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Evaluation of botanicals and bioagents against chickpea wilt complex pathogens

■ P.S. MORE* AND R.L. PARATE

Department of Plant Pathology, College of Agriculture (Dr.P.D.K.V.) NAGPUR (M.S.) INDIA

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*Corresponding author:

ABSTRACT

Botanicals and bioagents were evaluated by following poison food technique and dual culture technique against three pathogens. Data indicate significant difference on mean colony diameter at all the internal over uninoculated control. Fungicides carbendazim treatment recorded 100 per cent growth inhibition against *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*. Among botanicals *Azadirachta indica* at 20 per cent conc. Inhibits 84.44 per cent growth of *Fusarium oxysporum* f. sp. *ciceri*, 85.22 per cent growth of *Rhizoctonia bataticola* and 85.55 per cent growth of *Sclerotium rolfsii*. *Trichoderma viride* found most effective for restricting mycelial growth of *Fusarium oxysporum* f. sp *ciceri*. *Pseudomonas fluorescens* found most effective for inhibiting mycelial growth of *Rhizoctonia bataticola*. In case of *Sclerotium rolfsii*, *Trichoderma harzianum* was found most effective.

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INTRODUCTION

Chickpea [*Cicer arietinum* (L.)] is leguminous pulse crop which belongs to leguminaceae family. It was first domesticated in Middle East and is widely cultivated in world. It is third most important legume crop in world after beans and peas. It is first important pulse crop in India being grown on largest area in *Rabi* season.

The major fungal diseases are wilt complex caused due to *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Colletotrichum blight*, *Alternaria* blight, *Aschochyta blight*, Botrytis, gray mold and stunt etc.

Chickpea wilt, root rot and collar rot caused by *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia bataticola*, *Sclerotium rolfsii* (Nene, 1985 and Tewari and Mukhopadhyay, 2003) is the most serious and challenging diseases, which causes severe yield loss *i.e.* 60-70 per cent under favourable conditions. It was therefore the present study was conducted to study the soil borne fungi of chickpea.

MATERIAL AND METHODS

Isolation of pathogen:

Wilt complex pathogen *Fusarium oxysporium* f.sp.

ciceri, Rhizoctonia bataticola, sclerotium rolfsii were isolated by plating the infected cut piece of roots and stem of chickpea plant on potato dextrose Agar (PDA) medium after surface sterilization with 0.1 per cent, mercuric chloride ($HgCl_2$) solution. After seven days pathogens were isolated and transferred on PDA for growth. Pathogenicity test were confirmed by inoculating the respective pathogens mass multiplied on sand sorghum medium in soil.

In vitro evaluation of botanicals and bioagents against *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola, sclerotium rolfsii.*

Botanicals / plant extract :

To study effect of plant extract on growth of *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola, sclerotium rolfsii* Plant extract of *Lantana camera*, *Ocimum sanctum, Eucalyptus* spp, *Vinca rosea* and *Azadirachta indica* were prepared at different concentration as 5 per cent, 10 per cent, 20 per cent separately as follows.

Plant extract preparation :

The procedure for preparation of aqueous plant extract (w/v basis) was as followed by (Sarvamangala *et al.*, 1993) required quantity of selected plant leaves were placed in mercuric chloride ($HgCl_2$ 0.1 %) solution for 2 min and thoroughly washed with sterilized distilled water by three washing, equal weight of plant leaves and volume of water was grinded in mortar and pestle, then filtered through double layered muslin cloth to remove fibrous and suspended material, thus, filtrate prepared were treated as 100 per cent concentration from this extracts required concentration of botanicals were made by adding sterilized water.

