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Evaluation of effective chemical molecules, phyto-extracts and bio-agents against *Fusarium oxysporum* f. sp. *Radicis cucumerinum* causing root and stem rot of cucumber in Rajasthan

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Abstract
Root and stem rot of cucumber is one of the most destructive disease in the worldwide. For development of effective control measures of this disease, six chemicals that is Hexaconazole, Mancozeb, Copperoyxchloride, Carbendazim, SAAF (Carbendazim 12%+ Mancozeb 63%) and Azoxystrobin in three concentrations (250,500 & 750 ppm), six phyto-extracts (*Tagetes erecta*, *Occimum sanctum*, *Aloevera* sp., *Allium sativum*, *Eucalyptous globules* and *Azadiracta indica* at three different concentrations (10%, 20%& 30%) were evaluated by poison food Technique and three bio-control agent (*Trichoderma viride*, *T. harzianum* and *Bacillius subtilis*) were also tested against mycelial growth inhibition of *Fusarium oxysporum* f. sp. *radices cucumerinum* by dual culture techniques respectively. Observations of the inhibitory effects on mycelial growth of the pathogen were observed of these chemicals, phyto-extracts and bio-agents. Among chemicals, Carbendazim completely inhibited (100%) or no mycelial growth of the pathogen was observedat 500 and 750 ppm concentration and at 250 ppm concentration it was also found best by inhibited 72.22 percent with 25.0 mm mycelial growth followed by SAAF (Carbendazim 12%+ Mancozeb 63%).It gave 61.11, 88.89 and 94.44 percent mycelia growth inhibition with 5.0 mm, 10.0 mm and 35.0 mm mycelia growth at 250,500 and 750 ppm concentrations respectively. Minimum mycelial growth inhibition (15.28, 42.22 and 51.67 percent) or maximum mycelial growth (76.25 mm, 52.00 mm and 43.50 mm) was recorded with Mancozeb at all three concentrations respectively. Out of six phyto-extracts, *A. indica* leaf extract was found more effective than others by inhibited 30.83, 51.39 and 69.17 percent growth inhibition with 62.25 mm, 43.75 mm and 27.75 mm mycelial growth at 10, 20 and 30 percent concentrations respectively. Among bio-control agents, *Trichoderma viride* was found most effective by inhibited maximum growth inhibition (65.83% inhibition) with minimum (30.75 mm) mycelial growth of the pathogen. Thus, fungicide, Carbendazim, phyto-extract, *A. indica* and bio-control agent *T. viride* can be used for the management of root and stem rot of cucumber in field condition.

Keywords: *Fusarium oxysporum* f. sp. *radicis cucumerinum* (FORC), fungicides, phyto-extracts, bio-agents, growth inhibition, poison food technique

Introduction
Fusarium oxysporum is the most important and diverse phytopathogenic fungi, infecting nearly 150 plant species (Fourie *et al*., 2011). *Fusarium* species exhibit a high level of host specificity, and they are classified into more than 120 formae speciales and races based on plant species and cultivars infection (Armstrong and Armstrong,1981). It is a soil-dwelling and omni present phytopathogenic fungus that causes a variety of diseases such as root and stem rot, vascular wilt, and damping off (Mc Govern, 2015). Cucumber root and stem rot is a particularly serious disease. *Fusarium oxysporum f. sp. radices-cucumerinum* is the causative agent. It was the most damaging disease of glasshouse cucumber crops in Canada in 1994, France in 1998, China in 1999, and Spain in 2000, causing significant yield losses (Punja & Parker, 2000). It also caused significant losses (85.72 and 14.29 percent) in cucumber growing areas of Kathua, Jammu, Rajori, Udhampur, Doda, and Poonch districts of Jammu region in 2007 and 2008. (Pagoch *et al*., 2012). It is also causing significant cucumber yield losses in polyhouses in Rajasthan. In response to the significant losses caused by FORC in cucumber, fungicides were tested against pathogens *in vitro* and found to be effective. Phyto extracts and bio-control agents have also been used to control soil-borne plant pathogens in an environmentally friendly manner. Because the pathogen cannot be easily controlled by a single

control measure, multitasking may be tested *in vitro* against the FORC for disease management in the field. In this study, the efficacy of newer fungicides, phyto extracts, and bio-agents against the pathogen was tested *in vitro*.

