

Original Research Article

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Efficacy of Different Biocontrol Agents and Fungicides on Vigour Index of Lentil (*Lens culinaris* Medik) Infected with *Fusarium oxysporum* f. sp. *lentis* (Vasudeva and Srinivasan) Gordon

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ABSTRACT

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The present study was carried out *in vitro* to examine the efficacy of eight different biocontrol agents viz; *T. harzianum*, *T. viride*, *T. koningii*, *T. atroviride*, *T. virens*, *T. asperellum*, *T. hamatum*, and *Pseudomonas fluorescens* and eight fungicides viz; thiram, hexaconazole, carbendazim, copper oxychloride, propiconazole, mancozeb, thiophanate methyl and metalaxly against effect of wilt pathogen *Fusarium oxysporum* f.sp *lentis* and on the growth promotion of lentil against. The results of the observation revealed that all biocontrol agents and fungicides promote the growth of lentil *i.e.* germination percentage, plumule length, radical length and overall vigour index over control. Among the biocontrol agents, the maximum seedling vigour index (1073.29) was recorded in *T. harzianum* followed by *T. viride* (981.83) and *T. koningii* (897.30). However, minimum seedling vigour index was found in *T. atroviride* (579.54) followed by *T. virens* (636.80). Among the tested fungicides, carbendazim was found to be most effective and resulted in maximum seedling vigour index *i.e.* 966 which were followed by mancozeb (897.83), hexaconazole (857.70) and propiconazole (803.33). While copper oxychloride (616.34) was least effective in enhancing the vigour index of lentil.

Introduction

Pulses play an important role in human diet after cereals. It also enhances the productivity of soil. In the world, among pulses, lentil (*Lens culinaris* Medik) ranks third after chickpea and pea (FAO 2015). It is cultivated as an important component of diet over the last 8,000 years (Dhuppar *et al.*, 2012; Oplinger *et al.*, 1990) and also good source of proteins, minerals, carbohydrates, and fiber. It is an autogamous, annual, diploid crop

(2n=14) with genome size of approximately 4 Gbp in its haploid (Arumuganathan and Earle, 1991).

It fixes the atmospheric nitrogen thus improving the soil fertility, carbon sequestration, and also helps in management of diseases, weeds and insect pests (Kumar *et al.*, 2013). The major lentil producing countries are Australia, North America, Western Asia, China, Ethiopia, Syria, Bangladesh and India (FAOSTAT, 2014). In

India, main lentil growing states are Madhya Pradesh, Bundelkhand region of Uttar Pradesh, Bihar, Maharashtra and Rajasthan. Globally, its cultivated area is around 4.34 million hectares producing 4.95 million tons with an average production of 1140 kg/ha (FAOSTAT, 2014). In India it was grown in 1.89 mha with production of 1.13mt with an average production of 598 kg/ha during 2013-14.

However, yield of lentil remains low due to biotic and abiotic stresses. Biotic stresses such as fusarium wilt (*Fusarium oxysporum f.sp. lentis*), anthracnose (*Colletotrichum truncatum*), ascochyta blight (*Ascochyta lentis*), collar rot (*Sclerotium rolfsii*), root rot (*Rhizoctonia solani*), rust (*Uromyces viciaefabae*), stemphylium blight (*Stemphylium botryosum*), and white mold (*Sclerotinia sclerotiorum*), (Kumar *et al.*, 2013; Sharpe *et al.*, 2013) affect lentil and cause severe yield loss. Among them Fusarium wilt caused by *Fusarium oxysporum f.sp. lentis* is one of the major disease affecting lentil all over the world (Bayaa *et al.*, 1998; Khare 1981).