The effect of six botanicals plant extract, and three bioagents and one fungicides were used to evaluate against *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola, sclerotium rolfsii*. These botanicals and one fungicides were evaluated in *in vitro*, against test pathogens *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola, sclerotium rolfsii* separately by using Poisoned Food Technique (Nene and Thapliyal, 1979). The requisite quantity of plant extract was added to sterilized PDA by means of sterile pipette under aseptic conditions, so as to get desired concentration. The extract was thoroughly mixed before solidification of PDA and poured into sterilized Petri plates. The mycelial discs of 5 mm diameter of seven days old culture of respective test fungus were cut with the help of sterilized cork borer and transferred aseptically at the centre of each sterilized petri plates already poured with poisoned medium separately and antagonistic activity of bioagents *viz., Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* against pathogens *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola, sclerotium rolfsii* were tested on PDA by using dual culture technique. Bacterial antagonist was tested by streak plate method. Medium without fungicides, botanicals and bioagents served as control. Plates inoculated with respective test pathogen were incubated at 28 ± 2ºC and observation for colony diameter were recorded on viiith day after inoculation. Per cent inhibition of growth of test fungi was calculated.

RESULTS AND DISCUSSION

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

Botanicals :

From the Table 1(A) it was found that the radial mycelial growth was inhibited by carbendazim treatment 100 per cent. These treatment superior over all the treatment. Among botanicals *Azadirachta indica* was superior over all the treatment recorded growth inhibition at 5 per cent, 10 per cent, 20 per cent as 75.56 per cent, 80.00 per cent, 84.44 per cent, respectively. *Azadirachta indica* treatments followed by *Eucalyptus* spp. Recorded growth inhibition at 5 per cent, 10 per cent, 20 per cent as 54.88 per cent, 61.11 per cent, 82.66 per cent, respectively. And *Ocimum sanctum* at 5 per cent, 10 per cent, 20 per cent recorded growth inhibition as 55.22 per cent, 50.00 per cent, 80.44 per cent, respectively.

Among the botanicals as the concentration of botanicals increases from 5 to 20 per cent it was observed that botanicals @ 20 per cent concentration showed maximum growth of hihibition. *Azadirachta indica* @ 20 per cent concentration recorded maximum growth inhibition *i.e.* 84.44 per cent followed by *Eucalyptus* spp. 82.66 per cent, *Ociumum sanctum* 80.44 per cent. Similar finding have been reported by Sharma *et al.* (2003) *Azadirachta indica* had superior

antifungal activity on *Fusarium oxysporum* f. sp. *ciceri*.

From the Table 1 (B) carbendazim treatment is superior over all treatment as it inhibits *Rhizoctonia bataticola* 100 per cent. Among botanicals *Azadirachta indica* is superior over the treatments. As maximum growth of inhibition of *Rhizoctonia bataticola* at 5 per cent, 10 per cent, 20 per cent was recorded as 82.22 per cent, 87.44 per cent, 88.55 per cent, respectively. *Azadirachta indica* treatment followed by *Eucalyptus* spp. As it recorded growth of inhibition of *Rhizoctonia bataticola* at 5 per cent, 10 per cent, 20 per cent concentration such as 78.89 per cent, 85.56 per cent, 86.33 per cent, respectively. *Ocimum sanctum* at 5 per cent, 10 per cent, 20 per cent recorded growth of inhibition as 65.56 per cent, 70.00 per cent, 80.44 per cent, respectively. From the data of Table 1(B) it was found that maximum growth of inhibition of *Rhizoctonia bataticola* recorded by *Azadirachta indica* at 20 per cent concentration 88.55 per cent followed by *Eucalyptus* spp. @ 20 per cent concentration 86.33 per cent, *Ocimum sanctum* @ 20 per cent concentration 80.44 per cent. Similarly Tiwari and Shrivastava (2004) showed antifungal activity of *Azadirachta indica*, *Eucalyptus globutens*, *Ocimum sanctum*. Kaushik *et al.* (2002) showed that the antifungal activity of *Eucalyptus tereticornis* and *Ocimum sanctum* plant extrats increased significantly with increase in concentration.

