Research Article

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Silver nanoparticles elicited physiological, biochemical, and antioxidant modifications in rice plants to control *Aspergillus flavus*

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Abstract: This study was carried out to analyze the effects of biogenic silver nanoparticles (AgNPs) on physiological, biochemical, and enzymatic attributes of rice plants against *Aspergillus flavus*. The plant-based AgNPs were synthesized by using the aqueous extract of *Moringa oleifera* leaves. The characterization of AgNPs was accomplished through UV-visible spectrophotometry, SEM, and energy-dispersive X-ray (EDX) analysis, which confirmed that the nanoparticles are crystalline and are less than 100 nm in size. The exogenous applications of different concentrations of AgNPs (25, 50, 75, and 100 mg/L) on rice plants in field experiments were used to control the proliferation of *A. flavus*. The effects of biosynthesized AgNPs were evaluated for physiological (relative water content, membrane stability index, and chlorophyll content), nonenzymatic metabolites (total phenolic, total flavonoid, proline, soluble sugar, and protein contents), and enzymatic metabolites (superoxide dismutase, peroxidase, and catalase) in rice plants under biotic stress, and 50 mg/L concentration of AgNPs was found to be effective to elicit biochemical modifications to reduce biotic stress. The 50 mg/L concentration of AgNPs was also effective in controlling the proliferation of fungal pathogen. The applications of AgNPs reduced the biotic stress by decreasing the production level of osmolytes, enzymatic, and nonenzymatic compounds but significantly increased the protein content.

Keywords: AgNPs, rice, green synthesis, biochemical profiling, enzymatic assays

Abbreviations

AgNPs	silver nanoparticles
MSI	membrane stability index
TPC	total phenolic content
TFC	total flavonoid content
SOD	superoxide dismutase
POD	peroxidase
CAT	catalase
SEM	scanning electron microscope
EDX	energy-dispersive X-ray
UV-visible	ultra-violet visible spectrophotometer
SPR	surface plasmon resonance
RWC	relative water content
CC	chlorophyll content
Chl	chlorophyll

1 Introduction

Agriculture plays an important role in the economy of developing and developed countries, and it also contributes to feed and flourish the growing population [1]. The food safety of a society is greatly dependent on the increased production of crops. The rice belongs to the family Poaceae and is the most important staple food, and the entire grain is cooked. More than half of the world's population relies on rice to fulfill its nutritional requirements [2]. It is very important to increase the production and the nutritional value of rice grain to fulfill the needs of a growing population [3]. Keeping this in mind, the rice crop was selected to improve the crop's agromorphological and physiological parameters.

The rice crop is cultivated in a humid and tropical climate, which moist the rice panicle and provides a

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suitable substrate for the colonization of fungal species [4]. The fungal infection leads to the production of mycotoxins in rice grains. Grayish-green *Aspergillus* species are mainly considered responsible for the production of mycotoxins in rice grains [5]. Mycotoxins are secondary metabolites that are specifically produced by *A. flavus* and are carcinogenic in nature and health hazards. The basic secondary metabolites of *A. flavus* are aflatoxins G1, G2, B1, and B2, which contaminate the rice grains and other agricultural commodities. However, the aflatoxin, AFB1 is considered more toxic for both humans and mammals. These are extremely lethal and carcinogenic compounds that attack livestock and cause severe health-related issues such as hemorrhage, hepatitis, carcinoma, immunosuppression, and hepatitis [6]. In the last decade, scientists have considered numerous strategies such as suitable harvesting practices and storage conditions [7], climatic factors [8,9], or the applications of fungicides and other hazardous chemicals to prevent and inhibit the fungal growth [10–12] to manage aflatoxin contamination in rice plants. However, these strategies are not cost-effective, toxic, and pollute the environment, which results in affecting human health and other associated living organisms [13,14]. Therefore, there is an urgent need to develop a novel biocompatible method to inhibit the growth of aflatoxin-producing fungal species on rice plants [12,15,16].

