Lethal and Sub Lethal Effects of Plant Extracts and Green Silver Nanoparticles against *Culex pipiens*

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**ABSTRACT**

*Culex pipiens* are blood feeding mosquitoes which are responsible for transmission of various diseases such as filariasis, encephalitis and chikungunya. Various control measures have been used to control the mosquitoes but most important is the use of synthetic insecticides. The chemical control has disrupted natural biological control system, resulted in the development of resistance, interfered with natural food chains and had undesirable effects on the environment, non-target organisms and human health. Biopesticides such as plant extracts and green synthesized nanoproducts have received much attention as potentially useful bioactive compounds against mosquitoes. In the present study, the effects of extracts of four plants i.e. *Azadirachta indica*, *Zingiber officinale*, *Syzygium aromaticum* and *Datura stramonium* and their green synthesized silver nanoparticles (AgNPs) were evaluated against 3rd and 4th instar larvae of *C. pipiens*. The green synthesized AgNPs of these plants were characterized by UV-Multiskaner. LC⁰ and LC₂⁰ values were calculated through Probit and Logit analysis (POLO) software. All the plant extracts and their green synthesized AgNPs caused the maximum mortalities of 3rd and 4th larval instars of *C. pipiens* after 96 hours. With the increase in time intervals and concentrations, the mortalities increased showing direct positive relationships between mortalities and time intervals and concentrations. The lethal and sub lethal effects of plant extracts on the mean development of larvae was the maximum by *A. indica* while the effects on pupal period were the maximum by *A. indica* and *Z. officinale*. Similarly, female longevity was the maximum by *A. indica* and that of male by *S. aromaticum*. As regards green synthesized AgNPs, the lethal and sub lethal effects on the mean development of larvae and pupal period were the maximum by *A. indica* AgNPs. The female longevity was found to be the maximum by *A. indica* AgNPs and that of the male by *Z. officinale* AgNPs. The results were based on LC⁰ and LC₂⁰ values of plant extracts and green synthesized AgNPs. It is therefore, concluded that artificially synthesized AgNPs can be used as an environment friendly alternative insecticide for the management of *C. pipiens*.

**INTRODUCTION**

*Culex* mosquitoes (Diptera: Culicidae) are blood feeding mosquitoes which are present in urban, semi-urban and rural areas due to extending number of breeding sites. *Culex pipiens* and *C. quinquefasciatus* are the most important vectors of human and animal diseases. These species are responsible for the transmission of filariasis, rift valley, west Nile virus, bird malaria, dog heart worm, mosquito-borne flavivirus, hemorrhagic fever, encephalitis and chikungunya (Djeghader et al., 2018; Muturi et al., 2018). *C. quinquefasciatus* commonly known as the southern house mosquito is a major vector of lymphatic filariasis. It is estimated that more than 1.4 billion people from 73 countries are living in areas where lymphatic filariasis is present and population of these areas are at risk of being infected (Vincent et al., 2017). Mosquito bites result in the deaths of more than 1 million people every year (Toolabi et al., 2018; WHO, 2018). Various control measures have been used to manage mosquitoes. Among these, the most important is the chemical control, particularly the use of synthetic insecticides like methoprene, carbamates, pyriproxyfen, diflubenzuron, fenthion, Malathion and DDT (Abutaha et al., 2018). Several plant extracts like *Pelargonium graveolens*, *Cymbopogon flexuosus*, *Azadirachta indica*, *Melia
Azadirachta indica, Datura stramonium, Momordica charantia, Syzygium aromaticum, Cinnamomum camphora, Aloe vera, Tamarindus indica, Emblica officinalis, Allium sativum, Zingiber officinale and Eucalyptus camaldulensis have been successfully used against different insect pests including mosquitoes (Muturi et al., 2017). Green synthesis of silver nanoparticles is the subdivision of nanotechnology. Recently, biosynthetic strategies employing either biological microorganisms or fungus or vegetation extracts have emerged as an easy and feasible opportunity to complex chemical synthetic techniques to attain nanomaterials (Logeswari et al., 2015). Now a days, green synthesis of nanoparticles is one of the most interesting scientific areas of inquiry. The world health organization has facilitated the use of biopesticides which are less expensive, effective and environmentally friendly (Ullah et al., 2018). In the present study, the crude extracts of four plants (Azadirachta indica, Zingiber officinale, Syzygium aromaticum and Datura stramonium) and their green synthesized silver nanoparticles were evaluated against the third and fourth larval instars of Culex pipiens for their management.

