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Efficacy of Different Soil Amendments and Bio-Agents against *Fusarium Oxysporum* f. sp. *lentis* in Vitro & in Vivo

Khushboo Dubey & S.K. Singh
A.N.D. University of Agriculture and Technology

ABSTRACT

Five organic amendments were evaluated against wilt of lentil which were found more or less effective. Maximum disease control (42.85%) found in neem cake followed by mustard cake (38.23%), parthenium (32.90%), linseed cake (28.57%) and sawdust was least in reducing wilt (23.66%) in 2016-17. Similar results were also observed in the year 2017-18. 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. virens* (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: isolation, disease incidence, percentage disease control and bio-agents.

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ABSTRACT

Five organic amendments were evaluated against wilt of lentil which were found more or less effective. Maximum disease control (42.85%) found in neem cake followed by mustard cake (38.23%), parthenium (32.90%), linseed cake (28.57%) and sawdust was least in reducing wilt (23.66%) in 2016- 17. Similar results were also observed in the year 2017-18. 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. virens* (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: isolation, disease incidence, percentage disease control and bio-agents.

Author a& Department of Plant Pathology, A.N.D.University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.

I. INTRODUCTION

Food legumes are well-known part of diets worldwide and play an important and diverse role in the farming systems. Lentil (*Lens culinaris Medikus.*) is most important pulse crop grown in India, which suffers economic losses due to wilt complex. Legumes are also known as cost effective and an ideal crops for reducing poverty, improving human health, nutrition, and enhancing ecosystem resilience (Akibode and Maredia 2011). Lentil is cultivated as a rain fed crop in all India about 1.34 million ha area with 1.02MT production and 759 kg/ha productivity (Abraham, 2015). In India lentil is predominantly grown in the North, particularly in Uttar Pradesh, Madhya Pradesh, Bihar and West Bengal. In Uttar Pradesh, it is grown in 620.000 lakh/ha area with 452.000lakh tones production and 732.0 kg/ha productivity (Ahmad, *et al.*, 2018). They are low in fat, low in sodium, cholesterol free, and are an excellent source of both soluble and insoluble fibre, complex carbohydrates and vitamins (Market Outlook Report, 2010). *Fusarium* wilt disease is a widespread in almost every country where lentil is grown (Dikshit *et al.*, 2016). Sometime, this disease can cause complete failure of the crop, especially in a warm spring and dry and hot summer. *Fusarium* wilt is severe on lentil mainly grown on residual moisture in the highlands dominated with vertisols. *Fusarium oxysporum f sp. lentis* is an important soil borne fungus with limited host range (Sharfuddin *et al.*, 2012). It produces three types of spores; oval or kidney shaped micro conidia; thin walled, multicellular (4-6 cells) macro conidia with a definite foot cell and a pointed apical cell, and chlamydospores formed singly in macro conidia,

terminal or intercalary in the hyphae. In recent years, *Trichoderma* species have confirmed as effective biocontrol agents against Fusarium disease, caused by *Fusarium oxysporum* (Akrami *et al.*, 2011; Kashem *et al.*, 2016; Khaliquzzaman *et al.*, 2016). This wilt pathogen. This wilt pathogen survives in the soil as chlamydospores that can remain viable for several years (Erskine and Bayaa, 1996) and is capable of colonizing residues and roots of most crops grown in rotation with lentil. 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.* (1991), 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 (Bojdoa and Siskny, 1990).

There is much said about the role of organic amendments 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.

II. MATERIALS AND METHODS

2.1 Isolation of *Fusarium oxysporum* f.sp. *lentis*

Small pieces of infected root 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Pieces were dipped in 0.1per cent mercuric chloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water. The bits were then transferred to PDA medium in Petri plates with the help of inoculating needle under aseptic condition and incubated at 28 ± 10c. Pure culture was done by transfer of a pinch of mycelium on sterilized Potato Dextrose Agar medium in Petri plates and incubated in BOD.