Nanobiotechnology has attained a prominent position due to its various applications in agricultural ecosystem maintenance. Nanotechnology is gaining popularity in the field of agriculture, and scientists are trying to design nano-devices to manipulate agriculture on nanoplatforms [5,17]. It resulted in the manufacturing of fungicides that have nanoparticles and help to kill fungal species without contaminating or altering the environment. The physical and chemical methods of synthesis of nanomaterials involve the utilization of forces and hazardous chemical reactions, which results in the degradation of nature [18,19]. The plant-mediated synthesis of nanomaterials has advantages over routine physical and chemical methods of material synthesis as they are biocompatible and play a significant role in the biological control of the growth of fungal species and prevention of the production of aflatoxins [14,20]. The plant bodies have a special defense system that helps them to tolerate the biotic stress by the generation of reactive oxygen species (ROS). After the infection, the oxidative burst results in various biochemical and molecular adjustments to limit the further pathogenic proliferation and help plants to tolerate the infection [18].

Very few studies were carried out on the antifungal potential of the plant-based AgNPs to control the harmful effects of aflatoxins [5]. Herein, we report the use of the aqueous extract of plants to functionalize and stabilize biocompatible AgNPs and to explore their foliar applications on rice plants against aflatoxins contaminating fungal species. AgNP-elicited changes in rice plants were characterized in terms of biochemical attributes, antioxidant contents, and enzymatic and nonenzymatic potential. The aim of this study is to explore the effect of biogenic AgNPs on the physiological attributes and biochemical profiling of rice plants under the biotic stress of *A. flavus*.

2 Materials and methods

2.1 Preparation of plant extract

The plant extract was prepared from the leaves of *Moringa oleifera*. The plant was collected from the campus of PMAS-Arid Agriculture University Rawalpindi, Pakistan (33.649411, 73.082324). Fresh leaves were detached from the plant and washed with the tap water followed by the distilled water to remove all visible dust particles. About 30 g of leaves were chopped and boiled in 200 mL of distilled water until the watercolor change into dark yellow. The solution was then filtered through a Whatman No. 1 filter paper. The resulting plant extract was stored at 4°C to use later [21].

2.2 Phyto-synthesis of AgNPs

The AgNPs were phyto-fabricated by reducing the silver nitrate into AgNPs. The solution of AgNO₃ (1 mM) was prepared by dissolving 0.17 g of AgNO₃ into 1 liter of distilled water. The synthesis of AgNPs was carried out by mixing the aqueous extract of *Moringa* and AgNO₃ solution. The reaction solution was boiled for 5 min until the color of the solution turned dark brown. The characteristic dark brown color indicated the synthesis of AgNPs. The colloidal suspension was centrifuged at 4,500 rpm for 20–25 min to separate nanoparticles from the reaction mixture. The collected nanoparticles were re-dispersed in the distilled water to separate the plant metabolic components, and the process was repeated thrice. The nanoparticles were dried and stored in airtight vials for further experimentation [22].

2.3 Morphological and optical characterization of AgNPs

The nanoparticles were characterized morphologically and optically to determine their physical and chemical properties. The synthesis of AgNPs was confirmed by measuring the absorbance of the colloidal reaction mixture between 300 and 700 nm of the light wavelength [23]. The size and shape of the AgNPs were determined by using the scanning electron microscope (SEM). The drop coating method was used to prepare the analyte on the carbon-coated copper grids [24]. The sample was analyzed at various magnifications of the machine. The elemental composition of the AgNPs was determined by performing the energy-dispersive X-ray (EDX) analysis through detectors associated with the SEM machine [25].

2.4 Plant material and growth conditions

The rice seeds (super kernel) were obtained from Rice Research Institute Kala Shah Kaku, Pakistan. Super kernel rice variety was selected to perform experiments due to its high demand and susceptibility to fungal species. Before sowing the seeds, 1% solution of sodium hypochlorite was used for the sterilization of the seed surface followed by washing seeds three times with the distilled water. The paddy field experiments were established for 3 months from July to October 2020.

2.5 Preparation of *A. flavus* inoculum

The fungal strain of *A. flavus* was obtained from the mycology laboratory of PMAS-Arid Agriculture University. Its aflatoxin production potential was observed by growing it on potato dextrose agar media for 10 days at 25°C, and the fungal growth was examined. Then, sporulation spores were harvested from the plates by adding the distilled water. The required final concentration of 1,012 spores/mL was maintained by using a hemocytometer. The spore suspension was prepared a day before and then preserved at 4°C for further use.

