RESEARCH ARTICLE

IN-VITRO EVALUATION OF VARIOUS FUNGICIDES, PLANT EXTRACTS AND BIO CONTROL AGENTS AGAINST ROOT ROT OF AJWAIN

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Abstract: An incubation study was conducted at Department of Plant pathology, Rajasthan College of Agriculture, Udaipur to evaluation of different fungicides, plant extracts and bio-agents against ajwain (*Trachyspermum ammi* L.) root rot caused by *Rhizoctonia solani* and results revealed that treatment comprising of fungicide Bavistin, Plant extracts Neem oil and bio control agent *Trichoderma viride* at 7 days of incubation at 28±1^{0}C was found significantly superior over control and gave maximum percent growth inhibition of *R. solani.*

Keywords: *Ajwain, R. solani fungicide, Plant extracts, Bio-agents*

INTRODUCTION

Ajwain (*Trachyspermum ammi* L.) also known as Bishop's weed and Carom, is one of the most important seed spice crop it belongs to family *Apiaceae* and is believed to have originated from India and Egypt. In India it is widely distributed and its production is concentrated mainly in Rajasthan followed by Gujarat, Madhya Pradesh, Bihar, Utter Pradesh, Punjab, Tamil Nadu, Andhra Pradesh and West Bengal, respectively. Since ancient time the state of Rajasthan and Gujarat has emerged as "Seed spices bowl". Whose dried fruit of seeds are used as spices.

In Rajasthan, it is cultivated in the districts of Chittorgarh, Udaipur, Jhalawar, Baran, Rajsamand, Bhilwara and Kota covering an area of 11658 hectares with the production and productivity is 4672 tonnes per annum and 401 kg/ha, respectively (Anonymous, 2015-16). In India, Rajasthan contributes 73 per cent of total production of ajwain. The healthy seeds are having economic values in the market but the large number of diseases affects the ajwain crop in the field and a huge damage is caused by the pathogens carried to the harvested seeds during transit and storage. Root rot (*Rhizoctonia solani* Kuhn) and Powdery mildew (*Erysiphe polygoni* D.C.) are two major diseases of ajwain (Dhanbir, 2000 and Meena *et al.* 2009). Among these, the root rot disease is most and destructive common disease of ajwain, caused by *R. solani*, resulted losses in yield as well as quality of the crop. Madhusudhan *et al*. (2010) tested six fungicides viz., Carbendazim (50%WP), Propiconazole (25%EC), and Hexaconazole (5%EC) by poisoned food technique for their efficacy on *R. solani* compatibility different concentrations viz., 50, 100,250, 500 and 1000 ppm. *T. viride* and *T. harzianum* were reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey 2002, 2003; Poddar *et al*. 2004).

MATERIAL AND METHOD

***In vitro* efficacy of fungicides (Poison food technique):** The relative efficacy of different systemic and non-systemic fungicides evaluated against *R. solani* by using poisoned food technique (Schmitzs, 1930). Five fungicides *viz.*, Bavistin 50% WP [Carbendazim, Methyl-2-benzimidazole carbamate (MBC)] BASF India Ltd., Mumbai, Hexaconazole 5% EC [2-(2,4-dichlorophenyl) -1-(1H-1,2,4-triazol-1-yl) hexan-2-ol], Crop Life Science Ltd., Gujarat, Tebuconazole 25.9 w/w [1-(4-chlorophenyl) -4,4-dimethyl-3-(1,2,4-triazol-1-methyl) peptan-3-ol (Follicular 250 EC)] Bayer Crop Science, India Ltd., Mumbai, Dithane M-45 75% WP [mancozeb, Manganese ethylene bis-dithiocarbamate+zinc ions 2%] Indofil Chemicals Ltd., Mumbai and Saaf [(Carbendazim+Mancozeb) 75 WP (Methyl-2-benzimidazole carbamate (MBC +Manganese ethylene bis-dithiocarbamate + zinc ions 2%] United Phosphorus Limited, Mumbai were tested at two concentrations i.e. 0.1 and 0.2 per cent against *R. solani.* Desired quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized Petri plates and then allowed to solidify. For each treatment, three replications were taken and each plate was inoculated with 3 mm disc of *R. solani* and incubated at 28±1°C. The linear growth was measured after seven days and control treatment was also maintained (without fungicide). Per cent inhibition of radial growth of mycelium was calculated using formula as given below.

