

Optimizing Synthesis of *Citrus limetta* Peel Silver Nanocomposites Possessing Larvicidal Potential against Dengue Vector, *Aedes aegypti* L.

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Abstract *Aedes aegypti* L. is the major vector accountable for the spread of several diseases of medical importance. The control strategies primarily relying on chemical insecticides have caused negative impact on our environment and human health. Thus, current study employed *Citrus limetta* peel extracts (CLPE) against larvae of *Ae. aegypti*. Silver nanocomposites (AgNCs) from CLPE were synthesised and the process of synthesis was optimized by varying temperature; volume and concentration of silver nitrate solution; and the volume of catalyst. A conspicuous change in colour of the reaction mixture was noticed from pale yellow to dark brown. This indicated the synthesis of AgNCs which was traced by UV-Visible spectroscopy. The optimum synthesis of CLPE-AgNCs was obtained with the mixture of 4 mL AgNO₃ (3mM) and 3 mL CLPE. The larvicidal assay with these nanoparticles against *Ae. aegypti* resulted in LC₅₀ and LC₉₀ values of 26.82 µg/mL and 99.32 µg/mL after 24 h of exposure; which respectively decreased to 19.51 µg/mL and 71.99 µg/mL after 48 h of exposure. The results elucidate 1.3-fold higher larvicidal efficacy of nanoparticles with increased duration of larval exposure. We suggest that synthesis of AgNCs by utilizing peel extract of *C. limetta* is a cost-effective and eco-safe alternative to conventional insecticides; and can be utilized as the potent mosquito larvicide.

Keywords Green Synthesis, Silver Nanocomposites, *Citrus limetta*, Larvicidal, *Aedes aegypti*

1. Introduction

Aedes aegypti, the dreadful and global vector of fatal

diseases; like Yellow fever, Zika, Dengue fever and Chikungunya; has captivated attention of researchers, health organizations and vector control bodies. In the last few decades, the prevalence of *Aedes*-borne diseases; especially dengue; has augmented extensively at global level. According to World Health Organization, almost half of the world's population (3.9 billion) residing in 128 countries is prone to the dengue infection while more than 100 countries are endemic to dengue [1]. According to Union Health Ministry of India, the infections and fatalities caused by dengue virus are rising in India year by year raising serious concerns about the vector control.

It has been suggested that the rising dengue cases in India may be because of inappropriate vector control strategies, unplanned urbanization, variable environmental factors, host-pathogen interactions and immunological factors [2]. The radical shift of dengue from urban areas to rural areas is also considered one of the reasons for increase in incidences and severity of disease [3]. Though, Dengvaxia (R), a dengue vaccine, has been formulated and registered in several countries; it has not yet been approved by the Government of India and has recommended more clinical trials [4]. Because of lack of adequate medication and vaccines, the only measure to control the dengue is still based on the vector control.

Various measures have been attempted for *Aedes* control, yet major strategies still rely upon the use of chemical insecticides despite their harmful effects on the environment and human health. Therefore, researchers are nowadays exploring botanical extracts to formulate an eco-safe and effective remedy. Several botanicals, weeds and fruit wastes have been investigated as mosquito control agents and found effective [5-7]. The fruits, seeds, roots and leaves of the citrus plant have also been examined for their potential as mosquito control agents [8, 9]. The citrus

peels are being explored as a safe alternate to synthetic insecticides against mosquitoes as these are nutrients-rich and contain several secondary metabolites. Reports have revealed the larvicidal efficacy of the ethanolic extracts prepared from orange peel (*Citrus sinensis*) against *Ae. aegypti* [10]. The moderate efficacy of hexane leaf extracts of *C. sinensis* has been reported by Warikoo et al. [11] against early fourth instar of *Ae. aegypti*. They obtained LC₅₀ and LC₉₀ values of 446.84 and 1370.96 ppm, respectively, after 24 h of larvicidal bioassay. Earlier, essential oil from *Citrus limon* was found effective against larvae of *Ae. aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* [12]. Our earlier studies have established larvicidal efficacy of hexane and petroleum ether extract of *Citrus limetta* (Sweet Lime or *Mosambi*) peels against *Ae. aegypti* [13]. The study showed LC₅₀ values of 96.15 ppm and 145.50 ppm on 24 h larval exposure to hexane and petroleum ether extracts, respectively. The present study is an extension to our previous study and is aimed to formulate nanocomposites from *C. limetta* peel extracts, for probable use as more effective agents of mosquito control than crude extracts.

The formulation of nanoparticles (NP) using various agents has received substantial deliberation due to their exclusive features and potential applications in diverse fields [14]. The green synthesis of NPs using various biological agents is preferred over chemical and physical synthesis of NPs and, is considered more consistent, dependable and eco-friendly. This may be attributed to the biodegradable nature of agent used and absence of toxic chemicals. Therefore, utilization of botanicals and their secondary metabolites, such as flavonoids, tannins and ascorbic acid, is preferential for the formulation of NPs. Numerous plants have been investigated for the synthesis of silver nanocomposites; including gwarpatha (*Aloe vera*), neem (*Azadirachta indica*), green tea (*Camellia sinensis*), leguminous shrub (*Sesbania drummondii*), natural rubber (*Hevea brasiliensis*), lemongrass (*Cymbopogon citratus*) and prickly chaff flower (*Achyranthes aspera*), etc. [7, 15].

