Research Article

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Protective role of foliar application of green-synthesized silver nanoparticles against wheat stripe rust disease caused by *Puccinia striiformis*

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Abstract: Green-synthesized nanoparticles have a tremendous antimicrobial potential to be used as an alternative to hazardous fungicides. In this study, the green synthesis of silver nanoparticles (AgNPs) was performed by using *Moringa oleifera* leaf extract as a reducing and stabilizing agent. The synthesized AgNPs were subjected to different characterization techniques. UV-visible spectroscopy confirmed the surface plasmon resonance band in the range of 400–450 nm, and zeta analysis revealed that the synthesized AgNPs ranged 4–30 nm in size. Scanning electron microscopy depicted tiny fused rectangular segments and the crystalline nature of the synthesized AgNPs was confirmed using X-ray diffraction. Energy dispersive X-ray (EDX) detector confirmed the presence of metallic silver ions. Fourier-transform infrared analysis revealed the presence of phenols as main reducing agents in the plant extract. Foliar application of different concentrations (25, 50, 75, and 100 ppm) of AgNPs was applied on wheat plants inoculated with *Puccinia striiformis* to assess the disease incidence against stripe rust disease. AgNPs at a conc. of 75 ppm were found to be more effective against wheat stripe rust disease. Furthermore, the application of AgNPs enhanced morpho-physiological attributes and reduced nonenzymatic compounds and antioxidant enzymes in wheat. The present study highlights the potential role of the green-synthesized AgNPs as a biological control of yellow rust disease.

Keywords: silver nanoparticles, green synthesis, wheat, stripe rust

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AgNPs</td>
<td>silver nanoparticles</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>MG/L</td>
<td>part per million</td>
</tr>
<tr>
<td>TPC</td>
<td>total phenolic content</td>
</tr>
<tr>
<td>TFC</td>
<td>total flavonoid content</td>
</tr>
<tr>
<td>conc.</td>
<td>concentration</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>POD</td>
<td>peroxidase</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
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1 Introduction

Wheat is one of the most dominant and vital cereal crops. It is regarded as a staple food after rice because of its consumption by more than 40% of overall population of world [1]. The good nutritional profile of wheat enables it to provide more than 21% of proteins as well as carbohydrates to the entire human population [2,3]. Being foremost important crop of Pakistan, it accounts for 2.2% of the country’s gross domestic product (GDP) and adds 10% value to agriculture, but still the country is facing severe decline in meeting international average of wheat yield [4,5].
Wheat diseases are responsible for 12.5% of yield loss annually all over the globe, and among all these pathogens, the fungal pathogens are chief disease-causing mediators in wheat [5,6]. In Pakistan, major fungal diseases, such as rusts, smuts, and blights, are resulting in a huge decline in the wheat production in Pakistan [7]. Yellow or stripe rust triggered by a fungus _Puccinia striiformis_ is the chief biotic stress and destructive pathogen of wheat. It is the core cause for the decline in the crop production from a decrease between 20% and 50%, depending on the season, area, and the stage of wheat growth [8]. The disease has accounted to be the reason for huge economic losses within various regions of America, Europe, and Australia [9,10]. Yellow rust appears as a cluster of deep orange–yellowish urediniospores, which explode from the ripened pustules present on leaves and spikes of an infected plant [11–14]. About 71% of the total 9.1 million hectares of wheat growing area in Pakistan is targeted by this disease [15], being accountable for approximately 13 epidemics during various time periods, ultimately causing huge financial and economic losses in the country [5]. The most well-known methods to manage fungal diseases are utilization of chemical fertilizers and production of resistant wheat cultivars. Nevertheless, the fungicides proved to be effective against destructive fungal pathogens, but they cause collateral damage and are toxic to human health. The plant pathogens, particularly _P. striiformis_, vigorously generate new races that lead to epidemics in varieties that were regarded as pathogen or disease resistant [16]. The utilization of some modern technology in agriculture can be exploited to design new methods to lessen the use of destructive agro-chemicals and aid in enhancing yield. Nanotechnology deals with the fabrication of material with the size dimension of 1–100 nm. It has also been proved to enormously helping out the agricultural field and its narrated issues, resulting in an immense improvement in comparison to the conventionally used ways [17–20].

