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Comparative Analysis of Larvicidal Efficacy of Silver and Copper Nanoparticles Synthesized Leaf Extract of *Ocimum Basilicum* against *Epilachna Vigintioctopunctata*

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Abstract

The synthesis of green nanoparticles was accomplished using plant extract and environmentally friendly reducing agents. The goal of the current study was to evaluate the larvicidal activities of synthesized silver and copper nanoparticles against larvae and adult *Epilachna vigintioctopunctata* using an aqueous leaf extract of *Ocimum basilicum* (OB). For 24 hours, larvae and adults were exposed to various concentrations of an aqueous extract of *O. basilicum*, as well as an extract made with silver and copper nanoparticles. *O. basilicum* leaf extract was used to create AgNPs and CuNPs, and within 6 hours, nanoparticle formation was visible. FTIR, a particle size analyzer, and UV-vis spectrophotometer were used to confirm the successful synthesis of AgNPs and CuNPs. By using a GC-MS study, the phytochemicals found in leaf extract were investigated. *O.basilicum* leaf extract was used to create AgNPs and CuNPs, and larvicidal activity of the aqueous leaf extract's LC50 and LC90 values were calculated. The larvicidal efficacy of aqueous extract, AgNPs, and CuNPs synthesized leaf extract were

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compared. The larvicidal activity of the leaf extract produced by CuNPs was marginally inferior to that of the leaf extract produced by AgNPs.

Keywords: AgNPs, CuNPs, larvicidal activity, *Ocimum basilicum*, Probit analysis.

Introduction

The main phytophagous pest of the Solanaceae family, *Epilachna vigintioctopunctata* causes significant economic loss. Locally known as hadda beetles, epilachna beetles are widespread across the nation and significantly harm a number of solanaceous, cucurbitaceous, and leguminous crops (Islam *et al.,* 2011). Eco-friendly insecticides made from plant extracts are created for pest control because the use of synthetic insecticides against these phytophagous pests can reduce the quality of agricultural products and even have negative health effects. Plants can be used to quickly, cheaply, sustainably, and only require one step in the biosynthesis process in order to create nanoparticles (Huang *et al.,* 2007). According to Elemike et al., (2019). Plant-mediated nanoparticle synthesis is preferred among the available synthesis techniques because it is economical, environmentally friendly, and safe for therapeutic use in humans (Kumar and Yadav, 2009). Numerous studies on the biological control of *E. vigintioctopunctata* have been conducted. There have been no previous attempts to control the dangerous phytophagous pest E. *vigintioctopunctata* using leaf extract synthesized with nanoparticles. In the current study, the effectiveness of leaf extracts synthesized with copper nanoparticles (CuNPs) and silver nanoparticles (AgNPs) against the larva and adult of *E. vigintioctopunctata* was determined.

Materials and Methods

Plant materials:

From July 2019 to March 2020, the *O.basilicum* leaves were gathered in and around Meenampatti, Sivakasi Taluk. The study area is located between 9° 27' 2.6424" north latitude and 77° 48' 26.0496" east longitude. The plant species was verified by taxonomists at the Centre for Research and Post-Graduate Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu, India.

Ocimum basilicum leaf extract preparation:

The newly plucked leaves of the plant were thoroughly cleaned in deionized waster and running tap water to remove the dust. The leaves were pat dried with paper towels and allowed to dry in shade at room temperature. Using an electrical mixer, these dried leaves were mechanically ground into powder. Aqueous extract was produced by mixing 10g of dried leaf powder with 100 ml of double-distilled water. This mixture was well blended, left to stand undisturbed for five hours, and then filtered through Whatman No. 1 filter paper. Before the filtrate was used to determine the larvicidal activity against the target vector, *O. basilicum* leaves were washed with tap water and shade dried at room temperature from 5-7 days. Using an electric blender, the air-dried plant materials were powdered. For extraction, 10g of fine leaf powder that had been precisely weighed was taken and put in a beaker with 100ml of double-distilled water that had been sterilized. It was then boiled at 60 ^{o} C for 30 minutes. After being filtered through Whatman filter paper no. 1, the prepared leaf extract was kept at -20°C and could be used within a week.

Gas chromatography-mass spectrometry for phytochemical analysis:

Agilent GC7890A / MS 5975C and a capillary column Agilent DB5MS (30m × 0.25mm and 0.25 film thickness) were used in the GC-MS to analyze the leaf extract of *O. basilicum*. Set the MS (mass spectrometer) to 70 eV & computer libraries built into WILEY7, NIST05, and NIST05s were used to identify unknown compounds using probability-based matching.

