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Management of vascular wilt (*Fusarium oxysporum* f.sp. *lentis*) of Lentil (*Lens culinaria* Medic.) through botanicals and bioagents

Subhash Chandra, NK Rajvanshi, Ajay Kumar and Pankaj Kumar

Abstract
Lentil is an important pulse crop and plays very important role in the supply of the protein to under nourished vegetarian population of the country. *Fusarium* wilt of lentil caused by *Fusarium oxysporum* f. sp. *lentis* is one of the wide spread and destructive diseases in India under rice-based cropping system. Use of synthetic fungicides has led to the emergence of several problems like environment pollution, residual effect in grains and killing of non-target organism(s). Hence, for minimizing the losses caused by *Fusarium* wilt, four plant extracts such as Garlic, Neem, Eucalyptus, Tulsi and three antagonistic bio-agents *viz*. *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* were evaluated. The disease incidence was not much reducing by Neem (40.50 %) and Tulsi (34.45 %) at 10 per cent concentration of 60 days after sowing. The same trends were also found in per cent disease reduction. However, per cent disease reduction in between Eucalyptus (25.50 %) and Garlic (28.00 %), Tulsi (34.45 %) was at par to each other. The Maximum per cent disease reduction was found in Eucalyptus (25.50 %) and Garlic (28.00 %), Lowering the disease incidence and higher per cent disease reduction was also found in *Trichoderma viride* (19.64 %) as compared to *Trichoderma harzianum* (35.50 %) and *Pseudomonas fluorescence* (28.43 %) at 10 per cent concentration of 60 days after sowing.

Keywords: fenugreek, hematological, *O. niloticus*, serum

Introduction
Lentil (*Lens culinaris* Medik.) is an important pulse crop and the second major source of dietary proteins (25%) after soybeans in human and animal diet (Rahman *et al*., 2010) [10]. In India, it is the extensively grown cool season legume crop next to chickpea in terms of quality and quantity (Khan *et al*., 2001) [6]. The crop is infected by a number of fungal plant diseases and out of which vascular wilt is the most devastating caused by several *Fusarium* species. It is recognized the most important factor in reducing lentil production (Saxena, 1993; Erskine *et al*., 2009) [1]. The wilt disease occurs either at early (seedling) crop stage or at reproductive (adult plant) stage (Stoilova & Chavdarov, 2006) [13]. Presently, lentil production in India is facing continuous decrease as a result of several biotic stresses including wilt (Subhani *et al*., 2007) [14]. A number of management strategies aiming at controlling wilt disease are in practice. The cultural practices like crop rotation is common, however, it is not much effective because the pathogen is of seed or soil-borne nature and can survive in soil for extended period of time. Botanicals and biological management are considered to be the effective in reducing the inoculum present in soil. Among biological control agents, *Trichoderma* species are considered the most effective against several fungal pathogens including *F. oxysporum* (Sarhan *et al*., 1999) [11]. These antagonists are saprophytic filamentous fungi, easily growing and produce conidia having long survival period in large quantities (Mohamed & Haggag, 2006) [8]. Therefore, it is essential to determine the variability in the pathogen regarding its host plant resistance for a successful lentil breeding plan and replacing the low yielding and disease susceptible lentil varieties with those of high yielding and disease resistance ones. Considering these facts, this study was carried out to evaluate the available lentil germplasm, botanicals and bio-control agents against the disease in order to identify sources of resistance, effective botanicals and bio-control agents soil application for the management of lentil wilt

disease.

Materials and Methods

For the management of lentil wilt disease, two experiments viz., evaluation of botanicals and bioagants were carried out at Naredra Deva University of Agriculture and Technology, Narendra, Nagar, Kumarganj, Ayodhya (UP) during 2015-2016 cropping seasons. The effective concentration of plant extracts found effective *in vitro* will be further tested *in vivo*. Controlled pots were filled with soil without adding inoculum. After 4 days, plant extracts of Neem, Garlic, Tulsi and Eucalyptus, 10 per cent @ 100 ml per kg of soil was thoroughly mixed to determine the effect of plant extract *in vivo*. Twelve seeds of highly susceptible variety of lentil (L 9-12) were sown in each pot. The experiment was conducted in C.R.D. with 5 treatments including control in four replications. 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

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. These bio agents were multiplied *in vitro*. A suitable medium for growth of *T. viride*, *T. harzianum* and *P. fluorescence* were prepared by taking Sorghum bran (1000g), Carboxy methyl cellulose (10.00g), Sucrose (50.00), Lentil flour (4%) on dry weight basis, water (100 ml). Carboxy methyl, cellulose and sucrose were 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 ^{0}C for 10 days. The mass culture was used for soil treatment @ 10.00 per cent / 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 plants were maintained after germination. The experiment was conducted in CRD with 4 treatments (bio-agents including control) in 4 replication. Pots were kept in green house and per cent disease incidence and per cent disease reduction was calculated at 30 and 60 days after sowing.

