



Larvicidal and adulticidal potential of iron nanoparticles of *Eucalyptus citriodora* (L.) to control rural dengue vector *Aedes albopictus* (Skuse)

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Abstract

Development of insecticide resistance against the chemical synthesized insecticides and high operational cost of secondary plant resources as well as other vector controlling strategies, intake a step towards a new controlling tool with urgent basis. The present study was aimed to develop an alternative approach with low cost and healthy environmental aspects to control most prominent dengue vector in rural areas by using green synthesis of iron nanoparticles of *Eucalyptus citriodora* (L.) The synthesized iron nanoparticles (FeNPs) were found to be amorphous in morphology. While FeNPs were tested against *Aedes albopictus* (Skuse) third instar larvae and adult, it was found that LC₅₀ and LC₉₀ was recorded 11.269 and 23.076 ppm while KDT₅₀ and KDT₉₀ were calculated 14.253 and 34.657 minutes respectively. The study indicate that use of FeNPs as dengue vector control would be better approach with ecofriendly and economically.

Keywords: *Aedes albopictus* (Skuse), green synthesis, iron nanoparticles, larvicidal, adulticidal

Introduction

Mosquito borne diseases remain the world's most severe insect borne disease with excessive rates of morbidity and mortality. Currently a total of 3549 recognized mosquito species belonging to subfamily culicinae are reported all over the world. [1] Amongst these more than hundred species are capable for disease transmission to humans and the other animals. [2] Many of the studies indicated that 390 million people are in the world facing dengue infection per year with 96 million infections confirmed clinically. One third of the subfamily culicine is represented by genus *Aedes* of which *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) represent the two important mosquito vectors for arboviral diseases transmission at global scenario [3].

According to Dengue Bulletin, 2020 [4], dengue cases in the south-east-asia region rapidly increased in 2019, total 658301 clinical cases were reported in 2019 which was 46% increase from 2015. Dorta *et. Al*, 1993 reported three organophosphorus compounds such as folithion, malathion and temephose and two synthetic pyrethroids (Alphamethrin and Deltamethrin) were in trend to control the immature and mature stages (larvae and adults) of vector mosquitoes [5] but resistance against these insecticides was also reported from most of the countries including various states of India [6-11].

Development of insecticide resistance against the chemical synthesized insecticides and high operational cost of secondary plant resources as well as other vector controlling strategies, intake a step towards a new controlling tool with urgent basis.

The present study was aimed to develop an alternative approach with low cost and healthy environmental aspects to control most prominent dengue vector in rural areas by

using green synthesis of iron nanoparticles of *Eucalyptus citriodora* (L.).

Experimental

Mosquito rearing

Aedes albopictus (Skuse) mosquitoes were reared in the Laboratory of Public Health Entomology, Department of Zoology, University College of Science, M.L.S. University, Udaipur, Rajasthan, India.

The rearing of mosquitoes and experiments were carried out at 25±5°C and 70±10% relative humidity with a photoperiod of 12 h light followed by 12 h dark exposure [12].

Synthesis of nanoparticles

For the present study fresh plant leaves were collected from University College of Science, MLS University, Udaipur which lies in between 24°58' N and 73°68' E at 598 m above sea level. 10 g washed leaves were cut into fine pieces and crushed with the help of mortar and pestle and boiled in 100 ml double distilled water for 10 minute [13].

For the fabrication of FeNPs, 1 mM FeCl₃ solution was mixed to plant extract in 1:9 ratios and heated to 80°C for 15 min with continuously stirring on magnetic stirrer [14], change in color indicated the formation NPs.

