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COMPARATIVE PHYTOTOXIC ASSESSMENT OF SILVER NANOPARTICLES, HUMIC ACID-CAPPED AGNPS, AND CTAB-CAPPED AGNPS ON GERMINATION AND EARLY SEEDLING GROWTH OF DR-16 COARSE RICE (ORYZA SATIVA L) AT DIFFERENT CONCENTRATIONS

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ABSTRACT
The increasing application of silver nanoparticles (AgNPs) and its capping agents in agriculture raises concerns about their phytotoxic possessions on harvested plants. This research evaluated phytotoxic impact of AgNPs, Humic acid induced with synthesized silver nanoparticles, and AgNPs capped with CTAB , on the seedling growth of rice variety DR-16 (coarse) taken from Dokari Agricultural Research Institute Larkana . Seeds were treated with synthesized AgNPs, Humic acid-AgNPs, CTAB-AgNPs, at 5, 10, 15, and 20 ppm concentrations under controlled laboratory conditions. Growth parameters included shoot and root length, fresh and dry weight and germination percentage were recorded. Results indicated concentration-dependent respond, where low concentrations (5–10 ppm) showed growth stimulation, while higher concentrations (15–20 ppm) exhibited phytotoxic effects, indicated reduced root elongation and biomass. Capping agents significantly reduced toxicity by stabilizing nanoparticles and lowering reactive oxygen species (ROS) production. The study concluded, controlled use of capped AgNPs enhanced plant growth, whereas higher concentrations induced oxidative stress and phytotoxicity

KEYWORDS: Silver nanoparticles, Phytotoxicity, DR-16 (coarse), Capping agents, Seedling growth

INTRODUCTION

The rapid expansion of nanotechnology has increased the presence of engineered silver nanoparticles (AgNPs) in agricultural ecosystems, creating a critical need to evaluate their impact on plant physiology and food safety (Khan, et al., 2023; Siddiqi and Husen, 2022; Huang, et al., 2022). Because rice (*Oryza sativa* L.) is an essential global dietary staple, understanding how nanomaterials affect the highly sensitive stages of seed germination and early seedling growth—particularly in resilient cultivars like DR-16 coarse rice—is vital for ensuring crop yields and food security (Bin Rahman, and Zhang, 2023; Mohidem, et al., 2022; Zhao, et al., 2020).

Crucially, the phytotoxicity and environmental behavior of AgNPs are fundamentally governed by their surface chemistry and the capping agents used to stabilize them (Biba, et al., 2021; Xuet al., 2021; Ghobashy, et al., 2021). Capping layers alter the nanoparticle's surface charge and aggregation behavior, changing how it interacts with plant cell walls. By conducting a comparative assessment using different concentrations of natural, negatively charged humic acid-capped silver nanoparticles (AgNPs-HA) alongside synthetic, positively charged cationic surfactant-capped silver nanoparticles (AgNPs-CTAB), researchers can map out concentration-dependent thresholds (Jeon, et al., 2023: Milewska-Hendel, et al., 2021; Cartwright, et al., 2020). This helps define the precise boundary lines between potentially beneficial biostimulatory effects at low doses and severe cellular or mechanical toxicity at higher exposure levels.

The phytotoxicity of silver nanoparticles in crop plants has been the subject of various studies (Osterheld, 2017; Matras, et al., 2022: Hasan, et al., 2021), but less research is present about the relative effects of humic acid- and CTAB-capped AgNPs on the germination and early seedling development of the DR-16 coarse rice variety grown in District Larkana Sindh, Pakistan. Furthermore, not enough research has been done on how various capping agents affect the phytotoxicity caused by nanoparticles under controlled germination settings. This study was conducted in order to assess the concentration-dependent phytotoxic effects of synthesized AgNPs, humic acid-capped AgNPs, and CTAB-capped AgNPs on biomass accumulation, root and shoot growth, and seed germination in DR-16 rice seedlings.

Rice (*Oryza sativa L*) is one of the utmostessentialprimaryfoods crop worldwide, providing nourishment to the global population (Birla, et al., 2017). In countries like Pakistan, rice plays dynamicpart in nutrientssanctuary and economic stability, with coarse varieties such as DR-16 being widely cultivated due to their adaptability and yield potential at

District Larkana Sindh Province. However, increasing environmental stress and the introduction of emerging contaminants, particularly nanomaterial, have raised concerns regarding their impact on crop growth and productivity (Naeem, et al., 2023). Among engineered nanomaterial, silver nanoparticles (AgNPs) have gained significant attention due to their extensive applications in medicine, agriculture, textiles, and water treatment, primarily because of their strong antimicrobial properties (Bamal, et al., 2021). Despite their beneficial uses, the uncontrolled release of AgNPs into the environment has led concerns about their potential phytotoxic effects on plants. Phytotoxicity referred to the toxic response induced in organisms when exposed to light in the presence of certain chemicals or nanoparticles thatcan enhanced the generation of ROS (reactive oxygen species) and leads to oxidative stress (Yang, et al., 2017). Interaction of AgNPs with plant systems is complex and depends on several factors, including particle size, concentration, surface charge, and the presence of stabilizing agents, such as Humic acid and cetyltrimethylammonium bromide (CTAB) commonly used to stabilize nanoparticles and modify their physicochemical properties. These agents influence nanoparticle dispersion, bioavailability, and interaction with plant tissues, thereby altering their toxicity profiles. Recent studies demonstrated, AgNPs can significantly affect seed germination, root and shoot elongation, and biomass accumulation in various plant species (Siddiqi, and Husen, 2022). Thephytotoxic effects of AgNPs are particularly critical during early developmental stages, where light-mediated reactions can intensify oxidative damage, disrupt cellular structures, and impair physiological processes such as photosynthesis and nutrient uptake (Djanaguiraman, et al., 2024). However, the extent of these effects varies depending on the type of capping agent used, which may either mitigate or exacerbate nanoparticle-induced stress (Ramegowda, et al., 2024).
Despite growing research of nanoparticle on plant interactions, limited information is available regarding the combined phytotoxic effects of AgNPs and capping agents on coarse rice varieties, particularly DR-16. Understanding these interactions is essential for evaluating environmental risks and developing safer nanotechnology applications in agriculture (Yang, et al., 2017). Therefore, aims of this research is to explore the phytotoxic effects of silver nanoparticles and their associated capping agents, humic acid, and CTAB on the germination and early seedling growth of DR-16 coarse rice variety under varying concentrations (5, 10, 15, and 20 ppm). The outcomes of this study will contribute to well understanding of nanoparticle-induced stress mechanisms and provide insights into the safe use of nanomaterial in agricultural system

MATERIALS AND METHODS

The rice variety DR-16 Coarse were taken from Dokari Agricultural Research Institute District Larkana Sindh Province Pakistan,The experiment was conducted under controlled laboratory conditions at Shah Abdul Latif University Khairpur using DR-16 rice variety, healty,uniform and diseases free seeds stored at laboratory under dry condition before experimental work.

