Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 486-489

Received: 19-11-2020
Accepted: 21-12-2020

Mayur Bhujbal
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India
Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Raju Gawade
Department of Horticulture, College of Agriculture, V. N. M. K. V Parbhani, Maharashtra, India

Dhanshree Bachkar
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India
Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Chintamani Deokar
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India
Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Navanath Daingade
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India
Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Corresponding Author:
Mayur Bhujbal
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India
Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

In vitro efficacy of different biological agents against *Fusarium oxysporum* f.sp. *lycopersici* causing wilt of tomato

Mayur Bhujbal, Raju Gawade, Dhanshree Bachkar, Chintamani Deokar and Navanath Daingade

Abstract
F. oxysporum is so widespread, it is a significant problem in many crops. It is economically damaging to the banana industry, and the threat of more virulent strains or mutations to damage previously resistant crops is of major concern. *F. oxysporum* also causes damage to many crops from the family Solanaceae, including potato, tomato, and pepper. Yield losses of effected crops can be high, up to 45% yield loss of tomato crop has been reported in India. Other commercially important plants affected include basil, beans, carnation, chrysanthemum, peas, and watermelon. Woody ornamentals are infected, but are usually not killed by Fusarium wilt alone. Palms, however, are the exception, and there are many species that can die from *F. oxysporum* infection.
In vitro evaluation of the biological agents revealed that among the fungal bioagents *Trichoderma viride* was found to be superior as compared to remaining three species which found to inhibit 84.84 per cent growth of the pathogen followed by *T. harzianum* (72.54%), *T. hamatum* (69.93%), *T. koningii* (61.49%) inhibited growth of pathogen and among two bacterial species *B. subtilis* were found to be effective in inhibiting the growth of pathogen i.e. (79.25%) as compared to *P. fluorescens* which was found to inhibit (62.36%) growth of pathogen.

Keywords: Tomato, *Fusarium oxysporum* f.sp. *lycopersici*, biological agents, inhibition

Introduction
Tomato (*Lycopersicon esculentum* Mill., *Solanum lycopersicum* L.) from the family solanaceae is one of the most important of the popular vegetables in the world. It is a native of Peru in South America (Rick, 1976). The Nahuatl (Aztec language) word Tomatl gave rise to the Spanish word tomate, from which the English word tomato derieved. This crop spread to North America primarily by migrating birds and then to totally different countries. The largest concentration of wild tomatoes is found in North American country. Spanish priests introduced the tomato crop to Europe around 1550. In Europe it absolutely was conjointly reffered as *Poma amoris*-Amorous apple or love apple. It was also known as *Poma peruviana* apple of Peru. Only as late as 1880, did the British finally concede that tomato is edible. It was Robert Gibbon Johanson, an ordinary farmer in the U.S.A who first ate tomato on a hot day of August, 1820 to describe its edibleness. From then onwards, the tomato spread throughout the world. In India, it was introduced from Europe, in the seventeenth century (Kale and Kale, 1984) [4].
India ranks second in the area as well as in production of Tomato. The major tomato growing countries are China (30.7%), India (11.5%), USA (8.1%), Turkey (7.0%) and Egypt (5.3%). In India Andhra Pradesh is the leading state in both area and production of tomato which contributes 25.01% to the total production of tomato in India. MH ranks 8th by contributing 4.82% to the national production (Horticultural Statistics at a Glance 2017).
Annual production of tomato in India throughout 2018-19 was 20515.24MT with an area of about 814 thousand ha, and productivity of 27.8 MT/ha (Anonymous, 2018) [10], In Maharashtra state, it is grown on an area of about 43.64.thousand ha with production of 976.58.MT, and productivity of 22.07MT/ha (Anonymous, 2018) [10]
Chemical fungicides are generally used for the control of the disease, however, frequent and indiscriminate use of chemicals leads to ill effects such as environment pollution and development of resistance in pathogen, But complete eradication of chemical fungicides will

not practically be possible immediately hence use of both biocontrol and chemical have been advocated as the most promising alternative strategy to overcome this problems. Biocontrol agent has been successfully used to control various soil borne diseases. The mechanism involved in antagonism of bioagents, might be antibiosis, competition and mycoparasitism.

Material and Methods

Isolation, identification and pathogenicity of *Fusarium oxysporum* f.sp. *lycopersici.*

Isolation of pathogen associated with wilt of tomato was done by tissue isolation method on PDA. The fungus isolated from diseased specimens and maintained in pure form was tentatively identified on the basis of colony characters and morphological characters and later confirmed from the Department of Plant Pathology, College of Agriculture, Dhule.

