Int.J.Curr.Microbiol.App.Sci (2019) *8*(6): 1821-1826

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 06 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.806.217

In-vitro Efficacy of Botanical and Selected Bio-Agents in the Management of Fusarial wilts of Tomato (*Lycopersicon esculentum* L.)

Vinny John*, Sunil Zacharia, Amit Kumar Maurya, Anoop Kumar and Sobita Simon

Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, India

**Corresponding author*

Keywords

Carbendazim, *Fusarium oxysporum* f. sp. *lycopersici*, *Lycopersicon esculentum*, Neem leaf extract, *Pseudomonas fluorescens*, *Trichoderma harzianum*, *T. viride*

Article Info

Accepted:
15 May 2019
Available Online:
10 June 2019

A B S T R A C T

The study was conducted in the department of Plant Pathology SHUATS Allahabad (2014) to investigate mycoparasitism inhibitory effects of *Trichoderma* spp, *Pseudomonas* sp, neem leaves extract and carbendazim on the growth of the *Fusarium oxysporum* f.sp. *lycopersici* which causes wilt of tomato *in-vitro*, *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluoresens* were used @ 2% (dual culture technique) and carbendazim @0.1gm and Neem leaves extract in the ratio of 1:1(poison technique). Among all *Trichoderma viride* was the best inhibitor of test pathogen (87.44%) followed by carbendazim (87.11%). Result showed that all the treatments significantly inhibit the growth of the pathogen (%).

Introduction

Tomato is a most popular widely grown vegetable in the world. Both the species and its use as a food originated in Mexico, and spread around the world (Nem Pal Singh *et.al* 2004). Edible fruit, often red in color from the plant (*Lycopersicon esculentum*) from a member of family solanaceae is mainly cultivated as vegetable. It is a rich source of vitamin C; it also contains minerals like calcium, phosphorus (Linger and Hill 1991). As it is a short duration crop and gives high yield, it is important from economic Point of view and hence are under its cultivation is increasing day by day. *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a highly destructive pathogen of both green house and field grown tomatoes in worm vegetable production areas. The disease caused by this fungus is

characterized by wilted plants, yellowed leaves and minimal or no crop yield (Asha *et al.,* 2011). Wilt is one of the most economically important disease world-wide (Alexander and Tucker, 1945; Cal *et al.,* 2004; Srinon *et al.,* 2006). *Fusarium* wilt is soil- borne in nature; it produces two different kinds of conidia which is micro and macro conidia respectively. During unfavorable condition it survives in the form of chlamydospore. The fungus causes vascular wilts by infecting plants through the roots and growing internally through the cortex to the stele (Bowers and Locke 2000). Control of wilt diseases depends mainly on fungicides (Minton 1986). Several fungicides have been used for control of different plant pathogens including *Fusarium* (Liggit *et al.,* 1997) and the number of effective fungicides with negligible effect on the environment is rare. Fungicides are expensive, can cause environmental pollution and may cause the selection of pathogen resistance (Lumsden and Locke 1989).

Materials and Methods

The experiment was conducted in the research laboratory in the Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Deemed to be University, Allahabad (2013-2014), *in-vitro*. The infected plant, showing characteristic symptoms of wilt disease (infected plant collected from Plant Pathology Central Research Farm) was cut with healthy portion into small pieces (2mm), surface sterilized with 0.1 per-cent mercuric chloride (Hg Cl2) solution, thrice rinsed in sterilized distilled water and then transferred aseptically on PDA medium, and incubated at 25 ± 2^0C. After 3 days, a whitish colony growth was observed, from this whitish colony growth, a portion from the periphery having single hyphal tip were separated and transferred to other petri-plate shaving medium to get pure culture and slides were prepared for the identification, which showed the mycelium of the fungus which was both inter and intracellular within the mesophyll and vascular bundle (in xylem) tissues of the root. Macro conidia are usually relatively rare; fusiform mostly 2-3 septate while micro conidia were abundantly produced and are unicellular. The fungus isolated from infected tomato plants was identified as *F. oxysporum* f. sp. *lycopersici*.

