ANTIFUNGAL ACTIVITY OF *DRYOPTERIS NIGROPALEACEA* PLANT POWDER AGAINST *FUSARIUM OXYSPORUM* AND ITS EFFECT ON MORPHOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF TOMATO UNDER POT CONDITIONS

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(Received 22nd Dec 2023; accepted 19th Apr 2024)

Abstract. Fungal pathogens in crops are being controlled by using synthetic fungicides, but all these fungicides have adverse effects. Herein, the antifungal activity of *Dryopteris nigropaleacea* (Dn.) and its allelopathy was investigated first time by soil amendment in pots against *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in terms of morpho-biochemical attributes at conidial densities of Fol 1, 2 and 3 (1×10^7, 2×10^7, and 3×10^7 CFU/mL). Morphological, molecular, and phylogenetic analysis of fungal culture isolate (FI 3005) confirmed that our isolate resembled Fol with 98% similarity index with MW497420 *F. oxysporum*. High concentrations of plant powder of *D. nigropaleacea* were found to be inhibitory in nature. Incorporation of Dn 1 concentration (0.025%) of *D. nigropaleacea* was found to be the most feasible concentration as it resulted in an increase in morphological and biochemical parameters of tomato plants by decreasing the disease incidence (DI) and disease severity (DS) by 60 and 65.2%, respectively, and by improving antioxidants like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Area under disease progress curve (AUDPC) was also reduced by 50% for both DI and DS in Dn 1 treated plants, when compared with Fol 3, after 80 days of inoculation.
Keywords: *fern, antioxidants, allelopathy, soil amendment, disease incidence*

Introduction

Tomato (*Solanum lycopersicum* L.) is a very important crop all over the world, including Pakistan. Due to its high production and consumption, it is ranked second most important vegetable crop of the world (Wakil et al., 2017). About 177 and 2.9 million metric tons of tomato yield per annum is reported worldwide and in Pakistan, respectively (Hyder et al., 2019). The total area in Pakistan under tomato cultivation is 61 thousand hectares, with an average yield of 9.5 tons per hectare (Fatah et al., 2020).

Nowadays, plant diseases caused by phytopathogenic fungi are common which adversely affect the crop yield, leading to poor quality of agricultural products (Ashiq, 2015). The most common diseases of tomato are late blight, septoria leaf spot, anthracnose early blight, fruit rot and wilt. Among these, wilt disease caused by phytopathogenic fungus, *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is cosmopolitan (Javaid et al., 2018). This disease is estimated to cause 60% yield loss in tomato (de Rodriguez, 2019). Fusarium wilt has been reported all over the world. Fol is the causal agent of different diseases of many plants including cucurbits, tobacco, legumes, sweet potato, banana, and tomato. So, it is essential

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

to protect crops from this pathogen to meet food security (Savary et al., 2012). Besides crop yield losses, mycotoxins produced by Fol contaminate food and agricultural products (Gautier et al., 2020). Fusariosis produces potent mycotoxins and incurs negative impacts on crops due to reduced grain quality and overall yield (de Chaves et al., 2022).

Various management strategies like crop rotation (De Corato et al., 2020), cultural techniques (Ajilogba et al., 2013), use of chemical fungicides and resistant varieties of plants are adopted to cope with soil borne pathogens including *F. oxysporum* (Maurya et al., 2019). Use of resistant varieties proved to be effective against Fusarium wilt of tomato, but pathogens develop strategies to deal with different resistance mechanisms stimulated in plants against these different strains of pathogens. Although, fungal diseases in crops are being controlled by using synthetic fungicides (Poussio et al., 2021), but all these synthetic fungicides have numerous adverse effects on human health (Tao et al., 2020). Negative effects imposed by these synthetic fungicides are becoming critical day by day, so, there is a dire need to find alternatives to these synthetic fungicides.

In addition to the above-mentioned disease-suppressing techniques, there have been many successful attempts to suppress fungal diseases in plants using plant-based formulations e.g., plant residues or their extracts. Plants are full of natural resources and the use of these plants as antifungal sources is widespread. Along with other bioactive metabolites, there is evidence of the antifungal activity of these plants. The presence of antifungal compounds in plants proved to be potent natural fungicides (Kim et al., 2017) for the control of phytopathogens. In an experiment, 25.5 and 27.8% reduction in disease incidence in tomato was recorded by soil amendments with neem and willow aqueous extracts, respectively. Neem and willow enhanced the shoot length of tomato seedlings up to 21, 35.7% and root length up to 3.7 and 6.4%, respectively (Hanaa et al., 2011). Ethanol extract of *Juglans microcarpa*, *Juglans mollis* and *Carya ovata* (Family Juglandaceae) suppressed disease incidence up to 37.5% and 0.4 grades as compared to positive control. Moreover, there was marked increase in plant height (19%), weight of leaves (68%), stem (54%), and root (71%), respectively, over infested control (de Rodriguez et al., 2019). Antioxidant activities like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are affected by infection of Fol. Neem and willow extracts decreased Fusarium infection and 14, 97, and 38% increase was observed in SOD, POD, and CAT activities, respectively in willow extract-treated tomato seedlings after 7 days (Hanaa et al., 2011). In an investigation, *Ocimum bacilicum* extract decreased disease incidence from 95 to 18% and increased antioxidants as compared to negative control in tomato against Fol. *F. oxysporum* suppressed the morphological parameters and photosynthetic activity in tomato plants. Tomato plants pre-soaking in *O. bacilicum* extract increased morphological and biochemical parameters in healthy as well as infected plants. The activities of SOD, POD, and CAT were increased up to 18.4, 62.6, and 40% in infected plants without *O. bacilicum* extract, as compared to healthy control. While, plants soaked in the extract of *O. bacilicum* in infected treatments showed 29, 57.2, and 23.8% increase in SOD, POD, and CAT activities, respectively (Akladious et al., 2015). *Alternaria alternata* causes early blight disease in tomato genotypes and these genotypes exhibit variable resistance against *A. alternata*. There was an increase of 418.5, 21.5, and 152.8% in SOD, POD, and CAT activities as compared to non-infested healthy control (Alizadeh-Moghaddam et al., 2020). Resistant and susceptible varieties of tomato plants against Fusarium wilt show different response in terms of antioxidant activities. In a study, a remarkable increase was observed in peroxidase activity of Fusarium-treated resistant tomato plants as compared to susceptible plants where peroxidase activity increased after longer period of pathogen inoculation (Retig, 1974).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

Dryopteris nigropaleacea (Fraser-Jenk.) is a fern that belongs to family Dryopteridaceae. There are few studies on the antifungal activities of *Dryopteris* spp. (Alam et al., 2021), but studies regarding soil amendment and antifungal activity of plant powder of *D. nigropaleacea* are scarce. So, in the present study, the disease-suppressing ability of *D. nigropaleacea* was investigated under pot conditions against the wilt disease of tomato.

Materials and Methods

Test plant and plant pathogenic fungal isolate

Dryopteris nigropaleacea, whole plant samples were collected from Bara Gali, Khyber Pakhtunkhwa, Pakistan. *Fusarium oxysporum* f. sp. *lycopersici* (Fol) was isolated from diseased tomato plant.

Culturing of plant pathogenic fungal isolate and storage

Bits of diseased tomato plant were inoculated on to potato dextrose agar (PDA) medium contained in 9 cm glass Petri plates. When colonies of different fungal species appeared on PDA, these were transferred to fresh PDA Petri plates. The process was repeated until Fol pure culture was isolated and identified (Zehra et al., 2023).

Morphological identification

Fusarium culture was identified through color, texture, pigmentation, and margins of colony. Permanent slide of isolate was made for evaluation of spore size, mycelium characteristics and type examined under light microscope. Microconidia and macroconidia were examined on the basis of length and septation with the presence or absence of chlamydospores and its intercalary and terminal position on mycelium (Akbar et al., 2018).

DNA extraction

A pure culture of Fol was grown for 48 h at 28 °C on PDA. With the help of sterile spatula, fungal mycelial mats were collected and transferred into 5 ml Eppendorf tube and stored at -80 °C for DNA extraction. D Neasy 1 plant Mini kit (cat. No. 69104, Qiagen USA) was used with respect to manufacturer prescribed protocol for DNA extraction. To check the purity and concentration of extracted DNA, absorbance was recorded at 260 and 280 nm with micro volume plate and H_1 Hybrid multi-mode reader (Bio Tek). DNA samples were stored at -20 °C and -80 °C for further use (Akbar et al., 2018).

