

Influence of *Penicillium* spp. and *Chenopodium murale* application on histopathology of Fusarium wilt-affected tomato plants

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Abstract

Fusarium wilt of tomato is caused by a soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL). A pot experiment was carried out to examine the effect of two antagonistic fungi namely *Penicillium digitatum* and *P. expansum*, and dry plant material (1, 2, and 3% w/w) of *Chenopodium murale* on histopathology of tomato plants grown in the FOL contaminated soil. These treatments were applied to the soil either separately or in different combinations. Transverse sections of root and stem were stained in three reagents, namely Lugol's iodine to detect starch contents, ferric chloride to estimate phenolics, and safranin-fastgreen to detect lignin. Results showed that there were high concentrations of phenols, starch and lignin contents in root and stem sections of positive control (where only pathogen was inoculated) while concentrations of these compounds were very low in the negative control. Application of dry biomass of *C. murale* (DBC) and the two *Penicillium* spp. reduced the concentrations of these compounds to variable extents. The effect of *Penicillium* spp. was more pronounced than DBC. This study concludes that DBC and *Penicillium* spp. treated plants showed a resistance mechanism against FOL.

Keywords: Biocontrol, Fusarium wilt, Histopathology, Nettle-leaved goosefoot, *Penicillium* spp., Tomato.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and extensively consumed horticultural crops. It can be eaten in various ways and in a countless number of dishes (Ochilo *et al.*, 2019). Fungal pathogens cause huge economic losses due to reduced and lower-quality tomato production (AbdElfatah *et al.*, 2021; Khan and Javaid, 2022a; Jafri *et al.*, 2024). Among them, Fusarium wilt is a major disease of cultivated tomatoes caused by *F. oxysporum* f. sp. *lycopersici*, which is a soil-borne fungal pathogen (Khurshid *et al.*, 2016; Tetorya and Rajam, 2021). It can remain in the soil for many years and become more prevalent under warm and humid environmental conditions (Katan *et al.*, 1997). It often produces infectious structures and certain type of toxins (Murugan *et al.*, 2020) that penetrate through plant vascular system, which ultimately results in blockage of xylem vessels (Srinivas *et al.*, 2019). Moreover, mycelium laterally penetrates to the adjacent cells, resulting in clogging of vascular bundles and plant suffers from lack of water supply. In response to insufficient water supply, leaves start wilting and plant dies prematurely (Manikandan *et al.*, 2018; Malbrán *et al.*, 2020). The observed plant symptoms include browning, leaf yellowing, wilting, abscission, necrosis and chlorosis (Maurya *et al.*, 2019). Management of Fusarium wilt of tomato is quite challenging; however, many fungicides are being used against the pathogen (Akram *et al.*,

2018). However, these chemicals get accumulated in the environment and pose serious threat to the humans and animals (Gupta, 2018). Therefore, there is a strong need to develop alternative control measures that are easily biodegradable and environment friendly. Thus, many researchers are focusing on biocontrol agents and natural plant products for the control of soil-borne diseases (Ali *et al.*, 2020; Jabeen *et al.*, 2021).

Biological control of soil-borne pathogens by antagonistic microorganisms has been considered a good alternate to the chemical treatment methods (Akhtar and Javaid, 2018; Zohaib *et al.*, 2024). Many antagonistic microbes have been proved to be active under *in vitro* and *in vivo* conditions (Caulier *et al.*, 2018; Sharf *et al.*, 2021). Among them, *Penicillium* spp. are well-known that have the ability to produce secondary metabolites responsible for control of plant pathogens (Javaid *et al.*, 2020; Khan and Javaid, 2022b). These antagonists possess various mechanisms of action based on the production of antimicrobial compounds (Guijarro *et al.*, 2019; Khan and Javaid, 2021), induction of resistance (Carreras *et al.*, 2020), and establishment of mycoparasitic interactions (Villarino *et al.*, 2018). In a recent study, *F. oxysporum* f. sp. *lycopersici* was effectively managed by using conidial suspension of *Penicillium* species (Nigam *et al.*, 2021). Larena *et al.* (2003) reported that the application of *P. oxalicum* could inhibit the growth of soil-borne fungal pathogens of tomato under field conditions.

Several plant species belonging to family Chenopodiaceae are known to exhibit antifungal activity against many fungal pathogens (Ali *et al.*, 2017; Khan and Javaid, 2020). *Chenopodium murale* commonly known as Nettleleaf goosefoot, is a cosmopolitan fast-growing annual weed plant (Bajwa *et al.*, 2019). It is propagated through seeds and found abundantly in different habitat types. It grows under a wide range of environmental conditions including soil pH, temperature and moisture (Gupta and Narayan, 2012). It possesses certain antimicrobial, nematocidal and pesticidal properties (Naqvi *et al.*, 2020), and has been tested against a wide range of pathogens (Abdel-Aziz *et al.*, 2014; Naqvi *et al.*, 2019). Therefore, the present study was carried out to investigate the effect of *Penicillium* spp. and *C. murale* dry plant biomass on histopathology of tomato plants exposed to *F. oxysporum* f. sp. *lycopersici*.

Materials and Methods

Pot experiment

A pot experiment was carried out in earthen pots of 36 cm deep. Each pot was filled with 10 kg of fumigated soil. FOL inoculum was prepared on autoclaved pearl millet seeds and mixed in each pot @ 10 g kg⁻¹ of soil except in the negative control, where only sterilized seeds were mixed in the same concentration. Thereafter, pots were left for one week followed by the mixing of *P. digitatum* and *P. expansum* inocula in pot of respective treatments. *C. murale* dry plant biomass was added at different concentrations (1, 2 and 3%), while positive control was treated only with FOL inoculum. Fertilizers such as K₂O at 60 mg kg⁻¹, nitrogen at 30 mg kg⁻¹, and P₂O₅ at 10 mg kg⁻¹ were mixed to each pot. There were 11 treatments with four replications including soil amendment with 1, 2, and 3% *C. murale*, *P. digitatum*, *P. expansum*, and their combinations. Four-week-old seedlings @ 10 per pot were planted in each pot and watered on regular basis. After maturity, plants from each treatment were uprooted and washed carefully.

Histological studies

Fresh tomato plants from all the treatments were selected and hand-cut transverse sections of stem as well as root were prepared in radial plane of a cylindrical portion by using a blade. The selected sections from each treatment were stained in three different reagents. Firstly, specimens were dipped in Lugol's Iodine for 2 min to detect starch contents (Hinchman, 1973). Ferric chloride stain was used to estimate phenolic compounds (Pomer *et al.*, 2004). Likewise, samples were washed with safranin-fastgreen to detect lignin deposited areas (Johansen, 1940). Each treated section was mounted on clean glass slide and observed under light microscope. Digital photography was performed in different

directions to capture microscopic structures of plant tissues and to reveal the presence of physical barriers in response to pathogen attack.

Results

Effect of soil amendments on histology

Plants infected with FOL showed a significant reduction in plant growth due to the disintegration of internal tissues. Growth impairments were higher in stem as compared to root tissue. This was might be due to the pathogen colonization in vessels and adjacent tissues which resulted towards the blockage of xylem vessels. Moreover, infected tissues showed higher concentrations of lignin and phenolic compounds along with observable changings in morphology of diseased areas.

