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Research Article

Antimicrobial effect of green synthesized TiO_2 nanoparticles against *Cercospora canesces* growth in mung bean

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ABSTRACT

Mung bean is one of the economically important leguminous crops generally grown in arid region of Pakistan. Mung bean grows best at 30-35℃ and is a nutrient enriched crop with higher amounts of starch (50%), proteins (18-25%), fat (3%) and fiber (3-4.5%). Many biotic factors affect crop production. Yield losses due to biotic factors are up to 40-80% in mung bean. Cercospora leaf spot (CLS) is very common disease in mung bean caused by hemi-biotrophic fungal pathogen *Cercospora canescens.* For sustainable agrocontrol there is need to develop alternative solutions for conventional methods. Green synthesized nanoparticles offer promising solution to mitigate adverse effect of agrochemicals and fungicides. In this review green synthesized TiO_2 nanoparticles used to mitigate the disease severity by seed priming with 50mgL^{-1} and 150mgL^{-1} nanoparticles (NPs) suspension solution in mung bean. The result shows that 50mgL^{-1} Tio_2-NPs inhibit fungal growth by 87.85% in *in vitro* and 74.07% in *in vivo* experiment. Tio_2-NPs application improve root, shoot, leaf surface area, chlorophyll content, stomatal conductance and boost plant immunity by enhancing expression of the *Pathogenesis-related* (*PR-1)* gene in response stress. Therefore, present study indicates that green synthesized Tio_2-NPs is an effective way to enhance the growth and productivity of mung bean by inhibiting *C. canescens*.

Keywords: Mung bean, Cercospora leaf spot, hemi-biotrophic, *Cercospora canescens*, nanoparticles*, Pathogenesis-related* (*PR*-1) gene.

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INTRODUCTION

Pulses also referred as 'poor man's meat' are major source of natural dietary proteins and other essential micronutrients. Among pulses mung bean (*Vigna radiata* L.), also known as green gram or moong, is important leguminous crop. Higher amounts of carbohydrates (50%), proteins (18-25%), fat (3%) and fibers (3-4.5%) in mung bean seeds and sprouts make them anti- inflammatory, anti-diabetic and anti-obesity (Ganesan and Xu, 2018; Haider *et al*., 2020). Mung bean maintains soil health and fertility by nitrogen fixation (Kebede, 2021). Mung bean have short life cycle of about 70-75 days and mainly grown in rain-fed areas on sandy loamy soil with a pH between 6.2 and 7.2 and temperature ranges from 30–35 ℃. It is cultivated in rotation after wheat harvest in April/May (Hanumanth *et al*., 2016; Khan *et al*., 2018; Dahiya *et al.*, 2015). The major mung bean producers are South Asian countries like India, China, Myanmar and Thailand with total production of 5.3 million tons (ACIAR, 2022). In 2021-2022 the production of mung bean in Pakistan is about

0.000315 million tons (GOP, 2023). Many biotic factors like insect pests, bacteria, virus and fungi reduce yield up to 40-60% in mung bean. Mung bean is sensitive to about 26 fungal diseases in the world (Aziz-ur-Rehman *et al.*, 2019). Among these fungal pathogens, major yield lose in mung bean is due to the Cercospora leaf spot caused by *Cercospora canescens.* It is hemibiotrophic fungus. Globally, *C. canescens* causes qualitative and quantitative yield loss of up to 50-70% (Chand *et al*., 2012). In Pakistan maximum grain yield loss of 61 percent was observed (Iqbal *et al*., 1995). *C. canescens* produce semi-circular to irregular greyish-brown spots on leaves with diameter of 5-10 mm. Disease severity results in imperfect pods and seeds formation. As disease progress leaves become wrinkled or deshaped. Other parts of plant like petioles and stem are also affected (Jamadar, 1988; Poehlman, 1991). For germination of *C. canescens* warm temperature (25-30℃) and wet conditions with relative humidity 90-100% are required (Barbetti, 1985; Windels *et al*., 1998). Conidia produced by asexual reproduction are infectious agents and primary source of dispersal (Sumartini *et al*., 2017). *C. canescens* is air and soil born fungus so rain splashes also play role in dispersion of conidia (Kumar *et al*., 2011).

Chemical fungicides have been the most common method to control fungal infections but many efforts have been made to find alternatives to fungicides due to environmental concerns and their adverse effects on plants and humans health (Meena and Chattopadhyay, 2002). Nanotechnology is one of the most important technologies of modern science with particle size ranging from 0.1– 100 nm (Patel *et al.*, 2014). Various physical and chemical methods are used for the synthesis of nanoparticles but in recent years there has been lot of emphasis on the green synthesized nanoparticles due to their safe nature, environmental friendliness, easy production and low production costs. Nanoparticles synthesis by green synthesis method provide sustainable alternative approach to conventional synthetic approaches which involves the use of microbial sources such as bacteria, yeast, fungi, algae, plant extracts and hybrid materials (Naikoo *et al.*, 2021). The aim is to avoid problems related to yield, use of toxic chemicals and hazardous by-products unlike their physical and chemically synthesized counterparts (Naseer *et al.*, 2020).