Internal transcribed spacer (ITS) sequencing

Ribosomal DNA (rDNA) gene was analyzed through ITS sequencing (Internal Transcribed Spacer region) and was amplified by using ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50- TCCTCCGCTTATTGATATGC-30) primers (White et al., 1990). 10 µl of total volume was taken for amplification of ITS 1 and ITS4 which comprised of Taq DNA polymerase (0.625 U), dNTPs (0.2 mM each), DNA (0.5-5 ng), each primer (0.3 µM), 1X PCR buffer, and 2 mM $MgCl_2$. At first, in PCR amplification, denaturation step was carried out at 98 °C for 2 minutes followed by 35 cycles of denaturation at 98 °C for 10s, annealing at 55 °C for 30s, extension at 72 °C for 90s and final extension was done at 72 °C for 10 minutes. Negative control was kept as distilled water without DNA and positive control was DNA of known Fol isolate. PCR ITS reactions

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were run on agarose gel (1%) which contained SYBER green dye in 1XTAE buffer at 80 volts for 40 minutes. Gel was screened through visual assessment and photographed with Gel DOC XR software (Universal Hood II, USA). According to manufacturer recommended protocol of Qiang QIA quick gel extraction kit (cat# 28704) single approximately 500 bp long band from each lane were isolated after electrophoresis. The concentration of PCRed ITS DNA was quantified using ITS1 and ITS4 primers through Take3 Micro-volume plate with H1 Hybrid Multi Mode Reader. ITS DNA was sequenced on both strands using ITS1 and ITS4 primers by Sanger's Sequencing Method. Bioedit Software was used to check all the sequences of DNA chromatogram files manually and assembled ITS sequences of both strands into consensus contigs (Akbar et al., 2018).

Phylogenetic analysis

Sequence obtained from molecular analysis were subjected to BLAST in NCBI and all closely related sequences were downloaded and evaluated based on percent coverage, percent nucleotide identity and E value from all over the world. Reference sequences of ITS gene of Fol were downloaded from Gene bank of NCBI database which represented phylogenetic clades. *Trichoderma harzianum* was used as an outgroup. The ITS sequences were aligned using CLUSTAL W. The alignment was trimmed to sequences of about 543 bps. The phylogenetic tree was reconstructed using UPGMA with 500 bootstrap iterations (Tamura et al., 1993). Moreover, the model of evolution was selected based on a consensus of the Bayesian information criterion (BIC), the corrected Akaike information criterion (AICc), and the Neighbor-joining parameters using MEGA 11. The Hasegawa-Kishnio Yano (HKY85) model, with a gamma-distributed rate of variation among sites, was identified as the best fit model of evolution. The tree was visualized and edited using Tree Graph (Tamura et al., 2021).

Pots experiment

Inoculum preparation of Fusarium oxysporum f. sp. lycopersici (Fol)

For preparation of mass inoculum of Fol, procedure was adopted as described by Hanaa et al. (2011) with slight modifications. Chickpeas were soaked into water overnight at room temperature. These chickpeas were transferred to heat resistant polythene bags and autoclaved at 121°C for 20 min. After cooling, conidial suspension from single spore isolated *F. oxysporum* culture was transferred to chickpea bags under aseptic conditions, and incubated at 25 °C±1, until reasonable growth of hyphae/conidia on chickpeas (Ali et al., 2020). Conidial density of *F. oxysporum* on chickpeas was estimated with hemocytometer and 1×10^7, 2×10^7, and 3×10^7 colony forming units/mL (CFU/mL) were investigated in pot experiments.

Soil amendment bioassays

Pot experiment was carried out during January-April 2020. Soil was sterilized with 2% formalin solution and sun dried for 2 weeks to evaporate traces of formalin (Awan et al., 2018). Pots (23 cm diameter, length 23.5 cm) were filled with 10 kg/pot of sterilized soil. Soil was amended with dry powder of *D. nigropaleacea* with following three concentrations (w/w), 0.025%, 0.050% and 0.075%, watered, and left for one week in open. After that tomato seedlings with 4-5 leaves were transplanted into these pots @ 1 seedling/pot. *F. oxysporum* inoculum was introduced by mixing *Fusarium* inoculum in pot soil one week before transplanting seedlings of tomato (Alamri et al., 2019). Experiments were carried out

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by using Completely Randomized Design (CRD). Pots without inoculum and plant powder of *D. nigropaleacea* served as negative control. Pots were irrigated with water sprinkler.

Treatments

T1: Non infested control = NIC
T2: *F. oxysporum* 1×10^7 CFU/mL = Fol 1
T3: *F. oxysporum* 2×10^7 CFU/mL = Fol 2
T4: *F. oxysporum* 3×10^7 CFU/mL = Fol 3
T5: [*F. oxysporum* (dead) 1×10^7 CFU/mL] = DFol 1
T6: [*F. oxysporum* (dead) 2×10^7 CFU/mL] = DFol 2
T7: [*F. oxysporum* (dead) 3×10^7 CFU/mL] = DFol 3
T8: *D. nigropaleacea* (0.025%) = Dn 1
T9: *D. nigropaleacea* (0.050%) = Dn 2
T10: *D. nigropaleacea* (0.075%) = Dn 3
T11: *F. oxysporum* (1×10^7 CFU/mL)+*D. nigropaleacea* (0.025%) = Fol 1+Dn 1
T12: *F. oxysporum* (1×10^7 CFU/mL)+*D. nigropaleacea* (0.050%) = Fol 1+Dn 2
T13: *F. oxysporum* (1×10^7 CFU/mL)+*D. nigropaleacea* (0.075%) = Fol 1+Dn 3
T14: *F. oxysporum* (2×10^7 CFU/mL)+*D. nigropaleacea* (0.025%) = Fol 2+Dn 1
T15: *F. oxysporum* (2×10^7 CFU/mL)+*D. nigropaleacea* (0.050%) = Fol 2+Dn 2
T16: *F. oxysporum* (2×10^7 CFU/mL)+*D. nigropaleacea* (0.075%) = Fol 2+Dn 3
T17: *F. oxysporum* (3×10^7 CFU/mL)+*D. nigropaleacea* (0.025%) = Fol 3+Dn 1
T18: *F. oxysporum* (3×10^7 CFU/mL)+*D. nigropaleacea* (0.050%) = Fol 3+Dn 2
T19: *F. oxysporum* (3×10^7 CFU/mL)+*D. nigropaleacea* (0.075%) = Fol 3+Dn 3
Total 19 treatments were investigated in 95 pots having 5 replicates of each treatment.

Measurement of morphological growth parameters

Morphological attributes of tomato plants were recorded after 86 days of transplantation in pot experiments. Number of leaves per plant in all treatments were recorded when plants were still growing in pots. To measure shoot and root length, plants were uprooted, and their length was recorded with the help of measuring scale. For fresh weight of shoot and roots, plants were washed with tap water to remove all the dirt particles, extra moisture was removed from plants by placing washed plants under fan before fresh weight measurements. For dry weight, plants were placed in an electric oven, at 60 °C till constant weight (Akbar and Javaid, 2015). Fresh and dry weights were taken with the help of digital balance.

Calculation of disease incidence, disease severity and area under disease progress curve (AUDPC)

Disease incidence (DI) (%) was recorded two times, 45 days, and 80 days after seedling transplant (Khurshid et al., 2017; Devi et al., 2022; Attia et al., 2022). DI was calculated as number of diseased plants over total number of plants per treatment by following formula (de Rodriguez et al., 2019).

$$\%\ Disease\ incidence = \frac{No.\ of\ diseased\ plants}{Total\ number\ of\ Plants} \times 100 \qquad \text{(Eq.1)}$$

Disease severity (DS) was calculated through severity scale (0-5), devised by Kurabachew et al. (2013). Disease symptoms were determined by visual observations according to following scale;

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0 = no leaf wilted, 1 = one leaf wilted, 2 = two leaf wilted, 3 = three leaves wilted, 4 = wilting of all leaves without tip, and 5 = wilting of whole plant (dead plant).

The area under disease progress curve (AUDPC) was evaluated through % wilt incidence and severity (grades) using trapezoid integration of disease progress curve over time using following equation;

$$AUDPC = \sum_{n=1}^{n-1}((X_i + Xi - 1/2)(Ti - Ti - 1) \quad \text{(Eq.2)}$$

where X_i and X_{i-1} are disease severity or % incidence at time T_i-T_{i-1}, and T_i and T_{i-1} are consecutive evaluation dates with T_i-T_{i-1} is equal to 1 (Jeger et al., 2001).

