



EFFICACY OF BIMETALLIC COPPER-ZINC NANOPARTICLES AGAINST LARVAE OF MICROFILARIAE VECTOR IN LABORATORY

Zoology

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ABSTRACT

The present study was conducted to test the larvicidal efficacy of *Azadirachta indica* (Neem) leaf extract mediated synthesis of bimetallic nanoparticles. It is to control the mosquito vector population. The quantity of both precursor agents to synthesize bimetallic nanoparticles was assigned half in amount as designated for the synthesis of each monometallic nanoparticles forms. Characterization was performed via Microscan spectrophotometer and photomicrographs were obtained through TEM. Efficacy was conducted on 3rd instar larvae of filariasis vector *Culex quinquefasciatus* (Say). Characterization results supported the formation of nanoparticles in the solution. Lethal concentration (LC50) was recorded and found effective against selected mosquito larvae. For copper nanoparticles the LC50 for 3rd instar larvae after 24h was recorded at 7% of the test concentration, after 48h it was at 3.5% and after 72h it was at 2%. However, the selected concentration of prepared zinc nanoparticles was not found effective against the selected larvae. For copper-zinc bimetallic nanoparticles the LC50 for 3rd instar larvae after 24h was recorded at 7%, after 48h it was at 5% and after 72h it was at 4%. Conclusively, the efficacy of bimetallic nanoparticles showed drastic improvement over monometallic forms, no potential larvicidal activity of zinc nanoparticle test concentrations was observed at selected concentration range, but in combination with copper nanoparticles in an equal proportion i.e. half of the monometallic forms, were found efficient in larvicidal activity when compared with the activity of copper nanoparticles. This could be a possible new strategy to control filariasis in tropical countries where filariasis is abundant.

KEYWORDS

Efficacy; *Culex quinquefasciatus*; Bimetallic nanoparticles; Vector control; Filariasis

INTRODUCTION

Female mosquito *Culex quinquefasciatus* serves as the mechanical vector for many pathogenic agents responsible for disquieting diseases like Japanese encephalitis, Lymphatic filariasis, and West Nile fever [1]. Filariasis is one of the neglected tropical diseases (NTD), according to WHO over 1.23 billion people in 58 countries worldwide are threatened by the disease [2]. According to National Vector Borne Disease Control Programme (NVBDCP, India), Lymphatic filariasis is still a major public health problem in India after malaria and the indigenous cases have been reported from about 250 districts in 20 states/Union Territories. The two main parasites responsible for this disease in India are *Wuchereria bancrofti* and *Brugia malayi* [3]. Some of the basic strategies to control mosquito population employ primarily the use of synthetic chemical insecticides and secondary measures such as environmental management and biological control methods [4-6]. The use of synthetic chemical insecticides is potentially detrimental to public health and environment, as it affects non-target organisms which are responsible for biological control of mosquito resulting in the reappearance of insecticide-resistant vector population [7, 8]. Many insecticide resistant mosquito species were reported from 53 countries between the year 2010 and 2015, these mosquitoes were resistant to at least one class of synthetic chemical insecticide, therefore, threatening efficient vector control strategies through the chemical method and hampering the lowered mortality rate maintained by chemical control through the establishment of resistant species [9, 10]. New strategies to tackle the resurgence in vector-borne disease and thereby overall mortality rate in susceptible countries, led to an appeal for development of new tools to efficiently control vector population among these tools the use of nanoparticles is one [11-13]. Extensive research to check efficacies of different metal nanoparticles against insect vectors revealed the potency of nanoparticles in vector control. Several nanoparticles such as silver, gold, palladium, copper, zinc, silica and carbon nanoparticles, exemplified to be the best candidate for mosquito control [14-20]. Nanoparticles can be synthesized with the help of natural reducing and capping agents of biological nature, such as phytochemical extracts and other metabolites obtained from plants, fungi, algae, and microbes thus providing ecological soundness unlike synthetic chemicals [21-24]. New properties can also be studied with the induced changes in the geometry of nanoparticles with the help of LASER lights during synthesis [25]. Synthesis of a new class of material over the

monometallic nanoparticles i.e. bimetallic nanoparticles may have unique catalytic properties over their monometallic forms [26-28]. The best combination of bimetallic nanoparticle formulation can effectively control mosquito vector population. In our study, we are reporting the efficacy of bimetallic copper-zinc nanoparticles against *Culex quinquefasciatus* mosquito larvae taking a step towards efficient vector control strategy for filariasis in tropical countries.

