International Journal of Biotech Trends and Technology
ISSN: 2249 – 0183 /doi:10.14445/22490183/IJBTT-V11I2P601

Volume 11 Issue 2, 1-7, April-June 2021

Potential Antifungal Activity of Biosynthesized Copper nanoparticles Against *Colletotrichum Capsici In Chilly*

Chandrashekhara V.D[1], Lokesh, S[1]., Kavyashri V.V[2], Prasanna Kumar M.K[2]. and Sanggonda[3]

[1] *Department of Studies in Biotechnology, University of Mysore, Mysuru-570006*
[2] *Department of Plant Pathology, CoA, UAS, GKVK, Bangalore-560065*
[3]*Department of Seed Science & technology, CoA, UAS, Dharwad-580005*

Received Date: 02 March 2021
Revised Date: 05 April 2021
Accepted Date: 19 April 2021

Abstract

Nanotechnology is gaining more attraction in various fields, including Agriculture. Green synthesis of nanoparticles has emerged as a simple, viable, and alternative eco-friendly method for nanoparticle synthesis when compared with the chemical method. In the present study, Copper nanoparticles have been synthesized using aquatic weed Kappaphycus alvarezii extract, and characterization was done. The synthesized nanoparticles exhibited maximum absorption at 630 to 650nm, which is (SPR) for Copper nanoparticles. The Copper nanoparticles were characterized based on Scanning Electron Microscopy (SEM) and whose size was found to be in the ranges from 40 to 80nm. The XRD analysis of synthesized copper nanoparticles showed four peaks at 35.5, 38.6, 48.5, 61.5, and 68.02 angles it confirms the presence of Copper nano. The antifungal activity of the Copper nanoparticles was checked against Colletotrichum capsici at different concentrations, i.e., 10, 25, 50, 100, 200, 400, and 600ppm, in which the significant zone of inhibition was observed on PDA amended with 100ppm of copper nanoparticles in vitro. Further, the same resulted in reduced sporulation and Mycelial growth to a greater extent. This has promised that the newly synthesized Copper nanoparticles could be used as an antifungal agent for crop disease management, especially in the case of anthracnose.

Keywords: *Copper nanoparticles (CuNPs), Colletotrichum capsici, Chilly,*

Introduction

Nanotechnology has led to the development of new concepts and agricultural products with immense potential to manage the aforementioned problems. Nanotechnology has substantially advanced in medicine and pharmacology, but has received comparatively less interest for agricultural applications. The use of nanotechnology in agriculture is currently being explored in plant hormone delivery, seed germination, water management, transfer of target genes, nano barcoding, nanosensors, and controlled release of agrochemicals. It has become necessary to adopt the latest technologies, which primarily focus on achieving the better agriculture productivity. Artificially, Nanoparticles are synthesized by different approaches like thermal decomposing, Electrochemistry. Micro oven assisted process and green chemistry. Unfortunately, several of these methods include we of hazardous chemicals, high energy consumption &, in return, low material conversions. Therefore, the necessity to develop a more environmentally friendly process for nanoparticle synthesis is the need of the day & it is important is increasing every day. Such environmentally friendly method utilization of microorganisms, plant extracts are simple, viable & sustainable alternative to chemical ways of nanoparticle synthesis.

For a long time, its well-documented information regarding the plant is that, they can reduce the metal ions on their surface as well as in their organs and tissues that are far away from the site of ion penetration. This ability of plant is well utilized in the field of Phyto mining, where the plants or plant parts are used to extract precious metals from the earth, which are otherwise economically non-feasible to mine from earth. Such metals that accumulated in plants can be repeated after harvesting. Phyto mining revealed that plants store metals in the form of nanoparticles. Bio-reduction of such metals ions into nanoparticles in a plant is facilitated by several plant metabolites like terpenoids, polyphenols, sugars, phenolic acids and alkaloids, proteins, etc. [1]

