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Morphological and anatomical responses of *Vigna mungo* to *Fusarium oxysporum* infection and *Trichoderma* treatment

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ABSTRACT

Vigna mungo (L.) Hepper (black gram) is an economically important pulse crop whose productivity is severely constrained by *Fusarium* wilt, a destructive vascular disease caused by *Fusarium oxysporum*. Crop productivity is frequently constrained by biotic and abiotic stresses, among which fungal pathogens represent a major threat to legume cultivation. *Fusarium* oxysporum is a seed- and soil-borne pathogen capable of surviving in soil for several years in the absence of host plants, making disease management challenging. The present study investigates the morphological and anatomical changes that occur in *V. mungo* during *F. oxysporum* infection and evaluates the protective role of *Trichoderma* spp. as a biological control agent. Plants infected with *F. oxysporum* exhibited severe wilting, chlorosis, reduced growth, and extensive disruption of vascular tissues. In contrast, *Trichoderma*-treated plants showed improved growth performance, reduced disease severity, and restoration of normal anatomical structures. These findings demonstrate that *Trichoderma* effectively mitigates *Fusarium* wilt by preserving tissue integrity and enhancing host defense, highlighting its potential as an eco-friendly strategy for sustainable disease management in black gram.

Key Words - *Vigna mungo, Fusarium* wilt, *Fusarium oxysporum, Trichoderma*, morphology, anatomy, biocontrol.

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INTRODUCTION

Black gram (*Vigna mungo* (L.) Hepper) is an important legume crop valued for its high protein content and its role in improving soil fertility through nitrogen fixation. In addition to its nutritional importance, crop residues are widely used as cattle feed and green manure. According to Rawal (2019), pulses such as black gram are essential for global food security because of their high protein content and ability to improve soil fertility. Studies by Banerjee *et al.* (2021) and Gurumurthy *et al.* (2019) reported that the growth and productivity of black gram are strongly influenced by environmental stresses, which often reduce yield and weaken plant health. However, the productivity of black gram is severely affected by biotic stresses, particularly fungal diseases.

Among biotic stresses, Fusarium wilt has become a serious problem in black gram cultivation. Biswal *et al.* (2020) identified *Fusarium oxysporum* as a major cause of wilt in black gram, particularly in the western regions of Odisha, indicating that the disease is spreading to new areas. The pathogen shows high variability, which makes it difficult to manage. Nath *et al.* (2017) observed wide differences among *F. oxysporum* isolates in colony colour, growth pattern, and physiological behaviour. Similar findings were reported by Younesi *et al.* (2021), who showed that *Fusarium* species associated with chickpea wilt differ greatly in their morphology, molecular traits, and disease-causing ability. Several studies have reported the association of multiple fungal pathogens with legume seeds, including black gram, resulting in poor seedling vigor (Hussain *et al.,* 2007).

Fusarium oxysporum mainly infects plant roots and then spreads through the vascular system, blocking water movement and causing wilting symptoms. Sun *et al.* (2017) demonstrated that wilted plants infected with *Fusarium* do not always suffer from water shortage, suggesting that damage to vascular tissues is the primary reason for wilting. Sinha *et al.* (2018), in their review on wilt management in pulses, pointed out that the soil- and seed-borne nature of *Fusarium* allows it to survive in the soil for long periods, making chemical control less effective and environmentally unsafe.

Biological control using antagonistic fungi such as *Trichoderma* spp. has gained importance as an eco-friendly highly effective fungal biopesticide alternative to chemical control (Konappa *et al*., 2022). Mishra *et al*. (2021) isolated native *Trichoderma* strains from pulse rhizospheres and showed their strong antagonistic activity against *Fusarium oxysporum*. Sharma *et al.* (2020) also emphasized that secondary metabolites produced by beneficial microbes play a key role in suppressing plant pathogens. In the present study, the reduced vascular damage and limited fungal colonization observed in Trichoderma-treated plants suggest a combination of antagonistic activity and enhanced host defense responses.

Although many studies have focused on pathogen diversity, plant defense responses, and biocontrol strategies but limited information is available on the morphological and anatomical changes in black gram plants during *Fusarium* infection and after *Trichoderma* treatment. This gap highlights the need for detailed studies to understand how disease affects plant structure and biological control agents help in reducing tissue damage. Therefore, the present study aims to investigate the morphological and anatomical changes in *Vigna mungo* during *Fusarium oxysporum* infection and to evaluate the protective role of *Trichoderma* spp. in reducing disease-induced structural damage.

