



**BIOGENIC NANOPARTICLE SYNTHESIS FROM  
HERBAL EXTRACT AND FORMULATION OF ECO-  
CONSCIOUS BIOFERTILIZERS USING ORGANIC  
NUTRIENT SOURCE**

✉ [admin@reboin.com](mailto:admin@reboin.com)

🌐 [www.reboin.com](http://www.reboin.com)

# Biogenic Nanoparticle Synthesis from Herbal Extract and Formulation of Eco-Conscious Biofertilizers Using Organic Nutrient Source

Bharath K M<sup>1</sup>, S Nanjunda Swamy<sup>1</sup>, Annie Jessica Toppo<sup>2</sup>  
Department of Biotechnology, JSS Science and Technology University Mysuru<sup>1</sup>,  
Rapture Biotech Pvt. Ltd, Bengaluru<sup>2</sup>

\*Corresponding Author's Email- [rapturetrainer.bengaluru@gmail.com](mailto:rapturetrainer.bengaluru@gmail.com)

## ABSTRACT

Nanotechnology has recently gained attention as a potentially revolutionary invention. Chemical or synthetic fertilizer is used to give crops the essential minerals that are typically lacking in crops grown in this era. The environment is being negatively impacted by these synthetic fertilizers. In order to improve the plants nutrients absorption, this project focuses on combining biofertilizers like Jeevamrutha with herbally extracted nanoparticles like copper and silver. When plants are inoculated with nanobiofertilizers, their development and resistance are enhanced. By using plant based green synthesis to create nanobiofertilizers, metallic nanoparticles and organic materials like chitosan and polysaccharide can be encapsulated, avoiding the drawbacks of traditional chemical fertilizers. Although the use of nanobiofertilizers is still in its beginning stage, it has greater potential than other strategies for transforming traditional farming into high-tech, or "smart," farming. This study examines the innovative application of nanotechnology in agriculture, utilizing the special properties of materials at the nanoscale to tackle pressing issues like crop protection, nutrient delivery, and sustainable farming practices. By going over the creation, characterization, and formulation of nanobiofertilizers and clarifying the ways in which they interact with plants, this project aims to close some of the knowledge gaps related to nanobiofertilizers and their performance.

**Keywords-** *Azadirachta indica*, Silver nitrate, Copper nanoparticles, Nano-biofertilizer, Jeevamrutha, Black chana

## 1. INTRODUCTION

Nanotechnology is the emerging field of Science and Technology which deals with nanoparticles. The particle with dimension in nanometer are called as nanoparticles. Synthesis of nanoparticles is done due to its various application in the field of agriculture, textile industries, electronic sector, etc. This study focuses on the use of nanoparticles in agriculture. Nanoparticles play a variety of roles in plant growth; some are used as antimicrobial agents, while others are used as a component of nutrient sources. Copper nanoparticles are commonly used to increase plant growth by optimizing the absorption of nutrients present in the soil by the plant's roots, while silver nanoparticles are primarily used to avail themselves of their antimicrobial activity. One of the primary goals of many researchers is the synthesis of silver nanoparticles and their application to plants to prevent microbial infection. One of the reasons for using these nanoparticles is their reduced toxicity, environmental friendliness, stability, and affordability. Because of these advantages, the green synthesis of nanoparticles is carried out using different plant parts, such as the leaf, stem, and root; additionally, certain microorganisms can be employed to synthesize nanoparticles [1].

