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Evaluation of Bio Agents, de Oiled Cakes and new Generation Fungicides to control of *Fusarium oxysporum* Schlecht *in vitro*

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ABSTRACT: The present study was carried out to investigate the efficacy of microbial antagonist's bio-agents, de oiled cakes and fungicides under *in vitro* study against *Fusarium oxysporum* Schlecht caused wilt of Isabgol. Study results revealed that among all fungicides Bavistin 50WP recorded maximum inhibition percent of mycelium growth of test pathogen *Fusarium oxysporum* Schlecht at all four concentrations followed by Nativo-75WP. All fungicides were tested by poisoned food technique. Out of four bio-control agents *Trichodrma viride* showed highest mycelia growth inhibition (59.64%) followed by *T. harzianum* (58.25%). Among four de oiled cake neem cake showed highest mycelia growth inhibition (67.78%) followed by Groundnut cake (53.33%) found highly effective to control the mycelia growth of *Fusarium oxysporum* Schlecht. Application of bio control agents and de oiled cake will be an alternative to synthetic chemicals to control wilt of isabgol.

Keywords: Bio-control agents, Carbendazim 50 WP, De oiled cakes, *Fusarium oxysporum* Schlecht, Isabgol.

INTRODUCTION

Isabgol (*Plantago ovata* Forsk.) also known as blond psyllium. Isabgol is an annual herb with narrow linear rosette leaves and belongs to *Plantaginaceae* family. In India, *Plantago ovata* is grown for commercial scale of the western states namely Rajasthan, Gujarat and Madhya Pradesh as the dry climate of the states is best suited for the plant. Mandal (2010) reported that many pathogens were found in involve of causing severe yield losses and seed quality of isabgol *viz*., Fusarium wilt (*Fusarium oxysporum* Schlecht), damping off (*Pythium ultimum*), leaf blight (*Alternaria alternata* (Fr.) Keissler).

Fusarium wilt disease is most important disease of isabgol crop Rajastahn state causing damage to the crop. Meena and Roy (2020) reported that yield losses recorded 18-40% in isabgol crop by *Fusarium oxysporum* Schlecht. It occurs every year in severe form in the entire Isabgol growing areas of the state.

MATERIALS AND METHODS

Collection, Isolation and purification of test fungal pathogen. Isabgol plants showed drooping of leaves, yellowing and wilting symptoms were collected from plant pathology research field, R.C.A., Udaipur. The roots were washed under the tap water for removing all visible soil and other particles. The infected root portion was cut into small pieces of 3-4 mm which were surface sterilized using 0.1% Hg Cl_2 solution for one minute and followed by three time rinsed with sterile distilled water. Those bits were transferred on PDA media under aseptic condition and inoculated plates were kept under 28±2°C for two days and pure culture was obtained by single spore isolation method.

Pathogenicity assay. The pathogenicity of fungal isolate was established in a poly house under artificial inoculated condition by root inoculation. Pathogen isolate was subjected to the preliminary pathogenecity test on isabgol GI-2 cultivar. Earthen pots were filled with sterilized soil at 1 kg per pot. Corn meal sand medium grown inoculum of *Fusarium oxysporum* Schlecht was properly mixed with soil @15g/kg soil. Control pots were kept as sterilized soil. Twenty five seed of GI-2 were sown in each pot. Observation of number of wilted plants in each pot were observed at 30, 45, 60 days after sowing of seeds. Test pathogen was identified with the standard references description (Booth 1971) and pathogen was confirmed from (ID Number 10,953.18) ITCC New, Delhi.

***In vitro* efficacy of Bio agents antagonists against *Fusarium oxysporum* Schlecht.** Dual culture technique was applied to determine the effect of *Trichoderma* sp. on test pathogen (Dennis and Webester 1971). CRD design with four replications was applied for this study. *Trichoderma viride*, *T. harzianum P. fluorescence* and *Bacillus subtilis* were tested for antagonistic activity against *Fusarium oxysporum* Schlecht. Mycelia disc (five mm) were cut from edge of the seven to eight days culture of *Fusarium oxysporum* Schlecht werekept one centimetre away from the edge of plate and antagonistic *Trichoderma* sp. (five mm disk) was placed at the opposite of the Petri dish.. The plates that received only disc of *Fusarium oxysporum* Schlecht served as control and then plates were incubated in the laboratory at room temp. (25±2°C). Inhibition percentage of test

pathogen was calculated according to growth of the pathogen on PDA plates after seven days of incubation. The percentage inhibition was calculated by the formula (Vincent, 1927):

Percent growth inhibition = a-b/a × 100

Where, a is calculated as the growth of test pathogen in absence of antagonist (mm) and b is calculated as the growth of tested pathogen against antagonist (mm).