Freshly prepared neem leaves extract i.e,@10% concentration, were used in each conical flask of 250 ml; 90 ml PDA + 10ml prepared extract were added to it and sterilized.

In dual culture method, 9 mm width of petri dish of fifteen days old fungal culture were placed on a PDA medium one cm away from the edge of the plate. *T. harzianum, T. viride* and *P. fluorescens* were inoculated at opposite side of each petri-plate with the test pathogen.

In poison food technique, neem leaf extract 10% and carbendazim 0.1% with 10ml of PDA poured in each petriplates.

5 replicates of each treatment and control with normal PDA was incubated at 25 ± 20C. Per-cent inhibition over control was calculated by the following formula at 7 days of interval-

$$I = \frac{(C-T)}{C} X (100)$$

Where,

I=Per-cent reduction in growth of test pathogen

C = Radial growth (cm) in control

T =Radial growth (cm) in treatment

Radial growth (cm) at different hours was observed and recorded. Six treatments (including control) were replicated five times. Effect of bio-agents neem leaf extract and fungicide carbendazim (check) against test pathogen *F. oxysporum f. sp. lycopersici, in-vitro.*

Results and Discussion

To evaluate the efficacy of antagonist and neem leaf extract. Dual culture and poison food technique were respectively adopted in-vitro. The maximum reduction percentage of mycelia growth recorded in treatment *T. viride* which inhibited (87.44%) of the colony growth of test pathogen followed by carbendazim which was second most effective growth inhibitor (87.11%), *T. harzianum* T2 per-cent inhibition recorded (80.88%) and *P. fluoresens* inhibited (80.77%) of the radial growth of pathogen, neem leaves extract inhibited (80.66%) colony growth of the test pathogen in comparison to control (Table 1 and 2).

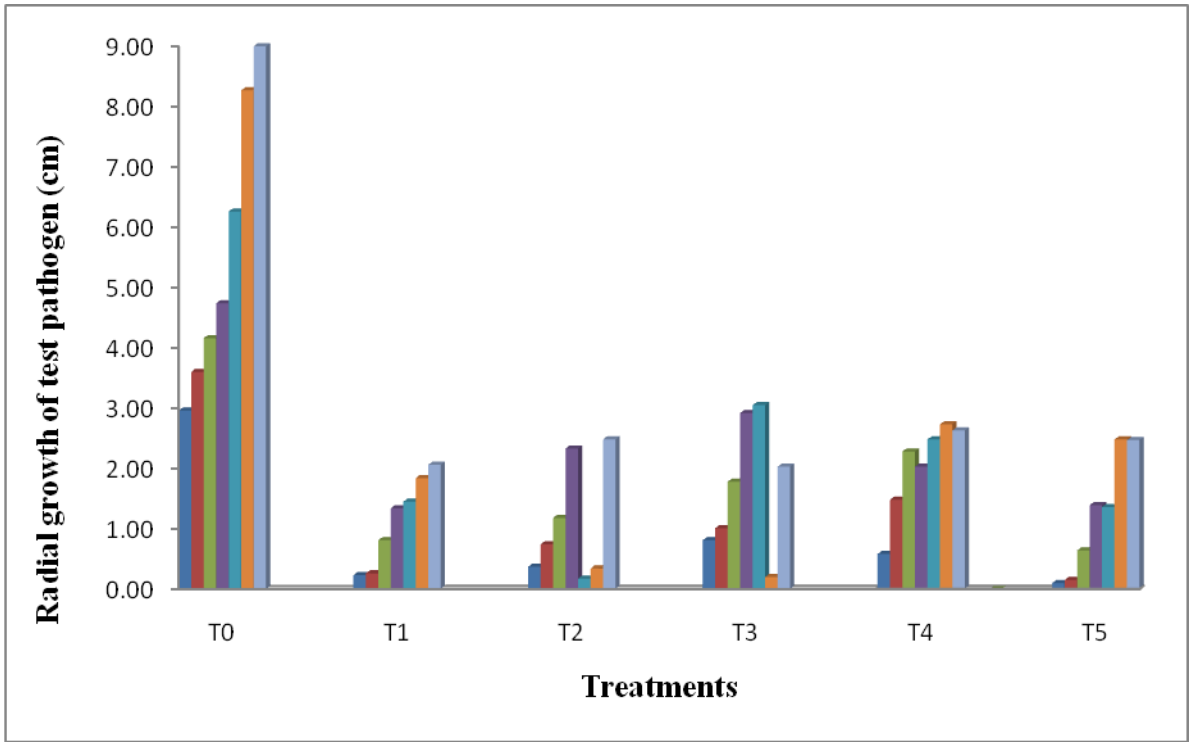
Table.1 Effect of *Trichodrema* spp and *P. fluorescens* carbendazim and neem leaf extract on growth of *F. oxysporum lycopersici* (L.)

