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Efficacy of Fungicides and Bio-agents against *Fusarium oxysporum* f.sp. *lentis* Causing vascular Wilt of Lentil (*Lens culinaris* Medik) *in-vitro*

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A B S T R A C T

Lentil (*Lens culinaris* Medik) is an important crop for farming systems in India, the major states producing lentil are Madhya Pradesh, Uttar Pradesh, Bihar and Punjab. It is one of the most important and nutritious *Rabi* pulse crop. The lentil crop is mostly affected by various fungal diseases e.g. vascular wilt (*Fusarium oxysporum* f.sp. *lentis*), Sclerotinia stem rot (*Sclerotinia sclerotiorum)* and collar rot (*Sclerotium rolfsii* Sacc.), in which vascular wilt is most destructive soil borne diseases of lentil growing area worldwide. In the present investigation the eight commercially available fungicides were tested *in vitro* for their efficacy in inhibiting the growth of the pathogen and evaluated the antagonistic efficacy of potent bio- control agents against *Fusarium oxysporoum* f.sp. *lentis.* It was observed that the 100 per cent inhibition of mycelial growth of pathogen was found in the treatment with Carbendazim 50 %WP, Cymoxanil 8%+ Mancozeb 64%, Tebuconazole 25.9% EC and Propiconazole 25% EC at all three concentrations (50ppm, 75ppm and 100ppm). The least inhibition per cent of mycelial growth was recorded (50.00%, 54.17% and 60.42%) in Mancozeb 75 % WP at all three concentrations (50ppm, 75ppm and 100 ppm) after 144 hrs. incubation. In the tested bio-agents, the maximum mycelial growth inhibition was recorded by *Trichoderma viride* (76.25 %)) which was significantly superior from all the tested bio-agents followed by *Chaetomium globosum* (75.00%) and *Pseudomonas fluorescens* (71.25%). The minimum mycelial growth inhibition was recorded by *Bacillus subtilis* (55.00%) followed by *Trichoderma harzianum* (68.75%) at 144 hours.

Introduction

Lentil (*Lens culinaris* L.) is an important crop in leguminous family (sub family "Papilionaceae"). This crop is a global importance and grown particularly in India, Pakistan, Bangladesh, Nepal and Iran. Lentil is an annual, bushy herb, erect or sub-erect, 15-75 cm tall, highly branched, softly hairy, stems slender, square and ribbed crop. It is adapted to cool temperate steppe through sub-tropical dry to moist ambience environment and it doesn't tolerate water logging condition. It is capable to withstand 4-12

week drought. Lentils are usually sown in those areas where temperature ranges between 20-30^{o}C. During 2016-17 cropping year lentil production was 6.71 million tones with 5.45 million hectares of area and productivity was 1105 kg/ha in worldwide while in India, total area under lentil crop is about1.276 million hectares, with production 0.976 million tones which contributes 6.18% share of total pulse production and productivity 764.9 kg/ha (FAOSTATS, 2016-17). Uttar Pradesh accounts for the maximum production in the country contributing to around 45% of the country's production as well as forth maximum area under lentil cultivation. In Uttar Pradesh, total area under lentil crop is about 0.44 mha with production 0.24 mt. and productivity 537 kg/ha (Agriculture statistics & amp; crop insurance, 2014–2015). The lentil production is greatly affected by many biotic and abiotic factors, among which diseases are the main constraint in the lentil production. The Lentil crop is affected by various fungal diseases e.g. Vascular wilt (*Fusarium oxysporum* f.sp. *lentis*), Sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) and collar rot (*Sclerotium rolfsii* Sacc.) is most destructive soil borne diseases of lentil growing area of worldwide.

