*= 0.012 *y*= -1.268	0.049 n.s

*The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test. LFL - Lower Fiducidal Limit; UFL - Upper Fiducidal Limit. x^2, Chi-square value. *Significant at P< 0.05 level, n.s. = not significant (α=0.05)*

Table 3. Larval and pupal toxicity of *T. trilobatum* leaf extract synthesized ZnO NPs against the dengue vector, *Ae. Aegypti*

Larval and pupal stage	Larval and pupal mortality (%) (Mean±S.D) Concentration (ppm)					LC_{50} (LC_{90})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	χ^2 (*d.f.*=4)
	2	4	6	8	10		Lower	Upper		
Larva I	58.32±2.1	72.30±2.6	84.64±0.8	96.54±1.4	100±0.0	1.555 (6.338)	0.480 (5.715)	2.290 (7.193)	*x*= 0.268 *y*= -0.417	3.389 n.s
Larva II	53.94±1.7	66.2±1.7	74.22±0.9	85.22±1.4	94.16±1.9	1.639 (9.134)	0.022 (8.073)	2.663 (10.825)	*x*= 0.171 *y*= -0.280	1.141 n.s
Larva III	40.16±1.2	52.08±2.4	63.4±1.9	75.58±1.3	85.58±1.3	3.667 (11.575)	2.585 (10.181)	4.453 (13.852)	*x*=0.162 *y*= -0.594	0.162 n.s
Larva IV	33.72±1.2	45.62±1.3	53.58±1.3	66.08±2.3	75.98±1.4	5.022 (14.273)	4.038 (12.230)	5.840 (17.915)	*x*= 0.139 *y*= -0.696	0.210 n.s
Pupae	25.92±2.1	35.82±2.0	43.84±1.5	55.26±1.3	66.78±1.6	6.906 (16.561)	6.055 (13.993)	7.926 (21.303)	*x*= 0.133 *y*= -0.917	0.151 n.s

*The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test. LFL - Lower Fiducidal Limit; UFL - Upper Fiducidal Limit. x^2, Chi-square value. *Significant at P< 0.05 level, n.s. = not significant (α=0.05)*

Table 4. Predation efficiency of *P. reticulata* against the dengue vector *Ae. aegypti* in an aquatic environment treated with Standard conditions

Targets	No. of. *Fish introduced*	Predation time (h)				Total Predation (Nos.)	Predatory Efficacy of Predation (%)
		No. of. *Mosquitoes larvae* introduced	Day Time (0-12 hours) (6 am to18 pm)	No. of. *Mosquitoes larvae* Introduced	Night Time (12-24 hours) (18 pm to 6a m)		
I instar	1	100	53.0±2.3	100	46.6±2.7	99.6	49.80
II instar	1	100	46.2±1.3	100	42.6±1.8	88.8	44.4
III instar	1	100	36.2±2.5	100	32.4±1.9	68.6	34.3
IV instar	1	100	27.8±2.1	100	24.0±2.9	51.8	25.9

Table 5. Predation efficiency of *P. reticulata* against the dengue vector *Ae. aegypti* in an aquatic environment treated with *T. trilobatum* leaf

Targets	No. of. *Fish introduced*	Predation time (h)				Total Predation (Nos.)	Predatory Efficacy of Predation (%)
		No. of. *Mosquitoes larvae* introduced	Day Time (0-12 hours) (6 am to 18 pm)	No. of. *Mosquitoes larvae* Introduced	Night Time (12-24 hours) (18 pm to 6 am)		
I instar	1	100	61.2±1.9	100	53.6±1.6	114.8	57.4
II instar	1	100	53.6±2.0	100	47.8±19	101.4	50.7
III instar	1	100	42.8±1.9	100	37±2.1	79.8	39.9
IV instar	1	100	33.2±2.3	100	28.2±1.4	61.4	30.7

Table 6. Predation efficiency of *P. reticulata* against the dengue vector *Ae. aegypti* in an aquatic environment treated with biosynthesized ZnO NPs