From the Table 1(C) Carbendazim inhibits 100 per

Table 1 (A) : Evaluation of botanicals against *Fusarium oxysporum* f.sp. ciceri

Sr. No.	Treatments	Conc.	Mean colony diameter (cm)VIIIth day	% growth inhibition	Conc	Mean colony diameter (cm)VIIIth day	% growth inhibition	Conc.	Mean colony diameter (cm) VIIIth day	% growth inhibition
1.	*Lantana camara*	5%	5.03	41.11	10%	4.70	47.77	20%	4.26	52.66
2.	*Ocimum sanctum*	5%	4.03	55.22	10%	4.50	50.00	20%	1.76	80.44
3.	*Eucalyptus* spp.	5%	4.06	54.88	10%	3.50	61.11	20%	1.56	82.66
4.	*Vinca rosea*	5%	8.80	2.22	10%	8.30	7.77	20%	8.23	8.55
5.	*Azadirachta indica*	5%	2.20	75.56	10%	1.80	80.00	20%	1.40	84.44
6.	*Alovea vera*	5%	5.70	36.67	10%	5.20	42.22	20%	5.00	44.44
7.	Carbendazim	0.1%	0.00	100.00	0.1%	0.00	100.00	0.1%	0.00	100.00
8.	Control		9.00			9.00			9.00	
	F test		Sig			Sig			Sig	
	S.E. ±		0.02			0.04			0.01	
	C.D. (P=0.01)		0.08			0.14			0.03	

Table 1 (B) : Evaluation of botanicals against *Rhizoctonia bataticola*

Sr. No.	Treatments	Conc.	Mean colony diameter (cm) VIIIth day	% growth inhibition	Conc.	Mean colony diameter (cm)VIIIth day	% growth inhibition	Conc.	Mean colony diameter (cm)VIIIth day	% growth inhibition
1.	*Lantana camara*	5%	4.03	55.22	10%	3.50	61.11	20%	2.53	71.88
2.	*Ocimum sanctum*	5%	3.10	65.56	10%	2.70	70.00	20%	1.76	80.44
3.	*Eucalyptus* spp.	5%	1.90	78.89	10%	1.30	85.56	20%	1.23	86.33
4.	*Vinca rosea*	5%	5.80	35.56	10%	5.30	41.11	20%	5.03	44.11
5.	*Azadirachta indica*	5%	1.60	82.22	10%	1.40	87.44	20%	1.03	88.55
6.	*Alovea vera*	5%	5.03	44.11	10%	4.53	49.66	20%	4.16	53.77
7.	Carbendazim	0.1%	0.00	100.00	0.1%	0.00	100.00	0.1%	0.00	100.00
8.	Control		9.00			9.00			9.00	
	F test		Sig.			Sig.			Sig.	
	S.E.±		0.04			0.01			0.04	
	C.D. (P=0.01)		0.2			0.03			0.11	

cent mycelial growth of *Sclerotium rolfsii* carbendazim treatment was found to be superior over all the treatment among the botanicals as the concentration of botanicals increases from 5 to 20 per cent maximum growth of

Table 1 (C) : Evaluation of botanicals against *Sclerotium rolfsii*

Sr. No.	Treatments	Conc.	Mean colony diameter (cm)VIIIth day	% Growh inhibition	Conc.	Mean colony diameter (cm)VIIIth day	% Growh inhibition	Conc.	Mean colony diameter (cm)VIIIth day	% Growh inhibition
1.	*Lantana camara*	5%	5.03	41.11	10%	4.53	49.66	20%	4.26	52.66
2.	*Ocimum sanctum*	5%	3.76	58.22	10%	3.40	62.22	20%	3.13	65.22
3.	*Eucalyptus* spp.	5%	2.86	68.22	10%	1.53	83.00	20%	1.86	79.33
4.	*Vinca rosea*	5%	8.03	10.77	10%	7.46	17.11	20%	7.13	20.77
5.	*Azadirachta indica*	5%	2.46	72.66	10%	1.50	83.33	20%	1.33	85.22
6.	*Alovea vera*	5%	6.46	28.22	10%	6.03	33.00	20%	5.66	37.11
7.	Carbendazim	0.1%	0.00	100.00	0.1%	0.00	100.00	0.1%	0.00	100.00
8.	Control		9.00			9.00			9.00	
	F test		Sig			Sig			Sig	
	S.E.±		0.04			0.05			0.04	
	C.D. (P=0.01)		0.11			0.15			0.11	

Table 2 (A) : Effect of antagonist against mycelial growth of *Fusarium oxysporum* f.sp. ciceri, *in vitro*

