EVALUATION OF SILICA, ZINC AND SILICA/ZINC COMPOSITE NANOPARTICLES LARVICIDAL ACTIVITY AGAINST MOSQUITO VECTOR CONTROL

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LAHORE, PAKISTAN

YEAR 2021-2023

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A THESIS SUBMITTED TO KINNAIRD COLLEGE FOR WOMEN IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCES

IN

ENVIRONMENTAL SCIENCES

By

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KINNAIRD COLLEGE FOR WOMEN, LAHORE

YEAR 2021-2023

KINNAIRD COLLEGE FOR WOMEN, LAHORE

OFFICE OF RESEARCH, INNOVATION & COMMERCIALIZATION

Thesis Approval Certificate

Date: —i – 2024

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Session: (2021-2023)

Registration No: W21MPES005

Title: Evaluation of Silic Zinc and Silica/Zinc Com osite Nano articles Larvicidal Activit ain st Mosquito Vector Control be accepted in partial fulfillment of the requirements for the award of MPhil degree.

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RESEARCH COMPLETION CERTIFICATE

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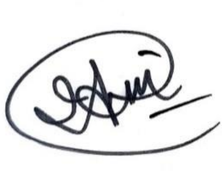

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ACKNOWLEDGEMENTS

First of all, we are thankful to Allah who is the most Beneficent and the most Merciful for giving us courage and strength for completing our research.

I want to express my gratitude to the Principal Prof. Dr. Rukhsana David for providing the opportunity to study in this prestigious institution. I am also thankful to Prof. Dr. Nighat Khan for her consistent support towards the research students. Her permission has let me go out for research work helped in completing the research on time.

I am thankful to our Head of Department Dr. Almas Hamid for always pushing me towards betterment. Without her support I would not be able to cherish 2 years of learning the concepts. She always motivates the students to take studies and co-curricular activities hand in hand and it really worked for increasing the efficiency of student work.

I am highly thankful to my supervisor Dr. Sadia Saif and co-supervisor Dr. Farzana Bashir (Principal Officer at Center for Environmental Protection Studies, PCSIR Labs Complex) for being there throughout the research and being so supportive. Their productive advice helped a lot in research.

I would like to thank Sir Haseeb and Mr. Shahbaz for providing the required equipment for lab analysis. I would also like to thank PCSIR staff and attendants for providing me with the required instruments for my research.

Lastly, I would like to thank my parents for their constant financial and emotional support. They have been always so generous throughout my life.

Iqra Amin

ABSTRACT

In this study silica (SiO_2), zinc (Zn) and silica/zinc (SiO_2/Zn) composite nanoparticles were synthesized using aqueous leaves extract of *Azadirachta indica* as a simple, cheap and ecofriendly green material as a simple, cheap and ecofriendly material. The present study is based on the investigation of SiO_2, Zn and SiO_2/Zn composite nanoparticles against 2^{nd} instar *Aedes aegypti* larvae. The green synthesized nanoparticles were characterized with scanning electron microscope (SEM) and Fourier transform infrared (FTIR) spectroscopy analysis. Larvicidal bioassay tests were conducted at various doses of NPs for 24 hours. Mortality data was subjected to probit analysis to determine the LC_{50}, LC_{90}, and LC_{99} values. Results demonstrated high potency of ZnO NPs against 2nd instar *A. aegypti* larvae. ZnO NPs gave a great larvicidal effect against *A. aegypti* larvae. Although, larvae treated with all the nanoparticles showed probit of mortality to be linear and R^2 <1. But green synthesized ZnO NPs showed more significant results as compared to silica, and silica/zinc composites nanoparticles. These findings suggest that green synthesized NPs could serve as an alternative potent technology for mosquito vector control.

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LIST OF ABBREVIATIONS

$AgNO_3$	Silver Nitrate
AgNPs	Silver Nanoparticles
bSNPs	Biogenic Silica Nanoparticles
CQD	Carbon Quantum Dot
DLS	Dynamic Light Scattering
FTIR	Fourier Transform Infrared
H_2SO_4	Sulphuric Acid
K_2SiF_6	Potassium Hexafluoro Silicate
LC	Lethal Concentration
NaOH	Sodium Hydroxide
Na_2SiO_3	Sodium Metasilicate
RCR	Relative Consumption Rate
RGR	Relative Growth Rate
SiO_2/Zn NPs	Silica/Zinc Nanoparticles
SEM	Scanning Electron Microscope
SiO_2 NPs	Silica Nanoparticles
TEM	Transmission Electron Microscopy
Zn $(NO_3)_2.6H_2O$	Zinc Nitrate Hexahydrate
Zn NPs	Zinc Nanoparticles

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CHAPTER 1

INTRODUCTION

Diseases that are transmitted by vectors especially arthropod vectors like mosquitoes are the main source of increasing infection globally. These are the vectors that are causing many diseases including dengue virus, yellow fever, malaria infection, and chikungunya etc. About 96 million cases per year of dengue virus are reported by World Health Organization (WHO). Over past 50 years, dengue virus is increasing at a rate of 30-fold in many countries in the world with 400 million people affected each year. Globally, one-third of the dengue cases are reported and 21,000 deaths are reported each year due to dengue virus. *Aedes aegypti* is the mosquito that has white and black spots on its legs and on the upper surface of its thorax causing dengue fever all around the world [1, 2].

Dengue infection may vary from asymptomatic infection to deadly complications such as last stage of dengue illness that is dengue shock syndrome. Moreover, misdiagnosis of dengue is also the common issue especially in resource constraint countries because the health budgets are not enough to focus on both control as well as treatment of disease. Most of the dengue cases remain undetected due to economic and technological constrains worldwide especially in developing countries like Pakistan. Hence, the current control of dengue virus is based on diminishing the larvicidal or adult mosquitoes of dengue using chemical and biological means. [3-5].

Dengue infection is caused by four different but antigenically related flavivirus stereotypes along with DENV i.e., dengue virus. DENV-2, that is actually dengue virus type 2, reported to one of the deadly stereotypes of all. Once the virus enters the body, the incubation period varies from 3-14 days. Headache, fever, muscle, and joint pain are the early symptoms of dengue virus. More severe case of dengue causes the hemorrhagic fever syndrome that ultimately results in dengue shock syndrome. Hence, symptoms of having dengue virus in human body include mild to severe complications in human body [6, 7].

Dengue virus is expanding at a faster rate and becoming a serious disease due to the health, socio-economic and environmental correlations especially in developing countries like Pakistan. Pakistan being a developing country has been facing the rising trend of dengue since 1994. There are several factors that contribute to rise in dengue virus in Pakistan mainly poor public health infrastructure, lack of sanitation facilities especially in rural areas, rapid urbanization, and lack of implementation of controlling plans. To cope with dengue virus, there is an emergent need to develop the possible applicable efficient technologies and proper action plans to achieve ways to vector-related disease challenges [8-13].

To diminish the mosquitoes population, larviciding is the powerful way to stop the growth of mosquitoes at larval stage in their breeding places. Due to this technique, changes in the midgut epithelium with gastric caeca occur that can also affect the malpighian tubules of mosquitoes larvae. Larviciding is actually the way that mostly relies on the use of conventional synthetic insecticides. Larviciding proves to be the effective method but repeated use of this method led to the development of resistance in mosquitoes that ultimately results in recommencing of vector borne diseases in many areas of the world. Moreover, their repeated use has disturbed the natural biological control systems [14-15].

Various control management strategies have been proven effective to control dengue viral infection. These strategies also include preventive measures such as avoiding contact with vectors, environmental cleanliness, and adequate drainage etc. Vector control approaches further consist of biological, chemical, and environmental control methods to control dengue vector infection [16-17].

Mosquitoes breed in water in their larval stage and are the main targets for pesticides as they are easy to handle them in this habitat. Use of conventional methods like chemical pesticides has resulted in the undesirable modification in the environment. Use of these methods also develops resistance against mosquitoes and several human health concerns. So, there is a need for herbal green preparations that do not have any harmful effects on non-target organisms and the environment. Herbal green synthesis of materials against mosquitoes control is the environmental friendly methods and is easily biodegradable. Hence, synthesis from herbal green materials becomes the main priority for scientists associated with the alternative green vector control approach [18-22].

Chemical methods for controlling dengue infection include the use of chemical solutions to repel or kill the mosquito vectors. But chemical methods cause environmental contamination that result in environmental pollution and toxicity. Biological vector control methods include the use of control agents that are not capable of transmitting viral pathogens. These methods also include genetically modified mosquitoes (GMM), sterile insect techniques (SITs) etc. Use of biological methods also causes environmental pollution such as radiations exposure. Environmental control methods include basic methods such as covering the water-filled containers, cleaning the vector-breeding and strategies to ensure environmental protection [23-26].

Use of green nanomaterial especially metal oxide nanoparticles play pivotal role in the field of pest and insect management Nanoparticles have various uses to enhance the agricultural productivity, pest control and herbicide delivery. Green synthesized nanoparticles are used to produce insect repellants, pesticides, and insecticides. To control the dengue infection, nanoparticles are used which are synthesized by plants or their leaves extracts as a greener route. Various green synthesized nanoparticles have excellent potential for controlling dengue infection and used as protecting agent to control the spread of dengue infection if applied by proper safety measures [27-29].

Hence, a proper technique is required to diminish the spread of mosquito-borne diseases. In order to increase the mosquito control, various types of new tools are being implemented. Among these tools, synthetic insecticide is the major tool that is used to control mosquito vector, but this has not been successful as it also creates toxicity problems. Moreover, regular use of synthetic insecticides has led to rejuvenation of mosquitoes. Due to these reason, an alternative approach must be needed that is resistive of developing resistance in mosquitoes [26].

Green synthesis of nanoparticles proves to be one of the most effective alternate to reduce and inhibit the spread of mosquito-borne diseases. There are various techniques to prepare nanoparticles but biosynthesis of nanoparticles is one of the synthetic method in which natural chemicals and biomaterials such as sugars, biodegradable polymers (chitosan, etc.), extracts of plants, and microorganisms are used. Nanoparticles are also synthesized by green sustainable approaches. So, green synthesis come up with advancement over various other methods because of many benefits such as they are cost-effective, less toxic, less harmful, environment friendly, reproducible and provide productive outcomes.

Many studies have reported the potential of some nanomaterial to lessen the dengue virus infection. These nanoparticles prove to be the most promising toxic agents that work against mosquito larvae. Nanoparticles that are synthesized from plants and plant extracts are less toxic and less harmful as compared to those nanoparticles that are synthesized from other methods. Nanoparticles such as zinc, silica, iron, silver, and iron-silica etc. have proved very effective against mosquito larvicidal activity and showed high mortality against mosquito larvae [19-21, 27-29].

The present study aims to fabricate green synthesized nanoparticles from plant extract and investigate the potential of these nanoparticles against selected mosquito larvae. In this research silica, zinc and silica/zinc composite nanoparticles was synthesized to investigate the larvicidal activity of mosquito vector. Nanoparticles were synthesized from greener approach using leaves extract of *Azadirachta indica*. The local name of this plant is neem. Neem tree belongs to the Meliaceace family. *Azadirachta indica* is prevalent in Pakistan, India, Africa, America, and Australia [30].

RATIONALE

Mosquito-borne diseases are going to be increase in number and their geographic incidence is also expanding. Over approximately 80% of the total population in the world is in danger of vector-borne disease and among this percentage, mosquito-borne disease is the major contributor to human disease. Various management strategies like chemical methods, biological methods etc. are used to control the dengue causing vector but are hazardous for human health. These methods are also affecting the economy of country at large scale. Therefore, there is needed to look for alternative approach that is readily available, affordable, and less toxic and less detrimental to the environment. The main focus of this study is to fabricate different nanoparticles by cost effective green method and application on selected mosquito larvae. The study focuses on comparative assessment of green synthesized nanoparticles such as silica, zinc and silica/zinc composite nanoparticles, focusing on the synthesis of new agent against mosquito larvae.

