Recent, Eco-friendly Approach for Controlling *Culex pipiens* (L.) using Novel Synthesized Cadmium Sulphide Nanoparticles of *Ocimum basilicum* Extract

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**ABSTRACT**

Mosquitoes are the key vector for the transmission of serious parasites and pathogens which affect many people worldwide. The massive overuse of synthetic mosquito insecticides had developed resistance in mosquitoes. Therefore, the need for reliable and eco-friendly control techniques became crucial. Nanoparticle fabrication is a cutting-edge and trending method. The green production of cadmium sulphide nanoparticles (CdS NPs) utilizing *Ocimum basilicum* leaves as a sophisticated and effective strategy against *Culex pipiens* was demonstrated in this study. CdS nanoparticles are characterized by a series of techniques including UV-Vis spectrophotometry, High Resolution Transmission Electron Microscope (HRTEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). CdS nanoparticles (CdS NPs) were highly effective against the larvae (3rd instars) of *Culex pipiens* after 24 hours of treatment. The LC$_{50}$ of CdS NPs was 0.0113 g/l. Meanwhile, the LC$_{50}$ of *Ocimum basilicum* aqueous extract of leaves was 52.36 g/l. There was no mortality in the control group. CdS NPs also increased larval and pupal duration significantly. Furthermore, it had a deleterious impact on pupation and adult emergence rates. Post-treatment imaging using a light microscope and scanning electron microscopy (SEM) results in larvae had deformities and constrictions in the head and thorax area. The abdomen (digestive tract and respiratory canal) was completely deformed, however, the syphon remained unaffected with typical spiracular valves and very minor abnormalities on the tracheal trunk surface and muscle fibre. Conclusion: Overall, this study suggested that the use of *Ocimum basilicum* leaves extract mediated synthesis of CdS nanoparticles can be used for controlling *Culex pipiens*. This is the first study to look at the larvicidal potential of CdS NPs synthesized by *Ocimum basilicum* leaves extract against *Culex pipiens* and the potential impacts.

**INTRODUCTION**

Mosquitoes represent a critical danger for millions of people around the world. They are significant vectors of human diseases such as malaria, filariasis, yellow fever, dengue fever and encephalitis, besides allergic responses include local skin and systemic reactions such as angioedema, and urticaria (Benelli, 2015; Benelli et al., 2016; Benelli & Mehlhorn, 2016). Mosquito-borne pathogens cause millions of deaths...
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every year. For instance, *Plasmodium* parasites, the causative agent of malaria, were responsible for an estimated 228 million cases worldwide and 405,000 fatalities in 2018 (WHO, 2019). *Culex pipiens* (Diptera: Culicidae) is one of the most common mosquito insect in rural and urban zones (Zahran et al., 2017).

Mosquito control mainly relied on the continuous use of conventional insecticides such as organophosphates and pyrethroids. Organophosphate insecticides are commonly used in the mosquito breeding sites as larvicides, meanwhile, pyrethroids are the most extensively used insecticides for the indoor adults’ mosquito control (Liu et al., 2012). However, the continuous increase of mosquito resistance to these insecticides is a major obstacle encountered ineffective control programs. As a result, there is a pressing need to create safer, more environmentally, friendly and effective alternatives to synthetic pesticides that are also simple to use (Tapondjou et al., 2005).

Bio-nanotechnology is a fast-growing field with a high impact on all spheres of human life (Vaidyanathan et al., 2009). More than a hundred studies have recently produced plant nanoparticles for their highly potent mosquitoicidal characteristics (Benelli et al., 2016). When contrasted to traditional physical and chemical synthesis methods, the green fabrication process uses low cost aqueous extracts of plant species because it does not require the use of extremely toxic chemicals or large energy inputs. (Govindarajan et al., 2016; Teimouri et al., 2018). The overall process is cheap, easy to do, and leads to the manufacturing of a wide array of nanoparticles.

Green-fabricated nanoparticles showing ovicidal, larvicidal, adulticidal, oviposition deterrent, growth and reproduction inhibitors and adult repellent activity against mosquitoes (Govindarajan et al., 2016). These NPs can be used as bio-insecticides to control mosquitoes. For instance, NPs have the potential to treat mosquito breeding sites. The utilization of nano-products has been widely investigated in relation to the control of infectious diseases (Barabadi et al., 2019).

