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RESEARCH ARTICLE

Effect of *Fusarium oxysporum* f. sp. *lentis* on Seed Quality Parameters in Lentil

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Abstract

The Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* (Fol) is recognized as a major seed and soil-borne disease of lentils worldwide. This disease not only affects yield but also cause deterioration of seed quality. The current study was carried out to determine the effect of Fol on seed quality parameters in lentils. Out of 120 samples collected from different agro-climatic regions of India, only 40 samples were used for seed quality parameter studies. The seed health testing method revealed the infection percentage of Fol-infected seed samples from 40 to 55%. We observed a significant reduction in the germination percentage (48.78%, Fol-102), seedling length (16.70%, Fol-125), and seedling vigour (882, Fol-135). In contrast, an increase in electrical conductivity (EC) (93.14 µS cm^{-1} g^{-1}) was observed in Fol-119 infected seeds as compared to healthy seeds. Our results showed that the seed quality is highly compromised in Fol-infected seeds, compared to healthy seeds and needs to be addressed through the development of different management practices.

Keywords: Electric conductivity, Fusarium wilt, Germination, Lentil, Seed borne disease, Seedling length, Seedling vigour.

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Introduction

Lentil (*Lens culinaris* Medik.), commonly known as Masur, is an important pulse crop, mostly grown in the northern plains, central and eastern parts of India. The major lentil producing states are Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and Bengal. The main constraints responsible for the low yield of lentils include lack of availability of good quality seeds, biotic and abiotic stresses etc., Among the biotic stresses, several fungal, bacterial and viral diseases affect the crop's quality and yield. The potential threats for lentil cultivation are wilt (*Fusarium oxysporum* f. sp. *lentis)*, collar rot (*Sclerotium rolfsii)*, root rot (*Rhizoctonia solani)*, rust (*Uromycis fabae)*, powdery mildew (*Erysiphe polygoni)* and downy mildew (*Peronospora lentis*) (Javeria *et al.,* 2020).

Among diseases, *Fusarium* wilt is the most widespread and important disease of lentil crop. Several species of *Fusarium* incite the disease but the most predominant fungus is *F. oxysporum* Schlecht. sp. *Lentis* (Hiremani *et al.,* 2016). It is one of the serious fungal diseases that cause maximum seed quality and yield loss in the crop by reducing crop stand in the field (Stoilova *et al*., 2006). Lentil diseases affect yield and cause deterioration of seed quality. The disease is estimated to cause economic yield losses in parts of South America, WANA, region, sub-Saharan Africa and South Asia. In Western Algeria, lentil was severely affected due to wilt (Tiwari *et al.,* 2018). The disease is both seed and soil borne and affects the seed germination, vigor to a great extent and attacks the crop both at the seedling and adult stages (Khare, 1996). In some fields, Fol

infection ranged from 25 to 95% and as high as 50 to 78% of wilt incidence has been reported in Madhya Pradesh (Agrawal *et al.,* 1991).

In India FoI is the main problem in states of Bihar, Madhya Pradesh, Uttar Pradesh, West Bengal and other areas where lentil is grown (Saxena *et al*. 2018). A survey of 116 districts in nine lentil-growing states of India recorded a plant mortality range of 0.7 to 9.3% at the reproductive stage. A loss of 37.5% grain yield per hectare and reduced marketability due to the discoloration of seed (Chaudhary *et al.,* 2012). The seed-borne pathogens and the mortality caused by them in major lentil growing areas of India during flowering to pod maturity are more crucial from a yield point of view and for carryover of the pathogens through seed (Chaudhary *et al.,* 2012). To increase the production of lentil qualitatively and quantitatively farmers requires quality seeds, with a high percentage of germination and purity. Hence, seeds must be tested before they are sown in the field. Another adverse effect of seed-borne pathogens is that they will contaminate areas that previously were disease-free. In this context, present studies were undertaken to determine the effect of FoI on seed quality parameters in lentils.