OBJECTIVES

The objectives of this study are:

- Synthesis of silica, zinc, and silica/zinc nanoparticles by *Azadirachta indica* (neem extract) and their characterization.
- Evaluation of efficacy of synthesized nanoparticles by mortality assay on selected mosquito larvae.

CHAPTER 2

LITERATURE REVIEW

Globally the loss of lives and health issues caused by the mosquito-borne diseases has pushed the world to focus on control strategies for the control of these diseases. Over 80% of the total population in the world is in danger of vector-borne disease and among this percentage; mosquito-borne disease is the major contributor to human disease [1]. With the passage of time, many mosquito-borne diseases are going to be increase in number and their geographic incidence is also expanding. To cope with these diseases, there is a need to develop the possible applicable technologies and proper action plans to reduce their geographic incidence [19, 20].

Various management strategies like chemical methods, biological methods etc. are used to control the dengue causing vector but are hazardous for human health. These methods are also affecting the economy of country at large scale. Therefore, there is need to search for alternative products that are readily available, affordable, less toxic to living being and less detrimental to the environment. Nanoparticles synthesized by greener approach are very effective. So, green synthesis produce advancement over many various methods because of various benefits such as they are cost-effective, one step, simple, environment friendly and reproducible and provide results in more stable materials [20].

In a study, Chinnathambi *et al.* [31] assessed the potential of Zn NPs nanoparticles synthesized from *Tarenna asiatica* methanol leaf extract against *Aedes aegypti* to assess the larvicidal and pupicidal potential of these nanoparticles. According to SEM analysis, nanoparticles that were synthesized by methanol leaf extract ranged from 22.35 to 31.27mm having spherical shape. At second, third and fourth larval stage of *Aedes aegypti,* these nanoparticles showed excellent larvicidal and pupicidal activity.

Nanoparticles synthesized from greener approach are less toxic and utilize fewer chemicals as compared to other methods. Das *et al.* [32] assessed three nanoparticles for controlling *Aedes aegypti* larvae. These were Si complexed with dsRNA, carbon quantum dot (CQD) and chitosan nanoparticles. Among these three nanoparticles, carbon quantum dot was the most effective nanoparticle as it contained dsRNA that causes major gene silencing and targeted two genes of mosquito i.e., (SNF7 and SRC). Hence, it resulted in mortality of *Aedes aegypti* larvae.

Roopan *et al*. [33] synthesized Zn NPs to check the larvicidal activity of *Aedes aegypti*. *Syzgium cumini* seed extract was used for the preparation of nanoparticles and it worked as a reducing agent. According to the results of FTIR, SEM and TEM, the particle size of zinc oxide nanoparticles synthesized by the green approach was found to be 50 to 60nm. Zn NPs synthesized from the plant extract proved effective resulting in LC_{50} and LC_{90} of 51.94ppm and 119.99ppm respectively.

Ishwarya *et al*. [34] also used *Pergularia daemia* unripe fruits to prepare *Pd*-Zn NPs and applied against *Aedes aegypti*. Characterization of these nanoparticles was done by instruments like SEM, FTIR, and TEM. Green synthesized Zn NPs was analyzed against third larval stage of *Aedes aegypti*. After 24 hours, larvicidal and mortality effect was evaluated. LC_{50} and LC_{90} of 11.11 and 21.20 µg/ml respectively clearly showed the mortality of *Aedes aegypti*. In *Aedes aegypti* at its third larval stage, results have clearly shown that level of enzymes especially phosphate, acetylcholine esterase; proteins were reduced effectively in the larvae treated with Zn NPs. Hence, these results have clearly highlighted the significance of *Pd*-ZnO NPs against the mosquito larvicidal activity.

In a study conducted by Loganathan *et al*. [35] synthesized Zn NPs using *Knoxia sumatrensis* aqueous leaf extract. SEM characterization showed that shape of Zn NPs was rod like with average size of 50-80nm. Nanoparticles were applied on *Ch. quinquefasciatus* to check the mosquito larvicidal activity. Results have shown LC_{50} value of 58.87 µg/mL and LC_{90} 19.46 mg/mL. At this range, larvicidal activity of Ch. *quinquefasciatus* was at a maximum rate. Hence, it also proves the efficiency of Zn NPs against pupicidal and larvicidal activity and these NPs exhibit a great role in pharmaceutical studies also.

Synthesis of nanoparticles from plant extract is the most convenient, quick, safe, and cost-effective way compared to other methods. Abdo *et al.* [36] biosynthesized Zn NPs using *Pseudomonas aeruginosa* plant extract. The plant extract was the biomass filtrate separated out from sediments of mangrove rhizosphere. Data analysis showed that green bio-synthesized Zn-NPs, analyzed by TEM, FTIR and XRD analyses, exhibited high efficacy for *Culex pipiens*. Comparison of Zn NPs and zinc acetate having same concentration showed that ZnO NPs have high efficacy and mortality against *Culex pipiens* as compared to zinc acetate. 100% mortality was reported at 200 ppm by using the nanoparticles in comparison to zinc acetate that showed 44% mortality.

Verma *et al.* [37] also prepared Zn NPs that were silver-doped by using the *Moringa oleifera* extract. Nanoparticles were characterized using the FESEM and TEM. Images from the FESEM and TEM revealed the flower-like and high crystallinity of the nanoparticles with 18mm inhibition zone against *C. albicans.* Observations have clearly shown the high antimicrobial and anti-larvicidal activity of these nanoparticles. Moreover, these NPs contained nanostructures having unique and desirable properties that work against mosquito larvae.

Similarly, Venotha *et al.* [38] evaluated the effects of green synthesized Zn NPs using *Elettaria cardamomumn* seed extract to check the larvicidal effect on *Aedes aegypti* and *Culex tritaeniorhychus* by the process of secure co-precipitation method. On larvae of *Aedes aegypti,* the LC_{50} and LC_{90} values were 13.27μg/ml and 25.36μg/ml respectively and that of *culex tritaeniorhychus* were 15.09μg/ml and 29.70μg/ml respectively. Comparison has shown that *Cx. tritaeniorhynchus* was less susceptible than *A. aegypti.*

Dengue virus not only increases in a faster rate, but its geographic incidence is also expanding at a faster rate especially in developing countries. Nityasree *et al.* [39] used the solution combustion synthesis method to biosynthesize the Zn NPs using *Solanum lycopersicum* leaf extract. Larvicidal efficacy was assessed against *Aedes aegypti.* To check the morphology of NPs, these were characterized by FTIR, SEM, XRD and EDX. Results have shown that NPs were of rod-like with average size of 40.93 nm without any impurities. Zn NPs showed high larvicidal activity as compared to the aqueous leave extract. $P < 0.05$ showed significant results within 48 hours. Bioactive constituents that were present in the leaf extract act as a capping agent for the preparation of Zn NPs and could be used to inhibit the growth of mosquito larvae especially *Aedes aegypti.*

Moreover, Baz *et al.* [40] used the sol–gel (A800) and sol–gel/combustion (B800) methods for the synthesis of silica nanoparticles (Si NPs) to be applied on *Culex pipiens* larvae. Different concentrations of silica nanoparticles i.e., 5, 25, 100, and 200ppm were applied on the *Culex pipiens* larvae. High larvicidal activity was observed after 24 and 48 hours of application because the LC_{50} values at first, second and third larval stage were 19.7, 37.4, 61.1, and 85.2, respectively. In first larval stage, high mortality was observed as compared to all other stages of mosquito larvae. Treatment with B800 and A800 silica nanoparticles, 100% and 90% mortality were observed in mosquitoes. In 1st larval stage, 81.7% mortality rate and in 4th larval stage, 63.3% mortality rate was observed. Si NPs could serve as one of the excellent ways to kill mosquito larvae especially *Culex pipiens* larvae.

In another study, Shehu *et al*. [41] successfully synthesized Zn-Cu nanoporous composite using Gum Arabic. Nano larvicidal activity of synthesized nanoparticles was assessed against 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval stages of malaria larvae. Different concentrations of NPs were added to check the mortality. The values of LC_{50} were 8.84, 8.73, 8.96 and 10.56 mg/l against the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval stages respectively. Moreover, the LC_{90} values were 19.06, 28.06, 40.88 and 79.56 ml/l against 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval stages respectively. Among all the larval stages, the values of correlation coefficients were in the range of 0.94 to .099 that shows strong concentration dependent larvicidal response.

Efficacy of Zn/Si nanocomposite was evaluated by Abba *et al*. [42] against *Culex quinquefasciatus* larvae. Three different concentrations of the nanocomposite were applied on these larvae. At concentration 10, mortality rates were 70%, 80% and 86% against 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval stages respectively. At concentration 20, mortality rates were 56%, 64% and 84% against 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval stages respectively. At concentration 25, mortality rates were 44%, 48% and 76% against 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval stages respectively. All of these conclusions mentioned above have shown that efficacy of green synthesized Zn/Si nanocomposites was highest in 1^{st} larval stage and lowest in 3^{rd} larval stage. Overall, Zn/Si nanocomposites exhibit high larvicidal activity at 1^{st} instar (LC_{50} 4.02 and LC_{90} 39.27 mg/l), 2^{nd} instar (LC_{50} 8.77 and LC_{90} 51.07 mg/l) and 3^{rd} instar stage (LC_{50} 13.76 and LC_{90} 81.81 mg/l).

Moreover, Abba *et al*. [43] synthesized the CuO/SiO_2 composite nanoparticles by green synthesis using Gum Arabic. SEM, EDX, UV-visible and FTIR analysis confirmed the formation of nanoparticles. Larvicidal test was conducted to check the larvicidal effect on 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} instar larvae of malaria vector. After 24 hours of treatment, LC_{50} and LC_{90} values for 2^{nd} instar larvae were 7.980 and 24.937 respectively by having 10, 20, and 25 mg/l concentrations. Results have shown that these nanoparticles are more significant for strong larvicidal response.

Additionally, Jaffri *et al*. [44] synthesized silver-doped Zn NPs using *Prunus cerasifera* leaf extract. In less than 15 minutes, degradation efficiencies of the NPs were 86% to 95%. NPs prepared by this plant extract exhibited active zones of inhibition against pathogenic nature. Silver doped Zn NPs were analyzed against *ampicillin* and *amphotericin B* in order to check the efficacy of nanoparticles. Incredible results occurred that clearly shown that Zn NPs exhibited great photolytic, anti-larvicidal and anti-microbial properties that ultimately signifies their importance on commercial and industrial scale.

Similarly, Chauhan *et al*. [45] prepared two types of Zn NPs using *Cannabis sativa* leaves. Pure Zn and Ag-doped Zn NPs were synthesized to analyze their efficiency against *Rosellinia nectarix* and *Fusarium spp*. The zone of inhibition obtained through Ag-doped NPs was 14.1mm and 23.25mm, which were 41.2mm and 38.3mm around the negative control group. In case of Pure Zn NPs, the zone of inhibition of about 21.7mm and 28.2mm was observed which were 41.2mm and 38.3mm in control group. Results have clearly shown that maximum inhibition was reported by Ag-doped Zn NPs as compared to Pure Zn NPs that clarified the potential of using green synthesized nanoparticles.

In another study, Al-Azawi *et al.* [46] synthesized the silica (Si) NPs by green synthesis method. Magnetic stirring and cold plasma methods were used to synthesize nanoparticles using aqueous leaf extract of *Thuja orientalis*. Nanoparticles formation was confirmed through FTIR analysis that showed the synthesis of hydrophilic functional groups in capping matrix. Presence of these functional groups can improve the stability of silica nanoparticles. Inhibition effect was analyzed against *E.coli* and *S.aureus* that was found to be highest compared with the control (0.1035nm, 0.07nm, 0.2773nm and 0.2nm) but in comparison to antilarvicidal activity, silica nanoparticles were not showing excellent results.

