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Efficacy of different bio-agents against Fusarium oxysporum f. sp. lentis in vitro and in vivo condition

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***Abstract*— Inhibitory effect of bioagents was tested against Fusarium oxysporum f. sp. lentis in vitro. Maximum(65.94%) mycelial growth was inhibited by Pseudomonas fluorescens followed by Bacillus subtilis (62.23%), T. viride (39.62%)and T. virense (39.22%).T. harzianum was found least effective inhibition of mycelia growth (35.65%) in dual plate technique. All five bio-agents evaluated against F. o. f. sp. lentis in vitro were also tested in vivo conditions, where they also were found effective in wilt management. Maximum disease control (42.10%) was recorded with P. fluorescens@ 10 g/kg seed followed by Bacillus subtilis (38.27%) @ 10 g/kg seed, T. viride (32.70%) @ 4 g/kg seed and T. virens (32.80%) @ 4 g/kg seed . T. harzianum was least effective in reducing wilt incidence (30.40%) @ 4 g/kg seed in 2016-17. Similar results were also observed in the year 2017-18. Disease incidence was maximum at 90 days after sowing as compared to 60 and 30 days after sowing in both the years.**

***Keywords*— Fusarium spp.; lentil; bioagents**

I. INTRODUCTION

Lentil (Lens culinaris Medikus.) is most important pulse crop in India, grown for dal making, culinary and for table purposes. It constitutes the main source of protein and several amino acids. It is a very cheap pulse and hence it is also referred as "Masur". The yield of lentil can be reduced considerably due to many diseases. Lentil is grown in diversified area and hence it succumbs to many fungal, bacterial and viral diseases in different geographical regions. The incidence of the wilt disease is increasing, causing substantial lentil yield losses. Yield losses due to lentil wilt reported by various workers, 50- 78 per cent yield loss under natural conditions at Madhya Pradesh by Khare et al. (1979 a, b) and Agrawal et al. (1993), upto 50 per cent at Madhya Pradesh by Khare, (1980 and 1991), 67 per cent wilt incidence reported by Vasudeva and Srinivasan (1952) at New Delhi, 25 to 50 per cent at Budelkhand region of Uttar Pradesh (Anonymous, 1999), 12 per cent at North west Syria (Bayaa et al., 1986 and 1994), 13.2 per cent at South Syria (El-Ahmed and Mouselli., 1986 and 1987) and 70 per cent at Czechoslovakia (Bojdova and Siskny, 1990).If you want healthy hairs and scalp, then include lentil in your diet at least 3-4 times a week because lentil are rich in folic acid (Anonymous, 2013). The total area under lentil in India was 1.47 m ha with a total production of 1.03 mt and 705 Kg/ha productivity (Anonymous, 2015-16). In Utter Pradesh, it is grown on 4.38 lakh hectare area with 4.8 million tonnes production and productivity 803 kg/ha (Anonymous, 2014-15). There is much said about the bio-agents in modification of physical, chemical and biological environment of soil through addition of decomposable organic matter. It improves the structure, texture, aeration and water holding capacity of soil and improves the development of root system. The biological environment also changes, due to intense microbial activities in the soil which is helpful for developing more antagonistic micro- organisms. The disease incidence is affected by various mechanisms operative in soil, host and pathogen. Considering the importance of these factors, the studies were carried out at the Department of Plant Pathology, College of Agriculture kumarganj, Ayodhya (20018-19) with a view to clarify the role of as a source of bio-agents, in reducing the severity and ultimately the losses caused by wilt causing organisms.

II. MATERIALS AND METHODS

A. Efficacy of different bio-agents against F. oxysporum f.sp. lentis in vitro

Table 1: List of bio-agents used for dual culture technique:

S. No.	Name of bio-agents
1	Trichoderma viride
2	Trichoderma harzianum
3	Trichoderma virens
4	Bacillus subtilis
5	Pseudomonas fluorescens