2.6 Exogenous applications of various concentrations of AgNPs and *A. flavus* spore suspension on rice plants

Various concentrations of AgNPs, i.e., 25, 50, 75, and 100 mg/L, were prepared and were applied separately

to the rice plants three to four times with an interval of 3 days through a foliar application at the stage when the rice plants panicle emerged from the leaf sheath (heading stage). After the 10 days of AgNP treatment, 20 mL (1,012 mL of *A. flavus*) of spore suspension of *A. flavus* was inoculated on all rice plants in an equal volume through a foliar spray. The plants without treatment of AgNPs and *Aspergillus* were maintained as positive and negative controls, respectively. The experiment was designed with three replicates of each treatment and repeated two times. The experimental treatment plan of this study is presented in Table 1.

2.7 Physiological mechanisms of rice plants under biotic stress

2.7.1 Relative water content

The fresh leaves were taken, and their fresh weight was calculated, followed by immersing leaves into the water. The turgidity of the immersed leaves was calculated as turgid weight after 24 h. The leaves were then placed in an oven at 70°C for 7 days, and the dry weight was noted after the end of 7 days. The relative water content was measured by using Eq. 1 [26]:

$$\text{Relative water content} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Saturated weight} - \text{Dry weight})} \times 100. \quad (1)$$

2.7.2 Chlorophyll content

The leaf chlorophyll content (CC) was measured by using the spectrophotometer. A total of 0.2 g of the leaves of each sample was ground in 10 mL of 80% acetone. The filtrate was poured into test tubes, and the absorbance was recorded by using the spectrophotometer at the light

Table 1: Experimental treatment plan for the foliar applications of AgNPs

Treatments	Conditions
T ₀	Control
T ₁	<i>A. flavus</i> (pathogen)
T ₂	25 mg/L of AgNPs + <i>A. flavus</i>
T ₃	50 mg/L of AgNPs + <i>A. flavus</i>
T ₄	75 mg/L of AgNPs + <i>A. flavus</i>
T ₅	100 mg/L of AgNPs + <i>A. flavus</i>

wavelength of 645, 652, and 663 nm. The CC was estimated by using Eq. 2–4 [27]:

$$\text{Chlorophyll } a \text{ } (\mu\text{g/mL}) = 12.7(A_{663}) - 2.7(A_{645}). \quad (2)$$

$$\text{Chlorophyll } b \text{ } (\mu\text{g/mL}) = 22.9(D_{645}) - 4.7(D_{663}). \quad (3)$$

$$\text{Total chlorophyll } (\mu\text{g/mL}) = (D_{645} \times 1,000/34.5). \quad (4)$$

2.7.3 Membrane stability index

Almost 100 mg of the fresh plant leaves were taken and cut into small discs. These small discs of the leaves were placed in test tubes containing double distilled water, and the test tubes were placed in a water bath at 40°C for 30 min. The electrical conductivity (C1) was measured after 30 min with the help of an electric conductivity meter. Test tubes were then placed in a water bath at 100°C for 10 min, and the second reading of electrical conductivity was noted (C2). The membrane stability index (MSI) was calculated with the help of Eq. 5 [28]:

$$\text{Membrane stability index} = [1 - (C1/C2)] \times 100. \quad (5)$$

2.8 Determination of the biochemical parameters

2.8.1 Total phenolic content

The total phenolic content (TPC) was calculated by using the protocol given in ref. [29]. A total of 0.75 mL of Folin–Ciocalteu reagent was used and mixed with 0.1 mL of the plant extract. Then, this mixture was allowed to incubate at 22°C for 5 min. Thereafter, 0.75 mL of sodium bicarbonate was added to this mixture and incubated at 22°C for 90 min. Finally, the spectrophotometer was used to record the absorbance at 725 nm.