A few studies have reported the utilization of citrus fruit extract as an effective, natural, renewable and low-cost bio-reducing agent for the synthesis of silver nanocomposites. Citrus peel represents an economic and cost-effective substrate for green synthesis of silver nanocomposites. It is known to comprise a variety of active constituents; such as vitamin C, flavonoids and poly methoxylated flavones etc.; which are often rare in other plant extracts [16-18]. It has also been reported that *Citrus* peel yields thousands-fold more phenolic compounds as compared to its pulp extracts.

Thus, keeping in mind the biodegradable nature, cost-efficacy and larvicidal potential of *C. limetta* peel extract against *Ae. aegypti*, the silver nanocomposites were synthesized using the extract. In order to obtain a significant yield, the biosynthetic process was optimized by varying factors; such as concentration and volume of

AgNO₃ (substrate), temperature, catalyst etc. The green nano-larvicides thus formed, were assayed against early fourth instar of *Ae. aegypti* to devise an eco-safe and effective formulation for the control of *Ae. aegypti* larvae. As 50% of the annual production of citrus fruits (31 million tons) is peel waste [19], the present study of using *Citrus* peels as a nano-larvicide against *Ae. aegypti* may be highly significant.

2. Materials and Methods

2.1. Rearing of *Aedes aegypti* L.

The present investigations were conducted on the early fourth instars of *Ae. aegypti* maintained in self-established Insect Pest and Vector Laboratory, Acharya Narendra Dev College (University of Delhi), India. The mosquito was reared under controlled conditions of 28 ± 1°C, 80 ± 5% RH and 14:10 L/ D photoperiod [20].

2.2. Collection of *Citrus limetta* Peels and Preparation of Extract

Fresh and healthy fruits of *C. limetta* were collected from a local market of New Delhi, India. The peels were removed and washed methodically with tap water followed by thorough rinsing in distilled water. The peels were cut into small pieces, weighed using electronic balance (Shimadzu, BL-220H) and ground using an electric blender (Philips, HL-1606). An aqueous extract of *C. limetta* peels was prepared by heating the mixture of 10% peel extract (10 g of the blended peels in 100 mL of distilled water) at 60 °C for 15-20 minutes [21]. The extract was kept undisturbed for 2-3 hours and filtered twice through Whatman No. 1 filter paper in order to make it particulate-free. In addition, the hexane extract of *C. limetta* was also prepared through cold percolation method [22]. The clear aqueous extract (CLPE) and hexane extract of citrus peels were stored at 4 °C for further investigations.

2.3. Synthesis and Optimization of *Citrus limetta* Silver Nanocomposites

The *C. limetta* Peel Extract (CLPE) and silver nitrate solutions (AgNO₃; Merck) were combined in different ratios to prepare the nanocomposites. In the mixtures, the volume of CLPE was kept constant (3 mL) while the volume of silver nitrate was varied (2, 4, 8 and 16 mL). The bio-synthesis was also optimized by varying concentration of silver nitrate (1 mM to 5 mM), temperature (27 °C, 37 °C and 60 °C), pH and catalyst (NaOH). The mixtures were incubated overnight and monitored intermittently to measure the bio-reduction of Ag⁺. The change in colour of nano-mixture from pale yellow to dark reddish brown served as a primary indicator for the synthesis of

nanocomposites.

2.4. UV-Vis Spectroscopy

The intensity of the nanocomposite mixtures was scanned and recorded in UV-visible spectra using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan); in the 200-700 nm wavelength range and 1 nm resolution. The aqueous solution of AgNO_3 was used as control during the spectrophotometry.

The optimal conditions for the biosynthesis of CLPE-mediated silver nanocomposites were selected on the basis of spectral scans. The resultant nano-colloidal solution was centrifuged (twice) at 10,000 rpm for 10 min in Remi refrigerated Centrifuge to remove any un-interacting biological molecules. The final pellet was collected, dried in a vacuum desiccator, and kept for future investigations.

2.5. Larvicidal Bioassay against *Aedes aegypti*

The standard WHO protocol with a slight modification was followed to estimate the larvicidal potential of the CLPE-AgNCs against the early fourth instars of *Ae. aegypti* [23]. Different test concentrations, in the range of 25 $\mu\text{g/mL}$ to 200 $\mu\text{g/mL}$, were prepared from the stored pellets of CLPE-AgNCs. A total of 20 active early fourth instars of *Ae. aegypti* were exposed to 1 mL of NCs solution added to 199 mL of distilled water. Five replicates were conducted simultaneously for each concentration.

Control sets were exposed to the respective concentration of aqueous extract of *C. limetta* and AgNO_3 solution. Simultaneous larvicidal bioassays were carried out with hexane extracts of *C. limetta* peels to analyze the larvicidal efficacy obtained with nano-formulations. Larval mortality was recorded after 24 h and 48 h of exposure and subjected to statistical analysis.

