Journal of Mines, Metals and Fuels, *71(11): 2084-2088; 2023. DOI: 10.18311/jmmf/2023/36098*

Print ISSN : 0022-2755

Journal of Mines, Metals and Fuels

Contents available at: *www.informaticsjournals.com/index.php/jmmf*

Phytotoxicity Study of Biogenic Mono-metallic and Bi-metallic (Au-Ag) Alloy NPs

Rupesh Dutta Banik[1], Rakesh Kumar Mandal[2], Bubai Das[3], Pritha Pal[4], Sibashish Baksi[5], and Sabyasachi Ghosh[5]*

[1]*Research Scholar, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Kolkata - 700121, India*

[2]*Research Scholar, Department of Physics, University of Kalyani, Kalyani, West Bengal - 741235, India*

[3]*Research Scholar, Department of Computer Science, West Bengal State University, Barasat, West Bengal - 700126, India*

[4]*Assistant Professor, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Kolkata - 700121, India*

[5]*Assistant Professor, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Kolkata - 700121, India; sabyasachig@svu.ac.in*

Abstract

Our findings of this study report the phytotoxicity assessment of biogenic bimetallic alloy (Au-Ag) NPs with monometallic counterparts (AgNPs and AuNPs) on Rice seed germination in respect of seedling growth and germination percentage. The phytotoxicity study of all three NPs on Rice seed indicates that seed germination percentage, root and shoot growth is not affected (upto 60 μg/ml) with biogenic (Au-Ag) NPs and AuNPs while, AgNPs had little phytotoxicity responses at higher concentration (40 μg/ml) level. So, ecological safe and sustainable biogenic alloy (Au-Ag) NPs promise potential nanocatalyst-based application for remediation of the hazardous dye from wastewater and other relevant areas.

Keywords: *Bimetallic, Germination and Seedling Growth, Gold-Silver Nanoparticles, Monometallic, Phytotoxicity Study*

1.0 Introduction

The world is currently confronted with the major challenge of environmental contamination. Various kinds of pollutants (metals, organic dyes, pesticides, pigments, etc.) containing cause an increased rate of effluence towards surface and groundwater[1]. Green methods using metallic nanoparticles (NPs) based catalytic process have exhibited to be potentially useful in the catalysis of environmental pollutant to nontoxic substances due to high efficiency required small amount, economical, and take less time to remove[2]. The NPs are clusters of atoms and their size ranging between 1-100 nanometers (nm) have huge number of active sites, large surface-to-volume ratios, and strong electron transfer abilities[3]. Of the several noble metal NPs, silver (Ag) and gold (Au) have been studied the maximum and have a wide range of important prospective applications[4]. Nevertheless, bimetallic nanoparticles (NPs) surpass the restricted characteristics of monometallic NPs and frequently exhibit increased catalytic activity in comparison to their monometallic equivalents[5].

*Author for correspondence

AuNPs is chemically inert and stable under atmospheric circumstances but AgNPs can form halides, oxides, and sulfide and also NPs are very reactive compared to their corresponding bulk materials. Therefore, metal NPs residues can be harmful for the environment, therefore determining their toxicity study is significant for the practical use of NPs[6,7]. The seed germination experiment is most commonly used method to assess the ecology safety of the NPs[8]. Rice is one of the important foods which feed more than half of the world's people it may be used to investigate the NPs phytotoxicity. Therefore, to protect the natural environment, it is of top priority to have a material that has potential applications for environmental remediation without any adverse impact. Numerous processes are available for production of metal NPs but employing plants is undoubtedly a well choice than other method due to are cost-effective, simple, eco-friendly, and fit for large-scale manufacture[9]. In our earlier article[10], we used *P. longifolia* leaf extracts to create monometallic and its bimetallic (Au–Ag) alloy NPs. Using rice (*Oryza sativa*) seeds as a model, we examine the ecological safety of these biosynthesized NPs against germination.

2.0 Materials and Methods

2.1 Biogenic Synthesis and Characterization of NPs

The comprehensive process of biosynthesis and its characterisation of bimetallic (Au–Ag) alloy NPs and its monometallic NPs were presented in our earlier study[10]. The produced nanoparticles (NPs) had a diameter ranging from 5 to 20 nm and exhibited a stable form for a period upto 3 weeks. Throughout the experiment, Milli-Q water and borosilicate/borosil glassware were employed.

