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Biological Management of *Fusarium* Wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*

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***Abstract*:** The *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a soil borne pathogen. Biological control of soil-borne plant pathogens through antagonist organisms can be a convenient method in control of disease. The fungal bioagents (*Trichoderma harzianum and T. asperellum*) and bacterial bioagents (*Pseudomonas fluorescens* and *Bacillus* sp.) were tested for the management of *Fusarium* wilt of tomato caused by FOL under greenhouse condition. *T. harzianum* was superior in controlling the fungal wilt, followed by *P. fluorescens* compared to biocontrol by *Bacillus* sp. and chemical control by Mancozeb (75% WP). The application of *T. harzianum* exhibited the least disease incidence (25.43%). *T. harzianum* treatment resulted in better plant growth (plant height 105.13 cm, number of leaves per plant 71.12, main stem girth2.55 cm, leaf area 45 cm^2) and increased yield contributing parameters and yields in tomato when compared to other treatments. *Trichoderma harzianum* is a potential alternate to chemical control of tomato wilt disease.

***Keywords*:** Biocontrol, *Fusarium* wilt, *Trichoderma harzianum,* Tomato

Tomato (*Solanum lycopersicum* L.) is an important solanaceous vegetable crop grown throughout the world, mainly in the warm seasons and can tolerate heat and drought reasonably, however it is grown under wide range of climatic conditions (Anonymous 2009). In India, estimated area, production and productivity during 2020-21 was about 840.33 thousand hectares, 20331 thousand tonnes, 24.20 tonnes/ha, respectively. Karnataka state ranks third in area (70.10 thousand hectares) and production (2104.68 thousand tonnes) with productivity of 30.00 tonnes/ha. In Karnataka, Bangalore and Kolar districts are the major tomato growing districts (Anonymous 2022), are affected by fusarium wilt caused by fungus, *Fusarium oxysporum* f. sp. *lycopersici* (Sayed Farooq Mahboobi et al 2023). The pathogen is very destructive causing 10 to 50 per cent yield loss in some tomato production areas (Ghazalibiglar et al 2016). It is very difficult to control fungal wilt of tomato, since the pathogen can progress within the vascular tissue by limiting the effectiveness of fungicides. However, biological control of this soil borne plant pathogen through antagonistic microorganisms can be an effective and alternative approach.

Different mechanism have been proposed to describe the biocontrol of plant pathogens by biocontrol agents, which include secretion of antibiotics, production of HCN, syderophores and, cell wall hydrolysing enzymes, competition for key nutrients, parasitism / stimulation of plant defence mechanisms and combination of all these possibilities (Taghdi et al 2015). Use of antagonist and plant growth promoting rhizobacteria (Schmidt et al 2004) such as *Bacillus, Enterobacter* and *Pseudomonas* strains, can encourage plant defenses. Similarly, fungal biocontrol agents, *Trichoderma* spp. are known to decrease the severity of plant disease by inhibiting plant pathogens in the soil through their great potent antagonistic and mycoparasitic activity (Viterbo and Horwitz 2010). Therefore, the evaluation of antagonists in the management of *Fusarium* wilt of tomato under greenhouse conditions was performed with fungal bioagents *viz*. *Trichoderma harzianum and Trichoderma asperellum* and bacterial bioagents *viz. Pseudomonas fluorescens* and *Bacillus* sp.

MATERIAL AND METHODS

The present study on biological management of *Fusarium* wilt of tomato was carried out in the polyhouse at University of Horticultural Sciences campus, Bengaluru situated at a Latitude 12º 581 North, longitude of 77º 351 East and altitude of 899 meters above mean sea level during the year 2019-20.

***In vitro* evaluation:** Sixty fungal and sixty bacterial biocontrol agents isolated from rhizosphere soils of tomato of three districts of Karnataka, namely Bengaluru rural, Chikkaballapur and Kolar. These isolates were screened for their efficiency to

inhibit *Fusarium oxysporum* f. sp. *lycopersici* by dual culture method on potato dextrose agar (PDA) for fungus and nutrient agar (NA)medium for bacteria against pathogen, After five days incubation, the zone of inhibition was measured to assess the efficacy of the biocontrol agents. The fungal cultures were maintained on PDA (Rahimi et al 2019) and the bacterial cultures on NA media. Two efficient fungal biocontrol agents *viz*., *T. asperellium* and *T. harzianum,* two efficient bacterial antagonistic isolates *viz*., *Psudomonas fluorescens* and *Bacillus spp.* (Sayed Farooq Mahboobi et al 2023) were selected for further scale up studies using grow bags culture against *Fusarium oxysporum* f. sp. *lycopesici* (*Fusarium* wilt of tomato).