During pathogenesis toxins are produced such as perylenequinone called cercosporin by the pathogen (Daub and Ehrenshaft, 2000). Recognition of toxins lead to rapid activation of defense reactions to limit pathogen growth which involves production of reactive oxygen species, release of hormones such as salicylic acid (SA) in case of hemibiotrophic fungus (Chen *et al*., 2022). Exposure to nanoparticles causing an increase in the intracellular levels of reactive oxygen species, ROS can damage fungal cells by causing oxidative stress. NPs alter plasma membrane dynamics of fungal cells resulting in loss of integrity, increased permeabilization and depolarization (Guilger-Casagrande *et al*., 2021; Bayat *et al*., 2019). Thus, it is concluded that NPs are effective way to control fungal stress.

MATERIALS AND METHODS

Titanium dioxide nanoparticles synthesis

Fresh leaves of Moringa oleifera was used for the synthesis of Titanium dioxide nanoparticles as instructed in Satti et al. (2021).

Preparation of spore suspension solution

Fungal culture of *Cercospora canescens* kindly provided by Ayub Agriculture Research Institute (AARI), Faisalabad which was refreshed and multiplied on potato dextrose agar (PDA) media in petri plates. These plates were incubated at 28± 1°C in incubator for 7 days. This fungal culture was then used to prepare spore suspension solution at concentration of $5x10^5$ spores mL^{-1} estimated by Hemacytometer. Koch's postulates were applied to confirm pathogenicity as described by Rafiq et al. (2022).

In vitro antifungal assay

The antifungal activity of TiO_2-NPs was analyzed by using the agar dilution method (Abdallah et al., 2022). TiO_2-NPs were autoclaved and mixed in sterilized PDA media at concentrations of 50 mgL-1 and 150 mgL-1 while control treatment have no nanoparticles. The fungi were inoculated in petri plates obtained from seven days old culture of *Cercospora canescens*. Antifungal activity was estimated by measuring the diameter of fungal colonies by a software of Image J.

In vivo antifungal assay

Seeds from mung bean varieties (NM- 51 and NM-2021) grown in pots for in planta characterization. The experiment was carried out under factorial CRD with three replicates.The seeds were sterilized and then soaked in TiO_2-NPs suspension solution of 50 and 150 mgL^{-1} for 4 hours at room temperature as described in Waqas Mazhar et al. (2022). Five presoaked seeds from each variety were sown in pots and thinned to 3 after 20 days.

Disease assessment

The disease incidence (%) was calculated 30 days of post-sowing as described by Awan and Shoaib (2019) and calculated using below given formula.

$$DI(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Measurement of Morphological, Physiological and Biochemical parameters

Morphological parameters of plants from each replicate such as root length (cm), shoot length (cm) and leaf surface area (cm^2) were measured at reproductive stage i.e. 60 DAS. Chlorophyll content and stomatal conductance was measured using 502-SPAD spectrum and leaf porometer respectively at flag leaf stage 30 DPS. For biochemical analysis, first trifoliate leaf samples were collected from all plants at 72-hour post inoculation (hpi) and stored at -80 °C. Superoxide dismutase (SOD), Peroxidase Dismutase (POD) and Catalase (CAT) activity were measured using spectrophotometer and absorbance was recorded as described in Maehly and Chance (1954).

Expression analysis

Total RNA was extracted from the third trifoliate leaves using Trizol reagent (Ke et al., 2021). The sequence of the PR-1 gene (Gene ID 106769349, Accession number NC_028357.1) was retrieved from NCBI (https://www.ncbi.nlm.nih.gov/gene/106769349) and primers were designed using Perl primer v1.1.21 (https://perlprimer.sourceforge.net/download.html) (Table S1). Gene UBQ (Accession number, XM 014636394.1) was selected as the housekeeping gene and the primer sequence was obtained from a previous study (Ke et al., 2021). Relative expression measured using real time qRT-PCR and 2-ΔΔCT method was used for relative gene expression as described by (Livak and Schmittgen 2001; Faisal *et al*., 2020).

Statistical analyses

The data of quantitative traits were statically assessed using analysis of variance ANOVA and Tukey's multiple comparison test using GraphPad (https://www.graphpad.com/).

