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"BIOCONTROL EFFICACY OF *TRICHODERMA* SPECIES AGAINST CHARCOAL ROT IN SUNFLOWER AND SYNTHESIS OF SILVER NANOPARTICLES FOR ENHANCED DISEASE MANAGEMENT"

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A B S T R A C T

Sunflower (*Helianthus annuus* L.) is an important cash crop in Pakistan, largely cultivated for its oil. However, its production is severely affected by a soil-borne fungal pathogen, *Macrophomina phaseolina* causing charcoal rot. The present study investigates the effectiveness of *Trichoderma* species, known antagonists to soil-borne pathogens for managing charcoal rot. The research also extends to the use of biologically synthesized silver nanoparticles (AgNPs) from *Trichoderma viride* for disease control. Isolates of both pathogen and antagonist from the Fungal Culture Bank of Pakistan were brought and cultured on PDA media, and their *in vitro* interactions with *M. phaseolina* were observed. Additionally, the *in vivo* efficacy of these species was tested on sunflower plants. Results showed that three *Trichoderma* spp. (*T. asperellum, T. harzianum* and *T. viride*) significantly reduced the prevalence of *M. phaseolina* and boosted plant growth. Moreover, AgNPs synthesized from *T. viride* were effective in halting the progression of charcoal rot, showing an increase in biochemical markers like Chlorophyll, Carotenoids, Phenol, and Flavonoids. This study demonstrates the potential of *Trichoderma* spp. and AgNPs in the sustainable management of charcoal rot in sunflower, thus contributing to improved crop yields.

Keywords: *Helianthus annuus* L., *M. phaseolina, Trichoderma* spp., Silver Nanoparticles (AgNPs), Biocontrol Agents.

INTRODUCTION

Sunflower (*Helianthus* L.) belonging to family Asteraceae is the most important oil crop which is grown over 22 million hectares, with a production of 26 million tonnes throughout the world (Shirshikar, 2005; Skoric *et al*., 2007). One race of sunflower is *Helianthus annuus* L. which is cultivated for edible seeds (Khan, 2007). In Pakistan, sunflower was introduced during 1960 with the object of bridging the gap between production and consumption of edible oil in the country (Burney *et al*., 1990). Over the years, sunflower has become an important crop for both farmers and consumers in Pakistan because it fits well in the local cropping system and is considered the most important cash crop in all parts of the country (Shah *et al*., 2005). Several reasons such as occasional adverse climate conditions and prevalence of diseases are the causes of low yield of sunflower (Mirza and Beg, 1983). An important fungal disease of sunflower is charcoal rot, caused by *M. phaseolina* (Tassi) Goid (Rayatpanah, 2012). It is considered most prevalent than the other diseases on sunflower. *M. phaseolina* is a soil born fungus and infects 500 species or more of 7 plant families worldwide which include legumes, fiber crops, vegetables and fruits. The fungus has a wide host range including both cultivated and wild plant species. Plants infected with charcoal rot generally have weak appearances and colourized leaves with fewer black secondary roots.

The term "biological control" and its abbreviated synonym "biocontrol" have been used in different fields of biology, most notably entomology and plant pathology. *Trichoderma* species are the successful antagonists, having the ability to act as biocontrol agents against soil-borne pathogens and plant parasites (Kushwaha and Verma, 2014; Shahid *et al*., 2014). These species attack on plant pathogens and reduce the infection of *M. phaseolina* on sunflower and promote the

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growth of plants and roots (Harman, 2006). The most important species of *Trichoderma* in the field are *T. harzianum* Rifai, *T. virens* (J. H. Mill., Giddens & A. A. Foster) Arx, *T. viride* Pers. and *T. asperellum* (Samuels *et al*., 1999). Many isolates of *T. viride* Pers. and *T. harzianum* Rifai were obtained from the soil rhizosphere of the plantation crops and forest soil (Chakraborty *et al*., 2010). *Trichoderma* species act as a guard for their hosts, and also fulfil the needs of their hosts and help them to increase their growth and protection from pathogens (Seidl *et al*., 2009). Recent studies showed that *Trichoderma* species induce localized and systemic resistance in plants against attack of variety of plant pathogens or damage caused by insects or treatment with different chemical inducers (Kuc, 2001; Oostendorp *et al*., 2001). Strains of *Trichoderma* are well known in their ability to colonize roots but conidia of *Trichoderma* when applied to fruits, flowers and foliage can control plant diseases (Harman, 2000). *T. viride* produces many hydrolytic enzymes like chitinases, proteases and glucanase and is used against the pathogenic fungi. It is also used to synthesize biogenic silver nanoparticles (AgNPs) because it is a non-pathogenic and environmental friendly fungus. The present study provides Sunflower disease research findings from Pakistan and a brief understanding of diseases incidence and management using different *Trichoderma* species as biocontrol agents. This research work suggested a potential for the large scale production of silver nanoparticles (AgNPs) from *T. viride* without the involvement of any toxic chemical or radiation and also deepens the understanding of molecular mechanism for the synthesis of AgNPs to control charcoal disease of sunflower.

MATERIALS AND METHODS

Culture collection: Cultures of three antagonists (*Trichoderma* spp.) and pathogen (*M. phaseolina*) used were obtained from Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences (IAS) Quaid-e-Azam Campus, University of the Punjab, Lahore.

Preparation of PDA medium and potato extract: For preparation of 1000 ml of PDA medium, 40 g of PDA powder was dissolved in 1000 ml of distilled water. Medium was sterilized at 15 psi for 20 minutes. For preparation of potato extract, potatoes were placed in a saucepan with approximately 1.2 litres of tap water and boiled on a stove for one hour. The saucepan was removed from the stove. Liquid potato extract was poured through a strainer or cheesecloth and collected in another container. The medium was mixed thoroughly and divided into smaller batches of 250 ml then poured into the four 500 ml flasks. Each flask was covered with a piece of loosely crimped aluminium foil (Kumar *et al*., 2019).

Sterilization: The sterilization process was performed in the autoclave at 121°C at the pressure of 103, 421 Pascal's (15PSI) for 15 minutes.

Pouring of media: After sterilization, the flasks were removed and left to cool down. Penicillin and streptomycin were added in sterilized stock medium just before pouring to inhibit the bacterial growth. Sterilized petri dishes were placed on a flat surface of Laminar flow. The medium was then transferred to sterilized petri dishes and left for some time for solidifying.