The pathogenicity of *Fusarium oxysporum* f.sp. *lycopersici* was done by soil inoculation technique (sick soil).

In vitro efficacy of biological agents

Following four fungal and two bacterial antagonist were evaluated *in vitro* against *F.oxysporum* f.sp. *lycopersici* given in Table 1.

Table 1: Details of biological agents used in the experiment.

Sr. No.	Bioagents used	Source
1.	*Trichoderma viride*	Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri
2.	*Trichoderma harzianum*	Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri
3.	*Trichoderma hamatum*	Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri
4.	*Trichoderma koningii*	Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri
5.	*Bacillus subtilis*	Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri
6.	*Pseudomonas fluorescens*	Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri

Antagonistic potential of four species of *Trichoderma* viz., *T. harzianum, T. viride, T. koningii, T.hamatum* and two bacterial species i.e *Bacillus subtilis* and *Pseudomonas fluorescens* was assessed against *F. oxysporum* f.sp. *lycopersici* by dual inoculation techniques (Upadhyay and Rai,1987). An experiment with seven treatment and three replications were carried out.

Experimental Details

For laboratory condition

i. Design - CRD
ii. Replication - 3
iii. Treatment – 7

Sr. No.	Treatment No.	Biological agents
1.	(T1)	*Trichoderma harzianum*
2.	(T2)	*Trichoderma viride*
3.	(T3)	*Trichoderma hamatum*
4.	(T4)	*Trichoderma koningii*
5.	(T5)	*Bacillus subtilis*
6.	(T6)	*Pseudomonas fluorescens*
7.	(T7)	Untreated control

To calculate the percent growth inhibition for four species of *Trichoderma* viz., *T. harzianum, T. viride, T. koningii, T. hamatum* following method was adopted. Mycelium discs of 5 mm diameter were cut from the margin of 7 days old cultures of test pathogen and antagonistic agents, respectively and placed opposite to each other on PDA in petriplates having diameter of 90 mm. The Discs were placed 30 mm away from each other. The petriplate inoculated with discs of *F. oxysporum* f.sp. *lycopersici* was measured to assess the antagonistic potential of *Trichoderma* spp. against pathogen. The percent growth inhibition of pathogen colonies was calculated by using formula given by Arora and Upadhyay (1978) [2].

$$\text{Percent growth inhibition} = \frac{D_1 - D_2}{D_1} \times 100$$

Where,

D_1 = Diameter of pathogen colony in control

D_2 = Diameter of pathogen colony in treatment

The antagonism of *Bacillus subtilis* and *Pseudomonas fluorescens* against fungal pathogen was tested *in vitro* by Dual culture technique on Potato Dextrose Agar (PDA) medium. The *Fusarium oxysporum* culture was placed at the centre of petriplate and after 48 hours when *F. oxysporum* f.sp. *lycopersici* germinate streaks of bacterial isolates were made equidistantly at the periphery of agar plates. Then the inoculated petriplates were incubated at 28 ℃ ± 5 ℃ for 7 days and the diameter of inhibition zones were measured by using formula given by Arora and Upadhyay (1978) [2].

$$I = C\text{-}T / C \times 100$$

whereas,

I = Percent inhibition

T = Colony growth (mm) in treatment plate

C = Colony growth (mm) in control plate

The data obtained was statistically analyzed (Panse and Sukhatme, 1978) [3] and the results were interpreted thereof.

Results and Discussion

In vitro efficacy of different biological agents against *Fusarium oxysporum* f.sp. *lycopersici*

The four *Trichoderma* spp. and two bacteria i.e. *Bacillus subtilis* and *Pseudomonas fluorescens* were tested *in-vitro* conditions for biological control of *Fusarium oxysporum* f.sp. *lycopersici*. The results (Table-2, Fig.-1, and Plate-1) revealed that the all the bioagents evaluated exhibited inhibitory effect on growth activity against *Fusarium oxysporum* f.sp. *lycopersici* and significantly inhibited its growth over untreated control.

It was observed that treatment T2 was significantly superior than rest of all the treatment and treatment T5, treatment T1 and treatment T3 significantly differ from each other but treatment T6 and treatment T4 were on par and doesn't have had significant difference.