RESULTS

Characterization of green synthesized Titanium dioxide nano particles:

For the synthesis TiO_2-NPs Moringa oleifera leaf extract was used. TheTiO_2-NPs synthesis was further confirmed by UV- spectroscopic technique. The TiO_2-NPs showed peaks in 200 to 800nm (Figure 1A). While the X-ray diffraction spectroscopy revealed average size of TiO_2- NPs which is around nm and also the crystalline structure of TiO_2-NPs. Various other elements were also present their presence was associated with presence of various organic compounds in leaf extract. These organic compounds used to reduce salt.

FTIR analysis was done to check the nature of organic compound present in TiO_2-NPs. This analysis showed the presence of different functional groups at various wavelengths which act as capping and reducing agents in the process of synthesis of nanoparticles. First peak was observed at 500 cm^{-1} due to the vibration of the Ti O-O bond. The broad-spectrum peak of TiO_2-NPs was observed at 3300 cm^{-1} which indicate the abundance of phenols and alcoholic compounds with O- H stretching. The peak at 1650cm^{-1} indicates the presence of acyclic compounds with C-C stretching (Figure.1B). SEM analysis of biosynthesized titanium dioxide nanoparticles appeared to be spherical in shape (Figure.1C).

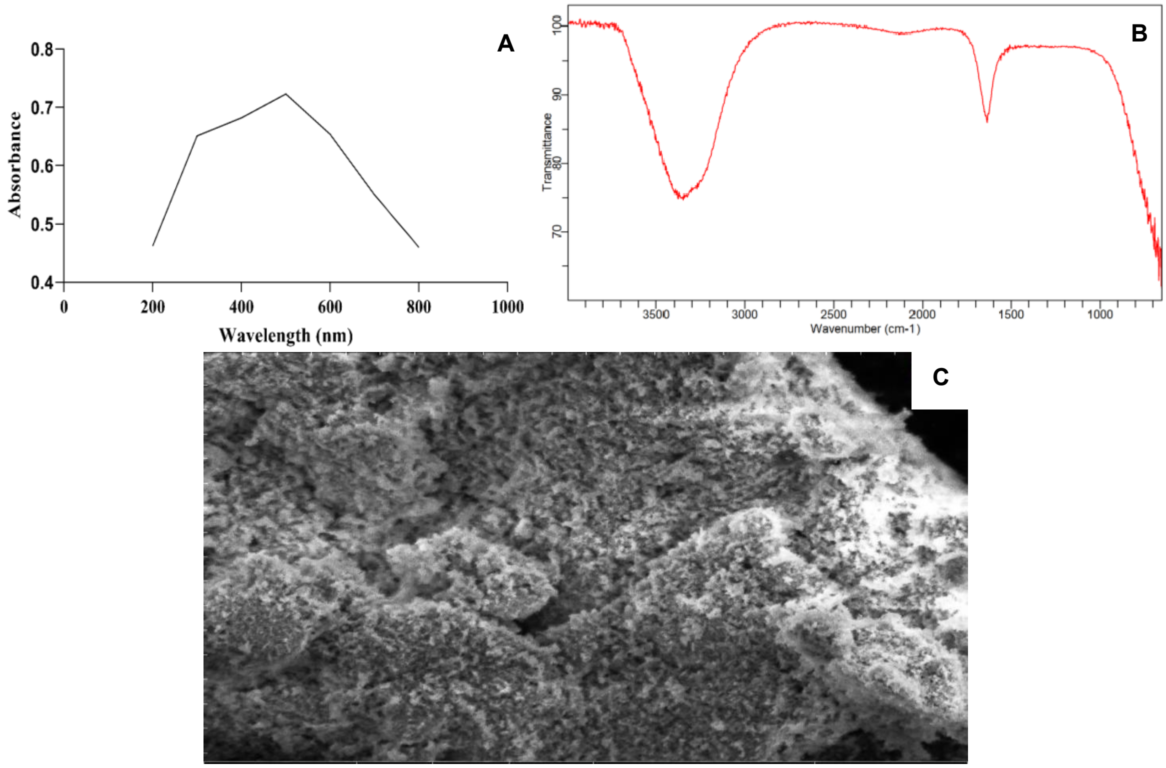

Figure 1. Characterization of green synthesized TiO_2-NPs: UV visible spectrophotometery (A) Fourier Transform Infrared Spectroscopy (FTIR) analysis (B) SEM analysis for the structure of TiO_2-NPs (C).

***In vitro* antifungal assay:**

Several studies reported that green synthesized TiO2-NPs shows antifungal potential by inhibiting the growth of fungal pathogens (Santos *et al.*, 2019; Silva *et al.*, 2019; Sreelekha *et al.*, 2021). In this study, we hypothesized that the application of TiO_2-NPs will inhibit the growth of *C. canescens*. To check the antifungal potential of TiO_2-NPs against *C. canescens* agar dilution method was performed *in vitro* (Ashraf *et al.*, 2021). The result of the current study indicates that the radial growth of *C. canescens* was inhibited by 87.85% to 57.5 % as compared to the control in *in vitro* experiment by 50 mgL^{-1} and 150 mgL^{-1} TiO_2-NPs respectively (Figure. 2). The results of current study are consistent with our hypothesis that green synthesized TiO_2-NPs has the ability to inhibit *C. canescens* growth.