Moreover, Gutierrez *et al.* [47] analyzed the larvicidal effects of *Jatropha curcas, Citrus grandis* and *Tinospora rumphii* leaf extracts against the larvae of *Aedes aegypti*. Among these three leaf extracts, *T. rumphii* leaf extracts showed highest mortality. After 24 and 48 hours of application, mortality rates were 90% and 93% respectively. LC_{50} and LC_{90} were 10 mg/ml and 60 mg/ml respectively. With the increase in concentrations of the leaf extracts, mortality rate was also increasing. At 60 mg/ml concentration, the larvicidal activity of *Aedes aegypti*, using these three leaf extracts, after 48 hours of exposure was in the following order i.e. *T. rumphii* leaves > *C. grandis* bark > *T. rumphii* stem > *C. grandis* leaves > *J. curaus* bark > *J. curcas* leaves.

Green synthesis of Zn NPs was also analyzed and prepared by Ghandi *et al.* [48]. These nanoparticles were prepared using *Momordica charantia* leaf extract against larvae of *Anopheles stephensi, Rhipicephalus microplus* and adults of *Pediculus humanus capitis*. Biosynthesized Zn NPs and aqueous leaf extract were analyzed against these vectors. Comparison has shown that LC_{50} values of biosynthesized Zn NPs were more accurate than aqueous leaf extract. These values were 6.87, 14.38, 5.42, and 4.87 mg/l against *R. microplus, P. humanus capitis, An. Stephensi* and *Cx. quniquefasciatus* respectively. Results have clearly shown that bio synthesized Zn NPs possess highest larvicidal effects among these viral species.

In addition to synthesis of Si NPs, Suresh *et al.* [49] prepared Si NPs using seed essential oil to check their effects on larvae of *A. aegypti* and *S. litura*. LC_{50} values were calculated and found to be in the range of 8.823-17.911 µl/l for *A. aegypti* and 24.610 -64.546 µl/l for *S. litura* larvae respectively. Overall results have clarified that green synthesized Si NPs act as strong larvicidal agents to control the spread of mosquito larvae that would ultimately help in reducing the mosquito causing human diseases.

Another study by Attia *et al*. [50] included the synthesis of mesoporous Si NPs and silica gel and their application on larvae of *Corcyra cephalonica*. To check the effectiveness of green synthesized nanoparticles, mosquito at its pupal stage was investigated by checking the following factors i.e. pupal percentage, pupal duration, pupal emergence and adult longevity. There was decrease in 28% in adult emergence by treating the pupae with mesoporous Si NPs and 78% decrease by using silica gel as compared to control group that have 87.5% adult emergence. Furthermore, when compared the effect of nanoparticles between male and female pupae, female pupae lived for about 15 days as compared to male pupae that lived for about 2.5 days. Hence, mesoporous Si NPs proved to be effective nanoparticles to control the spread of selected mosquito larvae.

Chamaria *et al*. [51] used aqueous essence of *Vernicia fordii* seed waste to prepare Zn NPs and copper (Cu) NPs. Mortality assay has shown significant mortality rate in *T. confusum* larvae within 24hours of application. As compared to Cu NPs, Zn NPs showed less mortality i.e. 50% than morality rate of Cu NPs i.e. 87.5%. 10 mg/l concentration of both NPs exhibited highest mortality rate. With the increase in dosages of both NPs, larvae deemed perished under increase amount. Thus, Cu NPs and Zn NPs both have an excellent potential to act a strong larvicidal agent against mosquito larvae.

Green synthesis of Si NPs was also analyzed by Khalifa *et al.* [52] against *Culex pipiens* larvae. To evaluate acute toxicity of deltamethrin and silica loaded deltamethrin, 14 days administration was carried out against selected larvae that were 25.35, 32.95 and 42.84 mg/kg in case of using deltamethrin and 57.8, 75.13 and 97.68 mg/kg in case of using silica loaded with deltamethrin respectively. Thus, it is concluded that deltamethrin with silica NPs possessed high efficacy even at low concentrations as compared to simple deltamethrin. The use of low concentration of silica loaded deltamethrin would also help to reduce the toxicity of chemicals in the environment.

Manoj *et al*. [53] prepared Zn NPs by using green synthesis method. Leaves extract of *Brassica loeracea* were used to synthesize the nanoparticles by co-precipitation method. Efficacy of Zn NPs was checked against 4^{th} instar of *Culex quinquefasciatus* mosquito larvae. The LC_{50} and LC_{90} values were 76.03 and 190.03 ppm respectively. Thus, green synthesized ZnO NPs proved to be effective and ecofriendly method for larvicidal approach.

Moreover, Rather *et al.* [54] synthesized Zn NPs through *Lavandula angustifolia* leaf extract. From 80mg/l to 160mg/l of NPs concentration, dengue causing vector was exposed to that concentrations. FESEM results have confirmed the formation of Zn NPs to be in aggregates with truncated octahedron morphology. After 24 hours of exposure, Zn NPs showed 100% mortality at 160mg/l of NPs concentration. LC_{50} and LC_{90} values were 118mg/l and 135mg/l respectively. The study also confirmed Zn NPs as a potent biomedical agent against mosquito vector diseases.

Nanoparticle synthesized by green method has become more emerging and useful approach as compared to conventional physio-chemical synthesis due to their vast applications and environmental benefits. Al-Dhabhi *et al*. [55] evaluated the effect of Zn NPs, prepared through *Scadoxus multiflorus* leaf extract, on *Aedes aeygpti* larvae and eggs. LC_{50} value was 34.04 and LC_{90} value was 78.06 after 24 hours of exposure that were considered significant. Moreover, TEM analysis showed irregular spherical shape of NPs with average particle size of 31± 2nm.

Abdel *et al*. [56] used green synthesized Zn NPs prepared through *Lantana camara* leaves against *Aedes aegypti.* TEM and UV-Vis spectroscopy showed formation of spherical shaped ZnO NPs. Comparison between aqueous extract of *L. camara* and green synthesized NPs from selected plan extract has done to check the mortality results. A large difference in concentrations was reported. Larvae assessed through aqueous extract showed 100% mortality at 400ppm concentration. While, larvae treated through green synthesized NPs showed 100% mortality at 35ppm concentration of synthesized NPs respectively. Moreover, LC_{50} and LC_{90} values were 14.85 and 30.41ppm using Zn NPs and were 182.39 and 356.69ppm using aqueous extract respectively. The results showed that significant efficacy was reported using green synthesized NPs rather than aqueous extract of leaves.

Another study by Manimaran *et al*. [57] prepared the Zn NPs through green synthesis method by using *Pleurotus djamor* extract. According to the results, Zn NPs have shown high antioxidant properties. Zn NPs had strong larval toxicity against 4th instar larvae of *Aedes aegypti* after 24 hours of exposure to nanoparticles. LC_{50} and LC_{90} values were 25.6 mg/l and 31.7mg/l respectively. Moreover, microscopic images have shown the morphological changes mainly damaged the anal area of larvae. Hence, overall results have proved that Zn NPs can be used as good alternative approach for reducing the larvae habitat.

Soni *et al*. [58] synthesized Zn NPs through *Cuscuta reflexa* leaves extract. SEM analysis verified that Zn NPs were spherical in shape and polydisperse in nature. Zn NPs were tested against *Anopheles stephensi* larvae. At concentration 25 mg/l, 50 mg/l, 100 mg/l, 150 mg/l, and 250 mg/l, mortality rates were 40%, 60%, 90%, 100%, and 100% respectively for 2nd instar larvae. Probit analysis showed that results were significant with $R^2<1$. Thus, Zn NPs prepared through plant extracts are more cost-effective and eco-safe approach.

CHAPTER 3

METHODOLOGY

3.1 Materials and chemicals

Sodium Metasilicate (Na_2SiO_3), Potassium Hexafluoro Silicate (K_2SiF_6), Sliver Nitrate ($AgNO_3$), Potassium Hexafluoro Silicate (K_2SiF_6), Zinc Nitrate Hexahydrate ($Zn(NO_3)_2.6H_2O$), Sodium Hydroxide (NaOH), Sulphuric Acid (H_2SO_4), Plant Neem Leaves.

3.2 Synthesis of Materials

3.2.1 Collection and preparation of *A. indica* extract

Leaves of neem plant were collected from Jillani Park, Lahore (Figure 3.1). After collection of neem leaves, these were washed with distilled water to remove impurity. After that, the leaves were air dried under shade for few days. Dried leaves were grinded to make powder by using domestic blender. Grinded powder of neem leaves were then store at room temperature in an air-tight glass container for further use.

For the preparation of neem extract, 10g of neem powder were taken in a beaker. 150ml of deionized water were added in it. Beaker containing neem powder and deionized were kept at $80^{o}C$ on hot plate with slight stirring for 2 hours. After 2 hours, the extract was filtered carefully to remove any plant residue (Figure 3.1).

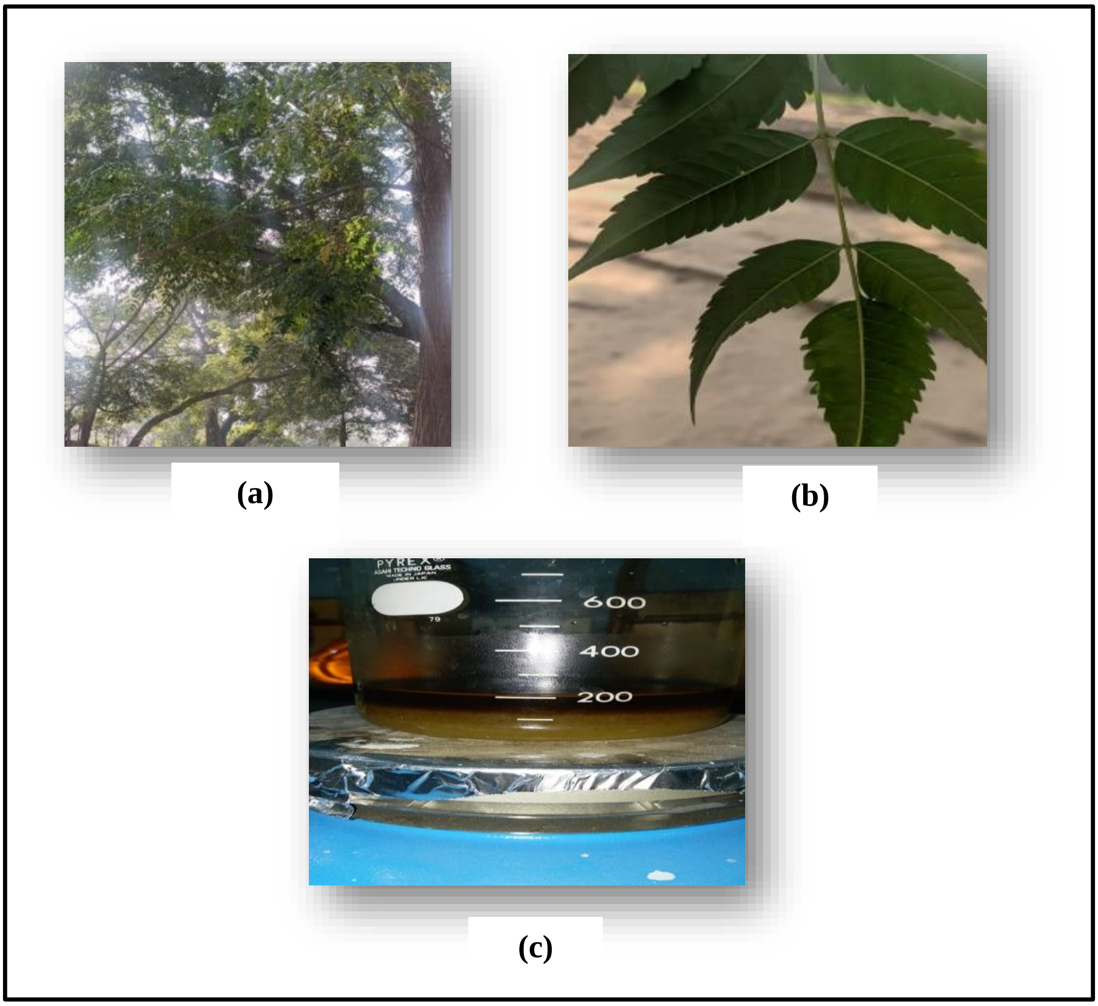

Figure 3.1 (a) *A. indica* tree (b) Leaves (c) Extract

3.2.2 Synthesis of zinc nanoparticles (NPs)

For the preparation of zinc NPs, the procedure of Hashemi *et al.* [59] was used with some modifications. 10 ml of *A. indica* extract was added in 0.1M Zinc nitrate solution in a beaker. Then, the beaker was put on magnetic stirrer at $80^{o}C$ for about two hours. During this phase, 2M solution of NaOH was slowly added in a drop wise manner to maintain the pH of solution to 10. Color changed from brown to yellow and zinc precipitates will then deposit at bottom of beaker.