Chemicals and Reagents

All chemical were analytical grade,used throughout the experiment. Silver nitrate ($AgNO_3$) and Sodium Borohydride ($NaBH_4$) were used as a precursor for the synthesis of silver nanoparticles; Humic acid and Cetyltrimethylammonium bromide (CTAB) were used as stabilizing agent. Double distilled water was used for the preparation of solutions.

Synthesis of Silver Nanoparticles

Preparation of Stock Solutions

Wet chemical reduction method used for the preparation of silver nanoparticles. All chemicals were analytical grade, 0.1 Molar (M) solution of silver nitrate was prepared with double distilled water and stored in the absence of sunlight. 0.1 Molar (M) solution of $NaBH_4$prepared with distilled water and stored in glass bottles.0.01 Molar (M) solution of CTAB (Cetyl-trimethy ammonium bromide), HA (Humic acid), were prepared with double distilled water and stored in reagent bottles. 1.5 mL of $NaBH_4$ (0.1 M), that has been chilled in ice bath, 2 folded molar excess of the reducing agents to which silver salt was used.0.5 mL of $AgNO_3$ (0.1 M) was poured drop wise into the solution of 1.5 mL of $NaBH_4$, stirred on magnetic stirrer at 60 ⁰C until the color of the solution turned to yellow indicating the formation of AgNPs.For capped nanoparticles, each capping agent, Humic acid, and CTAB was added separately during synthesis to stabilize the nanoparticles and prevent aggregation (Ijaz Hussain, et al., 2011).The synthesized AgNPs and silver Nanoparticles induced with Humic acid and CTAB were further characterized by UV-VIS Spectroscopy (A techniques that identify the ultraviolet or visible light absorbance, FT-IR analysis, (For identifying functional groups). X-RD (X-RAY Diffraction to analysis crystalline structure of substances) and zeta potential (Charge dispersion and stability of solutions) were used for analysis.

UV-VIS Spectroscopy Analysis

Shimadzu UV1800, Double beam Spectrophotometer were used from 200-800 nm, to analysissynthesis of silver nanoparticles by recording surface plasma Resonance band.Synthesized AgNPs displayed adsorption peaks at 400nm, confirmed the reduction of silver nitrate into silver ion, then into silver nanoparticles. While AgNPs induced with CTAB, Surface Plasmon resonance band recorded at 395nm confirmed the formation of capped silver nanoparticles. However AgNPs induced with Humicacid recorded to exhibited SPR band at 430nm.The different absorbance peaks were due to different capping agents, morphology and shape of molecules. The production of silver nanoparticles was successfully verified by UV-visible spectroscopy, which also showed that humic acid and CTAB had a major impact on the stability and optical characteristics of the nanoparticles. Because of surface coating, Humic acid produced broader peaks, whereas CTAB produced sharper peaks that showed improved nanoparticle dispersion and homogeneity (Jin, et al., 2024).

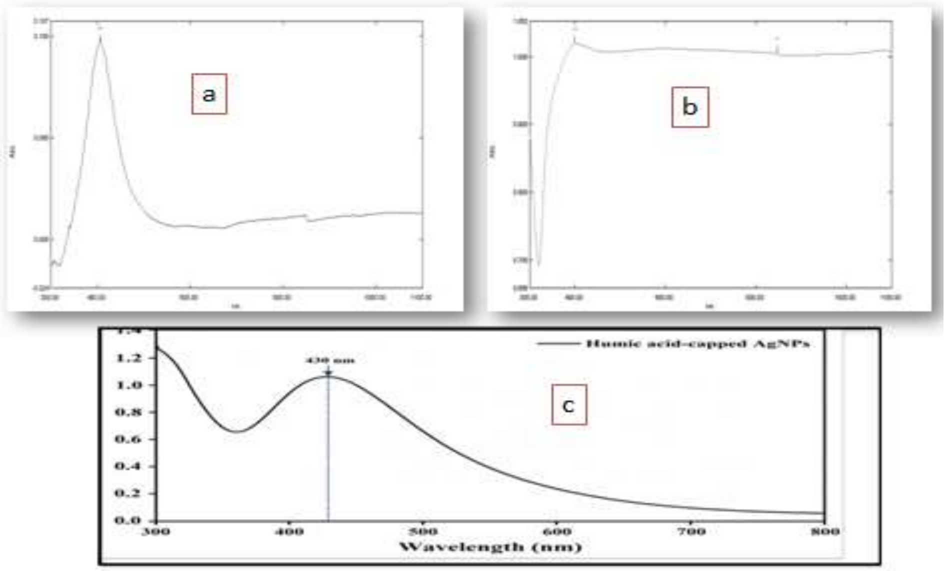

Figure: 1 (a) UV-VIS Spectrum of silver nanoparticles (b) UV-VIS Spectrum of AgNPs capped with CTAB (c) UV-VIS Spectrum of Humic acid capped AgNPs

FT-IR Analysis;

The functional groups convoluted in the manufacture and stability of silver nanoparticles were recognized by FTIR analysis. The occurrence of organic molecules in the manufacture of nanoparticles was attributed by the distinctive peaks in pure AgNPs that resembled to hydroxyl, carbonyl, and amine groups. The interaction of carboxylic and phenolic groups with silver nanoparticles was confirmed by the strong O–H and C=O stretching vibrations that humic acid-capped AgNPs showed with minor peak changes. Strong CH_2 stretching and C–N vibration bands were observed in CTAB-capped AgNPs, suggesting that surfactant molecules had successfully adhered to the nanoparticle surface. These spectrum changes demonstrated that CTAB and humic acid were efficient capping and stabilizing agents for AgNPs. FT-IR major peaks of AgNPs were recorded at1049, 1089, 1417, 2927, 2974, 3368 cm-1.Where as for AgNPs capped with Humic acid ,FTIR peaks were observed at 3429,2921, 1608 cm^{-1} confirmed induction of Humic Acid over AgNPs. The FTIR Spectrum of AgNPs-CTAB showed absorption band at 3422, 2921, 1240, 1043 cm^{-1}, that confirmed the formation of AgNPs capped with CTAB (Dharejo, et al., 2025).