2.2 Effect of different soil amendments in net house condition

Soil were collected and sterilized in autoclave, filled (3Kg /pot) in earthen pots separately. Neem cake (2.77 gm./kg soil), mustard cake(2.53 gm./kg soil), linseed oil cake(2.28 gm./kg soil), sawdust(1.64gm./kg soil) and *Parthenium* compost (5 gm./kg soil) were mixed individually in the sterilized soil filled pots, two weeks prior to sowing. Control pots were filled with soil without adding amendments. The seeds of wilt susceptible variety of lentil (L 9-12) were sown in each pot (15 seed per pot) where finally 10 plants will be 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.

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Percent disease control} = \frac{C - T}{C} \times 100$$

Where,

C = Per cent disease incidence of control pots

T = Per cent disease incidence in treated pots

*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.	<i>Trichoderma viride</i>
2.	<i>Trichoderma harzianum</i>
3.	<i>Trichoderma virens</i>
4	<i>Bacillus subtilis</i>
5	<i>Pseudomonas fluorescens</i>

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, Ayodhya (U.P.). The antagonistic potential of *Trichoderma viridae*, *Trichoderma harzianum* *Trichoderma virens*, *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 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 °C in BOD 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:

$$\% \text{ inhibition of radial growth (PIRG)} = (R_1 - R_2) / R_1 \times 100$$

Where, R_1 - radial growth of pathogen as control.

R_2 -radial growth of pathogen in dual culture experiments with antagonists (Sharfuddin and Chaudary, 2012).

*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 <i>Trichoderma harzianum</i> @ 4 g/kg seed
2.	Seed treatment with <i>Trichoderma viride</i> @ 4 g/kg seed
3.	Seed treatment with <i>Trichoderma virens</i> @ 4 g/kg seed
4	Seed treatment with <i>Bacillus subtilis</i> @ 10 g/kg seed
5	Seed treatment with <i>Pseudomonas fluorescens</i> @ 10 g/kg seed.
6	<i>Control</i>

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.

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Percent disease control} = \frac{C - T}{C} \times 100$$

Where,

C = Per cent disease incidence of control pots

T = Per cent disease incidence in treated pots

III. RESULTS

3.1 Efficacy of different soil amendment on disease incidence

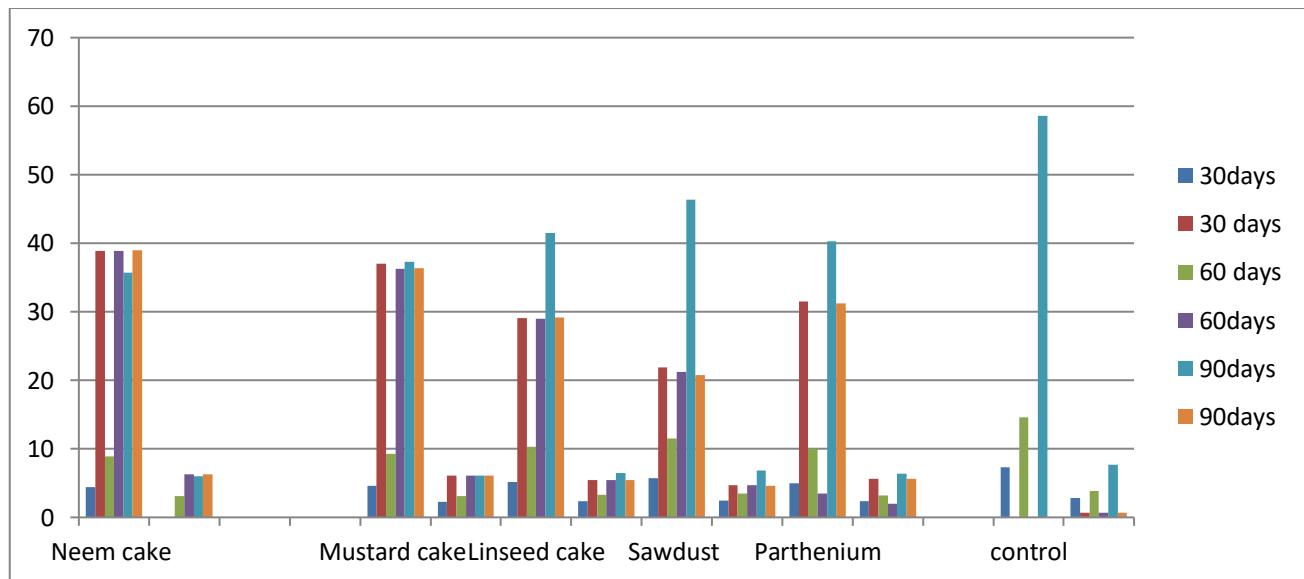
It is evident from the data that all five organic amendments tested reduced wilt incidence of lentil significantly over check and minimum disease incidence was recorded in Neem oil cake (31.71%) @ 2.77 gm./kg soil followed by mustard cake (34.29%)@ 2.53 gm./kg soil, parthenium compost (37.55%) @5 gm./kg soil, linseed cake (39.63%) @2.28 gm./kg soil and sawdust (42.33%) @1.64gm./kg soil and as compared to control (55.44%). Neem oil cake was found significantly superior over all other treatments except mustard cake after 90 days, maximum disease control (42.80%) was found in neem cake followed by mustard cake (38.14%), parthenium (32.81%), linseed cake (28.51%) and sawdust was least effective in reducing wilt (23.66%) in 2016- 17 (Table-3 and Fig. 1).

