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Investigation of antimicrobial activity of copper nanoparticle against plant pathogenic microorganisms

KD Padole, PK Lokhande, US Dalvi, BM Bhalerao, SG Mohite, DP Kachare, SV Damame and RM Naik

Abstract
Development of green nanotechnology is generating interest of researchers toward ecofriendly biosynthesis of nanoparticles. In this study, an attempt has been made to utilize a major bio waste product, pomegranate fruit peel (*Punica granatum* L.) to synthesize copper nanoparticle (CuNPs). The CuNPs were synthesized using the aqueous extract of pomegranate fruit peel extract as reducing and capping agent. The pomegranate fruit peel extract synthesized CuNPs were confirmed by X-ray diffraction (XRD), scanning electron microscope (SEM) as well as through the change of blue colour to dark brown solution. XRD pattern of copper nanoparticles demonstrated crystalline nature of the particles. The diffraction patterns observed three strongest peaks at 2ɵ = 26.75 (peak intensity 502), 25.72 (282) and 28.69 (380) were assigned to the reflection lines of copper nanoparticles. SEM micrographs of pomegranate fruit peel extract synthesized CuNPs. The pomegranate fruit peel extract synthesized CuNPs were screened for antimicrobial activity against two Gram-positive bacteria *Xanthomonas axonopodis* pv. *punicae* and *Xanthomonas axonopodis* pv. *malvacearum* and five fungi *viz*., *Penicillium chrysogenum*, *Macrophomina phaseolina*, *Rhizoctonia bataticola*, *Alternaria carthani* and *Fusarium oxysporum*. The antimicrobial activity of pomegranate fruit peel extract synthesized CuNPs was determined by paper disc diffusion method. The maximum zone of inhibition (17 mm) with 20 ppm concentration was exhibited against *Xanthomonas axonopodis* pv. *punicae* as compared with conventional antibacterial drug streptocycline. The application of 20 ppm CuNPs produced maximum mycelial growth inhibition zone of 18 mm *Alternaria carthani*. These results suggest that pomegranate fruit peel extract synthesized CuNPs could be used as effective control measure for bacteria and fungi.

Keywords: Green synthesis, pomegranate fruit peel extract, copper nanoparticle, antimicrobial activity

Introduction
Nanotechnology is an important field of modern research dealing with design, synthesis and manipulation of particle structure. Remarkable growth in this upcoming technology has opened novel fundamental and applied frontiers, including the synthesis of nanoscale materials and exploration or utilization of their exotic physiochemical and electronic properties (Wang, 1991) [32]. There is growing need to develop eco-friendly nanoparticles synthesis process without use of toxic chemicals to avoid adverse effects in bio medicals application. In the field of nano science and nanotechnology, the largest activity has been focused on synthesis of new nanoparticles with different sizes and shapes, which have strong effect on their widely varying properties. Green chemistry is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health (Gopalkrishnan *et al.*, 2012) [10]. There are various chemical methods (Murray *et al*., 2002) [20] and physical methods (Ayyub *et al.*, 2001) [3] to synthesize nano particles, but these routes for synthesis of particles/crystallites require tedious and environmentally challenging techniques. Hence, there is scope to develop new methods for the synthesis of nanoparticles which should require inexpensive reagent, less drastic reaction condition and eco-friendly. In recent, green synthesis of copper nanoparticles was achieved by using microorganism and plant extract. In producing nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way (Li *et al.*, 2011) [16]. The nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics (Dwivedi and Gopal, 2010) [8].

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity (Jiang *et al.*, 2009) [12]. Homogeneity of these properties is important in many applications. The common techniques of characterizing nanoparticles are as follows UV–Visible spectrophotometry, dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) (Feldheim and Foss, 2002 and Shahverdi *et al*., 2011) [9, 27].

Pomegranate (*Punica granatum* L.) fruits, fruit juice, its seeds, and peels are known to have higher contents of bioactive compounds *viz*., phenolic acids, flavonoids, and hydrolysable tannins. The peels of pomegranate fruits are the major by-products produced during food processing of pomegranate enriched in antioxidants and broad-spectrum antimicrobial agents Chen *et al.* (2020) [5]. Prashanth *et al.* (2001) [24] tested a number of extracts of pomegranates against a range of bacteria *S. aureus*, *E.coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella typhi*. Pomegranate extracts were able to inhibit not only the growth of *S. aures* but also the production of enterotoxin (Braga *et al.*, 2005) [4]. The CuNPs exhibited better antibacterial efficacy by inhibiting the growth and multiplication of pathogenic bacteria like *Escherichia coli* and *Pseudomonas* spp. In contrast, CuNPs exhibited outer membrane destabilization and splitting of plasma membrane there by causing reduction of intracellular ATP (Hajipour *et al.*, 2012) [11]. Bacterial membrane permeability increases with copper cations interaction results in severe changes in the membrane structure of bacteria (Lok *et al.*, 2006) [17]. Maximum zone of inhibition in *Xanthomonas* spp. were observed (*X. citri*-31.67 mm, *X. punicae*- 31.67 mm, *X. malvacearum*-32.33 mm) when the peel of Ganesh variety was extracted with methanol: water (80:20). When the pomegranate peel extract of same three solvents were tested for antifungal activity on *Fusarium oxysporum* f.sp. *ciceri* it was observed that only methanol: water (80:20) extract showed zone of inhibition (Manapure *et al.*, 2015) [19]. Currently, copper nanoparticles have received considerable attention because of their potential for use in nanomaterials, thermal conducting applications, lubrication, nano fluids, and catalysts (Lu *et al.*, 2001) [18]. In view of the above importance of copper nanoparticles the present research work is proposed with objectives *viz*., to characterize the copper nanoparticle synthesized from pomegranate peel extract and to assess antibacterial activity of green copper nanoparticle against plant pathogenic microorganisms.

