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RESEARCH ARTICLE

Application of Novel Biogenic nanoparticles for antimicrobial traits

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ABSTRACT
Nanotechnology is a better approach to dealing with the current challenges of phytopathogens in agriculture and the environment. Nanoparticles (NPs) are less time-consuming, non-toxic and environmentally friendly and provide a high yield compared to conventional synthesis of NPs. Using plant growth promoting (PGP) bacterial strain for the synthesis of nanoparticles can route out the challenges of using chemical-based fertilizers and pesticides for agriculture. In our study, silver nanoparticles (AgNPs) were synthesized by using *Burkholderia* sp. 15B, which were further characterized by various physical techniques. All the techniques suggested the formation of biogenic NPs. We found that the bacteria-based NPs were able to hamper the growth of phytopathogenic fungi by more than 80%, as examined by the inhibition of fungal growth in the presence of green NPs. In addition, the synthesized NPs efficiently rescued seedlings from phytopathogenic fungal invasion. The results implicate the use of microorganism-mediated AgNPs as pesticides over chemical-based pesticides.

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Introduction

Almost 60% of the population relies on agriculture for their livelihood, including farmers, food processing industries and other indirect beneficiaries (Brock *et al.*, 2011). In recent decades, the usage of chemical fertilizers and pesticides has expanded dramatically to address pathogen-related difficulties and meet the demand. The widespread use of these chemicals in agriculture harms the land and environment. Nanopesticides are being created to benefit the environment and increase crop yield in agriculture. They are made up of reformulations of registered active ingredients (AIs) with insecticidal, fungicidal, or herbicidal qualities (Walker *et al.*, 2017). Nanotechnology is a new scientific technique involving materials and equipment that can modify the physical and chemical properties of substances at the molecular level. Researchers have also made bio-based or green synthesized nanoparticles (NPs) for the benefit of agriculture. NPs synthesized by utilizing beneficial microbes are more useful than chemically-synthesized NPs (Vaseghi *et al.*, 2018). Conventional synthesis of NPs can be done by diverse physical, mechanical, and chemical methods such as radiolysis, microwave, ultra-sonication, sol-gel method, and electrospinning. These methods are time-consuming, produce less yield, and are an environmental hazard. Therefore, we must shift our focus toward safe methods for developing NPs (Fang *et al.*, 2019).

Over the last decade, research into nanotechnology for developing innovative plant-protection products has gottendeveloping innovative plant-protection products has gotten more attention compared to other fields, such as nanosensors and fertilizers. (Rodrigues *et al.*, 2017; Yin *et al.*, 2018). Recently, nanotechnology has advanced its hands in multiple areas, including diagnosis, therapeutics, pharmaceutical science and chemistry (Li *et al.*, 2020). Furthermore, it has an outstanding possibility to change the agricultural as well as related food industries by providing revolutionary techniques which not only enhance the capacity of crops to absorb more nutrients but also helpful in fast disease detection and their molecular management (Mittal *et al.*, 2021a). In addition, nano-biotechnology improves our understanding of the biology of diverse crops (Tarafdarn *et al.*, 2013). -based green synthesized NPs are a better option due to the presence of a biological capping agent, offering higher stability and acting as a covering against oxidation, aggregation and agglomeration (Capeness *et al.*, 2019). Transformation into precision agriculture with the help of new-age materials like nanoparticles help farmers to gain maximum output from available resources (Yaqoob *et al.*, 2020). In this study, culture filtrate (cell-free suspension) of plant growth-promoting rhizobacteria, *Burkholderia* sp. (15B-NPs) was used to synthesise AgNPs. Physical characterization was analysed by UV-Visible spectroscopy, EDS, XRD and FT-IR. Furthermore, the antifungal activity of 15B-NPs was tested against phytopathogens. The seedling experiment confirmed the plant growth is promoting trait bionanoparticles.

