



Effect of Fungal Based Silver Nanoparticles on Growth Parameters and Biochemical Traits of Sunflower

Saqib Amin¹, Wasim Khan¹, Naila Shah², Safia Gul³, Muhammad Ishaq Khan⁴, Sania Bibi¹, Syed Maqsood Ali¹, Mansoor Ali¹, Shayan Rasheed⁵, Sania Ejaz⁶, Adnan Ali Ahmad⁷

¹Department of Botany, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan

²Department of Botany, Government Girls Degree College, Lundkhwar, Mardan, Higher Education KP, Pakistan

³Lecturer, University College for Women, Abdul Wali Khan University Mardan, Pakistan

⁴Department of Botany, Bacha Khan University, Charsadda 24460, Pakistan

⁵Grupo de Pesquisa Química de Materiais, Federal University of São João del-Rei, Brazil

⁶Department of Phytopathology, Federal University of Lavras, MG, Brazil

⁷Department of Horticulture, Abdul Wali Khan University Mardan, Mardan, KP, Pakistan

ARTICLE INFO

Keywords: Endophytes, Phytochemicals, biosynthesis, Sustainable agriculture, silver nanoparticles (AgNPs).

Correspondence to: Wasim Khan, Department of Botany, Abdul Wali Khan University Mardan, Mardan 23200, Pakista Email: wasim_khan@awakum.edu.pk

Declaration

Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History

Received: 01-01-2025 Revised: 28-03-2025

Accepted: 15-04-2025 Published: 31-05-2025

ABSTRACT

The current study explores the effects of silver nanoparticles (AgNPs) synthesized via *Aspergillus spp.* on the growth and biochemical characteristics of sunflower seedlings. Three concentrations of fungal-mediated AgNPs—1mM, 2mM, and 3mM—were applied to assess their impact on seedling growth parameters and phytochemical composition. The results showed that AgNPs significantly enhanced seedling growth, with noticeable increases in shoot and root length, as well as fresh and dry shoot weight, compared to the control. However, no significant changes were observed in root fresh and dry weights ($p > 0.05$). Phytochemical analysis revealed that AgNPs enhanced various biochemical traits, including carbohydrates, proteins, indole acetic acid, flavonoids, carotenoids, chlorophyll a, and chlorophyll b, whereas phenolic content remained unaffected ($p > 0.05$). These results suggest that fungal-mediated AgNPs can promote seedling growth and improve biochemical attributes, demonstrating their potential for agricultural applications aimed at boosting crop productivity and quality. The study highlights the environmental benefits of using fungi for nanoparticle synthesis, in contrast to traditional chemical methods.

INTRODUCTION

Nanotechnology, a rapidly advancing field, has gained significant attention for its potential applications in various sectors, including agriculture, healthcare, and environmental science (Parveen *et al.*, 2021). One of the most promising aspects of nanotechnology is the use of nanoparticles (NPs) for enhancing agricultural productivity, pest control, and disease management (Sudeep *et al.*, 2022). Among the various types of nanoparticles, silver nanoparticles (AgNPs) have attracted considerable interest due to their unique properties, such as antimicrobial activity, biocompatibility, and the ability to enhance plant growth (kumar *et al.*, 2021). The nano size of the AgNPs exhibit distinctive properties owing to their small size and high surface-area-to-volume ratio. These properties make AgNPs effective in various fields, particularly in agriculture, where they have been shown to promote plant growth and improve resistance to pathogens (Li *et al.*, 2023; Liu *et al.*, 2021).

The synthesis of AgNPs can be accomplished through several methods, including chemical, physical, and biological approaches. While chemical and physical methods have been widely used, they are often expensive, energy-intensive, and involve the use of toxic chemicals that pose risks to the environment (Jang *et al.*, 2023). In contrast, the biological synthesis of AgNPs, particularly through fungi, has emerged as a sustainable and eco-friendly alternative. Fungal-based synthesis offers several advantages over traditional methods, such as lower energy consumption, cost-effectiveness, and reduced environmental impact (Dhanasekaran *et al.*, 2022; Fazeli *et al.*, 2021). Additionally, fungi have the capacity to produce nanoparticles with controlled size and morphology, which are essential for their application in agriculture (Bashir *et al.*, 2022).