Vascular wilt (*Fusarium oxysporum* f.sp. *lentis*) plays a major role in reducing lentil yield (Pouralibaba *et al.,* 2015), The pathogen can cause infection in all stages of plant growth with more incidences at flowering and pod stages than early vegetative stage (Chavdarov, 2006). Fusarium wilt is a vascular disease, is the most devastating of all lentil diseases worldwide that can cause extensive yield losses reaching up to 100% in prolonged favorable environments (Kumar *et al.,* 2010). Control of soil borne pathogens has become one of the major concerns in agriculture. However effective and efficient management of crop diseases is generally achieved by the use of synthetic pesticides. These pesticides are known to pollute the environment, soil and water besides causing deleterious effects on human health and biosphere. In the present investigation is related to the study of the management of wilt disease of lentil by different fungicides, bioagents and comparing the efficacy of both.

Materials and Methods

Isolation, purification and identification of antagonistic microorganisms

Isolation of bio-agents was done by serial dilutions technique. 10 gm of rhizospheric soil was dissolved in 100 ml of sterile distilled water to get 10^{-1} dilution. 1 ml of soil suspension was taken from stock and added to 9 ml of sterile distilled water to get 10^{-2} dilution. This is further repeated until a final dilution of 10^{-7} was obtained. 1 ml of each soil suspension was poured in sterilized petriplates containing nutrient medium and incubated at 25 ± 1°C and observed at frequent intervals for the development of colonies of bio-agents. Bio-agents were identified based on cultural and mycological characters described by Barnett and Hunter.

Isolation and purification of the pathogen

The diseased plant showing the symptoms were washed thoroughly with tap water, small pieces from infected parts 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with the help of sterilized blade. These pieces were surface sterilized with 1% sodium hypochlorite solution for 30 seconds and finally washed well in three changes of sterilized distilled water to remove trace of sodium hypochlorite. The pieces were then transferred aseptically to petri plates containing Potato Dextrose Agar. Inoculated Petri plates were incubated at 26 ±1^{o}C for three to five days and examined at frequently

intervals to see the growth of the fungus. The pure colonies were picked and inoculate in culture tube for further research work.

Dual culture technique

Antagonistic activities of bio control agent were tested against soil borne plant pathogen *F. oxysporum* f.sp. *lentis* by employing dual culture techniques of Morton and Stroube (1955) on PDA.

A mycelial disc (5 mm.), obtained from the peripheral region of 5-7 day old culture of pathogen on PDA, was placed on fresh PDA plate (3 cm from centre) then a 5 mm mycelial disc, obtained from the periphery of a 5-7 day old culture of fungal bio agents were placed 3cm away from the inoculum of the pathogen, for bacterial bio agents were streaked 3 cm away from the inoculum of the pathogen. Three replication of each treatment were maintained with one control set without inoculating the bio inoculants. Then the plates were incubated at 26 ± 1^0C. At the end of incubation period, radial growth was measured. The inoculated plates with culture discs of pathogen without bioagents served as control. After 48, 96 and 144 hrs of incubation at 26 ± 1^0C, radial growth of pathogen and percent inhibition was recorded.

Poisoned food technique

Bioassay test of fungicides were done by poisoned food technique (Schmitz, 1930) in the laboratory to find out the toxicity fungi of different fungicides against the pathogen. The efficacy of eight systemic and non-systemic fungicides was assayed against pathogen at different concentrations. The required concentrations of chemicals were prepared and incorporated into sterilized, cooled Potato Dextrose Agar. 20 ml of medium was poured into 90 mm sterilized petri plates and all plates were inoculated with actively growing 5 mm mycelial disc of *F. oxysporum* f.sp. *lentis*. Three replications were maintained for each treatment. These plates were incubated at 26±1°C for five days in an incubator and colony diameter was recorded (Table 1 and 2).

Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947).