Five bio-agents were used viz., Trichoderma viride, Trichoderma harzianum, Trichoderma virens, Bacillus subtilis and Pseudomonas fluorescens which were obtained from the Department of Plant Pathology, NDUA&T, Kumarganj, Faizabad (U.P.).The antagonistic potential of Trichoderma virdae, Trichoderma harzianum Trichoderma virense, Bacillus subtilis. and Pseudomonas fluorescens against F.oxysporum f. sp. lentis was assessed in dual culture technique. Measuring radial growth of the F. oxysporum f.sp. lentis as well as that of bio-agents. The mycelia disc of 3 mm diameter from the margin of 7 day old culture of bio-agents and F. oxysporum f.sp. lentis were placed on solid PDA (Potato Dextrose Agar) in paired combination at distance of 2.5 cm from each other in three replications. Control set was made by inoculating F. oxysporum f.sp. lentis singly on the medium. Dual Petri dishes were incubated at 28 0C in BOD (Biological oxygen demand) incubator and the extent of interaction was observed by measuring area covered in dual culture and in the control at 4 and 7 days of incubation. The per cent inhibition of the interacting fungi was calculated as follows:

% inhibition of radial growth (PIRG) =(R1-R2)/R1 X 100

Where,

R1- radial growth of pathogen as control. R2-radial growth of pathogen in dual culture experiments with antagonists (Sharfuddin and Chaudary, 2012).

B. Efficacy of different bio-agents against Fusarium wilt in vivo

Table 2: List of bio-agents used:

Treatment	Name of bio-agents
1	Seed treatment with Trichoderma harzianum@ 4 g/kg seed
2	Seed treatment with Trichoderma viride@4 g/ kg seed
3	Seed treatment with Trichoderma virens @4g /kg seed
4	Seed treatment with Bacillus subtilis @ 10 g/kg seed
5	Seed treatment with Pseudomonas fluoresens@10g /kg seed.
6	Control

Soil was collected and sterilized in autoclave, filled (3Kg/ pot) in earthen pots separately. Seed treated with bio-agents as per treatment mentioned above. Control pots were filled with soil without adding bio-agent. The seeds of susceptible variety of lentil (L 9-12) were sown in each pot (15 seed per pot) where finally 10 plants were maintained. The experiment was conducted in CRD with three replications. First appearance of disease, disease incidence and per cent disease control were observed 30 and 60 days after sowing. Per cent disease incidence and per cent disease control were calculated by using following formula.

Per cent disease incidence =Total number of plants/Number of infected plants X 100

Per cent disease control =C – T/C X100

Where,

C = Per cent disease incidence of control pots

T = Per cent disease incidence in treated pots

Table3: Efficacy of bio-agents against F. oxysporum f. sp. lentis on radial growth and growth inhibition using dual culture technique after 7 days incubation

Fungal antagonist	Inhibition (%) in 7 days Mycelial growth (mm)
T harzianum	35.65 (32.08 mm)
T. viride	39.62 (35.65 mm)
T. virens	39.22 (35.29 mm)
P. fluorescens	65.94 (59.34 mm)
Bacillus subtilis	62.23 (56.00 mm)

Efficacy of different bio-agents against Fusarium wilt of lentil in vivo:

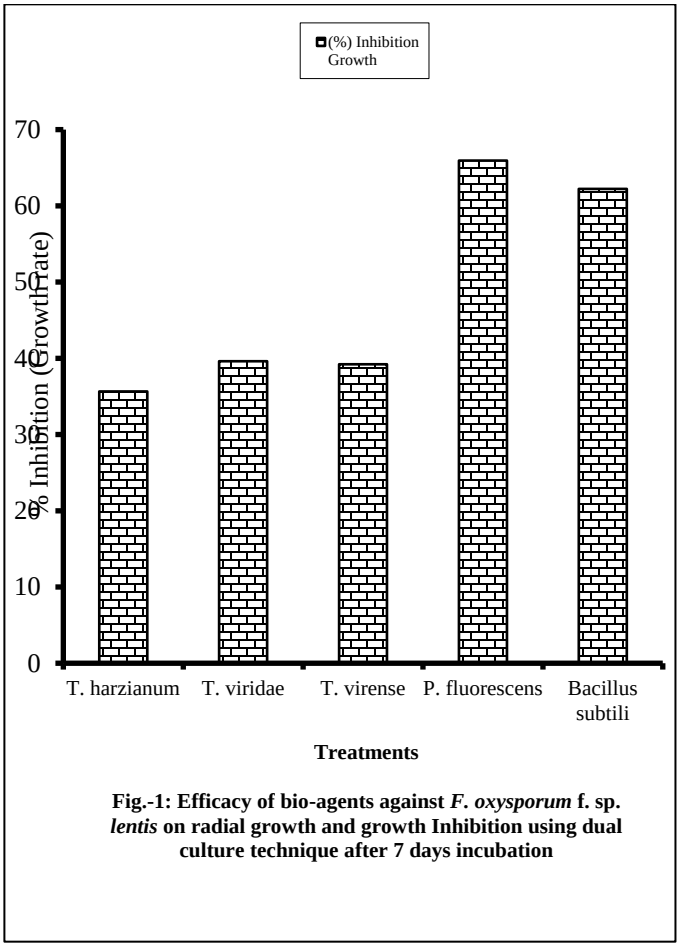

Fig.-1: Efficacy of bio-agents against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition using dual culture technique after 7 days incubation