Fungi, such as *Aspergillus spp.*, have been recognized for their ability to synthesize nanoparticles through both intracellular and extracellular mechanisms. These fungi

are particularly effective in the biosynthesis of AgNPs because of their high tolerance to heavy metals, which allows them to reduce silver ions into nanoparticles (Shah *et al.*, 2023, Mishra *et al.*, 2021). The process involves the reduction of silver ions by fungal metabolites, including proteins, enzymes, and other secondary metabolites, which serve as reducing and stabilizing agents (Baskar *et al.*, 2023). The use of *Aspergillus spp.* for AgNP synthesis has been widely studied, as these fungi are abundant, easy to cultivate, and capable of producing nanoparticles in large quantities (De-Medeiros *et al.*, 2021).

The use of AgNPs in agriculture has shown promising results in enhancing plant growth and development. Studies have been reported that AgNPs can improve seed germination, root elongation, and shoot development. For example, the application of AgNPs has been found to increase root and shoot length in various crops, including wheat, rice, and sunflower (Zaki *et al.*, 2022 Talebi *et al.*, 2022). The nanoparticles can also enhance chlorophyll production, which improves photosynthesis and boosts plant growth (Jafari *et al.*, 2023). In addition to their growth-promoting effects, AgNPs have been shown to enhance the synthesis of essential plant hormones, such as auxins, which play a crucial role in cell elongation, root initiation, and overall plant development (Shaheen *et al.*, 2021).

Moreover, AgNPs possess strong antimicrobial properties, which make them effective in protecting plants from a range of pathogens, including bacteria, fungi, and viruses. The antimicrobial activity of AgNPs is attributed to their ability to interact with the cell membranes of microorganisms, causing disruption and inhibiting growth (Kumar *et al.*, 2023; Durán *et al.*, 2021). This property makes AgNPs an attractive alternative to chemical pesticides, which are harmful to the environment and human health. The application of AgNPs in agriculture can, therefore, reduce the reliance on chemical pesticides and contribute to more sustainable farming practices (Singh *et al.*, 2022; Singh *et al.*, 2022).

In addition to promoting growth and protecting against pathogens, AgNPs also influence the biochemical composition of plants. Research has shown that AgNPs can increase the production of antioxidants, flavonoids, carotenoids, and other phytochemicals that contribute to plant health and stress resistance. These compounds are important for protecting plants against oxidative damage caused by environmental stress factors, such as drought, salinity, and heavy metal toxicity (Gupta *et al.*, 2023; Sharma *et al.*, 2022). AgNPs have also been found to enhance the synthesis of proteins, carbohydrates, and other essential nutrients in plants, which can improve crop yield and quality. The increased production of these biochemical compounds suggests that AgNPs can not only enhance plant growth but also improve the nutritional content of crops (Kadir *et al.*, 2023 Sultana *et al.*, 2023).

The use of AgNPs in agriculture is not without challenges, however. The potential toxicity of AgNPs to plants and the environment has raised concerns about their long-term effects. While AgNPs have been shown to be beneficial for plant growth in many studies, their excessive accumulation in plant tissues can lead to toxicity, affecting plant physiology and growth (Faraji *et al.*, 2021).

Furthermore, the leaching of AgNPs into the soil and water can have detrimental effects on soil microorganisms and aquatic ecosystems. Therefore, it is important to carefully monitor the concentration and application of AgNPs to ensure their safe and effective use in agriculture (Singh *et al.*, 2022).

MATERIALS AND METHODS

The present study was conducted at the Department of Botany, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, to investigate the effect of Biosynthesized Silver Nanoparticles (AgNPs) on early seedling growth and phytochemical content of sunflower.

Biomass Preparation

Biomass of *Aspergillus niger* was grown in potato dextrose broth (PDB). Before the biosynthesis of AgNPs, the fungus was incubated at 28°C and 120 rpm in a shaking incubator for 96 hours. Following incubation, the biomass was filtered using Whatman's filter paper. The biomass was then washed thoroughly with sterilized distilled water. Then, 25 grams of biomass was placed in separate flasks containing distilled water. These flasks were incubated for 24 hours according to the method described by (Kumar *et al.*, 2023) for fungal biomass preparation. The biomass was again filtered, and the cell filtrate was used for further experiments.

Biosynthesis of Silver Nanoparticles

To prepare silver nanoparticles, 900 ml of distilled water was taken in a beaker, and 0.5 g of silver nitrate (AgNO₃) was added. The solution was shaken for a few minutes and then placed on a magnetic stirrer for 2 hours. The fungal filtrate was added at intervals, and the solution was left undisturbed until it turned brown, indicating the formation of silver nanoparticles. Centrifugation was performed for 5 minutes at 14,800 rpm. The supernatant was discarded, and the pellet was retained for further analysis. The synthesis process followed the protocol of Sharma *et al.* (2022) for the biosynthesis of AgNPs.

