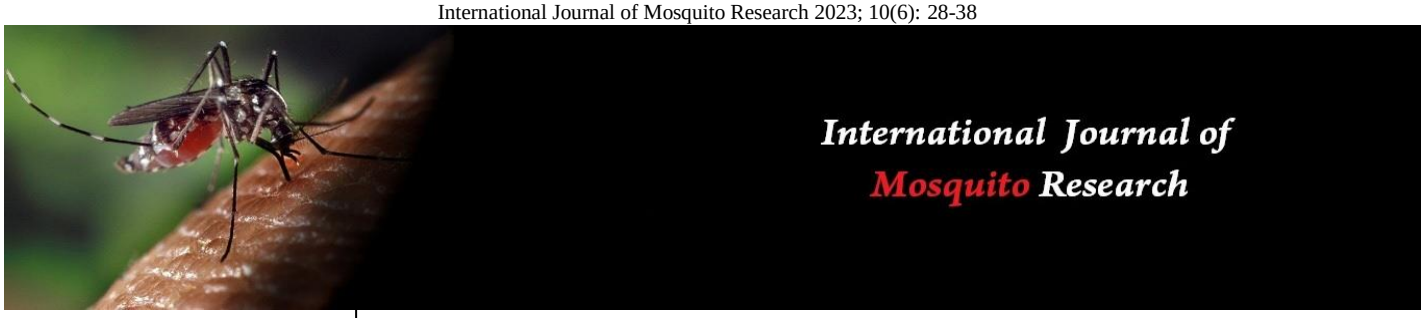


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# Developmental study on sustainable control of dengue vector *Aedes aegypti* using green-synthesized selenium nanoparticles

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**Abstract**
In this Research, we present a green synthesis method for selenium nanoparticles (SeNPs) using *Typhonium trilobatum* leaves and assess their potential applications. By utilizing different parts of *T. trilobatum* leaves as bio reductive agents, our research focuses on environmentally sustainable, safe, and socially responsible processes for the synthesis of selenium nanoparticles. Various biophysical techniques, including UV-Vis, FTIR, FESEM, TEM, EDAX, and XRD were employed to characterize the green-synthesized selenium nanoparticles (SeNPs). The toxicity of these biosynthesized SeNPs was evaluated against the dengue vector *Aedes aegypti* in its larval and pupal stages, indicating promising results at low concentrations (2 to 10 ppm). The study also examined the adulticidal activity, revealing significant mortality in adult *Ae. aegypti* following exposure to the biosynthesized SeNPs. Moreover, we exposed *Ae. aegypti* eggs to varying concentrations of leaf extract and biosynthesized SeNPs for 24 hours. After the treatment period, we transferred the eggs individually into distilled water cups and assessed their hatch rates 48 hours later. Moreover, both the *T. trilobatum* leaf extract and SeNPs demonstrated exceptional results in preventing the hatching of *Ae. aegypti* eggs aged 12 to 18 hours, achieving 100% mortality. Furthermore, the study investigated the predation effectiveness of *Poecilia reticulata* guppy fish on *Ae. aegypti* larvae in I to IV instar stages. The results demonstrated that biosynthesized SeNPs displayed significant larvicidal activity and enhanced the predatory potential of the guppy fish *P. reticulata*. These findings suggest that *T. trilobatum* leaf extract synthesized SeNPs as an eco-friendly strategy for controlling mosquito vectors during their early developmental stages.

**Keywords:** *Aedes aegypti*, *Typhonium trilobatum*, Mosquitocidal activity, Predation efficiency, Selenium Nanoparticles

## 1. Introduction
Over recent decades, metallic nanoparticles have undergone rapid synthesis through biological or green chemistry methods. These nanoparticles are manufactured in an environmentally friendly manner, characterized by purity and non-toxicity, by harnessing high-energy renewable materials. This approach not only promotes performance but also enhances safety in the development of nanoparticles [1, 2]. Moreover, the eco-friendly synthesis of metal nanoparticles from diverse plant-derived metabolites has taken on substantial roles in multiple sectors. These encompass medicinal applications, bio-catalytic functions, antioxidants, antibacterial properties, anticancer treatments, antibiotics, therapies for leishmaniasis, antifungal applications, solutions for mosquito control, and agents for pest control [3, 4].
Mosquitoes, particularly those of the Diptera: Culicidae family, represent a significant and pervasive threat to global human populations. They have been responsible for the deaths of thousands of individuals as carriers of various destructive parasites and pathogens. Mosquitoes play a pivotal role in the transmission of a range of diseases, including but not limited to malaria, filariasis, West Nile virus, Zika virus, dengue fever, Japanese encephalitis, chikungunya, and various other diseases that impact both humans and animals. These observations are supported by multiple studies [5-9]. Notably, *Aedes aegypti* mosquitoes are infamous for transmitting the viruses responsible for these diseases. Consequently, the implementation of effective mosquito control measures is absolutely crucial in the fight against the spread of these viral infections [10].

Recently, there has been growing interest in the use of nanoparticles, particularly those derived from plants, as promising toxic agents against the developmental stages of pre-adult mosquitoes. Various types of nanoparticles, including silver [11], silica [12], gold [13], iron, iron oxide [14], and selenium [15] nanoparticles, have been identified as effective toxic agents against different species of vector mosquitoes.

In recently, selenium nanoparticles (SeNPs) have gained acceptance among numerous enthusiastic researchers and are being recommended for various scientific applications due to their low toxicity and remarkable stability. The biologically mediated SeNPs are not only safer but also more environmentally friendly and economically viable when compared to alternative methods such as chemical and physical synthesis. Additionally, there are reports highlighting the successful biological synthesis of SeNPs using various parts of plants, including dried leaves, seeds, flowers, and bark, as demonstrated by studies such as those by Zhang *et al.* [16] and Alam *et al.* [17]

The successful preparation of selenium nanoparticles using plants owes much to the presence of active phytoconstituents that serve as both reducing and capping agents. Several plants, including fenugreek seed extract [18], hawthorn fruit extract [19], *Aloe vera* leaf extract [20], and *Vitis vinifera* [21], have proven effective in producing stable selenium nanoparticles.

