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MANAGEMENT OF *AEDES AEGYPTI* USING GREEN SILVER NANOPARTICLES AND BOTANICAL EXTRACTS

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ABSTRACT

The excessive use of insecticides has led to development of resistance in *Aedes aegypti* and negative impact on environment and non-target organisms. To overcome these problems, emphasis is being laid for alternatives, therefore, in the present study, the toxicity of eight plant extracts and their green synthesized nanoparticles were evaluated against *A. aegypti*. Clove extracts caused the maximum mortalities of 92% and 90% of 3rd and 4th instar larvae of *A. aegypti* followed by ginger causing 90% and 78% mortalities respectively. On the other hand, the minimum mortalities of these larvae were caused by neem and garlic extracts. In case of green silver nanoparticles, the maximum mortalities of 3rd and 4th instar larvae of *A. aegypti* were caused by clove followed by ginger while the minimum mortalities were caused by nanoparticles of datura followed by garlic. All the green silver nanoparticles caused mortalities of both the instars of the mosquito above 80% with few exceptions. Datura extracts showed the minimum LC_{50} values after 72 hours of application followed by neem against the 3rd and 4th instar larvae of *A. aegypti*. The highest LC_{50} value was observed in case of ginger followed by clove and eucalyptus. In case of silver nanoparticles, the minimum LC_{50} values after 72 hours were recorded in case of datura, neem and garlic while the values were the maximum in case of clove and ginger. The LC_{50} values decreased with the passage of time.

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INTRODUCTION

The yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae) is a vector of several arboviruses such as chikungunya virus (Leparc-Goffart et al., 2014), zika virus (Gutiérrez-Bugallo et al., 2019) and dengue virus (Morrison et al., 2019). Dengue is the most important mosquito borne disease (Li et al., 2019) and over 390 million humans are effected by dengue virus per year in the world (WHO, 2018). Dengue and zika fever do not have any specific medication as a cure, so the most important measure is to control the mosquitoes of these diseases. These control measures include the use of synthetic insecticides (Paul et al., 2006), essential oils (Pandiyan et al., 2019) and microorganism based insecticides (Vasantha-Srinivasan et al., 2019) against mosquitoes but the chemical based insecticides are commonly used (Manjarres-Suarez and Olivero-Verbel, 2013).

The major hindrance to the use of chemical insecticides is the development of resistance in the target mosquito (Kandel et al., 2019). The other problems associated with the application of synthetic insecticides are the adverse impact on the environment, non-target organisms and negative impact on human health (Hazra et al., 2017).

To overcome these problems, best alternative is the use of plant based insecticides which are environment friendly, safer for non-target organisms (Abbas et al., 2014) and less toxic to the ecosystem (Ghosh et al., 2012). Plant extracts have also been identified as strong repellent insecticides against mosquitoes (Misni et al., 2009). The plant extracts have been used in several ways for the management of mosquitoes. They have also been utilised as repellents in the form of oils against mosquitoes. Mosquito killing products like coils made from dried plant materials are also being used for the management of mosquitoes (Remia et al., 2017). In Java, plant synthesized incense has also been used routinely in order to repel mosquitoes (Sangat-Roemantyo, 1990).

Green synthesized nanobased insecticides are getting more attention due to many advantages such as enhancing the efficacy of botanical insecticides with very low quantity and without resistance against target pest (Pavunraj et al., 2017). Nanoparticles are substances possessing smaller particle size and are widely used in biological and medical science (Iravani, 2011). Among various metal nanoparticles, the silver nanoparticles (AgNPs) have got more attention due to broad applications in different fields (Fouad et al., 2018) and being less harmful to the ecosystem (Suresh et al., 2014). Green based silver nanoparticles are a modern practice which increases the insecticidal effectiveness of plant extracts (Barnawi et al., 2019).

To overcome the issues coupled with the use of pesticides, emphasis is being laid to quest alternative strategies such as use of biopesticides which are environmentally friendly and economical. As little research has been done to control the mosquito with green synthesized nanoparticles, therefore, the current study was carried out to manage *Aedes aegypti* with plant extracts and their green synthesized silver nanoparticles.

MATERIAL AND METHODS

Plant materials: Eight plants were evaluated in the present study for the management of *Aedes aegypti*. The test plants include neem, ginger, bakain, eucalyptus, garlic, clove, datura and bitter gourd. The lethal effects of these plants and their silver synthesized nanoparticles were tested against 3rd and 4th instars larvae of *A. aegypti*.