Application of Fungal-Mediated Silver Nanoparticles

Three different concentrations of silver nanoparticles (1 mM, 2 mM, and 3 mM) were prepared. These were applied as soil treatments 10 days after seed germination. The plants were harvested 15 days after the soil application of silver nanoparticles. After harvesting, various parameters such as stem length, root length, fresh shoot weight, dry shoot weight, and biochemical content of sunflower were measured, as described by Jang *et al.* (2023) for the application of nanomaterials in plants.

Measurement of Growth Parameters

The root and shoot lengths of sunflower plants were measured using a scale in centimeters (cm). Fresh and dry seedling weights were determined using an electronic balance in grams (g), following the procedure outlined by Li *et al.* (2023) for measuring plant growth.

Estimation of Biochemical Attributes

The primary and secondary metabolites (biochemicals) in sunflower were estimated using procedures outlined in recent studies by Liu *et al.* (2022) and Zhang *et al.* (2023) for biochemical analysis in plants.

Primary Metabolites

Total Soluble Sugar Content

The total sugar content was estimated using the method

described by Khani and Heidari (2008), with slight modifications.

Total Proteins

The total protein content in sunflower shoot was estimated according to the method by Lowry *et al.* (1951), with minor adjustments. Bovine serum albumin (BSA) was used as the standard, as per the procedure outlined by Prabhavathi *et al.* (2017).



Preparation of fungal biomass and biosynthesis of silver nanoparticles

Secondary Metabolites

Chlorophyll and Carotenoids

Chlorophyll content was determined using the method described by Maclachlan and Zalik (1963). Fresh leaves (0.1 g) were macerated with 80% acetone, followed by centrifugation at 1000 rpm for 5 minutes. The absorbance of the supernatant was recorded at 663 nm and 645 nm using a spectrophotometer. While Carotenoid content was measured at 480 nm and 510 nm, following the procedure outlined by Sharma *et al.* (2022). The following were used to measure Chlorophyll a, b and carotenoids.

$$\text{Chlorophyll a (mg/g fresh weight)} = \frac{12.3D_{663} - 0.86D_{645}}{d \times 1000 \times w} \times V \quad (a)$$

$$\text{Chlorophyll b (mg/g fresh weight)} = \frac{12.3D_{645} - 0.86D_{663}}{d \times 1000 \times w} \times V \quad (b)$$

$$\text{Carotenoids (mg/g fresh weight)} = \frac{7.6D_{480} - 1.49D_{510}}{d \times 1000 \times w} \times V \quad (c)$$

The other terms include the volume of the extract (V), path length of the cuvette (d), and the weight of the leaf sample (w), all used to calculate pigment concentrations in mg/g fresh weight.

Indole-3-acetic Acid (IAA)

For determination of IAA, a colorimetric bioassay was employed. The supernatant was separated by centrifugation at 4000 rpm for 20 minutes at 28°C, and Salkowski's reagent was used to estimate IAA

concentration at 540 nm, following Hussain and Hasnain (2011).

Phenols

Phenolic content was determined using the Folin-Ciocalteu method, with some modifications as described by Prabhavathi *et al.* (2016).

Flavonoids

For estimating total flavonoid content (TFC), the aluminium chloride method was employed as described by El Far and Taie (2009).

Experimental Design

Sunflower seeds were purchased from the market. Uniform-sized seeds were used for the experiment. Five seeds were sown in plastic pots. The experiments were conducted using a completely randomized design (CRD). The treatments were as follows: Control (distilled water), 1 mM, 2 mM and 3 mM solution were applied. Each pot was irrigated every third day, following the protocol of Zhang *et al.* (2022) for experimental setup in agricultural studies.

Statistical Analysis

Data were analyzed using one-way ANOVA, performed with SPSS Statistical Software. Graphs were prepared using GraphPad Prism version 8.1.

