

***In vitro* evaluation of new generation fungicides to control of *Fusarium oxysporum* Schlecht causing wilt of Isabgol**

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ABSTRACT

The present study was carried out to investigate the efficacy of fungicides under *in vitro* condition against *Fusarium oxysporum* Schlecht causing wilt of Isabgol. Out of selected nine fungicides *viz.*, Azoxystrobin 25% SC, Mancozeb-75% WP, Copper oxychloride-50% WP, Thiram-50% WP, Fosetyl-Al- 80% WP, Propineb-70% WP, Tebuconazole-50%+Trifloxystrobin-25% WG, Azoxystrobin 11% + Tebuconazole 18.3% SC and Hexaconazole 5% EC were used at 500 and 1000 ppm against wilt pathogen *F. oxysporum* Schlecht by poison food technique. The results revealed that Tebuconazole-50% + Tebuconazole-50% + Trifloxystrobin-25% WG showed maximum inhibition percent of mycelial growth of test fungus 98.04 and 100.00 at 500 ppm and 1000 ppm, respectively followed by Hexaconazole 5% EC.

Key words: *Fusarium oxysporum* Schlecht, Isabgol, Fungicides, Poison food technique, Tebuconazole-50%+Trifloxystrobin-25%WG, Hexaconazole,

Introduction

Isabgol (*Plantago ovata* Forsk.) is a short stemmed medicinal annual herb belongs to *plantaginaceae* family that is frequently referred to as 'Psyllium' in English and 'Isabgol' in Hindi. It is native to the West Asian and Mediterranean regions extending upto Sutlaj and Sindh in Pakistan. Isabgol grows up to a height of 10 to 40 cm. Ashwagolan, aspaghol, aspagol, bazarqutuna and blond Psyllium are some of its synonyms. Isabgol is mainly grown for the production of seed husk which contains colloidal mucilage that mostly consisting of xylose, arabinose and galacturonic acid with rhamnose and galactose (Jat *et al*, 2015). Psyllium husk is primarily used as traditional herbal medicine to treat constipation and other digestive system disorders (Voderholzer *et al.*,

1997). In India, area under isabgol cultivation was estimated at 448 thousand hectares with production of 432 thousand metric tonnes (Anonymous, 2018-19). The total export value of medicinal and aromatic plants in India was 200.89 thousand lakhs (Anonymous, 2019-20). In India, *Plantago ovata* is commercially cultivated in the western states of Gujarat, Madhya Pradesh and Rajasthan during winter season. At present, isabgol has acquired the name "Dollar earner" in north Gujarat and south western Rajasthan. In Rajasthan Isabgol is cultivated in about 3.49 lakh hectare with a production of 1.96 lakh tonnes and productivity about 563 kg per hectare (Agriculture statistics at a glance, 2020-21). In India, isabgol is commercially cultivated in North Gujarat, Rajasthan, Madhya Pradesh, Haryana and Punjab (Rathore and Pathak, 2002). The major

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Isabgol growing districts in Rajasthan include Chittorgarh, Barmer, Jalore, Jodhpur, Pali, Sirohi and Udaipur.

Many fungal pathogens, such as Fusarium wilt (*Fusarium oxysporum* Russel), damping off (*Pythium ultimum*), leaf blight (*Alternaria alternata* (Fr.) Keissler), downy mildews (*Peronospora plantaginis* Underwood), and powdery mildew (*Erysiphe cichoracearum* D.C.), are infecting the isabgol crop, causing significant yield losses and lowering the quality of the isabgol seed (Mandal, 2010). Isabgol wilt caused by *Fusarium oxysporum* Schlechtis one of the most serious soil-borne diseases which has been reported by Russel, 1975 and Mehta *et al.*, 1985. Meena and Roy (2020) reported that Fusarium wilt is the most important and wide spread disease of isabgol in Rajasthan which causes extensive damage to the isabgol crop. It occurs every year in severe form in the entire Isabgol growing areas.

Materials and Methods

Collection, Isolation and purification of pathogen
Isabgol plants showed drooping of leaves, yellowing and wilting symptoms were collected from plant pathology research field, Rajasthan College of Agriculture, Udaipur. The roots were washed under tap water to remove all visible soil particles. The infected root portion was cut into small pieces of 3-4 mm which were surface sterilized using 0.1% HgCl₂ solution for one minute and followed by three time rinsed with sterile distilled water. Those bits were transferred on PDA media under ascetic condition and inoculated plates were kept under 28±2 °C for 2-3 days and pure culture was obtained by single spore isolation method.