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

Where,
I= Per cent inhibition
C= Colony diameter in control

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T= Colony diameter in treatment

***In vitro* evaluations of plant extracts (Poison food technique)**: Efficacy of Neem oil, Karanj oil, Garlic oil and Neem formulation were evaluated at 1 and 2 per cent concentration against *R. solani* by using poison food technique. One and two ml of individual plant extracts was added to 100 ml sterilized PDA in the conical flasks so as to obtain the final concentration in the medium. The plant extracts amended medium was poured aseptically in 90 mm sterilized Petri plates @ 20 ml per plate and allowed to solidify. Three mm bits of *R. solani* removed from the periphery of seven days old cultures and aseptically inoculated at the centre of each plate. For comparison, plates having PDA without plant extracts were kept as control. For each treatment, three replications were maintained. The plates were incubated at 28±1°C for seven days and then colony diameters were measured along with control plates. The per cent inhibition zone was calculated using formula given as above.

***In vitro* efficacy of fungal biocontrol agents (dual culture technique**): The efficacy of biocontrol agents *i.e. Trichoderma viride and T. harzianum* were tested by using dual culture plate method on PDA medium (Johnson *et al*., 1959). The antagonistic effect of *T. viride, T. harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested against *R. Solani.* Three mm diameter mycelium bit of seven days old culture of *Rhizoctonia* was inoculated in center of 1st half of Petri plate and *Trichoderma* spp. in center of 2nd half of Petri plate containing sterilized PDA medium. For each treatment three replications were taken. Inoculated plates were incubated at 28±1°C temperature in incubator. Observations on colony diameter were recorded up to the complete coverage of control plates, which was inoculated with only pathogen. The linear growth after seven days of incubation was recorded and per cent inhibition zone was calculated.

***In vitro* efficacy of bacterial biocontrol agents (dual culture technique):** Dual culture method was used for assessing inhibition of radial growth of the pathogen by bacteria (biocontrol agents) inoculated on King's B agar medium in sterilized Petri dishes. A loopful of bacterial suspension from the 24 hours old cultures was streaked on two sides of each plate and then placed 3 mm disc of *R. solani* in the centre. Control plates were inoculated by pathogens individually. Three replications were maintained for each treatment and were inoculated at 28±1°C. The measurement of radial growth of the test pathogens was recorded after five days and compared with respective controls.

RESULT AND DISCUSSION

***In vitro* evaluation of fungicides (poisoned food technique):** Five fungicides Bavistin, Hexaconazole, Tebuconazole, Dithane M-45 and SAAF were evaluated at two concentrations *viz.*, 1000 and 2000 ppm with poisoned food technique against *R. solani*. All the test fungicides significantly inhibited the mycelial growth of *R. solani* at both concentrations.

The effect was more on *R. solani* where two fungicides Bavistin and Tebuconazole completely inhibited the growth both at 1000 and 2000 ppm concentrations. Further, the fungicides varied in their efficacy on the particular pathogen. Here Bavistin and Tebuconazole at 1000 and 2000 ppm exhibited 100 per cent growth inhibition of *R. solani*. This was followed by Hexaconazole which exhibited 98.1 per cent and 98.9 per cent growth inhibition at 1000 and 2000 ppm, respectively. The other test fungicides Dithane M-45 and SAAF were found to show very weak efficacy to control of *R. solani* at both 1000 and 2000 ppm (Table 1). The per cent growth inhibition for SAAF was 55.5 per cent each at 1000 ppm and 64.4 per cent growth inhibition at 2000 ppm and Dithane M-45 the per cent growth inhibition was 44.4 per cent each at 1000 ppm and 53.3 per cent as well as 2000 ppm as compared to control (Table 1).

Table 1: *In vitro* evaluation of different fungicides (systemic and non-systemic) against of *R.solani* at 1000 and 2000 ppm concentrations after 7 days of incubation at 28 ± 1°C (Poison food technique)

S.No.	Fungicides	Colony diameter (mm)*			Per cent growth inhibition*		
		1000 ppm	2000 ppm	Mean	1000 ppm	2000 ppm	Mean
1.	Bavistin	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
2.	Hexaconzole	1.7	1.0	1.35	98.1 (82.10)	98.9 (83.9)	98.5 (83.0)
3.	Tebuconazole	0.0	0.0	0.0	100.0 (90.00)	100.0 (90.0)	100.0 (90.0)
4.	Dithane M-45	50.0	42.0	46.0	44.4 (41.81)	53.3 (46.9)	48.9 (44.3)
5.	SAAF	40.0	32.0	36.0	55.5 (48.19)	64.4 (53.4)	59.9 (50.7)
6.	Control	90.0	90.0	90.0	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
		SEm ±	CD at 5 %		SEm±	CD at 5%	
Fungicides		0.366	1.076		0.237	0.695	

concentration		0.211	0.621		0.136	0.401	
F X C		0.518	1.52		0.335	0.983	

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

Table 2. *In vitro* evaluation of various plant extracts against of *R.solani* at 1% and 2% concentration after 7 days of incubation at 28 ± 1°C (Poison food technique)