*Typhonium trilobatum* is a perennial herb of small to medium size, cultivated extensively for its rhizomes, leaves, and petioles in various regions including India, Bangladesh, China, Thailand, Vietnam, Malaysia, and Sri Lanka. *T. trilobatum* is known to contain essential nutrients such as thiamine, niacin, carotene, folic acid, sterols, and β-sitosterol [22, 23]. Previous research has highlighted the antibacterial and nematocidal properties of several components of *T. trilobatum* [24], along with its larvicidal activity [25]. Moreover, *T. trilobatum* has demonstrated antibacterial effects against various pathogenic microorganisms [26].

In this research, we propose the utilization of *T. trilobatum* leaf extract for the synthesis of selenium nanoparticles (SeNPs) as an innovative approach to combat the *Ae. aegypti*. Comprehensive characterization of the SeNPs was performed using various biophysical techniques, including UV-vis, FT-IR, XRD, SEM, TEM and EDX. The *T. trilobatum* leaf extract synthesized SeNPs were subjected to rigorous testing for their larvicidal, pupicidal, adulticidal, and ovicidal toxicity against the *Ae. aegypti*. Additionally, we evaluated the efficacy of predation by *P. reticulata*, a larvivorous fish, on all larval stages under normal conditions, utilizing lower concentrations of T. trilobatum leaf extract-synthesized SeNPs.

## 2. Materials and Methods

### 2.1 Preparation of plant extract

The *Typhonium trilobatum* plant was sourced from the campus of Bharathiar University in Coimbatore, Tamil Nadu, India, located at coordinates 11.01 Latitude and 76.91 Longitude. Proper identification of the plant was conducted, and voucher specimens with the reference number BSI/SRC/5/23/2023-24/Tech-445 were deposited at the Botanical Survey of India, TNAU, Coimbatore, Tamil Nadu, India. The collected plant samples underwent a meticulous process, including washing, shade drying, grinding into a fine powder, and storage in a sterile container for future research purposes. To prepare the extract, 6 g of *T. trilobatum* leaf extract powder was incubated in 100 mL of deionized water for a period of 24 hours. Subsequently, the sample was filtered, and the resulting extract was employed as a stabilizing agent in the synthesis of nanoparticles.

### 2.2 Synthesis of SeNPs from plant extract

In the standard procedure, 5 mL of *T. trilobatum* leaf extract was mixed with 45 mL of double-distilled water (DDW) and then combined with 20 mL of 25 mM Sodium selenite at 60 °C. The resulting solution was stirred for 24 hours at room temperature (37 °C) until the color transformed from yellow to a vivid ruby red. Following this, the product underwent several rounds of washing with DDW via centrifugation at 10,000 rpm for 10 minutes each time to eliminate impurities. The red pellet obtained was subsequently freeze-dried for two days before further utilization in subsequent research.

### 2.3 Characterization of the biosynthesized SeNPs

The morphological and physico-chemical properties of the biosynthesized SeNPs were confirmed through several characterization techniques. These techniques included UV–visible spectra analysis, as well as examinations using different methods such as FESEM, TEM, and EDAX. Additionally, the phase purity of the synthesized SeNPs was investigated using XRD analysis. Furthermore, the particle size and the identification of relative functional groups were observed through FTIR spectroscopy, as detailed in the study by Ramimoghadam *et al.* [27]

### 2.4 *Ae. aegypti* rearing

The eggs of *Ae. aegypti* were supplied by the field station of the National Centre for Disease Control (NCDC) in Mettupalayam, Tamil Nadu, India. The eggs were cultivated in standard-sized plastic containers, with each container receiving 1 liter of distilled water. Larvae were provided with appropriate larval food, and for the adult mosquitoes, a diet consisting of a mixture of sucrose and honey solutions was supplied, as described in the study conducted by Murugan *et al.* [28]

### 2.5 Larvicidal and pupicidal toxicity on *Ae. Aegypti*

The culture and maintenance of *Ae. aegypti* mosquitoes were conducted in accordance with the protocol specified by Suresh *et al.* [29] In the toxicity experiments, 25 *Ae. aegypti* larvae at various larval stages (1st, 2nd, 3rd, and 4th instars) and pupae were subjected to a 24-hour exposure in conical flasks containing 250 mL of distilled $H_2O$, alongside biosynthesized SeNPs at concentrations of 2, 4, 6, 8, and 10 ppm. Each treatment was replicated five times, and control groups were included for comparison. The mortality rate (%) was subsequently calculated as follows:

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

### 2.6 Adulticidal activity

The adulticidal bioassay was conducted in adherence to the 1981 WHO technique, utilizing both plant extract and biosynthesized SeNPs. The leaf extract and SeNPs were applied in varying quantities onto Whatman no. 1 filter papers, while control papers were treated with Sodium selenite and distilled water. We carefully collected twenty female mosquitoes for each experiment and placed them in a

plastic containment tube. These mosquitoes were then exposed to the test paper for one hour. Following this exposure, a 24-hour recovery period was implemented, during which a mesh screen with a cotton pad soaked in a 10% glucose solution was introduced. Each trial included a series of control groups, comprising five replicates for every concentration. The lethal concentrations were identified through probit analysis.

