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Integrated Diseases Management of Lentil Wilt Caused by *Fusarium oxysporum* f. sp. *lentis* [L.] Medik

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Abstract

Lentil (*Lens esculenta* Monech) is an important Rabi pulse crop grown throughout India. Lentil plays an important role in the supply of the protein to under nourished vegetarian population of the country. It suffers from a number of diseases. Wilt of lentil caused by *F. oxysporum* f. sp. *lentis* is one of the wide spread and destructive diseases in India. The sequential development of disease symptoms were observed and recorded in inoculated wilt sick pots maintain in experiment field. The first incidence of disease was recorded after 30 days of showing. Effect of different concentration of fungicide and plant extract, bio agents against the pathogen *Fusarium oxysporum* f. sp. *lentis* on mycelial growth, Table 3, 4 and 5 illustrates the different concentration of fungicide and plant extract (Metalaxyl 8% + Mancozeb 64% @ 1000 ppm) against pathogen on per cent inhibition were recorded maximally i.e. 100 % followed by Neem Leaf extract (*Azadirachta indica* @ 10%) (84.11%), Garlic (*Allium sativum* @ 10%) (74.89 %) respectively, Percent wilt disease incidence was found at par in Seed treatments with neem leaf extract 10% (46.47%), *Trichoderma viride* and *Trichoderma harzianum* @ 4 g kg^{-1} seed (48.18), Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed + seed treatment with *Trichoderma harzianum* @ 4 g kg^{-1} seed (58.42).

Key words : Lentil, growth, neem and leaf.

The sequential development of disease symptoms were observed and recorded in inoculated wilt sick pots maintain in experiment field. The first incidence of disease was recorded after 30 days of showing. Fusarium wilt first appear as slight vein clearing on the outer portion of younger leaves, followed by epinasty (downward dropping) of older leaves. The seedling droop down followed by sudden death. The infected root, collar region and main stem showing the typical symptoms and browning of the vascular tissue is strong evidence of the disease. Further on older plant symptoms appear during flowering and pod maturation. One hundred ten genotypes screened in natural field condition with three replications during Rabi season of 2019. Two rows of 4 m length spaced 45 cm apart with plant to plant distance of 15 cm. After every genotypes, one row to L.9-12 (a Fusarium wilt of susceptible variety of Lentil) was planted and the experi-mental plot also surrounded by two rows of L.9-12 to ensure uniform spread of the disease.

Nine different combinations of various plant extracts, fungicides and bio-agents were evaluated under field conditions for their comparative efficacy against seed germination and wilt disease incidence, disease control against wilt disease of lentil by seed treatment, soil application and soil drenching methods. Effect of different concentration of fungicide and plant extract, bio agents against the pathogen Fusarium oxysporum f. sp. lentis on mycelial growth were evaluated and the different concentration of fungicide and plant extract (Metalaxyl 8% + Mancozeb 64% @ 1000 ppm) against pathogen on per cent inhibition were recorded maximally i.e. 100% followed by Neem Leaf extract (*Azadirachta indica* @ 10%) (84.11%), Garlic (*Allium sativum* @ 10%) (74.89%) respectively, Percent wilt disease

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incidence was found at par in Seed treatments with neem leaf extract 10% (46.47%), *Trichoderma viride* and *Trichoderma harzianum* @ 4 g kg^{-1} seed (48.18), Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatment with Trichoderma harzianum @ 4 g kg^{-1} seed (58.42).

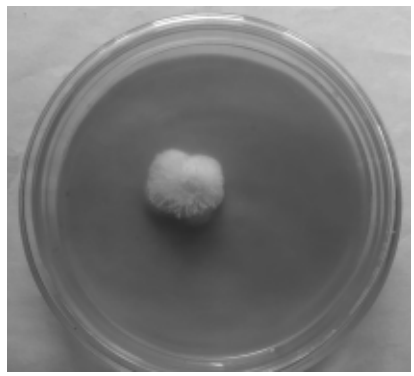
Lentil (*Lens culinaris* Medik L.) is ancient grain legume and one of the important cultivated pulse crop in India as well as its subcontinents, it contains proteins which helps to under nourished vegetarian population. Uttar Pradesh is one of the major lentil growing areas 0.46 million hectares with production of 0.45% with yield of 978 kg $hectare^{-1}$. Lentil crop cultivated at 1.32 million hectare and production of 1.18 million tonnes with the yield of 894 kg $hectare^{-1}$ in all over India. (Anonymous, 2020-21). It is a self-pollinated legume crop belongs to the Fabaceae family with diploid chromosome *viz*. 2n = 2X = 14. (Kumar *et al.*, 2021). It suffers from different kinds of diseases among these wild of lentil caused by Fusarium oxysporum f. sp. lentis is major destructive as well as widely spread disease. Different strategies have been tried to control the disease and its pathogen with the help of biological, chemical and cultural methods (Ram and Pandey, 2011; Sinnha and Sinha, 2004; Khan and Mehnaz, 2003; Srivastava *et al*. 1999).