2.2 Phytotoxicity effect of NPs

2.2.1 Germination and Seedlings Growth Assay

Initially, 10% (v/v) sodium hypochlorite solution was used to sterilise rice (*Oryza sativa*) seeds that were received from BCKV, Nadia, West Bengal, India. The seed was cleaned of any dust particles using distilled water, and then dried at room temperature in preparation for future use. In Petri plates with a 150 mm Whatman 42 Filter submerged in water at the bottom, ten robust seedlings were positioned. As the control treatment, sterile distilled water was used while solutions containing varying doses of biogenic nanoparticles (10, 20, 40, and 60 μg/ml) were added. The Petri plates were marked, coated, and wrapped with Parafilm tape when the treatment was finished. They were then put in a plant-growth incubator chamber with a temperature of 28 ± 2°C, 80% relative humidity, and 12-hour day and night cycles. Therefore, the germination data and seedling (shoot and root length) growth of seeds under hydroponic culture circumstances were recorded after 7 d throughout the testing for each treatment[11].

2.3 Statistical Analysis

Windows version of the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) Since a computer programme was utilized for the statistical study, one-way analysis of variance (ANOVA) was performed to look at the data that were acquired.

3.0 Results and Discussions

3.1 Nanoparticles Used

We previously reported on the biogenic synthesis of bimetallic alloy (Au–Ag) NPs and its monometallic NPs by using leaf extract from *Polyalthia longifolia* as suitable stabilising and reducing agents[10]. All three of the biosynthesized NPs have a lengthy half-life and are primarily spherical in form (5–20 nm).

3.2 Phytotoxicity Assessment of NPs

The phytotoxicity effect of the biosynthesized NPs was assessed through evaluation on germination of Rice (*Oryza sativa*) seeds as well as on seedling growth.

3.3 Effect on Seed Germination

AuNPs showed no detrimental effects on plants, as shown by the fact that rice (*Oryza sativa*) seeds applied with biogenic AuNPs (79–85%) didn't significantly differ from seeds applied with leaf (*P. longifolia*) extract (81–85%) or control (81%) (Figure 1a). Even though it is statistically insignificant, there is evidence that the extract and AuNPs treatments promote germination. Similar effects on seed germination after treatment with biosynthesized AuNPs

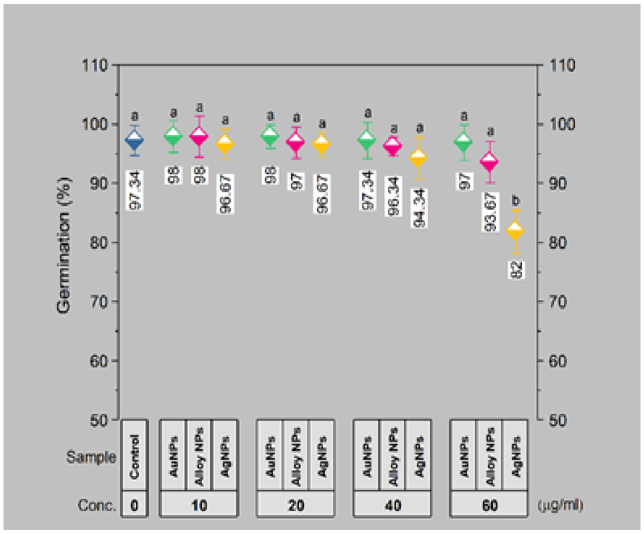

Figure 1. Phytotoxicity (*in-vitro*) of biogenic NPs assessed in terms seed germination of Rice (*Oryza sativa*). [Bars signify standard error (±SE)]. According to the Duncan's multiple (p < 0.05) range tests different letters indicate statistically significant variances.

have also been observed in previous investigations[12]. The germination of the alloy NPs treated rice (75-83%) seeds showed a minor effect as well (Figure 1).

Therefore, neither the bimetallic Au-Ag NPs nor the biosynthesized AuNPs had any influence on the germination of rice seeds upto 60 μg/ml concentration. Rice seed germination was significantly affected by AgNPs at higher doses (60 μg/ml), whereas normal seed germination being seen (Figure 1) at lower concentrations (40 μg/ml)[13]. The result indicated that the biogenic Au and alloy NPs (60 μg/ml) had no phytotoxic effects on rice seed germination. As compared to the control, AgNPs exhibited a little phytotoxic consequence on the seed germination at higher concentrations (100 g/ml).