Research Methodology

Source of microbes

The bacterial strains *Burkholderia* sp. abbreviated as 15B was tested against fungal pathogens i.e. *Fusarium oxysporum*, *Aspergillus niger*; *Aspergillus fumigates*, *Pythium* sp. and *Rosellinia* sp. were obtained from the microbial collection centre at ATC, SU which was isolated earlier (Mittal *et al.*, 2019). The pure cultures were maintained in the required medium subcultured from time to time to regulate their viability. We aim to use this strain to develop green nanoparticles and test them for biofertilizer and biocontrol traits. In the current study, culture filtrate (cell-free suspension) of plant growth-promoting rhizobacteria, *Burkholderia* sp. 15B was used for the synthesis of AgNPs.

Synthesis of green silver nanoparticles

The bacterial strains were seeded into 300 ml nutrient broth and incubated for 72 hours on an orbital shaker at 30°C with 150 rpm. After 72 hours of growth, the culture filtrate (CF) was centrifuged, and the supernatant was collected. Further, the CF was autoclaved at 121°C after filtering with a 0.45 um filter. Bionanoparticles were made the same way as previously described, with a few changes (Petatan-Sagahon *et al.*, 2011; Chandan *et al.*, 2021; Mittal *et al.*, 2022b). On a magnetic stirrer, sterilized CF was combined with silver nitrate (1 mM), and 1% PEG was added to the reaction mixture as a stabilizer. The reaction was carried out in the dark for 90 minutes with constant stirring. Further, the mixture was left overnight under dark conditions till the color changed to dark brown. The mixture was centrifuged, washed thrice with water and dried at 80 °C.

Characterization of green NPs

Various approaches were used to characterize the green NPs, as described by (Raizada *et al.*, 2016). A Shimadzu UV 2600 spectrophotometer was used to measure the UV–Vis spectra of the purified NPs suspension in the wavelength range of 300–800 nm. Following that, EDS analysis, scanning electron microscopy (SEM), X-ray diffraction (XRD) spectroscopy, and Fourier transform infrared spectroscopy (FTIR) were performed.

Biocontrol activity of NPs

The antifungal activity of green NPs was tested against phytopathogenic fungal strains. For this, potato dextrose agar (PDA) containing NPs was used. The fungal disc was placed in the center of PDA plate, and the growth of fungus in the presence and absence of NPs was compared after incubating the fungal disc *Fusarium oxysporum*; *Aspergillus niger*; *Aspergillus fumigatus*; *Pythium* sp. and *Rosellinia* sp. at 28 °C for 7 days as described earlier (Mittal *et al.*, 2021b). In addition, to study the impact of NPs in controlling the adverse effects of phytopathogenic fungi, the surface-sterilized gram (chick pea) seedlings were grown in the dark at 25 °C under moist conditions (Gupta *et al.,* 2016). On the fourth day, the germinated seedlings were incubated with fungal strain (1.8x 10^5 cells/ml) for 1 hr and grown at 25 °C under moist conditions. Further, on the sixth day, the seeds were treated with 100 µl of green NPs and kept at 25 °C under moist conditions. Seeds treated with normal water and fungal pathogens were taken as control. After 16 days of treatment, the fresh and dry weight was measured (Narayan *et al.,* 2017).

Results and Discussion

The development of a wide range of materials for nano-formulation of pesticides and fertilizers, is the critical concern to enhancing the crop productivity using nanotechnology. To counterpart the significant loss by the invasion of fungal pathogens to the food crops in tropical and subtropical countries, nanotechnology will play an important role. The focus of this study is to develop nano-biofungicides using microbes which can counterpart the invasion of fungal pathogens and enhance crop productivity.