Synthesis of silver nanoparticles from leaf extract:

Silver nitrate served as the precursor in the production of siler nanoparticles. Analytical-grade silver nitrate ($AgNO_3$) in a concentration of 16.96 mg was precisely measured out and was dissolved in 90 ml Milli-Q water. In an Erlenmeyer flask 90 ml of prepared 1 mM aqueous $AgNO_3$ Solution were combined 10 ml of aqueous leaf extract and before being incubated at ambient temperature in dark. The aqueous leaf extract containing 1mM of $AgNO_3$ allegedly led to the synthesis of AgNPs and a change in colour from pale yellow to dark brown, according to Lingarao and Savithramma (2013).

Synthesis of Copper nanoparticles from leaf extract:

A clear liquid was obtained after stirring an accurately weighed 0.49g of $CuSO_4.5H_2O$ for 30 minutes with a magnetic stirrer in 20ml of deionized water. 20 ml of leaf extract was added to the aforementioned solution and stirred for an additional 3 hours. The color was observed to have changed from a light green to a dark green. Using aqueous ammonia solution as a precipitating agent all the reaction's pH to remain constant. Following ethanol and water washing, the precipitated CuNPs were dried at 60 °C.

Nanoparticle Characterization:

The preliminary description of silver nanoparticles was completed using UV-Visible spectroscopy. To determine the various kinds of chemical bonds between the bioactive compounds of silver nitrate and copper sulphate synthesized leaf extract, FTIR measurements were performed using an FTIR-8400S-spectrophotometer (Shimadzu International Co. Ltd, Tokyo, Japan). The mean particle size was measured using Shimadzu SALD-2300 (WingSALD II: version 3.1.1.) for the silver and copper nanoparticle synthesized leaf extract. All measurements were carried out at a temp. of 25°C and a detection angle of 90°. The topographic make-up of the nanoparticles made from leaf extract was determined using SEM analysis. The created copper and silver nanoparticles were evenly distributed, platinum was sputter coated using an ion coated for 120s, and they were then examined with a SEM made by Carl Zeiss in the USA, model number EV018. Quantax 200 with X Flash 6130 was used for Energy Dispersive X-ray (EDX) spectroscopy, a part of SEM, to identify the elements arranged in the analyte sample.

Epilachna beetle rearing:

In order to obtain a huge number of epilachna larva continuously for experimental use a mass culture was maintained in laboratory at the department of zoology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu, India by providing the brinjal leaves as a feed. The adults of *E.vigintioctopunctata* were collected from the infested brinjal plant cultivated in the nearby agrifarm, Sivakasi. Bioassays were conducted using the laboratory-raised epialchna larvae, and the cultures persisted throughout the research period. The larvae hatched from the eggs laid in the laboratory culture were fed with fresh brinjal leaves, and they were allowed to grow until the 3rd instar stages for the study.

Larvicidal activity:

In a lab setting, the larvicidal abilities of the leaf extract were investigated. By dilution with de-ionized water, various concentrations of aqueous and synthetic silver & copper nanoparticles synthesized leaf extracts were made. The concentrations were 100, 50, 25, 12.5 and 6.25 mg/ml of each extract. A spray test was done to determine the leaf extract's larvicidal potential. Different concentration of extracts was prepared, sprayed on the brinjal leaf and given as the feed for larvae of epialchna beetles. Ten larvae per were exposed to observe the larvicidal activity of each concentration of extracts. Three replicates of each concentration were given to each group. 24 hours after the exposure period, the mortality rate was calculated. For each concentration, the overall percentage of larval mortality from the ten replicates of the dead larvae was calculated.

