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BIOSYNTHESIZED SILVER NANOPARTICLES AMELIORATE BIOTIC STRESS IN RICE (*ORYZA SATIVA*) BY INTRICATING BIOCHEMICAL AND MINERAL PROFILE

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Abstract

Due to its cost-effectiveness and eco-friendliness, plant-mediated nanomaterial production has been gaining prominence. The current study aimed to determine how biosynthesized AgNPs affected rice under biotic stress in terms of its biochemical characteristics, aflatoxin B1 levels, and mineral profiling. By an extract from *Moringa oleifera* leaves, AgNPs were biosynthesized and then characterized using UV-visible spectroscopy, energy dispersive X-Ray (EDX), X-ray powder diffraction (XRD), zeta analysis, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). Before applying the *Aspergillus flavus* inoculum, different concentrations of biosynthesized AgNPs (25, 50, 75, and 100 mg/kg) were exogenously administered to rice plants at the heading stage. High activities of superoxide dismutase (SOD) (2.91 nmol/min/mg), peroxidase (POD) (2.84 nmol/, min/mg), and catalase (CAT) (2.79 nmol/min/mg), and enhanced levels of proline (2.75 µg/ mL FW) and malondialdehyde (MDA) (8.5 µmol/g FW) were observed in rice plants exposed to biotic stress alone. However, the application of biosynthesized AgNPs significantly reduced the production of antioxidative enzymes and non-enzymatic compounds by inhibiting the intensity of biotic stress. Likewise, the aflatoxin B1 level (25.1 µg/kg FW) was maximum in rice grains of those rice plants which were only subjected to biotic stress; however, the application of biosynthesized AgNP sunder biotic stress significantly reduced the aflatoxin B1 level (10.6 µg/kg) at 50 mg/kg AgNPs. The application of 50 mg/kg biosynthesized AgNPs under the biotic stress proved to be the most suitable concentration for the biochemical response, aflatoxin B1, in rice. The concentrations exceeding 50 mg/kg of biosynthesized AgNPs induced toxic effects, thus increasing the levels of biochemical attributes, aflatoxin B1, and Ag content. Additionally, Ca (0.6%), Fe (0.25%), and P (0.06%) content of rice grains also exhibited improvement at 50 mg/kg biosynthesized AgNPs under biotic stress. In conclusion, applying biosynthesized AgNPs may act as an anti-fungal agent, controlling the aflatoxin B1 level in rice grains and improving mineral profiling.

Key words: *Oryza sativa*, Nanotechnology, Biosynthesis, Aflatoxin B1. Rice, *Moringa oleifera*.

Introduction

The advancement in the field of nanotechnology is opening new horizons in the synthesis and application of nanoparticles. The production of nanoparticles using plant parts is a prime attraction for researchers owing to their safety attributes and low cost (Prasad *et al*., 2016). Plant-based compounds such as flavonoids, aldehydes, quinones, etc., play a vital role in reducing ions, a key step in synthesizing the nanoparticles (Prabhu & Poulose, 2012). Advances in nanotechnology have opened to manage diseases at the molecular level, thus improving crop yield potential and nutritional attributes (Tarafdar *et al*., 2013). The action of nanoparticles may depend upon their size, increased surface ratio, and enhanced optical aspects (Ditta, 2012). The application of nanoparticles can be used as an effective strategy to improve various agricultural aspects of crop production in a state of stress (Prasad *et al*., 2014).

Silver nanoparticles' antibacterial and anti-fungal activity has been established (Lee *et al*., 2007; Lamsal *et al*., 2011). Nanoparticles, especially silver nanoparticles (AgNPs), after attacking the microorganisms, cause damage to their cell membrane (Dibrov *et al*., 2012; Pal *et al*., 2007). Silver has also been reported to interact with biomaterials having high phosphorus and sulfur content, DNA, and proteins, both structural and functional, and ultimately affect various metabolic processes of cells (Song *et al*., 2006). Biosynthesized AgNPs also increased the growth profile and biochemical attributes of *B. juncea*, common beans, and corn (Sharma *et al*., 2012).

The vitality of rice as an essential part of a balanced diet cannot be denied, as it is the source of providing carbohydrates (80%), fiber (3%), and protein (8%) (Juliano, 1985). The position of rice in terms of its nutritional value among other crops is because of the high protein efficiency ratio due to the presence of lysine in excess proportion. Rice grains are a source of several important minerals like copper, manganese, iron, magnesium and silicon (Hashmi & Tianlin, 2016).

The contamination of rice by aflatoxins as a consequence of temperature and moisture conditions results in annual losses of useful food bioresources and devastates the economy of rice-producing countries (Súarez-Bonnet *et al*., 2013; Lai *et al*., 2015; Naseer *et al*., 2014). Several genera have been reported producing mycotoxins, among which *Aspergillus* is worth mentioning (Berthiller *et al*.,

2013; Probst & Cotty, 2012). Aflatoxin B1 (AFB1) is the most dangerous among other classes of aflatoxins and affects the liver even at very low (µg/kg) levels (Bhat *et al*., 1999). The production of ROS in response to biotic stress inhibits the processes of growth and differentiation. Under such adverse conditions, plant cells use catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD) as oxidative enzymes and non-enzymatic components such as malondialdehyde (MDA), proline, phenolic compounds and flavonoids to cancel the negative role of ROS (Valko *et al*., 2007). The non-enzymatic components stimulate the immune system, apoptosis induction, gene expression, enzyme activation, and quenching of harmful free radicals (Joo *et al*., 2010).

We hypothesized that biosynthesized silver nanoparticles by *Moringa oleifera* leaf extracts ameliorate biotic stress in rice (*Oryza sativa*) by intricating biochemical and mineral profiles. The study's objectives were: (i) the impact of biosynthesized AgNPs on enzymatic and non-enzymatic components of rice under biotic stress (ii) whether biosynthesized AgNPs are effective in enhancing the mineral contents of rice under biotic stress.

Material and Methods

Biosynthesis of AgNPs: Fresh leaves of Moringa oleifera were taken from the plant growing in PMAS Arid Agriculture University Rawalpindi, Pakistan (33.6492° N, 73.0815° E) and used in the biosynthesis of AgNPs. Silver nitrate ($AgNO_3$) was obtained from Merck (Germany, CAS 7761-88-8). The leaves were washed adequately in sterilized water to remove dust, then dried at room temperature using blotting paper. 200 mL of distilled water was used to boil 30 g of dried leaves. Filtration was done as soon as the solution's color changed to a dark brown hue, and the resulting filtrate was stored for later use at 4°C in the refrigerator. The procedure (Hussain *et al*., 2017) described how to create a 5 mM solution of $AgNO_3$ by combining 0.85 g of $AgNO_3$ with 1 L of distilled water, heating for 10 minutes, and then adding gradually (10–20 mL of plant extract until the solution's color turns brown (point indication for AgNPs synthesis). The solution was centrifuged at 14,000 rpm for 10 minutes before separating the AgNPs pallet. The pallet was centrifuged at 14,000 rpm for 10 minutes in distilled water to remove any remaining $AgNO_3$ salt residues.