Effect on stem histology

Tomato stem sections from all the treatments were stained with ferric chloride, iodine and safranin-fastgreen solution as given in Fig. 1, 2 and 3, respectively to check differences among anatomical structures. Typical symptoms of Fusarium wilt were observed as browning of vascular tissue upon basal stem cut. Stem samples showed vibrant and sharp cell structures. A single layered epidermis with compactly arranged collenchyma cells followed by several layers of parenchymatous cells was visible (Fig. 2 A). In positive control treatment, whole cell structures from epidermis to pith was spoiled, vascular tissues were disintegrated which led towards deformed pith and disrupted epidermal cells (Figure 2 B).

Stem sections stained with ferric chloride yielded black color to confirm the presence of phenolic acid. In negative control treatment, phenolic acid was present in very low amount whereas in positive control, higher concentrations of phenolic acid turned the whole section into black color (Fig. 1 A and B). Soil amendment with DBC, *P. digitatum* and *P. expansum* alone or in different combinations changed the amount of phenolics. In treatments where soil was amended with *P. digitatum* only (Figure 1 F) and *P. digitatum* + 2 % DBC (Fig. 1 H), the phenolics were more visible around the vascular bundles whereas higher dose (3%) of dry biomass of *C. murale* along with *P. digitatum* and *P. expansum* exhibited very less deposition of phenolics, which depicts the inhibition of pathogen in these amendments (Fig. 1 I–M).

Iodine stained sections exhibited very less starch grains in negative control while these were observed mainly in positive control and in treatments where soil was amended with different concentrations of DBC alone. The soil amendment with biocontrol agents *P. digitatum* and *P. expansum* along with DBC exhibited very few starch grains (Fig. 2 F–K).

After staining with safranin-fastgreen, samples from positive control treatment showed a thick cuticle and an increased lignin concentration (Fig. 3 B). Elevated levels of lignin and phenolic compounds in response to pathogenic infections is a general plant response. On the other hand, no increase in lignin concentration or wall thickening observed in negative control treatments (Fig. 3 A). A slight thickening in cuticle was observed in treatments in which soil was amended with only 1% DBC + biocontrol agent or biocontrol agents alone (Fig. 3 F and G). Infection initiated by FOL resulted in disruption of overall stem anatomy. Conversely, visible positive effect on vascular bundles and no cell wall thickening was seen in plants where soil was amended with *Penicillium* species and various doses of DBC. In positive control treatment, cell wall thickening was considerably high. Cells with lyseginous cavities were also observed with slight thickening in plants of positive control treatment. Tomato has bicollateral vascular bundles which were highly disintegrated in positive control. In negative control treatments, vascular bundles were shown compact and no visible fungal traces were observed.

Effect on root histology

Root sections stained with ferric chloride, iodine and safranin-fastgreen solution showed different anatomical changes than stem as presented in Fig. 4, 5 and 6, respectively. In negative control treatment, roots were well-grown with plenty of root hairs. In positive control treatment, roots appeared stunted and dried. The stele was visible in the root cross sections in the form of ring comprising of four vascular bundles, well arranged in bicollateral position (Fig. 5). Fungal mycelial growth was observed in root cortical cells, pith and xylem cells. Phloem and xylem tissues were significantly reduced in the positive control. Inhibition of root elongation was also observed in positive control. Root elongation is the result of various processes including division of cells and cell enlargement which seems to hinder in positive control treatments. Growth of root hair was also less in positive control treatment (Fig. 6 B). Root hair were visible in negative control and other treatments with soil amendment of *P. digitatum*, *P. expansum* and different doses of DBC.

Sections stained with ferric chloride indicated the presence of phenolics. In the negative control, the amount of phenolics was very low whereas in positive control, black coloration showed higher accumulation of phenols (Fig. 4 A and B). Treatments in which soil was amended with the lowest concentration of DBC only, phenolic acid was more abundant around the vascular bundles. However, dry biomass of *C. murale* along with *P. digitatum* and *P. expansum* exhibited very less amount of phenolics depicting the suppression of FOL in these amendments (Fig. 4 G–M).

Iodine stained sections exhibited black colored starch granules which were formed by the presence of amylose in starch. In negative control, no grains were found, whereas, in positive control, blackish starch granules were observed with disintegration of parenchymatous cells (Fig. 5 A and B). Starch grains were more dense around the vascular bundles and in the cortical parenchymatous cells in the treatments where soil was amended with only 1% dry biomass of *C. murale* alone or in combination with *P. digitatum* (Fig. 5). In other treatments in which soil was amended with higher doses (2 to 3%) of DBC along with *P. expansum*, no starch grains appeared (Fig. 6 H–M).

In case of roots stained with safranin-fast green, both thin and thick walled lignified secretory cells were found. Lignification is a normal mechanism of cell differentiation, which is also triggered in response of pathogen attack. In negative control treatment, lignification was not seen, on the other hand, lignified cells were visible in positive control treatment (Fig. 5 A and B). In the case of positive control, higher ligno-suberization was observed in comparison to negative control. Disintegrated and narrow vessels were also appeared in positive control with their reduced number. Soil amendments with various doses of DBC along with *P. digitatum* and *P. expansum*, showed an increase in vessel number as compared to positive control. Moreover, in all other treatments, vessels appeared wider in diameter (Fig. 6 C–M).

Cell browning was also observed in all stem and root sections, which was possibly due to the production of tannins. These are the polyphenolic compounds like polyphenol oxidase (PPO) that can cause postharvest browning in plants. Varied amounts of tannin deposits were observed in stem and root sections. Browning was more commonly observed in the vascular region of both stem and root sections (Fig. 2 C–M and 5 C–M).

Discussion

In nature, plants are exposed to a wide range of fungal pathogens and as a result these have evolved the production of chemical defense related enzymes as well as physical barriers to protect themselves against pathogenic attacks (Pouzoulet *et al.*, 2014). In the present study, three histochemical strains were used to examine the differences in healthy and FOL infected plants treated with DBC, *P. digitatum* and *P. expansum*. Plant phenolics are secondary metabolites that have a broad range of physiological role in plants. These are considered as defense related compounds which are produced in excessive amounts against pathogenic infections (Vermerris and Nicholson, 2008). Plant sections from DBC and antagonistic fungi treated soils exhibited a significant increase in phenolic compounds as compared to negative control where it was present in lower concentrations. Previously,

Báidez *et al.* (2007) also reported the presence of excessive number of phenolic compounds in *Verticillium dahliae* infected olive plants. Moreover, in response to pathogenic infections phenolic compounds are produced excessively in xylem vessels as observed in *Ulmus minor* trees. These phenols induced resistance mechanism against *Ophiostoma novo-ulmi*, the causal agent of Dutch elm disease (Martín *et al.*, 2008). Mace *et al.* (1972) also reported the presence of phenols in parenchymatous cells of tomatoes infected with FOL whereas, it was absent in healthy plants. Similarly, Cooper and Williams (2004) found that different plant species restricted the movement of *Verticillium dahliae*, a xylem invading pathogen through the production of phenolics. *Ophiostoma novo-ulmi* sporulation and mycelial growth was also controlled by the production of phenols in elm plant xylem tissues (Martín *et al.*, 2010).