% inhibition of mycelial growth
Where,
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Results and Discussion

Efficacy of bio-control agents against *Fusarium oxysporum* f.sp. *lentis*

Five bio-agents were evaluated for their efficacy against *Fusarium oxysporum* f.sp.*lentis* through dual culture technique as explained in material and method. The result revealed that there was significant mycelial growth inhibition of *Fusarium oxysporum* f.sp.*lentis* by all the tested bio agents. The maximum (76.25%) mycelial growth inhibition of pathogen was recorded in *Trichoderma viride* which was significantly superior from all the tested bio-agents followed by *Chaetomium globosum* (75.00%) and *Pseudomonas fluorescens* (71.25%). Whereas, minimum mycelial growth inhibition was recorded by *Bacillus subtilis* (55.00%). *Trichoderma* spp. have potential antagonistic activity by parasitism, antibiosis and completion in inhibiting the growth of pathogen *Fusarium oxysporum* Choudhary *et al.,* (2012). Sivamani and Gnanamaniekan (1988) tested several strains of *Pseudomonas flurorescens* and reported the inhibitory effect-on *Fusarium oxysporum*. *Pseudomonas* comprises a large group and they produced wide range of antifungal metabolites active against *Fusarium* species (Ligoy *et al.,* 2000) (Fig. 1).

Effect of fungicides on inhibiting mycelial growth of *Fusarium oxysporum* f.sp. *lentis*

An experiment were conducted to determine the inhibitory effect of 8 fungicides *viz.* Mancozeb 75 % WP, Carbendazim 50 %WP, Cymoxanil 8%+ Mancozeb 64%, Thifluzamide 24%SC,Tebuconazole 25.9% EC, Hexaconazole 5% SC, Propiconazole 25% EC and Chlorothalonil 75%WP at three concentration 50,75 and 100 ppm. The results from (Table-3, 4 and 5) revealed that there is significant difference in per cent inhibition of mycelial growth of pathogen. Maximum 100 % inhibition of mycelial growth of pathogen was recorded with Carbendazim, Cymoxanil + Mancozeb, Tebuconazole and Propiconazole followed by 82.08%, 84.58% and 91.67% in Chlorothalonil at 50ppm, 75ppm and 100ppm respectively. The least inhibition per cent of mycelial growth was recorded 50%, 54.17% and 60.42% in Mancozeb all three concentrations i.e. 50ppm, 75ppm and 100ppm respectively after 144 hrs. incubation. Similarly, The results of our studies are similar to earlier of several workers Maheshwari *et al.,* (2008) tested seven fungitoxicants against *Fusarium oxysporum* f. sp. *lentis in vitro*. All these significantly checked the growth of the pathogen as compared to control. Carbendazim proved most effective fungitoxicant for checking the fungal growth, followed by Captan, Hexaconazole and Diniconazole. Singh *et al.,* (2010) obseved efficacy of six fungicides against *Fusarium oxysporum* f.sp.*lentis* revealed that carbendazim and carboxin completely inhibited the growth of *Fusarium oxysporum* f.sp. *lentis* whereas, thiram and captafol inhibited 87.5 and 83.1% of mycelial growth, respectively (Fig. 2).

Table.1 List of fungicide used

Common Name	Trade name(a.i)	Chemical name	Concentration (in ppm)
Mancozeb	DithaneM- 45 75% WP	Maganese + zincethylene Bisdithiocarbomate	50,75,100
Carbendazim	Bavistin50% WP	2-(methoxyphenyl)-Benzimidazole	50,75,100
Cymoxanil 8%+ Mancozeb 64%	Curzate M	1-(2-cyano-2-methoxyiminoacetyl)-3-ethyl urea;DPX3217M	50,75,100
Thifluzamide 24%SC	Pulsor	2',6'-dibromo-2-methyl-4'-trifluoromethoxy-4trifluoromethyl-1,3—thiazole-5 carboxamide	50,75,100
Tebuconazole	Folicur	1-(4-Chlorophenyl)-4,4-dimethyl-3	50,75,100
Hexaconazole	Contaf 5% EC	1-(2,4-Dichlorophenyl)-1	50,75,100
Propiconazole	Tilt 25% EC	1-{[2-(2,4 Dichlorophenyl)-4-propyl-1,3-dixolan-2- yl]methyl}-1H-1	50,75,100
Chlorothalonil	Bravo	2,4,5,6- Tetrachlorobenzene-1,3-Dicarbonitrile	50,75,100