Characterization of nanoparticles

For the morphological characterization, FeNPs were investigated by Nova Nano FE-SEM 450 (FEI) scanning electron microscope. The entire sample was coated with gold before SEM analysis [16]. (Sample was analyzed by AIIMS, New Delhi)

To determine the crystallinity and accountability of particle size, FeNPs were investigated using an XRD patterns. The

sample was recorded on a Philips X-Pert MMP diffractometer. The diffractometer was controlled and operated by a PC computer with the programs Pro-fit and used a MoK (source with wavelength 0.70930Å, operating with Mo-tube radiation at 50 kV and 40 mA) and data was analyzed with Origin Pro software. (Sample was analyzed by Department of Physics, M.L.S. University, Under the supervision of Prof. N. Laxmi

Larvicidal

For the larvicidal bioassay 250 ml plastic disposable cup were used. Total 600 same aged third instars laboratory reared larvae (including control replica treated with acetone) of *Aedes albopictus* (Skuse) were introduced to disposable cups containing 100 ml water [17]. 100 PPM stock solution of FeNPs in acetone was prepared with the help of ultrasonicator. To perform larvicidal bioassay three replicas stepped for each different concentration of 2.5, 5.0, 7.5, 10.0 and 12.5 ppm was applied after determining the mortality; results were analyzed statistically to calculate LC₅₀ and LC₉₀.

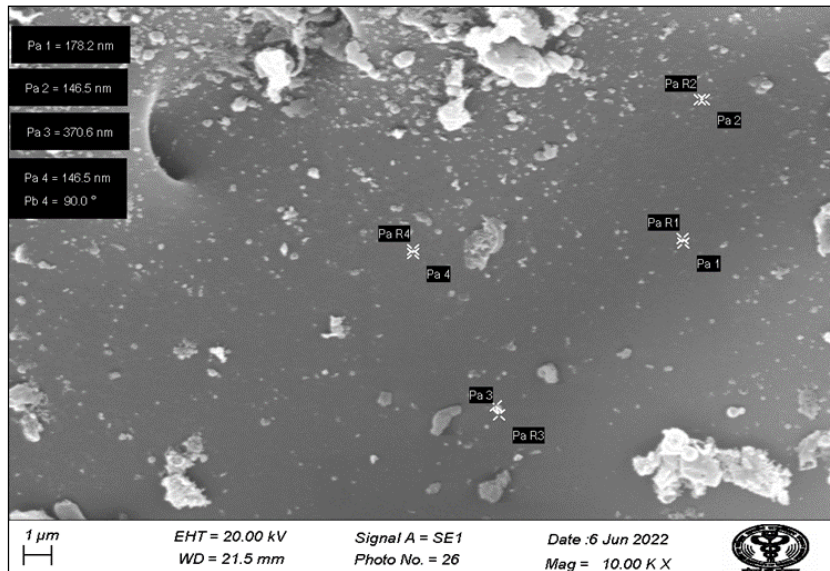
Adulticidal

The adulticidal assay was conducted according to the protocol of the Centers for Disease Control and Prevention(CDC) bottle bioassay [18]. The bioassay was done in 250 mL CDC bottle. Stock solution of 100 ppm was diluted to 2.5, 5.0, 7.5, 10.0 and 12.5 ppm as the mortality was recorded at the lowest dose after the 1 hour exposure.