Targets	No. of. *Fish introduced*	Predation time (h)				Total Predation (Nos.)	Predatory Efficacy of Predation (%)
		No. of. *Mosquitoes larvae* introduced	Day Time (0-12 hours) (6 am to18 pm)	No. of. *Mosquitoes larvae* Introduced	Night Time (12-24 hours) (18 pm to 6 am)		
I instar	1	100	81.2±1.3	100	75.4±1.6	156.6	78.3
II instar	1	100	72.2±1.6	100	62.8±1.4	135.0	67.5
III instar	1	100	57.0 ±1.5	100	52.6±2.0	109.6	54.8
IV instar	1	100	43.4±2.4	100	36.2±1.7	79.6	39.8

Predation rates are means ± SD of five replicates (1 predator vs. 200 *Aedes aegypti* larvae per replicate), Control was clean water, without mosquito predators. Within each column, values followed by different letter(s) are significantly different (generalized linear model, P<0.05)

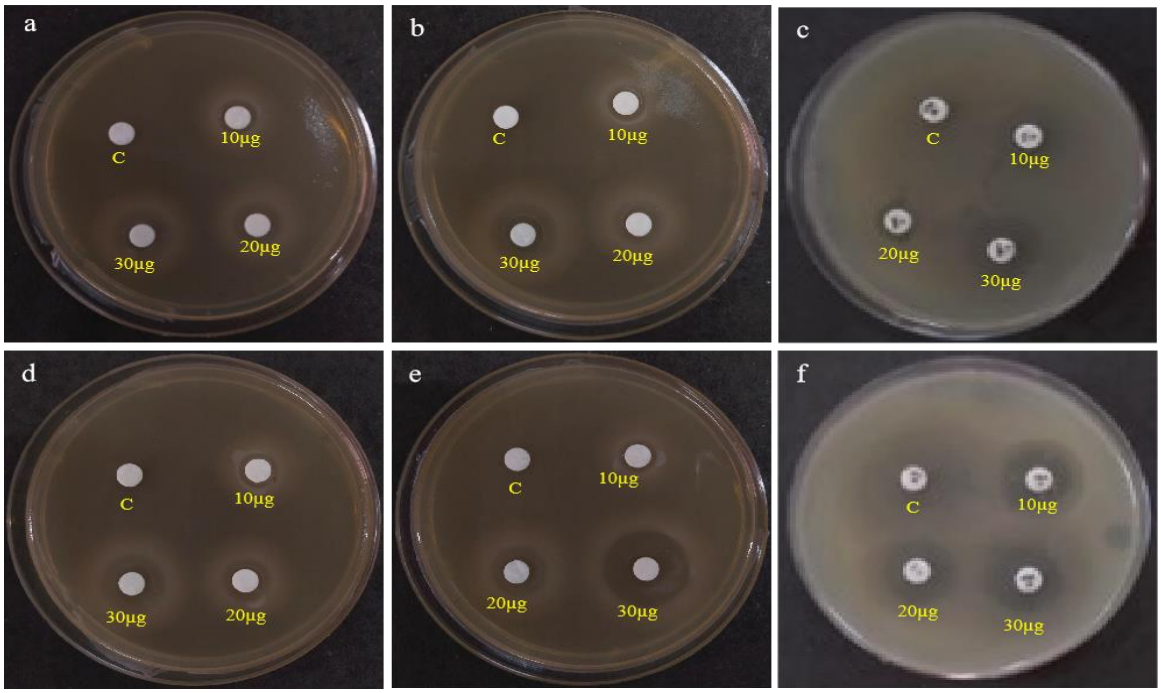

Fig. 8. Activity of *T. trilobatum* leaf extract (a-c) and Biosynthesized ZnO NPs (d-f) against the tested microbial species (*B. subtilis, S. typhi* and *K.pneumoniae*)

Table 7. Inhibition zone of the *T. trilobatum* leaf extract and biosynthesized ZnO NPs activity against the tested pathogenic microorganisms

