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Research Article

STUDIES ON BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* IN TOMATO (*SOLANUM LYCOPERSICUM* L)

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ABSTRACT

Biological control is a method of controlling plant diseases using other organisms. In this study the bio control of *Fusarium oxysporum* in Tomato (*Solanum lycopersicum* L.) under nursery experiments. The soil samples were collected from isolate fungal pathogens and antagonistic fungi. The various type of fungal colonies were identified from collected soil. Among the isolate the dominant fungal pathogen (*F. oxysporum)* and antagonistic fungi (*Trichoderma viride*) was selected based on antagonistic activity. The biocontrol agent resulted in increase in soil fertility, control of minor pathogens and increase in general health of plants, ultimately leading to an increase in plant metabolism. If enhanced the growth and development of the plant.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is largest vegetable crops in the world and healthy food for human, special nutritive value and widespread production. It is one of the most important nursery-based vegetable crops cultivated for its fleshy fruits. Root rot disease caused by *Fusarium oxysporium, Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii* are the most destructive disease of tomato (Benhamou e*t al.,* 1994; El-Mougy, 1995). Root and crown rot as well as wilt diseases of tomato caused by *Fusariun oxysporum* pathogens which survive in soil are responsible for serious losses in vegetables crop yield (Lobo and Silva, 2000; Radwan and AL-Masri, 2012; El-Mohamedy et al., 2013). *Trichoderma* sp. has proved to be useful in the control of phytopathogens affecting different crops (Benitez *et al.,* 2004). Also, tomato plants treated by *Trichoderma* sp. have shown biocontrol activity against damping-off and root rot disease and gave high yield of tomato (Morsy Ebtsam, 2005). Hence, the present manuscript deals with the investigation biological control of *Fusarium oxysporum* in Tomato (*Solanum lycopersicum* L.). Effects of biocontrol agent on the growth of plant were analyzed by pot culture experiment.

MATERIALS AND METHODS

The meteorological factors such as Relative Humidity (RH), Hours of Bright Sunshine (HBSS), Rainy Days (RD), Wind Velocity (WV) and Evaporation (EP) have been investigated. The field soil samples were collected in and around the rural area of Tiruchirapalli district, Tamilnadu, India. The collected soil sample kept in sterile polythene bags and stored in refrigerator at $4^{\circ}C$ for further studies. Standard serial dilution plate techniques were followed for the isolation of fungi using Rose Bengal agar medium. Isolated fungi were identified based on cultural and morphological character.

Antagonistic property of the fungal antagonist was studied by mycoparasitism. *F. oxyporum* was placed on one edge of petri dishes containing PDA and incubated at 25°C. Forty eight hours later, *Trichoderma viride* isolates were placed on the opposite side of *F. oxysporum* in previous Petri dishes and they were incubated in the same thermal condition. Interactions between *Trichoderma viride* isolates and *Fusarium* were evaluated based on radial growth of pathogen, overgrowth speed of *Trichoderma viride* on pathogen colony, production of yellow pigment in overlapped area of two colonies and hyper parasitism (mycelial coiling) (Dennis and Webester, 1971; Kucuk and Kivance, 2004).

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Pot culture experiment

Effects of biocontrol agent on the growth of plant were analyzed by pot culture experiment. The following treatment was made for this study.

T1 - Control
T2 - Pathogen (*F. oxyporum)*
T3 - Pathogen (*F. oxyporum)* + biocontrol agent (*Trichoderma viride*)
T4 - Pathogen (*F. oxyporum)* + fungicide
T5 - Pathogen (*F. oxyporum)* + biocontrol agent (*Trichoderma viride*) + fungicide

The sterile soil samples were mixed with biocontrol agent for above treatment methods respectively. This setup was incubated at room temperature for 5 days. After the incubation period the seeds were showed in the soil samples. The plant materials from the pot culture were collected after 30 days for the observation of following parameters.

The morphometric parameters such as Root, shoot length (cm), No. of branches, leaves (cm) and root, shoot fresh and dry weight of cultivated tomato plants (30, 60 and 90 days) were noted. The biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll (Arnon, 1949), protein (Lowry *et al.,* 1951), carbohydrate, amino acid, enzymatic and minerals (mg/ml) were analysed from 30, 60 and 90 days treatments unit Tomato leaves. Treatment plants were characterized for mineral contents (APHA, 1995) was analyzed. The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar (1984).