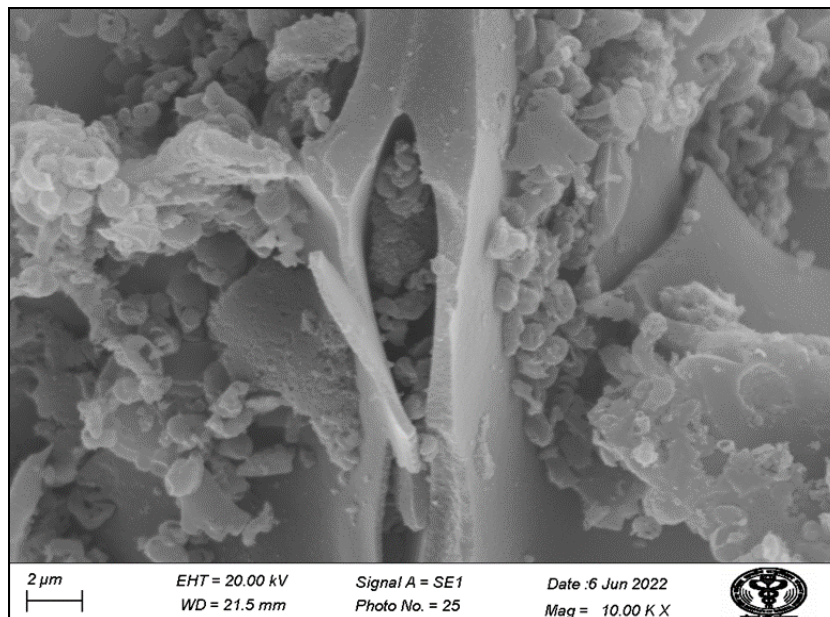
Results and Discussions

SEM and XRD

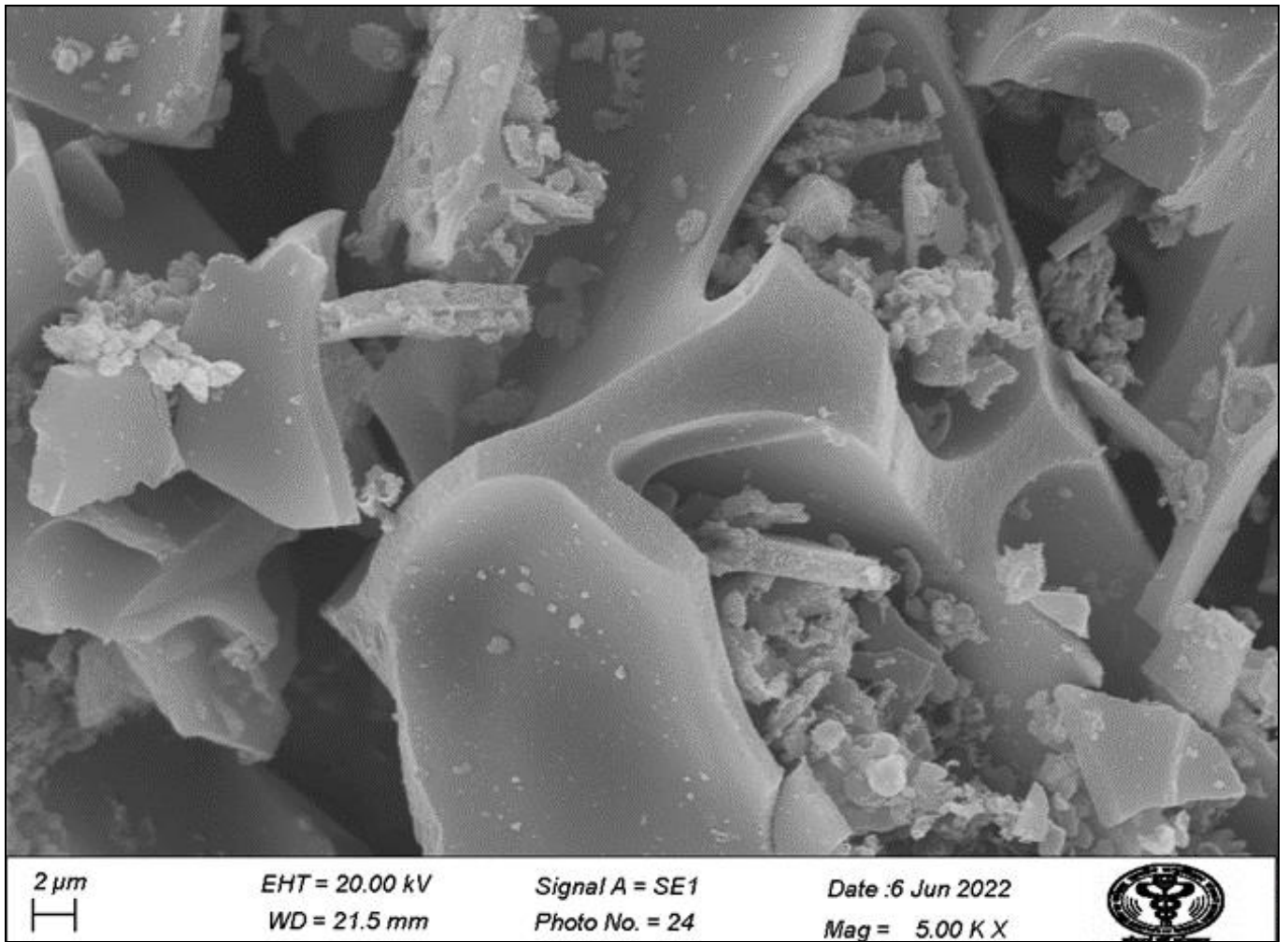
Scanning Electron Microscopy (SEM) results depicted the morphology of FeNPs which was found to be amorphous, aggregated light color was showing the metal element while dark color surrounding the metal was showing plant molecule aggregation. [Fig.1 (A-C)] The EDAX of FeNPs justified the purity of nanoparticles. [Fig.1 (D)] Similarly, XRD pattern also indicated the same results i.e. synthesized nanoparticles were amorphous in shape and the pattern was showing the non calculable data.