It affects lentil at every growth stage from seed upto maturity (Khare *et al.*, 1979; Vasudeva and Srinivasan, 1952). Disease is favoured by warm and dry condition (Bayaa and Erskine 1990). In India, fusarium wilt is the major factor which limits lentil production in the states of Madhya Pradesh, Rajasthan, Uttar Pradesh, Haryana, Punjab, Himachal Pradesh, Bihar, West Bengal and Assam (Agrawal *et al.*, 1993; Chaudhary *et al.*, 2009; 2010). In India, the incidence of this disease has been reported at seedling, flowering and pod stages at temperature 25°C or above (Kannaiyan and Nene, 1976).

Disease management by the application of fungicide and biocontrol agents are best way to overcome problem which ensures the stable lentil production and aided in increasing the vigour index (Taylor *et al.*, 2007).

Materials and Methods

Material used

The study was conducted in Pathology Lab, Department of Plant Protection, Aligarh Muslim University AMU, Aligarh (U.P) India, to study the efficiency of different *Trichoderma* spp. on growth promotion *in-vitro* (towel paper) using seed treatment method of application. The antagonists were procured from IARI (ITCC NO: *T. harzianum*: 2895), Division of Plant Pathology New Delhi. The fungal antagonists were maintained on potato dextrose agar (PDA) slant and stored in refrigerator for further studies. Pathogen was isolated from the root of lentil crop procured from agriculture field of Aligarh district. Lentil seeds were collected from Jamalpur market, Aligarh.

Treatment with biocontrol agents and fungicides on seedling vigour index of lentil crop

The experiments for seedling vigour index were conducted *in-vitro* (towel paper). The seeds of lentil (pusa vaibhav, IPL 316) were treated with seven *Trichoderma* spp. i.e. *T. harzianum*, *T. viride*, *T. koningii*, *T. atroviride*, *T. virens*, *T. asperellum*, *T. hamatum*, & *Pseudomonas fluorescense* and eight fungicides i.e Thiram, Hexoconazole, Bavistin, Copper Oxchloride, Propiconazole, Mencozeb, Thiophenate Methyl, Metalaxly, were tested over control for their growth promotion effect (*in vitro*) against *Fusarium oxysporum f.sp lentis*. @ 5g or ml/ kg seed @ 2g or ml/kg respectively. Paper towel method was used for observation of seedling vigour of lentil. Ten seeds of each treatment of biocontrols and fungicides with 3 replications were placed in moist towel paper in equal distance, thereafter folded in a circular way using butter paper. The folded towel papers were placed in a horizontal position in a

medium sized tray and incubated at 25°C for 10 days. Proper moisture was maintained by addition of sterilized distilled water in the tray as per requirements. Seeds treated with BCA and fungicide was kept as standard check and those without any treatment kept as control. The experiment was laid out in a completely randomized design with three replications. The observations on germination, plumule length and radical length were recorded at 10 days after incubation and seedling vigour index was calculated using following formula:

Seedling Vigour Index = (Plumule length + Radical length) x Germination (%)

Results and Discussion

The objective of this experiment was to investigate whether the potential of biological control agents and fungicides could be enhanced through the application of different *Trichoderma* spp. and fungicides. This *in-vitro* experiment showed that an application of *Trichoderma* spp. achieved more growth promotion (increased germination percentage plumule and radical length) (Fig. 1 and 2).

Effect of different *Trichoderma* spp. on seedling vigour of lentil *in-vitro* (Towel paper method)

Outcome of result clearly showed (Table 1) that among *Trichoderma* spp., when applied as seed treatment, *T. harzianum* @ 5 g/kg, significantly enhanced germination percentage (93.33%), plumule length (7.60cm), radical length (3.90cm) and seedling vigour index (1073.29) followed by *T. viride* [(93.33% germination, 6.84cm (plumule length) & 3.68 cm (radical length), 981.83 (vigour index)], and *T. koningii* (90%, 6.47cm, 3.50cm, 897.30 respectively). Other biocontrol agents also showed increase in Germination percentage, plumule length, radical length and vigour index viz: *T. hamatum*, *T. asperellum*,

Pseudomonas fluorescence [(86.66%, 83.33%, 80% (germination), 5.51cm, 5.8cm, 5.72cm (plumule length), 3.00cm, 2.66cm, 2.50cm (radical length), 737.47, 704.97, 657.60 vigour index respectively. Among all *Trichoderma* spp. *T. atroviride* followed by *T. virens* found to be least effective with 76.66% and 80% in germination, 5.46cm, 5.63cm (plumule length), 2.16cm, 2.33cm (radical length) and 579.54, 636.80 (vigour index) respectively.