Determination of antioxidants

Superoxide dismutase (SOD) activity

To calculate the antioxidant activity of SOD enzyme, nitro blue tetrazolium (NBT) method was used as described by Giannopolitis and Reis (1977). The level of SOD in the plant samples was estimated through the following formula (Gao, 2006).

$$SOD\ (\frac{U}{g}Fw) = \frac{Control - Sample}{Control} \times 100 = \text{X\% Inhibition} \quad \text{(Eq.3)}$$

Peroxidase (POD) activity

Guaiacol peroxidase activity was assessed according to the method described by Gao (2006). The enzyme activity of POD at 25±2 °C was calculated with the following formula:

$$POD\ activity\ \left(\frac{U}{g}FW\right) = \triangle A470 \times \frac{V1}{V2 \times 0.01 \times FW} \quad \text{(Eq.4)}$$

where, OD = Optical density of samples at 470 nm, V1 volume of sample and V2 is total volume in test tube.

Catalase (CAT) activity

Catalase (CAT) activity was determined by the method of Cai (2013). The CAT activity was determined by the consumption of H_2O_2 (extinction coefficient of 39.4 mM/cm at 240 nm for 30 s) using the following formula:

$$CAT\ activity\ (U/g\ FW) = \triangle A240 \times V1/\ (0.1 \times V2 \times FW) \quad \text{(Eq.5)}$$

where, OD = Optical density of samples at 240 nm, V1 = Volume of sample and V2 is total volume in test tube.

Confirmation of Koch's postulates

From pathogenicity tests conducted in pots experiment, the pathogen was reisolated from tomato plants and re-identified.

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Statistical analysis

For statistical analysis, ANOVA was done followed by Fisher's LSD test to delineate the treatment means at 5% probability by using computer software Minitab 20.2.

Results

Identification of fungal pathogen

Morphological identification of *Fusarium oxysporum* f. sp. *lycopersici* (Fol) showed the white, brown color of culture isolate with the presence of macro/microconidia and macroconidia septation (22.8, 4.7, 4-5) with no chlamydospores. Molecular analysis of culture isolate (FI3005) confirmed that our isolate resembled Fol with 98% similarity index with MW497420 *F. oxysporum*. The sequence of isolate FI3005 was submitted to the gene bank (NCBI) with accession number ON908610. All similar sequences including our isolate FI3005 resembled main clad (KT002000) Fol (*Fig. 1*).

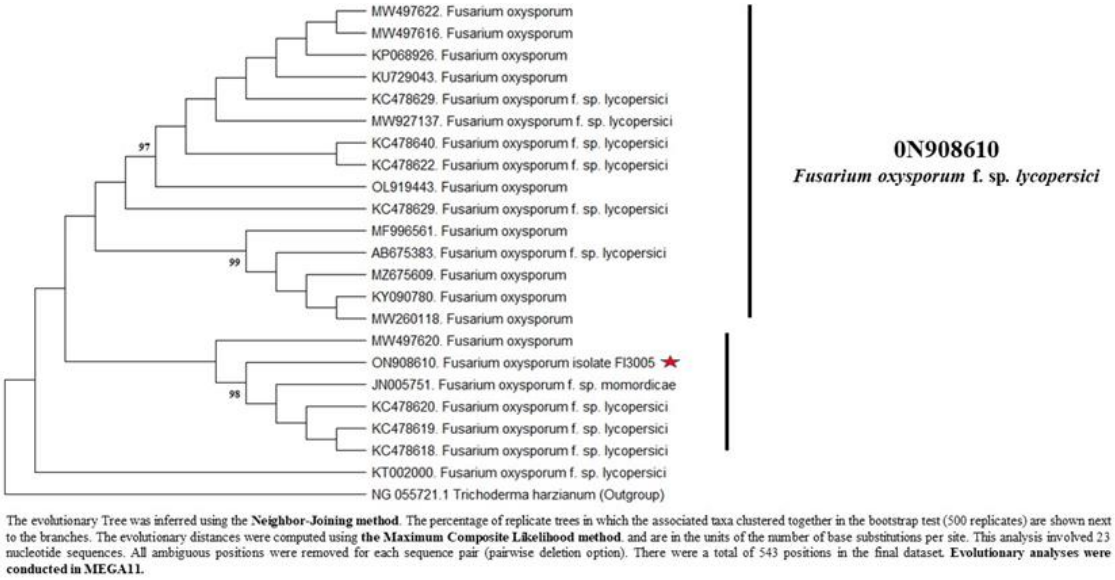

Figure 1. *Phylogenetic analysis of Fusarium oxysporum f. sp. lycopersici (FI3005) isolate (0N908610)*

Effect of soil amendments on the shoot length and shoot fresh weight of tomato plants

Data regarding effect of various treatments on the shoot length of pot grown tomato plants are presented in *Fig. 2A*. There was 10, 13, and 33% significant decrease in the shoot length of tomato plants when there was inoculation of concentration 1 (Fol 1), 2 (Fol 2), and 3 (Fol 3) of Fol, in comparison with non infested control (NIC). The effect of 3 concentrations of dead *F. oxysporum* (DFol 1), 2 (DFol 2), and 3 (DFol 3) was found nonsignificant when compared with NIC, as well as when compared with each other. The effect of 3 concentrations *viz*., (Dn 1), (Dn 2), and (Dn 3) of *D. nigropaleacea* on the shoot length of tomato plants was variable as there was nonsignificant effect of Dn 1 on the shoot length while, there was significant inhibitory effects (4 and 7%) by using higher concentrations, Dn 2, Dn 3, of *D. nigropaleacea*, respectively, as compared to NIC.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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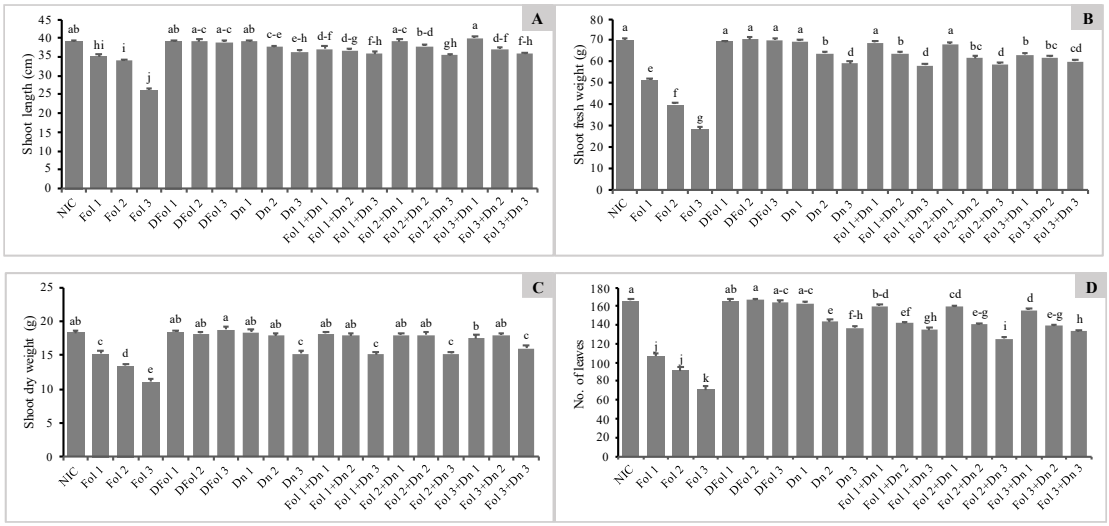

Figure 2. *Effect of treatments on (A) shoot length, (B) shoot fresh weight, (C) shoot dry weight and (D) number of leaves of tomato plants. Bars sharing common alphabets do not differ at P≤5%, as determined by Fisher's LSD on Minitab 20.2. Y-error bars reveal standard error of means of 5 replicas*

Soil amendment with *D. nigropaleacea* plant powder significantly overcome the fungal attack and subsequently increased the shoot length of tomato plants. There was an increase of 6, 5, and 3% in shoot length of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. There was a significant increase of 15, 12, and 5% in shoot length of tomato plants by the introduction of Fol 2+Dn 1, Fol 2+Dn 2, Fol 2+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant increase of 53, 41, and 36% in shoot length of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3. There was 27, 43, and 60% significant decrease in the shoot fresh weight of tomato plants when there was inoculation of Fol 1, Fol 2, and Fol 3 of *F. oxysporum*, respectively, when compared with NIC. The effect of 3 concentrations of dead *F. oxysporum* (DFol 1), 2 (DFol 2) and 3 (DFol 3) was found nonsignificant when compared with NIC, as well as when compared with each other. The effect of 3 concentrations namely, Dn 1, Dn 2, Dn 3 of *D. nigropaleacea* on the shoot fresh weight of tomato plants was variable as there was nonsignificant effect of Dn 1 on the shoot fresh weight while, there was significant inhibitory effects (9 and 16%) by using higher conc., Dn 2, Dn 3, of *D. nigropaleacea*, respectively.

