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BIOSYNTHESIS OF SILICA NANOPARTICLES FROM *SACCHARUM OFFICINARIUM* BAGASSE AND ITS EFFICACY AGAINST *CULEX QUIQUEFASICATUS* (SAY) LARVAE.

Zoology

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ABSTRACT

Major vector borne diseases are prevalent in tropical and subtropical parts of the world. Mosquitoes are the major vectors immensely creating health problems to mankind cattle as they carry several pathogens. In view of public health, environmental care and insecticide resistance, appropriate actions must be opted to reduce the growth of mosquitoes. A biosynthesized nanoparticles have potency against the target organisms without effecting the environment. In our present study we have biosynthesized ecofriendly and cost effective silica nanoparticles from non- edible plant waste of Saccharum officinarium, followed by characterization and bioassays. The formation of Silica nanoparticles were characterized and confirmed by Transmission Electron microscope (TEM), UV-Vis spectrophotometry, and X-Ray diffraction (XRD) analysis. Efficacy of biosynthesized Silica Nanoparticles was performed against the 2nd and 3rd Instar larvae of Culex quinquefasicatus and mortality responses were recorded after an exposure of 24h and 48h. The Lethal Concentration (LC50) was calculated by probit analysis. LC50 values for 2nd instars was 4.4 mg/ml after 24h and 3.8 mg/ml after 48 h. The synthesized silica nanoparticles showed effective larvicidal properties and thus can be used to control the mosquito population,especially Filaria and viral vector Culex quinquefasicatus.

This study is performed to specifically generate the efficacy from a hydrophobic Silica nanoparticles which can be used for many vectors globally.

KEYWORDS

Mosquito, *Culex quinquefasicatus*, Sugarcane bagasse, Silica nanoparticles.

INTRODUCTION

WHO in 2017 reported that more than 17% of infectious diseases are vector borne diseases, causing more than 7, 00, 000 annual deaths. Out of several pathogen carrying vectors like ticks, flies, bugs, and snails mosquitoes are the most common and potential vectors for spreading infectious diseases. Many infectious diseases like Malaria, Dengue, and Chikungunia, Lymphatic filariasis, caused by different species of mosquito. Lymphatic filariasis is a neglected tropical disease spreads by *Culex* species. Globally more than 1.3 million people are at risk. Many of these diseases are preventable only by controlling the disease spreading vectors. Larvicides have the potential to destroy the mosquito larvae in their breeding sites. Unfortunately, insecticide-resistant mosquitoes have been reported by WHO across many regions due to which the insecticides of chemical and biological origin are not as effective as they were before. Hence, it is necessary to develop new tools and technologies for the control of mosquito vector population and nanotechnology is one among them.

Plant and fungi mediated biosynthesis of nanoparticles showed a potential parasitological and mosquitocidal properties. (Giovanni *et al*., 2016). Biosynthesized metal nanoparticles like gold, silver, and zinc have previously showed potential larvicidal properties against mosquito species. Nanoparticles synthesized from fungus and its metabolites are very good potential larvicides against mosquito species. (Soni and Prakash, 2011, 2012, 2013) respectively. Silica nanoparticles have different applications in science. Porous hollow silica nanoparticles as delivery system for water soluble pesticides (FLiu *et a*l., 2006). Dispersible silica nanoparticles as carrier to enhance the pesticidial activity (Mei-rong song et al., 2012). Amorphous Silica nanoparticles have entmotoxic and insecticidal effects and found to be highly effective in controlling pests (Debanth *et al.,* 2011; Barik *et al*., 2012; Ziaee and Ganji 2016). It has been shown that Silica nanoparticles like other metallic nanoparticles show diverse effects as an effective pesticide. But the biosynthesized nanoparticles have great importance in vector control. As Silica is abundant element on earth and highly found in plant skeleton and the various natural sources from which it can be extracted by sequential procedures. (Thaudaij and Nuntiya 2008; Qisti *et a*l., 2016). Therefore, in our present study we have attempted to biosynthesize the silica nanoparticles from non- edible plant waste of *Saccharum officinarium* by following the precipitation method and the larvicidal potency against *Culex quinquefasicatus* Say was assessed.

1 MATERIALS AND METHODS

1.1 Collection of material

Sugarcane bagasse (*Saccharum officinarium*) were collected from the Dairy campus of Dayalbagh Educational Institute. (D.E.I). 2nd and 3rd instar larvae of *Culex quinquefasicatus* were collected from botanical garden of D.E.I campus.