Characterization of AgNPs: Based on the availability of technology and related expertise, UV-Vis spectrophotometer, X-ray diffraction analysis, energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), and transmission electron microscopy were used to characterize the resulting pallet (biosynthesized AgNPs) (TEM). Using a spectrophotometer to analyze the sample, the UV-Vis spectrum was captured. On a Rigaku D/max-2200 PC diffractometer operating at 40 kV/40 mA and employing CuKa1 radiation with a wavelength of 1.54 Å and an angle of 05°–55° on 2θ scale, XRD was conducted. EDS was used to verify AgNPs elemental analyses. While transmission electron microscopy (TEM) was performed by dropping the nanoparticle solution drop on a copper grid, and after drying, nanoparticles were seen at 100kV inside TEM, SEM was used to verify the amorphous nature of AgNPs. The Institute of Space and Technology (IST) Islamabad assisted in the characterization of biosynthesized AgNPs. The Debye–Scherrer equation was used to analyze AgNPs size and shape (Arokiyaraj *et al*., 2014).

$$D = k\lambda/\beta\cos\theta$$

where k is the shape factor, λ the X-ray wavelength, θ the Bragg's angle, and β the full width in radians at half maximum.

Plant material source, site description, and growth conditions: To achieve surface sterilization, rice seeds (var. Super basmati) were purchased from the Rice Research Institute Kala Shah Kaku, Pakistan, and exposed to a 10% sodium hypochlorite solution for 10 minutes (Iqbal *et al*., 2016). The seeds were thoroughly washed in water to get rid of the sterilizing agent. A field experiment was conducted in Pakistan's District Sargodha, Bhera (Chak Qazi). To prevent drought, the plants received regular irrigation.

Application of biosynthesized AgNPs and Aspergillus inoculum: Aspergillus inoculum was obtained from a laboratory at the University Institute of Biochemistry and Biotechnology (UIBB), Arid Agriculture University, Rawalpindi. Stock solutions of biosynthesized AgNPs at concentrations of 25, 50, 75, and 100 mg/L were prepared and kept in the refrigerator. Each stock solution's volume of biosynthesized AgNPs, measuring 16.7, 33.3, 50, and 66.7 mL, was taken separately and sprayed onto rice plants (foliar spray) at the heading stage. Silver nanoparticles (NPs) were used for 10 days before Aspergillus inoculum was sprayed onto rice plants as a foliar treatment. Plants not treated with AgNPs, Aspergillus, or Aspergillus alone served as controls.

Biochemical parameters

Preparation of leaf extract for enzymatic assays: The protocol (Nayyar & Gupta, 2006) was followed to prepare the extract of endogenous enzymes by utilizing fresh leaves of rice plants. The mixture of fresh rice leaves (0.1 g) and extraction buffer (10 mL) was sonicated thrice for 10 minutes, followed by centrifugation at 8000 rpm to remove the pallet, and the resultant supernatant was utilized for enzymatic assays.

Assessment of SOD activity: The technique from Ullah *et al*., (2013) was followed with a few minor adjustments to determine SOD activity. Phosphate buffer (390 L), 130 mM $C_5H_{11}NO_2S$ (100 L), 1 mM EDTA (100 L), 0.02 mM riboflavin (10 L), 0.075 mM NBT (100 L), and enzyme extract (300 L) were the components of the reaction mixture (1 mL), whereas phosphate buffer was used to prepare the blank. The blank and the reaction mixture were held for 7 minutes under fluorescent lighting, and spectrophotometer readings of the absorbance at 560 nm were made. The calculation of SOD activity was based on Lambert–Beer law $A = \varepsilon LC$ where ε represents the extinction coefficient, L is the length of the wall, and C stands for the concentration of enzymes.

Assessment of POD activity: The protocol (Lagrimini, 1991) with minor modifications was considered to assess POD activity. The reaction mixture (2 mL) containing 200 µl enzyme extract,400 µl phosphate buffer, 200 µl of 100 mM Guaiacol, 200 µl of 27.5 mM hydrogen peroxide (H_2O_2), and 1 mL distilled water, and the blank containing phosphate buffer instead of enzyme extract were placed for 7 min under fluorescent light and absorbance was recorded at 470 nm by utilizing a spectrophotometer.

Assessment of CAT activity: The determination of CAT activity was carried out by considering the protocol (Aebi, 1984). The reaction mixture (2 mL) contained 200 µl of 27.5 mM hydrogen peroxide (H_2O_2), 400 µL phosphate buffer, 500 µL enzyme extract, and 900 µl distilled water, while the blank contained phosphate buffer instead of enzyme extract. Both the blank and reaction mixture shifted for 7 minutes under fluorescent light, and absorbance was recorded at 240 nm using a spectrophotometer (model UV-1650 PC) Shimadzu.

Proline estimation: According to the procedure (Bates *et al*., 1973), freshly harvested rice leaves (0.2 g) were combined with 4 mL of sulfosalicylic acid (3.0%) and then ground. Separate test tubes containing 2 mL of filtrate, 2 mL of ninhydrin reagent, and 2 mL of glacial acetic acid were allowed to react before being heated in a water bath until the formation of color. The reaction was then stopped by placing the test tubes in ice. Toluene (4 mL) was then added, and the mixture was thoroughly mixed until the development of an upper-colored layer. This layer was then separated from the solution mixture and placed in separate test tubes, where its absorbance was measured at 520 nm wavelength. The following formula was used to calculate the proline contents.

$$\text{Total Proline (µg/mL)} = \frac{\text{(Sample absorbance} \times \text{Dilution factor} \times \text{K value)}}{\text{Fresh weight of plant tissue}}$$

Malondialdehyde (MDA) estimation: Malondialdehyde (MDA) content was recorded using the protocol (Velikova *et al*., 2000) with minor modifications. After the homogenization of 1 g of sample with 0.1% trichloroacetic acid, followed by centrifugation of the mixture at 181.76 G for 30 minutes, the supernatants were used for lipid peroxidation content. The reaction mixture included plant extract (1 mL) prepared by homogenizing 1 g of leaves with 0.1% of trichloroacetic acid followed by centrifugation at 181.76 G for 30 minutes, 3 ml of TCA and TBA (1:1) and heated for 30 min at 95ºC. The reaction mixture was then cooled immediately in an ice bath to stop the reaction. The centrifugation of the reaction mixture was done at 181.76 G for 10 minutes to collect the supernatant, and the absorbance was recorded at 532 nm and 600 nm. The value of nonspecific absorbance at 600 nm was then subtracted from that of 532 nm. MDA content was expressed in µM/L.

Evaluation of different concentrations of biosynthesized AgNPs to control aflatoxin against *Aspergillus flavus*: Rice grains collected from rice plants sprayed with various concentrations (25, 50, 75, and 100 mg/kg) of biosynthesized AgNPs thrice with an interval of 3 days followed by application of *Aspergillus flavus* inoculum were analyzed for Aflatoxin B1 estimation. A correlation was established between the appropriate concentration of biosynthesized AgNPs and aflatoxin B1 level (Table 1).

Table 1. Analysis of aflatoxins in rice plants in response to AgNPs treatment against *Aspergillus* application.