The solution obtained after precipitation of solution was filtered with Whatman's filter paper with continuous washing. After proper filtration and washing, the material collected on filter paper was kept in China dish. Then, material was kept in the furnace for 2 hours at $500^{\circ}C$. White material was kept in glass vial for further use.

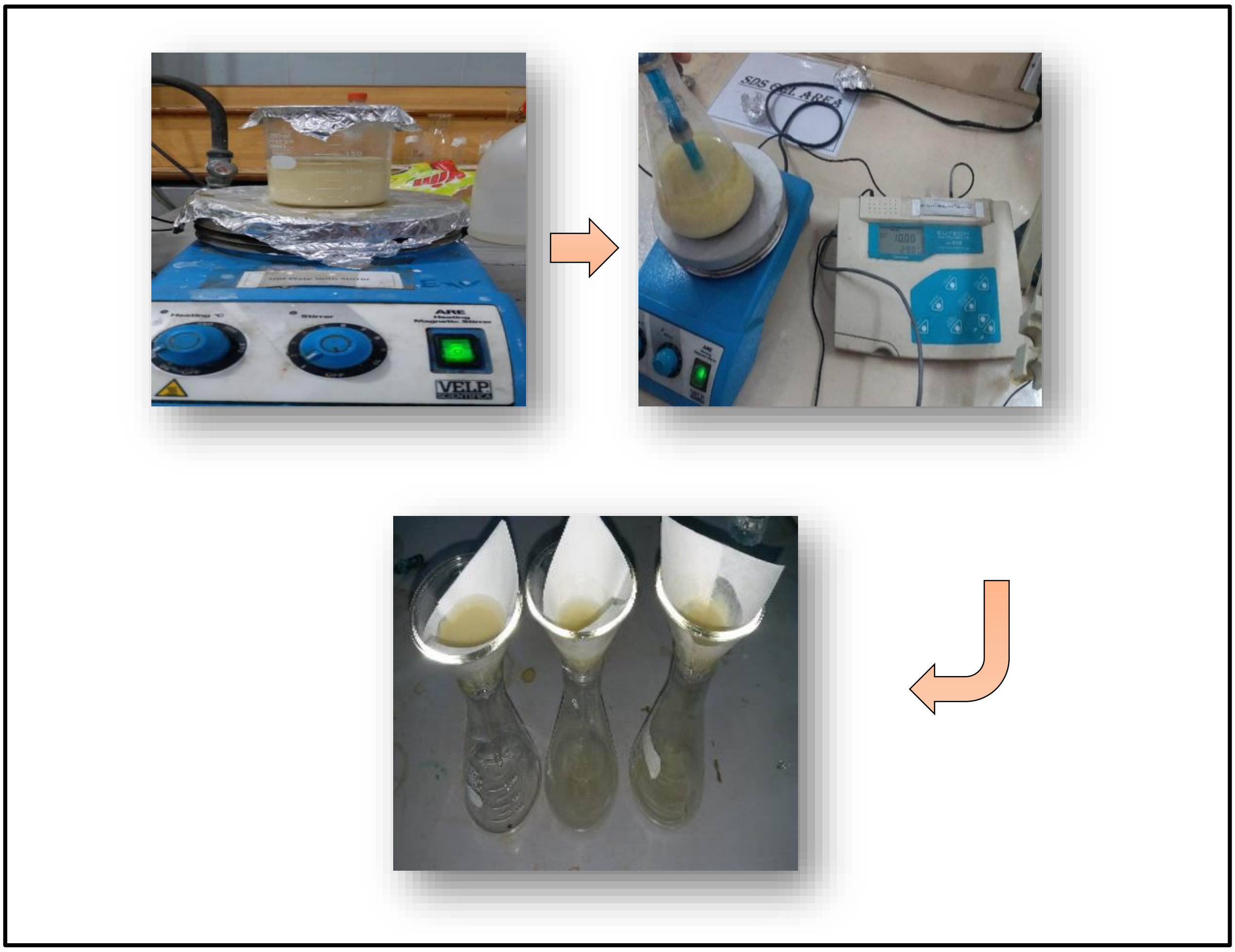

Figure 3.2: Synthesis of zinc nanoparticles

3.2.3 Synthesis of silica nanoparticles

For the preparation of silica NPs, the method adopted by Bankole *et al.* [60] was used with minor modifications required during experimentation. 25ml of neem extract was added in sodium metasilicate solution in a beaker. Beaker containing neem extract and sodium metasilicate solution was kept at 80^oC on hot plate with slight stirring for 2 hours. 0.1M H_2SO_4 was added drop wise in the solution until precipitates formed. After filtration, materials were calcinated in furnace at 600^oC for about 40 minutes. White silica nanoparticles were then obtain and preserved for further use.

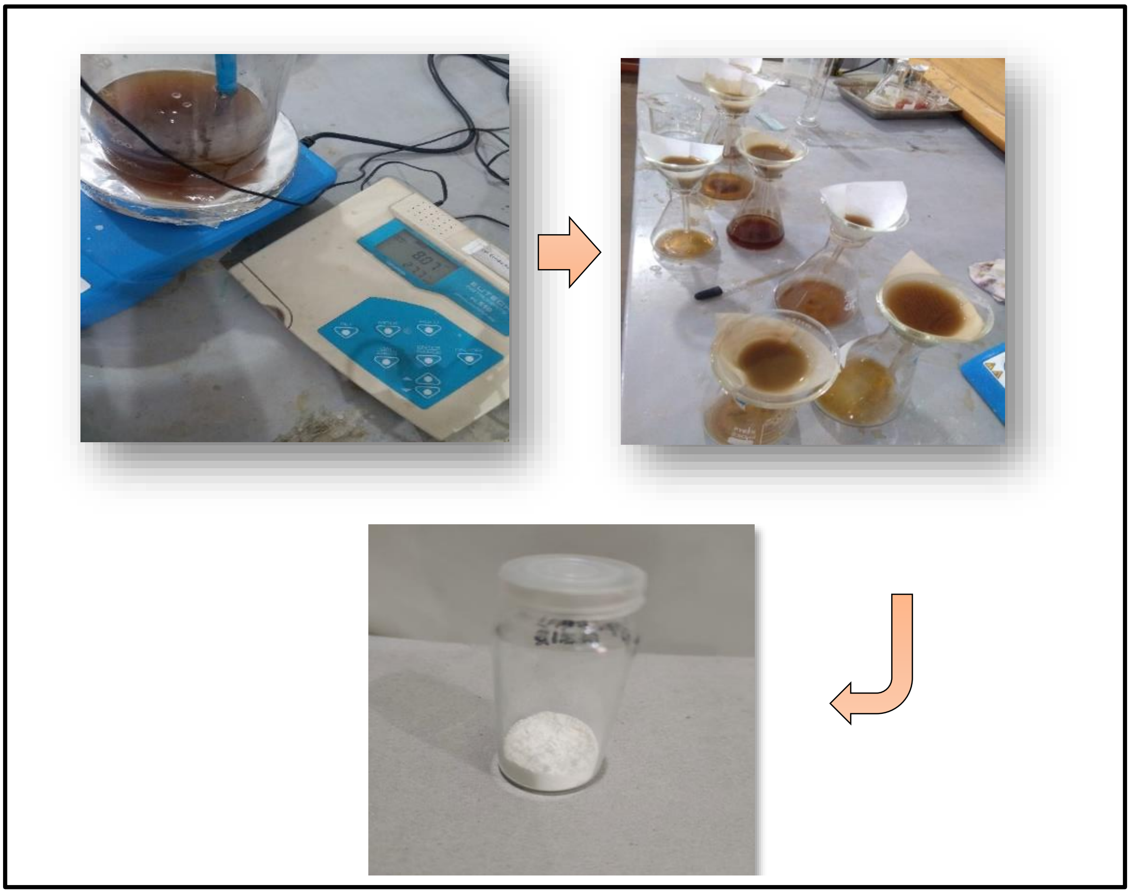

Figure 3.3: Preparation of silica nanoparticles

3.2.4 Synthesis of SiO_2/Zn composite nanoparticles

Method for the synthesis of SiO_2/Zn composite NPs was taken from the procedures used by Abba *et al*. [61] and Hassan *et al.* [62]. Neem extract was used for preparation of silica/zinc nanoparticles. 2 g of Zn $(NO_3)_6$ .6H_2O and 2 g of sodium metasilicate will be added and stirred for 30 minutes. Upon addition of Zn $(NO_3)_6$ .6H_2O and sodium metasilicate, the aqueous solution changed to milk color. With time, the solution became viscous. The material was placed in a furnace at 450 ^oC for two hours to obtain Si/Zn composite nanoparticles.

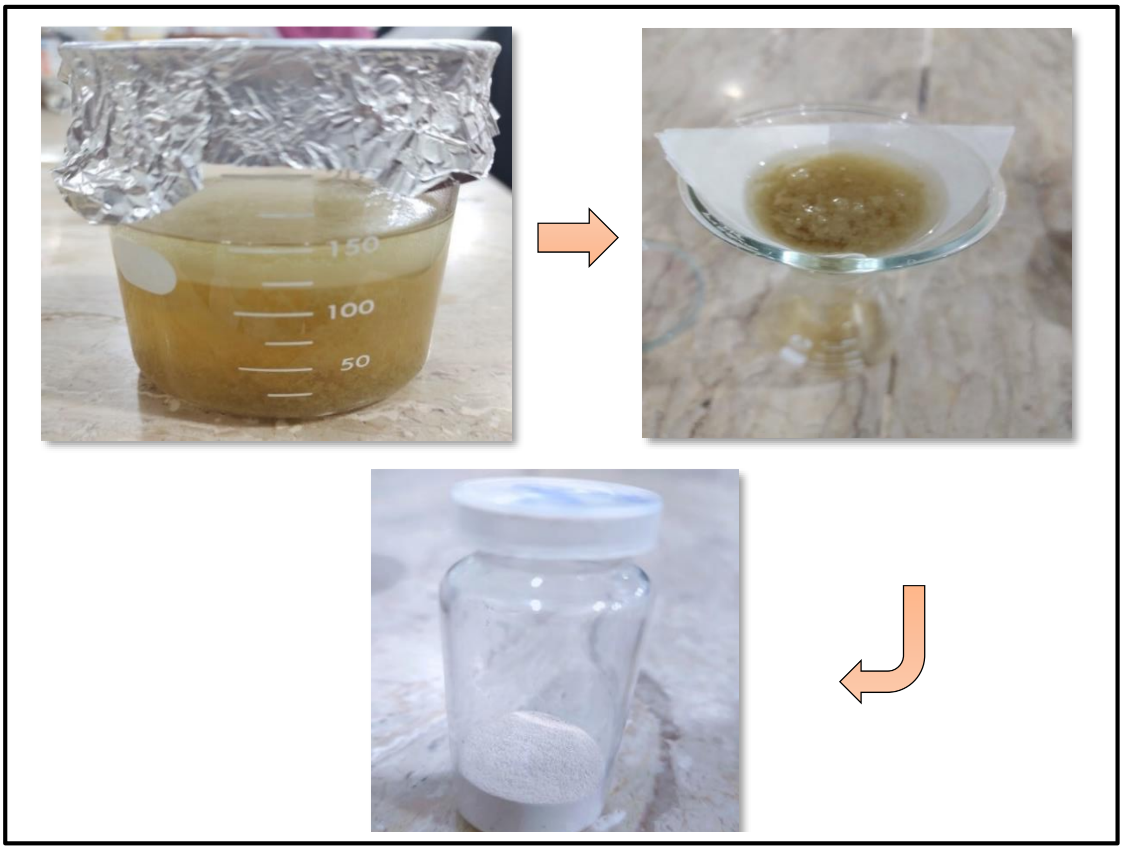

Figure 3.4: Preparation of SiO_2/Zn Composite Nanoparticles

3.3 Characterization of synthesized material

Green synthesized nanoparticles were characterized by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) in order to examine the functional groups, morphology and size of materials, respectively.

