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In vitro Evaluation of Fungicides and Bio-agents for the Management of Lentil Wilt caused by *Fusarium oxysporum* f. sp. *lentis*

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ABSTRACT: Lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis* is a significant disease and a major limiting factor for production of lentil. The present study on *in vitro* evaluation of fungicides and bio-agents against *Fusarium oxysporum* f. sp. *Lentis* revealed that fungicides provided better inhibition in growth of test pathogen than *Trichoderma* spp. Among the seven different fungicides, Thiophanate Methyl 450g/l + Pyraclostrobin 50g/l@800 ppm was recorded as best treatment which maximum inhibited the growth of test pathogen (92.60%). Tebuconazole 5.36% w/w FS when applied @800 ppm concentrations was identified another potential fungicide with 91.60% inhibition. Among the four different species of *Trichoderma, T. asperellum* was found most effective in inhibiting the growth of *Fusarium oxysporum* f. sp. *lentis*.

Keywords: Lentil, *Fusarium oxysporum* f. sp. *lentis*, *in vitro* evaluation, fungicides and bioagents.

INTRODUCTION

Lentil (*Lens culinaris* Medik) is one of the oldest legume crops and rich source of protein and some other amino acids like lysine and tryptophane, which is traced back to 13000- and 7000-years BC in the world and Asia, respectively (Sandhu and Singh 2007; Zapata *et al*., 2004; Kumar *et al*., 2021). It is commonly known as masoor or poor man's meat (Sen and Kapoor 1975).

Major lentil growing states in India are Madhya Pradesh (42.50%), Uttar Pradesh (31.25%), West Bengal (9.38%) and Bihar (8.75%). In India, lentil was grown in 1.49 mh with production of 1.61 mt with an average production of 1006 kg/ha (Anonymous, 2018; Ahmad, *et al*., 2018; Abraham, 2015).

Lentil production is challenged by a wide range of pathogens (Nelson *et al*., 1983; Dubey, 2020). Among the different diseases of lentil *viz*., Fusarium wilt (*Fusarium oxysporum*), Collar Rot (*Sclerotium rolfsii*), Root Rot (*Rhizoctonia solani*), Alternaria blight (*Alternaria alternata*), Aphanomyces Root Rot (*Aphanomyces euteiches*), Anthracnose (*Colletotrichum lindemuthianum*), Rust (*Uromyces fabae*), Ascochyta Blight (*Ascochyta fabae* f.sp. *lentis*), Botrytis gray mold (*Botrytis cinereal*) and Sclerotinia stem rot (*Sclerotinia sclerotiorum*), wilt is major limiting factor in its production and productivity (Lindbeck, 2009).

Fusarium oxysporum f. sp. *lentis* persists in soil through chlamydospores and remains viable for several seasons. It has a very narrow host range infecting only lentil, nevertheless it exhibits significant morphological as well as pathogenic variability (Vasudeva and Srinivasan 1952; Belabid *et al*., 2004; Bayaa *et al*., 1986).

The wilt disease affected both seedlings and flowering stage (Adult stage) and appears as spots/ patches in the field. *Fusarium oxysporum* f. sp. *Lentis* infection is considered by a sudden drooping of the lentil leaves, followed by dull green leaves with drying and the ultimate death of the seedling plants. The root system of infected plants shows brown discoloration of the vascular system (Lindbeck, 2009; Prasad *et al*., 2019).

The pathogen is known to produce three kinds of asexual spores; micro conidia, macro conidia and chlamydospores. Microconidia are usually single celled, ovoid or kidney-shaped and hyaline. Macroconidia are usually two to seven celled, long with

pointed apical cell and notched basal cell. Chlamydospores are single celled, oval or spherical shaped and thick walled, formed singly in macroconidia or apical or intercalary in the hyphae (Khare, 1980; Sugha *et al*., 1994).