On the other hand, *D. nigropaleacea* incorporation in soil significantly overcome the wilt disease and therefore, increased the shoot fresh weight of tomato plants. There was a significant increase of 34, 24, and 13% in shoot fresh weight of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. There was a significant increase of 71, 55, and 48% in shoot fresh weight of tomato plants by the introduction of Fol 2+Dn 1, Fol 2+Dn 2, Fol 2+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant enhancement of 123, 119, and 112% in shoot fresh weight of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3, as shown in *Fig. 2B.*

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DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

Effect of soil amendments on the shoot dry weight and number of leaves of tomato

Data regarding the effect of various treatments on the shoot dry weight of tomato plants are presented in *Fig. 2C*. There was 17, 26, and 40% decrease in the shoot dry weight of tomato plants by using (Fol 1), (Fol 2), and (Fol 3), as compared with NIC. The application of (Dn 1), (Dn 2), and (Dn 3) revealed variable effects on the shoot dry weight of tomato plants. There was an increasing trend in the shoot dry weight of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, and Fol 1+Dn 3 of *D. nigropaleacea*, in comparison with Fol 1. There was a significant increase of 34, 34, 13%, and 59, 63, 45% in shoot dry weight of tomato plants by the application of Fol 2+Dn 1, Fol 2+Dn 2, Fol 2+Dn 3 and Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3, when compared with Fol 2 and Fol 3, respectively. Soil amendment with Fol 1, Fol 2, and Fol 3 significantly reduced the number of leaves of tomato plants by 36.1, 45.1, and 57.2%, respectively, as compared to NIC. The effect of 3 concentrations *viz.*, Dn 1, Dn 2, Dn 3 of *D. nigropaleacea* on the number of leaves of tomato plants was found dose dependent as there was nonsignificant effect of Dn 1 while, there was a significant decrease in the number of leaves (13.6 and 17.5%) by using higher conc., Dn 2, Dn 3, of *D. nigropaleacea*, respectively. The introduction of Fol 1+Dn 1, Fol 1+Dn 2, and Fol 1+Dn 3 significantly increased the number of leaves by 51, 33.6, and 27.4%, respectively, in relation to Fol 1. Likewise, Fol 2+Dn 1, Fol 2+Dn 2, Fol 2+Dn 3 caused significant increase of 74.3, 53.7, and 37.1% in number of leaves, in comparison with Fol 2. There was a significant enhancement of 119.7, 95.8, and 88.2% in number of leaves of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3 (*Fig. 2D*).

Effect of soil amendments on the root length and root fresh weight of tomato plants

There was 22, 31, and 41% decrease in the root length of tomato plants when there was inoculation of concentration 1 (Fol 1), 2 (Fol 2), and 3 (Fol 3) of Fol, in comparison with NIC. The effect of 3 strengths of (DFol 1), (DFol 2), and (DFol 3) was found nonsignificant when compared with NIC. The effect of 3 concentrations namely, (Dn 1), (Dn 2), (Dn 3) of *D. nigropaleacea* on the root length of tomato plants was variable as there was nonsignificant effect of Dn 1 on the root length while, there was significant inhibitory effects (17 and 35%) by Dn 2 and Dn 3, of *D. nigropaleacea*, respectively.

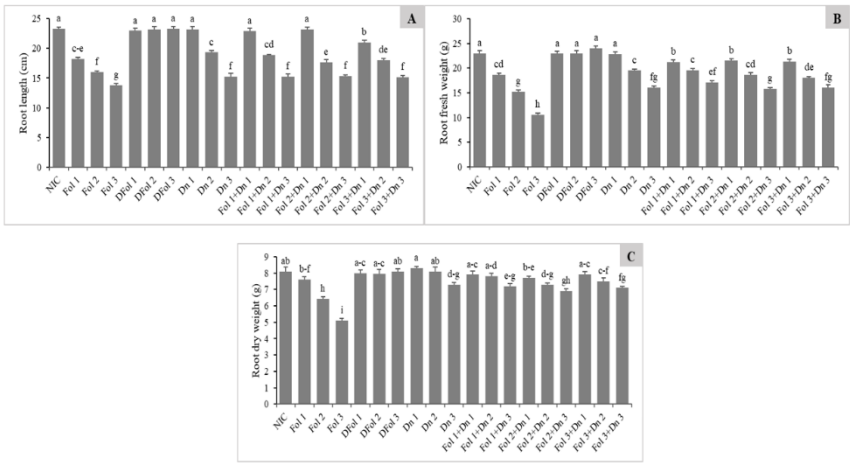

Figure 3. *Effect of treatments on (A) root length, (B) root fresh weight, and (C) root dry weight of tomato plants. Bars sharing common alphabets do not differ at P≤5%, as determined by Fisher's LSD on Minitab 20.2. Y-error bars reveal standard error of means of 5 replicas*

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DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

Soil amendment with *D. nigropaleacea* significantly overcome the deleterious effects of the pathogen and subsequently increased the root length. There was an increase of 26, 4 and, 17% in root length of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. There was a significant increase of 45 and 10% in root length of tomato plants by the introduction of Fol 2+Dn 1 and Fol 2+Dn 2 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant increase of 52, 30, and 9% in root length of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3 (*Fig. 3A*).

There was 19, 34, and 54% significant decrease in the root fresh weight of tomato plants when there was inoculation of Fol 1, Fol 2, and Fol 3, respectively, when compared with NIC. The effect of 3 concentrations of dead inoculum (DFol 1), 2 (DFol 2), and 3 (DFol 3) was found nonsignificant when compared with NIC, as well as when compared with each other. The effect of Dn 1, Dn 2, Dn 3 of *D. nigropaleacea* on the root fresh weight of tomato plants was variable as there was nonsignificant effect of Dn 1 on the root fresh weight while, there was significant inhibitory effects (15 and 30%) by using higher conc., Dn 2, Dn 3, of *D. nigropaleacea*, respectively. On the other hand, *D. nigropaleacea* incorporation in soil significantly overcome the wilt disease and therefore, increased the root fresh weight of tomato plants. There was a significant increase of 14 and 8% in root fresh weight of tomato plants by the introduction of Fol 1+Dn 1 and Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. There was a significant increase of 41 and 22% in root fresh weight of tomato plants by the introduction of Fol 2+Dn 1 and Fol 2+Dn 2 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant enhancement of 103, 71, and 52% in root fresh weight of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, and Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3 (*Fig. 3B*).

Effect of soil amendments on the root dry weight of tomato plants

Data regarding effect of various treatments on the root dry weight of pot grown tomato plants are presented in *Fig. 3C*. There was 6, 21, and 37% decrease in the root dry weight of tomato plants when there was inoculation of concentration 1 (Fol 1), 2 (Fol 2), and 3 (Fol 3) of Fol, in comparison with NIC. The effect of 3 concentrations of dead *F. oxysporum* (DFol 1), 2 (DFol 2), and 3 (DFol 3) was found nonsignificant when compared with NIC, as well as when compared with each other. The effect of (Dn 1), (Dn 2), and (Dn 3) of *D. nigropaleacea* on the root dry weight of tomato plants was variable as there was nonsignificant effect of Dn 1 and Dn 2 on the root dry weight while, there was significant inhibitory effect (10%) by using higher concentration, Dn 3, of *D. nigropaleacea*.

There was an increase of 4 and 3%, while there was a decrease of 5% in root dry weight of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. There was a significant increase of 20 and 14%, in root dry weight of tomato plants by the introduction of Fol 2+Dn 1 and Fol 2+Dn 2 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant increase of 55, 47, and 39% in root dry weight of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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The effect of Dryopteris nigropaleacea plant powder on defence related antioxidants in tomato

Data regarding SOD activity of tomato plants are presented in *Fig. 4A*. Fol 1, Fol 2, and Fol 3 showed 41, 57, and 84% increase in SOD concentration as compared to NIC. On the other hand, DFol 1, DFol 2, and DFol 3 showed nonsignificant results. However, with an increase in *D. nigropaleacea* concentration from 0.025 to 0.075%, a remarkable increase of 12, 46, and 54% was recorded with respect to NIC. While, treatments Fol 1+Dn 1, Fol 1+Dn 2, and Fol 1+Dn 3 depicted 73, 79, and 91% increase in SOD, as compared to NIC. Whereas, Fol 2+Dn 1, Fol 2+Dn 2, and Fol 2+Dn 3 treated plants showed significant increase of 75, 85, and 103%, as compared to NIC. There was a pronounced increase of 101, 114, and 123% in SOD activity of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with NIC.