RESULTS

***In vitro* culturing and interaction of antagonistic fungi and pathogen:** Cultures of *Trichoderma asperellum, T. harzianum, T. viride* and *M. phaseolina* were prepared on PDA medium. The culturing plates and line drawings are shown in figures 1–3. Their morphological, features were observed. Such as fungal colony, rings formed by colony, growth rate, culturability, habit, diameter of colony and specific characters like colour, odor and exudation were observed carefully. Growth rate of colony was determined by measuring the width of colony after fixed intervals of time. The culturability was noted on the basis of mycelial growth and enlargement of colony size whether it grows at slow, intermediate or fast rate. Furthermore, microscopic features like hyphae, shape of conidia etc. were also evaluated. The fungal colony was characterized and distinguished on the basis of colour, growth rate and texture. In order to study the interaction between antagonistic fungi and pathogen, a 5 mm inoculum disc of *T. harzianum* was placed at one side of a petri plate containing PDA amended with penicillin. A 5 mm disc of *M. phaseolina* was cut and placed at other side of petri plate. There were three replicates of each treatment. Same was done with other species of *Trichoderma* and their interaction with *M. phaseolina* was observed. The pathogen showed more growth than the antagonist and zone formation was observed. Antagonist and pathogen each colonized on one-half of the medium surface and neither organism appears to dominate the other. The pathogen colonized at least two thirds of the medium surface.

***In vivo* efficacy of *Trichoderma* spp. against *M. phaseolina*:** For pathogenicity test, half of the pots had artificial infestation of *M. phaseolina* and their effect on plant growth and root colonization was observed as represented in figure 4. The sunflower seeds were pelleted with *Trichoderma* spp. in such a way that 1 ml of conidial suspensions of *T. asperellum, T. harzianum and T.viride* were prepared separately and added to 10g of seeds in polythene bags that were shaken well to provide a uniform coating. The evenly coated seeds were

sown in pots containing 200 g soil separately and growth rate was checked. During pot experiment, those plants that were artificially infested with pathogen showed diseased symptoms in sunflower while other showed no symptoms. To monitor the efficacy of *Trichoderma* spp. against pathogen *M. phaseolina*, seeds were treated with *Trichoderma* spp., *M. phaseolina* and *Trichoderma* species + *M. phaseolina* (Antagonist + Pathogen) as shown in figure 5. *Trichoderma* spp. significantly suppressed the infection of *M. phaseolina*. The plant growth and morphological parameters such as plant height, weight, length, width and number of leaves per plant were noted and were recorded maximum in those plants that were treated with *Trichoderma* spp. and both *Trichoderma* species + *M. phaseolina* (Antagonist + Pathogen) as shown in table 1. It was observed that mean number of leaves per plant and mean fresh height were high in healthy and *Trichoderma* treated plants as compared to infected plants as shown in figure 6. Biochemical analysis of the infected and healthy plants was done to check the contents of Flavonoid, Phenol, Carotenoid and Chlorophyll (a, b) at 510nm, 765nm, 480nm and 663nm, 645nm (a, b) respectively. Results were recorded in the form of graphs as shown in figure 7. *T. asperellum*, *T. harzianum* and *T. viride* were experimentally proved to be environmental friendly biocontrol agents that showed effectiveness against a fungal pathogen (*M. phaseolina*). Silver Nanoparticles (AgNPs) were also synthesized biologically by *T. viride* at room temperature and then applied to infected plants as represented in figure 8. Its effect was observed and recorded for the period of 15 days. The results showed that *T. viride* extract stopped the spread of Charcoal rot on sunflower and its surface area did not increase further. The biochemical analysis was done again to analyze the Chlorophyll, Carotenoid, Phenol and Flavonoid contents after the spray of Silver Nanoparticles from *T. viride* and the results exhibited greater amount of all these parameters in those Sunflower diseased plants that were sprayed with AgNPs as compared to diseased plants before spray of *T. viride* AgNPs. Moreover, standard curves were also made for total phenolic contents (mg/100g) using Gallic acid and Calichine as shown in figure 9. *T. viride* free cell extract with AgNO3 showed dark brown colour in distilled water. The emergence of brown colour was due to the formation of AgNPs. This colour was mainly referred to the surface plasmon resonance of the AgNPs. No change in colour was detected in the control which was without AgNO3. Therefore, it was evident from the obtained results that the ability of *T. viride* to synthesize AgNPs was detected. The specimen was examined in the wavelength range from 420 to 640 nm; an extreme absorption was detected at 620 nm after 24 h of incubation. UV-Visible spectra of Silver Nanoparticles from *Trichoderma viride* were also constructed and represented in the form of graph as shown in figure 10.

DISCUSSION

Sunflower is a valuable crop from an economic as well as an ornamental point of view. It is considered as premium oil because of its high level of unsaturated fatty acid, light colour and lack of linolenic acid. Available varieties of sunflower contain oil in the seed from 39 to 49%. Various diseases pose severe threat to the production of this commercially important crop. Some major diseases include rust, powdery mildew, downy mildew, *Verticillium* wilt, *Sclerotinia* stalk and head rot, phoma black stem, charcoal rot and leaf spot, as their severity has adverse effects on crop yield (Vear, 2004). Among them, one of the most important disease is charcoal rot disease that is caused by a soil-borne fungus, *M. phaseolina.* Various chemical, biological, cultural and physical methods have been used to combat pathogenic fungi (Sharma, 1996; Katan, 2000). In recent year, biological control instead of using chemical treatment has proved to be more effective (Elad, 2000; Howell, 2003).

The antagonistic potentiality of *Trichoderma* species as biocontrol agents for plant diseases was first recognized in the early 1930s (Howell, 2003). In present research work, three species of *Trichoderma* (*T. harzianum, T. viride* and *T. asperellum*) have been found to be efficient against Charcoal disease and have shown their efficacy against *M. phaseolina* as shown in figure 5. These species attack on plant pathogens and reduce the infection of *M. phaseolina* by promoting the growth of plants and roots (Harman, 2006).

T. asperellum is a globally recognized soil fungus due to its broad-spectrum antimicrobial and plant growth promoting properties. One of its strain T34 has proved to be effective biocontrol agent against root rot caused by *Phytophthora capsici* in pepper (Segarra *et al*., 2013). Potential use of *T. asperellum* T8a has been observed as biocontrol agent against acanthrose in mango. Different strains of this species have been used to combat various fungal pathogenic diseases. In the current research work, it has demonstrated strong antagonistic behavior against charcoal disease of sunflower and its identity was confirmed by using molecular phylogenetic tools. It showed that studied sequence was 99.50% identical with *T. asperellum* and it is a new record for Pakistan. The distinguishing characters included ovoidal, globose, sub globose type conidia with radial hyphae as shown in figure 1. It has previously been reported from Asia, Africa, Europe, North America and South America (Samuels *et al*., 1999).