Table 3: Efficacy of organic amendment against *F. oxysporum* f. sp. *lentis* on disease incidence and per cent disease control *in vivo* at 30 days, 60 days and 90 days after sowing 2016-17

Treatment	Disease	%Disease	Disease	%	Disease	%
	incidence	control	incidence	Disease control	incidence	Disease control
	30 days	30 days	60 days	60 days	90 days	90 days
Neem oil cake @ 2.77 gm/kg soil	3.96 (2.11)	42.85 (6.58)	7.92 (2.90)	42.85 (6.58)	31.71 (5.67)	42.80 (6.57)
Mustard cake @ 2.53 gm./kg soil	4.28 (2.18)	38.23 (6.21)	8.57 (3.01)	38.16 (6.21)	34.29 (5.89)	38.14 (6.21)
Linseed cake@2.28 gm./ kg soil	4.95 (2.33)	28.57 (5.39)	9.90 (3.22)	28.57 (5.38)	39.63 (6.33)	28.51 (5.38)
Sawdust @ 1.64 gm./ kg soil	5.29 (2.41)	23.66 (4.91)	10.58 (3.33)	23.66 (4.91)	42.33 (6.54)	23.64 (4.91)

Parthenium@5 gm./kg soil	4.65 (2.27)	32.90 (5.77)	9.31 (3.13)	32.82 (5.77)	37.55 (6.14)	32.81 (5.76)
Control	6.93 (2.72)	0.00 (0.71)	13.86 (3.78)	0.00 (0.71)	55.44 (7.47)	0.00 (0.071)
SEM±	0.069	0.179	0.101	0.166	0.210	0.179
CD (0.05%)	0.211	0.552	0.312	0.510	0.646	0.553
CV	5.084	6.295	5.426	5.821	5.728	6.313