### 2.7 Ovicidal activity

We slightly adapted the method [30] to create various concentrations, using plant extracts ranging from 20 to 100 ppm and SeNPs ranging from 2 to 4 ppm. For each concentration, we exposed 100 mosquito eggs from distinct age groups (0-6 hours, 6-12 hours, and 12-18 hours old) to the leaf extracts and SeNPs. Subsequently, the treated eggs were individually transferred to separate cups of distilled water for hatching assessment, and egg counts were conducted under a microscope. This entire process was repeated six times, with each replication having its corresponding control group. Hatch rates were calculated 48 hours after treatment using the following formula:

$$\text{Egg hatchability (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total Number of eggs}} \times 100\%$$

### 2.8 Predatory potential of *P. Reticulata*

*P. reticulata* (guppy fish) were maintained in adequately sized fish tanks in the laboratory. These farmed guppy fish were used to evaluate their predatory ability. In the predatory bioassay, various larval stages of *Ae. aegypti* (I-IV) were used. 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 synthesized SeNPs. The trials were conducted five times, with a daily replacement of larvae. Throughout the experiments, close monitoring was maintained to evaluate the possible interactions between predators and prey. Predatory efficiency was determined using the formula originally established by Murugan *et al.* [28]

### 2.9 Statistical analysis

We used the SPSS software programme version 16.0 to conduct statistical analyses. The data from the mosquitocidal tests were analysed using Probit analysis, as described by Finney [31] To analyze fish predation data, a weighted generalized linear model in JMP 7 was utilized. A significance level of $P<0.05$ was employed to test for differences among the values.

## 3. Results and Discussion

### 3.1 Synthesis and characterization of biosynthesized SeNPs

#### 3.1.1 UV–Visible Spectroscopy

The confirmation of *T. trilobatum* leaf extract synthesized SeNPs was achieved through UV-Vis spectral studies, as evident from the color change observed in Figures 1 and 2. These SeNPs exhibited a broad absorption peak at 265 nm. Significantly, a previous investigation concerning SeNPs facilitated by *Nilgirianthus ciliates* leaf extracts indicated a UV-Vis spectral peak at 265 nm, ascribed to the Surface Plasmon Resonance (SPR) of the SeNPs [15]. Likewise, UV-Vis absorption spectra of SeNPs were documented at 261 nm during synthesis utilizing leaf extract of *D. Montana* [32] and at 270 nm during synthesis employing fruit extract of *E. officinalis* [33].


**Fig 1:** The visual observation of colour changes (a) *T. trilobatum* leaf and (b) synthesized selenium nanoparticle in *T. trilobatum* leaf extract.

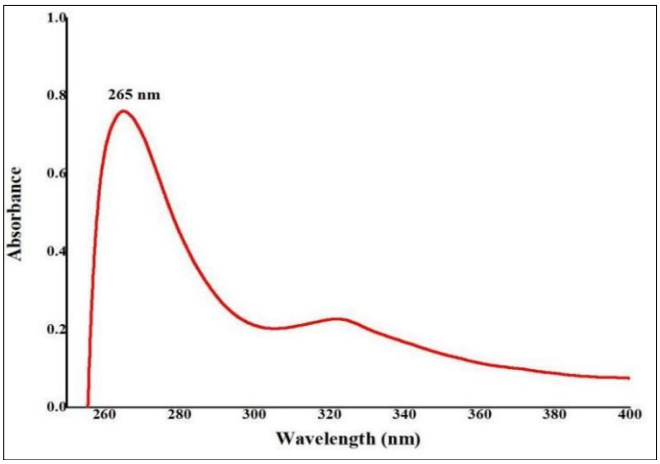


**Fig 2:** UV–Vis spectra for *T. trilobatum* leaf extract synthesized SeNPs

#### 3.1.2 FTIR-analysis

The confirmation of chemical bonds and the formation of *T. trilobatum* leaf extract synthesized SeNPs were investigated using Fourier Transform Infrared (FTIR) Spectroscopic analysis. This analysis aimed to identify any strong intensity peaks indicative of the elements involved in the biosynthesis of SeNPs within the range of 400–4000 $cm^{-1}$. The FT-IR spectrum revealed significant peaks at 3745.76 $cm^{-1}$, 3215.34 $cm^{-1}$, 2920.23 $cm^{-1}$, 1560.41 $cm^{-1}$, 1392.61 $cm^{-1}$, 1323.17 $cm^{-1}$, 1028.06 $cm^{-1}$, 881.47 $cm^{-1}$, 543.93 $cm^{-1}$, 507.28 $cm^{-1}$, and 406.98 $cm^{-1}$ (Figure. 2). The peaks at 3745.76 $cm^{-1}$ and 3215.34 $cm^{-1}$ were assigned to the O–H group. Peaks around 2920.23 $cm^{-1}$ were associated with alkene and aromatic C–H stretching frequencies, while 1392.61 $cm^{-1}$ represented C-H asymmetric bending in $CH_2$ and $CH_3$ groups. The peak at 1560.41 $cm^{-1}$ corresponded to N-O stretching, and 1028.06 $cm^{-1}$ represented C-N stretching. A strong peak at 881.47 $cm^{-1}$ was attributed to the C=C bending groups. Further bending vibrations of the Se-O bond were observed at 543.93 $cm^{-1}$ and 507.28 $cm^{-1}$. These FT-IR spectral observations are consistent with previous research on the production of SeNPs using plant extracts, revealing the existence of prominent and minor peaks associated with different molecules [15]. A preceding

investigation into the biogenic synthesis of SeNPs utilizing Aloe vera leaf extract demonstrated major and minor peaks resembling the vibrations of molecular bonds, partially resembling those observed in the current study [20]