Toxic effects of CdS NPs were reported by many authors (Brayner et al., 2011; Ma-Hock et al., 2012). Cytotoxic effects of CdS-based NPs in the laboratory, models showed a positive relationship with the release of Cd\(^{2+}\) ions from the particles (Derfus et al., 2004; Kirchner et al., 2005; Li et al., 2009). CdS nanoparticles made with a green procedure based on a cheap *Valoniopsis pachynema* seaweeds extract on immature instars of the malaria vectors *Anopheles stephensi* and *Anopheles sundicus*, CdS nanoparticles displayed substantial toxicity (Sujitha et al., 2017).

*Ocimum basilicum*, frequently referred to sweet basil, it belongs to the genus *Ocimum* of the family Lamiaceae. It is a commonly grown plant in a wide range of world regions. The presence of saponins, tannins and cardiac glycosides was revealed in a phytochemical screening of aqueous extract and elemental analysis of *O. basilicum*. It is a well-known herb planted in households and gardens for its pleasant odour, domestic and medicinal usage (Rubab et al., 2017). The natural volatile chemicals estragole, citronellal, limonene, and nerolidol found in basil leaves act as repellents. It is used as a mosquito repellent and has toxic properties (Ragavendran, 2015). So, the aim of this study is to evaluate the effect of synthesized cadmium sulphide using aqueous crude extract of *O. basilicum* in controlling *Cx. pipiens* and their biological and morphological effects.
MATERIALS AND METHODS

Mosquitoes rearing

*Culex pipiens* (Diptera: Culicidae) used in the bioassays was collected from some breeding sites in Qena, Egypt, and maintained in an insectary at Zoology Department, Faculty of Science. Egg rafts were maintained under laboratory conditions [27 ± 2 °C, R.H. 75–85 %, L:D 14:10] and placed in enamel pans [(30-35) cm in diameter and (8-10) cm in depth], half-filled with tap water. Larvae were fed daily with artificial diet Tetramin® fish food (Tetra GmbH, Melle, Germany). The breeding medium was renewed daily by pouring larvae into clean enamel pans to avoid scum formation.

Developed pupae were transferred daily to plastic cups containing tap water which were kept in wooden cages (30 × 30 × 30) cm until adult emergence. Emerged adults were provided daily with 10% glucose solution on cotton wicks and supplied with small water containers as oviposition sites; four old female adults were deprived of food for 24 h. and then allowed to feed on pigeons for a blood meal. Each egg raft was placed in a plastic cup containing fresh tap water for hatching.

Plant collection and extract preparation

Fresh leaves of *O. basillicum* were collected from the botanical garden, Qena, Egypt. Leaves were cleaned through tap water, dried in shade for 4 to 6 days then ground in a tissue grinder (IKAA 10, Germany). Dried Leaves powder (10 g) was mixed with 100 ml of distilled water and heated to 65 °C, then centrifuged at 6000 rpm for 5 mins. The obtained crude extract was stored at 4 °C for further use.

CdS nanoparticles synthesis

A volume of 50 ml of an aqueous solution of Cd(NO₃)₂ was prepared. 2H₂O (0.025 mole, 7.7 gm) and 50 ml of the aqueous solution of thiourea (0.025 moles, 1.9 gm) were added to 100 ml (0.05) M NaOH solution. The reaction mixture was kept under constant magnetic stirring. A white precipitate was produced in the beginning, after 10 min, which gradually changed to yellow precipitate then an action mixture was further stirred for 1 hr. After that, the precipitate was filtered and rinsed with distilled H₂O and dried at 100 °C.

Characterization of CdS nanoparticles

The formation of plant- mediated cadmium nanoparticles was explained by the spectral analysis. Synthesized CdS NPs were confirmed by sampling the reaction mixture at regular intervals by using a computerized SPECORD 200 PLUS spectrophotometer with 1 nm step, at ambient temperature and in the wavelength range with normal incidence 200-1100 nm. The phase purity and X ray powder diffraction were used to examine the structure of the produced samples X-ray diffraction (XRD), using Bruker D8 Advance with a copper sealed tube x-ray source producing Cu kα radiation (λ= 1.54056 Å). Furthermore, using Fourier transform infrared (FTIR) spectra, the functional groups in the produced samples were assessed. (Jasco Model 4100- Japan) with a resolution of 4 cm⁻¹ ranging from 4,000 to 400 in KBR pellet at room temperature. The particle characteristics and forms were also observed using a high- resolution transmission electron microscope ((HRTEM), model (JEOL, JEM- A 2100-Japan) operating at a 200 KV accelerating voltage.
Larval bioassay