2.6. Data Analysis

The data obtained was analyzed by SPSS Statistical Software Package (Version: 19.0). The lethal concentrations values were recorded at 30, 50 and 90 levels. Other statistical parameters including 95% confidential limits, chi-square, standard deviation and regression coefficient were calculated in order to compute the significance and estimate the difference between test samples.

3. Results

Present study attempted the formulation of silver nanocomposites from aqueous peel extract of *C. limetta* and assessed their possible use as a control agent of *Ae. aegypti*. Various factors in the reaction mixture were varied and ideal conditions for optimal synthesis were determined. The efficacy of synthesized green nanocomposites was evaluated against early fourth instars of dengue vector *Ae. aegypti*.

The incubation of the *C. limetta* peel extract-silver nitrate reaction mixture resulted in a significant colour change of the mixture from light yellowish-green to brown and finally dark brown (Figure 1). The change in the colour of reaction mixture is indicative of the synthesis of NPs which was confirmed by UV-Vis Spectrophotometry.



Preparation of *Citrus limetta* Peel Extract

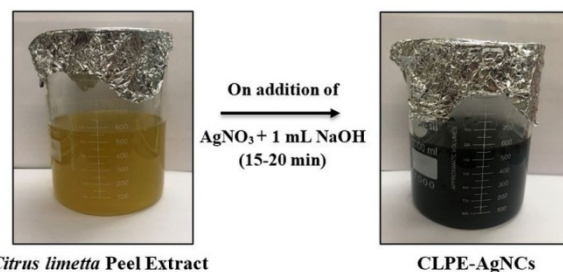


Figure 1. Synthesis of *Citrus limetta* peel extract-mediated silver nanocomposites depicted by a change in colour from light yellowish-green to dark brown

3.1. Optimization of CLPE-AgNCs Synthesis: UV-Vis Spectrophotometry

The synthesis of silver nanocomposites was monitored spectrophotometrically at a wavelength range of 200-700 nm (Figure 2, Table 1). The results showed a shift in the surface plasmon resonance (SPR) band to the higher wavelengths with increase in the volume of silver nitrate in the mixture.

