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EGYPTIAN ACADEMIC JOURNAL OF

# BIOLOGICAL SCIENCES

## MEDICAL ENTOMOLOGY & PARASITOLOGY

ISSN
2090-0783

WWW.EAJBS.EG.NET

**Vol. 13 No. 1 (2021)**

**Citation:** *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.13(1) pp* ***47-56****(2021)*
**DOI: 10.21608/EAJBSE.2021.188311**

Egypt. Acad. J. Biology. Sci., 13 (1):47–56 (2021)

Egyptian Academic Journal of Biological Sciences
E. Medical Entomology. & Parasitology

ISSN: 2090–0783
www.eajbse.journals.ekb.eg


# Larvicidal Activity of Green Synthesized Silver Nanoparticles Using Different Plant Extracts Against *Culex pipiens* L. (Diptera: Culicidae)

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**ARTICLE INFO**
**Article History**
Received:25/4/2021
Accepted:22/6/2021

***Keywords:***
Larvicidal, *Culex pipiens*, *Peganum harmala*, *Capparis sinaica*, *Salvia multicaulis*..

**ABSTRACT**

The activity of silver nanoparticles synthesized using aqueous extracts from leaves of *Peganum harmala*, *Capparis sinaica* and *Salvia multicaulis* against *Culex pipiens* third larval instar was tested. Biosynthesized silver nanoparticles (AgNPs) were characterized by Transmission Electron Microscope studies (TEM) and Ultraviolet-Visible (UV/VIS) spectroscopy. The TEM images revealed that the major population of the silver nanoparticles is found within the 10 to 22 nm range. Ultraviolet-Visible (UV/VIS) spectrophotometer analysis for synthesized AgNPs showed peaks between $\lambda_{max}$: 407-410 corresponding to the plasmon absorbance of the AgNPs. Results of larvicidal activity showed that, biosynthesized AgNPs have a great larvicidal activity against *C. pipiens* larvae at different concentrations ranging from 8.0 to 55.0 ppm, depending on plant extract used in synthesization and concentration of AgNPs. Complete larval mortality percent (100.0%) was recorded by AgNPs synthesized using aqueous extracts from leaves of *P. harmala*, *Ca. sinaica* and *S. multicaulis* at 20.0, 40.0 and 55.0 ppm, respectively. Based on $LC_{50}$ and $LC_{90}$ values, *P. harmala*-synthesized AgNPs was more effective against 3$^{rd}$ instar larvae of *C. pipiens* than those of *Ca. sinaica* and *S. multicaulis.*

## INTRODUCTION

Mosquitoes are carriers of many human and animal pathogenic agents worldwide causing millions of deaths annually and hundreds of millions of clinical cases (Dhanasekaran *et al.*, 2010; Roth *et al.*, 2010). In Egypt, *Culex pipiens* is the most widely distributed mosquito and is the main vector of lymphatic filariasis, *Wuchereria bancrofti* and Western Nile virus (Shehata, 2018). The indiscriminate use of chemical insecticides inducing several problems such as environmental pollution, insecticide resistance and toxic effects on human beings (Vijayakumar and Amirthanathn, 2014).

In controlling the continuous increase in mosquitos' population, Immature stages (eggs, larvae and pupae) are usually targeted by organochlorine, carbamates, pyrethrins and pyrethroids and organophosphourous (Shehata, 2019; Shehata *et al.*, 2019). However, the usage of these synthetic insecticides is not safer for the environment and non-target organisms, as well as, the development of resistance to these chemical insecticides (Shaalan, 2005; Liu *et al.*, 2006; Bilal *et al.*, 2012).

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Biosynthesis of nanoparticles and its wide applications in different areas such as medicine, environment, controlling various endemic diseases and biotechnology has become an area of concern (Elemike *et al.,* 2017; Hassanain *et al.,* 2019). Synthesized nanoparticles have several advantages of being eco-friendly, attributed to the absence of deadly chemicals in its synthesis (Bhosale *et al.,* 2014). Thus, efforts to use green-synthesized nanoparticles as new mosquitos' control agents remain indispensable.

