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EFFECT OF COMPOST APPLICATION ON TOMATO ROOT EXUDATES AND SUPPRESSION OF *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* (*FOL*)

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ABSTRACT

Fusarium oxysporium f. sp. *lycopersici* (*Fol*) is one of the major biotic stress factor damaging tomato productivity worldwide. Organic amendments such as compost has the ability to suppress soil borne pathogens. Compost originated from the domestic waste material (20% and 40%) were used to assess its effect on tomato growth and *Fol* tomato plants. Disease incidence was reasonably less in tomato plants growing at 20% compost as compared to plants with 40% compost. Spore germination was less in *Fol* treated plant root exudates as compared to non-treated except where 40% compost was used as growing medium. Changing the growing conditions has influenced the root exudates. Tomato plants grown on 40% compost showed significantly enhanced tomato growth and yield. Compost application in the potting mixture suppresses *Fol* and found to be effective when used as fertilizers. The better growth and yield of tomato can be achieved by using compost together with the inorganic fertilizer used in getting good growth and yield of tomatoes.

***Keywords*:** Compost, Tomato, *Fusarium Oxysporum*, Spore Germination

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1. INTRODUCTION

Solanum lycopersicum (tomato) is an important crop worldwide and its yield is greatly affected by soil-borne phytopathogens including *Fusarium oxysporum* (Saraf et al. 2014) because they survive in the soil for long period and remain dormant till they get in touch with an appropriate host. Amongst other soil-borne pathogens, the most devastating, well known and widely distributed fungi that induce necrosis, damping-off, vascular wilt and root rot in many cereal food crops is *F. oxysporum* (McGovern 2015). Different strains (pathogenic and non-pathogenic) of Fusarium fungi exist in agricultural soils and due to its high population in rhizosphere and uncultivated soils, it has received much attention from researchers (Gordon and Martyn 1997). It causes severe economic losses and several soil-borne diseases in host plants throughout the world (Steinkellner and Mammerler 2007).

Fusarium oxysporum causes veinal chlorosis and root cell collapse after secreting phototoxic compounds in plants. Moreover, during infection (necrotrophic phase), it also facilitates disease and senescence (Dong et al. 2014). *Fol* is an economically important soil borne pathogen, responsible for yield losses in tomatoes (Ramaiah et al. 2015). Under suitable environmental and weather conditions, this fungus has been pointed out to cause Fusarium wilt disease, accountable for heavy losses and damage for tomato crop. It has been documented that the root exudates activate the Fol microconidia germination (Steinkellner et al. 2005). To overcome or reduce the Fusarium wilt disease of tomato, farmers have indiscriminately used different chemical fungicides and pesticides in their fields. But in addition to disease control, these chemicals not only develop resistance in pathogens, but also persistently accumulate in plant tissues and may cause severe health hazards to humans and the wider environment (Bajwa et al. 2003). To control all these problems, one important strategy is the use of a safer and eco-friendly product like compost as a possible substitute to chemical fungicides (Paramanandham et al. 2017).

Compost is regarded as inexpensive, environmentally friendly and agronomically advantageous soil organic amendment (Senesi and Brunetti 1996). Compost is an important soil amendment, commonly used in agriculture for improving soil quality and suppressing different soil borne diseases like Fusarium wilt (Akhtar et al. 2015; Agegnehu et al. 2017; Antonangelo et al. 2021). Furthermore, the soil amended with compost, enhances the activities of different microbes like bacteria and fungi to improve plant growth, reduce the inoculums density and disease control (Salim et al. 2017). Compost has also the ability to efficiently suppress the fusarium wilt in tomato

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plants (Salim et al. 2017). The reported mechanism involved in disease control of plants in the course of compost application could be due to antibiotic production, pathways activation in plants (disease-resistance) and/or microbial competition for different resources (Taghdi et al. 2015). Combination of compost with other organic or biofertilizers was shown to be effective against Fusarium wilt of tomato (Noble and Conventry 2005; Srivastava et al. 2010; Anli et al. 2020; Awad-Allah et al. 2022) by activating soil microbes and biogeochemical cycles therein (Pascual et al. 1997).