Treatments	Aflatoxins, µg/kg
Control (without AgNPs and *Aspergillus*)	7.7 ± 0.1^{a}
0 mg/1 (without AgNPs + with *Aspergillus*)	8.3 ± 0.0^{b}
25 mg/1 (AgNPs *Aspergillus*)	5.9 ± 0.3^{c}
50 mg/1 (AgNPs *Aspergillus*)	4.2 ± 0.3^{c}
75 mg/1 (AgNPs + *Aspergillus*)	3.5 ± 0.1^{a}
100 mg/1 (AgNPs + *Aspergillus*)	3.1 ± 0.0^{b}

[a-c] The direct depiction of the statistical analysis done using statistic software. The data present are the statistical significance level in the form of superscripts

Extraction and estimation of aflatoxin B1: The extraction and subsequent estimation of aflatoxin B1 from grain samples were performed following the protocol (Ramesh *et al*., 2013) with slight modifications. The dried extract was prepared from 25 g grain samples treated with 106 mL acetone, 19 mL distilled water, 1.5 g of cupric carbonate, a solution of 85 mL of 0.2 N NaOH, 15 mL of 0.4 M $FeCl_3$, 100 mL of 0.03% H_2SO_4, 25 mL of chloroform, 1% KCl in 0.02 M KOH was re-dissolved in 0.2mL of chloroform and used for HPTLC spotting. The dried samples were applied as bands (spray-on technique) using a Linomat-5 sample applicator. After spraying, the plates were dried using a hair dryer. Finally, the plates were scanned in CAMAG HPTLC scanner-3 under 366nm wavelength to determine the levels of aflatoxin B1 contamination in the samples. The detection was done under fluorescence UV radiation.

Mineral profiling of rice grains: Each treatment's rice grain sample (0.5 g) was gathered and taken for analysis. The materials were thoroughly ground before being put through a 2-mm screen to be tested for calcium (Ca), iron (Fe), and phosphorus (P). Using the given protocols, the atomic absorption spectrophotometer method was used to determine the amounts of calcium, iron, and phosphorus (Anon., 2007).

Assessment of silver content: The acid digestion method measured Ag content in rice grains (Araujo *et al*., 2007) with slight modifications. Acid digestion of 0.5 g of dried and well-ground rice grains was followed by running the prepared samples against standards (Ag pure solutions 1, 2, 3, and 4 mg/l) in an atomic absorption spectrophotometer. A standard/sample calibration curve was considered to determine the silver content. An atomic absorption spectrophotometer (PERKIN-ELMER Model 1100 B) with a silver lamp was implied to measure the silver content at 328 nm. The burning of the samples was accomplished through a nebulizer, and absorbance was recorded directly from the screen.

Statistical analysis

Each treatment had three replicates in the experiment. This data was translated into mean and standard deviation. PAST 3 software was used to statistically analyze the experiment data by principal component analysis (PCA) (Ferrer-Galindo *et al*., 2018).

Results and Discussion

Biosynthesis of AgNPs: The extract of *M. oleifera* leaves utilized for the biosynthesis of AgNPs reduced the aqueous silver ions into AgNPs, thus acting as the main reducing and stabilizing agent. This action of the plant extract may be attributed to the presence of certain concentrations of active ingredients, such as plant flavonoids which are intended to play a key role in the synthesis of NPs (Hussain *et al*., 2019).

Characterization of AgNPs

AgNPs characterization by UV–Visible spectroscopy: The characterization of biosynthesized AgNPs using the *M. oleifera* leaves extract was carried out through UV–Visible spectroscopy. A set of characterization techniques was preferred to verify the biosynthesis of silver NPs because a single process cannot reveal all the characteristics of NPs. Ultraviolet (UV) –visible spectroscopy is the primary tool that confirmed the AgNPs biosynthesis based on characterization peaks ranging from 423-425 nm (Fig. 1). Some previous studies also revealed that SPR peaks for AgNPs ranged from 410 to 480 nm (Gogoi *et al*., 2015).

AgNPs characterization by XRD: X-ray diffraction spectroscopy confirmed the crystalline nature of biosynthesized AgNPs. The diffraction spectra of biosynthesized AgNPs showed crystalline silver (Fig. 2); however, some non-crystalline peaks were also visible, as presented in previous studies (Mie *et al*., 2014; Hussain *et al*., 2018). These diffraction peaks might be due to the presence of active constituents involved in the capping of AgNPs. The average size of silver NPs was ~ 22.56 nm, calculated by the Debye-Scherrer equation (Mie *et al*., 2014).

AgNPs characterization by zeta analyzer, SEM, and TEM: The zeta potential of biosynthesized AgNPs (Fig. 3) elucidated the size of AgNPs ranging from 8-28 nm. The structural analysis of biosynthesized AgNPs was illustrated through SEM (Fig. 4) and TEM (Fig. 5), which indicated that biosynthesized NPs were spherical fused segments. Similar morphology of the synthesized silver NPs was reported in a study that attempted to determine the shape using *Pedalium murex* leaf extract for AgNPs synthesis (Sathishkumar *et al*., 2012).

AgNPs characterization by EDX: Energy dispersive X-ray spectroscopy (EDX) confirmed the presence of Ag^{2+}in biosynthesized AgNPs for which the absorption peak ranged from 2-4 Kev (Fig. 6) as some other researchers reported the absorption of metallic silver using EDX detector (Jyoti *et al*., 2016; Mei *et al*., 2014).

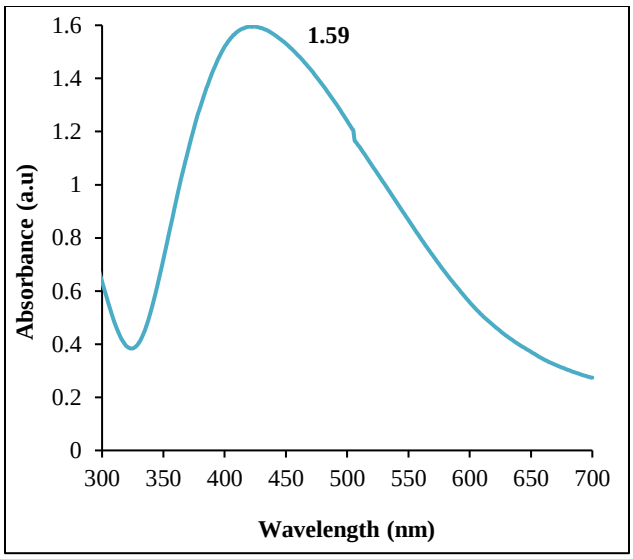

Fig. 1. Uv-visible of biosynthesized AgNPs (a.u. indicates absorption unit).

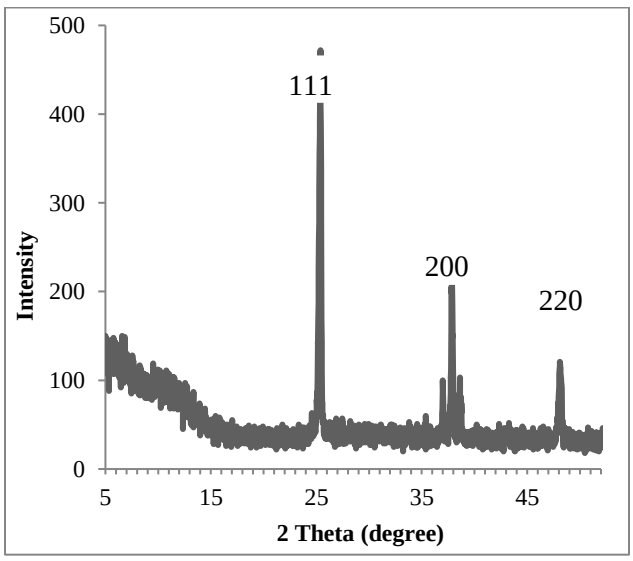

Fig. 2. X-ray diffraction of biosynthesized AgNPs.