I. RESULTS

Effect of bioagents was tested against inhibition of mycelial growth of Fusarium oxysporum f. sp. lentis. Maximum(65.94%) mycelial growth was inhibited by Pseudomonas fluorescens followed by Bacillus subtilis (62.23%), T. viride (39.62%) and T. virens (39.22%). T. harzianum was found least effective in inhibiting mycelia growth (35.65%) in dual plate technique. (Table3, Fig. 1 and Plate 1).

It is evident from the data (table-4) that seed treatment of all five bio-agents reduced wilt incidence of Fusarium wilt significantly over check. Minimum disease incidence (33.31%) was recorded with Pseudomonas fluorescens @ 10 g/kg seed , followed by Bacillus subtilis (35.50%) @ 10 g/kg seed, T.vd (38.70%) @ 4 g/kg seed, T.vs.(39.20%) @ 4 g/kg seed and T.h.-(40.10%) @ 4 g/kg seed, all bio-agents were significantly superior over control against Fusarium wilt. Maximum disease control (40.06%) was recorded with Pseudomonas fluorescens @ 10 g/kg seed followed by Bacillus subtilis (38.26%) @ 10 g/kg seed, T. viride (32.69%) @ 4 g/kg seed and T. virens (31.81%) @ 4 g/kg seed. T. harzianum was least effective in

reducing wilt incidence (30.26%) @ 4 g/kg seed in 2016-17 (Fig. 2).

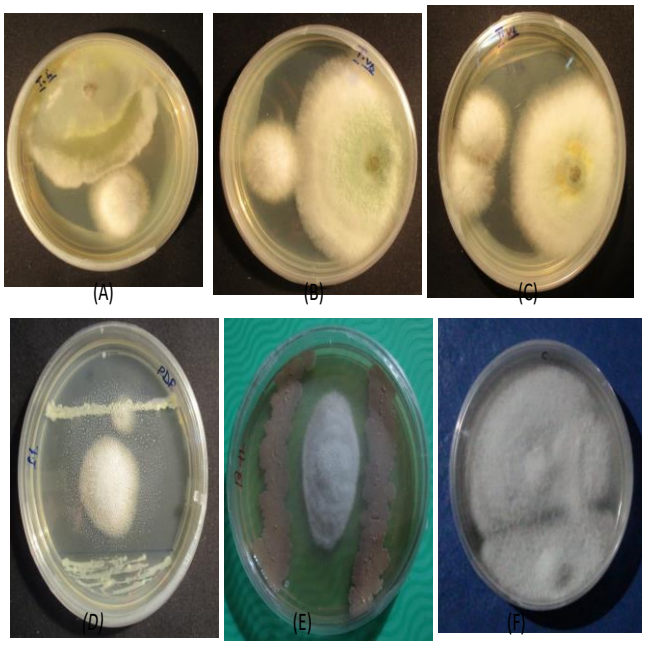

Plate 1. Inhibition of mycelial growth of F. oxysporum f. sp. lentis by different bio-agent on dual plate assay (a) T. harzianum (b) T. virens (c) T. viride (d) Pseudomonas fluorescens(e) B. subtilis (f) F. oxysporum f. sp. lentis (control)

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Similar results were also observed in the year 2017-18. Minimum disease incidence was recorded in Pseudomonas fluorescens (35.70%) @ 10 g/kg seed followed by Bacillus subtilis (39.70%) @ 4g /kg seed, T. viride (40.70%) 4g /kg seed, T. virens (42.20%) @ 4g /kg seed and T. harzianum (44%) @ 4g/kg seed as compare to control (61.50%). Maximum disease control (41.95%) was found in Pseudomonas fluorescens followed by Bacillus subtilis (35.44%), T. viride (33.82%) and T. virens (31.38%). T. harzianum was least effective in reducing wilt incidence (28.45%) (Table 5) (Plate 2 and Fig. 3).