***In vitro* evaluation of fungicides against *Fusarium oxysporum* Schlecht.** Eight fungicides namely Carbendazim-50WP, Mancozeb-75WP, Copper oxychloride-50WP, SAAF-75 WP (Carbendazim 12% + Mancozeb 63%), Thiram-50WP, Aliette -80 WP, Antracole -70 WP and Nativo-75 WP were tested against *Fusarium oxysporum* Schlecht. All fungicides were tested at 0.10%, 0.15%, 0.20% and 0.25% concentration in a autoclaved PDA media by poisoned food technique and 5 mm diameter agar disc of test fungi was cut from seven days old culture and placed in the middle of Petri plates containing different concentration of test fungicides. The plates without fungicides served as control plate. The inoculated plates were incubated @ 25±2°C temperature. The radial growth recorded after seven days of incubation. The percent inhibition of the fungus over control was calculated by using formula of Vincent (1927).

Efficacy of de oil cake against *Fusarium oxysporum* Schlecht under *in vitro* condition. One gm of each oil cake *viz.* Neem, Groundnut, Mustard and Cotton seed was made into powder form and then soaked in 1.25 ml of sterile distilled water overnight. The all material was ground using a pestle and mortar and filter through a muslin cloth and the filterate centrifuged at 10,000 rpm for 15 min. The supernatant served s that standard extract soutlion (100 percent) (Dubey and Patel 2000) and sterilized at 1.045 kg cm^{-3} pressure @ 20 minutes in autoclaving and subsequently cool down and used for *in vitro* experiments. The efficacy of oil extract was evaluated against *Fusarium oxysporum* Schlecht using the technique of Schmitz (1930). Fifty ml of freshly prepared PDA was placed in a conical flask. Aqueous extracts of oil cakes five ml were mixed with 45 ml of PDA medium to obtain a 5% concentration and sterilized. The sterilized PDA medium (15 ml per Petri dish) was poured into sterilized Petri dishes for allowed to solidifying. A 9 mm mycelial disc of *Fusarium oxysporum* Schlecht was taken from 15 days old culture and then placed centre of perti palte which was incubated at room temperature 25±2°C. The potato dextrose agar medium without extract of oil cake served as control. The radial growth of test pathogen was calculated after seven days of incubation. The percent inhibition of the fungus over control was calculated by using formula of Vincent (1927).

Observation and data collection. The observation for culture growth were recorded by measuring mycelial growth in diameter along with two diagonal axis moving through the centre of the culture plate (where five mm in diameter agar disc of test pathogen was put down) after seventh day of inoculation. Percent mycelia growth inhibition percent was calculated by formula I= {(C-T)/ C} × 100 Where, I= percent inhibition; C= colony diameter in control (mm); T= colony diameter in treatment (mm) (Bliss, 1934) and PDI was calculated by {total number of infected plants/ total number of plants×100. Percent efficacy of disease control (PEDC) was calculated by formula: {PD I in control- PDI in treatment// PDI control} × 100 (Chester 1959; Wheeler 1969). Colony forming unit of *T. viride*, *T. harzianum* and *P. fluorescence*, *Bacillus subtilis* and *Fusarium oxysporum* Schlecht was recorded by serial dilution plating (Warcup, 1955) on organism specific type medium.

RESULTS AND DISCUSSION

***In vitro* evaluation of antagonistic bio-control agents against *Fusarium oxysporum* Schlecht by dual culture technique.** The antagonistic activities of bio agents was screened *in vitro* against to *Fusarium oxysporum* Schlecht by dual culture technique by dual culture technique on PDA media for seven days. The bio-control agents namely as *T. harzianum*, *T. viride*, *P. fluorescence* and *Bacillus subtilis* were tested to recorded significant higher reduction in mycelia growth by *T. viride* (59.64%) followed by *T. harzianum* (58.25%). However, *P. fluorescens* were showed (56.10%) growth inhibition followed by *B. subtilis* (52.73%) growth inhibition (Table 1). Due to mycoparasitism and completion for space and nutrition growth of pathogen inhibited by the fast growth of antagonists.

