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# MOSQUITO LARVICIDAL EFFICACY OF LEAF EXTRACTS OF *TURNERA SUBULATA* AND GREEN SYNTHESIZED SILVER NANOPARTICLES ON *CULEX QUINQUEFASCIATUS*

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## ABSTRACT

Mosquitoes are responsible for the transmission of many vector borne diseases. Lymphatic filariasis, a diseases caused by the infection of *Wuchereria bancrafti* was transmitted by the *Culex quinquefasicatus* and around 100 million people are affected by these around the world. In the present study the larvicidal activity of the various plant extracts of *Turnera subulata* was estimated with concentrations varying from 50ppm to 250 ppm. The efficacy of plant mediated synthesized silver nanoparticles were tested against early 4$^{th}$ instar larvae of *Culex quinquefasciatus*. The observation of the results reveals the maximum larvicidal potential of 95.60 percentage with chloroform leaf extract with $LC_{50}$ and $LC_{90}$ values of 51.021ppm and 235.417ppm respectively. However, the larvicidal efficacy of the silver nanoparticles was maximum when compared to the extracts where a percentage mortality of 98% was recorded with 5ppm concentration with the $LC_{50}$ value of 45.499 ppm after 72 hours of experiment period. The results suggests that the bioactive compounds such as alkaloids and terpenoids were responsible for the larvicidal activity, while the bioactive compounds capped with the silver nanoparticles increased the potentiality of these particles against 4$^{th}$ instar larvae of *Culex quinquefasciatus*. To conclude, the application of green synthesized silver nanoparticles and the isolation and characterization of the bioactive compound from the chloroform leaf extract of *Turnera subulata* will pave way for the control of disease causing mosquitoes.

**KEYWORDS:** *Culex quinquefasciatus*, *Turnera subulata*, larvicidal, silver nanoparticles, phytochemicals.

## INTRODUCTION

Mosquitoes are the major vectors which transmit disease-causing organism for more than 700 million people annually and are responsible for the death of 1 in 17 people.[1] Mosquito borne disease is a major health problem among human and veterinary sector.[2] They are the disease transmitting agent for life-threatening diseases such as malaria, filariasis, dengue, chikungunya and encephalitis etc.[3] *C. quinquefasciatus* transmit *Wuchereria bancrofti* to cause filariasis. Japanese encephalitis diseases causing viruses is transmitted through *C. quinquefasciatus* by which around 10000 deaths were recorded around the world, especially in children aged less than five years.[4]

*C. quinquefasciatus* is a medium size brown mosquito. The body is about 3.96 to 4.25 mm long. Zoonotic diseases such as encephalitis and Nile fever were also transmitted by *C. quinquefasciatus*.[5] Filariasis is transmitted in many parts of the world by these mosquitoes. Lymphatic filariasis is the second most common cause of the long-term disability next to mental illness.[6] There are several types of mosquitoes and some have the ability to carry many different diseases. Female *Culex* is one of three most common mosquitoes worldwide. Vector-borne diseases account for more than 17% of all infectious diseases.[7] The synthetic organic insecticides were developed to control mosquitoes. Natural biosynthetic products were more preferred than synthetic chemicals for their rich source of chemicals with biological activities and their nontoxic nature to non-target organism and innate biodegradability. Plant-derived biosynthetic products are more effective over insect pests with resistance against pesticides.[8] *C.quinquefasciatus* is a familiar vector across urban and semi-urban areas of Asia and globally 15 million people are affected with Lymphoedema.[9] Nanoparticles were commonly used in the areas of biological sciences due to their novel properties, small size, eco-friendly and nontoxic nature. Green synthesized nanoparticles were produced and tested for the larvicidal efficacy.

*Turnera subulata* belonging to the family *Turneraceae* has been traditionally used in Asian medicine to cure cough and bronchitis. The genera *Turnera* consists of more than 10 species and their potential medicine values were under explored. Traditionally the plants possess antibacterial and antifungal properties. The silver nanoparticles were synthesized using aqueous leaf extract of *Turnera subulata* and particles were tested for their larvicidal potential against *Culex quiquefasciatus* along with other solvent extracts.

## MATERIALS AND METHODS

### Collection of plants

*Turnera subulata* plant were collected from the Queen Mary's College, Chennai, India and identified with the help of P.G Research Department of Plant Biology, Queen Mary's College, Chennai, India. The plants were washed and rinsed with tap water to remove dust and soil. Leaves were separated and allowed to shade dried for a period of 15 days and dried plant leaves was powdered using a blender and stored at room temperature for further use.