RESULTS AND DISCUSSION

In the present investigation suggested that the *T.viride* as a potential antagonistic fungi against *F.oxysporum* by the method of spot culture experiments with various treatments including fungicide corroboration results with potential T.viride by (Marco *et al*., 2003, Sanjay *et al*., 2008 and Indira, 2011) various strems of Trichoderma sp. Exhibit substantial variability with respective their antagonistic activity and host range. According to Sivan and Chet (1984) stated that the meteorically data was very important aspects for antagonistic activities were represented. In the current investigation on the growth and development of tomato were measured. The various treatments such as T1, T2, T3, T4 and T5 were analysed. Among the treatments T3 and T5 was excellent morphometeric analysis when compared to other treatment. Because T3 treatment (pathogen and biocontrol agent *T.viride*) ascertain development was recorded whereas T5 treatment also highly responsible with growth and development due to a minimize quantity of fungicide was altered wheather it may be stimulated with *T.viride* against *F.oxysporum* pathogen with 30, 60 and 90 the days recorded respectively. In the major aspects of root length, shoot length, no. of branches, no. of leaves, root fresh weight, root dry weight, shoot fresh weight and shoot dry weight were measured frequently at 30, 60 and 90th days of *Solanum lycopersicum* plant. Among the data of morphometeric analysis, the 90th day of data was highly significance with respective parameters recorded respectively (Table 2, 3 and 4).

Barani (2016) studied that the bicontrol of tomato Fusarium wilt by *Trichoderma* species under *invitro* and *invivo* conditions. *Trichoderma* sp. Have long been used as biocontrol agent *Trichoderma* sp. have long been used as biological control agents against plant fungal diseases, but the

Table 1 Meteorological data for the experimental period (January to December, 2015)

Month (2015)	Meterological data in 2015							
	Temperature (°C)		RH (%)	HBSS (hrs)	WV (km/h)	Rainfall (mm)	RD	EP (mm)
	Maximum	Minimum						
January	37.6	26.3	78	8.0	4.1	075.0	3	3.0
February	30.2	18.9	76	9.2	3.6	–	–	3.9
March	32.7	21.9	78	9.0	2.9	020.0	2	4.6
April	35.5	23.7	72	9.4	4.5	013.6	1	5.6
May	36.3	24.7	67	8.5	7.6	087.7	3	6.6
June	36.3	24.2	64	7.7	7.2	033.9	1	6.8
July	36.5	24.2	63	7.0	6.3	021.9	2	7.0
August	35.5	23.0	65	7.7	5.0	138.9	3	6.0
September	33.8	23.0	69	6.5	6.4	064.0	4	5.6
October	31.4	22.1	79	6.4	3.4	571.5	17	3.9
November	29.0	21.3	85	5.4	2.6	541.0	18	2.8
December	28.9	20.4	77	7.2	4.3	779.7	3	3.6
Total	394.7	266.7	873.0	91.8	57.9	1747.2	57.0	59.6
Mean	32.8	22.2	73.0	7.6	4.8	–	–	4.9

RH – Relative Humidity; HBSS – Hours of Bright Sunshine; RD – Rainy Days; WV – Wind Velocity; EP – Evaporation.

Table 2 Morphometric analysis of tomato (*Solanum lycopersicum* L.) at 30 days

Treatments	Root length (cm)	Shoot length (cm)	No of branches/Plant	No of leaves/Plant	Root fresh weight (gm)	Root dry weight (gm)	Shoot fresh weight (gm)	Shoot dry weight (gm)
T_1	5.00±015	10.00±0.30	4.00±0.12	10.00±0.30	0.70±0.02	0.30±0.009	5.90±0.17	0.70±00.02
T_2	4.39±013	8.74±0.26	3.00±0.09	7.00±0.21	0.88±0.02	0.32±0.009	4.88±0.14	1.20±0.03
T_3	7.00±0.21	13.11±0.39	7.00±0.21	12.00±	1.11±0.03	0.36±0.01	6.50±0.19	1.40±0.04
T_4	6.72±0.20	10.56±0.31	6.00±0.18	11.00±0.33	0.99±0.02	0.40±0.01	6.30±0.19	1.33±0.03
T_5	7.42±0.22	14.28±0.42	9.00±0.27	14.00±0.42	1.38±0.04	0.69±0.02	6.68±0.20	1.60±0.04

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 3 Morphometric analysis of tomato (*Solanum lycopersicum* L.) at 60 days