Similarly, Bardia and Rai (2007) recorded antagonistic effect of *Trichoderma viride* against *Fusarium oxyspoprum* f.sp. *cuminis* by 50.16% inhibition of mycelia growth. Cherkupally *et al*. (2017) tested to inhibiton percent of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f sp. *melongenae* found 78.88% and 81.11% inhibition percent respectively.

Trichoderma sp. produced extracellular proteolyyctic, glucanolytic, and chitinase enzymes which were responsibkle for the release of bio-active molicules likewise proteins, lysis of pathogen cells.

Table 1: *In vitro* efficacy of bio-agents against *Fusarium oxysporum* Schlecht isolate (UDP Fo-1) on PDA.

Sr. No.	Treatments	Mycelia growth (mm) *	Mycelial growth inhibition (%)
1.	*T. viride*	23.0	59.64 (74.44)
2.	*T. harzianum*	25.0	58.25 (72.22)
3.	*P. fluorescens*	28.0	56.10 (68.89)
4.	*Bacillus subtilis*	33.0	52.73 (63.33)
5.	Control	90.0	0.00 (0.00)
SEm ±		0.913	0.655
CD at 5%		2.812	2.018
C.V		4.59	2.89

*Mean of four replications; Figure in parentheses are arcsine percent angular transformed values

Plate 1: *In vitro* efficacy of bio agents against the mycelia growth (colony diameter) of the isolates of *Fusarium oxysporum* Schlecht.

These type released molecules and cell wall fragments were responsible for elicitation of induced systemic or localized resistance. The secondary metabolites are produced by *Trichoderma* spp. such as volatiles and antibiotics were responsible for antibiosis (Thangavelu and Mustaffa 2012).

***In vitro* evaluation of de oiled cakes against the *Fusarium oxysporum* Schlecht.** The efficacy of oiled cakes namely, Neem, Groundnut, Mustard and Cotton seed de oiled cake were tested @5, 10, 20 and 30 percent concentration. Neem cak @30 percent was found best effective in inhibition of mycelia growth (55.42%) followed by Ground nut cake @30 percent in inhibition of mycelia growth (46.91%), Mustard @30 percent in inhibition of mycelia growth was recorded (43.73%) while Cotton seed @30 percent was found least effective (36.59%) at all the concentration compare to other treatment. Similar results are found with the study conducted by Haseeb and Kumar (2007) reported that neem oil cake was effective against growth of *F. oxysporum*. Plant extracts of many plants like neem have been reported to exhibit antifungal, insecticidal and anti bacterial properties under laboratory condition (Satish *et al*., 1999).