Similarly, in negative control plants starch granules were not visible whereas in positive control these were present in higher concentrations. The plants grown in lower concentrations of DBC exhibited a significant amount of starch granules in comparison of higher doses and antagonistic treated plants. Pitino *et al.* (2018) found that starch produces in higher amounts in vascular bundles of citrus plant under pathogen stress. Pepper plants infected with *Verticillium dahliae* also showed the elevated levels of starch granules in xylem vessels (Goicoechea *et al.*, 2000). A similar finding was also given by Joyce *et al.* (2016) where sugarcane plants exhibited a higher concentration of starch granules in response to biotic stress. Plants evolved different sophisticated defense related mechanisms to protect themselves against biotic stresses (Miedes *et al.*, 2014). Previously, Zhang *et al.* (2017) reported that lignin plays a key role in plant defense mechanism through the formation of chemical and physical barriers to restrict or limit the colonization of pathogen growth. In the present study, stem and root sections from negative control plants exhibited no lignin deposits as compared to positive control plants. Earlier, Zhang *et al.* (2019) reported cell wall lignification in cotton plants as a defensive mechanism to restrict the movement of vascular wilt pathogen namely *Verticillium dahliae*. Hence, it is an important component which makes cell wall more resistant due to the toxins production to prevent pathogen invasions (Sattler and Funnell-Harris, 2013).

Cell browning was also observed in all stem and root sections which might be the result of tannin production in infected tissues. Walker (1971) reported vascular browning as one of the most common symptom of tomato Fusarium wilt disease. Tomato plants infected with FOL also showed the browning of parenchyma cells as well as xylem tissues in comparison of healthy tissues even at early stage of infection (Mace *et al.*, 1972). Moreover, in accordance to the present studies, *Arabidopsis thaliana* susceptible towards downy mildew exhibited distorted cell structures, pronounced sporulation, conidiophore production, mycelial growth and haustoria formation in comparison of negative control plants (Koch and Slusarenko, 1990). De Wit (1977) observed tomato plants infected with *Cladosporium fulvum* under microscope. The plant sections were completely collapsed, turned brown in color with abundant hyphal growth. Likewise, *Vigna radiata* infected with *Macrophomina phaseolina* showed the excessive accumulation of phenols, starch and lignin contents as a defensive mechanism in comparison of negative control plants. Moreover, plants grown in Plant growth-promoting rhizobacteria (PGPR) treated soils were diseased free with intact cell structures (Javed *et al.*, 2021).

Conclusion

The present study concludes that tomato plants roots and stem sections infected with FOL exhibited an increased production of phenols, starch and lignin contents. However, soils amended with DBC and antagonistic *Penicillium* species play a key role in arresting the growth of targeted pathogen. In addition to this, a significant reduction of phenols, starch and lignin was observed with intact plant tissues. Hence, it can be concluded that DBC and *Penicillium* spp. not only reduced the pathogen growth but also activated the resistance mechanism against FOL in tomato plants.

Contribution of authors

SFN did experimental work. IHK wrote the initial draft of paper. AJ supervised the work, and thoroughly revised and finalized the paper.

Conflict of interests

The authors declare no potential conflict of interest.

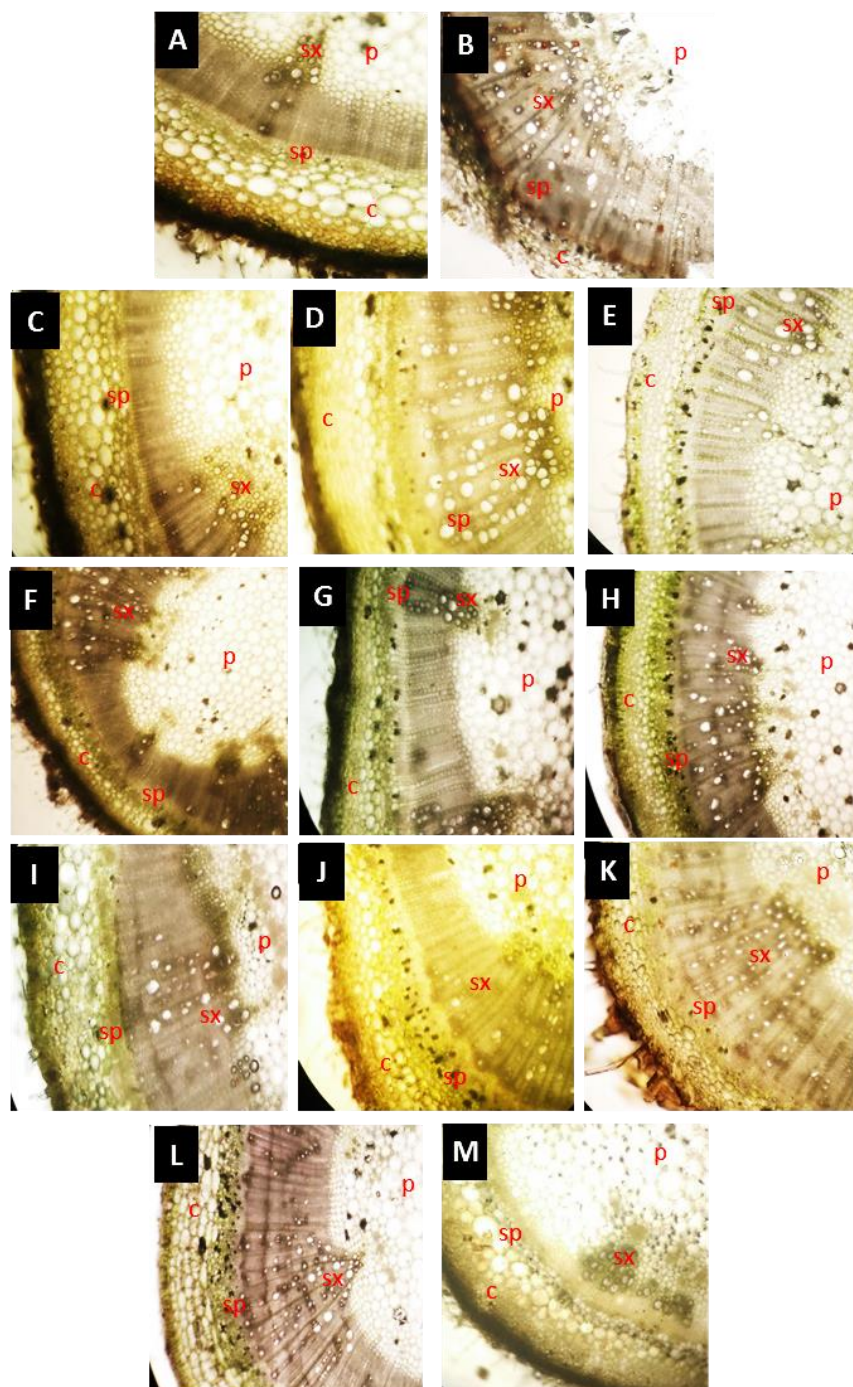


Fig. 1 (A–M): Cross sections of tomato stem stained with ferric chloride solution showing anatomical changes upon exposure to *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Penicillium digitatum* (PD) and *P. expansum* (PE), and different doses of dry biomass of *Chenopodium murale* (DBC).