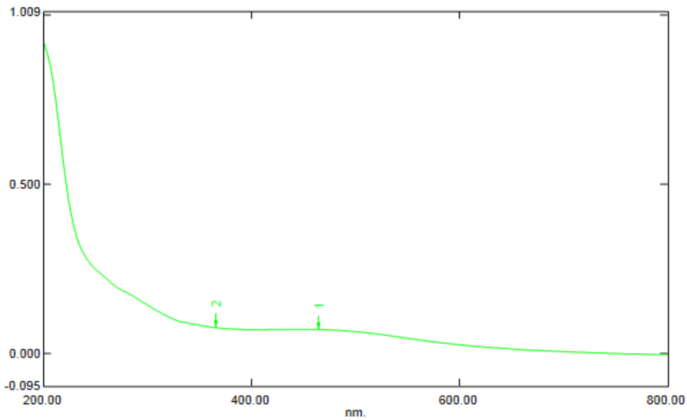
Statistical evaluation:

The results of the analysis were statistically examined to determine how reliable they were. The current experiment's data on larval mortality were analyzed using computer software using two statistical techniques: mean separation and standard deviation. The LC50 and upper and lower confidence limits at a 95% level were calculated using a probit analysis on the dose response mortality data. The values were computed using the SPSS 2007 software.

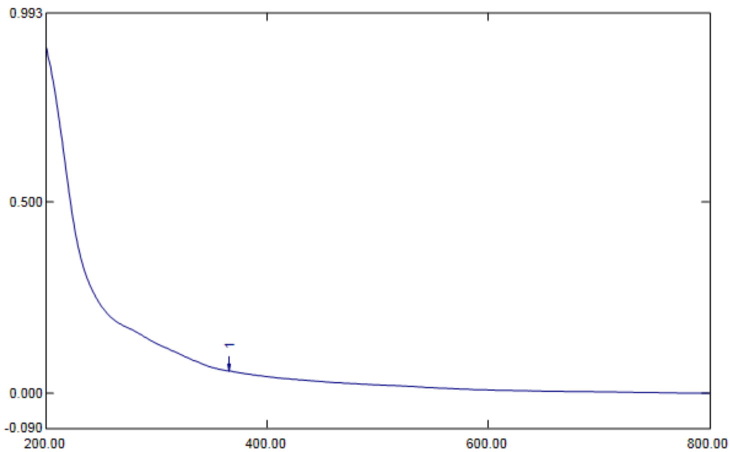
3. Results

GC-MS analysis of leaf extract:

O.basilicum leaf extract GC-MS spectra revealed 30 substances, 12 of which were recognized as phytochemicals and the remaining 14 were unidentified (Fig1).



(a)



(b)

Fig1: UV-Visible Spectral analysis of Nanoparticles synthesis leaf extract (a) AgNPs synthesis extract (b)CuNPs synthesized extract

Comparing mass spectra, retention times, and a library of common compounds allowed researchers to confirm the presence of phytochemical compounds. The discovered substances are listed in Table 1.

Sl. #	Retention time (RT)	% Area of Peak	Analyzed compounds	Mol. For.	Molecular weight (in g/mol)
1	6.153	5.50	4-Mercaptophenol	$C_6H_6O_4$	126.18
2	8.220	2.85	Heptanoic acid	$C_7H_{14}O_2$	130.187
3	9.875	1.74	Benzenemethanol	C_7H_8O	108.140
4	11.508	2.43	Piperazine	$C_4H_{10}N_2$	86.138
5	14.297	3.98	Benzenemethano	C_7H_8O	108.140
6	14.363	2.85	Propanedioic acid	$C_3H_4O_4$	104.061
7	16.241	2.15	Nonanoic acid	$C_9H_{18}O_2$	158.241
8	18.041	2.51	Benzyl .beta.-d-glucoside	$C_{13}H_{16}O_7$	284.264
9	19.429	4.24	Cyclohexanol	$C_6H_{11}OH$	100.158
10	19.518	2.17	2-Allylphenol	$C_9H_{10}O$	134.18
11	19.740	3.97	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258.40
12	20.107	2.94	5,6,7,8-Tetrahydroquinoxaline	$C_8H_{10}N_2$	134.1784

Table 1: Analyzed phytochemicals form leaf extract of *O.basilicum*

Visible observation:

Using *O.basilicum's* aqueous leaf extract silver and copper nanoparticles were made in a green manner. The visible observation of color change served as the initial confirmation of the silver nanoparticles in green manner. The initial suspension of Leaf extract was yellow in color. After the addition of silver nitrate and copper sulphate, the color changed to pale yellow and light brown, and from dark blue to dark brown, respectively, after incubating at room temperature for an entire night.

Characterization of leaf extract:

UV-visible spectral analysis:

The Surface Plasmon Resonance (SPR) is analyzed using an ultraviolet-visible spectrum. AgNPs and CuNPs both had UV-visible spectra that fell between 365 and 400 nanometers (nm) in wavelength, respectively (Fig. 1).