MATERIALS AND METHODS

Plants

In the current research, four plants (Azadirachta indica, Zingiber officinale, Syzygium aromaticum and Datura stramonium) were used for the synthesis of crude extracts and green silver nanoparticles.

Collection and rearing of C. pipiens

The larvae of C. pipiens were collected from still water in the standing areas, ponds and discarded tires. The larvae of C. pipiens were shifted to plastic storage jars containing 500 ml of water and were kept in Insect Molecular laboratory, Department of Entomology, University of Arid Agriculture Rawalpindi, under controlled conditions for rearing. A temperature of 25-27 °C and relative humidity of 85% were maintained. The plastic storage jars were placed in glass rearing cages with open mouth. The mouth of these glass rearing cages was covered with a muslin cloth for ventilation and transferring of food to the mosquito larvae. The culture of C. pipiens larvae was maintained as described by Kumar et al. (2018). The mosquito larvae were fed on dry chicken liver powder. Water of the plastic storage jars was changed after every 4 days. The larvae were fed until developed into pupae. The pupae were collected from the plastic storage jars with the help of manual glass pipette and transferred to a separate jar and was placed in another glass rearing cage for adult emergence. The adults were fed with 10% sugar solution for a period of three days. The larval period, adult period and adult longevity was assessed during the experimental bioassay. The female mosquito was fed on a chick trapped in glass rearing cage. A Petri dish containing water was kept inside the adult glass rearing cages for egg lying and eggs were collected after 2-3 days.

Preparation of plant extracts

Fresh parts of test plants were thoroughly washed with clean water to remove any dirt or other materials attached to them and dried up on a plastic sheet under shade for three weeks. The plant materials were crushed separately into powdered form and passed through a 20 mesh sieve. Hundred grams of each powdered material was dissolved into half liter of 99% ethanol serving as stock solution and different concentrations were made. The stock solution was poured into a conical flask and covered with aluminum foil. The solution was manually twice mixed every day by shaking for an entire week and then filtered through Whatman No.1 filter paper. The filtered extract solution was again collected in a separate conical flask.

The stock solution was stored at 5°C in a freezer. The extract was then converted into the crude extract form by placing it in an electric rotary evaporator at 78°C. After the ethanol had evaporated, the crude extract of the plant material left behind. This was collected in a beaker and placed at room temperature to ensure that the remaining moisture dried up. After 24 h, the extract was removed from the beaker, weighed, and then stored at 4°C in a freezer for use. The same procedure was repeated three times to obtain the crude plant extracts of all the plants. The entire procedure was conducted according to the methodology mentioned by Minjas and Sarda (1986).

Preparation of green silver nanoparticles

After the crude extracts of the plants had been prepared according to the above given procedure, these were then used to prepare the green silver nanoparticles tested in this study. Ten grams of the crude extract from each plant was taken and dissolved in 250 ml of distilled water, boiled for five minutes and passed through Whatman No. 1 filter paper. The filtrate was separately stored for further use. Silver nitrate (AgNO₃) was prepared by taking 100 ml of distilled water and then dissolving 1 mM of silver nitrate salt in it. The solution was poured into a beaker up to 80 ml and the remaining 20 ml was discarded. It was then mixed on a hot plate magnetic stirrer and the previously prepared crude extract filtrate was added to it. The hot plate was set to 100 °C for five minutes in order to boil the solution. After five minutes, the solution’s color changed to brown which indicated that the silver nanoparticle formation had concluded. The brown liquid was then poured into Falcon
tubes for centrifugation at 5000 rpm for a period of 15 minutes. Excess solution was removed from the falcon tubes and the remainder was transformed into pellets which were placed in a china dish. The dish was then heated in an oven at 50 °C for 24 h in order to remove moisture from the pellets. Once they were dried, the pellets were manually ground up using a pestle and mortar. Eppendorf tubes were used to store the pulverized powder after which they were covered with aluminum paper and labelled accordingly. The procedure was done according to the methodology described by Parashar et al. (2009).