Treatments	Root length(cm)	Shoot length(cm)	No of branches/Plant	No of leaves/Plant	Root fresh weight(gm)	Root dry weight(gm)	Shoot fresh weight(gm)	Shoot dry weight(gm)
T_1	9.00±027	11.00±0.33	27.00±081	16.00±0.48	0.34±1.02	0.80±0.02	6.61±0.19	0.80±0.02
T_2	7.00±021	13.00±0.39	23.00±0.69	13.00±0.10	0.25±0.007	0.70±0.02	1.58±0.04	0.11±0.003
T_3	11.65±0.34	16.54±0.49	30.00±0.90	18.00±0.54	0.95±0.02	0.95±0.02	1.80±0.05	0.28±0.008
T_4	10.32±0.30	15.22±0.45	29.00±0.87	17.00±0.51	0.32±0.009	0.88±0.02	1.77±0.05	0.30±0.009
T_5	13.20±0.39	19.74±059	33.00±0.99	19.00±0.57	1.65±0.54	0.99±0.02	1.98±0.05	0.32±0.009

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 4 Morphometric analysis of tomato(*Solanum lycopersicum* L.) at 90 days

Treatments	Root length(cm)	Shoot length (cm)	No of branches/Plant	No of leaves/Plant	Root fresh weight (gm)	Root dry weight (gm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	No of fruits /plant	Fresh weight of fruits (gm)	Dry weight of fruits (gm)
T_1	13.00±0.39	32.0±0.96	13.00±0.39	22.00±0.66	2.02±0.06	0.77±0.02	7.51±0.22	0.78±0.02	4.0±0.12	39.0±1.17	6.92±0.20
T_2	11.74±0.35	30.1±0.90	14.00±0.42	20.00±0.60	1.89±0.05	0.64±0.01	6.38±0.19	0.60±0.01	3.0±0.09	36.5±1.09	4.56±0.13
T_3	15.42±0.46	35.0±1.50	16.00±0.48	23.00±0.69	2.65±0.07	1.01±0.03	2.00±0.06	0.88±0.02	5.0±0.15	40.4±1.21	9.54±0.2
T_4	13.22±0.39	33.4±1.00	18.00±0.54	20.0±0.610	2.44±0.07	1.15±0.03	1.95±0.05	0.75±0.02	4.0±0.12	39.2±1.17	8.4±0.25
T_5	17.00±0.51	37.5±1.12	21.00±0.63	25.0±0.75	2.89±0.08	1.19±0.03	2.15±0.06	0.95±0.02	6.0±0.18	43.5±1.30	10.5±0.31

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 5 Biometric analysis of tomato (*Solanum lycopersicum* L.) at 30 days

Treatments	Chlorophyll "a" (ml/gm) fr.wt. basis	Chlorophyll "b" (ml/gm) fr.wt. basis	Total chlorophyll (ml/gm) fr.wt. basis	Carbohydrates (mg/ml) fr.wt. basis	Protein (mg/ml) fr.wt. basis	Amino acid (mg/ml) fr.wt. basis
T_1	0.600 ±0.018	0.411 ±0.012	1.011 ±0.030	3.280 ±0.098	6.000 ±0.180	4.000 ±0.120
T_2	0.592 ±0.008	0.400 ±0.012	0.992 ±0.029	3.110 ±0.933	6.228 ±0.186	3.748 ±0.112
T_3	0.611 ±0.018	0.418 ±0.012	1.029 ±0.030	3.411 ±0.102	6.447 ±0.193	4.128 ±0.123
T_4	0.581 ±0.017	0.388 ±0.011	0.969 ±0.029	3.012 ±0.090	5.928 ±0.177	3.556 ±0.106
T_5	0.632 ±0.018	0.422 ±0.012	1.054 ±0.031	3.569 ±0.107	6.784 ±0.147	4.612 ±0.138

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone

Table 6 Biometric analysis of tomato (*Solanum lycopersicum* L.) at 60 days

Treatments	Chlorophyll "a"(ml/gm) fr.wt. basis	Chlorophyll "b" (ml/gm) fr.wt. basis	Total chlorophyll(ml/gm) fr.wt. basis	Carbohydrates(mg/ml) fr.wt. basis	Protein(mg/ml) fr.wt. basis	Amino acid (mg/ml) fr.wt. basis
T_1	0.711 ±0.021	0.473 ±0.014	1.184 ±0.035	3.228 ±0.096	6.422 ±0.192	3.995 ±0.119
T_2	0.695 ±0.020	0.452 ±0.013	1.147 ±0.034	3.115 ±0.093	6.110 ±0.183	3.628 ±0.108
T_3	0.752 ±0.022	0.500 ±0.015	1.254 ±0.038	3.566 ±0.106	6.650 ±0.199	4.751 ±0.142
T_4	0.731 ±0.021	0.489 ±0.014	1.220 ±0.036	3.347 ±0.100	6.225 ±0.186	3.984 ±0.119
T_5	0.783 ±0.023	0.511 ±0.015	1.294 ±0.038	3.748 ±0.112	6.702 ±0.201	4.956 ±0.148