MATERIALS & METHODS

Plant material, pathogen, and biocontrol agent:

Black gram (*Vigna mungo*) variety 'WBU109' seeds were obtained from Birsa Agricultural University (BAU), Ranchi, Jharkhand, India. A pure culture of *Fusarium oxysporum* (wilt pathogen) was obtained from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, ICAR–Indian Agricultural Research Institute (IARI), New Delhi, India, for inoculation. *Trichoderma viride* (*CFU* $2*10^9$) was used as the biocontrol agent.

Growth conditions and stress treatments

Surface-sterilized seeds of *Vigna mungo* were grown under controlled conditions in sterilized soil. Fifteen-day-old seedlings were inoculated with potato dextrose agar (PDA) broth containing *Fusarium oxysporum*. Inoculation was repeated at three-day intervals to ensure successful pathogen establishment. Initial disease symptoms appeared approximately eight days after the first inoculation. The experiment consisted of three treatments: (i) control (healthy plants), (ii) plants inoculated with *Fusarium oxysporum*, and (iii) plants treated with *Trichoderma* spp. followed by *F. oxysporum* challenge.

Morphological Analysis

Morphological data were recorded fifteen days after inoculation. Morphological parameters including plant height, number of leaves, leaf size, number of infected leaves, leaf colour, nodes per plant and root length were recorded at regular intervals after inoculation.

Anatomical Studies

For anatomical and colonization studies, collected root, leaf, and stem samples were thoroughly washed under running tap water to remove adhering soil particles. Root samples were cut into approximately 1 cm segments and subjected to a clearing process using 10% potassium hydroxide (KOH) at 90^{o}C for 30–60 min. After clearing, the roots were rinsed with distilled water and acidified in 1% hydrochloric acid (HCl) for 5 min. The samples were then stained with lactophenol cotton blue for 1–2 h or overnight to visualize fungal colonization. Excess stain was removed by destaining the samples in glycerol. Finally, stained root segments were mounted on glass slides and examined under a compound microscope at 45× magnifications to assess anatomical features and fungal colonization.

RESULTS

Morphological Changes

Plants infected with *F. oxysporum* showed pronounced wilting, leaf yellowing, reduced root growth. In contrast, *Trichoderma viride* treated plants exhibited improved growth parameters and reduced disease symptoms, closely resembling healthy controls.

Table 1. Morphological parameters of *Vigna mungo* under different treatments

Parameter	Control	*F. oxysporum*–infected	*Trichoderma* + *F. oxysporum*
Plant height (cm)	15.24	13.46	21.08
Number of leaves per plant	5-6	5-6	6-8
Number of infected leaves	0	24 infected leaves in 15 plants	10 infected leaves in 15 plants
Leaf size (cm)	4.00	3.34	5.54
Leaf color	Green	Yellowing (chlorosis)	Chlorophyll loss
Nodes per plant	6-7	3-4	6-7
Root length (cm)	8-10	4.5-5.5	18-20
Disease symptoms	None	Severe wilting and chlorosis	Comparatively mild symptoms

Fig. 1- Initial signs observed at 8th day post-inoculation

Fig. 2- Symptoms at 15th day post-inoculation

Fig. 3- Showing *Vigna mungo* plants - A (*Fusarium oxysporum* infected), B (*Fusarium oxysporum + Trichoderma viridae*), C (showing wilting of plant)

Thin transverse sections of the root :

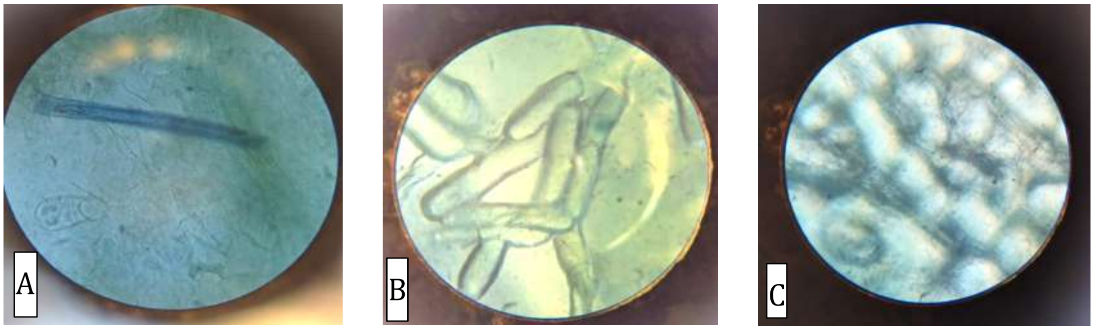

Fig: 4 (A & B) Presence of fungal hyphae colonizing the root tissues, indicating active pathogen invasion.