The disproportion in synthesis and quenching system in plants may result in the generation of reactive reactive oxygen species (ROS) [21]. Excessive ROS accumulation results in the loss of vital physiological functions and ultimately causes cell death [22,23]. Plants, being sessile organisms, hold a broad spectrum of defense mechanism to cope with the oxidative stress by accumulating several enzymatic and nonenzymatic antioxidant compounds [24,25]. Metallic nanoparticles (NPs) especially silver nanoparticles (AgNPs) have been reported to activate the antioxidant defense system of the plants. Only very few studies have been reported so far regarding the antifungal and antioxidant roles of AgNPs in the disease management as well as their impacts on morpho-physiological and biochemical attributes in response to the fungal disease stress in wheat. This study aims to estimate the antifungal and antioxidant effects of the green-synthesized AgNPs in response to _P. striiformis_ stress, morphological, physico-chemical profiling, and antioxidant enzymes to evaluate the resistance produced in wheat.

## 2 Materials and methods

### 2.1 Leaf extract preparation for the fabrication of AgNPs

The preparation of _Moringa oleifera_ leaf extract (MLE) was carried out to fabricate the AgNPs. Lush green leaves of _M. oleifera_ were collected, and then to remove all dust contaminants, they were washed with tap water and further soaked in distilled water for about 1 h. The properly cleaned leaves were air dried and ground to get a fine powder. About 25 g of leaf powder was homogenized in 100 mL of Millipore water. This mixture was stirred on magnetic stirrer for 45 min. The resultant solution was properly filtered and utilized as MLE.

### 2.2 Green synthesis of AgNPs

One molar prepared solution of AgNO₃ was boiled, and then its gradual reduction was done by adding about 25 mL of freshly prepared _Moringa_ leaf extract under continuous stirring. The change in color of the solution to dark brown revealed the synthesis of AgNPs. This obtained solution was subjected to 13,000 rpm centrifugation for 15 min. The collected pellet was dried in speedVac concentrator (THERMO FISHER) and utilized for characterization through different techniques as well as for the evaluation of disease resistance against stripe rust. The dried powdered AgNPs (10 g) were further dissolved in 1 L of deionized water to form the stock solution and various concentrations were made, that is, 25, 50, 75, and 100 ppm by dilution using equation \( C_fV_1 = C_iV_2 \), where \( C_i \) was the initial concentration, \( V_1 \) was the initial volume, \( C_f \) was the final concentration, and \( V_2 \) was the final volume.

### 2.3 Characterization of AgNPs

#### 2.3.1 UV-visible (UV-Vis) spectroscopy

The green synthesis of AgNPs was further confirmed initially by using UV-Vis spectroscopy at Islamic International
University, Islamabad, Pakistan. The dilution of the green-synthesized AgNPs was done with sterilized water and subjected to ultrasonication for about 10 min. They were further analyzed by observing the spectrum of UV-Vis in the range of 210–910 nm.

2.3.2 Determination of zeta potential and zeta size

To evaluate the range of size of AgNPs, zeta analysis was conducted using a zeta analyzer at the National Institute of Biotechnology and Genetics Engineering, Faisalabad. The zeta analysis involves the dynamic light scattering to estimate the size and potential of NPs. The prepared AgNPs were ultrasonicated for 20 min and further subjected to size determination by zeta potential.

2.3.3 X-ray diffraction (XRD) spectroscopy analysis

To find out whether the nature of synthesized AgNPs was crystalline or amorphous, XRD spectroscopy was used. The technique was used in the facility of National Centre for Physics (NCP), Islamabad. The fine powdered sample of NPs was placed on a Shimadzu XRD-6000 (Shimadzu), and the range was set in 5–50° at an angle of 2θ. The Debye–Scherrer equation, \( D = \frac{k\lambda}{\beta \cos \theta} \), was applied to calculate the average size of the prepared AgNPs. In this equation, \( k \) designates shape factor, \( \lambda \) symbolizes X-ray wavelength, and \( \beta \) specifies full width in radius at its maximum half, whereas \( \theta \) shows Bragg’s angle.

2.3.4 EDX spectroscopy analysis

The elemental composition of the green-synthesized AgNPs was done using EDX. The analysis was carried out at NCP, Islamabad. Elemental analysis of AgNPs was determined by putting the fabricated NPs on carbon-coated thin films.

2.3.5 Scanning electron microscopy (SEM)

SEM technique was utilized to study the structures of the green-synthesized AgNPs. The SIGMA model operated at 5 kV with a magnification set at \( \times 10,000 \) was used for analysis. The facility was used at NCP, Islamabad. To obtain the SEM image, the powdered AgNPs were dissolved in distilled water to get fine suspension. A drop of resultant suspension was taken and placed on the carbon-coated grid thin film and it is further allowed to dry under mercury lamp for almost 10 min. Excessive solution was removed, and the image was captured from the surface at various magnifications.

