

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

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ABSTRACT

Four plant extracts such as Garlic, Neem, Eucalyptus, Tulsi, and three antagonistic bio-agents viz., *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* were evaluated *in vitro* and *in vivo*. The results clearly showed that all plant extracts were more or less inhibitory to mycelial growth of *F. oxysporum* f. sp. *lentis*. The effectiveness of extracts increased with increase of their concentration and maximum inhibition was recorded at 10 per cent concentration after 7 days of incubation *in vitro*. The maximum growth inhibition was obtained in Eucalyptus followed by Garlic and Tulsi. While minimum per cent inhibition was recorded in Neem. The disease incidence was not much reduce by Neem and Tulsi at 10 per cent concentration of 60 days after sowing. The same trend was also found in per cent disease reduction. However, per cent disease reduction in between Eucalyptus and Garlic, Tulsi were at par to each other. The maximum per cent disease reduction was found in Eucalyptus and Garlic. *Trichoderma viride* was more effective in reducing radial growth as compare to *Trichoderma harzianum* and *Pseudomonas fluorescence* after 7 days of incubation. Lower disease incidence and higher per cent disease reduction was also found in *Trichoderma viride* as compared to *Trichoderma harzianum* and *Pseudomonas fluorescence* at 10 per cent concentration 60 days after sowing.

Keyword- Lentil, Fusarium, Biagents and Botanical Extract.

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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.* (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 (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 Uttar Pradesh, it is grown on 4.38 lakh hectare area with 4.8 million tones production and productivity 803 kg/ha (Anonymous, 2014-15). There is much said about the role of botanical and 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 some botanical as a source of bio-agents, in reducing the severity

and ultimately the losses caused by wilt causing organisms.

II. MATERIAL AND METHODS

A. Efficacy of plant extract against *Fusarium* wilt of lentil *in vitro*:

Table 1. List of plants with common name, English name, botanical name and their part used.

S. No.	Common name	English name	Botanical name	Part used
1.	Neem	Neem	<i>Azadirachta indica</i>	Leaves
2.	Lahsun	Garlic	<i>Allium sativum</i>	Bulbs
3.	Tulsi	Basil	<i>Ocimum sanctum</i>	Leaves
4.	Eucalyptus	Eucalyptus	<i>Eucalyptus globules</i>	Leaves

Fresh leaves and bulb were collected and wash thoroughly in clean water. Hundred ml of sterilized water and heated at 80°C for 10 minutes. The material was filtered through double layered muslin cloth.

All the plant extract were tested at 5 and 10 per cent concentration under *in vitro* condition by using food poison technique to study the inhibitory effect of these botanicals on radial growth of *Fusarium oxysporum*. Ten ml plant extracts of stock solution were added to the 90.00 ml of sterilized cooled PDA medium. The flasks were thoroughly taken to get uniform mix of the extracts under aseptic condition before pouring it into the Petri dishes. Twenty ml medium was poured into each Petri dish. Eight treatment having three replications were maintained Control treatment was maintained by pouring PDA medium without plant extracts. Five mm discs of 7 days old pure culture of *Fusarium oxysporum* f. sp. *lentis* were cut with sterilized cork borer and placed in the center of plant extracts amended Petri plates. These Petri plates were incubated at 25 ± 2°C. The observation was recorded on radial growth and per cent inhibition at 4 days and 7 days of incubation in plant extracts amended Petri plates as well as in control.

Per cent growth inhibition was calculated using formula:

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

I = Per cent inhibition of fungal growth

C = Radial growth of control

T = Radial growth of treated Petri plates.

b. Efficacy of bio-agents *Fusarium* wilt of lentil *in-vitro*:-

Table 2. List of bio-agents used for dual culture technique:

S.	Name of bio-
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In order of find out the efficacy of four plants extract against the *Fusarium* wilt viz., Neem, Garlic, Tulsi and Eucalyptus were used. Detailed description of plant and their part used are given in Table 1.

No.	agents
1.	<i>Trichoderma viride</i>
2.	<i>Trichoderma harzianum</i>
3.	<i>Pseudomonas fluorescence</i>

Three bio-agent were used viz., *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* which obtained from the Department of Plant Pathology, NDU&T, Kumarganj, Faizabad (U.P.).The antagonistic potential of *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* 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 four replication. 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 by 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:

$$I = \frac{A1 - A2}{A1} \times 100$$

Where as,

A₁ = Area covered by *Fusarium oxysporum* f.sp. *lentis* the in control.