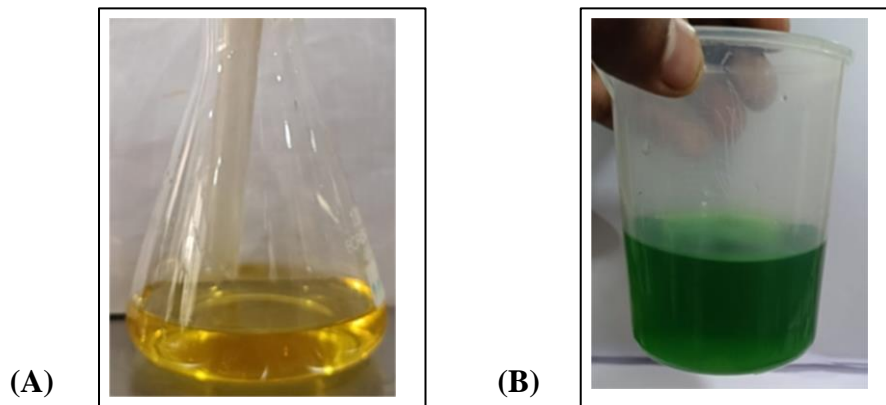
## 2. MATERIALS AND METHODS

**2.1. Sample collection-** Samples of Neem (*Azadirachta indica*) leaves were collected as shown in figure 1 from the local area of Bengaluru.



**Figure1. Neem leaves**

**2.2. Extract preparation-** For the synthesis of Silver nanoparticles the extract was prepared by boiling washed 10g neem leaves in 150ml distilled water at 80°C for 30 mins and for the synthesis of Copper nanoparticles the extract was prepared by boiling 100g washed and grinded neem leaves at 60°C for 30 mins. Both the extracts were filtered using whatmann filter paper. Figure 2 represents the filtered neem extract.



**Figure 2**

**(A) Neem extract for AgNp synthesis**

**(B) Neem extract for CuNP synthesis**

**2.3. Synthesis of nanoparticles-** Silver nanoparticles were prepared by adding 0.1M silver nitrate to the prepared neem leaves extract in 9:1 ratio. Copper nanoparticles were prepared by adding 0.1M copper sulphate to the prepared neem leaves extract in 1:3 ratio. These mixtures were then centrifuged at 3000rpm for 10mins. and dried in incubator to get nanoparticles [2].

**2.4. Antimicrobial susceptibility test:** Nutrient agar media was prepared by dissolving 0.6g of peptone, 0.16g of beef extract, 0.3g of NaCl, and 0.96g of agar agar in 60ml of distilled water. This media was then autoclaved and was poured into petri plates which were let to solidify. *P.aeruginosa* sample was taken and was poured and spreaded equally on solidified media using 'L' shape glass rod. Then the discs which were dipped in different concentrations of synthesized silver and copper nanoparticles like 0.1mg/ml, 0.2mg/ml, etc were placed on the media. A standard antibiotic disc i.e streptomycin disc was used as control. The area of inhibition was calculated by taking the diameter [3].

**2.5. Preparation of nano-biofertilizer-** Jeevamrutha was prepared by adding 1kg of cowdung, 1ltr of cowurine, 200g jaggery, 200g pulse powder, one handful of soil in 20ltrs of water and allowed to fermentation for 7 days in aerobic condition. Then to this prepared Jeevamrutha synthesized nanoparticles were added in the known concentration which is represented in table 1.

**Table 1. Concentration of nanoparticles added to Jeevamrutha to prepare nano-biofertilizers**

Nano-biofertilizer	Concentration of Silver nanoparticles	Concentration of Copper nanoparticles
Silver nano-biofertilizer	0.2 mg/ml	-
Copper nano-biofertilizer	-	0.4 mg/ml
Bi-metallic nano-biofertilizer	0.2 mg/ml	0.4 mg/ml

**2.6. Preparation and introduction of plantlets to highlight the effect of biofertilizer on them-** Black chana seeds were introduced to the autoclaved soil and watered once a day for 5-7 days to get the plantlets. Then the nano-biofertilizers were added to each tray and the plantlets were grown under similar condition for further observations.