Table 4: Efficacy of bio-agents against F. oxysporum f. sp. lentis on disease incidence and disease reduction in vivo at 30 days 60 days and 90 days after sowing 2016-17.

Treatment	Disease incidence	% Disease control	Disease incidence	%Disease	Disease incidence	% Disease control
	30 days	30 days	60 days	60 days	90 days	90 days
T. harzianum (T_1) @ 4 g/kg seed	5.00 (2.34)	29.57 (5.48)	10.00 (3.24)	30.40 (5.55)	40.10 (6.36)	30.26 (5.54)
T. viride (T_2) @ 4 g/kg seed	4.80 (2.30)	32.39 (5.73)	9.67 (3.19)	32.70 (5.76)	38.70 (6.25)	32.69 (5.75)
T.virens(T3) @ 4 g/kg seed	4.90 (2.32)	30.98 (5.61)	9.80 (3.20)	32.80 (5.76)	39.20 (6.30)	31.82 (5.68)
Bacillus subtilis (T4)@ 10 g/kg seed	4.40 (2.21)	38.02 (6.20)	8.87 (3.06)	38.27 (6.22)	35.50 (6.00)	38.26 (6.22)
Pseudomonas fluorescens (T5)@ 10 g/kg seed	4.16 (2.16)	41.40 (6.46)	8.32 (2.97)	42.10 (6.52)	33.31 (5.81)	40.06 (6.36)
Control	7.10 (2.75)	0.00 (0.71)	14.37 (3.85)	0.00 (0.71)	57.50 (7.61)	0.00 (0.71)
SEM±	0.072	0.176	0.103	0.165	0.214	0.176
CD(>0.05)	0.223	0.542	0.319	0.508	0.661	0.543
CV	5.343	6.055	5.511	5.608	5.815	6.055

* Figure in parenthesis is root transformed value

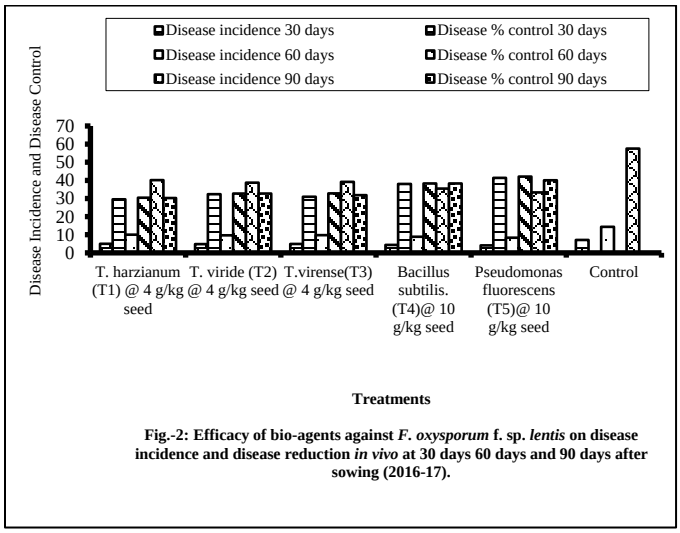

Fig.-2: Efficacy of bio-agents against *F. oxysporum* f. sp. *lentis* on disease incidence and disease reduction *in vivo* at 30 days 60 days and 90 days after sowing (2016-17).

Table 5: Efficacy of bio-agents against F. oxysporum f. sp. lentis on disease incidence and disease reduction in vivo at 30 days 60 days and 90 days after sowing 2017-18

Treatment	Disease incidence	%Disease control	Disease incidence	%Disease control	Disease incidence	%Disease control
	30 days	30 days	60 days	60 days	90 days	90 days
T. harzianum (T_1) @ 4 g/kg seed	5.50 (2.45)	27.63 (5.30)	11.00 (3.39)	28.10 (5.34)	44.00 (6.67)	28.45 (5.38)
T. viride (T2) @ 4 g/kg seed	5.80 (2.51)	23.68 (4.91)	10.17 (3.26)	33.52 (5.83)	40.70 (6.42)	33.82 (5.86)
T. virens (T3) @ 4 g/kg seed	5.25 (2.40)	30.92 (5.60)	10.50 (3.32)	31.37 (5.64)	42.20 (6.53)	31.38 (5.64)
Bacillus subtilis (T4) @ 10 g/kg seed	4.90 (2.32)	35.52 (5.99)	9.92 (3.22)	35.16 (5.96)	39.70 (6.33)	35.44 (5.99)
Pseudomonas fluorescens (T5) @ 10 g/kg seed	4.46 (2.22)	41.31 (6.46)	8.92 (3.07)	41.64 (6.48)	35.70 (6.01)	41.95 (6.51)
Control	7.60 (2.84)	0.00 (0.71)	15.30 (3.97)	0.00 (0.71)	61.50 (7.86)	0.00 (0.71)
SEM±	0.074	0.167	0.108	0.173	0.218	0.171
CD(>0.05)	0.229	0.515	0.333	0.534	0.671	0.526
CV	5.243	5.997	5.561	6.008	5.686	5.896