MATERIAL AND METHODS

Material

Fresh leaves of *Azadirachta indica* (Neem) were collected from the botanical garden of Dayalbagh Educational Institute, Agra, India. Zinc Nitrate Hexahydrate Purified was purchased from LOBA chemie and Copper (II) sulphate pentahydrate Pure (cupric sulphate) was purchased from Merk. Larvae of *Culex quinquefasciatus* were collected from the local waterbody in Dayalbagh location, Agra, India, and identified by an insect taxonomist in the department of zoology, D.E.I., Agra. Triple deionized water was used in the preparation of nanoparticles.

Preparation of Aqueous Extract

Fresh leaves of *Azadirachta indica*, were washed and air dried. These leaves were then finely chopped and 15 g were transferred in 250-ml flask containing 100 ml of triple deionized water to make 15% solution. The preparation was heated on the hot plate at 70C for 1h, and the resulting crude leaf extract was filtered using whatman-1 filter paper and the final filtrate was then stored at 4C.

Synthesis of Nanoparticles

Bimetallic nanoparticle solution was synthesized by mixing Copper Sulphate (20 mM) and Zinc Nitrate (20 mM) in a 250-ml flask containing 90 ml of triple deionized water, to this molar solution 10 ml of prepared aqueous extract of *Azadirachta indica* was added, to make the volume up to 100 ml in 9:1 ratio. Monometallic nanoparticles were synthesized in a similar manner with Copper Sulphate (40 mM) and Zinc Nitrate (40 mM). After adding leaf extract these preparations were left at room temperature in dark for overnight.

Characterization of Nanoparticles

Absorption spectra were recorded using Microscan reader spectrophotometer model no. Microscan MS5608A. Plant extract,

triple deionized water, and air were set as blank. The micrographs of copper-zinc nanoparticles, copper nanoparticles, and zinc nanoparticles were obtained by Transmission Electron Microscope (TEM) using TECNAI 200 KV, for this samples of monometallic and bimetallic nanoparticles were prepared on copper grids.

Mosquito Larvicidal Bioassay

Larvicidal efficacy of prepared bimetallic and monometallic nanoparticles was tested against larvae of mosquito *Culex quinquefasciatus*. The test was conducted as per W.H.O.'s guidelines for laboratory testing of mosquito larvicides [29]. 3rd instar larvae were selected for bioassay. Different test concentrations (3%, 4%, 5%, 6%, 7%, 8%, and 9%) were prepared for both mono- & bi-metallic nanoparticles in distilled water making volume up to 100ml. The test was conducted in two replicates. Twenty 3rd instars of *Culex quinquefasciatus* were added in each 100ml of test concentration. A common positive control was set in replicates containing 5ml plant extract and negative control was also set in replicates containing distilled water. Mortality was recorded after 24h, 48h, and 72h.

Data Management and Statistical Analysis

Obtained data on efficacy testing was subjected to corrected %mortality, using Abbott's formula, when the observed control mortality was more than 5%. (Abbott, 1925) [30]. The mean corrected percentage mortality was subjected to probit analysis (Finney, 1971) [31]. The relationship between probit of kill and concentrations was established, probit regression lines and probit equations were obtained from which value of LC50 was calculated.

RESULTS

Visible Color Change

Visible analysis of nanoparticles showed a change in color of the molar solution after the addition of aqueous leaf extract of *Azadirachta indica*, indicating the formation of nanoparticles. Copper Nanoparticles (Cu NPs.) and Copper-Zinc Nanoparticles (Cu-Zn NPs.) appeared greenish in color and Zinc Nanoparticles (Zn NPs.) appeared yellowish in color (Fig. 1).



Figure 1: Synthesized Zn NPs. Cu NPs., and Cu-Zn NPs. (from left to right)

Microscan Spectroscopy

Absorption spectra of prepared nanoparticles obtained through Microscan plate reader (Fig. 2), for monometallic copper nanoparticles the highest absorption was observed to be centered around 390nm, for monometallic zinc nanoparticles it was observed to be centered around 400nm, and for bimetallic copper-zinc nanoparticles observed at 400nm and another bump in absorption was observed around 550nm.

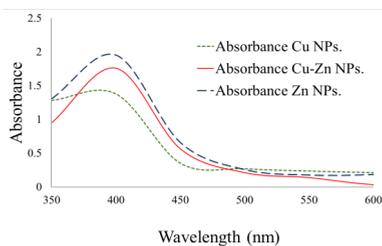


Figure 2: Microscan Spectroscopy Results

Transmission Electron Microscope (TEM)

Results of TEM micrographs showed the formation of poly-crystalline monometallic copper nanoparticles ranging in 20nm – 30nm in size.

The micrographs of synthesized monometallic zinc nanoparticles showed amorphous characteristics and the particle size observed between 40nm-100nm. The poly-crystalline bimetallic copper - zinc nanoparticles appeared in complex geometry and the particle size observed up to 50nm (Fig. 3-5).