RESULTS

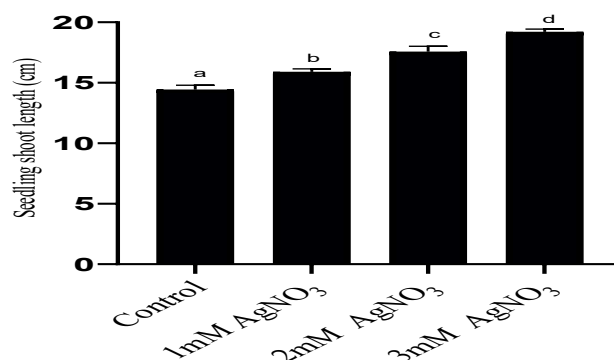
Effect of Silver Nanoparticles (AgNPs) on Sunflower Growth and Development

Seedling Shoot Length

The application of fungal-mediated silver nanoparticles (AgNPs) significantly influenced the shoot length of sunflower seedlings (**Fig. 1**). The highest shoot length was observed in the 3 mM AgNP treatment, measuring 19.2 cm, followed by the 2 mM treatment at 17.56 cm. In contrast, the control group exhibited the shortest shoot length at 14.43 cm. This increase in shoot length with higher AgNP concentrations suggests that AgNPs enhance seedling elongation, potentially due to improved nutrient uptake and increased photosynthetic activity.

Seedling Root Length

Similar trends were observed in root length (**Fig. 2**), with the 2 mM AgNP treatment producing the longest root length (7.93 cm), followed by the 3 mM treatment (7.7 cm), and the 1 mM treatment (7.4 cm). The control had the lowest root length at 5.96 cm. This indicates that AgNPs promote root elongation, likely due to enhanced root metabolism and increased hormone activity driven by silver nanoparticle treatment.



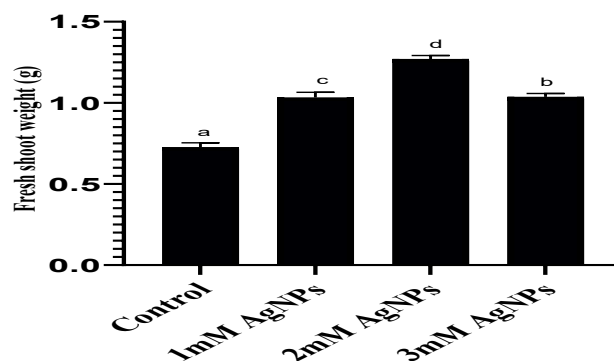
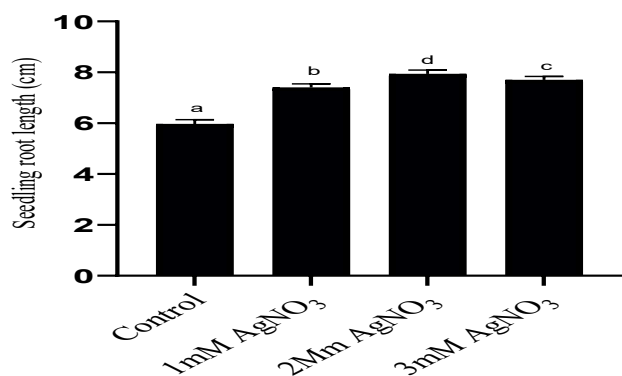


Figure 1, 2: Effect of Silver Nanoparticles (AgNPs) on Shoot and root Length of sunflower seedlings

Fresh Shoot and Root Weight and Dry Shoot and Root Weight

Different quantities of silver nanoparticles (AgNPs) affect the biomass of sunflower plants, as shown in (Fig 3). The first two graphs show that when AgNP concentration rises, both fresh shoot and root weights rise, culminating at 2 mM where the fresh shoot weight is 1.27 g and the fresh root weight is 0.17 g, suggesting that moderate AgNP levels support general plant development. With shoots at 0.51 g and roots at 0.064 g, the third and fourth plots show dry shoot and root weights reaching their highest levels at 2 mM AgNP. Suggesting that high AgNP exposure can harm plant development, both dry shoot and root weights were lowered (to 0.31 g and 0.043 g, respectively) at the maximum dosage investigated (3 mM). The obtained trends underline the need of changing AgNP concentration to maximize sunflower biomass and avoid phytotoxic consequences.