Effect of different fungicides on seedling vigour of lentil *in-vitro* (Towel paper method)

Outcome of result clearly showed (Table 2) that among different fungicide when applied as seed treatment @ 2g/kg seed. significantly enhanced germination percentage (96.66%), plumule length (7.4cm), radical length (2.6cm) and seedling vigour index (966.00) were recorded with Bavistin. followed by Mancozeb [(93.33% germination, 7.06cm (plumule length) and 2.56cm (radical length), 897.83 (vigour index)], and Hexaconazole (90%, 7.03cm, 2.50, 857.70 respectively).

Other fungicides also showed increase in germination percentage, plumule length, radical length and vigour index viz; Propiconazole, Thiram and Thiophanate methyl, [(86.66%, and 83.33%, (germination), 6.81cm, 6.80cm, 6.36cm, (plumule length), 2.46cm, 2.21cm, 2.16cm, (radical length), 803.33, 750.80, 709.97 vigour index respectively. Among all fungicides. Copper oxychloride followed by Metalaxyl found to be least effective with 76.66% and 80% in germination, 5.9cm, 6.24cm (plumule length), 2.14 cm, 2.15cm (radical length) and 616.34, 671.20 (vigour index) respectively. The overall outcomes of this experiment indicated that antagonistic fungus *Trichoderma harzianum* used as seed treatment for controlling lentil wilt which is one of the most destructive disease wherever lentil is grown.

Fig.1 Showing effect of different *Trichoderma* spp. on radical and plumule length of lentil seed



Fig.2 Showing effect of different *Trichoderma* spp. on radical and plumule length of lentil seed



Table.1 Effect of different biocontrol agents on seedling vigour index

Treatment	Germination (%)	Increase in germination (%)	Plumule length (cm)	Increase in plumule length (%)	Radical length (cm)	Increase in radical length (%)	Seedling vigour index
<i>T.harzianum</i>	93.33 (77.69)	38.08	7.60 (15.99)	80.95	3.90 (11.23)	85.71	1073.29
<i>T. viride</i>	93.33 (77.69)	33.32	6.84 (15.16)	62.85	3.68 (11.03)	79.00	981.83
<i>T. koningii</i>	90.00 (71.13)	28.57	6.47 (14.70)	54.04	3.50 (10.77)	66.66	897.30
<i>T. atroviride</i>	76.66 (61.19)	09.51	5.46 (13.50)	30.15	2.16 (8.44)	06.00	579.54
<i>T. virens</i>	80.00 (63.40)	19.04	5.63 (13.73)	34.12	2.33 (8.77)	10.95	636.80
<i>T.asperellum</i>	83.33 (66.11)	19.04	5.80 (13.92)	38.09	2.66 (9.38)	26.66	704.97
<i>T. hamatum</i>	86.66 (68.82)	23.80	5.51 (13.55)	31.19	3.00 (9.94)	42.85	737.47
<i>P.fluorescens</i>	80.00 (63.40)	14.20	5.72 (13.76)	36.19	2.50 (9.09)	19.04	657.60
Control	70.00 (56.76)		4.2 (11.78)		2.10 (8.32)		441.00
CD	9.74		1.68		0.73		
SE(m)	3.25		0.56		0.24		
SE(d)	4.60		0.79		0.34		
CV	8.36		6.97		4.40		

*Mean of three replications

Figures in the parenthesis are angular transformed values

Table.2 Effect of different fungicides on seedling vigour index.