On the other hand, treatments, Fol 1, Fol 2, and Fol 3 revealed 7, 11, and 20% increase respectively, in POD activity, as compared to NIC. Whereas, dead inoculum treatments showed nonsignificant results when compared with NIC, while tomato plants exhibited 8, 18, and 23% increase in POD activity when plants were treated with Dn 1, Dn 2, and Dn 3, respectively. However, Fol 1+Dn 1, Fol 1+Dn 2, and Fol 1+Dn 3 treated plants showed 15, 23, and 39% increase in POD activity, as compared to NIC. While, Fol 2+Dn 1, Fol 2+Dn 2, and Fol 2+Dn 3 treated plants exhibited 37, 41, and 51% increase, as compared to NIC. There was significant increase of 44, 52, and 61% in POD activity of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, and Fol 3+Dn 3 respectively, in comparison with NIC (*Fig. 4B*).

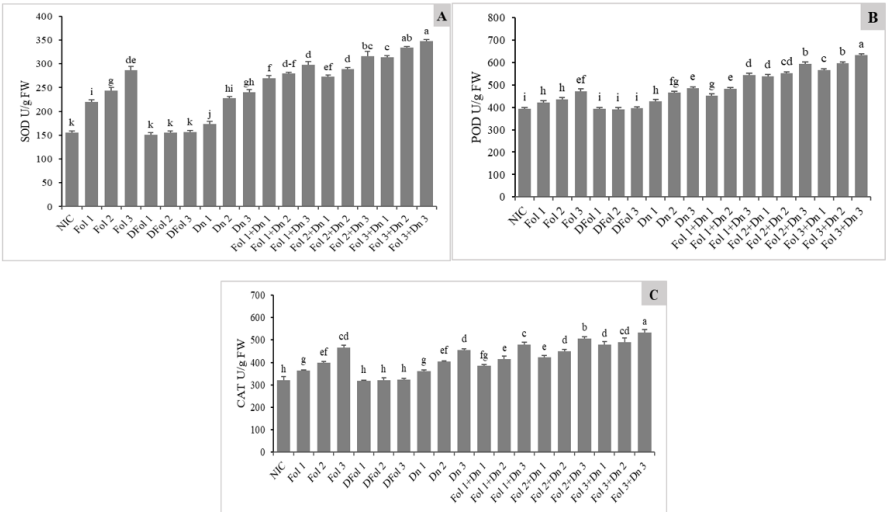

Figure 4. *Effect of treatments on (A) SOD, (B) POD, and (C) CAT of tomato plants. Bars sharing common alphabets do not differ at P≤5%, as determined by Fisher's LSD on Minitab 20.2. Y-error bars reveal standard error of means of 5 replicas. Fol=Fusarium oxysporum f. sp. lycopersici; colony forming units (CFU/mL); Dn=Dryopteris nigropaleacea; NIC=Non infested control; Fol 1=Fol 1×10^7; Fol 2= Fol 2×10^7; Fol 3=Fol 3×10^7; DFol 1=Fol (dead) 1×10^7; DFol 2=Fol (dead) 2×10^7; DFol 3=Fol (dead) 3×10^7; Dn 1=Dn (0.025%); Dn 2=Dn (0.050%); Dn 3=Dn (0.075%); Fol 1+Dn 1=Fol (1×10^7)+Dn (0.025%); Fol 1+Dn 2=Fol (1×10^7)+Dn (0.050%); Fol 1+Dn 3=Fol (1×10^7)+Dn (0.075%); Fol 2+Dn 1=Fol (2×10^7)+Dn (0.025%); Fol 2+Dn 2=Fol (2×10^7)+Dn (0.050%); Fol 2+Dn 3=Fol (2×10^7)+Dn (0.075%); Fol 3+Dn 1=Fol (3×10^7)+Dn (0.025%); Fol 3+Dn 2=Fol (3×10^7)+Dn (0.050%); Fol 3+Dn 3=Fol (3×10^7)+Dn (0.075%)*

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In case of CAT activity, Fol 1, Fol 2, and Fol 3 treated plants showed remarkable increase of 14, 24, and 46%, respectively, as compared to NIC. Just like SOD and POD activity, dead inoculum treatments expressed nonsignificant results with respect to NIC. However, individual effects of plant powder of *D. nigropaleacea* treatments showed 13, 26, and 42% increase with Dn 1, Dn 2, and Dn 3, respectively. On the other hand, Fol 1+Dn 1, Fol 1+Dn 2, and Fol 1+Dn 3 treated plants showed 20, 30, and 50% increase as compared to NIC. Whereas, Fol 2+Dn 1, Fol 2+Dn 2, and Fol 2+Dn 3 treated plants depicted 32, 41, and 58% increase in contrary to NIC. There was remarkable increase of 50, 54, and 67% in CAT activity of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with NIC (*Fig. 4C*).

Effect of soil amendments on disease incidence, disease severity and area under disease progress curve on tomato plants

Disease symptoms appeared after 45 days of inoculation and data regarding disease incidence (DI), disease severity (DS), and area under disease progress curve (AUDPC) are presented in *Table 1*. DI at the time of 1st observation was nonsignificant when there was inoculation of various concentrations of Fol. Fol 1 showed zero symptoms, while, Fol 2 and Fol 3 depicted (t^1=20%) DI, respectively. On the other hand, Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3, Fol 2+Dn 1 showed nonsignificant results when compared with Fol 1 and 2. A non-significant effect in DI of tomato plants was recorded by the introduction of Fol 2+Dn 2, Fol 2+Dn3, and Fol 3+Dn3 of *D. nigropaleacea*, as compared to Fol 2 also, Fol 3+Dn 1, Fol 3+Dn 2, revealed non-significant results, when compared with Fol 3. Inoculation of Fol 1, Fol 2, and Fol 3 in tomato plants showed 40, 60 and 100% DI, respectively, at the time of 2nd observation. On the other hand, *D. nigropaleacea* incorporation in soil significantly overcome the wilt disease in tomato plants. There was a nonsignificant decrease in DI in tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. Also, there was a nonsignificant decrease in DI of tomato plants by the introduction of Fol 2+Dn 1, Fol 2+Dn 2, Fol 2+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 2. However, there was a significant inhibition of 60, 80, and 80% in DI of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, and Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3. AUDPC for disease incidence in pot assays was 700, 1400, and 2100, when tomato plants were subjected to Fol 1, Fol 2, and Fol 3, respectively. While, soil amendment with *D. nigropaleacea* significantly overcome the fungal attack and subsequently decreased the Fusarium wilt of tomato plants. There was a nonsignificant decrease in AUDPC (DI) of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, and Fol 1+Dn 3 of *D. nigropaleacea*, when compared with Fol 1. There was a significant decrease of 75% in AUDPC (DI) of tomato plants by the introduction of Fol 2+Dn 2 and Fol 2+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant decrease of 50, 66.7, and 83% in AUDPC (DI) of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3. In case of DS at the time of 1st observation (t^1), Fol 1 exhibited zero symptoms while, Fol 2 and Fol 3 revealed 0.4 and 0.6 DS scores, respectively. There was a nonsignificant decrease in DS in Fol 2+Dn1, Fol 2+Dn 2, and Fol 2+Dn 3, when compared to Fol 2. In contrast to other treatments, Fol 3+Dn 3 showed 100% remarkable decrease in DS by using high concentration (Dn 3) of *D. nigropaleacea*. DS scores at the time of 2nd observation were 2, 3, and 4.6 in treatments *viz*., Fol 1, Fol 2, and Fol 3, respectively. On the other hand, *D. nigropaleacea* incorporation in soil significantly overcome the wilt disease in tomato

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plants. There was a significant decrease of 90% in DS in tomato plants by the incorporation of Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1, while the effect of lower concentrations of *D. nigropaleacea* was found nonsignificant. There was a significant decrease of 66.7, 73.3, and 86.7% in DS of tomato plants by the introduction of Fol 2+Dn 1, Fol 2+Dn 2, Fol 2+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant inhibition of 65.2, 82.6, and 87 % in DS of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, and Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3.

