ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 14, Issue, 03, pp.20953-20958, March, 2022

DOI: https://doi.org/10.24941/ijcr.43194.03.2022

RESEARCH ARTICLE

GREEN SYNTHESIS OF COPPER NANOPARTICLES USING MOSQUITO REPELLENT PLANT LEAVES FOR THE MANAGEMENT OF PULSE BEETLES

***Dhanalakshmi, R.L. and Lokesh, S.**

Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India

ARTICLE INFO

Article History:
Received 24th December, 2021
Received in revised form
19th January, 2022
Accepted 24th February, 2022
Published online 30th March, 2022

Keywords:

Plant extracts, CuNps, Pulse beetles, CuNps characterization, Green gram seeds, antimicrobial and antiinsecticidal assay.

****Corresponding author:***
Dhanlakshmi, R.L.

ABSTRACT

The biogenic CuNPs from *Ocimum tenuiflorum* and *Vitex negundo* were subjected for antimicrobial activity against 3 human pathogens *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* along suitable controls for comparison. Among CuNPs biosynthesized using *V. negundo* and *O. tenuiflorum*, *V. negundo* showed high inhibition zones than *O. tenuiflorum*. Nanoparticles treated seeds showed low incidence of seed mycoflora in which the incidence of *Aspergillus niger* and *Curvularia lunata* was reduced to 4%. *Aspergillus flavus* was depleted by 8% over control. Interestingly, the incidence of common field fungi in sorghum was reduced by 8% over control. In general the incidence of all the fungi remained low in CuNPs treated samples compared to control set. It indicated the antifungal effect of biogenic CuNps to some fungi which are commonly affecting the foliages in the sorghum fields during growth. Its occurrence was reduced in biosynthesized CuNPs through *Ocimum tenuiflorum* and *Vitex negundo* leaf extract. Insecticidal activity of the above biosynthesized CuNPs against *Callosobrushus chinensis* was assessed by treating the green gram seeds treated with 20mg/ml of CuNPs. The activity and mortality rate of *C. chinensis* was found decreased compared to control, thus, proving the insecticidal activity of the biosynthesized CuNPs from *Vitex negundo* and *Ocimum tenuiflorum*. Thus, it is proved the possibilities of biosynthesized nanoparticles as an ecofriendly approach to manage the field fungi and storage pests in pulses.

Citation: Dhanalakshmi, R.L. and Lokesh, S. "Green synthesis of copper nanoparticles using mosquito repellent plant leaves for the management of pulse beetles", *2022. International Journal of Current Research, 14, (03), 20953-20958.*

INTRODUCTION

The natural strains of microbes and plant extract secrete some natural chemicals that act as both reducing and capping or stabilization agent. Nowadays, researchers are focusing on the biosynthesis of nanoparticles using noble metals such as zinc, gold, silver, platinum, and palladium, because of their applications in medical and pharmaceutical products. Plants parts like leaf, stem, root, fruit and seed have been used for NPs synthesis because of the exclusive phytochemicals they produce when they react with metal salts. Synthesis of nanoparticles using plant and plant extract is also called as Green synthesis of nanoparticles. It is evident from earlier reports that plants are better candidates for synthesis of nanoparticles than microorganisms. Using plants for nanoparticles synthesis can have advantages over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis. The nanoparticles produced from plants parts are more stable and the rate of synthesis is rapid than in the case of microorganisms. Copper nanoparticles (CuNPs) have been used in various fields, including agricultural, industrial engineering and technological fields. Copper nanoparticles due to their unique biological, chemical, physical properties and the low cost of preparation have been of great interest. Copper is highly toxic to microorganisms and used for food packaging. Due to optical property of CuNPs, it is used in optical devices and optical materials. In recent years, Cu nanoparticles have attracted much attention of researchers due to application in wound dressings and biocidal properties. Copper based nanoparticles (Cu-based NPs) of different composition and sizes have been hydrothermally synthesized by varying the reaction temperature and in the presence of biocompatible surfactants; polyoxyethylene sorbitan laurate, polyethylene glycol 1000 and polyethylene glycol 8000.