*Peganum harmala, Capparis sinaica* and *Salvia multicaulis* used in the present study are medicinal plants found in Saint Catherine, South Sinai Governorate, Egypt and are used in folk medicine. *Peganum harmala* has different pharmacological effects due to the presence of alkaloids, especially harmine and harmaline including gastrointestinal, cardiovascular, antimicrobial, antidiabetic, osteogenic, emmenagogue, immunomodulatory and antitumor activity (Moloudizargari *et al.,* 2013). In addition, in vitro antiviral activity of *C. sinaica* against highly pathogenic avian influenza virus (H5N1) has been approved by Ibrahim *et al.*, (2013). On the other hand, Tavan *et al.*, 2020 approved the antimicrobial and antioxidant activities of *S. multicaulis* and attributed these activities to a wide variety of phenolic acids and flavonoids. Also, *S. multicaulis* extracts and essential oils isolated in Turkey approved to have antimicrobial and antioxidative activities (Tepe *et al.*, 2004).

The present study aimed at investigating the larvicidal activity of silver nanoparticles synthesized using aqueous extracts from leaves of *P. harmala*, *Ca. sinaica* and *S. multicaulis* against *Culex pipiens* larvae.

## MATERIALS AND METHODS

### 1.Colonization of *Culex pipiens:*

*Culex pipiens* larvae were provided by the Medical Entomology Institute, Dokki, Giza and reared for five generations in Mosquito insectary, Animal House, Faculty of Science, Al-Azhar University. The rearing procedure described by **Shehat *et al.,* (2020)** was applied.

### 2.Preparation of Tested Silver Nanoparticles (AgNPs):

#### Preparation of Aqueous Extracts:

Leaves of *P. harmala, Ca. sinaica* and *S. multicaulis* were washed and dried away from sun rays for five days at room temperature. Dried leaves were pulverized to powder separately using an electrical stainless-steel blender (Philips, HR2058). Four grams of *P. harmala, Ca. sinaica* and *S. multicaulis* leaves powder were boiled with 100 ml distilled water in a water bath for three minutes. Then, the solution was filtered and kept in the refrigerator at 4ºC until use (Mondal *et al.,* 2014).

#### Preparation of $AgNO_3$ Solution:

As described by Mondal *et al.,* (2014), 0.17g $AgNO_3$ (purchased from El-Go-mhouria Co. for Trading Pharmaceuticals, Chemicals & Medical Appliances, Cairo), was dissolved in 100 ml distilled water to prepare $AgNO_3$ stock solution.

#### Synthesis of Silver Nanoparticles (AgNPs):

The leaves extract of *P. harmala, Ca. sinaica* and *S. multicaulis* were mixed separately with $AgNO_3$ solution in the ratio of 1:9 and incubated at room temperature (26±2°C) for 72 hours until the appearance of reddish-brown color proving the formation of AgNPs (Shehata and Mahmoud, 2019).

### 3.Characterization of Tested Silver Nanoparticles (AgNPs):

#### Transmission Electron Microscope Studies (TEM):

The AgNPs suspensions were sonicated for 10 min and diluted to slight turbid suspensions. The AgNPs suspensions were subjected to JEOL, JEM-2100 high-resolution transmission electron microscope **(TEM)** at an accelerating voltage of 200 kV, respectively. Studied at NanoTech Egypt for Photo-Electronics, El-Wahaat Road, Dream Land City, Entrance 3, City of 6 October, Al Giza, Egypt.

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**Ultraviolet-Visible (UV/VIS) Spectroscopy:**

The AgNPs suspensions were diluted from 1 to 10 times by distilled water from colloidal solutions obtained from the synthesis process. The UV/VIS spectroscopy of suspension was carried out using UV-Vis spectrophotometer (Type: Evolution™ 300, Serial number: EVon 10600z, from Thermo Scientific, Fig. 5). The UV/VIS spectroscopy was carried out in the General analysis room in the Department of Chemistry, Faculty of Science, Ain shams University, Abbasia, Cairo, Egypt.

**4.Larvicidal Activity of Tested Silver Nanoparticles (AgNPs):**

Larvicidal activity of tested silver nanoparticles (AgNPs) was carried out using the previously described procedure of Hassanain *et al.,* (2019). All tested materials were prepared in 250 ml of dechlorinated tap water contained in 500 ml plastic cups. Then, twenty-five larvae from the third larval instar were put immediately into plastic cups that contained different concentrations of AgNPs. Three replicates were usually used. All values were calculated as Mean±SD.