2.3.6 Fourier-transform infrared (FTIR) spectroscopy

The FTIR analysis of AgNPs was conducted to locate the basic functional groups in the plant extract that are playing a role in the green synthesis of NPs. The powdered AgNPs were pelleted with the addition of potassium bromide. The Perkin Elmer FTIR spectrum was used to obtain spectrum with the wave number ranging 400–4,000 cm\(^{-1}\).

2.4 Inoculum preparation of \( P. striiformis \)

For the preparation of stripe rust inoculums, the urediniospores of \( P. striiformis \) strain 572432 were obtained from Crop Disease Research Institute, National Agriculture Research Council (NARC), Islamabad. The urediniospores were suspended in an isoparafin oil solution to prevent desiccation. This suspension was used as the inoculum of \( P. striiformis \). To determine the concentration of urediniospores in the inoculum, about 25 µL of inoculum was loaded on each side of hemocytometer, and spores were counted in each grid. Every spore seen on each total grid was taken as equal to 100 spores per mL. The concentration was adjusted to 600 spores per mL.

2.5 Glasshouse experiment

To determine the antioxidant and antifungal effects of the green-synthesized AgNPs in wheat against \( P. striiformis \), a glasshouse experiment was conducted. A number of earthen pots having capacity of almost 10 kg were filled with properly sterilized soil. The texture of soil was sandy loam with the composition by weight silt (20%), clay (40%), and sand (40%). Seeds of fungus prone wheat variety galaxy-13 were acquired from NARC, Islamabad. The proper surface sterilization of wheat seeds was carried with 0.1% of mercuric chloride. Sowing was done on 21 October 2016. Not more than five seedlings were kept maintaining in each pot. The completely randomized design was used to conduct experiment with each treatment having replicates. Initially, the experiments were conducted with low concentrations of AgNPs, and then on the basis of that, the referenced concentrations were chosen for the exogenous application to evaluate their
possible effects to restrict the rust fungus growth in comparison to control plants in the present glasshouse experiment. The detailed treatment layout is mentioned in Table 1.

### 2.6 Inoculation of wheat plants with pathogen *P. striiformis*

Uredioniospore suspension of *P. striiformis* was directly sprayed with the aid of an atomizer on wheat plant leaves at the flag leaf stage. The volume of the sprayed suspension was 60 mL for each plant. Autoclaved water was sprayed on wheat plant after inoculation, and polythene transparent bags were used to cover the plants to maintain the 95–100% humidity at a temperature of about 15–18°C. The covered plants were further covered with plastic layers for preventing the spread of spores. The AgNPs of various concentrations (25, 50, 75, and 100 mg L\(^{-1}\)) were sprayed to plants before and after inoculation. The first data were recorded after 1 week of disease inoculation, and then the data were recorded after every week.

### 2.7 Sample collection to evaluate disease severity

The leaf tissues were harvested randomly from the replicates for the study. The symptoms revealing the severity of stripe rust was assessed by the use of rating scale on visual basis (Tables 2 and 3). The disease severity was recorded using a standard scale of 0–5, recommended by Iqbal et al. [26], for yellow rust.

### 2.8 Evaluation of disease incidence

The disease incidence was estimated by using the following formula suggested in literature [27] (Eq. 1):

\[
\text{Disease incidence (\%) } = \frac{\text{Number of rust infected plants}}{\text{Sum of all plants}} \times 100
\]  

The disease severity in percent also known as disease index was calculated with the use of the following formula [27] (Eqs. 2 and 3):

\[
\text{Percent disease index (PDI)} = \frac{\text{Disease index}}{\text{Total infected plants}} \times 100
\]  

\[
\text{Disease index } = \text{(Stripes in scale one)} + \text{(Stripes in scale two)} + \ldots \text{(Stripes in the scale five)}.
\]

### 2.9 Growth attributes

#### 2.9.1 Estimation of surface area of leaf

The healthy flag leaves were collected from all plants to account the leaf area with the aid of CI-202, CID area meter for leaf.

#### 2.9.2 Measurement of surface area of roots

The roots were detached from the collected plants of every treatment, and the surface area of that detached roots was recorded with the use of area meter.