A₂ = Area covered by the *Fusarium oxysporum* f.sp. *lentis* in dual culture.

A. Efficacy of plant extracts against *Fusarium* wilt *in vivo*.

The effective concentration of plant extracts found effective *in vitro* were further tested *in vivo*. Control pots were filled with soil without adding inoculum. After 4 days, plant extracts 10 per cent @ 100 ml per kg of soil was thoroughly mixed to determine the effect of plant extract *in vivo*. The seeds of highly susceptible variety of lentil (L 9-12) were sown in each pots where finally four plants were maintained. The experimental was conducted in C.R.D. with 5 treatments including control. First appearance of disease, disease incidence and per cent disease control were observed at 30 and 60 days after sowing. Per cent disease incidence and per cent disease control were calculated by using following formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plant}}{\text{Total number of plant}} \times 100$$

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

Where,

C= Per cent disease incidence of control pots

T= Per cent disease incidence in treated pots

B. Efficacy of bio-agent against *Fusarium* wilt of lentil *in vivo*:

The bio-agents were also used for *in-vivo* reduction of *F. oxysporum* f.sp. *lentis* caused by wilt disease of lentil by adding *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* in soil. *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescence* were mass multiplied in vitro. A suitable medium for growth of *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescence* was prepared by taking following ingredients:

Sorghum bran	1000g
Carboxy methyl cellulose	10.00g
Sucrose	50.00g
Lentil flour	4% on dry weight basis
Water	100 ml.
Carboxy methyl cellulose and sucrose was thoroughly mixed with sorghum bran. Lentil flour	

was supplemented for source of organic nitrogen. All the substrate was moistened in fresh water for 1 to 2 hr. Then substrates are filled in polypropylene packets @ 100g/ packet filled with polypropylene rings and sterilized by autoclaving 15 *p.s.i.* pressure for 1.5 hr. Sterilized cooled substrates were then inoculated with 5 mm mycelia disc of the antagonists under aseptic condition. These packets were incubated at 28±10 °C for 10 days. The mass culture was used for soil treatment @ 10.00 per cent per kg soil. The mass culture was thoroughly mixed with sick soil maintained separately in sick pots. The seed of susceptible variety (L 9-12) was sown and only 10 plant were maintained after germination . The experiment was conducted in CRD with 3 treatments (bio-agents including control) in 4 replication. Pots were kept in glass house and per cent disease incidence and per cent disease reduction was calculated at 30 and 60 days after sowing as described above.

III. RESULT

A.Efficacy of botanicals against *F. oxysporum* f.sp. *lentis* on radial growth *in vitro*:

In present studies Four plant extract (Table 3) were used to evaluate under *in vitro* against *F. oxysporum* f.sp. *lentis* by poison food technique at 5 and 10 per cent concentration at 4 days and 7 days of inoculation.

After 4 days of incubation:

It may be seen from the data (Table 3) that minimum radial growth was obtained in Eucalyptus (4.0 mm) followed by Garlic (5.11 mm), Tulsi (3.50 mm) and Neem (9.10 mm). The radial growth in control was (22.0 mm). The radial growth significantly differed in different treatment with other expect Garlic and Tulsi, Neem which were at par to each other Similarly there was no significant difference observed in radial growth of Garlic and Tulsi at 5 per cent concentration. The maximum per cent growth inhibition was recorded in Eucalyptus (81.81%) followed by Garlic (76.77%), Tulsi (74.59%) and Neem (58.63%) at 5 per cent concentration.

Table 3. Effect of plant extracts against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition at 5 per cent concentration *in vitro* after 4 days incubation.