Grow bag culture studies: *Trichoderma asperellium* and *T. harzianum,* pure cultures grown in potato dextrose broth, mixed with sterilized talc powder to obtain 10^9 conidia per gram of talc powder. Similarly, the bacterial isolates *Pseudromons fluorescens* and *Bacillus* sp. multiplied on nutrient broth mixed with carrier material (talc) to have 10^9 CFU per gram. These biocontrol agents were tested along with one positive control treatment with Mancozeb 75% WP and another negative control treatment (without biologicals or chemical). Soil was mixed with FYM @ 3:1 (w/w), sterilized for 5 days by formaldehyde fumigation, filled to grow bags of 15 cm x 20 cm (10 kg). The soil medium was inoculated individually with biocontrol agents @ 2% or 20 ml per bag, followed by root dipping. Twenty-five days old tomato seedlings of variety Arka Vikas, were dipped in their respective formulation of biocontrol agents for 30 minutes transplanted at the rate of three seedlings per grow bag (10 kg capacity) (Patil et al 2011). Mancozeb 75% WP @ 2 ml per liter was used as foliar spray as well as soil drench. To test biocontrol by different agents, all the treated plants in grow bags were challenge inoculated by the wilt fungi, *Fusarium oxysporum* f. sp. *lycopesici*, @ 5 per cent (w/w) using talk based formulation as soil drenching at 25 and 45 days after transplantation. Before inoculation, the roots were slightly severed (wounded) by inserting a needle 1 cm away from the stem. Root severing was done to ensure pathogen penetration through roots. Observations were recorded on wilt symptoms up to 5 weeks. The soil was made sick by mixing of pathogen culture in upper 10-15 cm layer of the soil.

The disease incidence of wilt was measured at 30 , 60 and 90 DAT at harvest (PDI), based on number of plants that showed symptoms of wilting as a percentage of the total number of plants. Subsequently, the disease ratings were plotted over time to generate the disease progress curves. Symptoms of *Fusarium* wilt disease were assessed every 10 days using disease scale (0 – 4). The plant was considered infected when a rating of 2 was recorded. The disease incidence was based on the infection percentage [I (%) = (number of plants infected / total number of plants observed) x 100]. The assessment of the disease severity was calculated [(S (%) = (∑ [E.a] / N.T 100)]. where, E is the disease scale (0 to 4) for tomato wilt and a, is the number of plants infected at each symptomatological scale, N is the total number of plants observed and T is the maximum disease scale (4 for tomato wilt).

Plant height, number of leaves, diameter of stem, leaf area at 90 days after transplanting (DAT), average weight of fruits, number of fruits and yield per plant, fresh weight of shoot and root and dry weight of shoot and root per plant were recorded by following standard procedures. Each treatment was replicated twice in completely randomized design (the data was statistically analyzed using excel spread sheet for significance at 5 per cent critical difference as described by Gomez and Gomez (1983).

RESULTS AND DISCUSSION

***In vitro* evaluation:** The dual plate screening of fungal and bacterial biocontrol agents (BCA) against *Fusarium oxysporum* f. sp. *lycopersici* significantly inhibited the growth of the fungal wilt. (Table 1). The per cent inhibition in mycelial growth of the fungal wilt ranged between 39.10 and 49.86 per cent. *Trichoderma harzianum* was most effective and significantly inhibited the mycelial growth of the *Fusarium*(followed by *Pseudomonas fluorescens*. The chemical fungicide Mancozeb 75% WP showed inhibition of 37%.

Disease incidence under polyhouse: All treatments (biocontrol and chemical control) decreased the fungal wilt incidence significantly over control (Table 2). Soil application of *Trichoderma harzianum,* talc formulation was most effective than chemical, Mancozeb and resulted in significant reduction in *Fusarium* wilt incidence. The wilt incidence observed in the grow bags with *T. harzianum* treatment was 25.43 per cent as compared to 65.23 per cent in the control treatment. The *T. harzianum* provided 66.64 per cent more disease control over negative control treatment. Application of *Pseudomonas fluorescens* resulted in 42.45 per cent fungal wilt control. Application of Mancozeb @ 75 % WP showed lowest fungal wilt disease control (32.70 per cent) under protected cultivation. The disease control ability observed in *T. harzianum* could be because of its ability to induce resistance, suppression of pathogens by production of antibiotics. Similar findings were reported by earlier scientist (Srivastava et al 2010, Mwangi et al 2011, Oyetunji and Salami 2011).