Table I. Larvicidal toxicity of plant extracts against 3rd larval instar of Culex pipiens.

| Treatments         | Conc. (µg/ml) | Mean % mortality after 24 h | 48 h | 72 h | 96 h |
|--------------------|---------------|-----------------------------|------|------|------|
| Azadirachta indica | 0.5           | 20                          | 24   | 26   | 28   |
|                    | 1             | 32                          | 34   | 38   | 42   |
|                    | 2             | 38                          | 42   | 46   | 50   |
|                    | 4             | 56                          | 60   | 66   | 72   |
|                    | 8             | 84                          | 86   | 92   | 96   |
| Zingiber officinale| 12.5          | 16                          | 18   | 20   | 22   |
|                    | 25            | 32                          | 36   | 38   | 40   |
|                    | 50            | 46                          | 50   | 52   | 56   |
|                    | 100           | 64                          | 66   | 70   | 72   |
|                    | 200           | 82                          | 84   | 88   | 94   |
| Syzygium aromaticum| 5.5           | 16                          | 20   | 22   | 22   |
|                    | 11            | 34                          | 36   | 38   | 40   |
|                    | 22            | 44                          | 46   | 48   | 50   |
|                    | 44            | 64                          | 66   | 68   | 74   |
|                    | 88            | 86                          | 88   | 94   | 96   |
| Datura stramonium  | 102.5         | 14                          | 16   | 18   | 20   |
|                    | 205           | 32                          | 36   | 38   | 40   |
|                    | 410           | 44                          | 46   | 48   | 52   |
|                    | 820           | 66                          | 68   | 70   | 72   |
|                    | 1640          | 90                          | 92   | 92   | 96   |

Table II. Larvicidal toxicity of synthesized AgNPs of plant extracts against 3rd larval instar of Culex pipiens.

| Treatments         | Conc. (µg/ml) | Mean % mortality after 24 h | 48 h | 72 h | 96 h |
|--------------------|---------------|-----------------------------|------|------|------|
| Azadirachta indica | 0.1           | 18                          | 20   | 22   | 24   |
|                    | 0.2           | 34                          | 34   | 36   | 40   |
|                    | 0.4           | 52                          | 54   | 56   | 58   |
|                    | 0.8           | 70                          | 72   | 72   | 76   |
|                    | 1.6           | 92                          | 96   | 96   | 98   |
| Zingiber officinale| 2.5           | 18                          | 20   | 22   | 24   |
|                    | 5             | 32                          | 34   | 36   | 38   |
|                    | 10            | 56                          | 48   | 54   | 58   |
|                    | 20            | 64                          | 70   | 72   | 74   |
|                    | 40            | 88                          | 90   | 92   | 96   |
| Syzygium aromaticum| 1.1           | 18                          | 18   | 20   | 22   |
|                    | 2.2           | 36                          | 38   | 38   | 40   |
|                    | 4.4           | 48                          | 50   | 52   | 52   |
|                    | 8.8           | 66                          | 68   | 70   | 72   |
|                    | 17.6          | 86                          | 90   | 92   | 96   |
| Datura stramonium  | 20.5          | 18                          | 18   | 20   | 22   |
|                    | 41            | 34                          | 36   | 36   | 42   |
|                    | 82            | 46                          | 48   | 50   | 52   |
|                    | 164           | 66                          | 68   | 72   | 74   |
|                    | 328           | 92                          | 94   | 96   | 98   |

For the preparation of concentrations of each plant extract and their green synthesized AgNPs, the powder of the highest concentration of each treatment was dissolved into 1 ml of distilled water and further diluted by adding 499 ml of water to make stock solution. The subsequent concentrations were prepared by adding requisite amount of distilled water to the stock solution of each treatment. Two hundred and fifty larvae of each instar (3rd and 4th) were used for every concentration. Each concentration was replicated five times with ten individual larvae in a single replication. The mortality data were recorded after 24, 48, 72 and 96 h. All the bioassays were carried out under laboratory conditions in the lab. The larvicidal toxicity of A. indica extract was tested at the concentrations of 8, 4, 2, 1, 0.5 µg/ml and for their green synthesized AgNPs, the concentrations of 1.6, 0.8, 0.4, 0.2 and 0.1 µg/ml were used against both larval instars. The concentrations for Z. officinale extract were 200, 100, 50, 25 and 12.5 µg/ml and for their green synthesized AgNPs were 40, 20, 10, 5, 2.5 µg/ml. The concentrations in case of S. aromaticum extract were 88, 44, 22, 11 and 5.5 µg/ml while in case of their green synthesized AgNPs were 17.6, 8.8, 4.4, 2.2 and 1.1 µg/ml. Similarly, the contractions of D. stramonium fruit extract were 1640, 820, 410, 205 and 102.5 µg/ml and those for its green synthesized silver nanoparticles were 328, 164, 82, 41 and 20.5 µg/ml. A concentration of 0% was used as control for all the treatments.