Materials and Methods

Survey and Sample Collection of FoI Infected Seed

The present study was conducted in different geographical regions of India i.e. Madhya Pradesh, Rajasthan, Bihar, Uttar Pradesh, Jharkhand, New Delhi and Chandigarh during *rabi* season in the year of 2018-2019 (Table 1 and Figure 1). More than 120 disease samples (Infected plant and infected seeds) of lentil wilt were collected at podding and harvesting stage. Wilted lentil plants and seeds were identified based on their typical wilt symptoms like drooping, epinasty, yellowing, xylem necrosis, discoloration and shriveled seeds. The wilt incidence was recorded (Figure 2).

Seed Quality Analysis

The wilt-infected seeds were surface sterilized with sodium hypochlorite 1% for 1 to 2 minute. Sterilized seeds were placed on PDA and incubated for 7 days at 22 ± 1°C with a 12 hours alternate cycle of light and dark in BOD. Both healthy and infected seeds were subjected to seed health, standard germination test and seed vigor were evaluated by using ISTA rules, 2020.

Seed Health Testing

To know the efficacy of seed health testing methods i.e. 2,4-D blotter and agar plate methods, were used and evaluate the best seed health testing method. The infection percent of FoI-infected seed samples were recorded.

2,4-D Blotter Method

Total 400 infected seeds of lentil were tested by employing 2,4-D blotter method in 4 replications. Three pieces of blotting paper of 90 mm size were moistened with 0.2% solution of the sodium salt of 2, 4–dichlorophenoxy acetic acid and placed in 90 mm sterilized petri plates. seeds were placed at the rate of 25 seeds per petri plate at an

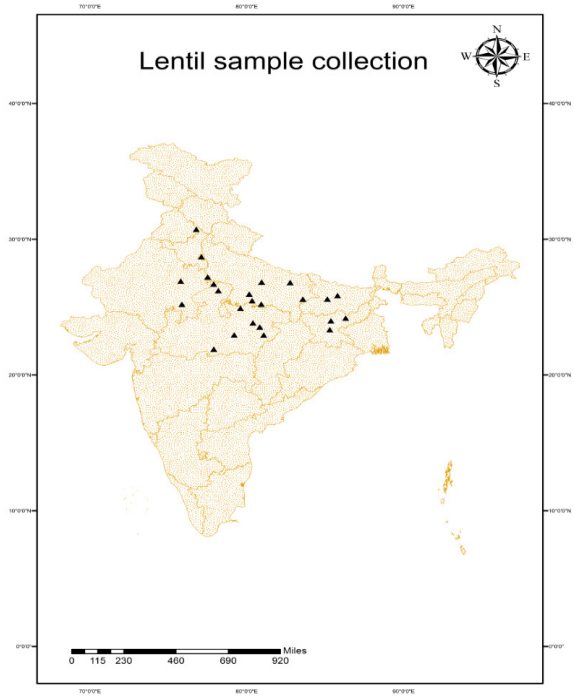


Figure 1: Map of India showing different states of collection of *Fusarium oxysporum* f. sp. *lentis* samples.

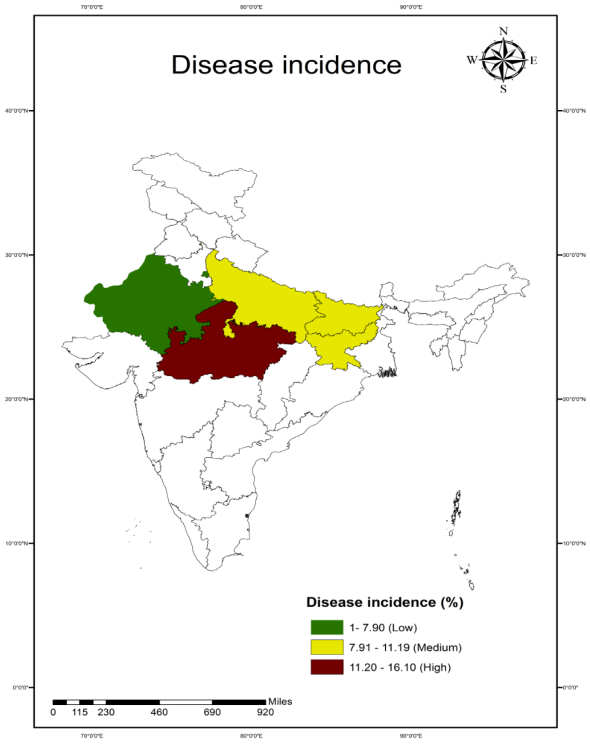


Figure 2: The disease incidence of lentil wilt was demarcated with different colors in these states.