A: Negative control, **B:** Positive control (FOL), **C:** 1% DBC+FOL, **D:** 2% DBC+FOL, **E:** 3% DBC+FOL, **F:** PD+FOL **G:** 1% DBC+PD+FOL, **H:** 2% DBC+PD+FOL, **I:** 3% DBC+PD+FOL, **J:** PE+FOL, **K:** 1% DBC+PE+FOL, **L:** 2% DBC+PE+FOL, **M:** 3% DBC+PE+FOL.

c: cortex, **sp:** secondary phloem, **sx:** secondary xylem, **p:** pith, **ep:** epidermis.

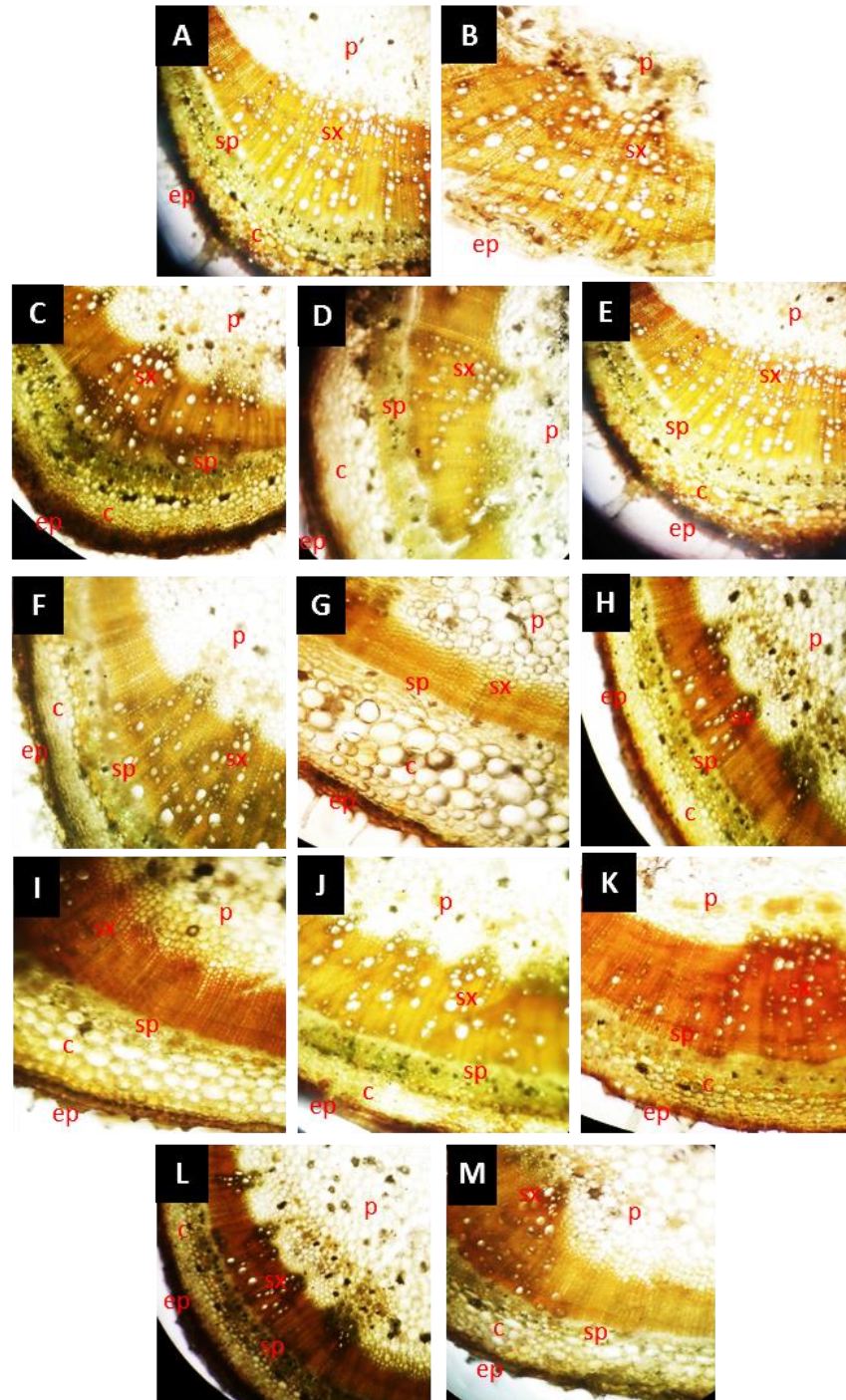


Fig. 2 (A–M): Cross sections of tomato stem stained with iodine solution showing anatomical changes upon exposure to *Fusarium oxysporum* f. sp. *lycopersici*, *Penicillium digitatum* and *P. expansum*, and different doses of dry biomass of *Chenopodium murale*. For details of treatments A–M, see Fig. 1.
c: cortex, **sp:** secondary phloem, **sx:** secondary xylem, **p:** pith, **ep:** epidermis.