The suppressive effect is mainly because of the soil properties (physical and chemical) and activation of microbes (Chef et al. 1983; Ueda et al. 1990; Hoitink et al. 1993; Hoitink and Boehm 1999; Bonanomi et al. 2022). Moreover, compost has disease suppressive effect (Corato 2020; Neher et al. 2022) when used as soil amendment against soilborne pathogens including Pythium spp. (Mandelbaum and Hadar 1990; Pascual et al. 2000), Phytophthora spp. (Hoitinkand Boehm 1999; Widmer et al. 1999) and Rhizoctonia spp. (Kuter et al. 1983; Tuitert et al. 1998).

This work is aimed to evaluate the effect of root exudates with combinations of compost on *Fol* growth and disease suppression. We hypothesized that it would suppress the *Fol*. The effect of compost on tomato growth is also discussed in this study.

2. MATERIALS AND METHODS

2.1. *Fusarium Oxysporum f. sp. Lycopersici* Culture

In order to develop workable *Fol* (isolate 007) culture, spores were cultivated at 25ºC in dark on CZD (Czapek Dox agar media) for 2–3 weeks. The microconidia were harvested, and a final concentration was adjusted at 1×10^7 microconidia/mL with a hemocytometer (Steinkellner et al. 2008).

2.2. Soil Preparation

A sterilized basic material comprised of soil, expanded clay, and sand. The basic material was used to prepare the combinations of compost 20% and 40% (v/v According to the Austrian compost regulation). *Fol* was either added (+*Fol*) or not added (-*Fol*) to the soil. Each treatment had five replicates and the experiment was repeated three times.

2.3. Plant Material and Root Exudate Extraction

The 4 weeks old tomato seedlings growing on perlite were transferred to the prepared potting mixes. The roots were clipped and immersed in the conidial suspension (1×10^5 microconidia/ml) for five minutes. The plants were grown in different treatments, which are as follows: 0% compost, 0% Compost + *Fol*, 20% Compost + *Fol* and 40% Compost + *Fol*. Un-inoculated plants were added as control treatments. The plants were under a complete randomized design (CRD) with long day conditions for 6 (Steinkellner et al. 2005). Root exudates were extracted in an acetate buffer (25mM, pH=5.5) for 6h (Hage-Ahmed et al. 2013a) and the final concentration was adjusted to 20mL/g of root fresh weight.

2.4. Agronomic and Physiological Parameters

Tomato (cv. 'Kremser Perle') seedlings were cultivated in pots under long day conditions with relative humidity 50% in a climate chamber for 6 weeks. The plants were grown in different treatments, which are as follows: 0% comp, 0% comp + *Fol*, 20% comp, 20% comp + *Fol*, 40% comp, 40% comp + *Fol*. Un-inoculated plants were added as control treatments. After 6 weeks, the plant roots were washed, and root exudates were extracted by submerging the roots in a buffer solution (acetate buffer) for 6hrs (Fig. 1). The root and shoot fresh weight and the phenological development stage (BBCH-scale) was recorded (Feller et al. 1995).

2.5. Disease Assessment

The disease confirmation was done as described by Steinkellner et al. (2012). Disease incidence was calculated by using the following equation:

$$\textbf{Disease incidence} = \frac{\textbf{Number of infected plants}}{\textbf{Total number of plants}} \times 100$$

The length percentage of discolored vascular tissue was measured and used to determine the disease severity (Hage-Ahmed et al. 2013). On the basis of tissue discoloration, plants were rated on a scale of 1 to 5 (c1=1-5%, c2=5-15%, c3=15-35%, c4=35-67.5%, and c5=67.5-100%). The disease severity was calculated by the following formula:

$$\textbf{Disease severity} = \frac{5 \times (nc1+2nc2+5nc3+10nc4+20nc5)}{\textbf{Number of infected plants}}$$

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Fig. 1: Extraction of root exudates of tomato plants by incubating in buffer solution.