The wilt appeared in field within three to four weeks after sowing under favorable condition, the wilt infection can damage the crop yield, and vascular wilt considerable important disease in India causing more than 50 per cent loss in some field (Khare, 1981; Vasudeva and Srinivason 1952; Kumar *et al.*, 2009).

Use of biocontrol agents is an eco-friendly management strategy for different plant disease management and plant growth promotion (Kumar *et al.*, 2013; Kumar *et al.*, 2014). The secondary metabolite produced by *Trichoderma* spp. are also helpful in plant disease management (Kumar *et al.*, 2010) and besides their role as antagonistic fungus, they also serve as potential bio-fertilizers leading to plant growth promotion (Srivastava *et al.*, 2009; Kumar *et al.*, 2019). However, sometimes, the efficacy of bio-control agents is not as effective as fungicides due to pre-vailing edaphic and environmental conditions. Therefore, in the present investigation, fungicides and different species of Trichoderma were evaluated for their efficacy in inhibiting the *F. oxysporum* f. sp. *lentis*.

MATERIAL AND METHOD

Isolation of *Fusarium.* The symptomatic diseased samples were collected from the lentil field and isolation of the test pathogen was conducted by tissue transfer technique. The diseased tissue (root) was cut into small bits (2-3 mm) with a sharp, sterilized blade so that each diseased tissue contained a portion of healthy tissue along with it. These bits were subjected to surface sterilization with 1% sodium hypochlorite solution under aseptic condition, followed by three rinses with distilled sterilized water to remove the remaining traces of sodium hypochlorite. The tissue pieces were blotted, dried, and later transferred aseptically to Potato Dextrose Agar (PDA) medium in sterilized Petri-plates (90mm) and incubated at 27±2°C for seven days. The fungal colonies originating from bits were examined after seven days of incubation, then transferred on fresh medium in Petri-plates for the purification and periodic observations (Booth, 1971).

Evaluation of Fungicides. Seven chemicals namely Azoxystrobin 23% SC, Carboxin 37.5% + Thiram 37.5% DS, Thiophanate Methyl 450g/l + Pyraclostrobin 50g/l, Tebuconazole 5.36% w/w FS, Azoxystrobin 11% + Tebuconazole 18.3%, Tebuconazole 50% + Trifloxystrobin 25% WG, and Flusilazole 12.5% + Carbendazim 25% SE were tested against the pathogen by employing poisoned food technique. The chemicals were incorporate in 20ml medium before pouring it into Petri plate separately, and a disc of 5mm of seven days old culture of fungus was transferred to each Petri plate and kept in BOD incubator for eight days for the full growth of fungus as observed in case of control where no chemicals were applied in medium. The inhibition in the radial growth of pathogen mycelium was recorded and percent inhibition in the mycelium of pathogen was recorded by comparing the mycelial growth of fungus in the treated plates with control. Per cent growth inhibition was calculated by using the formula given by (Vincent, 1947).

Observation Recorded

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

Where, PI = Inhibition percentage

C = Colony diameter in check plate (mm)

T = Colony diameter in treatments (mm)

The different concentrations of the chemicals taken for the *in-vitro* experiment was 100ppm, 200ppm, 400ppm, 600ppm and 800ppm.

Efficacy of *Trichoderma* spp. The antagonistic potential of *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma virens* and *Trichoderma asperellum* against *Fusarium oxysporum* f. sp. *lentis* was calculated in dual culture technique. The mycelial disc of 5 mm diameter from the margin of seven days old culture of bio-agents and test pathogen were placed on solid PDA in paired combination. Control set was made by inoculating test pathogen singly on the medium. Dual Petri dishes were incubated at 27°C in BOD incubator and the extent of interaction was observed by calculating per cent inhibition in growth of test fungi in respective treatments (Chandra *et al.*, 2020).

The per cent inhibition of the interacting fungi was calculated as follows:

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

C = Radial growth of *Fusarium oxysporum* f. sp. *lentis* in control.

T = Radial growth of *Fusarium oxysporum* f. sp. *lentis* in dual culture.