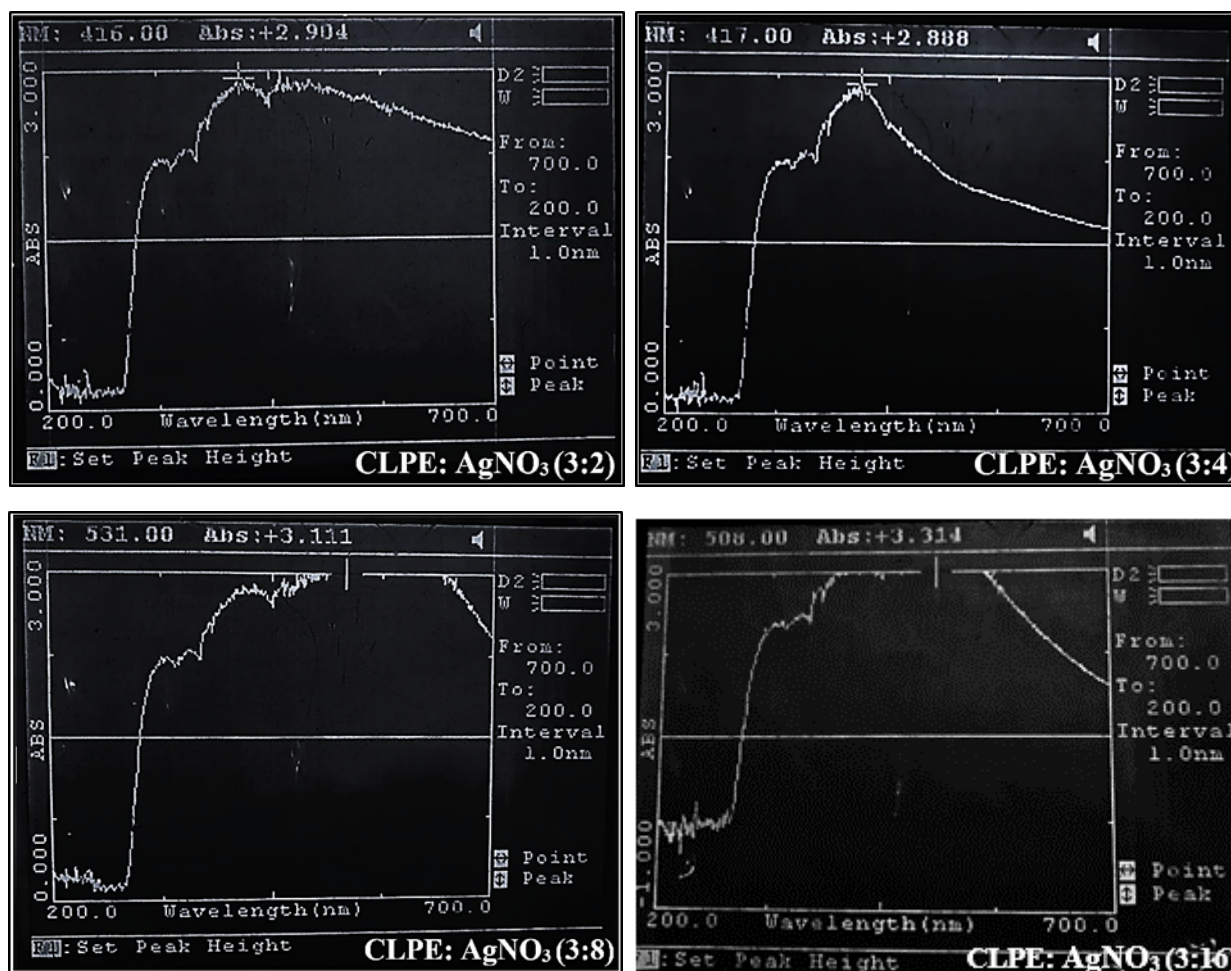


Figure 2. UV-Vis spectra of selected *Citrus limetta* peel extract-mediated synthesized silver nanocomposites in presence of 1 mL NaOH at 60 °C; 3 mL of CLPE with 3 mM silver nitrate of varied volumes; 2 mL, 4 mL, 8 mL, and 16 mL

Table 1. Optimization of *Citrus limetta* Peel Extract (CLPE)-mediated synthesis of silver nanocomposites at 60°C, in presence of 1 mL NaOH

CLPE (3mL): different volumes of AgNO ₃ solution (mL)	AgNO ₃ (1 mM)		AgNO ₃ (2 mM)		AgNO ₃ (3 mM)		AgNO ₃ (4 mM)		AgNO ₃ (5 mM)	
	λ (nm)	Abs. (a.u)	λ (nm)	Abs. (a.u)	λ (nm)	Abs. (a.u)	λ (nm)	Abs. (a.u)	λ (nm)	Abs. (a.u)
2 mL	408	2.982	407	2.747	416	2.904	374	4.095	420	2.796
4 mL	389	2.502	402	2.608	417	2.888	375	4.413	371	2.382
8 mL	386	2.319	412	2.660	531	3.111	419	4.482	352	2.225
16 mL	395	2.502	462	2.912	508	3.314	330	4.280	338	2.618

As it is clear from the Table 1, the bio-synthesis of silver nanocomposites attempted with 1 mM AgNO₃ occurred at wavelengths in the range of 386 nm - 408 nm. The nanocomposites synthesized with 2 mM AgNO₃ showed the peak in the range of 402 nm - 462 nm which reduced to 320 nm - 420 nm when 4 mM or 5 mM silver nitrate was added in the reaction mixture. The most optimal wavelength range (416-417 nm) of NPs was observed with the reaction mixture consisting of 3 mM AgNO₃.

The optimum biosynthetic combination was further confirmed by the observation of a sharp and narrow absorbance peak in UV-Vis spectra of nanocomposites

synthesized with 3 mM AgNO₃. The results are presented in Fig. 2 which shows the appearance of a noticeable, narrow and conspicuous absorbance peak at 417 nm with a mixture of 4 mL silver nitrate and 3 mL CLPE. The absorbance peaks obtained with other volumes of silver nitrate (2, 8 and 16 mL) in the reaction mixture were inconspicuous and broader indicating 4 mL of silver nitrate as the optimum volume.

In addition, varying the reaction temperature; 27 °C, 37 °C and 60 °C, showed the fastest reaction at 60 °C. Whole process of bioreduction completed within 2 h at 60 °C while at lower temperatures; the reaction took 24 h

for completion. Furthermore, addition of NaOH to reactant mixture at 60 °C increased the rate of reaction by 8-fold within 15-20 minutes.

3.2. Evaluation of Larvicidal Efficacy of *Citrus limetta* Silver Nanocomposites against *Aedes aegypti*

The *C. limetta* silver nanocomposites synthesized using the reaction mixture of 3 mL CLPE: 4 mL of AgNO₃ (3mM), were evaluated for larvicidal potential against *Ae. aegypti*. The results showed effective toxic potential of *C. limetta* peel extract-derived AgNCs against early fourth instars of *Ae. aegypti* (Table 2, Figure 3). Bioassay with just 25 µg/mL AgNCs resulted in 55% larval mortality after 24 h, while exposure to 125 µg/mL AgNCs resulted in

95% larval mortality (Table 2). Larval exposure to nanocomposites for an extended duration of 48 h increased the larval mortality by 1.3-fold (Figure 3).

The regression analysis of larvicidal data revealed the LC₅₀ and LC₉₀ values of 26.82 µg/mL and 99.32 µg/mL, respectively after 24 h of CLPE-AgNCs exposure to the *Aedes* larvae (Table 2). It is evident from the data that NPs synthesized from *C. limetta* peels exhibited approximately 2-fold toxic potential than that induced by crude hexane peel extract. In comparison to hexane crude extracts of *C. limetta*, CLPE-AgNCs showed higher larvicidal efficacy, effective at 1.73-fold and 2.03-fold lower LC₅₀ dosage. Moreover, no larval mortality was observed in the control assays, on exposure to the aqueous extract of *C. limetta* and silver nitrate solution.

Table 2. Larvicidal efficacy of CLPE- AgNCs against early fourth instars of *Aedes aegypti* L.