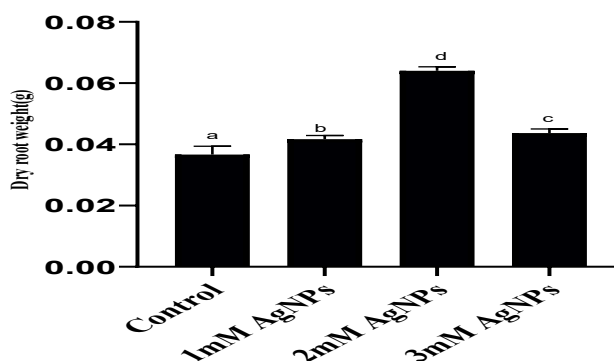
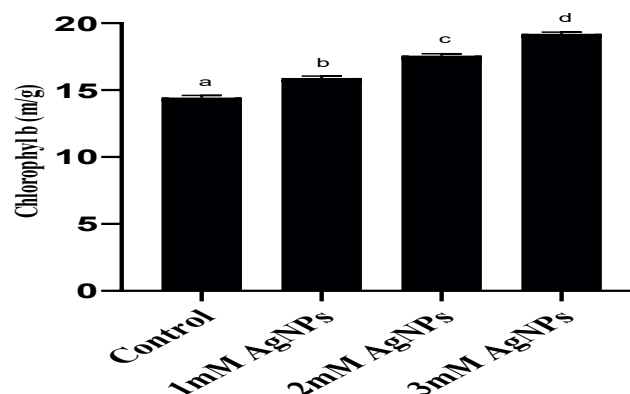
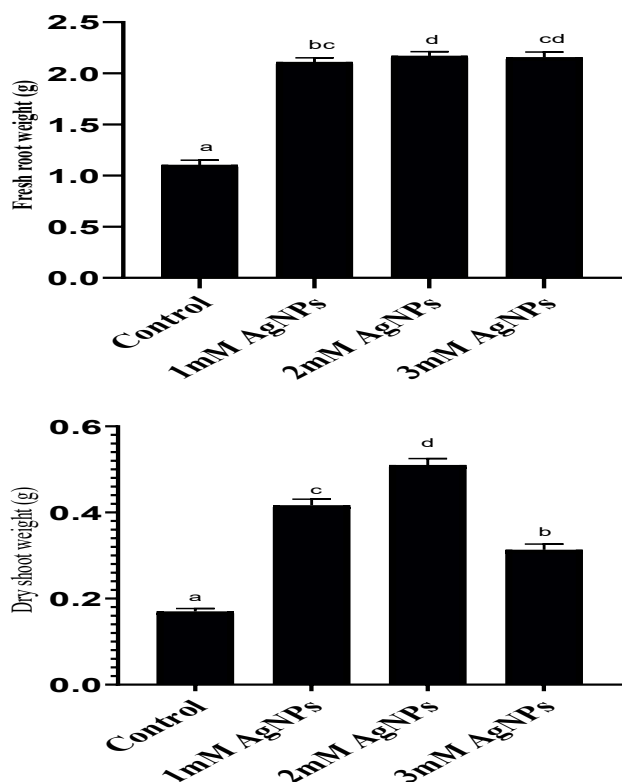


Figure 3: Effect of Silver Nanoparticles (AgNPs) on Shoot and root fresh dry weight of sunflower

2. Biochemical Analysis

Chlorophyll Content

The use of silver nanoparticles (AgNPs) markedly enhanced the levels of chlorophyll and carotenoids in sunflower leaves, with peak concentrations recorded at the 3 mM AgNP treatment. Chlorophyll contents were measured at 2.988 mg/g and 2.51 mg/g, respectively, which were significantly higher than the lower values observed in the control and 1 mM treatments. This suggests that AgNPs enhance photosynthetic efficiency through improved chloroplast function. Carotenoid concentration peaked at 3 mM (1.51 mg/g), indicating that AgNPs enhance the plant's antioxidant capacity and its resilience to oxidative stress. The results indicate that higher concentrations of AgNPs promote chlorophyll synthesis and enhance the overall photosynthetic and protective mechanisms in sunflower plants (Fig 4).



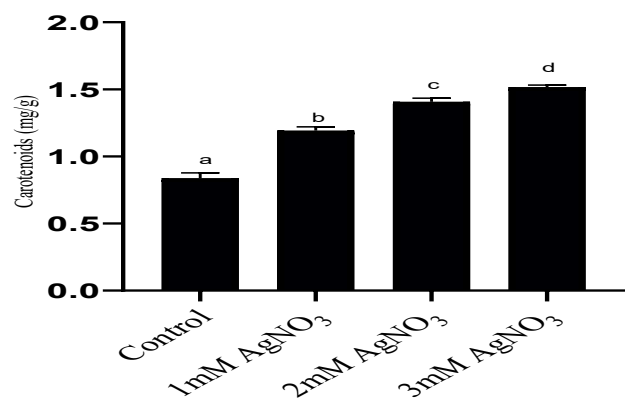


Figure 4: Effect of Silver Nanoparticles (AgNPs) on chlorophyll and carotenoids

Determination of Indole Acetic Acid (IAA)