**5.Statistical Analysis:**

All data were subjected to GraphPad InStat software, Inc. according to the method of lentner *et al.*, (1982) for the statistical analysis. Lethal concentrations ($LC_{50}$ and $LC_{90}$) were calculated using multiple linear regressions (Finney, 1971). All results represented as Mean±SD.

## RESULTS

**Characterization of synthesized silver nanoparticles (AgNPs):**

**1.Transmission Electron Microscopy (TEM) Analysis:**

Silver nanoparticles (AgNPs) synthesized using aqueous extracts from leaves of tested plants were subjected to TEM to find the information of AgNPs morphology and size. The TEM images showed the occurrence of individual silver nanoparticles and a number of aggregates in the test suspension. The sizes of AgNPs reported by TEM ranged between 9.31 and 15.99 nm for *P. harmala*- synthesized silver nanoparticles, respectively (Fig. 1). Also, the sizes of AgNPs ranged between 10.87 and 17.88 nm and were attained by using aqueous extract from leaves of *Ca. sinaica* (Fig. 2). Meanwhile, the sizes of AgNPs synthesized using aqueous extracts from leaves of *S. multicaulis* ranged from 20.15 and 21.80 nm, respectively (Fig. 3).

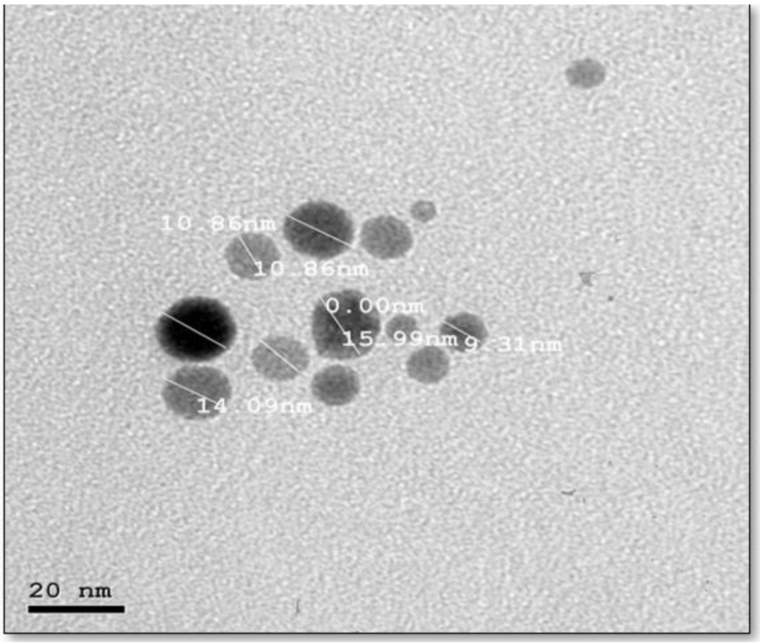


**Fig. 1**: TEM images of AgNPs synthesized using *Peganum harmala*

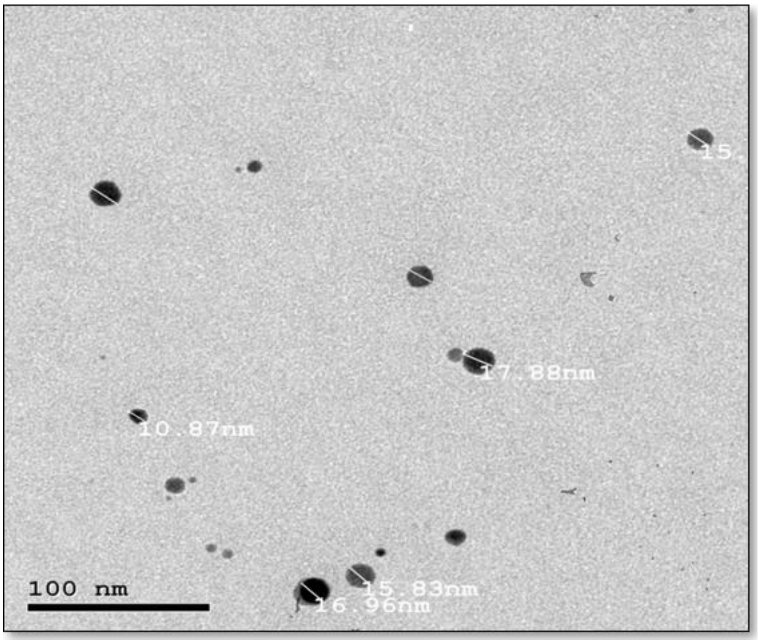


**Fig. 2**: TEM images of AgNPs synthesized using *Capparis sinaica.*

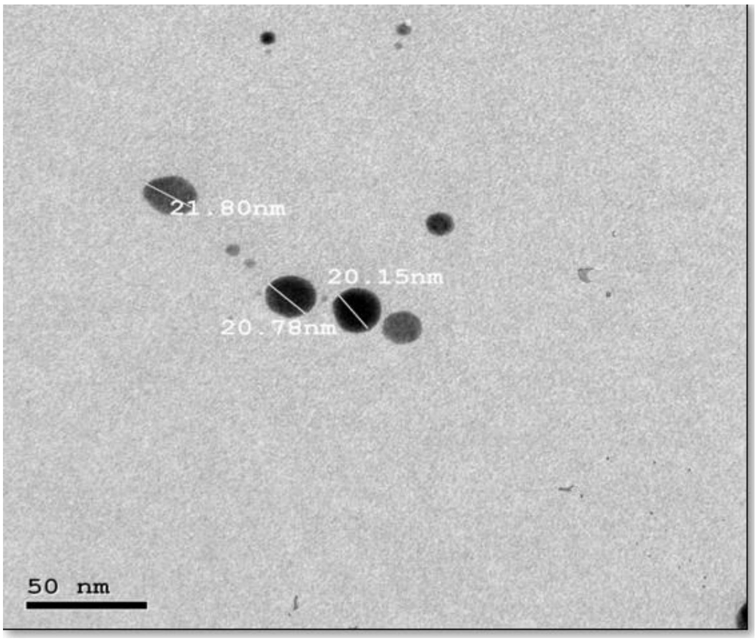


**Fig. 3:** TEM images of AgNPs synthesized using *Salvia multicaulis*

**2.Ultra-Violet (UV) - Visible:**

Localized Surface Plasmon Resonance (LSPR) phenomenon of synthesized-AgNPs using aqueous extracts from leaves of tested plants was tested. Results of LSPR showed that there was the occurrence of a single absorption peak at the range of 400 nm indicating the presence of spherical-shaped AgNPs (Figs. 4-6).

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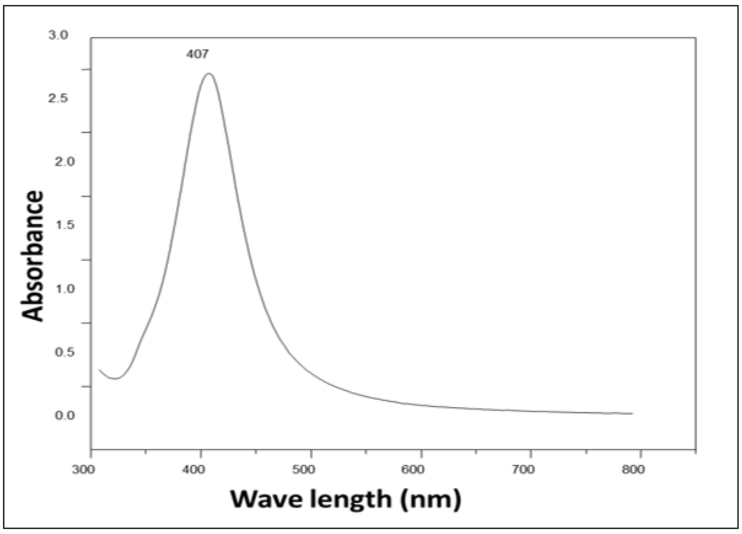


