Original Article

# Larvicidal Activity of Silver Nanoparticles Synthesized from *Azadirachta indica* Leaf Extract Against *Oryctes rhinoceros* (L.) Beetle.

**Ganga Gopalakrishnan*, Haritha Cheveliveli Mani, Sreekutty Thekkeveli Vijayan**

Assistant Professor in Microbiology Sree Ayyappa College Eramallikara, Nexgene Lab Cochin, Kerala, INDIA.

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## ABSTRACT

The coconut rhinoceros beetle, *Oryctes rhinoceros* (L), has been one of the major pests of coconut in India and other coconut producing countries causing direct or indirect losses to the crop. It can be controlled by eliminating the places where they breed and by manually destroying adult and immature (larvae). Botanical insecticides are relatively safer, degradable and are readily available source of biopesticides. Biosynthesis of pesticides from plant extracts is most promising approach for pest control. The present investigation aims to find out the possibility of eco-friendly management of the pest *Oryctes rhinoceros* by incorporating with silver nanoparticle synthesized from neem plant extracts. The silver nanoparticles (AgNPs) were synthesized by using aqueous extracts of *Azadirachta indica*. Formation of silver nanoparticles was observed by colour change of the solution, UV, FT-IR and SEM analysis. The Larvae is treated against different concentrations of plant extract and silver nanoparticles solution. 100% larval mortality was observed in higher concentration of silver nanoparticle solution. This result concluded that both silver Nanoparticles and leaf extract have larvicidal properties but Silver nanoparticles are more efficient and it serves as an alternative to synthetic insecticides for controlling harmful pest larvae. Hence the synthesized silver nanoparticles from the *A.indica* can be used as potential biolarvicidal agent against *Oryctes rhinoceros*.

**Key words:** *Oryctes rhinoceros* (rhinoceros beetle), *Azadirachta indica* (neem plant), Silver nanoparticles, FT-IR, SEM, Larvicidal activity.

**Correspondence:**
***Dr. Ganga Gopalakrishnan,***
Assistant Professor in Microbiology Sree Ayyappa College Eramallikara, Nexgene Lab Cochin, Kerala, INDIA.
Phone no: +91-7012656320

Email: gangaprasanth@gmail.com

## INTRODUCTION

*Oryctes rhinoceros* (L.), the coconut rhinoceros beetle, is a pest species occurring throughout many tropical regions of the world. Adults can spoil economically important wild and plantation palms. It causes disease in coconut and other palms by boring in to the centre of the crown. Where they injure the young, growing tissues and feed on the exuded sap. As they bore in to the crown, they cut though the developing leaves. When the leaves grow out and unfold, the damage appears as V-shaped cuts in the frond or holes through the midrib.

Rhinoceros beetle can be controlled by eliminating the places where they breed and by manually destroying adult and immature (larvae). Beetle infestation results in reduced yield and fatal to seedling, young or old palms in some situations. It makes burrows and resides between leaf sheaths, near the crown and cuts the leaf in unopened conditions. The affected frond, when fully opened. Show characteristic genometric cuts and holes are present on the unopened spindle leaves while unopened spathes show round to oblong holes. Yield loss of 5.5 to 9.1 percent due to rhinoceros beetle attack in coconut was reported.[1] Inflorescence damage by rhinoceros beetle under severe infestation and reported yield reduction up to 5.7%.[2]

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Botanicals (botanically derived pesticides) are reported to be safer than synthetic insecticides. They are easily degradable, environmentally safe, have broad spectrum in action, non-persistent and easily processed.[3,4] The botanicals offer desirable alternatives to synthetic chemicals in the agro-ecosystem where protection of the environment and preservation of beneficial organism are important.[5] More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programme.[6-8] The present investigation aims to find out the possibility of eco-friendly management of the pest *Oryctes rhinoceros* by incorporating with silver nanoparticle synthesized from neem plant extracts. Various neem products have been researched extensively for their phytochemistry and exploitation in pest control programmes.[9] But the investigator has made an attempt to study the larvicidal effects of silver nanoparticle from other plants and successfully used against some other type of pest larvae. Grown research attention is focused on Neem (*Azadirachta indica*, Meliaceae), exploring the utility of its products as insecticides and antibiotics. To the best of our knowledge, neem-borne products currently showed effective and eco-friendly features, including little non-target effects, multiple mechanisms of action, low cost, easy production in countries with limited industrial facilities. In particular, the potentiality of neem products as an ideal and affordable source for eradicating pests those are harmful to agriculture field. Overall, we propose the employability of neem-based products as an advantageous alternative to build newer and safer arthropod control tools.

