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Evaluation of different fungicide, bioagent and phytoextract against *Fusarium oxysporum* f. sp. *capsici*. under *in vitro* condition

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Abstract
Fusarium wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsici* has been emerging as one of the biotic threats in profitable cultivation of chilli crop. The fungicides evaluated *in vitro* against *Fusarium oxysporum* f. sp. *capsici* were significantly reduced the mycelial growth. Among that Benomyl 50% WP and Copper oxychloride 50% WP were found most effective with maximum growth inhibition (100%) and (86.65%). Antagonist tested against *Fusarium oxysporum* f. sp. *capsici in vitro* significantly reduced the growth of test pathogen. Among that *Trichoderma virens* inhibitited (92.38%) and *Trichoderma harzianum* (89.33%) found to be most effective with highest mycelial growth. Among the botanicals tested against *Fusarium oxysporum* f. sp. *capsici*, the *Allium sativum* @ 10% and 20% were found most effective with significantly highest growth inhibition 43.61 and 53.86% respectivly, then *Brassica juncea* L. @ 10% and 20% with growth inhibition 32.90% and 42.19%.

Keywords: *Fusarium* f. sp. *capsici*, wilt of chilli, phytoextract, bioagents

Introduction
Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop grown in almost all parts of tropical and subtropical regions of the world. It belongs to the family *Solanaceae* and originated from South and Central America where it was domesticated around 7000 BC. It was introduced in India by the Britishers in the 19th century in Shimla hills. Different varieties are cultivated for vegetable, pickles, spice and condiments. The genus *Capsicum* includes 30 species, five of which are cultivated: *Capsicum annuum* L., *C. frutescens* L., *C. chinense Jacq*, *C. pubescens* R. & P. and *C. baccatum* L. (Bosland and Votava, 2000; Wang and Bosland, 2006 and Ince *et al*., 2010) [4, 26, 9].
India is the leading producer and consumer of chilli. In India chilli is grown for vegetable purpose i.e. green chilli and also for spice i.e. red chilli. Area under green chilli cultivation is about 316 thousand hectares with production of 3634 thousand metric tonnes and area under dried chilli is about 840 thousand hectares with production of 2096 thousand metric tonnes (Anonymous, 2017) [1]. The important chilli growing states are Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu, Rajasthan, Orissa, West Bangal and Madhya Pradesh having more than 70 per cent acreage of India (Anonymous, 2017) [1].
The crop is susceptible to *Fusarium* wilt at all the stages of crop growth. Pathogen infect root, stem, leaves and fruit under favorable condition results in defoliation, drying off of the twigs and complete wilting of crop thereby causing heavy losses. Keeping in view the economic importance of chilli, the vegetable as well as spice crop and losses incurred by *Fusarium* wilt in chilli, present studies were conducted in scientific way.

Material and Methods
a) Evaluation of fungicides
Fungicides reported in Table 1 are effective against *Fusarium oxysporum* causing wilt in chilli were evaluated *in vitro* by applying poisoned food technique using Potato dextrose agar as basal medium. This Poisoned Food Technique was given by (Nene and Thapliyal 1993) [16]. An appropriate quantity of concentration of the fungicides was added in previously sterilized 100 ml PDA separately in 250 ml conical flasks. The flasks were shaken well to ensure uniform distribution of fungicides in the basal medium. Twenty ml of the medium containing fungicides was poured into sterilized petri dishes.

After solidification, the plates were inoculated by the fungal disc of 5 mm diameter cut out from seven days old culture and incubated at 27 ± 2 ºC for seven days. Observation on radial mycelia growth was recorded in all the replicated treatments. Per cent inhibition of the growth of the test pathogen was calculated by applying the formula given by Vincent (1927) [25] and the data obtained were averaged and analyzed statistically.