Bacterial Pathogens	Inhibition Zone (mm)					
	Leaf extract (µg mL^{-1})			Biosynthesized ZnONPs (µg mL^{-1})		
	10 µg mL^{-1}	20 µg mL^{-1}	30 µg mL^{-1}	10 µg mL^{-1}	20 µg mL^{-1}	30 µg m^{L-1}
B. subtilis,	8.16±0.40	12.96±0.56	16.86±0.35	11.1±0.2	18.13±0.35	24.16±0.40
S. typhi	8.9±0.2	15.23±0.40	18.86±0.15	10.06±0.15	20.13±0.35	31.8±0.36
K. pneumoniae	6.16±0.30	9.0±0.2	13.83±0.35	12.33±0.20	15.83±0.25	26.1±0.3

± Standard deviation (Three replicates)

3.8 Antimicrobial Assay

The antibacterial properties of *T. trilobatum* leaf extract synthesized ZnO NPs were examined. Different doses of *T. trilobatum* leaf extract synthesized ZnO NPs shown considerable bactericidal action against *Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae* cultured on nutritional agar media. In general, increasing *T. trilobatum* leaf extract and Biosynthesized ZnO NPs concentration (10, 20 and 30 µg mL^{-1} per well) considerably improved antibacterial activity. Additionally, the findings showed that a zone of inhibition against bacterial pathogens was established by *B. subtilis, S. typhi* and *K. pneumoniae* (Fig. 8). Table 7 shows the mean values of the zone of inhibition (mm) for three replicates.

Generally, the results revealed that the biosynthesized ZnO NPs using *T. trilobatum* leaf extract ossessed a significant antibacterial effect against all tested bacterial strains. The significant antibacterial zone of inhibition was recorded in *S. typhi* (31.8±0.36 mm) followed by *K .pneumoniae* (26.1±0.3 mm) and *B. subtilis* (24.16±0.40 mm). Furthermore, compared to *T. trilobatum* leaf extract, biosynthesized ZnO NPs displayed higher antibacterial activity. A similar trend was obtained by Ghdeeb and Hussain [48], who

stated that ZnO NPs synthesized from cinnamon and bay leaves displayed greater antibacterial agents against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). Hsueh [49] and Mastanaiah [50] demonstrated the anti-*B. subtilis* activity of green sunthesized ZnO NP respectively suggesting the antibacterial potential of ZnO NPs.