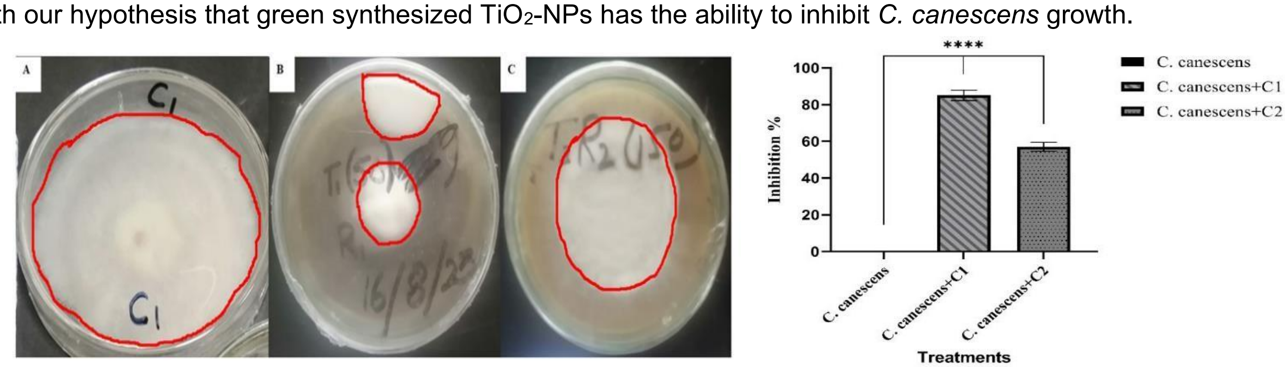

Figure 2. *In vitro* antifungal assay: Agar dilution method was performed in which fungal disc was placed on agar plate. Only fungal infected plate at 7th day **(A)** With fungal inoculum + 50mgL^{-1} NPs at 7th day **(B)** With fungal inoculum + 150mgL^{-1} $_{TiO2}$ NPs at 7th day (C). **C1**= 50mgL^{-1} NPs, **C2**= 150mgL^{-1} TiO_2-NPs (ns= non-significant, **** = Highly significant at < 0.0001 probability level, *** = significant at0.0004, ** = significant at 0.00128, * = significant at 0.0393).

Disease assessment:

Green synthesized metal oxide nanoparticles show great antifungal and antimicrobial activity (Javed *et al.*, 2021; Jebril *et al.*, 2020; Mallmann *et al.*, 2015). In current research we hypothesized that green synthesized TiO_2-NPs will mitigate the *C. canescens* in mung bean. To examine the effect of TiO_2-NPs on occurrence of *C. canescens* seed priming method was used and it was observed that biosynthesized TiO_2-NPs was not completely inhibit the *C. canescens* disease at any concentration but the intensity of disease was inhibited by different concentration of TiO_2- NPs at different level. DI data of *C. canescens* showed progressive decrease in disease symptoms in both varieties. Maximum disease incidence was observed in fungal stressed susceptible plants without any treatment of TiO_2-NPs and the lowest disease incidence was recorded in resistant verity with 50 mgL^{-1} concentration of green synthesizd TiO_2- NPs (Figure. 3). Results indicates that the effect of TiO_2-NPs on occurrence of *C. canescens* depend on genotype of mung and the concentration of TiO_2- NPs.

