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Screening Antimicrobial Potential of Copper Nanoparticles against *Pseudomonas fluorescens* and *Bacillus subtilis* and its Sustainability in Agriculture

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A B S T R A C T

Recent times copper nanoparticles (CuN) has gained attention due to its multifaceted action against several microbes. However, its impact on beneficial plant microbes has not much studied. To fill these knowledge gaps, copper nanoparticles were tested for antibacterial ability against well-known two plant biocontrol agents *Pseudomonas fluorescens* and *Bacillus subtilis* by an *in vitro* bioassay using disk diffusion methodology. Antibacterial activity of CuN was compared with botanicals like clove, pepper and standard antibiotics like ampicillin and streptomycin. Our study results revealed that CuN recorded higher eff ectiveness for both *P. fluorescens* and *B. subtilis* with maximum inhibition zone compared to others tested. Test results illustrate that, bio agents *P. fluorescens* and *B. subtilis* found to be sensitive to CuN. In conclusion, our research could help in determination of microbial strain specificity and to better understand nanoparticles usage for specific purposes. However, further research is needed to better understand CuN role, mechanisms and impact on beneficial plant microorganism in agriculture.

Introduction

Current advances and emergence of nanoscience or nanotechnology in last few decades showed rapid expansion, providing new opportunities in various fields include medical (Misra *et al.,* 2010; Mitra *et al.,* 2003; Liang *et al.,* 2008; Wang *et al.,* 2008), food industry (Caiyun *et al.,* 2005; Siegrist *et al.,* 2005), molecular diagnostics (Jain, 2003), paints, plastics and textiles, cosmetics, optical, electronic, mechanical and chemical fields etc. (Azam *et al.,* 2012; Mu and Sprando2010; Silva 2006; Goldhaber-Gordon *et al.,* 1997; Mnyusiwalla *et al.,* 2007). Furthermore, nanotechnology has gained a great deal of attention from the researchers in current times because of its multitasking in various fields.

In recent years increased chemical usage in management of insect pests and plant pathogens has impacted several environmental issues include environmental pollution (Dhaliwal *et al.,* 2010), pest resistance, pest resurgence and residual phytotoxicity problems etc. (Dhaliwal *et al.,* 2010). To reduce the environmental pollution impact caused by chemical pesticides or fungicide usage, there is an urgent need to develop an alternate strategy to reduce the concern. One such alternative could be use of metal nanoparticles. Metal nanoparticles has shown potent antibacterial capability against several microbes (Wang *et al.,* 2017).

Metal nanoparticles (MNPs) with antibacterial properties have unique qualities than bulk materials include greater ratio of surface area to volume with larger low-coordinate atoms that are easily available for microbial membranes interactions and further in release of metal ions (Choi *et al.*, 2008). Additionally, the bactericidal property of MNPs could be applied for surface coating, that can have wide application in various fields (Ruparelia *et al.*, 2008).

Furthermore, nanoparticles due its smaller particle size proved to act as good antimicrobial activity (Jones *et al.,* 2008). Moreover, the MNPs antibacterial activities depend mainly on two factors nanoparticle-physico chemical properties and bacteria type (Hajipour *et al.,* 2012). Because of special characters MNPs possess, MNPs could be a potential and sustainable alternative in plant pathogen or plant disease management in agriculture sector. However, thorough research is needed on MNPs usage or application in agriculture field.

Some of the MNPs shown potential antibacterial properties include ZnO (Sinha *et al.,* 2011), Ag (Morones *et al.,* 2005; Ruparelia *et al.,* 2008; Sinha *et al.,* 2011), CuO (Ruparelia, *et al.,* 2008, Azam *et al.,* 2012), Al_2O_3 (Jiang *et al.,* 2009), TiO_2 (Jiang *et al.,* 2009; Tsuang *et al.,* 2008); NiO (Wang *et al.,* 2010), Fe_2O_3 etc. (Azam *et al.,* 2012). Despite MNPs are well known for its antimicrobial ability, while in agriculture usage or application must be cautious on MNPs impact or side effect on beneficial microorganisms.

Antimicrobial activity of nanoparticles has mostly documented on human pathogenic bacteria such as *Escherichia coli* (Ruparelia *et al.,* 2008; Yoon *et al.,* 2007) and *Streptococcus aureus* etc. (Baek and An, 2011). Further antibacterial activity of nanoparticles mostly depends on MNPs size, MNPs stability and MNPs concentration used in the growth medium. Further, bacterial growth was inhibited due to MNPs interactions with bacteria in growing medium (Raghupati *et al.*, 2011). In comparison between bacterial cells and MNPs sizes, bacterial cells (size) are in micrometers while bacterial outer cell membranes pore (size) range are in nanometers. Whereas, nanoparticles sizeare much smaller than bacterial pore size. Because of MNPs unique ability of smaller size nature, helps MNPs in cell membrane crossing and interactions (Parisi *et al.,* 2015).

Current challenges impacted by climate change, food security motivated researchers in engage and exploration of new areas of science, one such discovery is investigation on nanotechnology as new tool in crop improvements for the agricultural sector. However, despite of numerous reports on nanotechnology advantages in recent times, still there is a lot of uncertainty in research and understanding (Parisi *et al.,* 2015).