Exposure Solution	LC ₅₀ (µg/mL)	95% fiducial limits	Fold Efficacy	LC ₉₀ (µg/mL)	Fold Efficacy	95% fiducial limit	Chi Square (df)	Slope ± SE
After 24 hours of exposure								
Control *	-	-	-	-	-	-	-	-
Crude Hexane extract of <i>C. limetta</i> peels	46.53	40.32-52.97	1.0	85.37	1.0	72.89-109.34	4.79 (5)	4.47 ± 0.4
<i>C. limetta</i> peels derived AgNCs	26.82	12.80-38.14	1.73	99.32	1.16	74.57-162.50	4.01 (5)	2.25 ± 0.49
After 48 hours of exposure								
Control *	-	-	-	-	-	-	-	-
Crude Hexane extract of <i>C. limetta</i> peels	39.57	35.22-43.66	1.0	61.92	1.0	54.77-74.83	2.23 (5)	6.49 ± 0.85
<i>C. limetta</i> peels derived AgNCs	19.51	6.51-30.21	2.03	71.99	1.16	52.57-114.18	3.282 (5)	2.26 ± 0.56

*Bioassays were discontinued as no mortality were recorded even at 1000 µg/mL. No mortality was observed in the control, LC₅₀ - Lethal Concentration that kills 50% of the exposed larvae, LC₉₀ - Lethal Concentration that kills 90% of the exposed larvae, S.E. = Standard Error, χ^2 = Chi-square, df = degree of freedom; Test samples were transformed into log covariant (log₁₀), $p > 0.05$, level of significance is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits, Values are mean of five replicates

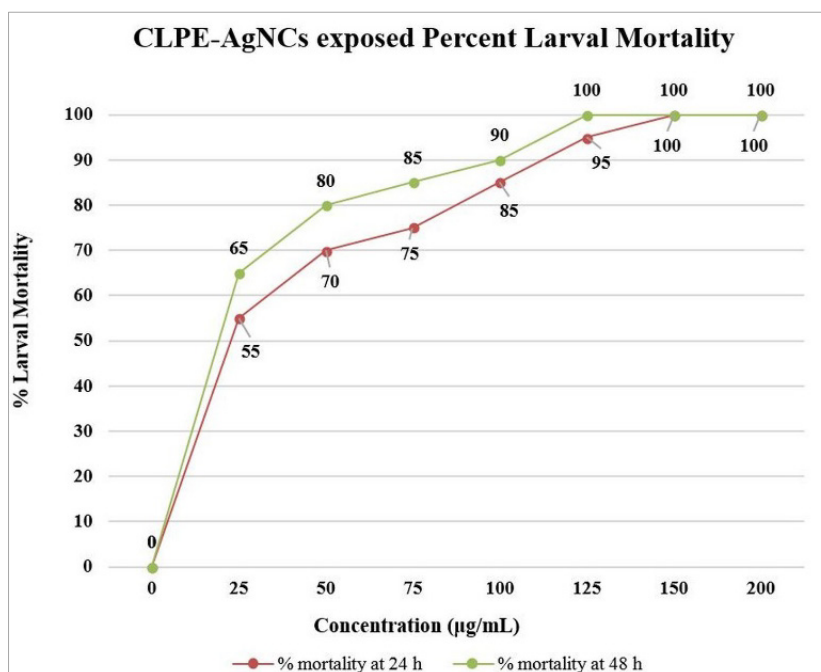


Figure 3. Percent mortality in early fourth instars of *Aedes aegypti* when exposed to CLPE-AgNCs synthesized with 3mM AgNO₃

4. Discussion

Rise in *Aedes*-borne diseases has created worldwide concerns since last few years. Dengue vector, *Ae. aegypti*, has attracted considerable attention of researchers and vector management organization due to the global occurrence of diseases transmitted by it. Despite multifarious control measures available, control of dengue vector has become a difficult task due to development of resistance to insecticides in use. The harmful effects on the environment and human health caused by the continued increasing doses of insecticides led to exploration of botanical extracts as alternate and eco-safe measures.

Our earlier studies have established the larvicidal efficacy of hexane and petroleum ether extract of *C. limetta* peels against *Ae. aegypti* [13]. In order to increase its efficiency at much lower concentrations, we attempted to formulate nanocomposites from *C. limetta* peel extracts as a toxic agent against *Ae. aegypti*. The synthesis of nanocomposites has engrossed extensive consideration due to their inimitable properties and budding applications [24]. Several methods have been attempted for their formulation; however, the green route of synthesis is considered more simplistic, reliable and eco-friendly as compared to the chemical and physical methods. The utilization of environmentally benign resources for the nanocomposites synthesis offers several benefits in pharmaceutical and biomedical applications [25]. Among these, utilization of NPs for their anti-mosquito potential is of great interest.

In the present investigations, the formation of silver nanocomposites in the aqueous solution of *C. limetta* peels was confirmed by visual colour change after 15-20 minutes of incubation at 60 °C. Similar observations have been documented by Trivedi et al. [16] who also recorded comparable colour change from pale yellow to brownish while synthesizing silver nanoparticles from peels of *C. limetta*. It is suggested that while formulation of NPs, change in colour of the reaction mixture is due to the excitation of Surface Plasmon Vibration in the silver nanoparticles which is positively correlated with AgNO₃ concentration in the solution [21, 26]. The current study also showed that a particular combination of high reductant volume, high temperature and high amount of NaOH led to rapid color change. This abrupt color change was also observed by Sahoo and Kalyan [27] and Trivedi et al. [16].