**Fig. 4:** Ultra-Violet (UV) - Visible curve of *P. harmala*-AgNPs

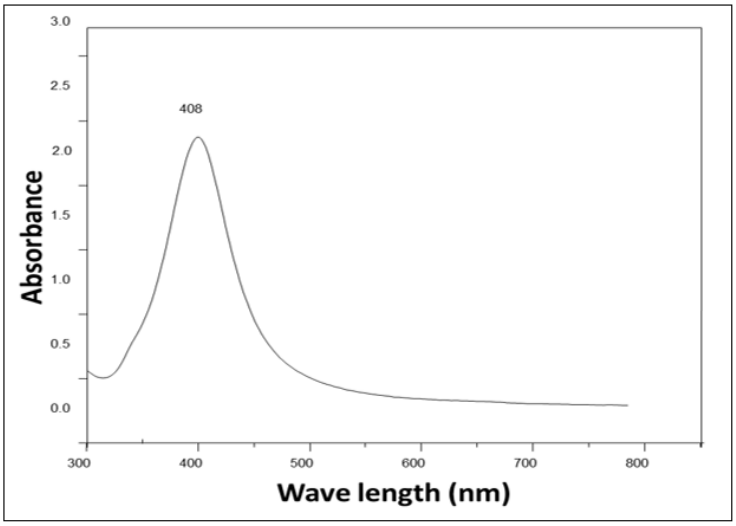


**Fig. 5**: Ultra-Violet (UV) - Visible curve of *Ca. sinaica*-AgNPs

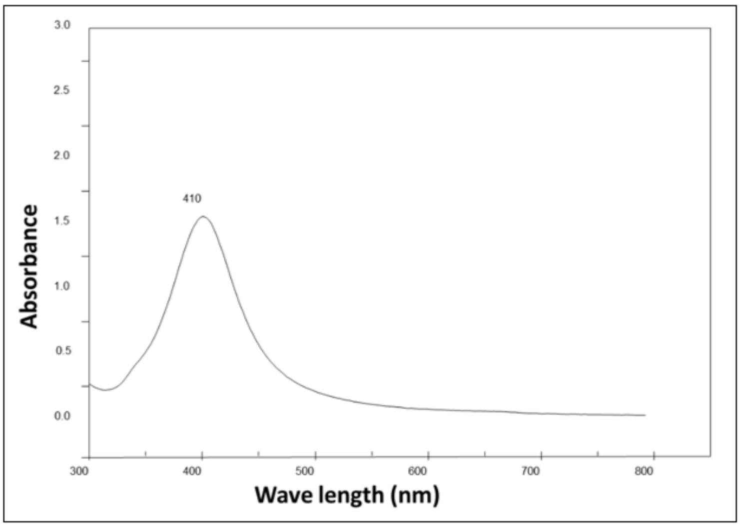


**Fig. 6**: Ultra-Violet (UV) - Visible curve of *S. multicaulis*-AgNPs.

**Efficacy of tested plant extracts-synthesized silver nanoparticles (AgNPs) against *Culex pipiens* larvae:**

**1. *Peganum harmala*-synthesized silver nanoparticles (*P. harmala*-AgNPs):**

The biological activity of biosynthesized silver nanoparticles using *Peganum harmala* extract against the 3$^{rd}$ instar larvae of *C. pipiens* is recorded in table 1. Complete larval mortality (100.0%) was attained at the highest concentration (20 ppm), meanwhile, the lowest mortality value (28.0%) occurred at the lowest concentration (8 ppm), respectively, compared with 0.0% for the control group. The mean larval period was significantly (P<0.001) prolonged by all concentrations used, as it ranged from 6.93±0.20 to 5.11±0.33 days, compared with 4.43±0.09 days for the untreated group. On the other hand, the pupal period was significantly (P<0.01) prolonged as compared with the untreated group, especially at the highest concentrations (18, 16 and 14 ppm), where, it recorded 3.41±0.02, 3.23±0.17 and 3.11±0.11 days, vs. 2.43±0.11 days for the untreated group. Also, the growth index affected by *P. harmala*- synthesized AgNPs at all concentrations used as compared with the control group (Table 1).