Table 1: List of different *F. oxysporum* f. sp. *lentis* sample collected from the different geographical location.

S. No. and state	*Isolate Name*	*District*	*Place*
Madhya Pradesh			
1	Fol-101	Sehore	Sehore
2	Fol-102	Chatarpur	Gulganj
3	Fol 103	Gwalior	Badnapur
4	Fol -104	Umariya	Chandiya
5	Fol-105	Betul	Sonman
6	Fol-106	Katani	Kataria
7	Fol-107	Narsingpur	Tendukheda
8	Fol-108	Dindori	Shahpur
Rajasthan			
9	Fol-109	Bharatpur	Nimayapur
10	Fol-110	Bharatpur	Lulhara
11	Fol-111	Kota	Dabarha
12	Fol-112	Dholapur	Sevapur
13	Fol-113	Bharatpur	Nimayapur
14	Fol-114	Jaipur	Durgapur
Bihar			
15	Fol 115	Patna PPPP	PCR Mokama
16	Fol-116	Patna	PCR Mokama
17	Fol-117	Samastipur	TCA Pusa
18	Fol-118	Samastipur	TCA Pusa
19	Fol-119	Bhagalpur	Rajpur
20	Fol-120	Bhagalapur	Sabour
Uttar Pradesh			
21	Fol-121	Mhoba	Chokasora
22	Fol-122	Band	Gureh ka purva
23	Fol-123	Band	Badusa
24	Fol-124	Karvi	Pahari
25	Fol-125	Hamirpur	Muskara
26	Fol-126	Lucknow	Rahmatnagar
27	Fol-127	Gazipur	Shadibadi
28	Fol-128	Basti	Saoonghat
Jharkhand			
29	Fol-129	Hazaribagh	Hazaribagh
30	Fol-130	Ranchi	PL-156
31	Fol-131	Giridih	Mehgaon
32	Fol-132	Chatra	Intaroti
Delhi			
33	Fol-133	Delhi	IARI field
34	Fol-134	Delhi	IARI field
35	Fol-135	Delhi	IARI field
36	Fol-136	Delhi	IARI field
Chandigarh			
37	Fol-137	Chandigarh	Local field
38	Fol-138	Chandigarh	Local field
39	Fol-139	Chandigarh	Local field
40	Fol-140	Chandigarh	Local field

equal distance. The petri plates were incubated at room temperature (22 ± 1°C) under alternate cycles of 12 hours NUV light and darkness. After 7 days of incubation, the fungal growth was examined under a stereoscopic binocular microscope (40X) (ISTA rules 2020).

Agar Plate Method

For 400 seeds of lentil wilt infected seeds were surface sterilized with 1% sodium hypochlorite solution for 1 to 2 min and then placed at the rate of 10 seeds per petri plate containing 20 mL of potato dextrose agar (PDA). The petri plates were incubated at room temperature (22 ± 1°C) under alternate cycles of 12 hours NUV light and darkness. After 7 days of incubation, the fungal growth was examined under a stereoscopic binocular microscope (40X) (ISTA rules 2020)

Standard Germination Test

Total 400 seeds were used for conducting a standard germination test. With the help of a counting board, hundred seeds for each replicate were placed between two moist germination papers. Then the germination papers were folded and rolled up carefully along one edge, ensuring no excess pressure was placed on the seeds. These were wrapped in wax paper to prevent surface evaporation and placed in an upright position in a germinator at 25 ± 1°C and 90% relative humidity. After 5 days of incubation, the seedlings were examined for normal, abnormal seedlings, fresh un-germinated and dead seeds following the International Rules for Seed Testing (2020). A second count of abnormal seedlings was made after the completion of 14 days. Seedling length and seed dry weights were recorded and seedling vigor index I and II were calculated, respectively as suggested by (Abdul Baki and Anderson, 1973).