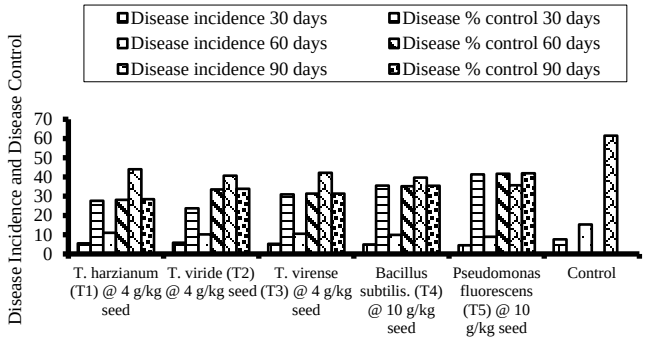

Fig.-3: Efficacy of bio-agents against *F. oxysporum* f. sp. *lentis* on disease incidence and disease reduction *in vivo* at 30 days 60 days and 90 days after sowing (2017-18)

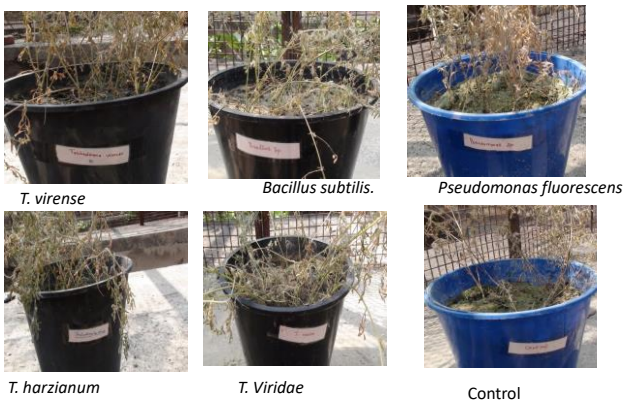

Plate 2. Effect of Antagonists on wilt disease

Disease incidence was maximum at 90 days after sowing as compared to 60 and 30 days after sowing in both the years

IV. Discussion

All five bio-agents evaluated against F. o. f. sp. lentis in vitro were also tested in vivo conditions, where they were also effective in wilt management. Minimum disease incidence (33.31%) was recorded with Pseudomonas fluorescens @ 10 g/kg seed , followed by Bacillus subtilis (35.50%) @ 10 g/kg seed, T. viride (38.70%) @ 4 g/kg seed, T. virens 39.20%) @ 4 g/kg seed and T. harzianum (40.10%) @ 4 g/kg seed, all bio-agents are significantly superior over control against Fusarium wilt. Maximum disease control (42.10%)was recorded with Pseudomonas fluorescens @ 10 g/kg seed followed by Bacillus (38.27%) @ 10 g/kg seed , T. viride (32.70%) @ 4 g/kg seed and T. virens (32.80%) @ 4 g/kg seed . T. harzianum was least effective in reducing wilt incidence (30.40%) @ 4 g/kg seed in 2016-17. Similar results were also observed in the year 2017-18. Disease incidence was maximum at 90 days after sowing as compared to 60 and 30 days after sowing in both the years.

V. Conclusion

All five bio-agents evaluated against F. o. f. sp. lentis in vitro were also tested in vivo conditions, where they also proved effective in wilt management. Maximum disease control (42.10%) was recorded with P. fluorescens@ 10 g/kg seed followed by Bacillus subtilis (38.27%) @ 10 g/kg seed , T. viride (32.70%) @ 4 g/kg seed and T. virens (32.80%) @ 4 g/kg seed T. harzianum was least effective in reducing wilt incidence (30.40%) @ 4 g/kg seed in 2016-17. Similar results were also observed in the year 2017-18. Disease incidence was maximum at 90 days after sowing as compared to 60 and 30 days after sowing in both the years.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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