Per cent inhibition (I) × 100

Where,
C= growth (mm) of test fungus in untreated control plate
T= growth (mm) of test fungus in treated plate

b) Evaluation of bio-control agents

Six antagonist's are in Table 2 *in vitro* against *Fusarium oxysporum* f. sp. *capsici* by dual culture method (Dennis and Webster, 1971) [6]. All the antagonist's and the pathogen were multiplied in PDA. Twenty ml of PDA was poured aseptically in each petri plates and allowed to solidify. Mycelial disc of 5 mm diameter of each antagonist and test fungus was placed on opposite ends of PDA containing petri plates. Each treatment was replicated three times. The plates were incubated at 27 ± 2 ºC for seven days. Observation on radial mycelia growth was recorded in all the replicated treatments. Per cent inhibition of the growth of the test pathogen were calculated by applying the formula. The data obtained were averaged and analyzed statistically.

Per cent inhibition (I) × 100

Where,
C= growth (mm) of test fungus in untreated control plate
T= growth (mm) of test fungus in treated control plate

c) Evaluation of botanicals

Seven locally available plants were used for their evaluation in Table 3 against *Fusarium oxysporum* f. sp. *capsici* by using Poison Food Technique (Nene and Thapliyal, 1993) [16]. Fresh leaves, rhizomes or cloves of respective plants were first washed with tap water followed by sterilized water. Each sample was homogenized in sterilized distilled water at the rate of 1 ml/g of tissues (1:1 v/w) with a pestle and mortar and filtered through fine muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 minutes and the supernatant was filtered through Whatman No. 1 filter paper, which forms the standard plant extract solution (100%). The extracts were individually incorporated into PDA medium at 10 and 20 per cent concentration in 250 ml conical flasks separately and sterilized at 1.2 kg/cm^2 for 15 minutes. These were poured in 90 mm sterilized petri dishes with three replications for each concentration of extract. PDA without extracts was maintained as control. All the petri dishes were inoculated with 5 mm disc of mycelium of the pathogen and incubated at 27 ± 2 ^{0}C. Seven days after inoculation, the radial growth of mycelium was recorded and per cent inhibition of fungal growth for each treatment and concentration was calculated as mentioned in fungicides test.

Per cent inhibition (I) × 100

Where,
C= growth (mm) of test fungus in untreated control plate
T= growth (mm) of test fungus in treated plate

Results and Discussion

a) Evaluation of Fungicides

The data presented in the Table 4, revealed that all of seven fungicides evaluated *in vitro* significantly inhibited mycelial growth of *F. oxysporum* f. sp. *capsici*, over untreated control. However, Benomyl 50% WP stood first with cent per cent mycelial growth inhibition (100%) @ 0.25%, followed by Copper oxychloride 50% WP @ 0.25% (86.65%), Bordeaux mixture @ 0.1% (65.22%), Captan 50% WP @ 0.25% (58.45%), Metalaxyl 72% WP @ 0.25% (50.19%), Propineb 75% WP @ 0.25% (43.60%) and Azoxystrobin 23% EC @ 0.1% (42.86%).

These results in consonance with the findings of Subhani *et al*. (2011) [24] and Sinha (1974) [23], who reported Benomyl as most effective against *F. oxysporum*, causing wilt in Chick pea and Pigeon pea crops. Similarly Jahanshir and Fevzi (2010) [10] and Yadav *et al*. (2014) [28] reported the effectiveness of Benomyl against *F. oxysporum*, causing wilt disease in tomato and onion crops, respectively. Rest of the fungicides *viz*., Azoxystrobin, Bordeaux mixture, Captan, Propineb, Copper oxychloride and Metalaxyl were also reported to cause significant mycelial growth inhibition of *F. oxysporum*, causing wilt in many crop hosts, earlier by several workers (Rajput *et al.*, 2006; Dar *et al*., 2013; Hegde *et al*., 2017; Manasa *et al*, 2017; Singh *et al*., 2017) [19, 5, 8, 13, 22].

b) Evaluation of bioagents

The results presented in Table 5, revealed that all of the six bioagents evaluated *in vitro* were antagonistic to the test pathogen, which significantly inhibited its mycelial growth, over untreated control. However, *Trichoderma virens* resulted with significantly highest mycelial growth inhibition (63.54%), followed by *T. harzianum* (57.94%), *T. koningii* (34.57%) and *T. viride* (28.97%). Whereas, *Bacillus subtilis* and *Pseudomonas fluorescens* were least effective with 20.55% and 19.24% inhibition, respectively.