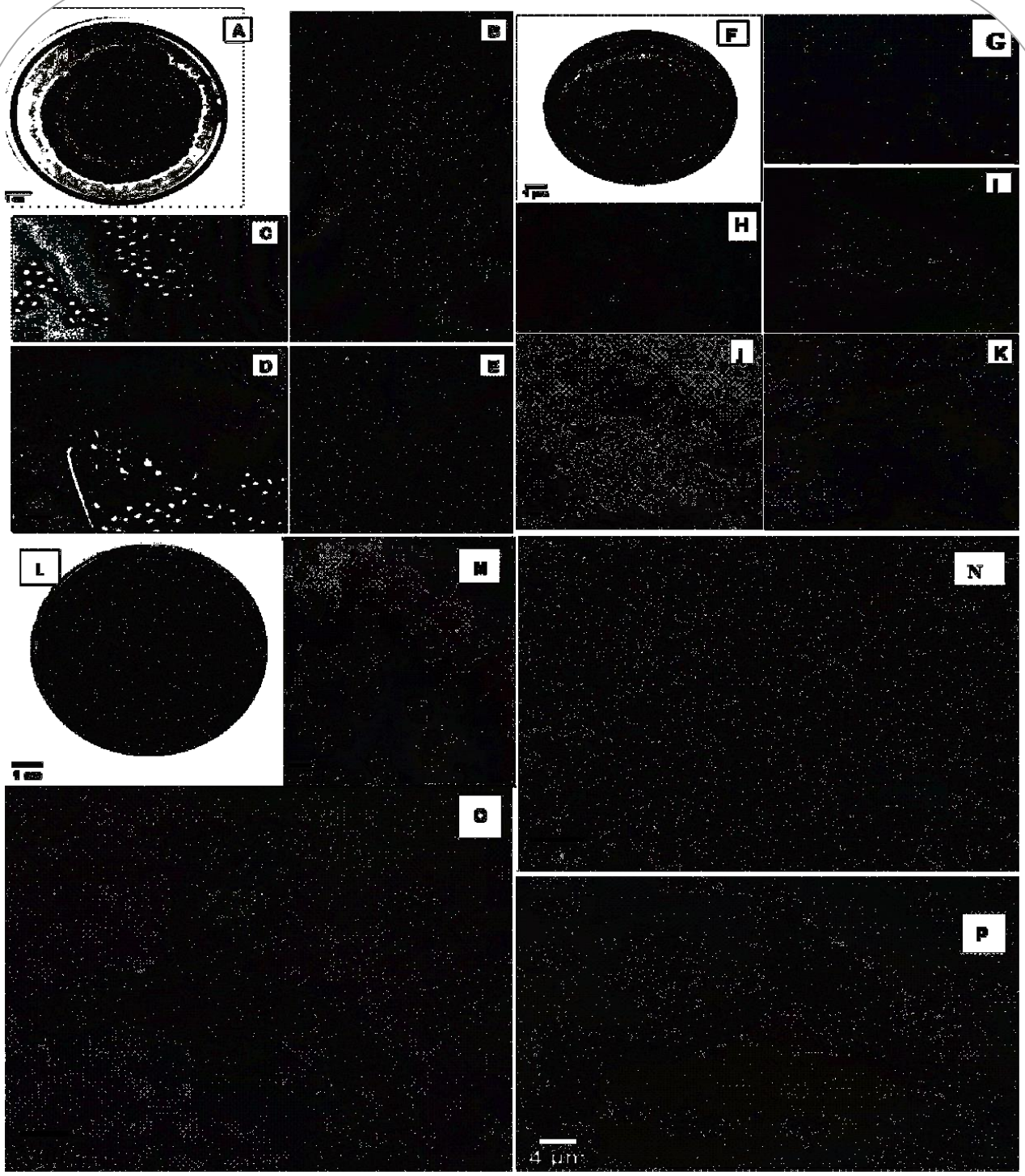


Figure 1: A- *Trichoderma harzianum* colony on PDA medium; B, C & E- Conidia; D- Mycelium; F- *Trichoderma viride* colony growth; G–I Conidia; J–K- Mycelium; L-*Trichoderma asperellum* colony growth on PDA medium; M–N- Conidia; O–P- Mycelium.