Among MNPs, copper nanoparticle is one such metal that has potential antimicrobial activity against several microbial species (Azam *et al.,* 2012). Among various MNPs, copper oxide (CuO) has unique properties such as photoconductive and photothermal applications (Rakhshani *et al.,* 1986), electric, catalytic, optical, nanofluid and photonic. Furthermore, CuN compared to other metal nanoparticles are cheaper, release Cu ions readily, has greater penetrating ability, cause cell wall disruption and nucleic acid damage (Yoon *et al.,* 2007; Raffi *et al.,* 2010; Rispoli *et al.*, 2010), however the exact mechanism of CuN antimicrobial potentiality is still not clear.

Moreover, CuO nanoparticles, can be prepared from several plant extracts include *Aloe vera* (Kumar *et al.*, 2015), *Tabernaemontana divaricate* (Sivaraj *et al.*, 2014), tea leaf and coffee powder extracts (Sutradhar, *et al.*, 2014), gum karay (Padil and Cernik, 2013) and brown algae, *Bifurcaria bifurcate* (Abboud *et al.*, 2014). Furthermore, Cu and CuO nanoparticles showed potential antimicrobial against several human pathogenic organisms include *Bacillus subtilis*, *E. coli*, *Vibria cholera*, *Syphillis typhus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Akhavan and Ghaderi, 2012; Hassan *et al.*, 2012; Stoimenov *et al.*, 2002), *Vibrio cholerae* non.0139 and *Shigella dysenteriae* 1 (Sutradhar, *et al.*, 2014).

We considered Cu nanoparticles for our study because of its unique characteristics and multi potent action against several microbes. Further investigated to better understand the CuN effect on beneficial microbes when applied in agriculture, we framed the experiment to determine Cu nanoparticles effect on two beneficial bioagents include *Bacillus subtilis* (gram-positive) and *Pseudomonas fluorescens* (gram-negative) bacterial strains. The objective of this study was to compare the bactericidal effect of copper nanoparticles with botanicals and other commercial antibiotics on beneficial microbial strains and to study the sensitivity of bioagents towards CuN.

Materials and Methods

All the experiments were carried out at Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad, India. Two bioagents, *Pseudomonas fluorescens* and *Bacillus subtilis* were procured from Directorate of Oil Seeds Research, Rajendranagar, Hyderabad. Nano copper from Osmania University, Hyderabad, India (fig. 2). Botanicals and antibiotics from local market, Hyderabad, India. Efficacy of copper nano, botanicals and antibiotics were evaluated against bacterial bioagents under *in vitro* condition by disk diffusion assay (Ruparelia *et al.*, 2008). Method followed summarized in the flow chart in the fig.1.

Isolation of bacterial bioagents

The procured bioagent bacterial cultures *P. fluorescens* and *B. subtilis* were cultured on nutrient agar medium (NA). The inoculated plates were incubated at 28 ± 2^{o}C for one week and were isolated and identified and were used for the further studies.

Disk diffusion assay

Disk diffusion method was used to evaluate *in vitro* antibacterial potentiality of copper nanoparticles against two bacterial strains.

Antibacterial activity

The bacterial suspension (10^4-10^5 CFUml^{-1}) was applied on nutrient agar medium surface uniformly.

To determine the antibacterial effect standard paper disk of uniform size (6mm diameter) were impregnated in 5 mgml^{-1} of copper nanoparticles, botanicals like clove, pepper and antibiotics like ampicillin and streptomycin, each disk containing 100µg.

Sterile water impregnated disks as a control. The nanoparticle amended filter paper was dried for approximately 1 hr. These disks were then placed on to the inoculated nutrient agar medium (4 disks per plate) containing bacteria.

P. fluorescens

Tested with copper nanoparticles, botanicals such as clove and pepper impregnated on sterile Whatman No. 1 filter paper disks was used. Not compared with standard antibiotics.

B. subtilis

Copper nanoparticles, botanicals such as clove, pepper and antibiotics like ampicillin and streptomycin impregnated on sterile Whatman No. 1 filter paper disks were used.

All the inoculated treatment plates were incubated at 35^{o}C for 24 hrs. Each treatment was replicated thrice. Average inhibition zone diameter (mm) surrounding the discs was measured.

Statistical Analysis

The experiment was Completely Randomized (CRD). The data obtained was transformed and was statistically analyzed by SAS-9.4 (SAS Institute, Cary, NC). Significant differences were further analyzed by the mean separation test by Least square means (LSD) (Table1.)