The larvicidal activity of the aqueous crude extract and CdS NPs from *O. basillicum* were carried out according to the standard protocol of the World Health Organization (WHO, 2005) with minor modifications. It was designed based on the wide-range and narrow-range to find out the activity range of the tested materials. Following the determination of larval mortality over a wide range of concentrations, a set of six concentrations yielding between 10% and 95% death in 24 hr was utilized to determine LC$_{50}$ and LC$_{90}$ values. For aqueous crude extract, we tested at 20, 30, 40, 50, 60, and 70 g/l concentrations and for CdS NPs we tested at 0.005, 0.0075, 0.01, 0.0125, 0.015, and 0.02 g/L concentrations.

The control group set up using distilled water only. For each concentration, five replicates were carried out of 20 late 3rd instar larvae which transferred to a 100 ml small plastic cup filled with 50 ml of the tested concentrations of *O. basillicum* extract or green CdS NPs. Larval mortality was recorded at 24 hr after exposure, no food was given to the larvae. Moribund larvae were confirmed by tapping them with a fine brush in the siphon or cervical area with no response.

No mortality was observed in the control. Percentage mortality was assessed using the formula (1) and mortality will be corrected using Abbot's formula (Abbott, 1925) if it occurred between 5-10% in control groups.

**Percentage mortality** = \( \frac{\text{Number of dead individuals}}{\text{Number of treated individuals}} \times 100 \)

**Corrected mortality** = \( \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{\text{Control mortality}} \times 100 \)

**Efficacy of green CdS nanoparticles on some biological aspects of Cx. pipiens**

For survival analysis, five replicates of the 3rd instar larvae after 24 hr CdS NPs post-exposure were placed in 100 mL small plastic cups half-filled with dechlorinated water and covered with nylon netting to prevent any cross breeding mosquitoes. Each replicate contains 20 individuals. The number of dead larvae was observed and recorded daily till pupation. Food was provided daily and breeding water was changed every other day. Pupae were collected every day and observed for mortality. Pupae were counted and transferred into a rearing cage.

Five fully engorged female mosquitoes (3-5 days old) were placed singly into cups (6 cm diameter and 10 cm height) covered with thin nylon cloth and supplied with a small plastic container (3 cm diameter and 3 cm height) half-filled with de-chlorinated water as oviposition media and a hanged cotton wick soaked in glucose 10% (W/V) solution. Egg patches were removed daily and reared separately in new white, enamel pans (25 cm diameter and 10 cm height) half-filled with de-chlorinated water. Larval hatching and pupation were followed up. Pupae from each pan were counted, transferred into a rearing cage and followed up for adult emergence and sex ratio.

**Parameters studied**

To estimate the following parameters, the larvae were monitored daily until pupation and the adult until emergence. Larval mortality, Larval duration, pupation rate, pupal mortality, pupal duration, adult emergence, sex ratio, fecundity, fertility, larval and pupal malformation. Failure to respond to mechanical stimulus indicates larval
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Larval mortality (Williams *et al.*, 1986). Larval mortality percent was estimated using the following equation (Briggs, 1960).

**Larval mortality** \( \% = \frac{A - B}{A} \times 100 \)

Where: A = number of tested larvae, B = number of tested pupa.

The larval duration was calculated as the intervals between the commencement of the 3rd instar larvae and the commencement of pupation. Pupation rate was estimated using the following equation:

**Pupation** \( \% = \frac{B}{A} \times 100 \)

(wher: B = number of pupae, A= number of tested larvae

Failure to respond to mechanical stimulation indicated pupal death. The pupal mortality percent was estimated using the following equation:

**Pupal mortality** \( \% = \frac{B - C}{B} \times 100 \)

Where: B = number of produced pupae, C = number of observed adults

The pupal duration was calculated as the interval between the commencement of pupation and the commencement of adult emergence. It was calculated for each one and then the mean value was considered. The adult emergence of males and females were counted and calculated using the following equation:

**Adult emergence** \( \% = \frac{C}{B} \times 100 \)

Where: C = number of emerged adults, B= number of tested pupae.

