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Efficacy of seed dresser fungicides, botanicals and bioagents against *fusarium oxysporum* f. sp. *Lentis in vivo*.

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Abstract
Lentil (*Lens culinaris* Medik) is a member of Leguminaceae family and it is commonly known as poor man's meat. Lentil suffers from attack of a number of diseases such as vascular wilt; collar rots, root rot, stem rot, rust, powdery mildew downy mildew and fusarium wilt which are caused by *Fusarium oxysporum* f. sp. *lentis*. The highest per cent disease control was found in T_8 Metalaxyl 8% + Mancozeb 64% + Neem leaf extract + *P. fluorescens* (87.75%) followed by T_6 Metalaxyl 8% + Mancozeb 64% + *P. fluorescens* (81.54%), T_7 Metalaxyl 8% + Mancozeb 64% *T. harzianum* (79.62%) T_5 Metalaxyl 8% + Mancozeb 64% Neem leaf extract 10% (73.88%), T_1 Metalaxyl 8% + Mancozeb 64% (71.50%), T_3 *P. fluorescens* (68.83%), T_4 *T. harzianum* (66.88%) and T_2 Neem leaf extract 10% (64.68%). as compared to T_9 control (0.0).

Keywords: Lentil, fusarium, fungicides, evaluation, neem

Introduction
Lentil (*Lens culinaris* Medik) is a member of Leguminaceae family and it is commonly known as poor man's meat. It has a high nutritional value and major source of dietary proteins (25%) after soybeans (Zia *et al.*, 2011) [10]. Lentil (*Lens culinaris* Medik L.) is the most important pulse crop in India and mostly grown in north east plain zone & central part of India. It is a diploid and self-pollinated crop which is grown in winter season and belonging to Order-Rosales, suborder-Rosnee, Family-Leguminaceae (fabaceae), subfamily- papilionaceae, genus-*Lens* and species- *culinaris* with chromosome number 2n=14. Among *rabi* pulses, lentil is next to chickpea. The total area under lentil in India was 1.49 m ha with a total production of 1.61 MT and 1006 Kg/ha productivity (Anonymous, 2018) [1]. In Uttar Pradesh, it is grown on 4.78 lakh hac. Area with 4.47 lakh tones production and productivity 936 kg/ha (Anonymous, 2018) [1].

Lentil suffers from attack of a number of diseases such as vascular wilt; collar rots, root rot, stem rot, rust, powdery mildew downy mildew and fusarium wilt which are caused by *Fusarium oxysporum* f. sp. *lentis*. Average yield of lentil is low due to various diseases. Among the disease, foot and root rot of lentil caused by *Fusarium oxysporum* and *Sclerotium rolfisii* are common and the most serious disease in India. The fusarium wilt of lentil caused by *Fusarium oxysporum* f. sp. *lentis* is one of the most important and destructive disease in India wilt pathogen minimize crop yield and deteriorate the seed quality (Khare, 1991) [6]. The disease was first reported from Hungary but in India it was first observed in 1941 from Delhi and Karnal. If infection takes place in early stage the plant do not produced seeds, formed few in number when infection occurred in later stage. Wilt causes more damage at flowering and pod formation stage of the crop. *Fusarium oxysporum* f. sp. *lentis*. Infection range from 25-95 % depending on the cultivars tested. Lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis* is a major disease in lentil growing areas in the country reported that the annual yield losses 10-15 % due to this disease alone which valued approximately Rs. 2000-2500 crores by (Chaudhary and Amarjit, 2002) [2].