Results and Discussion

The antibacterial activity of copper nanoparticles was investigated on two bioagents include *Pseudomonas fluorescens* (gram negative bacterium) and *Bacillus subtilis* (gram-positive bacterium) using the inhibition zonediameter (mm) in disk diffusion test. Generally, the diameter of inhibition zone (DIZ) reveal susceptibility of microorganism to the treatment. Microbes exhibiting larger DIZ reflect susceptibility to the disinfectant or treatment, whereas with smaller DIZ are considered as resistant strains. In our results we noted that disks with copper nanoparticles were surrounded by a larger DIZ (18mm) compared to botanicals such as clove and pepper against in both *P. fluorescens* and *B. subtilis* tested (fig. 2 and 3). Additionally, when tested with antibiotics like ampicillin and streptomycin against *B. subtilis*, copper nanoparticles were surrounded by a larger DIZ compared with others (fig. 4 and 5). The copper nanoparticles impregnated disks showed high effectiveness compared to other impregnated disks on both the strains of bacteria.

When tested on *P. fluorescens*, copper nanoparticles recorded larger DIZ followed byclove and least was by pepper (fig. 3). In *B. subtilis*, copper nanoparticles (CuN) recorded larger DIZ followed by antibiotics ampilicin and streptomycin, botanical clove and least was by pepper (fig. 4 and 5). Results are summarized in the fig. 6.

The test copper nanoparticles were found effective on both *P. fluorescens* and *B. Subtilis* compared to others tested. Thus, our results put forth that CuN have potential inhibitory property towards target bacteria. We also noted that beneficial microbes i.e. the two bioagents tested were sensitive to CuN. Although our results show that CuN has antimicrobial potentiality, it clearly illustrates from our results that in agriculture application or usage, special caution needed, as CuN could be deleterious to beneficial microbes present in soil or beneficial microbes present in plant ecosphere etc. In summary according to our investigation output clarifies that beneficial bioagents tested are sensitive to CuN. Our results of antibacterial property of CuN are in accordance with the results of Ruparelia *et al.,* 2008; Bogdanovic *et al.,* 2014; Cioffi *et al.,* 2005; Yoon *et al.,* 2007. Similarly, CuN showed antibacterial activity (DeAlba-Montero *et al.,* 2017) against several microbes include *Staphylococcus aureus, Escherichia coli* and *Candida albicans* (Bogdanovic *et al.,* 2014).

Fig.1 Diagrammatic representation of disk diffusion assay followed in the study

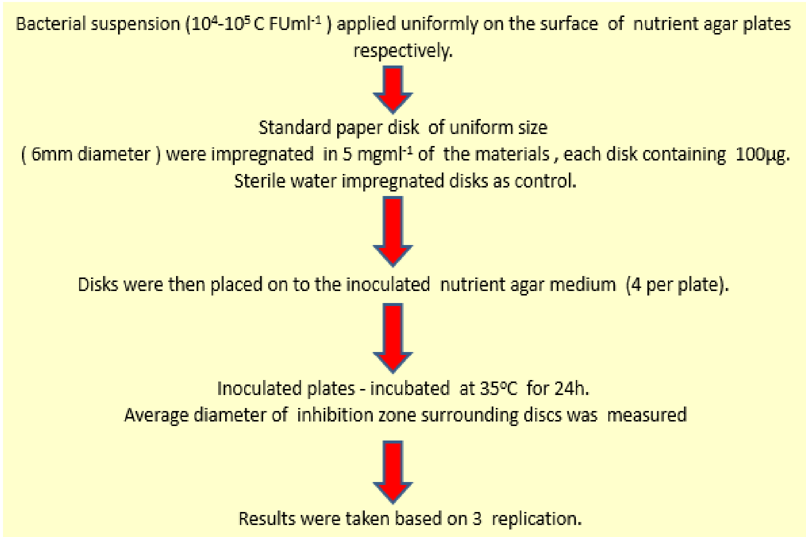


Fig.2 Copper nanoparticle powder

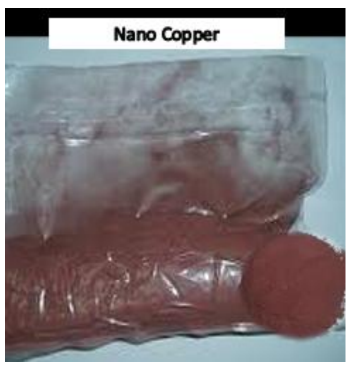


Fig.3 Comparison of copper nanoparticles versus botanicals efficacy on bioagent *Pseudomonas fluorescence*

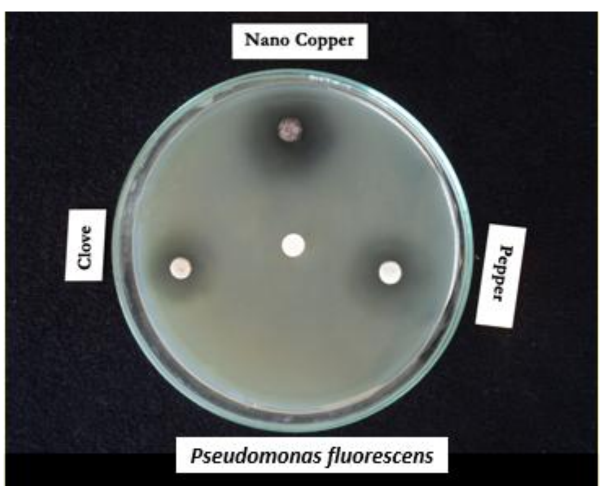


Fig.4 Comparison of copper nanoparticles versus standard antibiotics efficacy onbioagent *Bacillus subtilis*