### Extracts

50g of dried leaves were packed into the soxhlet apparatus and the cycles were continued for a period of 6-8 hours to collect the extract using different solvents such as Ethanol, Methanol, Ethyl acetate and Chloroform. The collected extracts were vacuum evaporated and stored at 4ºC until further use.

### Phytochemical analysis

Qualitative estimation for the presence of phytoconstituents were analysed in various extracts of *Turnera subulata* using the method of Harborne.[10]

### Synthesis of silver nanoparticles

Fresh leaves of *Turnera subulata* were boiled with water at 80 ºC for 15 minutes and the filtrate was used as a reducing agent in the process of silver nanoparticle synthesis. The aqueous extract was mixed with the 1mM solvent of $AgNO_2$ and kept at room temperature. The formation of silver nanoparticles was primarily confirmed by the color change when the colorless solution turned to yellow and finally to yellowish brown color.[11] Further UV-Visible spectroscopic analysis of the spectrum was carried out to confirm the presence of silver nanoparticles. The solution was centrifuged at 15000 rpm for 15 minutes and the pellets were re-suspended in double distilled water were further centrifuged for further purification and the collected pellets were stored at room temperature until further use.

### Characterization of nanoparticles

The AgNPs were subjected to Scanning electron microscopy (SEM) to observe the surface morphology of the particles, Energy Dispersive Spectroscopy (EDX) was performed for the analysis of elements and the samples were further subjected to Fourier Transform Infrared Spectroscopy (FTIR) for the presence of different chemical groups.

**Larvicidal bioassay**

Larvicidal activity of *Turnera subulata* was studied as per the method followed (Karthikeyan *et al.,*)[12] in accordance with the guidance of WHO. Laboratory-reared 4th instar larvae of *C. quinquefasciatus* were used to analyze the larvicidal potential of plant extracts and AgNPs. Five replicates with 20 larvae per replicates were used in the study. Different concentrations ranging from 50ppm to 250ppm of plant extracts and silver nanoparticles with the concentration of 1,2,3,4 and 5ppm were evaluated for the larvicidal potentiality.

**Statistics**

The average and mortality were calculated by Probit analysis for calculating $LC_{50}$ and $LC_{90}$ using SPSS software. $p<0.05$ was considered to be statistically significant.

**RESULTS**

The present study shows (Table 1) the presence of the bioactive compounds such as Alkaloid, Flavonoids, Phenols, Terpenoids, Tannins and Cumarins in all the extract studied. The biological properties of the plant extract purely depends on the presence of various bioactive compounds in the plant extracts.

**Table 1: Qualitative Phytochemical profiling of different leaf extracts of *Turnera subulata.***

| Phytoconstituents | Name of solvent | | | | |
|---|---|---|---|---|---|
| | Aqueous | Methanol | Ethanol | Ethyl acetate | Chloroform |
| Steroid | - | - | + | ++ | + |
| Terpenoid | - | ++ | + | ++ | ++ |
| Tannin | - | ++ | ++ | ++ | + |
| Saponin | - | - | - | + | - |
| Cumarin | + | + | + | ++ | +++ |
| Phenol | + | + | ++ | ++ | - |
| Alkaloid | + | + | + | ++ | ++ |
| Flavonoids | + | + | + | ++ | ++ |
| Cardiac glycosides | - | + | + | ++ | + |
| Carbohydrate | - | - | - | - | ++ |
| Protein | - | - | - | - | - |

*Turnera subulata* leaf extract was used as a reducing agent for synthesis of AgNPs. The formation of AgNPs was confirmed by the color change from a colorless solution to yellowish brown. The formation of yellowish brown color is due to the coupling of visible range with collation oscillation and conduction of electrons in an electromagnetic field. The

synthesized AgNPs show an absorption peak at 418nm. While in a typical absorption peak of spherical silver nanoparticles was formed due to the surface plasmon vibration. The UV-Vis spectra recorded with the leaf broth of *Turnera subulata* shows maximum absorption at 420nm and it steadily increases its intensity as a function of reaction time.

The scanning electron micrograph of AgNPs reveals its size range between 20 to 30nm (Fig 1). The EDX profile shows a strong signal for silver along with weak signal for carbon and oxygen (Fig 2).