Being called as a wonder of modern medicine, Nanoparticle possesses antimicrobial properties against pathogenic microorganisms like Bactria, Fungi, and Viruses. The metal oxide ions in nanoparticles exhibit broad-spectrum biocidal activity towards the micro-organism & have more advantage over conventional chemical pesticides [2]. With the progress of the application aspect of Nanotechnology in Agriculture, the use of NPs for disease management may become a novel approach in the future days. Nano-fungicides may become an effective alternative to conventional synthetic

fungicides in successful plant disease management. With an ever-increasing demand for staple food production, it is important to improve the productivity of crops to feed the growing world population. Commercializing the metal NPs may play a vital role in sustainable agriculture [3]

Biosynthesized Copper nanoparticles inhibit the growth of phyto bacteria and pathogenic fungi. Also, copper nanoparticles are known to improve the germination and growth of plants when it used at a lower concentration. Copper nanoparticle-based fungicides are known to produce highly reactive hydroxyl radical can damage lipids, proteins, DNA & other biomolecules [4]. This makes copper nanoparticles as an important option in disease prevention and management. In recent times there is a sharp increase in the interest in the use of Copper fungicides because copper nanoparticles are much cheaper compare to other nanoparticles.

MATERIAL AND METHODS

Collection of seaweeds:

Marine algae *Kappaphycus alvarezii* were collected from the coastal area of Gujarat in India. The seaweed *K. alvarezii* was washed thoroughly under running tap water followed by distilled water, air-dried, and was powdered with the help of mortar and pestle. About 10g of powdered sample was then mixed with 100ml of double distilled water in Erlenmeyer flasks (1:10 ratio), and the mixture was boiled for 5 minutes. The extract was then cooled and filtered through Whatman no 1 filter paper. The resultant filtrate was refrigerated at 4^{o}C for future use.

Green synthesis of copper nanoparticles

100 ml of Copper sulfate (1 mM) was prepared in double-distilled water. For this solution, 50 ml of polyethylene glycol 8000 (0.01M) was added. Finally, 50 ml of plant sample was added with vigorous stirring. The solution color changed from blue to white. 0.1M sodium hydroxide was added in drops to the solution under continuous rapid stirring to increase the solution pH to 10. After this, the solution was moved to a hot plate (80-85°C) with 800 rpm stirring. The solution color changed to black. The same observation was reported [5] in *Magnolia Kobus* leaf extract.

The solution was centrifuged at 11000 rpm for 20 min, and the solid black product was collected. The final products were obtained by drying at room temperature. The resulting dried sample was crushed into powder and stored in an air-tight container for further analysis.

Fig-1: Biosynthesis of Copper nanoparticles from the aqueous extract of seaweed

Isolation and identification of the pathogen

Small bits of infected tissue (2-3 mm size) were cut at the juncture of diseased and healthy portions with the help of disinfected. These bits were transferred to potato dextrose agar (PDA) medium in sterilized Petri plates and incubated for three days at 27±1^{0}C. The culture thus obtained was subjected to purification.

Fig-2: Cultural and morphological characteristics of *Colletotrichum capscici*

Pathogenicity of fungal pathogen:

Healthy chili fruits were collected from the fields and washed under tap water, and then surface sterilized with 70% ethyl alcohol. Then 0.7 cm agar plug containing mycelia from 10 days old culture of *C. capsici* was placed on the pierced area on chili fruit. Fruits inoculated with sterilized water served as control. Inoculated fruits were kept in moistened polythene bags to maintain humidity and incubated at 28 ±2°C and observed daily for the disease symptoms (Fig.3) [6]. Pathogen was re-isolated from the infected fruits and compared with the original culture.

Fig-3: Pathogenicity test of *Colletotrichum capscici*

Poisoned-food technique

Biosynthesized nanoparticles and other treatments were incorporated into 2 percent sterilized unsolidified Potato dextrose agar and shaken well to make it homogenous. Medium was then poured in 90 mm Sterilized Petri dishes with three replications of each treatment and allowed to solidify. These dishes were then inoculated with 4 mm diameter circular discs of inoculums were placed in the center of each Petri dish. The Petri dishes were incubated at 28±1°C for 10 days.