S.No.	Plant extracts	Colony diameter (mm)*			Per cent growth inhibition*		
		1%	2%	Mean	1%	2%	Mean
1.	Neem oil	68.0	65.0	66.5	24.4 (29.5)	27.8 (31.7)	26.1 (30.6)
2.	Karanj oil	87.0	85.0	86.0	3.33 (10.2)	5.60 (13.5)	4.46 (11.9)
3.	Garlic oil	82.0	79.0	80.5	8.90 (17.0)	12.2 (20.2)	10.5 (18.7)
4.	Neem formulation	75.0	72.0	73.5	16.7 (24.0)	22.2 (26.5)	19.4 (25.3)
5.	Control	90.0	90.0	90.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm ±		1.956	1.879	1.917	1.970	1.654	1.787
CD at 5 %		6.379	6.127	6.253	6.425	5.396	5.829

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

Table 3. *In vitro* evaluation of different biocontrol agents (fungal and bacterial) against on growth of *R.solani* after 7 days of incubation at 28 ± 1°C (Dual culture technique)

S. No.	Biocontrol agents	Colony diameter of *R. solani* (mm)*	Per cent growth inhibition* of *R. solani*
1.	*Trichoderma viride*	16.2	82.0 (64.9)
2.	*T. harzianum*	35.0	61.1 (51.4)
3.	*Bacillus subtilis*	20.0	77.8 (61.8)
4.	*Pseudomonas fluorescence*	42.0	53.3 (46.9)
5.	Control	90.0	0.00 (0.00)
SEm ±		0.571	0.422
CD at 5%		1.863	1.376

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

On the other hand two fungicides bavistin and tebuconazole could inhibit the growth (100%) of *R. solani* both at 500 and 1000 ppm concentrations. These were followed by hexaconazole where per cent growth inhibition was 98.1 per cent and 99.9 per cent at 1000 and 2000 ppm, respectively. SAAF fungicide was found at par in inhibiting of *R. solani* at 1000 ppm, where per cent growth inhibition was 55.5 per cent and 2000 ppm 64.4 per cent, respectively. Dithane M-45 was found to be the weakest fungicide both at 1000 (44.4 per cent) and 2000 ppm (53.3 per cent) as compared to the control (Table 1). Various fungicides were reported effective against root rot pathogen in different crops, but larger of such information on these diseases in ajwain is inadequate. Similar observations were made by Nikam *et al.* (2007), Mukhtar (2007), Christian *et al.* (2007), Mddhusudhan *et al.* (2010), Subhani *et al.* (2011), Andrabi *et al.* (2011), Tetarwal *et al*. (2013) and Padamini (2014).

***In vitro* evaluation of botanicals (poisoned food technique):** Four phyto-extracts viz., Neem oil, Karanj oil, Garlic oil and Neem formulation were evaluated at 1.0 and 2.0 per cent concentration with poisoned food technique against *R. solani.* Neem oil best effective caused 24.4 per cent inhibition at 1.0% concentration and 27.8 per cent inhibition at 2.0% growth of *R. solani* followed by Neem formulation caused 16.7 per cent growth inhibition at 1.0% concentration and 22.2 per cent inhibition at 2.0% concentration while, Karanj oil was less effective, and caused 3.33 per cent inhibition at 1.0% concentration and 5.60 per cent inhibition at 2.0% concentration of linear growth of *R. solani*. In general, both the botanical were not much effective in this study and therefore these were not taken forward for further pot culture experiments (Table 2). Fungicides can effectively control the disease but the residual problems are increasing and this is causing health hazards in human beings and animals. Similar results were obtained by Nwachukewe and

Umechuruba (2001), Singh and Chand (2004), Sitara *et al*. (2008), Tetarwal *et al.* (2013) and Padamini (2014).

***In vitro* evaluation of biocontrol agents (Dual culture technique)**: Efficacy of biocontrol agents the local isolates of *T. viride*, *T. harzianum* and bacteria *Bacillus subtilis*, *Pseudomonas fluorescence* as studied *in vitro* as described in Materials and Methods, using dual culture technique. Data revealed that *T. viride* and bacteria (*Bacillus* spp.) were potential antagonists of *R. solani*.

Maximum and significant high per cent inhibition of growth (82.0%) by *T. viride* was observed in dual culture method for *R. solani*, followed by *Bacillus subtilis* (77.8%), respectively. Efficacy of *T. viride* was found comparatively lower than bacteria *Bacillus subtilis*. The minimum per cent inhibition of growth of *Pseudomonas fluorescence* (53.3%) followed by *T. harzianum* (61.1%), respectively (Table 3). This result was similar with the results obtained by Meki *et al*. (2011) and Subhani *et al*. (2013).

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