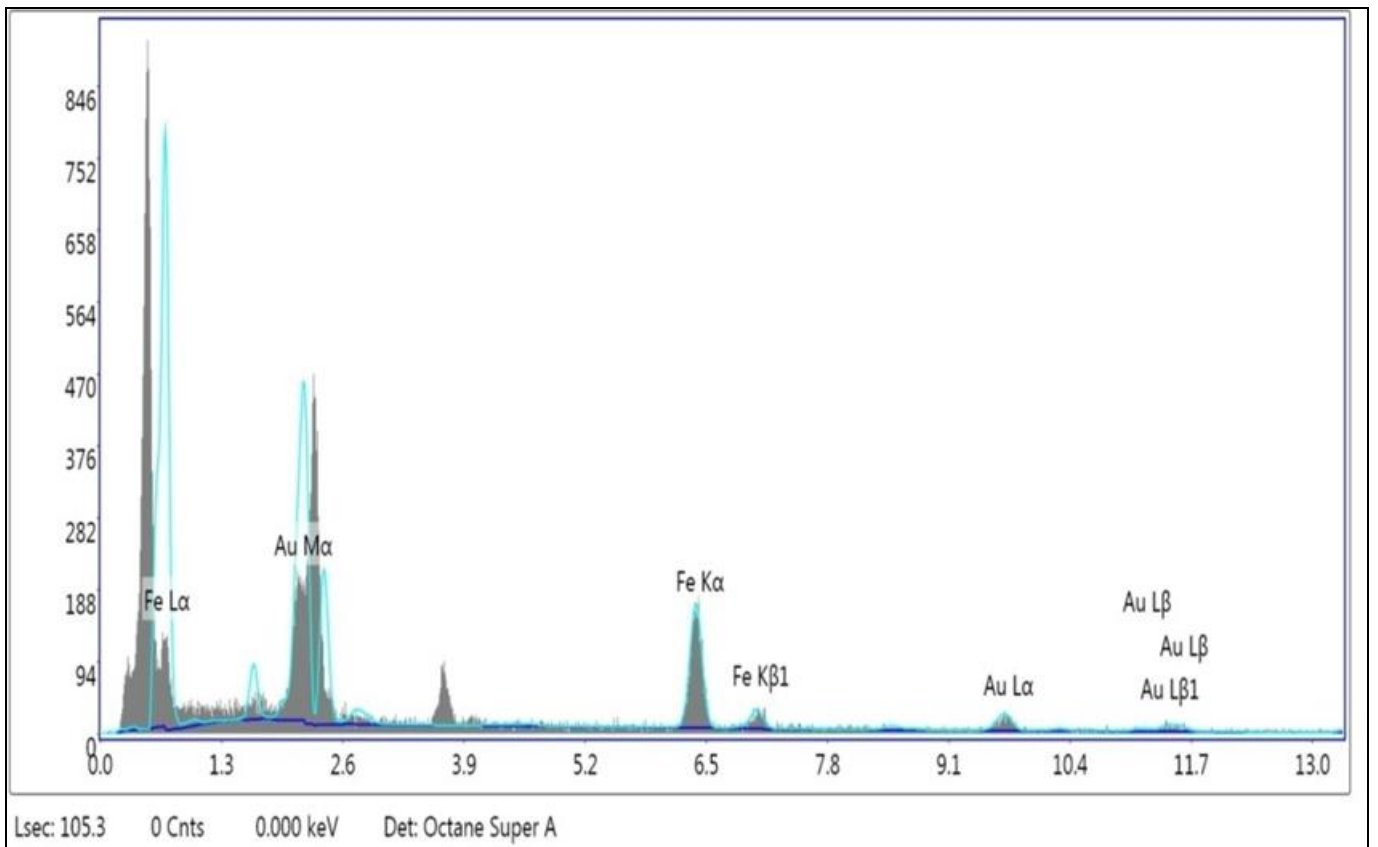
(A)