Table.2 Effect of Bio-agents on radial growth of *Fusarium oxysporum* f.sp. *lentis*

Treatments	Inhibition after 48 hours		Inhibition after 96 hours		Inhibition after 144 hours	
	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition
Trichoderma viride	18.00	10.00	19.00	52.50	19.00	76.25
Trichoderma harzianum	20.00	00.00	27.00	32.50	25.00	68.75
Chaetomium globosum	17.00	15.00	17.00	57.50	20.00	75.00
Bacillus subtilis	20.00	00.00	32.00	20.00	36.00	55.00
Pseudomonas fluorescence	13.00	35.00	16.00	60.00	23.00	71.25
Control	20.00	00.00	40.00	00.00	80.00	00.00
SE(m)	**0.639**	**-**	**0.866**	**-**	**0.864**	**-**
CD 5%	**1.991**	**-**	**2.698**	**-**	**2.691**	**-**

Table.3 Effect of different fungicides on the radial growth of *Fusarium oxysporum* f.sp.*lentis* at 50 ppm concentration

Treatments	Inhibition after 48 hours		Inhibition after 96 hours		Inhibition after 144 hours	
	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition
Mancozeb	10.00	50	25.33	36.67	40.00	50
Carbendazim	0.00	100	0.00	100	0.00	100
Cymoxanil 8%+ Mancozeb 64%	0.00	100	0.00	100	0.00	100
Thifluzamide 24%SC	8.00	60	25.00	37.50	35.66	55.42
Tebuconazole	0.00	100	0.00	100	0.00	100
Hexaconazole	0.00	100	0.00	100	15.00	81.25
Propiconazole	0.00	100	0.00	100	0.00	100
Chlorothalonil	0.00	100	7.00	82.50	14.33	82.08
Control	20.00	0	40.00	0	80.00	0
SE(m)	**0.357**	**-**	**1.098**	**-**	**0.826**	**-**
CD 5%	**1.069**	**-**	**3.289**	**-**	**3.552**	**-**

Table.4 Effect of different fungicides on the radial growth of *Fusarium oxysporum* f.sp.*lentis* at 75 ppm concentration

Treatments	Inhibition after 48 hours		Inhibition after 96 hours		Inhibition after 144 hours	
	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition
Mancozeb	8.66	56.70	22.33	44.17	36.66	54.17
Carbendazim	0.00	100	0.00	100	0.00	100
Cymoxanil 8%+ Mancozeb 64%	0.00	100	0.00	100	0.00	100
Thifluzamide 24%SC	8.00	60	22.00	45	33.33	58.33
Tebuconazole	0.00	100	0.00	100	0.00	100
Hexaconazole	0.00	100	0.00	100	12.00	85
Propiconazole	0.00	100	0.00	100	0.00	100
Chlorothalonil	0.00	100	0.00	100	12.33	84.58
Control	20.00	0.00	40.00	0.00	80.00	0.00
SE(m)	**0.420**	**-**	**0.709**	**-**	**0.788**	**-**
CD 5%	**1.259**	**-**	**2.123**	**-**	**2.359**	**-**

Table.5 Effect of different fungicides on the radial growth of *Fusarium oxysporum* f.sp.*lentis* at 75 ppm concentration

Treatments	Inhibition after 48 hours		Inhibition after 96 hours		Inhibition after 144 hours	
	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition
Mancozeb	6.33	68.35	19.00	52.50	31.66	60.42
Carbendazim	0.00	100	0.00	100	0.00	100
Cymoxanil 8%+ Mancozeb 64%	0.00	100	0.00	100	0.00	100
Thifluzamide 24%SC	7.00	65.00	20.00	50.00	29.00	63.75
Tebuconazole	0.00	100	0.00	100	0.00	100
Hexaconazole	0.00	100	0.00	100	11.00	86.25
Propiconazole	0.00	100	0.00	100	0.00	100
Chlorothalonil	0.00	100	0.00	100	6.66	91.67
Control	20.00	0	40.00	0	80.00	0
SE(m)	**0.320**	**-**	**0.673**	**-**	**0.651**	**-**
CD 5%	**0.960**	**-**	**2.016**	**-**	**1.948**	**-**

