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RESEARCH ARTICLE

INVITRO AND *IN VIVO* EVALUATION OF BOTANICALS, BIOAGENTS AND FUNGICIDE AGAINST LEAF SPOT OF SAFFLOWER

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ABSTRACT

The efficacy of two botanicals viz., neem oil and castor oil, one fungicide I.e., carbendazim and three bioagents were tested *in vitro* and *in vivo* against *Alternaria carthami* inciting leaf spot safflower leaf spot/blight. *In vitro* efficacy of botanicals and fungicide was evaluated by poison food technique against *Alternaria carthami. In vitro* efficacy of bioagents was evaluated by dual culture technique against *Alternaria carthami*. In *in vitro* evaluation of fungicide and botanicals carbendazim found to be most effective and showed maximum inhibition of mycelial growth (43.33%) followed by neem oil (30.53%). Among the bioagents maximum inhibition of radial growth of the test pathogen was noticed in *P. fluorescens* (87.36 per cent) which was found on par with *T. virde* (86.22 per cent). Mycelial growth of test pathogen was inhibited to an extent of 81.08 per cent in in *T. harzianum*. In *invivo* evaluation, combined seed treatment with of *P. fluorescens* (10 g kg^{-1} seed) + carbendazim (2 g kg^{-1} seed)+ neem oil (10 ml kg^{-1} seed) was effective in controlling Alternaria leaf spot/blight.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the important oilseed crops of the world. It is popular not only for seed and oil, but also for its brightly coloured petals, high levels of linoleic acid (75-80%) in oil and amino acids (Nagaraj, 2009).Alternaria leaf spot/blight is one of the most serious diseases of safflower reducing yield by 50%. Infected seed also often smaller with reduced oil content. It mostly infects leaves, stems, heads and seeds. Plant extracts are Known to posses antifungal properties (Nene and Thapliyal, 1993). The presence of antifungal compounds in higher plants is well recognized and considered valuable for plant disease control. Neem has attracted special interest of scientists due to presence of variety of bioactive compounds. of *Trichoderma* sp. *Pseudomonas fluorescens* are commercially applied as biocontral agents against many fungal pathogens. So keeping in view the present study undertaken to know the efficacy of Two botanicals and three bioagents for the control of *Alternaria carthami* inciting leaf spot safflower leaf spot/blight.

MATERIALS AND METHODS

Two botanicals viz., neemoil, castor oil one fungicide i.e., carbendazim were evaluated by poisoned food technique with three replications on Potato Dextrose Agar (PDA) and incubated at 28 ± 2 ^{o}C for seven days. The fungal and bacterial antagonists were evaluated against the test pathogen *Alternaria carthami* in laboratory by dual culture technique. Petri dishes (90 mm) containing PDA was inoculated with 5 mm diameter mycelia disc of 7 days old culture of *Alternaria carthami* and fungal/bacterial antagonists at equal distance from periphery. Inoculated plates were at 28 ± 2 ^{o}C. Each treatment was replicated four times. After required period of incubation *i.e.,* in the control plate growth reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was assessed.

$R = \{(C - T)/C\} \times 100$
Where, R= Per cent inhibition
C = Radial growth of pathogen colony in control
T = Radial growth of pathogen colony in treatments

Under glass house studies the healthy seeds of safflower (*cv*.Nira) were surface sterilized and artificially inoculated with the test pathogen by rolling the seeds in 10 days old sporulating culture grown on PDA. The inoculated seeds were kept for 8 h in Petri plates having moistened blotter papers. After incubation, inoculated seeds were treated separately by coating with potential botanicals and bioagents by imposing different treatments. Seeds from each treatment were then sown in pots (20 cm diameter) filled with sterilized soil @ five seeds per pot. Observations on pre emergence mortality, post emergence mortality, per cent seedling emergence were recorded after 35 days.

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Treatment Details

Design: CRD
Replications: 4
Treatments: 8
T1- Seed inoculation with test pathogen followed by seed treatment (10 g kg^{-1}seed) with potential bioagent
T2- Seed inoculation with test pathogen followed by carbendazim seed treatment @ 2 g kg^{-1}seed.
T3- Seed inoculation with test pathogen followed by seed treatment with neem oil @ 10ml kg^{-1}seed
T4= T1+T2
T5= T2+T3
T6= T1+T3
T7 = T1+T2+T3
T8= Inoculated control

RESULTS AND DISCUSSION

In *in vitro* evaluation, out of two botanicals tested neem oil was found to be effective in inhibiting the mycelia growth (30.53%).