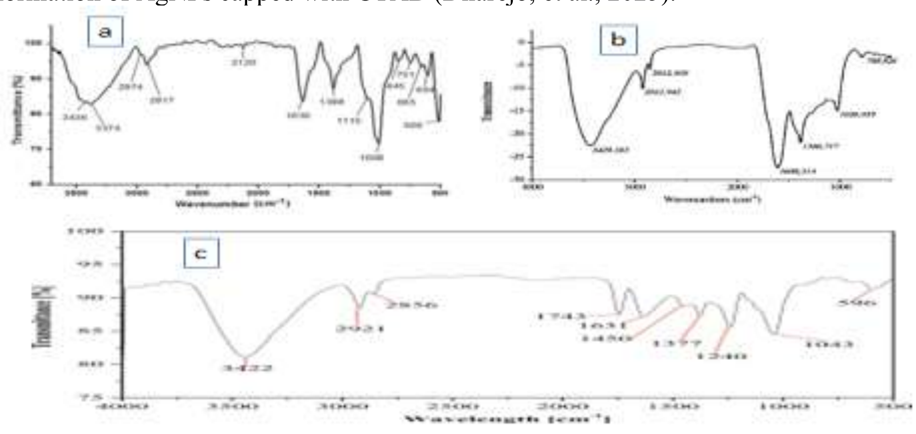

Figure: 2 (a) FTIR Spectrum of AgNPs (b) FTIR Spectrum of AgNPs-Humic acid (c) FTIR Spectrum of AgNPs-CTAB

X-RAY Diffraction Analysis:

The crystalline nature of the produced silver nanoparticles was verified by XRD analysis. The (111), (200), (220), and (311) planes of face-centered cubic silver were represented by the diffraction peaks seen at roughly 38.1°, 44.3°, 64.4°, and 77.4°, respectively. AgNPs' strong crystallinity was evidenced by the prominent (111) reflection. Because of the organic coating around the nanoparticles, humic acid-capped AgNPs displayed a small peak broadening and decreased intensity. AgNPs capped with CTAB showed relatively strong diffraction peaks, indicating better stability and dispersion of the nanoparticles. The absence of notable impurity peaks verified the synthetic silver nanoparticles' cleanliness.The effective production of crystalline silver nanoparticles with an FCC structure was confirmed by XRD analysis. Because of the organic surface coating, humic acid slightly broadened the peaks, while CTAB enhanced the stability and dispersion of the nanoparticles while preserving their crystallinity. Humic acid and CTAB both successfully functioned as capping agents without changing the metallic silver crystal structure, according to the diffraction patterns.

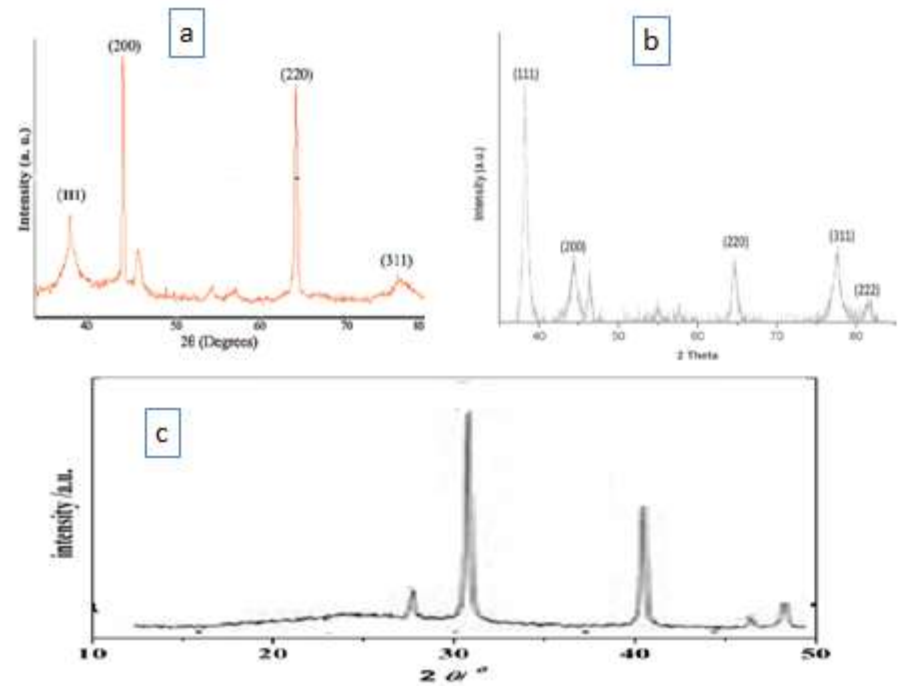

Figure: 3 (a) XRD Pattern of AgNPs (b) XRD of Pattern AgNPs-Humic Acid (c) XRD Pattern of AgNPs-CTAB

Zeta Potential Analysis

The surface charge and colloidal stability of the produced silver nanoparticles were assessed using zeta potential analysis. Because of the electrostatic repulsion between the particles, pure AgNPs showed a negative zeta potential value at,-28 mvindicating moderate stability. The presence of carboxyl and phenolic functional groups on the surface of humic acid-capped AgNPs was found at-32 mv that is responsible for their noticeably increased negative zeta potential. This higher negative charge decreased aggregation and improved the stability of the nanoparticles. On the other hand, because CTAB's positively charged quaternary ammonium groups were adsorbed, CTAB-capped AgNPs showed a positive zeta potential approximately .at 38mv.The high positive result demonstrated enhanced colloidal stability and efficient surfactant coating. These results showed that silver nanoparticles were effectively stabilized by humic acid and CTAB via electrostatic interactions (Francioso, et al., 2019). The solution of Synthesized AgNPs, Humic acid and CTAB capping agents were furtherapplied at DR-16 Coarse Rice variety to check the Agricultural effect and production stability.