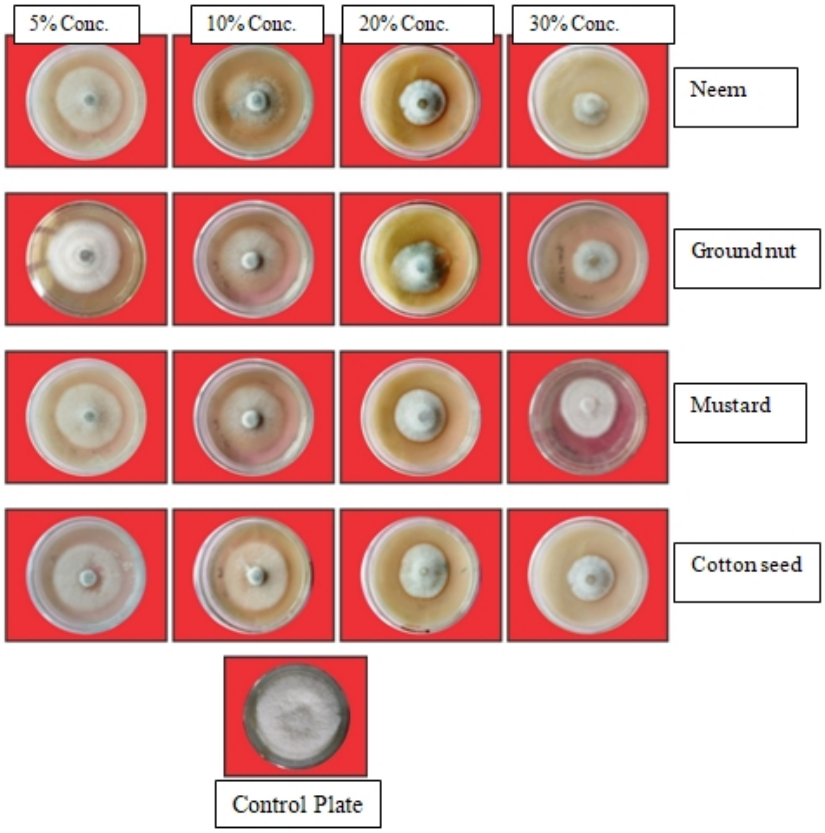

Plate 2: Effect of different de oiled cake on the mycelia growth of the isolates of *Fusarium oxysporum* Schlecht.

Table 2: Effect of different de oiled cakes on the mycelial growth of *Fusarium oxysporum* Schlecht isolate at various concentrations *in vitro.*

Sr. No.	Treatments De oiled cakes	Radial growth of pathogen (mm)* at different conc. (%)				Percent growth inhibition			
		5	10	20	30	5	10	20	30
1.	Neem	55.00	45.00	38.00	29.00	38.58 (38.89)	45.00 (50.00)	49.48 (57.78)	55.42 (67.78)
2.	Groundnut	76.00	65.00	53.00	42.00	23.12 (15.56)	31.81 (27.78)	39.88 (41.11)	46.91 (53.33)
3.	Mustard	81.00	70.00	58.00	47.00	18.29 (10.00)	28.08 (22.22)	36.60 (35.56)	43.73 (47.78)
4.	Cotton seed	88.00	79.00	68.00	58.00	8.57 (2.22)	20.35 (12.22)	29.62 (24.44)	36.59 (35.56)
5.	Control	90.00	90.00	90.00	90.00	(0.00)	(0.00)	(0.00)	(0.00)

	SEm±	CD at 5%	CD at1%	SEm ±	CD at 5%	CD at 1%
De oiled cakes	0.507	1.453	1.936	0.424	1.215	1.619
Concentration	0.454	1.299	1.732	0.379	1.086	1.448
Cake ×C	1.015	2.906	3.873	0.848	2.430	3.238

*Mean of three replications; Figure in parentheses are arcsine percent angular transformed values

***In vitro* evaluation of selected fungicides against *Fusarium oxysporum* Schlecht.** The efficacy of selected fungicides namely, Carbendazim-50WP, Mancozeb-75WP, Copper oxychloride-50WP, SAAF-75WP (Carbendazim 50WP 12% + Mancozeb 63%), Thiram-50WP, Aliette-80WP, Antracole-70WP and Nativo-75WP were tested @ 0.1, 0.15, 0.20 and 0.25 percent concentration against *Fusarium oxysporum* Schlecht. All the chemicals at various concentrations inhibited the fungal mycelia growth and all the fungicides were significantly superior over to control at all the concentrations. The maximum mycelial inhibition at 0.25% was recorded in Carbendazim-50WP (90%) followed by Nativo-75WP (90%), Antracole-70WP (68.58%), Aliette-80WP (62.64%), SAAF-75 WP (54.06%) and Copper oxychloride-50WP (49.475). The fungicide Mancozeb-75WP was found less effective with 44.36% inhibition of the pathogen over untreated control. Results were similar with the studies conducted by Behrani *et al.* (2015); Gahlot *et al*. (2022), who reported that Carbendazim 50WP followed by Antracole-70WP appeared as the most effective fungicides.