Plant extracts	Concentration (5%)	
	Radial growth (mm)	Growth inhibition (%)
Neem	9.10	58.63 (49.95)
Garlic	5.11	76.77 (61.14)
Tulsi	5.60	74.59 (59.67)
Eucalyptus	4.0	81.81 (64.75)

Control	22.0	00.00
SEm±	0.82	1.02
CD at 5%	2.60	3.21

Figure given in parenthesis are transformed value

The radial growth was recorded at 10% concentration of Eucalyptus (2.48 mm) followed by Garlic (3.25 mm), Tulsi (3.50 mm) and Neem (6.08 mm). The maximum radial growth observed in control (22.0 mm). The radial growth significantly differed in different treatments with other expect, Garlic and Tulsi which were at par to each other. Similarly there was no significant difference

observed in radial growth of Garlic and Neem after 4 days of incubation. In 10 per cent concentration in growth inhibition of *F. oxysporum* f.sp. *lentis* was recorded in Eucalyptus (88.72%), Garlic (85.22%), Tulsi (84.09%) and Neem (72.36%). The per cent growth inhibition in between Garlic and Tulsi were significantly to each other. However, per cent growth inhibition in rest of the treatments was different (Table 4).

Table 4. Effect of plant extracts against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition at 10 per cent concentration *in vitro* after 4 days incubation.

Plant extracts	Concentration (10%)	
	Radial growth (mm)	Growth inhibition (%)
Neem	6.08	72.36 (58.89)
Garlic	3.25	85.22 (57.37)
Tulsi	3.50	84.09 (66.42)
Eucalyptus	2.48	88.72 (70.36)
Control	22.0	00.00
SEm±	0.50	0.77
CD at 5%	1.59	2.42

Figure given in parenthesis are transformed value

After 7 days incubation:

The radial growth was minimum recorded in Eucalyptus (5.23 mm) followed by Garlic (5.77 mm), Neem (6.20 mm), and Tulsi (12.28 mm). The radial growth in control was (42.0 mm). The radial growth significantly differed in different treatment with other expect Garlic and Neem which were at

par to each other. There was no significantly differences in radial growth were at par to each other at 5 per cent concentration (Table 5). The per cent inhibition in Eucalyptus (87.54%) followed by Garlic (86.26%), Neem (85.23%) and Tulsi (70.76%) at 5 per cent concentration. The per cent growth inhibition in between, Eucalyptus and Garlic and Neem were at par to each other (Table 5).

Table 5. Effect of plant extracts against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition at 5 per cent concentration *in vitro* after 7 days incubation.

Plant extracts	Concentration (5 %)	
	Radial growth (mm)	Growth inhibition (%)
Neem	6.20	85.23 (57.37)
Garlic	5.77	86.26 (68.19)
Tulsi	12.28	70.76 (57.23)
Eucalyptus	5.23	87.54 (69.30)
Control	42.0	00.00
SEm±	0.58	0.64
CD at 5%	1.82	2.04

Figure given in parenthesis are transformed value

The radial growth minimum was recorded in Eucalyptus (3.36 mm) and Garlic (3.75 mm) followed by Neem (4.87 mm) and Tulsi (7.12 mm).

The radial growth in control was (42 mm) at 10 per cent concentration. The radial growth significantly differed in different treatment with each other Garlic, Tulsi and Neem which were at par to each other. Similarly there was no significant difference

observed in radial growth Eucalyptus and Garlic, Tulsi and Neem (Table 6). Thus, result clearly indicated that plant extracts reduced the mycelia growth of *F. oxysporum* f.sp. *Lentis* and the effectiveness of extracts increase of their concentration. The maximum growth inhibition was recorded in Eucalyptus (92.0 %) and Garlic (91.11

%) followed by Neem (88.40 %) and Tulsi (83.04 %) at 10 per cent concentration. The per cent inhibition in between Eucalyptus and Garlic, Neem and Tulsi were at par to each other. However, per cent inhibition in rest of the treatment differed significantly to each other (Table 6)

Table 6. Effect of plant extracts against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition at 10 per cent concentration *in vitro* after 7 days incubation.

Plant extracts	Concentration (10%)	
	Radial growth (mm)	Growth inhibition (%)
Neem	4.87	88.40 (70.09)
Garlic	3.75	91.11 (72.64)
Tulsi	7.12	83.04 (65.65)
Eucalyptus	3.36	92.00 (73.57)
Control	42.0	00.00
SEm±	0.32	0.38
CD at 5%	1.02	1.22

Figure given in parenthesis are transformed value

The per cent inhibition in radial growth was higher at 10 per cent concentration as compared to 5 per cent concentration after 4 days and 7 days of incubation.