The Cu nanoparticles are synthesized from various methods such as vapor deposition, electrochemical reduction radiolysis reduction, thermal decomposition, chemical reduction of copper metal salt and room temperature synthesis using hydrazine hydrate and starch. Mosquitoes are important vectors of diseases and nuisance pests. Repellents minimize contact with mosquitoes. Some of the plants which are also medicinally important have ability to act as mosquito repellents such as lavender, marigold, basil, citronella grass, catnip, rose merry. The juice of the leaves is used for the treatment of foetid discharges and maggots in ulcers. Smoke from dried leaves relieves catarrh and headache. Root-bark is useful in rheumatism and irritable bladder. Roots are also beneficial in boils, cholic, dyspepsia, leprosy. *Ocimum tenuiflorum* commonly known as holy basil is an aromatic perennial plant in the family Lamiaceae. *Callosobruchus chinensis* is a common and major pest of stored legumes. It is one of the serious stored grain insect pests infesting gram, cowpea, beans, lentil and other pulses. Its grubs are the infective stages and cause 32-64% loss under storage conditions during April to October. These make holes in grains and consume inner part leaving empty kernel. Damaged grains become unpalatable for human, incapable of producing sprout and thus, lose its market value. In the present work, mosquito repellent plants (*Vitex negundo* and *Ocimum tenuiflorum*) have been used to synthesize copper nanoparticles and were used to test their anti-microbial as well insecticidal activity against *Callosobruchus chinensis* in the stored green gram seeds.

MATERIALS AND METHODS

The young leaves of mosquito repellent plants like *Ocimum tenuiflorum and Vitex negundo* were collected from in and around Gundlupet taluk of Karnataka, India.

Preparation of Leaf extract: Fresh plant leaf samples collected and selected for study were first washed thoroughly with running tap water for 2 times and then with distilled water for 2 times to remove the debris and other contaminants. Such samples were shade dried for 4-6 days and were made into fine powder using electric blender and stored under dark place for further preparation of extracts. For the preparation of extract 15-20g of powdered leaf sample was taken in to 100ml distilled water and mixed well and boiled for 20 minutes. Then it was filtered using muslin cloth followed by repeated filtration using Whatman No.1 filter paper to get fine leaf extract. Such extracts were used for further studies *in vitro*.

Preparation of Copper Sulphate ($CuSO_4$) solution: For the preparation of 100ml of 3mM $CuSO_4$ solution, 100ml of double distilled water was taken in a series of 250ml beaker added with 47.9mg of $CuSO_4$ salt to each flask and allowed to dissolve completely.

Biosynthesis of Copper nanoparticles: 15ml of each aqueous leaf extracts was taken in 250mL Erlenmeyer flask and stirred at 70°C. To this solution 100mL of 3mM aqueous $CuSO_4$ solution was added slowly with continuous stirring and heating for 20 to 30 minutes. The color of the solution changed from light brown to blackish brown/ black on incubation in dark condition at room temperature overnight and the copper nanoparticles formed were separated out and allowed to settle. The Copper nanoparticles solution thus obtained after incubation was purified by repeated centrifugation at 12,000rpm for 15 minutes, followed by re-dispersion of the pellet using de-ionized water. The slurry obtained was dried in hot air oven at 80°C to get brown powder (Fig. 1).

a b

(a) CuNPs biosynthesized using *Vitex negundo* (b) CuNPs biosynthesized using *Ocimum tenuiflorum*

Fig. 1 . Plants mediated synthesis of CuNps *in vitro*

Characterization of Biosynthesized Copper Nanoparticles

These green synthesized copper nanoparticles were further characterized by UV-Visible spectroscopy, SEM and XRD analysis.

UV-Vis spectroscopy: The reduction of copper ions and stability of copper nanoparticles was monitored by measuring the UV-Vis spectrum of the reaction. UV-Vis absorption spectrum of the samples (Copper nanoparticles synthesized from plant extracts) was performed in a Hitachi U-3000 UV-Vis spectrophotometer. Then, the sample was sonicated and measured in the wavelength range from 200 to 800 nm to determine maximum absorption [λ_{max}]. Stability of copper nanoparticles was determined using UV-Vis Spectroscopy.

Antibacterial Activity of Copper Nanoparticles: The test organisms *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were collected from stock culture collection in the Department of Studies in Biotechnology, Manasagangothri, University of Mysore and were used for the antibacterial assay.