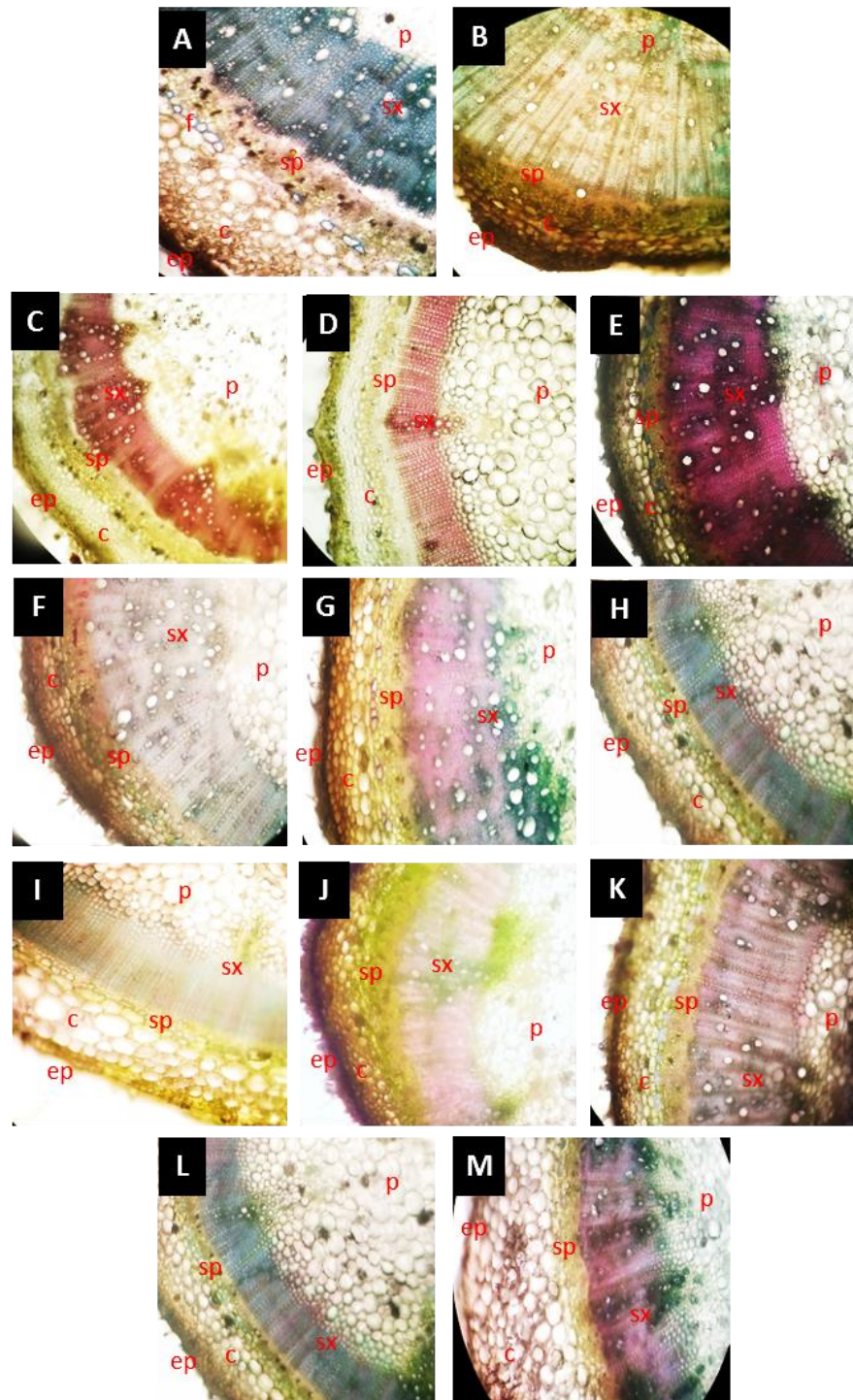


Fig. 3 (A–M): Cross sections of tomato stem stained with safranin-fast green solution showing anatomical changes upon exposure to *Fusarium oxysporum* f. sp. *lycopersici*, *Penicillium digitatum* and *P. expansum*, and different doses of dry biomass of *Chenopodium murale*. For details of treatments A–M, see Fig. 1.

c: cortex, **sp:** secondary phloem, **sx:** secondary xylem, **p:** pith, **ep:** epidermis.

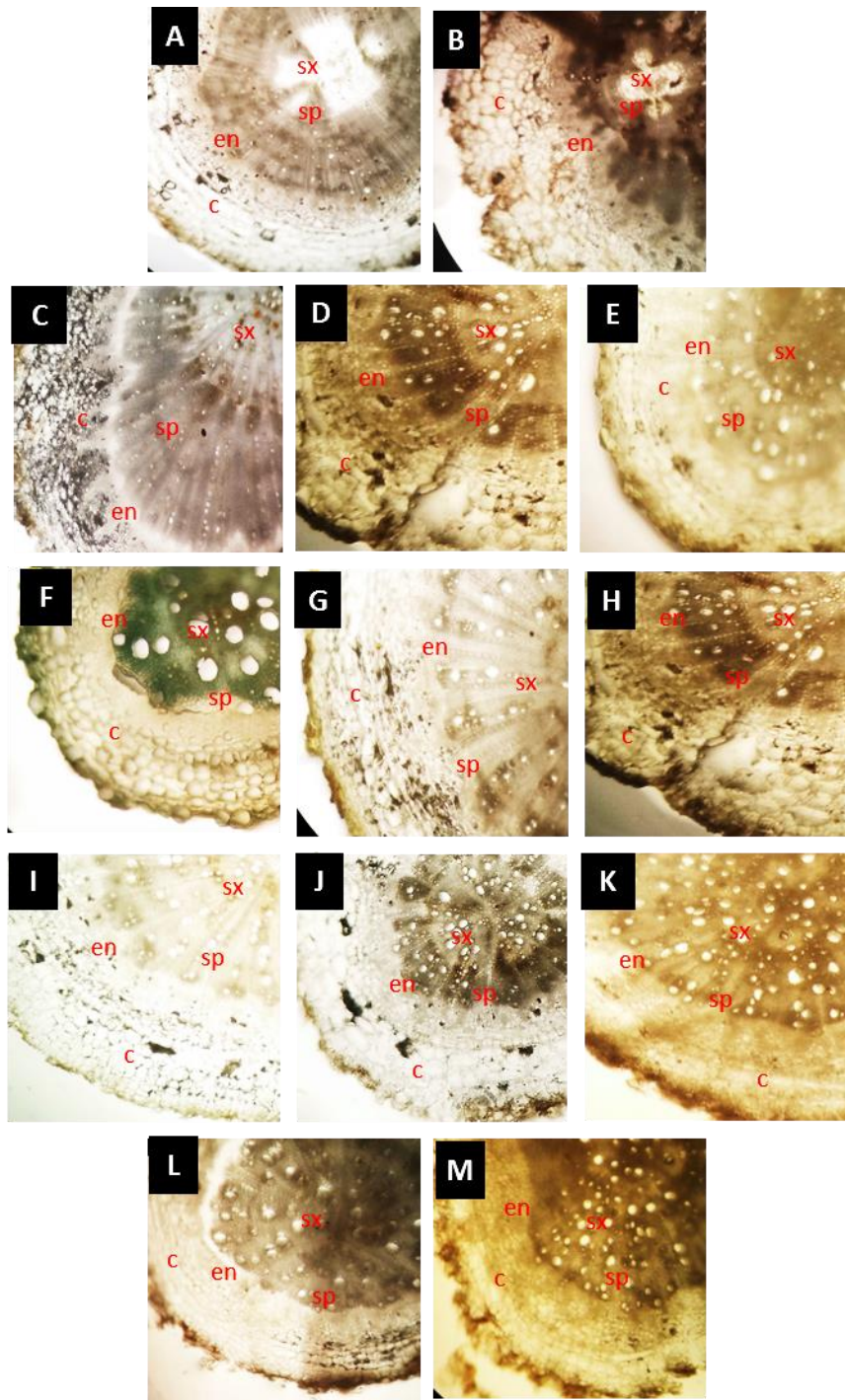


Fig. 4 (A–M): Cross sections of tomato root stained with ferric chloride solution showing anatomical changes upon exposure to *Fusarium oxysporum* f. sp. *lycopersici*, *Penicillium digitatum* and *P. expansum*, and different doses of dry biomass of *Chenopodium murale*. For details of treatments A–M, see Fig. 1. **c:** cortex, **sp:** secondary phloem, **sx:** secondary xylem, **p:** pith, **en:** endodermis.