B. Efficacy of bio-agent against *F. oxysporum* f.sp. *lentis* on radial growth and per cent inhibition using dual culture technique:

The efficacy of bio-agent *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescense* were tested for radial growth and per cent inhibition of *F. oxysporum* f.sp. *lentis* by using dual culture technique.

After 4 days of incubation:

The radial growth was recorded maximum in *Trichoderma harzianum* (13.50 mm) followed by *Pseudomonas fluorescense* (11.51 mm) and *Trichoderma viride* (7.00 mm). The radial growth in control was (22.0 mm). The radial growth significantly differed with each other in all the treatments after 4 days of incubation. *Trichoderma viride* showed maximum per cent growth inhibition (68.18 %) followed by *Pseudomonas fluorescense* (47.68 %) and *Trichoderma harzianum* (38.63 %) at 4 days of incubation, respectively which significantly differed with each other (Table 7)

Table 7. Efficacy of bio-agents against *F. oxysporum* f. sp. *lentis* on radial growth and growth Inhibition using dual culture technique after 4 days incubation.

Fungal antagonism	Radial growth (mm)	Growth inhibition (%)
<i>Trichoderma viride</i>	7.00	68.18 (55.61)
<i>Trichoderma harzianum</i>	13.50	38.63 (38.41)
<i>Pseudomonas fluorescense</i>	11.51	47.68 (43.62)
Control	22.0	00.00
SEm±	0.88	0.80
CD at 5%	2.89	2.62

Figure given in parenthesis are transformed value

4.3.3.2 After 7 days incubation:

The similar result were obtained at 7 days of incubation, however, the radial growth were *Trichoderma harzianum* (18.96 mm), *Pseudomonas fluorescense* (15.10 mm) and *Trichoderma viride* (9.67 mm). The radial growth in control was (42.0

mm). The radial growth significantly differed with each other in all the treatments after 7 days of incubation (Table 8). *Trichoderma viride* showed maximum per cent inhibition (76.97 %) followed by *Pseudomonas fluorescense* (64.04 %), *Trichoderma harzianum* (54.85 %) after 7 days of incubation, respectively which significantly differed with each other (Table 8).

Table 8 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	Radial growth (mm)	Growth inhibition (%)
<i>Trichoderma viride</i>	9.67	76.97 (61.27)
<i>Trichoderma harzianum</i>	18.96	54.85 (47.75)
<i>Pseudomonas fluorescense</i>	15.10	64.04 (53.13)
Control	42.0	00.00
SEm±	0.64	0.32
CD at 5 %	2.09	1.05

Figure given in parenthesis are transformed value

Thus, it is very clear that *Trichoderma viride* was better in reducing radial growth of *F. oxysporum* f.sp. *lentis* as compared to *Trichoderma harzianum* and *Pseudomonas fluorescense* in vitro.

A. Efficacy of plant extracts against *F. oxysporum* f. sp. *Lentis* on per cent disease incidence and growth reduction in vivo:

Ten per cent concentration was found most effective in vitro was further tested in vivo at 30 days and 60 days after sowing.

At 30 days after sowing:

The minimum disease incidence was found in Eucalyptus (21.50 %) followed by Garlic (23.41 %), Tulsi (31.25 %), Neem (34.50 %) and control (80.0 %). The per cent disease incidence in between Eucalyptus and Garlic, Tulsi and Neem were at par to each other (Table 9). The maximum disease reduction was found in Eucalyptus (73.12 %) followed by Garlic (70.73 %), Tulsi (60.93 %) and Neem (56.87 %). The per cent disease reduction in between Eucalyptus and Garlic, Tulsi and Neem were at par to each other. Rest of the treatments significantly differed from each other with respect to per cent disease control (Table 9).

Table 9. Effect of plant extracts on disease incidence and disease reduction against *F. oxysporum* f.sp. *Lentis* in vivo at 30 days after sowing.