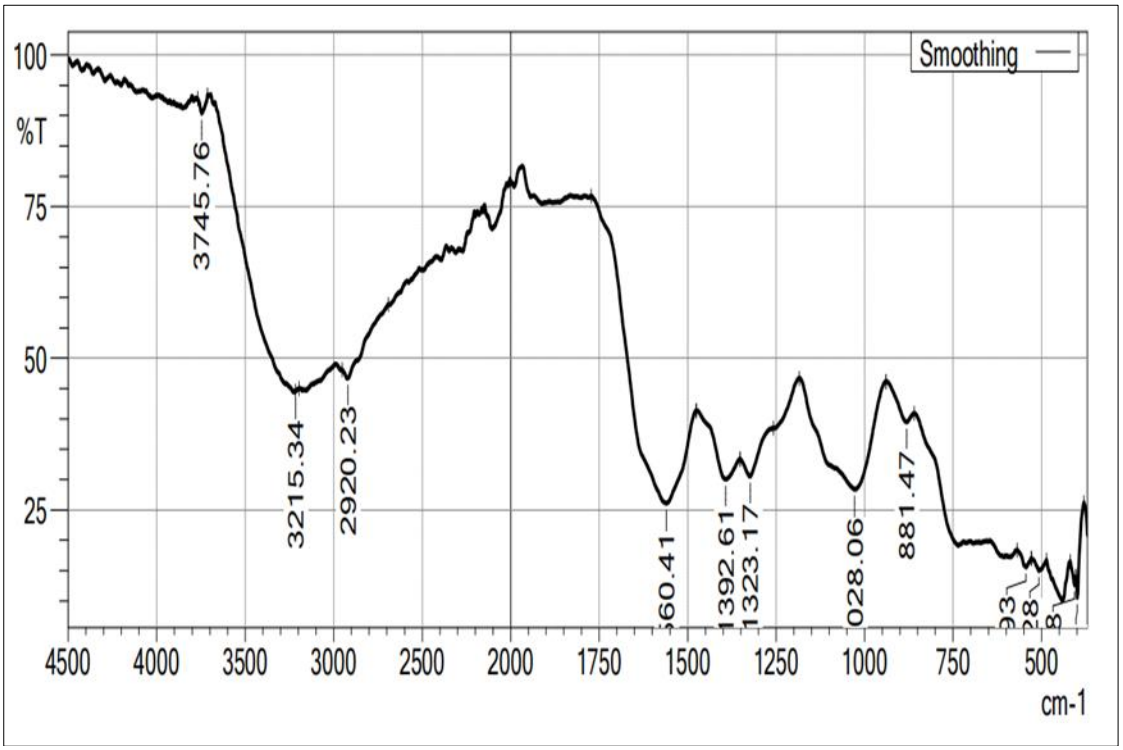


**Fig 3:** FTIR spectrum of SeNPs of leaf extracts of *T. trilobatum*

### 3.1.3 X-ray diffraction studies

X-ray diffraction analysis was employed to investigate the phase and crystalline structure of SeNPs biosynthesized using *T. trilobatum* leaf extract, as illustrated in Figure 4. The X-ray diffraction pattern of the biosynthesized SeNPs exhibited diffraction peaks that corresponded to the characteristic phases of selenium with distinct lattice structures. Consistent with the findings of Ealia and Saravanakumar [34], which reference JCPDS No. 04-0783, it was evident that the selenium nanoparticles were crystalline in nature, showcasing a cubic shape and the absence of impurities.

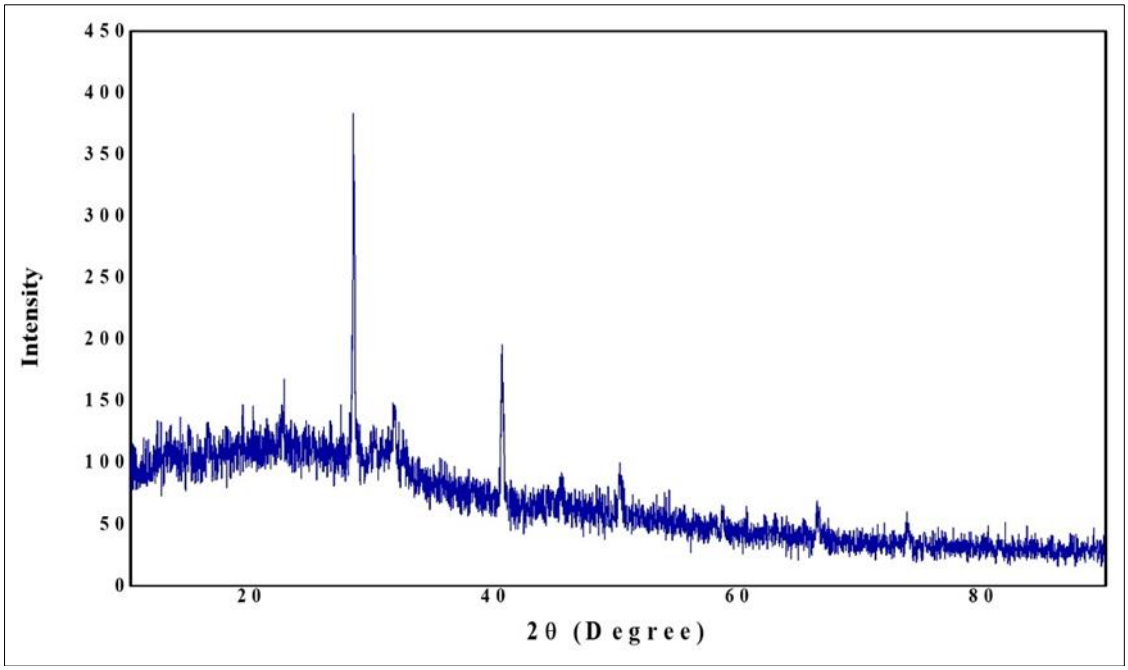


**Fig 4:** XRD pattern of biosynthesized SeNPs via *T. trilobatum* leaf extract

### 3.1.4 Microscopic analysis

The scanning electron microscope (SEM) was employed to

examine the morphological structure of *T. trilobatum* leaf extract synthesized SeNPs at various magnifications (Figure. 5). The SEM images of *T. trilobatum* leaf extract synthesized SeNPs revealed spherical and bulk shapes, consistent with previous findings, as observed in the majority of the micrographs, which depicted mostly spherical particles with a small proportion of elongated particles [35]. Previous studies have suggested that spherical and clustered nanoparticles exhibit superior biological activity compared to deformed nanostructured counterparts [36]. Additionally, it has been suggested that a smaller number of nucleation events involving the most accessible metal ions contribute to the agglomeration of the metal [37]