**Table 1:** Effect of biosynthesized silver nanoparticles using *Peganum harmala* extract against *Culex pipiens* different stages.

| Conc. (ppm) | Larval mort. (%) | Larval Period | Pupal Mort. (%) | Pupal Period | Adult Emergence (%) | Development Period | Growth Index |
|---|---|---|---|---|---|---|---|
| 20 | 100.0±0.0 | ---- | ---- | ---- | ---- | ---- | ---- |
| 18 | 89.33±4.62 | 6.93±0.20$^{d}$ | 6.62±6.11 | 3.41±0.02$^{d}$ | 93.38±6.11 | 10.34±0.20$^{d}$ | 9.03±0.53$^{d}$ |
| 16 | 78.66±4.63 | 6.62±0.31$^{d}$ | 5.33±2.31 | 3.23±0.17$^{d}$ | 94.97±2.31 | 9.75±0.40$^{d}$ | 9.47±0.32$^{d}$ |
| 14 | 62.66±8.33 | 6.11±0.40$^{d}$ | 4.0±0.01 | 3.11±0.15$^{d}$ | 96.0±0.0 | 9.22±0.43$^{d}$ | 10.41±0.11$^{d}$ |
| 12 | 46.66±11.50 | 5.62±0.43$^{d}$ | 2.62±2.31 | 2.83±0.11$^{d}$ | 97.18±2.31 | 8.55±0.43$^{d}$ | 11.36±0.50$^{d}$ |
| 10 | 37.33±12.82 | 5.33±0.40$^{d}$ | 0.0±0.0 | 2.73±0.04$^{d}$ | 100.0±0.0 | 8.06±0.40$^{d}$ | 12.40±0.62$^{d}$ |
| 8 | 28.0±0.0 | 5.11±0.33$^{d}$ | 0.0±0.0 | 2.62±0.11$^{c}$ | 100.0±0.0 | 7.73±0.33$^{d}$ | 13.93±0.62$^{d}$ |
| Control | 0.0±0.0 | 4.43±0.09$^{a}$ | 0.0±0.0 | 2.43±0.11$^{a}$ | 100.0±0.0 | 6.86±0.11$^{a}$ | 14.57±0.20$^{a}$ |

No. of tested larvae = 25 per one replicate; Conc. = Concentration; ppm = particle per million; SD = standard deviation; mort. = mortality; a = non-significant (P>0.05); b = significant (P<0.05); c = highly significant (P<0.01); d = very highly significant (P<0.001). Means followed by the same letter in the same column are not statistically significant.

**2.*Capparis sinaica*-Synthesized Silver Nanoparticles (*Ca. sinaica*-AgNPs):**

Data given in table 2 showed the biological activity of *Ca. sinaica* synthesized-AgNPs against 3$^{rd}$ instar larvae of *C. pipiens.* The highest larval mortality percent (100.0%) occurred at 40 ppm and the lowest mortality percent (24.0%) occurred at 10 ppm, while at 35, 30, 25, 20 and 15 ppm the mortality percent reached 96.0, 87.67, 58.67, 44.0 and 34.67%, respectively, compared with 0.0% for the untreated group. Also *Ca. sinaica* synthesized-AgNPs significantly (P<0.001) prolonged larval period all concentrations used as compared with the control group.

On the other hand, the mean pupal period recorded 3.52±0.15, 3.22±0.21, 3.0±0.16, 2.83±0.11, 2.73±0.09 and 2.63±0.12 days at 35, 30, 25, 20, 15 and 10 ppm, compared with 2.31±0.11 days for the untreated group. The adult emergence percent recorded 96.0 and 97.18% at 35 and 30 ppm, respectively, compared with 100.0% for the control group (Table 2). The growth index recorded 9.27, 10.07, 11.92, 11.96, 12.77 and 13.22 at 35, 30, 25, 20, 15 and 10 ppm, respectively, compared with 15.26 for the control group (Table 2).

**Table 2:** Effect of biosynthesized silver nanoparticles using *Capparis sinaica* extract against *Culex pipiens* different stages.