FTIR (Fourier Transform-Infrared) analysis:

The vibrational spectra of leaf extract synthesized with both AgNPs and CuNPs were displayed in Fig 2.

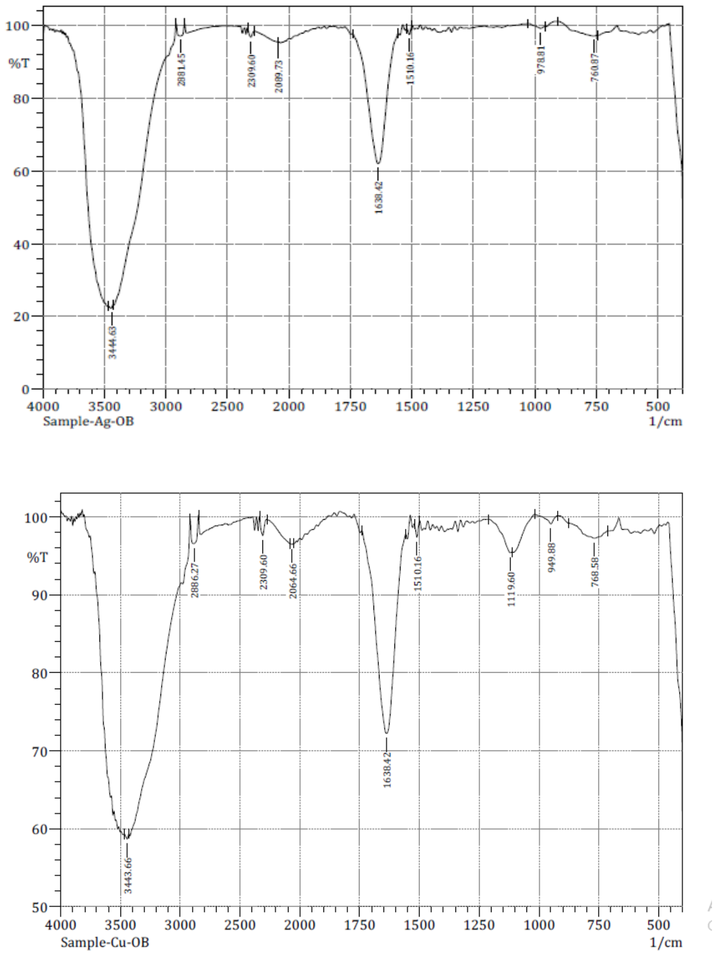


Fig2: FTIR analysis of Nanoparticles synthesis leaf extract (a) AgNPs synthesis extract (b)CuNPs synthesized extract

The water molecule found in the plant extract was responsible for the distinctive peak that appeared at 3453.31 and 3443.66. The peak at 760.87 was allocated to the vibrational stretching group of C-CL. The alkenes group is present in the AgNPs synthesized extract as indicated by the values 978.81 and 1638.42. The peat at 1510.16 proves the presence of aromatic compounds. The absorption peaks at 2089.73 and 2881.45 with C-H stretch and C-H stretch, respectively, indicate the presence of the alkanes group. The presence of alkynes in the AgNPs-synthesized leaf extracts with a C–C stretch is indicated by the peak at 2309.6. (Table 2a).

Sl. #	Absorption (cm^{-1})	Class of compounds	Bond
1	760.87	Alkyl halide	C-CL stretch
2	978.81	Alkenes	C-H bend
3	1510.16	Aromatic compound	C=C stretch
4	1638.42	Alkenes	C=C stretch
5	2089.73	Alkanes	C-H stretch
6	2309.6	Alkynes	C≡C stretch
7	2881.45	Alkanes	C-H stretch
8	3453.31	Alcohol	O-H stretch

Table 2a. Functional groups present in AgNPs synthesized leaf extract of OB revealed by FT-IR

In CuNPs synthesized leaf extracts nine peaks were appeared at 768.58, 949.88, 1119.6, 1510.16, 2084.91, 2064.66, 2309.6, 2886.27, 3443.66 indicates the presence of alkyl halide, alkenes, alkyl halide, aromatic compound, alkanes, alkanes, alkynes, alkanes, alcohol respectively with C-CL stretch, C-H bend, C-F stretch, C=C stretch, C-H stretch, C-H stretch, C≡C stretch, C-H stretch, O-H stretch. (Table 2b).