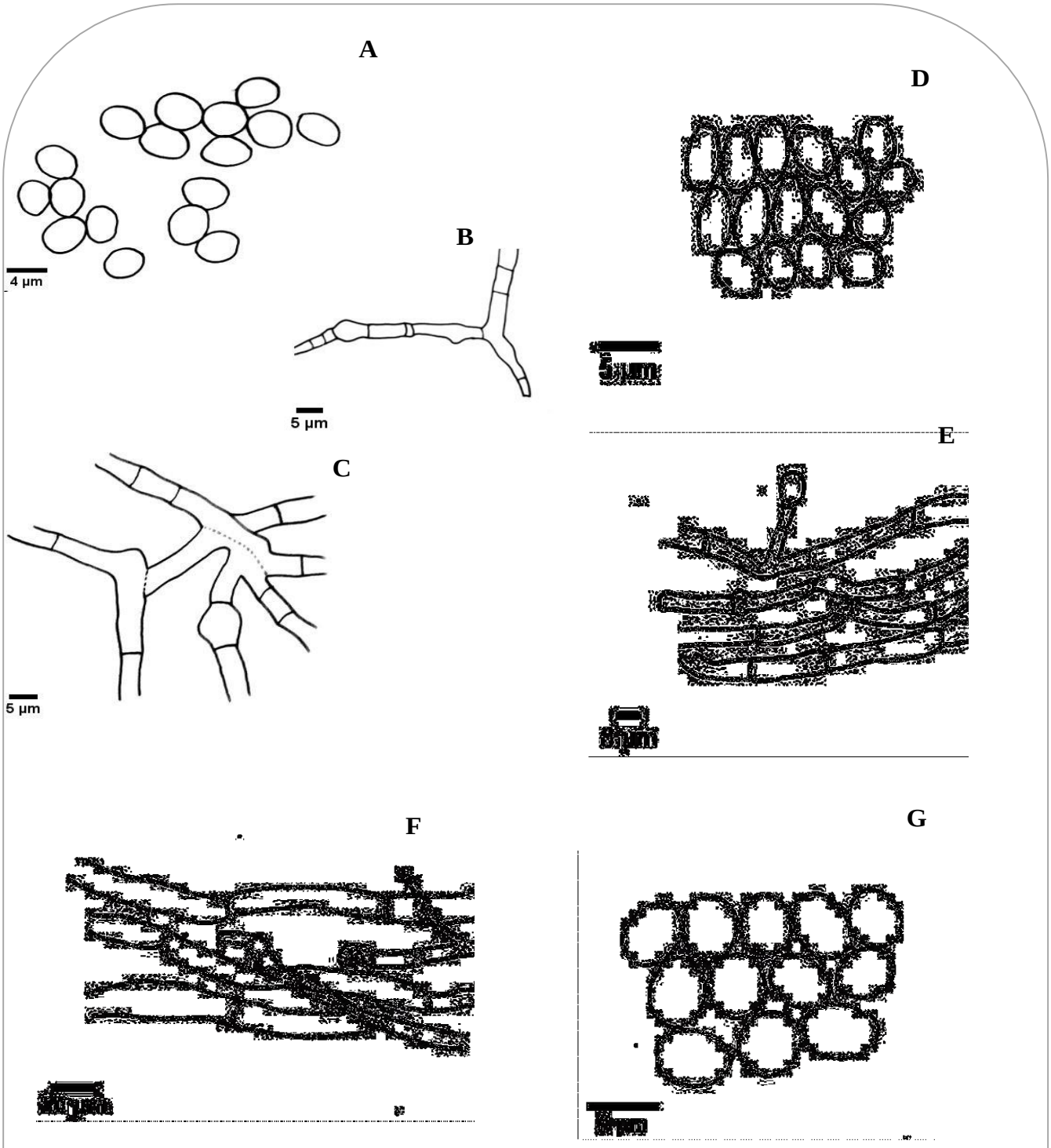


Figure 2: Line drawings of *Trichoderma* spp.; A–C- *T. asperellum*; A-Conidia; B- Hyphae; C- Mycelium; D–E- *Trichoderma harzianum;* **D-** Conidia; **E-** Mycelium; F–G- Line drawings of *Trichoderma viride*; **F-** Mycelium; G- Conidia.

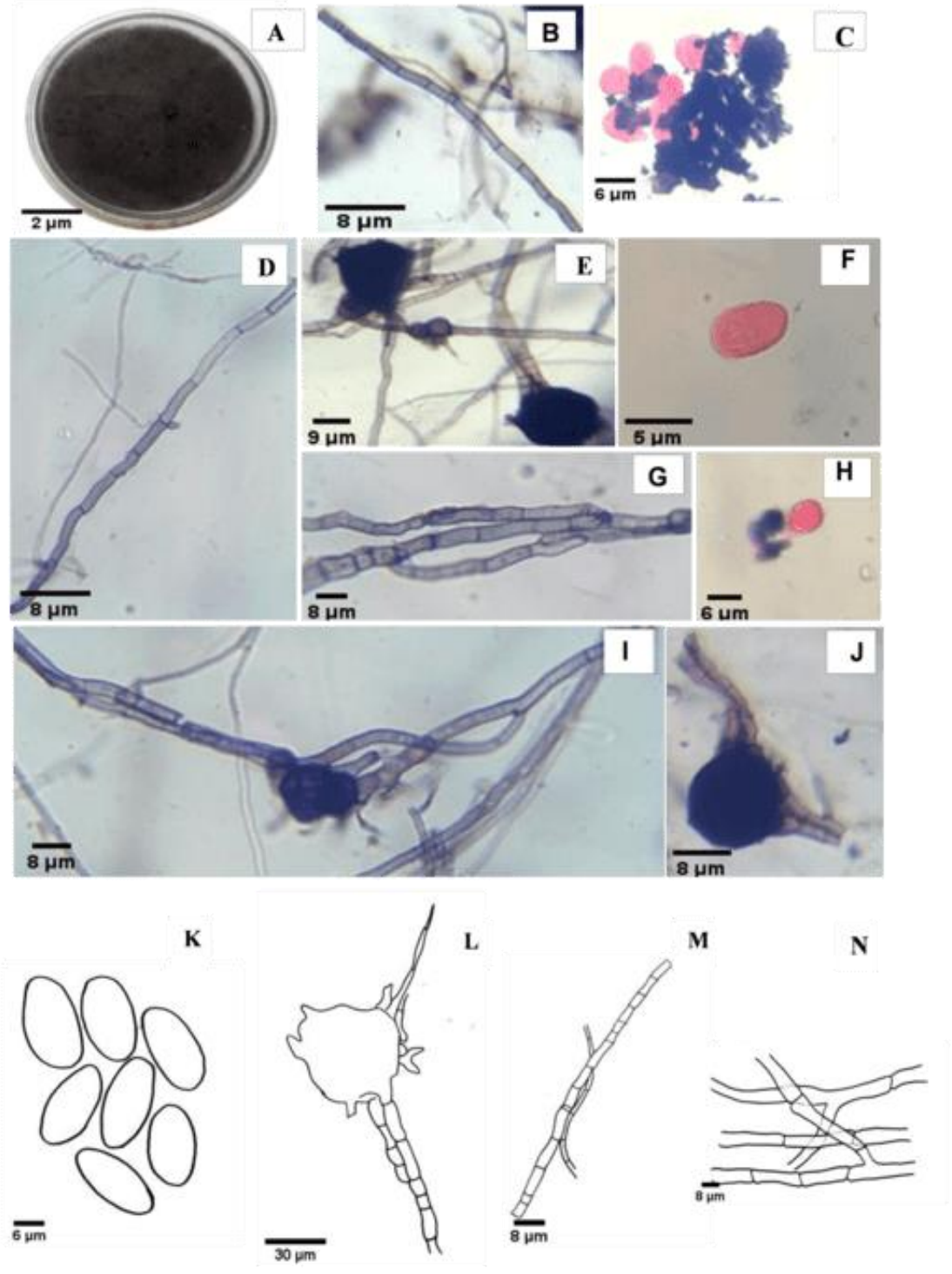


Figure 3: Plate and line drawings of *Macrophomina phaseolina* on *Helianthus annuus*. A - Colony on PDA medium; B, D & G- Hyphae; C, F & H- Conidia releasing from sporangium; E, I & J-Sporangium of *M. phaseolina*; K- Conidia; L- Sporogonium; M-N- Hyphae.