4. CONCLUSIONS

Overall, this research concludes that *T. trilobatum* leaf extract synthesized ZnO NPs proved high insecticidal efficacy against mosquito species of dengue vector, *Ae. aegypti*. Fishes are the very good predators in the aquatic and it was found that *P. reticulata* fish had highest predation efficacy against *Ae. aegypti* after the treatment of *T. trilobatum* leaf extract synthesized ZnO NPs when compared standard laboratory condition. Furthermore, the bactericidal property of the *T. trilobatum* leaf extract synthesized ZnO NPs suggested that these nanoparticles were diffusible through growth medium.They can also be considered as an eco-friendly alternative to synthetic insecticides which are in now in practice. Once implied, our option will certainly be a relatively safe, biodegradable with no secondary pollution and harmful side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Morejon B, Pilaquinga F, Domenech F, Ganchala D, Debut A, Neira M. Larvicidal activity of silver nanoparticles synthesized using extracts of *Ambrosia arborescens* (Asteraceae) to control *Aedes aegypti* L.(Diptera: Culicidae). Journal of Nanotechnology. 2018;2018.
2. Ga'al H, Fouad H, Mao G, Tian J, Jianchu M. Larvicidal and pupicidal evaluation of silver nanoparticles synthesized using *Aquilaria sinensis* and *Pogostemon cablin* essential oils against dengue and zika viruses vector *Aedes albopictus* mosquito and its histopathological analysis. Artificial Cells, Nanomedicine, and Biotechnology. 2018;46(6):1171-9.
3. Govindarajan M, Rajeswary M, Senthilmurugan S, Vijayan P, Alharbi NS, Kadaikunnan S, Khaled JM, Benelli G. Larvicidal activity of the essential oil from *Amomum subulatum Roxb*.(Zingiberaceae) against *Anopheles subpictus, Aedes albopictus* and *Culex tritaeniorhynchus* (Diptera: Culicidae), and non-target impact on four mosquito natural enemies. Physiological and Molecular Plant Pathology. 2018;101:219-24.
4. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food and Chemical Toxicology. 2008;46(2):446-75.
5. Ali K, Ashraf A, Biswas NN. Analgesic, anti-inflammatory and anti-diarrheal activities of ethanolic leaf extract of *Typhonium trilobatum* L. Schott. Asian Pacific Journal of Tropical Biomedicine. 2012 Sep 1;2(9):722-6.
6. Tran K. Medicinal Plants in Viet Nam. WHO Regional Office for the Western Pacific Manila and Institute of Material Medica Hanoi, Science and Technology Publishing House, Hanoi. 1990;271.
7. Chattopadhyay PR, Mukhopadhyaya MC. Comparative studies on the nematicidal properties of *Typhonium trilobatum* and *Melia azedarach*. Indian Journal of Nematology. 1989;19(1):5-9.
8. Haldar KM, Ghosh P, Chandra G. Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* against *Culex quinquefasciatus* Say. Asian Pacific Journal of Tropical Biomedicine. 2011;1(2):S199-203.
9. Kandhasamy M, Arunachalam KD. Efficacy of *Typhonium trilobatum* (L.) Schott tuber extracts on pathogenic bacteria. Elect J Nat Subs. 2008;3:1-7.
10. Lima SL, Dill LM. Behavioral decisions made under the risk of predation: A review and prospectus. Canadian Journal of Zoology. 1990;68(4):619-40.
11. Houston A, Clark C, McNamara J, Mangel M. Dynamic models in behavioural and evolutionary ecology, Nature. 1988; 332(6159):29-34.
12. Lima SL. Nonlethal effects in the ecology of predator-prey interactions. Bioscience. 1998;48(1):25-34.
13. Cavalcanti LP, Pontes RJ, Regazzi AC, Paula Júnior FJ, Frutuoso RL, Sousa EP, Dantas Filho FF, Lima JW. Efficacy of fish as predators of *Aedes aegypti* larvae,

under laboratory conditions. Revista de Saude Publica. 2007;41:638-44.

14. Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Dinesh D, Kumar PM, Chandramohan B, Suresh U, Rajaganesh R, Alsalhi MS. Multipurpose effectiveness of *Couroupita guianensis*-synthesized gold nanoparticles: High antiplasmodial potential, field efficacy against malaria vectors and synergy with *Aplocheilus lineatus* predators. Environmental Science and Pollution Research. 2016;23:7543-58.
15. Morgan DL, Gill HS, Maddern MG, Beatty SJ. Distribution and impacts of introduced freshwater fishes in Western Australia. New Zealand Journal of Marine and Freshwater Research. 2004;38(3):511-23.
16. Fernando GK, Jayakody S, Wijenayake WM, Galappaththy GN, Yatawara M, Harishchandra J. A comparison of the larvivorous habits of exotic *Poecilia reticulata* and native *Aplocheilus parvus*. BMC ecology. 2018;18(1):1-2.
17. Phukon HK, Biswas SP. An investigation on larvicidal efficacy of some indigenous fish species of Assam, India. Adv Biores. 2013;4(3):22-5.
18. Salata OV. Applications of nanoparticles in biology and medicine. Journal of Nanobiotechnology. 2004;2(1):1-6.
19. Dias AM, Hussain A, Marcos AS, Roque AC. A biotechnological perspective on the application of iron oxide magnetic colloids modified with polysaccharides. Biotechnology Advances. 2011;29(1):142-55.
20. Rawani A, Ghosh A, Chandra G. Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L. (Solanaceae: Solanales). Acta Tropica. 2013;128(3):613-22.
21. Ramimoghadam D, Bin Hussein MZ, Taufiq-Yap YH. Hydrothermal synthesis of zinc oxide nanoparticles using rice as soft biotemplate. Chemistry Central Journal. 2013;7:1-0.
22. Murugan K, Benelli G, Panneerselvam C, Subramaniam J, Jeyalalitha T, Dinesh D, Nicoletti M, Hwang JS, Suresh U, Madhiyazhagan P. *Cymbopogon citratus*-synthesized gold nanoparticles boost the predation efficiency of copepod *Mesocyclops aspericornis* against malaria and dengue mosquitoes. Experimental Parasitology. 2015;153:129-38.
23. Suresh U, Murugan K, Benelli G, Nicoletti M, Barnard DR, Panneerselvam C, Kumar PM, Subramaniam J, Dinesh D, Chandramohan B. Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae). Parasitology Research. 2015; 114:1551-62.
24. Murugan K, Dinesh D, Paulpandi M, Subramaniam J, Rakesh R, Amuthavalli P, et al. Mangrove helps: *Sonneratia alba*-synthesized silver nanoparticles magnify guppy fish predation against *Aedes aegypti* young instars and down-regulate the expression of envelope (E) gene in dengue virus (serotype DEN-2). Journal of Cluster Science. 2017;28:437-61.
25. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. International Journal of Nanomedicine. 2012;6003-9.
26. Finney DJ. A statistical treatment of the sigmoid response curve. Probit analysis. Cambridge University Press, London. 1971;633.
27. Abdelbaky AS, Abd El-Mageed TA, Babalghith AO, Selim S, Mohamed AM. Green synthesis and characterization of ZnO nanoparticles using *Pelargonium odoratissimum* (L.) aqueous leaf extract and their antioxidant, antibacterial and anti-inflammatory activities. Antioxidants. 2022;11(8):1444.
28. Ashokan AP, Paulpandi M, Dinesh D, Murugan K, Vadivalagan C, Benelli G. Toxicity on dengue mosquito vectors through *Myristica fragrans*-synthesized zinc oxide nanorods, and their cytotoxic effects on liver cancer cells (HepG2). Journal of Cluster Science. 2017 Jan;28: 205-26.
29. Lakshmi NJ, Anandakumar S, Sampathkumar V. Green synthesis and characterisation of ZnO nanoparticles from *Manihot esculenta* (cassava) peel and their antibacterial study. Global Nest Journal, 2023;25(3):56-62.
30. Jamdagni P, Khatri P, Rana JS. Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity. Journal of King