3.4 Collection of mosquitos larvae

The 2nd instar larvae of *Aedes aegypti* were collected from septic tank, contaminated water area of Lahore the method used by Govindan *et al.* [63] with little required changes. They were collected and preserved in the laboratory till use as per process described by Deshpande *et al.* [40]. Plastic tray containing water was used to preserve larvae at 25-27°C. 75-85% humidity rate will be maintained during the process. Meanwhile, egg rafts was collected to maintain for the new generation.

Figure 3.5: Collection of larvae of *Aedes aegypti*

3.5 Bioassay for Larvicidal Activity

The synthesized silica, zinc and silica/zinc composites nanoparticles were used at various concentrations by the procedure followed by Govindan *et al.* [64]. Each batch had a set of each control group with three replicates of same concentration. LC_{50} values were found out by using dose-response data after every 24 hours of gap at constant conditions. By maintaining 3 replicates, larvae were exposed to synthesized nanoparticles. Similarly, control was also maintained without nanoparticles exposure for 24 hours. For the assessment of mortality rate, triplicate setup was arranged for the bioassay of nanoparticles using Equation (1) as mentioned below. Nanoparticles that exhibit higher mortality rates were considered for further studies:

% Mortality= Total no. of dead larvae /Total no. of larvae introduce × 100………… (1)

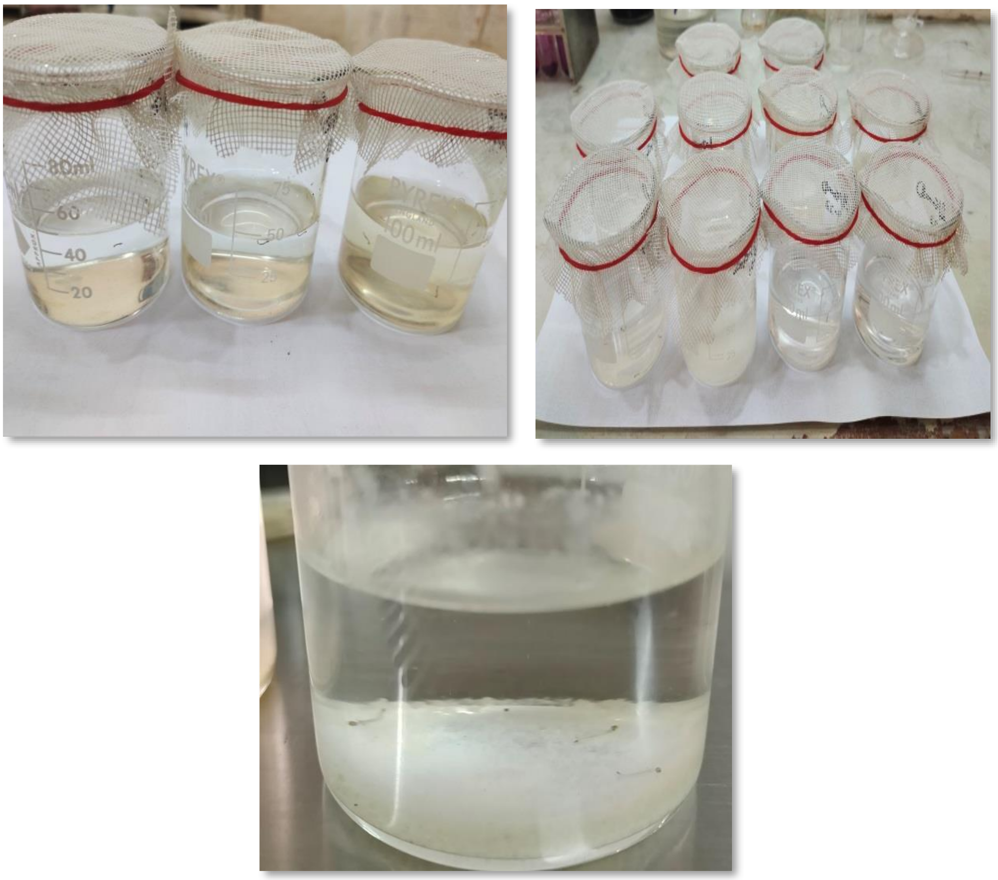

Figure 3.6: Bioassay for larvicidal activity

3.5.1 Histopathalogical and Stereomicroscopic Analysis of *Aedes aegypti* Larvae

The stereomicroscopic visualization of control group 2^{nd} instar larvae of *Aedes aegypti* is shown in figure 3.5.1 (a, b), in which no changes in the anatomy of 2^{nd} instar larvae occurred. Moreover, 2^{nd} instar larvae under treatment of Zn NPs are shown in figure (c). In this figure, Zn treated larvae showed the loss of epithelial hairs and outer cuticle. The gastric caeca, muscles, nerve cord and ganglia looked distorted and damaged as well and anus separated apart. In figure 3.5.1 (d), *Azadirachta indica*-fabricated SiO_2 NPs showed the larvicidal effect by having high shrinkage in the abdominal region and loss of antenna and mouth brush of 2^{nd} instar larvae. Also, in figure 3.5.1 (e), *Azadirachta indica*-fabricated SiO_2/Zn composite NPs showed shrinkage of mid-gut, hind-gut along with shrinkage of head region. Analysis of all three NPs have shown that Zn NPs had more anatomical changes as compared to changes cauSi NPs and SiO_2/Zn composites NPs.

Figure 3.7 (a)Stereomicroscopic images of 2nd instar larvae of *Aedes aegypti:* (b) Control (c) *Azadirachta indica*-fabricated Zn NPs (d) *Azadirachta indica*-fabricated SiO_2 NPs (e) *Azadirachta indica*-fabricated SiO_2/Zn composite NPs.

3.6 Data analysis and Thesis Writing

The data was arranged in a tabulated form and noted down. The mortality assay with different doses of silica, zinc and silica/zinc composite nanoparticles was represented in Tables using Microsoft Excel. Percentage mortality data from the three bioassay replicates were subjected to dose-response regressions using a log-probit model in order to calculate LC_{50}, LC_{90}, and LC_{99} values.

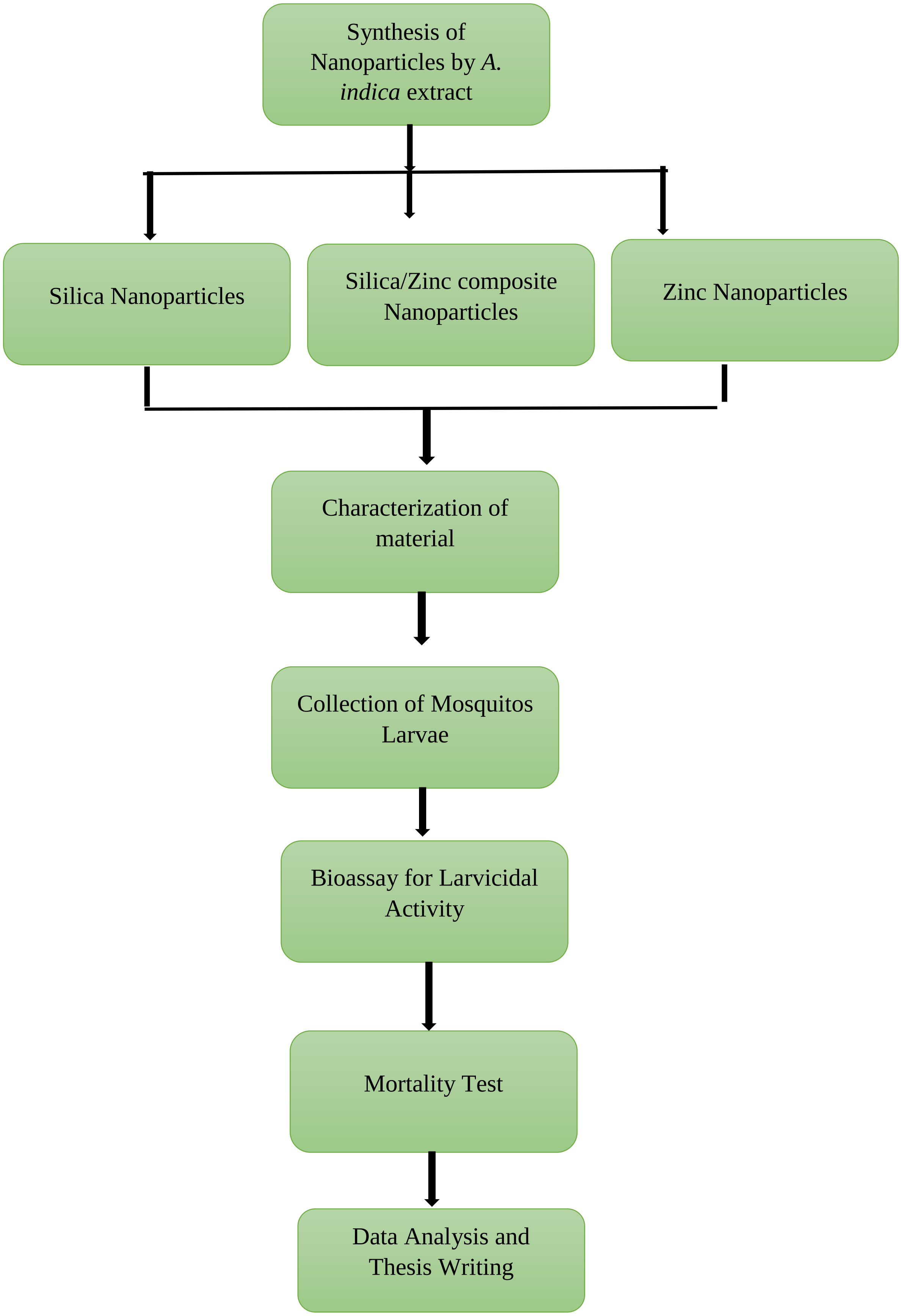

Figure 3.8: Flow Diagram of Methodology

CHAPTER 4

RESULTS

Bioassay of silica, zinc, silica/zinc composites nanoparticles, required to control dengue causing vector, were found out by checking the mortality of *Aedes aegypti*. The results obtained after larvae culture experiment include the mortality effects of silica, zinc silica/zinc composites nanoparticles on *Aedes aegypti* larvae.

4.1 Characterization Results

4.1.1 Scanning Electron Microscope (SEM)

Morphology of synthesized Zn samples was also characterized by scanning electron microscope SEM). SEM Image of Zn is shown in Figure 4.1. This shows that material was aggregated and required proper segregation before subject to analysis.

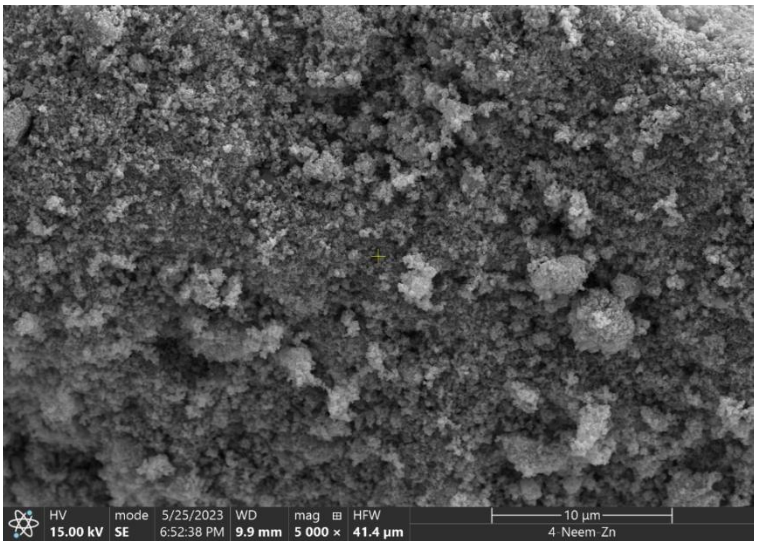

Figure 4.1: SEM image of Zn nanoparticles synthesized from *A. indica* extract

Azadirachta indica synthesized silica nanoparticles were subjected to SEM analysis. Figure 4.2 shows that average size of nanoparticles was found to be less than 40nm and spherical but somewhat aggregated.