(C) Extensive tissue damage with dense fungal colonization, reflecting severe infection and disruption of normal cellular organization

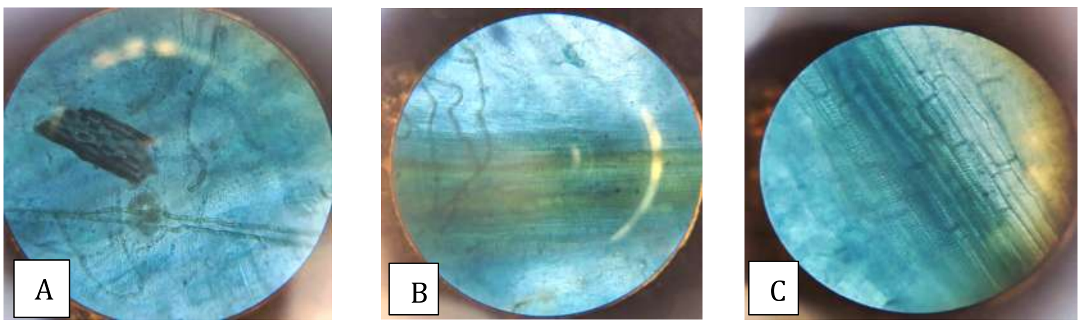

Fig. 5 (A) Presence of *Trichoderma* hyphae in close association with root tissues, indicating successful colonization without causing structural damage.

(B) Well-organized vascular tissues with intact xylem elements, showing absence of vascular blockage and improved tissue continuity.

(C) Restored cellular organization and enhanced tissue integrity, suggesting protective effects of *Trichoderma* against pathogen-induced damage.

Thin transverse sections of the shoots:

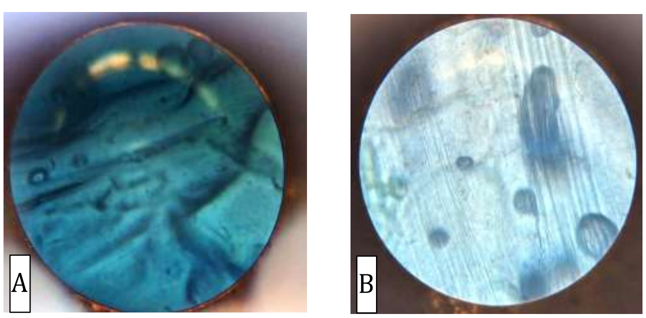

Fig. 6- Microscopic analysis of thin shoot sections from *Fusarium oxysporum*–infected plants at 45× magnification.

(A) Disorganization of shoot tissues with visible cellular distortion caused by pathogen invasion.

(B) Extensive structural degeneration with distorted vascular tissues and loss of normal cellular integrity, indicating advanced infection and impaired shoot architecture.

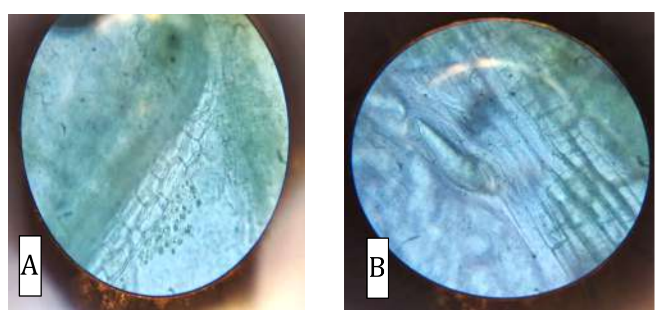

Fig. 7- (A) Transverse section of the stem of a *Trichoderma*-treated plant under *Fusarium* infection showing intact vascular bundles and limited fungal hyphal penetration.

(B) *Trichoderma*-treated stem section showing reduced cell wall degradation and suppressed pathogen spread within cortical tissues.

