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Synthesis, Characterization and Toxicity Analysis Of Silver Nano-Particle Synthesized From Widely Available Indian Xerophyte, *Opuntia Stricta.*

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Abstract:

The field of nanotechnology is one of the most active researches nowadays inmodern material science and technology. Eco friendly methods of green mediatedsynthesis of nanoparticles are the present research in the limb of nanotechnology.Now-a-day in modern sciences, Nanotechnology is a burning field for the researchers. Eco-friendly green synthesis of nanoparticles is evergreen branch and limb of Nanotechnology. The main features of this potential alternative option are inexpensive, safe and efficient which make it more attractive to biomedical field and elsewhere. The present work leads to the synthesis of silver nanoparticle, its characterization by UV-Visible and TEM analysis. Furthermore, various extracts and synthesized nanoparticle evaluated for their toxicity against malaria vector, Anopheles stephensi. Based on the results obtained, it can be concluded that plant resources can efficient used in the silver nanoparticle production and it could be utilized in various biomedical fields.

Key-words: Nanolarvicide, mosquito,Opuntia,Xerophyte

Introduction:

Today, the major challenge faced by the world's vector control technocrats is the increase incidences of deadly mosquito borne disease time to time. Major factor for this is vector develop resistant to the existing insecticides. Day by day, the ongoing emergence of multi insecticide resistant vector and the diseases caused by them is on the rise very steeply. This is alarming and a global threat. Gone are the days of popular belief that insecticides are a boon and a ready weapon to treat any type of vector. Earlier they were the most powerful weapons to fight against any type of vector and it work as the main therapy to prevent all type of vector transmission. But gradually, overuse or misuse of insecticides has reduced their efficacy and correspondingly vector resistance increased. This increasing incidence of insecticides resistance among the vector organisms necessitates an alternate therapy to curb the resistant mosquito species. A new approach to prevent or combat mosquito species is by the use of silver nanoparticles especially synthesized with the help of natural plants. Plants are already known for many therapeutic values and have been used since ages for curing many diseases and disorders including infectious diseases. This is because of the phytoconstituents present in them. The

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phytoconstituents or secondary metabolites present in them can be used for synthesizing silver nanoparticles. The synthesis of silver nanoparticles by means of using aqueous extracts of plants is simple, efficient, eco friendly, inexpensive, safe and it does not require any sophisticated instrumentation (Sarah et al., 2012). Any part of the plant like leaf, root, stem, peel or fruit can be utilized for the synthesis of silver nanoparticles. The synthesized silver nanoparticles can be used individually or used in combination therapy or synergistic therapy. The synthesized silver nanoparticles are generally characterized by UV-vis spectroscopy, Transmission electron microscopy (TEM), etc. The present research work describes larvicidal activity of xerophytes plant, *Opuntia stricta* by the help of which silver nanoparticles have been synthesized which can be used as new novel source of antimosquitocidal agent to combat multiple insecticide resistant tough vector borne diseases.

Material and Methods

1. Preparation of the extract

Indian xerophyte plant, *Opuntia stricta* (Naagphani) was selected from Agra region, India, on the basis of cost effectiveness, ease of availability, medicinal property and insecticidal activity. Fresh and healthy phylloclades were collected locally and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature.

Aqueous extract: About 10 g of these finely incised phylloclades were weighed and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 20 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Other extracts: The Phylloclades were washed with tap water, chopped, shade-dried, and finely grounded. The finely ground powder was placed in a Soxhlet apparatus (Borosil, Mumbai, India) and extracted in different solvents, petroleum ether, hexane and methanol, subsequently, for up to 72 h in each solvent for complete extraction. Thereafter, each extract was subjecting to rotary vacuum evaporator to remove solvent and get concentrated crude. These semisolid crude extracts stored at 4°C for further experimentation.

2. Silver nanoparticle (Ag NP) synthesis

Aqueous solution (1 mM) of silver nitrate (AgNO3) was prepared in 250 mL Erlenmeyer flasks and phylloclade aqueous extract was added for reduction into Ag+ ions and incubated at room temperature. In the mean time, the colour change of the mixture from light green to reddish brown colloidal form was formed, indicating formation of silver nanoparticle. Then, the colloidal mixture was sealed and stored properly for future use. The formation of Ag NPs was furthermore confirmed by spectrophotometric analysis.

