Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(4): 359 – 377 (2021)
www.ejabf.journals.ekb.eg

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

Mohammed Z. Y. Aly , Khaled S. M. Osman, Eman M. Omar , Mervat A. Mahmoud*
Department of Zoology, Faculty of Science, South Valley University- Qena 83523, Egypt
***Corresponding Author: Mervat.mahmoud@sci.svu.edu.eg**

ARTICLE INFO
Article History:
Received: July 10, 2021
Accepted: July 19, 2021
Online: Aug. 12, 2021

Keywords:
Mosquito control,
CdS nanoparticles,
Ocimum basillicum,
Larvicidal activity,
Biological parameters,
SEM analysis,
Culex pipiens

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 basillicum* 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 basillicum* 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 basillicum* 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 basillicum* 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

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 mosquitocidal 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 sundaicus*, CdS nanoparticles displayed substantial toxicity **(Sujitha *et al*., 2017).**

Ocimum basillicum, 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. basillicum*. 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 ^{o}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 ^{o}C, then centrifuged at 6000 rpm for 5 mins. The obtained crude extract was stored at 4 ^{o}C for further use.

CdS nanoparticles synthesis

A volume of 50 ml of an aqueous solution of $Cd(NO_3)_2$ was prepared. $2H_2O$ (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 H_2O and dried at 100 ℃.

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= (Number of dead individuals/ Number of treated individuals) × 100

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

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

mortality **(Williams *et al*., 1986).** Larval mortality percent was estimated using the following equation **(Briggs, 1960)**.

Larval mortality % = A – B / A × 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 % = B / A × 100

(where: 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 % = B – C / B × 100

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 % = C / B × 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 % = C / B × 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 (LC_{50}) and five from the control group (non-treated) prepared by washing in dist. H_2O 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 LC_{50} and LC_{90} 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, LC_{50} and LC_{90} 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.

$$D=K\lambda/\beta Cos\theta$$

Where D is the crystallite size, λ is the wavelength of the used X- ray radiation (0.15406 mm for Cu k α), 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.

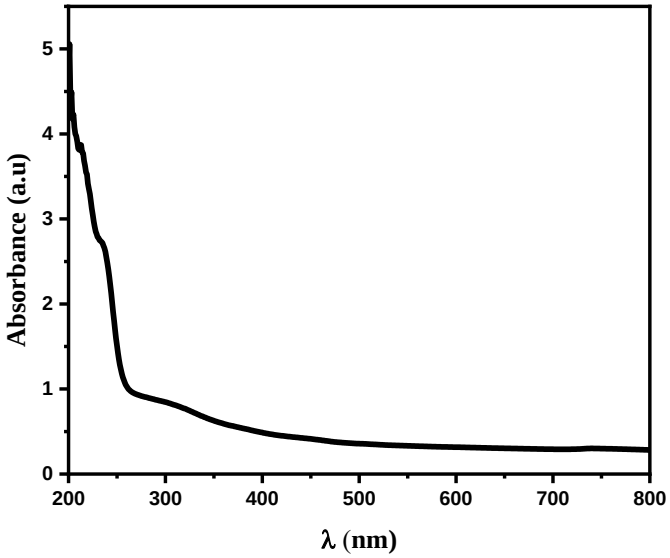

Fig. 1a. The absorbance spectra for synthesized CdS NPs.