Electrical Conductivity

Four subsamples of 50 seeds were weighed (0.001 g accuracy) and put in plastic cups containing 75 mL deionized water in a BOD-type germination chamber at 25°C for 24 hours. The electrical conductivity (EC) of soaking solution was measured after this period, and the findings were represented in $\mu Scm^{-1}g^{-1}$ of seed (AOSA, 1983).

Statistical Analysis

The data recorded were subjected to statistical analysis by adopting a complete block design using OPSTAT and the percentage data were transformed into arcsine value for analysis.

Results and Discussion

In the present study, a survey of lentil growing areas of Madhya Pradesh, Rajasthan, Bihar, Uttar Pradesh, Jharkhand, New Delhi and Chandigarh states lentil wilt indicated that the wilt incidence varied in different areas (Figure 2). Average of 16.2% wilt incidence was found in Madhya Pradesh

Table 2: Effect of *F. oxysporum* f. sp. *lentis* on seed quality parameters of lentil.

S No.	Isolate Name	Germination (%)		Seedling length (cm)		Seedling vigor		Electrical conductivity (μS cm^{-1} g^{-1})	
		HS	IS	HS	IS	HS	IS	HS	IS
1	Fol-101	82.34	67.32	26.22	18.34	2159	1235	73.56	88.78
2	Fol-102	78.23	48.78	28.04	21.54	2194	1051	64.87	87.57
3	Fol-103	79.45	59.77	27.46	19.56	2182	1169	75.18	89.39
4	Fol-104	81.44	69.32	23.34	20.33	1901	1409	64.86	82.67
5	Fol-105	80.21	58.34	21.43	17.34	1719	1012	59.35	72.13
6	Fol-106	77.34	63.50	27.44	19.68	2122	1250	78.96	88.08
7	Fol-107	85.42	68.21	24.32	22.39	2078	1527	61.55	76.14
8	Fol-108	83.32	59.33	25.20	19.56	2100	1161	50.73	67.61
9	Fol-109	79.85	70.22	27.97	17.44	2234	1226	67.02	81.11
10	Fol-110	76.12	61.50	24.20	18.40	1842	1133	61.37	79.51
11	Fol-111	79.80	68.78	25.12	21.97	2005	1511	68.42	81.05
12	Fol-112	81.34	63.22	25.47	20.40	2072	1290	63.64	83.94
13	Fol-113	76.45	58.45	23.26	20.56	1779	1202	41.71	62.31
14	Fol-114	76.45	56.66	27.79	21.65	2125	1227	53.86	81.69
15	Fol-115	76.45	53.21	29.34	19.80	2243	1054	50.11	68.86
16	Fol-116	76.45	63.22	27.47	18.12	2101	1146	60.80	71.43
17	Fol-117	78.72	57.33	27.89	19.45	2196	1115	80.69	91.99
18	Fol-118	83.32	69.32	24.35	20.85	2029	1446	71.66	81.87
19	Fol-119	81.12	58.67	29.22	19.34	2371	1135	88.55	93.14
20	Fol-120	82.78	69.80	21.45	20.85	1776	1456	87.58	91.54
21	Fol-121	77.23	61.32	26.43	18.23	2041	1118	89.28	91.54
22	Fol-122	79.52	68.34	25.08	16.75	1995	1145	59.06	72.16
23	Fol-123	81.76	67.53	24.04	18.90	1966	1277	70.24	80.73
24	Fol-124	79.80	56.45	22.78	17.56	1818	991	63.50	80.87
25	Fol-125	82.43	58.45	24.48	16.70	2018	976	57.63	69.28
26	Fol-126	82.23	71.43	23.80	22.67	1958	1619	69.97	81.67
27	Fol-127	78.89	68.23	27.83	17.54	2196	1197	52.07	68.22
28	Fol-128	75.75	73.23	29.89	21.78	2265	1595	52.96	73.72
29	Fol-129	89.76	63.22	23.20	18.32	2082	1158	50.42	71.14
30	Fol-130	83.21	54.32	26.23	19.00	2183	1032	53.54	74.31
31	Fol-131	87.43	65.35	22.86	17.54	1999	1146	64.75	78.09
32	Fol-132	79.01	62.24	28.57	19.04	2257	1185	67.79	77.07
33	Fol-133	86.87	57.43	23.56	18.23	2047	1047	79.19	91.86
34	Fol-134	83.45	54.42	23.56	16.20	1966	882	78.61	89.51
35	Fol-135	78.41	65.78	27.34	19.01	2144	1250	62.37	71.23
36	Fol-136	79.40	66.20	26.19	18.34	2080	1214	56.55	75.52
37	Fol-137	86.21	70.78	28.37	16.00	2446	1132	67.42	85.35
38	Fol-138	82.33	54.32	26.51	18.09	2183	983	69.56	87.39
39	Fol-139	82.32	52.43	23.16	22.00	1907	1153	75.51	91.04
40	Fol-140	78.85	52.91	27.83	21.61	2195	1143	69.92	82.30
	C.D.	3.566	3.928	1.133	1.472	97.782	51.104	1.965	2.395
	SE (m)	1.265	1.393	0.402	0.522	34.678	18.124	0.697	0.85
	C.V.	2.712	3.877	2.706	4.69	2.896	2.616	1.92	1.831