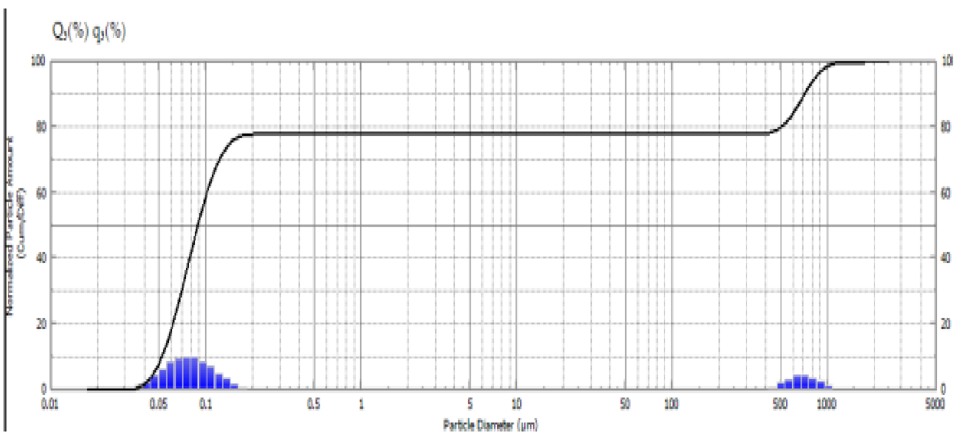
Sl. #	**Absorption (cm^{-1})**	**Class of compounds**	**Bond**
1	768.58	Alkyl halide	C-CL stretch
2	949.88	Alkenes	C-H bend

Sl. #	Absorption (cm^{-1})	Class of compounds	Bond
3	1119.6	Alkyl halide	C-F stretch
4	1510.16	Aromatic compound	C=C stretch
5	2084.91	Alkanes	C-H stretch
6	2064.66	Alkanes	C-H stretch
6	2309.6	Alkynes	C≡C stretch
7	2886.27	Alkanes	C-H stretch
8	3443.66	Alcohol	O-H stretch

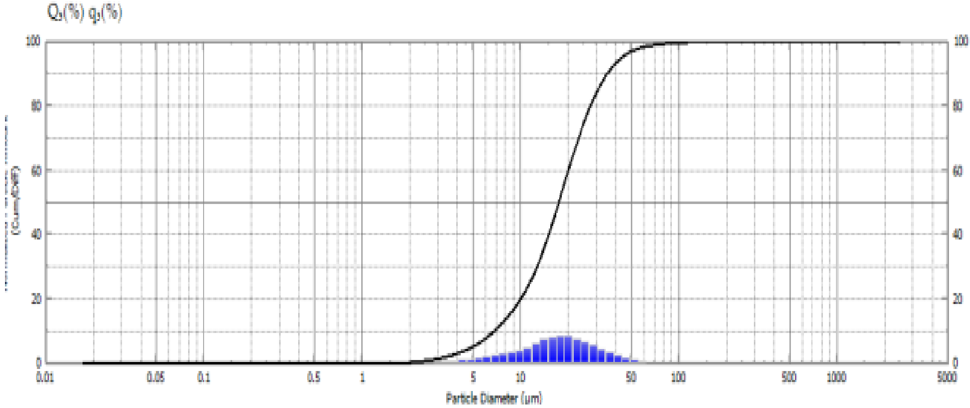
Table 2b. Functional groups present in CuNPs synthesized leaf extract of OB revealed by FT-IR

Particle size analysis:

Particle size of the synthesized AgNPs and CuNPs leaf extract was determined using dynamic light scattering as shown in Fig 3. In general, the diameter of OB-AgNPs and OB-CuNPs were both 15 nm (Fig 3).



(a)



(b)

Fig 3: Particle size analysis of Nanoparticles synthesis leaf extract (a) AgNPs synthesis extract (b)CuNPs synthesized extract

Larvicidal activity:

Probit analysis was used to determine the LC50 values for the AgNPs and CuNPs synthesized leaf extracts against 3rd instar larvae. (Table 3). Larval mortality improved with increase in the concentration of leaf extract. The LC_{50} of the AgNPs synthesized leaf extract of *O.basilicum* was 186.21 ppm and LC_{50} of the CuNPs synthesized against *O.basilicum* was 412.09 ppm (Table 3). When comparing the AgNPs and CuNPs synthesized leaf extracts AgNPs were more efficient than CuNPs synthesized leaf extract.