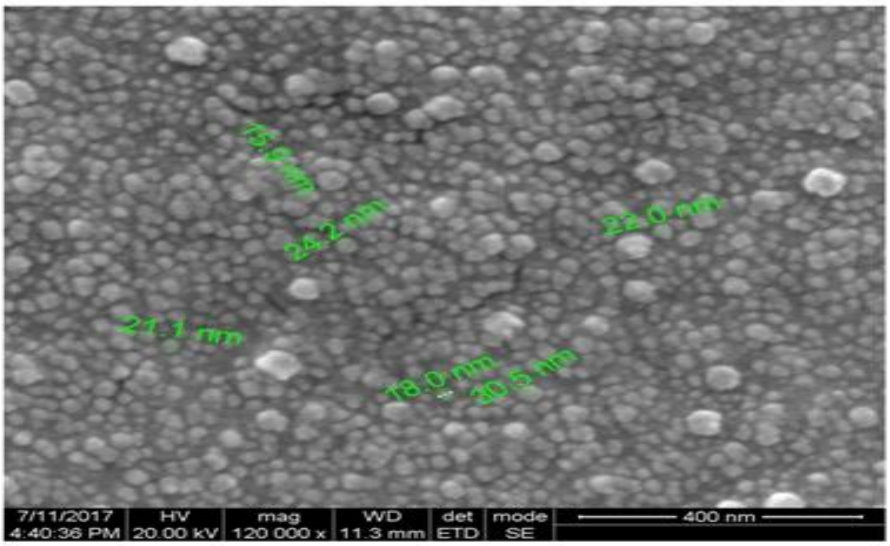


**Fig 1: Scanning Electron Micrograph of Green Synthesized Silver Nanoparticles.**

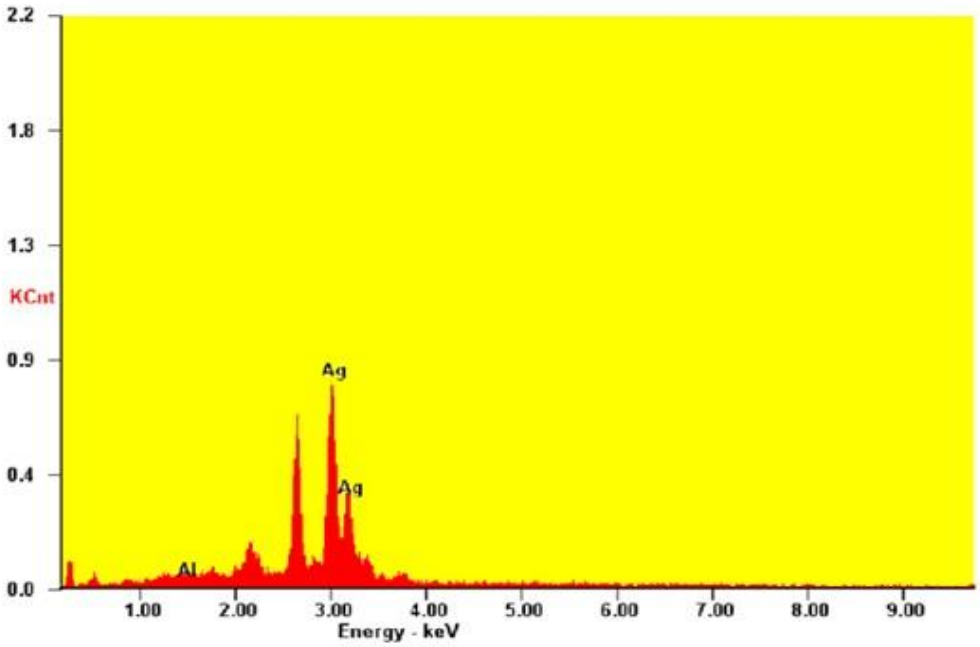


**Fig 2: Shows the Energy Dispersive Spectroscopy of green synthesized AgNPs.**

FTIR measurement was performed to identify various functional groups present in the different plant extracts. All the extracts tested in the present study shows the presence of various functional groups such as Carboxylic acids, Phenols, Alkynes, Alkanes and Primary amines.