Table 1: **Treatment detail**

Treatment details	
T-1	Plant sample- 2 %
T-2	Cupper sulphate- 0.1mM
T-3	Bavistin-2g/ltr
T-4	Raw Copper Nano solution
T-5	10 ppm Biosynth. Copper Nano
T-6	25 ppm Biosynth Copper Nano
T-7	50 ppm Biosynth Copper Nano
T-8	75 ppm Biosynth Copper Nano
T-9	100 ppm Biosynth Copper Nano
T-10	200 ppm Biosynth Copper Nano
T-11	400 ppm Biosynthd Copper Nano
T-12	600 ppm Biosynthd Copper Nano
T-13	Control

RESULTS AND DISCUSSION:

CHARACTERIZATION OF COPPER NANOPARTICLES

UV–Vis analysis

One nm resolution between 200 and 700 nm with a scanning speed of 500 nm/min was performed with UV-Vis spectral analysis. By measuring the UV-Vis spectrum of the reaction medium in which the bell shape peak was observed, the reduction of Cu ions was monitored. Surface plasmon resonance (SPR) was obtained in the same way for copper at 630-650 nm (Fig 4). No peak was observed in the solution of plant and Copper sulfate alone.

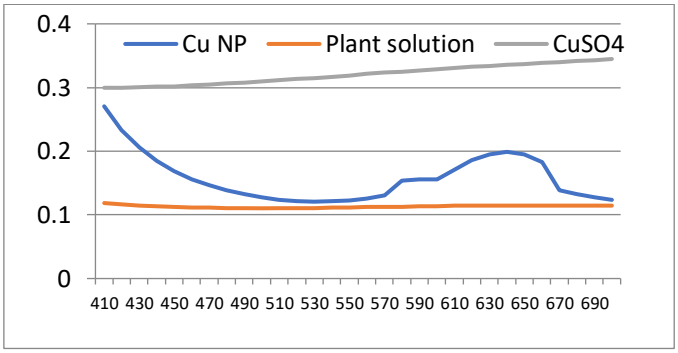


Fig 4: **UV-Vis reading of synthesized copper nanoparticles**

Scanning Electron Microscope

The surface morphology of the CuNPs was studied further using scanning electron microscopy (SEM). The substance appears to be mostly made up of particle-like Cu-NP crammed together to resemble collective cauliflower in SEM pictures **(Fig. 5).** With a higher magnification, these crowded Cu-NPs are shown to be groups of smaller nanoparticles with uniform crystal

structures. The scale of copper nanoparticles synthesized by algae was found to be in the range of 60-90 nm. Stable copper nanoparticles were synthesized by [7] using *Ocimum sanctum* leaf extract and characterized the same.

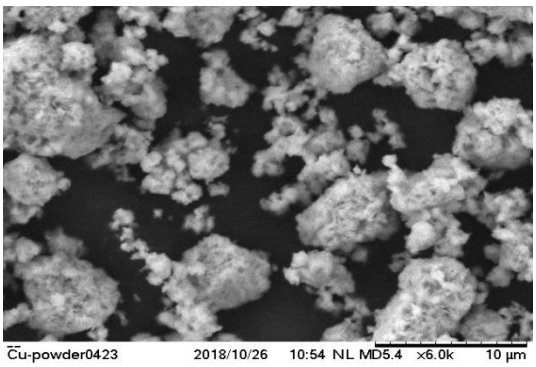


Fig 5- Scanning Electron Microscopy of biosynthesized Copper nanoparticles

XRD analysis:

The development of diffraction patterns is used in XRD research to examine molecular and crystal structures, qualitative and quantitative resolution of different molecules, particle sizes, and so on. XRD analysis (Fig-6) of biosynthesized Copper nanoparticles revealed distinct diffraction peaks at 34.2, 38.12, 47.15, 53.15, 56.27, 62.31, 66, and 69 degrees, which correspond to lattice planes at (254), (231), (186), (170), (158), (150), (140), and (137) planes. The highest peak on the curve, at 38.12 = 2, suggested the crystal structure of nanoparticles.

The Debye Scherer equation calculates an average crystalline size of 89 nm. Furthermore, the main XRD peaks at 30.8°, 38.66°, 48.66°, and 68.26° diffracted from the (110), (111), (200), and (220) planes of the face-centered cubic structure are visible in algal-synthesized copper nanoparticles. JCPDS card no. (71–4610) is used to index it. Furthermore, three other peaks were discovered at 2 of 32.3°, 35.4°, and 58.1°, which can be assigned to (002), (111), and (202), respectively, suggesting the existence of the oxide shell around the copper nanoparticles. The average size of synthesized copper nanoparticles was measured using the Debye Scherer equation and was found to be about 58 nm, which is consistent with what [8] found from SEM photos.