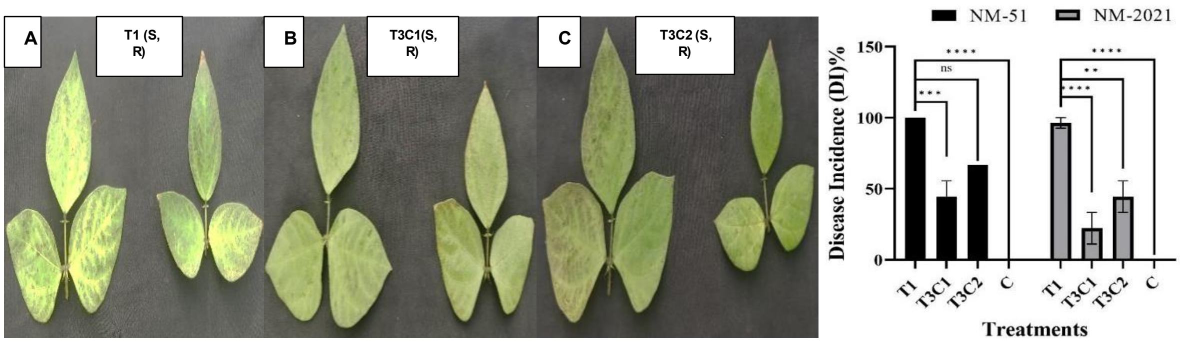

Figure 3. Measurement of Disease Incidence: In this study two mung bean verities (susceptible and resistant) were used respectively. T1 is only fungus infecte d plants **(A)** T3C1 is fungal inoculated + 50mgL^{-1} NPs treated plants **(B)** T3C2 is fungal inoculated + 150mgL^{-1} NPs treated plants **(C)**. **C1**=control, **T1**= Fungal treatment, **T3C1**= fungus + 50mgL^{-1} TiO_2-NPs, **T3C2**= fungus + 150mgL^{-1} TiO_2-NPs (**ns**= non-significant, ******** = Highly significant at < 0.0001 probability level).

Effect of green synthesized TiO_2- NPs on morphological and physiological parameters:

C. canescens infection effect morphological parameters such as defoliation, reduced root length and shoot length, reduced fruit quality and yield, stem lesions that can weaken the structural integrity of the plant, making it more susceptible to breakage, thus overall growth of a plant can be stunted due to *C. canescens* infection (Praveen *et al.*, 2018; Sahoo *et al.*, 2020). In following research, we hypothesized that TiO_2-NPs mitigate disease severity at different concentrations and boost plant resistant against fungal infection. The recorded data indicate that at 50 mgL^{-1} concentration both genotypes have positive impact on root length, shoot length and leaf surface area maximum increase in shoot length was observed in only nanoparticles treated plants which was about 8.7% (NM-51) while 3.6% (NM-51) increase in *C. canescens* plus TiO_2-NPs infected plants was observed, 13% (NM-51) decrease in shoot length was observed in *C. canescens* infected plants.

While the 150 mgL^{-1} concentration reduce the shoot length in both verities. In case of root length maximum increase of 46.3% (NM-51) and 9% (NM-2021) *C. canescens* plus TiO_2-NPs infected plants was observed while 27% (NM-51) decrease in shoot length was observed in *C. canescens* infected plants. Although the 150 mgL^{-1} concentration reduce the root length in both verities (Figure: 4). Similarly green synthesized TiO2-NPs treated plants shows outstanding increase of about 16.5% and 46.3% in chlorophyll content and stomatal conductance at 50 mgL^{-1} concentration respectively in susceptible variety and 15.5% decrease in fungal treated plant of susceptible variety.

TiO2-NPs shows increase in chlorophyll content in susceptible variety from 37.5 ug/ml to 49.7 ug/ml and 40.2 ug/ml to 51.7 ug/ml in resistant variety at 50 mgL^{-1} concentration (Figure: 5). Lowest stomatal conductance was recorded in fungal infected plants (10 mmol m^{-2} s^{-1}). TiO_2-NPs have positive impact on stomatal conductance in both fungus plus nanoparticles and only nanoparticles treated plant as TiO_2-NPs shows photocatalytic activity which boost plant immunity against fungus, stomatal conductance of NPs treated plant in susceptible and resistance variety was (17.88 mmol m^{-2} s^{-1}; 18.77 mmol m^{-2} s^{-1}) respectively at 50 mgL^{-1} concentration which is significantly higher than stomatal conductance of control plants. While fungus and nanoparticles treated plant shows stomatal conductance in susceptible and resistance variety (16.44 mmol m^{-2} $s^{-1}$and 17.55 mmol m^{-2} s^{-1}) respectively at 50 mgL^{-1} concentration which is much higher than fungal infected plants (Figure. 4).