Materials and Methods

Isolation and purification of the pathogen and its pathogenicity tests

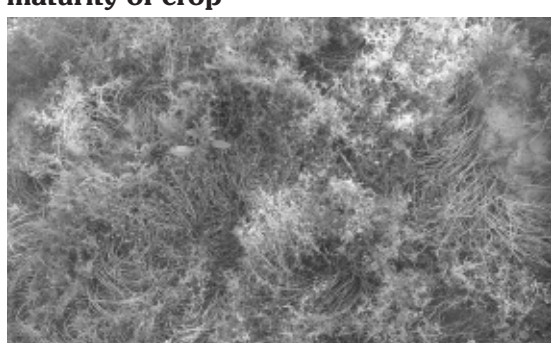
Symptomatology : The sequential development of disease symptoms were observed and recorded in inoculated wilt sick pots maintain in experiment field. The first incidence of disease was recorded after 30 days of showing. Fusarium wilt first appear as slight vein clearing on the outer portion of younger leaves, followed by epinasty (downward dropping) of older leaves. The seedling was droop down followed by sudden death. The infected root, collar region and main stem showing the typical symptoms and browning of the vascular tissue is strong evidence of the disease. Further on older plant symptoms appear during flowering and pod maturation.

Isolation



Evaluation of lentil genotypes against fusarium wilt of lentil the source of resistance : One hundred ten genotypes were screened in natural field condition with three replications during Rabi season of 2019. Two rows of 4 m length spaced 45 cm apart with plant to plant distance of 15 cm. After every genotypes, one row to L.9-12 (a *Fusarium wilt* of *lentil* susceptible variety of Lentil) was planted and the experimental plot was also surrounded by two rows of L.9-12 to ensure uniform spread of the disease.

Observation on disease severity were recorded at 15 days interval, starting with first appearance of symptoms till the maturity of crop



Infected plant of lentil Fusarium wilt Healthy Plant of lentil

Efficacy of botanicals against in *Fusarium oxysporum* f. sp. *Lentis vitro*: The locally available 5 plants which have antifungal properties was selected for this study. The extract of these plants were screened at 5 and 10 per cent concentration against *Fusarium oxysporum* f. sp. *Lentis* by using poison food technique. The data on mycelial growth were recorded at and 7 days of incubation.

List of plants with common name, English name, botanical name, family and their part used are given in table.

Common name	English name	Botanical name	Family	Part used
Neem	Neem	*Azadirachta indica*	Meliaceae	Leaves
Lahsun	Garlic	*Allium sativum*	Liliaceae	Bulb
Pyaz	Onion	*Allium cepa*	Liliaceae	Bulb
Adarakh	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome

Efficacy of bioagents against *Fusarium oxysporum* f. sp. *Lentis in vitro* : The efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *Lentis* were assessed by using dual culture technique by measuring the radial growth of against *Fusarium oxysporum* f. sp. *Lentis* as well as that of *Trichoderma* spp.

List of bio-agents used in management of *Fusarium oxysporum* f. sp. *Lentis*:

1. *Trichoderma viride*
2. *Trichoderma harzianum*
3. *Trichoderma virens*
4. *Psedomonas fluorescens*
5. *Bacillus subtilis*

Efficacy of chemicals against *Fusarium oxysporum* f. sp. *Lentis in vitro* : The 2 fungicides *viz*., benomyl were selected and evaluated at 100, 200, and 500, ppm concentration against *Fusarium oxysorum* f. sp. *Lentis* by using poison food technique. The data on mycelial growth were recorded at and 7 days of incubation.

List of fungicides, trade name, chemical name and their source of supply used in management Lens culinaris is as-

Common name - Benomyl, Trade name - Benlate, Chemical name - Dithane M45 and Source - Florida

Per cent inhibition over control was calculated by applying the following formula:

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

Where, I = Per cent inhibition, C = Colony diameter in control (mm) and T = Colony diameter in treated (mm)

Management of *Fusarium oxysporum* f. sp. *lentis. in vivo* : Efficacy of fungicide against Fusarium oxysporum f. sp. lentis. Efficacy of plant extracts against Fusarium oxysporum f. sp. lentis. Efficacy of bio-agents against Fusarium oxysporum f. sp. lentis.