3.4 Effect on Seedling Growth

In order to examine differences in shoot and root length seven days following NPs treatment, pre-germinated rice seeds were used in this investigation. The lengths of the roots (3.75–3.90 cm) and shoots (4.90–5.10 cm) following AuNP treatment were comparable to those of the control (shoots: 4.92 cm; roots: 3.73 cm) and extract (shoots: 4.75–5.35 cm; roots: 3.50–4.40 cm) treatments (Figure 2). Such AuNP-mediated seedling development beginning has been documented in earlier studies[14]. In comparison to extract and control treatment, a comparable outcome was also detected for treatment with alloy NPs in terms of shoots (4.72-5.13 cm) and roots (3.63-4.07 cm) length (Figure 2). Consequently, it was discovered that bimetallic alloy (Au-Ag) nanoparticles had no detrimental impacts on the growing of rice seedlings (root and shoot). This could be explained by the way that AuNPs prevent cellular damage caused by AgNPs, as well as how the two metals work in concert and how they are coated with a variety of phytochemicals[15,16]. No inhibitory effect was detected by AgNPs treatment up to 40 μg/ml on shoot and root length (Figure 2). Though, the inhibitory effect of AgNPs at 60 μg/ml was significant (<0.05) but at higher (≥ 60 μg/ml) concentrations, shoot and root elongation was penetrating treated with biogenic AgNPs.

Micronutrients are well recognized to have nontoxic effects at lower quantities, but at greater concentrations they can have considerable negative impacts on seed germination and seedling growth[2,17]. Throughout the course of the exposure period, a number of studies have demonstrated a strong link between the concentration of AgNPs and their phytotoxicity on plants. AgNPs can be phytotoxic to plants when applied in doses greater than a certain threshold. The plant cells have built-in defensive mechanisms that enable them to counteract the

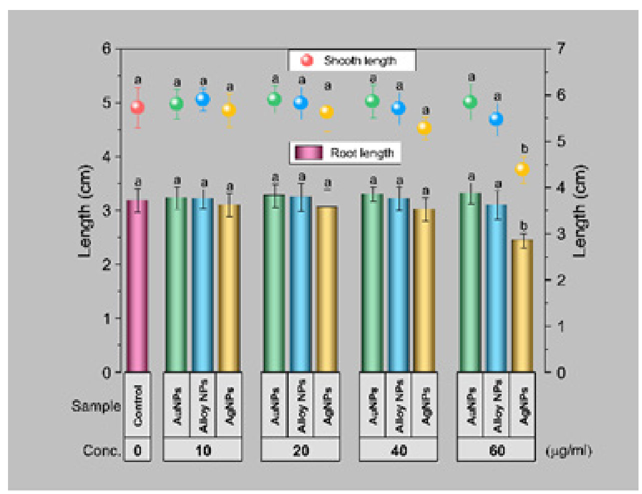

Figure 2. Phytotoxicity (*in-vitro*) of biogenic NPs assessed in terms of seedling growth (Root and Shoot length) of Rice (*Oryza sativa*). [Bars signify standard error (±SE)]. According to the Duncan's multiple (p < 0.05) range tests different letters indicate statistically significant differences.

toxic effects of stressful situations, such as those caused by NPs, by turning on a variety of enzymatic and non-enzymatic detoxifying procedures. When stress levels reach a certain level, the plant cells die because the internal defense mechanism is unable to resist the toxin[15]. AgNPs accumulate in roots or shoots and its changing endogenous mechanisms and causing stress in plants, increase ascorbate, glutathione, and carotenoid contents, as well as increase superoxide dismutase activity, reactive oxygen species formation, proline, total chlorophyll, and H_2O_2 levels. Such endogenous processes restrict transpiration and/or photosynthesis and cause aberrant morphological and structural alterations, among other symptoms[17,18]. Overall, the results showed that biogenic AuNPs and alloy NPs had no phytotoxic outcome on rice seed germination upto that point, whereas AgNPs showed reduced phytotoxicity at higher (60 μg/ml) levels concentration. Further comprehensive research is needed to study the phytotoxicity of NPs to different animal models and their ecologies.

4.0 Conclusions

The current study assesses the biogenic bimetallic alloy (Au-Ag) NPs and its mono (Ag, Au) NPs phytotoxicity with regard to rice seed germination. The findings showed that, when utilising biogenic alloy NPs and AuNPs, seed germination and seedling growth were nontoxic (upto 60 μg/ml), whereas AgNPs showed little phytotoxicity reactions at higher concentrations (40 μg/ml). The results of this work contribute to the expanding body of research on NPs' environmental risks and the investigation of bimetallic alloy NPs from sustainable sources that may be used in a variety of environmental applications.

