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Evaluation of Bio-Agents, Essential Oils and Chemicals against *Fusarium* Wilt of Tomato

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A B S T R A C T

Tomato (*Lycopersicon esculentum* L.) is the world's largest vegetable crop after potato and sweet potato, belongs to family Solanaceae. It was universally treated as "protective food" because of its special nutritive value. *Fusarium* wilt of tomato is one of the most devastating diseases, caused by *Fusarium oxysporum* f.sp. *lycopersici.* It is a soil borne pathogen which causes serious loss in tomato production and attacks plants through roots at all stages of plant growth. So, the present study was been undertaken to evaluate the effects of some eco-friendly bio-agents, chemicals and essential oils against *Fusarium oxysporum* f.sp. *lycopersici*, by which we can improve the agricultural production of tomato. Out of all bioagents, *Trichoderma harzianum* showed maximum zone of inhibition of test pathogen followed by *Trichoderma viride* and *Trichoderma koningii*. During the evaluation of agrochemicals against the test pathogen, carbendazim showed best results followed copper oxychloride and mancozeb. As we know that the essential oils are the best alternative in place of chemicals. So, in this context, five essential oils *viz.*, thyme oil, clove oil, eucalyptus oil, neem oil and cinnamon oil is used to manage the test pathogen of tomato wilt, in which thyme oil inhibit the growth of test pathogen completely followed by clove oil.

Introduction

Tomato (*Lycopersicon esculentum* L.) is the world's largest vegetable crop after potato and sweet potato, belongs to family Solanaceae (Kumar *et al.*, 2018). The tomato is well consumed in diverse ways, including raw, processed items like paste, puree, syrup, sauce, ketchup, chutney and soup. Botanically it was a fruit but it also considered as a vegetable for culinary purposes. It was highly nutritive crop and it's having powerful antioxidants and rich in lycopene (bright red carotenoid hydrocarbon) and lycopene nutritional value (20-50/100g) its having at 21-24ºC temperature. It helps to improve skin ability to protect from harmful environmental effects like ultra violet rays. The consumption of tomato the nutritional value were supported to decrease the heart risks, weight loss

healthful skin, head and neck cancers and might be strongly protective against blood pressure, diabetes and neurodegenerative disorders (Arab and Steck, 2000). The Ascorbic acid content 16-65 Mg/ 100g, Amino acid content 100-300 Mg/100g, water 95.0 grams, Vitamin C 22.0 mg and Vitamin A 300 IU (Taylor, 1993).

Fusarium wilt of tomato is one of the most destructive diseases of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici.* It is a soil borne pathogen and causes serious loss in tomato production by attacking plants through roots at all stages of plant growth, it was considered as one of the main soil-borne systemic disease and the major limiting factor in the production of tomato both in green house and field-grown (McGovern, 2015; Khiareddine *et al.,* 2019).

Trichoderma has long been considered as one of the most promising bio-control agent for several plant pathogens (Srivastava *et al.,* 2010). The species of *Trichoderma* have been evaluated against the wilt pathogens and have exhibited greater potential in managing wilt under field condition (Podder *et al.,* 2004). Soil drenching with fungicides are generally used to control of this disease, however, frequent and indiscriminant use of its leads to ill effects on environment causing soil and water pollution and development of new strain with more virulence, hence bio-control and essential oils has been advocated as one of promising alternative strategy to overcome these problems (Calo *et al.,* 2015; Chouhan *et al.,* 2017).

So, by keeping the above views in mind, the present study was been undertaken to evaluate the effects of some eco-friendly bio-agents, effects of chemicals and essential oils against *Fusarium oxysporum* f.sp. *lycopersici*, by which we can improve the agricultural production of tomato.

Materials and Methods

All the experiments of the present study were conducted at the Department of Plant Pathology, School of Agriculture, Lovely Professional University, Punjab. The management of *Fusarium* wilt was mainly followed biological method and chemical method. In biological method we used bio-agents and essential oils. In chemical method we used various systemic and contact fungicides and we followed poisoned food technique for chemicals and essential oils and dual culture technique for bio-agents. The pure culture of *Fusarium oxysporum* f.sp. *lycopersici* was purified from isolated Petri plates and sub-cultured on PDA slants and incubated at 25±2ºC for 7 to 8 days and slants were preserved in refrigerator at 7ºC for 25 days (Aneja, 2007). Effect of different temperature ranges and different growth media on the growth of *Fusarium oxysporum* f.sp. *lycopersici* were also evaluated.