2. Materials and Methods

2.1 Efficacy of different chemical molecules against *Fusarium oxysporum* f.sp. *radicis cucumerinum In vitro*

Cucumber root and stem disease is a very destructive disease in polyhouses and fields in Rajasthan. At the moment, no appropriate control measures for disease management are available. In order to determine the importance of the pathogen on the crop, an experiment was carried out in 2017 at the Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan. A pathogen was isolated on PDA medium for the experiment, and the pathogen was identified in the Department of Plant Pathology after further purification using the hyphal tip method. Furthermore, the isolated culture passed the pathogenicity test using the soil inoculation technique. The pathogen was re-isolated and cultures were preserved for future research. Six fungicides, including Hexaconazole (5 percent SC), Mancozeb (75 percent WP), Copper oxychloride (50 percent WP), Carbendazim (50 percent WP), SAAF (Carbendazim 12 percent + Mancozeb 63 percent), and Azoxystrobin (23 percent SC), were tested *in vitro* against mycelial growth of F. *oxysporum* f. sp. *radicis cucumerinum* using (250, 500, 750 ppm). Calculated amounts of fungicides were weighed/measured and thoroughly mixed in the molten PDA medium in a conical flask before pouring into Petri plates to achieve the desired concentration of each fungicide separately. In each 90 mm sterilised petri plate, 20 ml of fungicide-adjusted medium was poured and allowed to solidify. Each Petri plate was inoculated with one piece of 5 mm diameter 7-day-old culture in the centre of the plate and incubated at 282oC in a BOD incubator. For each treatment, four replications were kept in order to compare them. Colony diameter was measured in each case by measuring the diameter of the colony when the control plates were full of fungal growth. The percent inhibition of mycelial growth over control was calculated using Vincent's Formula (1927) as:

$$I = \frac{C - T}{C} \times 100$$

Where

I = Percent inhibition

C = Colony diameter in control; T = Colony diameter in treatment

2.2 Efficacy of phyto-extracts against *Fusarium oxysporum* f.sp. *radicis cucumerinum In vitro:-*

Six phytoextracts, including Eucalyptus globules (Eucalyptus), Tagetus erectus (marigold), Aloe-vera spp. (Aloe), Azadiracta indica (neem), Ocimum sanctum (tulsi), and Allium sativum (garlic), were tested *in vitro* for antifungal efficacy against F. oxysporum f.s Fresh samples were washed in tap water to remove dust particles and saprophytes attached to them before being washed three times with sterilised distilled water. They were crushed in a sterilised pestle and mortar with just enough sterile distilled water to crush the sample easily. Filtering the extract through two layers of Whatman filter paper was used to collect it. Finally, the filtrate obtained from the leaves was used as a stock solution for aqueous crude extracts. The poisoned food technique, as suggested by Nene and Thapliyal, was used to investigate the antifungal mechanism of phyto-extracts (1993). Separately, 10, 20, and 30 ml of each extract's stock solution were taken and mixed in 100 ml of sterilised molten potato dextrose agar medium to obtain 10, 20, and 30% concentrations of each extract. The medium was thoroughly shaken to ensure that the Phytoextracts were evenly mixed. Each 90 mm sterilised Petri plate received 20 ml medium and was kept for solidification. After solidification, a 5 mm diameter mycelial bit was aseptically removed from the periphery of a 7-day-old culture of *Fusarium oxysporum f. sp. radicis cucumerinum* was incubated at 282ºC in a BOD incubator until the colony reached the control plate's periphery. For each treatment, four replications were kept, as well as control plates. In each case, the mean redial colony diameter (mm) was measured. Phytoextract efficacies were expressed as a percentage inhibition of mycelial growth using the Vincent (1927) Formula.