These results are in conformity with the earlier findings of Rudresh *et al*. (2005) [21] who reported *Trichoderma virens* as most effective against *F. oxysporum* f. sp. *ciceris* causing wilt disease in Chick pea crop. Similarly, Jamwal *et al*. (2011) [11], Dar *et al.* (2013) [5], Bharari (2015) [3], Malathi (2015) [12] and Mishra *et al*. (2017) reported the efficacy of *T. harzianum* against *F. oxysporum*, causing wilt disease in safflower, tomato, fir, tomato, onion and chilli crops.

c) Evaluation of Phyto extract

It was revealed from the data depicted in Table 6 that, the antifungal activity of seven aqueous phytoextracts was evaluated (each @ 10 & 20%) *in vitro* against *F. oxysporum* f. sp. *capsici*.

The results *Allium sativum* resulted with significantly highest mycelial growth of 43.67% and 53.86% @ 10 and 20% respectively, followed by *Azadirachta indica* (42.93%), *Zingiber officinale* (42.75%), *Brassica juncea* L. (42.19%), *Jatropha curcas* (39.17%), *Chrysanthemum morifolium* L. (38.80%) and *Ocimum sanctum* (37.67%) @ 20%.

Many workers, Nisa *et al*. (2011) [17], Wani *et al*. (2011) [27], Prasannath *et al*. (2011) [18], Hegde *et al*. (2017) [8] have found similar results as the *Allium sativum* extract is most effective for mycelial growth inhibition of the *Fusarium oxysporum* pathogen. Similarly, Ganie *et al*. (2003), Ramaiah and Grampalli (2006), Mengal *et al.*, (2017) reported the effectiveness of *Azadirachta indica* against *F. oxysporum* fungus causing wilt in various crop hosts.

Table 1: List of fungicides used to check their efficacy against *F. oxysporum* f. sp. *Capsici*

Tr. No.	Treatments	Conc. (%)
T_1	Azoxystrobin 23% EC	0.1
T_2	Bordeaux mixture	0.1
T_3	Benomyl 50% WP	0.25
T_4	Captan 50% WP	0.25
T_5	Propineb 70% WP	0.25
T_6	Copper Oxychloride 50%WP	0.25
T_7	Metalaxyl M 72% WP	0.25
T_8	Control (Untreated)	-

Table 2: List of bioagents used to check their efficacy against *F. oxysporum* f. sp. *Capsici*

Treatment	Bioagents
T1	*Trichoderma harzianum*
T2	*Trichoderma viride*
T3	*Trichoderma virens*
T4	*Trichoderma koningii*
T5	*Pseudomonas fluorescens*
T6	*Bacillus subtilis*
T7	Control (Untreated)

Table 3: List of phytoextract used to check their efficacy against *F. oxysporum* f. sp. *Capsici*

Tr. No.	Treatments	Common name	Plant part used	Concentration	
T1	*Azadirachta indica*	Neem	Leaf extract	10%	20%
T2	*Allium sativum* L.	Garlic	Clove extract	10%	20%
T3	*Ocimum sanctum*	Tulsi	Leaf extract	10%	20%
T4	*Zingiber officinale*	Ginger	Rhizome extract	10%	20%
T5	*Jatropha curcas*	Jatropha	Leaf extract	10%	20%
T6	*Brassica juncea* L.	Mustard	Mustard oil	10%	20%
T7	*Chrysanthemum morifolium* L.	Chrysanthemum	Leaf extract	10%	20%
T8	Control (Untreated)	-	-	-	-

Table 4: *In vitro* efficacy of various fungicides against mycelial growth of *F. oxysporum* f. sp. *Capsici*

Tr. No.	Treatments	Conc. (%)	Mean Colony Diameter (mm)*	Per cent Inhibition Over control
T1	Azoxystrobin 23% EC	0.1	50.66	42.86 (40.89)
T2	Bordeaux mixture	0.1	30.83	65.22 (53.86)
T3	Benomyl 50% WP	0.25	00.00	100.00 (90.00)
T4	Captan 50% WP	0.25	36.83	58.45 (49.86)
T5	Propineb 70% WP	0.25	50.00	43.60 (41.32)
T6	Copper oxychloride 50%WP	0.25	11.83	86.65 (68.56)
T7	Metalaxyl M 72% WP	0.25	44.16	50.19 (45.10)
T8	Control (Untreated)	-	88.66	-
S. E. (m) ±		0.51		
C.D (P = 0.01)		1.48		