Preparation of inoculum: Stock cultures of the selected microbes were maintained at 4°C on nutrient agar. Active cultures for experiment were prepared by transferring a loopful of cells from the stock culture to the conical flask containing 20ml containing the nutrient broth, which were incubated with agitation for 24 hours at 37°C on a rotary shaker.

Preparation of nanoparticles solution as antibacterial agents: 1, 5 and 10mg of copper nanoparticles powder was taken in 3 different 15ml falcon tubes and dissolved in 1ml distilled water. The solutions were then sonicated at 40% frequency for 15minutes to get homogenized solution and used for further tests.

Antibacterial activity test by Agar disc diffusion method: Kirby-Bauer method was followed for disc diffusion assay. *In vitro* antibacterial activity was screened using nutrient agar (NA) media. The sterile pertiplates before adding medium was marked into 3 parts using a glass marker and labelled each division as positive control, negative control and test sample, respectively. Such plates were poured with sterilised 15ml of molten nutrient medium. The plates were allowed to solidify for 5 minutes and 300µl inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Different concentrations of copper NPs discs were prepared by loading 1mg/ml, 5mg/ml and 10mg/ml on to 5mm sterile individual Whatman No. 1 filter paper discs. Negative control discs was prepared using sterile distilled water and positive controls with Azithromycin (1mg/ml), Ampicillin (1mg/ml) and Tetracycline (1mg/ml) for *E. coli, S. aureus* and *B. subtilis,* respectively. Thus prepared discs were placed on inoculum swabbed pertiplates on their respective division. These plates were then incubated for 24hours at 37^{o}C. After incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeter.

Antifungal Activity of Copper Nanoparticles: The antifungal activity of biosynthesied copper nanoparticles from *Ocimum tenuiflorum* and *Vitex negundo* leaf extract was tested by seed treatment through standard blotter method (SBM). The sorghum seeds were soaked in sonicated nanoparticles (10mg/ml of water) for 2-4 hours, air dried and was plated on petriplates at the rate of 25 seeds per pertriplates containing 3-4 wet blotter discs for maintaining moisture condition.

Along with test samples control plates i.e. sorghum seeds soaked in distilled water was also plated. These plates were incubated for -7 days at room temperature under alternating cycle of light/dark condition. After incubation individual seeds were examined for the presence or absence of fungi under a stereomicroscope and identification was confirmed by examining mycelium and or conidia under a compound microscope. The fungal species present on each seed were recorded and the percentage incidence of each fungus per plate was computed and analysis of the antifungal activities of copper nanoparticles was performed.

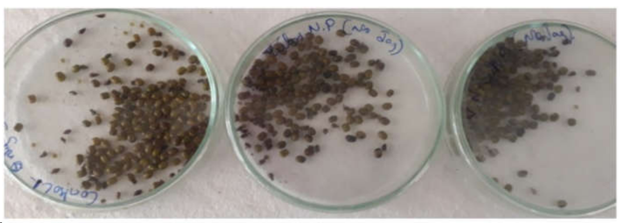
Assessment of Insecticidal Activity of Biosynthesized CuNPs against *Callosobruchus chinensis*

Culturing of *Callosobruchus chinensis*: The culture bottle of *Callosobruchus chinensis* with green gram as feed was collected from Food Protectants and Infestication Control Department, CFTRI, Mysuru. The culture bottle contained around 100 pairs of adult beetles which were further cultured in fresh green gram feed for increased population under semi dark condition. These cultures were used for further experimental purpose.

Insecticidal Activity of CuNPs against *Callosobruchus chinensis* (Pulse Beetles): 5g of fresh green gram seeds were treated with sonicated biosynthesized copper nanoparticles (20mg/ml) taken in clean petridishes. To these petridishes 10-15 pairs of *Callosobrushus chinensis* were added and closed to check the activity of copper nanoparticles on beetles. Along with test samples control i.e. only seeds with pulse beetle was kept for comparison. These pertridishes were kept in dim light area for better growth of pulse beetles. These petridishes were kept for 3-4 weeks for their life cycle. The mortality rate and antifeedant activity of the beetles was checked at regular intervals (Fig. 2).