Plant extract	Concentration (%)	Disease incidence (%)	Disease Reduction (%)
Neem	10	34.50(35.97)	56.87 (48.91)
Garlic	10	23.41(28.93)	70.73 (57.23)
Tulsi	10	31.25(33.95)	60.93 (51.30)
Eucalyptus	10	21.50(27.63)	73.12 (58.76)
Control	-	80.0	00.00
SEm±	-	0.40	0.55
CD at 5 %	-	1.30	1.79

Figure given in parenthesis are transformed value

At 60 days after sowing:

The minimum disease incidence was obtain in Eucalyptus (25.50 %) followed by Garlic (28.0 %), Tulsi (34.45 %), Neem (40.50 %) and

control (95 %) was recorded at 10 per cent concentration of 60 days after sowing. The disease incidence in between Eucalyptus and Garlic were at par to each other (Table 10). Thus, the minimum disease incidence was observed in Eucalyptus and Garlic and it was maximum in Neem and Tulsi.

Table 10. Effect of plant extracts on disease incidence and disease reduction against *F. oxysporum* f. sp. *Lentis* in vivo at 60 days after sowing.

Plant extract	Concentration (%)	Disease incidence (%)	Disease reduction (%)
Neem	10	40.50 (39.52)	57.36 (49.20)
Garlic	10	28.00 (31.95)	70.52 (57.10)
Tulsi	10	34.45 (35.91)	63.73 (52.95)
Eucalyptus	10	25.50 (30.33)	73.15 (58.76)
Control	-	95.0	00.00
SEm±	-	0.258	0.49
CD at 5 %	-	0.84	1.62

Figure given in parenthesis are transformed value

The maximum disease reduction was obtained in Eucalyptus (73.15 %) followed by Garlic (70.52 %), Tulsi (63.73 %) and Neem (57.36 %) at 10 per cent concentration of 60 days after sowing. The per cent disease reduction in between Eucalyptus and Garlic, Tulsi and Neem were at par to each other. Similarly there was no significant difference observed of disease reduction in Garlic and Tulsi at 10 per cent concentration of 60 days after sowing. Per cent disease reduction in rest of the treatment differed significantly (Table 10). Thus, the disease reduction was maximum in Eucalyptus and Garlic and minimum in Neem, Tulsi at 30 days and 60 days after sowing.

B. Efficacy of bio-agents against *F. oxysporum* f.sp. *lentis* on disease incidence and disease reduction in vivo:

The results revealed that *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescense* significantly reduced disease incidence at 30 and 60 days after sowing.

At 30 days after sowing:

The disease incidence was minimum in *Trichoderma viride* (16.50 %) followed by *Pseudomonas fluorescense* (25.50 %) and *Trichoderma harzianum* (31.17 %). The disease incidence in control was (80.0 %) at 30 days after sowing, respectively which differed (Table 11) significantly to each other.

Table 11. Efficacy of bio-agents against *F. oxysporum* f. sp. *Lentis* on disease incidence and disease reduction in vivo at 30 days after sowing:

Fungal antagonist	Concentration (%)	Disease incidence (%)	Disease reduction (%)
<i>Trichoderma viride</i>	10	16.50 (23.97)	79.37 (62.65)
<i>Trichoderma harzianum</i>	10	31.17 (33.89)	61.03 (51.35)
<i>Pseudomonas fluorescense</i>	10	25.50 (30.33)	68.12 (55.61)
Control	-	80.0	00.00
SEm±	-	0.116	0.46
CD at 5 %	-	0.44	1.63

Figure given in parenthesis are transformed value

The maximum disease control was obtained in *Trichoderma viride* (79.37 %) followed by *Pseudomonas fluorescense* (68.12%), *Trichoderma harzianum* (61.03 %) at 30 days after sowing which significantly, differed with each other.

At 60 days after sowing:

The maximum disease incidence was recorded in control (95.0 %) followed by *Trichoderma harzianum* (35.50%), *Pseudomonas fluorescense* (28.43 %) and *Trichoderma viride* (19.64 %) which were significantly different to each other (Table 12).