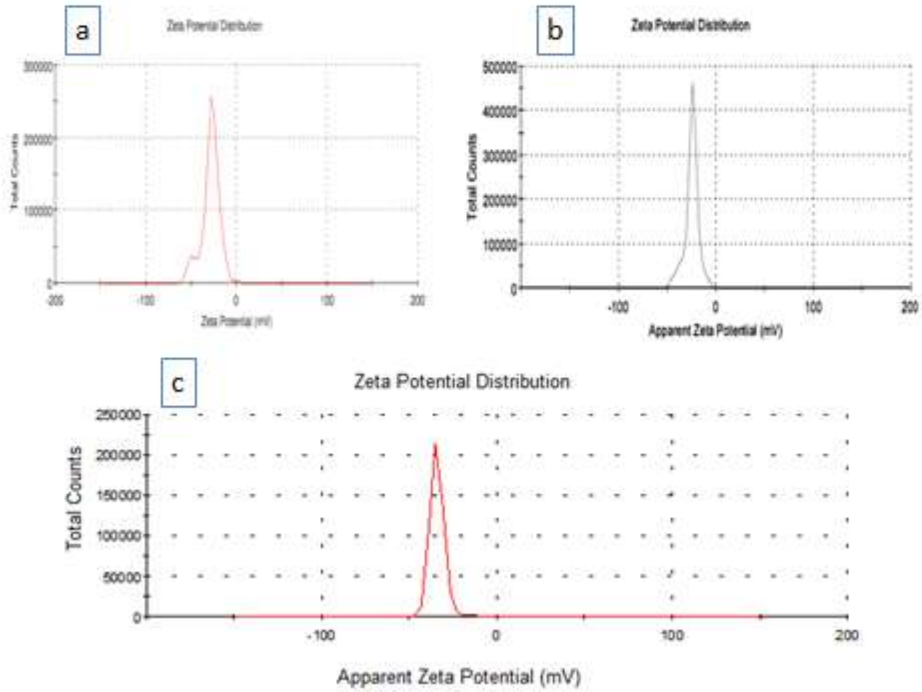

Figure 4: (a) Zeta potential of AgNPs (b) Zeta potential graph of AgNPs-CTAB (c) Zeta potential graph of AgNPs-Humic acid''

Preparation of Treatment Solutions

Stock solutions of AgNPs and capping agents CTAB-AgNPs, Humic acid-AgNPs solution were stored then 0.01M solution of each of synthesized AgNPs and capping agents were prepared and stored at laboratory of Shah Abdul Latif University Khairpur, among all stock solution, 5,10,15,20 ppm solution were applied for further treatment at the DR-16 Coarse rice variety .A control treatment (double distilled water) was utilized for comparison growth of shoot and root.

Seed Sterilization and Germination Setup:

Rice seeds of DR-16 Coarse were surface sterilized using 0.1% Bleaching powder ($CaOCl_2$) for 2–5 minutes followed by thoroughly washed withsterilized distilled water to eliminate any residues. Sterilized seeds were placed in Petri dishes in triplicate form lined with Whitman filter paper. Each Petri dish contained 10 seeds and was treated with respective solutions. All treatments were performed in triplicates to analysis germination rate of Shoot, root and effect on fresh and dry weight by treating seeds at 5, 10, 15, and 20 ppm of synthesized AgNPs and stabilizing agents induced with synthesized AgNPs. The experiment was arranged in a completely randomized design (CRD) with the following factors: Factor A: Treatment type (AgNPs and different capping agents) Factor B: Concentration levels (5, 10, 15, and 20 ppm), all treatment was repeated three times (Khamsen, et al., 2016).

Growth Conditions: Petri dishes were incubated under controlled environmental conditions: Temperature: 25 ± 2°C, Photoperiod of 12 h light / 12 h dark. Light intensity at standard laboratory illumination **(**Daryaei, et al., 2016) .These conditions were maintained to evaluate the phytotoxic effects of nanoparticles and Data Collection were recorded from 3rd day of germination and following parameters were recorded the germination percentage by Number of germinated seeds} × Total number of seeds ×100. Shoot and Root length was measured using ruler at 3rd day of germination recorded. Fresh weight of seedlings was recorded immediately after harvesting using a digital balance.Dry Weigh**t of** Seedlings were dried in an oven at 70°C for 48 hours and weighed to obtain dry biomass (Achten, et al., 2010**).**

Safety and Handling

All nanoparticle preparations and chemical handling were performed following standard laboratory safety protocols. Proper disposal methods were adopted for nanoparticle-containing waste to minimize environmental contamination. (Amoabediny, et al., 2009).

RESULTS AND DISCUSSION

Effect of AgNPs on seed Germination

The germination percentage of DR-16 coarse rice variety seed showed a concentration-dependent response to silver nanoparticles. The germination of shoot started at third day of arrangement. At lower concentrations (5 ppm), a slight stimulation in germination was observed compared to the control, suggesting a hermetic effect. While from 10 to15 ppm of AgNPs concentration showed high response towards germination, while at highest concentrations of 20 ppm, a significant decline in germination of shoot length was recorded as compared to the control. Below chart showed mean record of shoot length in centimeter.

Table 1: Effect of varying concentrations (5, 10, 15, and 20 ppm) of uncapped silver nanoparticles (AgNPs) on the shoot growth of DR-16 coarse rice (*Oryza sativa* L.) seedlings over time

control	5 ppm	10 ppm	15 ppm	20 ppm
0.19	0.15	0.26	0.27	0.24
0.24	0.29	0.34	0.34	0.25
0.34	0.34	0.36	0.38	0.28
0.39	0.41	0.45	0.47	0.33
1.43	0.48	0.57	0.66	0.47
1.5	0.64	0.64	0.66	0.62
2.97	1.53	1.8	1.83	1.7
3.93	2.56	2.56	2.68	3.11