* Mean of three replications
Figures in parentheses are Arcsine values

Table 5: *In vitro* efficacy of various bio-agents against mycelial growth of *F. oxysporum* f. sp. *Capsici*

Tr. No.	Bio-agents	Radial Growth (mm)*	Per cent Inhibition* of mycelial growth
T1	*T. harzianum*	8.75	89.33 (70.73)
T2	*T. viride*	6.25	92.38 (73.98)
T3	*T. koningii*	20.25	75.30 (60.20)
T4	*B. subtilis*	9.50	88.41 (70.10)
T5	Control	82.00	-
SE (m) ±		1.04	
CD@ 1%		4.35	

* Mean of three replications
Figures in parentheses are Arcsine values

Table 6: *In vitro* efficacy of various phytoextract against mycelial growth of *F. oxysporum* f. sp. *Capsici*

Tr. No.	Treatments	Mean Colony Diameter (mm)* at Conc.		Per cent Inhibition Over control at Conc.	
		10%	20%	10%	20%
T1	*Azadirachta indica*	64.16	50.50	28.44 (32.22)	42.93 (40.93)
T2	*Allium sativum* L.	50.50	40.83	43.67 (41.36)	53.86 (47.21)
T3	*Ocimum sanctum*	67.50	55.16	24.71 (29.80)	37.67 (37.86)
T4	*Zingiber officinale*	64.66	50.66	27.88 (31.87)	42.75 (40.83)
T5	*Jatropha curcas*	69.16	53.83	22.86 (28.56)	39.17 (38.74)
T6	*Brassica juncea* L.	60.16	51.16	32.90 (35.00)	42.19 (40.50)
T7	*Chrysanthemum morifolium* L.	68.33	54.16	23.78 (29.18)	38.80 (38.52)
T8	Control (Untreated)	89.66	88.50	-	-
S. E. m ±		0.53	0.61	-	-
C.D (P = 0.01)		1.54	1.78	-	-