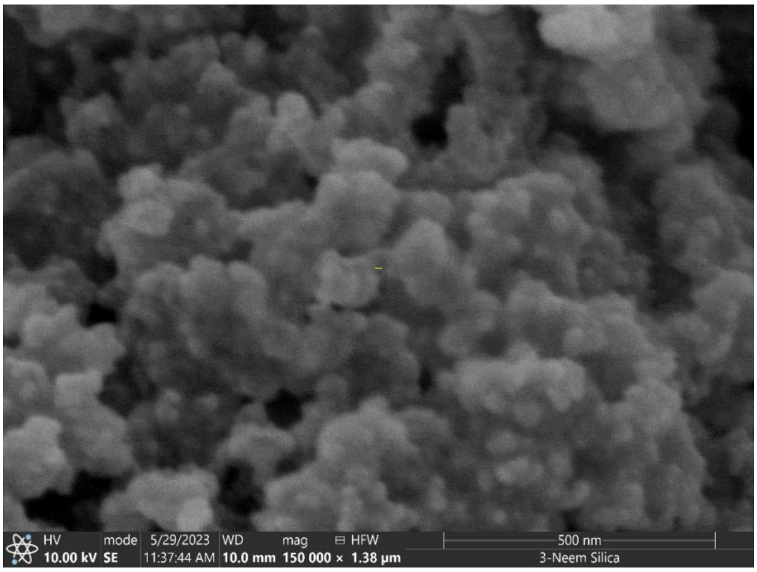

Figure 4.2: SEM images of SiO_2 nanoparticles synthesized from *A. indica* extract

The morphology and surface structure of SiO_2/Zn composite nanoparticles were analyzed by SEM analysis. The Figure 4.3 showed that dumb-bell shaped monodisperse spheres having smooth surfaces with no aggregation showed Zn nanoparticles and the average size was 500nm. Si NPs were present as tiny particle dispersed and coated the zinc particles [64].

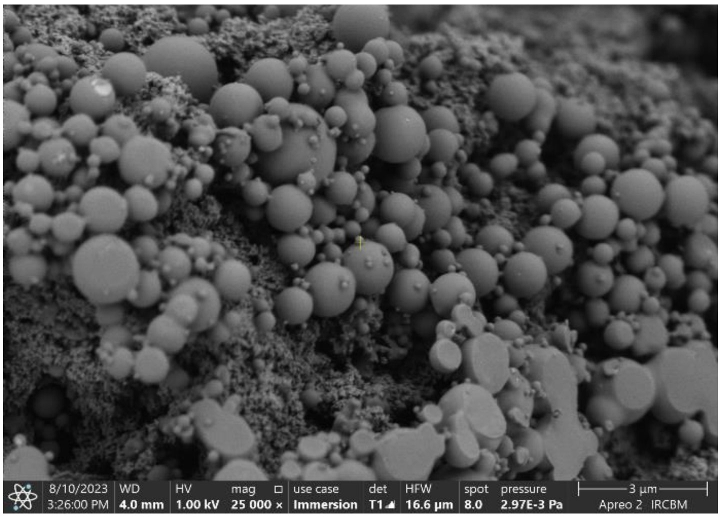

Figure 4.3 SEM images of SiO_2/Zn nanoparticles synthesized from *A. indica* extract

4.1.2 Fourier Transform Infrared (FTIR) Spectroscopy

The functional groups of silica nanoparticles attained from neem leaves extract were observed by using FTIR spectroscopy over the range of 400-4000 cm^{-1}. Presence of absorption bands cm^{-1} indicated the bands at 471.16 cm^{-1}, 799.26 $cm^{-1,}$ 1100 cm^{-1}, 1416 cm^{-1} ,1629 cm^{-1} , and 3431 cm^{-1}. Figure 4.4 depict the FTIR spectra of Silica nanoparticles synthesized by *Azadirachta indica* leaves extract. Asymmetric vibration and stretching of Si-O can be found in between 790-800 cm^{-1}. So, peak 799 cm^{-1} was attributed to Si-O stretching. Peak intensity in between 700-1100 cm^{-1} increased significantly as shown in figure 4.4. Formation of peak at 1416.43 cm^{-1} identified the CH_2 functional group bending vibration. These peaks indicated the formation of Si nanoparticles [65].

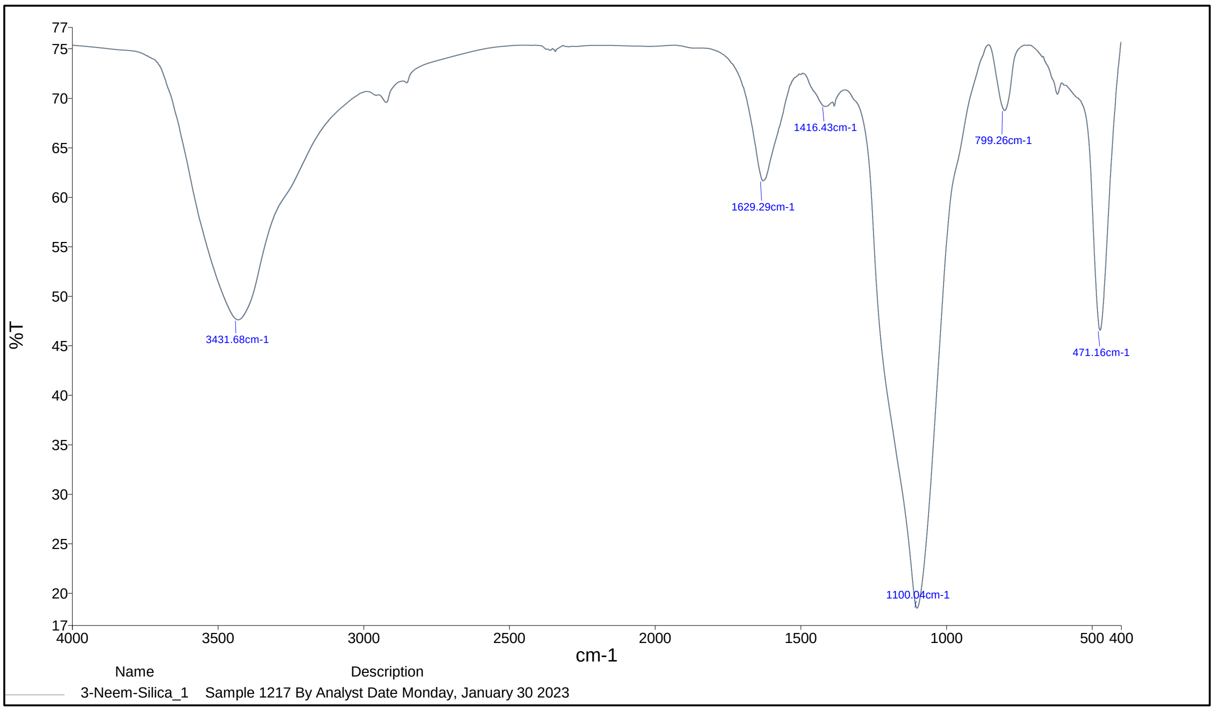

Figure 4.4: FTIR Spectrum of silica nanoparticles syntheszied from *A. indica* extract

The FTIR spectrum of *A. indica* as shown in figure 4.5 showed the absorption bands at 466.94 cm^{-1}, 707.57 cm^{-1}, 841.60 cm^{-1}, 870.15 cm^{-1}, 1066.14 cm^{-1}, 1464.01 cm^{-1}, 2339.18 cm^{-1}, and 3434.54 cm^{-1} respectively. These peaks depicted the characteristics of –OH stretching vibration and –CH stretching vibration. The band peak at 870.15 cm^{-1} was because of the symmetrical and asymmetrical of zinc carboxylates. Absorption bands at 466.94 cm^{-1} confirmed the ZnO stretching. The intensity of the peak 1400-1600 cm^{-1} was because of COOH group that showed the elimination of organic compounds from Zn NPs [66, 67].

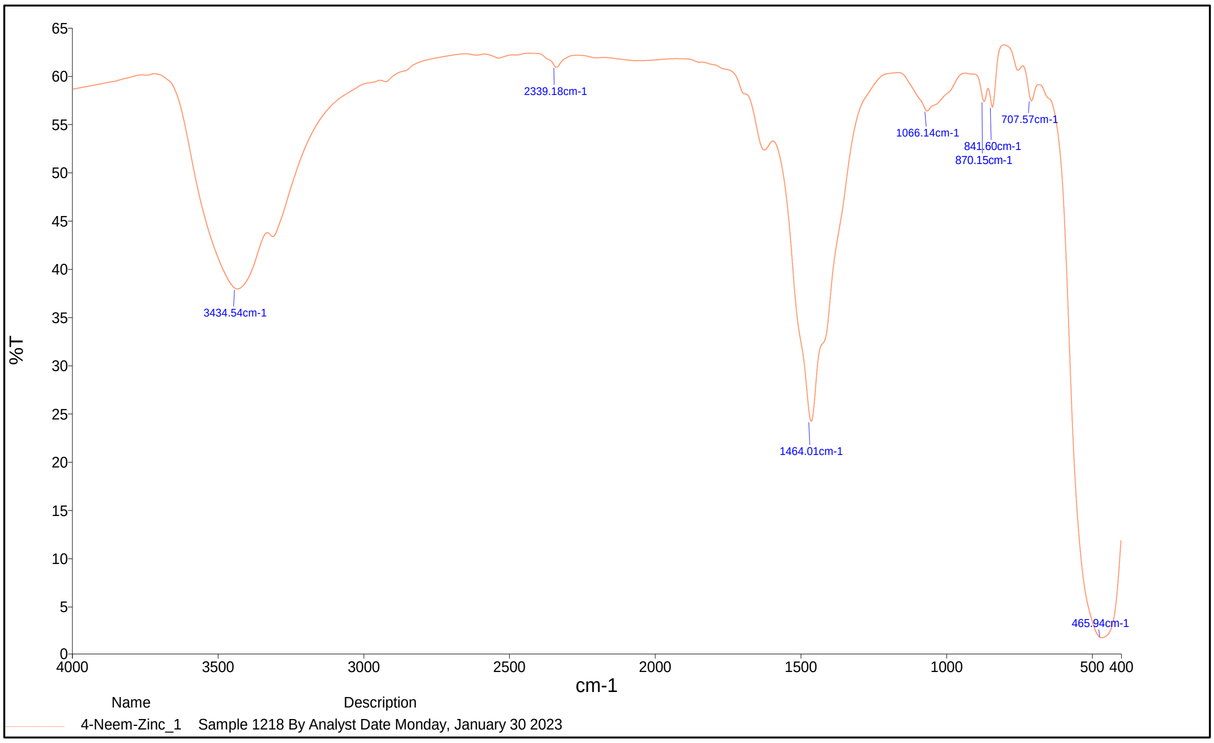

Figure 4.5: FTIR Spectrum of Zinc nanoparticles syntheszied from *A. indica* extract

The nature of biomolecules that were invovled in the reduction and formation of SiO_2/Zn composite nanoparticles were studied by FTIR analysis. Figure 4.6 depict the FTIR spectra of SiO_2/Zn composite nanaoparticles synthesized by *Azadirachta indica* leaves extract. The FTIR analysis revealed the bands at 493.23, 801.02, 1095.78, 1384.42, 1645.65, and 3441.86 cm^{-1} in the region of 400-4000 cm^{-1}. The peak absorption at 801.02 cm^{-1} was attributed to Si-OH bands. The peak at 493.23 cm^{-1} was related to Zn-O stretching. Thus, this information confirmed the formation of Si/Zn composite nanoparticles [37, 68].