Culture filtrate 15B-CF strain exhibits antifungal activity

The bacterial strain 15B was cultured to produce the biomass for biosynthesis in nutrient broth. It was reported that the formulation of nanoparticles using LB medium maximizes the synthesis of silver nanoparticles. Earlier, microbial production of silver nanoparticles with *E. coli* as biocomponent was done extracellularly (Natarajan *et al.*, 2010). We use the NB medium for the synthesis of nanoparticles as it was reported that the strain shows better growth in the basal NB medium (Mittal *et al.*, 2019). The biomass was harvested after 72 h of growth and centrifuged. The supernatant material (culture filtrate) was collected and autoclaved at 121 °C for the nanoformulation of nanobiopesticide. It was found that culture filtrate of bacterial strain (termed herein as 15B-CF) showed antifungal activity against the tested phytopathogen by more than 70% (data not shown) compared to the control. Our results suggest that 15B-CF contained important bacteria-based metabolites that could control tested phytopathogen growth. It is also important to mention that the culture filtrate is devoid of any bacteria since it was autoclaved. Also, we did not see any growth when the 15B-CF was plated on solid agar media. Culture filtrate was further used to determine antifungal activity.

Physical characterization of Biogenic 15B-NPs

Further, we made the NPs by using the CF of 15B bacterial strainmade the NPs by using the CF of 15B bacterial strain described above in the methods section. During the stirring, we observed a color change (due to the phenomenon of surface plasmon resonance) in the solution owing to the formation of silver nanoparticles in the presence of 15B-CF. Previously, Gupta *et al.* used *Bacillus amyloliquefaciens* and *B. subtilis*, to prepare Ag-NPs. In another study, silver NPs were synthesized using *Stenotrophomonas* sp. BHU-S7 (Mishra *et al.*, 2017). How the NPs were synthesized in the presence of 15B-CF. It could be possible that strain 15 B must be secreting certain enzymes like nitrate reductase or other reductases that could help transfer electron to Ag^+, leading to the synthesis of biogenic NPs (Gupta *et al.*, 2016; Mishra *et al.*, 2017). Another possible mechanism could be that the C=O groups of specific proteins or secreted enzymes could interactwith the surface and stabilize the metal-NPs. Besides C=O, other functional groups like amine, hydroxyl, carboxyl etc, on secreted metabolites could help in the stabilization of NPs by reduction (Grasso *et al.*, 2019). Similar to previous results (Fafal *et al.*, 2017), we found the SPR peak for microbial nanoparticles at 465 nm, indicating that microbial culture filtrate has the potential to reduce Ag^+ ions (Fig. 1).

FT-IR, SEM-EDS and XRD analysis of NPs

The FT-IR spectra (Fig. 2) of the 15B-NPs were analyzed to determine the functional groups involved in reducing the silver nanoparticles. We observed a peak at 1627 cm^{-1} with BC-15B spectrum, whereas in 15B-NPs, this peak shifted to 1634 cm^{-1} (Fig. 2), indicating that the ether (C=C) functional group are involved in

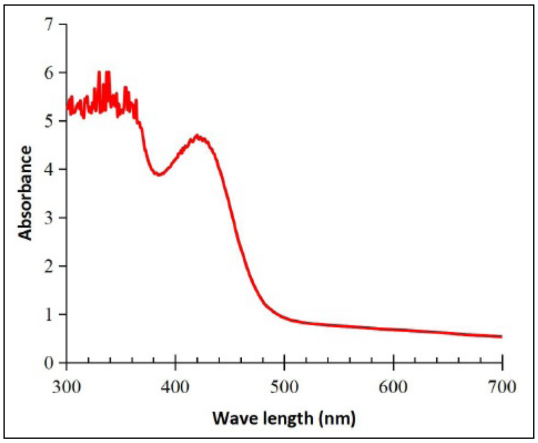

Figure 1. Figure illustrates UV-Vis Spectroscopy for 15B-NPs. The λ max was around 465 nm for 15B-NPs.

the synthesis. Similarly, the 15B-CF spectrum showed a peak at 3334 cm^{-1} that reflects O-H stretching from the polyphenols. This peak shifted to 3280 cm^{-1} in 15B-NPs (Fig. 2), suggesting hydroxyl groups' role in reducing silver. The stretching at 1627 cm^{-1} in BC-15B shifted to 1634 cm^{-1} in the 15B-NPs spectrum, exhibiting the amine groups' vibrations (NH_2). A peak at 1106 in CF shifted to 1073 cm^{-1} in 15B-NPs (Fig. 2), indicating the involvement of C-O-C alkyl-substituted ether stretch. The presence of aliphatic chloro compounds can be estimated at the peak range of 800 - 900 cm^{-1}.