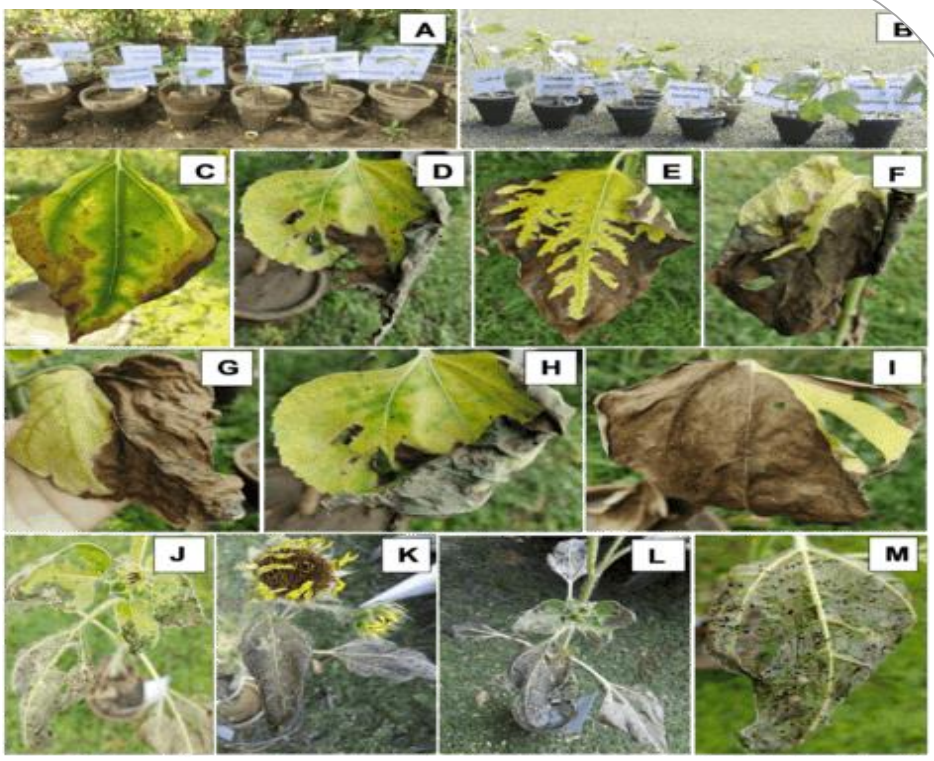


Figure 4: Pathogenecity Test.: Different stages of development of charcoal rot disease on Sunflower (*Helianthus annuus* L.) after pelleting of sunflower seeds with *Macrophomina phaseolina*

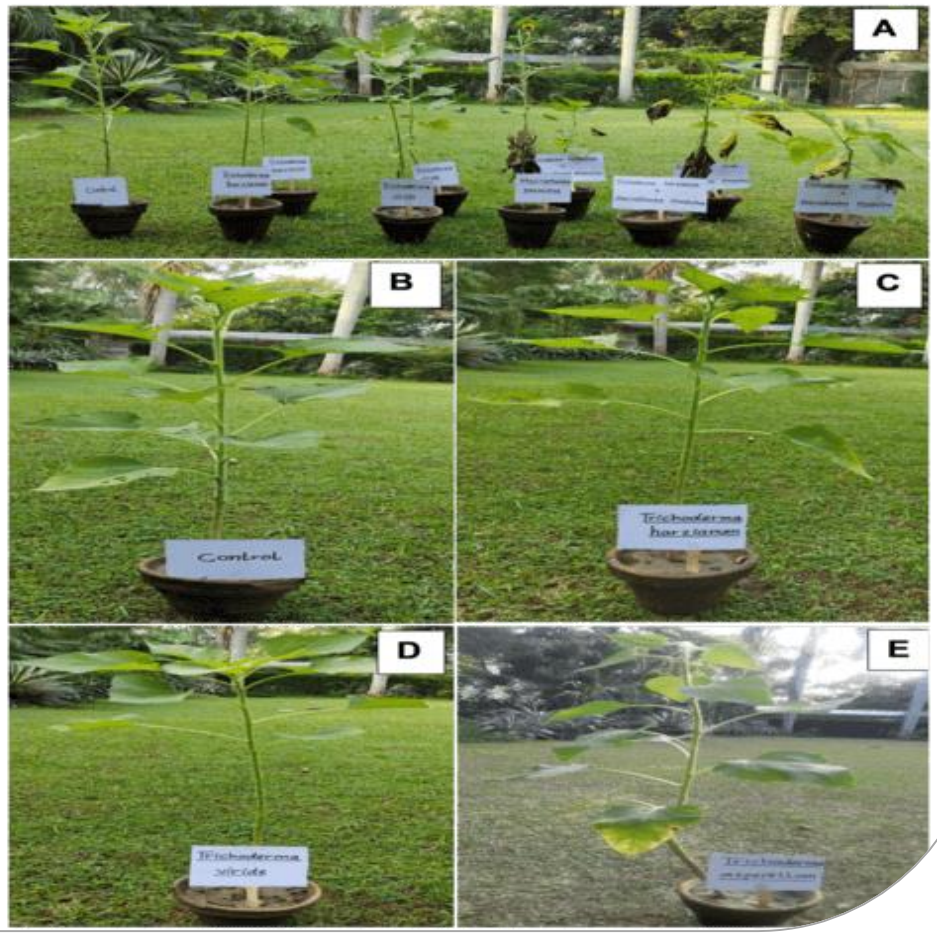


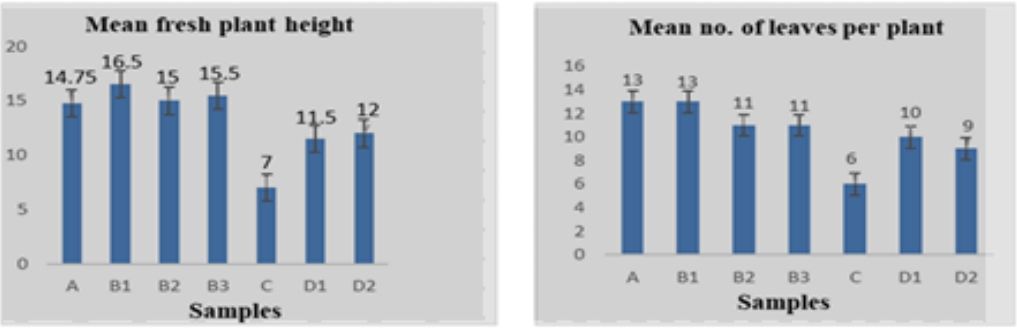
Figure 5 Pathogenecity Test: Different stages of development of charcoal rot disease on Sunflower (*Helianthus annuus* L.) after pelleting of sunflower seeds with control plant *and Trichoderma spp.*