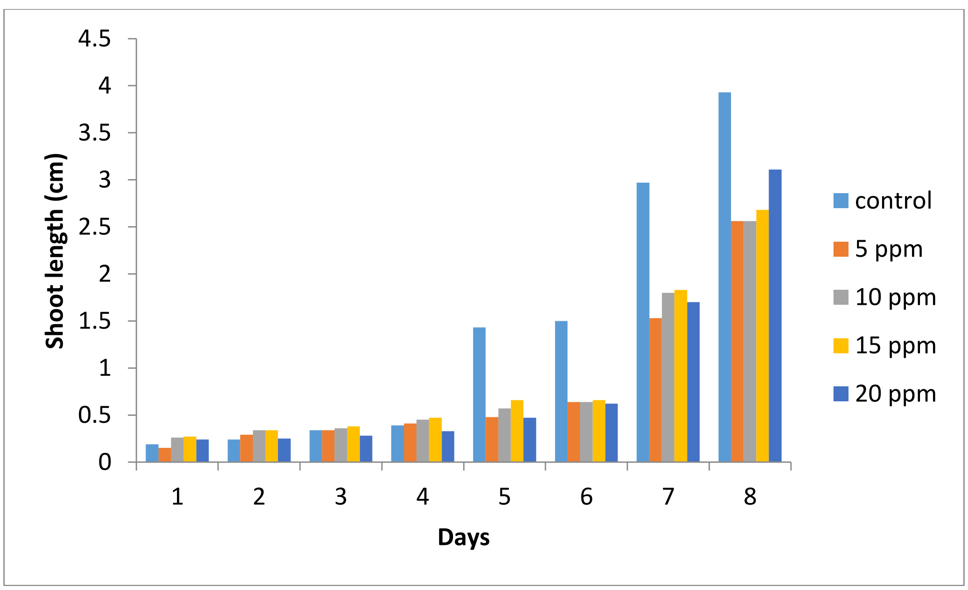

Figure 5: Concentration-dependent effect of synthesized silver nanoparticles (AgNPs) on the shoot length extension of DR-16 coarse rice (*Oryza sativa* L.) seedlings measured over an 8-day observation period. Data series represent control (double distilled water) alongside 5 ppm, 10 ppm, 15 ppm, and 20 ppm treatment concentrations.

AgNPs effect on Root vs. Concentration

A significant reduction in root length was observed with increasing nanoparticle concentration. Root growth was observed at fourth day of setting. Below chart is mean record of Root length. Root length became morepretentious than shoot length, indicated greater sensitivity to root tissues by nanoparticle exposure From 5ppm to 15 ppm,slightly growth of root were recorded while as concentration increased at 20 ppm, reduction of root length were appeared as compared to the control. The reduction in root length may be due to:

- Disruption of cell division in meristemetic tissues
- Oxidative stress-induced damage
- Impaired water and nutrient uptake

Table: 2 Effect of varying concentrations (5, 10, 15, and 20 ppm) of uncapped silver nanoparticles (AgNPs) on the root elongation (cm) of DR-16 coarse rice (*Oryza sativa* L.) seedlings recorded over consecutive observation days

Control	**5 ppm**	**10 ppm**	**15 ppm**	**20 ppm**
0	0	0	0	0
0.12	0.12	0.12	0.12	0.12
0.12	0.16	0.16	0.16	0.16
0.48	0.23	0.23	0.23	0.23
0.34	0.26	0.26	0.26	0.26
0.36	0.35	0.35	0.35	0.39
1.08	0.61	0.61	0.64	1.05
2.44	1.68	1.68	1.71	1.62

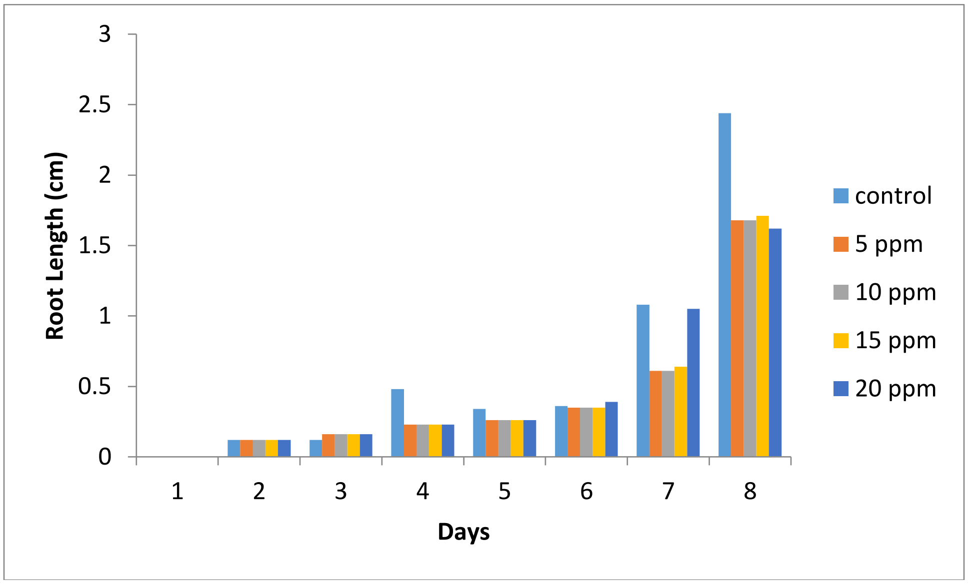

Figure 6: Concentration-dependent effect of synthesized silver nanoparticles (AgNPs) on the root elongation of DR-16 coarse rice (*Oryza sativa* L.) seedlings evaluated over an 8-day observation period. Data series represent the control treatment alongside 5 ppm, 10 ppm, 15 ppm, and 20 ppm nanoparticle concentrations, highlighting root sensitivity and growth variations over time.

AgNPs-Humic acid effect on shoot vs. Concentration

Humic acid is favorable capping agent for plants growth, among the treatments, AgNPs capped with Humic acid showed relatively higher germination rates, indicating a protective role, possibly due to its antioxidant properties.Humic acid: Least toxic, protective role. The protective effect of humic acid suggests its potential use in modifying nanoparticle toxicity. Below chart showed the AgNPs capped humic effect on Shoot growth of DR-16 Coarse variety taken from Dokari Research Center Larkana. The table showed that AgNPs-Humic effect showed increased respond on Shoot length from 5ppm to 20 ppm compared to control due to least toxic nature as compared to CTAB, PEG, EDTA and Phenylalanine .The germination of Shoot started form 3rd day of assembly.