Results and Discussions

At 30 days after sowing the data present in Table 1 shows that 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 1). 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 1).

The minimum disease incidence was obtained 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 in 60 days after sowing. The disease incidence in between Eucalyptus and Garlic were at par to each other (Table 1). Thus, the minimum disease incidence was observed in Eucalyptus and Garlic, it was maximum in Neem and Tulsi. 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 1). Then, the disease reduction was maximum in Eucalyptus and Garlic while minimum in Neem and Tulsi at 30 and 60 days after sowing (Table 1). Present studies are in conformity with the reports of Agrawal *et al*., (1977) reported that *Trichoderma harizanum* and *Bacillus subtilis* has been more effective when applied to seed rather than soil. The three decades the followed the pioneering work of Weindling 1934 and 1937 of *Trichoderma* and *Gliocladium* were effective agents for bio-control. There are many recent advances indicate that biocontrol of plant pathogens can be successfully exploited. (Papavizes, 1985) [9]. Upadyayay and Mukhopadyayay, (1986) [15] *Trichoderma viride* and *Trichoderma hariziamum* effective against *Fusarium oxysporum, Sclerotium rolfsi* and *Rizoctonia solnai* amoung the soil borne pathogens of chickpea. Dhedhi *et al*., (1990) reported the *Trichoderma viride*, *Trichoderma hariziamum* and *Glioclodium virens* inhibit the pathogen by competion, mycoparasitism and antibioses. (De and Mukhopadhyay, 1995., Papavizas *et al*., 1985.) [9]. Mandhore and Kumar *et al*., (2002) Evaluated several plants extracts but he found effective against wilt of lentil, Ganja (*Cannbis sativa*) and Lantana (*Lantana camara*). Dolatabadi *et al.*, (2010) reported that the pathogen was inoculated in soil then he found most effective combination of antagonistic fungi *Sebacina vermifera* + *T. harzinum* was observed. Garkoti *et at*., (2010) tested plant extract and he was observed that all the botanicals were effective. The results revealed that *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* significantly reduced disease incidence at 30 and 60 days after showing. The disease incidence was minimum in *T. viride* (16.50 %) followed by *P. fluorescence* (25.50 %) and *T. harzianum* (31.17 %). The disease incidence in control was (80.0 %) at 30 days after sowing, respectively which differd (Table 2). The maximum disease control was obtained in *T. viride* (79.37 %) followed by *P. fluorescence* (68.12%), *T. harzianum* (61.03 %) the result sowing which significantly, differed with each other (Table 2).

Sixty days after sowing the maximum disease incidence was recorded in control (95.0 %) followed by *T. harzianum* (35.50%), *P. fluorescence* (28.43 %) and *T. viride* (19.64 %) which were significantly different to each other (Table 2). Similar result were found in 60 days after sowing where maximum disease reduction was recorded in *T. viride* (79.32 %) followed by *P. fluorescence* (57.79 %), *T. harzianum* (52.30 %) which were at par to other (Table 2). Results

clearly indicated that *T. viride* was better as compare to *T. harzianum* and *P. fluorescence* in reducing disease and enhancing plant disease reduction.

Table 1: Effect of plant extracts on disease incidence and disease reduction against *F. oxysporum* f. sp. l*entis in vivo* at 30 and 60 days after sowing at 10% concentration.

Plant extract at 10% concentration	At 30 days after sowing		At 60 days after sowing	
	Disease incidence (%)	Disease reduction (%)	Disease incidence (%)	Disease reduction (%)
Neem	34.50(35.9)	56.87(48.91)	40.50 (39.52)	57.36 (49.20)
Garlic	23.41(28.9)	70.73(57.2)	28.00 (31.95)	70.52 (57.10)
Tulsi	31.25(33.9)	60.93(51.3)	34.45 (35.91)	63.73 (52.95)
Eucalyptus	21.50(27.63)	73.12(58.7)	25.50 (30.33)	73.15 (58.76)
Control	80.0	00.00	95.0	00.00
SEm±	0.40	0.55	0.258	0.49
CD at 5 %	1.30	1.79	0.84	1.62

Figure given in parenthesis are transformed value.