followed by the moderate incidence of wilt 7.09, 9.23, 11.19 and 9.99% in Rajasthan, Bihar, Uttar Pradesh and Jharkhand, while 4.8% average wilt incidence was noticed in Delhi. Chaudhary et al. (2010) have reported average mortality 6.3% due to soil-borne pathogens where in, *F. oxysporum* f.sp. *lentis* was associated in 62% cases. *F. oxysporum* f. sp. lentis has also remained the most crucial limiting variable for decreasing yield levels of lentil (Parihar *et al.* 2017).

Among the 120 of samples, 40 samples were selected for further study. Out of the 40 samples , 8 were from Madhya Pradesh, 8 from Uttar Pradesh, 4 from Jharkhand, 6 each from Bihar and Rajasthan and four from Delhi and Chandigarh were used for analysis of seed quality parameters (Table 1). The seed health testing was conducted for both infected and healthy seed samples by using 2-4 D blotter method and Agar plate method (Figure 3). The results of this study indicate that more than 52.55% (Fol- MP (102) infection was seen in 2-4 D blotter method as compared to agar plate method (33.11%). The lowest infection per cent was 29.18% in 2-4 D method but in case of agar plate method lowest infection percent was (19.19%) (Figure 4). It indicate that the 2, 4- D blotter method is an effective method for the detection of Fol fungi. The moistening of blotter paper with 0.2% 2,4-D solution, which enhances the growth of slow-growing seed pathogen, suppress the germination of seed and suppress saprophytic fungi which impair the detection of pathogenic fungi on seed (Naimuddin and Chaudhary.

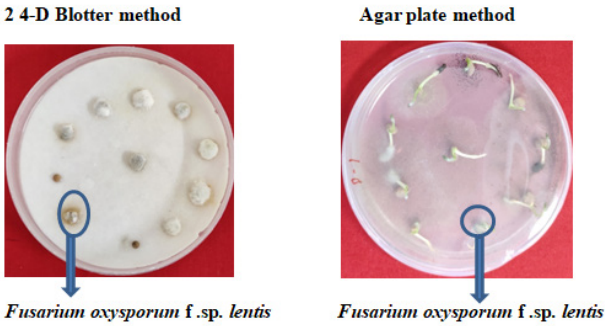
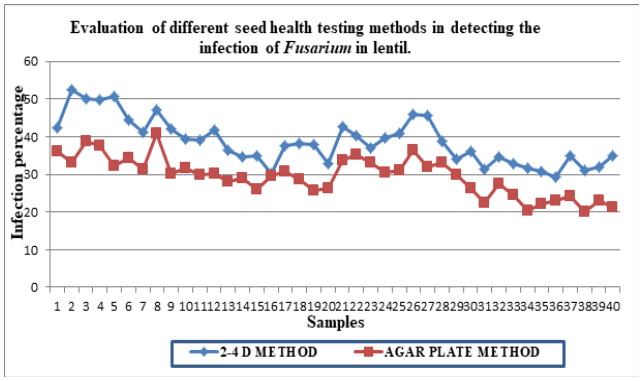


Figure 3: Seed health testing method



Supplementary Figure 1: Evaluation of different seed health testing methods in detecting the infection of *Fusarium* in lentil.