Table 12. Efficacy of bio-agents against *F. oxysporum* f. sp. *Lentis* on disease incidence and disease reduction in vivo at 60 days after sowing:

Fungal antagonist	Concentration (%)	Disease incidence (%)	Disease reduction (%)
<i>Trichoderma viride</i>	10	19.64 (26.28)	79.32 (62.85)
<i>Trichoderma harzianum</i>	10	35.50 (36.57)	52.30 (62.63)
<i>Pseudomonas fluorescense</i>	10	28.43 (32.29)	57.79 (70.07)
Control	-	95.0	00.00
SEm±	-	0.66	0.47
CD at 5 %	-	2.31	1.65

Figure given in parenthesis are transformed value

Similar result were found in 60 days after sowing where maximum disease reduction was recorded in *Trichoderma viride* (79.32 %) followed by *Pseudomonas fluorescense* (57.79 %), *Trichoderma harzianum* (52.30 %) which were at par to other (Table 12). Results clearly indicated that *Trichoderma viride* was better as compare to

Trichoderma hrzianum and *Pseudomonas fluorescense* in reducing disease and enhancing plant disease reduction.

IV. DISCUSSION

A. Efficacy of botanicals against *F. oxysporum* f.sp. *lentis* in vitro:

During the present investigation extracts of four plants namely Neem, Garlic, Tulsi and

Eucalyptus were evaluated for fungi toxicity against *Fusarium oxysporum* f.sp. *lentis* by using poison food technique. The result showed that all plant extracts were more or less inhibitory to mycelia growth and increasing per cent inhibition in growth of *Fusarium oxysporum* f.sp. *lentis*. The effectiveness of the extracts increased with an increase in concentration and the maximum inhibition was recorded at 10 per cent concentration. The radial growth ranged from (0.00) to (7.12 mm) as compared to control (42.0 mm) at 7 days of incubation at 10 per cent concentration. The similar trends were also observed in 5 per cent concentration. The radial growth was recorded minimum in eucalyptus (3.36 mm) and Garlic (3.75 mm) followed by Neem (4.87 mm), Tulsi (7.12 mm). The radial growth in control was (42.0 mm) at 7 days of incubation at 10 per cent concentration. The radial growth significantly differed in different treatment with each other except Garlic which were at par to each other. The effect of Neem (4.87 mm) were least with respect to radial growth of pathogen after 7 days of incubation at 10 per cent. In 10 per cent concentration of plant extracts against *Fusarium oxysporum* f.sp. *lentis* maximum radial growth inhibition was recorded in Eucalyptus (92.0 %) and Garlic (91.11 %) followed by Neem (88.40 %) and Tulsi (83.04 %). The per cent inhibition in between Eucalyptus and Garlic, Neem and Tulsi were at par to each other. However, per cent inhibition in rest of the treatment differed significantly to each other. The similar patterns were also observed in 5 per cent concentration at 4 days of incubation. Thus, it is very clear that Eucalyptus and Garlic were most effective and Tulsi and Neem least effective in suppressing the growth of *Fusarium oxysporum* f.sp. *lentis* *in vitro*.

The several plant are known to possess antifungal properties against *F. oxysporum* f.sp. *lentis* leaf extract of Eucalyptus, Tulsi, Garlic and Neem have been found effective against wilt of lentil caused by *F. oxysporum* f.sp. *lentis*. The evaluation of plant extract tested *in vitro* had considerable inhibitory effect on the radial growth of *F. oxysporum* f.sp. *lentis*. The results also indicated the relatively higher fungi toxicity of Neem extract to control mycelium growth of the *F. oxysporum* f.sp. *lentis*, showing more than 5 per cent inhibition at lowest concentrations 10 per cent. The Plant extracts of Garlic and Tulsi had nearly equal effective behavior against the fungus, showing 80 to 90 % inhibition of the radial growth at different concentration. These reported are also inconformity with the present findings.

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

Biological control of plant disease is an integral part of disease management and its aim is to eradicate or manage the pathogen through the activity of other microorganism(s). It can be carried out by either manipulating the existing soil mycoflora to the disadvantage of the pathogen or by adding of non-resident antagonists. Biological management of soil and seed borne diseases are increasing and gaining stature as a possible practical and safe approach (Patel and Anahosur, 2001).