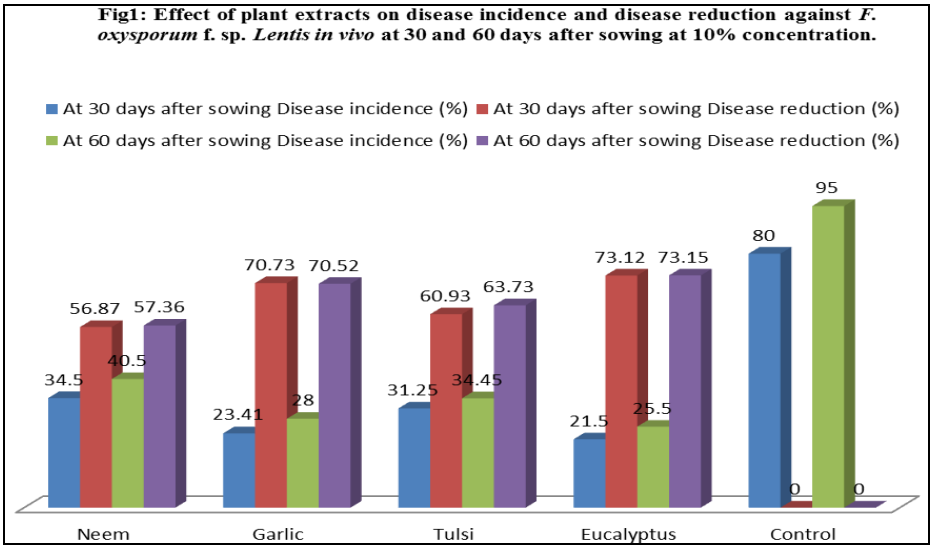
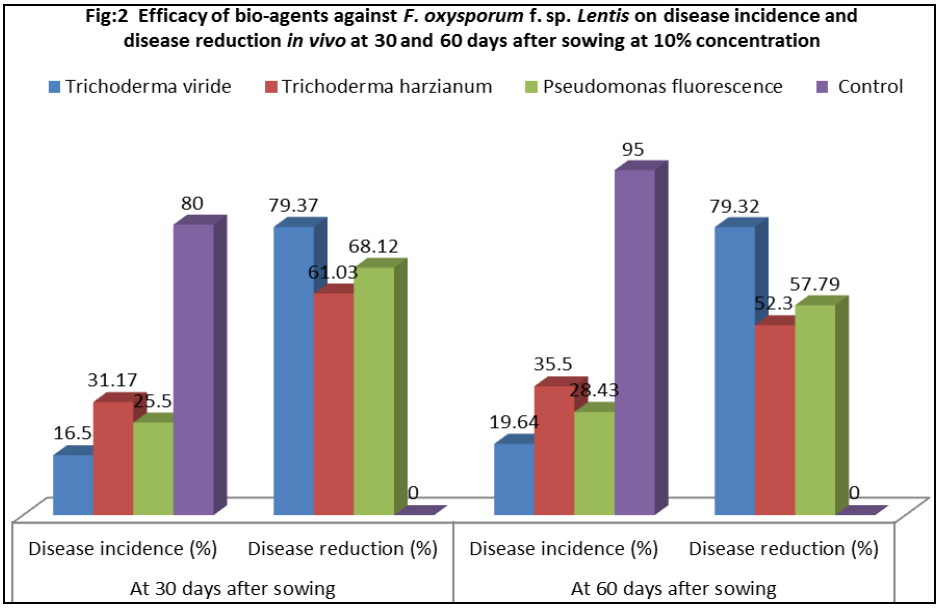


Table 2: Efficacy of bio-agents against *F. oxysporum* f. sp. *lentis* on disease incidence and disease reduction *in vivo* at 30 and 60 days after sowing at 10% concentration

Fungal antagonist at 10% concentration	At 30 days after sowing		At 60 days after sowing	
	Disease incidence (%)	Disease reduction (%)	Disease incidence (%)	Disease reduction (%)
Trichoderma viride	16.50 (23.97)	79.37 (62.65)	19.64 (26.28)	79.32 (62.85)
Trichoderma harzianum	31.17 (33.89)	61.03 (51.35)	35.50 (36.57)	52.30 (62.63)
Pseudomonas fluorescence	25.50 (30.33)	68.12 (55.61)	28.43 (32.29)	57.79 (70.07)
Control	80.0	00.00	95.0	00.00
SEm±	0.116	0.46	0.66	0.47
CD at 5 %	0.44	1.63	2.31	1.65

Figure given in parenthesis are transformed value.



Summary and Conclusion

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. The minimum 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 (Table 1 & Fig 1). Thus 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 and rest of the treatment differed significantly (Table 1&Fig.1).

Trichoderma viride, Trichoderma harzianum and *Pseudomonas fluorescence* were also tested *in vivo* where the disease incidence was minimum in *Trichoderma viride* (19.64%) followed by *Pseudomonas fluorescence* (28.43%) and *Trichoderma harzicinum* (35.50 %) which were significantly different to each other at 60 days after sowing. 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 (Table 2 & Fig.2).

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