Preparation of botanical 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^{o}C in a freezer. The extract was then converted into the crude extract form by placing it in an electric rotary evaporator at 78^{o}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 hours, the extract was removed from the beaker, weighed, and then stored at 4^{o}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).

Synthesis of green silver nanoparticles:

After the crude extracts of eight 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. This was then 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 hours 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).

Rearing of larvae of *A. aegypti:* The larvae of *A. aegypti* were collected from the National Institute of Health Sciences Islamabad and brought to the Insect Molecular Biology laboratory, Department of Entomology, Arid Agriculture University, Rawalpindi. The larvae were reared in plastic trays which were maintained in rearing jars. The front side of rearing cage was covered with a muslin cloth for aeration and feeding. The larvae were fed on commercial based beef liver powder. A temperature of 25°C and a relative humidity of 85% were maintained in the laboratory.

Assessment of botanical extracts and green synthesized Ag-nanoparticles against *A. aegypti:* A total of six concentrations of plant extracts with five replications were assessed against 3rd and 4th larval instars of *A. aegypti*. The bioassays were performed in plastic dishes and mortalities were recorded after 24, 48, and 72 hours of treatment. The concentrations were recorded as C1 to C5 and control was referred to as C0. C1 denoted the lowest concentration value while C5 represented the highest one. The same procedure was followed for the evaluation of green synthesized Ag-nanoparticles.

Statistical analysis: LC_{50} values were calculated using probit analysis. The percentage corrected mortality of mosquito larvae was calculated using Abbot's formula (Abbott, 1925). R statistical software was used for data analysis. Probit analysis was conducted using Polo-plus software.

RESULTS

Larvicidal potential of plant extracts against 3rd and 4th instar larvae of *A. aegypti:* Clove extracts caused the maximum mortalities of 92% and 90% of 3rd and 4th instar larvae of *A. aegypti* followed by ginger causing 90% and 78% mortalities respectively. On the other hand, the minimum mortalities of these larvae were caused by neem and garlic extracts (Table 1).

Table 1: Percent mortality of 3rd and 4th instars of *Aedes aegypti* with the highest concentrations of plant extracts and green synthesized nanoparticles after 72 hours.

Botanical used	Mortality of *A. aegypti* with botanical extracts against		Mortality of *A. aegypti* with green nanoparticles against	
	3rd instar	4th instar	3rd instar	4th instar
Neem	72%	72	94	82%
Ginger	90%	78	96	88%
Bitter gourd	84%	76	92	72%
Eucalyptus	80%	76	90	82%
Datura	84%	72	74	80%
Clove	92%	90	98	90%
Bakain	80%	74	92	82%
Garlic	72%	70	86	80%

Larvicidal potential of plant based nanoparticles against 3rd and 4th instar larvae of *A. aegypti:* In case of green silver nanoparticles, the maximum mortalities of 3rd and 4th instar larvae of *A. aegypti* were caused by clove followed by ginger while the minimum mortalities were caused by nanoparticles of datura followed by garlic. All the green silver nanoparticles caused mortalities of both the instars of the mosquito above 80% with few exceptions (Table 1).

LC_{50} values of plant extracts against 3rd and 4th instar larvae of *A. aegypti:* The LC_{50} values of plant extracts against 3rd instar larvae are shown in Table 2.

Datura extracts showed the minimum LC_{50} values after 72 hours of application followed by neem against the 3rd instar *A. aegypti* larvae. The LC_{50} values decreased with an increase in the time. The highest LC_{50} value was observed in case of ginger followed by clove and eucalyptus as shown in Table 2. Similar trend of LC_{50} values was observed in case of 4th instar larvae (Table 3).