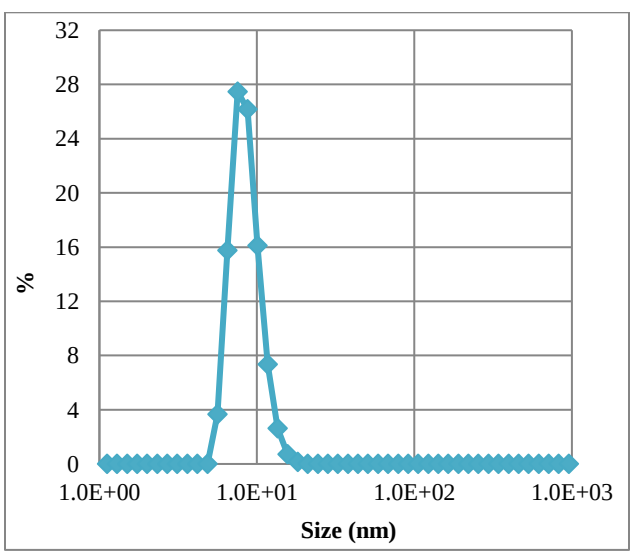

Fig. 3. Size distribution of biosynthesized AgNPs.

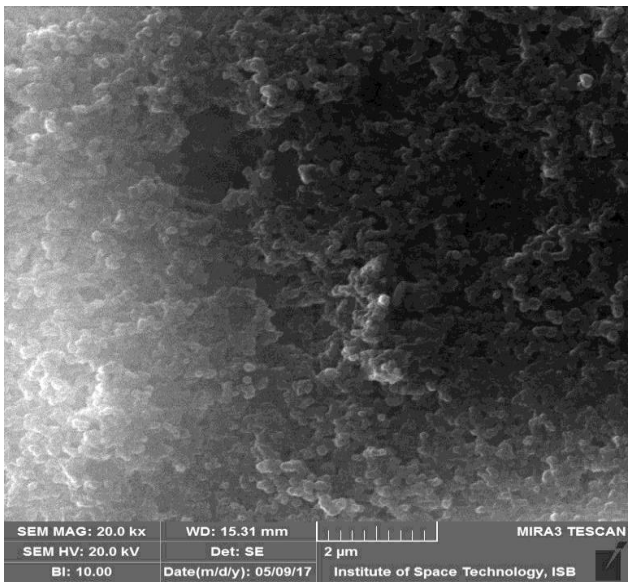

Fig. 4. SEM micrograph of biosynthesized AgNPs.

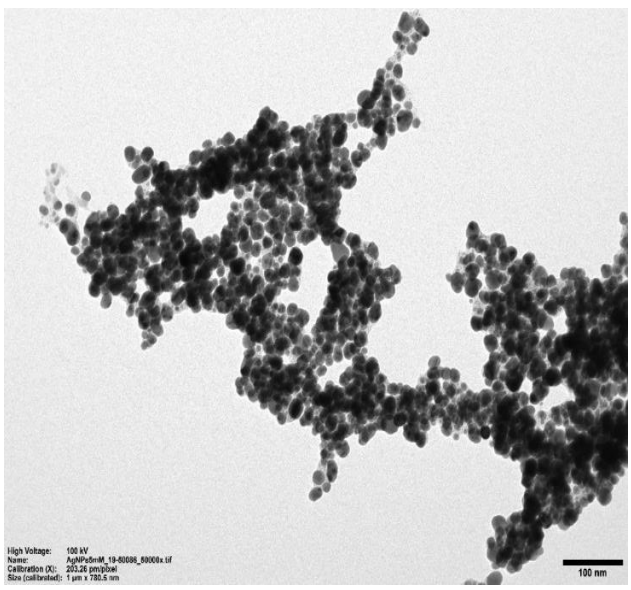

Fig. 5. TEM micrograph of biosynthesized AgNPs.

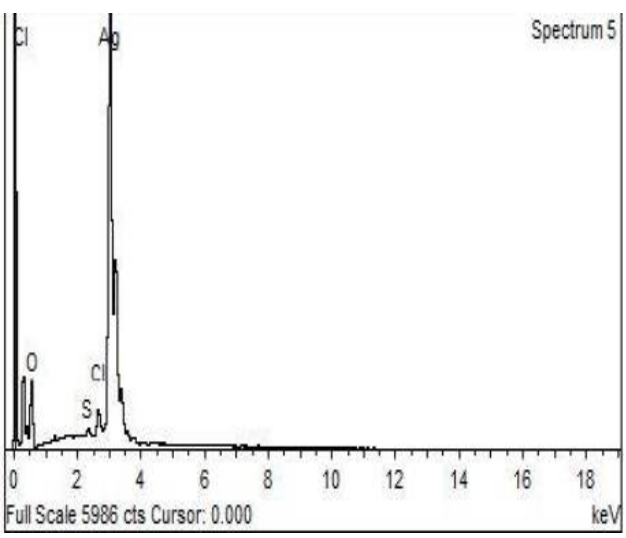

Fig. 6. EDX spectrum of biosynthesized AgNPs.

Enzymatic and non-enzymatic compounds: In the present study, the activities of major anti-oxidative enzymes (SOD, POD, and CAT) of rice leaves were investigated in response to different concentrations of biosynthesized AgNPs under biotic stress. A linear correlation of SOD with POD was apparent, highlighting the dependency of SOD on POD. High activities of SOD, POD and CAT production were illustrated in the rice plants exposed to biotic stress; however, the application of biosynthesized AgNPs significantly reduced the production of anti-oxidative enzymes by decreasing the intensity of biotic stress. The minimum SOD (2.03 U/mg FW) and POD (2.21 U/mg FW) levels were recorded in the rice plants which were neither treated with biosynthesized AgNPs nor with an inoculum of *Aspergillus flavus*. The maximum POD level (2.91 U/mg FW) was recorded in rice plants subjected to *Aspergillus flavus* inoculum only. The increased production of CAT (2.79 U/mg FW) was also noticed in the rice plants, which were only exposed to biotic stress. Among all the treatments (AgNPs and *Aspergillus flavus*), the minimum levels of SOD, POD, and CAT were observed in the rice plants exposed to an exogenous spray of biosynthesized AgNPs (50 mg/kg) under biotic stress. Biotic stress is the main factor impacting the enhanced production of endogenous enzymes. The increased levels of antioxidant enzymes were reported in several plants by researchers (Gupta & Datta, 2003; Varjovi *et al*., 2015; Fazal *et al*., 2016). These enzymes are involved in scavenging reactive oxygen species (Ahmad *et al*., 2010). The principal component analysis (PCA) was carried out for observed values of enzymatic and non-enzymatic parameters elucidating six PCs. However, significant results were recorded in the case of PCA 3 and PCA 5 instead of other components (Fig. 7). The scree plot (Fig. 8) about % eigenvalues indicated high variations between PC1 and PC2 whereas fewer variations were observed between PC2 and PC3 and so on. The eigenvalues for PC3 and PC5 were 0.0396 and 0.00671, with 0.756 and 0.1282 percent variance, respectively.

The non-enzymatic osmolytes, i.e., proline and malondialdehyde production under biotic stress in response to various concentrations of biosynthesized AgNPs in rice plants, were also investigated. The investigation revealed that proline concentration (0.55 µg/mL) was maximum in the rice plants treated with biotic stress only. The application of biosynthesized AgNPs (50 mg/kg) decreased proline levels (0.28 µg/mL). The findings are in line with the researchers who stated that environmental factors are the main cause behind proline accumulation in plants (Dar *et al*., 2016). In plants, intracellular proline levels have been found to increase by >100-fold during stress (Handa *et al*., 1983; Verbruggen & Hermans, 2008). Beyond 50 mg/kg concentration of AgNPs increased level of proline content was manifested, which may be due to the toxicity effect of silver, as it has previously been reported that proline accumulation in plants occurs during exposure to various stresses, including heavy metal ions (Chen *et al*., 2001) and pathogens (Fabro *et al*., 2004). Correlations among biochemical compounds showing relationships with one another in the form of positive or negative loadings in component (PC3) illustrated that SOD, POD, and proline have positive loadings correlation (Fig. 9), whereas CAT and MDA presented negative loadings. A maximum positive correlation was observed in proline and POD for others in PC3. Likewise, SOD, CAT, and proline illustrated

positive loadings correlation in component (PC5), and POD and MDA showed negative loadings concerning correlation. A maximum positive correlation was observed in proline and POD for others (Fig. 10).

