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Biogenic Silver Nanoparticles Modulates Heavy Metal Toxicity in Moringa Oleifera

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Abstract

This research explores the antioxidative and adsorptive potential of biogenic silver nanoparticles (AgNPs) in reducing heavy metal toxicity in Moringa oleifera. Utilizing DPPH, ferric reducing, and hydrogen peroxide scavenging assays, we analyzed antioxidant levels in M. oleifera grown in soils treated with cadmium and lead, both with and without the addition of AgNPs. The silver nanoparticles, synthesized through a green process using cocoa pod extracts, demonstrated a significant reduction in heavy metal toxicity effects, enhancing growth metrics such as germination rate, shoot/root length, and antioxidant activity. These findings indicate that AgNPs effectively mitigate heavy metal stress, providing a sustainable approach to enhancing plant resilience in contaminated soils and suggesting potential applications for AgNPs in phytoremediation.

Keywords: Biogenic Silver Nanoparticles, Heavy Metal Toxicity, Moringa oleifera, Antioxidant Activity and Phytoremediation

Introduction

Industrial and agricultural endeavors are intimately associated with the extensive use of a wide array of chemicals. Historically chemical wastes generated through industrial processes were disposed of through flagrant release into the environment. Heavy metals uptake by plants and successive accumulation in human tissues and biomagnifications through the food chain cause both human health and environmental concerns (Wong J.W.C et al.,2006). Heavy metals are considered one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals, especially Cu, Ni, Cd, Zn, Cr, and Pb [1]. Some heavy metals (like Fe, Zn, Ca, and Mg) have been reported to be of bio-importance to man and their daily medicinal and dietary allowances have been recommended. However, some others (like As, Cd, Pb, and methylated forms of Hg) have been reported to have no known bio-importance in human biochemistry and physiology, and consumption even at very low concentrations can be toxic [2].

Heavy metals exert toxic effects on soil microorganisms hence resulting in a change in the diversity, population size, and overall activity of the soil microbial communities [3]. Gasses quickly dispersed into the atmosphere; liquids were diluted into receiving waters and efficiently transported away from the site of generation. Similarly, pesticides and other agricultural chemicals revolutionized farm and forest productivity. Potential adverse effects of the application of such chemicals to the environment were viewed as insignificant relative to the benefits bestowed by such practices. Appropriate regulation of the release of chemicals into the environment without applying unnecessarily stringent limitations on industry and agriculture requires a comprehensive understanding of the toxicological properties and consequences of release of the chemicals into the environment. It was from this need that modern environmental toxicology evolved. Appropriate regulation of the release of chemicals into the environment without applying unnecessarily stringent limitations on industry and agriculture requires a comprehensive understanding of the toxicological properties and consequences of the release of the chemicals into the environment. It was from this need that modern environmental toxicology evolved.

Materials and Method

Chemicals/ Reagents

Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Aluminiun trichloride, Ferric (ii) chloride, Cadmium chloride, Lead chloride, Sodium Hydroxide, Methanol, iron(iii)chloride, Sodium tricarbonate, 10ml Folin Caocalteu Acetone, KH_2PO_4, and

K_2HPO_4 was used to prepare [Phosphate buffer pH (7.4), Phosphate buffer saline pH (6.6)], 2,2-diphenyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), Silver nanoparticles, Distilled Deionized water.

Apparatus/ Equipment

Analytical Weighing Balance, UV-visible spectrophotometer, Beakers, Pipette 10ml, Micropipette, Standard Volumetric Flask, Conical flask Water Bath with shaker, Centrifuge Bucket, Centrifuge Tubes, Molinux Blender, Oven, Measuring Cylinder, Spatula, Sample Bottles, Cuvettes.

Synthesis of Silver Nanoparticle from Cocoa Pod

Biologically synthesized AgNps from cocoa pods were obtained from the biotechnology unit of the biological science department at Ladoke Akintola University of Technology, Ogbomosho

Planting of Moringa Oleifera

Moringa Oleifera seeds were procured from a seed vending shop at Oja-Oba market in Osogbo, Osun state Nigeria, the seeds were selectively placed in the buckets containing solutions of water, AgNPs, Cadmium chloride (Cd), Lead chloride (Pb), Cadmium + AgNPs, Lead + AgNPs, Cadmium + AgNPs equal proportion, Lead + AgNPs equal proportion designated as A, B, C, D, E, F, G and H respectively. These concentrations were prepared from the stock of each solution using distilled water. Forty 7-litre capacity buckets were filled each with 25 g of 2 mm wire mesh filtered soil containing eight groups (five buckets for each group). Each group was soaked with the prepared solution designated for it, followed by the sowing of an average of one hundred and sixty (160) Moringa Oleifera seeds on the soil. The seeds were watered daily with 20 ml solution prepared for each group and all groups were subjected to the same environmental conditions. The Moringa Oleifera plants were grown for two weeks before harvesting.

Aqueous Methanolic Extraction of Moringa Oleifera

Moringa Oleifera leaves were harvested after two weeks of planting and oven-dried at the temperature (110oC). Thereafter, weight according to the proportion of yield of leaves from each group was ground into powder using a laboratory Morter and Pestle. The extraction of phytochemicals was done twice for each group by dissolving proportionate mass of the powder into 1litre of 70 % aqueous methanol for 2 hours. The solution was afterwards filtered using Whatman No 1 filter paper. The residues from the previous filtration were extracted with proportionate volume of aqueous methanol of 70 % for two hours and then filtered. The filtrates were combined and concentrated and thereafter evaporated to dryness using water bath at 700C. The dried concentrate was dissolved in absolute methanol for analysis.