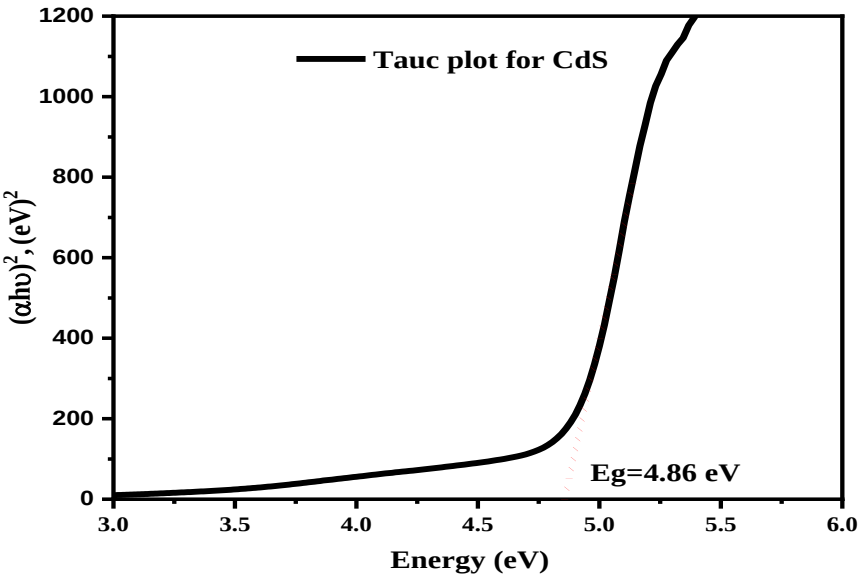

Fig. 1b The absorption coefficient vs. photon energy (hν) for synthesized CdS NPs

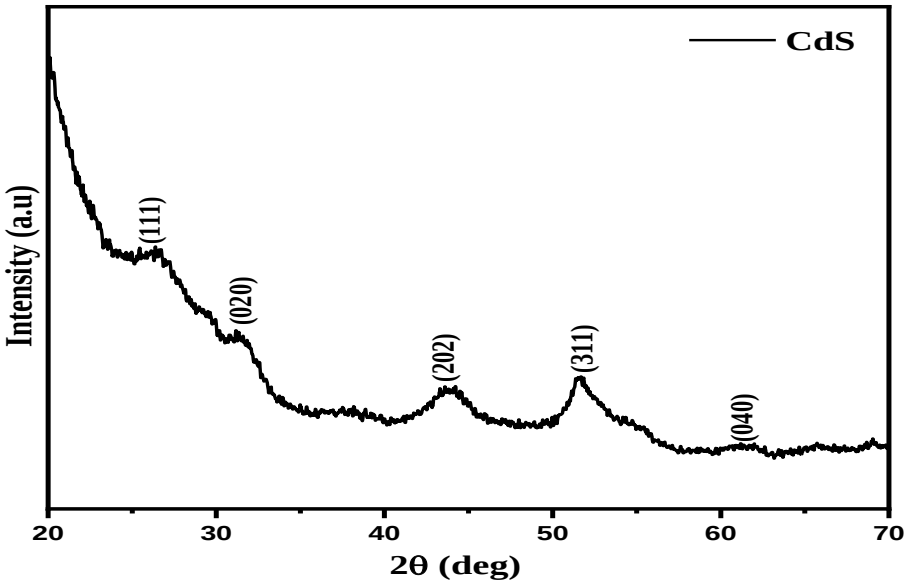

Fig. 2 XRD pattern for CdS NPs

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.

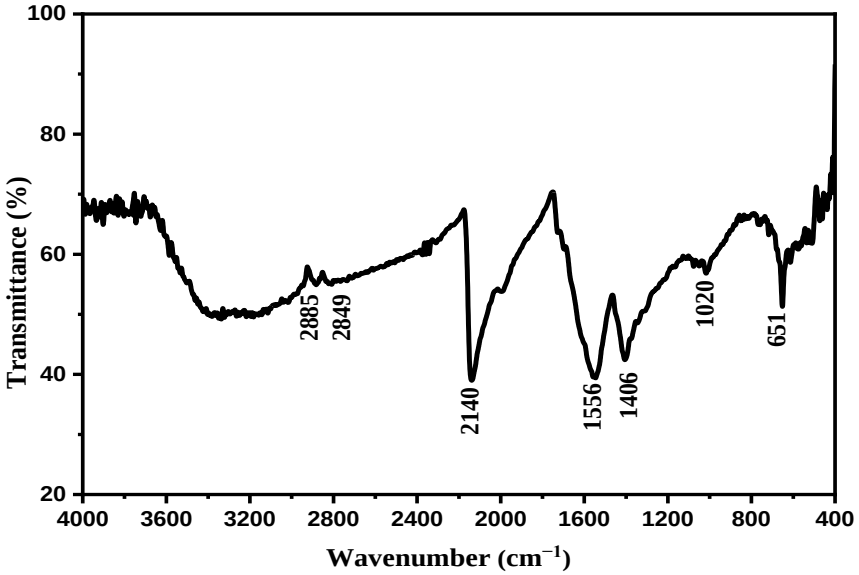

Fig. 3 FTIR profile of green synthesized CdS NPs

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.