1.2 Preparation and synthesis of Silica Nanoparticles

The synthesis of silica nanoparticles was done by the precipitation method. Sugarcane bagasse samples were chopped down into small pieces. 5 grams of the sample was dissolved in 3.0 N 150 ml of sodium hydroxide solution in an Erlenmeyer flask and boiled at 100°C for 3h on hot plate. After 3h the solution was cooled down and filtered into another flask using whatman filter paper. The pH of the filtrate was adjusted to pH 3 by adding 5N sulphuric acid and after the solution reached the pH 3 then the solution was further changed to pH 8.25 by adding ammonium hydroxide solution and the solution was stored in room temperature for over-night. The filtrate solution was dried in an oven for 4h per day for 3 days. The dried filtrate was transferred into a round bottomed flask containing 250 ml of 6 N HCL and further refluxed for 4h on a heating mantle at the temperature of 100°c. The particles formed from refluxing process were again filtered and the resulting residue was washed with distilled water repeatedly until the solution was acid free and allowed to dry on the Whatman filter paper. The resulting acid free residue was again dissolved in 100ml of 1N sodium hydroxide solution in a 250 ml glass beaker and was stirred on magnetic stirrer at 500 rpms for 5h per day for 2 days. The resulting solution was adjusted to pH 6.2 by adding sulphuric acid drop-wise which resulted in the precipitate formation. The precipitant was filtered and washed with distilled water until it was alkali free. The washed precipitant was allowed to dry for 1 day at room temperature and then dried powder of nanosilica was collected into a clean petridish.

1.3 Characterization of Silica Nanoparticles

A sample of silica nanoparticles diluted in deionized water and characterized through UV-Vis spectroscopy using UV-1800 Shimadzu UV-Vis spectrophotometer (Fig.2). A finely grinded moisture free powder was dispersed on 1cm x 1cm sq. glass slide to perform XRD analysis using D8 Advance Bruker Diffractometer (Fig.3).A small amount of sample dispersed with deionized water sonicated further subjected to TEM analysis using Technai G 20 (FEI) TEM (Fig.1).

1.4 Larvicidal bioassay

The bioassay of biosynthesized Silica nanoparticles was performed against 2nd and 3rd instar larvae of *Culex quinquefasicatus* based on standard WHO, (2005) protocols. Mixed population of collected larvae which were kept in the beakers contained distilled water were separated instar wise. Experimental set up was made where beakers containing 100ml of distilled water were arranged in replicates. Selected 20 larvae of both instars separately placed each beaker containing 100ml of distilled water which were arranged earlier. Treated test concentrations ranges from 0.5µl to 3 µl. The mortality of larvae were recorded after time interval of 24 and 48h respectively. The percent mortality and LC_{50} values were calculated using Finney's table.

2 RESULTS

2.1 Characterization

The TEM results showed polyamorphous nanosilica agglomerates (fig. 1), which are globular and spherical in shape and measured in the range of 200 to 500 nm. The UV-Vis spectra showed the highest absorption of biosynthesized Silica nanoparticles (fig. 2) at 282 nm. Diffractogram (fig. 3) obtained from XRD analysis of Nanosilica showed broad peaks between 20° and 25°(2), suggested the characteristic of amorphous SiO_2 nature of prepared silica nanoparticles.

2.2 Efficacy results

The LC_{50} values (Table-1) obtained from probit analysis after 24h and 48h of exposure of test concentrations. LC_{50} after 24h for 2nd instars was observed at 4.4 mg/ml and LC_{50} after 48 h was observed at 3.8 mg/ml. LC_{50} for 3rd Instar larvae after 24h was observed at 6.3 mg/ml and LC_{50} after 48 h was observed at 10.6 mg/ml. An increased % mortality with the increasing concentrations were observed for both 2nd and 3rd instar larvae.

3 DISCUSSIONS

While conducting the study of biosynthesized Silica Nanoparticles from *Sacchrum officinarium* bagasse and testing their efficacy against mosquito larvae of *Culex quiquefasicatus*. We found that Barik et.al (2012) for the first time reported the effectiveness of purchased custom-made 99 % silica nanoparticles as larvicidal, pupicidal, growth inhibition, ovi-deterrence activity against *Anopheles stephensi, Aedes aegpti, and Culex quiquefasicatus.* They have reported that the hydrophobic nature of nanosilica was most effective than other forms. The larvicidal activity of hydrophobic nanosilica on the mosquito species was in the order of ***Anopheles stephensi>Aedes aegypti>Culex quinquefasciatus.*** Debnath *et al., (*2011) found that nanosilica particles shows the entomotoxic effects against rice weevil *Sitophilus oryzae.* Diatomaceous earth used as source for the formation of amorphous Silica nanoparticles particles and found to be highly effective against the rice weevil.