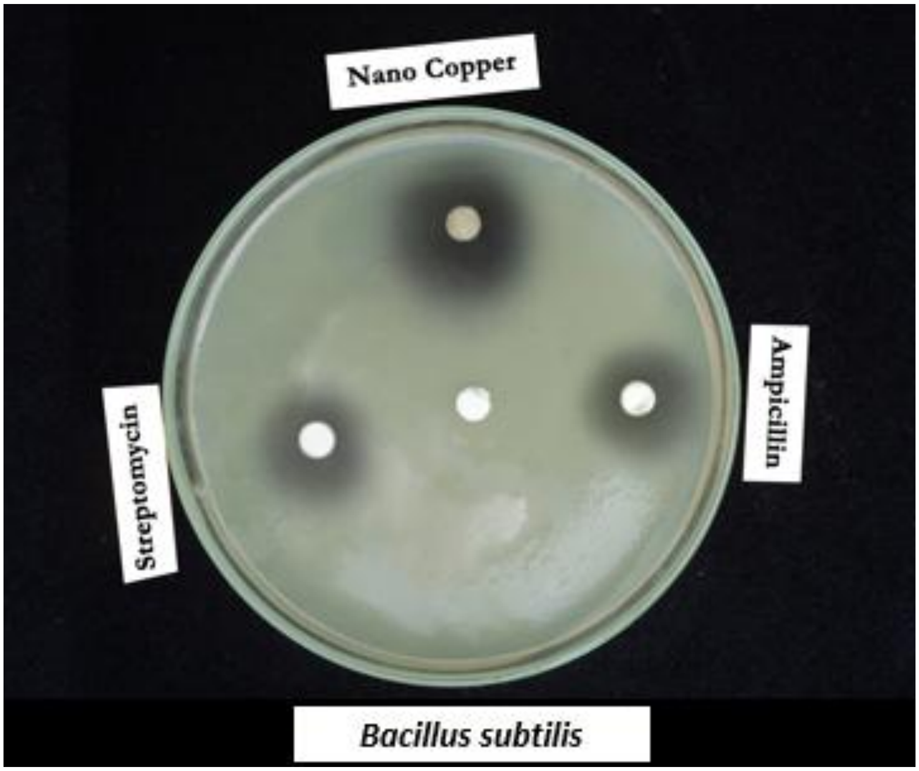


Fig.5 Comparison of copper nanoparticles versus botanicals efficacy on bioagent *Bacillus subtilis*

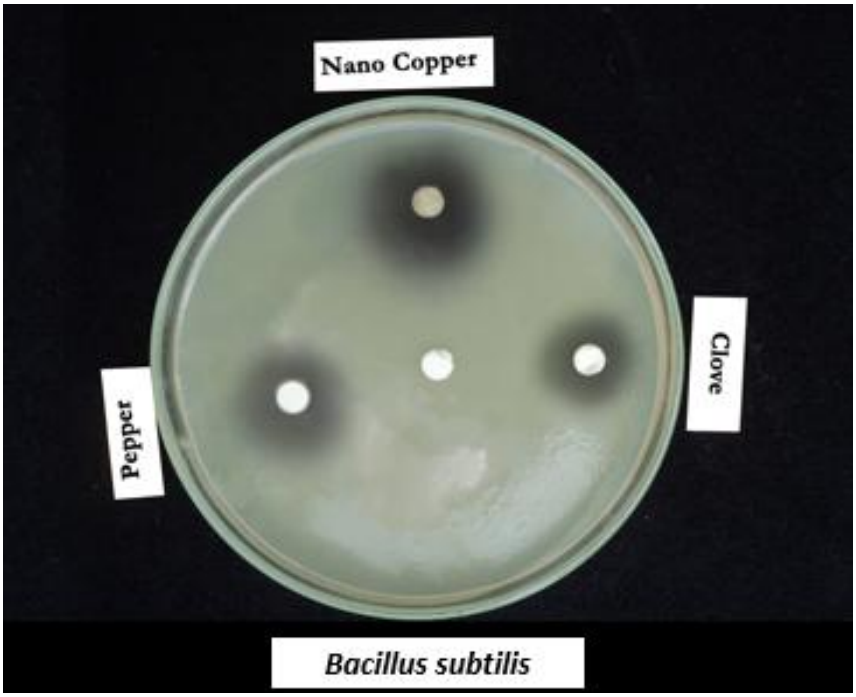
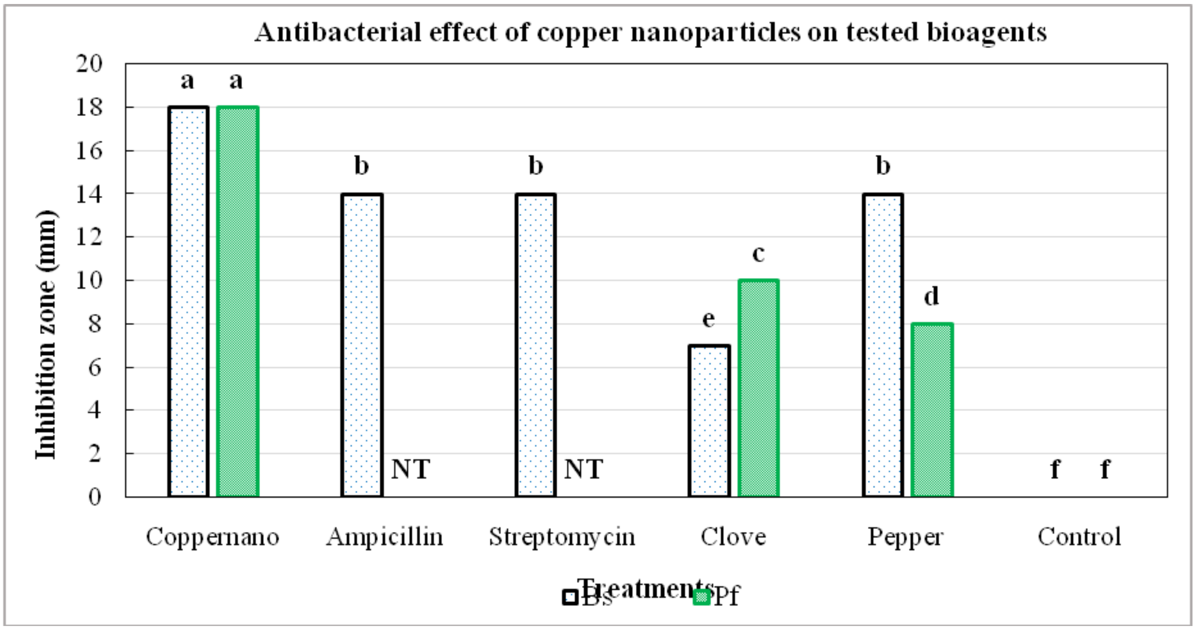