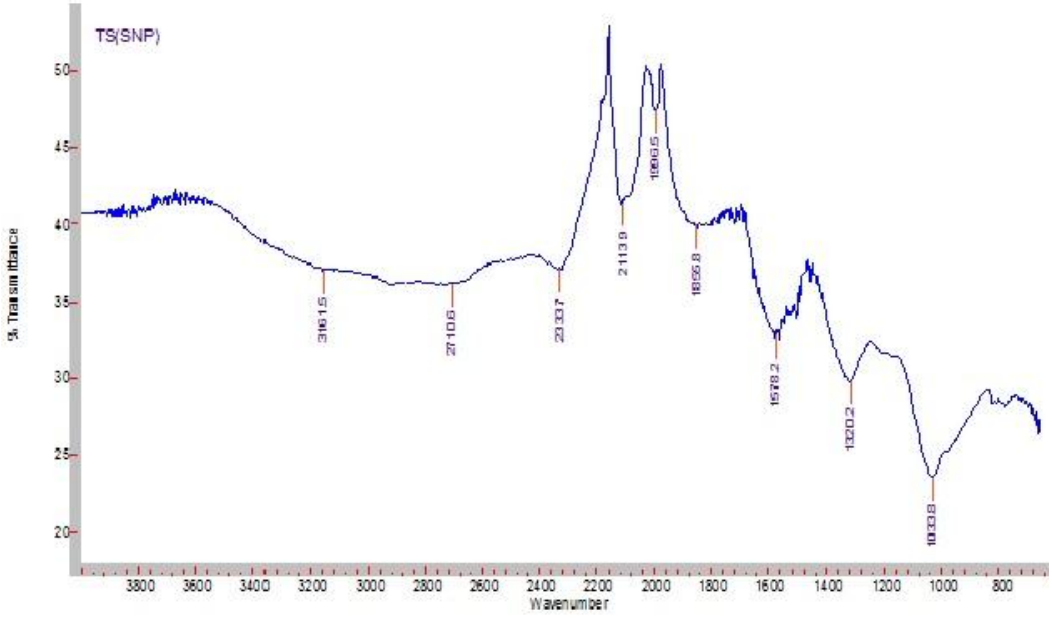


**Fig 3: FTIR spectrum of *Turnera Subulata* mediated synthesized silver nanoparticles.**

Figure 3 shows the FTIR spectrum of silver nanoparticles with the presence of a broad peak at 3161.5cm$^{-1}$ representing the functional group of carboxylic acids (O-H Stretching). A peak at 2710.6 cm$^{-1}$ represents aldehydes H-C=O (C-H Stretch). The functional group Phosphine (P-H) was confirmed with a peak at 2333.7 cm$^{-1}$. The peak at 2113.9 cm$^{-1}$ and 1996.5 cm$^{-1}$ represents the functional group of Alkynes (C=C stretch) and Alkanes (C-H) respectively. The presence of primary amines (C-N) was observed with the peak at 1578.2 cm$^{-1}$, 1320.2 cm$^{-1}$ and 1033.8 cm$^{-1.}$

The Methanol, Ethanol, Ethyl acetate, Chloroform and Aqueous extract of *Turnera subulata* were screened for larvicidal bioassay using water has a vehicle. The larvicidal activity of the extracts was calculated as per the procedure described by WHO. The experiment was carried out with 5 replicates of 20 larvae each of early 4$^{th}$ instar of *C. quinquefasciatus*. A total of 100 larvae were exposed to different concentrations for each extract. Five different concentrations of plant extracts ranging from 50ppm to 250ppm were prepared with different extracts. The silver nanoparticles were dissolved in distilled water with the concentration ranging from 1ppm to 5ppm. The observation of data presented in the table 2 shows the $LC_{50}$

and $LC_{90}$ values (95% confidence interval) of *Turnera subulata* were 103.33 (77.419-122.75) and 310.53 (271.34-376.01) for aqueous extracts, 82.02 (56.97-101.92) and 262.42 (233.52-307.51) for methanol extract, 81.09 (51.17-101.96) and 279.85 (246.06-335.27) for ethanol extract and 58.79 (26.29 – 80.69) and 238.64 (211.64 – 279.35) for ethyl acetate extract and with reference to chloroform extracts the $LC_{50}$ and $LC_{90}$ values were 51.02 (14.93 – 74.62) and 235.417 (208.59 – 277.81) after 96 hrs of exposure. The percentage of mortality amounts to 95.60 ± 0.748 which represents chloroform extract of *Turnera subulata* (Table 3).