Types of leaf extract	**Test solution**	**LC_{50} values in ppm**
Silver nanoparticles of *O.basilicum*	AgNPs synthesized from leaf extract of *O.basilicum* leaves	186.21
Copper nanoparticles of *O.basilicum*	CuNPs synthesized from leaf extract of *O.basilicum* leaves	412.09

Table 3. LC_{50} value of the test solutions AgNPs and CuNPs synthesized leaf extract of OB against larvae of epilachna.

4. Discussion

Recent years have been a significant increase in biosynthesis of nanoparticles as a result of the pressing need for material scientists to create environmentally friendly technologies. For instance, a lot of work has gone into using plants to biosynthesize nanoparticles, particularly metal nanoparticles (Lloyd *et al.,* 2011). Silver, gold, and platinum have been synthesized into metallic nanoparticles that are typically used in pharmaceuticals and insect vector control (Mittal *et al.,* 2013).

According to the results of the current study, *O. basilicum*'s aqueous leaf extract has the ability to reduce copper ions and transform copper sulphate into copper nanoparticles while also having larvicidal effects on epilachna beetles. By reducing (Ag+ into Ag0), it has the potential to also turn silver nitrate into silver nanoparticles. Kaviya *et al.,* (2011) reported similar research on samples of *Polyalthia longifolia*, whose color changes from nearly colorless to brown.

Comparing mass spectra, retention times, and a library of common compounds helped to confirm the presence of phytochemical compounds in leaf extract. The molecular formula of the discovered phytochemicals is shown in Table 1. Mishra and Patnaik's 2020 methanol extract of the *Withania somnifera* whole plant produced results that were comparable. They stated that Athere are 12 different bioactive compounds in the *W. somnifera* extract.

A popular method for describing the optical characteristics of the created NPs is UV-Vis spectroscopy. A typical AgNP absorption peak is centered at 43-20 nm and depends on the size and polydispersity of the NPs (Anandalakshmi *et al.,* 2016). According to Rajagopal *et al.,* (2021), the UV-Vis spectra of the CuNPs produced using *Wrightia tinctoria* showed 357 nm as the maximum absorption peak.

In order to pinpoint the potential biomolecules in charge of silver nanoparticles reduction, capping and effective stabilization, FTIR measurements are made (Padalia *et al.,* 2015). It was demonstrated that NPs were synthesized by the presence of functional groups like alcohol, halides, alkanes, alcohol, and aromatic compounds (Prabha et al., 2020). The biomolecules that these functional groups serve to reduce, cap and stabilize NPs (Sharon *et al., 2018).*

Size, shape and morphology are all significantly influences by surface plasmon resonance bands (Philio, 2010). Lalitha *et al.*, (2013) reported similar findings in Azhadirachta indica. According to Rajagopal *et al.,* (2021), the *Wrightia tinctoria* based CuNPs had an average diameter of 15 nm.

The larvicidal activity of aqueous extract, silver and copper nanoparticles made from *O.basilicum* against epilachna beetle was observes in this study. According to the LC50 values determined in this study, epilachna beetles are highly susceptible to the larvicidal effects of both AgNPs and CuNPs. Similar findings from 72-hour dose-mortality data on the third instar larvae of *E. vigintioctopunctata* were reported by Islam *et al.,* (2011). The LC50 values were 18.40%, 23.70%, and 29.61% for *R.communis, C.procera*, and *D.metal,* respectively. India, which has a wide variety of medicinal plants, can use these plants for this purpose because they are not only effective pest and insecticides but also effective antiparasitic, antifungal, antimalarial, antimicrobial agents. and As a result, it has been widely used for the above said qualities, in the current research, synthesized AgNPs and CuNPs from the plant source *O.basilicum* to act as a larvicidal agent against *E.vigintioctopunctata.*

5. Summary

Based on the results of the current research work, *O.basilicum* leaf extract has larvicidal efficacy against the epilachna beetle when used as an aqueous extract and also when used as a nanoparticle synthesized extract. AgNPs were more effective than CuNPs against the epilachna beetle when comparing the effectiveness of the two nanoparticles made from leaf extract. As a result, the current study provided evidence that silver nanoparticles mediated by *Ocimum basilicum* could be effective larvicidal agents against *E. vigintioctopunctata.*

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Conflicts of interest

The authors have no conflict of interest.

Funding disclosure

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