3. Characterization of the synthesized silver Nanoparticle

a.) **UV-Vis spectroscopy:** Leaves extract were challenged to 100 ppm AgNO3 solution. The mixture were observed visually for any colour change and one mL of reaction mixture were withdrawn periodically for analysis of surface Plasmon resonance of silver nanoparticles using a UV-Vis spectrophotometer (Perkin Elmer Lambda 15) at the resolution of 1 nm in range of 200-850 nm.

b.) **Transmission electron microscopy (TEM):** Samples for TEM studies were prepared by placing a drop of the silver nanoparticles colloidal suspension on carbon–coated grids. TEM images were obtained by using a transmission electron microscope FEI Philips Tecnai 12.

4. **Test organisms:** The malarial vector, *Anopheles stephensi*, were reared in the laboratory, maintained continuously at 27±2°C and 70–80% relative humidity under a photoperiod of 14:10 h (light/dark) without exposure to pathogens or insecticides. The larvae were fed with powdered brewer's yeast. Freshly molted larvae were continuously available for the mosquito larvicidal experiments.
5. **Bioassay Larvicidal:** The larval mortality test was assessed by standard WHO procedure (WHO, 1996). For bioassay test, the Twenty late 3rd instars larvae were taken in five batches of 25 in 100 mL test solution with dechlorinated tap water. Five different working concentrations were prepared individually for all the extracts tested. The control was set up with respective solvent and distilled water. The number of dead larvae was counted after 24 and 48 hr of exposure and the percentage of mortality was reported from the average of five replicates. Yeast was offered as food during treatment. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within reasonable period of time) or showing the characteristic diving reaction when the water was disturbed.

$$\text{Molarity}(\%)=\frac{\text{Number of dead larvae}}{\text{Number of introduced larvae}}\times 100$$

Corrected molarity=[(molarity In treatment – molarity In control /100)-Control Molarity] X100

All experiments were tested at room temperature (28 ± 2°C) for the all solvent extracts.

6. **Pupicidal activity** The pupicidal activity of extract was assessed by using the standard method as prescribed by WHO (2005). A laboratory colony of the pupae was used to observe the pupicidal activity of extracts. Twenty five individuals of freshly emerged pupae were kept in 300mL plastic cups containing 100mL of de-chlorinated water with different concentrations. Five replicates were maintaining with each concentration of treatment. The control was maintained with distilled water and ethanol. The numbers of dead pupae were counted after 24 and 48 h

of treatment exposure and percentage of mortality was reported from the average of five replicates. The percentage of mortality was calculated by using Abbott's formula (1925).

7. **Calculation of LC50, LC90 and statistical analysis:** Larval and pupal mortality data were corrected by Abbott's formula (1925), LC50 values (the concentration at which 50% of the larvae were immobilized) and LC90 values (the concentration at which 90% of the larvae were immobilized) were calculated by probit analysis using the PROBIT software Statistical Package. 95% of upper confidence limit (UCL), lower confidence limit (LCL) and Chi square were also calculated. LC50 and LC90 were calculated from toxicity data by using Probit analysis (Finney, 1971). Results with $p<0.05$ were considered to be statistically significant.

Results

1. **Characterization of the synthesized silver Nanoparticle**

a. UV-Vis spectroscopy

Phylloclade extracts from *Opuntia* showed rapid conversion of silver nitrate into silver nanoparticles indicated by color changes from light green to red brown within few minutes of extract addition in 100 ppm AgNO3 solution. A representative scheme of biosynthesis and UV-Vis spectrum is given in Figure . Synthesized silver nanoparticles primarily characterized by UV-visible spectroscopy. AgNPs give typical spectrum having maximum absorption in range of 420-450 nm. This absorption is unique property of metal nanoparticles called SPR (surface Plasmon resonance) arises due to conduction of electrons on surface of AgNPs. After adding phylloclade extract in AgNO3 solution, the biomolecules are stabilized in medium, interact with each other, and with silver salt, after initial interaction silver salt are consumed and the process of nucleation, reduction and capping starts leading nanoparticles synthesis. Similar observations were also reported by other researchers (Fig.1).

b. TEM analysis

TEM analysis reveals that silver nanoparticles are predominately ellipsoids, as can be observed in micrograph correspond to silver nanoparticles formed after 24 hours of reaction, using 5 ml of 10^{-3} M of aqueous solution $AgNO_3$. Nanoparticles sizes are between 8 and 50 nm, with an average of 23 nm ± 5 nm (Fig.2).