## MATERIALS AND METHODS

### Collection and preparation of *Azadirachta indica* leaves extract

Fresh plant leaves of *Azadirachta indica* (Neem plant) was collected from the defferent House hold areas in Ernakulam district, Kerala. Plant material was surface sterilized with running tap water and followed by distilled water to remove all dust and debris and all inorganic maters from leaf surface. The leaf extracts used for synthesis of nanoparticles were prepared by placing 20 g of fresh Neem leaves in a beaker along with 200 ml of distilled water. The aqueous mixture was then boiled for 25-30 min until the colour of aqueous solution changes to light yellow colour. The aqueous extracts were cooled in room temperature and filtered with Whitman filter paper. After filtering the leaf extract were stored in refrigerator in order to be used for further studies as per the method.

### Synthesis of silver nanoparticles

Silver Nanoparticles were synthesized from plant extract. For synthesis of silver nanoparticles, 1mM silver nitrate solution was prepared by adding 0.017 gm of $AgNO_3$ in 100 ml distilled water and 10 ml of 1mM aqueous solution of silver nitrate ($AgNO_3$) (sigma Aldrich) were mixed with 6 ml of plant leaf extract *Azadirachta indica* in a conical flask and it put it in to the dark condition for 1-2day. After 1-2 days, the resulting light solution changed to dark brown in colour indicating the formation of silver nanoparticles (AgNps).

### Characterization of Green synthesized silver Nanoparticle (AgNps) of *Azadirachta indica*

The preliminary detection of AgNps was carried out by visual observation of colour change of the filtrate. These samples were later subjected to an optical measurement, characterization of green synthesized AgNps of *Azadirachta indica* were done by the furier transform infrared spectroscopy (FTIR) and SEM analysis. The furier transform infrared (FTIR) analysis, used for the characterization of functional groups on the surface of AgNps by plant extracts. The analysis was done by shimadzu 8400s instrument in the range 750-4000 $cm^{-1}$ at a resolution of 1 $cm^{-1}$. Which is usually helps to identify the possible biomolecules responsible for reduction, capping and sterilization of silver Nanoparticles. This is a good method for characterization of Nanoparticles. SEM gives size and shape of formed Nanoparticle. By using this methodology we can identify the surface morphology, shape and size of formed Nanoparticles.

### Determination of the larvicidal activity of silver nanoparticle

To determine the larvicidal activity, the larvae were collected directly from the fields, the larvae were maintained in different petridishes containing 1ppm, 2ppm, and 3ppm test concentrations of silver Nanoparticles coated with *Azadirachta indica* leaf extract and the same number of larvae are placed in the another petridishes containing only leaf extract without silver Nanoparticles in 1ppm, 2ppm and 3ppm concentrations. Control Larvae were placed in another petridishes containing distilled water. The mortality rate was recorded after 24 and 48 hr of exposure. Larvicidal mortality (LM) was calculated using the Abbott's formula given below.[10]

$$LM\ (\%) = \frac{\text{Number of dead larvae}}{\text{Initial number of larvae}} \times 100$$

## RESULTS

In the present study silver nanoparticles were prepared by adding silver nitrate into aqueous leaf extract of *Azadirachta indica*. After 24hr of incubation it showed a colour change from light yellow to dark brown. The appearance of dark brown color in leaf extract indicates silver nanoparticle formations. Characterization should be done measuring with the following analysis.