Any change in colour, size, shape or failure to develop into the adult stage (pupal-adult intermediate) indicated pupal abnormality. All malformed pupae were counted and removed immediately. The pupal malformation percent was calculated using the following equation:

**Pupal malformation** \( \% = \frac{C}{B} \times 100 \)

Where: C = number of malformed pupae, B = number of tested pupae.

**Morphological malformation of larvicidal efficacy of CdS nanoparticles Light Microscope**

To detect signs of morphological changes that occurred in the 3rd instar larvae of *Cx. pipiens* mosquitoes due to green-synthesized CdS NPs applications, Only five instantly dead larvae were prepared using a serial dilution of alcohols, xylene for clearing and transferred to glass slides using a fine brush and well-mounted for microscopic analysis using (AxioLab- Carl Zeiss, Germany) light microscope.

**Scanning Electron Microscope (SEM) study**

Assessment of CdS NPs larvicidal effect on *Cx. pipiens* was performed by using SEM (JEOL JSM-5500 LV Scanning Electron Microscope (JEOL, Japan). Five instantly dead 3rd instar larvae were chosen from each green CdS NPs treatment (LC50) and five from the control group (non-treated) prepared by washing in dist. H2O then stored using 70% ethyl alcohol until analyzed.

**Statistical analysis**

IBM SPSS statistics (V. 25.0, IBM Corp., USA, 2015) was used for data analysis. To determine the LC50 and LC90 values, dose response data were subjected to probit analysis after 24 hr exposure. The control mortality was corrected by using Abbott’s formula. The obtained toxicity data were fitted to the log-probit model according to (Finney, 1971) using an LDP line program, LC50 and LC90 were determined for each material. The results are the mean ± SD of at least three independent replicates.
RESULTS

1. Characterization of the green synthesized CdS nanoparticles

1.1. Ultraviolet-visible spectroscopy (UV) analysis

The green synthesized CdS NPs using basil leaf extract appeared as a yellow powder, and its successive formation was monitored and confirmed by UV–visible spectrophotometer as shown in (Fig. 1a). The resultant UV–visible spectrum of CdS NPs ranged from 200–1100 nm clearly showed two peaks at ~ (246 and 300 nm) indicating the successful formation of CdS NPs. The energy band gap with direct transition can be measured using the Tauc equation (Pankove, 1971). Fig. (1b) showed the Tauc plot for CdS QDs and the energy band gap for CdS nano-crystals that were calculated from Tauc equation and that equal 4.86 eV.

1.2. X-ray diffraction (XRD) analysis

The observed peaks were indexed with Bruker D8 Advance standards and all the reflections shown in (Fig. 2) can be indexed with cubic CdS. The XRD pattern exhibited five prominent peaks at 2θ values of 26.6, 31.17, 44.13, 51.8 and 61.8; these corresponded to the (111), (020), (202), (311) and (040), respectively. The prominent positions and intensities of the diffraction peaks are well-matched with COD card no. 1011251. The relatively broad peaks indicated the fine size of the grains. Crystalline size of CdS sample was estimated using the Debye-Scherrer’s formula.

\[ D = \frac{K \lambda}{\beta \cos \theta} \]

Where D is the crystallite size, λ is the wavelength of the used X-ray radiation (0.15406 mm for Cu kα), K is constantly based on the particle shape and β is the line width at half-maximum height. The average crystallite size calculated from XRD analysis was 3 nm and it can be said CdS nanoparticles form in quantum dots.

![Fig. 1a](image.png)

Fig. 1a. The absorbance spectra for synthesized CdS NPs.
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Fig. 1b The absorption coefficient vs. photon energy (hv) for synthesized CdS NPs

Fig. 2 XRD pattern for CdS NPs

1.3. Fourier transform infrared (FTIR) analysis

Fourier transform infrared (FT-IR) spectra were used to identify the functional biomolecules that may be responsible for the reduction and capping of the aqueous plant extract formed CdS NPs. Results in (Fig. 3) showed that a number of absorption bands are present in the region between 400 and 4000 cm\(^{-1}\). The two weak peaks at 2885 and 2849 cm\(^{-1}\) show C-H asymmetric stretching vibration.
1.4. High-resolution transmissions electron microscopy (HR-TEM) analysis

The progress in the formation and morphological structure of CdS NPs was also monitored using a High-Resolution Transmission Electron Microscope (HRTEM) Fig. (4a). Magnification part of TEM micrographs revealed CdS NPs were homogeneous morphology and spherical in shape (Fig. 4b). A histogram of the particle size distribution determined using the HRTEM images is presented in (Fig. 4c). The average particle size was 3.84 nm and the particles size is consistent with sizes measured from XRD.