Management of Culex pipiens with green AgNPs

After preparation of the green synthesized nanoparticles, their absorbance and the corresponding wavelengths were measured using a UV-Vis spectrophotometer at the Alpha Genomics Laboratory, Islamabad.

Larvicidal toxicity of plant extracts and green AgNPs

The larvicidal potential of four plant extracts and their green silver nanoparticles (AgNPs) with six concentrations was tested against 3rd and 4th larval instars of C. pipiens using the standard method (WHO, 2017).
Table III. Lethal and sub lethal effects of plant extracts on 3rd instar larvae of Culex pippins.

| Treatments               | Hours | Slope | \( LC_{20} \) (95% FL) | Lower limit | Upper limit | \( LC_{50} \) (95% FL) | Lower limit | Upper limit |
|--------------------------|-------|-------|-------------------------|-------------|-------------|-------------------------|-------------|-------------|
| **Azadirachta indica**   | 24    | 0.229 | 0.29                    | 1.720       | 3.48        | 2.751                   | 4.314       |
|                          | 48    | 0.228 | 0.18                    | 2.295       | 3.06        | 2.322                   | 3.853       |
|                          | 72    | 0.262 | 0.16                    | 2.218       | 2.48        | 1.813                   | 3.156       |
|                          | 96    | 0.299 | 0.10                    | 2.164       | 2.04        | 1.414                   | 2.627       |
| **Zingiber officinale**  | 24    | 0.009 | 21.7                    | 180.223     | 80.97       | 32.836                  | 142.294     |
|                          | 48    | 0.009 | 20.62                   | 224.575     | 71.85       | 16.705                  | 130.442     |
|                          | 72    | 0.010 | 18.20                   | 176.284     | 62.79       | 14.255                  | 107.350     |
|                          | 96    | 0.012 | 10.98                   | 108.506     | 53.55       | 19.765                  | 83.286      |
| **Syzygium aromaticum**  | 24    | 0.023 | 6.15                    | 47.83       | 34.03       | 18.043                  | 52.826      |
|                          | 48    | 0.023 | 4.45                    | 22.575      | 30.75       | 23.172                  | 38.521      |
|                          | 72    | 0.027 | 3.77                    | 17.223      | 27.29       | 20.737                  | 33.923      |
|                          | 96    | 0.030 | 3.00                    | 17.35       | 23.97       | 17.77                   | 30.03       |
| **Datura stramonium**    | 24    | 0.001 | 380.74                  | 621.678     | 612.07      | 353.834                 | 916.938     |
|                          | 48    | 1.797 | 78.05                   | 302.732     | 1966.29     | 352.234                 | 513.448     |
|                          | 72    | 0.001 | 75.60                   | 176.350     | 520.63      | 253.449                 | 792.263     |
|                          | 96    | 0.001 | 15.99                   | 586.865     | 455.45      | 246.103                 | 658.956     |

Table IV. Lethal and sub lethal effects of synthesized AgNPs of plant extracts on 3rd instar larvae of Culex pippins.