Evaluation of bio control agents against *Fusarium oxysporum* f.sp. *lycopersici*

The efficacy of three bio-agents against *Fusarium* wilt by applying dual culture method. The fungal bio-agents were grown in PDA media and poured in Petri plates (90mm), the plates were kept in B.O.D incubated and maintained temperature at 25±2ºC and observed the radial mycelial growth of the test pathogen and bio-agents. The tested pathogen growth was controlled by bio-agent and formed the inhibition zone, and also observed the untreated (control) pathogen growth for seven days. The inhibition percentage was calculated by using the formulae (Vincent, 1947):-

$$I=C-T/Cx100$$

Where, I – inhibited growth; C – control; T – the radial growth of the pathogen

Evaluation of essential oils against *Fusarium oxysporum* f.sp. *lycopersici*

The different concentrations were taken by all five plant oils (thyme, clove, eucalyptus, neem and cinnamon). The oils were emulsify with acetone at 1ml/10ml were mixed and make stock solution. The different concentrations (0.1%, 0.5%, 1%, 5%, 10%) were tested by using poison food technique method. The maximum and minimum efficacy of essential oils was observed in percentage inhibition of radial growth over the control. The formula were used to calculate the radial growth of the pathogen is I=C-T/CX100 (Nene and Thapliyal, 2000).

Evaluation of fungicides against *Fusarium oxysporum* f.sp. *lycopersici*

The efficacy of systemic and non-systemic fungicides were used against *Fusarium* wilt and evaluated in in vitro conditions by using poisoned food technique. The two systemic fungicides were (Carbendazim 50% WP) and non- systemic fungicides were (Copper oxychloride 50% WP, Mancozeb 75% WP, Captan 50%WP). These fungicides were evaluated in various concentrations (10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm) against *Fusarium oxysporum.* The efficacy of chemical were expresses as percentage of inhibition of growth over control. The formula *i.e.*, I=C-T/CX100 and measured the radial growth of the pathogen until the control were covered the edges of the Petri plates (Nene and Thapliyal 2000).

Results and Discussion

Effect of different temperatures on growth of *Fusarium oxysporum* f.sp. *lycopersici*

Study was undertaken to find out the optimum as well as the best temperature for the growth of *Fusarium oxysporum* f.sp. *lycopersici* by growing at different temperatures (5, 15, 20, 25, 30, 35, 40°C temperature) on Potato Dextrose agar medium. After 7 days of incubation the average radial growth (mm) was recorded and presented in Table 1. From the perusal of the data presented in Table 1, it is evident that maximum growth of the test pathogen was recorded at 30°C temperature followed by 25±2°C. So, it is clear from the above findings that room temperature which ranges between 25±2°C is very much suitable for the growth of *Fusarium oxysporum* f.sp. *lycopersici*.

Hibar *et al.,* 2006 were evaluated the effect of different temperature range on mycelial growth of *Fusarium oxysporum* f.sp. *radicis-lycopersici*, on potato dextrose agar (PDA) media, revealed that this pathogen grows well at temperatures ranged from 20 to 30°C.

However, the optimum of mycelial growth was recorded at 25°C. Pal *et al.*, 2019 also showed that after 9 days of incubation, the maximum growth of the fungus was 88.33mm at 24°C with highest growth rate of 9.81mm per day.

Growth pattern of *Fusarium oxysporum* f.sp. *lycopersici* on different culture media

For growth and physiological studies of antagonistic five different culture media were tested and present in Table 2. All the culture media used showed significant effect at on mycelium growth, sporulation of the *Fusarium oxysporum* f.sp. *lycopersici* but the potato dextrose agar medium supporting maximum growth of37.66mm followed by the growth observed on Czapeck's Dox agar medium (36.33 mm). Minimum growth rate of the test pathogen was observed on Richard's agar (13.50 mm). Same results was also proved earlier by many scientists *viz.*, Paulkar and Raut (2004), Gupta *et al.,* (2010), Sonkar *et al.,* (2013), Manikandan *et al.,* (2018).