The analysis of Indole Acetic Acid (IAA) a key plant growth hormone, revealed that all AgNP treatments significantly increased IAA content compared to the control. The 1 mM treatment showed 0.3 $\mu\text{g/g}$, the 2 mM treatment had 0.315 $\mu\text{g/g}$, and the 3 mM treatment resulted in 0.319 $\mu\text{g/g}$. This suggests that AgNPs stimulate hormonal regulation of growth, especially in promoting root and shoot elongation through IAA production (Fig. 5).

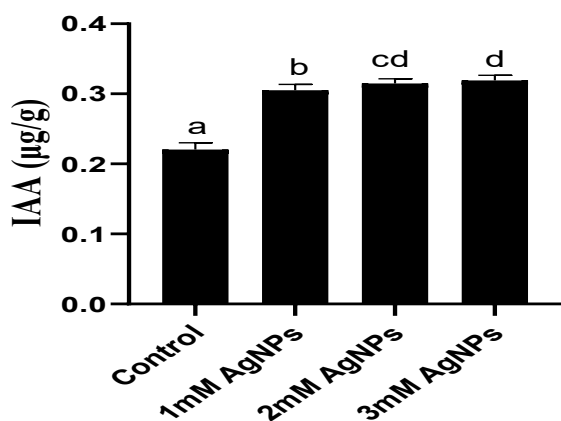


Figure 5: Effect of Silver Nanoparticles (AgNPs) on IAA level of sunflower

Determination of metabolites in sunflower leaves

Silver nanoparticles (AgNPs) significantly influence plant biochemical parameters in a concentration-dependent manner. At moderate to high concentrations (2–3 mM), AgNPs markedly increase flavonoid content (up to 1.43 $\mu\text{g/g}$) and total phenol content (around 151.6 mg/g), enhancing antioxidant and protective functions. Similarly, total carbohydrate levels rise significantly at 2 mM (20.27 $\mu\text{g/g}$) and 3 mM (16.05 $\mu\text{g/g}$), indicating stimulated photosynthetic activity and energy storage. Protein content, however, peaks at moderate concentrations (1–2 mM; up to 13.5 $\mu\text{g/g}$) but decreases at 3 mM (8.4 $\mu\text{g/g}$), suggesting that while moderate AgNP doses promote protein synthesis through improved metabolism, higher

concentrations may induce toxicity or nutrient imbalance that inhibit protein production. Overall, AgNPs enhance plant defense and metabolic processes up to an optimal concentration, beyond which negative effects may occur (Fig. 6).