RESULTS AND DISCUSSION

When copper nanoparticles were synthesized using plant extracts *Ocimum tenuiflorum* and *Vitex negundo* as reducing and stabilizing agent, the aqueous copper sulphate solution turned to brownish black color within 30 min on heating.



Control 1: Green Gram + Pulse Beetles	Test set up1:Green Gram treated with V.negundo Cu NPs + Pulse Beetles	Test set up2:Green Gram treated with O.*tenuiflorum* Cu NPs + Pulse Beetles

Fig. 2. Insecticidal Activity Assessment Test (First day)

Later obtained dried black powder was characterized using the Spectrophotometer. Spectrophotometry is a technique that uses the absorbance of light by an analyte (the substance to be analyzed) at a certain wavelength to determine the analyte concentration. UV/VIS (ultra violet/visible) spectrophotometry uses light in UV and visible part of the electromagnetic spectrum. Stability of the sonicated CuNPs biosynthesized from *Ocimum tenuiflorum* and *Vitex negundo* leaf extract was confirmed using UV-Vis spectroscopic analysis. When the sonicated sample was analyzed in the wavelength range between 200nm to 900nm, the maximum absorbance was noticed around 290nm to 300nm shown in figure 3(a) and3(b). When the spectral analysis was performed after 24, 48, 72 and 96h of incubation, the maximum absorbance was recorded at the same wavelength for all incubation periods as shown with wavelength on x-axis and absorbance on y-axis and the absorbance above 400nm was found to be negative. UV-VIS spectroscopy has provided confirmation for the presence of nanoparticles and their stability (Fig. 3a & b).

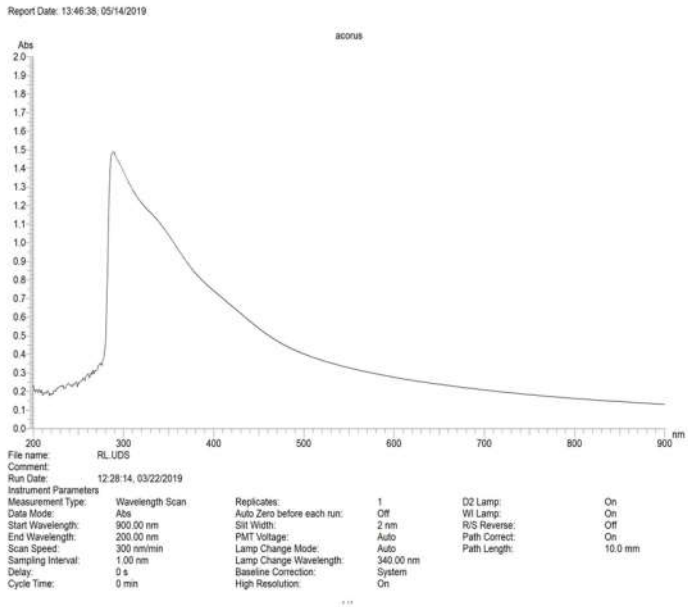


Fig.3(a). UV-Vis spectrum of aqueous medium during biosynthesis of copper nanoparticles using *V. negundo*

Antibacterial (Agar well Diffusion) assay of biosynthesized CuNPs from *Vitex negundo* leaf extract

Antibacterial (Agar well Diffusion) assay of biosynthesized CuNPs from *Ocimum tenuiflorum* leaf extract: The above antibacterial assay information suggested that the minimal inhibitory concentration of these nanoparticle was 10mg/ml and can be used as antibacterial agents at the same concentration.

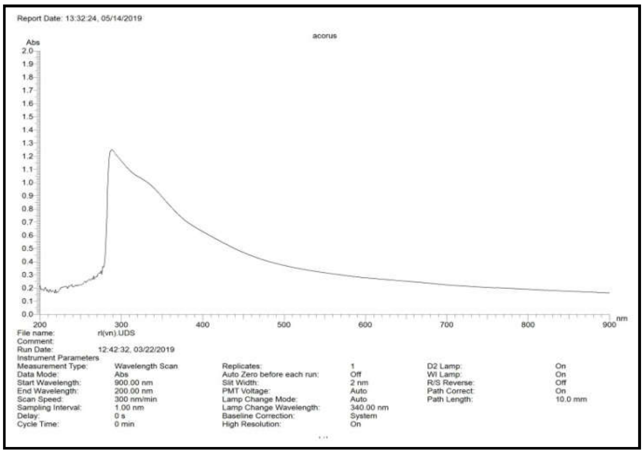


Fig. 3(b). UV-Vis spectrum of aqueous medium during biosynthesis of copper nanoparticles using *O. tenuiflorum*

The increased antibacterial effect may expect with increase in concentration of CuNPs. The best antibacterial activity was found in CuNPs biosynthesized by *Vitex negundo* compared to *Ocimum tenuiflorum* leaf extract (Table 1 & 2; Fig. 4).