Fig. 4 (a) HR_TEM micrograph of green synthesized CdS NPs (b) Enlarged particle (c) Particle size distribution
2. Larvicidal potential of *Ocimum basilicum* and green CdS NPs

The larvicidal activities were assessed using various concentrations of both *O. basilicum* leaf extract and CdS NPs on the 3\textsuperscript{rd} instar of *Cx. pipiens* larvae as shown in (Fig. 5 & Table1). Results revealed that green CdS NPs of *O. basilicum* extract exhibited higher toxicity against *Cx. pipiens* when compared to the leaf aqueous extract of *O. basilicum* alone, with LC\textsubscript{50} and LC\textsubscript{90} values of (0.0113 g/L and 0.0354 g/L) and (52.67 g/L and 109.99 g/L), respectively. Furthermore, CdS NPs showed a higher larvicidal effect even at lower concentrations compared to plant derivatives alone which denote that CdS NPs potentially increases the bioactivity of plant products as shown in (Fig. 6 and Tables 2 & 3).

**Table 1.** The larvicidal efficacy of *Ocimum basilicum* extract on mortality (%) of 3\textsuperscript{rd} larval instar of *Culex pipiens*:

<table>
<thead>
<tr>
<th>Conc. (g/L)</th>
<th>No. of larvae Tested</th>
<th>No. of dead larvae</th>
<th>Percent mortality</th>
<th>LC\textsubscript{50} (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>1</td>
<td>(\ldots)</td>
<td>(\ldots)</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>10</td>
<td>(10.00)</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
<td>17</td>
<td>(17.00)</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>100</td>
<td>22</td>
<td>(22.00)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>40</td>
<td>(40.00)</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>100</td>
<td>64</td>
<td>(64.00)</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>100</td>
<td>76</td>
<td>(76.00)</td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 5 Probit analysis curve of mortality rate after exposure to aqueous leaves extract of *Ocimum basilicum*](image-url)
Table 2. Effect of CdS NPs synthesized by *Ocimum basillicum* extract on mortality (%) of 3rd larval instar of *Cx. pipiens*:

<table>
<thead>
<tr>
<th>Conc. (g/L)</th>
<th>No. of tested larvae</th>
<th>No. of dead larvae</th>
<th>Percent mortality</th>
<th>LC_{50} (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.005</td>
<td>100</td>
<td>21</td>
<td>21.00</td>
<td>0.005</td>
</tr>
<tr>
<td>0.0075</td>
<td>100</td>
<td>31</td>
<td>31.00</td>
<td>0.011</td>
</tr>
<tr>
<td>0.01</td>
<td>100</td>
<td>42</td>
<td>42.00</td>
<td></td>
</tr>
<tr>
<td>0.0125</td>
<td>100</td>
<td>54</td>
<td>54.00</td>
<td></td>
</tr>
<tr>
<td>0.015</td>
<td>100</td>
<td>60</td>
<td>60.00</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>100</td>
<td>78</td>
<td>78.00</td>
<td></td>
</tr>
</tbody>
</table>

![Probit analysis curve of mortality rate after exposure to CdS NPs synthesized by *Ocimum basillicum* extract](image)

Table 3. Toxicity of CdS NPs synthesized by *Ocimum basillicum* extract against *Culex pipiens* 3rd instar larvae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LC_{50} (g/L) (95% confidence limits)</th>
<th>LC_{90} g/L</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. basillicum</em> Extract</td>
<td>52.671 (67.297 - 44.743)</td>
<td>109.99</td>
<td>4.008 ± 0.368</td>
<td>14.668</td>
<td>0.005</td>
</tr>
<tr>
<td>CdS NPs</td>
<td>0.011 (0.012 - 0.010)</td>
<td>0.035</td>
<td>γ², γ²±, γ²±²</td>
<td>2.067</td>
<td>0.723</td>
</tr>
</tbody>
</table>