Treat. No.	Antagonist	Average mycelial growth (mm)	% Inhibition
T_1	*Trichoderma viride*	12.42	70.95
T_2	*Trichoderma harzianum*	14.32	60.50
T_3	*Pseudomonas fluorescens*	21.35	50.05
	Control	42.75	
	'F' test	Sig.	
	S.E.±	0.10	
	C.D. (P = 0.01)	0.50	

Table 2 (B) : Effect of antagonist against mycelial growth of *Rhizoctonia bataticola in vitro*

Treat. No.	Antagonist	Average mycelial growth (mm)	% Inhibition
T_1	*Trichoderma viride*	20.11	71.15
T_2	*Trichoderma harzianum*	20.33	77.01
T_3	*Pseudomonas fluorescens*	14.23	83.83
	Control	88.00	
	'F' test	Sig.	
	S.E.±	0.40	
	C.D. (P = 0.01)	1.94	

Table 2 (C) : Effect of antagonist against mycelial growth of *Sclerotium rolfsii in vitro*

Treat. No.	Antagonist	Average mycelial growth (mm)	% Inhibition
T_1	*Trichoderma viride*	12.95	85.15
T_2	*Trichoderma harzianum*	12.62	85.53
T_3	*Pseudomonas fluorescens*	12.94	85.16
	Control	87.22	
	'F' test	Sig.	
	S.E.±	0.41	
	C.D. (P = 0.01)	1.93	

inhibition recorded. *Azadirachta indica* at 5 per cent, 10 per cent, 20 per cent recorded growth inhibitions of *Sclerotium rolfsii* as 72.66 per cent, 83.33 per cent, 85.22 per cent, respectively. These treatment followed by *Eucalyptus* spp. At 5 per cent, 10 per cent, 20 per cent concentration it recorded 68.22 per cent, 83.00 per cent, 79.33 per cent, respectively, growth of inhibition of *Sclerotium rolfsii*. *Ocimum sanctum* recorded growth of inhibition of *Sclerotium rolfsii* at 5 per cent, 10 per cent, 20 per cent as 58.22 per cent, 62.22 per cent, 65.22 per cent, respectively. From the data of table it was found that as the concentration of botanicals increases per cent inhibition also increase. *Azadirachta indica* is superior over all the treatments @ 20 per cent concentration it recorded 85.22 per cent growth of inhibitions of *Sclerotium rolfsii* followed by *Eucalyptus* spp. @ 10 per cent concentration 83.00 per cent and *Ociumum sanctum* @ 20 per cent concentration 65.22 per cent similar result shown by Lakshmanan and Mohan (1990) that water extract of *Azadirachta indica* significantly inhibitied mycelial growth and Sclerotium germination of *Thanatephorus cucumeris* in *in vitro*. Shitole *et al*. (2007) showed plant extract of *Eucalyptus* spp, garlic, ginger were significantly effective and showed maximum per cent inhibition of mycelial growth of pathogen as compared to the untreated control.

The per cent inhibition of radial growth of *Sclerotium rolfsii* is directly proportional to the concentration of plant extract as the concentration increases the per cent inhibition also increases as compared to control.

Bioagents :

Effect of antagonist on mycelial growth of *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola, Sclerotium rolfsii in vitro* presented in Table 2 A, B and C, respectively.

The data of Tables 2(A) showed that *Trichoderma viride* was superior in restricting the mycelial growth *Fusarium oxysporum* f.sp. *ciceri* by 70.95 per cent inhibition followed by *Trichoderma harzianum* and *Pseudomonas fluorescens* of 60.50 per cent and 50.05 per cent, respectively.

The data of Table 2(B) showed that *Pseudomonas fluorescence* was superior in restricting the myclieal growth of *Rhizoctonia bataticola* by 83.83 per cent inhibition followed by *Trichodarma viride and Trichodarma harzianum* of 71.15 per cent and 77.01 per cent, respectively.

The data of Tables 2(C) showed that *Trichoderma harzianum* was superior in restricting the mycelial growth of *Sclerotium rolfsii* by (85.53%), inhibition followed by *Pseudomonos fluorescens* and *Trichodarma viride* of 85.16 per cent and 85.15 per cent, respectively. Kandoliya and Vakharia (2013) and Padghan and Baviskar (2009).

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