Plant growth: There was significant influence on the growth of tomato plants in response to treatment of biocontrol agents

(Table 3). Plant growth in terms of height (105.13 cm), number of leaves (71.12), stem girth (2.55 cm), leaf area (45 cm^2), fresh weight of shoot (740 g), fresh weight of root (110 g), dry weight of shoot (190.26 g) and dry weight of root (22.30 g) were significantly higher in the treatment of *Trichoderma harzianum*. Followed by *Pseudomonas fluorescens*. The Mancozeb 75% WP treatment plants although superior over control showed significantly lower plant growth characters. Plant growth parameters revealed that soil application of *Trichoderma harzianum* (talc based formulation) was most effective treatment. The better performance of plants to antagonists' inoculation perhaps be due to the production of growth promoters along with increased microbial activity and biocontrol of fusarium wilt increased growth parameters in plants *viz*. plant height and yield of plant (Narayan et al 2017). Application of *Trichoderma harzianum* may have influenced in efficient uptake of micro nutrients that could also have contributed for better growth of plant, throughout the crop growth period along with the improvement in soil physical and chemical properties which might have resulted in increased leaf area as reported by Baset et al (2010).

Fruit yield: The yield parameters such as number of fruits and yield per plant were significantly high in *Trichoderma harzianum* treated plants (Table 4). Maximum number of fruits (16), fruit yield per plant (1.89 kg) and average fruit weight (96.40 g) were in *Trichoderma harzianum* treated plants. *P. fluoresceins* although on par with *Trichoderma harzianum* treated plants but yields d were on the lower side . These two treatments are significantly superior over chemical and control treatments. Similar increase in fruit yield due to application of *Trichoderma harzianum* was reported by Nirmalkar et al (2018) in brinjal. The higher fresh weight and dry weight of shoot and root per plant due to biocontrol agents were also observed by Cornejo et al (2009) and by Lamour et al (2012) in tomato

Table 1. *In vitro* efficacy of bio-control agents against *Fusarium oxysporum* f. sp. *lycopirsici* (*Fusarium* wilt of tomato)

Treatments	Mean growth of mycelium (mm)	Inhibition in mycelial growth (%)
Trichoderma asperellum	52.12	42.08
Trichoderma harzianum	45.12	49.86
Bacillus sp.	47.00	39.11
Pseudomonas fluorescens	56.60	47.78
Mancozeb 75% WP	46.00	37.00
Control	90.00	-
CD (p=0.05)	1.62	1.80

Table 2. Effect of bio control agents on incidence and control of *Fusarium* wilt of tomato under poly house condition

Treatments	Disease incidence (%)	Disease control (%)
T1-Sick soil + *Trichoderma asperellum*	39.60	37.76
T2-Sick soil + *Trichoderma harzianum*	25.43	66.64
T3-Sick soil + *Bacillus* sp.	44.32	33.65
T4-Sick soil + *Psuedomonas florescens*	35.67	42.45
T5-Sick soil + Mancozeb 75 % WP	31.64	32.71
T6-Sick soil (Control)	65.23	0.00
CD (p=0.05)	1.69	1.21

Table 4. Effect of biocontrol agents on yield of tomato under poly house conditions

Treatments	Fruits per plant (No.)	Yield per plant (kg)	Average fruit weight per plant (g)
T1	12	1.31	76.30
T2	16	1.89	96.40
T3.	11	1.14	73.41
T4	13	1.61	85.61
T5	10	0.90	71.50
T6	8	0.81	56.41
CD (p=0.05)	1.29	0.36	18.87

See Table 2 for details

Table 3. Effect of biocontrol agents on growth parameters at harvest (PDI) of tomato

Treatments	Plant height (cm)	Leaves per plant (No.)	Main stem girth (cm)	Leaf area (cm^2)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of roots (g)
T1	85.25	58.93	1.68	35	600	90	176.71	17.41
T2	105.13	71.12	2.55	45	740	110	190.26	22.30
T3.	79.71	54.62	1.56	31	570	70	167.50	15.61
T4	96.11	67.87	2.31	40.50	700	110	183.60	20.23
T5	73.08	53.10	1.45	29	540	70	160.41	14.38
T6	56.96	46.35	1.33	23	410	60	128.53	11.61
CD (p=0.05)	5.72	2.18	0.24	0.75	12.02	1.92	3.54	0.77

See Table 2 for details

CONCLUSION

The fusarium wilt incidence can be effectively controlled by treating the plants with fungal biocontrol agent, *Trichoderma harzianum* and bacterial biocontrol agent, *Pseudomonas florescens.* Growth parameters of tomato *viz*. Plant height, number of leaves, stem girth, leaf area, fresh and dry weights of shoot and root as well as yield parameters, fruit yield and number had enhanced in response to biocontrol agents inoculation.