\( LC_{50} \) = The lethal concentration killing 50% of treated larvae; \( \chi^2 \) = Chi square value; \( P \) = Probability value.
Data in Table 4 indicated the biological activity of green synthesized CdS NPs against the 3rd instar larvae of Cx. pipiens. The data showed a higher larval mortality percentage (51%) for the synthesized CdS NPs treated group in comparison to (5%) for the control group. So, pupation percent in the control was higher than CdS NPs treated with (95%) and (49%), respectively. In general, CdS NPs showed weak toxicity against pupae, our results revealed the pupal mortality recorded (18.36%) and (6.62 %) for CdS NPs and control, respectively. Also, adult mortality was noticed; it was recorded (0.0%) in the control group but was (6.25%) in CdS NPs which indicated that adult mortality was not affected directly. On the other hand, the malformation percentage not highly affected in pupae; it recorded 8.16% in CdS NPs while in the control group no malformed pupae were observed.

Table 4. Effect of CdS NPs on some biological features of Culex pipiens.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval mortality (%+SD)</th>
<th>Pupal mortality (%+SD)</th>
<th>Pupation (%+SD)</th>
<th>Malformed Pupae (%+SD)</th>
<th>Adult mortality (%+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5± 0.640\textsuperscript{a}</td>
<td>6.62± 0.10\textsuperscript{a}</td>
<td>95±0.640\textsuperscript{a}</td>
<td>0.0± 0.0\textsuperscript{a}</td>
<td>0.0± 0.0\textsuperscript{a}</td>
</tr>
<tr>
<td>CdS NPs</td>
<td>51± 1.019\textsuperscript{b}</td>
<td>18.36±0.249\textsuperscript{b}</td>
<td>49±1.019\textsuperscript{b}</td>
<td>8.16± 0.267\textsuperscript{b}</td>
<td>6.25±0.20\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Mean values with different letters are significantly different at the 0.05 probability level.

Our results in Table 5 revealed that the mean larval duration was significantly affected by CdS NPs treatment where the larval duration prolonged to 7.26±0.117 days larvae treated with CdS NPs of O. basillicum compared to 5.65±0.082 days in the control. The pupal duration was affected because the mean duration significantly increased to 2.12±0.0203 days at CdS NPs vs. 1.784±0.0191 days for the control group (P<0.05). A significant reduction in adult longevity was observed. In the control group, it recorded 48.81 ±0.194 vs. 20.631±0.099 for green CdS NPs treatment. In addition, the adult emergence was significantly decreased where it recorded (97.36%) in the control that reduced to (81.63%) in green CdS NPs treatment.

Table 5. Effect of CdS NPs on longevity of Cx. pipiens different stages.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval Longevity (days ± SE)</th>
<th>Pupal Longevity (days ± SE)</th>
<th>Adult Longevity (days ± SE)</th>
<th>Adult Emergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.65±0.082\textsuperscript{a}</td>
<td>1.784±0.019\textsuperscript{a}</td>
<td>48.81 ±0.194\textsuperscript{a}</td>
<td>97.36\textsuperscript{a}</td>
</tr>
<tr>
<td>CdS NPs</td>
<td>7.26 ± 0.117\textsuperscript{b}</td>
<td>2.12±0.020\textsuperscript{b}</td>
<td>20.631±0.099\textsuperscript{b}</td>
<td>81.63\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Mean values with different letters are significantly different at the 0.05 probability level.
3. Morphological malformation of green CdS nanoparticles on *Cx. pipiens*

3.1. Using light microscope

Morphological changes in *Cx. pipiens* larvae were meant to show the damage of the body parts after the treatment with green synthesized CdS NPs at LC$_{50}$ comparing to the control. **Fig. (7)** shows an overview of the morphological alterations. Control larvae showed no signs of damage in their bodies displaying clear head, thorax, abdomen, and breathing siphon regions as in (**Fig. 7A**). After treating larvae with CdS NPs and comparing with control, they exhibited external morphological abnormalities as shrinking in body size, damaged cuticles and some detached setae feathers as well as decreased body size and discoloration. Meanwhile, some internal structural changes in the larvae were observed, such as swollen or lysed digestive tract with some dark spots, digestive lumen and muscles, narrowed and highly folded respiratory tubes as in (**Fig. 7B**).