AUDPC for disease severity in pot assays was 35, 59.5, and 91, when tomato plants were exposed to Fol 1, Fol 2, and Fol 3, respectively. While, soil amendment with *D. nigropaleacea* significantly overcome the fungal attack and subsequently decreased the Fusarium wilt of tomato plants. There was nonsignificant decrease in AUDPC (DS) of tomato plants by the introduction of Fol 1+Dn 1, Fol 1+Dn 2, Fol 1+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 1. There was a significant decrease of 76.5 and 88.2% in AUDPC (DS) of tomato plants by the introduction of Fol 2+Dn 2 and Fol 2+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 2. There was a significant decrease of 50, 73.1, and 88.5% in AUDPC of tomato plants by the introduction of Fol 3+Dn 1, Fol 3+Dn 2, Fol 3+Dn 3 of *D. nigropaleacea*, respectively, when compared with Fol 3.

Table 1. *Effect of different treatments on disease incidence, disease severity and area under disease progress curve on tomato plants*

Treatments	Disease incidence (%)		AUDPC (DI)	Disease severity (Scores)		AUDPC (DS)
	t^1	t^2		t^1	t^2	
Fol 1	0±0a	40±24.5bc	700±428b-d	0±0b	2±1.2bc	35±21.4b-d
Fol 2	20±20a	60±24.5ab	1400±350ab	0.4±0.4ab	3±1.2ab	59.5±25ab
Fol 3	20±20a	100±0a	2100±350a	0.6±0.24a	4.6±0.24a	91±6.5a
Fol 1+Dn 1	0±0a	40±24.5bc	700±428b-d	0±0b	0.6±0.4cd	10.5±7de
Fol 1+Dn 2	0±0a	20±20bc	350±350cd	0±0b	0.4±0.4cd	7±7de
Fol 1+Dn 3	0±0a	20±20bc	350±350cd	0±0b	0.2±0.2d	3.5±3.5de
Fol 2+Dn 1	20±20a	20±20bc	700±428b-d	0.2±0.2ab	1±1cd	31.5±19.5b-e
Fol 2+Dn 2	0±0a	20±20bc	350±350cd	0±0b	0.8± 0.8cd	14±14c-e
Fol 2+Dn 3	0±0a	20±20bc	350±350cd	0±0b	0.4±0.4cd	7±7de
Fol 3+Dn 1	20±20a	40±24.5bc	1050±700bc	0.6±0.6a	1.6±1.02b-d	45.5±19.6bc
Fol 3+Dn 2	20±20a	20±20bc	700±700b-d	0.4±0.4ab	0.8±0.8cd	24.5±15c-e
Fol 3+Dn 3	0±0a	20±20bc	350±350cd	0±0b	0.6±0.6cd	10.5±10.5de

Note: Disease symptoms did not appear in NIC, DFol 1, DFol 2, DFol 3, Dn 1, Dn 2, Dn 3, so data were omitted from table for these treatments. Data presented represent means ± standard error of 5 replicates. Values sharing common alphabets do not differ at $P \leq 5\%$, as determined by Fisher's LSD on Minitab 20.2. Significance level was calculated within each column. t^1 (after 45 days of inoculation); t^2 (after 80 days of inoculation) are two times at which data regarding disease incidence and severity were recorded. Disease severity was calculated according to scale (0-5). Fol=*Fusarium oxysporum* f. sp. *lycopersici*; colony forming units (CFU/mL); Dn=*Dryopteris nigropaleacea*, NIC=Non infested control; Fol 1=Fol 1×10^7; Fol 2= Fol 2×10^7; Fol 3=Fol 3×10^7; DFol 1=Fol (dead) 1×10^7; DFol 2=Fol (dead) 2×10^7; DFol 3=Fol (dead) 3×10^7; Dn 1=Dn (0.025%); Dn 2=Dn (0.050%); Dn 3=Dn (0.075%); Fol 1+Dn 1=Fol (1×10^7)+Dn (0.025%); Fol 1+Dn 2=Fol (1×10^7)+Dn (0.050%); Fol 1+Dn 3=Fol (1×10^7)+Dn (0.075%); Fol 2+Dn 1=Fol (2×10^7)+Dn (0.025%); Fol 2+Dn 2=Fol (2×10^7)+Dn (0.050%); Fol 2+Dn 3=Fol (2×10^7)+Dn (0.075%); Fol 3+Dn 1=Fol (3×10^7)+Dn (0.025%); Fol 3+Dn 2=Fol (3×10^7)+Dn (0.050%); Fol 3+Dn 3=Fol (3×10^7)+Dn (0.075%)

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Confirmation of Koch's postulates

From pathogenicity tests conducted in pot experiments, the pathogen was reisolated from tomato plants and re-identified as *F. oxysporum* f. sp. *lycopersici*.

Discussion

In the present study, *Dryopteris nigropaleacea* (Dn.) was found to be a potent antifungal agent, where it significantly suppressed the wilt disease of tomato and consequently increased the growth of tomato plants. Soil amendment with *D. nigropaleacea* at 0.025%, and Fol at 3×10^7, (Fol 3+Dn 1) showed an increase in shoot and root length, shoot fresh and dry weight, and the number of leaves of pot-grown tomato plants by 53, 52, 123, 63, and 120% in *Fusarium* infested plants (3×10^7), as compared to a positive control (3×10^7). Fol 3+Dn 1 reduced DI and DS up to 60 and 65.2%, as compared with Fol 3 treated plants. Area under disease progress curve (AUDPC) was also reduced to 50 and 50%, for disease incidence (DI) and disease severity (DS), respectively, when compared with Fol 3.

Although Fol 3+Dn 2 and Fol 3+Dn 3, also exhibited a marked decrease in DI, DS, and AUDPC, these concentrations were found inhibitory to the growth of tomato plants also, so Dn 1 was found the optimum dose of *D. nigropaleacea* amendment that significantly controlled the wilt disease of tomato, thereby, increased growth parameters of tomato plants. Previously, Khurshid et al. (2017) reported that plant powder of *Cenchrus pennisetiformis* reduced DI up to 10% from 60% and DS was reduced to 1 from 3 grade against *F. oxysporum*, causing wilt disease of tomato. In another study, soil amendment with *Brassica carinata* reduced the Fusarium wilt DS up to 74-84%, 30 and 60 days after inoculation, respectively (Gilardi et al., 2018). In another study, soil amendment with pineapple residue alleviated the pathogen pressure by increasing the relative abundance of antagonistic fungi, causing a negative effect on pathogen growth and DI (Yuan et al., 2021).

Soil amendment with plant residues is a favorable tool to suppress soil-borne pathogens. Soil amendment increases the water-holding capacity of the soil, soil fertility, and beneficial microbes, ultimately helping in improving the morphology and physiology of diseased plants. It also provides a healthy environment for antagonistic microbes against fungal pathogens which increases plant growth regulators. Soil amendment creates competition for fungal pathogens because plant residue contains different bioactive components like phenols, diterpenes, phloroglucinols, etc., which kill the devastating pathogens (Panth et al., 2020).

Different plants affect the growth of other plants either by enhancing the growth of recipient plant or by retarding the growth of the recipient plant. So, in the present investigation, treatments of *D. nigropleacea* with different concentrations (0.025, 0.050, and 0.075%) were also included in pot experiments to evaluate the effect of *D. nigropaleacea* on the plant growth parameters of tomato. Moreover, in pot experiments, treatments also comprised dead inoculum of Fol. These treatments were included to evaluate the effects of dead Fol on the growth parameters of tomato. In the present study, 0.025% (Dn 1) concentration of *D. nigropaleacea* did not cause any negative or positive effect on the growth of tomato plants, but the higher concentrations of 0.05 (Dn 2) and 0.075% (Dn 3), of *D. nigropaleacea*, were found to be inhibitory to the growth of tomato plants also. Dn 2 and Dn 3, declined the growth parameters of tomato plants, and this inhibition was dose-dependent. In previous studies, soil

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amendment with the powder or extraction residue of *Acacia albida*, at dose of 40 g/pot, induced improvements in tomato plant growth parameters *viz*., plant height, root biomass, and shoot biomass by 9.8, 50, and 34.3%, respectively, in tomato plants (Schinzoumka et al., 2016).

