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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. LC_{20} and LC_{50} 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. officinal* AgNPs. The results were based on LC_{20} and LC_{50} 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*.

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AJ, MT and TM designed the study. AJ conducted the experiments. MT, TM and AG supervised the work. AG and TZ helped in data analysis. AJ, MT and AG wrote the manuscript.

Key words
Biopesticide, Green synthesized nanoparticles, *Culex pipiens*, LC_{50} and LC_{20}, Male and female longevity

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*

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azedarach, 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 ($AgNO_3$) 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	20	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

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 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.

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	0.756	3.48	2.751	4.314
	48	0.228	0.18	2.295	0.391	3.06	2.322	3.853
	72	0.262	0.16	2.218	0.204	2.48	1.813	3.156
	96	0.299	0.10	2.164	0.079	2.04	1.414	2.627
Zingiber officinale	24	0.009	21.7	180.223	36.067	80.97	32.836	142.294
	48	0.009	20.62	224.575	29.348	71.85	16.705	130.442
	72	0.010	18.20	176.284	23.252	62.79	14.255	107.350
	96	0.012	10.98	108.506	16.595	53.55	19.765	83.286
Syzygium aromaticum	24	0.023	6.15	47.83	14.023	34.03	18.043	52.826
	48	0.023	4.45	22.575	4.115	30.75	23.172	38.521
	72	0.027	3.77	17.223	4.898	27.29	20.737	33.923
	96	0.030	3.00	17.35	3.76	23.97	17.77	30.03
Datura stramonium	24	0.001	380.74	621.678	288.388	612.07	353.834	916.938
	48	1.797	78.05	302.732	476.855	1966.29	352.234	513.448
	72	0.001	75.60	776.350	202.892	520.63	253.449	792.263
	96	0.001	15.99	586.865	156.004	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	0.215	0.50	0.234	0.789
	48	1.684	0.08	0.255	0.101	0.46	0.353	0.569
	72	1.628	0.06	0.315	0.069	0.43	0.324	0.547
	96	1.786	0.03	0.321	0.052	0.38	0.274	0.482
Zingiber officinale	24	0.053	3.32	18.485	5.668	14.41	7.867	21.609
	48	0.055	3.02	17.240	4.481	13.20	7.230	19.326
	72	0.056	2.16	20.918	3.70	11.60	4.954	17.763
	96	0.064	1.42	9.33	0.914	10.10	7.163	12.86
Syzygium aromaticum	24	0.110	1.39	13.113	2.412	6.29	2.525	10.316
	48	0.121	1.30	11.329	2.254	5.71	2.224	9.209
	72	0.128	1.22	9.205	1.815	5.30	2.338	8.129
	96	0.145	1.01	3.672	0.678	4.79	3.528	6.027
Datura stramonium	24	0.007	13.85	54.666	25.898	112.92	88.293	138.738
	48	0.007	7.30	53.774	23.551	105.12	81.542	129.422
	72	0.008	7.10	51.390	21.494	95.91	73.770	118.380
	96	0.009	6.08	59.450	14.886	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}, LC_{20} 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.

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 I and II). The lethal and sub lethal effects of four plant extracts and their AgNPs on 3rd instar larvae of *C. pippins,* LC_{50} and LC_{20} values have been given in Tables III and IV.

Table V. Larvicidal toxicity of plant 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	0.5	22	22	24	24
	1	34	34	36	38
	2	48	50	52	58
	4	72	72	74	76
	8	92	94	94	96
Zingiber officinale	12.5	20	22	22	24
	25	32	34	36	38
	50	50	50	52	52
	100	70	72	72	74
	200	88	90	92	94
Syzygium aromaticum	5.5	20	20	22	24
	11	34	36	36	38
	22	48	48	50	52
	44	66	68	70	72
	88	90	92	92	94
Datura stramonium	102.5	18	20	20	24
	205	32	34	36	36
	410	48	50	50	52
	820	66	68	70	72
	1640	88	90	92	92

The effects of four plant extracts and their concentrations at different time periods against 4th instar larvae of mosquito showed significant effects. All the plant extracts and their AgNPs gave the maximum mortalities of the 4th instar larvae after 96 hs. It was also observed that 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	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	36	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	36	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_{20} and LC_{50} 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. The female longevity was found to be the maximum by *A. indica* AgNPs and that of the 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	0.59	0.401	1.682	2.49	3.110	1.880
	48	0.308	0.55	0.433	1.519	2.39	2.980	1.815
	72	0.302	0.41	0.255	1.832	2.22	2.813	1.624
	96	0.327	0.33	0.558	3.184	1.98	2.915	0.936
Zingiber officinale	24	0.010	15.86	26.375	123.640	67.53	107.31	28.77
	48	0.011	15.21	19.987	99.104	62.66	93.96	30.48
	72	0.011	15.06	17.481	89.809	59.12	87.118	29.631
	96	0.012	13.12	4.877	48.872	54.62	69.33	39.20
Syzygium aromaticum	24	0.024	7.02	19.526	4.982	30.06	22.910	37.408
	48	0.026	6.05-	18.429	4.803	28.41	21.570	35.348
	72	0.025	2.57	20.896	3.357	26.96	19.964	33.874
	96	0.027	1.28	21.7	2.210	24.48	17.62	31.05
Datura stramonium	24	0.001	145.55	796.406	228.430	591.65	317.562	892.342
	48	0.001	99.42	746.608	180.160	541.78	289.950	798.441
	72	0.001	92.42	670.076	167.617	511.77	278.944	743.831
	96	0.001	68.22	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	1.244	0.07	0.662	0.216	0.61	0.380	0.891
	48	1.417	0.06	0.311	0.103	0.53	0.411	0.661
	72	1.526	0.06	0.302	0.091	0.48	0.371	0.606
	96	1.748	0.05	0.307	0.064	0.40	0.295	0.506
Zingiber officinale	24	0.051	4.36	26.391	6.464	14.74	6.489	24.056
	48	0.053	4.28	10.110	1.559	12.98	9.653	16.310
	72	0.054	2.94	11.889	0.267	11.12	7.736	14.296
	96	0.062	1.79	11.239	0.062	9.23	6.103	12.043
Syzygium aromaticum	24	0.122	1.43	3.423	1.287	6.30	4.901	7.789
	48	0.119	1.41	4.560	0.644	5.71	4.233	7.185
	72	0.125	1.36	4.529	0.507	5.29	3.858	6.688
	96	0.132	0.56	4.399	0.457	4.98	3.602	6.320
Datura stramonium	24	0.006	23.81	325.250	58.609	123.68	37.807	224.463
	48	0.006	22.05	231.543	46.085	110.75	40.892	182.554
	72	0.007	19.51	175.619	36.047	104.63	48.290	160.156
	96	0.008	13.98	116.999	32.306	91.14	48.996	132.257

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

Treatments	Hours	Control	LC_{20} (95% FL)	LC_{50} (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 (±0.2) ^{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	LC_{20} (95% FL)	LC_{50} (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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