Table 1. Antibacterial activity of biosynthesized CuNPs from *Vitex negundo* leaf extract

Microorganisms	Positive control(1mg/ml) (mm)	Negative control (mm)	CuNPs from *Vitex negundo* leaf extract (10mg/ml) (mm)
Escherichia coli	8	-	4
Bacillus subtilis	10	-	2
Staphylococcus aureus	10	-	3

Table 2. Antibacterial activity of biosynthesized CuNPs from *Ocimum tenuiflorum* leaf extract

Microorganisms	Positive control (1mg/ml) (mm)	Negative control (mm)	CuNPs from *Ocimum tenuiflorum* leaf extract (10mg/ml) (mm)
Escherichia coli	7	-	3
Bacillus subtilis	10	-	3
Staphylococcus aureus	8	-	2

1.Positive control = Azithromycin, Ampicillin and Tetracycline for *E. coli, S. aureus* and *B. subtilis*
2.Negative control = Sterile Distilled Water

Antifungal Activity assay: Antifungal activity of biosynthesized CuNPs from *Ocimum tenuiflorum* and *Vitex negundo* leaf extract was performed using standard blotter method. Nanoparticles treated seeds showed low incidence of seed mycoflora in which the incidence of *Aspergillus niger* and *Curvularia lunata* was reduced to 4%. *Aspergillus flavus* was depleted by 8% over control. Interestingly, the incidence of common field fungi in sorghum was reduced by 8% over control. In general the incidence of all the fungi grown in control compared in CuNPs treatment plates, there was decrease upto 50% mycoflora.

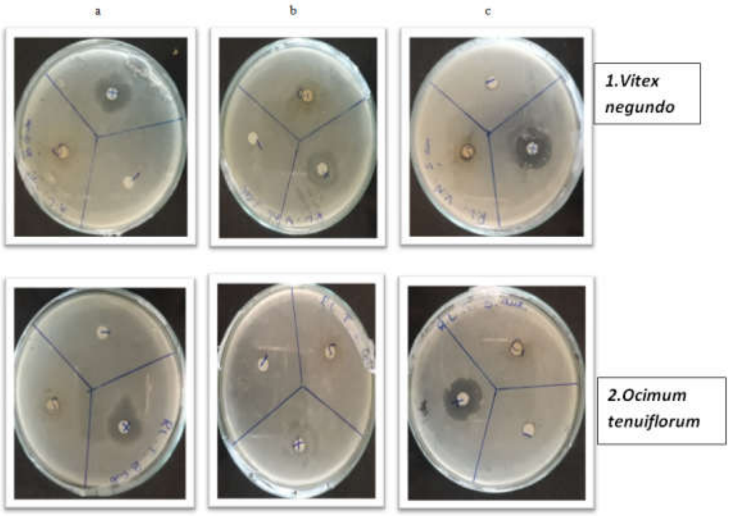
It indicated the antifungal effect to some fungi which are commonly known to affect the foliages in the field during growth, whose occurrence was reduced in biosynthesized CuNPs from *Ocimum tenuiflorum* and *Vitex negundo* leaf extract plates. However, *Aspergillus niger* was reduced in CuNPs treated seeds. The increased antifungal effect may be expected with increase in concentration of CuNPs (Table. 3). The above information suggests the possible application of CuNPs for seed treatment.