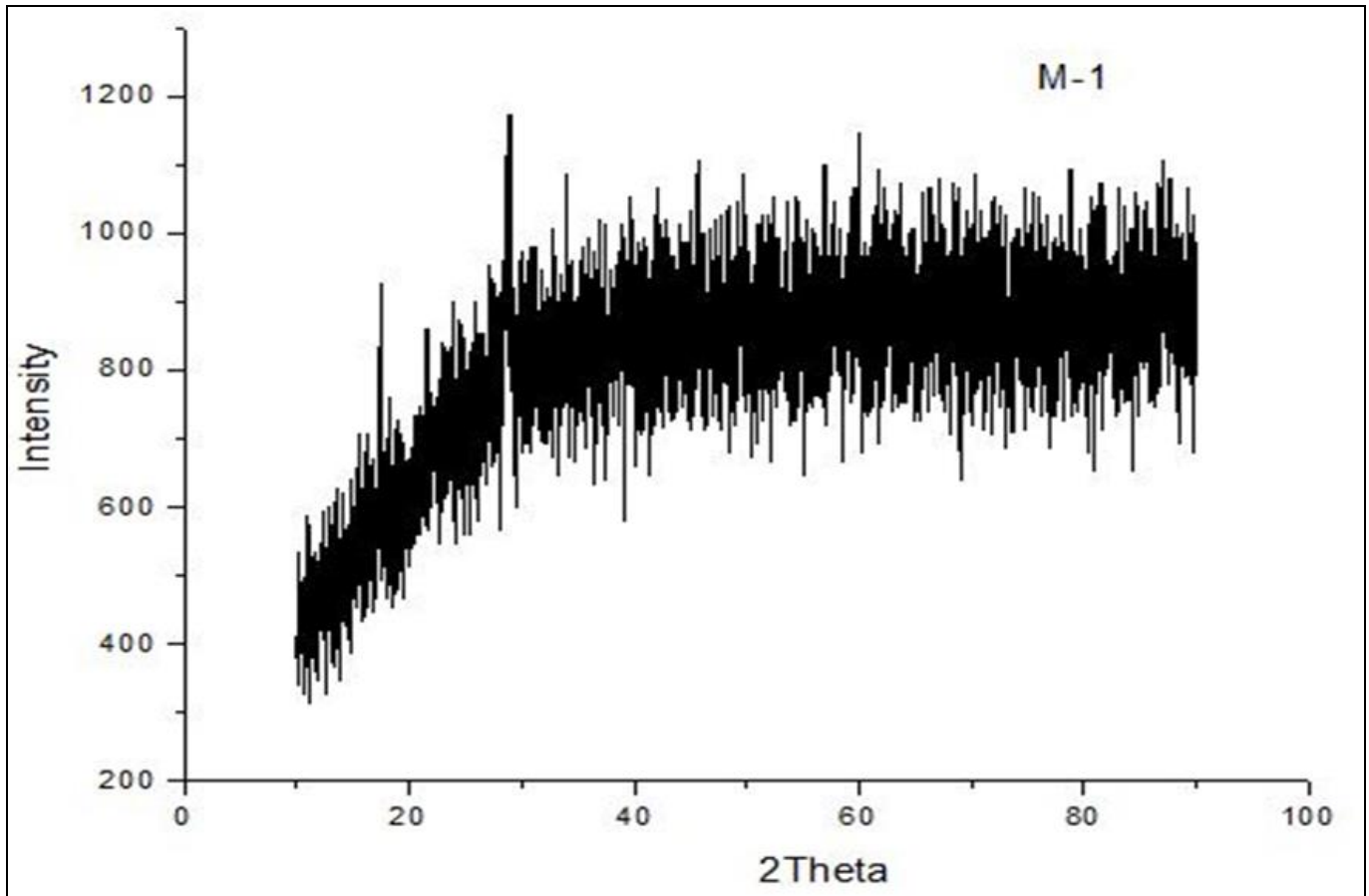
(B)