| Treatments               | Hours | Slope | \( LC_{20} \) (95% FL) | Lower limit | Upper limit | \( LC_{50} \) (95% FL) | Lower limit | Upper limit |
|--------------------------|-------|-------|-------------------------|-------------|-------------|-------------------------|-------------|-------------|
| **Azadirachta indica**   | 24    | 1.467 | 0.09                    | 0.796       | 0.50        | 0.234                   | 0.789       |
|                          | 48    | 1.684 | 0.08                    | 0.255       | 0.46        | 0.353                   | 0.569       |
|                          | 72    | 1.628 | 0.06                    | 0.315       | 0.43        | 0.324                   | 0.547       |
|                          | 96    | 1.786 | 0.03                    | 0.321       | 0.38        | 0.274                   | 0.482       |
| **Zingiber officinale**  | 24    | 0.053 | 3.32                    | 18.485      | 14.41       | 7.867                   | 21.609      |
|                          | 48    | 0.055 | 3.02                    | 17.240      | 13.20       | 7.230                   | 19.326      |
|                          | 72    | 0.056 | 2.16                    | 20.918      | 11.60       | 4.954                   | 17.763      |
|                          | 96    | 0.064 | 1.42                    | 9.33        | 10.10       | 7.163                   | 12.86       |
| **Syzygium aromaticum**  | 24    | 0.110 | 1.39                    | 13.113      | 6.29        | 2.525                   | 10.316      |
|                          | 48    | 0.121 | 1.30                    | 11.329      | 5.71        | 2.224                   | 9.209       |
|                          | 72    | 0.128 | 1.22                    | 9.205       | 5.30        | 2.338                   | 8.129       |
|                          | 96    | 0.145 | 1.01                    | 3.672       | 4.79        | 3.528                   | 6.027       |
| **Datura stramonium**    | 24    | 0.007 | 13.85                   | 54.666      | 112.92      | 88.293                  | 138.738     |
|                          | 48    | 0.007 | 7.30                    | 53.774      | 105.12      | 81.542                  | 129.422     |
|                          | 72    | 0.008 | 7.10                    | 51.390      | 95.91       | 73.770                  | 118.380     |
|                          | 96    | 0.009 | 6.08                    | 59.450      | 84.11       | 62.424                  | 105.302     |

Lethal and sub lethal effects of plant extracts and green AgNPs on C. pipiens

Biological study (larval period, pupal period and adult longevity) of C. pipiens was observed at \( LC_{50} \) and sub-lethal \( LC_{20} \) values of different plant extracts and green AgNPs. \( LC_{50} \) and \( LC_{20} \) values were calculated in the previous bioassay. The \( LC_{50} \) and \( LC_{20} \) concentrations prepared by using the stock powder of plant extracts and green AgNPs. The \( LC_{50} \) and control were used with five replications for both the larval instars. Fifty larvae of every instar of C. pipiens were used and there were ten individuals in one replication. Adult females of C. pipiens were fed on chick and sugar solution of 10% while the male adults were fed on 10% sugar solution.
Management of Culex pipiens with green AgNPs

Statistical analysis
Percent mean mortality was calculated using SPSS software. Mortality data were corrected with Abbot’s formula (Abbott, 1925) and respective LC values were calculated through Probit and Logit analysis (POLO) software. Means for the parameters were compared using t-test at 5% probability.

RESULTS

All the plant extracts and their AgNPs gave the maximum mortalities after 96 hours. With the increase in time intervals and concentrations, the mortalities increased showing a direct positive relationship between mortalities and time intervals and concentrations (Tables V and VI). The lethal and sub lethal effects of four plant extracts and their AgNPs on 4th instar larvae of C. pippins, their LC$_{50}$ and LC$_{20}$ values have been given in Tables VII and VIII.

Table VI. Larvicidal toxicity of synthesized AgNPs of different plants extracts against 4th larval instar of Culex pipiens.

| Treatments          | Conc. (µg/ml) | Mean % mortality after |
|---------------------|---------------|------------------------|
|                     |               | 24 h  | 48 h  | 72 h  | 96 h  |
| Azadirachta indica  |               |       |       |       |       |
| 0.1                 | 18            | 20    | 24    |       |
| 0.2                 | 32            | 34    | 36    | 40    |
| 0.4                 | 44            | 48    | 50    | 54    |
| 0.8                 | 64            | 66    | 70    | 74    |
| 1.6                 | 86            | 96    | 94    | 98    |
| Zingiber officinale | 2.5           | 18    | 22    | 26    | 28    |
| 5                   | 30            | 34    | 36    | 40    |
| 10                  | 48            | 50    | 54    | 58    |
| 20                  | 68            | 68    | 72    | 76    |
| 40                  | 86            | 90    | 92    | 96    |
| Syzygium aromaticum | 1.1           | 20    | 22    | 24    | 24    |
| 2.2                 | 32            | 36    | 38    | 38    |
| 4.4                 | 46            | 48    | 50    | 52    |
| 8.8                 | 64            | 68    | 70    | 70    |
| 17.6                | 90            | 90    | 92    | 94    |
| Datura stramonium   | 20.5          | 16    | 18    | 20    | 20    |
| 41                  | 34            | 36    | 38    | 38    |
| 82                  | 46            | 48    | 50    | 54    |
| 164                 | 68            | 70    | 70    | 72    |
| 328                 | 84            | 88    | 90    | 96    |