It is well known now that UV-Visible spectroscopy is a simple and rapid confirmatory technique for the silver nanocomposite synthesis. The absorbance peak values, ranging between 400 to 500 nm wavelengths, denote the occurrence of silver nanocomposites in the solution and confirm their synthesis [28, 29]. In current study, optimum synthesis of silver nanocomposites in the solution of *C. limetta* peel extracts was observed at a wavelength ranging from 416-417 nm. Earlier, Konwarh et al. [30] had reported the synthesis of green silver nanoparticles using aqueous peel extract of *C. sinensis* at 404 nm; while Nair et al. [31]

observed the synthesis at 429 nm - 432 nm. The absorption peak of silver nanoparticles synthesized from fresh peels of *C. limetta* observed in the range of 390 nm - 450 nm by Trivedi et al. [16] is in agreement with our study. In contrast, when Shanmugavadivu and Kuppasamy [32] recorded UV-Vis spectrum of silver nanoparticles synthesized from the fresh peels of *Citrus reticulata*, they found the surface plasmon broad peak at 466 nm after 6 h of incubation; which is much higher than observed by us. Nevertheless, Jalani et al. [33] recorded UV-Vis spectra of synthesized AgNCs from pomelo peel extract (*Citrus grandis*) and found standard surface plasmon resonance band in the range of 401 nm - 433 nm.

The rapid green synthesis of AgNCs using 3 mL of *C. sinensis* peel extract with 40 mL of 1mM AgNO₃ solution has been shown by Nair et al. [31]. They obtained NPs within a short period of just two minutes; as against present studies in which the fastest NP synthesis was observed with 3 mM silver nitrate solution. Moodley et al. [34] suggested that raising the temperature can enhance the rate of NPs synthesis thus resulting in smaller-sized NPs. We obtained most stable NPs when reaction mixture was heated up to 60 °C, while Nair et al. [31] obtained the best NPs at 80 °C. The variable conditions reported in AgNCs synthesis may be attributed to the bioreducing agent which may possess different bioactive constituents due to species as well as geographical variations.

Earlier reports have shown that citrus peel extracts possess appreciable larvicidal potential against different mosquito vectors. Current investigations revealed LC₅₀ and LC₉₀ values of 46.53 µg/mL and 85.37 µg/mL when *C. limetta* peel extracts were assayed against *Ae. aegypti* larvae. In contrast, similar larvicidal assays with hexane peel extracts of *C. limetta* and hexane leaf extracts of *C. sinensis* against *Ae. aegypti* resulted in much higher LC₅₀ and LC₉₀ values of 96.15 µg/mL and 163.27 µg/mL; and 446.84 and 1370.96 ppm, respectively [11, 16]. The considerable toxicity of ethanol extract of *C. sinensis* peels has been reported by Murugan et al. [35] against each instar of *An. stephensi*. They obtained respective LC₅₀ values of 182.24, 227.93, 291.69 and 398.00 ppm against I, II, III, IV and V instar of *An. stephensi*. Bagavan et al. [36] investigated the chloroform and methanol extracts of *C. sinensis* peels against *An. subpictus* and *Cx. tritaeniorhynchus* and obtained LC₅₀ values of 58.25 ppm and 38.15 ppm, respectively; which are in agreement with the values obtained by us. The aqueous extract of *C. grandis* peels was found to be ineffective when assessed against *Ae. aegypti* larvae as no mortality were recorded at even 1000 µg/mL [37]. On the other hand, aqueous peel extract of *C. limetta* was effective against fourth instars of *Cx. quinquefasciatus* but at much higher concentration (LC₅₀ = 0.08%) [38].

The nanoformulations have been shown to exhibit higher larvicidal potential in comparison to hexane crude extracts of citrus peels. In present study, CLPE-AgNCs

showed 1.73-fold higher larvicidal potential against *Ae. aegypti* as compared to crude extracts. In addition, it also exhibited delayed effects with increased efficacy on increased time of exposure against early fourth instars of *Ae. aegypti*. The citrus peel-derived nanocomposites have not been yet assessed as potential control agent for mosquitoes. This study is first of its kind till date, to our knowledge, though such NPs have been explored for potential use against *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative), *Salmonella typhimurium* and *Staphylococcus aureus* [24, 39]. Nevertheless, our results are in accordance with studies conducted by Sharma et al. [7] who assessed the impact of *Achyranthes aspera*-mediated silver nanocomposites against early fourth instars of *Ae. aegypti* and also reported the delayed toxic impact of green silver nanocomposites.