Table 3: Effect of varying concentrations (5, 10, 15, and 20 ppm) of humic acid-capped silver nanoparticles (AgNPs-HA) on the shoot length (cm) of DR-16 coarse rice (*Oryza sativa* L.) seedlings recorded from day 3 to day 10 of germination.

Day	**Control**	5 ppm	10 ppm	15 ppm	20ppm
3	0.32	0.32	0.39	0.4	0.42
4	0.35	0.35	0.46	0.47	0.5
5	0.38	0.37	0.72	0.75	0.8
6	0.59	0.63	1.1	1.25	1.35

7	1.94	1.8	2.1	2.45	2.7
8	3	2.9	2.2	3.4	3.6
9	3.83	3.5	3.8	4.1	4.5
10	4.93	4.8	5.1	5.3	5.6

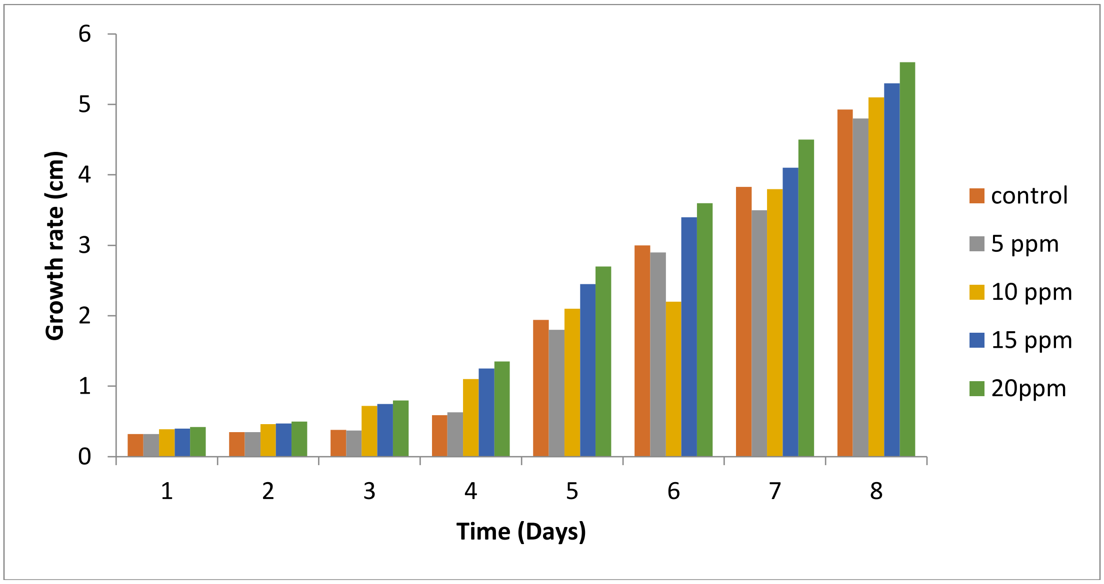

Figure: 7 Effect of varying concentrations (Control, 5, 10, 15, and 20 ppm) of AgNPs-Humic acid on Shoot on growth rate across different days (3-10)

AgNPs-Humic acid effect on Root vs. concentration;
The AgNPs capped with humic acid have significantly role on root growth. Germination of root started at 4^{rth} day of assembly.From 5ppm to 20ppm, dramatically increment in root length recorded as compared to control. At 20ppm concentration, highest root length were observed

Table: 4 Time-dependent changes in Root under varying concentration exposures of AgNPs-Humic acid (5–20 ppm) compared to the control group, measurements taken daily from Day 3 to Day 10.

Day	control	5ppm	10ppm	15ppm	20ppm
3	0	0	0	0	0
4	0.27	0.29	0.28	0.43	0.44
5	0.45	0.45	0.26	0.35	0.4
6	0.64	0.71	0.33	0.37	0.45
7	0.67	0.7	0.55	0.6	0.65
8	1.07	0.9	0.8	0.73	0.85
9	2.74	2.7	1.9	2.3	2.6
10	4.04	4.4	2.9	3	3.1

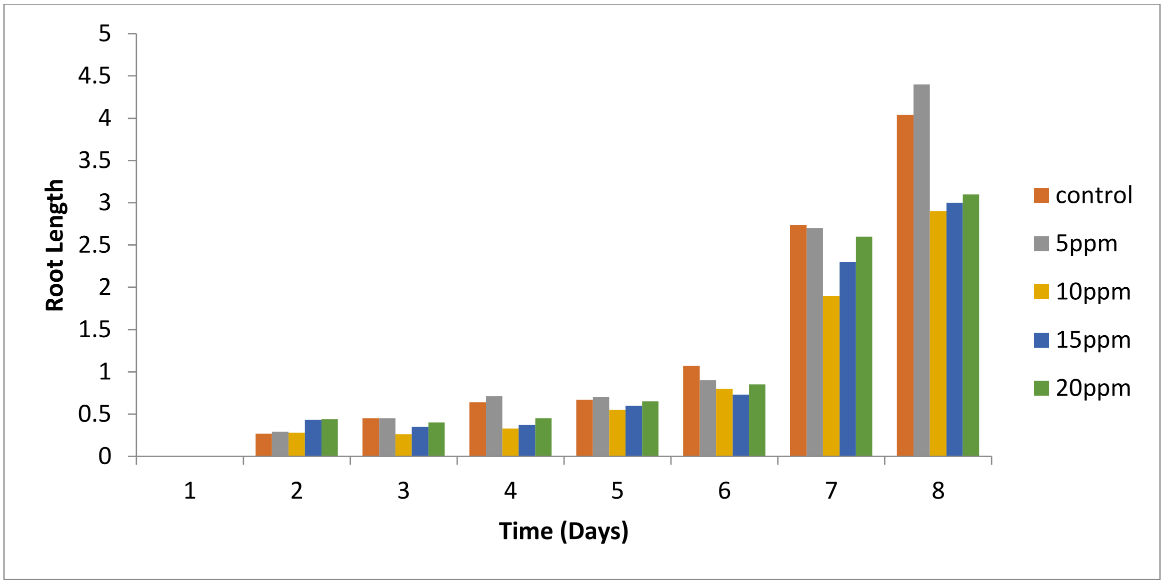

Figure: 8 Effect of silver nanoparticles (AgNPs) and humic acid mixtures at varying concentrations (Control, 5, 10, 15, and 20 ppm) on root development, monitored daily from Day 3 to Day 10.