Materials and Methods

Pomegranate fruit peel extract

The pomegranate fruit peel of Ganesh variety were shade dried and powdered in grinder to 40-mesh. Five gram dried powder of fruit peels was heated in 100 ml of water at 100 °C for 20 minutes. The extract was then filtered through Whatman no. 42 filter paper to remove the fine particles. The volume of extract was made to 100 ml with sterile distilled water.

Synthesis of copper nanoparticle form pomegranate fruit peel extract

Pomegranate peel extract were used as reducing agent for synthesis of copper nanoparticles. Twenty milliliter pomegranate peel extract taken in beaker and 80 ml $CuSO_4$ (100 mM) solution was added in it and kept it for precipitation for 24 hrs. After 24 hrs at room temperature 25 °C, the colour of solution turned to deep green colour that indicates the systhesis of copper nanaparticles. The content was then centrifuged the content at 10,000 rpm for 10 min. The precipitated semi-solid sample was collected using spatula and kept it for drying in hot air oven. After few minutes black coloured crystalline particles were formed as copper nanoparticles.

Characterization of pomegranate peel extract synthesized CuNPs

X-Ray Diffraction (XRD) and scanning electron microscopy (SEM) techniques were used for characterization of pomegranate peel extract synthesized CuNPs.

Antimicrobial activity of pomegranate peel extract synthesized CuNPs

Antimicrobial potential of copper nanoparticles was evaluated by recording the diameter of zone of inhibition.

Culturing of bacteria on nutrient agar media

Nutrient agar medium was used for culture of bacterial samples. The media was prepared by dissolving all the media components in appropriate quantity in distilled water and the volume made up to 1 liter. This was then autoclaved at 121 °C and 15-psi pressure for 15-20 minutes. Bacterial cultures were incubated on nutrient agar medium at 37 °C for 24 hrs. Composition of nutrient agar media: peptone (5g), yeast extract (3 g), NaCl (5 g), agar agar (20 g), distilled water (1000 ml) and pH adjusted to 6.8.

Culturing of fungi on potato dextrose agar media

Potato dextrose agar (PDA) medium was used for culture of fungal samples. The 200 g peeled potato slices boiled and filtered using muslin cloth. To this 20 g of dextrose and agar powder added and volume made up to 1 liter. This was then autoclaved at 121 °C and 15-psi pressure for 15-20 minutes. Fungal cultures were incubated on potato dextrose agar (PDA) medium at 26-28 °C for 7-10 days. Composition of potato dextrose agar media: peeled potato (200 g), dextrose (20 g), agar agar (20 g) and distilled water (1000 ml)

Evaluation of bacterial minimum inhibitory concentration

The antibacterial activity of the copper nanoparticles synthesized from pomegranate peel extract was tested on different strain of *Xanthomonas* spp. using the disc diffusion method. Active culture for the experiments were prepared by taking suspension on bacterial strain and mixed with sterilized Nutrient Agar (NA). This NA containing bacterial strains was poured in petri plates. The sterile disc deeped in different sample extract having 10-20 ppm volume of extract placed on surface on any medium. Plates were incubated at 25±2 °C. After 48 hours the inhibition zones around the disc were measured. Two disc with streptocycline place in 6-7 and 4-5 disc of $CuSO_4$ and one disc of control also placed on surface of NA in petri plates.

Evaluation of fungal minimum inhibitory concentration

The above same procedure was followed for *Penicillium chrysogenum* using Potato Dextrose Agar (PDA) to determine antifungal activity. The antifungal activity of extract also tested on *Macrophomina phaseolina*, *Rhizoctonia bataticola*

by mixing different concentration of copper nanoparticles synthesizing pomegranate peel extract in PDA. Poured this mixture in petri plates and a bit of culture placed in petri plates. Allow it to grow in incubator at 25±2 °C. After 4 days growth of zone measured in mm and same procedure was followed for control.