The lethal and sub lethal effects of plant extracts on the mean development of larvae was the maximum by A. indica while the effects on pupal period were the maximum by A. indica and Z. officinale. Similarly, female longevity was the maximum by A. indica and that of male by S. aromaticum. The results were based on LC$_{50}$ and LC$_{20}$ values of plant extracts (Table IX). As regards green synthesized AgNPs, the lethal and sub lethal effects on the mean development of larvae and pupal period were the maximum by A. indica AgNPs and that of male by Z. officinal AgNPs. The results were based on LC$_{20}$ and LC$_{50}$ values of green synthesized AgNPs (Table X).
Table VII. Lethal and sub lethal effects of four plants on 4th instar larvae of *Culex pippins*.

| Treatments                  | Hours | Slope | LC_{20} (95% FL) | Lower limit | Upper limit | LC_{50} (95% FL) | Lower limit | Upper limit |
|-----------------------------|-------|-------|------------------|-------------|-------------|------------------|-------------|-------------|
| *Azadirachta indica*        | 24    | 0.289 | 0.401            | 1.682       | 2.49        | 3.110            | 1.880       |             |
|                             | 48    | 0.308 | 0.433            | 1.519       | 2.39        | 2.980            | 1.815       |             |
|                             | 72    | 0.302 | 0.255            | 1.832       | 2.22        | 2.813            | 1.624       |             |
|                             | 96    | 0.327 | 0.558            | 3.184       | 1.98        | 2.915            | 0.936       |             |
|                             | 24    | 0.010 | 26.375           | 123.640     | 67.53       | 107.31           | 28.77       |             |
| *Zingiber officinale*       | 48    | 0.011 | 19.987           | 99.104      | 62.66       | 93.96            | 30.48       |             |
|                             | 72    | 0.011 | 17.481           | 89.809      | 59.12       | 87.118           | 29.631      |             |
|                             | 96    | 0.012 | 4.877            | 48.872      | 54.62       | 69.33            | 39.20       |             |
| *Syzygium aromaticum*       | 24    | 0.024 | 19.526           | 4.982       | 30.06       | 22.910           | 37.408      |             |
|                             | 48    | 0.026 | 18.429           | 4.803       | 28.41       | 21.570           | 35.348      |             |
|                             | 72    | 0.025 | 20.896           | 3.357       | 26.96       | 19.964           | 33.874      |             |
|                             | 96    | 0.027 | 21.7             | 2.210       | 24.48       | 17.62            | 31.05       |             |
| *Datura stramonium*         | 24    | 0.001 | 796.406          | 228.430     | 591.65      | 317.562          | 892.342     |             |
|                             | 48    | 0.001 | 746.608          | 180.160     | 541.78      | 289.950          | 798.441     |             |
|                             | 72    | 0.001 | 670.076          | 167.617     | 511.77      | 278.944          | 743.831     |             |
|                             | 96    | 0.001 | 435.887          | 35.847      | 475.16      | 341.342          | 603.624     |             |

Table VIII. Lethal and sub lethal effects of synthesized AgNPs of plant extracts on 4th instar larvae of *Culex pippins*.

| Treatments                  | Hours | Slope | LC_{20} (95% FL) | Lower limit | Upper limit | LC_{50} (95% FL) | Lower limit | Upper limit |
|-----------------------------|-------|-------|------------------|-------------|-------------|------------------|-------------|-------------|
| *Azadirachta indica*        | 24    | 0.124 | 0.662            | 0.216       | 0.61        | 0.380            | 0.891       |             |
|                             | 48    | 1.417 | 0.311            | 0.103       | 0.53        | 0.411            | 0.661       |             |
|                             | 72    | 1.526 | 0.302            | 0.091       | 0.48        | 0.371            | 0.606       |             |
|                             | 96    | 1.748 | 0.307            | 0.064       | 0.40        | 0.295            | 0.506       |             |
|                             | 24    | 0.051 | 26.391           | 6.464       | 14.74       | 6.489            | 24.056      |             |
| *Zingiber officinale*       | 48    | 0.053 | 10.110           | 1.559       | 12.98       | 9.653            | 16.310      |             |
|                             | 72    | 0.054 | 11.889           | 0.267       | 11.12       | 7.736            | 14.296      |             |
|                             | 96    | 0.062 | 11.239           | 0.062       | 9.23        | 6.103            | 12.043      |             |
| *Syzygium aromaticum*       | 24    | 0.122 | 3.423            | 1.287       | 6.30        | 4.901            | 7.789       |             |
|                             | 48    | 0.119 | 4.560            | 0.644       | 5.71        | 4.233            | 7.185       |             |
|                             | 72    | 0.125 | 4.529            | 0.507       | 5.29        | 3.858            | 6.688       |             |
|                             | 96    | 0.132 | 4.399            | 0.457       | 4.98        | 3.602            | 6.320       |             |
| *Datura stramonium*         | 24    | 0.006 | 325.250          | 58.609      | 123.68      | 37.807           | 224.463     |             |
|                             | 48    | 0.006 | 231.543          | 46.085      | 110.75      | 40.892           | 182.554     |             |
|                             | 72    | 0.007 | 175.619          | 36.047      | 104.63      | 48.290           | 160.156     |             |
|                             | 96    | 0.008 | 116.999          | 32.306      | 91.14       | 48.996           | 132.257     |             |
Management of *Culex pipiens* with green AgNPs