Malondialdehyde (MDA) quantity was recorded on the application of various treatments of biosynthesized silver NPs in rice. The rice plants exposed only to biotic stress (*A. flavus*) produced maximum MDA content (9.55 µM/g FW), which may result from over-lipid peroxidation of the membranes. The rice plants subjected to 50 mg/kg AgNPs under biotic stress indicated a minimum MDA level (6.36 µM/g FW). The production of MDA is believed to be an indicator of the peroxidation of lipids and indicates cell membrane damage by pathogenic infection (Hameed & Iqbal, 2014; Aly *et al*., 2012).

Assessment of aflatoxin B1: The rice grains treated with biosynthesized AgNps and biotic stress were assessed for aflatoxin B1 quantification. The results revealed the lower level of aflatoxin B1 grains of plants exogenously sprayed with 50 mg/kg concentration of AgNPs. The plants exposed to 25, 50, 75, and 100 mg/kg biosynthesized AgNPs produced grains exhibiting aflatoxin levels of 13.6, 10.6, 12.1, 13.7 µg/kg compared to plants (25.1 µg/kg) only exposed to biotic stress. The inhibition of AFB_1 production by *A. parasiticus* resulted from fungal growth inhibition (Mousavi & Pourtalebi, 2015). It has also been proved experimentally that silver nanoparticles pave the way to significantly decreasing aflatoxin secretion in *A. flavus* (Zhao *et al*., 2017). The Principal Component Analysis of two PCs observed for values of aflatoxin B1 and silver content highlighted more explanatory results in PCA 1 and PCA 2 for each parameter separately (Fig. 11). The scree plot about % eigenvalues also indicated level of variations (Fig. 12).

Silver content estimation in rice grains elucidated that rice plants treated with 25 mg/kg biosynthesized AgNPs under biotic stress produced grains having a silver content of 0.0032 µg/g, whereas the plants treated with 50 mg/kg, 75 mg/kg, and 100 mg/kg AgNPs raised grains accumulating 0.0047 µg/g, 0.0064 µg/g and 0.0081 µg/g silver content respectively. The optimum conc. Biosynthesized AgNPs (50 mg/kg) resulting in significant enzymatic and non-enzymatic components and mineral profile in rice plants manifested 0.0047µg/g content, which may be considered a safe level for human consumption (Tripton *et al*., 1966).

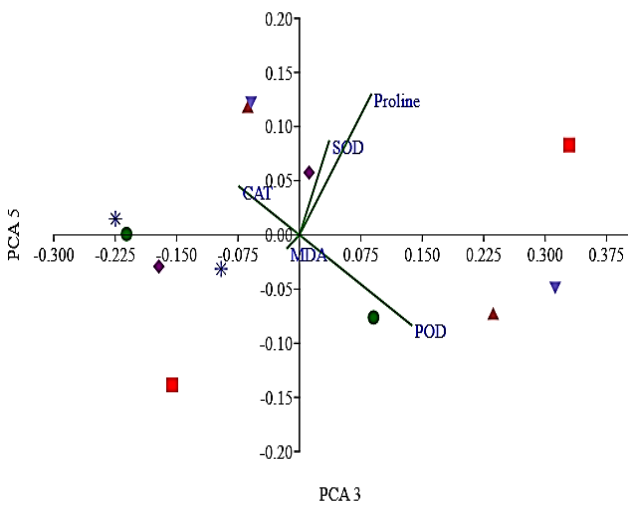

Fig. 7. Principal Component Analysis (PCA) of enzymatic and non-enzymatic compounds.

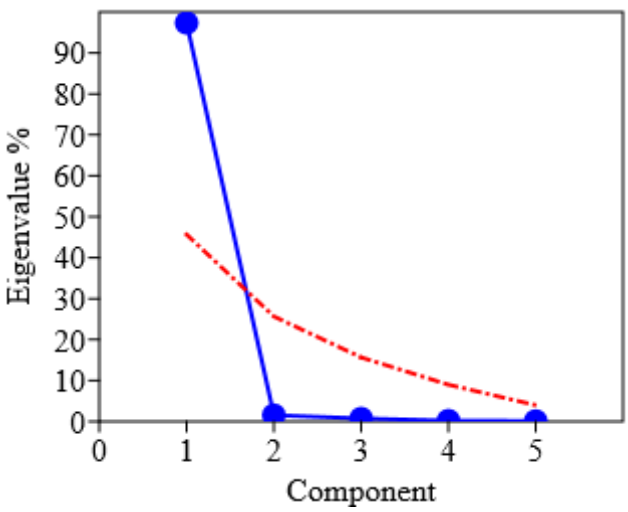

Fig. 8. Scree plot of obtained PCs produced based on percent eigenvalue.

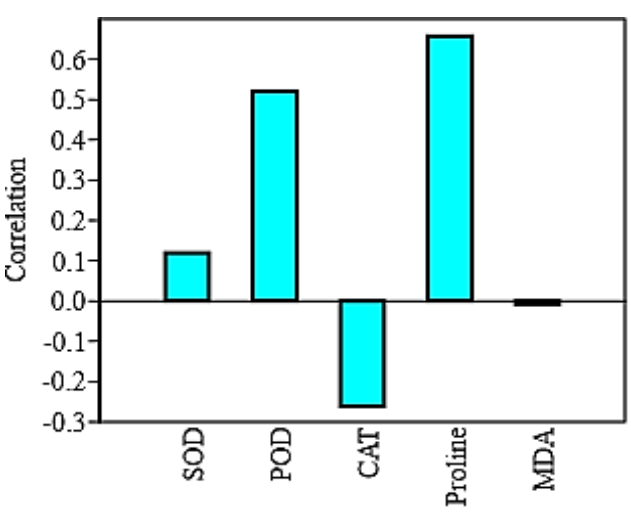

Fig. 9. Correlation analysis within biochemical attributes in PC3.

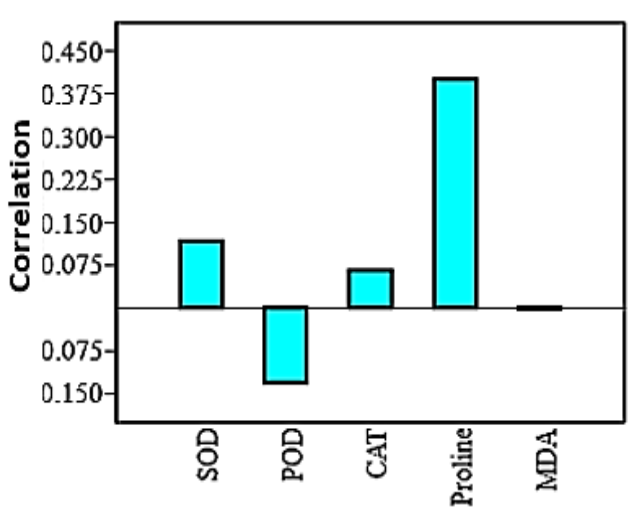

Fig. 10. Correlation analysis within biochemical attributes in PC5.