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**Table 1:** Treatment layout for the evaluation of disease severity index as well as foliar application of AgNPs in experiment

<table>
<thead>
<tr>
<th>T0</th>
<th>Control (healthy wheat plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Only pathogen (<em>P. striiformis</em>)</td>
</tr>
<tr>
<td>T2</td>
<td>25 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
<tr>
<td>T3</td>
<td>50 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
<tr>
<td>T4</td>
<td>75 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
<tr>
<td>T5</td>
<td>100 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
</tbody>
</table>

**Table 2:** Treatment layout for evaluation of disease severity and AgNPs concentrations

<table>
<thead>
<tr>
<th>T0</th>
<th>Control (healthy wheat plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Only pathogen (<em>P. striiformis</em>)</td>
</tr>
<tr>
<td>T2</td>
<td>25 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
<tr>
<td>T3</td>
<td>50 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
<tr>
<td>T4</td>
<td>75 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
<tr>
<td>T5</td>
<td>100 mg L(^{-1}) of AgNPs + pathogen</td>
</tr>
</tbody>
</table>

**Table 3:** Rating scale for yellow rust disease

<table>
<thead>
<tr>
<th>0</th>
<th>No symptoms</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1–5% stripes on the leaves</td>
<td>Moderately resistant</td>
</tr>
<tr>
<td>2</td>
<td>6–20% stripes on the leaves</td>
<td>Moderately resistant</td>
</tr>
<tr>
<td>3</td>
<td>21–40% stripes on the leaves</td>
<td>Moderately susceptible</td>
</tr>
<tr>
<td>4</td>
<td>41–60% stripes on the leaves</td>
<td>Moderately susceptible</td>
</tr>
<tr>
<td>5</td>
<td>&gt;61% stripes on the leaves</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>
2.9.3 Measurement of length of the shoot

The entire plants were uprooted after taking samples for the disease index. Shoots along with roots of the plants for each treatment were separated. All plants were washed thoroughly. The length of the shoot for each treatment was measured in centimeters (cm).

2.9.4 Quantification of plant fresh and dry weight

The fresh plants of each treatment were collected and weighed with roots to calculate fresh weight and then were dried in an oven at 65°C for 7 days for dry weight estimation.

2.10 Physiological parameters

2.10.1 Estimation of membrane stability index (MSI)

The MSI of leaves was estimated by following the method proposed by Sairam [28], with little modification. The leaves were cut into tiny discs of about 100 mg then properly washed and heated at 41°C for about 35 min in a water bath filled with 15 mL of double-distilled water. Electrical conductivity (EC) of these discs was measured using an EC meter. The same discs were boiled once again at 110°C for about 15 min, and the EC was again estimated. The MSI computed as follows (Eq. 4):

\[
MSI = (1 - \frac{C_1}{C_2}) \times 100.
\]  

2.10.2 Estimation of chlorophyll contents

Estimation of chlorophyll a, b, and total chlorophyll content was done using a methodology suggested in an earlier study [29]. For this purpose, fresh leaves were harvested from the plants of each and every treatment. The collected leaf tissues were grounded in mortar with 15 mL acetone solution. After grinding, this solution was filtered thrice and taken in labeled test tubes for each treatment separately. The absorbance for all these samples was checked at 647, 660, and 675 nm respectively.

\[
\text{Chlorophyll a (µg mL}^{-1}) = 12.7(A_{663}) - 2.7(A_{645})
\]  

\[
\text{Chlorophyll b (µg mL}^{-1}) = 22.9(D_{665}) - 4.7(D_{645})
\]  

Total chlorophyll content (µg mL\(^{-1}\))
\[
= (D_{645} \times 1,000/34.5).
\]  

2.11 Biochemical attributes

2.11.1 Estimation of soluble sugar

The methodology presented in Dubois et al. [30] was used to estimate the soluble sugar content of each sample with a minor modification. In accordance with that protocol, about 0.6 g of fresh leaves of each treatment was taken and placed in separate test tubes and 12 mL of 80% ethanol was added. The leaves were heated in a water bath for about 1 h, and after heating, 18% phenol was added in the attained mixture. This mixture was kept intact for 60 min at the room temperature. After 1 h, 2.5 mL of H\(_2\)SO\(_4\) was added to that resulted mixtures and vigorously stirred the absorbance was recorded at 490 nm:

\[
\text{Sugar (µg mL}^{-1}) = \frac{\text{Absorbance of sample} \times \text{Dilution factor} \times K \text{ value}}{\text{Fresh tissue weight in grams}}.
\]  

2.11.2 Determination of total proteins

To estimate the sum of leaf protein, the methodology was followed with certain modifications [31], and the bovine serum albumin was used as a standard. For that purpose, about 10 mg of fresh disc of leaf was ground well in mortar with the addition of 0.9 mL of phosphate buffer with a pH of 7.1. The centrifugation of the homogenized solution was done for 20 min at 25,000 rpm at room temperature. Further, the collected supernatant was added in the test tubes and with the addition of distilled water, the final volume was made to 1 mL. About 1 mL of alkaline copper sulfate and 0.2 mL of Folin’s reagent was added to the mixture and shaken. The test tubes were further incubated for 30 min. The absorbance of each sample was checked against 0.1 mL of 0.5 M NaOH as blank. The concentration of total soluble proteins was estimated using bovine serum albumin (BSA) curve as a reference.