**Table 2 Dose - response larvicidal activity of different leaf extracts of *Turnera subulata* against 4th instar larvae of *C.quinquefasciatus.***

| | $LC_{50}$ | Lower limit | Upper limit | $LC_{90}$ | Lower limit | Upper limit | Chi square |
|---|---|---|---|---|---|---|---|
| **Aqueous** | 103.33 | 77.419 | 122.785 | 310.531 | 271.345 | 376.01 | 3.846 |
| **Methanol** | 83.023 | 56.973 | 101.927 | 262.423 | 233.524 | 307.501 | 5.248 |
| **Ethanol** | 81.091 | 51.178 | 101.963 | 279.859 | 246.06 | 335.276 | 5.117 |
| **Ethyl acetate** | 58.791 | 26.29 | 80.696 | 238.083 | 211.638 | 279.357 | 4.095 |
| **Chloroform** | 51.021 | 14.931 | 74.621 | 235.417 | 208.596 | 277.813 | 3.943 |
| **AgNPs** | 45.499 | 16.045 | 65.710 | 190.580 | 171.549 | 217.785 | 6.931 |

**Table 3: Larvicidal activity (% mortality) of early 4th instar larvae of *Culex quinquefasciatus* exposed to various leaf extracts of *Turnera subulata.***

| Dosage | Aqueous | Methanol | Chloroform | Ethylacetate | Ethanol |
|---|---|---|---|---|---|
| **50ppm** | 38.00 ± 0.447 | 41.80 ± 0.374 | 51.60 ± 0.400 | 51.00 ± 8.894 | 42.80 ± 0.663 |
| **100ppm** | 48.20 ± 0.374 | 55.60 ± 0.400 | 63.40 ± 0.748 | 63.60 ± 1.077 | 54.20 ± 1.020 |
| **150ppm** | 62.60 ± 0.678 | 67.60 ± 0.400 | 77.80 ± 0.663 | 73.80 ± 0.860 | 65.00 ± 0.632 |
| **200ppm** | 76.40 ± 0.510 | 77.80 ± 0.374 | 86.00 ± 0.894 | 79.60 ± 2.993 | 77.20 ± 0.583 |
| **250ppm** | 82.60 ± 0.927 | 91.40 ± 0.510 | 95.60 ± 0.748 | 93.80 ± 0.583 | 88.00 ± 0.447 |

The larvicidal potential was due to the various secondary metabolites present in the extracts. It is evident from the present results that most of the bioactive compounds were absent in aqueous extract when compared to the chloroform extract were the maximum percentage mortality of 96% was observed after 96 hrs of exposure.

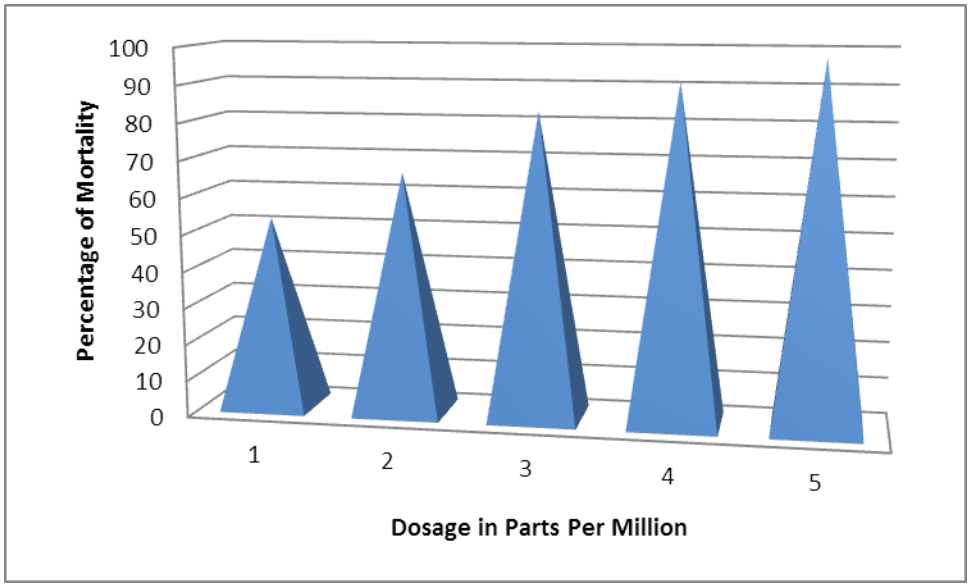


**Fig 4: shows larvicidal activity (% Mortality) of AgNPs on 4th instar larvae of *Culex quinquefasciatus.***

However, when compared to $LC_{50}$ and $LC_{90}$ values of silver nanoparticles against the 4th instar larvae of *C. quinquefasciatus* was 45.499 (16.04-65.71) and 190.58 (171.54-271.785) and 98% mortality (Fig4) was evident at 5ppm concentration of green synthesized silver nanoparticles.