### FTIR analysis

FTIR analysis aids in identify the possible biomolecule especially functional groups which are responsible for the capping and efficient stabilization of the metal nanoparticles synthesized by *Azadirachta indica* leaf extract. It provides information about different functional groups such as amide linkages (C=O), COO-, N-H, OH groups (Figure 1). They found in amino acid residues in protein and synthesized Silver nanoparticles. Here have got a broad band's or peaks in between 1000 to 3000cm$^{-1}$. The FTIR spectrum gives a wide range of absorption peaks which includes1338.6, 1487.12, 1799.59 cm$^{-1}$. The present FTIR analysis of silver nanoparticle synthesized from *Azadirachta indica* leaf extract showing a strong peak at 1338.6 cm$^{-1}$ could assigned to C-O vibrations of alcohol, phenols and C-N vibration of amides. The band at 1487.12 cm$^{-1}$ and 1799.59 cm$^{-1}$ indicate fingerprinting region of O-H and C-O groups. And the peak 2378.23 cm$^{-1}$ results N-H stretching of amine and amide groups. The peak observed at 1070.49 cm$^{-1}$ showing C-O stretching of ether linkages. So, the result is always giving the information's about dual role of plant extract as a reducing and capping agent.

### SEM analysis

The SEM analysis was usually carried out to understand the topology, characteristic particle size and morphology of the silver nanoparticles (Figure 2). SEM is attached with energy dispersive x- Ray spectroscopy (EDS). The EDS analysis silver nanoparticle was revealing the presence of phase. The result gives average size of Silver nanoparticle was 10-80 nm and the shape of the nanoparticle proved to be spherical in nature. The particles show different size ranges in between the above value. The rough morphology always of the silver nanoparticles provides excellent larvicidal activity of the synthesized nanoparticle.

### Larvicidal activity of silver nanoparticle and leaf extract against the larvae of *Oryctes rhinoceros.*

Present study revealed that the efficiency of green synthesis of silver nanoparticle from *A.indica* and leaf extract at different concentrations (1ppm, 2ppm, 3ppm and control) was tested against *Oryctes rhinoceros* larvae. Larvicidal activity of silver nanoparticle and leaf extract was recorded in the table (Table 1). Mortality rate were comparatively very low in leaf extract when compared with the action of silver nanoparticle against the larvae. Here larvae are treated separately with defferent concentration of AgNPs and leaf extract and are recorded defferent mortality rates at 24 and 48hr of reactions. Higher mortality was recorded at 48hr bioassay than those at 24hr. There is no mortality observed in control up to 48 hr.

Larvicidal activity of plant extract shows comparatively low efficacy against larvae than silver nanoparticles. The mortality rate of Larvae in 3ppm plant extract results 62% and it is significantly higher than the mortality at 1ppm, 2ppm concentrations which results25%, 37.5% respectively at 48 hr reaction. At the same time the mortality rates of Larvae in 3ppm of

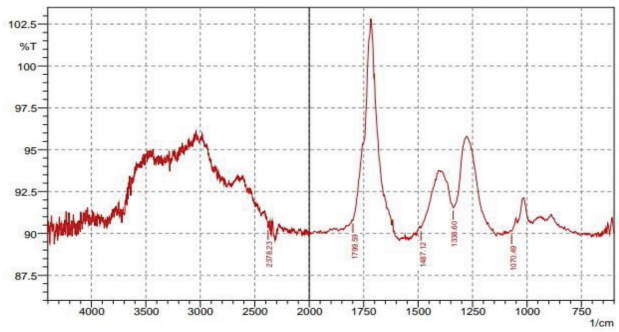


**Figure 1: FTIR spectrum of silver nanoparticle synthesized reduction of Ag+ ions by *Azadirachta indica* leaf extract.**