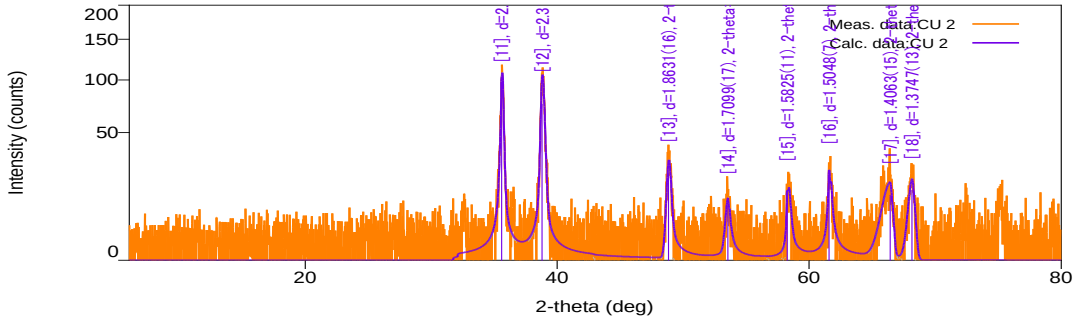


Fig 6- XRD pattern of the biosynthesized copper nanoparticles

Particle size analysis

CuNPs, particle size analysis, showed that 80% of particle sizes fell between 36 and 60 nm, according to the size distribution. The 85.90 nm channel was used by 98.53 percent of the particulates. Via 36.4 nm channels, 50% of the particles moved. It indicates that the biosynthesized copper nano solution contains particles with a size of 30-86 nm. It can be deduced from this that biosynthesized Copper nano solution contains nanoparticles of various sizes. [7] Copper nano from *Ocimum sanctum* leaf extracted and observed the results. The average particle size of copper nanoparticles was 25 nm, with a total concentration of 30.29 particles/frame and 13.53 particles/ml, in the same way, that [9] observed between 5 nm and 28 nm size with an average size of 28 nm synthesized from Citrus limon fruits extract.

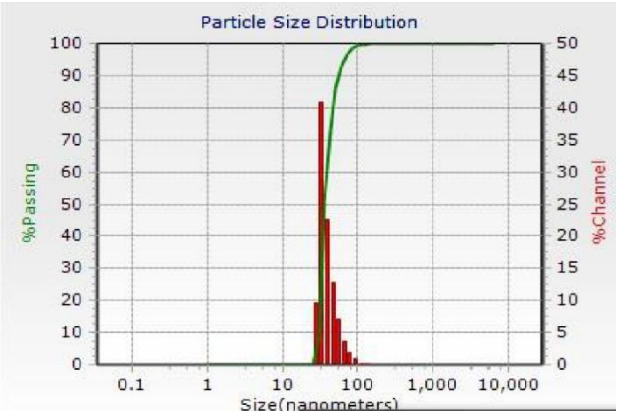


Fig 7- Particle size distribution of biosynthesized Copper nanoparticles

A. FTIR study

The active biofunctional major groups present in the seaweed *Kappaphycus alvarazii* were identified using Fourier transform infrared (FTIR) spectrum measurements to investigate their potential involvement in the synthesis of copper nanoparticles. FTIR peaks were observed for copper nanoparticle solution at 3344.10 cm^{-1}, 1640.04 cm^{-1}, and 1045.34 cm^{-1}, which correspond to alcohols, polyphenols, proteins, and amino acids, respectively.