Results and Discussion

Characterization of pomegranate peel extract synthesized CuNPs X-Ray Diffraction (XRD) analysis of pomegranate peel extract synthesized CuNPs

The two theta peak intensities of the pomegranate peel extract synthesized CuNPs was analyzed by XRD; the peak intensities were measured at voltage 40kV and current 30 mA. XRD patterns of pomegranate peel extract synthesized CuNPs is depicted in Plate 1. The XRD pattern of pomegranate peel extract synthesized CuNPs demonstrates crystalline nature of the particles. The diffraction patterns were observed strongest three peaks at 2θ = 26.75 (integrated intensity 502), 25.72 (282) and 28.69 (380). These peaks intensity are characteristic of the face centered cubic (FCC) structure of CuNPs synthesized from pomegranate peel extract. The totals of 63 peak with the different integrated intensity were observed. The present experimental result where found to be in agreement with reported diffraction pattern of CuNPs prepared by Das *et al*. (2013) [7] and Shende *et al*. (2016) [28].

Scanning Electron Microscope analysis of pomegranate peel extract synthesized CuNPs

The surface morphology of the pomegranate peel extract synthesized CuNPs was analyzed by SEM; image was captured and magnification using a 5kV current is depicted in Fig. 1 and Table 1. The morphology of the synthesized CuNPs using pomegranate peel extract was observed through SEM. The observations revealed that the CuNPs were crystalline nature.

The findings the present research work is in agreement with earlier report of Salam *et al*. (2012) [26] (size 30 to 55um). SEM played a key role to explore the size and morphology of the NPs because of biological behavior of NPs strongly depends on size and shape (Laurent *et al*., 2008) [15].

In vitro antimicrobial bioefficiency of pomegranate peel extract synthesized CuNPs

The bioefficacy of antimicrobial activity of the pomegranate peel extract synthesized copper nanoparticles was analyzed on the basis of the zone of inhibition obtained by disc diffusion method. Copper nanoparticles exhibited strong antimicrobial activity against bacterial pathogen such as *Xanthomonas axonopodis* pv. *punicae*, *Xanthomonas axonopodis* pv. *malvacearum*, and fungal plant pathogens *viz*., *Penicillium chrysogenum*, *Macrophomina phaseolina*, *Rhizoctonia bataticola*, *Alternaria carthami* and *Fusarium* oxysporum f. sp. *carthami*.

Antibacterial activity of pomegranate peel extract synthesized CuNPs

Plant-disease management using biological culture methods and conventional breeding to generate disease-tolerant varieties has been practiced for many years, but it has given only mediocre results. This could be because of environmental conditions, which ultimately play a role in disease spread. This problem has been successfully tackled with synthetic chemicals, such as the antibiotic streptocycline. However, continuous use of antibiotics has led to the development of resistant bacterial species, making streptocycline less effective (Thayer and Stall, 1962) [30]. In recent years, nanotechnology has emerged as an intriguing alternative to overcome pest menace in the agricultural sector.

Xanthomonas axonopodis pv. *punicae* (Xap)

Pomegranate (*Punica granatum* L.) is one of the major cash fruit crops grown in India and particular in Maharashtra as well as Gujarat, and Xap is known to hamper its production. Very little information is available on the disease management of the bacteria by antibiotics, chemicals or biological practices (Kumar *et al.*, 2009) [14].

The pomegranate peel extract synthesized CuNPs *in vitro* antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* was evaluated by disk diffusion method and it is presented in Plate 2. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 11, 14 and 17 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 6 and 7 mm at 10 and 20 ppm concentrations, while the positive control, streptocycline, yielded inhibition zone of 5.5 and 7 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 17 mm at 20 ppm was observed against *Xanthomonas axonopodis* pv. *punicae*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and streptocycline showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs.

The *Ocimum sanctum* leaf extract synthesized copper nanoparticles (CuNPs) recorded the maximum zone of inhibition (17.25±0.95 mm) against *Xanthomonas axonopodis* pv. *punicae* (Shende *et al*., 2016) [28]. AgNP treatment of *Xanthomonas axonopodis* pv. *punicae* generated an inhibition zone of 13.5±0.7 mm at MBC (12.5 µg mL^{-1}AgNPs), and 18±1 mm at 50 µg mL^{-1} concentration (Vanti *et al.*, 2020) [31]. The chitosan-CuNPs were effectively inhibited the growth of Xap at 1000 ppm concentration and remained on par with standard antibiotic check streptocycline of 500 ppm (Chidanandappa and Nargund, 2020) [6]. The results obtained in the present research work are in agreement with the earlier work of other researcher.