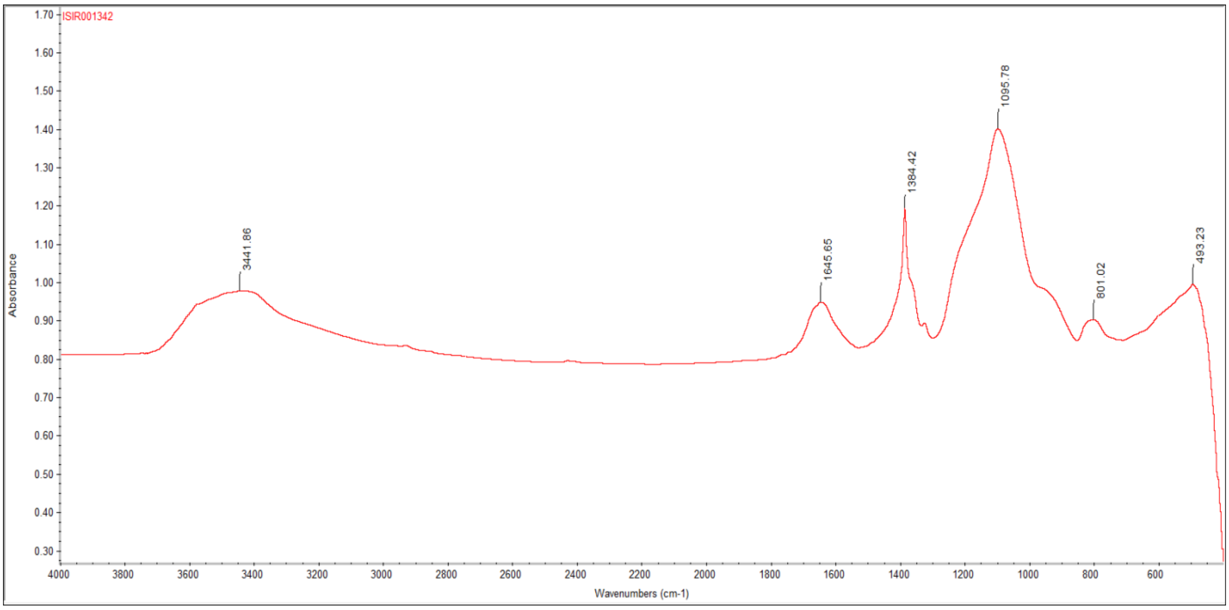

Figure 4.6: FTIR Spectrum of SiO_2/Zn Composite Nanoparticles

4.2 Bioassay Results

4.2.1 Mortality effect of Zn NPs on dengue vector (*Aedes aegypti*)

Table 4.2.1 showed the % Mortality of *Aedes aegypti* at different concentrations of Zn NPs i.e. 0.0005, 0.001, 0.0015 and 0.002%. It was obvious from the table 4.1 that larval mortality significantly increased with the increase in concentrations of Zn NPs. The mortality rates of concentrations 0.0005, 0.001, 0.0015 and 0.002% for 2nd instar were 30%, 75%, 98% and 99% respectively. The results have revealed that green synthesized Zn NPs larvicidal activity have shown significant results at 2nd instar larval stage.

Table 4.1: % Mortality of *Aedes aegypti* treated with green synthesized Zn NPs

Instars	Conc. (%)	Conc. (mg/l)	Mortality (%)	LC_{50} (mg/l)	LC_{90} (mg/l)	LC_{99} (mg/l)	R^2
2nd Instar	0.0005	5	30% ± 0.02	6.61	12.02	19.5	0.971
	0.001	10	75% ± 1.0				
	0.0015	15	98% ± 2.0				
	0.002	20	99% ±1.0				

(Mortality %- percentage mortality; LC_{50}- lethal concentration that killed 50% exposed larvae; LC_{90}- lethal concentration that killed 90% exposed larvae; LC_{99}- lethal concentration that killed 99% exposed larvae; R^2- R squared)

The larvae showed the dose-dependent mortality with significant differences in the results of against the 2nd instar larvae of *Aedes aegypti*. No mortality was recorded in the control group. Exposure to Zn NPs prepared from neem leaves extract at a concentration 20 mg/l showed maximum mortality i.e. 99% of 2nd instar larvae. However, lethal concentrations of the NPs on the 2nd instar larvae of *Aedes aegypti* were found to be LC_{50}=6.61mg/l, LC_{90}=12.02mg/l and LC_{99}=19.5mg/l. Figure 4.7 showed the probit of mortality to be linear and R^2<1 that showed that the results are significant.

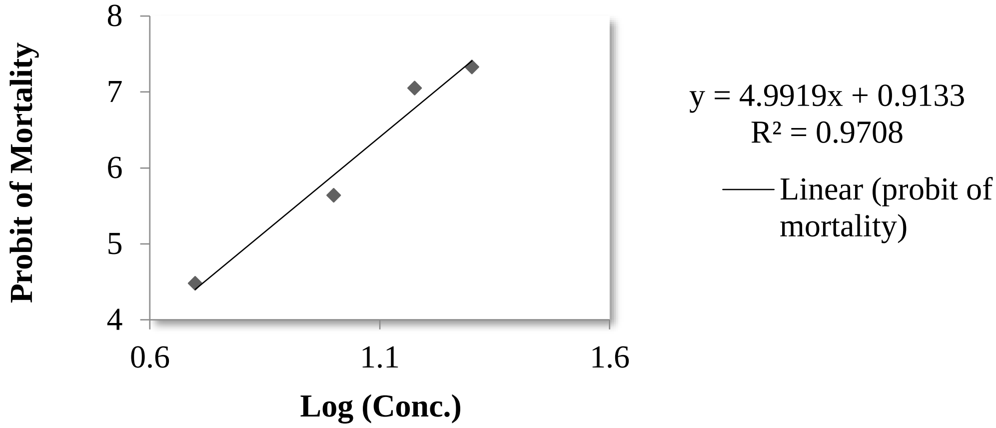

Figure 4.7: Larvicidal activity of Zn NPs on *Aedes aegypti* larvae

4.2.2 Mortality effect of SiO_2 NPs on dengue vector (*Aedes aegypti*)

Table 4.2.2 showed the % Mortality of *Aedes aegypti* at different concentrations of SiO_2 NPs i.e. 0.01, 0.02, 0.03 and 0.035%. It was evident from the table 4.2 that larval mortality increased with the increase in concentrations of SiO_2 NPs. The mortality rates of concentrations 0.01, 0.02, 0.03 and 0.035% for 2nd instar were 25, 37, 50, and 65% respectively. The results have revealed that green synthesized SiO_2 NPs larvicidal activity has increased with increase in NPs concentration.

In comparison to Zn NPs, SiO_2 NPs showed less mortality than Zn NPs to 2nd instar larvae. Moreover, increased dose of SiO_2 NPs were required to kill the larvae as shown in table 4.2.

Table 4.2: % Mortality of *Aedes aegypti* treated with green synthesized SiO_2 NPs

Instars	Conc. (%)	Conc. (mg/l)	Mortality (%)	LC_{50} (mg/l)	LC_{90} (mg/l)	LC_{99} (mg/l)	R^2
2nd Instar	0.01	100	25% ± 0.02	263	1380	5370	0.904
	0.02	200	37% ± 0.04				
	0.03	300	50% ± 1.0				
	0.035	350	65% ± 0.02				

(Mortality %- percentage mortality; LC_{50}- lethal concentration that killed 50% exposed larvae; LC_{90}- lethal concentration that killed 90% exposed larvae; LC_{99}- lethal concentration that killed 99% exposed larvae; R^2- R squared)

The larvae showed the dose-dependent mortality with significant differences in the results of against the 2nd instar larvae of *Aedes aegypti*. No mortality was recorded in the control group. Exposure to SiO_2 NPs prepared from neem leaves extract at a concentration 350 mg/l showed 65% mortality. Increased in concentrations of NPs had not significantly increased the % mortality as compared to Zn NPs that required much less concentration to obtain 100% mortality.

However, lethal concentrations of the SiO_2 NPs on the 2nd instar larvae of *Aedes aegypti* were found to be LC_{50}=263mg/l, LC_{90}=1380mg/l and LC_{99}=5370mg/l. Figure 4.8 showed the probit of mortality to be linear and R^2<1 that showed that the results are significant.

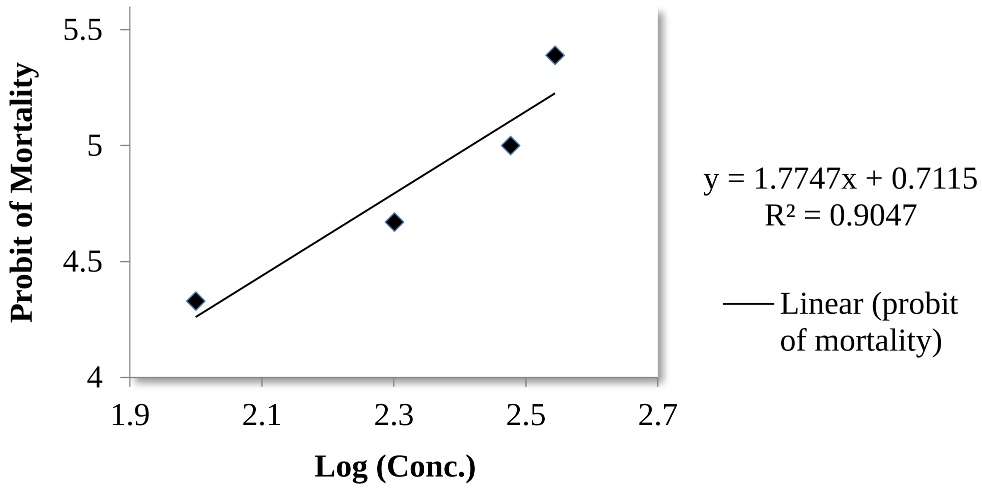

Figure 4.8: Larvicidal activity of SiO_2 NPs on *Aedes aegypti* larvae

4.2.3 Mortality effect of SiO_2/Zn composite NPs on dengue vector (*Aedes aegypti*)

Table 4.2.3 showed the % Mortality of *Aedes aegypti* at different concentrations of SiO_2/Zn composite NPs i.e. 0.02, 0.04, 0.05 and 0.055%. It was obvious from the table 4.3 that larval mortality significantly increased with the increase in concentrations of SiO_2/Zn NPs. The mortality rates of concentrations 0.02, 0.04, 0.05 and 0.055% for 2nd instar were 45%, 75%, 80% and 89% respectively. The results have revealed that green synthesized SiO_2/Zn NPs larvicidal activity have shown significant results at 2nd instar larval stage.

Table 4.3: % Mortality of *Aedes aegypti* treated with green synthesized SiO_2/Zn Composite NPs

Instars	**Conc. (%)**	**Conc. (mg/l)**	**Mortality (%)**	**LC_{50} (mg/l)**	**LC_{90} (mg/l)**	**LC_{99} (mg/l)**	**R^2**
2nd Instar	0.02	200	45% ± 0.0	224	646	1513	0.960
	0.04	400	75% ± 0.02				
	0.05	500	80% ± 0.0				
	0.055	550	89% ± 0.0				

(Mortality %- percentage mortality; LC_{50}- lethal concentration that killed 50% exposed larvae; LC_{90}- lethal concentration that killed 90% exposed larvae; LC_{99}- lethal concentration that killed 99% exposed larvae; R^2- R squared)

The larvae showed the dose-dependent mortality with significant differences in the results of against the 2nd instar larvae of *Aedes aegypti*. No mortality was recorded in the control group. Exposure to SiO_2/Zn composite NPs prepared from neem leaves extract at a concentration 550 mg/l showed maximum mortality i.e. 89% of 2nd instar larvae.