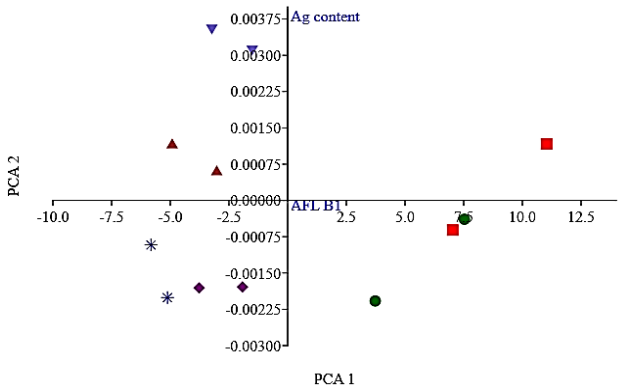

Fig. 11. Principal Component Analysis (PCA) of Aflatoxin B1 and Silver content.

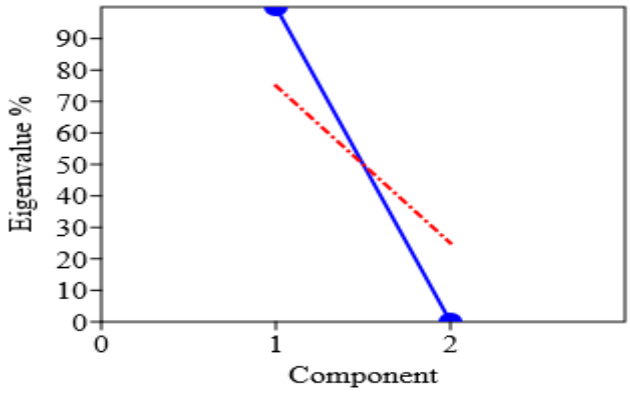

Fig. 12. Scree plot of obtained PCs produced based on % eigenvalue.

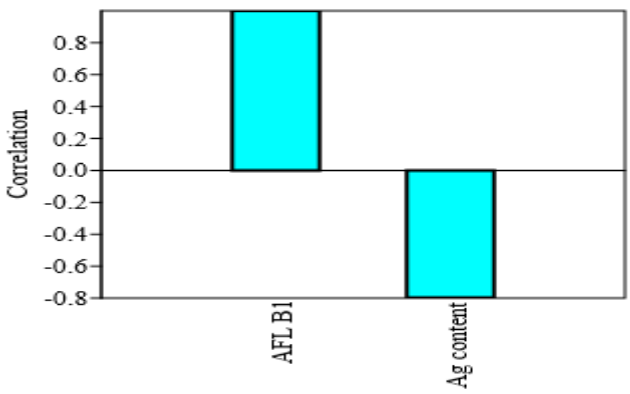

Fig. 13. Correlation analysis within biochemical attributes in PC1.

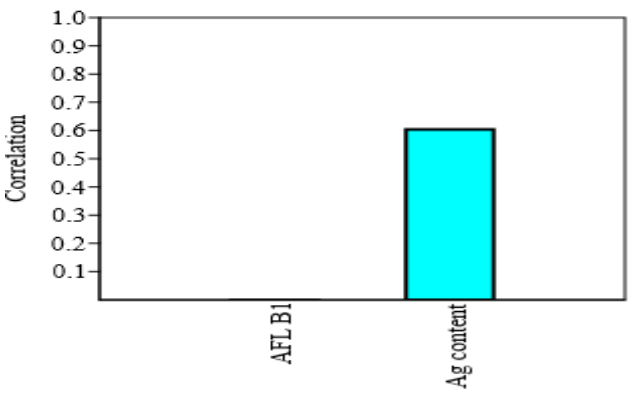

Fig. 14. Correlation analysis within biochemical attributes in PC2.

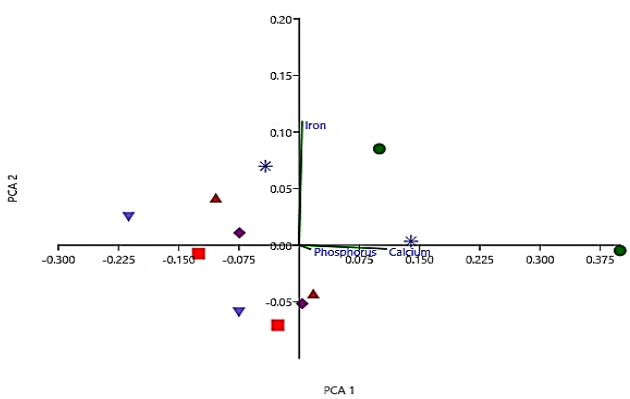

Fig. 15. Principal Component Analysis (PCA) of Calcium, Iron and Phosphorus content.

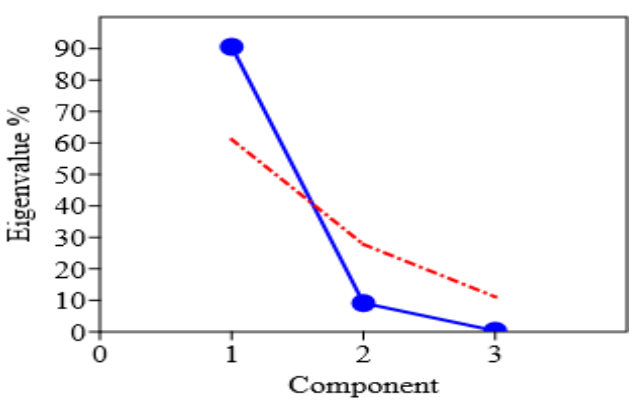

Fig. 16. Scree plot of obtained PCs produced based on % eigenvalue.

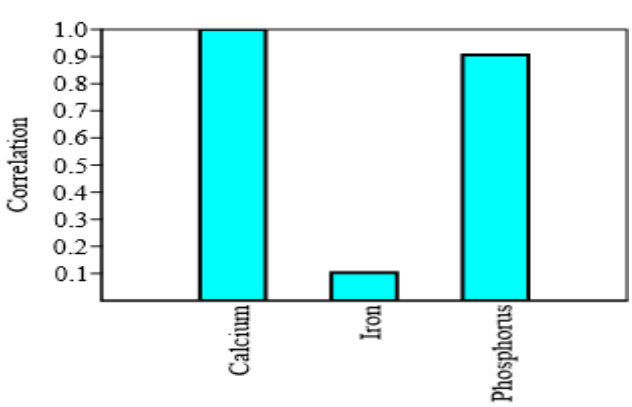

Fig. 17. Correlation analysis within grain quality attributes in PC1.

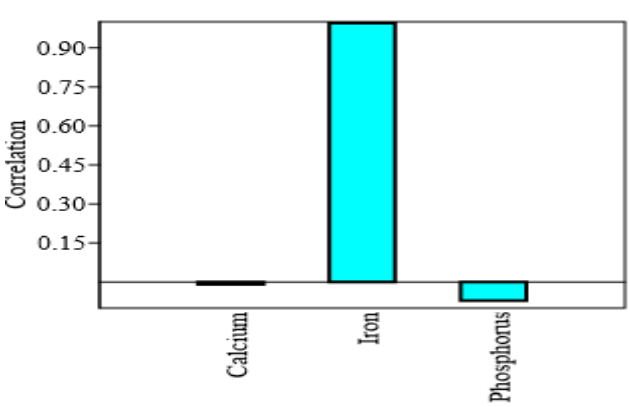

Fig. 18. Correlation analysis within grain quality attributes in PC2.

