Journal of Applied and Advanced Research 2017, 2(3): 179–183
http://dx.doi.org/10.21839/jaar.2017.v2i3.81
http://www.phoenixpub.org/journals/index.php/jaar
ISSN 2519-9412 / © 2017 Phoenix Research Publishers

Research Article – Biotechnology

Chitosan mediated nanoparticles from *Saccharomyces cerevisiae* and its mosquito larvicidal activity

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Abstract

Mosquitoes are one of the most medically significant groups of vectors, having an ability to transmit parasites and pathogens that can have devastating impacts on humans. In this study, chitosan nanoparticles were synthesized from chitosan polymer by ionic gelation method. The chitin was first extracted from *Saccharomyces cerevisiae* and then deacetylated to chitosan. Silver nanopartcals were also prepared and the presence and characterization was investigated by scanning electron microscopy (SEM). The comparative study of the larvicidal activity of chitosan nanoparticles and silvers was also studied which shows chitosan nanoparticles started mortality at higher concentrations, it showed uniform rise in mortality of mosquito larvae than silver nanoparticles.

Key words: Chitosan, Nanoparticles, *Saccharomyces cerevisiae,* SEM, Food preservative

Introduction

Nanotechnology is the one of the most promising and new areas research in modern science. As nanoparticle posses new and improved properties of material which is mainly based on size, shape, distribution and morphology than large particles from which the nanoparticles are made [71]. Mosquitoes are the principal vectors of many vector-borne diseases affecting human beings and animals, in addition to being nuisance pests. Vector-borne diseases in India, e.g., malaria, dengue, chikungunya, filariasis, Japanese encephalitis, and leishmaniasis, cause thousands of deaths per year. India reports 1.48 million malarial cases and about 1173 deaths, 1.4 million suspected and 1985 confirmed chikungunya cases, 5000 Japanese encephalitis cases and approximately 1000 deaths, and 383 dengue cases and 6 deaths during 2006 and 2007 (Kumar *et al.,* 2007; WHO, 2007; Gopalan & Das, 2009; Dhiman *et al.,* 2010). India had 1.5 million confirmed malaria cases in 2009 with over 1000 deaths (WHO, 2010). Anopheles stephensi is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed (Burfield & Reekie, 2005; Mittal *et al.,* 2005). India is endemic to mosquito-borne diseases due to favorable ecological conditions. Aedes albopictus, a vector for the transmission of many viral pathogens poses serious threat to human health and has proven to be very difficult to control due to their remarkable ability to adapt to various environments, their close contact with humans, and their reproductive biology.

However, significant problems exist with all of these. Silver nanoparticles are emerging as one of the fastest growing materials due to their unique physical, chemical and biological properties; small size and high specific surface area (WHO, 1999). Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis. Chitin is a characteristic compound found in fungi and some animals. Chitosan acts as water binding agent and inhibits various enzymes. It inhibits bacterial activity by

Received: 01-05-2017; Accepted 10-06-2017; Published Online 12-06-2017

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inhibiting RNA and protein synthesis (El-Diasty, *et al*., 2012). Various factors play role in antimicrobial activity of chitosan.

Till date, no report has present about bio-synthesis of NPs utilizing Chitason of Yeast and their Mosquito Larvicidal Activity. The use of environmentally benign materials such as nano-particles offers numerous benefits of eco-friendliness and compatibility for larvicidal application. In these circumstances, an improvised method using the biologically synthesized nanoparticles using Chitosan form yeast was evaluated for the destruction of the mosquito larvae.

Materials and Methods

Materials

Baker'syeast (*Saccharomyces cerevisiae*) was purchased from local market (Mumbai, India). All other chemicals and medium used were of analytical grade.

Isolation of chitosan from yeast cells

Dry yeasts were activated by adding them into warm water for 5 mins. The froth indicated that yeast cells were in active condition. An active yeast cells were suspended in 1 mol l M NaOH solution (1 : 30 w/v) and autoclaved at 121°C for 15 min. Alkali-insoluble fractions were collected after centrifugation at 12 000 g for 15 min, washed with distilled water and recentrifuge to a neutral pH (pH 7). Further extracted the residues using 2% acetic acid (1 : 40 w/v) at 95°C for 8 h. Centrifuged the extract slurry at 12 000 g for 15 min and insoluble acid were discarded.

The pH of supernatant fluid were adjusted to 10 with 2 M NaOH, the solution was centrifuged at 12 000 g for 15 min and washed the precipitated chitosan with distilled water, 95% ethanol (1:20 w/v) and acetone (1: 20 w/v), respectively and dried at 60°C to a constant weight.

Determination of chitosan percentage yield

The percentage yield of chitin was determined by taking the dry weight of *Saccharomyces cerevisiae* before treatment and the dry weight of prepared chitosan in percentage. The percentage yield was calculated from the weight of chitosan obtained as a percentage of chitin before deacetylation.