| Conc. (ppm) | Larval mort. (%) | Larval Period | Pupal Mort. (%) | Pupal Period | Adult Emergence (%) | Development Period | Growth Index |
|---|---|---|---|---|---|---|---|
| 40 | 100.0±0.0 | ---- | ---- | ---- | ---- | ---- | ---- |
| 35 | 96.0±0.0 | 6.83±0.11[d] | 4.0±0.0 | 3.52±0.15[d] | 96.0±0.0 | 10.35±0.20[d] | 9.27±0.23[d] |
| 30 | 78.67±2.31 | 6.33±0.20[d] | 2.62±2.31 | 3.22±0.21[d] | 97.18±2.31 | 9.65±0.43[d] | 10.07±0.21[d] |
| 25 | 58.67±4.63 | 5.82±0.20[d] | 0.0±0.0 | 3.00±0.16[d] | 100.0±0.0 | 8.92±0.20[d] | 11.92±0.82[d] |
| 20 | 44.0±0.0 | 5.33±0.31[d] | 0.0±0.0 | 2.83±0.11[d] | 100.0±0.0 | 8.36±0.43[d] | 11.96±0.61[d] |
| 15 | 34.67±2.31 | 5.0±0.20[d] | 0.0±0.0 | 2.73±0.09[c] | 100.0±0.0 | 7.83±0.20[d] | 12.77±0.63[d] |
| 10 | 24.0±0.0 | 4.93±0.20[d] | 0.0±0.0 | 2.63±0.12[b] | 100.0±0.0 | 7.56±0.11[d] | 13.22±0.52[d] |
| Control | 0.0±0.0 | 4.23±0.28[a] | 0.0±0.0 | 2.31±0.11[a] | 100.0±0.0 | 6.54±0.35[a] | 15.26±0.80[a] |

See footnote of table 1.

***3.Salvia multicaulis*-Synthesized Silver Nanoparticles (*S. multicaulis*-AgNPs):**

The biological activity of *S. multicaulis* synthesized-AgNPs against *C. pipiens* larvae is shown in table 3. Obtained results revealed that complete larval mortality (100.0%) was caused by the highest concentration (55 ppm) and the lowest mortality percent (24.0%) caused by the lowest concentration (25 ppm), respectively, compared with 0.0% for the untreated larvae. Treatment of larvae with *S. multicaulis* synthesized-AgNPs induced prolongation in larval period, where it recorded 6.83±0.20, 6.22±0.11, 5.83±0.11, 5.33±0.31, 5.12±0.33 and 4.80±0.20 days, vs. 4.23±0.20 days for the control group. On the other hand, the mean pupal period was significantly ($P<0.05$) affected by *S. multicaulis* synthesized-AgNPs at all concentrations used as compared with the untreated group (Table 3).

As shown from the results, the growth index for larvae and pupae recorded 9.27, 10.07, 11.92, 11.96, 12.77 and 13.22 at 50, 45, 40, 35, 30 and 25ppm, respectively, compared with 15.26 for the untreated group (Table 3).

**Table 3:** Effect of biosynthesized silver nanoparticles using *Salvia multicaulis* extract against *Culex pipiens* different stages.

| Conc. (ppm) | Larval mort. (%) | Larval Period | Pupal Mort. (%) | Pupal Period | Adult Emergence (%) | Development Period | Growth Index |
|---|---|---|---|---|---|---|---|
| 55 | 100.0±0.0 | ---- | ---- | ---- | ---- | ---- | ---- |
| 50 | 89.33±6.12 | 6.83±0.20[d] | 6.62±6.12 | 3.54±0.31[d] | 93.38±6.12 | 10.47±0.52[d] | 8.91±0.71[d] |
| 45 | 77.33±6.12 | 6.22±0.11[d] | 5.33±2.31 | 3.33±0.11[d] | 94.97±2.31 | 9.45±0.12[d] | 10.04±0.33[d] |
| 40 | 64.0±0.0 | 5.83±0.11[d] | 4.0±0.0 | 3.11±0.11[d] | 96.0±0.0 | 9.04±0.21[d] | 10.61±0.51[d] |
| 35 | 49.33±10.0 | 5.33±0.31[d] | 2.62±2.31 | 2.93±0.20[d] | 97.18±2.31 | 8.36±0.52[d] | 11.62±0.20[d] |
| 30 | 38.67±6.12 | 5.12±0.33[d] | 0.0±0.0 | 2.73±0.82[d] | 100.0±0.0 | 7.55±1.11[d] | 13.24±0.54[d] |
| 25 | 28.0±4.0 | 4.80±0.20[d] | 0.0±0.0 | 2.53±0.12[a] | 100.0±0.0 | 7.43±0.11[d] | 13.45±0.21[d] |
| Control | 0.0±0.0 | 4.16±0.20[a] | 0.0±0.0 | 2.43±0.11[a] | 100.0±0.0 | 6.59±0.25[a] | 15.17±0.60[a] |

See footnote of table 1

From the aforementioned results, it is obvious that the toxicity values of tested plant extracts-synthesized silver nanoparticles (AgNPs) based on $LC_{50}$ and $LC_{90}$ values (Table 4) may be arranged in descending order as follows: *P. harmala*-synthesized AgNPs > *Ca. sinaica*-synthesized AgNPs > *S. multicaulis*-synthesized AgNPs.