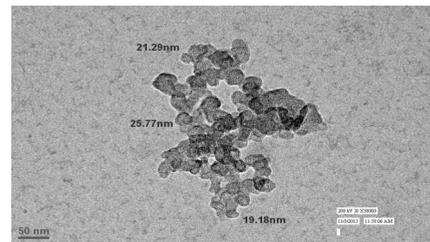


Figure 3: TEM photo-micrograph of monometallic copper nanoparticles

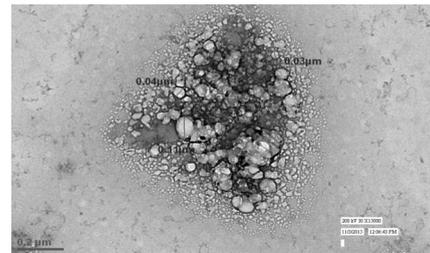


Figure 4: TEM photo-micrograph of monometallic zinc nanoparticles

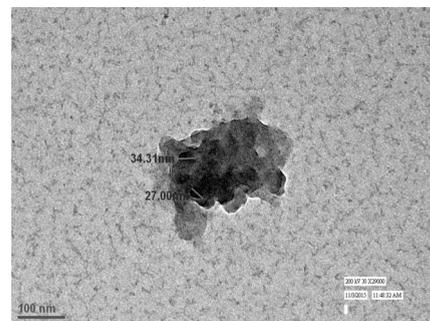


Figure 5: TEM photo-micrograph of bimetallic copper-zinc nanoparticles

Bioassay

Efficacy testing results of different test concentrations of prepared nanoparticles on 3rd instar larvae of *Culex quinquefasciatus*, were recorded after 24h, 48h and 72h (Table 1). For test concentrations containing monometallic copper nanoparticles the LC50 where 50% mortality was achieved at 7% after 24h, 3.5% after 48h and 2% after 72h. For monometallic zinc nanoparticles test concentrations, the data showed insignificant results suggesting the non-potential activity of synthesized nanoparticles concentrations. For bimetallic copper-zinc nanoparticle test concentration LC50 was recorded at 7% test concentration same as copper nanoparticles but since the molarity of copper in bimetallic solution is less than its molarity in monometallic solution, hence it showed increased potential activity as compared to the monometallic forms. However, this efficacy has been decreased after 48h and 72 h. No mortality was observed in positive and negative control with 5ml aqueous leaf extract and distilled water after 72h.

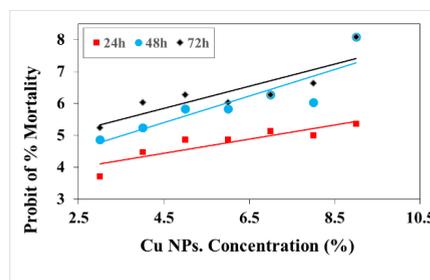


Figure 6: Probit analysis of Cu NPs. test concentrations

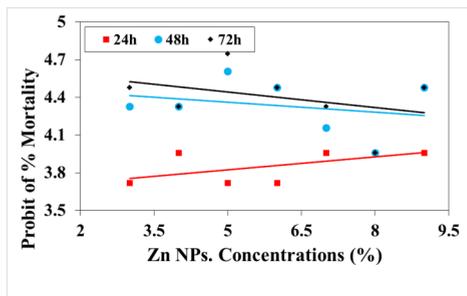


Figure 7: Probit analysis of Zn NPs. test concentrations

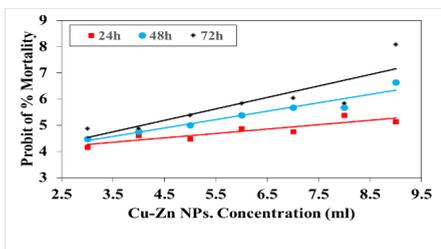


Figure 8: Probit analysis of Cu-Zn NPs. test concentrations

TABLE – 1 EFFICACY RESULTS AFTER 24h

NPs.	Probit equation	R2	LC50 (%)a
Cu NPs.	24h	$y = 0.2221x + 3.4429$	0.795 7
	48h	$y = 0.4171x + 3.5271$	0.769 3.5
	72h	$y = 0.3471x + 4.2914$	0.746 2
Zn NPs.	24h	$y = 0.0343x + 3.6514$	0.333 NS*
	48h	$y = -0.0264x + 4.4943$	0.067 NS*
	72h	$y = -0.0414x + 4.65$	0.139 NS*
Cu-Zn NPs.	24h	$y = 0.1693x + 3.7543$	0.797 7
	48h	$y = 0.3211x + 3.445$	0.929 5
	72h	$y = 0.4375x + 3.2236$	0.745 4