Synthesis of Chitosan Nanoparticles

Chitosan solution was prepared of 2.5 mg/ml by dissolving the polymer in 1% (w/v) acetic acid aqueous solution for 0.5 hrs under magnetic stirring. The pH of solution was adjusted to 5.0-6.0 using 1 mol/L NAOH. Chitosan solution was stirred for 0.5 hr at room temperature. Finally, dissolved sodium tripolyphosphate (TPP), the counter ion in pure water to prepare a 1 mg/ml solution, added in to the chitosan solution under mild magnetic stirring to form chitosan nanoparticles. Centrifuged the nanoparticles solution at 18000 rpm and 4°C for 30 minutes, after which the nanoparticles were collected at the bottom, extensively washed 3 times with water to remove the TPP and the acetic acid, and finally lyophilized and Stored at 4°C- 8°C (Yu-Lan H *et al.,* 2011).

Characterization of Chitosan Nanoparticles

Chitosan nanoparticles were characterized by SEM (Scanning Electron Microscopy) by Philips XLD 3D model, CIRCOT, Matunga East, Mumbai, to examine the particle size and surface morphology. Where CSNPs are coated with gold metals film and magnified under 15000X.

Synthesis of AgNps

The *Saccharomyces cerevisiae* was grown in 250-mL Erlenmeyer flasks containing 100 ml Nutrient broth at 37° C and 150 rpm for 24 hours. After incubation, biomass was separated by centrifugation and washed with sterile distilled water to remove the traces of media components, and, challenged with AgNO3 solution (1 mM). Incubate the solution for 48 hr and after that centrifuged the solution and separate out the biomass and supernatant solution.

Characterization of Ag-NPs

After 48 hours of incubation of the above mixture, the preliminary detection of Ag-NPs was carried out by visual observation of color change of the cell filtrate. These samples were later subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Shimadzu 1650 PC) and scanning the spectra between 430 nm at the resolution of 1 nm. A Transmission electron microscopy (SEM) was used to record the micrograph images of synthesized Ag-NPs. The silver nanoparticles synthesized showed sharp adsorption peak at 430 nm and which is the characteristic property of surface plasmon resonance of the silver nanoparticles.

Mosquito larvicidal activity of Chitosan nanoparticles and silver nanoparticles

Mosquito larvae were collected from the stagnant water and maintained under laboratory conditions for 48 hours. During this time larvae were fed with yeast extract suspension (1%) in water. For the experiment, 3rd and 4th instar larvae of *Culexpipens fatigans* were used. Chitason nanoparticles were dissolved in water and Tween 80 for preparing the test solutions.

Irrespective of the percentage mortality the test concentrations were ranged between 0.05% and 0.5% pertaining to the objective of collecting preliminary data. A batch of 25 larvae was placed in 500 ml water. Definite concentrations of test material in water in case of silver nanoparticles and Tween 80 saline in case of Chitosan nanoparticles was added to a beaker. The vehicle was used according to the solubility of test material. These beakers were covered with muslin cloth and maintained at room temperature (320C.) away from direct sun light.

The mortality of larvae was observed after 24 hours. The mortality was recorded by combining dead and moribund larvae. The dead larvae were those that could not be induced to move when they were probed with needle. Moribund larvae were those incapable of rising to the surface (with reasonable time or showing characteristics diving reaction when the water was disturbed). The larvae that were pupated during the test were discarded. Two negative controls were maintained, one of the water and other of the vehicle (Tween80) in a similar manner.

Tests with control mortality of 20% or more were unsatisfactory and discarded. All the experiments were carried out in triplicate. The mean value was recorded as mortality after 24 hours. The corrected mortality was calculated by Abbot's formula.

Corrected mortality = Test mortality – control mortality/100 – control mortality X 100.

LC_{50} value was calculated graphically by dosage mortality line.

Results and Discussion

Chitosan synthesis from Saccharomyces cerevisiae

In this study chitosan has been successfully prepared from *Saccharomyces cerevisiae*. The synthesis of chitosan involves various chemical steps. Pretreatment methods were done using 1N NaOH and 2% acetic acid. The alkali and acid treatments remove proteins and minerals from chitin respectively and deacetylates simultaneously. These methods give advantages for obtaining of higher quality chitosan. Chitin is not soluble but chitosan, the deacetylated product of chitin, is soluble in very dilute acids like acetic acid, lactic acid, formic acid etc. the deacetylation experiment using 2 N NaOH was done to reduce acetyl group from molecular structure, because the presence of acetyl group prevents to make the solution of chitosan. 10 gm (dry weight) of *Saccharomyces cerevisiaegives* 0.9 gm chitosan and percentage yield of chitosan is 0.81%.