Xanthomonas axonopodis pv. *malvacearum* (Xam)

The pomegranate peel extract synthesized CuNPs *in-vitro* antibacterial activity against *Xanthomonas axonopodis* pv. *malvacearum* was evaluated by disk diffusion method and it is presented in Plate 2. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 7, 9 and 12 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 7 and 8 mm at 10 and 20 ppm concentrations, while the positive control, streptocycline, yielded inhibition zone of 6 and 10 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 12 mm at 20 ppm was observed against *Xanthomonas axonopodis* pv. *malvacearum*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and streptocycline

showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs, except 20 ppm streptocycline.

The silver nanoparticles (SNP) synthesized from aqueous extract of fresh leaves from *Leea coccinea* L. showed a promising antibacterial activity against Xanthomonas phaseoli pv. *phaseoli* (Novelles *et al*., 2021) [21]. Biogenic AgNPs showed substantial antibacterial activity (24.21±1.01 mm) for *Xanthomonas oryzae* pv. *oryzae* bacterial leaf blight (BLB) disease of rice (Ahmed *et al*., 2020) [2]. The results obtained in the present research work are in agreement with the earlier work of other researcher.

Antifungal activity of pomegranate peel extract synthesized CuNPs

Penicillium chrysogenum

The pomegranate peel extract synthesized CuNPs *in-vitro* antifungal activity against *Penicillium chrysogenum* was evaluated by disk diffusion method and it is presented in Plate 3. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 7, 12 and 15 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 7 and 8 mm at 10 and 20 ppm concentrations, while the positive control, carbendazim, yielded inhibition zone of 8 and 10 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 15 mm at 20 ppm was observed against *Penicillium chrysogenum*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and carbendazim showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs.

The green synthesis of AgNPs using *H. leucopus* mushroom extract showed the highest inhibition in spore germination at highest concentrations of AgNPs against *Penicillium chrysogenum* followed by *Aspergillus niger* and *Alternaria alternata* (Talie *et al*., 2020) [29]. *Moringa oleifera* seeds extract synthesized iron nanoparticles (FeNPs) showed higher zones (17 mm) for *Penicillium chrysogenum* (Pradhipa and Iyer, 2019) [23]. The results obtained in the present research work are in agreement with the earlier work of other researcher.

Macrophomina phaseolina

The pomegranate peel extract synthesized CuNPs *in-vitro* antifungal activity against *Macrophomina phaseolina* was evaluated by disk diffusion method and it is presented in Plate 3. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 7, 9 and 12 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 6 and 6.5 mm at 10 and 20 ppm concentrations, while the positive control, carbendazim, yielded inhibition zone of 5.6 and 11 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 12 mm at 20 ppm was observed against *Macrophomina phaseolina*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and carbendazim showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs.

Silver nanoparticles from *Yucca shilerifera* leaf extract (AgNPs) showed broad spectrum antagonism against *Fusarium solani* (83.05%) and *Macrophomina phaseolina* (67.05%) when compared to the control after nine days of incubation (Ruiz-Romero *et al*., 2018) [25]. Abdelmoteleb *et al*. (2020) [1] reported that the silver nanoparticles synthesized by biological method using aqueous extract of *Abronia villosa* recorded highest inhibition of *Macrophomina phaseolina* (86.06 ± 0.92%). The results obtained in the present research work are in agreement with the earlier work of other researcher.

Rhizoctonia bataticola

The pomegranate peel extract synthesized CuNPs *in-vitro* antifungal activity against *Rhizoctonia bataticola* was evaluated by disk diffusion method and it is presented in Plate 3. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 5, 7 and 10 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 6 and 7 mm at 10 and 20 ppm concentrations, while the positive control, carbendazim, yielded inhibition zone of 6 and 8 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 10 mm at 20 ppm was observed against *Rhizoctonia bataticola*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and carbendazim showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs.

The *Ocimum sanctum* leaf extract synthesized copper nanoparticles (CuNPs) recorded the zone of inhibition (10±0.81 mm) against *Rhizoctonia solani* (Shende *et al*., 2016) [28]. Chitosan-copper nanoparticles (Ch-CuNPs) showed 63.6 ± 3.5% and 94.3 ± 2.1% mycelial growth inhibition for *Rhizoctonia solani* at 0.05 and 0.1%, respectively in potato dextrose broth (Vanti *et al*., 2020) [31]. The results obtained in the present research work are in agreement with the earlier work of other researcher.

Alternaria carthami

The pomegranate peel extract synthesized CuNPs *in-vitro* antifungal activity against *Alternaria carthami* was evaluated by disk diffusion method and it is presented in Plate 3. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 10, 16 and 18 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 6 and 7 mm at 10 and 20 ppm concentrations, while the positive control, carbendazim, yielded inhibition zone of 6 and 8 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 18 mm at 20 ppm was observed against *Alternaria carthami*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and carbendazim showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs. The *Ocimum sanctum* leaf extract synthesized copper nanoparticles (CuNPs) recorded the maximum zone of inhibition (18.5±1.7 mm) against *Alternaria carthami* (Shende *et al*., 2016) [28]. The results

obtained in the present research work are in agreement with the earlier work of other researcher. The results obtained in the present research work are in agreement with the earlier work of other researcher.