* Figure in parenthesis is root transformed value



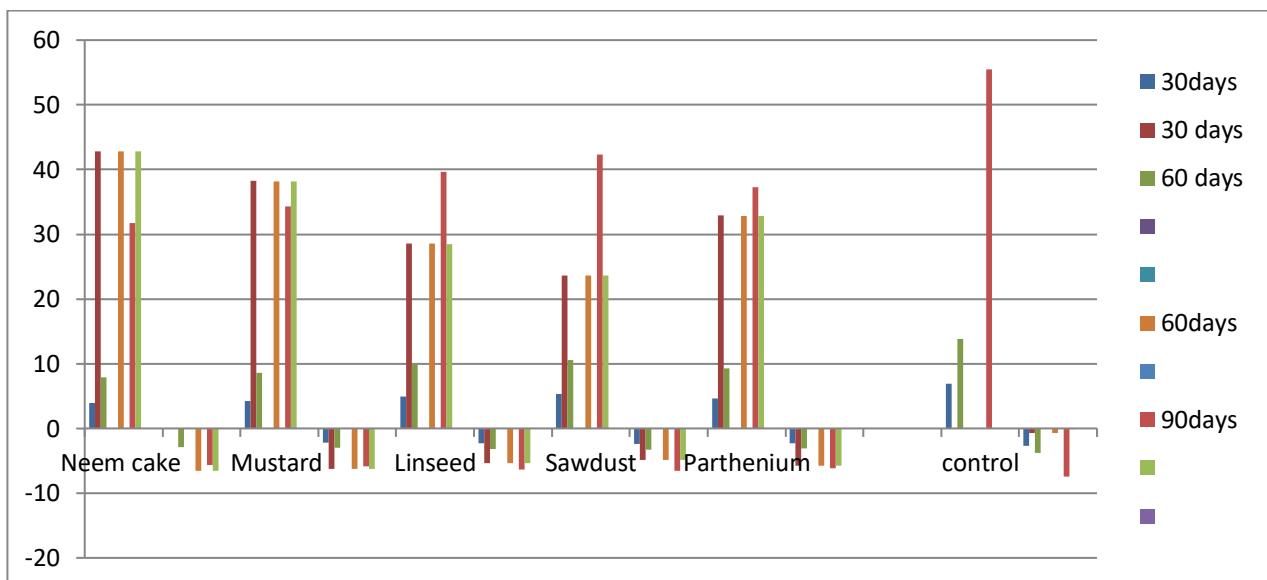
Similar results were also observed in the year 2017-18, Neem oil cake was found significantly superior over all other treatments. Minimum disease incidence was recorded in Neem cake (35.71%) followed by mustard cake (37.26%) parthenium compost (40.25%), linseed cake (41.48%) and sawdust (46.38%) as compared to control (58.55%). Maximum disease control was obtained in neem cake (39.00%) followed by mustard cake(36.36%), parthenium (31.25%) and linseed cake (29.15%). sawdust was least effective in reducing wilt (20.78 %) (Table 4 and Fig. 2).

Table 4: Efficacy of organic amendment against *F. oxysporum* f. sp. *lentis* on disease incidence and per cent disease control *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
Neemcake@ 2.77 gm/kg soil	4.46 (2.23)	38.90 (6.27)	8.92 (3.06)	38.90 (6.27)	35.71 (6.01)	39.00 (6.28)
Mustardcake@ 2.53 gm./ kg soil	4.60 (2.26)	36.98 (6.12)	9.31 (3.13)	36.23 (6.06)	37.26 (6.14)	36.36 (6.07)
Linseed cake @2.28 gm./ kg soil	5.18 (2.38)	29.04 (5.43)	10.31 (3.29)	28.97 (5.43)	41.48 (6.47)	29.15 (5.44)

Sawdust @ 1.64 gm./kg soil	5.70 (2.49)	21.91 (4.73)	11.50 (3.46)	21.23 (4.66)	46.38 (6.84)	20.78 (4.61)
Parthenium@5 gm./kg soil	5.00 (2.34)	31.50 (5.65)	10.00 (3.24)	3.50 (2.00)	40.25 (6.38)	31.25 (5.63)
Control	7.30 (2.79)	0.00 (0.71)	14.60 (3.88)	0.00 (0.71)	58.55 (7.67)	0.00 (0.71)
SEM±	0.074	0.158	0.107	0.147	0.219	0.157
CD	0.229	0.485	0.331	0.452	0.674	0.482
CV	5.333	5.663	5.562	6.074	5.752	5.662

* Figure in parenthesis is root transformed value



Disease incidence was maximum at 90 days after sowing as compared to 60 and 30 days after sowing in both the years (Plate 1).



Plate 1: Efficacy of soil treatment with various organic amendments on wilt of lentil

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

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. (Table5, Fig. 3 and Plate 2).

Table 5: 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)
<i>T. harzianum</i>	35.65 (32.08 mm)
<i>T. viride</i>	39.62 (35.65 mm)
<i>T. virens</i>	39.22 (35.29 mm)
<i>P. fluorescens</i>	65.94 (59.34 mm)
<i>Bacillus subtilis</i>	62.23 (56.00 mm)