Liu et al. (2013) reported the enhancement in morphological growth parameters of tomato plants due to soil amendments with *Dryopteris crassirhizoma* concentrations *viz*., 10, 30, and 50 grams/pot. There was an increase of 19, 10, 29, 41%, in the shoot length, root length, shoot weight and root weight of tomato plants by the addition of *D. crassirhizoma* (50 grams/pot), respectively. However, there are several reports that depicted the negative effects of the addition of biomass or isolated compounds of one plant over the recipient plants. As an example, *Leucaena leucocephala* contains certain allelochemicals such as phenolic acids, flavonoids, and mimosine, which are released into the rhizosphere soil during the decomposition process of the plant residues. The most allelopathic compound, mimosine, was reported to range from 0.11 to 6.4% of the dry weight of *L. leucocephala*. Mimosine showed growth inhibitory activity against *Petunia hybrida*, by blocking cell division of protoplasts in cells between G_1 and S phases, and it also disturbed the activity of antioxidant enzymes such as indole acetic acid oxidase, peroxidase, and catalase (Kato-Noguchi and Kurniadie, 2022). Aldinary et al. (2021) also showed a declining trend of up to 40% in the shoot length of tomato due to Fol infection. A decline in the shoot length of tomato plants is the characteristic feature of Fol-infected tomato plants (Cai et al., 2003). In the present investigation, Fol was identified on the basis of morphology (Akbar et al., 2018) and molecular data, based on phylogenetic analysis of ITS1 and ITS4 gene sequences showing agreement with Singha et al. (2016).

Fol+Dn 1 treated plants showed 107, 44, and 50% increases in SOD, POD, and CAT activities, as compared to negative control. Moreover, the antioxidants analysis also revealed that defensive enzymes SOD, POD, and CAT also increased when the pathogen was inoculated. It was also examined that treatments of *D. nigropaleacea* showed positive effects on defensive enzymes and marked increase was observed when different treatments were analyzed. Dead pathogen treatments showed nonsignificant results at all concentrations. ROS (Reactive oxygen species) are produced in different crops under stress conditions (Miller et al., 2010). It produces negative effects and damage macromolecules and other contents (Zhang et al., 2009). Naturally, chemical antioxidants like SOD, POD, and CAT are produced in response to ROS production under oxidative stress conditions for protection against the overproduction of ROS (Gill and Tuteja, 2010). To meet plant security against oxidative stress, the overproduction of antioxidants is a naturally occurring phenomenon in plants under stressful conditions (Nawaz et al., 2015). Active defense mechanism in plants produce resistance against diseases. Antioxidant enzymes like SOD, POD, and CAT increase tolerance against infection. Antioxidants scavenge or control ROS production which is ultimate result of infection or biotic stress (Hasanuzzaman et al., 2020). Antioxidant enzyme activities provide defensive enzyme's production against biotic stress. It produces resistance against plant stress along with production of phenolic compounds. Our results suggest that antioxidant activities increased due to *Fusarium* infection. Hence defensive enzymes SOD, POD, and CAT were increased to keep ROS at lower level when plants were inoculated with Fol. There was 23, 28%, and 56% increase observed in SOD, POD, and CAT activity in *Fusarium* inoculated tomato seedlings as compared to negative control while, 14, 97, and 38% increase was also observed in SOD, POD, and CAT activities in willow extract treated tomato seedlings (Hanaa et al., 2011). In the present investigation, defense related

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enzymes were increased due to Fol infection and *D. nigropaleacea* amendment. The increase in antioxidants suppress oxidative stress and detoxify ROS which are produced due to Fol infection (Vellosillo et al., 2010). Similarly, 69, 108, and 45% increase was observed in SOD, POD, and CAT activities, as compared to negative control due to biochar rice straw soil amendment (Shahkolaie et al., 2020).

In our study, increased morphological and biochemical attributes of tomato plants can be visualized as increased resistance in *D. nigropaleacea* treated tomato plants as compared to Fol treated plants without incorporation of *D. nigropaleacea*, under pot conditions. Moreover, the presence of antifungal compounds in *D. nigropaleacea* may also contributed in lowering the effect of Fol by directly killing Fol due to its antifungal properties.

Conclusion

The findings of this study suggest the antifungal efficacy of *Dryopteris nigropaleacea* against Fusarium wilt of tomato. In pot bioassays, soil amendment with *D. nigropaleacea* at lower concentration (0.025%) was found to be the most effective concentration as it resulted in significant decrease in disease incidence (DI) and disease severity (DS), when compared with *F. oxysporum* f. sp. *lycopersici* (Fol) infested control, thereby significantly increasing the morphological growth of tomato. The increased morphological growth can be due to improved biochemical attributes of tomato plants by the introduction of *D. nigropaleacea*.

Acknowledgements. The authors acknowledge Prof. Dr. Amna Shoaib, Department of Plant Pathology, University of the Punjab, Lahore, Pakistan for morphological identification of *Fusarium* culture and Prof. Dr. Muhammad Zia ur Rehman, Government Science College, Lahore for molecular identification of *Fusarium*.

REFERENCES

[1] Ajilogba, C. F., Babalola, O. O. (2013): Integrated management strategies for tomato Fusarium wilt. – Biocontrol Science 3: 117-127.

[2] Akbar, M., Javaid, A. (2015): Management of *Rumex dentatus* L. (Toothed Dock) by fungal metabolites under field conditions. – International Journal of Agriculture and Biology 17(1): 187-192.

[3] Akbar, A., Hussain, S., Ullah, K., Fahim, M., Ali, G. S. (2018): Detection, virulence, and genetic diversity of Fusarium species infecting tomato in northern Pakistan. – Plos One 13(9): e0203613.

[4] Akladious, S. A., Isaac, G. S., Abu-Tahon, M. A. (2015): Induction and resistance against Fusarium wilt disease of tomato by using sweet basil (*Ocimum basilicum* L.) extract. – Canadian Journal of Plant Science 95(4): 689-701.

[5] Alam, F., Ali Khan, S. H., Asad, M. H. H. B. (2021): Phytochemical, antimicrobial, antioxidant, and enzyme inhibitory potential of medicinal plant *Dryopteris ramosa* (Hope) C. Chr. – BMC Complementary Medicine and Therapies 21: 1-10.

[6] Alamri, S. A., Hashem, M., Mostafa, Y. S., Nafady, N. A., Abo-Elyousr, K. A. (2019): Biological control of root rot in lettuce caused by *Exserohilum rostratum* and *Fusarium oxysporum* via induction of the defense mechanism. – Biological Control 128: 76-84.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

[7] Aldinary, A. M., Abdelaziz, A. M., Farrag, A. A., Attia, M. S. (2021): Biocontrol of tomato Fusarium wilt disease by a new Moringa endophytic *Aspergillus* isolates. – Materials Today: Proceedings.
[8] Ali, A., Javaid, A., Shoaib, A., Khan, I. H. (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. – Egyptian Journal of Biological Pest Control 30(1): 1-9.
[9] Alizadeh-Moghaddam, G., Rezayatmand, Z., Nasr-Esfahani, M., Khozaei, M. (2020): Bio-genetic analysis of resistance in tomato to early blight disease, *Alternaria alternata*. – Phytochemistry 179: 112486.
[10] Ashiq, S. (2015): Natural occurrence of mycotoxins in food and feed: Pakistan perspective. – Comprehensive Reviews in Food Science and Food Safety 14(2): 159-175.
[11] Attia, M. S., Abdelaziz, A. M., Al-Askar, A. A., Arishi, A. A., Abdelhakim, A. M., Hashem, A. H. (2022): Plant growth-promoting fungi as biocontrol tool against Fusarium wilt disease of tomato plant. – Journal of Fungi 8(8): 775.
[12] Awan, Z. A., Shoaib, A., Khan, K. A. (2018): Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease. – Scientia Horticulturae 239: 216-223.
[13] Cai, Q. S. (2013): Experiment of plant physiology. – China Agricultural University Press, Beijing (in Chinese).
[14] Cai, Y., Sun, M., Corke, H. (2003): Antioxidant activity of betalains from plants of the Amaranthaceae. – Journal of Agricultural and Food Chemistry 51(8): 2288-2294.
[15] de Chaves, M. A., Reginatto, P., da Costa, B. S., de Paschoal, R. I., Teixeira, M. L., Fuentefria, A. M. (2022): Fungicide resistance in *Fusarium graminearum* species complex. – Current Microbiology 79(2): 62.
[16] De Corato, U., Patruno, L., Avella, N., Salimbeni, R., Lacolla, G., Cucci, G., Crecchio, C. (2020): Soil management under tomato-wheat rotation increases the suppressive response against Fusarium wilt and tomato shoot growth by changing the microbial composition and chemical parameters. – Applied Soil Ecology 154: 103601.
[17] de Rodriguez, D. J., Gaytán-Sánchez, N. A., Rodríguez-García, R., Hernández-Castillo, F. D., Díaz-Jiménez, L., Villarreal-Quintanilla, J. A., Flores-López, M. L., Carrillo-Lomelí, D. A., Peña-Ramos, F. M. (2019): Antifungal activity of *Juglans* spp. and *Carya* sp. ethanol extracts against *Fusarium oxysporum* on tomato under greenhouse conditions. – Industrial Crops and Products 138: 111442.
[18] Devi, N. O., Tombisana Devi, R. K., Debbarma, M., Hajong, M., Thokchom, S. (2022): Effect of endophytic Bacillus and Arbuscular Mycorrhiza Fungi (AMF) against Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. – Egyptian Journal of Biological Pest Control 32(1): 1-4.
[19] Fatah, S. A., Mubarik, A., Aqsa, Y. (2020): Tomato cluster feasibility and transformation study. – Cluster Development Based Agriculture Transformation Plan Vision-2025 Project 131: 434.
[20] Gao, J. F. (2006): Experimental guidance for plant physiology. – China Higher Education Press: Beijing, China.
[21] Gautier, C., Pinson-Gadais, L., Richard-Forget, F. (2020): Fusarium mycotoxins enniatins: An updated review of their occurrence, the producing Fusarium species, and the abiotic determinants of their accumulation in crop harvests. – Journal of Agriculture and Food Chemistry 68: 4788-4798.
[22] Giannopolitis, C. N., Ries, S. K. (1977): Superoxide dismutases: I. Occurrence in higher plants. – Plant Physiology 59(2): 309-314.
[23] Gilardi, G., Cucu, M. A., Pugliese, M., Gullino, M. L., Garibaldi, A. (2018): Effect of different organic amendments on lettuce Fusarium wilt and on soilborne microorganisms. – In IX International Symposium on Soil and Substrate Disinfestation 1270: 117-126.