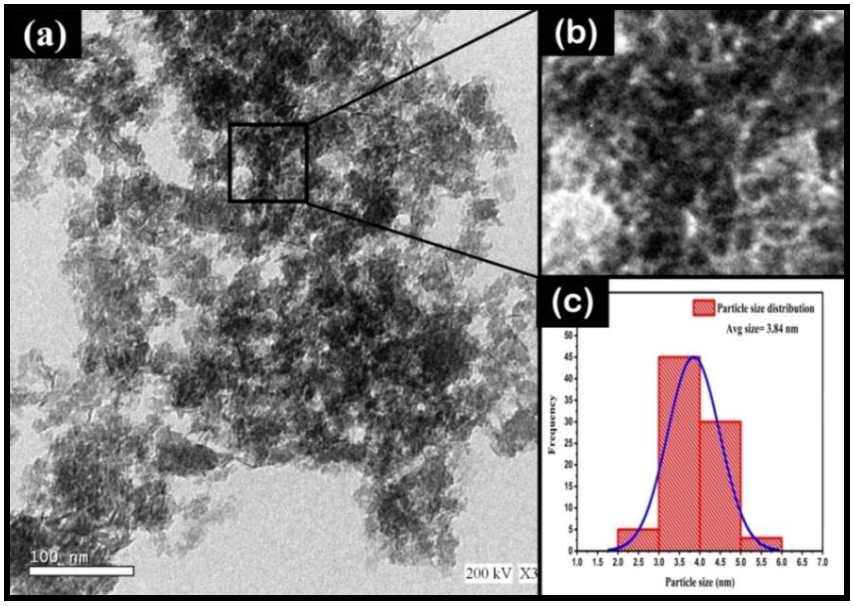

Fig. 4 (a) HR_TEM micrograph of green synthesized CdS NPs (b) Enlarged particle (c) Particle size distribution

2. Larvicidal potential of *Ocimum basillicum* and green CdS NPs

The larvicidal activities were assessed using various concentrations of both *O. basillicum* leaf extract and CdS NPs on the 3rd instar of *Cx. pipiens* larvae as shown in (Fig. 5& Table1). Results revealed that green CdS NPs of *O. basillicum* extract exhibited higher toxicity against *Cx. pipiens* when compared to the leaf aqueous extract of *O. basillicum* alone, with LC_{50} and LC_{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 basillicum* extract on mortality (%) of 3rd larval instar of *Culex pipiens*:

Conc. (g/L)	No. of larvae Tested	No. of dead larvae	Percent mortality	LC_{50} (g/L)
Control	100	1	١.٠٠	
٢٠	100	10	١٠.00	
٣٠	100	17	١٧.00	٥٢.٦٧
٤٠	100	22	٢٢.00	
٥٠	100	40	٤٠.00	
٦٠	100	64	٦٤.00	
٧٠	100	76	٧٦.00	

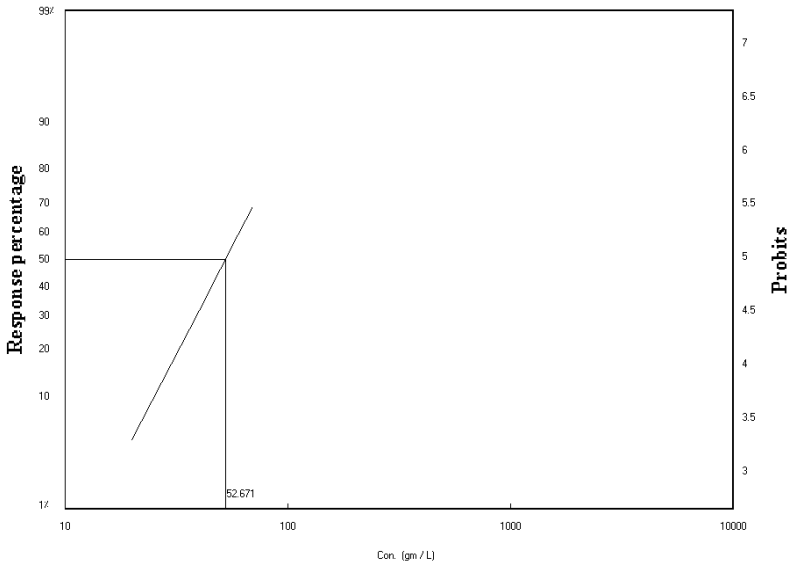

Fig. 5 Probit analysis curve of mortality rate after exposure to aqueous leaves extract of *Ocimum basillicum*