(C)



(D)



(E)

Fig 1-5: (A-E) Characterization of iron nanoparticles of *Eucalyptus citridora* (L.); [A-C] SEM of FeNPs at 5000x,10000x; [D] EDAX; [E] XRD pattern

Larvicidal

When different dose concentrations i.e. 2.5, 5.0, 7.5, 10.0 and 12.5 ppm of iron nanoparticles of *Eucalyptus citridora* (L.) were tested against third instars larvae of *Aedes albopictus* (Skuse) and analyzed statistically, it was observed that mortality increased with time and dose. The highest dose concentration 12.5 ppm obtained the percent mortality of 58.89 ± 0.06 after 24 hours which increased to 96.67 ± 0.03 after 48 hours while at the lowest applied dose

of 2.5 ppm the observed percent mortality was 5.00 ± 0.01 and 10.00 ± 0.00 at 24 hours and 48 hours respectively, other applied concentrations viz. 5.0, 7.5 and 10.0 ppm encompassed the percent mortality 9.00 ± 0.02 , 24.44 ± 0.03 and 38.89 ± 0.05 after 24 hour respectively. There was no mean mortality recorded in control during the experiment [Table 3]. The regression equation for larvicidal bioassay is $Y = 4.8 (\text{Dose}) + (-4.88)$ and LC_{50} and LC_{90} was calculated as 11.269 and 23.076 ppm.

Table 1: Larvicidal activity of iron nanoparticles against *Aedes albopictus* (Skuse)

Concentrations (ppm)	24 h mortality (%) \pm SD ^a	48 h mortality (%) \pm SD ^a	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ^2 (df)
Control	0.00 \pm 0.00	0.00 \pm 0.00	11.269 (9.687-17.527)	23.0761 (15.872-234.322)	0.137 (1) *
2.5	5.00 \pm 0.01	10.00 \pm 0.00			
5.0	9.00 \pm 0.02	15.00 \pm 0.04			
7.5	24.44 \pm 0.03	56.67 \pm 0.06			
10.0	38.89 \pm 0.05	78.89 \pm 0.05			
12.5	58.89 \pm 0.06	96.67 \pm 0.03			

SD standard deviation, LCL-lower confidence limits, UCL- upper confidence limits, χ^2 -chi-squared test

* $P < 0.05$, level of significance.

a. value are mean \pm SD of three replicates.