2009; Singh *et al*., 2010; Dawar *et al.,* 2007).The finding of the present study was in concurrence with Mohamed *et al*. (2011), where they find that 2.4- D blotter method is an effective method for the detection of some important seed-borne pathogenic fungi (*Cephalosporium* sp., *Fusarium verticilloides*, *F. oxysporum*, *F. solani* and *Verticillium* spp.) in peanut seeds. Hence 2,4-D blotter method is most effective for the detection of infection of seed borne *Fusarium* spp.

No published information is available from our country regarding seed quality deterioration studies in Fol. The effect of Fol on seed quality parameters like germination, seedling length, dry seedling weight, seedling vigor and EC were recorded. The present study revealed the infected seeds significantly reduced the seed quality parameters compared to healthy seeds (Table 2). Based on the experimentation, the germination percentage of the infected seed sample ranged from 48.78 to 70.78% and minimum germination was recorded in the sample Fol-102 (48.78%) which was collected from Madhya Pradesh. In case of a healthy seed lot germination percentage ranged from 75.75 to 89.76% (Figure 5a). It indicates that the 30% reduction was observed in infected seed samples as compare to healthy seed samples (Figure 5b). Similarly, the reduction in the seedling length was observed in infected seed samples as compare to healthy seed. The seedling length of infected seed samples ranges from 16.00 to 22.67 cm and minimum

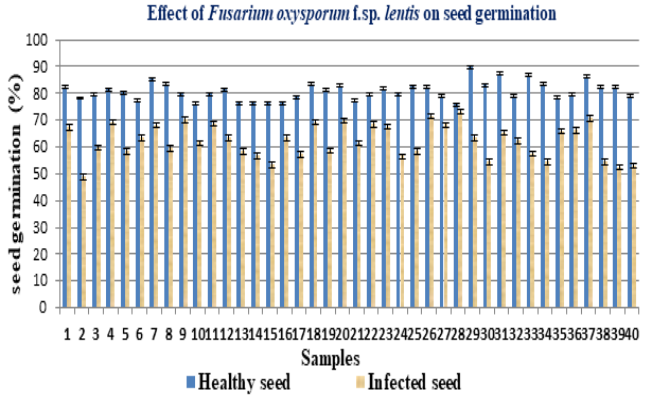


Figure 5 (a): Effect of *Fusarium oxysporum* f.sp. lentis on seed germination

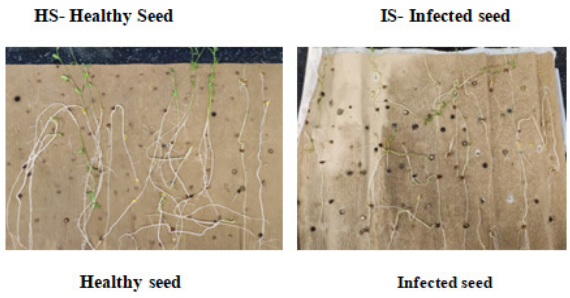


Figure 5 (b): Effect of *Fusarium oxysporum* f.sp. *lentis* on seed germination.