Table 2. Effect of CdS NPs synthesized by *Ocimum basillicum* extract on mortality (%) of 3rd larval instar of *Cx. pipiens*:

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

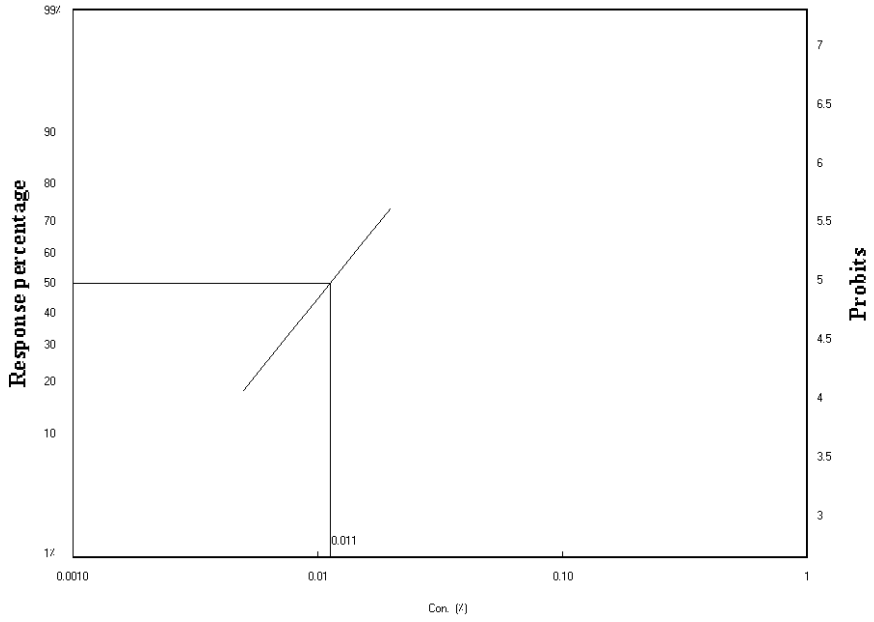

Fig. 6 Probit analysis curve of mortality rate after exposure to CdS NPs synthesized by *Ocimum basillicum* extract

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

Treatment	LC_{50} (g/L) (95% confidence limits)	LC_{90} g/L	Slope ± SE	χ^2	*P*
***O. basillicum* Extract**	52.671 (67.297- 44.743)	109.99	4.008±0.368	14.668	0.005
CdS NPs	0.011 (0.012- 0.010)	0.035	٢.٥٧±٠.٢٨٦	2.067	0.723

LC_{50} = The lethal concentration killing 50% of treated larvae; χ^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.*

Treatment	Larval mortality (%+SD)	Pupal mortality (%+SD)	Pupation (%+SD)	Malformed Pupae (%+SD)	Adult mortality (%+SD)
Control	5± 0.640[a]	6.62± 0.10[a]	95±0.640[a]	0.0± 0.0[a]	0.0± 0.0[a]
CdS NPs	51± 1.019[b]	18.36±0.249[b]	49±1.019[b]	8.16± 0.267[b]	6.25±0.20[b]

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.

Treatment	Larval Longevity (days ± SE)	Pupal Longevity (days ± SE)	Adult Longevity (days ± SE)	Adult Emergence (%)
Control	5.65±0.082[a]	1.784±0.019[a]	48.81 ±0.194[a]	97.36[a]
CdS NPs	7.26 ± 0.117[b]	2.12±0.020[b]	20.631±0.099[b]	81.63[b]

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)**.

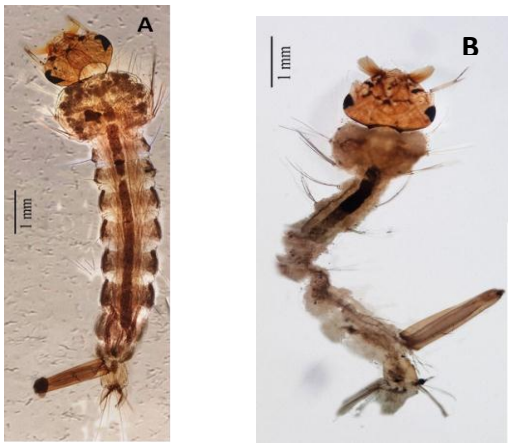

Fig. 7 Light microscopic images of *Culex pipiens* 3rd instar larvae of A. control and B. treated with green synthesized CdS NPs

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)**.