Fusarium oxysporum

The pomegranate peel extract synthesized CuNPs *in-vitro* antifungal activity against *Fusarium oxysporum* was evaluated by disk diffusion method and it is presented in Plate 3. The pomegranate peel extract synthesized CuNPs showed inhibition zone of 8, 12 and 14 mm at 10, 15 and 20 ppm concentrations. The negative control $CuSO_4$ showed inhibition zone of 6 and 8 mm at 10 and 20 ppm concentrations, while the positive control, carbendazim, yielded inhibition zone of 8 and 11 mm at 10 and 20 ppm concentrations. The absolute control, sterile distilled water did not show any zone of inhibition. The maximum zone of inhibition 14 mm at 20 ppm was observed against *Fusarium oxysporum*. As concentration of pomegranate peel extract synthesized CuNPs increased from 10 to 20 ppm the corresponding increase in zone of inhibition was observed. Copper sulphate and carbendazim showed less zone of inhibition as compared to pomegranate peel extract synthesized CuNPs.

The absolute inhibition (100%) was observed on PDA medium against *Alternaria solani*, *Cylindrocarpon destructans*, *Fusarium* sp., *Pythium aphanidermatum* and *Pythium spinosum* when treated with a 100 ppm concentration of AgNPs (Kim *et al.*, 2012) [13]. The green-synthesized Cu-NPs are potential fungicidal action at different concentrations as restricting mycelial growth of *Fusarium solani*, *Neofusicoccum* sp., and *Fusarium oxysporum* (Pariona *et al.*, 2019) [22]. The results obtained in the present research work are in agreement with the earlier work of other researcher.

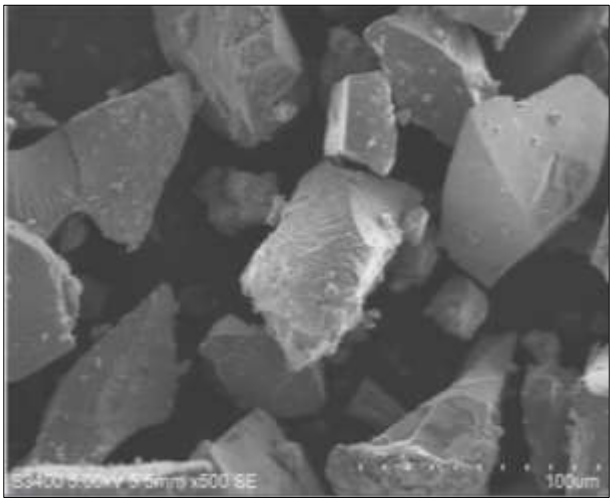


Plate 1: TEM image from 100 µm magnification of pomegranate peel extract biosynthesized copper nanoparticle (CuNPs)