Among these four species of *Trichoderma*, *Trichoderma viride* was found to be most effective in inhibiting the growth of *Fusarium oxysporum* of about 84.84 per cent growth of test fungus, followed by *Trichoderma harzianum* inhibited 72.54 per cent, *Trichoderma hamatum* which inhibited 69.93 per cent and *Trichoderma koningii* which inhibited 61.49 per cent growth of test fungus which was less effective than above species.

The antagonistic activity of *Bacillus subtilis* and *Pseudomonas fluorescens* were examined on potato dextrose agar plates by dual culture method. The results revealed that, both bacterial species were found effective against *Fusarium oxysporum* f.sp. *lycopersici*, *B. subtilis* inhibited 79.25 per cent and *P. fluorescens* inhibited 62.36 per cent growth of the pathogen.

The results of the present studies clearly indicated that the biological agents viz., *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum* and *Trichoderma koningii* were found to be effective in inhibiting the growth of *Fusarium oxysporum* f.sp. *lycopersici*. Further it could be seen that *Trichoderma viride* was most effective biocontrol agents against *Fusarium oxysporum* f.sp. *lycopersici* which recorded 84.84 per cent inhibition of the growth of pathogen followed by *T. harzianum*, *T. hamatum*, *T. koningii* which inhibited 72.54, 69.93 and 61.49 per cent. Two bacterial species viz., *Bacillus subtilis* and *Pseudomonas fluorescens* were found effective against *Fusarium oxysporum* f.sp. *lycopersici*. *B. subtilis* inhibited 79.25 per cent and *P. fluorescens* inhibited 62.36 per cent growth of pathogen.

The results are in conformity with those obtained by Calvet *et al.* (1990) [6], Narnavar and Kalekar (1997) [8], Eo Monda (2002) [9].

Table 2: Evaluation of biocontrol agents against *Fusarium oxysporum* f.sp. *lycopersici in-vitro*

Sr. No.	Biological agents/ Treatment	Mean colony diameter (mm) *after 7 days	Percent inhibition of growth **
1.	*T. harzianum* (T1)	22.1	72.54 (58.39)
2.	*T. viride* (T2)	12.2	84.84 (67.08)
3.	*T. hamatum* (T3)	24.2	69.93 (56.74)
4.	*T. koningii* (T4)	31.0	61.49 (51.64)
5.	*Bacillus subtilis* (T5)	16.7	79.25 (62.90)
6.	*Pseudomonas fluorescens* (T6)	30.3	62.36 (52.15)
7.	Control (T7)	80.5	-
	SE ±	0.06	0.52
	C.D. at 1%	0.19	1.61

Note: * Average of three replication
** Figure sin parenthesis are square arc sin value