Thin transverse sections of the leaves:

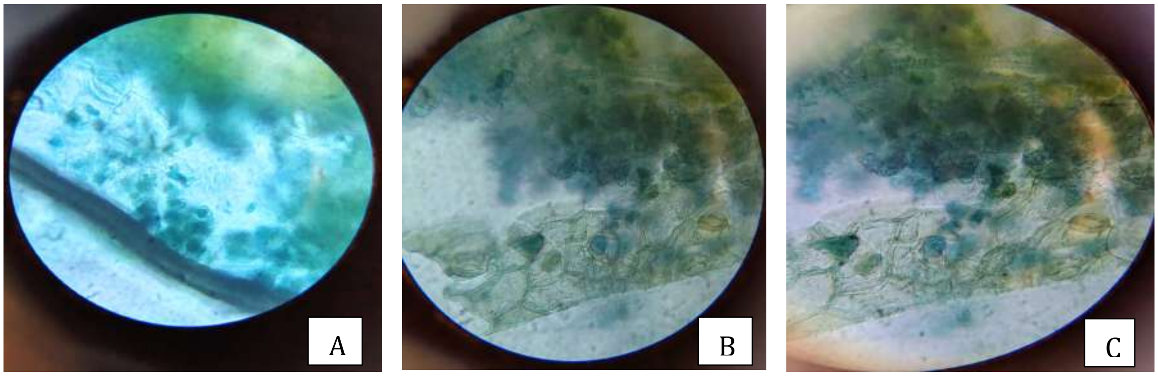

Fig. 8 (A–C)- Microscopic sections of leaves infected with *Fusarium* spp. showing progressive tissue damage, cellular disorganization, and fungal colonization within leaf tissues.

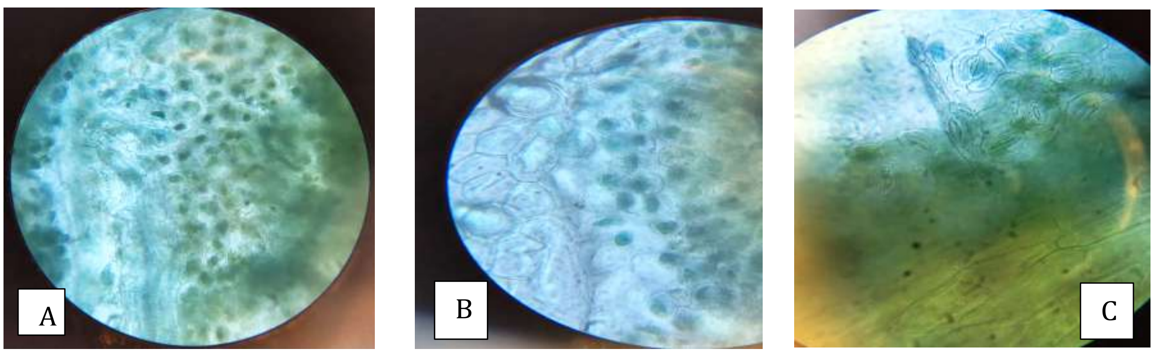

Fig. 9- Representing microscopic view of leaf thin sections from *Vigna* mungo plants treated with *Trichoderma* and infected with *Fusarium*.

(A) Leaf epidermal and mesophyll cells appear well organized with less damage caused by the pathogen.

(B) Enlarged view of the epidermis showing clear cell walls and stomata, indicating better cell condition in *Trichoderma*-treated plants.

(C) Transverse section of the leaf showing tightly arranged mesophyll cells with less tissue breakdown compared to the infected control.

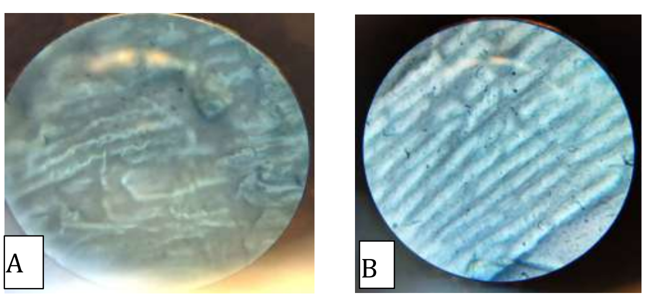

Fig. 10 (A &B)- Showing microscopic observation of root and shoot tissues from wilt-affected plants.

Anatomical Changes

Thin transverse sections of the root, shoot, and leaf tissues were prepared and examined under a compound microscope at 45× magnification. Microscopic analysis of *Fusarium oxysporum*–infected plants revealed extensive structural damage. The cortical tissues were severely disrupted, and the xylem vessels showed marked deformation. Fungal hyphae were clearly observed colonizing the vascular tissues, leading to blockage of xylem elements. This vascular obstruction likely impaired water and nutrient transport, contributing to the overall decline in plant health.