* Mean of three replications
Figures in parentheses are Arcsine values

Reference

1. Anonymous. National Horticultural Board Database, India, 2017.
2. Bai AT, Ruth Ch, Arunodhayam GK, Tanuja P, Ramkrishna M. Survey and Identification of *Fusarium* Wilt Disease in Chilli (*Capsicum annuum* L.). Int. J Curr. Microbiol. App. Sci. 2018; 7(6):1073-1078.
3. Barari H. Biocontrol of tomato *Fusarium* wilt by Trichoderma species under *in vitro* and *in vivo* conditions. Cercetari Agronomice în Moldova. 2015; 19(165):91-98.
4. Bosland PW, Votava E. Peppers, vegetables and spices. CABI Publishing, New York, 2000, 204.
5. Dar WA, Beig MA, Ganie SA, Bhat JA, Shabir-u-Rehman, Razvi SM. *In vitro* study of fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *pini* causing root rot of Western Himalayan fir (Abies pindrow). Sci. Res. Essays. 2013; 8(30):1407-1412.
6. Dennis KL, Webster J. Antagonistic properties of species group of *Trichoderma* and hyphal interaction. Trans. British Mycol. Soc. 1971; 57:363-396.
7. Granie SA, Pant VR, Ghani MY, Ashiq Hussain Lone, Qaisar Anjum, Razvi SM. *In vitro* evaluation of plant extracts against *Alternaria brassicae* (Berk.) Sacc. Causing leaf spot of mustard and *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. I.J.P.S. 2013; 8(37):1808-1811.
8. Hegde KT, Narayanaswamy H, Kavitha Veeraghanti S, Manu TG. Efficacy of bio-agents, botanicals and fungicides against *Fusarium oxysporum* f. sp. *dianthi* causing wilt of carnation. Int. J of Chemical Studies. 2017; 5(6):139-142.
9. Ince AG, Karaca M, Onus AN. Genetic relationship within and between Capsicum species. Biochem Genet. 2010; 48:83-95.
10. Jahanshir A, Fevzi SD. The effect of fungicides on *Fusarium oxysporum* f. sp. *lycopersici* associated with *Fusarium* wilt of tomato. Journal of Plant Protection Research. 2010; 50(2):172-178.
11. Jamwal S, Jamwal A, Verma VS. Effect of biocontrol agents on wilt management and plant growth of tomato. Indian Phytopath. 2011; 64(4):381-382.
12. Malathi S. Biological control of onion basal rot caused by *Fusarium oxysporum* f. sp. cepae. Asian J Bio. Sci. 2015; 10(1):21-26.
13. Manasa BG, Somashekara YM, Shankara K, Swamy C. Efficacy of Fungicides in Control of *Fusarium oxysporum* f. sp. *dianthi*, the Cause of Wilt in Carnation. Int. J Curr. Microbiol. App. Sci. 2017; 6(10):2559-2565.
14. Mengal AS, Hussain S, Abro MA, Khalid A, Maari SA, Jatoi GH *et al*. Evaluation of different botanical extracts on the linear colony growth of the fungus *Fusarium* wilt of mango nursery and its *in-vitro* control. European J of Biotech and Biosci. 2015; 3(11):07-14.
15. Mishra A, Ratan V, Subha Trivedi, Dabbs MR, Supriya Dixit, Yatindra Srivastava. Identification and evaluation of potential *Trichoderma* strains against *Colletotrichum capsici* and *Fusarium oxysporum* f. sp. *capsici* causing anthracnose and wilt disease in Chilli. Int. J Curr. Microbiol. App. Sci. 2015; 6(9):1159-1166.
16. Nene YL, Thapliyal RN. Evaluation of fungicides In: Fungicides for Plant Disease Control (3rd ed). Oxoford, IBHPub. Co. New Delhi, 1993, 331.
17. Nisa TU, Wani AH, Bhat MY, Pala SA, Mir RA. *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. Journal of Biopesticides. 2011; 4(1):53-56.
18. Prasannath K, Karunakaran S, Mahendran S. Control of *Fusarium* wilt of chilli (*Capsicum annum* L.) by crude plant extracts. East. Univ. S. L. Proceedings of the 10th Annual Research Session, 2011, 291-294.
19. Rajput AQ, Arain MH, Pathan MA, Jiskani MM, Lodhi AM. Efficacy of different fungicides against *Fusarium* wilt of cotton caused by *Fusarium oxysporum* f. sp. vasinfectum. Pak. J Bot. 2006; 38(3):875-880.
20. Ramaiah KA, Garampalli RK. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*. Asian J of Pl. Sci. and Res. 2016; 5(1):22-27.
21. Rudresh DL, Shivaprakash MK, Prasad RD. Potential of *Trichoderma* spp. as biocontrol agents of pathogens involved in wilt complex of chickpea (*Cicer arietinum* L.). J Biol. Control. 2005; 19(2):157-166.
22. Singh JK, Kumar Manoj, Kumar Sanjeev, Kumar Anil, Mehta Naresh. Effect of chemicals and antagonists on growth and sporulation of *Fusarium oxysporum* causing wilt of chilli (*Capsicum annuum* L.). I.J.C.S. 2017; 5(5):2175-2181.
23. Sinha AK. The use of a benomyl fungicide against *Fusarium* wilt of pigeon pea. Journal of Plant Diseases and Protection. 1974; 81(10):571-574.
24. Subhani MN, Ali A, Sahi ST, Iqbal J. Evaluation of various fungicides for the control of gram wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. African J of Agril. Res. 2011; 6(19):4555-4559.
25. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors Nature. 1927; 59:850.
26. Wang D, Bosland P. The genus of Capsicum. Hort. Science. 2006; 41:1169-1187.
27. Wani AH, Bhat yaqub M, Pala SA, Mir RA. *In vitro* inhibitory effect of fungicide and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. Journal of biopesticides. 2011; 4(1):53-56.
28. Yadav SL, Ahir RR, Rathore BS, Yadav SM. Efficacy of different fungicides and organic amendments against basal rot of onian caused by *Fusarium oxysporum in vitro.* Plant Pathology Journal. 2014; 13(1):56-58.