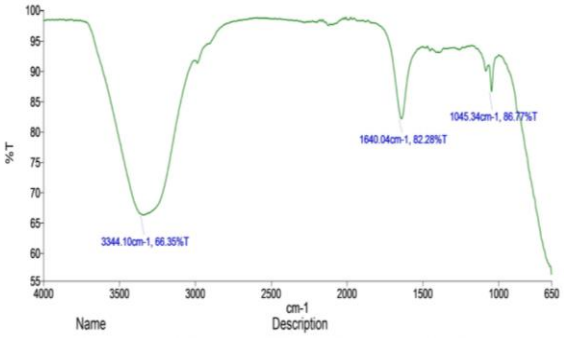


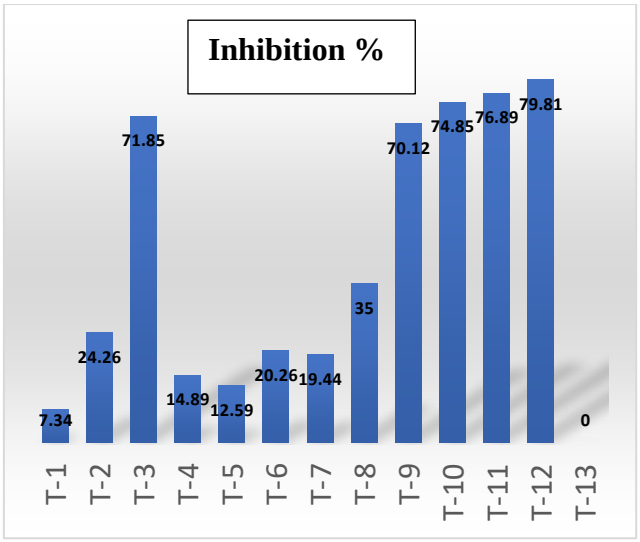
Fig 8- FTIR study of copper nanoparticles

Proteins play a significant role in the biosynthesis of nanoparticles [10]. Cysteine residues in proteins have free amine groups that bind to nanoparticles and thus stabilize AgNPs [11]. The role of NADH-dependent nitrate reductase in the silver ion reduction process [3] has been documented. Proteins' carbonyl groups and peptides have a greater tendency to bind silver ions [12].

ANTIFUNGAL ACTIVITY OF THE COPPER NANOPARTICLES:

Table 2: Effect of Biosynthesized nanoparticles on Colony diameter and inhibition.

	Colony diameter	Inhibition %
T-1	8.39	7.34
T-2	6.82	24.26
T-3	2.62	71.85
T-4	7.66	14.89
T-5	7.87	12.59
T-6	7.18	20.26
T-7	7.25	19.44
T-8	5.85	35
T-9	2.79	70.12
T-10	2.53	74.85
T-11	2.35	76.89
T-12	2.27	79.81
T-13	9.00	0



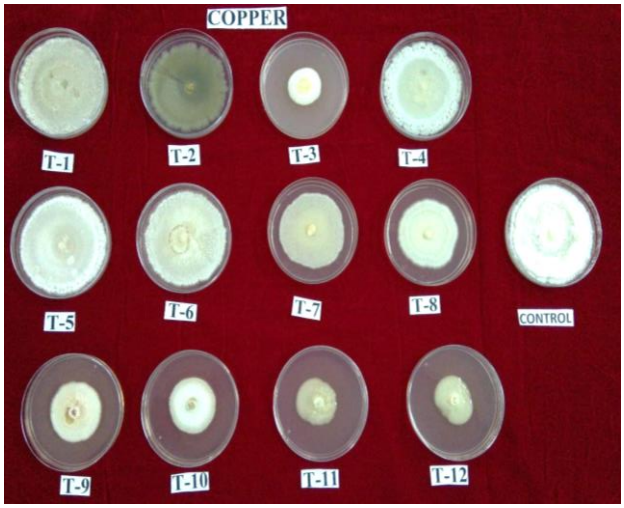


Fig-9: Antifungal activity of biosynthesized Copper nanoparticles against *C. capsici*

To check the antifungal activity of the Copper nanoparticles against *Colletotrichum capsici* at different concentrations, i.e., 25, 50, 100, 200, 400, and 600ppm. Along with these nanoformulation Plant sample 2%, Copper sulfate- 0.1 mM, Copper raw solution which is used as it is without separating nanoparticles, in which the significant zone of inhibition was observed in PDA amended with 100ppm of copper nanoparticles (78.5%) which is on par with Bavistin 2% which is used as a positive control in this the inhibition percentage is 80% (Table-2) . Apart from this highest zone of inhibition, 81.25%, 83.125%, and 84.375% was observed at 200 ppm, 400 ppm & 600 ppm, respectively, but these inhibition rates are on par with the 100 ppm (78.5%) whereas, 80 percent inhibition was observed in Bavistin (0.2%) treated plates. 100 ppm Bio synthesized copper nano solution, and Bavistin (2%) is almost equal. Further, the same resulted in reduced sporulation and Mycelial growth to a greater extent. There was no much deviation in colony and mycelia morphological changes observed.