Table 1: Effect of *Trichoderma* spp. a nd *Macrophomina phaseolina* on morphological parameters of sunflower plants

Sr. No	Factors	Control (A)	Antagonist (B1)	Antagonist (B2)	Antagonist (B3)	Pathogen (C)	D1 (B1+C)	D2 (B2+C)
1	Mean fresh plant height	14.75	16.5	15	15.5	7	11.5	12
2	Mean fresh plant weight (g)	30	33	32	30	15	25	27
3	Mean no. of leaves per plant	13	13	11	11	6	10	9
4	Mean length ot leaf (cm)	9	9.5	8.5	9.5	5.5	7.5	6.5
5	Mean width of leaf (cm)	8	9	8.5	9	5	6	6

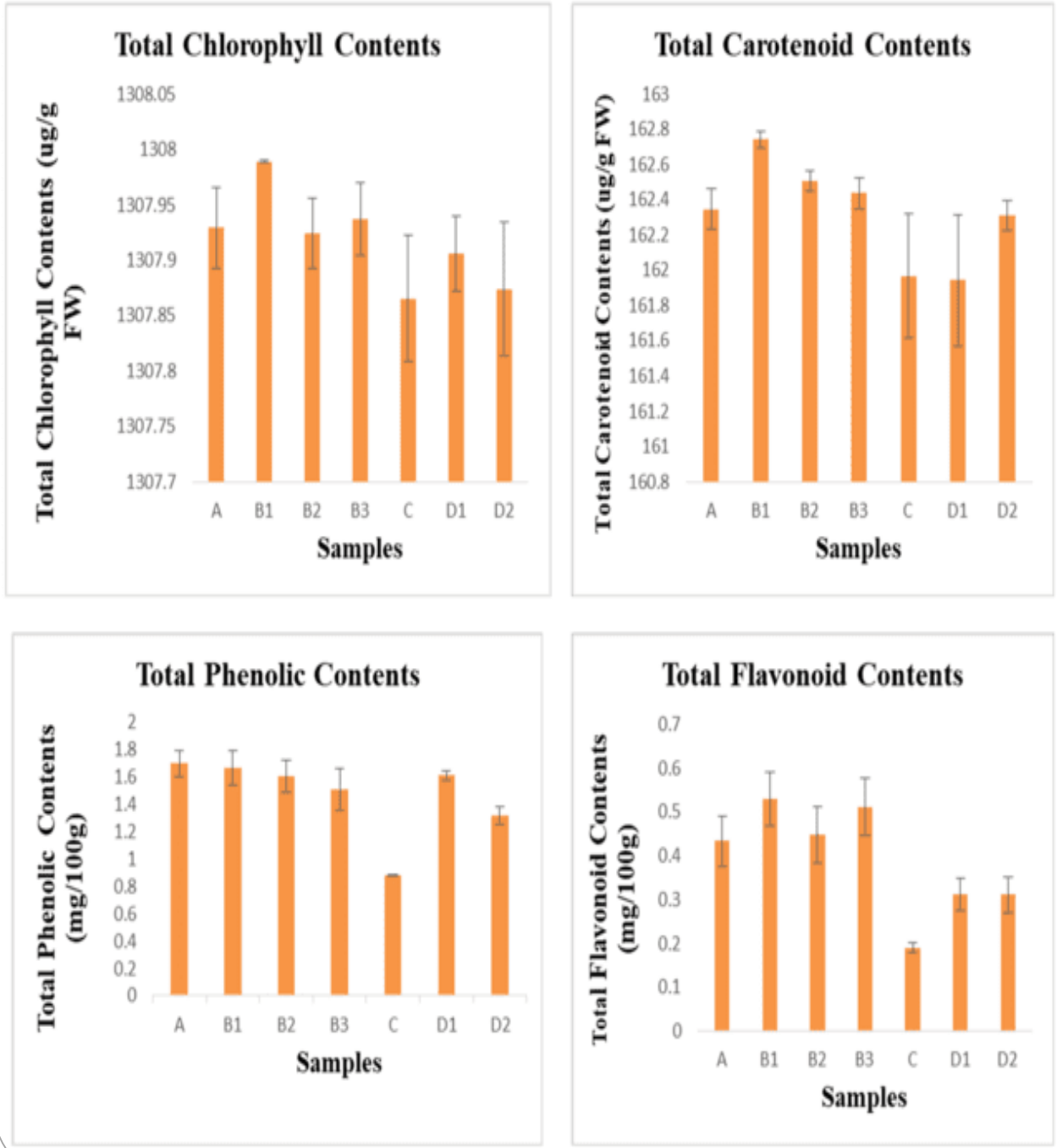
Antagonist B1= *Trichoderma harzianum*, **Antagonist B2** = *T. viride*, **Antagonist B3**= *T. asperellum*, **Pathogen C**= *Macrophomina phaseolina*, **D1 (B1 + C)** = *T. harzianum* + *M. phaseolina*, **D2 (B2 + C)**= *T. viride* + *M. phaseolina.*

Figure 06: Efficacy of *Trichoderma* spp. against *Macrophomina phaseolina* (Effect on mean fresh plant height and no. of leaves of sunflower).



A =Control, **B1**= Antagonist (*Trichoderma h arzianum*), **B2**= Antagonist (*T. viride*), **B3**= Antagonist (*T. asperellum*), **C**= Pathogen (*Macrophomina phaseolina*), **D1**= Antagonist + Pathogen (*T. harzianum* + *M. phaseolina*), **D2**= Antagonist + Pathogen (*T. viride* + *M.phaseolina*).