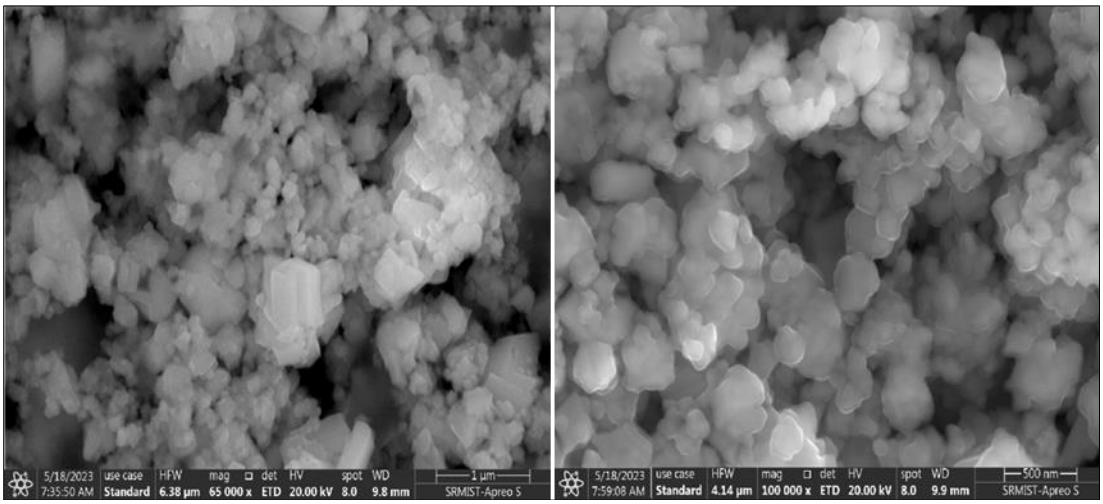

**Fig 5:** FE-SEM image of biosynthesized SeNPs.

The surface structure and particle size of the SeNPs synthesized using *T. trilobatum* leaf extract were further analyzed using transmission electron microscopy (TEM), as illustrated in Figure 6 (a-c). The TEM image of the synthesized SeNPs revealed varying sizes and shapes, with many exhibiting a spherical morphology. This spherical shape aligns with previous studies, which have established that the presence of SeNPs often results in a spherical shape [38]. In this study, the sizes of biosynthesized SeNPs observed through TEM fell within the range of 20-80 nm [39].

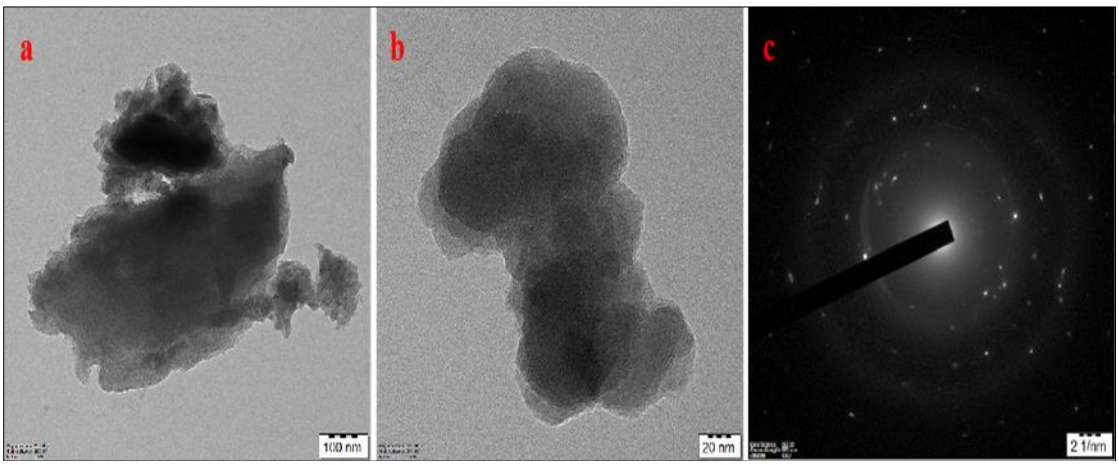


**Fig 6:** (a-c) TEM analysis of biosynthesized SeNPs

### 3.1.5 Energy Dispersive X-ray Analysis (EDX) Spectrum of SeNPs

Figure 7 and Table 1 provide insights into the elemental composition of *T. trilobatum* leaf extract synthesized SeNPs, as determined by Energy Dispersive X-ray Spectroscopy (EDS). The results indicate the presence of various elements, with selenium (Se) exhibiting the highest value at 47.71% weight and 48.359% atomic composition at 20 keV. This prominent presence of selenium in the peak confirms the formation of SeNPs. Notably, recent research has shown that a high selenium content in the peak indicates that the synthesized nanoparticles are predominantly composed of selenium and are free from other elements [50].