Statistical analysis. The data was analysed statistically using analysis of variance (ANOVA) at probability level 0.05 by using OPSTAT software (Sheoran *et al.*, 1988).

RESULTS

Isolation and identification of *F. oxysporum.* *Fusarium oxysporum* f.sp. *lentis*, was isolated from the infected root parts of lentil. The identification of *F. oxysporum*on the basis of (cultural and morphological characterization) was confirmed by compound microscope and trinocular microscopic at Seed Technology Research Center, JNKVV, Jabalpur, Madhya Pradesh, India. The wilted plant sample used for isolation of test pathogen, pure culture of isolated pathogen and microscopic identification through the presence of macro and micro conidia and formation of chlamydospores are given in Fig. 1.

Fig. 1. (a) Wiled plant of lentil; (b) Pure culture of *Fusarium oxysporum* f. sp. *lentis*; (c) Macro- and micro conidia and (d) Chlamydospores.

***In-vitro* evaluation of different fungicides against *F. Oxysporum*.** Under *in vitro* evaluation of different fungicides against *Fol* revealed that all the fungicides significantly inhibited the growth of test pathogen. However, per cent inhibition in growth of test pathogen significantly varied not only in different fungicides but at different concentrations also. Further, besides fungicides, different species of Trichoderma also exhibited great impact on growth of test pathogens. However, fungicides exhibited more impact on inhibition of growth of test pathogen in comparison to *Trichoderma* spp. The radial growth and per cent inhibition in growth of *Fol* in different fungicidal treatments are presented in Table 1 (Fig. 2 & 3).

Table 1: Efficacy of different concentration of fungicides against *Fusarium oxysporum* f. sp. *lentis*

Treatments		Mycelial radial growth after 8 days (in mm) and percent inhibition in mycelial growth of test fungus*, **									
	Dose	100ppm		200ppm		400ppm		600ppm			800ppm
Fungicides	Active ingredient	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition
Amistar	Azoxystrobin 23% SC	31.80^{d}	61.80 (7.908^{b})	30.33^{cd}	63.60 (7.968^{bc})	19.67^{d}	76.40 (8.79^{a})	13.33^{de}	84.00 (9.22^{ab})	8.183^{c}	90.20 (9.544^{a})
Vitavax power	Carboxin 37.5% + Thiram 37.5% DS	55.70^{bc}	33.20 (5.848^{cd})	53.17^{b}	36.20 (6.094^{d})	40.00^{b}	52.00 (7.276^{c})	31.00^{b}	62.80 (7.97^{d})	14.67^{b}	82.40 (9.132^{b})
Xelora	Thiophanate Methyl 450g/l + Pyraclostrobin 50g/l	21.70^{e}	74.00 (8.66^{a})	19.17^{de}	77.00 (8.832^{ab})	17.00^{d}	79.60 (8.978^{a})	12.50^{de}	85.00 (9.27^{ab})	6.17^{c}	92.60 (9.674^{a})
Raxil Easy	Tebuconazole 5.36% w/w FS	19.00^{e}	77.20 (8.843^{a})	17.50^{e}	79.00 (8.944^{a})	15.50^{d}	81.40 (9.077^{a})	10.67^{e}	87.20 (9.39^{a})	7.00^{c}	91.60 (9.623^{a})
Coustodia	Azoxystrobin 11% + Tebuconazole 18.3%	53.70^{c}	35.60 (6.05^{c})	38.33^{c}	54.00 (7.416^{c})	29.33^{c}	64.80 (8.111^{b})	23.83^{c}	71.40 (8.51^{c})	9.83^{c}	88.20 (9.445^{a})
Nativo	Tebuconazole 50% + Trifloxystrobin 25% WG	61.00^{b}	26.80 (5.269^{e})	41.50^{bc}	50.20 (7.155^{c})	28.33^{c}	66.00 (8.185^{b})	17.83^{d}	78.60 (8.92^{b})	8.00^{c}	90.40 (9.56^{a})
Luster	Flusilazole 12.5%+ Carbendazim 25% SE	59.30^{bc}	28.80 (5.457^{de})	42.00^{bc}	49.60 (7.113^{cd})	28.67^{c}	65.60 (8.16^{b})	17.00^{d}	79.60 (8.98^{ab})	8.00^{c}	90.40 (9.56^{a})
Control	-	83.30^{a}	-	83.33^{a}	-	83.33^{a}	-	83.33^{a}	-	83.33^{a}	-
CD at 5%		5.724	0.475	11.397	0.923	5.068	0.384	5.271	0.419	4.199	0.282
SE (m)		1.893	0.155	3.769	0.301	1.676	0.125	1.743	0.137	1.389	0.092