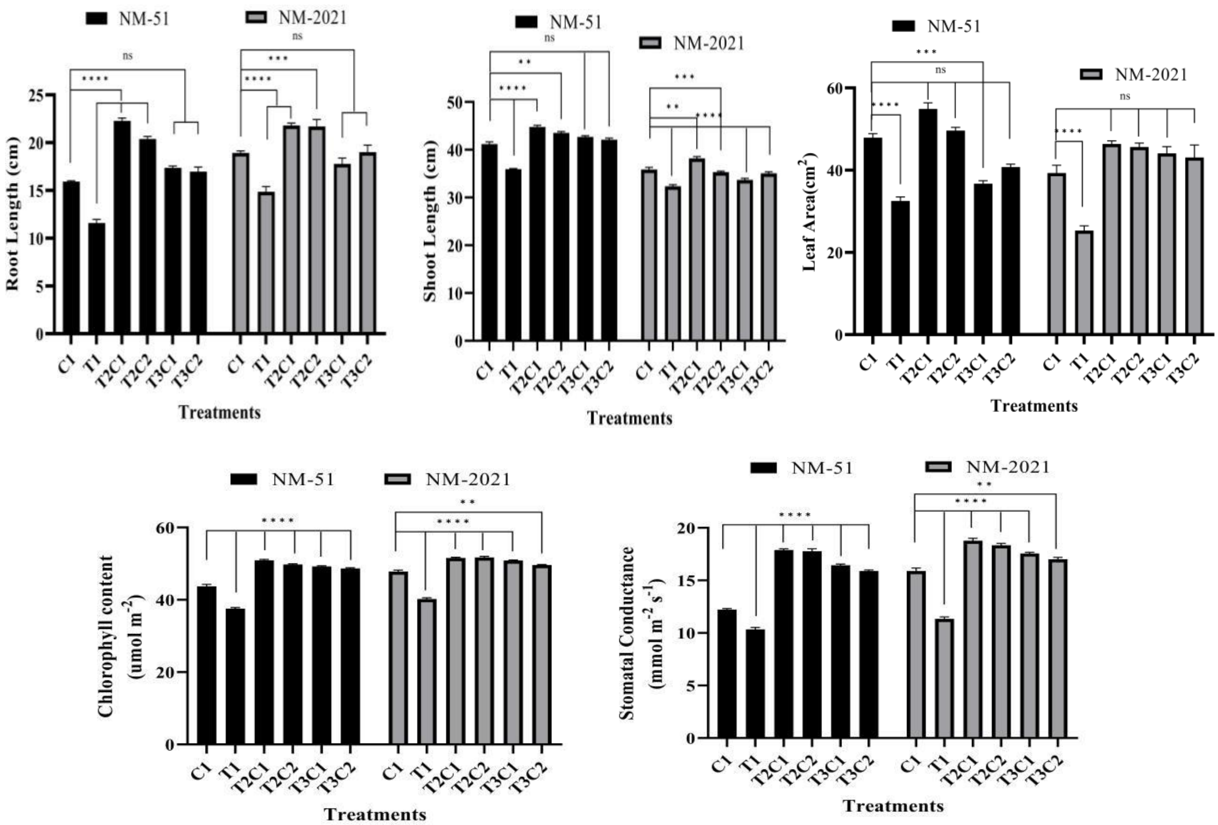

Figure 4. Morphological Parameters (Root length, Shoot length, Leaf area) Physiological Parameters (Chlorophyll content and Stomatal conductance) among all treatments: C1=control, T1= Fungal treatment, T2C1= only 50mgL^{-1} TiO_2-NPs, T2C2= only 150mgL^{-1} TiO_2- NPs, T3C1= fungus + 50mgL^{-1} TiO_2-NPs, T3C2= fungus + 150mgL^{-1} TiO_2-NPs. Two-way ANOVA and Tukey's multiple comparison test was applied to test data statistically. All treatments are compared with C1. Data are presented as means of three replicates ± standard error. *Represent that the results are significantly different ($p < 0.05$).

Effect of green synthesized TiO_2- NPs on biochemical parameters:

The pathogen interaction with plant is one of the biotic stresses that induces the production of pathogenesis-related proteins, like phenylalanine ammonia lyase (PAL), super oxide dismutase (SOD), peroxidase (POX) are produced by the host plant system to subordinate the rate of spread of the disease (Ahmad *et al.*, 2015; Biczak, 2016; Thakur *et al.*, 2019). In present line of work we hypothesized that defense system of *C. canescens* infected mung bean plants were enhanced by the application of green synthesized TiO_2-NPs. The results of CAT activity indicate one-fold increase in susceptible and half fold increase in fungal infected resistant plants as compare to control and two folds increase and four folds increase in fungal plus nanoparticles infected susceptible and resistant plants respectively. The results of peroxidase activity indicate one and half fold increase in susceptible and three folds increase in resistant variety of only fungus treated plants while only nanoparticles treated plants of both verities shows quarter fold increase at 50mgL^{-1} concentration and almost half fold increase at 150mgL^{-1} concentration and fungus plus nanoparticles treated plants shows half folds increase at both concentrations in susceptible verity while resistant verity show quarter fold increase at 50mgL^{-1} and two folds increase at 150mgL^{-1}. The results of superoxide dismutase activity indicate three and half fold increase in susceptible and two and half folds increase in resistant variety of only fungus treated plants while only nanoparticles treated plants of both verities shows one and half fold increase at 50mgL^{-1} concentration and almost two folds increase at 150mgL^{-1} concentration and fungus plus nanoparticles treated plants shows one quarter and three folds increase at 50mgL^{-1} and 150mgL^{-1} concentrations in susceptible verity respectively while resistant verity shows one and half fold increase at 50mgL^{-1} and two folds increase at 150mgL^{-1} (figure. 5).