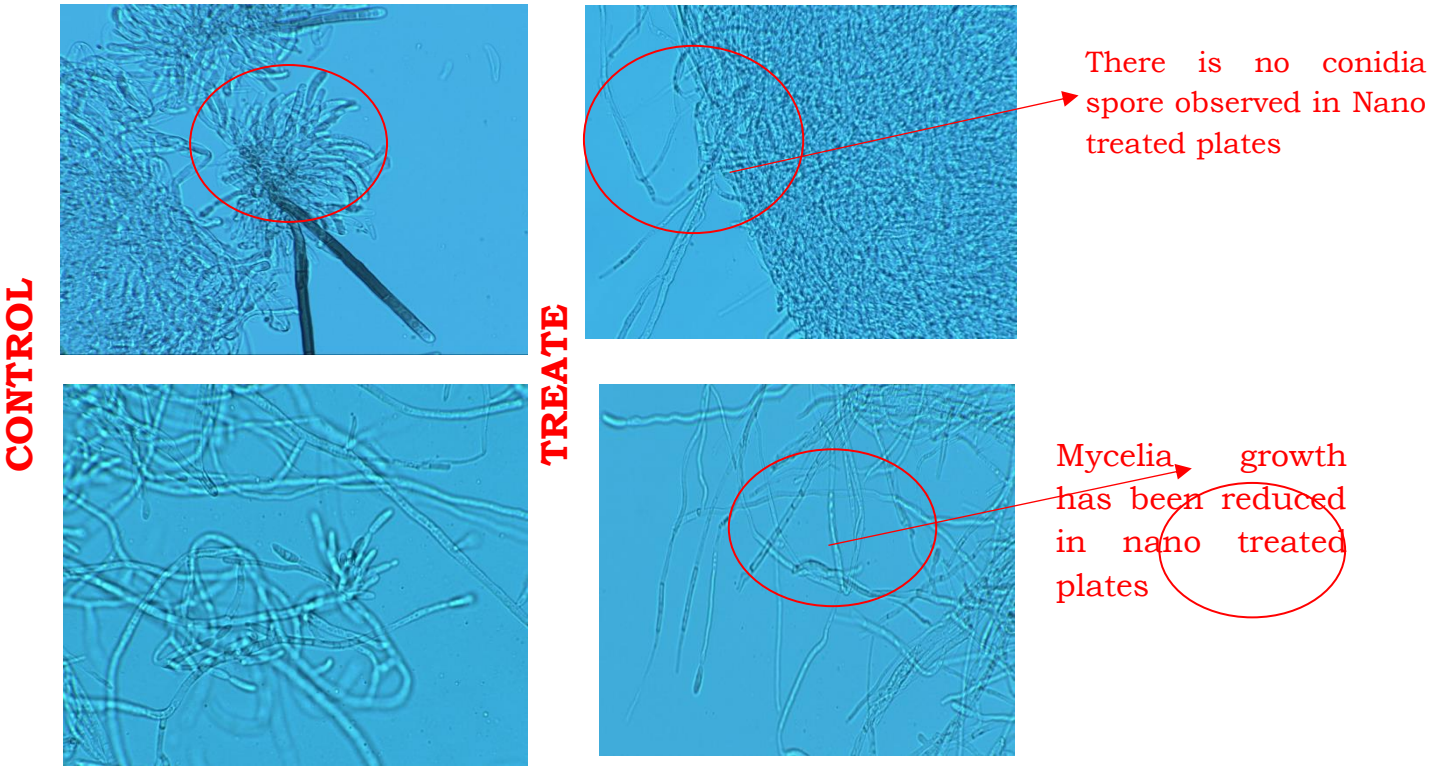


Fig-10 Microscopic observations on the sporulation and mycelial growth of *C. capsici*