The EDS spectrum of the nanoparticles showed characteristic absorption of silver nano-crystallites owing to SPR with higher counts of silver

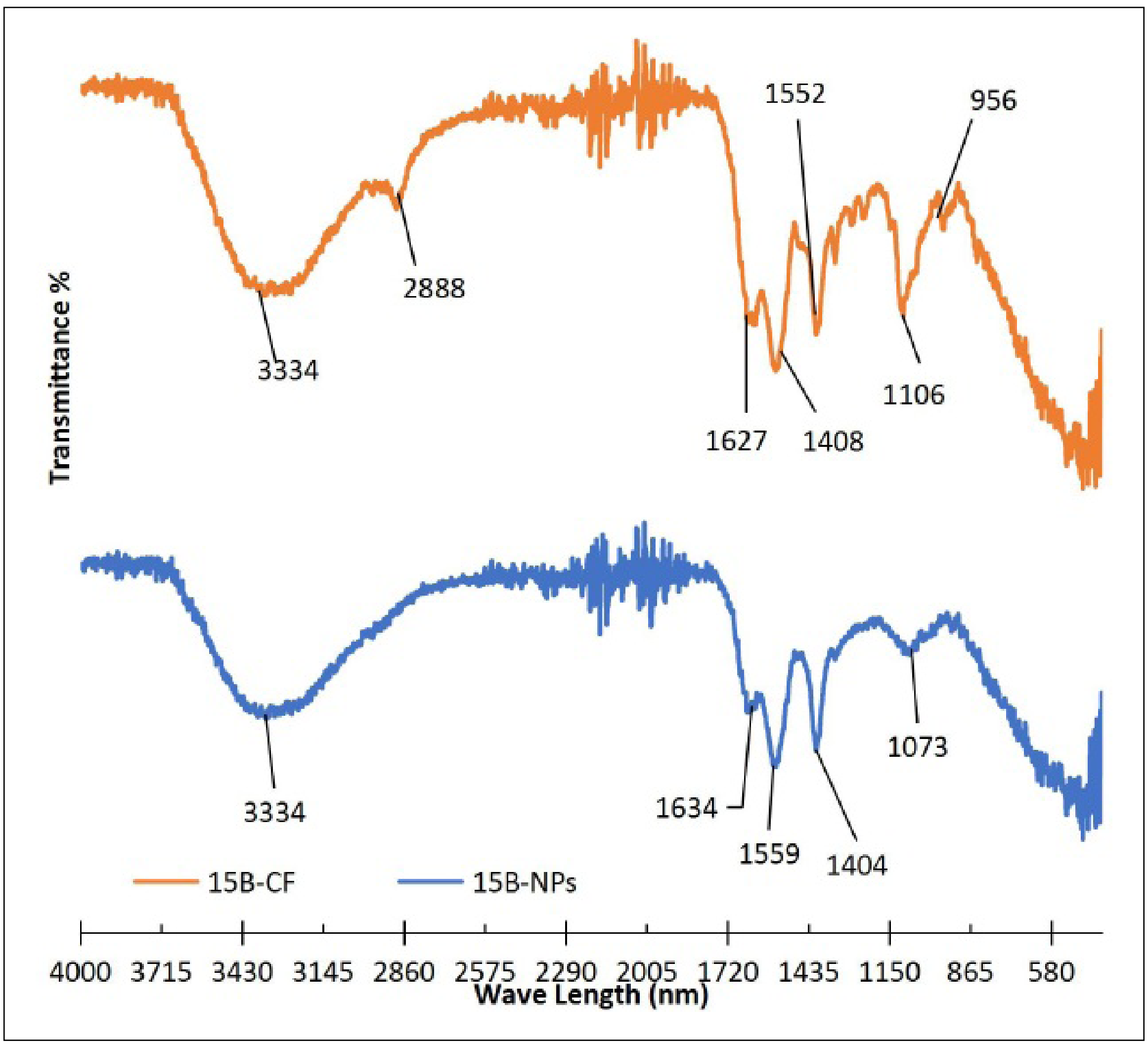

Figure 2. Figure illustrates (A) FTIR spectra of 15B-NPs (red) and culture filtrate 15B-CF (blue).

at 3 keV, thus approving the synthesis of the nanoparticles (Fig. 3). The presence of other elements like aluminium, carbon, chlorine, iron, nitrogen, sodium, oxygen, and phosphorus was also determined by EDS (Fig. 3).

The X-Ray diffractogram of 15B-NPs (Fig. 4) showed a crystalline structure. The peaks demonstrated at 32.4°, 46.3°, 55.1°, 57.7° and 76.9° in the 2θ range, concerning the (200), (220), (311), (222) and (311) reflection planes, respectively, confirming face-centered cubic