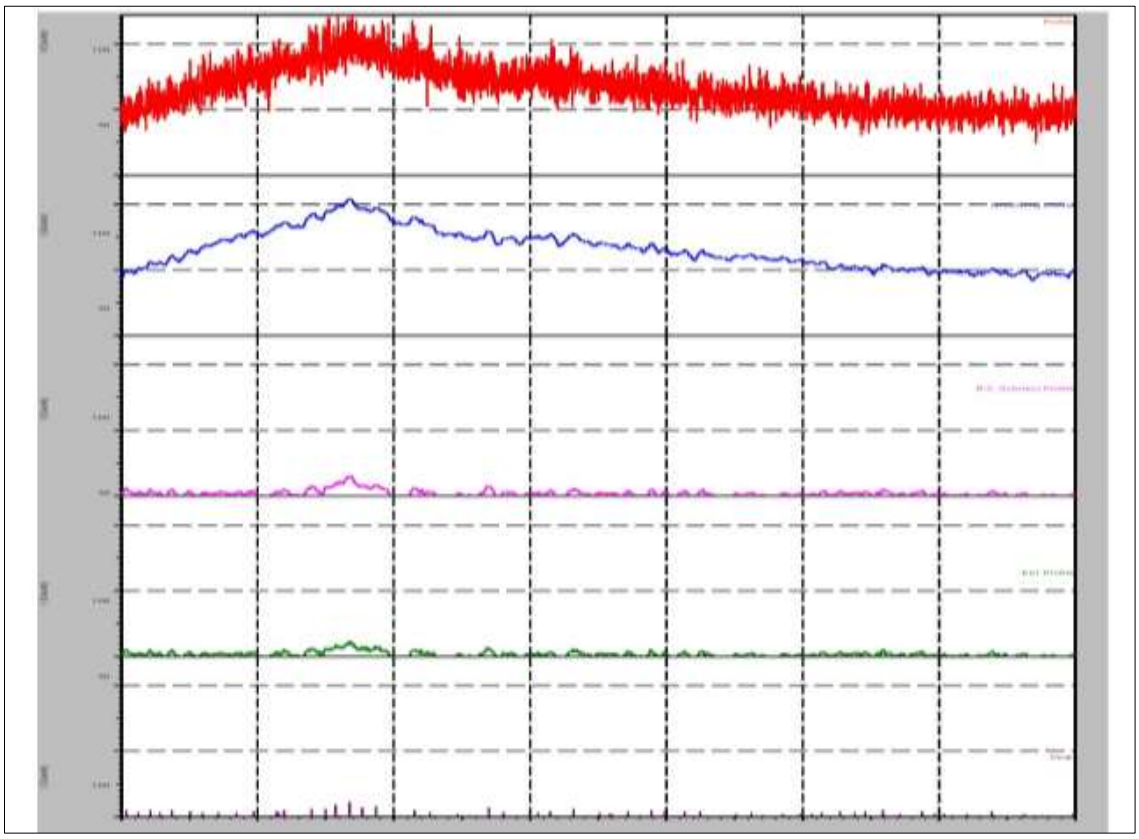
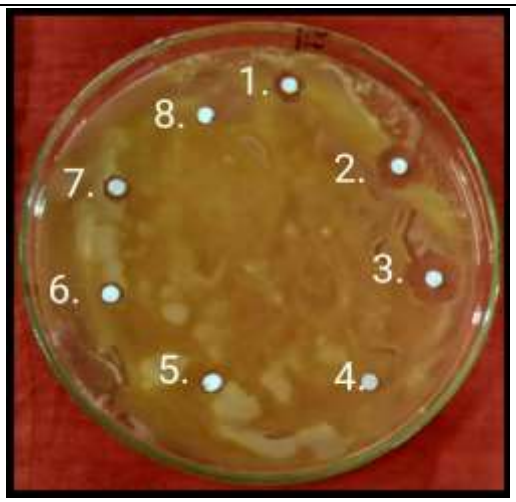


Fig 1: X-ray diffraction pattern of CuNPs biosynthesized from pomegranate peel extract.

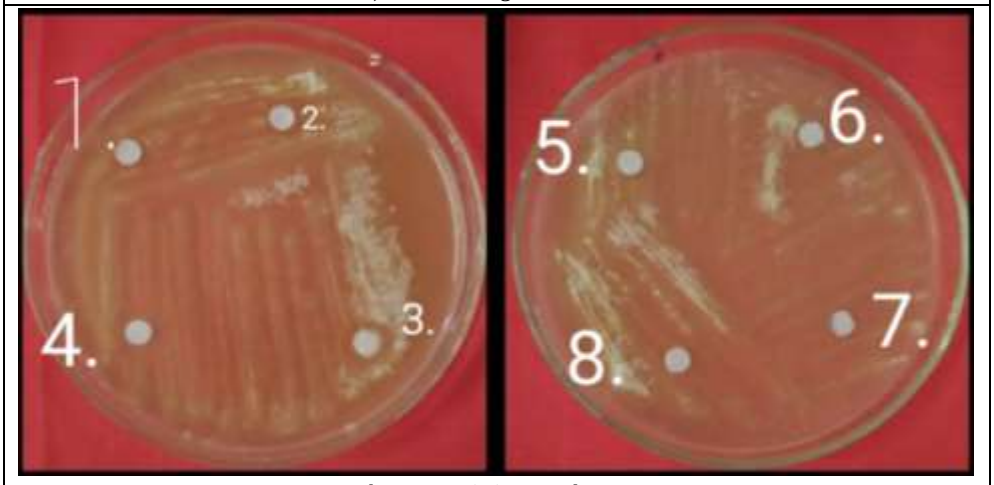
Table 1: XRD strongest peak data of pomegranate peel extract biosynthesized copper nanoparticle (CuNPs)

Sr. No.	Peak No.	2 Theta (deg)	D (A)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Intensity (Counts)
1.	17	26.7500	3.32997	100	0.86000	11	502
2.	16	25.7200	3.46094	82	0.44000	9	282
3.	19	28.6900	3.10906	73	0.78000	8	380