Resistant sources are available against wilt disease of lentil but they are unstable. At present wilt is being managed by using fungicide through seed and soil treatment. However, fungicides are more costly and pollutant to environment. Many plant extract are known to have antifungal activity. Therefore, keeping in view the importance of the crop, seriousness of the disease, non-availability of suitable management practices and gaps in our knowledge

about this disease

Materials and Methods

The present investigation were carried out during *Rabi* 2018-2019 at students instructional farm of A. N. D. University of Agriculture and Technology Kumarganj, Ayodhya (U.P.) India located at latitude 26.47 ^{0}N, longitude 82.12 ^{0}E and altitude 113m above the sea level. The cultivar 'L9-12' was selected to conduct the experiments in three replications. The experiment was laid out in randomized block design (RBD) with nine treatments *viz*.

T_1: Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed.
T_2: Seed treatments with neem leaf extract (10%).
T_3: Seed treatment with *Pseudomonas fluorescens* @ 5g/kg seed.
T_4: Seed treatment with *Trichoderma harzianum* @ 4g/kg seed.
T_5: Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed + Seed treatment with neem leaf extract (10%),
T_6: Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed + Seed treatment with *Pseudomonas fluorescens* @ 5g/kg seed.
T_7: Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed + Seed treatment with *Trichoderma harzianum* @ 4g/kg seed.
T_8: Seed treatment with Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed + Seed treatment with neem leaf extract (5%) + Seed treatment with *Pseudomonas fluorescens* @ 5g/kg seed.
T_9: Control.

Efficacy of fungicide against *F. oxysporum* f. sp. *lentis*

The effective fungicides used for seed treatment *in vivo*. Seeds of lentil variety L9-12 were moist for 12 hrs. prior to showing and then treated with the Metalaxyl 8% + Mancozeb 64% @ 2.5g/kg seed, treated seeds of susceptible variety 'L9-12' were sown in each wilt sick plot. Per cent wilt incidence was recorded at 30, 45 and 60 days after sowing.

Efficacy of plant extracts against *F. oxysporum* f. sp. *lentis*

The effective concentration of plant extracts found effective *in vitro* were further tested *in vivo*. After 7 days plant extracts (10 per cent) @ 100 ml per kg of soil was thoroughly mixed to determine the effect of plant extract *in vivo*. Twelve seeds of a highly susceptible variety of lentil (L 9-12) were sown in each plot where finally 10 plants were maintained. The experiment was conducted in RBD with 9 treatment including control.

First appearance of disease, disease incidence and per cent disease control were recorded at 30, 45 and 60 days after sowing. Per cent disease incidence and per cent disease control was calculated by using following formula.

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

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

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

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

The pure culture of antagonists was used for seed treatment. Pure culture of *Trichoderma harzianum* and *Pseudomonas fluorescence* was prepared with 15 days old culture multiplied in Potato Dextrose Agar and Nutrient agar medium. Potato Dextrose Agar medium and Nutrient agar medium was filled in the Petri plates, approximately up to 1/3 of their total capacity. The medium of each flask were sterilized at15 *p.s.i.* for 20 minutes. After sterilization, broth was inoculated with mm disc of actively growing culture of most effective isolate of *Trichoderma harzianum* and *Pseudomonas fluorescence*. Flasks were incubated at 25 ± 2 ^{0}C for 14-15 days till the whole surface was completely covered by mycelial mat. The liquid culture of the bio-agents were filtered through Whatman filter paper No. 44. Mycelial mat was desiccated at room temperature for three days. Dried mycelial mats were grinded by pestle and mortar to get pure powder of these bio-agents. This preparation was used for the seed treatment @ of 4gm per Kg seed of lentil. The seed was treated with bio-agents separately and sown in wilt sick plots as described earlier in three replications. The sowing of untreated seeds served as check. Per cent wilt incidence was recorded at 30, 45 and 60 days after sowing.