2.8.2 Total flavonoid content (TFC)

The total flavonoid content (TFC) was quantified by using the procedure given in ref. [30]. A total of 0.01 g of Quercetin reagent was dissolved into 80% of ethanol. Then, the solution was prepared by mixing 0.1 mL of plant extract with 0.1 mL of aluminum chloride, 0.1 mL of 1 M potassium acetate, 1.5 mL of 95% ethanol, 2.8 mL of distilled water, and 0.4 mL of diluted Quercetin, the solution was incubated at room temperature for 30 min. The spectrophotometer was used to record the absorbance at 415 nm.

2.8.3 Proline content

To determine the proline content, 0.2 g of fresh leaves were ground using 3% of sulfosalicylic acid. The mixture was filtered by using the Whatman filter paper. The filtrate was reacted with 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent. Finally, 4 mL of toluene was mixed in this mixture and shaken properly until the upper layer of varied color appeared. The micropipette was used for the separation of the layer, and the absorbance was calculated at 520 nm. The proline content was calculated by using Eq. 6 [31]:

$$\begin{aligned} \text{Total proline content} \\ = \frac{(\text{Sample absorbance} \times \text{Dilution factor} \times K \text{ value})}{\text{Fresh weight of plant tissue}}. \end{aligned} \quad (6)$$

2.8.4 Soluble sugar content

The phenol sulfuric acid method was used for the estimation of the soluble sugar content. A total of 0.5 g of fresh leaves were ground into 10 mL of 80% ethanol. The mixture was filtered by using the Whatman filter paper. The filtrate was later incubated at 80°C for 1 h. After 1 h, 0.5 mL of sample was mixed with 1 mL of 18% phenol and incubated at room temperature for 1 h. Then, 2.5 mL of sulfuric acid was added into the mixture and shaken properly. In the end, the absorbance was recorded at 490 nm of the light wavelength. The soluble sugar content was estimated by using Eq. 7 [32]:

$$\begin{aligned} \text{Sugar } (\mu\text{g/mL}) \\ = \frac{\text{Absorbance of sample} \times \text{Dilution factor} \times K \text{ value}}{\text{Weight of fresh tissue in grams}}. \end{aligned} \quad (7)$$

2.8.5 Protein content

The protein content was measured by mixing 0.5 mL of the plant extract with 0.5 mL of distilled water. This was followed by adding 3 mL of bio rad dye to the mixture. After proper shaking of the mixture, the spectrophotometer was used to measure the absorbance at 595 nm of the light wavelength [33].

2.9 Antioxidant enzymatic activities of rice plants under biotic stress

2.9.1 Superoxide dismutase (SOD) assay

The SOD activity was examined according to the method described in ref. [34]. The mixture was prepared by using

0.6 mL of plant extract, 0.075 mM of NBT, 130 mM of methionine, 1 mM of EDTA, 0.02 mM of riboflavin, and 0.78 mL of phosphate buffer (pH 7). After proper mixing, the solution was subjected to fluorescent light for 7 min. Finally, the absorbance was recorded at 560 nm of the light wavelength of each sample.

SOD activity was calculated by using the Lambert–Beer law (Eq. 8):

$$A = \varepsilon LC \quad (8)$$

2.9.2 Peroxidase (POD) activity

The POD activity was calculated according to the method presented in ref. [35]. The mixture (2 mL) was prepared by adding 0.2 mL of the plant extract, 27.5 mM of hydrogen peroxide, 100 mM of Guaiacol, 0.4 mL of phosphate buffer, and 1 mL of distilled water. The absorbance of the solution was observed at 470 nm.

2.9.3 Catalase activity

The catalase activity was measured by preparing the mixture after adding 0.5 mL of plant extract, 27.5 mM of hydrogen peroxide, 0.9 mL of distilled water, and 0.4 mL of phosphate buffer. The absorbance of the resultant mixture was noted at 240 nm by using the spectrophotometer [36].

2.10 Statistical analysis

The experiments were performed in triplicate and repeated twice. The mean and the standard deviation were used to represent the results. The analysis of the variance (ANOVA) was used to determine the significance of the results. $P < 0.05$ was considered statistically significant.