***In Vivo* Study :** The present investigation were carried out during Rabi 2019 at students instructional farm of A. N. D. University of Agriculture and Technology Kumarganj, Ayodhya (U.P.) India located at latitude 26.47 0N, longitude 82.12 0E and altitude 113m above the sea level.

Design and Layout : The experiment was laid out in randomized block design (RBD) with Nine Treatments and three replications.

Date of Sowing : *Rabi* : 7-11-2020

Plot size : 4.0 m x 3 m^2

Spacing : 30 x10 cm

Variety : L.9-12. A susceptible Variety L.9-12 was used in this experiment.

Land preparation : The land was prepared by through ploughing and harrowing and soil was brought to a fine tilth.

Application : DAP / 5 kg = 600 m^2 was applied as broadcasting method at the sowing.

Treatments : T_1 : Seed treatment with Benomyl @ 2 gm kg^{-1} seed + neem leaf extracts (10%), T_2 : Seed treatment with Benomyl @ 2 gm kg^{-1} seed + garlic bulbs extract (10%), T_3 : Seed treatment with Benomyl @ 2 gm kg^{-1} seed + onion bulbs extract (10%). T_4 : Seed treatment with Benomyl @ 2 gm kg^{-1} seed + zinger rhizome extracts (10%), T_5 : Seed treatment with Trichoderma viride @ 4 gm kg^{-1} seed, T_6 : Seed treatment with *Trichoderma harzianum* @ 4 gm kg^{-1} seed, T_7 : Seed treatment with Pseudomonas fluorescens @ 6 gm kg^{-1} seed, T_8: Seed treatment with Bacillus subtilis @ 4 gm kg^{-1} and T_9 : Control.

1. Isolation, identification of the pathogen and its pathogenicity : The infected plant showing the typical symptoms of the fusarium wilt of lentil will be collected and brought to the laboratory. The pathogen will be isolated on Potato Dextrose Agar (PDA) from the diseased plants collected from field.

2. Screening of lentil genotypes against *Fusarium oxysporum* f. sp. *lentis* in field condition : Available 100 genotypes of lentil will be evaluated for source of resistance against Fusarium wilt in field condition. The appearance of disease sympotoms, the percentage of dead plants was recorded following the method proposed by Bayaa and Erskine (1990). The following formula was used to calculate wilt disease incidence

3. Efficacy of different botanicals and bio-agents against *Fusarium oxysporium* f. sp. *lentis* under *in vitro* condition : The effectiveness of the fungicide Benomyl (500 and 1000 ppm), plant extract like neem leaf (*Azadirachta indica*), garlic bulbs (*Allium sativum*), onion bulbs (*Allium cepa*), and zinger rhizome (*Zingiber officinale*) in vitro at 5% and 10% concentration evaluated in food poison technique under in vitro condition.

4. Management of fusarium wilt of lentil : The effectiveness of benomyl @ 2 gm kg^{-1} seed + neem leaf extract 10%, 2.5 g kg^{-1} seed, bio-agent *Pseudomonas fluoresens* @ 6 g kg^{-1} seed and *Trichoderma harzianum* and Trichoderma viride @ 4 g kg^{-1} seed. will be applied as seed treatment at Students" Instructional Farm, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodha. And all recommended agronomical practices will be followed while conducting the experiment

Expermental Findings

First appearance of disease : Disease intensity was recorded at 15 days interval. Rating was done on 1-9 scale (Mayee and Datar, 1986). These numerical ratings were used to calculate the per cent disease intensity (PDI) as follows:

$$PDI = \frac{\text{Sum of all numeric rating}}{\text{Total no. of leaves examined x Maximum grade}} \times 100$$

Per cent disease control was calculated by using the following formula:

$$PDC = \frac{\text{PDI in unprotected plot x PDI in protected plot}}{\text{PDI in unprotected plot}} \times 100$$

Per cent increase in yield was calculated by using the following formula:

$$\text{Avoidable loss in yield (\%)} = \frac{\text{Yield in protected plot - Yield in unprotected plot}}{\text{Yield in protected plot}} \times 100$$

Per cent disease incidence formula

$$\text{Per cent disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Table 1. Effect of different concentration of plant extract, and fungicide against *Fusarium oxysporum* f. sp. *lentis* on mycelial growth *in vitro* at 7 days