5.0 References

1. Mukherjee A, Mondal R, Biswas S, Saha S, Ghosh S, Kole RK. Dissipation behaviour and risk assessment of fipronil and its metabolites in paddy ecosystem using GC-ECD and confirmation by GC-MS/MS. Heliyon. 2021 May 1; 7(5).
2. Falco WF, Scherer MD, Oliveira SL, Wender H, Colbeck I, Lawson T, Caires AR. Phytotoxicity of silver nanoparticles on *Vicia faba*: evaluation of particle size effects on photosynthetic performance and leaf gas exchange. Science of The Total Environment. 2020 Jan 20; 701:134816.
3. Mandal RK, Ghosh S, Majumder TP. Comparative study between degradation of dyes (MB, MO) in monotonous and binary solution employing synthesized bimetallic (Fe-CdO) NPs having antioxidant property. Results in Chemistry. 2023 Jan 1; 5:100788.
4. Bootharaju MS, Pradeep T. Understanding the degradation pathway of the pesticide, chlorpyrifos by noble metal nanoparticles. Langmuir. 2012 Feb 7; 28(5): 2671-9.
5. Zaleska-Medynska A, Marchelek M, Diak M, Grabowska E. Noble metal-based bimetallic nanoparticles: the effect of the structure on the optical, catalytic and photocatalytic properties. Advances in Colloid and Interface Science. 2016 Mar 1; 229:80-107.
6. Drake PL, Hazelwood KJ. Exposure-related health effects of silver and silver compounds: a review. Annals of Occupational Hygiene. 2005 Oct 1; 49(7):575-85.
7. Levard C, Hotze EM, Lowry GV, Brown Jr GE. Environmental transformations of silver nanoparticles: impact on stability and toxicity. Environmental Science and Technology. 2012 Jul 3; 46(13):6900-14.
8. Barrena R, Casals E, Colón J, Font X, Sánchez A, Puntes V. Evaluation of the ecotoxicity of model nanoparticles. Chemosphere. 2009 May 1; 75(7):850-7.
9. Botha TL, Elemike EE, Horn S, Onwudiwe DC, Giesy JP, Wepener V. Cytotoxicity of Ag, Au and Ag-Au bimetallic nanoparticles prepared using golden rod (Solidago canadensis) plant extract. Scientific Reports. 2019 Mar 12; 9(1):4169.
10. Ghosh S, Roy S, Naskar J, Kole RK. Process optimization for biosynthesis of mono and bimetallic alloy nanoparticle catalysts for degradation of dyes in individual and ternary mixture. Scientific Reports. 2020 Jan 14; 10(1):277.
11. Kim DY, Saratale RG, Shinde S, Syed A, Ameen F, Ghodake G. Green synthesis of silver nanoparticles using Laminaria japonica extract: characterization and seedling growth assessment. Journal of Cleaner Production. 2018 Jan 20; 172:2910-8.
12. Mahakham W, Theerakulpisut P, Maensiri S, Phumying S, Sarmah AK. Environmentally benign synthesis of phytochemicals-capped gold nanoparticles as nanopriming agent for promoting maize seed germination. Science of the Total Environment. 2016 Dec 15; 573:1089-102.
13. Budhani S, Egboluche NP, Arslan Z, Yu H, Deng H. Phytotoxic effect of silver nanoparticles on seed ger-

mination and growth of terrestrial plants. Journal of Environmental Science and Health, Part C. 2019 Oct 2; 37(4):330-55.

14. Kumar V, Guleria P, Kumar V, Yadav SK. Gold nanoparticle exposure induces growth and yield enhancement in Arabidopsis thaliana. Science of the Total Environment. 2013 Sep 1; 461:462-8.
15. Tripathi DK, Singh S, Singh S, Pandey R, Singh VP, Sharma NC, Prasad SM, Dubey NK, Chauhan DK. An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. Plant Physiology and Biochemistry. 2017 Jan 1; 110:2-12.
16. Zaka M, Abbasi BH. Effects of bimetallic nanoparticles on seed germination frequency and biochemical characterisation of Eruca sativa. IET nanobiotechnology. 2017 Apr; 11(3):255-60.
17. Kumari R, Singh JS, Singh DP. Biogenic synthesis and spatial distribution of silver nanoparticles in the legume mungbean plant (*Vigna radiata L.*). Plant Physiology and Biochemistry. 2017 Jan 1; 110:158-66.
18. Ghosh S, Rana D, Sarkar P, Roy S, Kumar A, Naskar J, Kole RK. Ecological safety with multifunctional applications of biogenic mono and bimetallic (Au–Ag) alloy nanoparticles. Chemosphere. 2022 Feb 1; 288:132585.