*-the values in the parenthesis are square root transformed values and values are means of three replicates

**-the values with different alphabets are significantly superior at P <0.05 level.

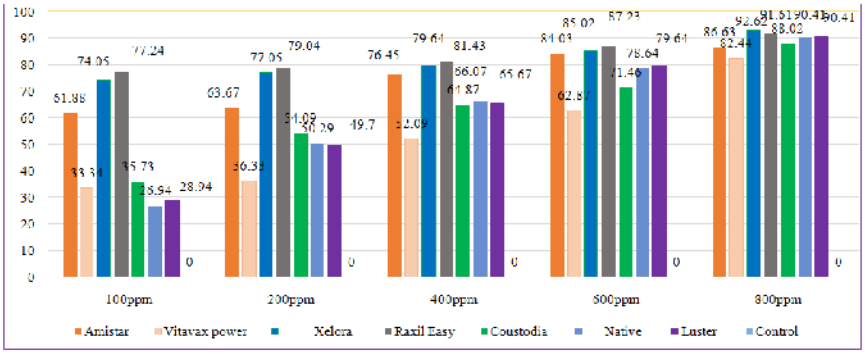

Fig. 2. The per cent inhibition in growth of Fol at different concentration of fungicides.

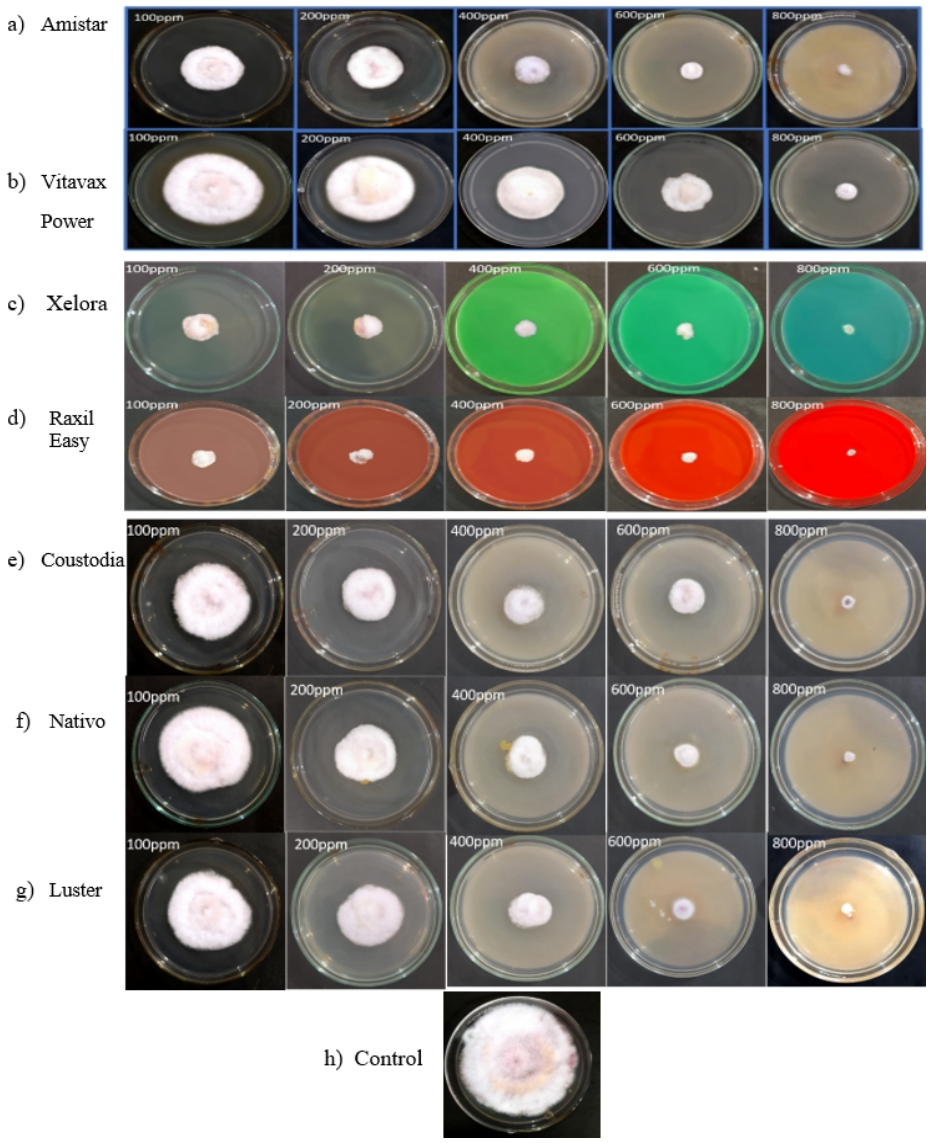

Fig. 3. Evaluation of different fungicides against *Fol* at different concentrations.

The *in-vitro* evaluation of seven different fungicides revealed that all the tested fungicides were significantly superior over control at different concentrations (100, 200, 400, 600 and 800 ppm). At 100, 200, 400 and 600 ppm, Raxil easy showed maximum inhibition in mycelial growth of *Fol i.e.,* 77.2, 79.0, 81.4 and 87.2 per cent at 100, 200, 400 and 600 ppm respectively. However, at 800 ppm, Xelora showed the maximum inhibition *i.e.,* 92.6 per cent. The minimum mycelial inhibition was showed by Vitavax power at all the concentrations (33.2, 36.2, 52.0, 62.8 and 82.4% at 100, 200, 400, 600 and 800 ppm respectively).

***In-vitro* evaluation of different bio-agents against *F. Oxysporum*.