Plant extract/Fungicide	Dose	Mycelial growth (mm)
Benomyl 500ppm	500 ppm	00.00
Neem leaf extract (*Azadirachta indica*)	5%	10.60
Neem	5%	11.13
Garlic	5%	10.80
Onion	5%	11.30
Ginger	5%	11.90
Control	5%	80.12
SEm±	0.42	
CD at 5%	1.31	

Table 2. Effect of different concentration of fungicide and plant extract, bio agents against *Fusarium oxysporum* f. sp. *lentis* on mycelial growth *in vitro* at 7 days

Plant extract/Fungicide	Dose	Mycelial growth (mm)
Benomyl	1000 ppm	00.00
Neem Leaf extract (*Azadirachta indica*)	10%	10.60
Garlic (*Allium sativum*)	10%	10.80
Onion (*Allium cepa*)	10%	11.30
Ginger (*Zingiber officinale*)	10%	11.90
Control	10%	80.12
SEm±	0.42	
CD at 5 %	1.31	

Table 3. Effect of different concentration of fungicide and plant extract against *F. oxysporum* f. sp. *lentis* on per cent inhibition *in vitro* at 7 days

Plant extracts/ Fungicide	Dose	Per cent inhibition
Metalaxyl 8% + Mancozeb 64%	1000 ppm	100
Neem Leaf extract (*Azadirachta indica*)	10%	84.11
Garlic (*Allium sativum*)	10%	74.89
Onion (*Allium cepa*)	10%	72.44
Ginger (*Zingiber officinale*)	10%	70.89
Metalaxyl 8% + Mancozeb 64% + *Trichoderma harzianum*	10%	66.22
Parthenium (*Parthenium hysterophorus*)	10%	61.56
Metalaxyl 8% + Mancozeb 64%	10%	55.33
Control		0.00
SEm±	1.72	
CD at 5 %	5.21	

Table 4. Effect of different concentration of fungicide and plant extract against *F. oxysporum* f. sp. *lentis* on per cent inhibition *in vitro* at 7 days

Metalaxyl 8 % + Mancozeb 64 %	1000 ppm	100
Neem Leaf extract (*Azadirachta indica*)	10%	84.11
Garlic (*Allium sativum*)	10%	74.89
Onion (*Allium cepa*)	10%	72.44
Ginger (*Zingiber officinale*)	10%	70.89
Metalaxyl 8% + Mancozeb 64% + *Trichoderma harzianum*	10%	66.22
Parthenium (*Parthenium hysterophorus*)	10%	61.56
Metalaxyl 8% + Mancozeb 64%	10%	55.33
Control		0.00
SEm±	1.72	
CD at 5 %	5.21	

Results and Discussion

Nine different combinations of various plant extracts, fungicides and bio-agents were evaluated under field conditions for their comparative efficacy against seed germination and wilt disease incidence, disease control against wilt disease of lentil by seed treatment, soil application and soil drenching methods. Different bio-agents and plant extracts were used

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	Radial growth (mm)	Growth inhibition (%)
Trichoderma harzianum	20.24	56.86 (48.94)
Pseudomonas fluorescence	11.34	79.91 (63.39)
Trichoderma virence	22.76	47.67 (43.66)
Trichoderma viride	17.27	67.11(55.01)
Bacillus subtilis	21.13	52.37 (46.35)
Control	42.00	00.00 (0.28)
SEm±	0.84	1.33
CD at 5 %	3.63	4.89

at their different doses and lentil grain yield was also recorded.

The evident states from the Table 1 and Table 2 that the Effect of different concentration of fungicide and plant extract, bio agents against the pathogen *Fusarium oxysporum* f. sp. *lentis* on mycelial growth, Table 3, 4 and 5 illustrates the different concentration of fungicide and plant extract (Metalaxyl 8% + Mancozeb 64% @ 1000 ppm) against pathogen on per cent inhibition were recorded maximally i.e. 100% followed by Neem Leaf extract (*Azadirachta indica* @ 10%) (84.11%), Garlic (*Allium sativum* @ 10%) (74.89%) respectively, Percent wilt disease incidence was found at par in Seed treatments with neem leaf extract 10% (46.47%), *Trichoderma viride* and *Trichoderma harzianum* @ 4 g kg^{-1} seed (48.18), Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatment with *Trichoderma*

Table 6. Effect of seed dresser fungicides, botanicals and bio-agents against *F. oxysporum* f. sp. *lentis* on per cent disease incidence *in vivo*