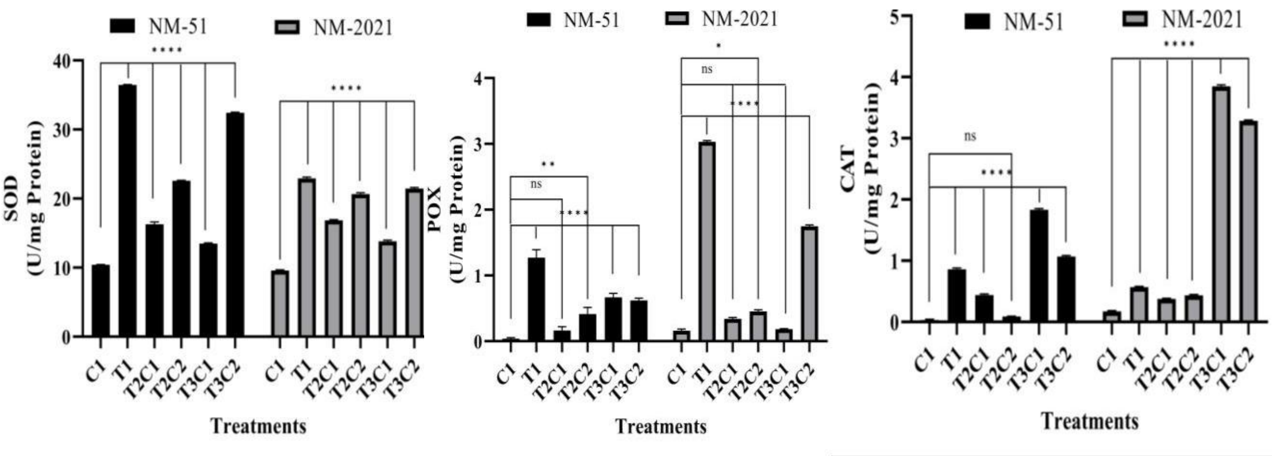

Figure 5. Biochemical Parameters (SOD, POX, CAT) among all treatments: C1=control, T1= Fungal treatment, T2C1= only 50mgL^{-1} TiO_2-NPs, T2C2= only 150mgL^{-1} TiO_2-NPs, T3C1= fungus + 50mgL^{-1} TiO_2-NPs, T3C2= fungus + 150mgL^{-1} TiO_2-NPs. Two-way ANOVA and Tukey's multiple comparison test was applied to test data statistically. All treatments are compared with C1. Data are presented as means of three replicates ± standard error. *Represent that the results are significantly different ($p < 0.05$).

Gene expression analysis:

As pathogen interact with plants it triggers complex mechanism of upregulation and down regulation of pathogenicity related genes. These genes induce defense mechanism of plants by initiating signaling pathways of plants (Liu and Ekramoddoullah, 2006). In recent study we hypothesized that application of green synthesized TiO_2-NPs upregulates pathogenicity related *PR*-1 gene. The results of present study indicate almost one-fold increase in susceptible and two folds increase in resistant variety of only fungus treated plants while fungus plus nanoparticles treated plants of both verities shows two and half and three folds increase at 50mgL^{-1} concentration and almost two folds increase at 150mgL^{-1} concentration and only nanoparticles treated plants shows very little variation of *PR*-1 gene expression at 50mgL^{-1} and at 150mgL^{-1} concentration. All above results indicates that green synthesized titanium nanoparticles upregulate expression of pathogenicity related *PR*-1 gene (figure. 6).

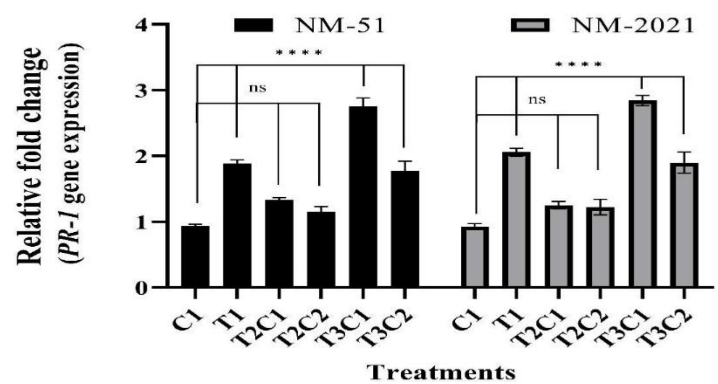

Figure 6. Gene expression analysis among all treatments: C1=control, T1= Fungal treatment, T2C1= only 50mgL^{-1} TiO_2-NPs, T2C2= only 150mgL^{-1} TiO_2-NPs, T3C1= fungus + 50mgL^{-1} TiO_2-NPs, T3C2= fungus + 150mgL^{-1} TiO_2-NPs. Two-way ANOVA and Tukey's multiple comparison test was applied to test data statistically. All treatments are compared with C1. Data are presented as means of three replicates ± standard error. *Represent that the results are significantly different ($p < 0.05$).