Trichoderma viride, *Trichoderma harzianum* and *Pseudomonas fluorescense* have been used against soil borne pathogen causing diseases in different crops. But only limited information is available on the management of *Fusarium oxysporum* f. sp. *lentis* causing wilt of chickpea. Studies have indicated that *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescense* have tremendous bio control potential for *Fusarium oxysporum* f. sp. *lentis*. The native isolate of were tested by dual culture technique against *Fusarium oxysporum* f. sp. *lentis* *in vitro* for inhibiting the mycelial growth and percent inhibition. It was found that *Trichoderma viride* was highly effective in reducing mycelial growth and increasing percent inhibition of *Fusarium oxysporum* f. sp. *lentis* at 4 and 7 days after incubation. The radial growth of 18.96 mm, 15.10 mm, 9.67 mm and 42.00 mm in *Trichoderma harzianum*, *Pseudomonas fluorescense*, *Trichoderma viride* and control. The per cent of disease inhibition *Trichoderma viride* (76.97%) as compared to *Pseudomonas fluorescense* (64.04%) and *Trichoderma harzianum* (54.85%) at 7 days after incubation.

Ramezani Hesamedin (2010) to test efficacy of two fungal bio agents viz., *Trichoderma harzianum*, *Trichoderma viride*, were evaluated against the lentil vascular wilt pathogen, *Fusarium oxysporum* f. sp. *lentis* *in vitro* condition using dual culture technique. Among the fungal bio-agents, *Trichoderma viride* produced the maximum inhibition zone of 17 mm compared to the minimum of 7 mm *Trichoderma harzianum*. Soil application of talc- based formulation of *Trichoderma harzianum* and *Trichoderma viride* effectively controlled the vascular wilt of lentil under glasshouse condition.

A. Efficacy of botanicals against *Fusarium oxysporum* f. sp. *lentis* *in vivo*.

Ten per cent concentration of plant extract was found most effective in reducing radial growth *in vitro* which was further tested *in vivo* to find out the effectiveness of the ten plant extracts at 30 and

60 days after sowing for Fusarium wilt management. The results were also found at 60 days after sowing. The minimum disease incidence was obtained in Eucalyptus (25.50%) followed by Garlic (28.0%), Tulsi (34.45%) and Neem (40.50%). In check, 95.0 per cent disease incidence was recorded. The disease incidence in between Tulsi and Neem were at par to each other.

Thus, the minimum wilt incidence was observed in Eucalyptus and Garlic it was maximum in Neem and Tulsi. The similar results were also found at 30 days after sowing. Disease incidence was not much reduced Tulsi and Neem. The results were also found at 60 days after sowing. The maximum disease reduction was obtained in Eucalyptus (73.15%) followed by Garlic (70.52%), Tulsi (63.73%), Neem (57.36%). The per cent disease reduction in between Eucalyptus and Garlic, Tulsi and Neem were at par to each other. Per cent disease reduction in rest of the treatment differed significantly. Thus, disease reduction was highest in Eucalyptus and Garlic and minimum in Neem and Tulsi at 30 and 60 days after sowing. Singh *et al.*, 2000 also found that Garlic showed maximum wilt disease control *in vivo*.

B. Efficacy of bio-agents against *Fusarium oxysporum* f. sp. *lentis* *in vivo*.

Trichoderma viride, *Trichoderma harzianum* and *Pseudomonas fluorescens* were also tested *in vivo* in sick pot against *Fusarium oxysporum* f. sp. *lentis*. The disease incidence was minimum in *Trichoderma viride* (19.64%) followed by *Pseudomonas fluorescens* (28.43%), *Trichoderma harzianum* (35.50) and control (95.0%) at 60 days after sowing which differed significantly to each other. The similar results were also obtained in 30 days after sowing. Disease reduction was maximum in *Trichoderma viride* (79.37%) followed by *Pseudomonas fluorescens* (68.12%) and *Trichoderma harzianum* (61.03%) at 30 days after sowing. The similar results were also found 60 days after sowing where maximum disease reduction was recorded in *Trichoderma viride* (79.32%) followed by *Pseudomonas fluorescens* (57.79) and *Trichoderma harzianum* (52.30%).

Rani S. Ranjitha and Mane S. S. (2014) to test efficacy of two species of fungal bio agents such as *Trichoderma viride*, *Trichoderma harzianum*, were evaluated against *Fusarium* wilt *in vitro* condition using dual culture technique. The highest disease incidence of observed in *Trichoderma harzianum* (35.50%) and *Pseudomonas fluorescens* (28.43%).

