Studies on Seed Borne Fungi of Lentil

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ABSTRACT

Studies on seed-borne mycoflora of four lentil varieties (DPL 15, DPL 62, K 75 and Sehore 74-3) were made using untreated and NaOCI treated seeds during 1998-99. In agar plate and blotter paper methods, 6.50 - 22.00 and 5.75 - 14.00% of seeds respectively were found to carry seed-borne fungi 10 days after incubation. Six different fungal genera were detected in 4 varieties. *Fusarium oxysporum* and *F. solani* were found in 1.75 - 4.25, 0.75 - 2.00% of untreated and 0.75 - 2.25, 0.50 - 1.00% of treated seeds, respectively. *Aspergillus niger, A. fumigatus, Penicillium* sp., *Alternaria alternata, Curvularia* sp. and *Dreschlera* sp. were detected in untreated and treated lentil seeds. Agar plate method with PDA was better than blotter paper method in terms of percentage recovery of fungi. Treatment of seeds with NaOCI was effective in reducing seed-borne fungi in the all 4 varieties. *F. oxysporum* f.sp. *lentis* was associated only with the testa or seed coat of 4 varieties. Testa of 6.00 - 22.00% of untreated and 0 - 12.00% of treated seeds of wilted plants carried *F. oxysporum f. sp. lentis.*

Key words : Lentil, Mycoflora, Seed borne fungi

Lentil (*Lens culinaris* Medikus) is grown as a major *rabi* pulse crop in India with an annual 1.44 million hectare area and 1.05 million tonnes production. It is grown mainly in the states of Uttar Pradesh, Madhya Pradesh, Bihar, Rajasthan and West Bengal, which contribute 90% of production and 85% of area of lentil. Contribution of lentil alone to pulse production accounts to 6.0% in India. The national productivity of lentil is 732 kg/ha (during 1999-2000) which is far below the average yield (1500-2000 kg/ha) of modern improved varieties. Among various factors responsible for the low yield of lentil the seed and soil borne diseases, especially wilt, are the major constraints in the country. Lentil wilt incited by *Fusarium oxysporum* f. sp. *lentis* (hencefourth *F. o. lentis*) is both seed and soil borne disease causing yield loss of 25-50 % (Khare 1980; 1991).

In order to study the seed borne pathogen, especially, wilt causing *F. o. lentis* in lentil, the present investigation was carried out.

Materials and Methods

Four varieties of lentil, namely, DPL 15, DPL 62, K 75 and Sehore 74-3 were used for study. Surface disinfestation of seeds was done by immersing in the sodium hypochlorite (NaOCI) solution containing 1.0% chlorine, for 2-5 min. as per requirement of study. Untreated seeds were also examined. The germination test was done using towel paper soaked with water.

Sample size of 400 seeds was taken randomly from seed lot of each variety. In blotter paper test, blotter papers were soaked in sterile distilled water and placed in Petriplates after draining excess water. Agar plate method using sterile Petriplate containing PDA medium was also employed. The seeds were kept on PDA or blotter paper with or without pre-treatment of NaOCI. Ten seeds were plated in each Petriplate at equidistance. These plates were incubated at 24 ± 1°C for 5 - 10 days. Seed borne fungi were identified based on growth, colour of mycelium conidiophores, chain formation and conidial

morphology (Neergaard, 1979; Agrawal & Sinclair, 1987).

One hundred seed of each variety were taken from wilt infected lentil plants from the field. Each seed was dissected into different components, namely, testa, cotyledon, and embryo. Ten such seed components were placed with or without pre treatment in each Petriplate containing blotter paper or PDA media. Identification of *F. o. lentis* was done after 7 days incubation at 24 ± 1°C.

Results and Discussion

A total of six fungal genera, namely, *Fusarium, Aspergillus, Penicillium, Alternaria, Curvularia* and *Dreschlera* was observed in four cultivars of lentil. In agar plate and blotter paper methods, 6.50 - 22.00 and 5.75 - 14.00% of seeds respectively were found to carry seed-borne fungi 10 days after incubation. The seeds had 89 - 95% germination.