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

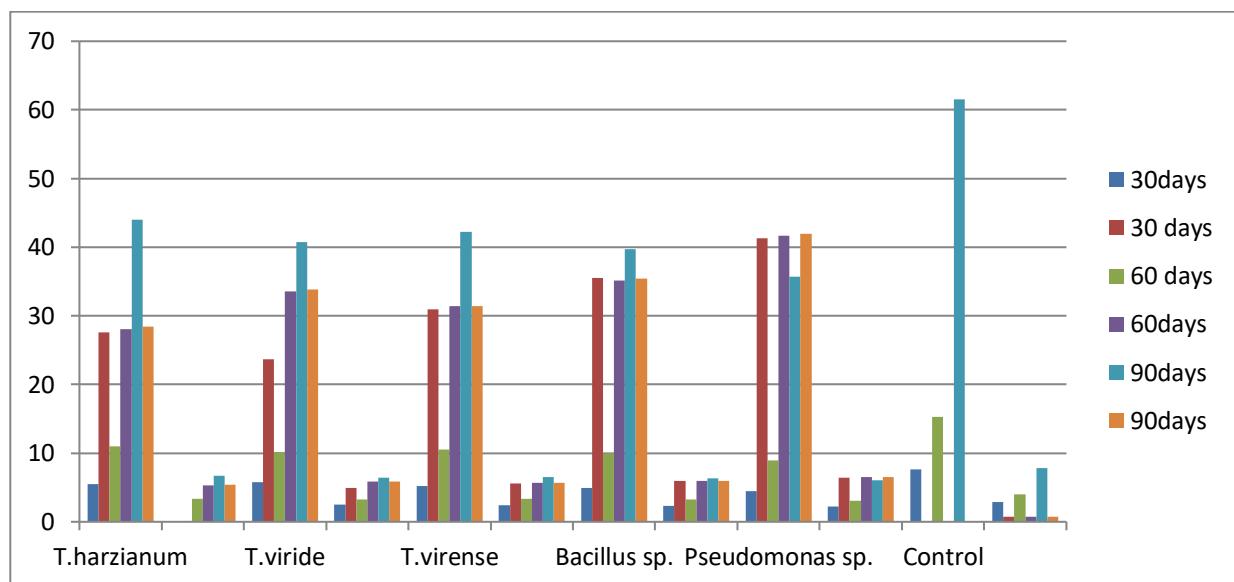
It is evident from the data (table-12) that seed treatment of all five bio-agents reduced wilt incidence of *Fusarium* wilt significantly over check. Minimum disease incidence (33.31%) was recorded with *Pseudomonas fluorescens* @ 10 g/kg seed , followed by *Bacillus subtilis* (35.50%) @ 10 g/kg seed, *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. 3).

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 2016-17.

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
<i>T. harzianum</i> (<i>T₁</i>) @ 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)
<i>T. viride</i> (<i>T₂</i>) @ 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)
<i>T.virens</i> (<i>T₃</i>) @ 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)

<i>Bacillus Subtilis</i> (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)
<i>Pseudomonas fluorescens</i> (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 \pm	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