![Fig. 7](image1.jpg) Light microscopic images of *Culex pipiens* 3rd instar larvae of A. control and B. treated with green synthesized CdS NPs

3.2. Using SEM analysis

The entire body of the control 3rd *Cx. pipiens* larvae have normal morphology. They exhibited a common appearance of the typical structure with well-developed distinct head, thorax and abdomen. Although SEM analysis showed significant shrinkage in the overall morphology after treatment with green synthesized CdS NPs. Post-treatment larvae showed distortions in the head region, especially with seta as in (**Fig. 8B**). Significant changes were not noticed in the antenna region. In control larvae, the thorax region showed normal structures, but in the treated larvae, thorax region was completely deformed with numerous wrinkles as in (**Fig. 9B**). Midgut was entirely malformed and the anal papillae were utterly disrupted as in (**Fig. 10B**). Prominent changes were not observed in the siphon with normal spiracular valves in the post-treatment larvae as in (**Fig. 11**).
Controlling *Culex pipiens* using Cadmium Sulphide Nanoparticles of *Ocimum basilicum* Extract

**Fig. 8** SEM analysis of *Cx. pipiens* 3\textsuperscript{rd} instar larvae (Head region) A. control B. treated with green synthesized CdS NPs

**Fig. 9** SEM analysis of *Cx. pipiens* 3\textsuperscript{rd} instar larvae (Thorax region) A. control B. treated with green synthesized CdS NPs

**Fig. 10** SEM analysis of *Cx. pipiens* 3\textsuperscript{rd} instar larvae (Abdomen region) A. control B. treated with green synthesized CdS NPs

**Fig. 11** SEM analysis of *Cx. pipiens* 3\textsuperscript{rd} instar larvae (siphon) A. control B. treated with green synthesized CdS NPs
Mosquito-borne diseases are one of the main public health dilemmas in developing countries. Usage of synthetic insecticides as mosquito controlling agent has become complicated due to mosquito resistance, toxicity to humans and non-target beings, all this stimulate interest in investigating novel control methods (Benelli, 2018). Plant extracts have been regarded as an alternative agent insecticide (Isman, 2006; Pavela, 2008).

Recently, increasing evidence suggests that green-fabricated mosquitocidal nanoparticles may be more effective than the plant extract tested alone (Murugan et al., 2016). Few researches on nanoparticle toxicity concerning the filariasis vector Cx. pipiens (Murugan et al., 2017). However, there is little information available on CdS-based nano-pesticides. Concerning the mechanism of action, we hypothesized that the toxicity of green-fabricated nanoparticles on mosquitoes could be owing to the nanoparticles’ small size, which allows them to penetrate through the insect cuticle and into individual cells, where they interfere with molting and other biological processes. This is the first study to evaluate the larvicidal activity of CdS NPs produced using O. basillicum aqueous leaves against Cx. pipiens and its potential consequences.

In this investigation, the obtained UV–visible spectrum approved the successive formation of CdS NPs owing to the resultant maximum two peaks at 246 and 300 nm. It has been established that CdS NPs showed spectra of optical absorption in the range of 200–1100 nm. Similarly, Sujitha et al., (2017) demonstrated that the synthesized CdS NPs displayed a maximum absorption peak at 295 nm. The XRD pattern revealed characteristic results of diffraction planes of the cubic zinc blende phase of cadmium sulphide. The average size of the synthesized crystallites CdS NPs was found to be 3 nm that calculated using Debye-Scherrer’s equation (Klug & Alexander, 1974). Earlier, similar reports on XRD analysis of bio-synthesized CdS NPs were published (Singh & Chauhan, 2009). FTIR was used to identify the potential biomolecules accountable for the reduction of Cd²⁺ ions and the capping agent that responsible for binding the cadmium nanoparticles. The transmittance peaks at 2141, 1556, 1406 and 1020 are corresponding to C≡C stretching mode, C=O stretching mode, aromatic C-H bending mode and C-N stretching vibration of amine groups, respectively (Kandasamy et al., 2019). However, variation of peaks may be due to some metabolites such as tannins, flavonoids, alkaloids and carotenoids which are abundant in basil extract and produce cadmium nanoparticles (Ragab & Saad-allah, 2020). TEM micrographs also revealed that the synthesized CdS NPs had a sphere-like structure with a mean range size of 3.84 nm that remarked these nanoparticles are in quantum dots range and perfectly matched with the XRD analysis results. Also, (Borovaya et al., 2015) the quantum dots obtained were spherical and ranged in size from 4 to 5 nm.