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

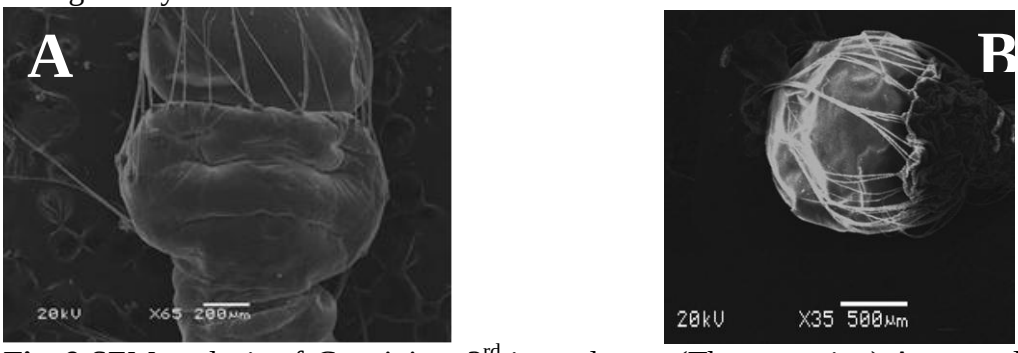

Fig. 9 SEM analysis of *Cx. pipiens* 3rd instar larvae (Thorax region) A. control B. treated with green synthesized CdS NPs

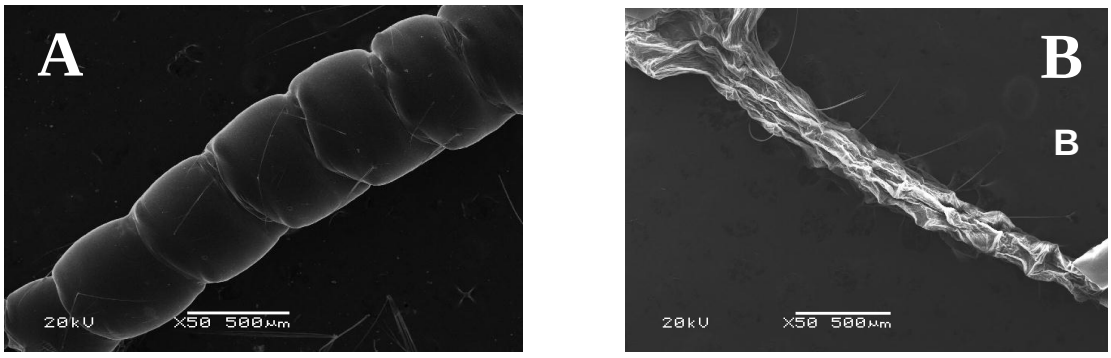

Fig. 10 SEM analysis of *Cx. pipiens* 3rd instar larvae (Abdomen region) A. control B. treated with green synthesized CdS NPs

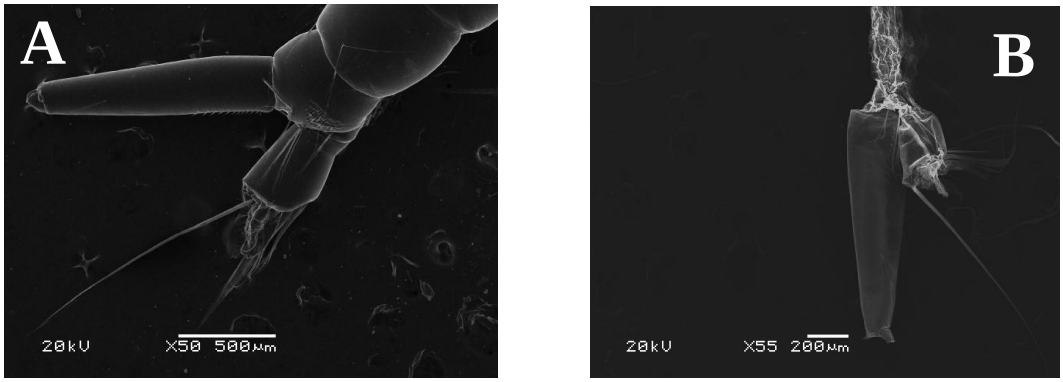

Fig. 11 SEM analysis of *Cx. pipiens* 3rd instar larvae (siphon) A. control B. treated with green synthesized CdS NPs

DISCUSSION

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 Cd^{2+} 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 LC_{50} values 52.67 and 0.011 g/L, respectively (Table 1). Very few articles discussed the effect of green CdS NPs toxicity on mosquitos; 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 LC_{50} 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 LC_{50} and LC_{90} 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. pipenes* 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.