3 Results and discussion

3.1 Green synthesis of AgNPs

In the present study, the biogenesis of AgNPs was carried out by using the aqueous leaf extract of *M. oleifera* to reduce silver nitrate (AgNO_3) into AgNPs. The plant aqueous leaf extract act as a reducing and capping agent of AgNPs. The secondary metabolites of the plant play a

potential role in reducing the silver salt in AgNPs in various redox chemical reactions, while the potential functional groups provide bioactivity and also prevent the agglomeration of the nano-silver core. Initially, the synthesis of the AgNPs was recorded by observing the change in the color of the reaction mixture that was later confirmed by measuring the absorbance of the reaction mixture by using an ultra-violet visible (UV-visible) spectrophotometer. A characteristic surface plasmon resonance (SPR) band was observed between 410 and 480 nm, and the highest peak was recorded at 450 nm to confirm the synthesis of AgNPs (Figure 1a). The characteristic SPR band represents the interaction of the oscillating colloidal AgNPs with the electromagnetics light waves [37].

3.2 Morphological and optical characterization of AgNPs

The structure analysis of AgNPs was performed by collecting the micrographic images of AgNPs by using the scanning electron microscope (SEM). SEM images represented that the AgNPs are irregular in shape, while some nanoparticles were cubical and rectangular (Figure 1b). Ref. [38] also documented a similar result. The elemental composition analysis of biosynthesized AgNPs was performed by using the EDX. The EDX analysis confirmed the presence of Ag. The highest characteristic peak of the elemental silver [39] was observed at 3 keV, while the intensity of the Ag signal was very high (Figure 1c). The elemental oxygen was also observed in trace amounts, which originate from plant secondary metabolites and bind to the Ag core as a potential stabilizing agent.

3.3 Effects of AgNPs on plant's physiological attributes

3.3.1 Measurement of the relative water content, membrane stability index, and chlorophyll content of the rice plants treated with AgNPs

In the present study, different concentrations of biogenic AgNPs were applied to rice plants against *A. flavus* (biotic stress). The effects of AgNPs on physiological attributes of rice plants under biotic stress were studied in terms of relative water content (RWC), membrane stability index (MSI), and chlorophyll content (CC).

Figure 1: Morphological and optical characterization of bio-fabricated AgNPs: (a) UV-visible spectrum, (b) SEM image, and (c) elemental composition analysis.

Physiological parameters of the rice plants in terms of relative water contents, membrane stability index, and chlorophyll content were determined in response to various concentrations of *M. oleifera* leaves extract-mediated AgNPs under the biotic stress of *A. flavus*. RWC is an important physiological parameter that measures the water level of plants and is involved in the performance of metabolic activities of plants tissues. According to ref. [40], plant membrane stabilization also plays a prominent role in maintaining the plant's cell integrity.

A. flavus applications on rice plants act as biotic stress and significantly decrease the plant physiological activity. The pronounced decrease in plant physiological attributes in terms of RWC (22%), MSI (18%), chl *a* (12%), chl *b* (11%), and TCC (17%) was observed in control plants that were exposed to the biotic stress of *A. flavus* (Figure 2a and b). It was reported [41] earlier that the wheat plants under biotic stress have decreased water content due to a decrease in the water potential of the plant. It was confirmed experimentally that the biotic stress results in decreasing the plant physiological process, which ultimately affects the plant growth, yield, and development. The foliar application of 25 mg/L concentration

of AgNPs resulted in improving the RWC of the infected rice plants compared to the control plant. The increase in the concentration of AgNPs at 50 mg/L expressed a remarkable increase in the plant physiological parameters, but the increase in the concentration beyond optimum (50 mg/L) resulted in a decline in RWCs (Figure 2a).