Results and Discussion

Effect of fungicide, plant extracts and bio-agents was tested alone and in combination to see their individual as well as combined effect on wilt disease management in field condition. Nine treatment were under taken in this study, among them least per cent lowest disease incidence was found in T_8 Metalaxyl 8% + Mancozeb 64% + Neem leaf extract 10% + *P. fluorescens* (12.25%) followed by T_6 Metalaxyl 8% + Mancozeb 64% + *P. fluorescens* (18.46%), T_7 Metalaxyl 8% + Mancozeb 64% *T. harzianum* (20.38%), T_5 Metalaxyl 8% + Mancozeb 64% Neem leaf extract 10% (26.12%), T_1 Metalaxyl 8% + Mancozeb 64% (28.50%), T_3 *P. fluorescens* (31.17%), T_4 *T. harzianum* (33.12%) and T_2 Neem leaf extract 10% (35.32%) as compared to control (80.00%). The similar disease incidence was also reported in same manner at 45 and 60 days after sowing.

The highest per cent disease control was found in T_8 Metalaxyl 8% + Mancozeb 64% + Neem leaf extract + *P. fluorescens* (87.75%) followed by T_6 Metalaxyl 8% + Mancozeb 64% + *P. fluorescens* (81.54%), T_7 Metalaxyl 8% + Mancozeb 64% *T. harzianum* (79.62%) T_5 Metalaxyl 8% + Mancozeb 64% Neem leaf extract 10% (73.88%), T_1 Metalaxyl 8% + Mancozeb 64% (71.50%), T_3 *P. Fluorescens* (68.83%), T_4 *T. harzianum* (66.88%) and T_2 Neem leaf extract 10% (64.68%). as compared to T_9 control (0.0). The similar per cent disease control was also reported in same manner at 45 and 60 days after sowing. De *et al.* (2003) [4] reported that seed treatment with carbendazim + thiram and *Gliocladium virens* + *Pseudomonas fluorescens* + carboxin were effective and controlling 48.8 per cent and 44.2 per cent lentil wilt in field condition. Dolatabadi *et al.* (2012) [5] reported that *Trichoderma viride, Trichoderma hariziamum* and he found effective against Fusarium wilt of lentil. *Trichoderma viride*, *Trichoderma harziamum* and *Gliocladium virens* inhibit the pathogen by competition, mycoparasitism and antibiosis (De and Mukhopadhyay, 1995., Papavizas *et al*., 1985) [3, 7].

Singh *et al.* (2017) [8] reported the effect of seed treatment with *Trichoderma harzianum* (4g/kg seed) + Carbendazim (1g/kg seed) + intercropping with linseed (2:1 ratio) found minimum wilt incidence (11.67%), maximum disease control (72.06%) as compared to check.

Sinha and Sinha (2004) [9] reported that Neem leaf extract

(*Azadirachta indica*), Marigold leaf extract (*Tagetes erecta*`) and Garlic bulb extract (*Allium sativum*) at 5 per cent as seed treatment reduce wilt incidence and increase yield compared with the untreated control.

Singh *et al.* (2017) [8] the fungicides carbendazim 50% and carbendazim 12% + mancozeb 63% were quite effective which completely inhibited the mycilial growth and the sporulation at all the concentrations (50ppm to 1000ppm).

Table 1: Effect of seed dresser fungicides, botanical and bio-agents against *F. oxysporum* f. sp. *lentis* on per cent disease incidence *in vivo*

Treatment		gram/kg seed	Per cent disease incidence		
			At 30 DAS	At 45 DAS	At 60 DAS
T_1	Metalaxyl 8 % + Mancozeb 64 %	2.5	28.50 (32.25)	30.36 (33.42)	34.42 (35.26)
T_2	Neem leaf extracts (10%)	2	35.32 (36.45)	39.12 (38.71)	46.38 (42.92)
T_3	*Pseudomonas fluorescence*	5	31.17 (33.93)	33.14 (35.14)	35.58 (36.61)
T_4	*Trichoderma harzianum*	4	33.12 (35.12)	36.16 (36.96)	40.58 (39.56)
T_5	Metalaxyl 8% + Mancozeb 64% + Neem leaf extract (10%)	2.5	26.12 (30.72)	29.67 (32.99)	33.58 (35.40)
T_6	Metalaxyl 8% + Mancozeb 64% + *P. fluorescens*	2.5	18.46 (25.39)	21.56 (27.65)	25.13 (30.06)
T_7	Metalaxyl 8% + Mancozeb 64% + *T. harzianum*	2.5	20.38 (26.82)	23.12 (28.72)	28.19 (32.05)
T_8	Metalaxyl 8% + Mancozeb 64% + Neem leaf extract (5%) + *P. fluorescens*	2.5	12.25 (20.45)	15.17 (22.91)	22.29 (28.15)
T_9	Control		80.00 (63.50)	90.00 (71.69)	90.00 (71.75)
	SEm±		1.07	1.07	1.39
	CD at 5%		3.20	3.22	4.17