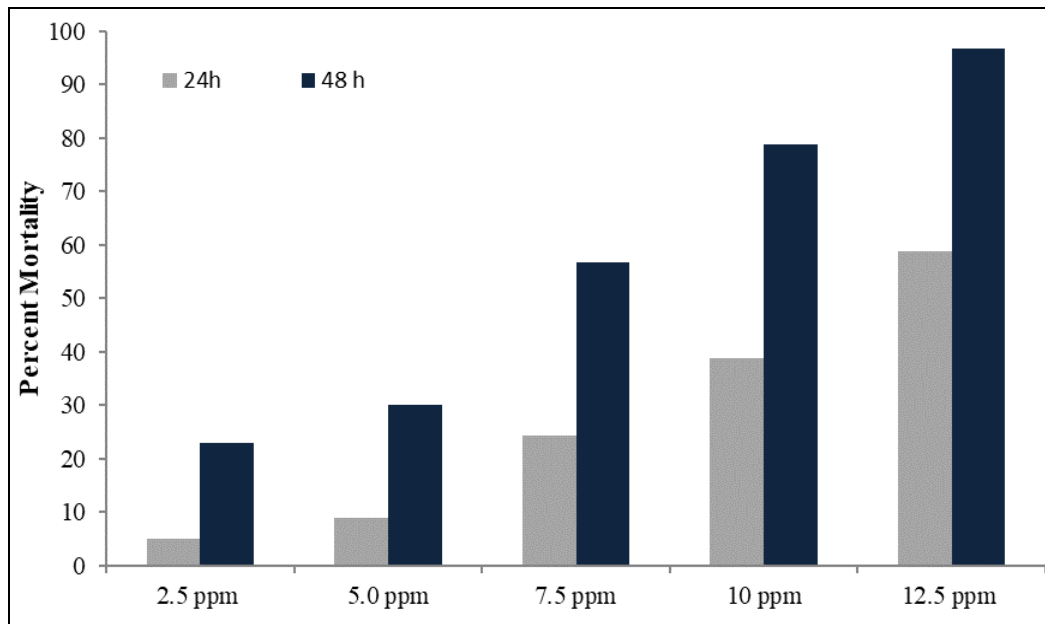


Fig 6: Graph showing percent mortality at different concentrations after 24h and 48h.

Adulticidal

Different doses concentrations 2.5, 5.0, 7.5, 10.0 and 12.5 ppm of iron nanoparticles of *Eucalyptus citridora* (L.) were tested against *Aedes albopictus* (Skuse) 3-4 days old females using CDC Bottle Bioassay method and results were analyzed statistically. It was observed that the highest dose concentration 12.5 ppm was showing 100%

knockdown at 50 minutes exposure period while the concentration of 7.5 ppm showed 100% mean knockdown at 55 minutes. It indicated that each half of the dose brought cent percent mortality with less than 5 minutes interval. There was no knockdown recorded in control while the control was coated with acetone. [Table. 2]

Table 2: Adulticidal activity of FeNPs against *Aedes albopictus* (Skuse) adult.

Concentrations (ppm)	100% KDT (Minutes)	Regression equation	KDT ₅₀ (LCL-UCL) ^a	KDT ₉₀ (LCL-UCL) ^b	Statistical Summary
Control	0.00	Y = 2.8X + (-3.68)	14.253(5.678-36.467)	34.657 (12.356-54.687)	P < 0.05, χ^2 (df) = 3.646 (34)
2.5	-				
5.0	60				
7.5	55				
10.0	50				
12.5	50				

- a. Duration of time at which 50% Knockdown occurs.
- b. Duration of time at which 90% Knockdown occurs.
- c. LCL- Lower confidence limit, UCL- Upper confidence limit.