The biotic stress affects the photosynthetic apparatus, which in turn alters the physiological attributes of plants [42]. Oxidative stress is also responsible for decreasing the photosynthesis rate by the disruption of the thylakoid membrane and the chlorophyll pigment in plant cells [43]. The determination of the contents of the photosynthetic pigments plays a significant role in determining efficiency of plants to produce carbohydrate metabolites. Chlorophyll is an essential component of the chloroplast and is directly involved in carbohydrate metabolism during the process of photosynthesis. Photosynthesis plays a crucial role in the plant growth and development, and the growth of plants improves by increasing the chlorophyll content [44]. The content of chlorophyll decreases under stress, which disturbs the major components of photosynthesis, and as a result reduces the photosynthetic quotient that affects plant's

growth, yield, and biochemical processes and causes oxidative stress [45]. In the present study, a clear decline in chlorophyll *a* and *b* and total chlorophyll content was reported in rice plants in response to pathogen stress (Figure 2b). It was confirmed earlier that stress decreases the chlorophyll content in wheat plants against stress conditions [20]. The synthesis of chlorophyll *a* and *b* decreases under stress conditions due to the injuries to the thylakoid, which results in reducing the exposure area of the leaf [42].

An increase in RWC (6%), MSI (16%), chl *a* (5%), chl *b* (9%), and TCC (5.8%) was estimated when rice plants were treated with a 50 mg/L concentration of AgNPs. However, the further increase in the concentration of AgNPs above 50 mg/L resulted in the reduction of the plant physiological parameters. It was reported earlier that the biotic stress reduces the plant physiological attributes in terms of water relation such as relative water content, rate of transpiration, and water potential of crops, but the AgNPs maintained the water balance by reducing the stress [20].

AgNPs inhibit the growth of *Aspergillus* and give protection to rice plants. The antifungal potential of AgNPs might be attributed to the loss of DNA replication ability and denaturation of enzymes, which are responsible for the fungal growth [46], degradation of cellular proteins leading to inactivation of enzyme expression and other proteins that are helpful in ATP synthesis, and eventually, AgNP hampers the aflatoxin biosynthetic pathways [47].

According to the present study, the optimum concentration of AgNPs enhances physiological attributes under biotic stress conditions. Ref. [48] mentioned that the treatment of plants with AgNPs increases the physiological

activity in crops by hampering the growth of fungi and controlling the aflatoxin production. Gupta *et al.* [49] reported similar results who stated that the green synthesized AgNPs at optimum concentration increase the carotenoid and chlorophyll contents in rice plants.

3.4 Effects of AgNPs on plant's biochemical attributes

3.4.1 Measurement of the enzymatic components of the rice plants treated with AgNPs

In the present study, the enzymatic activities in terms of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of rice plants were investigated in response to various concentrations of AgNPs under biotic stress. It was observed that the SOD activity (2.91 nM/min/mg) significantly enhanced (32%) in plants that were exposed to the biotic stress (Figure 3a). The action of the SOD activity is directly correlated with POD and CAT activities (Figure 3a). The highest POD (2.84 nM/min/mg) (42%) and CAT (2.79 nM/min/mg) (22%) activities were recorded under biotic stress conditions. The concentration of the SOD (2.64 nM/min/mg) (20%), POD (2.3 nM/min/mg) (15%), and CAT (2.47) (8%) was decreased in plants that were treated with 50 mg/L of AgNPs under stress. Ref. [17] manifested that biotic stress contributes to the highest enzymatic activities in plants under stress conditions. The environmental and pathogenic stress damages the plant cells because of the production of reactive oxygen species (ROS) [50]. However, the application of AgNPs

Figure 2: Effect of different treatments of AgNPs on the physiological parameters of rice plants under biotic stress: (a) relative water content and membrane stability index and (b) chlorophyll contents. Note: T0: control; T1: *A. flavus* (pathogen); T2: 25 mg/L of AgNPs + *A. flavus*; T3: 50 mg/L of AgNPs + *A. flavus*; T4: 75 mg/L of AgNPs + *A. flavus*; T5: 100 mg/L of AgNPs + *A. flavus*.

Figure 3: Effect of different treatments of AgNPs on enzymatic and nonenzymatic parameters of rice plants under biotic stress: (a) superoxide dismutase, peroxidase, and catalase activity and (b) total phenol and flavonoid contents. Note: T0: control; T1: *A. flavus* (pathogen); T2: 25 mg/L of AgNPs + *A. flavus*; T3: 50 mg/L of AgNPs + *A. flavus*; T4: 75 mg/L of AgNPs + *A. flavus*; T5: 100 mg/L of AgNPs + *A. flavus*.

resulted in lowering the SOD, POD, and CAT activities in rice plants under biotic stress.