2.11.3 Total phenol content

The leaf extract for phenolic content was prepared by using the method in literature with a few alterations [32]. About 0.8 g of fresh leaf of each treatment was obtained. The leaves were homogenized in about 12 mL of methanol and placed overnight. To get the stock solution, the resultant mixture was thoroughly filtered and diluted with the addition of 90 mL of water. The resultant extract was used to assess the phenolic content. About 200 µL of the stock solution
was added in a test tube, and 1.2 mL of distilled water and 0.1 mL of 50% Folin–Ciocalteu phenol reagent were added. The mixture was allowed to settle for 3 min, and then 20% (w/v) of sodium carbonate 0.4% was added. This solution was placed undisturbed for about 3 h and gently shaken. The absorbance of each sample was recorded at 766 nm. As a standard, the gallic acid was used to check the total phenolic content (TPC) of the samples.

### 2.12.4 Flavonoid content estimation

The total flavonoid content (TFC) was calculated using the methodology suggested in an earlier study [33] with some alterations. About half gram of leaves from each treatment was cut into tiny discs. The leaf pieces were placed in 80% methanol, and a homogenized mixture was made with the volume of about 8 mL. Centrifugation of this resultant suspension was done at 20,000×g for about 20 min. The supernatant was collected, and 0.5 mL was added into a volumetric flask. Further, about 0.6 mL of 5% sodium nitrate and 0.7 mL of 12% aluminum chloride were also added. The resulting solution was placed at rest for 10 min, and then 2 mL of 1 M sodium hydroxide and about 2.8 mL of distilled water were added and gently shaken. The absorbance was checked at 510 nm.

### 2.12 Antioxidant enzyme assays

#### 2.12.1 Preparation of leaf extract for enzyme assays

To perform the antioxidant assays, wheat leaf extract was needed to be prepared. For that purpose, fresh leaves were harvested for every treatment and extracts were made. Around 150 mg of fresh leaves were dissolved in 15 mL extraction buffer containing 10% polyvinyl pyrrolidone and about 0.2 M ethylene diamine tetra acetic acid (EDTA) and centrifuged at 12,000 rpm for 15 min at 5°C, and the supernatant was taken for further analysis of endogenic enzyme assays [34].

#### 2.12.2 Peroxidase (POD) activity

For POD assay, around 0.2 mL enzyme extract was added with 1.35 mL of 0.2 M 2-(N-morpholino) ethanesulfonic acid (MES) buffer of pH not more than 5.6, 0.05% hydrogen peroxide, and 0.2% phenylene diamine. The absorbance was observed at 485 nm for 3 min for each replicate of each treatment [35].

#### 2.12.3 Superoxide dismutase (SOD) activity

The SOD enzyme activity was measured using the methodology reported previously with some changes [36]. About 600 µL of the prepared enzyme extract was mixed with 200 µL of 0.076 mM nitro blue tetrazolium chloride (NBT), 250 µL of 135 mM methionine, 250 µL of 1.5 mM EDTA, 25 µL 0.04 mM of riboflavin, and 800 µL of phosphate buffer. This reaction mixture was tested against the blank, which contained the same compounds as in reaction mixture except for the addition of phosphate buffer instead of enzyme extract. Both reaction mixture and the blank were kept under the fluorescent light for 7 min, and the absorbance was observed at 560 nm. The Lambert–Beer law was followed to estimate the SOD enzyme activity, \(A = εL/C\). In this equation, \(ε\) is the coefficient of extinction, \(L\) represents the wall length, and \(C\) shows the concentration of enzyme.

#### 2.12.4 Catalase (CAT) activity

To determine the CAT activity, the methodology suggested by Aebi [37] was used with certain modifications. To prepare a 200 mL of reaction mixture, 500 µL prepared enzyme extract was dissolved in 250 µL of 28 mM hydrogen peroxide, 450 µL of phosphate buffer, and 950 µL of distilled water. Blank used was containing all compounds as in reaction mixture except the addition of phosphate buffer in place of the enzyme extract. Both the reaction mixture and blank were placed under the fluorescent light for 8 min, and the absorbance was recorded at 250 nm via a spectrophotometer.

### 2.13 Statistical analysis of data

The experiments were carried out in triplicates. Duncan’s multiple range test \(p < 0.05\) was used to assess the difference in treatments and control. SPSS 18.0 version software (IBM) was used to perform all calculations in the study.