Table 1: Principal growth stages (BBCH-scale)

Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth / shoot development(main shoot)
4	Development of harvestable vegetative plant parts or vegetatively
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

2.6. Fungal Growth Assay

The *Fol* spore germination assay was carried out in 96-well plates. Each well contained 35µL of a conidial spore suspension and of 175µL of root exudates. After 20 hours of incubation at 24°C in the dark, the germination rate (%) was assessed microscopically by counting 200 spores. The optical density (600nm) was measured after 24h for 5 days to determine the mycelial growth (Steinkellner and Mammerler 2007).

2.7. Statistical Analysis

Two-way analysis of variance (ANOVA) and Tukey's test ($P<0.05$) were used to analyze the data.

3. RESULTS

Estimation of phenotypic growth parameters was as under:

3.1. Effect of Root Exudates on Mycelial Growth of *Fol*

The effect of root exudates on mycelial growth of *Fol* was studied for a total of 120h. Acetate buffer and CZD broth were used as control treatments. The maximum growth was recorded in root exudates from 20% compost (0.75) whereas, minimum growth was observed in 0% Comp + *Fol* (0.34) (Fig. 2). There was a significant reduction in mycelial growth of *Fol* containing 40% compost + *Fol* treatments with (0.72) optical density. However, the growth was not significantly different in plant root exudates taken from the 0% compost and 0%Comp + *Fol* treatment.

3.2. *Fol* Spore Germination Rate

Germination rate (%) of *Fol* microconidia was analyzed in root exudates from plants grown in different soil substrates 0% comp, 0% comp + *Fol*, 20% comp, 20% comp + *Fol*, 40% comp, 40% comp + *Fol* after 20hrs at 24°C (Fig. 3). Data revealed that the maximum spore germination rate of 58.89% was obtained with the treatments of 20% Compost. The lowest spore germination rate 42.67% of *Fol* was observed in root exudates of growing plants containing 20% Compost + *Fol* treatment. However, the treatment with 0% Compost + *Fol* was also highly effective with 43.17% spore germination rate. Other treatments with 40% Compost + *Fol*, showed more than 56.94% spore germination. Spore germination was less in *Fol* treated plant root exudates as compared to non-treated except where 40% compost was used as a growing medium.

3.3. Disease Incidence and Severity in *Fol* Inoculated Treatments

The incidence and severity of *Fol* were assessed on tomato plants after six weeks of transplantation. Tomato plants treated with 40% Compost + *Fol* were found to be effective in decreasing the disease severity and incidence by 17.3 and 80%, respectively. Notably, treatment 20% Compost + *Fol* was the best performing and showed 7.2 and 46.6% disease severity and incidence, respectively (Fig. 4). In contrast, the treatments containing 0% Compost + *Fol*, disease incidence and severity were recorded maximum such as 66.1and 93.07%, respectively. Control plants inoculated with pathogens showed the typical symptoms observed caused by *Fol*. The treatments without compost and with *Fol* revealed a relatively high disease severity and incidence. Disease incidence was reasonably less in plants growing at 20% compost. Moreover, plants growing at 20% compost tolerated the disease attack in a better way than the rest of the treatments and yielded better biomass under disease stress.

3.4. Effect of Treatments on Root and Shoot Dry Mass

Both root and shoot weight of tomato plants grown on different soil substrates were assessed. The lowest dry weight was observed in plants grown in soil without compost inoculated with *Fol* which was 0.17g of root weight

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and 0.16g shoot weight (Fig. 5). The maximum root biomass recorded was 2.51g of plants grown in 20% compost soil. The maximum shoot biomass recorded was 10.31g of plants grown in 40% compost soil. The treatment T6 40% compost soil inoculated with (*Fol*) revealed that the root and shoot dry weight were affected by a pathogen, followed by 2.31 and 5.91g, respectively.