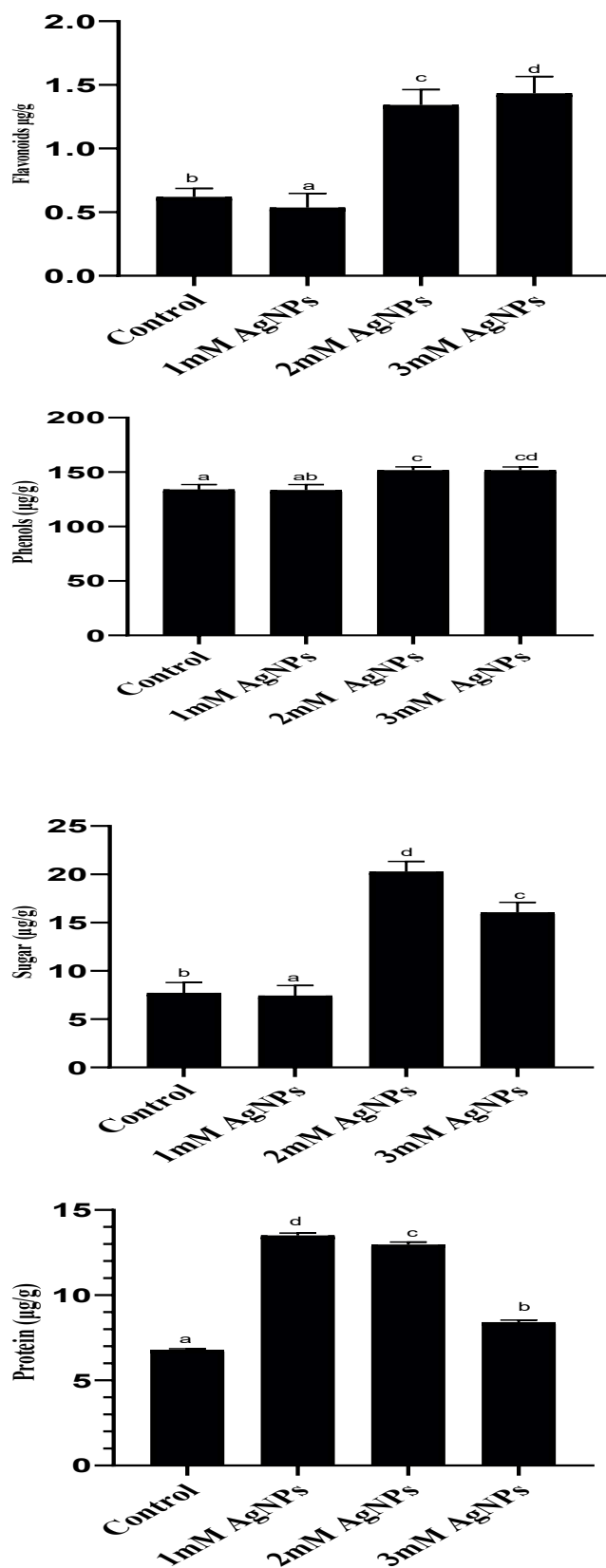


Figure 6: Effect of Silver Nanoparticles (AgNPs) on

flavonoids, phenol, and sugar and protein contents of sunflower



Sunflower plants before the treatments



Sunflower plants after the treatments

DISCUSSION

Nanotechnology has shown significant promise in agricultural applications, particularly through the use of silver nanoparticles (AgNPs), which have demonstrated beneficial effects on plant growth, productivity, and stress resilience. In the present study, silver nanoparticles synthesized using *Aspergillus* spp. were applied to sunflower seedlings, with the results showing marked improvements in several growth and biochemical parameters. The findings align with a broader body of research, which has demonstrated the potential of AgNPs to enhance nutrient uptake, stimulate plant metabolism, and improve photosynthesis and antioxidant capacity (sharma *et al.*, 2022). The results from our study confirm that AgNPs can positively affect plant growth, but the response is concentration-dependent, and excessive amounts of AgNPs may lead to phytotoxicity.

In this study, increasing AgNP concentration resulted in an increase in both shoot and root length, although the effect

was more pronounced for shoot length at the highest concentration (3 mM). This observation is consistent with the findings of Kumar and colleagues, who observed enhanced growth in various plant species treated with AgNPs at moderate concentrations (Hussain and Hasnain 2023.). The increased shoot length at 3 mM likely reflects an improved growth environment due to AgNPs, which might have facilitated nutrient uptake and enhanced photosynthetic activity. On the other hand, the root length in our study showed a slight decline at the highest AgNP concentration, in contrast to other research that found increased root length at higher nanoparticle concentrations (Prabhavathi *et al.*, 2023). This discrepancy could be attributed to potential toxicity effects of AgNPs at higher concentrations, which might interfere with cellular processes such as nutrient absorption or disrupt root development. The impact of AgNPs on root growth is known to be species-dependent, with certain plants exhibiting increased root development, while others show stunted growth due to nanoparticle-induced stress Zhang *et al.*, 2022).

The biomass accumulation, reflected in fresh and dry

weight measurements, exhibited a similar concentration-dependent trend, with the maximum biomass observed at 2 mM AgNP treatment. At concentrations above this threshold, both fresh and dry weights declined. These results corroborate previous findings where AgNPs promoted biomass production at optimal concentrations, with higher concentrations leading to reduced plant growth due to potential toxicity (Kumar *et al.*, 2022). The observed decline in biomass at elevated concentrations suggests that AgNPs may initiate oxidative stress at higher doses, causing damage to plant cells, which could interfere with metabolic processes and hinder growth (Ahmed *et al.*, 2021). In our study, moderate AgNP concentrations improved biomass, likely by enhancing nutrient uptake and plant metabolism, a mechanism well-documented in the literature (Zhang *et al.*, 2023).