In vitro evaluation of different biocontrol agents against *F. oxysporum* f. sp. *lycopersici*

Biological management of plant pathogens by employing potential bioagents has been an important component of non-chemical plant disease management. Extensive study was undertaken through *in vitro* screening of three bioagents to ascertain their potential as suitable bio-pesticides against *Fusarium oxysporum* f. sp. *lycopersici*. Analysis of data presented in Table 3 indicated that *Trichoderma harzianum* was very effective in controlling *F. oxysporum* f. sp. *lycopersici* where inhibition zone formation was highest (54.73%) followed by *Trichoderma viride and Trichoderma koningii* with inhibition zone of 48.18 and 43.28%, respectively.

Maximum growth inhibition (54.73%) against the tested isolate of *Fusarium oxysporum* f.sp. *lycopersici* was shown by the *Trichoderma harzianum*. A large variety of volatile secondary metabolites could be produced by *Trichoderma* spp. such as ethylene, hydrogen cyanide, aldehydes and ketones, which play an important role in controlling various plant pathogens (Faheem *et al.,* 2010; Siddiquee *et al.,* 2012; Chen *et al.,* 2105).

Several researches of Calistru *et al.,* (1997) revealed that volatile metabolites produced by *Trichoderma harzianum* species can significantly suppress the growth of *Aspergillus flavus* and *Fusarium moniliforme* rather than mycoparasitism (Calistru *et al.,* 1997; Srivastava *et al.,* 2011; Singh *et al.,* 2018; Kumar *et al.,* 2019).

Table.1 Radial growth (mm) of *Fusarium oxysporum* f.sp. *lycopersici* at Different temperature range

Temperature (^{o}C)	Mean colony diameter (mm) of test Pathogen after 7 days of inoculation
5	0.00 (0.00)^{f}
15	18.16 (4.26)$^{d\,d}$
20	26.67 (5.16)^{C}
25	32.16 (5.67) ^{b}
30	37.66 (6.13) ^{a}
35	15.0 (3.86) ^{c}
40	0.00 (0.00)^{f}
Average	18.52 (4.30)
S.E(m)±	0.51
C.D (0.05%)	1.55 %

Table.2 Radial growth (mm) of *Fusarium oxysporum* f.sp. *lycopersici* on different media

Media	Mean colony diameter (mm) of test Pathogen after 7 days of inoculation
Potato Dextrose Agar	37.66(6.14)^{a}
Czapeck's Dox Agar	36.33(6.03)^{ab}
Malt Extract Agar	33.83(5.82)^{b}
Oat Meal Agar	34.83(5.90)^{ab}
Richard's Agar	13.50(3.67)^{d}
Average	31.23
S.E(m)±	0.53
C.D (0.05%)	1.56

Table.3 *In vitro* antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici*

Treatments		12 HOURS		48 HOURS		72 HOURS	
		Growth (mm)		Growth (mm)		Growth (mm)	
		Fusarium	I.O.C. %	*Fusarium*	I.O.C. %	*Fusarium*	I.O.C.%
T1	***T. harzianum***	9.33 (3.03)[a]*	34.96 (36.24)	13.33 (3.65)[b]*	43.66 (41.35)	15.00 (3.87)c*	66.66 (54.73)
T2	***T.viride***	9.70 (3.10)[a]	32.16 (34.54)	15.50 (3.93)[b]	34.48 (35.95)	20.00 (4.47)[b]	55.55 (48.18)
T3	***T.koningii***	12.83 (3.58)[a]	11.18 (19.53)	19.50 (4.41)[a]	17.58 (24.28)	23.85 (4.88)[a]	47.00 (43.28)
T4	**Control**	14.30	-	23.66	-	45.00	-
	S.E.(m)±	1.42	-	0.73	-	0.44	-
	CD (0.05)	4.28	-	2.20	-	1.39	-

Table.4 *In vitro* activity of essential oils against *Fusarium oxysporum* f. sp. *lycopersici*