ACKNOWLEDGMENT

Authors are grateful to Prof. Dr. Khaled Ebnalwaled A.bdel- Fatah, Professor of applied physiscs, Department of Physics, Faculty of Science, South Valley University, the director of the END Lab for providing lab facilities and also recording UV spectra.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal* of *Economic Entomology*, *18*(2)**:**265-267.

Amutha, V.; Deepak, P.; Kamaraj, C.; Balasubramani, G.; Aiswarya, D.; Arul, D.; Santhanam, P.; Ballamurugan, A. M. and Perumal, P. (2019). Mosquito-Larvicidal Potential of Metal and Oxide nanoparticles Synthesized from Aqueous Extract of the Seagrass, *Cymodocea serrulata*. *Journal of Cluster Science*, *30*(3): 797–812. https://doi.org/10.1007/s10876-019-01542-7

Barabadi, H.; Alizadeh, Z.; Rahimi, M.T.; Barac, A.; Maraolo, A.E.; Robertson, L. J.; Masjedi, A.; Shahrivar, F. and Ahmadpour, E. (2019). Nanobiotechnology as an emerging approach to combat malaria: A systematic review. *Nanomedicine: Nanotechnology, Biology and Medicine*, 18: 221–233. https:// doi.org/ 10.1016/j.nano.2019.02.017.

Benelli, G. (2015). Research in mosquito control: current challenges for a brighter future. *Parasitology Research*, 114 (8): 2801–2805. https://doi.org/10.1007/s00436-015-4586-9

Benelli, G. (2018). Mode of action of nanoparticles against insects. *Environmental Science and Pollution Research*, *25*(13)**:**12329–12341. https://doi.org/10.1007/ s11356-018-1850-4

Benelli, G.; Lo Iacono, A.; Canale, A. and Mehlhorn, H. (2016). Mosquito vectors and the spread of cancer: an overlooked connection? *Parasitology Research*. *115*(6)**:** 2131–2137. https://doi.org/10.1007/s00436-016-5037-y

Benelli, G. and Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitology Research*, *115*(5): 1747–1754. https://doi.org/10.1007/s00436-016-4971-z

Bibi, R.; Tariq, R. M. and Rasheed, M. (2020). Toxic assessment, growth disrupting and neurotoxic effects of red seaweeds' botanicals against the dengue vector mosquito *Aedes aegypti* L. *Ecotoxicology and Environmental Safety*, 195: 110451. https://doi.org/10.1016/j.ecoenv.2020.110451

Borovaya, M.; Pirko, Y.; Krupodorova, T.; Naumenko, A.; Blume, Y. and Yemets, A. (2015). Biosynthesis of cadmium sulphide quantum dots by using Pleurotus ostreatus (Jacq.) P. Kumm. *Biotechnology and Biotechnological Equipment*, *29*(6): 1156–1163. https://doi.org/10.1080/13102818.2015.1064264

Brayner, R.; Dahoumane, S. A.; Nguyen, J. N. L.; Yéprémian, C.; Djediat, C.; Couté, A. and Fiévet, F. (2011). Ecotoxicological studies of CdS nanoparticles on photosynthetic microorganisms. *Journal of Nanoscience and Nanotechnology*, *11*(3): 1852–1858. https://doi.org/10.1166/jnn.2011.3564

Briggs, J. D. (1960). Reduction of adult house-fly emergence by the effects of *Bacillus spp*. on the development of immature forms. *Journal of Insect Pathology*, *2*(4): 418–32.

Derfus, A. M.; Chan, W. C. W. and Bhatia, S. N. (2004). Probing the Cytotoxicity of Semiconductor Quantum Dots. *Nano Letters*, *4*(1): 11–18. https://doi.org/10.1021/nl0347334

Finney, D. (1971). Probit analysis. 3rd. Ed. Cambridge University Press*:* Cambridge, UK.