Fig.6 Graphical representation of comparison between copper nanoparticles versus botanicals versus standard antibiotics effect on bioagents *Bacillus subtilis* and *Pseudomonas fluorescence*



*Mean of three replications, means followed by the same letter in a column are non-significant, at 0.05 level of significance according to LSD. Highest mean is assigned the letter A. NT= not tested, Bs *Bacillus subtilis* and Pf *Pseudomonas fluorescence.*

Likewise, Azam *et al.,* 2012 recorded that ZnO and CuO nanoparticles showed greatest antibacterial ability against gram-negative bacteria and gram-positive compared to Fe_2O_3 nanoparticles.

Antibacterial property of CuN against *Pseudomonas* spp. was documented by Longano *et al.*, 2012. Similarly, Kumar *et al.*, 2015 recorded that CuN exhibited enhanced antibacterial activity against *P. fluorescens* even at lower concentrations i.e. above 20 µg/ml (Kumar *et al.*, 2015). In our studies we found that *P. fluorescens* was sensitive to CuN. Possibly the reason for *P. fluorescens* sensitivity towards CuN could be attributed by the fact *P. fluorescens* is a gram-negative bacterium consists of special cell membrane structure (Shahmiri *et al.*, 2013).

Sensitive of *B. subtilis* to copper nanoparticles in our results are in agreement with Yoon *et al.,* 2007, who reported that copper nanoparticles showed greater antibacterial than silver nanoparticles on *B. subtilis* and *E. coli*. Probable reason for *B. subtilis* susceptibility to CuN could be attributed to the fact that gram-positive bacterial cell wall consists of thick peptidoglycan layer (Scott and Barnett, 2006) and bacterial cell surface with higher amounts of amines and carboxyl groups (Shahmiri *et al.*, 2013). However, size of CuN are much smaller that cell pore size. Additionally, Azam *et al.*, 2012 recorded in their findings that gram-positive bacteria showed higher sensitivity than gram-negative towards nanoparticles.

The antibacterial property of CuN could be attributed by several factors, firstly due to ready release of copper ions in the medium (Cioffi *et al.*, 2005) or by direct nanoparticles interaction on bacterial cell membrane (Ruparelia *et al.*, 2008). Another possibility could be released ions could have attached to cell wall of that bacteria finally leading to cell membrane disruption (Chatterjee *et al.,* 2014) or thereby leading to protein denaturation, disruption of biochemical pathway (Kim *et al.,* 2000; Stohs and Bagchi, 1995; Azam *et al.,* 2012). Further released copper ions inside the bacterial cells, get bonded and cross linked to nucleic acid molecule strands internally, resulting in helical structure disorganization (Azam *et al.,* 2012), nucleic acid degradation (Chatterjee *et al.,* 2014), bacterial cell filamentation, reactive oxygen species generation, protein oxidation, lipid peroxidation (Chatterjee *et al.,* 2014) cell killing and ultimately cell death (Yu-sen *et al.,* 2000). The nanoparticles (NPs) can change or manipulate bacteria microenvironment and generate reactive oxygen species and increase NPs solubility, that finally cause bacteria death (Heinlaan *et al.,* 2008)

The toxicity MNPs ions also depends on the properties of heavy metals. Further MNPs toxicity are in relation to the microbial colony size, colony number and the concentration of nanoparticles (NPs) used (Baek and An, 2011). Furthermore, other factors such as nanoparticle diffusion ability may also influence on its effect on target bacteria or microorganism. Nevertheless, further research is needed to confirm the process. However, the mechanism involved for CuN antimicrobial ability has not been fully understood.