S.No.	Treatment Details	Mycelial growth (mm)					Average (mm)	Percent inhibition growth (%)					Average growth of percent inhibition
		0.1 %	0.5 %	1 %	5 %	10 %		0.1 %	0.5 %	1 %	5 %	10 %	
1	Thyme oil	00.00^{a}	00.00^{a}	00.00^{a}	00.00^{a}	00.00^{a}	00.00	100(90)	100(90)	100(90)	100(90)	100(90)	100 (90)
2	Clove oil	39.16^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	7.83	13 (21.13)	100 (90)	100(90)	100(90)	100(90)	82.60 (65.35)
3	Eucalyptus oil	34.00^{a}	23.50^{b}	17.83^{c}	0.00^{e}	0.00^{e}	15.07	24.44 (29.63)	47.77 (43.72)	60.37 (50.99)	100(90)	100(90)	66.51 (54.64)
4	Neem oil	40.00^{a}	26.83^{b}	15.16^{d}	12.00^{d}	0.00^{e}	18.80	11.11 (19.47)	40.37 (39.45)	66.28 (54.50)	73.33 (58.91)	100(90)	58.21 (49.73)
5	Cinnamon oil	39.83^{a}	35.83^{b}	32.83^{b}	0.00^{e}	0.00^{e}	21.70	11.48 (19.81)	20.37 (26.83)	27.04 (31.33)	100(90)	100(90)	51.77 (46.01)
0	Control	45.00	45.00	45.00	45.00	45.00	45.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	0.00 (00.00)
	Average	33.00	21.86	18.47	9.50	7.50		26.67 (31.09)	51.41 (45.80)	58.94 (50.14)	78.88 (62.64)	83.33 (65.90)	
	S.E(m)±	(T) 0.192	(C) 0.175	(TxC) 0.429									
	C.D (0.05 %)	0.544	0.497	1.217									

T=Treatment; C=Control; TxC=Treatment x Control

Table.5 *In vitro* effect of fungicides against *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Mycelial Growth (mm)					Average	Percent Growth Inhibition (%)					Average growth of percent inhibition
	10 ppm	25 ppm	50 ppm	75 ppm	100 ppm		10 ppm	25 ppm	50 ppm	75 ppm	100 ppm	
T1 (Carbendazim)	0.00^{a}	0.00^{b}	0.00^{bc}	0.00^{c}	0.00^{d}	0.00	100.00 (90.00)	100.00(90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 % (90.00)
T2 (Copper oxychloride)	6.00^{a}	2.83^{b}	1.83^{bc}	1.00^{c}	0.00^{d}	2.33	86.66 (68.58)	93.71 (75.48)	95.93 (78.36)	97.77 (81.41)	100.00 (90.00)	94.78 % (76.79)
T3 (Mancozeb)	8.23^{a}	5.66^{b}	2.83^{c}	1.66^{c}	0.00^{d}	3.67	81.70 (64.67)	87.40 (69.21)	93.71 (75.48)	96.28 (78.88)	100.00 (90.00)	91.81 % (73.37)
T4 (Captan)	38.50^{a}	32.83^{b}	28.66^{b}	23.66^{c}	18.50^{d}	28.4	14.44 (22.33)	27.00 (31.31)	36.28 (37.04)	47.40 (43.51)	58.88 (50.11)	36.78 % (37.33)
T0 (Control)	45.00	45.00	45.00	45.00	45.00	45.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 % (00.00)
Average	19.55	17.27	15.67	14.27	12.70		56.58 (48.78)	61.63 (51.73)	65.18 (53.83)	68.29 (55.72)	71.77 (57.90)	
S.E (m)	(T) (0.23)	(C) (0.23)	(T x C) (0.52)									
CD (0.05 %)	0.64	0.64	1.45									