5. Conclusions

In the current study, we have optimized the conditions for ideal synthesis of AgNCs. The synthesized CLPE-AgNCs exhibited significant larvicidal potential against early fourth instars of *Ae. aegypti*. Thus, CLPE-AgNCs, we believe, can be recommended as an eco-friendly nano-larvicide to control mosquito population.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

REFERENCES

- [1] WHO (World Health Organization), Dengue and severe dengue. Online available: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue>, 2019
- [2] S. R. Mutheneni, A. P. Morse, C. Caminade, S. M. Upadhyayula. Dengue burden in India: recent trends and importance of climatic parameters, *Emerging Microbes and Infections*, Vol 6, No. 8, e70
- [3] N. Arunachalam, U.S. Murty, L. Kabilan, A. Balasubramanian, V. Thenmozhi, D. Narahari, A. Ravi, K. Satyanarayana (2004). Studies on dengue in rural areas of Kurnool District, Andhra Pradesh, India. *Journal of the American Mosquito Control Association*, Vol. 20, No. 1: 87-90, 2004
- [4] WHO (World Health Organization), Dengue vaccine: WHO position paper. *Weekly Epidemiological Record*, Vol. 91, No. 30: 349-364, 2016.
- [5] S. Kumar, N. Wahab, M. Mishra, R. Warikoo. Evaluation of fifteen local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Frontiers in Physiology*, Vol. 3, No. 104: 1-6, 2012a.
- [6] M.C. Rathy, U. Sajith, C.C. Harilal. Larvicidal efficacy of medicinal plant extracts against the vector mosquito *Aedes albopictus*. *International Journal of Mosquito Research*, Vol. 2, No. 2: 80-82, 2015.
- [7] A. Sharma, S. Kumar, P. Tripathi. A facile and rapid method for green synthesis of *Achyranthes aspera* stem extract-mediated silver nanocomposites with larvicidal potential against *Aedes aegypti* L. *Saudi Journal of Biological Sciences*, Vol. 26, No. 4: 698-708, 2017.
- [8] A.F. Traboulsi, S. El-Haj, M. Tueni, K. Taoubi, N.A. Nader, A. Mrada. Repellency and toxicity of aromatic plant extracts against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). *Pest Management Science*, Vol. 61: 597-604, 2005.
- [9] W. Akram, H.A.A. Khan, F. Hafeez, H. Bilal, Y.K. Kim, J-J. Lee. Potential of citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pakistan Journal of Botany*, Vol. 42: 3343-3348, 2010.
- [10] A.A. Amusan, A.B. Idowu, F.S. Arowolo. Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (*Citrus sinensis*) oil extract on larvae of the yellow fever mosquito *Aedes aegypti*. *Tanzania Health Research Bulletin*, Vol. 7: 174-178, 2008.
- [11] R. Warikoo, A. Ray, J.K. Sandhu, R. Samal, N. Wahab, S. Kumar. Larvicidal and irritant activities of hexane leaf extracts of *Citrus sinensis* against dengue vector *Aedes aegypti* L. *Asian Pacific Journal of Tropical Biomedicine*, Vol. 2, No. 2: 152-155, 2012.
- [12] A. Amer, H. Mehlhorn. Persistency of larvicidal effects of plant oil extracts under different storage conditions. *Parasitology Research*, Vol. 99: 473-477, 2006.
- [13] S. Kumar, R. Warikoo, M. Mishra, A. Seth, N. Wahab. Larvicidal efficacy of the *Citrus limetta* peel extracts against Indian strains of *Anopheles stephensi* Liston and *Aedes aegypti* L. *Parasitology Research*, Vol. 111, No. 1: 173-178, 2012b.
- [14] B. Swathy. A review of metallic silver nanoparticles. *IOSR Journal of Pharmacy*, Vol. 4, No. 7: 38-44, 2014.
- [15] K. Vijayaraghavan, K.S.P. Nalini, P.N. Udaya, D. Madhankumar D. Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*. *Colloids and Surfaces B: Biointerfaces*, Vol. 75: 33-35, 2012.
- [16] P. Trivedi, M. Khandelwal, P. Srivastava. Statistically optimized synthesis of silver nanocubes from peel extracts of *Citrus limetta* and potential application in waste water treatment. *Journal of Microbial and Biochemical Technology*, Vol. S4: 004, 2014.
- [17] R.R. Gada, U. Padalia. Biosynthesis of silver nanoparticles using citrus fruits. *International Journal of Pharma and*

Biosciences, Vol. 8, No. 1: 146-149, 2018.