Botanicals tested clove, pepper showed an inhibition effect on the both bacteria tested, however we noted differential inhibition rates. Pepper was highly effective on *B. subtilis* compared to *P. fluorescens*. While clove was highly effective on *P. fluorescens* compared to *B. subtilis.* However, clove and pepper both have bactericidal properties that have already been documented by many researchers (Nascimento *et al.,* 2000; Karsha and Lakshmi, 2010).

Antibiotics, ampicillin and streptomycin showed effective against *B. subtilis*, and both were on par with each other with inhibition rate and were inferior than CuN in inhibition rate. The antibacterial property of standard antibiotics ampicillin and streptomycin already has been well established by many research scholars (Schatz *et al.,* 1944; Dyas and Wise, 1983).

In conclusion, in our studies we found that copper nanoparticles have a greater antimicrobial affinity against both bacteria *P. fluorescens* and *B. subtilis*, in comparison with botanicals like clove, pepper and commercial antibiotics ampicillin and streptomycin. Susceptibility of the bioagents tested *P. fluorescens* and *B. subtilis* to copper nanoparticles was recorded. Our study could help to determine microbial strain specificities and provide knowledge on better application of metal nanoparticles for specific purposes. However, further research needed to better understand the relationship between the nanoparticle size and microbe susceptibility index. Before commercialization of CuN, detailed research and comparative study needed to understand CuN impact on beneficial microorganisms when applied in agriculture.

Future work

Detailed research should be conducted on metal nanoparticles implications on different beneficial bioagents before utilization in agricultural application. Further metal nanoparticles effect on different bacterial and fungal plant pathogens must be investigated by following effective, inexpensive and eco-friendly technology usage.

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References

Abboud, Y., Saffaj, T., Chagraoui, A., El Bouari, A., Brouzi, K., Tanane, O., and Ihssane, B. 2014. Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcariabifurcata*). *Applied Nanoscience*, 4(5), 571-576.*https://goo.gl/MKJN4h*

Akhavan, O., and Ghaderi, E. 2010. Cu and CuO nanoparticles immobilized by silica thin films as antibacterial materials and photocatalysts. *Surface and Coatings Technology*, 205(1), 219-223. *https://doi.org/10.1016/j.surfcoat.2010.06.036*

Azam, A., Ahmed, A. S., Oves, M., Khan, M. S., Habib, S. S., and Memic, A. 2012. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *International journal of nanomedicine*, 7, 6003.*https://doi.org/10.2147/IJN.S35347*

Baek, Y. W., and An, Y. J. 2011. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb 2 O 3) to *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus aureus*. *Science of the Total Environment*, 409(8), 1603-1608. *https://doi.org/10.1016/j.scitotenv.2011.01.014*

Bogdanović, U., Lazić, V., Vodnik, V., Budimir, M., Marković, Z., and Dimitrijević, S. 2014. Copper nanoparticles with high antimicrobial activity. *Materials Letters*, 128, 75-78. *https://doi.org/10.1016/j.matlet.2014.04.106*

Caiyun, L., Wei, Z., Yang, B., Bo-zhong, G., and Wei-qiang, L. 2005. The applications of nanotechnology in food industry [J]. *Science and Technology of Food Industry*, 4, 061.*https://goo.gl/5iF9J9*

Chatterjee, A. K., Chakraborty, R., and Basu, T. 2014. Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology*, 25(13), 135101.*goo.gl/6t298Q*

Choi, O., and Hu, Z. 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environmental science & technology*, 42(12), 4583-4588. *https://goo.gl/bsxJgD*

Cioffi, N., Ditaranto, N., Torsi, L., Picca, R. A., Sabbatini, L., Valentini, A., and Zambonin, P. G. 2005. Analytical characterization of bioactive fluoropolymer ultra-thin coatings modified by copper nanoparticles. *Analytical and bioanalytical chemistry*, 381(3), 607-616.*https://goo.gl/pAV4nT*

Cioffi, N., Torsi, L., Ditaranto, N., Tantillo, G., Ghibelli, L., Sabbatini, L., and Traversa, E. 2005. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. *Chemistry of Materials*, 17(21), 5255-5262. *https://goo.gl/45rWjg*

DeAlba-Montero, I., Guajardo-Pacheco, J., Morales-Sánchez, E., Araujo-Martínez, R., Loredo-Becerra, G. M., Martínez-Castañón, G. A., and Compeán Jasso, M. E. 2017. Antimicrobial properties of copper nanoparticles and amino acid chelated copper nanoparticles produced by using a soya extract. *Bioinorganic chemistry and applications*. Article ID 1064918, 6 pages. *https://doi.org/10.1155/2017/1064918*