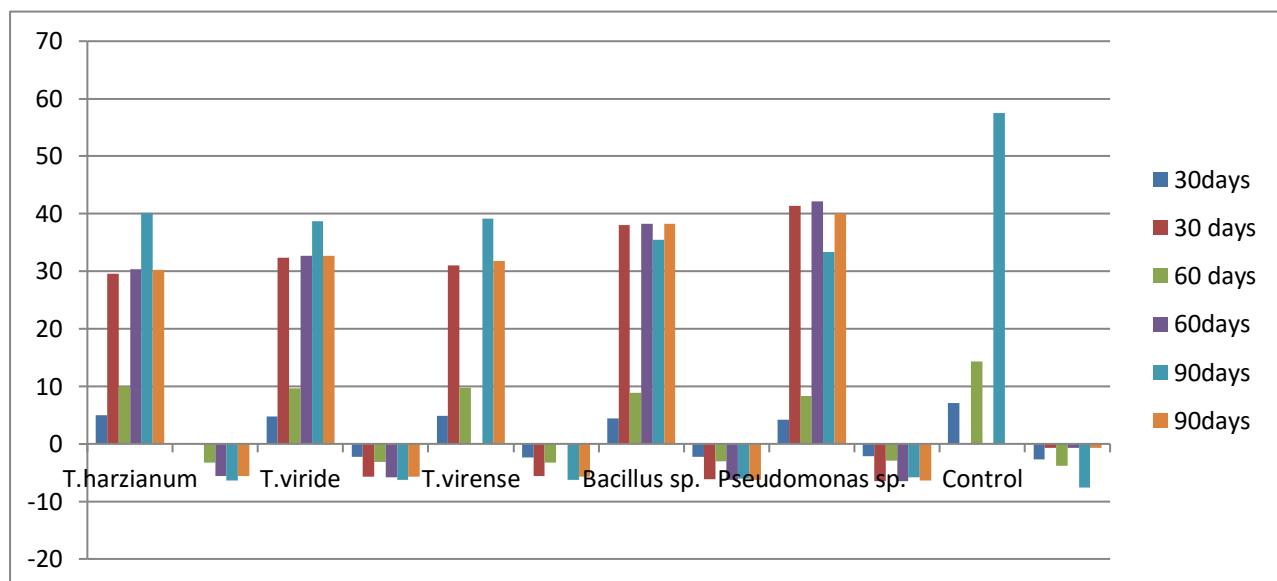


Similar results were also observed in the year 2017-18. Minimum disease incidence was recorded in *Pseudomonas fluorescens* (35.70%) @ 10 g/kg seed followed by *Bacillus subtilis* (39.70%) @ 4g /kg seed, *T. viride* (40.70%) 4g /kg seed, *T. virens* (42.20%) @ 4g /kg seed and *T. harzianum* (44%) @ 4g/kg 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 6) (Plate 2 and Fig. 4).

Table 6: 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
<i>T. harzianum</i> (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)
<i>T. viride</i> (T ₂) @ 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)
<i>T. virens</i> (T ₃) @ 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)
<i>Bacillus subtilis</i>	4.90	35.52	9.92	35.16	39.70	35.44
(T ₄) @ 10 g/kg seed	(2.32)	(5.99)	(3.22)	(5.96)	(6.33)	(5.99)
<i>Pseudomonas fluorescens</i> (T ₅) @ 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