T=Treatment; C=Control; TxC=Treatment x Control

Volatile metabolites produced by *Trichoderma* strains displayed inhibitory effects on *R. solani* and *P. ultimum* pathogens growth (Raut *et al.,* 2014). In India, *Trichoderma viride* and other several bacterial species such as *Streptomyces gougeroti* were found to be antagonistic to *Fusarium oxysporum* f. sp. *lycopersici* (Mehrotra and Caludius, 1972). Bioagents like *Aspergillus niger*, *Trichoderma* sp. and *Penicillium citrinum* and some bio-dynamic antagonists have shown their effectiveness towards the control of wilt pathogens of guava (Srivastava *et al.,* 2011). Several reports indicated that *Trichoderma* species can effectively suppress *Fusarium* wilt pathogens (Kumar *et al.,* 2016; Tomar *et al.,* 2017). *Trichoderma* species has multiple mechanisms of action, including coparasitism via production of chitinases, β-1-3glucanases and β-1-4glucanases, antibiotics, competition, solubilization of inorganic plant nutrients, induced resistance and inactivation

of the pathogen's enzymes involved in the infection process (Altomare *et al.,* 1999).

In vitro evaluation of different essential oils against *F. oxysporum* f. sp. *lycopersici*

The antifungal effects of five essential oils *viz*., thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus globulus*), neem (*Azadirachta indica*) and cinnamon (*Cinnamomum zeylanicum*) oil were evaluated against tomato wilt causing fungus, *Fusarium oxysporum* f. sp. *lycopersici* in Table 4. The inhibitory effect of oils showed dose-dependent activity on the tested fungus. Most active being the thyme oil, exhibiting complete inhibition of mycelial growth and spore germination at 0.1, 0.5, 1, 5, 10%, respectively. Clove oil is second most effective essential oil against test pathogen by showing 82.60% inhibition followed by eucalyptus and neem oil by showing 66.51% and 58.21% inhibition respectively. The Minimum inhibitory concentration (MIC) was shown by cinnamon oil was 51.77%.

There is an increasing demand to reduce the use of chemicals as antimicrobial agents in the field of nutrition and to combat various infections due to increasingly aggressive and increasingly endogenous microorganisms that are resistant to the use of synthetic antimicrobials. In this direction, substances derived from plants, such as hydro-alcoholic extracts or essential oils, can certainly play a fundamental role (Nazzaro *et al.,* 2017). Essential oils are employed in agriculture, medicine and food industries among others, due to their antimicrobial, antiviral, insecticidal and anti-fungal properties. Recent studies show anti-fungal effects of many essential oils against plant pathogenic fungi, which make them candidates for the development of new fungicidal agents (Arraiza *et al.,* 2018). Sharma *et al.,* 2017 also reported that clove oil as potent antifungal agent that could be used as biofungicide for the control of *F. oxysporum* f. sp. *lycopersici* in both preventive and therapeutic manner.

In vitro evaluation of different fungicides against *F. oxysporum* f. sp. *lycopersici*

In presented research, four fungicides *viz.,* carbendazim, copper oxychloride, mancozeb and captan, were evaluated for their efficacy against the disease casual agent *Fusarium oxysporum* f. sp. *lycopersici* under *in vitro* conditions (Table 5). Five different concentration (10, 25, 50, 75, 100ppm) were used for assessment of their inhibitory activities against the test pathogen through mycelial growth inhibition on PDA media. Observations revealed that carbendazim was the most effective fungicides by showing 100% inhibition at all the concentrations against the pathogen under *in vitro* condition, followed by copper oxychloride (94.78 %) and mancozeb (91.81%), respectively. From the above findings it is also evident that captan is least effective against *F. oxysporum* f. sp. *lycopersici.*

The effective control of tomato wilt can be done by seed treatment with Thiram 75 WDP before sowing followed by 10 minute dipping of seedlings roots in 0.3% solution of Carbendazim 50 WP before transplanting and plant roots drenched with Copper oxychloride 50 WP @ 0.3 % solution+0.01 % Streptomycin solution one month after transplanting (Singh and Kamal, 2012).

Out of all the three bioagents, *Trichoderma harzianum* showed maximum zone of inhibition of test pathogen followed by *Trichoderma viride* and *Trichoderma koningii*.

During the evaluation of agrochemicals against the test pathogen, carbendazim

showed best results followed copper oxychloride and mancozeb. As we know that the essential oils are the best alternative in place of chemicals. So, in this context, five essential oils *viz*., thyme oil, clove oil, eucalyptus oil, neem oil and cinnamon oil is used to manage the test pathogen of tomato wilt, in which thyme oil inhibit the growth of test pathogen completely followed by clove oil.

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