2.3 Efficacy of bio-agents against *Fusarium oxysporum* f.sp. *radicis cucumerinum In vitro*

In vitro efficacy of three bioagents *viz*., *Trichoderma harzianum, T. viride, Bacillus subtilis* were evaluated their antagonistic potential against mycelial growth of *F. oxysporum f.sp. radicis cucumerinum* by dual culture technique (Dennis and Webster, 1971). The methodology used in described below.

2.3.1 Dual culture Technique

The dual culture method was used to assess antagonist inhibition of pathogen radial growth. After pouring approximately 20 ml Luke warm PDA into each of 90 mm diameter sterilised Petri plates, 5 mm bits of pathogen and antagonist, Trichoderma species, were placed on the PDA surface at equal distances from each other. In the case of bacterial antagonist, pathogen mycelial discs were inoculated at the Petri plate's periphery, and bacterial antagonist was streaked in the centre of the same plate. The control plates were inoculated with one piece of pathogen in the centre. For each treatment, four replications were kept. The plates were incubated in a BOD incubator at 282oC. The pathogen's growth was monitored on a regular basis. After 7 days of inoculation, measurements of the pathogen's radial growth were taken.

3. Results and Discussion

The findings of the present study as well as relevant discussion have been presented under the following heads:

3.1 Effect of fungicides against mycelial growth of *F. oxysporum* f.sp. *radicis cucumerinum*

Five systemic and contact fungicides such as Hexaconazole, Carbendazim, Copper oxychloride, SAAF, Azoxystrobin and Mancozeb at three different concentrations (250, 500, 750 ppm) were tested *in vitro*. The results are reveals that all the fungicides were found effective to inhibition of mycelial growth of the pathogen. Among them,Carbendazim was found best by gave 100 percent mycelial growth inhibition of the pathogen at both 500 and 750 ppm concentration and at 250 ppm concentration it was also found best by inhibited

72.22 percent mycelial growth inhibition with 25 mm mycelial growth followed by SAAF growth inhibition with 5.0 mm,10 mm and 35 mm mycelial growth at 250,500 and 750 ppm concentration respectively. Minimum mycelial growth inhibition (15.28, 42.22 and 51.67 percent) or maximum mycelial growth (76.25 mm, 52.00 mm and 43.50 mm) was recorded with Mancozeb at same concentrations. Our findings are in accordance to earlier workers (Kumari *et al.*, 2014). They evaluated fungicides (*in vitro*) and found that Carbendazim was most effective at its all three concentrations (0.01%, 0.02% and 0.03%) followed by Azoxystrobin and SAAF against *F. oxysporum* f. sp. *cubense*. Harender Raj *et al.,* (2005) found that Carbendazim and SAAF were more effective against *Fusarium oxysporum* f. sp. *gladioli* under *in vitro* conditions. Under *in vitro* conditions, Singh and Jha (2003) discovered that carbendazim was the most effective fungicide against *Fusarium oxysporum* f. sp. ciceris. Many other researchers discovered similar observations on different Fusarium oxysporum formae specials (Raju *et al*., 2008 and Srivastava *et al.*, 2011), because carbendazim is systemic in nature and inhibits the pathogen's synthesis of B-tubulin, whereas mancozeb is a contact fungicide that inhibits the formation of germ tubes. SAAF is a combination of Mancozeb (63%) and Carbendazim (12%), and it inhibited both mechanisms. Thus, FORC, the causal pathogen for cucumber root and stem rot, can be effectively controlled in the field by using the fungicide Carbendazim.