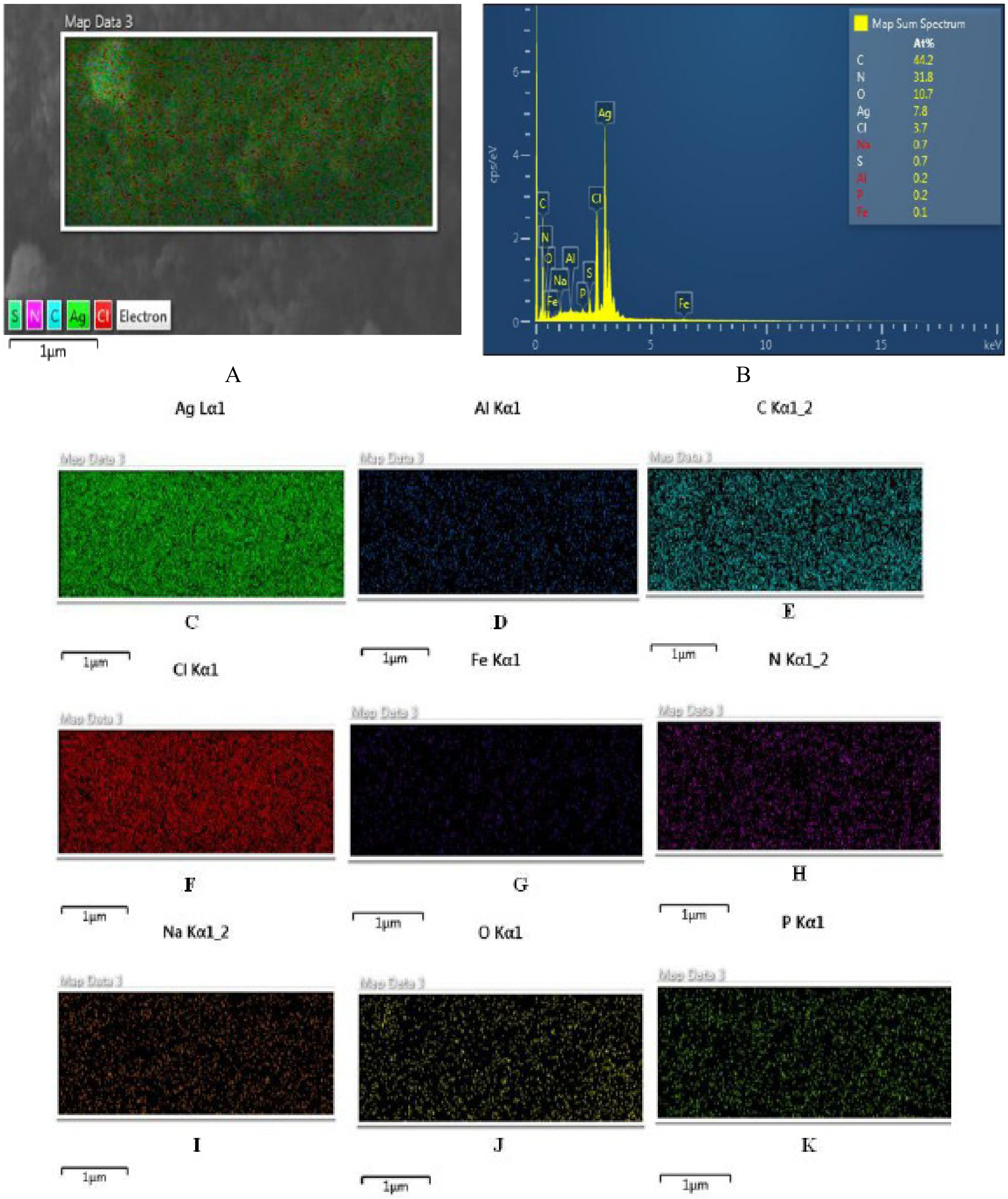

Figure 3. EDS Analysis of 15B-NPs. **(A)** SEM image for 15B-NPs and **(B)** Elemental composition of 15B-NPs. Different elements present are shown in other panels. **(C)** Ag (Silver), **(D)** Aluminum, **(E)** Carbon, **(F)** Chlorine, **(G)** Iron, **(H)