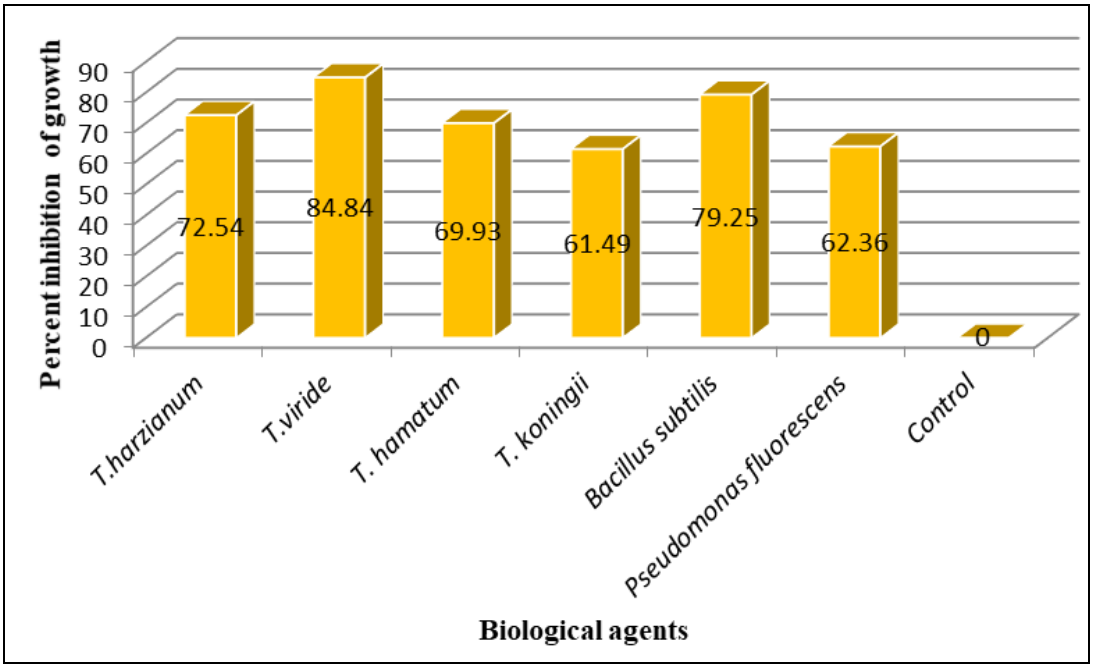
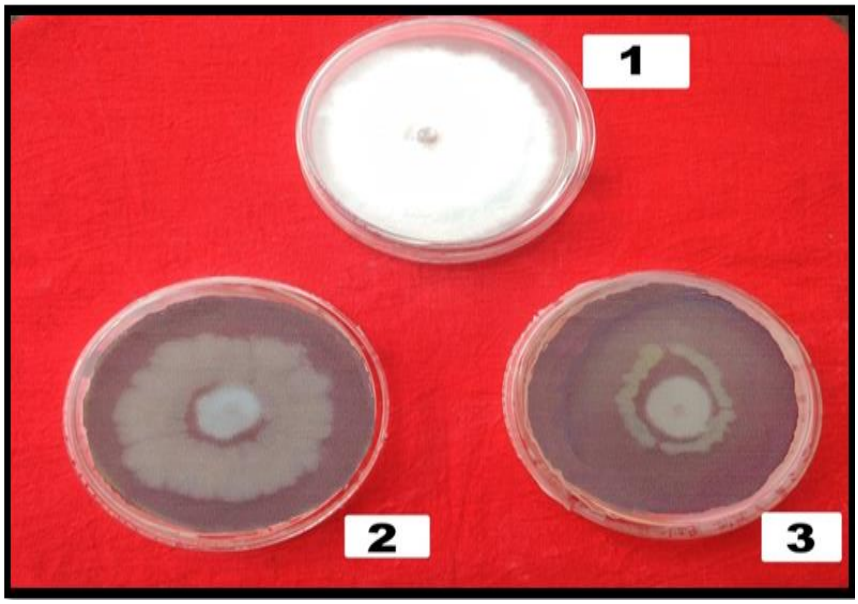


Fig 1: Evaluation of biocontrol agent against *Fusarium oxysporum* f.sp. *lycopersici*

(1) *Trichoderma harzianum* (2) *Trichoderma viride* (3) *Trichoderma hamatum* (4) *Trichoderma koningii* (5) Control



(1) Control (2) *Psuedomonas fluorescens* (3) *Bacillus subtilis*

Plate 1: Evaluation of Biological agents *in-vitro*

4. Conclusion

Hence, from ongoing results and discussion, it is concluded that *in vitro* efficacy of biological control the *Trichoderma viride* was found to be most effective for inhibiting the growth of *Fusarium oxysporum* f.sp. *lycopersici* followed by *T. harzianum*, *T. hamatum* and *T. koningii* in order of merit. In bacterial bioagent species, the *Bacillus subtilis* was found to be more effective for control of test pathogen followed by *Pseudomonas fluorescens*.

5. References

1. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;150:850.
2. Arora DK, Upadhyay RK. Plant and soil. 1978;49(3):685-690.
3. Panse VG, Sukhatme PV. Statistical methods for Agricultural Workers. I. C. A. R., New Delhi 1978.
4. Kale PN, Kale SP. Bhajipalautpadan continental publishing, Pune, India 1984, 29.
5. Upadhyay RS, Rai B. Studies in antagonism between *F. udum* basal and root region miroflora of pigeonpea. Pl. Soil 1987;101(1):79-93.
6. Calvet C, Pera J, Barea TM. Interaction of *Trichoderma* spp. with two wilt pathogenic fungi. Agric. Ecosyst. and Environ 1990;29(1-4):59-65.
7. Nene YL, Thapliyal RN. Evaluation of fungicides in: Fungicides for Plant Disease Control (3rd Ed.). Oxford, IBH Pub. Co., New Delhi: 1993, 331.
8. Narnavar, Kalekar. Studies on wilt disease complex in tomato. M.Sc. (Agri.) thesis submitted to M.P.K.V., Rahuri 1997.
9. Monda EO. Biological control of *Fusarium* wilt of tomato. J. Tropical Microbiology Biotech 2002. ISSN : 1607-4106.
10. Anonymous. National Horticulture Board, Ministry of Agriculture Government of India 2018.