### 3 Results and discussion

#### 3.1 Green synthesis and characterization results of AgNPs

The green synthesis of AgNPs was performed utilizing the MLE. The leaf extract was used to reduce AgNO₃ salt to
AgNPs. Upon mixing leaf extract to AgNO₃ solution, it turned to dark brown color. The newly synthesized AgNPs were capped and stabilized by phytochemicals present in the plant leaf extract (Figure 1). The synthesis of AgNPs was confirmed using UV-Vis spectroscopy technique. The AgNPs showed surface plasmon resonance (SPR) characterization peak in the range 400–450 nm (Figure 2).

The green-synthesized AgNPs were also characterized through zeta potential to evaluate their size range. Zeta potential revealed that the AgNPs were 4–30 nm in size range (Figure 3).

The crystalline nature of the green-synthesized AgNPs was analyzed through XRD spectroscopy. XRD pattern revealed that the AgNPs have a centered cubic structure. The average crystalline size of AgNPs was evaluated about 25.46 nm, respectively (Figure 4).

The elemental analysis of AgNPs was performed using EDX, which established the presence of AgNPs in the plant samples. The peaks of absorption for silver were in the range 2–4 keV (Figure 5). Presence of other elements in the plant sample was associated with the presence of various organic compounds present in plant leaf extract used to reduce the silver salt to NPs. SEM analysis image of biosynthesized AgNPs appeared to be rectangular to spherical in shape and fused together (Figure 6). The green-synthesized NPs of similar shapes were also reported in literature [38].
The FTIR spectroscopy analysis of the green-synthesized AgNPs revealed the absorbance peaks at various wavelengths of several functional groups of prepared AgNPs showed the existence of different functional groups that were recorded at various wavelengths. The broader peak of absorption was recorded at 3,416 cm\(^{-1}\) that depicts the significant higher amount of O–H functional groups such as phenols and alcohols. The peak at 2,920 cm\(^{-1}\) reveals N–H (ammonium ions functional groups). A less broad peak at 1,629 cm\(^{-1}\) shows the C–C acyclic group. A lower peak at 1,046 cm\(^{-1}\) represents C–I aliphatic iodo-compounds (Figure 7).

### 3.2 Estimation of disease severity

The disease incidence and the percentage disease index were assessed under P. striiformis against different conc. of biosynthesized AgNPs at different interval of days for instance on day 5, 10, 15, 20, 25, and day 30. Possible effects of the green-synthesized AgNPs on the prevalence of stripe rust disease revealed immense variation in response to number of days passed after the application of various concentrations of the green-synthesized AgNPs. First data were collected after 15 days of inoculation and then after every 15 days till the harvest. It was revealed that none of the concentration of the green-synthesized AgNPs inhibited, absolutely, the stripe rust disease infection. Nevertheless, the disease severity was greatly influenced by the application of various conc. of AgNPs. A progressive decline in the disease incidence of stripe rust was revealed by the passage of time in response to all the concentrations of the green-synthesized AgNPs till the day 25.

The significantly higher disease incidence and disease index were recorded in wheat plants under stripe rust stress infection and with no application of AgNPs.
treatment. The significantly lowest disease incidence and disease index were depicted in the plants under stripe rust stress applied with 75 mg·L⁻¹ conc. of biosynthesized AgNPs. Percentage disease index values were considerably decreased by 80% in yellow rust-infested plants, respectively, when the application of the concentration of 75 mg·L⁻¹ AgNPs was carried out. Percentage disease index was also decreased at all the concentrations of AgNPs and most significant at 75 mg·L⁻¹ AgNPs (Figure 8). It was also revealed that 75 mg·L⁻¹ conc. of the biosynthesized AgNPs caused a significant decline in the disease incidence and percentage disease index under stripe rust disease stress at various intervals of time in comparison to other conc. of green AgNPs. An earlier study supports our findings that the green-synthesized AgNPs by using Boerhavia procumbens extract revealed concentration-dependent strong antimicrobial activity against several pathogenic bacterial strains and seven fungal species. Another report also supports our results in which AgNPs act as effective and strong antimicrobial agents against numerous plant pathogens Magnaporthe grisea, Colletotrichum gloeosporioides, Pythium ultimum, Rhizoctonia solani, Alternaria alternata, Xanthomonas axonopodis, and Botrytis cinerea [39].