Table 3. Antifungal activity of biosynthesized CuNPs

Fungal Species	% Incidence of fungi in Sorghum seeds treated with CuNPs	
	Control(H_2O)	CuNPs
Aspergillus niger	24	20
Curvularia lunata	12	4
Drechslera sorghicola	12	4
Aspergillus flavus	24	16
Chaetomium globosum	4	0
Alternaria alternata	9	0
Nigrospora oryzae	4	0
Penicillium sp.	4	0
Fusarium moniliforme	1	0
Phoma spp.	1	0

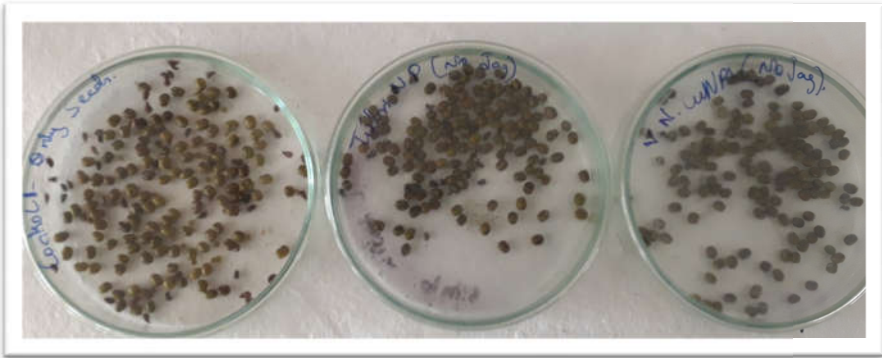
Through, the occurrence of *F. moniliforme* and *Phoma* sp. are common in sorghum it has been reduced in NPs treatment. In parallel the % of seed germination was enhanced by 20% in NPs treated sorghum seeds. The results also indicated the phytostimulation effect in which the seedling vigor also enhanced due to increased root and shoot growth compared to control. The antifungal property of NPs may be due to possible interference with the growth and establishment of fungi, in turn it might have reduced competition by fungi for nutrition and hence, there was enhanced germination and stimulated growth of the seedling with increased vigor. Insecticidal activity of biosynthesized CuNPs using *O. tenuiflorum* and *V. negundo* was assessed by treating green gram with sonicated CuNPs in petridishes. Along with CuNPs samples control pertidishes containing only green gram were also taken. To these petridishes 10-15 pairs of Pulse beetle were allowed to feed on. The petridishes were kept in dark place for 3 to 4 weeks to check the mortality rate and anti-feedant activity of pulse beetle on the green gram (Fig.5). The mortality of the insects in both control and test samples were increased at the initial days of first week due to adaption time of insects, later in further weeks there was increase in population in control pertidishes, but, where in petridishes containing green gram treated with biosynthesized CuNPs using *V.negundo and O.tenuiflorum* showed gradual increased death rate due to their insecticidal activity (Fig. 6a and 6b).

The anti-feedant activity of the insects was also gradually increased and lead to the death of the Pulse beetles in the petridishes containing green gram treated with biosynthesized CuNPs using *V. negundo* and *O. tenuiflorum* due o their insecticidal activity. Using higher concentration of synthesized CuNPs can give better results as insecticides. Hence, the above tests showed that the biosynthesized CuNPs act as insecticides and can be used for further studies lead to its commercialization. It is known that copper (II) sulphate salt when dissolved in water, dissociates into Cu^{2+} and SO_4^{2-}. These Cu^{2+} ions thus formed are unstable and so are reduced to Cu° by reducing action of components present in plant extracts, forming metallic copper nuclei that can further be reduced in size to form nanoparticles. Thus, copper sulphate favors formation of copper nanoparticles. Also the growing nanoparticles are stabilized by several molecules of plant extracts distributed around them. These agglomerates grow to a certain size and eventually get precipitated. Copper ions released may also interact with DNA molecules and intercalate with nucleic acid strands. This encouraged more and more researchers to exploit biological systems as possible eco-friendly nano-factories leading to green synthesis of nanoparticles (Hameed and Al-Samarrai 2012; Adugna and Haile, 2014; Caroling *et al*., 2015; Chung *et al*., 2017).



(a)*Bacillus subtilis* (b) *Escherichia coli* (c) *Staphylococcus aureus*

Fig. 4: Petriplates showing zone of inhibition by biosynthesized CuNPs from 1. *Vitex negundo* and *2.Ocimum tenuiflorum* leaf extract against human pathogens *E. coli, S. aureus* and *B. subtilis* along with positive and negative control.