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DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

[24] Gill, S. S., Tuteja, N. (2010): Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – Plant Physiology and Biochemistry 48(12): 909-930.
[25] Hanaa, R. F., Abdou, Z. A., Salama, D. A., Ibrahim, M. A., Sror, H. A. (2011): Effect of neem and willow aqueous extracts on Fusarium wilt disease in tomato seedlings: Induction of antioxidant defensive enzymes. – Annals of Agricultural Sciences 56(1): 1-7.
[26] Hasanuzzaman, M., Bhuyan, M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., Fujita, M., Fotopoulos, V. (2020): Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. – Antioxidants 9(8): 681.
[27] Hyder, S., Inam-ul-Haq, M., Fatima, N., Hannan, A., Alam, M. M., Iqbal, M. (2019): First report of root rot of tomato caused by *Phytophthora syringae* in Pakistan. – Plant Disease 103(3): 590.
[28] Javaid, A. R., Latif, U. M., Akhtar, N., Ahmed, D., Perveen, S. H. (2018): Molecular characterization of *Fusarium moniliforme* and its management by methanolic extract of *Coronopus didymus*. – Pakistan Journal of Botany 50(5): 2069-2075.
[29] Jeger, M. J., Viljanen-Rollinson, S. L. (2001): The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. – Theoretical and Applied Genetics 102: 32-40.
[30] Kato-Noguchi, H., Kurniadie, D. (2022): Allelopathy and allelochemicals of *Leucaena leucocephala* as an invasive plant species. – Plants 11(13): 1672.
[31] Khurshid, S., Shoaib, A., Javaid, A., Akhtar, F., Shafiq, M., Qaisar, U. (2017): Management of Fusarium wilt of tomato by soil amendment with *Cenchrus pennisetiformis* under chromium stress. – Physiological and Molecular Plant Pathology 97: 58-68.
[32] Kim, K. H., Kabir, E., Jahan, S. A. (2017): Exposure to pesticides and the associated human health effects. – Science of the Total Environment 575: 525-535.
[33] Kurabachew, H., Stahl, F., Wydra, K. (2013): Global gene expression of rhizobacteria-silicon mediated induced systemic resistance in tomato (*Solanum lycopersicum*) against *Ralstonia solanacearum*. – Physiological and Molecular Plant Pathology 84: 44-52.
[34] Liu, J., Xie, S., Feng, J., Cai, J. (2013): Protective effect of *Dryopteris crassirhizoma* extracts in the control of the root-knot nematode *Meloidogyne incognita*. – Journal of Plant Diseases and Protection 120: 34-40.
[35] Maurya, S., Dubey, S., Kumari, R., Verma, R. (2019): Management tactics for Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.): A review. – International Journal of Research in Pharmacy and Pharmaceutical Sciences 4(5): 1-7.
[36] Miller, G. A., Suzuki, N., Ciftci-Yilmaz, S. U., Mittler, R. O. (2010): Reactive oxygen species homeostasis and signalling during drought and salinity stresses. – Plant, Cell & Environment 33(4): 453-467.
[37] Nawaz, F., Ashraf, M. Y., Ahmad, R., Waraich, E. A., Shabbir, R. N., Bukhari, M. A. (2015): Supplemental selenium improves wheat grain yield and quality through alterations in biochemical processes under normal and water deficit conditions. – Food Chemistry 175: 350-357.
[38] Panth, M., Hassler, S. C., Baysal-Gurel, F. (2020): Methods for management of soilborne diseases in crop production. – Agriculture 10(1): 16.
[39] Poussio, G. B., Abro, M. A., Syed, R. N., Khaskheli, M. I., Jiskani, A. M. (2021): In vitro chemical management of Fusarium wilt of tomato in Sindh, Pakistan. – International Journal on Emerging Technologies 12(1): 162-169.
[40] Retig, N. (1974): Changes in peroxidase and polyphenol oxidase associated with natural and induced resistance of tomato to Fusarium wilt. – Physiological Plant Pathology 4(2): 145-150.
[41] Savary, S., Ficke, A., Aubertot, J. N., Hollier, C. (2012): Crop losses due to diseases and their implications for global food production losses and food security. – Food Security 4(4): 519-537.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_26852703

[42] Schinzoumka, P., Jean, A. N., Valère, T. (2016): Effects of *Acacia albida* and *Crotalaria retusa* on the growth and development of tomato. – Journal of Agriculture and Ecology Research International 8(3): 1-9.
[43] Shahkolaie, S. S., Baranimotlagh, M., Dordipour, E., Khormali, F. (2020): Effects of inorganic and organic amendments on physiological parameters and antioxidant enzymes activities in *Zea mays* L. from a cadmium-contaminated calcareous soil. – South African Journal of Botany 128: 132-140.
[44] Singha, I. M., Kakoty, Y., Unni, B. G., Das, J., Kalita, M. C. (2016): Identification and characterization of Fusarium sp. using ITS and RAPD causing Fusarium wilt of tomato isolated from Assam, North East India. – Journal of Genetic Engineering and Biotechnology 14(1): 99-105.
[45] Tamura, K., Nei, M. (1993): Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. – Molecular Biology and Evolution 10(3): 512-526.
[46] Tamura, K., Stecher, G., Kumar, S. (2021): MEGA11: Molecular evolutionary genetics analysis version 11. – Molecular Biology and Evolution 38(7): 3022-3027.
[47] Tao, H., Bao, Z., Jin, C., Miao, W., Fu, Z., Jin, Y. (2020): Toxic effects and mechanisms of three commonly used fungicides on the human colon adenocarcinoma cell line Caco-2. – Environmental Pollution 263: 114660.
[48] Vellosillo, T., Vicente, J., Kulasekaran, S., Hamberg, M., Castresana, C. (2010): Emerging complexity in reactive oxygen species production and signaling during the response of plants to pathogens. – Plant Physiology 154(2): 444-448.
[49] Wakil, W., Brust, G. E., Perring, T. (2017): Sustainable Management of Arthropod Pests of Tomato. – Academic Press.
[50] White, T. J., Bruns, T. D., Lee, S. B., Taylor, J. W., Innis, M. A., Gelfand, D. H., Sninsky, J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – PCR Protocols: A Guide to Methods and Applications 18(1): 315-322.
[51] Yuan, X., Hong, S., Xiong, W., Raza, W., Shen, Z., Wang, B., Li, R., Ruan, Y., Shen, Q., Dini-Andreote, F. (2021): Development of fungal-mediated soil suppressiveness against Fusarium wilt disease via plant residue manipulation. – Microbiome 9(1): 1-5.
[52] Zehra, A., Aamir, M., Dubey, M. K., Ansari, W. A., Meena, M., Swapnil, P., Upadhyay, R. S., Ali, M. A., Al-Ghamdi, A. A., Lee, J. (2023): Enhanced protection of tomato against Fusarium wilt through biopriming with *Trichoderma harzianum*. – Journal of King Saud University-Science 35(2): 102466.
[53] Zhang, H., Ye, Y. K., Wang, S. H., Luo, J. P., Tang, J., Ma, D. F. (2009): Hydrogen sulfide counteracts chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage against osmotic stress. – Plant Growth Regulation 58: 243-250.

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