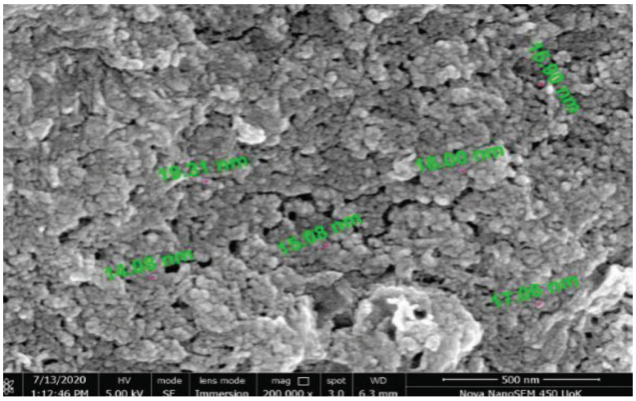

**Figure 2: High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs).**

**Table 1: Larval mortality rates of AgNPs and leaf extract against *Oryctes rhinoceros* at 24hr and 48hr of reaction.**

| Materials | Percentage of LM (24hr) | | | Percentage of LM (48hr) | | |
|---|---|---|---|---|---|---|
| | 1ppm | 2ppm | 3ppm | 1ppm | 2ppm | 3ppm |
| Distilled water | 0 | 0 | 0 | 0 | 0 | 0 |
| Plant extract | 12.5% | 25% | 50% | 25% | 37.5% | 62% |
| AgNps | 37.5% | 50% | 75% | 50% | 62% | 100% |

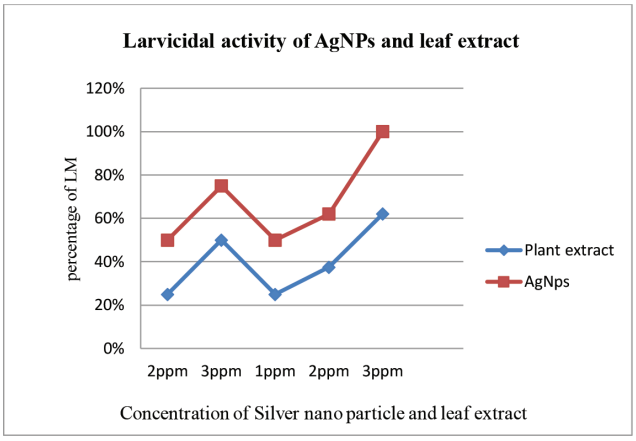


**Figure 3: Graphical representation of larvicidal activity of AgNPs and leaf extract against *Oryctes rhinoceros*.**

silver nanoparticles (AgNPs) provide 100% mortality and 50% in 1ppm, 62%in 2ppm at 48hr of reaction (Figure 3). Results of larvicidal activity clearly indicate the percentage of mortality being directly proportional to the concentration of extract and time of exposure. The smaller size of the nanoparticle gave higher efficacy than leaf extract.

## DISCUSSION AND CONCLUSION

The present study is mainly focused on simple process of green synthesis by using aqueous leaf extract of *Azadirachta indica* leaf for biosynthesis of silver nanoparticle, characterization and larvicidal activity against larvae of *Oryctes rhinoceros*. Visual observation in photosynthesis of silver nanoparticle using aqueous leaf extract *of A. indica* provide a colour change from Yellow to dark brown in 24hr of reaction. Colour change of leaf extract is clearly an indication for the formation of silver nanoparticles. Present findings agreement with previous studies.[11,12]

Characterization of obtained nanoparticle is done by using a variety of techniques including FTIR (furier transform infrared spectroscopy), SEM analysis. The FTIR analyses are carried out to identify the possible biomolecules responsible for reduction, capping and stabilization of Silver nanoparticle.[13] In the present study FTIR analysis of silver nanoparticle synthesized from *A. indica* leaf extract showing a strong peak at 1338.6 $cm^{-1}$ could assigned to C-O vibrations of alcohol, phenols, and C-N vibration of amides. The band at 1487.12 $cm^{-1}$ and 1799.59 $cm^{-1}$ indicate fingerprinting region of O-H and C-O groups. And the peak 2378.23 $cm^{-1}$ results in N-H stretching of amine and amide groups. The peak observed at 1070.49 $cm^{-1}$ showing C-O stretching of ether linkages. The similar and almost equal results are reported in the article.[14] According to this FTIR analysis of medicinal plant especially *A. indica* exhibit the peak 1395.52 gives phenol C-O, peak 2359.59 gives N-H stretching of amine and amides. So, the IR analysis also provided an idea about biomolecules present in leaf extracts.