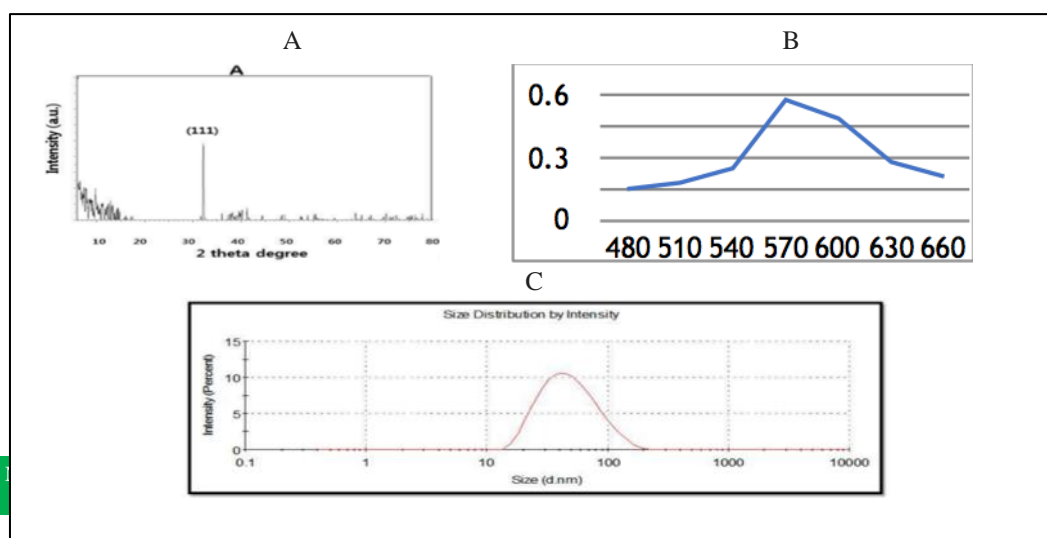
### 3. RESULT AND DISCUSSION

**3.1. Characterization of nanoparticles-** Both the nanoparticles were characterized using UV-Visible spectrometer, DLS, and XRD.

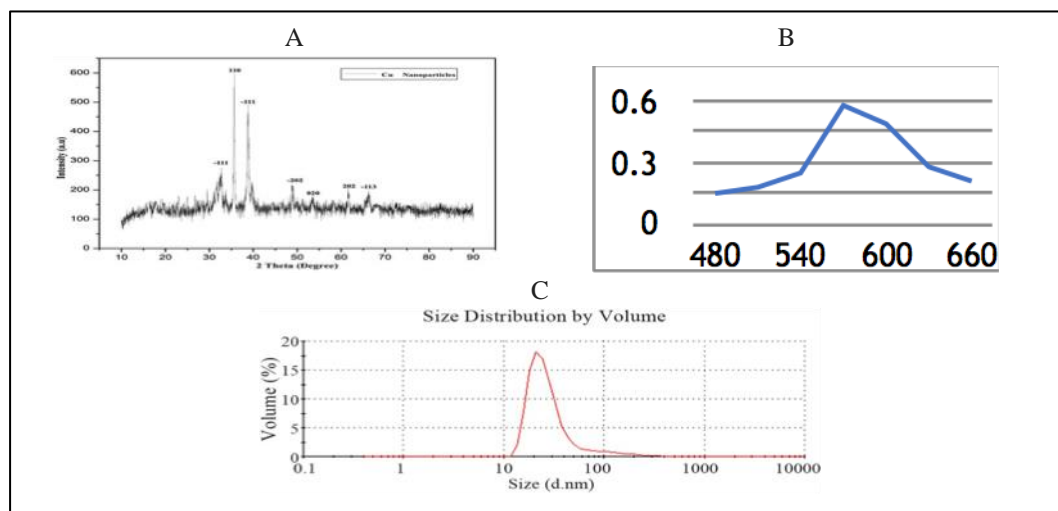
**3.1.1. X-ray diffraction-** The samples were then characterized with XRD to identify the structure of nanoparticles. XRD report of AgNP had sharp peak at  $32.12^\circ$  which was indexed and matched with the JCPDS card no. 04-0783. This result (Figure 3 (A),4 (A)) indicates a strongly oriented crystalline structure that corroborates with the results of Banerjee et al., [2]. Similarly the XRD report of CuNP had sharp peaks at  $36.5^\circ$  and  $38.5^\circ$  which was indexed and matched with the JCPDS card no. 04-0836. This result indicates a good crystallographic structure which resembles the result of Gurudevi et al., [4]