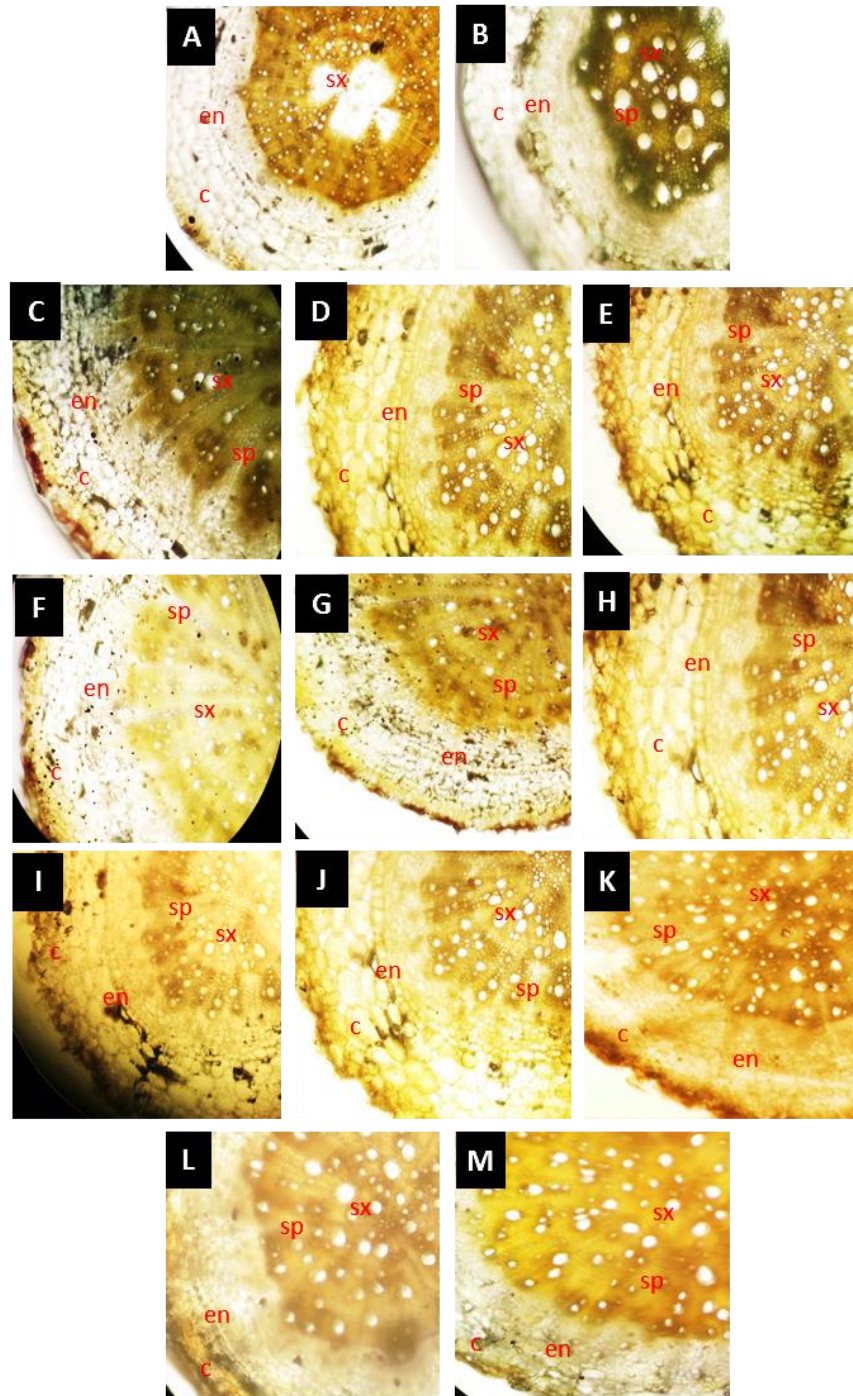


Fig. 5 (A–M): Cross sections of tomato root stained with iodine solution showing anatomical changes upon exposure to *Fusarium oxysporum* f. sp. *lycopersici*, *Penicillium digitatum* and *P. expansum*, and different doses of dry biomass of *Chenopodium murale*. For details of treatments A–M, see Fig. 1. **c:** cortex, **sp:** secondary phloem, **sx:** secondary xylem, **p:** pith, **en:** endodermis.