Control 1: Green Gram + Pulse Beetles	Test 1: Green Gram treated with V.negundo Cu NPs + Pulse Beetles	Test 2: Green Gram treated with O.tenuiflorum Cu NPs + Pulse Beetles

Fig. 5. Insecticidal Activity Assessment Test (24th day)

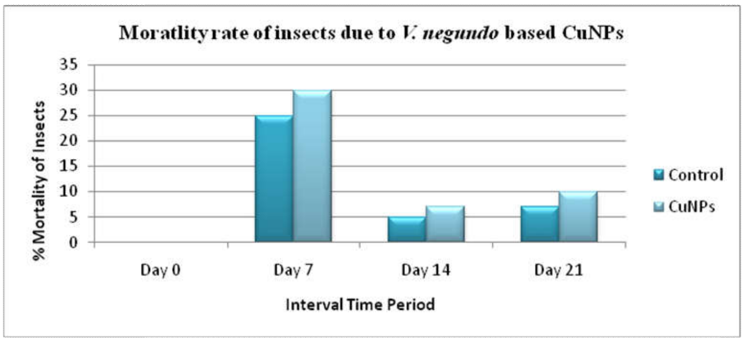


Fig. 6a. Effect of *V. negundo* based CuNPs on the population of Pulse beetles

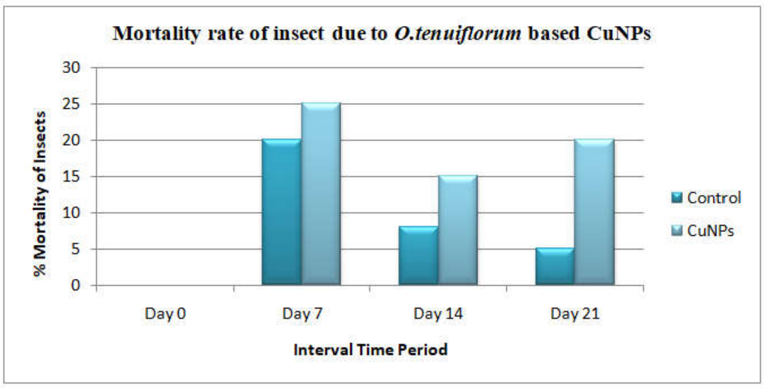


Fig. 6b. Effect of *O. tenuiflorum* based CuNPs on the Pulse beetles

Antimicrobial activity of CuNPs was taken as base effect to check the insecticidal activity of them. Mode of action of copper nanoparticles on pulse beetle have not been studied before, hence, it is not clear how these copper nanoparticles reduce the activity and increase the mortality rate of pulse beetle when pulses like cow pea, green gram etc., are treated with biosynthesized copper nanoparticles. These CuNPs may interact with the intestinal enzymes of Pulse beetles which may results in pathetic situation lead to their death.

CONCLUSION

In conclusion, here we report eco-friendly synthesis of Cu nanoparticles using leaf extract of *Ocimum tenuiflorum* and *Vitex negundo*. Result of our study suggested that mosquito repellent plant extracts are of great medicinal use and copper nanoparticles synthesized from them has natural biocidal activity with broad spectrum for many microbes and pests.

REFERENCES

Hameed, M. and Al-Samarrai 2012. Nanoparticle as alternative to pesticides in management of plant disease- A Review. *International Journal of Scientific and Research Publications*, 2(4).

Adugna and Haile 2014. Eco-friendly management of chickpea storage pest, *Callosobruchus chinensis* L. (Coleoptera; Bruchidae) under laboratory conditions in Eritrea. *Journal of Stored Products and Postharvest Research,* 6(8): 66-71.

Caroling, G., Vinodhini, E., Ranjitham, A. M., and Shanthi, P. (2015). Biosynthesis of copper nanoparticles using aqueous Phyllanthus embilica (Gooseberry) extract-characterisation and study of antimicrobial effects. *Int. J. Nano. Chem*, *1*(2), 53-63.

Chung, I. M., Abdul Rahuman, A., Marimuthu, S., Vishnu Kirthi, A., Anbarasan, K., Padmini, P., & Rajakumar, G. (2017). Green synthesis of copper nanoparticles using Eclipta prostrata leaves extract and their antioxidant and cytotoxic activities. *Experimental and therapeutic medicine*, 14(1), 18-24.

****