### Table IX. Lethal and sub lethal effects of plant extracts on the mean development time (Days ± SE) of *Culex pipiens*.

| Treatments          | Hours       | Control      | LC₉₀ (95% FL) | LC₅₀ (95% FL) |
|---------------------|-------------|--------------|---------------|---------------|
| *Azadirachta indica* | Larval period | 3.1 (±0.1) c | 3.9 (±0.2) b  | 4.3 (±0.4) a  |
|                     | Pupal period | 1.9 (±0.3) c | 2.2 (±0.4) b  | 2.5 (±0.5) a  |
|                     | Female longevity | 33 (±0.6) a | 22 (±0.8) b   | 11 (±0.2) c   |
|                     | Male longevity | 13 (±0.2) a  | 9 (±0.3) b    | 5 (±0.5) c    |
| *Zingiber officinale* | Larval period | 3 (±0.4) c   | 3.4 (±0.5) b  | 4.2 (±0.2) a  |
|                     | Pupal period | 1.9 (±0.3) c | 2.2 (±0.1) b  | 2.5 (±0.7) a  |
|                     | Female longevity | 34 (±0.2) a | 24 (±0.3) b   | 11 (±0.1) c   |
|                     | Male longevity | 14 (±0.5) a  | 11 (±0.8) b   | 7 (±0.6) c    |
| *Syzygium aromaticum* | Larval period | 3.0 (±0.1) c | 3.7 (±0.2) b  | 4.2 (±0.1) a  |
|                     | Pupal period | 1.9 (±0.3) c | 2.2 (±0.4) b  | 2.4 (±0.5) a  |
|                     | Female longevity | 33 (±0.6) a | 23 (±0.3) b   | 12 (±0.8) c   |
|                     | Male longevity | 16 (±0.2) a  | 12 (±0.3) b   | 6 (±0.5) c    |
| *Datura stramonium*  | Larval period | 2.5 (±0.6) c | 3.7 (±0.8) b  | 4.3 (±0.5) a  |
|                     | Pupal period | 1.9 (±0.1) c | 2.2 (±0.3) b  | 2.4 (±0.2) a  |
|                     | Female longevity | 34 (±0.3) a | 20 (±0.1) b   | 11 (±0.7) c   |
|                     | Male longevity | 15 (±0.2) a  | 11 (±0.5) b   | 7 (±0.4) c    |