Xanthomonas axonopodis pv. *punica*

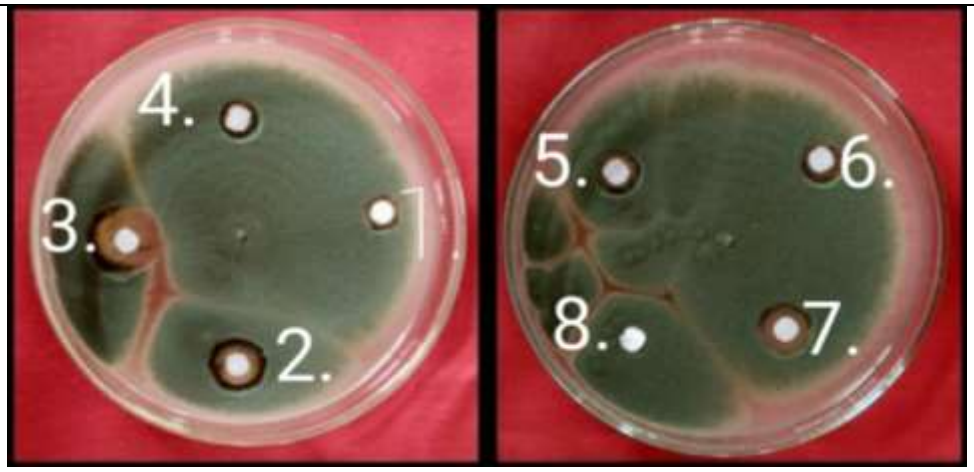
The number on plate represents:
1) 10 ppm CuNPs = 11 mm
2) 15 ppm CuNPs = 14 mm
3) 20 ppm CuNPs =17 mm
4) 10 ppm $CuSO_4$ = 6 mm
5) 20 ppm $CuSO_4$ = 7 mm
6) 10 ppm (anti) = 5.5 mm
7) 20 ppm (anti) = 7 mm
8) Water = Negative control



Xanthomonas citri pv. *malvacearum*

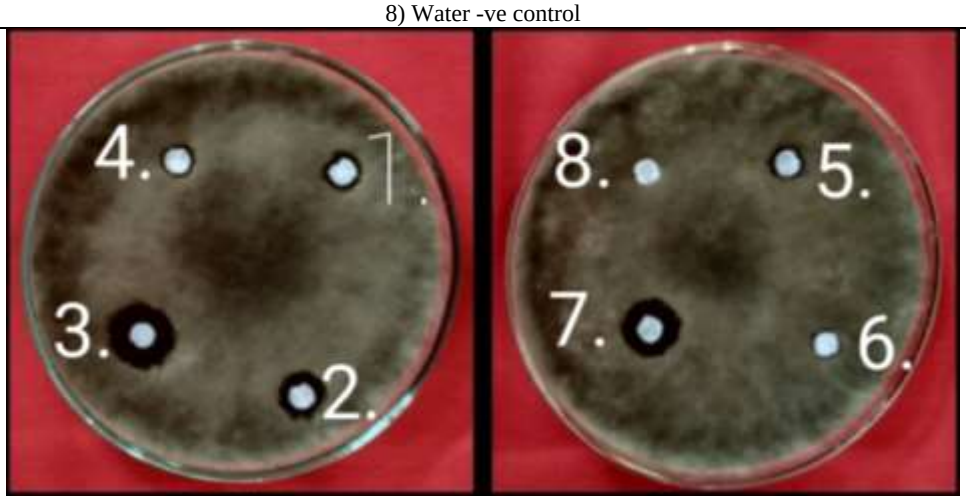
1) 10 ppm CuNPs = 7 mm
2) 15 ppm CuNPs = 9 mm
3) 20 ppm CuNPs = 12 mm
4) 10 ppm $CuSO_4$ = 7 mm
5) 20 ppm $CuSO_4$ = 8 mm
6) 10 ppm (anti) = 6 mm
7) 20 ppm (anti) = 10 mm
8) Water = Negative control

Plate 2: Antibacterial activities of pomegranate peel extract biosynthesized copper nanoparticle (CuNPs) against plant bacteria



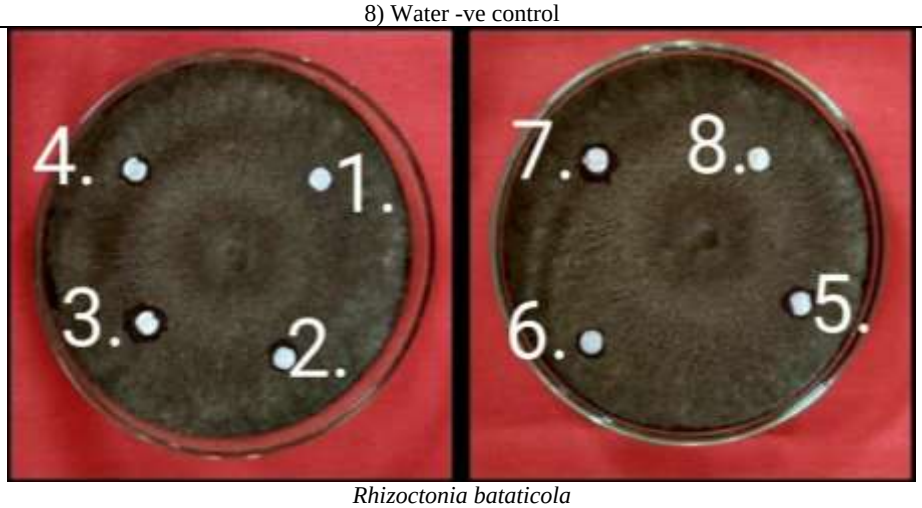
Penicillium chrysogenum

The number on plate represents:
1) 10 ppm = 7 mm
2) 15 ppm = 12 mm
3) 20 ppm = 15 mm
4) 10 ppm $CuSO_4$ = 7 mm
5) 20 ppm $CuSO_4$=8 mm
6) 10 ppm (anti) = 8 mm
7) 20 ppm (anti) = 10 mm
8) Water -ve control