Dpph Scavenging Assay

The free radical scavenging ability of the extract was determined using the stable radical DPPH as previously determined. One ml of various concentrations of the homogenates was added to 4 ml of 0.1 mmol L-1 methanolic solution of DPPH. Blank was obtained by preparing 1 ml of methanol in 4 ml of DPPH. The samples were incubated in the dark at room temperature for 30 min. The absorbance was read at 517 nm against the prepared blank.

Inhibition of free radicals by DPPH in percent (I%) was calculated using this formula:

$$Inhibition\ (\%) = \frac{(A_{control} - A_{Sample})}{A_{Control}} \times 100$$

where Acontrol is the absorbance of the control reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound.

Hydrogen Peroxide Scavenging Assay

4 ml of graded concentrations of AgNPs with 0.6 ml of 40 mM H_2O_2 prepared in phosphate buffer (pH 7.4) at room temperature (30oC) for 20 min. While distilled water was used as blank, the H_2O_2 solution was used as the control, and the absorbance readings were read at 610 nm. The percentage peroxide scavenging activity was calculated thus:

$$Peroxide\ scavenging\ activity = \frac{(A_{control} - A_{Sample})}{A_{Control}} \times 100$$

Ferric Reducing

1ml of each extract was added to 250uL of phosphate buffer of PH (6.6), 2.5ml of potassium ferricyante was added to the solution of each extract, and the solution of each extract was incubated at 50oc for 20 minutes, after the incubation, the solution of each extract was centrifuged at 500rpm for 10 minutes. After the centrifugation 250uL of the supernatant of each of the centrifuged extracts was added to 250uL of deionized distilled H_2O and 500uL of $FeCl_3$, the blank was obtained by using Deionized water, and the absorbance was read at 700nm against the prepared blank.

Determination of Growth Parameters and Moisture Content

Moringa oleifera were harvested after 2 weeks of planting and different growth parameters were determined. The germination percentage was determined using equation 1. Root and shoot lengths were measured using a metric rule.

The Vigour index was calculated using equation 2 while the moisture content was determined as reported by Azeez et al. (2012)

$$\% \text{ Germination} = \left(\frac{\text{number of germinated seeds}}{\text{total number of seeds}} \right) \times 100 \quad \text{—— Eq. 1}$$

Vigour index = (root length + shoot length) x % Germination ------- equation 2

Results

The results of antioxidant analysis of DPPH scavenging assay, Ferric reducing, and H_2O_2 scavenging are presented in figure.1 Highest antioxidant activity in Moringa oleifera was obtained in soil treated with silver nanoparticles and also assisted in modulating heavy metal toxicity induced in M. oleifera planted on contaminated soil, the M. oleifera contaminated with heavy metals (lead and cadmium) reduced the antioxidant activity in M. oleifera while he one with AgNPs boost the antioxidant activity. Following the trend AgNPs: DPPH>H_2O_2>Ferric.

Growth Parameters

Soils treated with AgNPs showed improved germination rates, root and shoot lengths, and overall plant vigor compared to plants exposed solely to Cd or Pb. The plant grown with cadmium + AgNPs, lead + AgNPs, cadmium + AgNPs equal proportion, and lead + AgNPs equal proportion also had improvement in antioxidant activity. Therefore, it was deduced that silver nanoparticles (AgNPs) helped in modulating heavy metal toxicity induced in Moringa oleifera by reducing the heavy metal toxicity via adsorption. These results support the potential of AgNPs as a protective agent that enhances plant growth in heavy metal-contaminated environments.

Antioxidant Activity

The DPPH, hydrogen peroxide, and ferric-reducing assays indicated elevated antioxidant activity in M. oleifera treated with AgNPs. Specifically, the DPPH scavenging activity showed the highest antioxidant enhancement in plants treated with AgNPs combined with heavy metals, suggesting that AgNPs contribute to enhanced oxidative stress defense mechanisms in plants under metal-induced toxicity.

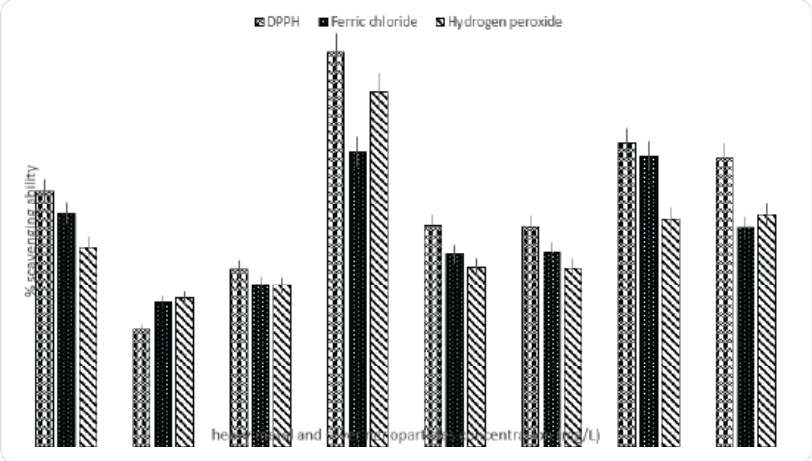

Figure 1: Antioxidant Result

Conclusion

Biogenic silver nanoparticles significantly modulate heavy metal toxicity effects in Moringa oleifera, promoting better growth and enhancing antioxidant defenses. This study demonstrates the potential for AgNPs in reducing environmental heavy metal toxicity and highlights their application in sustainable agriculture and phytoremediation [4].

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