Evaluation of different fungicides against *F. oxysporum* in *In-vitro* condition

Efficacy of different fungicides was examined by using poisoned food technique (Nene and Thapliyal, 1993). Nine fungicides viz., Azoxystrobin 25% SC, Mancozeb-75% WP, Tebuconazole-50%+Trifloxystrobin-25%WG, Propineb-70%WP, Fosetyl-Al-80%WP, Thiram-50%WP, Azoxystrobin 11% + Tebuconazole 18.3% SC, Copper oxychloride-50%WP and Hexaconazole 5% EC were used for *in-vitro* bioassay at 500 and 1000 ppm against wilt pathogen *F. oxysporum* Schlecht.

Required quantity of each fungicides were measured as per ppm to get 500 and 1000 ppm added

separately into 100 ml sterile molten and warm PDA media taken in 250 ml conical flasks and thoroughly mix to dissolve the chemical and get appropriate concentrations of each fungicide. 20 ml of poisoned medium from each specific concentration were poured into each 90 mm sterilized Petri plate separately. Petri plates without amended fungicide was served as control. Three replications were maintained for each treatment. Each plate was placed with 5 mm mycelial bit aseptically taken from the periphery of 7 days old culture and incubated at 28±1°C in BOD incubator till the growth of colony touched the periphery in control plate. Mean colony diameter for each treatment was recorded after the fungus in control plates attained full growth. The per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1927) as follows

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

Where,

I = Per cent growth inhibition zone of pathogen

C = Growth of test fungus in control in (mm)

T = Growth of test fungus in treatment in dual culture (mm)

Results

In vitro evaluation of fungicides against wilt of isabgol pathogen *F. oxysporum* Schlecht

In vitro efficacy of selected nine fungicides viz., Azoxystrobin 25% SC, Mancozeb-75% WP, Copper oxychloride-50% WP, Thiram-50% WP, Fosetyl-Al-80% WP, Propineb-70% WP, Tebuconazole-50%+Trifloxystrobin-25% WG, Azoxystrobin 11% + Tebuconazole 18.3% SC and Hexaconazole 5% EC were used at 500 and 1000 ppm against wilt pathogen *F. oxysporum* Schlecht by poison food technique described in the materials and methods.

Among all fungicides, at 500 ppm and 1000 ppm concentration tested, Tebuconazole-50% + Trifloxystrobin-25% WG showed minimum radial mycelial growth of the pathogen (mm) 1.76 and 0.00 respectively followed by Hexaconazole 5% EC 6.00 and 2.82 mm at 500 ppm and 1000 ppm, respectively. Azoxystrobin 11% + Tebuconazole 18.3% SC recorded radial mycelial growth of the pathogen (mm) 15.35 and 6.73 at 500 ppm and 1000 ppm, respectively. Azoxystrobin 25% SC recorded mycelial growth of the pathogen (mm) 21.30 and 14.97.

Table 1. Fungicides used for *In vitro* evaluation against *F. oxysporum* Schlecht

S. No.	Technical name	Common name	Manufacturing company
1	Azoxystrobin 23% SC	Parizox	Parijat Industries India Pvt. Ltd.
2	Mancozeb-75%WP	Dithane M-45	DOW Agro sciences India Pvt. Ltd.
3	Copper oxychloride-50%WP	Blitox	Rallis India Limited TATA Enterprises
4	Thiram-50%WP	Thirox	Crop Chemicals India Limited
5	Fosetyl-Al-80%WP	Aliette	Bayer Corporation USA
6	Propineb-70%WP	Antracole	Bayer Corporation USA
7	Tebuconazole 50%+Trifloxystrobin 25% WG	Nativo	Bayer Corporation USA
8	Azoxystrobin 11% + Tebuconazole 18.3% SC	Spectrum	Dhanuka Agritech Ltd.
9	Hexaconazole 5% EC	Trigger	Agriplex Private Limited

Fosetyl-Al- 80% WP recorded radial mycelial growth of the pathogen (mm) 45.92 and 16.2 at 500 ppm and 1000 ppm, respectively. Likewise, Propineb-70% WP, Mancozeb-75% WP, Copper oxychloride-50% WP and Thiram-50% WP recorded radial mycelial growth of the pathogen (mm) observed 27.12 and 21.37, 65.01 and 32.89, 45.07 and

36.43, 50.18 and 39.8 at 500 ppm and 1000 ppm, respectively.