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 7 Biometric analysis of tomato (*Solanum lycopersicum* L.) at 90 days

Treatments	Chlorophyll "a"(ml/gm) fr.wt. basis	Chlorophyll "b" (ml/gm) fr.wt. basis	Total chlorophyll (ml/gm) fr.wt. basis	Carbohydrates (mg/ml) fr.wt. basis	Protein (mg/ml) fr.wt. basis	Amino acid (mg/ml) fr.wt. basis
T_1	0.733 ±0.021	0.482 ±0.014	1.215 ±0.036	4.122 ±0.123	5.784 ±0.173	4.625 ±0.138
T_2	0.731 ±0.021	0.456 ±0.013	1.187 ±0.035	4.000 ±0.120	5.987 ±0.174	4.176 ±0.125
T_3	0.753 ±0.022	0.510 ±0.015	1.263 ±0.037	4.330 ±0.129	6.112 ±0.183	4.998 ±0.149
T_4	0.728 ±0.21	0.522 ±0.015	1.308 ±0.039	3.987 ±0.119	5.890 ±0.176	3.995 ±0.119
T_5	0.786 ±0.023	0.522 ±0.015	1.308 ±0.0039	4.522 ±0.135	6.219 ±0.186	5.628 ±0.168

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 8 Enzymatic studies of tomato (*Solanum lycopersicum* L.) at various sampling days

Treatments	Catalase (min/mg/protein)			Peroxidase (min/mg/protein)		
	30th day	60th day	90th day	30th day	60th day	90th day
T_1	7.652±0.229	7.848±0.235	8.100±0.243	13.995±0.419	15.228±0.456	16.541±0.496
T_2	7.628±0.228	7.428±0.222	7.658±0.229	13.654±0.409	15.110±0.453	15.781±0.473
T_3	7.958±0.238	8.123±0.243	8.200±0.246	14.550±0.436	16.665±0.499	16.739±0.502
T_4	7.844±0.235	7.685±0.230	8.000±0.240	13.752±0.412	15.338±0.460	15.987±0.479
T_5	8.100±0.243	8.420±0.252	8.500±0.255	14.685±0.440	15.722±0.471	17.382±0.521

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 9 Enzymatic studies of tomato (*Solanum lycopersicum* L.) at various sampling days

Treatments	Catalase (min/mg/protein)			Peroxidase (min/mg/protein)		
	30th day	60th day	90th day	30th day	60th day	90th day
T_1	7.652±0.229	7.848±0.235	8.100±0.243	13.995±0.419	15.228±0.456	16.541±0.496
T_2	7.628±0.228	7.428±0.222	7.658±0.229	13.654±0.409	15.110±0.453	15.781±0.473
T_3	7.958±0.238	8.123±0.243	8.200±0.246	14.550±0.436	16.665±0.499	16.739±0.502
T_4	7.844±0.235	7.685±0.230	8.000±0.240	13.752±0.412	15.338±0.460	15.987±0.479
T_5	8.100±0.243	8.420±0.252	8.500±0.255	14.685±0.440	15.722±0.471	17.382±0.521

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 10 Mineral contents of tomato (*Solanum lycopersicum* L.) on 60[th] days

Treatments	N	P	K	Ca	Mg	Zn	Cu	I	Mn
T_1	192.41±5.77	24.88±0.74	13.711±0.41	14.00±0.420	45.551±0.136	28.651±0.855	18.628±0.558	170.00±5.100	27.338±0.820
T_2	192.0±5.760	24.652±0.739	13.521±0.40	13.952±0.41	45.118±1.34	28.33±0.34	18.44±0.55	169.74±5.09	27.11±0.81
T_3	192.78±5.78	125.11±0.75	14.38±0.43	14.330±0.42	46.11±1.38	29.32±0.87	19.28±0.57	171.320±5.13	28.11±0.84
T_4	192.52±5.77	24.98±0.74	13.94±0.41	14.11±0.44	45.74±1.37	28.77±0.80	18.74 ±0.56	170.33±5.10	27.55±0.82
T_5	193.00±5.79	25.330±0.75	14.52±0.43	14.55±0.43	46.321±1.38	29.55±0.88	19.220±0.57	171.55±5.14	28.33±0.84