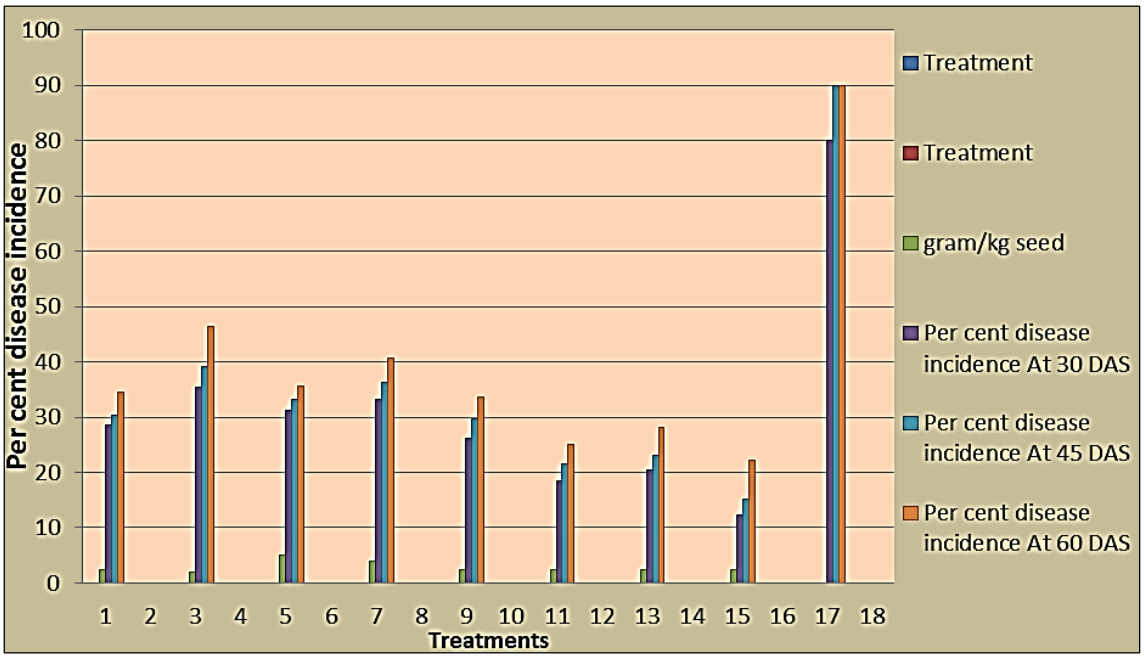

Fig 1: Effect of seed dresser fungicides, botanical and bio-agents against *F. oxysporum* f. sp. *lentis* on per cent disease incidence *in vivo*

Table 2: Effect of seed dresser fungicides, botanicals and bio-agents against *F. oxysporum* f. sp. *lentis* on per cent disease control *in vivo.*