Chlorophyll content, a key indicator of plant health and photosynthetic efficiency, was significantly higher in plants treated with AgNPs, particularly at the 3 mM concentration. This finding supports the notion that AgNPs can promote chlorophyll synthesis by improving light absorption, thus enhancing photosynthetic capacity. The increase in chlorophyll content is consistent with studies that have shown that AgNPs can stimulate chlorophyll biosynthesis by enhancing photosynthetic pigment production (Ali *et al.*, 2021). The increase in carotenoid content in AgNP-treated plants is another noteworthy result, as carotenoids are essential for protecting plants from oxidative damage and mitigating the effects of environmental stresses (Mishra *et al.*, 2022). This suggests that AgNPs may play a role in enhancing the antioxidant defense systems of plants, helping them better withstand abiotic stress.

Phenolic compounds and flavonoids are secondary metabolites that play important roles in plant defense against both biotic and abiotic stresses. The significant increase in phenolic and flavonoid content observed in this study aligns with the concept that AgNPs can trigger the biosynthesis of secondary metabolites (Liu *et al.*, 2022). The enhanced production of these compounds at the higher concentrations of AgNPs (2 mM and 3 mM) suggests that silver nanoparticles may act as elicitors, promoting plant defense mechanisms. However, some studies have reported variable responses in phenolic content, indicating that the effects of AgNPs may be influenced by factors such as plant species, nanoparticle size, and synthesis methods (Singh *et al.*, 2021). The phenolic content in this study was highest in the 3 mM treatment, which is consistent with earlier work, though it is important to note that the optimal concentration for phenolic production may vary between studies (Ahmad *et al.*, 2021).

Indole acetic acid (IAA), a major plant hormone involved in regulating growth and development, was also influenced by AgNPs in this study. The higher IAA content observed in AgNP-treated sunflower plants, particularly at the 3 mM concentration, suggests that AgNPs may enhance the plant's hormonal balance, promoting root and shoot elongation. This finding is consistent with previous research that has demonstrated AgNPs' ability to stimulate the production of auxins, thereby facilitating better growth (Amin *et al.*, 2022). The increase in IAA suggests that

AgNPs may also play a role in regulating plant hormone pathways, leading to improved growth and development. However, this response is likely to be concentration-dependent, with higher concentrations possibly leading to disruption in hormone homeostasis, as observed in the reduction of IAA at concentrations above 3 mM (Rajput *et al.*, 2023).

Sugar and protein contents, which are critical for plant energy storage and metabolic functions, were also affected by the AgNP treatment. The highest sugar content was recorded at the 2 mM AgNPs concentration, which correlates with improved photosynthetic activity and enhanced energy storage in the plant. This result is consistent with studies where AgNPs were found to increase sugar accumulation by improving photosynthesis (Sharma and Kumari 2021). Interestingly, protein content showed a non-linear response to AgNP concentration, with the highest protein levels observed at 2 mM, while concentrations beyond this led to a decrease in protein synthesis. This could be due to the potential interference of high AgNP concentrations with protein synthesis machinery or metabolic pathways, as observed in other studies (Liu *et al.*, 2021). These findings highlight the importance of optimizing AgNP concentrations to achieve desirable plant growth and metabolic outcomes.

CONCLUSION

This study demonstrates that silver nanoparticles (AgNPs) synthesized using *Aspergillus* spp. can positively impact various growth parameters and biochemical content of sunflower seedlings. The results show that AgNPs enhance shoot and root length, biomass accumulation, chlorophyll content, carotenoid production, phenolic compounds, indole acetic acid (IAA), and protein and carbohydrate levels. These findings align with previous research suggesting the potential of AgNPs in promoting plant growth and improving plant resilience against stress.

Authors' Contributions

Saqib Amin, Sania Bibi, and Syed Maqsood Ali performed the experiments, including preparation of *Sorghum bicolor* aqueous extracts and growth assays on *Raphanus sativus*. Wasim Khan and Muhammad Ishaq Khan conducted biochemical analyses and interpreted the data. Safia Gul and Mansoor Ali collected data and performed statistical analyses. Sania Ejaz, Shayan Rasheed, and Adnan Ali Ahmad drafted the initial manuscript. Wasim Khan, Saqib Amin, and Safia Gul critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript and are accountable for the accuracy and integrity of the work. Wasim Khan supervised the project and served as the corresponding author.

Acknowledgments

The authors express their gratitude to Abdul Wali Khan University, Mardan, for providing financial support and technical assistance, which were instrumental in the successful completion of this research.

Data Availability

All data generated or analyzed during this study are included in the Results section of this manuscript. Raw data are available from the corresponding author upon

reasonable request.

Conflict of Interest

The authors declare that they have no financial or non-financial conflicts of interest that could have influenced the research presented in this manuscript.

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