Table 4. Effect of botanicals on growth of *A. carthami in vitro*

Treatments	* Radial Growth (mm)	Per cent inhibition
Neem oil	20.83	30.53
(Azadirachta indica)		(33.50)
Castor oil	26.2	12.66
(Ricinus communis)		(20.77)
Carbendazim	17	43.33
		(41.14)
Control	40	0.00
		(0.00)
C.D at 0.05 %		3.696
SE (m)±		1.116

*Mean of three replications; Figures in parenthesis are angular transformed values

Table 1 Effect of bioagents on growth of *A. carthami in vitro*

Treatments	* Radial Growth (mm)	Per cent inhibition
Trichoderma viride	12	86.22
		(66.192)
Trichoderma harzianum	15	81.02
		(64,20)
Pseudomonas fluorescens	11.3	87.36
		(69.17)
Control	90	0.00
		(0.00)
C.D at 0.05 %		1.803
SE (m)±		0.544

*Mean of three replications; Figures in parentheses are angular transformed values

Table 2 Effect of seed treatment of botanicals/bioagents/fungicide on Pre emergence mortality of safflower cv. Nira against *A. carthami* under glass house conditions

S.No	Treatment	*Pre emergence mortality (%)	Per cent decrease over control
1	Pseudomonas fluorescens (10 g kg^{-1} seed)	13.27	75.62
		(21.35)	
2	Carbendazim	20.20	62.89
	(2 g kg^{-1} seed)	(26.69)	
3	Neem oil	26.833	50.71
	(10 ml kg^{-1} seed)	(31.17)	
4	Pseudomonas fluorescens (10 g kg^{-1} seed) + Carbendazim (2	5.08	90.66
	g kg^{-1} seed)	(12.97)	
5	Pseudomonas fluorescens (10 g kg^{-1} seed) + Neem oil	6.19	88.62
	(10 ml kg^{-1} seed)	(14.40)	
6	Carbendazim (2 g kg^{-1} seed)+ Neem oil(10 ml kg^{-1} seed)	13.01	76.10
		(21.12)	
7	Pseudomonas fluorescens (10 g kg^{-1} seed) + Carbendazim (2	1.20	97.97
	g kg^{-1} seed)+ Neem oil (10 ml kg^{-1} seed)	(6.29)	
8	Control	54.44	-
		(47.54)	
	C.D at 0.05 %	2.552	
	SE (m)±	0.844	

*Mean of three replications Figures in parentheses are angular transformed values.

Table 3 Effect of seed treatment of botanicals/bioagents/fungicide on Post emergence mortality of safflower cv. Nira against *A. carthami* under glass house conditions

S.No	Treatment	*Post emergence mortality(%)	Per cent decrease over control
1	Pseudomonas fluorescens (10 g kg^{-1} seed)	6.637	88.5
		(14.91)	
2	Carbendazim	13.51	76.59
	(2 g kg^{-1} seed)	(21.55)	
3	Neem oil	19.00	67.08
	(10 ml kg^{-1} seed)	(25.82)	
4	Pseudomonas fluorescens (10 g kg^{-1} seed) + Carbendazim	6.67	88.44
	(2 g kg^{-1} seed)	(14.95)	
5	Pseudomonas fluorescens (10 g kg^{-1} seed) + Neem oil (10	6.88	88.20
	ml kg-1 seed)	(15.19)	
6	Carbendazim (2 g kg^{-1} seed)+ Neem oil(10 ml kg^{-1} seed)	13.30	96.96
		(21.38)	
7	Pseudomonas fluorescens (10 g kg^{-1} seed) + Carbendazim	0.753	99.43
	(2 g kg^{-1} seed)+ Neem oil (10 ml kg^{-1} seed)	(4.69)	
8	Control	57.73	-
		(49.44)	
	C.D at 0.05 %	2.287	
	SE (m)±	0.756	

*Mean of three replications Figures in parentheses are angular transformed values

Similar results were obtained by Ghewande (1989) and Usman *et al*. (1991) who reported the Antifungal properties of neem based products. All the antagonists viz., *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens* inhibited mycelia growth of the pathogen. *Pseudomonas fluorescens* inhibited maximum mycelia growth with a mean inhibition of (87.36 per cent) which was found on par with *T. virde* (86.22 per cent). Mycelial growth of test pathogen was inhibited to an extent of 81.08 per cent in in *T. harzianum*. Similar results were obtained by Amaresh (2000) who reported that among fungi *T.viride* and *T.harzianum* overgrew and inhibited the growth of *A.helianthi,* while the bacterium *P.fluorescens* produced maximum inhibition zone. all the seed treatments were significantly superior in reducing the pre emergence and post emergence mortality seed treatment with combined treatment of with *Pseudomonas fluorescens* (10 g kg^{-1} seed) + carbendazim (2 g kg^{-1} seed) + neem oil (10 ml kg^{-1} seed) resulted in high per cent reduction of pre and post emergence mortality (97.97 per cent and 99.43 per cent, respectively) followed by *P.fluorescens* (10 g kg^{-1} seed) + carbendazim (2 g kg^{-1} seed) when compared to control (54.44 per cent). The beneficial effect of seed treatments with bioagents and fungicides in minimising the pre and post emergence mortalirty is in accordance with Govindappa *et al*. (2011) in safflower

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