* Figure in parenthesis is root transformed value



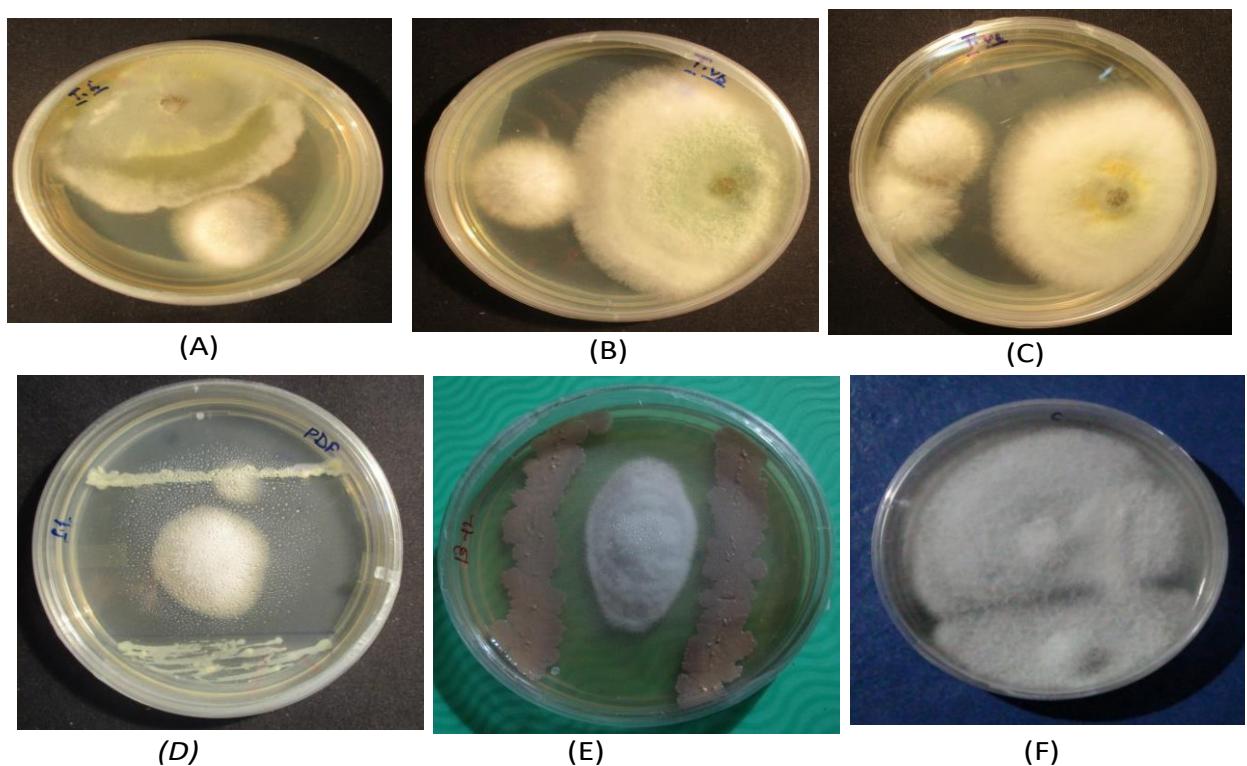


Plate 2: Inhibition of mycelial growth of *F. oxysporum* f. sp. *lenti* by different bio-agent on dual plate assay (a) *T. harzianum* (b) *T. virens* (c) *T. viride* (d) *Pseudomonas fluorescens* (e) *B. subtilis* (f) *F. oxysporum* f. sp. *lenti* (control)

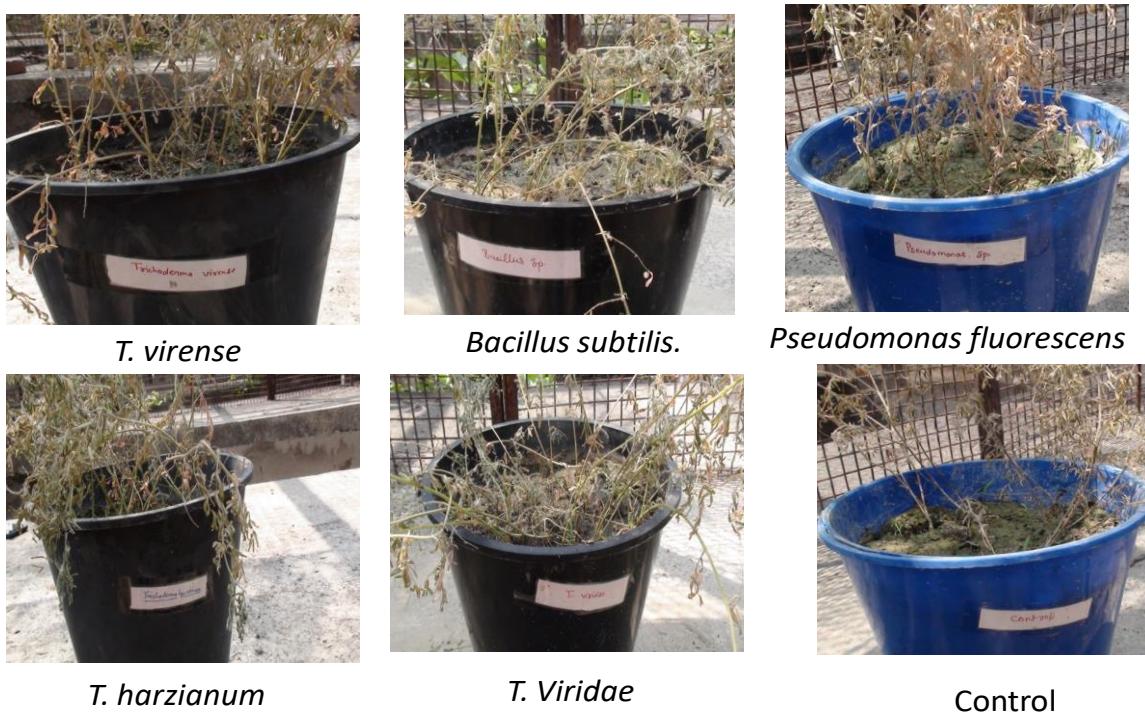


Plate 2: Effect of Antagonists on wilt disease

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

IV. DISCUSSION

4.1 Efficacy of different soil amendments on disease incidence

Five organic amendments were evaluated against wilt of lentil which were found more or less effective. Minimum disease incidence was recorded in neem oil cake (31.71%) @ 2.77 gm./kg soil followed by mustard cake (34.29%)@ 2.53 gm./kg soil, Parthenium compost (37.55%) @5 gm./kg soil, linseed cake (39.63%) @2.28 gm./kg soil and sawdust (42.33%) @1.64gm./kg soil and as compared to control (55.44%). Neem oil cake has found significantly superior over all other treatments except mustard at 90 days. Maximum disease control (42.85%) found in neem cake followed by mustard cake (38.23%), parthenium (32.90%), linseed cake (28.57%) and sawdust was least effective in reducing wilt control (23.66%) in 2016- 17.