** The *in-vitro* evaluation of bio-agents, *Trichoderma viride, Trichoderma harzianum, Trichoderma virens* and *Trichoderma asperellum* showed that all the tested species of *Trichoderma* were able to inhibit the growth of test pathogen. However, maximum inhibition in radial growth of *Fol* was exhibited by *Trichoderma asperellum* where only 30.80mm radial growth could be witnessed with a per cent inhibition of 64.06% However, the minimum inhibition of 42.59% could be recorded in *T. harzianum* with maximum colony growth of 49.17 mm. The average growth rate of *Fol* was calculated which was found to be maximum in control (10.71 mm per day) and among the different treatments, minimum growth rate of 3.85 mm per day was recorded in treatment with *T. asperellum* (Table 2, Fig. 4).

Table 2: Per cent inhibition in growth of *F. oxysporum* f. sp. *lentis* using different species of *Trichoderma* under *in vitro* conditions

Fungal antagonist	Radial growth after 8 days (mm) **	Per cent inhibition*	Radial growth (mm/day)
Trichoderma viride	33.33[d]	61.14 (7.88[a])	4.16
Trichoderma harzianum	49.17[b]	42.59 (6.599[c])	6.15
Trichoderma virence	38.67[c]	54.84 (7.474[b])	4.83
Trichoderma asperellum	30.83[d]	64.06 (8.063[a])	3.85
Control	85.67[a]	0.00	10.71
C.D. at 5%	4.35	0.386	
SEm±	1.36	0.117	

*-the values in the parenthesis are square root transformed values and values are means of three replicates
**-the values with different alphabets are significantly superior at P <0.05 level.