- [18] S. Rafiq, R. Kaul, S.A. Sofi, N. Bashir, F. Nazir, G.A. Nayik. Citrus peel as a source of functional ingredient: A review. *Journal of the Saudi Society of Agricultural Sciences*, Vol. 17, No. 4: 351-358, 2018.
- [19] C.S. Sharma, S. Yadav. Green processes to use extract from citrus peel waste for novel applications (direct polystyrene recycling to natural solvent to source of carbon). Conference proceeding at AIChE Annual Meeting. Available at <https://www.aisc.org/conferences/aisc-annual-meeting/2017/proceeding/paper/25c-green-processes-use-extract-citrus-peel-waste-novel-applications-direct-polystyrene-recycling>, 2017.
- [20] A. Sharma, S. Kumar, P. Tripathi. Effects of *Achyranthes aspera* extracts on the survival and midgut histo-architecture of *Aedes aegypti* L. early IV instars. *Open Parasitology Journal*, Vol. 6: 41-51, 2018.
- [21] D. Elumalai, P.K. Kaleena, K. Ashok, A. Suresh, M. Hemavathi. Green synthesis of silver nanoparticles using *Achyranthes aspera* and its larvicidal activity against three major mosquito vectors. *Engineering in Agriculture, Environment and Food*, Vol. 9, No. 1: 1-8, 2016.
- [22] A. Sharma, S. Kumar, P. Tripathi. Evaluation of the larvicidal efficacy of five indigenous weeds against an Indian strain of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Journal of Parasitology Research*, Vol. 2016: 1-8, 2016.
- [23] A. Sharma, S. Kumar, P. Tripathi. Impact of *Achyranthes aspera* leaf and stem extracts on the survival, morphology and behaviour of an Indian strain of Dengue Vector, *Aedes aegypti* L. (Diptera: Culicidae). *Journal of Mosquito Research*, Vol. 5, No.7: 1-9, 2015.
- [24] M. Reena, A.S. Menon. Synthesis of silver nanoparticles from different citrus fruit peels extracts and a comparative analysis on its antibacterial activity. *International Journal of Current Microbiology and Applied Sciences*, Vol. 6, No. 7: 2358-2365, 2017.
- [25] D. Jain, H.K. Daima, S. Kachhwaha, S.L. Kothari. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti-microbial activities. *Digest Journal of Nanomaterials and Biostructures*, Vol. 4, No. 3: 557-563, 2009.
- [26] U.K. Parashar, S.P. Saxena, A. Srivastava). A bio-inspired synthesis of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures*, Vol. 4: 159-166, 2009.
- [27] P.K. Sahoo, K.S.S. Kalyan. Synthesis of silver nanoparticles using facile wet chemical route. *Defence Science Journal*, Vol. 59: 447-455, 2009.
- [28] A.R. Vilchis-Nestor, V. Sánchez-Mendieta, M.A. Camacho-López, R.M. Gómez-Espinosa, Camacho-M.A. López, J.A. Arenas-Alatorre. Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. *Materials Letters*, Vol. 62, No. 17-18: 3103-3105, 2008.
- [29] P. Logeswari, S. Silambarasan, J. Abraham. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. *Journal of Saudi Chemical Society*, Vol. 19, No. 3: 311-317, 2015.
- [30] R. Konwarh, B. Gogoi, R. Philip, M.A. Laskar, N. Karak. Biomimetic preparation of polymer-supported free radical scavenging, cytocompatible and antimicrobial “green” silver nanoparticles using aqueous extract of *Citrus sinensis* peel. *Colloids and Surfaces B: Biointerfaces*, Vol. 84, No. 2: 338-345, 2011.
- [31] S.S. Nair, S.T. Rizvi, P.D. Anthappan. Optimization of rapid green synthesis of AgNPs using *Citrus sinensis* peel extract for antibacterial activity. *International Journal of Advanced Scientific Research and Management*, Vol. 3, No. 8: 274-281, 2018.
- [32] M. Shanmugavadivu, S. Kuppusamy. Green synthesis of silver nanoparticles using *Citrus reticulata* fruit peels aqueous extract and their antibacterial activity. *International Journal of Advanced Research*, Vol. 5, No. 4: 700-708, 2017.
- [33] N.S. Jalani, W. Michell, W.E. Lin, S.Z. Hanani, U. Hashim, R. Abdullah. Biosynthesis of silver nanoparticles using *Citrus grandis* peel extract. *Malaysian Journal of Analytical Sciences*, Vol. 22, No. 4: 676-683, 2018.
- [34] J.S. Moodley, S.B.N. Krishna, K. Pillay, Sershen, P. Govender. Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, Vol. 9, No. 015011: 1-9, 2018.
- [35] K. Murugan, M.P. Kumar, K. Kovendan, D. Amerasan, J. Subramaniam. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, Vol. 111, No. 4: 1757-1769, 2012.
- [36] A. Bagavan, C. Kamaraj, A.A. Rahuman, G. Elango, A.A. Zahir, G. Pandiyan. Evaluation of larvicidal and nymphicidal potential of plant extracts against *Anopheles subpictus* Grassi, *Culex tritaeniorhynchus* Giles and *Aphis gossypii* Glover. *Parasitology Research*, Vol. 104: 1109-1117, 2008.
- [37] M.S. Manorenjitha, M. Jamil, N. Hashim, L.S. Kiong, Z. Jaal. Toxicity of white flesh *Citrus grandis* Osbeck fruit peel extracts against *Aedes aegypti* (Linnaeus) larvae and its effect on non-target organisms. *International Journal of Mosquito Research*, Vol. 4, No. 4: 49-57, 2017.
- [38] S. Mallick, U. Adhikari, A. Rawani, G. Chandra. Phytochemical analyses and larvicidal potentiality of fruit peel extracts of *Citrus limetta* against filarial vector *Culex quinquefasciatus*. *Journal of Mosquito Research*, Vol. 6, No. 22: 1-7, 2016.
- [39] S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary, K. Srinivasan. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol. 79, No. 3: 594-598, 2011.