** Nitrogen, **(I)** Sodium, **(J)** Oxygen, **(K)** Phosphorus.

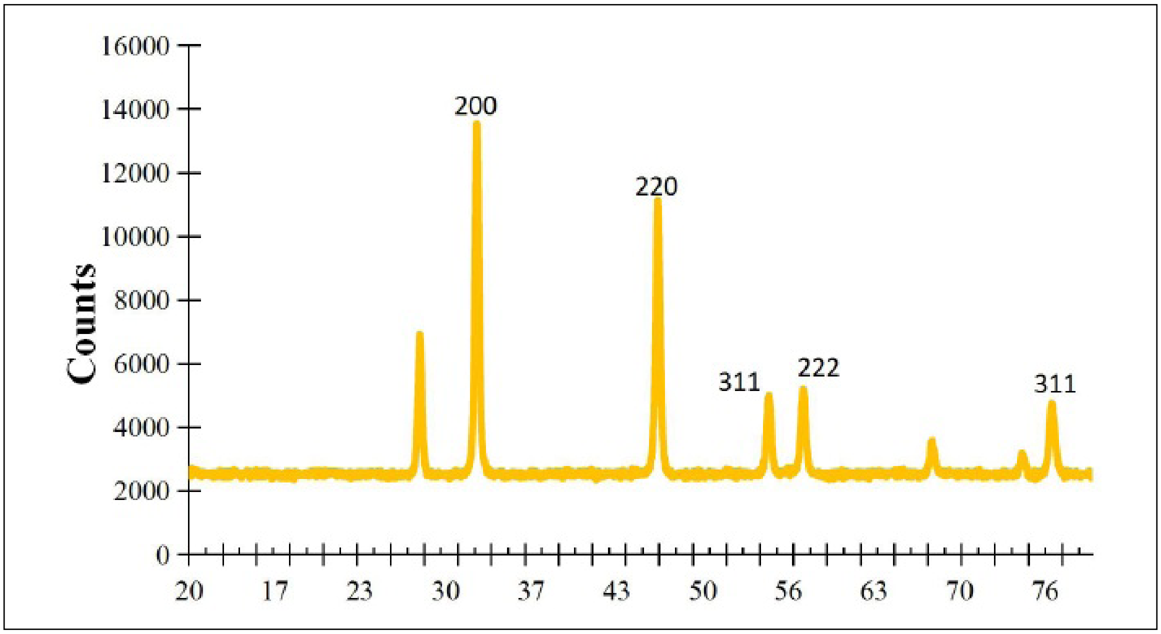

Figure 4. Figure represented XRD-Analysis of 15B-NPs

structure of silver as nanocrystals as per standards of JCPDS, Ag-04-0783 (File number) (Kumari *et al.*, 2020; Kumari *et al.*, 2021).