2. Larvicidal activity of crude extracts:

The results of the present study indicate the crude solvent extract of *Opuntia stricta* phylloclades to possess larvicidal activity against *Anopheles stephensi* and observations are interpreted in Table and . No larval mortality was observed in control.

Among the extracts tested, the crude petroleum ether extract was found to be most effective with LC_{50} and LC_{90} values was 18.18±1.15 ppm and 33.95±3.08 ppm after 24 hours, respectively and LC_{50} was 17.13±0.98 ppm and LC_{90} was 28.55±2.02 ppm after 48 hours of exposure respectively.

The LC_{50} and LC_{90} values for Hexane extract were 28.62±2.52 ppm and 66.19±9.42 ppm along after 24 hours and LC_{50} was 26.09±2.42 ppm and LC_{90} was 60.51±8.28 ppm after 48 hours of treatment, respectively.

The LC_{50} of Methanol extract was 62.62±4.44 ppm and LC_{90} was 124.22±19.37 ppm after 24 hours and LC_{50} was 59.45±4.03 ppm and LC_{90} was 116.81±17.42 ppm after 48 hours of exposure (Table.1).

3. Larvicidal activity of synthesized silver nanoparticle:

Third instar larvae of *An. stephensi* was treated with biosynthesized silver nanoparticles and the percentage mortality was assessed against various concentrations ranging between (0.5-7 ppm). The LC_{50} and LC_{90} values Phytonanoparticle (silver) were 1.70±0.26 ppm and 4.92±1.62 ppm after 24 hours and LC_{50} 1.42±0.26 ppm and LC_{90} was 4.06±1.14 ppm after 48 hours of exposure periods against anopheline larvae (Table.2).

4. Pupicidal activity of plant crude extract and synthesized silver nanoparticle:

Among the extracts tested, the highest pupicidal activity was observed in synthesized silver nanoparticle against *An. stephensi* followed by Petroleum ether extract with the LC50 and LC90 values were 93.53±9.61ppm and 215.53±27.03 ppm after 24 hrs. And 85.46±8.60 ppm and 184.09±21.92 ppm after 48 hrs of treatment, respectively. (Table 5). Petroleum ethar extract showed highest LC50 and LC90 values i.e. 248.55±60.03 ppm and 578.25±34.08 ppm after 24 hrs. of exposure and LC_{50} and LC_{90} values 215.52±43.05 ppm and 562.72±72.84 ppm after 48 hrs. of exposure. This value indicates very lowest mortality towards *An. stephensi*. The lethal concentration data clearly showed that synthesized silver nanoparticle of *Opuntia stricta* was the most lethal treatment since its LC50 and LC90 were significantly less (chi-square was 75.65 and 0.90; significant at *p* 0.05) than other treatments (Table.3).

Results of the present research reveals that toxicity of all the extracts was susceptible to anopheline larvae and increases as concentration and time increases.

Discussion

Synthesis of silver nanoparticles has the potential to be utilized as a good, eco-friendly approach for the control of mosquito population. In the present study, synthesis of silver nanoparticles using aqueous extract from *Opuntia stricta* against mosquito larvae was carried out. The synthesized Ag NPs were characterized by UV and TEM. However, present work revealed that all the extracts of *Opuntia* possess the larvicidal activity but the highest mortality was found in synthesized Ag NPs against the larvae of *An. Stephensi.* Current work results have been supported by number of researchers:

Rawani, 2017 stated that Synthesized Ag nanoparticles from buds of *Polianthus tuberosa* were effective larvicidal agents against *Culex vishnui* and *Culex quinquefasciatus*. In larvicidal bioassay with synthesized AgNPs, highest mortality was observed at 20 ppm against *Cx. vishnui* with LC50 and LC90 values of 8.25 and 17.99 ppm; 7.46 and 23.26 ppm against 3rd and 4th instars respectively. While in *Cx. quinquefasciatus* the LC50 and LC90 values are 9.65 and 27.18; 7.94 and 22. 47 ppm against

3rd and 4th instars respectively. Non target organisms were also exposed to respective lethal concentrations (to mosquito larvae) of dry nanoparticles which was tested against *Toxorhynchites* larvae (mosquito predator), *Diplonychus annulatum* (predatory water-bug) and there is no abnormality seen in the non target organisms. These results suggest that the synthesized AgNPs of *P. tuberose* have the potential to be used as a supreme environmental friendly compound for the control of the mosquito larvae. **Velayutham and Ramanibai (2016)** found the larvicidal activity of twelve fractions from *Annona squamosa* and synthesized Ag NPs was noted; however, the highest mortality was found in synthesized Ag NPs against the first to fourth instars larvae of *A. aegypti* and *C. quinquefasciatus* with the values of (LC50 =2.50, 2.78, 3.02, 3.05 lg/L; LC90= 7.52, 8.34, 9.06, 9.15 lg/L) and (LC50= 2.75, 3.00, 3.21, 3.48 lg/L; LC90= 8.25, 9.01, 9.63, 10.44 lg/L), respectively. The bioassay water samples decreased Optical density and increased the concentration of synthesized Ag NPs from 2 to 10 lg/ml. This result indicates that the level of nitrite was decreased after using the synthesized AgNPs.

Prusty et al. 2016 were tested the synthesized silver nanoparticles from leaf extract of *Aponogeton natans* for larvicidal activity against the 3rd instar larvae of *Culex bitaeniorhynchus* and the recorded 50% and 90% lethal concentration (LC50) and (LC90) 565.65 ppm and 1052.86 ppm respectively. From the results it may be concluded that the silver nanoparticles synthesized by leaf extract of *Aponogeton natans* has the potential to be used as an ideal eco-friendly approach in reducing growth of mosquitoes. Larvicidal activity of synthesised AgNPs from *Arachis hypogaea* peels was tested by **Velu et al. 2015** for their larvicidal activity against the fourth instar larvae of *Aedes aegypti* (Yellow fever), *Anopheles stephensi* (Human malaria). The results suggest that the synthesised AgNPs have the potential to be used as an ideal eco-friendly resource for the control of *A. aegypti* and *An. stephensi*.

Veerakumar et al. (2014) synthesized Ag NPs using *Heliotropium indicum* leaves against late third instar larvae of *A. aegypti* (LC50= 20.10; LC90 =35.97 mg/mL) and *C. quinquefasciatus* (LC50= 21.84; LC90= 38.10 mg/mL). The synthesized Ag NPs using aqueous extract of *Ficus racemosa* against *C. quinquefasciatus* LC50 =12.00 mg/L **(Velayutham et al., 2013).**

Dhanasekaran and Thangaraj (2013) evaluate the larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector. The mortality rate of *Agaricus bisporus* biogenic nanoparticles against Culex larvae are 5 mg/L (100%), 2.5 mg/L (81%), 1.25 mg/L (62%), 0.625 mg/L (28%) and 0.312 mg/L (11%).

To the best of our knowledge there is no report in the literature for the control of mosquito population by using synthesized Ag NPs using Phylloclades of *Opuntia stricta*. This is an ideal eco-friendly approach for the control of malaria vectors.