(%)a – percentage of the NPs. in the solution; NS* - Result Not significant

DISCUSSION

In our study, we have synthesized bimetallic copper-zinc nanoparticles and monometallic copper and zinc nanoparticles. Reduction of precursor agent into nanoparticles in the solution was first estimated by a visible color change, later analyzed by Microscan spectrophotometer. After assigning three blanks i.e. aqueous leaf extract, deionized water, and air, the formation of nanoparticles in the solution was confirmed with the presence of absorption spectra. Photomicrographs were obtained through an electron microscope (TEM) further confirmed the formation of polycrystalline and amorphous nanoparticles in the solution. The positive control containing 5 ml of aqueous leaf extract of Azadirachta indica, has no larvicidal efficacy, hence the efficacy recorded in the test concentrations was due to nanoparticles. Test concentrations of zinc nanoparticles were found non-effective at the selected range for the prepared concentration, while for the same concentration 50-90% mortality was observed in copper nanoparticle and copper-zinc nanoparticle test concentrations. As only half of the respective precursor agents were added in bimetallic formulation, it was proved to be effective over monometallic copper and zinc nanoparticles efficacies, where highest set concentration of monometallic zinc nanoparticle could not reach 50 % mortality, but bimetallic form containing half of each quantity was able to give an approximate result yielding 50-90% mortality after 24 h in filariasis vector larvae. Extracts of different part of plant, fungi, and microbes which alone have the potential larvicidal efficacy can be used for nanoparticles

synthesis which could possibly increase the insecticidal effectiveness of such prepared larvicide. Soni and Prakash (2012a), synthesized silver and gold nanoparticles from an insecticidal pathogenic fungus Chrysosporium tropicum that has higher larvicidal efficacy in lower concentration after 1 hour [32]. Soni and Prakash (2012b), synthesized silver nanoparticles with the help of mosquito adulticidal fungus Chrysosporium keratinophilum, Verticillium lecanii, and Fusarium oxysporum f.sp. pisi, and evaluated its adulticidal efficacy on adults of Culex quinquefasciatus [33]. Giovanni Benelli (2015) reviewed the problem of mosquito vector borne diseases, biosynthesis of nanoparticles and their use as larvicides and adulticides for mosquito vector, and explored that more inputs in the field of vector control through nanoparticles are from India and USA [34]. Minal and Prakash (2016) synthesized copper-zinc bimetallic nanoparticles with the help of leaf extract of Ocimum sanctum (Linn.) and tested its efficacy against 3rd instar larvae of Anopheles stephensi [35].

We have reported the use of bimetallic nanoparticles in mosquito control nano-formulations, as the effectiveness of bimetallic nanoparticles has so far not been investigated for mosquito control. As for the effectiveness of nanogold and nanosilver being found highly effective, however, our attempt was to ensure the use of cost-effective novel formulation for mosquito control. Therefore, specific experimental investigation with the dual mode of bimetallic nanoparticle formulation may be a solution to the problem in specific cases of insecticide-resistant mosquito species where the biological and chemical control are less effective against third instar larvae of mosquito, however the real state problem in water reservoir is to tackle the third instars which are ecologically more tolerant and difficult to control. Therefore, we have selected and tested a new larvicide of bimetallic nature and it appears that the bimetallic nanoparticles could enhance efficacy by providing two kinds of interfaces and can have better properties as larvicides.

CONCLUSION

In the present investigation bimetallic copper-zinc nanoparticles, and monometallic copper and zinc nanoparticles were synthesized using aqueous leaf extract of Azadirachta indica, commonly known as 'Neem' tree. We have recorded that bimetallic copper-zinc nanoparticles differ in their effectiveness from their monometallic forms and can also be used for controlling mosquito vector at specific developmental stages particularly the 3rd instar larval stage in the life cycle of Culex quinquefasciatus (Say). Prepared bimetallic copper-zinc nanoparticle formulation was found effective against the 3rd instars of Culex quinquefasciatus (Say) and can be used as mosquito larvicidal agent. The future study aimed to find out other combination of metal precursor agents for synthesizing bimetallic or multi-metallic nanoparticles to find a potentially effective couple with higher efficacy, than their respective monometallic forms, and also in combination of extracts with potential larvicidal efficacy, and the effect of synthesized metal nanoparticles on non-target organisms.

ACKNOWLEDGMENT

We sincerely express our gratitude to Prof. P. S. Satsangi Sahab, Chairman of the Advisory Committee on Education, Dayalbagh Educational Institute, Agra, India. We are thankful to Prof. P.K. Kalra, Director, Dayalbagh Educational Institute, Agra, India. We are also grateful to Dr. Shashi Wadhawa and S.A.I.F. staff for TEM facility (AIIMS, New Delhi, India).

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