Ziaee and Ganji (2016) evaluated the insecticidal efficacy of nanosilica against *R. dominica* and *T. confusum* adults on wheat and barley, and to assess initial and delay mortality of adults after a post-treatment period with or without food.

As Silica is one of the most abundant element in nature with various applications. In our present study we have conducted investigation on biosynthesized nanosilica. The biosynthesized nanosilica of our study is cost-effective. It encourages recycling of plant waste to synthesis low cost silica nanopartilces. We have bio synthesized the polyamorphous nanosilica from *Sacchrum officinarium* bagasse by precipitation method which was characterized through TEM, XRD and UV-Vis spectrophotometry analysis. Nanosilica particles observed were globular and spherical in geometry of 200 - 500 nm.

We have conducted the efficacy study against 2nd and 3rd instar larvae of *Culex quinquefasciatus* (Say) treated with biosynthesized polyamorphous nanosilica and observed mortality at 24h and 48h of time interval. The LC_{50} response exceeds the time interval as well as the tested concentrations.

Significantly we have observed cuticular desiccation of dead larvae during the mortality count, which is the notable features of uptake of Nanosilica reported by Tiwari and Behari (2009). Therefore, it appears to be a novel attempt to find out the efficacy of these nanoparticles on major vectors in India. Since it is found everywhere mainly marine, aquatic and terrestrial ecosystem,it can further be tested in fields also.

4 CONCLUSIONS

It has been concluded from our investigation that Silica nanoparticles from *Saccharum officinarium* showed potent efficacy against 2nd and 3rd instar larvae of *Culex quinquefasicatus* Say. The calculated LC_{50} values showed that the larvicidal activity was increased slowly with the increase in exposure time of the test concentration. The concentrations of LC_{50} decreased in 48h exposure time for 2nd instar larvae as compare to 24h. In future, we recommended further studies which can be conducted to find the efficacy of these nanoparticles on major vectors causing dreadful diseases. It can be a good model for vector control in rural, urban, aquatic, terrestrial environment.

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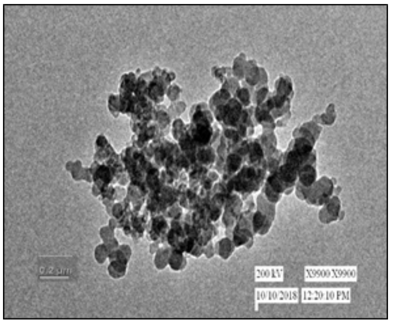

Fig. 1 TEM images of Silica nanoparticles

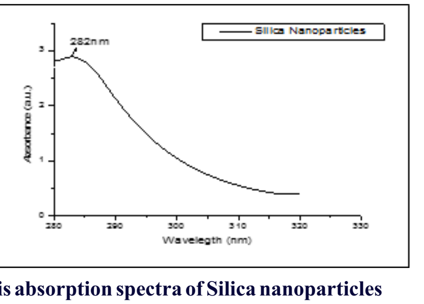

Fig.2 UV-Vis absorption spectra of Silica nanoparticles

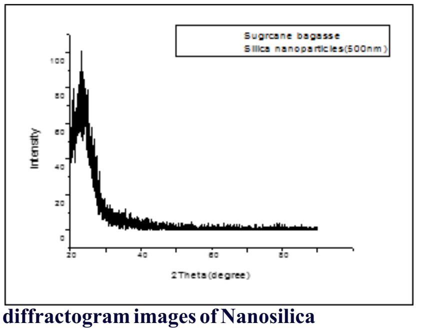

Fig.3 XRD diffractogram images of Nanosilica

Table 1: The LC50 values of 2nd and 3rd instar larvae of *Culex quinquifasicatus* after exposure of 24h and 48h of time interval.

Time	2nd Instar Probit equation (y)	R^2	LC_{50} (mg/ml)	3rd Instar Probit equation (y)	R^2	LC_{50} (mg/ml)
24h	y =0.2636x+ 3.8318	0.4795	4.4	y= 0.1502x+ 4.0462	0.2173	6.3
48h	y =0.234x+ 4.1048	0.5215	3.8	y= 0.0462x+ 4.5071	0.0228	10.6

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