**3.1.2. UV-Visible spectrophotometer-** The results (Figure 3 (B),4 (B)) obtained from UV-Visible spectrophotometer for silver nanoparticles corroborates with the results of Jervin Ananth et al., [5] and the results of copper nanoparticles corroborates with the results of Banerjee et al., this results are shown in table 2. The UV-Visible spectrometer records a maximum peak at 420nm wavelength for silver nanoparticles and records maximum peak at 570nm wavelength for copper nanoparticles.

**3.1.3. Dynamic light scattering-** The results (Figure 3 (C),4 (C)) obtained from DLS gives the size of nanoparticles where it identifies the size of AgNP as 34nm which resembles with the study of Chika Iwuji et al., [6] similarly the DLS results of CuNP gives the size of nanoparticles as 23nm which is similar to the study reported in Gurudevi et al., [4]

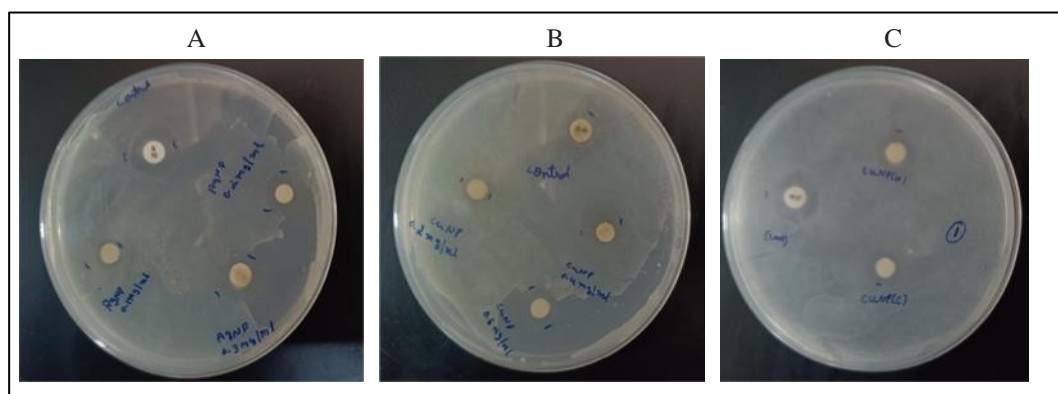


**Figure 3. A) XRD report of AgNP B) UV-Visible spectrometer reading of AgNP C) DLS report of AgNP**



**Figure 4. A) XRD report of CuNP B) UV-Visible spectrometer reading of CuNP C) DLS report of CuNP**

**3.2. Antimicrobial susceptibility test-** Chemically synthesized silver nanoparticles generally exhibit their maximum antimicrobial activity in the range of 0.3 mg/ml [6]. Herbally synthesized nanoparticles exhibited antimicrobial activity when they are treated in the range 0.2 mg/ml. Similarly copper nanoparticles exhibit optimum antimicrobial activity in the range of 0.6 mg/ml [7] when synthesized chemically. In contrast green synthesized nanoparticles exhibited antimicrobial activity when they are treated in the range 0.4 mg/ml. This result shows us that green synthesized nanoparticles are more efficient than the chemically synthesized nanoparticles. This statement also corroborates with the study by Banerjee et al., [2,8,9] which is shown in figure 5.