3.3 Morphological attributes of plant against P. striiformis stress in response to AgNPs

The data of morphological parameters revealed noticeable decline in the shoot length in wheat plants with P. striiformis stress. The impacts of various conc. of AgNPs on morphological parameters such as shoot and root length as well as leaf surface showed varied results depending on the concentration of AgNPs as well as time interval (days passed after inoculation). The data of morphological attributes depicted that among all concentrations of AgNPs, the 75 ppm conc. of AgNPs showed significantly a positive impact on the morphological attributes of wheat under stripe stress. The highest value of the shoot length (51 cm), leaf surface area (16.8 cm²), and root surface area (28.4 cm²) was recorded when the stripe rust infested wheat plants were sprayed with 75 ppm conc. of biosynthesized AgNPs at the end of the experiment. The highest conc. of 100 ppm AgNPs reduced the shoot length, leaf surface area, and root surface area. Fresh and dry weight of plants were also considerably declined in plants infested with stripe rust without NPs. Although, the application of AgNPs enriched both fresh weight as well as dry weight of plants. Their maximum

Figure 6: SEM of AgNPs showing morphology of AgNPs.

Figure 7: FTIR spectroscopy analysis of AgNPs revealing absorption peaks of phytochemicals in plant extract.

Figure 8: Disease incidence and percent disease index of wheat plants inoculated with P. striiformis and treated with green-synthesized AgNPs.
values were recorded at 75 ppm AgNPs and lowest values for these such as 1.78 and 0.89 g were revealed in fungus-infected plants without NPs. Fresh and dry weight of plants were noticeably reduced in the stripe rust-infected plants. Results indicated that the application higher conc. 100 ppm of AgNPs reduced fresh and dry weight in plants as compared to other treatments due to its toxic effects at higher concentrations (Figure 9). A previous study reported similar concentration-dependent effects of AgNPs, the lower concentrations of AgNPs considerably elevated the root biomass, fresh weight, and dry weight of Fenugreek, whereas the higher concentrations of NPs had imposed a total opposite effect [40]. Another study also reported a similar dose-dependent impact of AgNPs on wheat where higher concentration of AgNPs showed inhibitory effects on growth parameters and altered protein expression [41].

3.4 Wheat physiological parameters against *P. striiformis* stress in response to biosynthesized AgNPs

The physiological attributes such as MSI and chlorophyll contents of wheat plants were assessed to evaluate the antimicrobial and antioxidant effects of biosynthesized AgNPs under stripe disease in wheat. The recorded data for physiological attributes revealed that stripe rust-stressed plants without NPs had lower MSI as compared to control and treated plants. The foliar application of AgNPs shows that among all conc., 75 mg·L⁻¹ conc. of AgNPs increased the MSI up to 30% in stripe rust-stressed plants.

The stripe rust biotic stress also caused reduction in chlorophyll a content, chlorophyll b content, and total chlorophyll content. The wheat plants that were applied with 75 mg·L⁻¹ conc. of AgNPs revealed enhancement in chlorophyll a content, chlorophyll b content as well as total chlorophyll content. The total chlorophyll content was improved from 23 µg·mL⁻¹ in stripe rust-stressed plants to 32 µg·mL⁻¹ in wheat plants applied with 75 mg·L⁻¹ biosynthesized AgNPs (Figure 10). These findings were supported by previous study in which the foliar application of AgNPs enhanced photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in fenugreek plants [42].

3.5 Nonenzymatic attributes of wheat plants against *P. striiformis* stress in response to green-synthesized AgNPs

The data revealed that a significant quantity of soluble sugars were stored in the plants under the stress of stripe