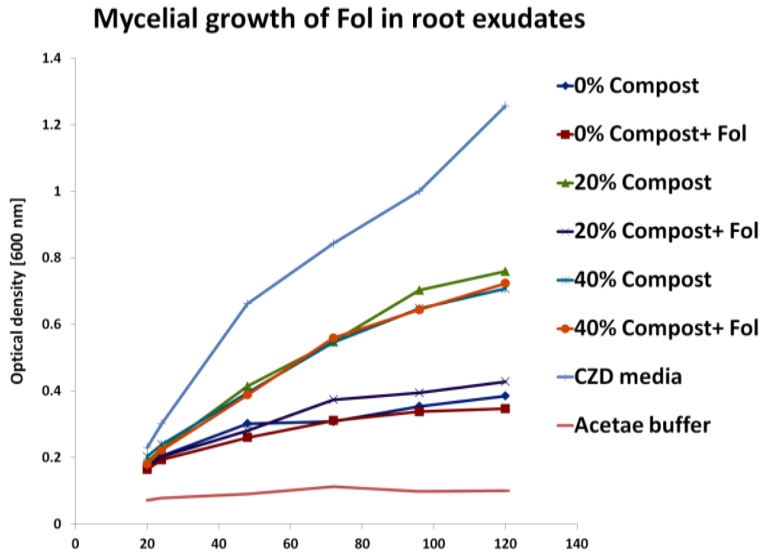

Fig. 2: Effect of root exudates on mycelial growth of *F. oxysporum* f.sp. *lycopersici*. Root exudates were extracted from plants grown in different soil substrates comprising 0% comp, 0% comp + *Fol*, 20% comp, 20% comp+ *Fol*, 40% comp, 40% comp + *Fol* after 20 hrs at 24°C.

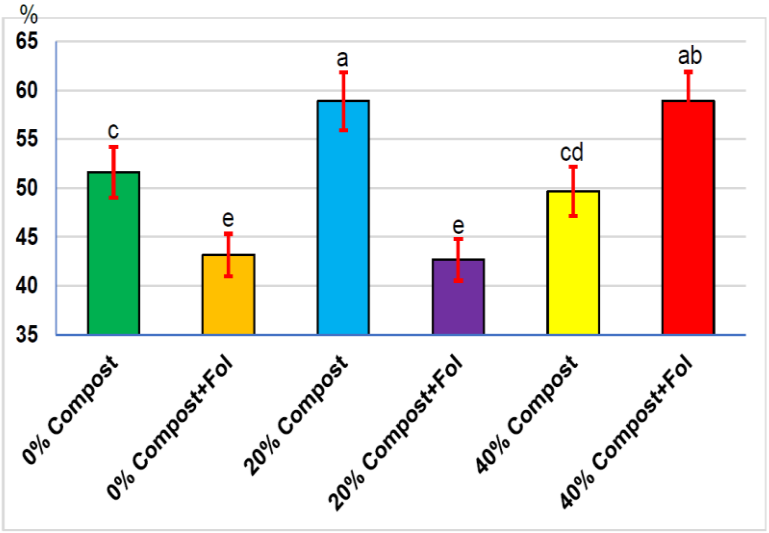

Fig. 3: Effect of root exudates on spore germination (%). Spore germination rate of *F. oxysporum* f.sp. *lycopersici* spore germination rate on root exudates of plants grown in different soil substrates comprising 0% comp, 0% comp + *Fol*, 20% comp, 20% comp+ *Fol*, 40% comp, 40% comp + *Fol* after 20 hrs at 24°C.

3.5. Effect of Treatments on Shoot Length of Tomato Plants

The shoot length of plants grown on different soils were shown in Fig. 6. The treatments without compost and with *Fol* had a relatively small shoot length with 5.28 and 2.44cm, respectively. While the plants grown in 20% compost and *Fol* showed better growth. *9The average shoot length '19.42cm' was observed in T3, followed by T4 which is18.03cm. Maximum shoot length '21.53cm' was observed in tomato plants grown in 40% compost. However, a reduction in shoot length was observed in plants grown in 40% compost and *Fol* inoculation.