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

Inhibitory effect of bioagents were 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. virens* (39.22%).*T. harzianum* was found least effective in the inhibition of mycelia growth (35.65%) in dual plate technique.

All five bio-agents evaluated against *F. oxysporum* 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. CONCLUSIONS

Five organic amendments were evaluated against wilt of lentil which were found more or less effective. Maximum disease control (42.85%) found in neem cake followed by mustard cake (38.23%), parthenium (32.90%), linseed cake (28.57%) and sawdust was least effective in reducing wilt control (23.66%) in 2016- 17.

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. virens* (39.22%) *T. harzianum* was found least effective in 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 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.

REFERENCES

1. Abraham, R. (2015). Lentil (*Lens Culinaris Medikus*) Current Status and Future Prospect of Production in Ethiopia. *Adv. Plants Agric Res* 2 (2): 00040.
2. Agarwal, S. C., Singh, K. and Lal, S. S. (1993). Plant protection of lentils in India. In: Erskine, W. and Saxena, M. C. (eds). pp. 147-165.
3. Anonymous (2014-15). Project Coordinator's Report (Rabi). All India Coordinated Research Project on MULLARP. IIPR, Kanpur. 1. 19-22 pp.
4. Bayaa, B., Erskine W, (1994). Diseases of lentils. In: The Pathology of Food and Pasture Legumes (pp. 423-471).
5. Bayaa, B., Erskine, W. and Khoury, L. (1986). Survey of wilt damage on lentils in north "vest Syria. *Arab J. Pl. Protec.* 4(2): 118-119.
6. Bojdova, J. and Sinsky, T. (1990). Species spectrum of the *Fusarium* genus on lentil in Czechoslovakia. *LENS Newsletter* 17(2): 29-31
7. EI-Ahmad, M. and Mouselli, N. (1986). Lentil wilt in south Syria. *Arab J. PI. Pro tee.* 4(1): 30.
8. EI-Ahmad, M. and Mouselli, N. (1987a). Wilt and root rot of lentil (*Lens culinaris*). *LENS Newsletter*, 14: 10-14.
9. EI-Ahmad, M. and Mouselli, N. (1987b). Wilt and root rot of lentil *LENS Newsletter*, 14(1-2): 31-37.
10. Khare, M. N. (1980). Wilt of lentil. Technical Bulletin. Jabalpur, India: Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, M.P. India, 155.
11. Khare, M. N. (1991) . Lentil diseases variety spetal reference to seed quality. *Indian J. Mycol. Pl. Patho1.* 21 (1): 1-13.
12. Khare, M.N., Agrawal, S.C. and Jain, A.C. (1979 a). Diseases of lentil. *Indian Phytopathol.* .. 21: 455
13. Khushboo Dubey, S.K. Singh, (2018). Efficacy of different soil amendments on disease incidence wilt of lentil. *International Journal of Chemical Studies* 2018; 6 (5): 72-74
14. Khushboo Dubey, S.K. Singh, (2021). Efficacy of different bio-agents against *Fusarium oxysporum* f. sp. *lentis* in vitro and in vivo condition. *Journal of Agricultural Science & Engineering Innovation (JASEI) U.S. ISSN 2694 -4812 Vol. 2, No. 1, 2021.*
15. Khushboo Dubey, S.K. Singh, (2021). Integrated Disease Management wilt of lentil caused by *Fusarium oxysporum* F. Sp. *Lentis*. *Int. J. Sci. Res. in Biological Sciences.* 8, (2) pp.41-48.
16. Sharfuddin, C. and Mohanka, R. (2012). In vitro antagonism of indigenous *Trichoderma* isolates against phytopathogen causing wilt of Lentil. *Int J L Sci. Phar. Res..* 2: 195-202.
17. Vasudeva, R.S. and Srinivasan, K.V. (1952). Studies on the wiltdisease of lentil (*Lens esculenta* Moench). *IndianPhytopath.* 5(1): 23-32