**Table 4:** Relative efficiency of tested plant extracts-synthesized silver nanoparticles (AgNPs) against larvae of *Culex pipiens*.

| Synthesized AgNPs | $LC_{50}$ ($LC_{90}$) (ppm) | 95% Confidence limits | | Slope |
|---|---|---|---|---|
| | | Lower | Upper | |
| *P. harmala*-synthesized AgNPs | 11.3 (18.0) | 9.8 (17.8) | 12.7 (18.6) | 6.2857 |
| *Ca. sinaica*-synthesized AgNPs | 20.5 (35.0) | 19.6 (34.1) | 21.3 (35.8) | 2.7524 |
| *S. multicaulis*-synthesized AgNPs | 34.3 (50.5) | 32 (49.5) | 36.5 (51.6) | 2.4667 |

## DISCUSSION

### 1.Characterization of Synthesized Silver Nanoparticles (AgNPs):

The Transmission Electron Microscopy (TEM) images showed that sizes of AgNPs ranged between 9.31 and 15.99 nm for *P. harmala*- synthesized silver nanoparticles. Also, the sizes of AgNPs ranged between 10.87 and 17.88 nm and were attained by using aqueous extract from leaves of *Ca. sinaica* in synthesis. Meanwhile, the sizes of AgNPs synthesized using aqueous extracts from leaves of *S. multicaulis* ranged from 20.15 and 21.80 nm, respectively. The sizes of synthesized AgNPs confirm the previously reported by Priyadarshini *et al.*, (2012) using *Euphorbia hirta* in AgNPs preparation, Kumar *et al*, (2015) using *Morinda tinctoria* leaf extract and Shehata and Mahmoud, (2019) using aqueous extract from leaves of *L. siceraria.* Also, in agreement with Susilowati *et al.*, (2019), UV-Visible curves showed the occurrence of a single absorption peak at the range of 400 nm indicating the presence of spherical-shaped AgNPs.

2.Efficacy of synthesized silver nanoparticles (AgNPs) against *Culex pipiens* larvae:

As shown from the results, the activity of synthesized AgNPs using aqueous extracts from leaves of tested plants against *C. pipiens* increased as the concentration increased. Based on $LC_{50}$ and $LC_{90}$ values, *P. harmala*-synthesized AgNPs was more effective against *C. pipiens* larvae than those of *Ca. sinaica* and *S. multicaulis*. In 2015 Subramaniam *et al.*, attributed the high efficacy of plant synthesized AgNPs against mosquito larvae to its ability in permeating the exoskeleton, penetrating into insects' cells, where they restrict macromolecules like proteins and DNA, changing their structure and therefore their function. Also, these results are in consistent with results recorded by Jayaseelan *et al.*, (2011) for AgNPs synthesized using *Tinospora cordifolia* against *C. quinquefasciatus*, Roni *et al.*, (2013) for AgNPs synthesized using *Nerium oleander* aqueous extract against An. *stephensi*, Morej´on *et al.*, (2018) for AgNPs synthesized using *Ambrosia arborescens* against *Ae. aegypti* and Shehata and Mahmoud, (2019) for AgNPs synthesized using *L. siceraria* leaves aqueous extract against *C. pipiens* and *An. pharoensis* larvae.

**Conclusion:**

Silver nanoparticles (AgNPs) synthesized using aqueous extracts from leaves of *P. harmala*, *Ca. sinaica* and *S. multicaulis* have a significant efficacy against *C. pipiens* larvae; however, *P. harmala*-synthesized AgNPs was more effective against *C. pipiens* larvae than those of *Ca. sinaica* and *S. multicaulis.*

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