DISCUSSION

The current research aimed to develop a unique approach against one of the major fungal pathogens *Cercospora canescens* which cause cercospora leaf spot disease in mung bean. Nanotechnology is one of the most important technologies of modern science with very small particles in nonorange with different physical and chemical properties (Patel *et al.*, 2014). Various physical and chemical methods are used for the synthesis of nanoparticles but in recent years green synthesized nanoparticles approaches gained more attention which involves the use of microbial sources such as bacteria, yeast, fungi, algae and plant extracts (Ghormade *et al.*, 2011; Crane & Scott, 2012). This work illustrated the successful synthesis, characterization and ecofriendly management of *C. canescens* fungus.

In this research TiO_2-NPs was synthesized by *Moringa oleifera* leaf extract and antifungal activity was checked against *Cercospora canescens in vitro* and *in vivo* and the results of recent research are comparable with the results of Irshad *et al.* (2020) the plant extract of *Trianthema portulacastrum* and *Chenopodium quinoa was reported to be* used for the production of TiO_2-NPs and showed 79.8% antifungal activity against wheat rust which is higher than their chemically synthesized TiO_2-NPs. Saka *et al.* (2022) reported antifungal activity of TiO_2-NPs prepared by *Caricaceae* (Papaya) Shell extracts, inhibitions ratio was 60.5% for S. *sclerotiorums.* Ahmad *et al.* (2020) synthesize TiO_2-NPs by using leaf extract of *Mentha arvensis* which act as the reducing agent and showed excellent antimicrobial and antifungal activity which was around 60% against *Aspergillus niger*. Antioxidant and antimicrobial properties of TiO_2-NPs formed by reducing alcohols, primary and aromatic amines present in *Psidium gujava* leaf extract, these nanoparticles show 90.6% antimicrobial activity against *E.Coli* and 98.4% against S. aureus (Santhoshkumar *et al.*, 2014). The mango leaf extract was also used for the production of titania nanoparticles because of its rich components of terpenoids and other proteins which shows 79% inhibition against *P. steckii* for 120 min of UV light induction (Xing *et al.*, 2023). The antifungal activity was also shown by TiO_2-NPs synthesized by Fenugreek plant extract (Roopan *et al.*, 2012). Rajamehala *et al.*, 2022 reported that TiO_2-NPs synthesize by *Calotropis gigantea* extract reduce pathogenic disease impressively. Rafique *et al.* (2014) suggested that the application of TiO_2-NPs increase chlorophyll content which has direct effect on photosynthetic rate and lead to the enhanced production of carbohydrates. Sheikh Mohamed and Sakthi Kumar (2016) recommended that TiO_2- NPs have positive impact on photosynthetic pigments like chlorophyll “a” and chlorophyll “b” similar results were shown by Morteza *et al.*, 2013 in *Zea mays*.

The TiO_2-NPs when come in contact with microbial cells, it causes oxidative damage to cell wall and cell membrane by increasing the cell permeability and oxidative stress of intracellular compartments accelerates cell death (Desai and Meenal, 2009). Interaction with nanoparticles results in better performance for enhanced antioxidant potential for plant stress resistance by increasing radical scavenging potential and antioxidant enzymatic activities of plants that help plants by optimizing yields (Wang *et al.*, 2016). The result of the current study resembles with previous results as described above the TiO_2-NPs inhibited the growth of *C. canescens* by 87.85% as compared to the control *in vitro* experiment at $50mgL^{-1}$ but $150mgL^{-1}$ concentration was proved to be toxic as described by Satti *et al.* (2021) that higher concentration of TiO_2-NPs has negative impact on morphological and physiological parameters while 40 ppm concentration have positive impact on these characteristics. The maximum improvement was observed in SOD, POD and CAT activity at 40 ppm concentration while higher concentration of TiO_2-NPs also has negative impact on these parameters.

CONCLUSION

In the present study, the application of green synthesized TiO_2-NPs significantly enhanced plant growth and development. These nanoparticles also have shown to have upregulated pathogenesis- related genes, which reinforce that they have elevated plant immunity. Additionally, TiO_2-NPs prepared by geen synthesis method could act as ecofriendly control for CLS and protect humans from hazardous effects. Due to the environmental changes from past few years, there is need to control diseases with such a method that have “NO” or less impact on environment. Many environments friendly alternative approaches were developed by scientists and use of nanotechnology is one of them but traditional methods for synthesizing nanoparticles are costly and produce very toxic products so there is need to do more on green synthesis of nanoparticles, as they are safer, cheaper and are used in small amount as compared to fungicides. Fungicides and chemical control of fungus has adverse effect on human health as well as environment so there is severe need to rush toward safe, biotic or ecofriendly methods as with the passage of resistance against fungicides aggregated. More research and field trials are essential to fully explore the practical applications of TiO_2-NPs.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare no competing interests.

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