Fig.1 Efficacy of bio-agents on inhibition mycelial growth of pathogen

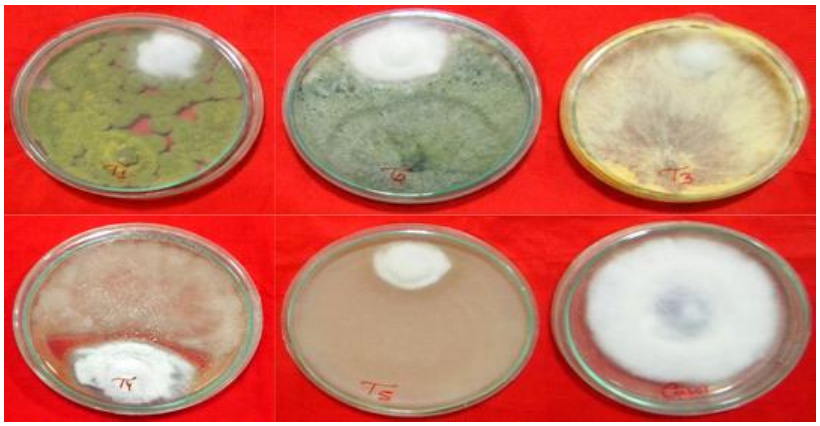
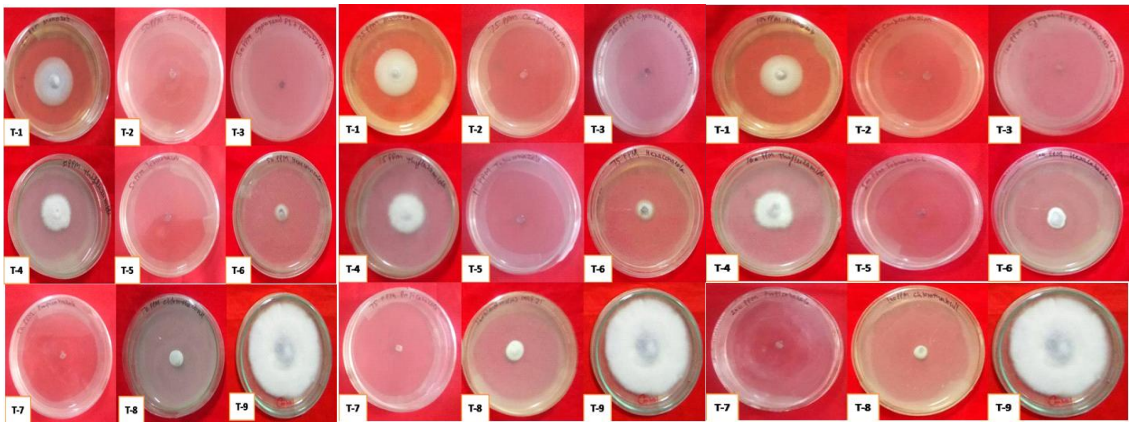


Fig.2 Efficacy of different fungicide at different concentration, inhibition on mycelial growth of *Fusarium oxysporum* f.sp.*lenti*



In conclusion, *Trichoderma viridae, Pseudomonas fluorescence* and *Bacillus subtilis* have capability to inhibit the mycelial growth of pathogen by completion, parasitism and antibiosis mechanisms. In the fungicide, Carbendazim 50% WP, Cymoxanil 8%+ Mancozeb 64%, Thifluzamide 24% SC, Tebuconazole 25.9% EC, and Propiconazole 25% EC are most effective fungicide for inhibition of mycelial growth of pathogen. The results reported here suggest that above bio-agents and fungicide were more capable of influencing the growth of pathogens in dual culture under controlled condition, and may be used as broad spectrum under field condition.

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