V. CONCLUSION

Four plant extracts *viz.*, Neem, Garlic, Eucalyptus and Tulsi were tested *in vitro* against *Fusarium oxysporum* f. sp. *lentis* follow poison food technique. All the Four plant extract showed significant reduction in radial growth of test fungus at different concentration (5% and 10%). The effectively extracts increased with an increase in concentration and time of incubation (4 days and 7 days). In 10 per cent concentration of plant extracts radial growth was minimum recorded in Eucalyptus (3.36 mm) followed by Garlic (3.75 mm), Neem (4.87mm) and Tulsi (7.12 mm). The radial growth in check was (42.00 mm). The radial growth significantly differed in different treatment with each other except Eucalyptus, Garlic which were at par to each other. Similarly there was no significant difference observed in radial growth Eucalyptus and Garlic, Neem of at 10 per cent concentration of 7 days incubation. In 10 per cent concentration growth inhibition of *F. oxysporum* f. sp. *lentis* was recorded in Eucalyptus (92.0 %), Garlic (91.11 %), Neem (88.40 %) and Tulsi (83.04 %). The per cent inhibition in between Eucalyptus and Garlic were significantly to each other. However, per cent inhibition in rest of the treatments was not significantly to each other. The after 4 days of incubation but the radial growth was higher and percent inhibition was lower. The efficacy of bio-agents *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* were tested for growth and per cent inhibition of *Fusarium oxysporum* f. sp. *lentis* by using dual culture technique. *Trichoderma viride* was more effective as compare to *Pseudomonas fluorescens* and *T. harzianum* in inhibiting the radial growth of *Fusarium oxysporum* f. sp. *lentis* at 4 and 7 days after incubation. The radial growth was minimum in *Trichoderma viride* (9.67 mm) following by *Pseudomonas fluorescens* (15.10 mm) and *Trichoderma harzianum* (18.96) as compared to control (42.00 mm) which were statistically different to each other at 7 days after incubation. *Trichoderma viride*, *Pseudomonas fluorescens* and *Trichoderma harzianum* significantly inhibited the radial growth of *Fusarium oxysporum* f. sp. *lentis* as compared to control after 7 days of incubation. *Trichoderma viride* showed higher percent inhibition (76.97 %) as compared to *Trichoderma harzianum* (54.85 %) and *Pseudomonas fluorescens* (64.04 %) after 7 days of incubation, respectively which significantly differed with each other. The similar results were also found at 4 days after incubation, however, the radial growth was more and per cent inhibition was less. Ten per cent concentration of plant extracts was found most effective *in vitro* and was further tested *in vivo* to find out the effectiveness of the four plant extract at 30 and 60 days after sowing. The disease

incidence was obtained in Eucalyptus (25.50 %) followed by Garlic (28.0%), Tulsi (34.45 %) and Neem (40.50 %) at 60 day after sowing. In check, 95.0 per cent disease incidence was recorded. The disease incidence in between Tulsi and Neem, were at par to each other. Thus, the minimum wilt incidence was observed in Eucalyptus and Garlic and it was maximum in Neem .The maximum disease control was obtained in Eucalyptus (73.15 %) followed by Garlic (70.52 %), Tulsi (63.73 %) and Neem (57.36 %) at 60 days after sowing. The percent disease control in between Tulsi and Neem were at par to each other. Percent disease control in rest of the treatment differed significantly .The per cent disease incidence and disease control was similar at 30 days after sowing, however, per cent disease incidence was higher while disease control was lower. *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* were also tested *in vivo* for biological management against *F. oxysporum* f. sp. *lentis* following soil treatment under glass house condition. The disease incidence was minimum in *Trichoderma viride* (19.64%) followed by *Pseudomonas fluorescence* (28.43%) and *Trichoderma harzianum* (35.50 %) which were significantly different to each other at 60 days after sowing. The similar results were also obtained at 30 days after sowing but the disease incidence was lower. The disease reduction was higher in *Trichoderma viride* (79.32 %) as compared to *Pseudomonas fluorescence* (57.79%) and *Trichoderma harzianum* (52.30 %) which differ significantly to each other at 60 days after sowing. However, the per cent disease reduction at 30 days after sowing was (79.37%), (68.12 %) and (61.03 %) in *Trichoderma viride*, *Pseudomonas fluorescence* and *Trichoderma harzianum* respectively and were at par to each other.

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