Treatment	Germination (%)	Increase in germination (%)	Plumule length (cm)	Increase in plumule length (%)	Radical length (cm)	Increase in radical length (%)	Seedling vigour index
Thiram	83.33 (66.1)	19.04	6.80 (15.12)	65.06	2.21 (8.50)	43.72	750.80
Hexaconazole	90.00 (71.53)	28.57	7.03 (15.35)	69.07	2.50 (9.06)	62.30	857.70
Bavistin	96.66 (83.84)	38.08	7.40 (15.80)	71.88	2.60 (9.26)	68.83	966.00
Copperoxychloride	76.66 (61.19)	9.51	5.90 (14.04)	41.82	2.14 (8.40)	38.96	616.34
Propiconazole	86.66 (68.82)	23.80	6.81 (15.11)	63.78	2.46 (9.03)	60.17	803.33
Mancozeb	93.33 (77.69)	33.32	7.06 (15.40)	69.87	2.56 (9.19)	66.66	897.83
Topsin M	83.33 (66.11)	19.04	6.36 (14.50)	52.88	2.16 (8.43)	40.25	709.97
Metalaxyl	80.00 (63.40)	14.28	6.24 (14.46)	50.08	2.15 (8.42)	39.61	671.20
Control	70.00 (56.76)		4.16 (11.70)		1.54 (7.11)		399.00
CD	10.11		2.23		1.05		
SE(m)	3.37		0.74		0.35		
SE(d)	4.77		1.05		0.50		
CV	8.55		8.84		7.11		

*Average of three replications.

Figures in the parenthesis are angular transformed values

In this investigation, we used seven antagonist of *Trichoderma* spp. viz; *T. harzianum*, *T. viride*, *T. koningii*, *T. atroviride*, *T. virens*, *T. asperellum*, *T. hamatum* and one bacterial antagonist *Pseudomonas fluorescens* under the lab conditions for growth promotion (germination percentage, plumule length and radical length) against lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis*.

Now a days *Trichoderma harzianum* widely used and easily available in market, marketed as biopesticides, biofertilizers and soil amendments. It has intense potential to protect plants, help in enhancing vegetative growth and reduce pathogen populations

under various agricultural conditions (Nicot, 2011).

The present study results revealed that all biocontrol agents were significantly increased (more or less) germination percentage and growth (plumule length and radical length) of lentil plants as compared to control.

This may conclude that *Trichoderma*-treated plants enhance potential to uptake nutrient also increased nutrient solubility (Kaya *et al.*, 2009). *Trichoderma* spp. improved root and shoot growth, and hence enhanced seedling vigour to grow more rapidly (Harman, 2006). The root system enhanced nutrient and water

use efficiency (Lopez-Bucio *et al.*, 2005). Thus facilitated, increased radical length which in turn increased plumule length, which results into growth promotion and seedling development. The enhanced seedling growth by *Trichoderma* (*T. harzianum*) might be due to secondary metabolites released by biocontrol agents like auxin and cytokinin like compound which help in development of root and shoot system (Vinale *et al.*, 2008). The results obtained for the growth promotion experiments support the finding of Srivastava *et al.*, (2008) who reported that *T. harzianum* significantly increased vigour of lentil cultivar IPL 316. Entesari *et al.*, (2013) reported that the seed bio-priming with *Trichoderma* strains increases seed germination, seedling growth and chlorophyll content in plant. Kumar *et al.*, (2014) reported that percentage of seed germination was higher in *T. harzianum* and *Trichoderma viride* treated seeds. Results showed that, almost all *Trichoderma* and *pseudomonas fluorescens* performed effectively and reduced the disease.

The results of this study indicate that use of antagonistic fungi such as *T. harzianum* followed by *T. viride* and among fungicides carbendazim followed by mencozeb can be use for controlling lentil wilt which is a major disease of lentil and can lead to yield increase in yield.

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