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Received: 1 January 1970 Accepted: 1 January 1970 Published: 1 January 1970

Abstract

Index terms—

1 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, 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.

2 II. MATERIALS AND METHODS

3 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.

4 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.

5 Percent diseaseincidence = Number of infected plants x 100

Total number of plants

6 Percent diseae control= C -T x100 C

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Where, C = Per cent disease incidence of control pots T = Per cent disease incidence in treated pots 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 0 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: % inhibition of radial growth (PIRG) =(R 1 -R 2)/R 1 X 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).

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

8 Efficacy of different bio-agents against Fusarium wilt in vivo

9 Table-2:

10 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 List of bio-agents used 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.

Where, C = Per cent disease incidence of control pots T = Per cent disease incidence in treated pots III. RESULTS

11 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. **??**). 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. **??**).

12 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. **??** and Plate 2).

13 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. **??**). 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.

Figure 1:

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Figure 2: Plate 2 :Plate 2 :

Figure 3:

1

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, Ayodhya (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.

Figure 4: Table 1 :

3

Treatment	Disease incidence 30 days	%Disease control 30 days	Disease incidence 60 days	% Disease control 60 days	Disease incidence 90 days	% Disease control 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)

* Figure in parenthesis is root transformed value

Figure 5: Table 3 :

4

70
0 10 20 30 40 50 60 Neem cake Mustard cakeLinseed cake Sawdust Parthenium control 30days 30 days 60 days 60days 90days 90days London Journal of Research in Science: Natural and Formal

Treatment	Disease incidence 30 days	%Disease control 30 days	Disease incidence 60 days	%Disease control 60 days	Disease incidence 90 days	%Disease control 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)

* Figure in parenthesis is root transformed valueDisease 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

Figure 6: Table 4 :

5

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

Figure 7: Table 5 :

5

Treatment	Disease incidence 30 days	% Disease control 30 days	Disease incidence 60 days	%Disease control 60 days	Disease incidence 90 days	% Disease control 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)

Figure 8: Table 5 :

6

Treatment	Disease incidence 30 days	%Disease control 30 days	Disease incidence 60 days	%Disease control 60 days	Disease incidence 90 days	%Disease control 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)

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Figure 9: Table 6 :

.1 ACKNOWLEDGMENT

The authors are thankful to Dr. Sushil Kumar Singh, Department of Plant Pathology, N. D. University of Agriculture and Technology, Kumarganj, Ayodhya -224229, U.P., India for his help in molecular studies and data analysis for providing the lab facilities to carry out the work.

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

.2 IV. DISCUSSION

.3 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 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.

.5 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 London Journal of Research in Science: Natural and Formal

.6 Conflict of Interest

The authors declare that they have no conflict of interest.

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