Treatments		gram/ kg seed	Per cent disease control		
			At 30 days	At 45 days	At 60 days
T_1	Metalaxyl 8 % + Mancozeb 64 %	2.5	71.50 (57.74)	69.64 (56.56)	65.58 (54.08)
T_2	Neem leaf extracts (10%)	2	64.68 (53.54)	60.88 (51.28)	53.62 (47.07)
T_3	*Pseudomonas fluorescens*	5	68.83 (56.06)	66.86 (54.86)	64.42 (53.38)
T_4	*Trichoderma harzianum*	4	66.88 (54.86)	63.84 (53.04)	59.42 (50.43)
T_5	Metalaxyl 8% + Mancozeb 64% + Neem leaf extract (10%)	2.5	73.88 (59.27)	70.33 (56.99)	66.42 (54.59)
T_6	Metalaxyl 8% + Mancozeb 64% + *P. fluorescens*	2.5	81.54 (64.56)	78.44 (62.35)	74.87 (59.91)
T_7	Metalaxyl 8% + Mancozeb 64% + *T. harzianum*	2.5	79.62 (63.17)	76.88 (61.26)	71.81 (57.93)
T_8	Metalaxyl 8% + Mancozeb 64% + Neem leaf extracts (5%) + *P. fluorescens*	2.5	87.75 (69.54)	84.83 (67.08)	77.71 (61.83)
T_9	Control		00.00 (0.28)	00.00 (0.28)	00.00 (0.28)
	SEm±		0.75	0.90	0.47
	CD at 5%		2.24	2.69	1.41

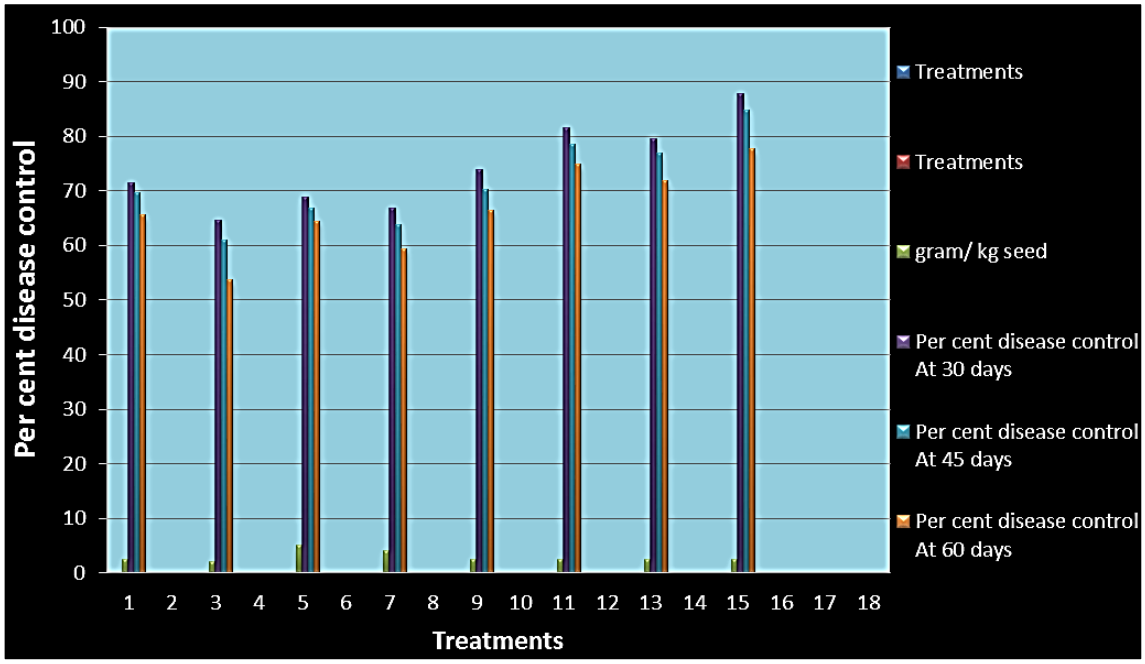

Fig 2: Effect of seed dresser fungicides, botanicals and bio-agents against *F. oxysporum* f. sp. *lentis* on per cent disease control *in vivo*.

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