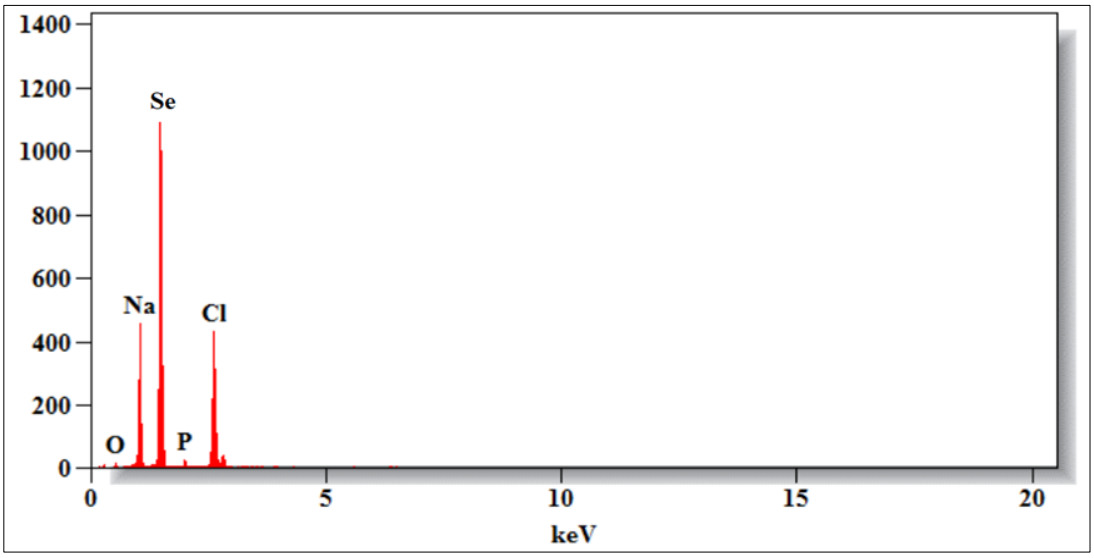


**Fig 7:** Energy dispersive X-ray analysis of *T. trilobatum* leaf extract synthesized SeNPs

**Table 1:** Elemental constituents of *T. trilobatum* leaf extract synthesized SeNPs

| Element | Net Counts | Weight % | Atom % |
|---|---|---|---|
| *O* | 93 | 3.62 | 6.19 |
| *Na* | 3235 | 18.64 | 22.16 |
| *Se* | 9174 | 47.71 | 48.35 |
| *P* | 175 | 1.19 | 1.05 |
| *Cl* | 4396 | 28.85 | 22.25 |
| *Total* | | 100.00 | 100.00 |

### 3.2 Larvicidal and Pupicidal Toxicity

Table 2 and Figure 8 represents the mosquitocidal efficacy of *T. trilobatum* leaf extract-synthesized selenium nanoparticles (SeNPs) at various concentrations (2, 4, 6, 8, and 10 ppm) against the larval and pupal stages of the dengue vector, *Ae. aegypti*. The highest mortality rate (100%) was observed in 1st instar larvae when treated with 10 ppm of *T. trilobatum* leaf extract synthesized SeNPs. Conversely, when exposed to SeNPs synthesized with T. trilobatum leaf extract at a concentration of 2 ppm, the lowest mortality rate (28.6%) was observed during the 4th instar larval stage. The median $LC_{50}$ values of *T. trilobatum* leaf extract synthesized SeNPs against *Ae. aegypti* larval instars ranged from 2.338 ppm for the 1st instar to 6.188 ppm for the 4th instar. For the pupal stage, the $LC_{50}$ value was 7.857 ppm and the $LC_{90}$ values for the larval instars ranged from 7.274 ppm for the 1st instar to 14.870 ppm for the 4th instar, while the pupal stage had an $LC_{90}$ value of 17.421 ppm. In a recent study by Shobana and Ayyappadas, [10] it was found that *T. trilobatum* leaf extract contained median $LC_{50}$ of 36.633 ppm (I instar), 48.733 ppm (II instar), 65.580 ppm (III instar), 85.159 ppm (IV instar), and 102.436 ppm (Pupa). These concentrations suggest a positive correlation between mortality rate and dosage level, which aligns with the findings reported by Sowndarya *et al.* [35] In their research, SeNPs derived from *Castanea dentata* leaf extract had median lethal concentrations of 240.714 mg/L, 104.13 mg/L, and 99.60 mg/L. The observed mortality of *Ae. aegypti* larvae resulting from *T. trilobatum* leaf extract synthesized SeNPs can be attributed to the penetration of SeNPs through the cell membrane and subsequent interaction with membrane proteins, thereby disrupting their normal functioning. Furthermore, Krishnan *et al.* [15] characterized SeNPs and found that they exhibited strong insecticidal efficacy against Ae. aegypti during the early larval stages. These findings underscore the potential of SeNPs as an effective means of controlling *Ae. aegypti* mosquito populations.

**Table 2:** Larval and pupal toxicity of *T. trilobatum* leaf extract synthesized SeNPs 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 | $\chi^2$ (*D.F.*=4) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | | Lower | Upper | | |
| Larva I | 51.08±0.7 | 63.4±1.8 | 79.84±1.2 | 91.92±1.3 | 100±0.0 | 2.338 (7.274) | 1.461 (6.610) | 2.980(8.192) | *x*= 0.260 *y*= -0.607 | 4.539 N.S |
| Larva II | 47.54±1.2 | 55.48±1.1 | 65.96±1.0 | 80.12±2.9 | 93.34±1.5 | 2.961 (10.081) | 1.843 (8.969) | 3.751 (11.815) | *x*= 0.180 *y*= -0.533 | 3.660 N.S |
| Larva III | 37.2±0.9 | 47.04±1.6 | 55.22±0.9 | 71.46±2.1 | 82.8±1.2 | 4.449 (12.580) | 3.504 (11.009) | 5.194 (15.185) | *x*=0.158 *y*= -0.701 | 1.268 N.S |
| Larva IV | 28.64±1.5 | 36.06±2.1 | 46.86±2.0 | 60.82±1.8 | 72.4±1.6 | 6.188 (14.870) | 5.402 (12.828) | 7.004 (18.390) | *x*= 0.148 *y*= -0.914 | 0.464 N.S |
| Pupae | 21.76±1.3 | 30.48±1.4 | 38.46±1.0 | 52.94±1.8 | 60.48±2.7 | 7.857 (17.421) | 6.974 (14.683) | 9.087 (22.488) | *x*= 0.1334 *y*= -1.053 | 0.343 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)