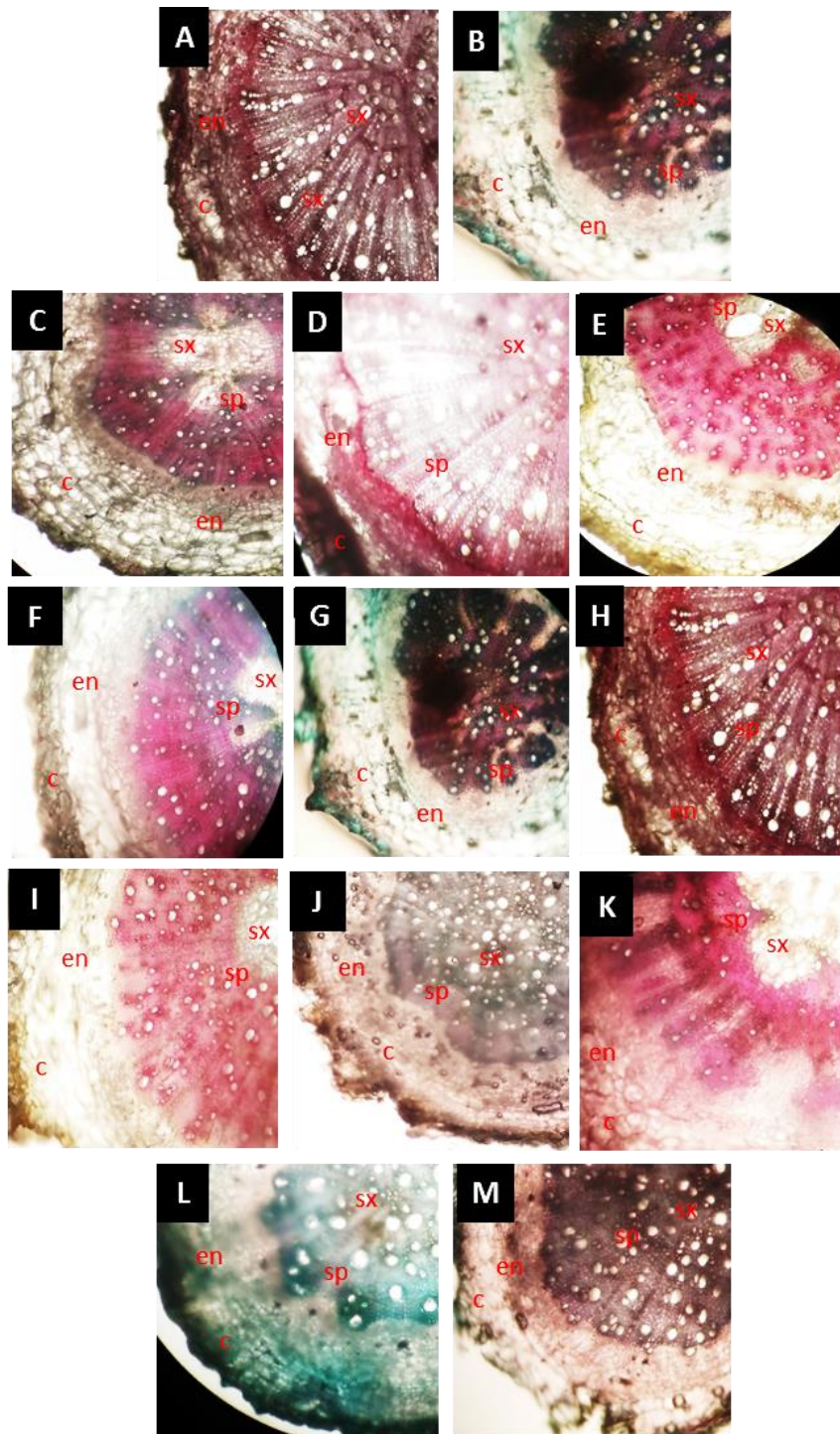


Fig. 6 (A–M): Cross sections of tomato root stained with safranine-fast green solution showing anatomical changes upon exposure to *Fusarium oxysporum* f. sp. *lycopersici*, *Penicillium digitatum* and *P. expansum*, and different doses of dry biomass of *Chenopodium murale*. For details of treatments A–M, see Fig. 1.

c: cortex, **sp:** secondary phloem, **sx:** secondary xylem, **p:** pith, **en:** endodermis.

References

Abdel-Aziz MS, Shaheen MS, El-Nekeety AA, Abdel-Wahhab MA, 2014. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. *J. Saudi Chem. Soc.*, **18**: 356-363.
AbdelFatah HAS, Sallam NM, Mohamed MS, Bagy

HMK, 2021. *Curvularia lunata* as new causal pathogen of tomato early blight disease in Egypt. *Mol Biol Rep* **48**: 3001-3006.

Akhtar R, Javaid A, 2018. Biological management of basal rot of onion by *Trichoderma harzianum* and *Withania somnifera*. *Planta Daninha* **36**:

- Article e017170507.
- Akram S, Khan SM, Khan MF, Khan HU, Tariq A, Umar UU, Gill A, 2018. Antifungal activity of different systemic fungicides against *Fusarium oxysporum* F. sp. *lycopersici* associated with tomato wilt and emergence of resistance in the pathogen. *Pak. J. Phytopathol.*, **30**: 169-176.
- Ali A, Javaid A, Shoaib A, 2017. GC-MS analysis and antifungal activity of methanolic root extract of *Chenopodium album* against *Sclerotium rolfsii*. *Planta Daninha*, **35**: Article e017164713.
- Ali A, Javaid A, Shoaib A, Khan IH, 2020. Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. *Egypt. J. Biol. Pest Control*, **30**: Article 102.
- Báidez AG, Gómez P, Del Río JA, Ortuño A, 2007. Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. role of phenolic compounds in plant defense mechanism. *J. Agric. Food Chem.*, **55**: 3373-3377.
- Bajwa AA, Zulfiqar U, Sadia S, Bhowmik P, Chauhan BS, 2019. A global perspective on the biology, impact and management of *Chenopodium album* and *Chenopodium murale*: two troublesome agricultural and environmental weeds. *Environ. Sci. Poll. Res.*, **26**: 5357-5371.
- Carreras M, Espeso EA, Gutierrez-Docio A, Moreno-Fernandez S, Prodanov M, Hernando MD, Larena I, 2020. Exploring the extracellular macromolecular composition of crude extracts of *Penicillium rubens* Strain 212 for elucidation its mode of action as a biocontrol agent. *J. Fungi*, **6**: Article 131.
- Caulier S, Gillis A, Colau G, Licciardi F, Liépin M, Desoignies N, Bragard C, 2018. Versatile antagonistic activities of soil-borne *Bacillus* spp. and *Pseudomonas* spp. against *Phytophthora infestans* and other potato pathogens. *Front. Microbiol.*, **9**: Article 143.
- Cooper RM, Williams JS, 2004. Elemental sulphur as an induced antifungal substance in plant defense. *J. Exp. Bot.*, **55**: 1947-1953.
- De Wit PJ, 1977. A light and scanning-electron microscopic study of infection of tomato plants by virulent and avirulent races of *Cladosporium fulvum*. *Neth. J. Plant Pathol.*, **83**: 109-122.
- Goicoechea N, Aguirreolea J, Cenoz S, Garcia-Mina JM, 2000. *Verticillium dahliae* modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. *Eur. J. Plant Pathol.*, **106**: 19-25.
- Guijarro B, Larena I, Casals C, Teixidó N, Melgarejo P, De Cal A, 2019. Compatibility interactions between the biocontrol agent *Penicillium frequentans* Pf909 and other existing strategies to brown rot control. *Biol. Control*, **129**: 45-54.
- Gupta PK, 2018. Toxicity of fungicides. In: Veterinary toxicology. Academic Press, pp. 569-580
- Gupta S, Narayan R, 2012. Phenotypic plasticity of *Chenopodium murale* across contrasting habitat conditions in peri-urban areas in Indian dry tropics: Is it indicative of its invasiveness? *Plant Ecol.*, **213**: 493-503.
- Hinchman RR, 1973. A permanent iodine stain-mountant combination for starch in plant tissues. *Stain Technol.*, **48**: 344-346.
- Jabeen N, Javaid A, Shoaib A, Khan IH, 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel*. *J. Plant Pathol.*, **103**: 901-913.
- Jafri FI, Akbar M, Andolfi A, 2024. Antifungal activity of *Dryopteris nigropaleacea* plant powder against *Fusarium oxysporum* and its effect on morphological and biochemical attributes of tomato under pot conditions. *Appl. Ecol. Environ. Res.*, **22**: 2685-2703.
- Javaid A, Afzal R, Shoaib A, 2020. Biological management of southern blight of chili by *Penicillium oxalicum* and leaves of *Eucalyptus citriodora*. *Int. J. Agric. Biol.*, **23**: 93-102.
- Javed S, Javaid A, Hanif U, Bahadur S, Sultana S, Shuaib M, Ali S, 2021. Effect of necrotrophic fungus and PGPR on the comparative histochemistry of *Vigna radiata* by using multiple microscopic techniques. *Microsc. Res. Technol.*, **84**: 2737-2748.
- Johansen DA, 1940. Plant microtechnique. New York: McGraw-Hill Book.
- Joyce P, Don NH, Sousa M, Olsen D, 2016. Starch accumulation in sugarcane in response to stress. *Proc. Aust. Soc. Sugar Cane Technol.*, **38**: 20-28.
- Katan T, Shlevin E, Katan J, 1997. Sporulation of *Fusarium oxysporum* f. sp. *lycopersici* on stem surfaces of tomato plants and aerial dissemination of inoculum. *Phytopathology*, **87**: 712-719
- Khan IH, Javaid A, 2020. Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina*. *Int. J. Agric. Biol.*, **24**: 441-446.
- Khan IH, Javaid A (2021). *In vitro* screening of *Aspergillus* spp. for their biocontrol potential against *Macrophomina phaseolina*. *J. Plant Pathol.*, **103**: 1195-1205.
- Khan IH, Javaid A, 2022a. *Penicillium echinulatum* causing blue mold on tomato in Pakistan. *J.*