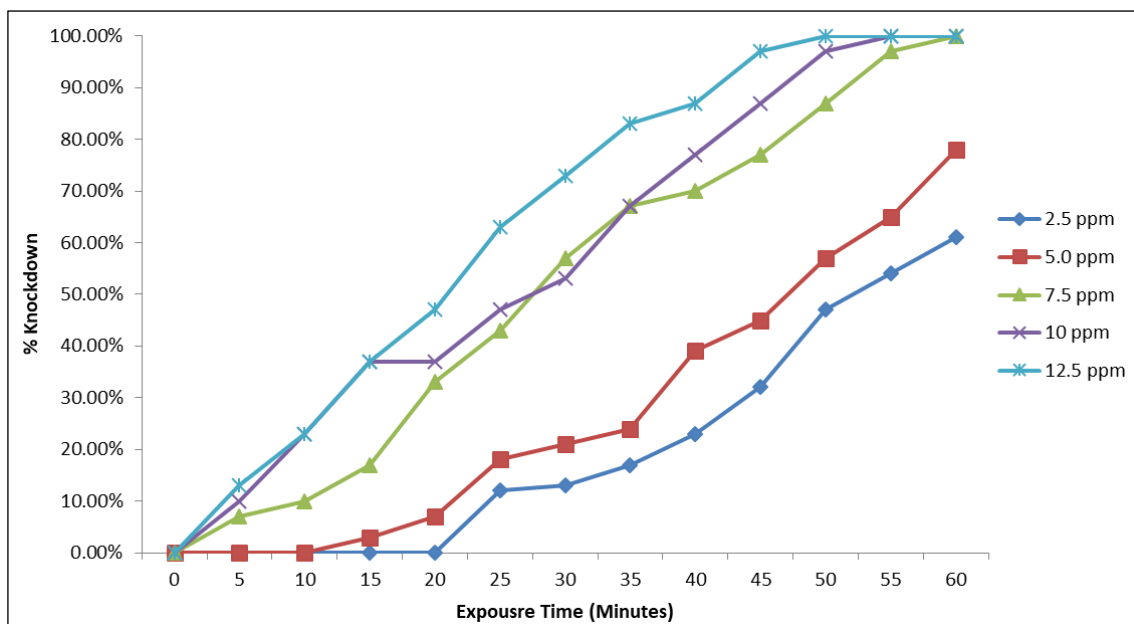


Fig 7: Graph showing percent knockdown after different dose exposure at different interval FeNPs against *Aedes albopictus* (Skuse) adult.

Discussion

The present study is aim to develop an effective and eco-friendly tool for rural dengue vector control. In this study iron nanoparticles were synthesized by using aqueous leave extract of *Eucalyptus citridora* (L.), after the synthesis to identify the morphology and crystalline nature scanning electron microscopy (SEM) and X-ray diffraction (XRD) was depicted with the results that the synthesis was amorphous in structure which was supported by Wang, 2013 [19]. Simultaneously, some of studies depicted that plant mediated iron nanoparticles were in pure crystalline in morphology with was evident by XRD [20-22].

Use of plant mediated silver nanoparticles is most abundantly in trends from last few years although it have a high attention in scientific society due to its high toxicity on targeted animals such as *Culex*, *Anopheles* and *Aedes* mosquitoes and other pests [23-36].

In present study iron nanoparticles of *Eucalyptus citridora* (L.) were screened against third instar and adult of *Aedes albopictus* (L.), results depicted that LC₅₀ against larvae was recorded 11.269 ppm where LC₉₀ was 23.0761 ppm. Mondal *et al.*, 2022 supported the present investigation i.e. gold and silver nanoparticles of *Moringa oleifera* (Lam.) evident the LC₅₀, LC₉₀ as 8.235 ppm and 5.664 ppm respectively [37]. Silver nanoparticles of *Bauhinia variegata* *Berberis tinctoria* and *Mimusops elengi* also screened against *Ae. albopictus*, LC [50] for these plants viz. 46.16 ppm [38], 9.65 ppm [39], 11.71ppm [40].

Adulticidal activity was also evaluated in present study and concluded that KDT₅₀ (50% knockdown time for adult) was resulted i.e. 14.253 minutes while the KDT₉₀ (90% knockdown time for adult) was 34.567. Veerakumar *et al.* 2014c [41] supported the present study that silver nanoparticles of *H. indicum* with LD₅₀ 29.56 ppm against *Ae. aegypti*, similarly, *F. elephantum* leaf extract mediated silver nanoparticles were reported toxic for adult with 20.399 ppm LD₅₀. [42] Subramaniam *et al.* 2015 [12], concluded his work against the adults of *Ae. albopictus* with LD₀ of 14.7 ppm [43].

Conclusion

Development of new insecticides for the control of vector is major issues throughout the world; there is an urgent need of effective and safe vector controlling methods. Therefore, plant derived nanoparticles play a remarkable role as an effective insecticide.

Our results clearly show that FeNPs is effectively control against rural dengue vector *Ae. albopictus*. FeNPs has strong insecticidal activity at very low concentrations against both larvae and adults of *Aedes albopictus*. Therefore, we suggest that the selection of nano formulation based insecticide more effective to control vector, also reducing environmental pollution and resistance from the continuous usage of conventional insecticides.

Author contributions

MJ conceived the idea, perform the experiments and observed the results. AP contributed materials, reagents and facility for experimental work. MJ analyzed and interpreted the data of statistics. CS analysis of statistical data and manuscript writing. MJ edited compiled and finalized the draft. Finally, authors were gone through and approved it for publication.

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Declaration of competing interest

The author (s) declare (s) that there is no conflict of interest regarding the publication of this paper.

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