Figures 7: Graphs showing total chlorophyll, caretenoids, phenolic and flavonoid Contents (mg/100g) in Sunflower (*Helianthus annuus).*



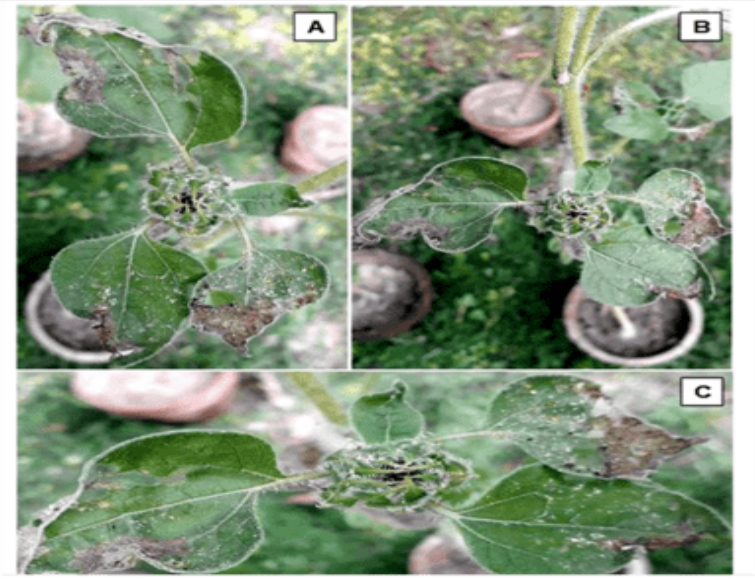


Figure: 8 A-C: Application of Silver Nanoparticles prepared from *Trichoderma viride* on sunflower plants infected with *Macrophomina phaseolina*

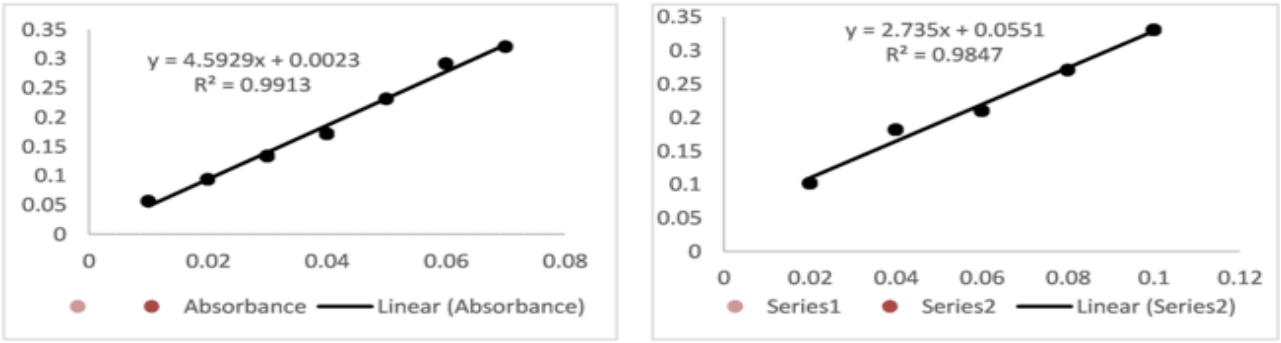


Figure 9: Standard Curve for Total Phenolic Contents (mg/100g) using Gallic acid and calichine as Standard respectively.

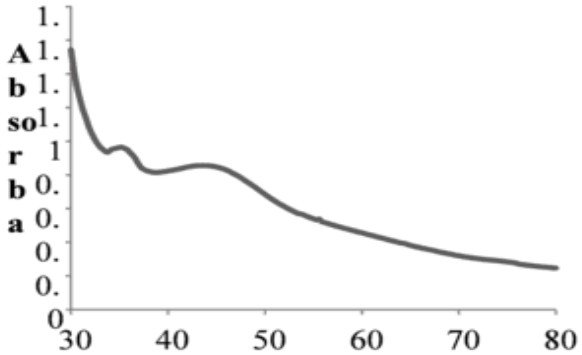


Figure 10: UV-Visible spectra of Silver Nanoparticles from *Trichoderma viride*

T. harzianum is being used as a fungicide. These act as bioprotectants, biofertilizers, biostimulants, and bio pesticides (Harman *et al*., 2004) to control foliar, soil borne and post-harvest plant fungal pathogens. Balasubramanian (2003) investigated that *T. harzianum* enhanced rice and tomato shoot weight and plant length. *Rhizoctonia solani* infection was completely inhibited by *T. harzianum* (Malik *et al*., 2005). Commercial biotechnological products such as 3Tac have been useful for treatment of *Botrytis*, *Fusarium* and *Penicillium* sp. Furthermore, *in vitro* antagonistic effects of *T. harzianum* against wheat-borne fungal pathogens has been observed (Bibi *et al*., 2023). It has been previously reported from Pakistan (Ahmad *et al*., 1997) on the grains of *Sorghum vulgare* from coniferous forest soil (Matsushima, 1993). In our investigation, it has shown strong efficacy against *M. phaseolina.* The anatomical features that were noticed included globose to subglobose conidia and smooth conidial wall with septate, smooth, branched and thick wall mycelium as shown in figure 1. (Samuels *et al*., 2002). *T. viride* is considered as important plant protective biological agent. Efficiency of *T.viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants was observed by (Morsy *et al*., 2009). In our research analysis, it showed biocontrol behavior against charcoal rot disease of sunflower. The characteristic features of *T. viride* included smooth, septate and thick wall hyphae, globose conidia with rough or smooth conidial wall as demonstrated in figure 1. Previously two reports have been found from Pakistan on *Phoenix dactylifera* (Ahmad, 1969; Rizvi, 1966; Qureshi, 1966; Hussain *et al*., 1966). This has also been reported from New Zealand, Australia, Europe, America, Africa and Asia on different plants. During our current investigation, nanoparticles were biosynthesized by mixing 20 mL silver nitrate suspension (10 mmol/L) with 100 mL cell filtrate (biomass) of *T. viride*. Erlenmeyer flask was incubated at 25 C for 24 h in darkness. A flask without AgC was used as control. Ag NPs were intensified by centrifugation (Universal 320R, Hettich, Germany) at 11,000 rpm twice for 15 min and collected for additional tests. The prepared samples were analyzed through UV-Visible spectrophotometer which confirmed the synthesis of silver nanoparticles (AgNPs) from *Trichoderma* sp. showing absorbance at around 420-450 nm as shown in figure 10. Furthermore, biochemical analysis was also done to check chlorophyll, caretenoids, chlorophyll, phenols contents. According to results, infected plants of sunflower treated with AgNPs from *T. viride* showed maximum amount of all these contents than infected plants without treatment of AgNPs. The results showed that *T. viride* extract stopped the spread of Charcoal rot on sunflower and its surface area did not increase further as shown in figure 8.