Figure 9: Morphological responses: (a) shoot length, (b) plant fresh weight, (c) plant dry weight, and (d) root surface area of wheat plants under *P. striiformis* stress in response to various concentrations of AgNPs: 25, 50, 75, and 100 mg·L⁻¹. The data is presented as the mean ± S.D from three independent biological replicates. The error term is mean square error = p ≤ 0.05.
rust (21.1 µg·mL⁻¹) as compared to control plants. The results revealed that a significant quantity of soluble sugars were stored in the plants under the stripe rust stress (21.1 µg·mL⁻¹) in comparison to control plants. Nevertheless, the exogenous foliar application of AgNPs caused a noticeable decrease in soluble sugars in stripe rust-infected plants. The foremost significant decline in the soluble sugars (15.5 µg·mL⁻¹) was observed in wheat plants applied with 75 ppm conc. of AgNPs (Figure 10). Data also showed a decline in the total protein content in plants infected with yellow rust. But the foliar application of AgNPs enriched the protein content in stressed plants. The most considerable rise in total protein content was illustrated in wheat plants that were applied with 75 ppm conc. of AgNPs. A significant higher proline concentration was recorded in wheat plants under biotic stress of stripe rust. However, the application of AgNPs reduced the proline content by combating with biotic stress with great effectiveness. Proline content was considerably higher (10.3 µg·mL⁻¹) in stripe rust-stressed plants and reduced (6.8 µg·mL⁻¹) in the plants applied with 75 ppm conc. of biosynthesized AgNPs. The phenolic content and flavonoid content were also studied in plants under stripe rust stress in response to the green-synthesized AgNPs (Figure 11). It was recorded that phenolic content were raised in plants under stripe rust stress (5.6 µg·mg⁻¹ FW). However, the application of AgNPs decreased the phenolic content, and the maximum decrease (3.7 µg·mg⁻¹ FW) was observed in plants treated with 75 ppm of AgNPs. The total flavonoid content was also increased in plants under stripe rust stress and declined significantly in plants treated with AgNPs. The most considerable decline in flavonoid content was recorded in plants treated with 75 ppm of biosynthesized AgNPs. The linear correlation and dependency were observed for total flavonoid and phenolic content. The findings of present study are supported by previous report [43], and the effect of biologically synthesized AgNPs on Bacopa monnieri observed that AgNPs showed a noteworthy effect on seed germination and persuaded the synthesis of protein and carbohydrate reduced the total phenol content as well as CAT and POD activities. Another study supports our results in which AgNPs improved plants growth profile and biochemical attributes (chlorophyll, carbohydrate, and protein content, and

![Figure 10: Physiological parameters: (a) chlorophyll b content, (b) total chlorophyll content, (c) chlorophyll a content (d), and MSI of wheat plants under P. striiformis stress in response to various concentrations of AgNPs: 25, 50, 75, and 100 mg·L⁻¹. The data is presented as the mean ± S.D from three independent biological replicates. The error term is mean square error = p ≤ 0.05.](image-url)
antioxidant enzymes) of *Brassica juncea*, corn, and common bean [44].

### 3.6 Antioxidant enzymes of wheat plants against *P. striiformis* stress in response to green-synthesized AgNPs

In present study, the chief antioxidant enzymes, including SOD, POD and CAT, were also investigated in response to various concentrations of AgNPs applied on fungus-infected wheat plants. The increased production of SOD, POD, and CAT was recorded in the wheat plants infected by fungal pathogens (Figure 12). The SOD activity (0.57 nM·min⁻¹·mg⁻¹ FW) and POD activity (0.56 nM·min⁻¹·mg⁻¹ FW) was evident in wheat plants affected by stripe rust disease stress. Both POD and SOD showed dependency and correlated in linear manner with CAT. The CAT activity was also evident (0.16 nM·min⁻¹·mg⁻¹ FW) in wheat plants infected with stripe rust disease and without the exogenous application of AgNPs. The AgNP application evidently reduced the endogenous enzyme production. The minimum activity of SOD (0.25 nM·min⁻¹·mg⁻¹ FW), POD (0.23 nM·min⁻¹·mg⁻¹ FW), and CAT (0.07 nM·min⁻¹·mg⁻¹ FW) was recorded at 75 ppm AgNPs. Hence, the most considerable decline in enzyme concentration was recorded in infected plants applied with 75 ppm conc. of biosynthesized AgNPs. These results were in line with previous report suggested that the foliar application of AgNP-enhanced plant growth in *Trigonella foenum-graecum* and improved antioxidant activity [42].
4 Conclusion

Present study revealed the easy and eco-friendly approach for the synthesis of AgNPs by using MLE that act as a reducing and capping agent. The green-synthesized AgNPs were spherical in shape with an average size range of 3–27 nm, respectively. The NPs were stabilized by phenols and other functional groups in plant extract. The estimation of disease index as well as disease incidence revealed that severity of yellow rust caused by *P. striiformis* disease reduced in response to the exogenous foliar application of AgNPs. The green-synthesized AgNPs improved the antioxidant defense system in wheat plants ultimately enhanced the disease resistance against stripe rust disease. The morphological parameters recorded in terms of leaf and root area, plant fresh and dry weight, physiological parameters such as MSI and chlorophyll contents, the nonenzymatic attributes, such as soluble sugars, total protein, proline, phenolic and flavonoid contents, major antioxidant enzymes such as SOD, POD, and CAT, were stabilized and provided resistance to wheat plants in response to 75 mg L$^{-1}$ conc. of the green-synthesized AgNPs. The findings of the present study provide foundations for the more in-depth research about the effectiveness and toxicity of the metal NPs as well as changes they make at molecular level in response to biotic stress.

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References