4. DISCUSSION

Soil variables not only influence plant growth but also interfere with the activities of soil-borne microbial communities either beneficial or pathogenic to plants. In the recent past, soil amendment with compost has gained the interest of researchers because of their ability to improve plant growth, soil characteristics and microbial behavior. However, knowledge is limited about the complex interactions between soil-borne pathogenic or beneficial microbial communities and soil-organic amendments. This study highlighted the role of soil organic

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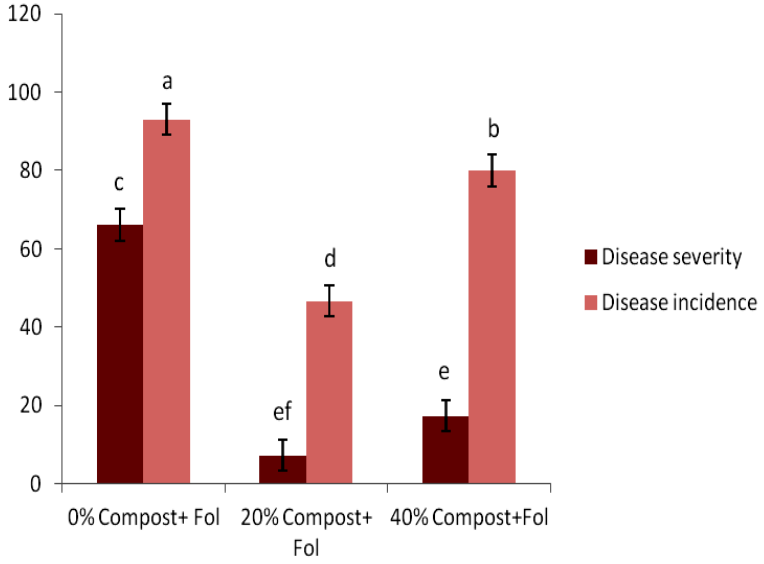

Fig. 4: Effect of treatment on disease incidence and severity. Treatments includes 0% comp + *Fol*, 20% comp+ *Fol* and 40% comp + *Fol*

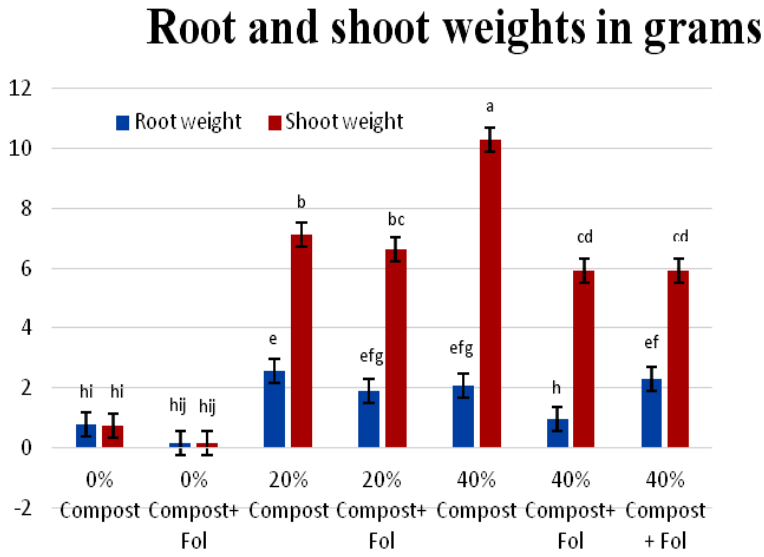

Fig. 5: Effect of treatments on root and shoot dry mass of *Solanum lycopersicum* and the treatments includesT1(0% comp), T2(0% comp + *Fol*), T3 (20% comp), T4 (20% comp+ *Fol*), T5 (40% comp), T6 (40% comp + *Fol.*).

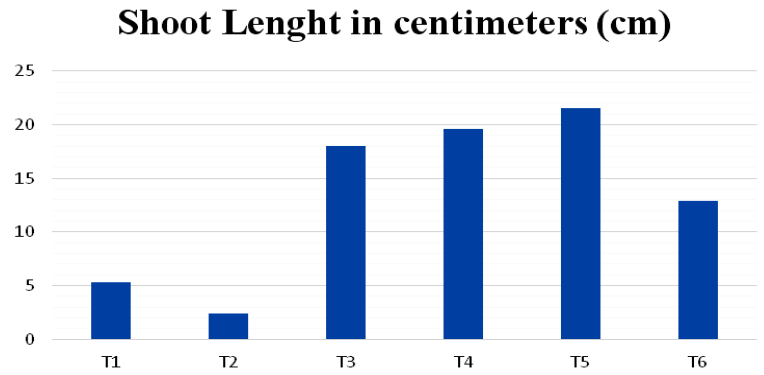

Fig. 6: Effect of treatments on shoot length of *Solanum lycopersicum* and the treatments includes T1 (0% comp), T2 (0% comp + *Fol*), T3 (20% comp), T4 (20% comp+ *Fol*), T5 (40% comp), T6.

additives such as compost in altering the infectivity of pathogenic fungal propagules (microconidia and chlamydospores), in addition to its influence on plant health.