Correlations between aflatoxin B1 and silver content were obtained to look into their relationship with each other in the form of positive or negative loadings in one component (PC1). Aflatoxin B1 has a maximum positive loadings correlation, while Ag content shows a maximum negative loadings correlation (Fig. 13). Overall insignificant correlation was observed between aflatoxin B1 and Silver content in PC1.In PC2, Ag content indicates a highly positive loadings correlation (Fig. 14), but no loadings (either positive or negative) were noticed for AFL B1 concerning correlation.

Grain quality parameters: Calcium, iron, phosphorus, and silver contents of rice were assessed under the application of biosynthesized AgNPs and biotic stress. The present study revealed significant calcium (0.6%), iron (0.21%), and phosphorus (0.06) contents in the rice grains of plants treated with 50 mg/kg AgNPs in response to biotic stress. This response can be attributed to the antifungal action of AgNPs and growth promoting capability of AgNPs (Alavi, & Dehpour, 2009; Rathnayake *et al*., 2012; Salama, 2012; Zahra *et al*., 2015; Ejaz *et al*., 2018).

The Principal Component Analysis (PCA) for Ca, Fe, and P indicate significant outcomes in terms of two components (PC1 & PC2). The analysis stated more explanatory results in PCA 1 and PCA 2 for each parameter separately (Fig. 15). The scree plot (Fig. 16) about % eigenvalues of obtained PCs illustrates high variations between PC1 and PC2 whereas low variation was noted between PC 2 and PC 3. Correlations between calcium, iron, and phosphorus content were obtained to know their relationships with one another in the form of positive loadings or negative loadings in one component PC1, which indicated that Ca and P had maximum positive loadings correlation. At the same time, Fe showed minimum positive loadings (Fig. 17). Likewise, in PC2, Fe indicates maximum positive loadings correlation, while Ca and P show negative loadings (Fig. 18).

Conclusion

The biochemical characteristics of rice plants were negatively impacted by biotic stress, which increased the degree of oxidative stress; however, the application of biosynthesized AgNPs protected rice plants from *Aspergillus flavus.* As a result, biosynthesized AgNPs tend to change rice's biochemical and mineral profiles and develop resistance to *Aspergillus flavus*. In reaction to biosynthesized AgNPs, the rice grain quality parameters considerably improved. Thus, it can be said that rice plants produce grains of higher quality under biotic stress thanks to biosynthesized AgNPs. These results serve as the foundation for more in-depth research on the ecotoxicity of NPs and molecular changes brought on by biotic stress in the future.

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References

Aebi, H. 1984. Catalase *In vitro*. *Methods Enzymol*., 105: 121-126.

Ahmad, P., C.A. Jaleel, M.A. Salem, G. Nabi and S. Sharma. 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit. Rev. Biotechnol*., 30: 161-175.

Alavi, S.V. and A.A. Dehpour. 2009. Evaluation of the nanosilver colloidal solution in comparison with the registered fungicide to control greenhouse cucumber downy mildew disease in the North of Iran. *VI Int. Postharvest Symp*., 877: 1643-1646.

Aly, A.A., M.T. Mansour, H.I. Mohamed and K.A. Abd-Elsalam. 2012. Examination of correlations between several biochemical components and powdery mildew resistance of flax cultivars. *Plant Pathol. J*., 28: 149-155.

Anonymous. 20227. Association of Official Analytical Chemists (AOAC) Officials Methods of Analysis 17th ed. Revision 2 AOAC International: Gaithersburg MD USA. 2007.

Araujo, R.G., F.D.S. Dias, S.M. Macedo, W.N. dos Santos and S.L. Ferreira. 2007. Method development for the determination of manganese in wheat flour by slurry sampling flame atomic absorption spectrometry. *Food Chem*., 101: 397-400.

Arokiyaraj, S., M.V. Arasu, S. Vincent, N.U. Prakash, S.H. Choi, Y.K. Oh and K.H. Kim. 2014. Rapid green synthesis of silver nanoparticles from Chrysanthemum indicum L and its antibacterial and cytotoxic effects: an *In vitro* study. *Int. J. Nanomed*., 9: 379.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.

Berthiller, F., C. Crews, C. Dall'Asta, S.D. Saeger, G. Haesaert, P. Karlovsky and J. Stroka. 2013. Masked mycotoxins: A review. *Mol. Nutr. Food Res*., 57: 165-186.

Bhat, R.V. 1999. Vasanthi. S. Mycotoxin contamination of foods and feeds-an overview. Third Joint FAO/WHO/UNEP Intl.

Chen, C.T., L.M. Chen, C.C. Lin and C.H. Kao. 2001. Regulation of proline accumulation in detached rice leaves exposed to excess copper. *Plant Sci*., 160: 283-290.

Dar, M.I., M.I. Naikoo, F. Rehman, F. Naushin and F.A. Khan. 2016. Proline accumulation in plants: roles in stress tolerance and plant development. In Osmolytes and plants acclimation to changing environment: emerging omics technologies (pp. 155-166). Springer, New Delhi.

Dibrov, P., J. Dzioba, K.K. Gosink and C.C. Häse. 2002. Chemiosmotic mechanism of antimicrobial activity of Ag+ in Vibrio cholerae. *Antimicrob. Agents Chemother*., 46: 2668-2670.

Ditta, A. 2012. How helpful is nanotechnology in agricultur? *Adv. Nat. Sci: Nanosci. Nanotechnol*., 3: 3-5.

Ejaz, M., N.I. Raja, M.S. Ahmad, M. Hussain and M. Iqbal. 2018. Effect of silver nanoparticles and silver nitrate on growth of rice under biotic stress. *IET Nanobiotechnol*., 12: 927-932.

Fabro, G., I. Kovács, V. Pavet, L. Szabados and M.E. Alvarez. 2004. Proline accumulation and AtP5CS2 gene activation are induced by plant-pathogen incompatible interactions in *Arabidopsis*. *Mol. Plant Microb. Interact*., 17: 343-350.

Fazal, H., B.H. Abbasi, N. Ahmad and M. Ali. 2016. Elicitation of medicinally important antioxidant secondary metabolites with silver and gold nanoparticles in callus cultures of *Prunella vulgaris* L. *Appl. Biochem. Biotechnol*., 180: 1076-1092.

Ferrer-Galindo, L., A.D. Sañu-Ginarte, N. Fleitas-Salazar, L.A. Ferrer-Moreno, R.A. Rosas, M. Pedroza-Montero and R. Riera. 2018. Denoising and principal component analysis of amplified raman spectra from red blood cells with added silver nanoparticles. *J. Nanomater.*, Article ID 9417819, https://doi.org/10.1155/2018/9417819

Gogoi, N., P.J. Babu, C. Mahanta and U. Bora. 2015. Green synthesis and characterization of silver nanoparticles using alcoholic flower extract of *Nyctanthes arbortristis* and *In vitro* investigation of their antibacterial and cytotoxic activities. *Mat. Sci. Eng.*, 46: 463-469.

Gupta, S.D. and S. Datta. 2003. Antioxidant enzyme activities during *In vitro* morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. *Biol. Plant.*, 47: 179-183.

Hameed, A. and N. Iqbal. 2014. Chemo-priming with mannose, mannitol and H 2 O 2 mitigate drought stress in wheat. *Cereal Res. Comm.*, 42: 450-462.

Handa, S., R.A. Bressan, A.K. Handa, N.C. Carpita and P.M. Hasegawa. 1983. Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiol.*, 73: 834-843.