3.2 Effect of phytoextracts against mycelial growth of *F. oxysporum f. sp. Radicis-cucumerinum* (*in vitro*)

The efficacy results of six phytoextracts are revealed that none of the phytoextracts could completely inhibited the mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum* even at 30 per cent concentration. Out of them, *Azadiracta indica* leaf extract was found significantly effective in inhibiting themycelial growth of pathogen suchas 30.83, 51.39 and 69.17 percent growth inhibition at 10, 20 and 30 percent concentrations, respectively. *Allium sativum*, *Eucalyptus globulus*, *Tagetus erecta*, *Occimum sanctum* and *Aloevera* spp. extracts were caused 22.22, 8.61, 9.44, 9.72, 16.67 percent inhibition of mycelial growth of *FORC* at 10 percent concentration, respectively. At 20 per cent concentration, *A.sativum*, *T. erecta*, *O.sanctum*, *Aloevera* spp. and *E.globulus* caused 33.06, 11.11, 17.50, 22.22 and 25.00 percent inhibition of mycelial growth of FORC, respectively. At 30 per cent concentration, *A.sativum*, *E.globulus*, *T.erecta*. *Aloevera* spp. and *O.sanctum* caused 41.11, 34.4, 27.78, 30.56 and 32.78 percent inhibition of mycelial growth of *FORC*, respectively.The present results are similar to earlier results suggested by many other workers (Ramaiah *et al.,* 2015). They have tested some phyto- extracts against mycelial growth of *F.oxysporum* f.sp. *lycopersici* and resulted that growth of pathogen were obtained significantly with *Solanum indicum* (78.33%), *Oxalis latifolia* (70.33%), *Azadirachta indica* (75.00%). Patil in 2003, evaluated different botanicals against the *F.oxysporum* causing wilt of patchouli in Lab, and found that 76.72 per cent growth inhibition were recorded with garlic extract and tulsi leaf extract at 10% concentration. Likewise, Thakare in 2003, evaluated various kind of botanicals *in vitro* against *Fusarium oxysporum*, and showed that 100 per cent growth inhibition were recorded in *Allium sativum* followed by 100 percent with *Azadirachta indica at* 10 per cent concentration. Similarly, Riaz *et al*., in 2008 in Pakistan, evaluated some leaf extracts like *Allium cepa*, *Tagetes erectus* for their antifungal activity at different concentrations *viz.* 2, 4, 6 and 8% w/v against *Fusarium oxysporum* f. sp. *Gladioli* caused corm rot disease of gladiolus and found that extract of *Tagetes erectus* were found highly effective where all the employed extract concentrations significantly reduced fungal biomass by 54-79%. Likewise, Tahon *et al.,* in 2014, studied the efficacy of medicinal plant extract *i.e, Eucalyptus globules, Lantana camera, Nerium oleander* and *Ocimum basilicum* against *F.oxysporum* f.sp. *lycopersici* in Egypt *in vitro,* and found that extract of *O. basilicum and E. globulus* were most effective to inhibiting the growth of the pathogen.

3.3 Effect of bio agents against mycelial growth of *F. oxysporum* f. sp. *Radicis-cucumerinum* (*in vitro*)

Biocontrol agents manage the soil borne plant pathogen in an eco-friendly manner as compared to hazardous fungicides (Saleem *et al*., 2004). Efficacy of bio control agents like *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* were evaluated *in vitro* by dual culture techniques as described in material and method. The results are revealed that *Trichoderma spp*. were potential antagonists against *Fusarium oxysporum* f.sp. *radices cucumerinum.* Efficacy of *T.viride* was found comparatively more effective than *T. harzianum* and *Bacillus subtilis*. Maximum and significant reduction in mycelial growth of *FORC* was observed with 65.83 per cent by *T. viride*, while *T. harzianum* caused 55.00 per cent growth inhibition followed by *Bacillus subtilis* with 51.11 per cent growth inhibition. Anu Rajan *et al.,* (2013) reported that these biocontrol agents use either antibiosis or mycoparasitism against fungal pathogen. *Trichoderma species* use mycoparasitism and *Baccilus subtilis* use antibiosis mechanism against the pathogen. Thus these results obtained are in accordance to the earlier studies carried out by the several workers Javid *et al.,* in 2016, evaluated the effectiveness of three isolates i.e. T-22, T-9 and T-6 of *Trichoderma harzianum* against isolate *Fusarium oxysporum* f. sp. *radicis cucumerinum* and found that isolate T-22 of *T. harzianum* had the greatest effective on the pathogen. Likewise, Barari in 2016, tested bio-efficacy of the isolates of *Trichoderma* species against *F. oxysporum* f. sp. *lycopersici* under *in vitro* and *in vivo* condition. Under *in vitro* condition, isolate N-8 of *T. harzianum* gave 68.22 percent growth inhibition of mycelium of the pathogen.