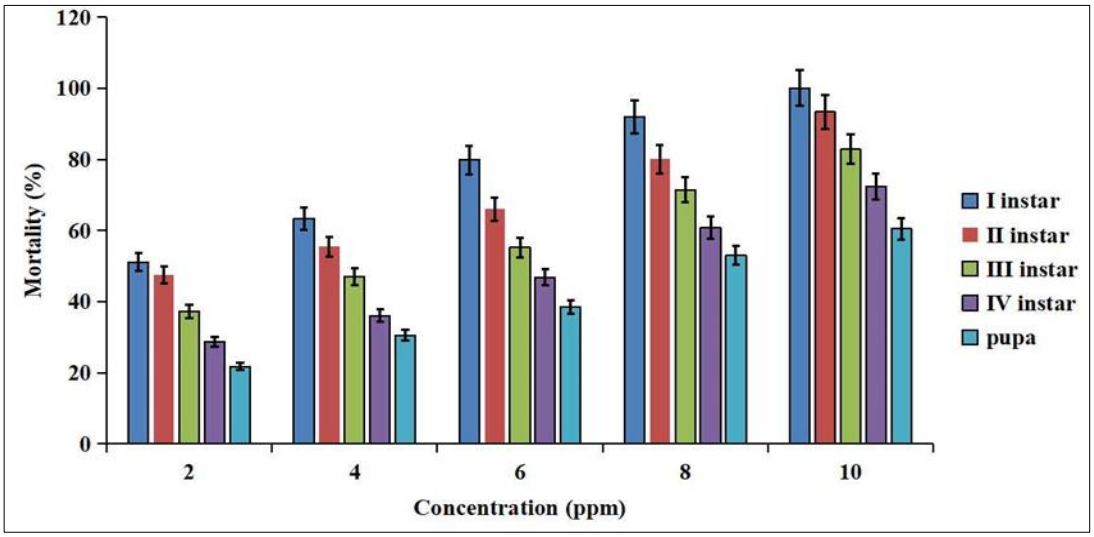


**Fig 8:** Larval and pupal toxicity of *T. trilobatum* leaf extract synthesized SeNPs against the dengue vector, *Ae. Aegypti*

### 3.3. Adulticidal activity

The results of the adulticidal activity of *T. trilobatum* leaf extract and biosynthesized SeNPs against adult *Ae. aegypti* mosquitoes are summarized in Table 3. The *T. trilobatum* leaf extract exhibited effective adulticidal properties, with calculated $LC_{50}$ and $LC_{90}$ values of 74.101 ppm and 162.356 ppm, respectively, against *Ae. aegypti* (Table 3). Similarly, the biosynthesized SeNPs using *T. trilobatum* leaf extract displayed substantial adulticidal activity against *Ae. aegypti*, with calculated $LC_{50}$ and $LC_{90}$ values of 3.697 ppm and 11.992 ppm, respectively (Table 3). In contrast, the control group showed no mortality in the concurrent assay. In a study conducted by Govindarajan and Sivakumar, [41] the methanol extract of *A. paniculata* exhibited the highest rates of adult mortality against *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. The $LC_{50}$ and $LC_{90}$ values were determined as 149.81 ppm and 172.37 ppm for *Cx. quinquefasciatus*, and 288.12 ppm and 321.01 ppm for *Ae. aegypti*, respectively. Similarly, nanoparticles synthesized from three other plant species, namely *C. asiatica*, *Z. gracilis*, and *H. indicum*, using the same method, exhibited a range of $LC_{50}$ values, ranging from 8.48 to 32.23 µg/mL, respectively [42, 8, 43]. In the case of *Ae. albopictus*, *Chenopodium ambrosioides*-synthesized AgNPs exhibited an $LC_{50}$ value of 14.29 µg/mL, as determined by Subramaniam *et al.* [44] These findings collectively highlight the potential of various plant-derived extracts and nanoparticles in effectively combating different mosquito species and emphasize the value of natural resources in developing mosquito control strategies.

**Table 3:** Adulticidal activity of *T. trilobatum* leaf extract and biosynthesized SeNPs against dengue fever mosquito, *Aedes aegypti*

| Treatment | Concentration (ppm) | Mortality (%) (mean±SD) | $LC_{50}$ (LCL-UCL) | $LC_{90}$ (LCL-UCL) | $x^2$ |
|---|---|---|---|---|---|
| *T. trilobatum* leaf extract | Control | 0.0 ±0.0 | 74.101 (66.145-84.248) | 162.356 (139.016-203.366) | 0.350 |
| | 20 | 22.8±2.1 | | | |
| | 40 | 29.8±2.1 | | | |
| | 60 | 42.0±2.2 | | | |
| | 80 | 51.8±1.6 | | | |
| | 100 | 66.1±1.7 | | | |
| *T. trilobatum* leaf extract synthesized SeNPs | Control | 0.0 ±0.0 | 3.697 (2.557- 4.515) | 11.992 (10.484- 14.517) | 0.492 |
| | 2 | 37.6±1.2 | | | |
| | 4 | 54.4±0.9 | | | |
| | 6 | 64.6±1.5 | | | |
| | 8 | 74.0±2.2 | | | |
| | 10 | 83.1±1.7 | | | |

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. $LC_{50}$ = lethal concentration killing 50% of the insects $LC_{90}$ = lethal concentration killing 90% of the insects. Chi-Square D.F. = degrees of freedom N.S. = Not Significant (A = 0.05).

### 3.4 Ovicidal activity

The ovicidal activity of both *T. trilobatum* leaf extract and biosynthesized SeNPs was evaluated by assessing the mean percent of egg hatchability in *Ae. aegypti*. The results, presented in Tables 4 and 5, revealed a clear relationship between the concentration of the treatment and the percent hatchability of the eggs. Interestingly, the *T. trilobatum* leaf extract synthesized SeNPs exhibited remarkable ovicidal activity, resulting in 100% mortality (zero hatchability) for eggs aged between 12 to 18 hours when treated at concentrations of 6, 8, and 10 ppm against *Ae. aegypti*. Compared to *T. trilobatum* leaf extract alone, the biosynthesized SeNPs proved to be more effective in preventing the hatching of *Ae. aegypti* eggs. In contrast, the control group eggs displayed a 100% hatchability rate. These findings underscore the potency of *T. trilobatum* leaf extract synthesized SeNPs as a highly effective means of controlling the hatching and development of *Ae. aegypti* mosquito eggs, which could significantly contribute to the management of mosquito populations in dengue vector control programs.