Table 2: LC_{50} values of plant extracts at three time intervals against 3rd instar larvae of *Aedes aegypti.*

Plant extract (mg/L)	LC_{50} with fiducial limit at		
	24 hours	48 hours	72 hours
Ginger	276.640	236.788	209.632
	248.324-309.933	213.058-258.178	181.439-230.950
Garlic	68.09	61.75	46.04
	61.17-78.74	54.56-72.43	38.30-54.03
Neem	24.475	22.494	19.206
	22.477-27.510	20.340-25.431	16.433-21.670
Bakain	37.415	30.681	24.911
	32.972-43.269	25.622-35.891	18.690-29.8473
Bitter gourd	195.892	158.703	125.985
	171.394-234.192	137.804-185.192	106.103-146.131
Clove	378.741	317.073	225.291
	339.008-429.678	274.980-362.059	174.681-265.229
Eucalyptus	322.550	254.184	208.353
	281.748-381.839	216.854-298.708	170.724-245.883
Datura	0.439	0.378	0.305
	0.394-0.505	0.333-0.438	0.263-0.348

Table 3: LC_{50} values of plant extracts at three time intervals against 4th instar larvae of *Aedes aegypti.*

Plant extract (mg/L)	LC_{50} with fiducial limit at		
	24 hours	48 hours	72 hours
Ginger	307.758	262.717	216.522
	281.047-350.246	235.843-293.170	178.071-243.943
Garlic	72.92	63.34	52.33
	65.98-8.399	56.82-7.276	45.84-6.013
Neem	26.394	22.566	19.565
	24.184-30.153	20.495-25.357	17.117-21.867
Bakain	44.165	35.130	27.063
	38.913-52.711	29.261-42.978	20.552-32.618
Bitter gourd	223.356	183.159	138.195
	194.825-273.252	150.940-241.615	113.353-166.423
Clove	443.163	302.493	230.907
	392.380-524.221	258.802-346.784	177.686-272.912
Eucalyptus	371.031	307.650	209.013
	323.842-447.876	261.255-377.914	166.707-251.618
Datura	0.473	0.422	0.341
	0.427 -0.547	0.369-0.505	0.290-0.403

LC_{50} values of plant-based Ag-nanoparticles against 3rd and 4th instar larvae of *A. aegypti:* The LC_{50} values of the green synthesized silver nanoparticles used against 3rd instar larvae are shown in Table 4. The minimum LC_{50} values after 72 hours were recorded in case of datura, neem and garlic while the values were the maximum in case of clove and ginger. A similar trend was found in case of 4th instar larvae. On the other hand, the maximum values were observed with clove and ginger. The LC_{50} values decreased with the passage of time. The values were the maximum after 24 hours and decreased as the time interval increased (Table 5).

Table 4: LC_{50} values of green synthesized Ag-nanoparticles at three time intervals against 3rd instar larvae of *Aedes aegypti.*

Plant extract (mg/L)	LC_{50} with fiducial limit at		
	24 hours	48 hours	72 hours
Ginger	46.798	39.743	31.696
	41.417-51.481	31.46-44.338	22.901-36.953
Garlic	11.023	9.687	6.769
	9.475-13.136	8.210-11.332	4.904-8.162
Neem	4.109	3.501	2.795
	3.709-4.533	3.006-3.881	2.170-3.189
Bakain	6.141	4.427	3.916
	5.068-7.251	3.139-5.371	2.641-4.813
Bitter gourd	34.408	24.253	15.011
	28.983-42.703	18.841-29.510	8.554-19.560
Clove	61.574	46.570	33.899
	53.442-69.973	36.377-54.720	9.017-46.885
Eucalyptus	52.344	38.279	17.973
	43.965-62.950	28.809-47.029	2.947-27.305
Datura	0.076	0.061	0.044
	0.068-0.087	0.051-0.072	0.030-0.054

Table 5: LC_{50} values of green synthesized Ag-nanoparticles at three time intervals against 3rd instar larvae of *Aedes aegypti.*

Plant extract (mg/L)	LC_{50} with fiducial limit at		
	24 hours	48 hours	72 hours
Ginger	53.729	39.738	35.790
	48.249-60.363	30.891-45.312	27.100-41.105
Garlic	13.052	11.218	6.769
	11.657-15.167	9.885-12.977	4.904-8.162
Neem	4.595	3.862	3.165
	4.203-5.130	3.404-4.282	2.437-3.618
Bakain	7.688	5.841	3.594
	6.548-9.425	4.512-7.108	1.222-4.888
Bitter gourd	39.183	30.952	22.810
	33.280-49.486	25.450-38.443	14.799-29.712
Clove	79.183	53.106	37.943
	68.167-96.217	39.637-64.372	23.551-47.609
Eucalyptus	63.474	49.399	31.173
	54.262-77.609	40.969-59.492	19.322-40.270
Datura	0.085	0.072	0.054
	0.075-0.100	0.061-0.087	0.042-0.064

DISCUSSION

In the current study, all the plant extracts caused 50% mortalities of 3rd and 4th instar larvae of *A. aegypti* while Ag-nanoparticles of these plants gave even higher mean mortality values. 4th instar larvae were found resistant to plant extracts and nanoparticles as compared to 3rd instar larvae of the mosquito.