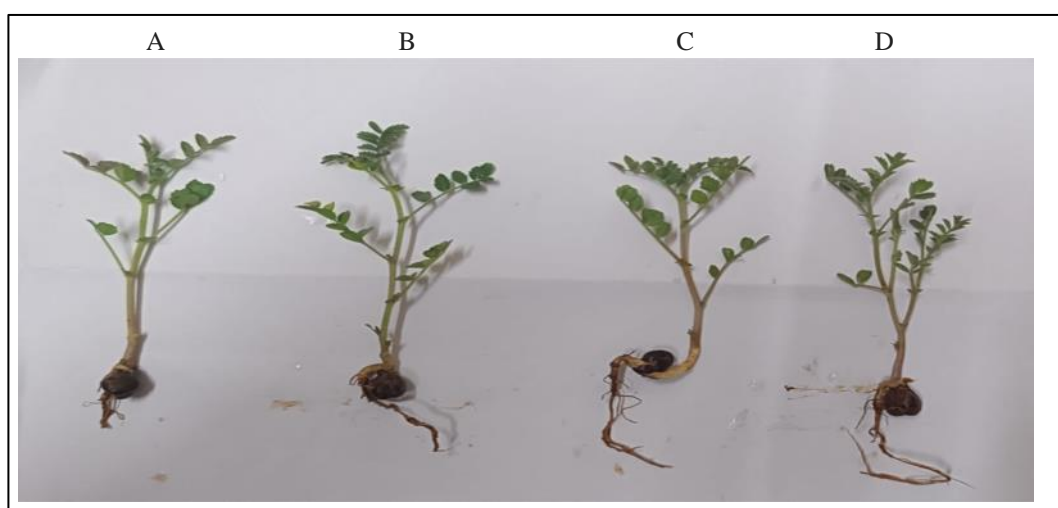
**Figure 5. A) Antimicrobial susceptibility test for chemically synthesized AgNP vs green synthesized AgNP B) Antimicrobial susceptibility test for chemically synthesized CuNP**

**vs green synthesized CuNP C) Antimicrobial susceptibility test for green synthesized AgNP vs green synthesized CuNP**

**3.3. Effectiveness of nano-biofertilizers-** Nano-biofertilizers showcased their ability of improving biofertilizer (Jeevamrutha). Increased branching in black chana directly improves the plant yield. The experimental data reveals (Table 2 and Figure 6) that the nanoparticles alone in Jeevamrutha can increase the branching in black chana by 17% in contrast when Jeevamrutha was added with both nanoparticles the effectiveness of Jeevamrutha was increased by 46% on black chana plants.

**Table 2. Effectiveness of nano-biofertilizers on plants**

Day	No. of branches on feeding Jeevamrutha	No. of branches on feeding silver nano-biofertilizer	No. of branches on feeding copper nano-biofertilizer	No. of branches on feeding bimetallic nano-biofertilizer
Day 12	3	3	3	3
Day 15	4	5	5	5
Day 18	5	6	6	8
Day 21	7	7	7	11
Day 24	7	9	9	13



**Figure 6. A) Plant grown using Jeevamrutha B) Plant grown using Jeevamrutha with AgNPs C) Plant grown using Jeevamrutha with CuNPs D) Plant grown using bimetallic nano-biofertilizer**

This study highlights the potential of stabilized and green synthesized silver and copper nanoparticles added into Jeevamrutha which improved the plant growth in black chana. Despite the addition of nanoparticles in Jeevamrutha improved the vegetative development of black chana plant the study had some limitations as this study was done on a single crop and in controlled condition. Long term impacts of this nano-biofertilizers is yet to be explored and as it is a laboratory experiment the field trail need to be done. Overall, this work contributes in advancing organic and sustainable farming to avoid synthetic farming.

## CONCLUSION

In recent years, the demand for food is very high so the use of synthetic fertilizers is increasing day to day. These chemical fertilizers can cause many infections to plants and animals which leads to the imbalance in the ecological cycle. These synthetic fertilizers degrading the soil quality and ground water quality. The maximal use of synthetic fertilizers killing the essential and farmer friendly insects like honey bees, butterfly, etc and worms like earthworms. Nanotechnology improves the efficiency of biofertilizers by improving the nutrient absorption and nitrogen fixation. The increase in efficiency was visibly observed through the development of plants. Yield of the plant mainly depends on the growth of vegetative parts of plant. When the plants were provided with silver and copper nanobiofertilizers the growth of the plant branches will be increased by 14% against to the control Jeevamrutha. This indicates that by providing nanoparticles with biofertilizers potentially increases the yield of the crop. The use of bimetallic nanobiofertilizers helped us to study the effects of using two different nanoparticles combined with Jeevamrutha on plants. The use of type of nanobiofertilizer visibly increased the growth of plants. The plant growth was increased by 46% than the control Jeevamrutha. This confirms that the use of two different nanoparticles in combination with a biofertilizer boosts the yield of the plant in high proportion.