AgNPs-CTAB effect on Shoot vs. concentration

Cetyltrimethylammonium bromide (CTAB) has highest toxicity due to surfactant nature and membrane disruption.CTAB-capped nanoparticles caused drastic inhibition of root elongation, with almost no root formation at 20 ppm.In contrast, (cetyltrimethylammonium bromide) CTAB-capped AgNPs exhibited the highest toxicity, resulting in maximum germination inhibition at 20 ppm.This inhibitory effect may be due to reactive oxygen species (ROS)production under light exposure, leading to membrane damage and enzyme inactivation during germination. Below chart is the mean of shoot length from 5ppm to 20ppm compared with control. It is clear observed that a slightly germination of shoot started from 3rd day of triplicate setting, At 10 ppm to 15 ppm,significant growth were recorded ,while at high concentration of 20 ppm,Inhhibition or controlled growth of shoot were appeared, as compared to the growth at control, while control is double distilled water.

Table: 5 Effect of varying concentrations (5, 10, 15, and 20 ppm) of CTAB-capped silver nanoparticles (AgNPs-CTAB) on the shoot length extension (cm) of DR-16 coarse rice (Oryza sativa L.) seedlings recorded daily from Day 3 to Day 10 of germination.

Day	**control**	5ppm	10ppm	15ppm	20ppm
3	0.33	0.21	0.22	0.23	0.23
4	0.35	0.26	0.27	0.27	0.27
5	0.4	0.35	0.35	0.36	0.37
6	0.59	0.36	0.37	0.47	0.47
7	2	0.39	0.49	0.53	0.53
8	2.93	1.49	1.57	2.21	2.21
9	3.87	1.64	2.33	2.79	2.65
10	4.95	2.31	1.65	3.1	3.7

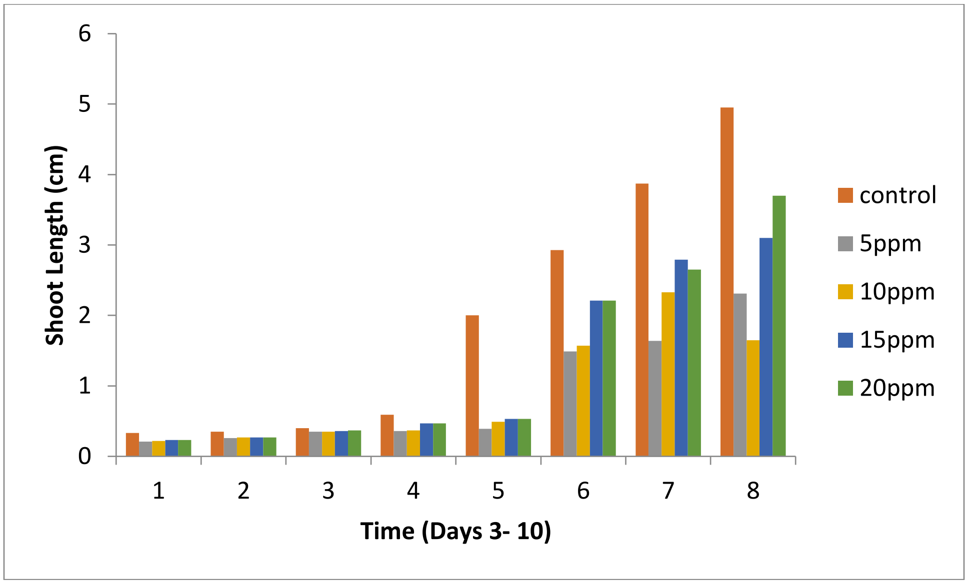

Figure: 9 Concentration-dependent effect of CTAB-capped silver nanoparticles (AgNPs-CTAB) on the shoot length extension of DR-16 coarse rice (*Oryza sativa* L.) seedlings evaluated from Day 3 to Day 10

AgNPs-CTAB effect on Root vs. concentration

AgNPs induced CTAB have greater impact on the root length of DR-16 Coarse from 5 ppm to 20ppm respectively. Root growth initiated at 4rth day of triplicate setting of petri dishes. At 5ppm slightly germination initiated ,While at 10ppm some extended root length recorded ,but from 15ppm to 20ppm decline in root length as compared to control ,were measured that showed highly toxicity of CTAB over concentration**.**

Table: 6 Effect of varying concentrations (5, 10, 15, and 20 ppm) of CTAB-capped silver nanoparticles (AgNPs-CTAB) on the root elongation (cm) of DR-16 coarse rice (Oryza sativa L.) seedlings recorded daily from Day 3 to Day 10 of germination.

Day	Control	5ppm	10ppm	15ppm	20 ppm
3	0	0	0	0	0
4	0.27	0.23	0.23	0.21	0.23
5	0.45	0.23	0.26	0.22	0.21
6	0.51	0.24	0.27	0.23	0.21
7	0.68	0.27	0.33	0.3	0.41
8	1.07	0.33	0.32	0.36	0.32
9	2.71	0.42	0.41	0.41	0.43
10	4.09	0.72	0.72	0.74	0.73