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

Table 11 Mineral contents of tomato (*Solanum lycopersicum* L.) on 90[th] days

Treatments	N	P	K	Ca	Mg	Zn	Cu	I	Mn
T_1	184.44±5.53	18.52±0.55	101.91±3.05	108.74±3.26	38.65±1.15	24.75±0.74	13.12±0.39	162.54±4.87	122.74±0.68
T_2	183.751±5.512	17.99±0.539	102.771±3.083	108.33±3.24	38.11±1.14	24.55±0.73	12.78±0.38	162.33±4.86	22.54±0.67
T_3	185.00±5.55	19.11±0.57	103.11±3.09	109.22±3.27	39.41±1.18	25.33±0.75	13.50±0.40	163.79±4.91	23.74±0.71
T_4	184.71±5.54	18.74±0.56	102.95±3.08	109.00±3.27	38.92±1.16	25.00±0.75	13.40±0.40	162.79±4.88	23.11±0.69
T_5	185.32±5.55	19.32±0.57	103.56±3.10	110.00±3.30	39.74±1.19	25.74±0.77	13.78±0.41	164.11±4.92	23.95±0.71

T_1-Control, T_2-Pathogen, T_3-Pathogen+Biocontrol agent, T_4- Pathogen+Fungicide, and T_5-Pathogen+Biocontrol agent+Fungicide alone.

mechanisms by which the fungi confer protection are not well understood. Our goal in this study was to isolate species of *Trichoderma*, that exhibit high levels of biocontrol efficacy from natural environments and to investigate the mechanisms by which these strains confer plant protection. In this study, efficacy of the native isolates of *Trichoderma* species to promote the growth and yield parameters of tomato and to manage Fusarium wilt disease under in vitro and in vivo conditions were investigated. The dominant pathogen, which causes Fusarium wilt of tomato, was isolated and identified as *Fusarium oxysporum f. sp. lycopersici* (FOL). Twenty eight native *Trichoderma* antagonists were isolated from healthy tomato rhizosphere soil in different geographical regions of Mazandaran province, Iran. Under in vitro conditions, the results revealed that *Trichoderma harzianum*, isolate N-8, was found to inhibit effectively the radial mycelial growth of the pathogen (by 68.22%). Under greenhouse conditions, the application of *T. harzianum* (N-8) exhibited the least disease incidence (by 14.75%). Also, tomato plants treated with *T.harzianum* (N-8) isolate showed a significant stimulatory effect on plant height (by 70.13 cm) and the dry weight (by 265.42 g) of tomato plants, in comparison to untreated control (54.6 cm and 195.5 g). Therefore, the antagonist *T. harzianum* (N-8) is chosen to be the most promising bio-control agent for *F. oxysporum f. sp. lycopersici*.

Titi Thongkamngam and Tanimnun Jaenaksorn (2017) studied that the Efficacy of non-pathogenic *Fusarium oxysporum* (F221-B) was assessed as a possible biocontrol agent against fungal pathogens, namely *Curvularia lunata* (C11, C12), *F. semitectum* (F113), *F. oxysporum f.sp. lactucae* (F221-R, F442-G), *Rhizoctonia solani* (R11, R12), *Rhizoctonia* sp. (R111, R112, R113) *in vitro*, while F221-B showed a moderate ability to inhibit the mycelial growth of tested fungi about 36–56%. Then, F221-B was further evaluated for its ability of controlling lettuce root rot and wilt caused by F442-G in hydroponics. It was revealed that F221-B reduced disease incidence and severity about 60–80% compared to the inoculated control and significantly promoted the growth of 3 lettuce varieties. Interestingly, using only F221-B gave the significantly highest fresh weight (twice over the healthy control). Conclusively, this study provides an important suggestion for further development of F221-B since it showed the ability of biocontrol agent and plant growth promoting fungus.

Metrological data also carried out such as, temperature, relative humidity, hours of bright sunshine, rainy days, wind velocity and evaporation were measured from January to December 2015. The reliable data were promoted for the growth and development of *Solanum lycopersicum* (Table 1). Some of the parameters at an adequate level and be capable of effectively interacting with the pathogen or host plant to provide acceptable with disease control measure. The wilt disease inducing strains of *Fusarium oxysporum* cause serious losses of many economically important agricultural crops. Biocontrol is considered a safer pest management strategy involving the use of natural enemies to maintain pest population levels under economic threshold (Beasley *et al*., 1996 and Parra *et al*., 2002).

CONCLUSION

Effectiveness of native *Trichoderma viride* antagonistic on wilt disease and yield parameters under pot culture experiments.

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