Table 1: Effect of different fungicides on the mycelial growth of *F. oxysporum f.sp. radicis cucumerinum* at various concentrations by *in vitro* technique

Treatments (Fungicides)	Colony growth (mm)* at different concentrations (ppm)			Per cent growth inhibition at different concentrations (ppm)		
	250	500	750	250	500	750
Hexaconazole(5%SC)	39.00	29.25	21.50	56.67 (48.84)	67.50 (55.25)	76.11 (60.76)
Mancozeb(75%WP)	76.25	52.00	43.50	15.28 (22.93)	42.22 (40.41)	51.67 (45.96)
Copper oxychloride (50%WP)	71.50	70.00	61.75	20.56 (26.95)	22.22 (28.13)	31.39 (34.04)

Carbendazim(50%WP)	25.00	0.00	0.00	72.22 (58.19)	100.00 (90.00)	100.00 (90.00)
SAAF (Carbendazim 12% + Mancozeb 63%)	35.00	10.00	5.00	61.11 (51.44)	88.89 (70.53)	94.44 (76.37)
Azoxystrobin (23%SC)	61.75	40.00	0.00	31.39 (34.06)	55.56 (48.11)	100.00 (90.00)
Control	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEM±	1.21	2.26	2.24	1.55	2.52	0.84
CD (P=0.05)	3.58	6.67	0.76	3.97	7.41	2.48

*Mean of four replications; Figures in parentheses are arcsine√ per cent angular transformed values.

Table 2: Effect of different phytoextracts on the mycelial growth of *F. oxysporum* f. sp. *radicis cucumerinum* by *in vitro* technique

Treatments (Botanicals)	Common name	Part used	Radial growth of Pathogen (mm)*at different conc. (%)			Growth Inhibition Per cent (%) at different conc. (%)		
			10	20	30	10	20	30
Eucalyptus globulus	Eucalyptus	Leaves	82.25	67.50	59.00	8.61 (17.04)	25.00 (29.97)	34.44 (35.93)
Tagetus erecta	Marigold	Leaves	81.50	80.00	65.00	9.44 (17.88)	11.11 (19.47)	27.78 (31.79)
Aloe vera spp.	Aloe	Leaves	75.00	70.00	62.50	16.67 (24.09)	22.22 (28.13)	30.56 (33.54)
Azadiracta indica	Neem	Leaves	62.25	43.75	27.75	30.83 (33.71)	51.39 (45.80)	69.17 (56.28)
Ocimum sanctum	Tulsi	Leaves	81.25	74.25	60.50	9.72 (18.13)	17.50 (24.71)	32.78 (34.92)
Allium sativa	Garlic	Cloves	70.00	60.25	53.00	22.22 (28.13)	33.06 (35.09)	41.11 (39.88)
Control	-	-	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em ±			0.67	0.88	1.03	0.51	0.56	0.63
CD (P=0.05)			1.99	2.60	3.04	1.52	1.66	1.87

*Mean of four replications; Figures in parentheses are arcsine √ per cent angular transformed values

Table 3: *In vitro* efficacy of bio-agent against mycelial growth of *F. oxysporum* f. sp. *Radicis cucumerinum* by dual culture technique

Treatments (Bio agents)	Mycelial growth (mm)*	Mycelial growth Inhibition (%)
Trichoderma. viride	30.75	65.83
Trichoderma harzianum	40.50	55.00
Bacillus subtilus	44.00	51.11
Control	90.00	0.00
S.Em±	0.67	0.43
CD (P=0.05)	2.07	1.33

*Mean of four replications

4. Conclusion

Cucumber root and stem rot is a serious disease in southern Rajasthan caused by *Fusarium oxysporum* f. sp. radicis cucumerinum. It is a soil-borne pathogen that cannot be easily managed by single measures, so multitask has been tested *in vitro* against the pathogen. Among the fungicides tested, carbendazim was found to be the most effective at all three concentrations (250,500 and 750 ppm) *Azadirecta indica* leaf extract outperformed the other phytoextracts in terms of maximum inhibition of pathogen mycelial growth. Trichoderma viride outperformed the other two by inhibiting pathogen mycelial growth the most.

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