Dhaliwal, G. S., Jindal, V., and Dhawan, A. K. 2010. Insect pest problems and crop losses: changing trends. *Indian Journal of Ecology*, 37(1), 1-7. *https://goo.gl/4Xmahx*

Dyas, A., and Wise, R. 1983. Ampicillin and alternatives. *British medical journal (Clinical research ed.)*, 286(6365), 583. *https://goo.gl/VsdBBR*

Goldhaber-Gordon, D., Montemerlo, M. S., Love, J. C., Opiteck, G. J., and Ellenbogen, J. C. (1997). Overview of nanoelectronic devices. *Proceedings of the IEEE*, 85(4), 521-540. *https://goo.gl/gNAvxr*

Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., de Aberasturi, D. J., de Larramendi, I. R., Rojo, T., and Mahmoudi, M. 2012. Antibacterial properties of nanoparticles. *Trends in biotechnology*, *30*(10), 499-511.*https://doi.org/10.1016/j.tibtech.2012.06.004*

Hassan, M. S., Amna, T., Yang, O. B., El-Newehy, M. H., Al-Deyab, S. S., and Khil, M. S. 2012. Smart copper oxide nanocrystals: synthesis, characterization, electrochemical and potent antibacterial activity. *Colloids and Surfaces B: Biointerfaces*, 97, 201-206. *https://doi.org/10.1016/j.colsurfb.2012.04.032*

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H. C., and Kahru, A. 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO 2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalusplatyurus. *Chemosphere*, 71(7), 1308-1316. *https://doi.org/10.1016/j.chemosphere.2007.11.047*

Jain, K. K. 2003. Nanodiagnostics: application of nanotechnology in molecular diagnostics. *Expert review of molecular diagnostics*, 3(2), 153-161. *https://doi.org/10.1586/14737159.3.2.153*

Jiang, W., Mashayekhi, H., and Xing, B. 2009. Bacterial toxicity comparison between nano-and micro-scaled oxide particles. *Environmental pollution*, 157(5), 1619-1625. *https://doi.org/10.1016/j.envpol.2008.12.025*

Jones, N., Ray, B., Ranjit, K. T., and Manna, A. C. 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS microbiology letters*, 279(1), 71-76. *https://doi.org/10.1111/j.1574-6968.2007.01012.x*

Karsha, P. V., and Lakshmi, O. B. 2010. Antibacterial activity of black pepper (Piper nigrum Linn.) with special reference to its mode of action on bacteria. *Indian Journal of Natural Products and Resources*. 1 (2), pp. 213-215. *https://goo.gl/D8mmdz*

Kim, J. H., Cho, H., Ryu, S. E., and Choi, M. U. 2000. Effects of metal ions on the activity of protein tyrosine phosphatase VHR: highly potent and reversible oxidative inactivation by Cu 2+ ion. *Archives of Biochemistry and Biophysics*, 382(1), 72-80. *https://doi.org/10.1006/abbi.2000.1996*

Kumar, P. V., Shameem, U., Kollu, P., Kalyani, R. L., and Pammi, S. V. N. 2015. Green synthesis of copper oxide nanoparticles using Aloe vera leaf extract and its antibacterial activity against fish bacterial pathogens. *BioNanoScience*, 5(3), 135-139.*https://goo.gl/eQ5Pzc*

Liang, H. Y., Hu, D., and Xiao, H. M. 2008. Application of Nanotechnology for Preservation of Fruit and Vegetable. *Storage & Process*, 5, 021. *https://goo.gl/bSwSLp*

Longano, D., Ditaranto, N., Cioffi, N., Di Niso, F., Sibillano, T., Ancona, A., and Torsi, L. 2012. Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging. *Analytical and bioanalytical chemistry*, 403(4), 1179-1186. *https://doi.org/10.1007/s00216-011-5689-5*

Misra, R., Acharya, S., and Sahoo, S. K. 2010. Cancer nanotechnology: application of nanotechnology in cancer therapy. *Drug Discovery Today*, 15(19), 842-850. *https://doi.org/10.1016/j.drudis.2010.08.006*

Mitra, S. B., Wu, D., and Holmes, B. N. 2003. An application of nanotechnology in advanced dental materials. *The Journal of the American Dental Association*, 134(10), 1382-1390.*https://doi.org/10.14219/jada.archive.2003.0054*

Mnyusiwalla, A., Daar, A. S., and Singer, P. A. 2003. 'Mind the gap': science and ethics in nanotechnology. *Nanotechnology*, 14(3), R9. *https://goo.gl/BGtcn3*

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., and Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10), 2346. *https://goo.gl/W843gH*

Mu, L., and Sprando, R. L. 2010. Application of nanotechnology in cosmetics. *Pharmaceutical research*, 27(8), 1746-1749. *https://goo.gl/1TGFqA*

Nascimento, G. G., Locatelli, J., Freitas, P. C., and Silva, G. L. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, 31(4), 247-256. *http://dx.doi.org/10.1590/S1517-83822000000400003*

Padil, V. V. T., and Černík, M. 2013. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. *International Journal of Nanomedicine*, 8, 889.*https://doi.org/10.2147/IJN.S40599*

Parisi, C., Vigani, M., and Rodríguez-Cerezo, E. 2015. Agricultural nanotechnologies: what are the current possibilities?.*Nano Today*, 10(2), 124-127. *https://doi.org/10.1016/j.nantod.2014.09.009*

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), 75-80. *https://goo.gl/kfjgnx*

Raghupathi, K. R., Koodali, R. T., and Manna, A. C. 2011. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*, 27(7), 4020-4028.