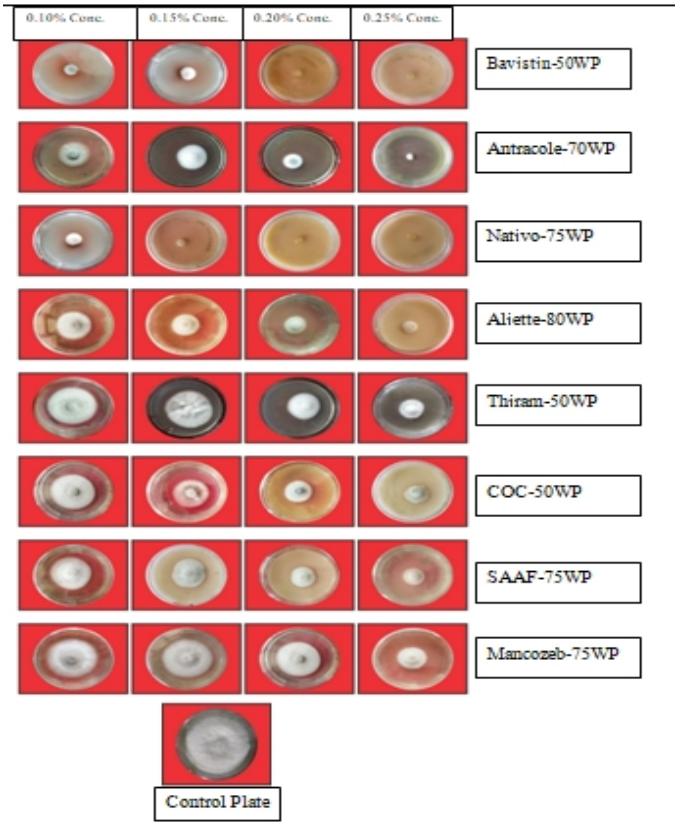

Plate 3: Comparative efficacy of different fungicides on the mycelia growth (colony diameter) of the isolates of *Fusarium oxysporum* Schlect at various concentrations *in vitro.*

Table 3: Comparative efficacy of different fungicides against *Fusarium oxysporum* Schlecht isolate at various concentrations *in vitro.*

Sr. No.	Treatments Fungicides	Colony growth (mm)* at different concentration (%)				Percent growth inhibition			
		0.10	0.15	0.20	0.25	0.10	0.15	0.20	0.25
1.	Carbendazim-50WP	5.00	3.00	0.00	0.00	76.35 (94.43)	79.48 (96.66)	90.00 (100.00)	90.00 (100.00)
2.	Mancozeb-75WP	62.00	58.00	50.00	46.00	33.65 (30.82)	36.61 (35.57)	41.77 (44.39)	44.36 (48.88)
3.	Copper oxychloride-50WP	50.00	45.00	43.00	38.00	41.71 (44.28)	44.93 (49.87)	46.26 (52.21)	49.47 (57.77)
4.	SAAF-75 WP (Bavistin 12% +Mancozeb 63%)	45.00	41.00	36.00	31.00	44.95 (49.91)	47.54 (54.42)	50.73 (59.94)	54.06 (65.55)
5.	Thiram-50WP	56.00	52.00	48.00	42.00	37.79 (37.59)	40.45 (42.12)	43.05 (46.60)	46.89 (53.30)
6.	Aliette-80WP	35.00	28.00	24.00	19.00	51.34 (60.95)	56.10 (68.90)	58.89 (73.30)	62.64 (78.87)
7.	Antracole-70WP	26.00	23.00	20.00	12.00	57.42 (70.97)	59.62 (74.42)	61.86 (77.75)	68.58 (86.66)
8.	Nativo-75WP	8.00	5.00	0.00	0.00	72.66 (91.11)	76.37 (94.44)	90.00 (100.00)	90.00 (100.00)
9.	Control	90.00	90.00	90.00	90.00	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)

	SEm±	CD at 5%	CD at 1%	SEm ±	CD at 5%	CD at 1%
Fungicides	0.576	1.625	2.199	0.607	1.713	2.317
Concentration	0.384	1.083	1.466	0.404	1.142	1.544
F×C	1.152	3.251	4.397	1.214	3.426	4.634

*Mean of three replications; Figure in parentheses are arcsine percent angular transformed values

Gupta *et al.* (1983) tested the efficacy of the fungicides against *F. oxysporum* f. sp. *cepae* causing the basal rot of onion *in vitro* and found that Benlate (Benomy) performed the best (250 ppm) followed by Bavistin (Carbendazim), Thiram and Vitavex (Carbonil) (2000 ppm). Amini and Sidovich (2010) tested Carbendazim 50WP and some other fungicides for their inhibitory activities against the wilt pathogen *F. oxysporum* f.sp *lycopersici.*

CONCLUSIONS

From the findings it is concluded by *in vitro* study application of bio control agents and de oiled cake will be significantly promising and applicable as an alternative to synthetic chemicals and low efficiency and harmful methods for control of Fusarium wilt disease of Isabgol caused by *Fusarium oxysporum* Schlecht. Microorganisms that have fast growth in the rhizosphere are best for antagonism of pathogen.

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Conflict of Interest. None.

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