## DISCUSSION

Mosquitoes are the most important pest affecting the health of people and animals. The Culucidae family of Diptera order includes around 2500 species of mosquitoes all around the world. Vector-borne diseases cause more damages to the public health in developing countries. The plant extract contain complex of chemicals with potential biocidal activities, which serves as a suitable alternative to synthetic insecticides.[13,14] The plant-based insecticides were considered ecofriendly, safe and easily biodegradable.[15] The plant metabolites cause several physiological damages in the body of insects.[16] The treatment of *Turnera subulata* on the 4th instar larvae of *Culex quinquefasciatus* might result in the inhibition of enzymes for the cuticular oxidative process as suggested by Vinayagam et al.[17] The morphogenic changes of the mosquito larvae are attributed to the interaction of bioactive compounds with the endocrine system of insects.[18] The larvicidal activity recorded in the present study may be due to the interaction of plant extracts with the target systems of the mosquitoes. Basu and Basu[19] suggested that the biological and larvicidal efficacy of the plant extract were due to the presence of alkaloids. The present study also reveals the same

trend as the chloroform leaf extract shows the maximum larvicidal activity against *Culex quinquefasciatus* which may be attributed due to the maximum amount of alkaloids present in the crude leaf extract.

The biocidal activities of the various metabolites of the plant extracts increases the larvicidal potential.[20] It is evident that the alcoholic extracts possesses a moderate amount of alkaloids and terpenoids along with other metabolites showing more than 90% mortality in 4$^{th}$ instar larvae of *Culex quinquefasciatus*. The dose dependent increase in the percentage mortality was supported by the findings of Manzoor et al.,[21] They reported a dose-dependent larvicidal activity of essential oils against *Aedes aegypti* and *Culex quinquefasciatus*. *R.cordifolia* root extract shows larvicidal activity against *Culex quinquefasciatus* with $LC_{50}$ and $LC_{90}$ values of 95.69 and 349.62ppm while in the present study the chloroform leaf extract of *Turnera subulata* shows maximum larvicidal activity with $LC_{50}$ and $LC_{90}$ values of 51.21 and 233.42ppm with a percentage mortality of 95.60 It is suggested from the present study that the different extracts of *Turnera subulata* show a moderate to high percentage of activity against *Culex quinquefasciatus*, the variation in the percentage of mortality among the different extracts may be due to the combination of bioactive secondary metabolites in the extracts.

The green synthesized silver nanoparticles (AgNPs) were tested against the early 4$^{th}$ instar larvae of *Culex quinquefasciatus* and the maximum efficacy was with the $LC_{50}$ and $LC_{90}$ values of 74.419 and 190.58ppm with the percentage mortality of 98%. Rajkumar and Rahuman[22] reported the $LC_{50}$ value of 27.49 with silver nanoparticles against the third instar larvae of *Culex quinquefasciatus*. The FTIR spectrum of AgNPs suggests the presence of phytocompounds which are bound free to the silver nanoparticles. The presence of various functional groups such as alcohols and alkenes was evident in the silver nanoparticles as the compounds were responsible for the stabilization and capping agents for the green synthesized silver nanoparticles.[23] Observations of the present study indicates the effect of chloroform mediated crude leaf extract and *Turnera subulata* aqueous extract mediated silver nanoparticles over the early 4$^{th}$ instar larvae of *Culex quinquefasciatus*.

## CONCLUSION

*Turnera subulata* leaf extracts shows a potential larvicidal activity against *Culex* mosquitoes of which the chloroform leaf extracts shows more than 95 percent of mortality in the 4$^{th}$ instar larvae. It is required to identify the compound with potential larvicidal activity from the

chloroform extract of the plant and the application of eco-friendly green synthesized silver nanoparticles for the control of mosquitoes thus will pave the alternative natural way for the synthetic larvicidal agents.

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