Among all fungicides, Tebuconazole-50% + Trifloxystrobin-25% WG showed maximum Per cent growth inhibition 98.04 and 100.00 at 500 ppm and 1000 ppm, respectively followed by Hexaconazole 5% EC 93.33 and 96.86 at 500 ppm and 1000 ppm, respectively. Azoxystrobin 11% + Tebuconazole 18.3% SC showed Per cent growth inhibition 82.94 and 92.51 at 500 ppm and 1000 ppm, respectively. Azoxystrobin 25% SC recorded Percent growth inhi-

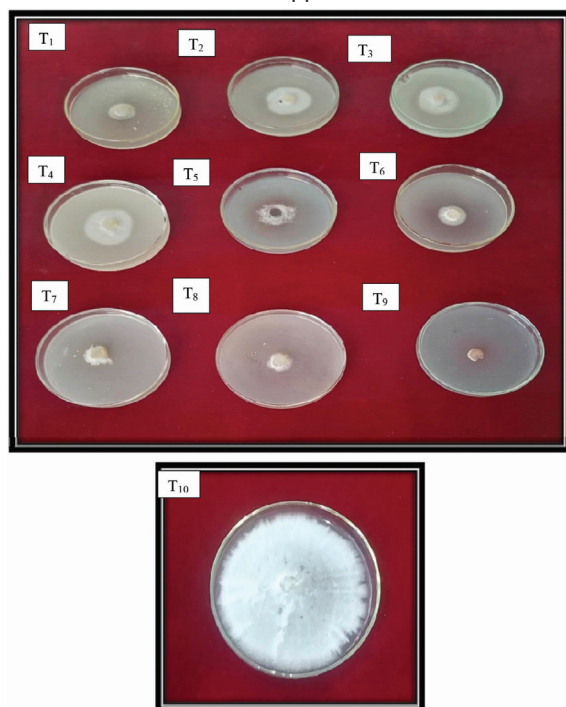


Plate 1a. Comparative efficacy of different fungicides on the mycelial growth of *F. oxysporum* at 500 ppm concentrations *in vitro*

T₁- Azoxystrobin 11% + Tebuconazole 18.3% SC, T₂- Propineb-70% WP, T₃-Fosetyl-Al-80% WP, T₄- Mancozeb-75% WP, T₅- Azoxystrobin 25% SC, T₆- Copper oxychloride-50% WP, T₇- Thiram-50% WP, T₈- Hexaconazole 5% EC, T₉- Tebuconazole 50%+Trifloxystrobin 25% WG, T₁₀- Control

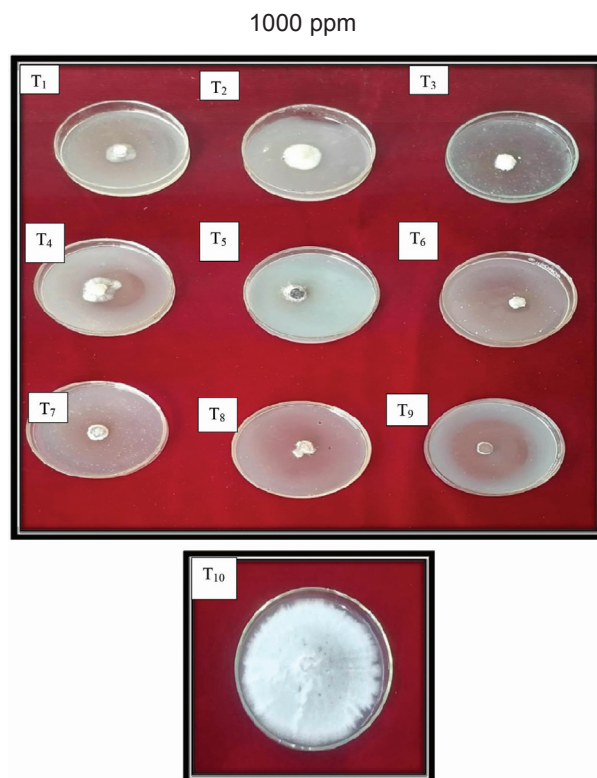


Plate 1b. Comparative efficacy of different fungicides on the mycelial growth of *F. oxysporum* at 1000 ppm concentrations *in vitro*

Table 2. Comparative efficacy of different fungicides against *F. oxysporum* Schlect *In vitro*