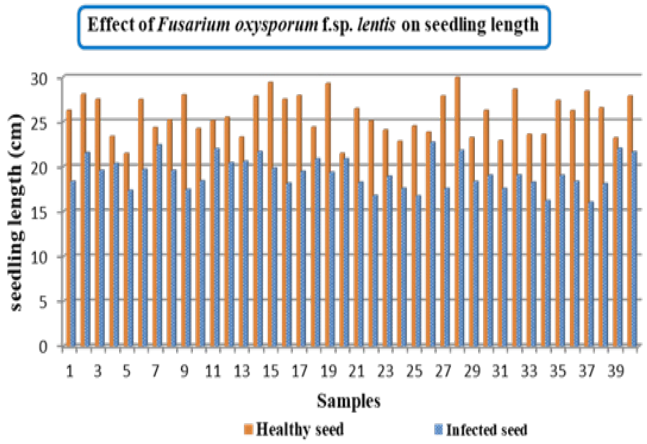


Figure 6a: Effect of *Fusarium oxysporum* f.sp. lentis on seedling length

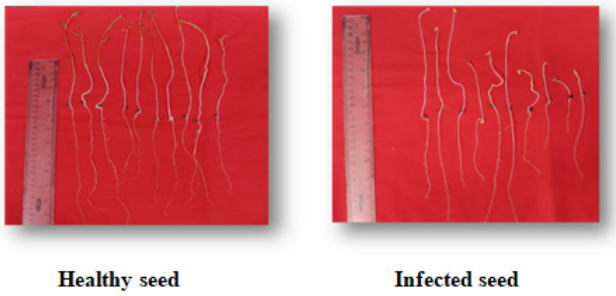


Figure 6b: Effect of *Fusarium oxysporum* f.sp. lentis on seedling length

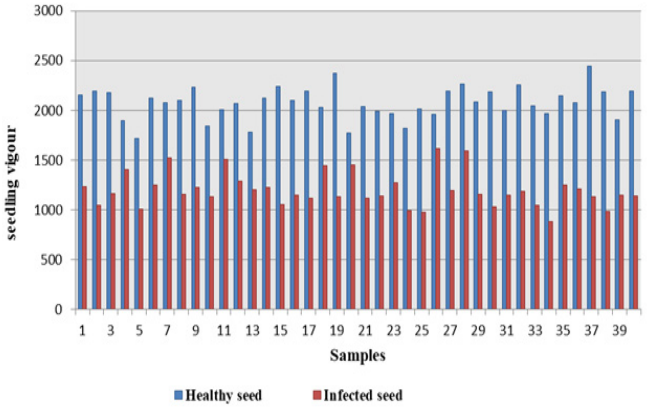


Figure 7a: Effect of *Fusarium oxysporum* f. sp. lentis on seedling vigor

Figure 7b: Effect of *Fusarium oxysporum* f. sp. lentis on seedling vigor

seedling length was observed in sample Fol-122 (16.75 cm) which was collected from Uttar Pradesh (Figure 6a). The highest seedling length was observed in healthy seed samples and ranges from 21.43 to 29.34 cm (Figure 6b). The minimum seedling vigor was observed in infected seed samples, it ranges from 882 to 1619 (Figure 7a). In case of healthy seed, seedling vigor ranges from 1719 to 2446. The highest seedling vigor was observed in Delhi isolate Fol-137 (2446) (Figure 7b). The highest EC was observed in the infected seed sample, it ranges from 62.31 to 93.14 µS cm^{-1} g^{-1}. The lowest EC was recorded in healthy seed samples it ranging from 41.71 to 89.28 µS cm^{-1} g^{-1}. The result of our study indicated that healthy seeds performed better, as compared to infected seeds in terms of seed quality parameters. The reduced seed quality parameters in infected seeds are due to shriveled seed, seed rotting, and seed infection. It makes the seeds vulnerable to seed deterioration it, ultimately causes reduced germination and poor crop establishment. Same variation of results was observed by Sadhu *et al.* (2014) who reported the *Fusarium moniliforme* drastically reduces seed germination (70%), root length (3.8 cm), shoot length (3.0 cm) and seedling emergence (70%) in green gram. Seed associated with *A. niger, A. tenuis, Pencillium spp, Cladopsorium spp.* and *F. moniliforme,* reduced the seedling length, seed germination and seed vigor drastically in pigeon pea also reported by Prasanna Kumar (2004).

Conclusion

Lentil wilt had a significant ($p < 0.05$) effect on seed quality parameters. The germination percentage, seedling length, and seedling vigor index I and II, reduces drastically while EC was increased in Fol-infected seeds. The noteworthy information produced would be helpful in analyzing the effect of Fol on seed quality parameters of lentil.

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