Rakhshani, A. E. 1986. Preparation, characteristics and photovoltaic properties of cuprous oxide-a review. *Solid-State Electronics*, 29(1), 7-17. *https://doi.org/10.1016/0038-1101(86)90191-7.*

Rispoli, F., Angelov, A., Badia, D., Kumar, A., Seal, S., and Shah, V. 2010. Understanding the toxicity of aggregated zero valent copper nanoparticles against Escherichia coli. *Journal of Hazardous Materials*, 180(1), 212-216. *https://goo.gl/tK58Wq*

Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., and Mukherji, S. 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia*, 4(3), 707-716. *https://doi.org/10.1016/j.actbio.2007.11.006*

Schatz, A., Bugle, E., and Waksman, S. A. 1944. Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria.∗. *Proceedings of the Society for Experimental Biology and Medicine*, 55(1), 66-69. *https://goo.gl/1Z9brF*

Scott, J. R., and Barnett, T. C. 2006. Surface proteins of gram-positive bacteria and how they get there. *Annu. Rev. Microbiol.*, 60, 397-423. *https://goo.gl/fCMXSQ*

Shahmiri, M., Ibrahim, N., Shayesteh, F., Asim, N., and Motallebi, N. 2013. Preparation of PVP-coated copper oxide nanosheets as antibacterial and antifungal agents. *Journal of Materials Research*, 28(22), 3109-3118.*doi:10.1557/jmr.2013.316*

Siegrist, M., Cousin, M. E., Kastenholz, H., and Wiek, A. 2007. Public acceptance of nanotechnology foods and food packaging: The influence of affect and trust. *Appetite*, 49(2), 459-466.*https://doi.org/10.1016/j.appet.2007.03.002*

Silva, G. A. 2006. Neuroscience nanotechnology: progress, opportunities and challenges. *Nature Reviews Neuroscience*, 7(1), 65-74. *https://goo.gl/SHwwz4*

Sinha, R., Karan, R., Sinha, A., andKhare, S. K. 2011. Interaction and nanotoxic effect of

ZnO and Ag nanoparticles on mesophilic and halophilic bacterial cells. *Bioresource technology*, 102(2), 1516-1520. *https://doi.org/10.1016/j.biortech.2010.07.117*

Sivaraj, R., Rahman, P. K., Rajiv, P., Salam, H. A., and Venckatesh, R. 2014. Biogenic copper oxide nanoparticles synthesis using *Tabernaemontana divaricate* leaf extract and its antibacterial activity against urinary tract pathogen. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 133, 178-181. *https://doi.org/10.1016/j.saa.2014.05.048*

Stohs, S. J., and Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free radical biology and medicine*, 18(2), 321-336. https://goo.gl/e1nqdi

Stoimenov, Peter K., *et al.*, "Metal oxide nanoparticles as bactericidal agents." *Langmuir* 18.17 (2002): 6679-6686. *DOI:10.1021/la0202374*

Sutradhar, P., Saha, M., and Maiti, D. 2014. Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity. *Journal of Nanostructure in Chemistry*, 4(1), 86.*https://goo.gl/2FCvAs*

Tsuang, Y. H., Sun, J. S., Huang, Y. C., Lu, C. H., Chang, W. H. S., and Wang, C. C. 2008. Studies of photokilling of bacteria using titanium dioxide nanoparticles. *Artificial Organs*, 32(2), 167-174.*https://DOI: 10.1111/j.1525-1594.2007.00530.x*

Wang, L., Hu, C., and Shao, L. 2017. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine*, *12*, 1227.*https://doi.org/10.2147/IJN.S121956*

Wang, X., Yang, L., Chen, Z. G., and Shin, D. M. 2008. Application of nanotechnology in cancer therapy and imaging. *CA: a cancer journal for clinicians*, 58(2), 97-110. *https://DOI: 10.3322/CA.2007.0003*

Wang, Z., Lee, Y. H., Wu, B., Horst, A., Kang, Y., Tang, Y. J., and Chen, D. R. 2010. Anti-microbial activities of aerosolized transition metal oxide nanoparticles. *Chemosphere*, 80(5), 525-529. *https://doi.org/10.1016/j.chemosphere.2010.04.047*

Yoon, K. Y., Byeon, J. H., Park, J. H., and Hwang, J. 2007. Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles. *Science of the Total Environment*, 373(2), 572-575. *https://doi.org/10.1016/j.scitotenv.2006.11.007*

Yu-sen, E. L., Vidic, R. D., Stout, J. E., McCartney, C. A., and Victor, L. Y. 1998. Inactivation of Mycobacterium avium by copper and silver ions. *Water Research*, 32(7), 1997-2000.*https://doi.org/10.1016/S0043-1354(97)00460-0*

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