Govindarajan, M.; Rajeswary, M.; Veerakumar, K.; Muthukumaran, U.; Hoti, S. L.; Mehlhorn, H.; Barnard, D. R. and Benelli, G. (2016). Novel synthesis of silver nanoparticles using *Bauhinia variegata*: a recent eco-friendly approach for mosquito control. *Parasitology Research*, *115*(2): 723–733. https://doi.org/10.1007/s00436-015-4794-3

Hajra, A.; Dutta, S. and Mondal, N. K. (2016). Mosquito larvicidal activity of cadmium nanoparticles synthesized from petal extracts of marigold (*Tagetes sp.*) and rose (*Rosa sp.*) flower. *Journal of Parasitic Diseases*, *40*(4): 1519–1527. https://doi.org/10.1007/s12639-015-0719-4

Ishwarya, R.; Vaseeharan, B.; Anuradha, R.; Rekha, R.; Govindarajan, M.; Alharbi, N. S.; Kadaikunnan, S.; Khaled, J. M. and Benelli, G. (2017). Eco-friendly fabrication of Ag nanostructures using the seed extract of *Pedalium murex*, an ancient Indian medicinal plant: Histopathological effects on the Zika virus vector *Aedes aegypti* and inhibition of biofilm-forming pathogenic bacteria. *Journal of Photochemistry and Photobiology B: Biology*, 174: 133–143. https://doi.org/10.1016/j.jphotobiol.2017.07.026

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 51(1): 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146

Kandasamy, K.; Venkatesh, M.; Syed Khadar, Y. A. and Rajasingh, P. (2019). One-pot green synthesis of CdS quantum dots using *Opuntia ficus*-indica fruit sap. *Materials Today: Proceedings*, 26: 3503–3506. https://doi.org/10.1016/j.matpr.2019.06.003

Kirchner, C.; Liedl, T.; Kudera, S.; Pellegrino, T.; Javier, A. M.; Gaub, H. E.; Stölzle, S.; Fertig, N. and Parak, W. J. (2005). Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano Letters*, *5*(2): 331–338. https://doi.org/ 10.1021/nl047996m

Klug, H. P. and Alexander, L. E. (1974). X-ray diffraction procedures: for polycrystalline and amorphous materialse. 2. Auflage. John Wiley & Sons, New York-Sydney-Toronto.

Li, K. G.; Chen, J. T.; Bai, S. S.; Wen, X.; Song, S. Y.; Yu, Q.; Li, J. and Wang, Y. Q. (2009). Intracellular oxidative stress and cadmium ions release induce cytotoxicity of unmodified cadmium sulfide quantum dots. *Toxicology in Vitro*, *23*(6): 1007–1013. https://doi.org/10.1016/j.tiv.2009.06.020

Liu, Z. L.; Liu, Q. Z.; Du, S. S. and Deng, Z. W. (2012). Mosquito larvicidal activity of alkaloids and limonoids derived from *Evodia rutaecarpa* unripe fruits against *Aedes albopictus* (Diptera: Culicidae). *Parasitology Research*, *111*(3): 991–996. https://doi.org/10.1007/s00436-012-2923-9

Ma-Hock, L.; Brill, S.; Wohlleben, W.; Farias, P. M. A.; Chaves, C. R.; Tenório, D. P. L. A.; Fontes, A.; Santos, B. S.; Landsiedel, R.; Strauss, V.; Treumann, S. and Van Ravenzwaay, B. (2012). Short term inhalation toxicity of a liquid aerosol of CdS/$Cd(OH)_2$ core shell quantum dots in male Wistar rats. *Toxicology Letters*, *208*(2): 115–124. https://doi.org/10.1016/j.toxlet.2011.10.011

Murugan, K.; Anitha, J.; Suresh, U.; Rajaganesh, R.; Panneerselvam, C.; Aziz, A. T.; Tseng, L. C.; Kalimuthu, K.; Alsalhi, M. S.; Devanesan, S.; Nicoletti, M.; Sarkar, S. K.; Benelli, G. and Hwang, J. S. (2017). Chitosan-fabricated Ag nanoparticles and larvivorous fishes: a novel route to control the coastal malaria vector *Anopheles sundaicus*? *Hydrobiologia*, *797*(1): 335–350. https://doi.org/ 10.1007/s10750-017-3196-1

Murugan, K.; Panneerselvam, C.; Subramaniam, J.; Madhiyazhagan, P.; Hwang, J. S.; Wang, L.; Dinesh, D.; Suresh, U.; Roni, M.; Higuchi, A.; Nicoletti, M. and Benelli, G. (2016). Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. *Environmental Science and Pollution Research*, *23*(16): 16671–16685. https://doi.org/10.1007/s11356-016-6832-9

Pankove, J. (1971). Optical processes in semiconductors Prentice-Hall. New Jersey, 92.