Treatment	Gram kg^{-1} seed	Per cent disease incidence		
		At 30 DAS	At 45 DAS	At 60 DAS
T_1 - Seed treatment with Metalaxyl 8% + Mancozeb 64 % @ 2.5 g^{-1} kg seed	2.5	27.90 (31.87)	29.85 (33.11)	34.03 (35.68)
T_2 - Seed treatments with neem leaf extract (10%).	2	35.00 (36.25)	37.45 (37.73)	42.69 (40.79)
T_3 - Seed treatment with *Trichoderma viride* @ 4 g^{-1} kg seed.	4	30.90 (33.75)	33.06 (35.10)	37.69 (37.84)
T_4 - Seed treatment with *Trichoderma harzianum* @4 g^{-1} kg seed	4	32.80 (34.93)	35.10 (36.33)	40.01 (39.23)
T_5 - Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatments with neem leaf extract (10%)	2.5	26.30 (30.85)	28.14 (32.03)	32.08 (34.49)
T_6 - Seed treatment with Trichoderma viride @ 4 g^{-1} kg seed	2.5	18.60 (25.55)	19.90. (26.47)	22.69 (28.44)
T_7 - Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatment with *Trichoderma harzianum* @ 4 g kg-1 seed	2.5	20.40 (26.85)	21.83 (27.83)	24.88 (29.92)
T_8 - Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatment with neem. Leaf extract (5%) + seed treatment with *Trichoderma viride* @ 4 g kg^{-1} seed	2.5	12.30 (20.53)	13.16 (22.27)	15.00 (22.79)
T_9 - Control		80.00 (63.56)	90.00 (71.57)	90.00 (72.25)
SEm±		1.13	0.67	1.69
CD at 5%		3.39	2.03	5.11

Table 7. Effect of seed dresser fungicides, botanicals and bio-agents against *F. oxysporum* f. sp. *lentis* on per cent disease control *in vivo*

Treatment	Gram kg^{-1} seed	Per cent disease incidence		
		At 30 DAS	At 45 DAS	At 60 DAS
T_1 - Seed treatment with Metalaxyl 8% + Mancozeb 64 % @ 2.5 g^{-1} kg seed	2.5	65.13 (53.82)	66.83 (54.88)	62.19 (52.08)
T_2 - Seed treatments with neem leaf extract (10%).	2	56.25 (48.59)	58.39 (49.85)	52.56 (46.47)
T_3 - Seed treatment with *Trichoderma viride* @ 4 g^{-1} kg seed.	4	61.38 (51.58)	63.26 (52.74)	58.12 (48.18)
T_4 - Seed treatment with *Trichoderma harzianum* @4 g^{-1} kg seed.	4	59.00 (50.19)	61.00 (51.37)	55.55 (48.18)
T_5 - Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatments with neem leaf extract (10%)	2.5	67.13 (55.04)	68.73 (56.00)	64.35 (53.37)
T_6 - Seed treatment with *Trichoderma viride* @ 4 g^{-1} kg seed	4	76.75 (61.24)	77.89 (61.98)	74.79 (60.03)
T_7 - Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatment with *Trichoderma harzianum* @ 4 g kg^{-1} seed	2.5	74.50 (60.12)	765.75 (60.51)	72.35 (58.42)
T_8 - Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5 g kg^{-1} seed + seed treatment with neem. Leaf extract (5%) + seed treatment with *Trichoderma viride* @ 4 g kg-1 seed	2.5	84.63 (67.14)	85.38 (67.59)	83.33 (66.09)
T_9 - Control		00.00 (0.00)	00.00 (0.00)	00.00 (0.00)
SEm±		1.92	1.62	2.00
CD at 5%		5.82	4.92	6.08

harzianum @ 4 g kg^{-1} seed (58.42). These similar findings have been recorded by the Ansari (2003) who reported that Integrated disease management strategies including the use of alum, zinc and biocontrol agents in vitro studies revealed that the maximum increase in seed germination, disese control was observed in *Trichoderma harzianum* + zince and *Pseudomonas fluorescens* + alum + zinc also similar observations with Singh *et al.* 2017.

Conclusion

Lentil (*Lens esculenta* Monech) is an important *Rabi* pulse crop grown throughout India. Lentil plays an important role in the supply of the protein to under nourished vegetarian population of the country. It suffers from a number of diseases. Wilt of lentil caused by *F. oxysporum* f. sp. *lentis* is one of the wide spread and destructive diseases in India. Use of botanicals plant extracts and bio-agents are environment safe, pollution free for management of wilt disease. Many plant extracts are known for their antifungal activity.

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