**Table 4:** Ovicidal activity of *T. Trilobatum* leaf extract against *Aedes aegypti*

| *Aedes aegypti* | Age of theegg raft/eggs (h) | Percentage of egg hatchability | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentration (ppm) | | | | | |
| | | Control | 20 | 40 | 60 | 80 | 100 |
| *T. trilobatum* leaf extract | 0-6 | 100±0.0 | 38.2±0.6 | 22.8±1.4 | 11.6±1.8 | NH | NH |
| | 6-12 | 100±0.0 | 51.6±1.4 | 38.2±0.8 | 19.2±1.6 | NH | NH |
| | 12-18 | 100±0.0 | 71.2±1.2 | 54.6±1.0 | 39.8±1.4 | 21.4±1.3 | NH |

NH - No Hatchability

**Table 5:** Ovicidal activity of *T. Trilobatum* leaf extract synthesized SeNPs against

| *Aedes aegypti* | Age of theegg raft/eggs (h) | Percentage of egg hatchability | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentration (ppm) | | | | | |
| | | Control | 2 | 4 | 6 | 8 | 10 |
| *T. trilobatum* leaf extract synthesized SeNPs | 0-6 | 100±0.0 | 26.2±1.8 | 13.6±1.6 | NH | NH | NH |
| | 6-12 | 100±0.0 | 38.4±1.2 | 22.4±1.0 | 11.6±0.8 | NH | NH |
| | 12-18 | 100±0.0 | 44.2±0.8 | 28.6±1.4 | 17.2±0.8 | NH | NH |

NH - No Hatchability

In a previous study, Veerakumar *et al.* [45] found that the aqueous leaf extract and AgNPs exhibited complete mortality at various concentrations when tested against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* mosquitoes. Additionally, Kamakshi *et al.* [46] demonstrated the lethal effect of *C. hildmannianus* extracts on Aedes aegypti eggs. The petroleum ether extract showed a moderate impact, resulting in a 52.8% mortality rate after a 96-hour post-treatment period at a concentration of 1000 mg/L. At a lower concentration of 62.5 mg/L, the petroleum ether extract caused the mortality of 28.8% of *Ae. aegypti* eggs. Furthermore, at the same concentration, the carbon tetrachloride extract exhibited a mortality rate of 38.4%, while the hexane, ethyl acetate, and aqueous extracts demonstrated mortality rates of 21.6%, 24.8%, and 20%, respectively.

### 3.5 Impact of nanoparticles on *P. reticulata* predation

The predation of mosquito larvae by mosquito fish is a well-documented natural phenomenon at breeding sites [47]. Predatory fish are known for their ability to effectively reduce larval mosquito populations in standing water ecosystems [48]. In a standard laboratory experiment was conducted by Shobana and Ayyappadas [10] mosquito fish demonstrated substantial predatory efficacy against *Ae. aegypti* after a 24-hour post-treatment with *T. trilobatum* leaf extract. In the current study, following the treatment with *T. trilobatum* leaf extract and biosynthesized SeNPs against *Ae. aegypti*, predation rates against larval instars of *Ae. aegypti* were observed to be 72.1% (I$^{st}$ instar), 62.3% (II$^{nd}$ instar), 46.3% (III$^{rd}$ instar), and 35.5% (IV$^{th}$ instar) (Table 6). These findings highlight the potential of fish as natural predators of mosquito breeding sites, which can significantly contribute to mosquito population control. Interestingly, it was observed that *P. reticulata* fish exhibited the highest predation efficacy following treatment with *T. trilobatum* leaf extract synthesized SeNPs against *Ae. aegypti*. Similarly, it was reported that *S. alba*-synthesized silver nanoparticles did not have a negative impact on mosquito fish concerning *Ae. aegypti* control, as noted by Murugan *et al.* [14] In alignment with our results, *Pergularia daemia*-synthesized AgNPs were found to be non-toxic to non-target fish *P. reticulata* while effectively reducing mosquito vectors *An. stephensi* and *Ae. aegypti* populations, as reported by Patil *et al.* [49] These findings emphasize the potential use of predatory fish as an environmentally friendly strategy for managing mosquito populations.

**Table 6:** Predation efficiency of *P. reticulata* against the dengue vector *Ae. aegypti* in an aquatic environment treated with *T. trilobatum* leaf extract synthesized SeNPs

| 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) (6am to18pm) | No. of *Mosquitoes larvae* introduced | Nighttime (12-24 hours) (18pm to 6am) | | |
| I instar | 1 | 100 | 76.2±2.1 | 100 | 68.0±1.8 | 144.2 | 72.1 |
| II instar | 1 | 100 | 68.4±2.0 | 100 | 56.2±1.4 | 124.6 | 62.3 |
| III instar | 1 | 100 | 52.0 ±1.5 | 100 | 40.6±2.6 | 92.6 | 46.3 |
| IV instar | 1 | 100 | 40.2±1.7 | 100 | 30.8±202 | 71 | 35.5 |

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, $p<0.05$)

### 4. Conclusions

In conclusion, selenium nanoparticles (SeNPs) were successfully synthesized using *T. trilobatum* leaf extract through a green synthesis approach. The characterization of these biosynthesized SeNPs was carried out using various techniques, including UV-vis spectroscopy, FTIR, XRD, SEM, TEM, and EDaX. The results of our studies demonstrate that these biosynthesized SeNPs exhibit potent mosquito larvicidal, pupicidal, adulticidal, and ovicidal activities in a dose-dependent manner. Notably, treatment with *T. trilobatum* leaf extract synthesized SeNPs proved to be more effective in mosquito control. Additionally, we observed that non-target aquatic organisms, particularly *P. reticulata* fish, displayed high predation efficacy against *Ae.*

*aegypti* following treatment with *T. trilobatum* leaf extract synthesized SeNPs. This study highlights the promising potential of next-generation SeNPs synthesized using plant bioactive molecules for more effective mosquito control. Importantly, these nanoparticles offer the advantage of being relatively safe, biodegradable, with no secondary pollution or harmful side effects, making them an environmentally friendly option for mosquito control management.

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The author (s) declare (s) that there is no conflict of interest regarding the publication of this paper.

**8. Abbreviations**
UV-Vis: Uv-Vis Spectroscopy; FTIR: Fourier Transform Infrared Spectroscopy; FESEM: Field Emission Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; EDAX: Energy Dispersive X-Ray Analysis; XRD: X-Ray Diffraction; SeNPs: Selenium Nanoparticles

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