### Table X. Lethal and sub lethal effects of AgNPs on the mean development time (Days ± SE) of *Culex pipiens*.

| Treatments          | Hours       | Control      | LC₉₀ (95% FL) | LC₅₀ (95% FL) |
|---------------------|-------------|--------------|---------------|---------------|
| *Azadirachta indica* | Larval period | 3.1 (±0.1) c | 3.9 (±0.2) b  | 4.5 (±0.4) a  |
|                     | Pupal period | 1.9 (±0.3) c | 2.3 (±0.4) b  | 2.6 (±0.5) a  |
|                     | Female longevity | 34 (±0.6) a | 21 (±0.2) b   | 8 (±0.8) c    |
|                     | Male longevity | 16 (±0.2) a  | 10 (±0.3) b   | 3 (±0.5) c    |
| *Zingiber officinale* | Larval period | 3.2 (±0.4) c | 3.9 (±0.5) b  | 4.4 (±0.2) a  |
|                     | Pupal period | 2.0 (±0.3) c | 2.1 (±0.1) b  | 2.6 (±0.7) a  |
|                     | Female longevity | 32 (±0.2) a | 20 (±0.3) b   | 9 (±0.1) c    |
|                     | Male longevity | 18 (±0.5) a  | 9 (±0.8) b    | 6 (±0.6) c    |
| *Syzygium aromaticum* | Larval period | 3.0 (±0.3) c | 3.9 (±0.1) b  | 4.4 (±0.7) a  |
|                     | Pupal period | 1.9 (±0.4) c | 2.2 (±0.5) b  | 2.6 (±0.2) a  |
|                     | Female longevity | 33 (±0.5) a | 20 (±0.8) b   | 9 (±0.6) c    |
|                     | Male longevity | 13 (±0.2) a  | 10 (±0.3) b   | 5 (±0.1) c    |
| *Datura stramonium*  | Larval period | 3.0 (±0.5) c | 3.5 (±0.8) b  | 3.9 (±0.6) a  |
|                     | Pupal period | 1.9 (±0.2) c | 2.2 (±0.3) b  | 2.6 (±0.1) a  |
|                     | Female longevity | 34 (±0.3) a | 20 (±0.1) b   | 10 (±0.7) c   |
|                     | Male longevity | 12 (±0.4) a  | 8 (±0.5) b    | 5 (±0.2) c    |

**DISCUSSION**

Results of the current study showed that larval period, pupal period and adult longevity were affected when treated with crude plant extracts and silver nanoparticles. The results are comparable with those reported by Vincent *et al.* (2017) against *Culex* species. In another study, the larval and pupal period increased up to 1 day when treated with plant extracts and AgNPs of *A. indica*, *M. azedarach* and *D. stramonium* (Ullah *et al.*, 2018). The larvicidal
toxicity of plant extracts against *C. quinquefasciatus* has also been reported by Al-Mehmadi and Al-Khalaf (2010).

The percentage of adult emergence reduced when *C. pipiens* larvae were treated with the aqueous leaf extract of *A. indica* and their green AgNPs. Similarly, low percentage of adult emergence of *Aedes aegypti* and *C. quinquefasciatus* was obtained when treated with aqueous leaf extract of *Adiantum raddianum* and green synthesized AgNPs (Govindarajan et al., 2017).

The adult longevity of male and female mosquitoes reduced when treated with *Momordica charantia* AgNPs and plant extract of *M. azedarach*. The reduction in adult longevity was the same as reported by Velayutham et al. (2013). In the present study, the mean % mortality of larval instars increased with increasing of the concentration and exposure time of both plant and green AgNPs. Similar results were also reported by Marimuthu et al. (2011).

In the present study, the lethal and sub lethal values decreased with the exposure time and concentration. The minimum lethal and sub lethal values were recorded at high concentration and at high exposure time for both the larval instars after the application with each treatment. The findings are similar to those described by Benelli et al. (2018) who studied the lethal and sub lethal toxicity of *Mentha piperita, M. spicata, Ocimum basilicum, Helichrysum italicum, Achillea ligustica, Pelargonium odoratissimum, Cinnamomum verum* and *Lippia alba* extracts against 4th larval instar of *C. quinquefasciatus* and adults of *Musca domestica*. Results of the current study showed that the leaves of *A. indica* and *M. azedarach* have shown toxicity against mosquitoes because their leaves are highly rich in metabolic compounds as reported by Poopathi et al. (2015).

In the present study, the color of green synthesized AgNPs was brown when placed at room temperature. Similar results were also reported by Velayutham et al. (2013). The results also showed that garlic plant extracts caused higher % mortality of 3rd and 4th larval instar of *C. pipiens* as compared to other plant extracts. Similarly, *A. indica* based AgNPs gave higher % mortality as compared to other green synthesized AgNPs. The larval and pupal period of *C. pipiens* increased when the plant extracts and green synthesized AgNPs were applied and similar results were described by Vincent et al. (2017). It is therefore, concluded that plant extracts of *Azadirachta indica, Zingiber officinale, Syzygium aromaticum* and *Datura stramonium* and their artificially synthesized AgNPs can be used as an environmentally friendly alternative insecticides for the management of *Culex pipiens*.

**Statement of conflict of interest**

The authors have declared no conflict of interest.

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