Microscopic observations are recorded to know the possible mode of action of biosynthesized nanoparticles and observed that damaged hyphae in treated plates than control plates it clearly indicates that supply of nutrients is affected, also it may be due to physical damage (Fig 10). No sporulation was observed in treated plates, but more sporulation was observed on control plants. It shows the physical damage of nanoparticles against fungi mycelia and Spores. This may be due to the Sporulation of has been arrested due to binding of nanoparticles to the mycelium, and it may damage the cell wall due to leakage of ATP, finally leads to cell death. Also, it may also be due to positively charged ions (Ag^+) act with phosphorus and sulfur present in DNA and RNA. This is perhaps due to such attachment results in the disruption of DNA and RNA functions [13].

The same type of observation recorded by several researchers against many pathogens like Application of silver nanoparticles synthesized from *Acalypha indica* produced significant inhibition of growth of *C. capsici* as well as conidial germination in vitro and recorded 54 and 70% reduction in growth at 100 and 200ppm concentrations of silver nanoparticles, respectively. Sporulation of has been arrested due to the binding of nanoparticles to the mycelium, and it may damage the cell wall due to leakage of ATP, finally leads to cell death. Also, it may also be due to positively charged ions (Ag^+) act with phosphorus and sulfur present in DNA and RNA [14]. This is perhaps due to such attachment results in the disruption of DNA and RNA functions. With respect to reduced sporulation and mycelial growth was observed in nanoparticles amended medium plates, in which ruptured and compressed mycelium. The alterations in membrane permeability and respiration of the silver nanoparticles treated bacterial cells, *Escherichia coli* and *Vibrio cholera*, remained evident from the activity of silver nanoparticles [15].

Copper nanoparticles antimicrobial action may be due to their affinity for the carboxyl group on the microbial surface, CuNPs interact with the microbial cell wall. The antimicrobial action of nanoparticles is attributed to the generation of reactive oxygen species (ROS), membrane damage, loss of enzyme activity, protein dysfunction, and other factors. CuNPs' antibacterial properties were investigated [13]. When CuNPs come into contact with a bacterial cell, they release Cu ions, which are absorbed on the cell wall, causing the development of reactive oxygen species (ROS) and the loss of membrane integrity. CuNPs are also involved in the destruction of cellular metabolic pathways, the creation of pits in membranes, and the growth of oxidative stress, both of which lead to cell death. Cu polymer nanocomposites have been suggested as a potential antibacterial agent. The authors discovered that nanocomposites' bactericidal effect was due to the release of Cu ions and CuNPs [15]. The released Cu ions associate with amines and carboxyl groups in the peptidoglycan layer, as well as sulfhydryl groups, leading to denaturation of the protein when they come into contact with an outer bacterial membrane. Cu ions (Cu^{2+}) bind to DNA and cause cross-linking of nucleic acid strands, causing the helical structure to disorganize. CuNPs released in this manner adhere to the cell membrane and enter the bacterium through endocytosis.

Microbe susceptibility to CuNPs' microbicidal action is largely determined by particle size, electrostatic attraction between microbial cells and nanoparticles, microbial cell wall and membrane composition, and the nanoparticles' hydrophobic or hydrophilic existence [12] .

CONCLUSION:

In conclusion, these nanoparticles were synthesized and showed significant antifungal activity against a fungal pathogen *C. capsici* at 100 ppm concentrations. The various effective concentrations of CuNPs showed good significant results against *C. capsici*. The biosynthesized copper nanoparticles in this study will presumably useful in the formulation of various biopesticides and ecological feasible, effective management strategy against *Colletotrichum capsici*.

The same resulted in reduced sporulation and Mycelial growth to a greater extent. This has promised that the newly synthesized Copper nanoparticles could be used as an antifungal agent for crop disease management, especially in the case of anthracnose.

Acknowledgment: Authors are thankful to the Institute of Excellence (IOE), University of Mysore, Manasagangothri, Mysuru, 570006, Karnataka, India. Authors are also thankful to the Department of Studies in Biotechnology, University of Mysore, Manasagangothri, Mysuru, 570006, Karnataka, India.