3.4.2 Measurement of the nonenzymatic components of the rice plants treated with AgNPs

3.4.2.1 Total phenol and flavonoid contents of the rice plants treated with AgNPs

TPC and TFC were also measured in the rice plants under biotic stress. The concentration of TFC (2.74 μg/mg; 24%) and TPC (1.59 μg/mg; 45%) were increased in plants under biotic stress (Figure 3b). However, the application of AgNPs reduced the quantity of TFC (2.33 μg/mg; 4%) and TPC (1.25 μg/mg; 10%) in plants subjected to 50 mg/L

of AgNPs. It was also reported earlier that nonenzymatic activities increase significantly in crops when the plants were in stress conditions, while they decrease after the application of AgNPs [20]. TFC and TPC play a promising role in defending the plants against stresses by scavenging the ROS [51]. They also maintain the homeostasis and the structures of organelles by protecting the enzymes at the cellular level and help in osmotic adjustment [52].

3.4.2.2 Proline and soluble sugar contents of the rice plants treated with AgNPs

The osmolyte concentration, such as proline and soluble sugar, was also estimated in response to the different concentrations of AgNPs against biotic stress. The proline

Figure 4: Effect of different treatments of AgNPs on nonenzymatic parameters of rice plants under biotic stress: (a) total proline content and total soluble sugar contents and (b) total protein content. Note: T0: control; T1: *A. flavus* (pathogen); T2: 25 mg/L of AgNPs + *A. flavus*; T3: 50 mg/L of AgNPs + *A. flavus*; T4: 75 mg/L of AgNPs + *A. flavus*; T5: 100 mg/L of AgNPs + *A. flavus*.

concentration (22%) and sugar content (21%) were increased in plants under the pathogen stress. However, the plants treated with 50 mg/L of AgNPs expressed a reduction in the proline (38%) and sugar (5%) levels of rice plants (Figure 4a). Our outcomes are similar to ref. [28] that confirmed that plants can produce various kinds of osmolytes under biotic stress conditions, which help plants to tolerate stress by detoxifying and scavenging the ROS and help in osmotic adjustments.

3.4.2.3 Protein content of the rice plants treated with AgNPs

Protein content was also studied in rice plants under biotic stress and AgNPs treatment. It was confirmed that the protein content was minimum (1.34 µg BSAE/mg; 26%) under biotic stress and maximum (1.74 µg BSAE/mg; 4%) protein content was measured in plants that were treated with 50 mg/L of plant-based AgNPs (Figure 4b). It was observed that stress reduces the production of primary metabolites in plants. Our findings are aligned with ref. [53] that explained that the application of AgNPs increases the protein content against the biotic stress by maintaining the membrane integrity.

This experimental evidence successfully draws a direct relationship between the treatment of AgNPs and biotic stress caused by *A. flavus*. Biotic stress in rice plants drastically decreased the RWC, MSI, chlorophyll *a* and *b*, total chlorophyll, and protein contents, while the SOD, POD, CAT, TFC, TPC, proline, and soluble sugar contents increased in infected plants. However, the exogenous applications of AgNPs on rice plants in the field conditions resulted in osmotic, biochemical, and enzymatic adjustments to tolerate the biotic stress and control the further proliferation of fungal pathogen.

4 Conclusion

The present study confirms that the biosynthesized silver nanoparticles have a positive effect on rice plants under biotic stress caused by saprophytic fungi (*A. flavus*). The biotic stress shows an adverse effect on physiological, biochemical, and growth aspects of rice plants by producing aflatoxins that contaminate the quality of the rice grain. AgNPs played a promising role in regulating the production of osmolytes (proline and soluble sugar), enzymatic (SOD, POD, and CAT) and nonenzymatic (TPC and TFC) metabolites, and protein contents in plants under stress.

The foliar application of silver nanoparticles at 50 mg/L prepared plants to cope with the biotic stress. However, the usage of higher concentrations of colloidal solutions decreased the plant's physiological and biochemical parameters. The findings of this study established a foundation for the in-depth analysis of the effectiveness and toxicity of the nanoparticles and alterations at the molecular level in response to the biotic stress.

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