The mortalities caused by plant extracts and green sliver nanoparticles could be attributed to certain insecticidal and insect repellant compounds found in the green synthesized silver nanoparticles of the plants. The clove plant contains the chemical eugenol which is known for its medicinal and antibiotic potential. Medeiros et al. (2013) evaluated the larvicidal potential of clove extracts and eugenol against two mosquito species i.e. *Anopheles darlingi* and *A. aegypti*. The results showed that *A. aegypti* was 63 times more susceptible to clove extracts as compared to *A. darlingi* larvae. Similarly, bakain showed good larvicidal potential and required a relatively large concentration to kill 50% of the mosquito population in the present study. The results are supported by the study of Selvaraj and Mosses (2011) who evaluated the efficacy of bakain on three different mosquito species i.e. *A. aegypti, Culex quinquefasciatus,* and *A. stephensi*. The dengue mosquito species was found to be the most resistant to bakain extracts. The other two species showed very similar levels of susceptibility.

Ginger was found to cause the maximum mortality of mosquitos. Similar results were obtained by Nasir et al. (2017) who tested different plant extracts and confirmed that *Zingiber officinale* had the highest effectiveness in inducing mean mortality in immature *A. albopictus* larvae. The 3rd instar larvae were the least resistant to plant extracts as compared to 4th instar larvae. It was also found that plant oils of eucalyptus gave the highest mean mortality and that of ginger the lowest. The study also showed that *Azadirachta indica* extracts gave the highest LC_{50} values of all the test plants.

In the present study, bitter gourd and eucalyptus extracts proved to be less effective against *A. aegypti* larvae. The present results were in contrast to those reported by Singh et al. (2006) in which they treated the 4th instars of three different mosquito species with crude fruit extracts of *Momordica charantia* and obtained good results.

Eucalyptus was also an average performer in the current study, giving similar results to bitter gourd. A study conducted by Nair et al. (2014) also reported the larvicidal potential of leaf extracts of eucalyptus as well as of the medicinal plant *Centella asiatica*. According to these authors, eucalyptus was not as effective as its counterpart in inducing mean mortality in the targeted mosquito species i.e. *A. aegypti* and *A. stephensi*.

The extracts and Ag-nanoparticles of garlic did not perform well in this study against *A. aegypti* mosquito larvae. Garlic had by far the highest amount required to reach the LC_{50} value. These results conformed to the findings of Selvam and Durai (2018)Selvam and Durai (2018) who evaluated the larvicidal potential of 11 different plant extracts against *A. albopictus* and an unspecified species of *Anopheles*. In their study, *Allium sativum* bulb extracts in ethyl alcohol gave the worst performance out of all the 11 test plant extracts. Conversely, the extracts of *A. indica* showed the best larvicidal potential. As these were the only two plants out of the 11 used in this study which were the same as the plants used in the current study. In another study, Ali et al. (2017) tested five plants against *A. aegypti* larvae. Neem showed the highest larvicidal potential whereas eucalyptus did not prove effective and confirmed the present findings.

In all cases, the Ag-nanoparticles outperformed their plant extract counterparts in causing larval mean mortality of both the larval instars. The effectiveness of Ag-nanoparticles against *A. albopictus* larvae has previously been demonstrated by Ga'al et al. (2018). They utilized three different types of silver nanoparticles: first was artificially fabricated while the other two were synthesized using Salicylic acid and 3, 5-dinitrosalicylic acid respectively. It was found that the artificially synthesized Ag-nanoparticles proved to be highly toxic to dengue mosquito larvae as compared to the ones derived from Salicylic acid whereas the ones derived from 3, 5-dinitrosalicylic acid did not cause significant mortality. It is therefore, concluded that the artificially synthesized Ag-nanoparticles can be used as an environmentally friendly alternative insecticide for the management of *A. aegypti*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

HS, MT and AG designed the study, HS and MSB conducted the experiments and collected data, MT and ZRM analyzed the data, MT supervised the work, HS and MT wrote the manuscript and all the authors edited and approved the final manuscript.

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