Preparation and characterization of chitosan nanoparticles

Chitosan nanoparticles can be prepared using many methods such as ionic gelation method, emulsion cross- linking, and spray drying. In this study, ionic gelation method was applied because the method is easy and fast to be carried out. This simple technique involves electrostatic interaction between positively charged amino group of chitosan and negatively charged polyanions. Formation of nanoparticles occurs spontaneously through the formation of intra- and intermolecular cross- linkages under a constant stirring at room temperature (Liang *et al*, 2012).

The chitosan nanoparticles prepared in the experiment exhibit a white powdered shape and are soluble in deionized water. The synthesized chitosan nanoparticles were characterized by scanning electron microscopy. SEM was used for the determination of the particle size and the morpho-logical structure of the prepared polymer matrix. The coating of chitosan nanoparticles were done by gold metal and magnified under 15000X. It was observed that chitosan/ TPP has average particle size of 60-110 nm. Fig.1 shows the size of chitosan nanoparticles. Particle size of chitosan nanoparticles is depending on concentration of chitosan and TPP, their mass ratios, and drying methods. Fig.1 shows the SEM picture of chitosan nanoparticles.

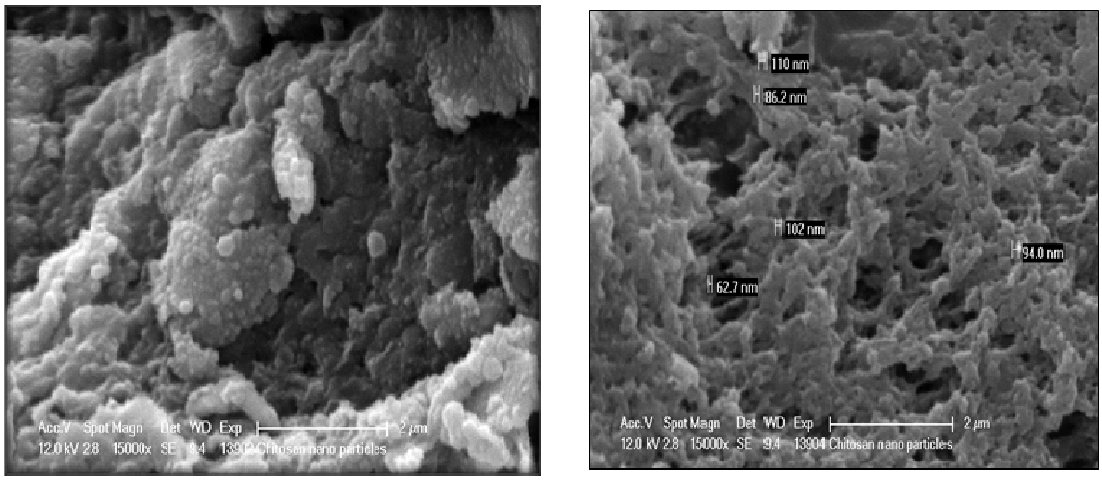

Fig. 1. Scanning electron microscopy photograph of chitosan nanoparticles

Characterization of silver nanoparticles

After UV–Visible spectrophotometer, further characterization was carried out by scanning electron microscopy, transmission electron microscopy and X–ray diffraction pattern generated by transmission electron microscope. On analysis, Spherical silver nanoparticles were observed and the size of silver nanoparticles synthesized by *Saccharomyces cerevisiae* 38–42 nm. The silver nanoparticles were found to be capped by sodium citrate. On X – ray diffraction pattern generated by transmission electron microscope, it was confirmed that the silver nanoparticles are crystalline in nature.

Mosquito larvicidal activity of Chitosan nanoparticles and its comparison with silver nanoparticles

Table 1 and 2 presents the range of mortality in the mosquito larvae due to two different test substances. The dosage mortality lines are drawn on the basis of these observations. LC_{50} value for both the substances was mentioned at the bottom of the tables.

Table 3. Larvicidal activity of silver nanoparticles

% Concentration	Corrected % mortality	
	Silver nanoparticles	Chitosan nanoparticles
0.05	14.28	19.04
0.1	28.57	33.33
0.2	42.85	38.09
0.3	52.38	52.38
0.4	71.32	61.90
0.5	89.0	80.95
LC_{50}	0.21%	0.24%

Silver nanoparticles showed uniform range of mortality with increase in percent concentrations and has the lowest LC_{50} value of 0.21%. In Chitosan nanoparticles though the mortality starts at higher concentrations, it also showed uniform rise in mortality of mosquito larvae. This substance showed 0.24% LC_{50}.

Conclusion

In the present study the Silver nanoparticles and Chitasan nanoprticles were evaluated for mosquito Larvicidal activity. The prospect of utilizing Chitason for synthesizing nanoparticles and testing its efficacy in controlling mosquitoes as larvicides is a recent phenomenon facilitating the development of a more potent and environmentally safe pesticide. Identification of the bioactive principles involved and their mode of action and field trials are necessary to recommend an effective formulation as an anti-mosquito product in control programs

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