Sl. No.	Treatments	Colony growth (mm)*		Per cent growth Inhibition (%)		
		500 ppm	1000 ppm	500 ppm	1000 ppm	
1	Azoxystrobin 25% SC	21.30	14.97	76.32(60.91)	83.42(65.93)	
2	Thiram 50% WP	50.18	39.8	44.23(41.69)	55.83(48.32)	
3	Fosetyl AL 80%WP	45.92	16.2	48.98(44.41)	81.96(64.87)	
4	Mancozeb 75% WP	65.01	32.89	27.76(31.80)	63.45(52.81)	
5	Copper oxychloride 50% WP	45.07	36.43	49.91(44.95)	59.52(50.49)	
6	Propineb 70% WP	27.12	21.37	69.86(56.71)	76.25(60.84)	
7	Azoxystrobin 11% + Tebuconazole 18.3% SC	15.35	6.73	82.94(65.61)	92.51(74.13)	
8	Hexaconazole 5% EC	6	2.82	93.33(75.13)	96.86(79.80)	
9	Tebuconazole 50%+Trifloxystrobin 25% WG	1.76	0	98.04(83.43)	100(90.00)	
10	Control	90	90	0(0.00)	0(0.00)	
	SEm±	CD at 5%	CD at 1%	SEm±	CD at 5%	CD at 1%
Fungicides	0.869	2.489	3.318	0.675	1.932	2.575
Concentration	0.388	1.133	1.484	0.301	0.864	1.152
F×C	1.229	3.521	4.692	0.954	2.733	3.642

*Mean of three replications

Figure in parentheses are arcsine percent angular transformed values

bition 76.32 and 83.42 at 500 ppm and 1000 ppm, respectively. Likewise, Fosetyl-Al- 80% WP, Propineb-70% WP, Mancozeb-75% WP, Copper oxychloride-50% WP and Thiram-50% WP recorded Percent growth inhibition 48.98 and 81.96, 69.86 and 76.25, 27.76 and 63.45, 49.91 and 59.52, 44.23 and 55.83 at 500 ppm and 1000 ppm, respectively.

T₁- Azoxystrobin 11% + Tebuconazole 18.3% SC, T₂- Propineb-70% WP, T₃-Fosetyl-Al-80% WP, T₄- Mancozeb-75% WP, T₅- Azoxystrobin 25% SC, T₆- Copper oxychloride-50% WP, T₇- Thiram-50% WP, T₈- Hexaconazole 5% EC, T₉- Tebuconazole 50%+Trifloxystrobin 25% WG, T₁₀- Control

Discussion

The results of current *in vitro* studies indicated that out of nine fungicides tested, *viz.*, Azoxystrobin-25% SC, Mancozeb-75% WP, Tebuconazole-50% + Trifloxystrobin-25% WG, Propineb-70% WP, Fosetyl-Al-80% WP, Thiram-50% WP, Azoxystrobin 11% + Tebuconazole 18.3% SC, Copper oxychloride-50% WP and Hexaconazole 5% EC. Overall the minimum colony growth of pathogen was observed in Tebuconazole-50%+Trifloxystrobin-25% WG at concentration of 500 ppm and 1000 ppm, the colony growth was 1.76 and 0.00 in mm, respectively. Whereas, maximum colony growth of pathogen (*Fusarium oxysporum*) was observed in Mancozeb-75% WP at @ concentration of 500 ppm and 1000

ppm, the colony growth was 65.01 and 32.89 in mm, respectively and maximum Per cent growth inhibition of Tebuconazole-50%+Trifloxystrobin-25% WG was 98.04 and 100.00 per cent at 500 ppm and 1000 ppm, respectively. The findings of present investigation are similar to (Salman *et al.*, 2021) where, maximum mycelial growth inhibition of *Fusarium* spp. reported by Tebuconazole-50% + Trifloxystrobin-25% WG with the 81.10 and 88.00 per cent at 750 and 1000 ppm, respectively. Similarly, Mengal *et al.*, 2015 and Kewal and Singh, 2021 tested efficacy of different nine fungicides against *Fusarium oxysporum in vitro* and found that fungicides Tebuconazole 50% + Trifloxystrobin 25% WG showed best effective against pathogen.

Conclusion

Among all fungicides, at 500 ppm and 1000 ppm were tested, Tebuconazole-50% + Trifloxystrobin-25% WG showed maximum Per cent growth inhibition 98.04 and 100.00 at 500 ppm and 1000 ppm, respectively followed by Hexaconazole 5% EC 93.33 and 96.86 at 500 ppm and 1000 ppm, respectively.

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Conflict of Interest: None

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