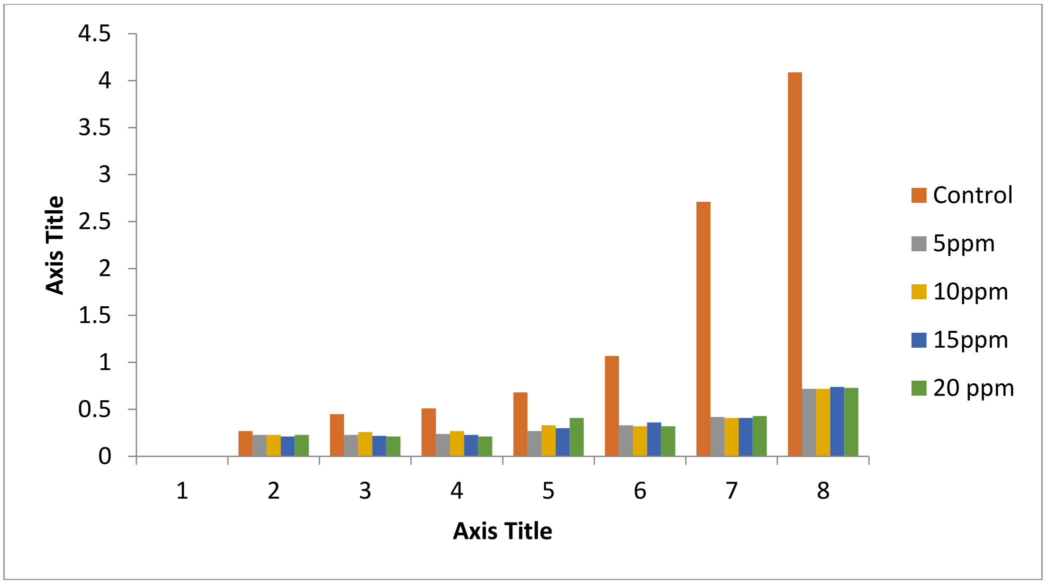

Figure: 10 Concentration-dependent effect of CTAB-capped silver nanoparticles (AgNPs-CTAB) on the root elongation of DR-16 coarse rice (*Oryza sativa* L.) seedlings evaluated from Day 3 to Day 10.

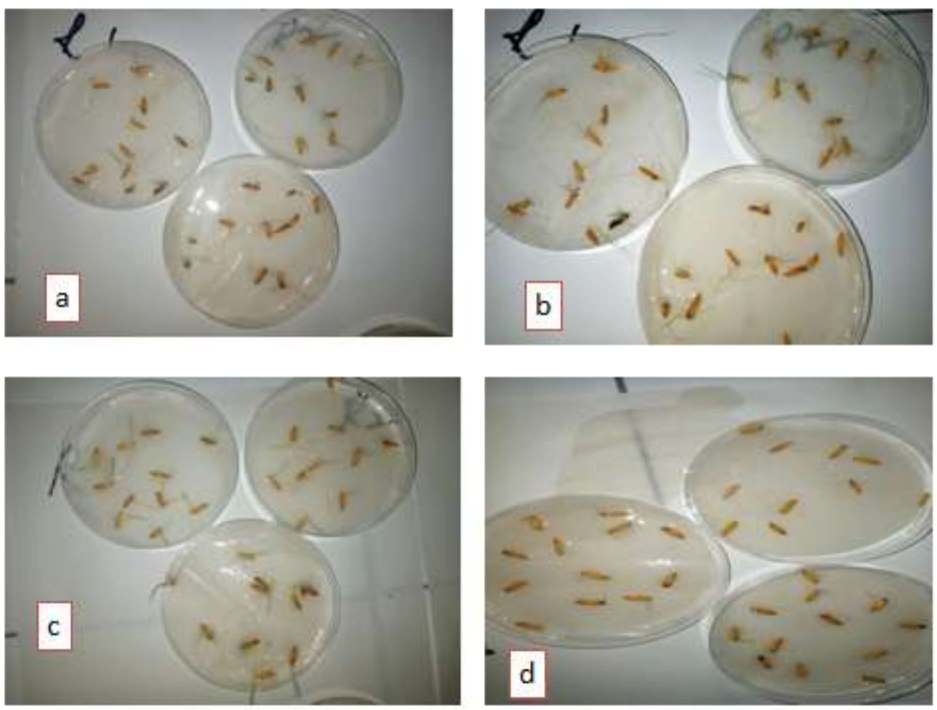

Figure: 11 (a) Phenotypic response and growth performance of DR-16 coarse rice (Oryza sativa L.) shoots and roots treated with silver nanoparticles (AgNPs).

(b) *Early seedling development showing shoot and root growth of DR-16 coarse rice (Oryza sativa L.) under control conditions (double distilled water) in triplicate Petri dishes.*
(c) *Photographic representation of seed germination and early seedling development under varying AgNPs-humic acid treatment conditions. Visible variations in elongation can be observed in both the root systems and emerging shoots across the experimental plates.*
(d) *Visual observation of early-stage seedling development and germination performance of DR-16 rice grains exposed to experimental concentration gradients of synthesized AgNPs-CTAB in a controlled laboratory environment.*

Effect on Fresh and Dry Biomass

Fresh and dry weights of seedlings decreased significantly with increasing concentrations of AgNPs. At 5 ppm, a slight increase in biomass was observed in some treatments (especially Humic acid-capped AgNPs), suggesting enhanced metabolic activity. However, at 15 and 20 ppm, a sharp decline in biomass was recorded across all treatments. CTAB-capped AgNPs again showed the highest reduction, indicating severe phytotoxicity.
The decrease in biomass can be linked to:

- Reduced photosynthetic efficiency
- Increased oxidative stress
- Cellular damage and reduced metabolic activity

CONCLUSION

The present study demonstrated that silver nanoparticles induce significant phytotoxic effects on the sprouting and early seedling evolution of DR-16 coarse rice (*Oryza sativa*). The results clearly indicate a dose-dependent response, where lower concentrations (5 and 10 ppm) exhibited slight stimulatory effects, while higher concentrations (15 and 20 ppm) caused substantial inhibition in germination percentage, root and shoot elongation, and biomass accumulation. The study further highlights the critical role of Humic acid and CTAB capping agents in modulating nanoparticle toxicity. Among the tested agents, Humic acid demonstrated a protective effect, likely due to its antioxidant properties and ability to reduce oxidative stress. In contrast, cetyltrimethylammonium bromide-capped AgNPs exhibited the highest level of toxicity, severely affecting root development and overall seedling vigor. The observed phytotoxicity is primarily attributed to enhanced generation of reactive oxygen species (ROS) under light conditions, leading to oxidative damage, disruption of cellular structures, and impairment of physiological processes such as water uptake and photosynthesis. The stronger inhibition of root growth compared to shoot growth suggests that root tissues are more sensitive to nanoparticle-induced stress. Overall, this study emphasizes that both nanoparticle concentration and surface modification significantly influence phytotoxic responses. While the use of AgNPs in agriculture holds potential benefits, their uncontrolled application may pose risks to crop productivity and environmental safety. The findings contribute to a better understanding of nanoparticle–plant interactions and provide a scientific basis for the safe and sustainable application of nanotechnology in agriculture.

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