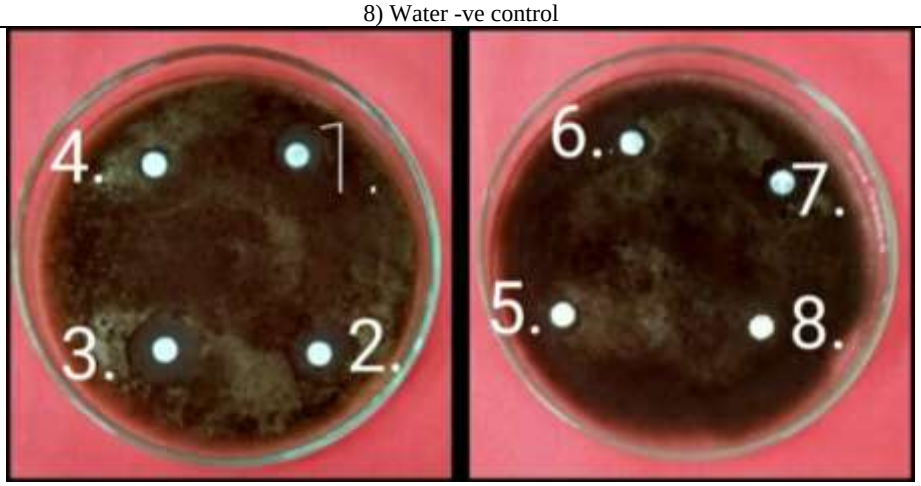
Macrophomina phaseolina

1) 10 ppm =7 mm
2) 15 ppm = 9 mm
3) 20 ppm =12 mm
4) 10 ppm $CuSO_4$=6 mm
5) 20 ppm$CuSO_4$= 6.5 mm
6) 10 ppm(anti) =5.6 mm
7) 20 ppm (anti)=11 mm
8) Water -ve control



Rhizoctonia bataticola

1) 10 ppm = 5 mm
2) 15 ppm = 7 mm
3) 20 ppm =10 mm
4) 10 ppm $CuSO_4$ = 6 mm
5) 20 ppm $CuSO_4$ = 7 mm
6) 10 ppm (anti) = 6 mm
7) 20 ppm (anti) = 8 mm
8) Water -ve control



Alternaria carthami

1) 10 ppm = 12 mm
2) 15 ppm = 16 mm
3) 20 ppm = 18 mm
4) 10 ppm $CuSO_4$ = 6 mm
5) 20 ppm $CuSO_4$ = 8 mm
6) 10 ppm (anti) = 9 mm
7) 20 ppm (anti) = 11 mm
8) Water -ve control

Fusarium oxysporum

1) 10 ppm =8 mm
2) 15 ppm =12 mm
3) 20 ppm=14 mm
4) 10 ppp $CuSO_4$ = 6 mm
5) 20 ppm $CuSO_4$ = 8 mm
6) 10 ppm anti = 8 mm
7) 20 ppm anti = 11 mm
8) Water -ve control

Plate 3: Antifungal activities of pomegranate peel extract biosynthesized copper nanoparticle (CuNPs) against plant fungi

Conclusion

The pomegranate fruit peel extract synthesized copper nanoparticles were characterized by SEM revealed the crystalline nature of NPs. X-ray diffraction patterns were observed a strongest three peaks at 2θ = 26.75 (peak intensity 502), 25.72 (282) and 28.69 (380) were assigned to the reflection lines of copper nanoparticles. The antimicrobial activity of CuNPs was determined by disc diffusion method against some selected species of plant pathogenic bacteria such as *Xanthomonas axonopodis* pv. *punicae* (causal agent of bacterial blight of pomegranate) and *Xanthomonas axonopodis* pv. *malvacearum* (causal agent of bacterial blight of cotton) and fungi such as *Penicillium chrysogenum*, *Macrophomina phaseolina*, *Rhizoctonia bataticola*, *Alternaria carthami* and *Fusarium oxysporum*. It was observed that the synthesized CuNPs demonstrated a

significant inhibitory activity at 20 ppm concentration against selected bacteria and fungi as compared to antibiotic and copper sulphate.

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