REFERENCE

[1] Jha, A.K.; Prasad, K.; Prasad, K. & Kulkarni, A.R. (2009). Plant system: Nature's nanofactory. Colloids and Surfaces B: Biointerfaces (inpress) doi:10.1016/ j.colsurfb.2009.05.018.

[2] Ahmed A., Mukherjee P., Senapati S., Mandal D., Khan M.I., Kumar R., and Sastry M. (2003) Colloids and surfaces B: Biointerfaces, 28(4), 313-318.

[3] Yaqub, A., Malkani, N., Shabbir, A., Ditta, S.A., Tanvir, F., Ali, S., Naz, M., Kazmi, S.A.R. and Ullah, R., 2020. Novel Biosynthesis of Copper Nanoparticles Using Zingiber and Allium sp. with Synergic Effect of Doxycycline for Anticancer and Bactericidal Activity. Current Microbiology, pp.1-13.

[4] Samuel, U. and Guggenbichler, J.P., 2004. Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter. International journal of antimicrobial agents, 23, pp.75-78.

[5] Lee, H. J., Lee, G., Jang, N. R., Yun, H. J., Song, Y. S. and Kim, B. S., 2011, Biological synthesis of copper nanoparticles using plant extract. Nanotech., 3 (1) : 371-73

[6] Intana, W., Suwanno, T., Chamswarng, C., Chantrapromma, K. and Ngamriabsakul, C. (2007). Increased Efficacy for Controlling Anthracnose of Chili Using Antifungal Metabolites from Mutant Strains of Trichoderma harzianum. Thai Journal of Agricultural Science, 40(1-2): 65-72.

[7] Shende, S., Gaikwad, N. and Bansod, S., 2016. Synthesis and evaluation of the antimicrobial potential of copper nanoparticles against agriculturally important phytopathogens. Synthesis, 1(4), pp.41-47.

[8] Sathiyabama, M., Indhumathi, M. and Amutha, T., 2020. Preparation and characterization of curcumin functionalized copper nanoparticles, and their application enhances disease resistance in chickpea against wilt pathogen. Biocatalysis and Agricultural Biotechnology, p.101823.

[9] Amer, M.W. and Awwad, A.M., 2021. Green synthesis of copper nanoparticles by Citrus limon fruits extract, characterization, and antibacterial activity. Chem Int, 7, pp.1-8.

[10] Ovais, M., Khalil, A.T., Islam, N.U., Ahmad, I., Ayaz, M., Saravanan, M., Shinwari, Z.K. and Mukherjee, S., 2018. Role of plant phytochemicals and microbial enzymes in the biosynthesis of metallic nanoparticles. Applied microbiology and biotechnology, 102(16), pp.6799-6814.

[11] Ibrahim, H.M., 2015. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. Journal of Radiation Research and Applied Sciences, 8(3), pp.265-275.

[12] Chen, J., Wu, L., Lu, M., Lu, S., Li, Z. and Ding, W., 2020. Comparative study on the fungicidal activity of metallic MgO nanoparticles and macroscale MgO against soilborne fungal phytopathogens. Frontiers in microbiology, 11, p.365.

[13] Raffi, M., Mehrwan, S., Bhatti, T.M., Akhter, J.I., Hameed, A., Yawar, W. and ul Hasan, M.M., 2010. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. Annals of microbiology, 60(1), pp.75-80.

[14] Zhao, H., Su, H., Ahmeda, A., Sun, Y., Li, Z., Zangeneh, M.M., Nowrozi, M., Zangeneh, A. and Moradi, R., 2020. Biosynthesis of copper nanoparticles using Allium Eriophyllum Boiss leaf aqueous extract; characterization and analysis of their antimicrobial and cutaneous wound-healing potentials. Applied Organometallic Chemistry, p.e5587.

[15] Miyake, K., Underhill, C.B., Lesley, J., and Kincade, P.W., 1990. Hyaluronate can function as a cell adhesion molecule, and CD44 participates in hyaluronate recognition. Journal of Experimental Medicine, 172(1), pp.69-75.