Saud University-Science. 2018;30(2):168-75.
31. Das RK, Gogoi N, Bora U. Green synthesis of gold nanoparticles using *Nyctanthes arbortristis* flower extract. Bioprocess and Biosystems Engineering. 2011;34:615-9.
32. Awwad AM, Salem NM, Abdeen AO. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International Journal of Industrial Chemistry. 2013;4:1-6.
33. Umamaheswari A, Prabu SL, John SA, Puratchikody A. Green synthesis of zinc oxide nanoparticles using leaf extracts of *Raphanus sativus var. Longipinnatus* and evaluation of their anticancer property in A549 cell lines. Biotechnology Reports. 2021;29:e00595.
34. Venkateasan A, Prabakaran R, Sujatha V. Phytoextract-mediated synthesis of zinc oxide nanoparticles using aqueous leaves extract of *Ipomoea pes-caprae* (L). R. br revealing its biological properties and photocatalytic activity. Nanotechnology for Environmental Engineering. 2017;2:1-5.
35. Chaudhuri SK, Malodia L. Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: characterization and its evaluation on tree seedling growth in nursery stage. Applied Nanoscience. 2017;7(8):501-12.
36. Chithiga A, Manimegalai K. Biosynthesis of zinc oxide nanoparticles using *Indigofera tinctoria* and their efficacy against dengue vector, *Aedes aegypti* (Diptera: Culicidae). Experimental Parasitology. 2023;249: 108513.
37. Jayachandran A, Aswathy TR, Nair AS. Green synthesis and characterization of zinc oxide nanoparticles using *Cayratia pedata* leaf extract. Biochemistry and Biophysics Reports. 2021;26:100995.
38. Abdullah FH, Bakar NA, Bakar MA. Comparative study of chemically synthesized and low temperature bio-inspired *Musa acuminata* peel extract mediated zinc oxide nanoparticles for enhanced visible-photocatalytic degradation of organic contaminants in wastewater treatment. Journal of Hazardous Materials. 2021 Mar 15;406: 124779.
39. Pillai AM, Sivasankarapillai VS, Rahdar A, Joseph J, Sadeghfar F, Rajesh K, Kyzas GZ. Green synthesis and characterization of zinc oxide nanoparticles with antibacterial and antifungal activity. Journal of Molecular Structure. 2020;1211:128107.
40. Amuthavalli P, Hwang JS, Dahms HU, Wang L, Anitha J, Vasanthakumaran M, et al. Zinc oxide nanoparticles using plant *Lawsonia inermis* and their mosquitocidal, antimicrobial, anticancer applications showing moderate side effects. Scientific Reports. 2021 Apr 23;11(1):8837.
41. Chinnathambi A, Alharbi SA, Lavarti R, Jhanani GK, On-Uma R, Jutamas K, Anupong W. Larvicidal and pupicidal activity of phyto-synthesized zinc oxide nanoparticles against dengue vector *aedes aegypti*. Environmental Research. 2023; 216:114574.
42. Manna B, Aditya G, Banerjee S. Vulnerability of the mosquito larvae to the guppies (*Poecilia reticulata*) in the presence of alternative preys. J Vector Borne Dis. 2008;5(3):200-6.
43. Seng CM, Setha T, Nealon J, Socheat D, Chantha N, Nathan MB. Community-based use of the larvivorous fish *Poecilia reticulata* to control the dengue vector *Aedes aegypti* in domestic water storage containers in rural Cambodia. Journal of Vector Ecology. 2008;33(1):139-44.
44. Anogwih JA, Makanjuola WA. Predator-Prey Density of *Poecilia reticulata* (guppy) under laboratory investigation. The Zoologist. 2010;8:47-51.
45. Ghosh SK, Chakaravarthy P, Panch SR, Krishnappa P, Tiwari S, Ojha VP, Dash AP. Comparative efficacy of two poeciliid fish in indoor cement tanks against chikungunya vector *Aedes aegypti* in villages in Karnataka, India. BMC Public Health. 2011;11:1-8.
46. Warbanski ML, Marques P, Frauendorf TC, Phillip DA, El-Sabaawi RW. Implications of guppy (*Poecilia reticulata*) life-history phenotype for mosquito control. Ecology and Evolution. 2017;7(10):3324-34.
47. Patil CD, Borase HP, Patil SV, Salunkhe RB, Salunke BK. Larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex against *Aedes aegypti* and *Anopheles stephensi* and nontarget fish *Poecillia reticulata*. Parasitology Research. 2012;111:555-62.
48. Ghdeeb NJ, Hussain NA. Antimicrobial activity of ZnO Nanoparticles prepared using a green synthesis approach. Nano Biomedicine and Engineering. 2023;15(1): 14-20.

49. Hsueh YH, Ke WJ, Hsieh CT, Lin KS, Tzou DY, Chiang CL. ZnO nanoparticles affect *Bacillus subtilis* cell growth and biofilm formation. PloS One. 2015;10(6): e0128457.

50. Mastanaiah J, Prabhavathi NB, Varaprasad B. *In vitro* antibacterial activity of leaf extracts of *Lawsonia Inermis*. International Journal of PharmTech Research. 2011;3(2):1045-9.