Pavela, R. (2008). Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus Say* larvae (Diptera: Culicidae). *Parasitology Research*, *102*(3): 555–559. https://doi.org/10.1007/s00436-007-0821-3

Priyadarshini, K. A.; Murugan, K.; Panneerselvam, C.; Ponarulselvam, S.; Hwang, J. S. and Nicoletti, M. (2012). Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi Liston* (Diptera: Culicidae). *Parasitology Research*, *111*(3): 997–1006. https://doi.org/10.1007/s00436-012-2924-8

Ragab, G. A. and Saad-allah, K. M. (2020). Ecotoxicology and Environmental Safety Green synthesis of sulfur nanoparticles using *Ocimum basilicum* leaves and its prospective e ff ect on manganese-stressed *Helianthus annuus* (L .) seedlings. *Ecotoxicology and Environmental Safety*, 191: 110242. https://doi.org/10.1016/ j.ecoenv.2020.110242

Ragavendran, C. (2015). Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. *International Journal of Mosquito Research*, *2*(2): 01–08.

Rubab, S.; Hussain, I.; Ali, B.; Ayaz, K.; Unar, A.; Abbas, 3K. A. and Khichi, Z. H. (2017). Biomedical Description of *Ocimum basilicum L* . Journal *of* Islamic International *Medical* College, *12*(1): 59–67.

Singh, V. and Chauhan, P. (2009). Structural and optical characterization of CdS nanoparticles prepared by chemical precipitation method. *Journal of Physics and Chemistry of Solids*, *70*(7): 1074–1079. https://doi.org/10.1016/j.jpcs.2009.05.024

Sujitha, V.; Murugan, K.; Dinesh, D.; Pandiyan, A.; Aruliah, R.; Hwang, J. S.; Kalimuthu, K.; Panneerselvam, C.; Higuchi, A.; Aziz, A. T.; Kumar, S.; Alarfaj, A. A.; Vaseeharan, B.; Canale, A. and Benelli, G. (2017). Green-synthesized CdS nano-pesticides: Toxicity on young instars of malaria vectors and impact on enzymatic activities of the non-target mud crab *Scylla serrata*. *Aquatic Toxicology*, 188: 100–108. https://doi.org/10.1016/j.aquatox.2017.04.015

Tapondjou, A. L.; Adler, C.; Fontem, D. A.; Bouda, H. and Reichmuth, C. (2005). Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais Motschulsky* and *Tribolium confusum du Val*. *Journal of Stored Products Research*, *41*(1): 91–102. https://doi.org/10.1016/j.jspr.2004.01.004

Teimouri, M.; Khosravi-Nejad, F.; Attar, F.; Saboury, A. A.; Kostova, I.; Benelli, G. and Falahati, M. (2018). Gold nanoparticles fabrication by plant extracts: synthesis, characterization, degradation of 4-nitrophenol from industrial wastewater, and insecticidal activity – A review. *Journal of Cleaner Production* 184: 740–753. https://doi.org/10.1016/j.jclepro.2018.02.268

Vaidyanathan, R.; Kalishwaralal, K.; Gopalram, S. and Gurunathan, S. (2009). Nanosilver-The burgeoning therapeutic molecule and its green synthesis. In Biotechnology Advances, *27*(6): 924–937. https://doi.org/ 10.1016/j.biotechadv.2009.08.001

WHO. (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization, 1–41. http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?ua=1

WHO. (2019). Guidelines for Malaria Vector Control. In Guidelines for Malaria Vector Control. https://apps.who.int/iris/bitstream/handle/10665/310862/9789241550499-eng.pdf?ua=1%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/30844152

Williams, K. A.; Green, D. W. J.; Pascoe, D. and Gower, D. E. (1986). The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera: Chironomidae) and its ecological significance for pollution regulation. *Oecologia*, *70*(3): 362–366. https://doi.org/10.1007/BF00379498

Zahran, H. E. D. M.; Abou-Taleb, H. K. and Abdelgaleil, S. A. M. (2017). Adulticidal, larvicidal and biochemical properties of essential oils against *Culex pipiens* L. *Journal of Asia-Pacific Entomology*, *20*(1): 133–139. https://doi.org/ 10.1016/j.aspen.2016.12.006