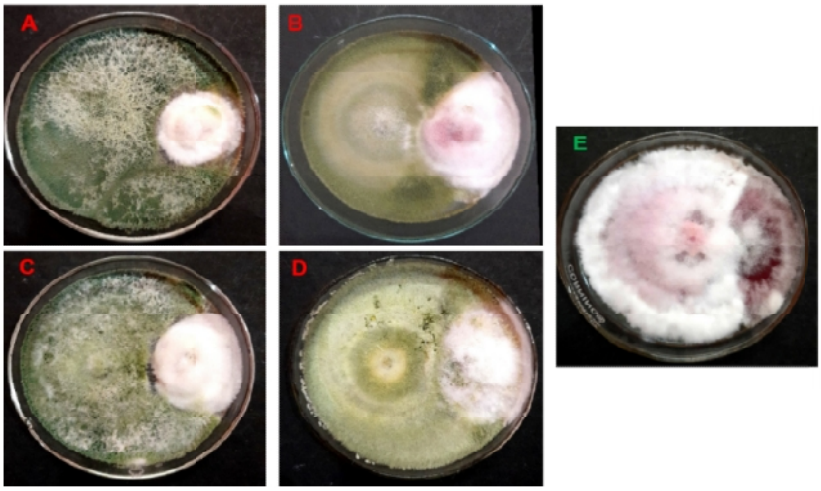

Fig. 4. Dual culture assay of different species of Trichoderma with *Fusarium oxysporum* f. sp. *lentis* (a) *Trichoderma viride* (b) *Trichoderma harzianum* (c) *Trichoderma virens* (d) *Trichoderma asperellum* and (e) Control.

In the present investigation, Tebuconazole 5.36% w/w FS showed maximum inhibition in growth of *Fol* at all the tested concentrations except at 800 ppm. However, Thiophanate Methyl 450g/l + Pyraclostrobin 50g/l was found best treatment at 800 pm and exhibited 92.60% growth inhibition of *Fol*. The findings of present investigation are in agreement of Maheshwari *et al*. (2008); Chanu *et al*. (2019), where they also reported the efficacy of these fungicides in controlling Fusarium wilt pathogen in lentil and pea respectively. Further, *Trichoderma* has been reported to be an effective bioagent against various plant pathogens under *in vitro and in vivo* conditions (Jain *et al.,* 2017; Kumar and Sahu 2015; Kumar *et al.,* 2013; Singh *et al*., 2014). The findings of present investigations are similar to the findings of Tiwari *et al.* (2018); Bana *et al*. (2017); Singh *et al*. (2017; Choudhary and Mohanka 2012) where they reported variable degree of per cent inhibition in growth of test pathogen using different species of *Trichoderma*.

CONCLUSION

All the tested fungicides were significantly superior over control in inhibiting the growth of *Fol*. However, a combination product Thiophanate Methyl 450g/l + Pyraclostrobin 50g/l @ 800ppm exhibited maximum per cent inhibition across all the tested fungicides and concentrations. Tebuconazole 5.36% w/w FS could be identified as second-best fungicide at 800 ppm and best fungicides at all the other tested concentrations. These identified fungicides could be useful in management of Fusarium wilt of lentil. Further, among the different species of *Trichoderma*, *T. asperellum* was identified as better antagonist for *Fol* and can be recommended for management of Fusarium wilt in lentil and organic/ commercial cultivation of lentil.

FUTURE SCOPE

The fungicides identified in present investigations could be utilized for management of wilt of chickpea. The identified chemicals and *T. asperellum* can be used in natural field conditions as seed treatment and/or soil drenching individually or in combination to develop a

suitable integrated approach for the management of lentil wilt.

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Conflict of Interest. None.

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