	Treatments	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	Percent inhibition (%)
T0	**Control**	2.960	3.600	4.160	4.740	6.260	8.270	9.000	
T1	***Trichoderma viride***	0.230^{bc}	0.260^{b}	0.810^{ab}	1.340^{b}	1.450^{a}	1.840	2.060	87.44
T2	***Trichoderma harzianum***	0.370^{a}	0.740	1.180	2.330^{a}	0.170	1.50^{a}	2.480^{b}	80.88
T3	***Pseudomonas fluoresens***	0.810^{bc}	1.010^{a}	1.780	2.920	3.050	1.60^{a}	2.970^{a}	80.77
T4	**neem leaf extract**	0.580^{ab}	1.480^{a}	2.280^{a}	2.030^{a}	2.480	2.730^{a}	2.630^{ab}	80.66
T5	**Carbendazim**	0.100^{c}	0.150^{b}	0.640^{b}	1.390^{b}	1.360^{a}	2.480^{a}	2.470^{b}	87.11
	S. Ed. (±)	0.416	0.112	0.096	0.088	0.084	0.037	0.085	
	C. D. (P = 0.05)	0.108	0.433	0.408	0.384	0.375	0.237	0.375	

Note: Data are average of five replications. Values followed by same letters in the column are non-significant.

Pure culture of *F. oxysporum* f. sp. *lycopersici*

Table.2 Effect of *Trichodrema* spp and *P. fluorescens*, carbendazim and neem leaf extract on growth of *F. oxysporum* f. sp. *lycopersici*



The most effective treatment among all was *Trichoderma viride* which inhibited the growth of test pathogen completely. The antagonist *Trichoderma viride* grew very fast than *F. oxysporum lycopersici* and did not allowed the test pathogen to grew on the PDA medium, carbendazim, which was also effective in suppressing the growth of pathogen followed by *T. harzianum*, *P. flourescens* and neem extract. The following

mentioned data were obtained by recorded continuous observation for seven days, after inoculation of pathogen on media treated with different bio-agents, cardendazim and neem leaves extract compared with control. Similar findings were observed Amini and Sidovich. (2010).

Summary and Conclusions are as follows:

T. viride was the best among all treatments followed by carbendazim, *T. harzianum, P. fluorescens* and neem leaf extract in comparison to control. *In-vitro* trails also found in the findings of Nikam *et al.,* (2007), Sivakumar *et al.,* (2008), Chakraborty *et al.,* (2009)

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How to cite this article:

Vinny John, Sunil Zacharia, Amit Kumar Maurya, Anoop Kumar and Sobita Simon. 2019. *In-vitro* Efficacy of Botanical and Selected Bio-Agents in the Management of Fusarial wilts of Tomato (*Lycopersicon esculentum* L.). *Int.J.Curr.Microbiol.App.Sci.* 8(06): 1821-1826.
doi: https://doi.org/10.20546/ijcmas.2019.806.217