AUTHORS CONTRIBUTION

Sayed Farooq Mahboobi conducted the laboratory and field experiments. T.H. Shankarappa did conceptualization of the experiment and analysis. V. Devappa provided the technical and laboratory facilities. R. Manjunath supervised the work and J.S. Aravind Kumar reviewed the work and manuscript.

REFERENCES

Anonymous 2009. Botanical classification of cherry tomato. (*www.lose-weight-ithus.com/cherry tomato- nutrition.html*).

Anonymous 2022. Agricultural statistics at a glance 2022. (*https://agriwelfare.gov.in/Documents/CWWGDATA/Agricultural_Statistics_at_a_Glance_2022_0.pdf*).

Baset Mia MA, Shamsuddin ZH, Wahab Z and Marziah M 2010. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. *Australian Journal of Crop Science* **4**(2): 85-90.

Chavan AA, Deshpande GD and Zagade SN 2011. Varietal screening against *Pythium ultimum* trow by sick soil method in heavy black cotton soil. *An Asian Journal of Soil Science* **6**(1): 74-76.

Cornejo CHA, Macias-Rodríguez L, Cortes-Penagos C and Lopez-Bucio J 2009. *Trichoderma virens,* a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. *Plant Physiology* **149**(3): 1579-1592.

Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH and Stampfer MJ 1999. Lower prostate cancer risk in men with elevated plasma lycopene levels: Results of a prospective analysis. *Cancer Research* **59**(6):1225-1230.

Ghazalibiglar H, Kandula DRW and Hampton JG 2016. Biological control of *Fusarium* wilt of tomato by *Trichoderma* isolates. *New Zealand Plant Protection* **69**: 57-63.

Gomez KA and Gomez AA 1983. *Statistical procedure for agriculture research* (2nd Ed.). John Willey and Sons, New York 680.

Lamour KH, Stam R, Jupe J and Huitema E 2012. The oomycete broad-host-range pathogen *Phytophthora capsici*. *Molecular Plant Pathology* **13**(4): 329-337.

Mwangi MW, Monda EO, Okoth SA and Jefwa JM 2011. Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian Journal Mycrobiology* **42**(2): 508-513.

Narayan PV, Inderdeep K, Harison M, Ajay KS and Ankit S 2017. Efficacy of *Trichoderma* in controlling *Fusarium* wilt in tomato (*Solanum lycopersicum* L.) *Research in Environment and Life Sciences* **10**(7): 636-639.

Nirmalkar VK, Tiwari RKS and Singh S, 2018. Efficacy of bio-agents against damping off in Solanaceous crops under nursery conditions. *International Journal of Plant Protection* **11**(1): 1-9.

Oyetunji OJ and Salami AO 2011. Study on the control of *Fusarium* wilt in the stem of mycorrhizal and trichodermal inoculated pepper (*Capsicum annum* L.) *Journal of Applied Biosciences* **45**: 3071- 3080.

Patil S, Sriram S and Savitha MJ 2011. Evaluation of non-pathogenic *Fusarium* for antagonistic activity against *Fusarium* wilt of tomato. *Journal of Biological Control* **25**(2): 118-123.

Rahimi BA, Shankarappa TH, Sahel NA and Patil SV 2019. Effective edible coatings on control of microbial growth in strawberry fruits. *Indian Journal of Ecology* Special Issue **46**(7): 91-95.

Sayed Farooq Mahboobi, Shankarappa TH, Devappa V and Arvinda Kumar JS 2023. Incidence of fungal wilt in tomato and characterization of pathogen. *Indian Journal of Ecology* **50**(5): 1479-1482.

Schmidt CS, Agostini F, Leifert C, Killham K and Mullins CE 2004. Influence of soil temperature and matric potential on sugar beet seedling colonization and suppression of *Pythium* damping off by the antagonistic bacteria *Pseudomonas fluorescens* and *Bacillus subtilis*. *Phytopathology* **94**(4): 351-363.

Srivastava R, Khalid A, Singh US and Sharma AK 2010. Evaluation of arbuscular mycorrhizal fungus, *Pseudomonas fluorescent* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. *Biological Control* **53**(1): 24-31.

Taghdi Y, Hermosa R, Dominguez S, Rubio MB, Essalmani H, Nicolas C and Monte E 2015. Effectiveness of composts and *Trichoderma* strains for control of *Fusarium* wilt of tomato. *Phytopathology Mediterranea* **54**(2): 232-240.

Viterbo A and Horwitz BA 2010. Mycoparasitism. In: *Cellular and molecular biology of filamentous fungi.* Eds. Borkovich KA and Eggole DJ, American Society of Microbiology Washington, 676-693.

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