Our results revealed that larvicidal effects of both O. basillicum extract and green CdS NPs conducted on Cx. pipiens mosquitoes had strong toxicity with LC₅₀ values 52.67 and 0.011 g/L, respectively (Table 1). Very few articles discussed the effect of green CdS NPs toxicity on mosquitoes; however, Hajra et al., (2016) reported that larvae of Aedes albopictus exposed to marigold and rose flower-synthesized CdS NPs were highly effective against mosquito vectors which partially in agreement with our results. According to the current research, larvicidal treatments with CdS NPs had also toxicity
against pupae and adults of *Cx. pipiens*. However, the poisoning of CdS NPs against pupae and adults was less than larvae. The high toxicity of CdS NPs against larvae could be related to two reasons, first, the nanoparticles were applied directly on water-containing larvae, and second, larvae are feeding stage while pupae non-feeding stage. The weak nanoparticles toxicity towards adults may be due to the absence of direct contact between nanoparticles and adults.

Interestingly, CdS NPs affected larval and pupal longevity and prolonged their larval stages than control by 2 days meanwhile pupal duration significantly increased from 1.78 days in control to 2.12 days in CdS NPs. Moreover, it strongly shortened *Cx. pipiens* adult’s longevity. The decline of the mature stage longevity is highly important for mosquito control as adult is the injurious stage. As result, the exposure times of humans to adults, blood-feeding periods and insect oviposition will be reduced and lead to a decrease in mosquitoes population. CdS NPs application resulted in a marked decrease in developmental stages of *Cx. pipiens*. Similar toxic and developmental effects have been observed when *Cx. pipiens* exposed to other green nanoparticles. 

*Priyadarshini et al., (2012)* observed that the synthesized Ag NPs had the maximum larval mortality against first- to fourth-instar larvae and pupae with LC50 values of 10.14, 16.82, 21.51, and 27.89 ppm, respectively. *Govindarajan et al., (2016)* synthesized Ag NPs using *Bauhinia variegata* leaf extract showed toxic effect with 41.96, 46.16, and 51.92 μg/mL and 82.93, 89.42, and 97.12 μg/mL LC50 and LC90 values, respectively, for *An. subpictus, Ae. albopictus,* and *Culex. tritaeniorhynchus*.

Results of microscopic observations confirmed that the tested nanoparticles have the potential to cause morphological abnormalities in larvae of *Cx. pipiens* (Fig. 7) showed the disintegration of the epithelial layer and the outer cuticle. Limited studies have described morphological deformities in mosquito larvae caused by nanoparticles. *Ishwarya et al., (2017)* observed morphological abnormalities of green Ag NPs synthesized from *Pedalium murex* seeds extract against *Aedes aegypti*. *Amutha et al., (2019)* in *Cx. quinquefasciatus* the 4th instar larvae were exposed to CS-Ag NPs showed darkening or blackening of the abdomen and twisted abdomen. Also, *Bibi et al., (2020)* reported that red seaweeds against the dengue vector mosquito *Ae. aegypti* led to aberrations in the treated larval internal structure as well as chitin synthesis-related effects such as ‘inhibiting impact on adult emergence.

Overall, the potential of *O. basillicum* extract as a reducing agent for the manufacture of CdS nanoparticles that are efficient mosquitocidal agents is highlighted in this study. *O. basillicum* caused a decline in CdS ions in water, which could be helpful in the suppression of bancroftian filariasis vectors, outlining also significant impacts on the biological and morphological activities.

**CONCLUSIONS**

In the current study, we focused on the insecticidal effect of sweet basil aqueous leaves extract and the green synthesized cadmium sulphide nanoparticles from its leaves. The physical properties of synthesized nanoparticles confirmed that the particles nano-size was in quantum dots. Promising results of the insecticidal effect CdS NPs more than the plant extract were showed in *Cx. pipines* larvae. Also, it affected negatively the larval
duration, pupal duration, and adult longevity. Besides, the deformities of the body parts affected negatively the life quality of the mosquito. These results consider an addition to the IPM field by using alternatives technology in mosquito control. More studies will be needed on the effect of the nanoparticles on the non-target organisms.

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Controlling *Culex pipiens* using Cadmium Sulphide Nanoparticles of *Ocimum basilicum* Extract


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