Hashmi, M.I. and J.S. Tianlin. 2016. Minerals contents of some indigenous rice varieties of Sabah Malaysia. *Int. J. Agric. For. Plant.*, 2: 31-34.

Hussain, M., N.I. Raja, M. Iqbal and S. Aslam. 2019. Applications of plant flavonoids in the green synthesis of colloidal silver nanoparticles and impacts on human health. *Iran J. Sci. Technol. Trans. Sci.*, 43: 1381-1392.

Hussain, M., N.I. Raja, M. Iqbal, M. Ejaz and F. Yasmeen. 2017. *In vitro* germination and biochemical profiling of Citrus reticulata in response to green synthesized zinc and copper nanoparticles. *IET Nanobiotechnol.*, 11: 790-796.

Hussain, M., N.I. Raja, Z.U.R. Mashwani, M. Iqbal, S.K. Chaudhari, M. Ejaz and F. Yasmeen. 2018. Green Synthesis and Characterization of silver nanoparticles and their effects on disease incidence against canker and biochemical profile in *Citrus reticulata* L. *Nanosci. Nanotech. Lett.*, 10: 1348-1355.

Iqbal, M., S. Asif, N. Ilyas, N.I. Raja, M. Hussain, S. Shabir and A. Rauf. 2016. Effect of plant derived smoke on germination and post germination expression of wheat (*Triticum aestivum* L.). *Amer. J. Plant Sci.*, 7: 806-813.

Joo, S.S., Y.B. Kim and D.I. Lee. 2010. Antimicrobial and antioxidant properties of secondary metabolites from white rose flower. *Plant Pathol. J.*, 26: 57-62.

Juliano, B.O. 1985. Criteria and tests for rice grain qualities.

Jyoti, K., M. Baunthiyal and A. Singh. 2016. Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. *J. Radiant Res. Appl. Sci.*, 9: 217-227.

Lagrimini, L.M. 1991. Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase. *Plant Physiol.*, 96: 577-583.

Lai, X., H. Zhang, R. Liu and C. Liu. 2015. Potential for aflatoxin B1 and B2 production by *Aspergillus flavus* strains isolated from rice samples. *Saudi J. Biol. Sci.*, 22: 176-180.

Lamsal, K., S.W. Kim, J.H. Jung, Y.S. Kim, K.S. Kim and Y.S. Lee. 2011. Application of silver nanoparticles for the control of Colletotrichum species *In vitro* and pepper anthracnose disease in field. *Mycobiol.*, 39: 194-199.

Lee, H.Y., H.K. Park, Y.M. Lee, K. Kim and S.B. Park. 2007. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. *Chem. Commun.*, 28: 2959-2961.

Mie, R., M.W. Samsudin, L.B. Din, A. Ahmad, N. Ibrahim and S.N.A. Adnan. 2014. Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum*. *Int. J. Nanomed.*, 9: 121.

Mousavi, S.A.A. and S. Pourtalebi. 2015. Inhibitory effects of silver nanoparticles on growth and aflatoxin B1 production by *Aspergillus Parasiticus*. *Iran. J. Med. Sci.*, 40: 501.

Naseer, R., B. Sultana, M.Z. Khan, D. Naseer and P. Nigam. 2014. Utilization of waste fruit-peels to inhibit aflatoxins synthesis by *Aspergillus flavus*: a biotreatment of rice for safer storage. *Biores. Technol.*, 172: 423-428.

Nayyar, H. and D. Gupta. 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: association with oxidative stress and antioxidants. *Environ. Exp. Bot.*, 58: 106-113.

Pal, S., Y.K. Tak and J.M. Song. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol.*, 73: 1712-1720.

Prabhu, S. and E.K. Poulose. 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int. Nano Lett.*, 2: 32.

Prasad, R., R. Pandey and I. Barman. 2016. Engineering tailored nanoparticles with microbes: quo vadis? *Nanomed. Nanobiotechnol.*, 8: 316-330.

Prasad, R., V. Kumar and K.S. Prasad. 2014. Nanotechnology in sustainable agriculture: present concerns and future aspects. *Afr. J. Biotechnol.*, 13: 705-713.

Probst, C. and P.J. Cotty. 2012. Relationships between *In vivo* and *In vitro* aflatoxin production: reliable prediction of fungal ability to contaminate maize with aflatoxins. *Fungal Biol.*, 116: 503-510.

Ramesh, J., G. Sarathchandra and V. Sureshkumar. 2013. Survey of market samples of food grains and grain flour for Aflatoxin B1 contamination. *Int. J. Curr. Microbiol. App. Sci.*, 2: 184-188.

Rathnayake, W.G.I.U., H. Ismail, A. Baharin, A.G.N.D. Darsanasiri and S. Rajapakse. 2012. Synthesis and characterization of nano silver based natural rubber latex foam for imparting antibacterial and anti-fungal properties. *Polym. Testing*, 31: 586-592.

Sathishkumar, G., C. Gobinath, K. Karpagam, V. Hemamalini, K. Premkumar and S. Sivaramakrishnan. 2012. Phyto-synthesis of silver nanoscale particles using *Morinda citrifolia* L. and its inhibitory activity against human pathogens. *Colloids and Surf. B: Biointerfaces*, 95: 235-240.

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.*, doi:10.1155/2012/217037.

Song, H.Y., K.K. Ko, L.H. Oh and B.T. Lee. 2006. Fabrication of silver nanoparticles and their antimicrobial mechanisms. *Eur. Cells Mater*, 11: 58.

Suárez-Bonnet, E., M. Carvajal, I. Méndez-Ramírez, P. Castillo-Urueta, J. Cortés-Eslava, S. Gómez-Arroyo and J.M. Melero-Vara. 2013. Aflatoxin (B1, B2, G1, and G2) contamination in rice of Mexico and Spain, from local sources or imported. *J. Food Sci.*, 78: 1822-1829.

Tarafdar, J.C., S. Sharma and R. Raliya. 2013. Nanotechnology: Interdisciplinary science of applications. *Afr. J. Biotechnol.*, 12: 219-226.

Tipton, I.H., P.L. Stewart and P.G. Martin. 1966. Trace elements in diets and excreta. *Health Phys.*, 12: 1683-1690.

Ullah, N., I.U. Haq, N. Safdar and B. Mirza. 2015. Physiological and biochemical mechanisms of allelopathy mediated by

the allelochemical extracts of *Phytolacca latbenia* (Moq.) H. Walter. *Toxicol. Ind. Health*., 31: 931-937.

Valko, M., D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur and J. Telser. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol*., 39: 44-84.

Varjovi, M.B., M. Valizadeh and A. Bandehagh. 2015. Primary antioxidant enzymes and their important role in oxidative stress in plants and mammalian. *Biol. Forum-Int. J*., 7: 148-154.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci*., 151: 59-66.

Verbrugge, N. and C. Hermans. 2008 Proline accumulation in plants: a review. *Amino Acids*, 35: 753-75

Zahra, Z., M. Arshad, R. Rafique, A. Mahmood, A. Habib, I.A. Qazi and S.A. Khan. 2015. Metallic nanoparticle (TiO2 and Fe3O4) application modifies rhizosphere phosphorus availability and uptake by *Lactuca sativa*. *J. Agric. Food Chem*., 63: 6876-6882.

Zhao, J., L. Wang, D. Xu and Z. Lu. 2017. Involvement of ROS in nanosilver-caused suppression of aflatoxin production from *Aspergillus flavus*. *RSC. Adv*., 7: 23021-23026.

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