Biocontrol activity of green nanoparticles

Formulated nanobiopesticides were examined for their biocontrol activity (Fig. 5). For the analysis, PDA was used with 10 µl/ml of the AgNPs and 15B-NPs against *Fusarium oxysporum*; *Aspergillus niger*; *Aspergillus fumigatus*; *Pythium* sp. *Rosellinia* sp. and allowed to grow for 5-6 days at 27 °C along with control in which we have not added Nps into the medium. We found that Phytopathogenic fungus could not grow in the presence of Nps. The percentage inhibition was 59% for AgNPS and 88% for 15B-NPs. We have observed that culture filterate (CF) of bacterial strains could inhibit the growth of fungal strains in the present formulation with Ag.

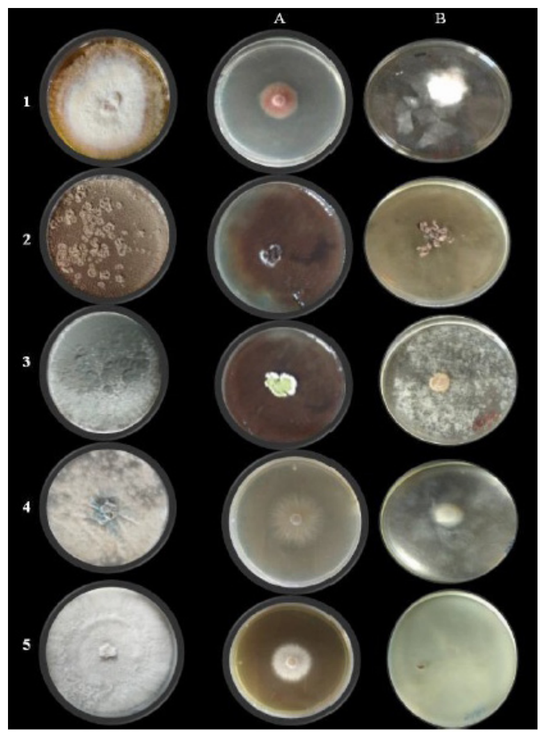

Figure 5. The figure showing antifungal activity of **(A)** 15B-NPs **(B)** Ag-NPs againstdifferent phytopathogens. Row 1 *Fusarium oxysporum,* row 2 *Aspergillus niger* , row 3 *Aspergillus fumigatus,* row 4 *Pythium* sp. and row 5 *Rosellinia* sp. on PDA agar plates were supplemented with NPs (fungi were allowed to grow without NPs was taken as control shown in first lane).

Green NPs control the growth of the Gram seedlings

We previously found that bacterial strains could reduce the influence of phytopathogens on seedling growth. We treated sterilised seedlings with 15B-NPs and studied the influence of phytopathogens to show that microbial CF-derived AgNPs might reduce the impact of fungal diseases. Seeds that had only been exposed to normal water were used as controls. Compared to water-treated control seedlings, NPs-treated seedlings did not allow the growth of *Fusarium oxysporum* (Fig. 6A).

When the seedlings were allowed to grow further for 12 days, we found that Nps considerably reduced the growth of the fungal pathogen. The seeds infected with phytopathogen could not grow as compared to uninfected seedlings. In addition to this, we have observed that the seeds incubated with 15B-NPs not only grow better but also able to rescue the effect of fungus. The average shoot length of untreated seedlings and AgNPs treated seedlings was 11.75 cm and ~7 cm, indicating a negative impact of AgNPs. Similarly, the root length of untreated and AgNPs treated seedlings was 10 cm and ~7 cm (Fig. 6B). The average root length and shoot length of 15B-NPs were 15 cm and 11 cm, respectively. Our results indicated a considerable biocontrol activity of the 15B-NPs and their ability to enhance the growth as a biofertilizer.