Mortality rate increased with increase in concentration of SiO_2/Zn composite NPs. At concentration 200 mg/l, morality rate reported was about 45% which was higher than Si NPs of the same concentration (200mg/l) that was 37%. As compared to SiO_2 NPs and SiO_2/Zn composite NPs, Zn NPs have shown maximum mortality with less concentration of nanoparticles. At 0.013% concentration of Zn NPs, almost 100% mortality of 2nd instar larvae occurred and at this range of concentrations, SiO_2 NPs and SiO_2/Zn composite NPs had less than 30%.

However, lethal concentrations of the NPs on the 2nd instar larvae of *Aedes aegypti* were found to be LC_{50}=224mg/l, LC_{90}=646mg/l and LC_{99}=1513mg/l. Figure 4.9 showed the probit of mortality to be linear and $R^2<1$ that showed that the results are significant.

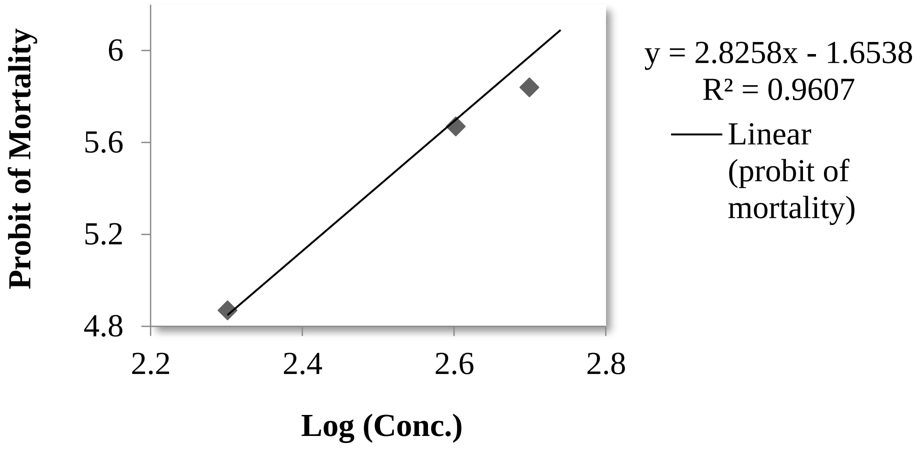

Figure 4.9: Larvicidal activity of SiO_2/Zn Composite NPs on *Aedes aegypti* larvae

CHAPTER 5

DISCUSSION

About 96 million cases per year of dengue virus are reported by World Health Organization (WHO). Over past 50 years, dengue virus is increasing at a rate of 30-fold in many countries in the world with 400 million people affected each year. One-third of the dengue cases are reported and 21,000 deaths are reported each year due to dengue virus globally. The use of conventional chemical pesticides has resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concerns. So, there is a need of green synthesized nanoparticles that have excellent potential for controlling dengue infection and used as protecting agent to control the spread of dengue infection if applied by proper safety measures [1, 2, 27-29, 18-22].

The present study aims to fabricate green synthesized nanoparticles from plant extract and investigate the potential of these nanoparticles against selected mosquito larvae. In this study research silica, zinc and silica/zinc composite nanoparticles will be synthesized, to investigate the larvicidal activity of mosquito vector, using neem leaves extract. Different doses of Zn, SiO_2, and SiO_2/Zn NPs were used to evaluate the effect of green synthesized nanoparticles on mosquito larvae.

In this regard, the present work focuses on the formation of Zn, SiO_2, and SiO_2/Zn composite NPs through neem leaves extract. The SEM analysis confirmed of all these NPs. The results of SEM of Zn NPs confirmed that particles are somewhat spherical and have spherical morphology but aggregated with average size between 140-160nm as shown in Figure 4.2. These results aligned with the studies of Rather *et al.* [26]. Moreover, the results of SEM also aligned with the studies of Baz *et al*. [42].

Moreover, Absorption bands at 466.94 cm-1 confirmed the Zn stretching and the intensity of the peak 1400-1600 cm-1 was because of COOH group that showed the elimination of organic compounds from Zn NPs as shown in Figure 4.5. The results are aligned with the studies of Roopan *et al.* [26].

Figure 4.4 depict the FTIR spectra of Silica nanoparticles synthesized by *Azadirachta indica* leaves extract. Asymmetric vibration and stretching of Si-O can be found in between 790-800 cm-1. So, peak 799 cm-1 was attributed to Si-O stretching. These results coincide with the reported result of green synthesized Silica nanoparticles using *Ethyl oleate* extract in Zhou *et al.* [72].

The use of conventional chemical pesticides has resulted in the development of resistance and undesirable effects on non-target organisms. Chemical methods cause environmental contamination that result in environmental pollution and toxicity. Use of biological methods also causes environmental pollution such as radiations exposure. Continual use of synthetic insecticides for mosquito control has led to resurgences in mosquito population. Green synthesized nanoparticles proved to be one of the most effective alternate to reduce and inhibit the spread of mosquito-borne diseases. Varieties of nanoparticles have been used such as silica nanoparticles against *Culex pipiens* larvae by Baz *et al.* [40]. Similarly, effect of green ZnO/Si nanocomposite was evaluated by Abba *et al.* [42] against *Culex quiquefasciatus* larvae [23, 25-27].

In case of *Aedes aegypti* exposure to Zn NPs, very low concentration i.e. 20 mg/l was required to bought 99 % mortality after 24 hours of exposure (Table 4.1). As compared to the study of Rather *et al.* [62], after 24 hours of investigation, 100% mortality was reported at concentration 160 mg/l against *Aedes albopictus*. It means that Zn NPs have shown more significant results against *Aedes aegypti* larvae at low concentration also as compared to *Aedes albopictus* larvae. Moreover, the lethal concentrations of the NPs on the 2nd instar larvae of *Aedes aegypti* were found to be LC_{50}=6.61 mg/l and LC_{90}=12.02 mg/l and the lethal concentrations of ZnO NPs on *Aedes albopictus* larvae were LC_{50}=118mg/l and LC_{90}=135mg/l. The findings of the present study show that green synthesized Zn NPs are more effective to kill selected mosquito larvae than the previous study related to it.

Bioassay of green synthesized SiO_2/Zn composites NPs was investigated to check the % Mortality against 2nd instar *Aedes aegypti*. Different doses of nanoparticles were applied by setting apparatus. In case of SiO_2/Zn composite NPs, the mortality rate of concentration 550mg/l, mortality rate was 89% against 2nd instar *Aedes aegypti* (Table 4.3), but in case of Abba *et al*. [42], 48% of mortality was reported at 25mg/l concentration of SiO_2/Zn nanocomposite against 2nd instar *Culex quinquefasciatus* larvae. The results have clarified that increased dose of Si/Zn composite NPs exhibit greater larvicidal effect on *Aedes aegypti* larvae.

At concentration 20 mg/l, Zn NPs showed 99 % mortality after 24 hours of exposure of dengue larvae of 2nd instar stage and LC_{50} and LC_{90} values of Zn NPs were 6.61mg/l and

12.02mg/l respectively (Table 4.1). Present results relate to the study of Manimaran *et al.* [65] that also prepared Zn NPs through green synthesis approach to check their efficacy against 4th instar larvae of dengue larvae. At concentration 20 mg/l, 81 % mortality was reported with the LC_{50}, LC_{90} values 10.1mg/l and 14.4mg/l respectively. This shows that results obtained through present study are more significant than the previous studies that are related to it.

2nd instar larvae treated with Zn NPs have shown mortality 30%, 75%, 98% and 99% at concentrations 5ppm, 10ppm, 15ppm and 20ppm respectively after 24 hours of treatment. Similar results were obtained by Thakur *et al.* [66], in which Zn NPs were assessed on *Macrosiphum euphorbiae*. Nanoparticles at concentration 200ppm, 300ppm, 400ppm and 500ppm showed 23%, 37%, 47% and 57% respectively after 24 hours of treatment. Analysis between the current results and previous results have clearly shown that Zn NPs have more effective larvicidal response against *Aedes aegypti* larvae as compared to larvae of *M. euphorbiae* .

Larvicidal efficacy of SiO_2/Zn composite nanoparticles on 2nd instar larvae of *Aedes aegypti* aligned with the previous study of Abba *et al.* [43] in which after 24 hours of treatment of Cu/Si composite nanoparticles, LC_{50} and LC_{90} values for 2nd instar larvae were 7.980 and 24.937 respectively by having 10, 20, and 25 mg/l concentrations. It was obvious from the table 4.2.3 that larval mortality significantly increased with the increase in concentrations of SiO_2/Zn NPs. LC_{50} and LC_{90} values for 2nd instar larvae were 224mg/l and 646 mg/l respectively. It means that green synthesized Cu/Si composite NPs shows more significant results on malaria vector as compared to green synthesized SiO_2/Zn composite NPs on *Aedes aegypti.*

SEM analysis of Zn NPs in the present research has shown that Zn NPs are spherical in shape and have polydisperse nature (Figure 4.1). These results are similar with the previous research of Soni *et al*. [58] in which *Cuscuta reflexa* leaves extract was used to synthesize Zn NPs. These nanoparticles were also spherical in shape with polydisperse nature.

Similarly, larvicidal activity against *Aedes aegypti* larvae was assessed by Venotha *et al*. [38] to check the effect of green synthesized Zn NPs using *Elettaria cardamomumn* seed extract by the process of secure co-precipitation method. On larvae of *Aedes aegypti,* the LC_{50} and LC_{90} values were 13.27μg/ml and 25.36μg/ml respectively. Table 4.1 showed that lethal concentrations of the NPs on the 2nd instar larvae of *Aedes aegypti* were found to be

LC_{50}=6.61mg/l, LC_{90}=12.02mg/l and LC_{99}=19.5mg/l. Figure 4.2.1 showed the probit of mortality to be linear and $R^2<1$ that showed that the results are significant than previous study.

In various studies, NPs proved as a potential larvicidal agent for many types of viruses. Under both storage and laboratory conditions, NPs of Zn, SiO_2 and SiO_2/Zn composite NPs emerged as effective way for controlling the spread of *Aedes aegypti* infection [38,47]. So, present results of the study also support the evidence for efficacy of especially ZnO NPs against 2nd instar of *Aedes aegypti* with 99% mortality at 0.002% concentration of nanoparticles. This study also highlights the Zn, Si, and Si/Zn composite NPs as an effective alternative to chemically synthesized materials. This study will also provide baseline information required for further detailed study.

CONCLUSION

The present study investigated the evaluation of Silica, Zinc and Silica/Zinc composite nanoparticles against mosquito vector control i.e., 2^{nd} instar *Aedes aegypti* larvae. For this purpose, Silica, Zinc and Silica/Zinc composite NPs were prepared through green synthesized approach with the help of *Azadirachta indica* (neem leaves) extract. Green synthesized NPs were characterized through SEM, FTIR analysis. Different doses of these green synthesized nanoparticles were applied on larvae. Bioassay results have shown that Zn NPs proved more effective as compared to Silica, and Silica/Zinc composite nanoparticles. Results demonstrated high potency of Zn NPs against 2^{nd} instar *A. aegypti* larvae. Zn NPs gave a great larvicidal effect against *A. aegypti* larvae. Mortality data was subjected to probit analysis to determine the LC_{50}, $LC_{90,}$ and LC_{99} values. Although, larvae treated with all the nanoparticles showed probit of mortality to be linear and $R^2 <1$. But green synthesized Zn NPs showed more significant results as compared to Silica, and Silica/Zinc composites nanoparticles. The study confirmed that green synthesized nanoparticles proved to be a safe and useful technology for mosquito vector control.

RECOMMENDATIONS

The recommendations are as follows:

- Further investigate the effects of green synthesized nanoparticles on selected mosquito vector by conducting large-scale randomized controlled trial.
- Identify potential areas for future research based on the gaps in current literature and findings of the study.
- Conduct a replication study to validate the results of this study and further establish the generalizability of the findings.
- After validation of the results, apply the study on a larger commercial scale.
- By following these approaches, this would ultimately help to control the growth of selected mosquito vector on a larger scale.

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