- Plant Pathol.*, **104**: 1143.
- Khan IH, Javaid A, 2022b. DNA cleavage of the fungal pathogen and production of antifungal compounds are the possible mechanisms of action of biocontrol agent *Penicillium italicum* against *Macrophomina phaseolina*. *Mycologia*, **114**: 24-34.
- Koc E, Slusarenko A, 1990. Arabidopsis is susceptible to infection by a downy mildew fungus. *Plant Cell*, **2**: 437-445.
- Khurshid S, Shoaib A, Javaid A, 2016. Fungicidal potential of allelopathic weed *Cenchrus pennisetiformis* on growth of *Fusarium oxysporum* f. sp. *lycopersici* under chromium stress. *Planta Daninha*, **34**: 453-463.
- Larena I, Sabuquillo P, Melgarejo P, De Ca A, 2003. Biocontrol of Fusarium and Verticillium wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. *J. Phytopathol.*, **151**: 507-512.
- Mace ME, Veech JA, Beckman CH, 1972. Fusarium wilt of susceptible and resistant tomato isolines: histochemistry of vascular browning. *Phytopathology*, **62**: 1-654.
- Malbrán I, Mourellos CA, Lori GA, 2020. First report of *Fusarium oxysporum* f. sp. *lycopersici* race 3 causing Fusarium wilt of tomato in Argentina. *Plant Dis.*, **104**: 978-978.
- Manikandan R, Harish S, Karthikeyan G, Raguchander T, 2018. Comparative proteomic analysis of different isolates of *Fusarium oxysporum* f. sp. *lycopersici* to exploit the differentially expressed proteins responsible for virulence on tomato plants. *Front. Microbiol.*, **9**: Article 420.
- Martín JA, Solla A, Domingues MR, Coimbra MA, Gil L, 2008. Exogenous phenol increase resistance of *Ulmus minor* to Dutch elm disease through formation of suberin-like compounds on xylem tissues. *Environ. Exp. Bot.*, **64**: 97-104.
- Martín JA, Solla A, Gil L, García-Vallejo MC, 2010. Phenological and histochemical changes of *Ulmus minor* due to root absorption of phenol: Implications for resistance to DED. *Environ. Exp. Bot.*, **69**: 175-182.
- Maurya S, Dubey S, Kumari R, Verma R, 2019. Management tactics for Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*: A review. *Management*, **4**: 1-7.
- Miedes E, Vanholme R, Boerjan W, Molina A, 2014. The role of the secondary cell wall in plant resistance to pathogens. *Front. Plant Sci.*, **5**: Article ID 358.
- Murugan L, Krishnan N, Venkataravanappa V, Saha S, Mishra AK, Sharma BK, Rai AB, 2020. Molecular characterization and race identification of *Fusarium oxysporum* f. sp. *lycopersici* infecting tomato in India. *Biotech*, **10**: 1-12.
- Nadeem SM, M Ahmad, ZA Zahir, A Javaid, M Ashraf, 2014. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.*, **32**: 429-448.
- Naqvi SF, Javaid A, Qureshi MZ, 2019. Evaluation of antifungal potential of leaf extract of *Chenopodium murale* against *Fusarium oxysporum* f. sp. *lycopersici*. *Planta Daninha*, **37**: Article e019199050.
- Naqvi SF, Khan IH, Javaid A, 2020. Hexane soluble bioactive components of *Chenopodium murale* stem. *Pak. J. Weed Sci. Res.*, **26**: 425-432.
- Nigam R, Singh J, Khan RU, Singh R, 2021. Antagonistic efficacy of *Penicillium oxalicum* against wilt and root rot pathogens of Pea. *J. Hort. Pest Sci.*, **1**: 58-62.
- Ochilo WN, Nyamasyo GN, Kilalo D, Otieno W, Otipa M, Chege F, Lingeera EK, 2019. Characteristics and production constraints of smallholder tomato production in Kenya. *Sci. Afr.*, **2**: Article e00014.
- Pitino M, Allen V, Duan Y, 2018. LasΔ5315 effector induces extreme starch accumulation and chlorosis as Ca. *Liberibacter asiaticus* infection in *Nicotiana benthamiana*. *Front. Plant Sci.*, **9**: Article 113.
- Pomar F, Novo M, Bernal MA, Merino F, Barcelo AR, 2004. Changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during *Verticillium* wilt in *Capsicum annuum*. *New Phytol.*, **163**: 111-123.
- Pouzoulet J, Pivovarovoff AL, Santiago LS, Rolshausen PE, 2014. Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. *Front. Plant Sci.*, **5**: Article 253.
- Sattler S, Funnell-Harris D, 2013. Modifying lignin to improve bioenergy feedstocks: strengthening the barrier against pathogens?. *Front. Plant Sci.*, **4**: Article 70.
- Sharf W, Javaid A, Shoaib A, Khan IH, 2021. Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis*. *Egypt. J. Biol. Pest Control*, **31**: Article 16.
- Srinivas C, Devi DN, Murthy KN, Mohan CD, Lakshmeesha TR, Singh B, Srivastava RK, 2019. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity – a review. *Saudi J. Biol. Sci.*, **26**: 1315-1324.
- Tetorya M, Rajam MV, 2021. RNAi-mediated silencing of PEX6 and GAS1 genes of *Fusarium oxysporum* f. sp. *lycopersici* confers resistance against Fusarium wilt in tomato. *3*

- Biotech*, **11**: 1-8.
- Vermerris W, Nicholson R, 2008. The role of phenols in plant defense. In Phenolic compound biochemistry. Springer, Dordrecht. pp. 211-234
- Villarino M, Espeso EA, Melgarejo P, Larena I, 2018. Transformation of *Penicillium rubens* 212 and expression of GFP and DsRED coding genes for visualization of plant-biocontrol agent interaction. *Front. Microbiol.*, **9**: Article 1653.
- Walker JC, 1971. Fusarium wilt of tomato. Fusarium wilt of tomato. Phytopathological Monograph No. 6. *The American Phytopathology Society*, St. Paul, Minnesota. p. 56.
- Zhang Y, Wang X, Rong W, Yang J, Li Z, Wu L, Ma Z, 2017. Histochemical analyses reveal that stronger intrinsic defenses in *Gossypium barbadense* than in *G. hirsutum* are associated with resistance to *Verticillium dahliae*. *Mol. Plant-Microbe Interact.*, **30**: 984-996.
- Zhang Y, Wu L, Wang X, Chen B, Zhao J, Cui J, Ma Z, 2019. The cotton laccase gene GhLAC15 enhances Verticillium wilt resistance via an increase in defence-induced lignification and lignin components in the cell walls of plants. *Mol. Plant Pathol.*, **20**: 309-322.
- Zohaib KA, Bashir U, Khan IH, Javaid A, Anwar W, 2024. Synergistic effect of plant growth promoting rhizobacteria and *Cirsium arvense* against black scurf disease of potato. *Sains Malays.*, **53**: 3071-3083.