M. phaseolina infection on Sunflower was first reported from Sri Lanka in 1927, then from Uruguay, Australia and Yugoslavia in1966, Argentina and Senegal (1967), Hungary (1970), USA (1971), India (1973), France (1976), Egypt (1980) (Bhutta, 1998). In Pakistan it has previously been reported on stems of *Citrullus colocynthis*, *Corchorus capsularis*, *Dalbergia sissoo* and on pods of *Gynandropsis gynandra* (Ghaffar and Abbas, 1972). Here it has caused charcoal rot disease of sunflower as shown in figure 4. The microscopic characters showed single celled, hyaline, and elliptical or oval type conidia and is characterized by thin-walled hyphae with light brown to dark brown colour as represented in figure 3.

The ability of *Trichoderma* isolates to inhibit the mycelial growth of *M. phaseolina* in dual culture (Antagonist & Pathogen) was determined on PDA medium. Dual culture plate technique was used to study the antagonistic effects of the *Trichoderma* isolates on *M. phaseolina* observing the zone of inhibition at the point of pathogen and the antagonist and measuring their colony diameter on the third and fifth day after inoculation, served as an indicator of them in vitro biocontrol activity. *T. harzianum*, *asperellum* and *T. viride* isolates were found better potential control agents against *M. phaseolina in vitro*. This work of *in vitro* plate assays showed that *T. harzianum, T. asperellum* and *T.viride* were more effective in suppressing the growth of *M. phaseolina* (Patel and Anahosur, 2001). The biochemical analysis was done separately for each *Trichoderma* species, pathogen, Antagonist + Pathogen. The chlorophyll and carotene contents in (µg/fW) were calculated by putting the absorbance values into equation $(0.0202 * OD - 645) + (0.0082 * OD - 663)$ and $[100 * OD - 480 - 1.29 (OD - 663) - 53.78(OD - 645)/220]$ respectively. The total phenolic contents of the plant extract were determined by following Iqbal *et al*. (2008) and comparing standard equation of Gallic acid as $Total\ Phenolic\ Content = Absorbance\ (at\ 765\ nm) - 0.0023/4.5929$. Concentrations were expressed in mg /10g of fresh weight of the plant. The flavonoid contents were determined by following Ardekani *et al*. (2011) and

putting the absorbance values in the equation $Total\ Falonoid\ Content = 2.735 * Absorbance\ (at\ 510nm) + 0.0551$. The data is being represented in the form of graphs as shown in figure 7. The specimens were named A for control, B1 for *Trichoderma harzianum*, B2 for *T. viride,* B3 for *T. asperellum*, C for *M. phaseolina*, D1 (B1+C) for *T. harzianum* + *M*. *phaseolina* D2 (B2+C) *T*. *viride* + *M*. *phaseolina*. Maximum results were recorded in control plant followed by *Trichoderma* spp. (Antagonist + Pathogen) and pathogen. The biochemical analysis for determination of Chlorophyll, Carotenoid, Phenols, Flavonoid and morphological parameters such as mean fresh height, mean fresh plant weight (g), mean no. of leaves per plant, mean length of leaf (cm), mean width of leaf (cm) were observed maximum in *Trichoderma* spp. followed by (Antagonist + Pathogen) and pathogen as shown in table 1.

Seeds of sunflower treated with different *Trichoderma* species, produced various effects on growth parameters when observed at different time intervals. Seeds of Sunflower treated with *T. harzianum* showed maximum germination (98%) followed by *T. viride* (97%) and *T. asperellum* (93 %) significantly higher than the control. Plant length and weight were significantly enhanced due to seed treatment with *Trichoderma* species in comparison to the control. In both observations, plants were larger when seeds were treated with *T. viride*, *T. harzianum* and *T. asperellum* after 30- and 60-days' interval ranked second highest (243 cm) in their response towards increment of plant length. Seeds of Sunflower were also treated with fungal plant pathogen (*M. phaseolina*) which reduced and inhibited the growth of the plant and cause a disease Charcoal rot. All *Trichoderma* species significantly reduced *M. phaseolina* colonization after 30- and 60-days' interval.

Furthermore, control of charcoal rot on sunflower using biocontrol agents is more environment friendly approach and needs attention in Pakistan. Biocontrol agents are environmental friendly as they cause no pollution and effects only target (invasive) plant. They are self-sustaining, cost effective as well. Moreover, this research may help in future for providing baseline data for further research on the use of other *Trichoderma* spp. as biocontrol agents. Additionally, the results support the development of environmentally friendly alternatives to chemical pesticides, potentially reducing the negative impacts of chemical pesticides on the environment, human health, and non-target organisms. In nutshell, the study provides significant insights into the potential use of *Trichoderma* spp. as natural biocontrol agents against drastic pathogen of sunflower and contributes to the ongoing search for sustainable, environmentally friendly alternatives to chemical pesticides.

Significance of the Study: The significance of this study lies in its potential to revolutionize sustainable agricultural practices, particularly in regions where sunflower crops are a significant part of the economy. By examining the combined effects of *Trichoderma* species and silver nanoparticles (AgNPs) on sunflower growth and soil health, the research offers valuable insights into alternative, environmentally friendly ways to enhance crop yield and quality. Moreover, the study has implications for soil conservation efforts, given that soil health is an often-overlooked factor affecting agricultural productivity. These outcomes not only benefit local farmers but could also serve as a model for sustainable farming practices globally, as the world grapples with the challenges of climate change and food security. The knowledge generated could be instrumental in informing agricultural policy and best practices, potentially transforming how we approach both crop management and environmental sustainability.

CONCLUSIONS

In conclusion, this research highlights the significant potential of *Trichoderma* species and biologically synthesized silver nanoparticles (AgNPs) in managing the challenging charcoal rot disease in sunflower. These biocontrol agents not only reduce disease incidence but also promote plant growth, offering a sustainable and eco-friendly alternative to chemical treatments. The study provides valuable insights into environmentally friendly methods for enhancing crop yield and quality, with implications for sustainable agricultural practices in regions where sunflower cultivation is economically vital. Moreover, the findings have the potential to influence agricultural policy and practices, contributing to the crop management and oil conservation efforts. This research serves as a key step toward a more sustainable and reliable agricultural future.

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