It is generally agreed that compost application to control the pathogens should be at least 20 % v/v (Pugliese et al. 2015). Low or high levels of compost application may have negative effects through phytotoxicity, poor disease suppression and deterioration of the soil's physical and chemical properties. The effectiveness of compost in suppressing phytopathogens depends on many factors such as microbial and nutritional profile, physicochemical

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properties, maturity of the compost along with the raw material and conditions used for the composting determine the outcome of the plant response to disease stress. The characteristics of the compost play a vital role in the successful suppression of different plant pathogens, for example higher pH of the compost had a suppressive effect on the Fusarium wilt of tomatoes, whereas lower pH suppress the potato scab development caused by Streptomyces scabies (Noble and Conventory 2005; Pugliese et al. 2015). Results presented in this study support the previously reported results that organic amendment improves growth and helps in controlling fungal infection. 20% compost amendment helps in controlling the *Fol* infection (Fig. 4) as well as improves the plant growth visible from root and shoot dry mass (Fig. 5).

Composts are known to harbor millions of colonies forming units of microorganisms per gram (Borrero et al. 2004). Among these several microorganisms was reported previously to induce either systemic acquired resistance (SAR) or induced systemic resistance (ISR) in plants against a wide range of pathogens (Vallad et al. 2003). In SAR, pathogen attack or bio-protective microbial strain triggers a defense response, signal transferred systemically and the entire plant becomes resistant to future pathogens attacks. SAR requires salicylic acid (Delaney et al. 1994) Whereas ISR is dependent on jasmonic acid and ethylene (ET) (Verhagen et al. 2004).

Kavroulakis et al. (2006) reported the expression of Pathogenesis Related (PR) genes in tomato plants grown in a substrate amended with compost and the mechanism was associated with compost associated microbial community. Compost water extracts also have the ability to activate ISR against plenty of soil-borne pathogens (El-Masry et al. 2002). Later on, Sang and Kim (2011) suggested ISR mediated pepper and cucumber plants protection from anthracnoses of leaves caused by Colletotrichum coccodes and Colletotrichum orbiculare respectively. There are also reports associating the compost suppressiveness towards plant diseases through an improved nutritional status and volatile organic or antioxidant compounds present in compost (Noble and Conventory 2005; Mehta et al. 2013; El Nour et al. 2020; Debode et al. 2020).

A soil substrate dependent modification of root exudation has been reported previously (Neumann et al. 2014). The study was designed to investigate the effect of different soil substrates on root exudates by analyzing *Fol* growth and development. We found that *Fol* microconidia and mycelium act differently and also have a variable growth and development response in the root exudates obtained from different treatments. This indicates the responsiveness of fungal spores and mycelium to the specific components of the root exudates. The stimulation of spore germination is not necessarily associated with enhanced pathogenesis. The germination of fungal propagules and establishment of infection is reliant on the successful completion of all steps involved in pathogenesis such as spore germination, formation of infecting peg, penetration, and growth in the host (Akhter et al. 2016; Rasool et al. 2021). Root exudate compounds required for germination and to complete the sequence of pathogenesis may not be the same. The alteration of specific metabolites in the root exudates required for the pathogenicity possibly hinders the pathogen ability to cause infection (Schroth and Hilderbrand 1964).

5. Conclusion

Concisely, we can conclude from the data presented in this study that soil substrate consisting of compost exhibits great potential in suppressing *Fol* inoculum infectivity and wilt development in tomatoes. The soil amendments induced physiological changes in tomato plants which might enhance the resistance against *Fusarium* wilt of tomato. *In vitro* studies have revealed that the alterations in tomato root exudation and in the *Fol* growth and development due to soil amendments could also contribute to the plant response to disease stress associated with soil-borne fungi.

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