In contrast, plants treated with *Trichoderma* exhibited a much healthier tissue structure. The overall organization of cortical and vascular tissues was largely restored, with minimal xylem blockage. Cellular integrity was well maintained, and signs of fungal invasion were significantly reduced. These observations suggest that *Trichoderma* treatment effectively protected plant tissues from pathogen-induced damage and helped preserve normal vascular function.

DISCUSSION

Fusarium oxysporum infection in *Vigna mungo* resulted in morphological changes characteristic of *Fusarium* wilt in legumes. Structural damage to internal tissues, particularly the vascular system, disrupts water and nutrient transport, leading to physiological dysfunction and plant wilting. In contrast, *Trichoderma* treated plants exhibited reduced disease severity, improved growth parameters, and restoration of near normal anatomical organization.

The protective effect of *Trichoderma* is likely associated with its ability to suppress pathogen colonization and enhance host defense responses, thereby preserving tissue integrity and vascular function. These findings support the potential application of *Trichoderma* spp. as an effective biological control agent for sustainable management of *Fusarium* wilt.

CONCLUSION

Fusarium oxysporum infection induces severe morphological and anatomical damage in *Vigna mungo*. Application of *Trichoderma* significantly reduces disease severity and preserves tissue integrity. The study provides structural evidence for the biocontrol efficacy of *Trichoderma* and supports its integration into *Fusarium* wilt management strategies.

REFERENCES

Banerjee P., Venugopalan V.K., Nath R., Althobaiti Y.S., Gaber A., Al-Yasi H., Hossain A. 2021 Physiology, growth, and productivity of spring-summer black gram (*Vigna mungo* L. Hepper) as influenced by heat and moisture stresses under different sowing dates and nutrient management conditions. *Plants* 11(11): 23-29

Biswal K., Sahu V., Ranasingh N., Moharana R.L. 2020. *Fusarium oxysporum* causing black gram wilt: a new threat in the western undulated zones of Odisha. *International Journal of Current Microbiology and Applied Sciences.* 9(6): 3027-3031

Gurumurthy S., Sarkar B., Vanaja M., Lakshmi J., Yadav S.K., Maheswari M. 2019. Morpho-physiological and biochemical changes in black gram (*Vigna mungo* L. Hepper) genotypes under drought stress at flowering stage. *Journal of Plant Physiology*. 41(42): 1-14

Hussain A., Iqbal S.M., Ayub C.M., Iqbal J. 2007. Seed-borne fungi associated with legume crops and their effect on seed germination and seedling vigor. *Pakistan Journal of Phytopathology.* 19: 38-43

Konappa N., Dhamodaran N.D., Shanbhag S.S., Sampangi M.A., Krishnamurthy S., Arakere U.C., Chowdappa S. and Jogaiah S. 2022. *Trichoderma*: a potential biopesticide for sustainable management of wilt disease of crops. *Science Direct.* 2(823355): 261-275.

Mishra R.K., Mishra M., Pandey S., Saabale P.R., Naimuddin. 2021. Exploring indigenous *Trichoderma* strains from pulse rhizosphere and their biocontrol potential against *Fusarium oxysporum* f. sp. ciceri in chickpea. *Journal of Plant Diseases and Protection.* 75: 273-278

Nath N., Ahmed A.U., Aminuzzaman F.M. 2017. Morphological and physiological variation of *Fusarium oxysporum* f. sp. ciceri isolates causing wilt disease in chickpea. *International Journal of Environment, Agriculture and Biotechnology.* 2(1): 202-212

Rawal V. 2019. The global economy of pulses. Food and Agriculture Organization of the United Nations, Rome, pp 4-190

Sharma D., Gupta S., Gupta M., Summuna B. 2020. Exploration of secondary metabolites for management of chickpea diseases. *Journal of Applied and Natural Science.* 8(2): 15-33

Sinha P., Rizvi G., Parashar R. 2018. Management of wilt disease of pulses: a review. *Journal* *of Pharmacognosy and Phytochemistry.* 6(4): 696-708

Sun Y., Wang M., Li Y., Gu Z., Ling N., Shen Q., Guo S. 2017. Wilted cucumber plants infected by *Fusarium oxysporum* f. sp. cucumerinum do not suffer from water shortage. *Annals of Botany.* 120(3): 427-436.

Younesi H., Darvishnia M., Bazgir E. and Chehr K. 2021. Morphological, molecular and pathogenic characterization of *Fusarium* spp. associated with chickpea wilt in western Iran. *Journal of plant protection research.* 61(4):402-413.

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