The surface morphology, shape and size of the silver Nanoparticle were analyzed by using SEM. The SEM images shows individual Nanoparticle which is predominantly spherical as well as number is of aggregates with no defined morphology. The present study shows the size of the Silver Nanoparticles ranging from 10-80 nm. Similar result of the silver Nanoparticle size was reported by using *Aloe vera* extract[15] and by using *Euphorbia hirta* leaves.[16] However, we found very little variations in the sizes of the Nanoparticle. It is observed that most of the AgNPs were of various spherical shapes, which fall closer to many of the Silver Nanoparticle produced by other plant materials.[17-19] SEM reveals information about the sample including morphology, chemical composition and crystalline structure of Nanoparticle. SEM provides detailed high resolution images of the sample by restoring a focused electron beam across the surface and detecting secondary or back scattered electron signal.

Many of the reported tropical plants are leading to extraction and characterization of their active components and its nanoparticle for various uses. A large number of plant products have been reported to have repellent activity.[20,21] Keeping these in mind the present study was undertaken to evaluate the larvicidal activity of *A.indica* leaf extract and its Silver nanoparticle against *Oryctes rhinoceros* larvae. The present work revealed that more larvicidal activity showed by silver nanoparticles compare with Neem leaf extract at 24 and 48 hr of reaction. Larvicidal activity higher at 48hr in 3ppm concentrated silver nanoparticle which

indicates 100% mortality. But in leaf extract the mortality shows just 62% at 48hr of reaction. From these results, nanoparticles have more efficacy than aqueous *A. Indica* leaf extract. Similar larvicidal activity of silver nanoparticles is reported from defferent plant extracts includes *Plumeria rubra* plant latex against Aedes larvae[22] and *Eclipta prostrata* leaf extract against filarial and malarial vectors larvae.[23]

So present study results showing that silver nanoparticles synthesis from *Azadirachta indica* has a great promising application in agriculture fields to reduce the number of harmful pests by its larvicidal activity.

The present study concluded that the leaf extract of *Azadirachta indica* has the potential to reduce silver ions to make silver nanoparticles and it can be used as an eco-friendly larvicidal agent against *Oryctes rhinoceros* (palm attacking beetles). The main cause of larval mortality is due to their unique characteristics of silver Nanoparticles and its smaller size. So, they can interact very easily with the fat body of larvae and produce either genotoxicity or protein degradative effects on its body lead to their death. This is the major reason for death.

This method of synthesis and their activities, efficiencies are providing to be an eco-friendly, rapid greening approach for the synthesis of providing a cost effective and an efficient way for the Silver Nanoparticles formation and is satisfied all the conditions of a 100% green chemical process. The main benefits of using plant extracts are high energy efficiency, cost effective, and safer to humans.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**%:** percentage; **A.indica:** *Azadirachta indica* (Neem plant); **O. rhinoceros:** Oryctes rhinoceros beetle; **SEM:** Scanning electron microscope; **FTIR:** Fourier Transform Infrared Spectroscopy; **ppm:** Parts Per Million; **AgNO3:** Silver Nitrate; **AgNPs:** Silver Nanoparticles.

## SUMMARY

The present study deals with the biosynthesis of silver nanoparticles from aqueous leaf extract of *Azadirachta indica* and its larvicidal activity against *Oryctes rhinoceros* beetle. The larvicidal actions of silver Nanoparticles exhibit more efficacy than aqueous leaf extract against the larvae in the same concentration (3ppm) and time of exposure (48 hrs). From the results, both silver nanoparticles and leaf extract have larvicidal properties. Silver nanoparticles are more efficient hence; it serves as an alternative to synthetic insecticides for controlling harmful pest larvae. The results obtained during the present study gave an idea that, developing natural products for synthesizing silver nanoparticles and testing their efficiency in controlling of pest larvae is an advanced technique for making a more potential environmentally safe biopesticides.

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