## ACKNOWLEDGEMENT

I would like to show my gratitude to all my guides, mentors, lab mates, friends and my family. To start of I sincerely thank the management of JSS Science and Technology University for providing an opportunity to identify and work on a topic which we are interested in. I extend my thanks to my HOD (Department of Biotechnology). Dr. Sanjay K R and my Intenal guide Dr. S Nanjundaswamy for their guidance and support.

I would like to express my gratitude to Mr. Jaspal Singh, Director of Rapture Biotech, Bengaluru for his support and for providing the necessary facilities and a conducive research environment to conduct the study.

My deepest thanks to Dr. Annie Jessica Toppo, my research guide, for her exceptional mentorship, insightful feedback, and unwavering support throughout my research journey. Her guidance has been instrumental in refining and shaping this paper.

I also extend my gratitude to my lab mates who helped, encouraged, supported and created a good atmosphere for the research. I have got full support from Indrajeeth and family, organic farmers from Sakaleshpura in the preparation process of Jeevamrutha I would be pleased to express my gratitude towards them.

## REFERENCES

1. Singh KA, Singh B, Pavithran N, Fayaz A, Kaundal M. Panchagavya: a novel approach for the sustainable production of crops. *Curr J Appl Sci Technol.* 2023;42(48):137-147.
2. Banerjee P, Satapathy M, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour Bioprocess.* 2014;1(1):3.
3. Kulkarni SS, Gargelwar AP. Production and microbial analysis of Jeevamrutham for nitrogen fixers and phosphate solubilizers in the rural area from Maharashtra. *IOSR J Agric Vet Sci.* 2019;12:85-92.
4. Gurudevi MD, Sirisha C. Green synthesis of copper nanoparticles using neem (*Azadirachta indica*) leaf extract and their antimicrobial activity. *DR S Res J.* 2023;13:383-389.

5. Nzekekwa AK, Abosede OO. Green synthesis and characterization of silver nanoparticles using leaves extracts of neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*). *J Appl Sci Environ Manag.* 2019;23(4):695-699.
6. Iwuji C, Saha H, Ghann W, et al. Synthesis and characterization of silver nanoparticles and their promising antimicrobial effects. *Chem Phys Impact.* 2024;9:100758.
7. Devi RS, Gayathri RJ. Green synthesis of zinc oxide nanoparticles by using *Hibiscus rosa-sinensis*. *Int J Curr Eng Technol.* 2014;4(4):2444-2446.
8. Ajayan AS, Hebsur N. Green synthesis of zinc oxide nanoparticles using neem (*Azadirachta indica*) and tulasi (*Ocimum tenuiflorum*) leaf extract and their characterisation. *Int J Curr Microbiol Appl Sci.* 2020;9(2):277-285.
9. Ansilin S, Nair JK, Aswathy C, Rama V, Peter J, Persis JJ. Green synthesis and characterisation of copper oxide nanoparticles using *Azadirachta indica* (neem) leaf aqueous extract. *J Nanosci Nanotechnol.* 2016;:221-223.



# **HARNESSING HERBAL POWER FOR SUSTAINABLE NANO- AGRICULTURE**

Plot no 977, GMS Road, near Balliwala Flyover, opposite Cubic Plaza,  
Dehradun, Uttarakhand 248001

✉ [admin@reboin.com](mailto:admin@reboin.com)

🌐 [www.reboin.com](http://www.reboin.com)