Table.1: Efficacy of different phylloclade extracts of *Opuntia stricta* against *Anopheles stephensi* after 24 and 48 hrs. of exposure

Solvent Extract	Expo -sure (Hrs)	Regression equation	X^2	LC_{50} ± S.E.	95% Fiducial limits	LC_{90} ± S.E.	95% Fiducial limits
Petroleum ether	24	119.33-18.86X	101.14	18.18±1.15	17.50 & 18.89	33.95±3.08	27.92 & 39.99
	48	130.0-20.0X	62.57	17.13±0.98	16.48 & 17.81	28.55±2.02	24.60 & 32.49
Hexane	24	115-17.86.0X	45.77	28.62±2.52	26.54 & 30.88	66.19±9.42	47.72 & 84.65
	48	117.67-17.43X	60.70	26.09±2.42	24.11 & 28.23	60.51±8.28	44.28 & 76.75
Methanol	24	89.33-14.57X	3.26	62.62±4.44	58.88 & 67.02	124.22±19.37	86.26 & 162.19
	48	94-14.71X	2.98	59.45±4.03	55.97 & 63.14	116.81±17.42	82.67 &150.95

Table.2: Efficacy of synthesized silver phyto-nanoparticle of *Opuntia stricta* against *Anopheles stephensi* after 24 and 48 hrs. of exposure

Nanoparticle	Exposure time	Regression equation	Chi square	LC50 (ppm)	95% Fiducial limits	LC90 (ppm)	95% Fiducial limits
Silver	**24**	2.78X+1.57	2.04	1.70±0.26	1.19 & 2.22	4.92±1.62	1.73 & 8.10
	48	2.82X+1.75	1.53	1.42±0.26	0.92 & 1.93	4.06±1.14	1.83 & 6.30

Table.3: Pupicidal activity of Potent Phytoextract and synthesized silver phyto-nanoparticle of *Opuntia stricta* against *Anopheles stephensi* after 24 and 48 hrs. of exposure

Extracts	Exposure time	Regression equation	Chi square	LC50 (ppm)	95% Fiducial limits	LC90 (ppm)	95% Fiducial limits
Plant Extract	24	3.61X-7.26	0.42	248.55±60.03	130.90 & 366.20	578.25±34.08	511.45 & 645.05
	48	2.99X-4.97	5.79	215.52±43.05	131.15 & 299.90	562.72±72.84	419.95 & 705.49
Synthesized silver	24	129.46-21.20X	75.65	93.53±9.61	87.15 & 100.37	215.53±27.03	162.54 & 268.52
	48	133.33-19.55X	0.90	85.46±8.60	77.96 & 93.67	184.09±21.92	141.12 & 227.06

Conclusion

The development of a more potent and environmentally safe pesticide by utilizing natural products for synthesizing silver nanoparticles and testing their efficacy in controlling mosquito borne diseases as larvicide is a very recent experience. From the present study it is found that the application of silver nanoparticles synthesized from Phylloclades of Opuntia stricta as potential larvicide to control the vector population and thereby reduces the causative diseases while protecting the environment. Therefore, this technique is very economically feasible and viable to apply on such habitats where mosquito is preferably growing. Therefore, to overcome the application of conventional synthetic chemicals methods, synthesized nanoparticles are better substitute in order to make environment benevolent.

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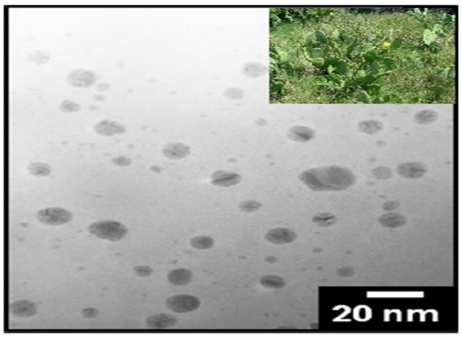

Fig1: Tem image of Synthesized AgNPs From Phylloclade Of *Opunita Stricta*

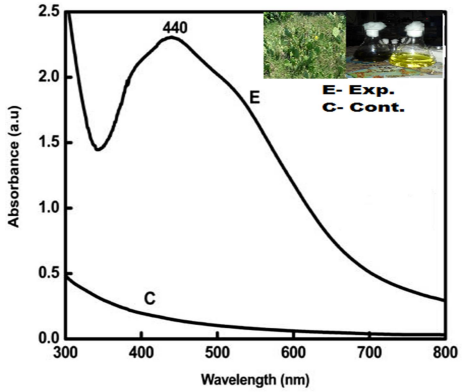

Fig2: UV-vis spectra of synthesized AgNPs from Phylloclade Of *Opunita Stricta*

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