Previously researchers showed that chemically prepared zinc oxide NPs when primed to wheat improved the growth of plantwheat seeds improved plant growth in terms of health

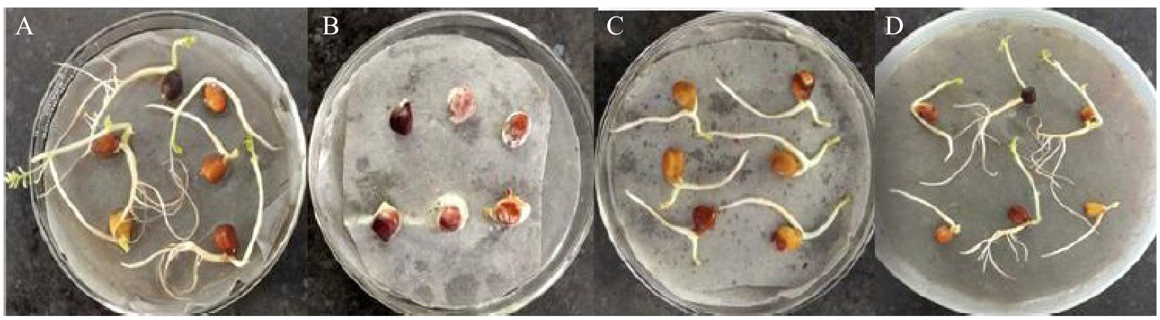

Figure 6A. Effect of Ag-NPs and 15B-NPs on gram seeds **(A)** Normal control seeds, **(B)** Fungus (*Fusarium oxysporum*) treated seeds, **(C)** Ag-NPs treated seeds and **(D)** 15B-Nps treated seeds.

Figure 6B. The figure represents the growth of seedlings after 16 days **(A)** control seeds, **(B)** AgNPs treated seeds, **(C)** 15B-NPs treated seeds, **(D)** fungus treated seeds, **(E)** AgNPs and fungal treated seeds and **(F)** 15B-NPs +fungal treated seeds. A representative among 25 seedlings is shown here.

and yield when primed to seeds of wheat. Significantly, the treatment with NPs also mitigated the accumulation of heavy metals like Cadmium in wheat grains (Rizwan *et al.*, 2019). Similarly, Hira *et al.* discovered eco-friendly Zinc nanoparticles exhibit anticancer activity against A549 lung adenocarcinomas (Hira *et al.*, 2021). Another study showed that the application of Silica nanoparticles on Ginseng restricted fungus growth, causing root rot disease (Abbai *et al.* 2019). It was shown that the NPs of silicon regulated the gene expression involved in sugar efflux and thus contributed to the decreased pathogenesis. In an interesting report, the use of nanoscale foliar exposure of Carbon nanotubes, C60-fullerenes, TiO_2, and Ag showed the plant's resistance against the Tobacco Mosaic Virus. The treatment of NPs on the plants helped them to maintain photosynthesis process by keeping the chloroplast unaffected (Adeel *et al.*, 2021).

Conclusion

Biogenic NPs have a lot of advantages, and they improve the application in comparison to chemically synthesized NPs. Different types of bio-based materials can be utilized, including microbes. One of our previously isolated bacterial strains *Burkholderia* sp (15B), carrying beneficial agricultural traits, is utilized to prepare the Ag-NPs. The application of the nanopesticides on the seeds not only controls the phytopathogen fungal strains but also augments their growth which is a remarkable outcome of the synthesized nanoparticles from the microbes. Generally, these microbes are found in the soil exhibiting biofertilizer activity. Biocontrol mechanism is yet unknown for now but more research in the area is requisite to find out the possible mechanism of their activity and understand their impact on environment.

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Data statement

Not applicable

Conflict of interest

The authors declare to have no conflict of interest.

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