F. oxysporum was found to be associated with 4.25% of DPL, 15 to 2.75% of K 75 in untreated seeds and 2.25% of DPL, 62 to 1.0% of K 75 in treated seeds. *F solani* was found in 2.00% of DPL, 15 to 0.75% in Sehore 74-3 in untreated seeds and 1.0% in DPL, 62 to 0.50% in K 75 in treated seeds. Other species of *Fusarium* was observed in 4.75% of Sehore 74-3 to 2.75% of DPL 62 in untreated seeds. *Aspergillus niger* was identified in 2.75 - 1.50% of untreated seeds and in 1.50 - 0.50% of treated seeds. *A. fumigatus* was noted in 2.00 - 1.25% of untreated seeds and 1.50 - 0.25% of treated seeds. Other *Aspergillus* spp. were found in 2.00 - 0.25% seeds. *Penicillium* sp. and *Alternaria alternata* were carried by 3.25 - 0.75 and 2.75 - 0.25% seeds, respectively. *Curvularia* sp. and *Dreschlera* sp. were borne respectively by 1.50 - 0.25 and 1.00 - 0.25% seeds of four varieties (Table 1).

In seeds of untreated category, 2.25% of DPL 15, 1.75% of K 75, 2.75% of DPL 62 and 1.75% of Sehore 74-3 and in treated category, 1.00% of DPL 15 and DPL 62, 1.25% of Sehore 74-3 and 0.75% of K 75 seeds carried *F. oxysporum, F. solani* was found in untreated seeds of DPL 15

Table 1. Frequency of different seed mycoflora of lentil using agar plate method

Features	Untrated seeds				Treated seeds			
	DPL 15	K 75	DPL 62	Sehore 74-3	DPL 15	K 75	DPL 62	Sehore 74-3
No. of seeds tested	400	400	400	400	400	400	400	400
% germination	92	94	95	91	93	90	89	94
No. of seeds with fungi	88	83	78	78	38	29	47	40
Fungi detected on (%) seeds								
F. oxysporum	4.25	2.75	4.00	3.25	1.75	1.00	2.25	1.50
F. solani	2.00	1.00	1.50	0.75	0.75	0.50	1.00	0.75
Other *F.* spp.	4.00	4.50	2.75	4.75	2.25	1.50	1.75	3.50
Aspergillus niger	1.75	2.0	2.75	1.50	0.75	0.75	1.50	0.50
A. fumigatus	2.00	1.75	1.25	2.25	0.50	0.25	0.50	1.50
Other *A.* spp.	1.25	2.00	1.50	1.00	0.50	0.25	1.75	0.75
Penicillium sp.	2.25	3.25	2.50	2.00	1.50	0.75	1.25	1.00
Alternaria alternaria	2.00	1.75	2.50	2.75	0.50	0.75	0.50	0.25
Curvularia sp.	1.50	1.50	1.00	0.75	0.25	0.50	0.75	0.50
Drechslera sp.	1.00	0.75	0.75	0.50	0.75	0.25	0.50	0.50
Total	22.00	21.25	20.50	19.50	9.50	6.50	11.75	10.75

(1.50%), K 75 (1.00%), DPL 62 (1.25%) and sehore 74-3 (0.75%) and 0.75 - 0.50% of treated seeds. Other *Fusarium* spp. were observed in 3.00 - 2.25% of seeds in untreated categtory and 2.00 - 1.25% of treated category. *Aspergillus niger* was recorded in 1.75 - 0.50% of seeds. *A. fumigatus* was found in 1.25 - 0.50% of seeds. Other species of *Aspergillus* were observed in 1.50 - 0.59% of untreated seeds and in 1.00 - 0.25% of treated seeds. *Penicillium* sp. was present in 2.00 - 0.50% of seeds in four cultivars of lentil. *Alternaria alternata* was noted in 1.00 - 0.50% of untreated seeds. *Dreschlera* sp. was recorded in 0.50 - 0.00% of seeds (Table 2).

Agar plate method was better for detection of seed borne fungi in lentil than blotter paper method as the % of seed carrying fungi was higher in the former. Gupta and Gupta (1991) reported that agar plate and 2,4 - D method were best for detection of *Fusarium* spp. Hashmi *et al.* (1992) isolated 21 fungal species, including *F. moniliforme, F. oxysporum* and *F. semitectum* from 4 cultivars of lentil using blotter technique. Moslem and Sarwat (1993) reported that out of 38 genera isolated from lentil seeds, *Aspergillus, Urocladium, Curvularia, Alternaria, Dreschlera* and *Penicillium* were predominant.

F. o lentis was not detected in the embryo of any of the four varieties in both untreated and treated seeds under both agar plate and blotter paper methods. The cotyledons of the four varieties of treated and untreated seeds also did not carry *F. o. lentis. F. o. lentis* was only associated in the testa or seed coat of four varieties of lentil. In the agar plate method, testa of 14 - 22% of untreated seeds and 8 - 12% of treated seeds carried *F. o. lentis.* In the blotter paper method, testa of 6 - 9% untreated seeds and 0 - 3% of treated seeds were found to carry *F. o. lentis.* This shows the importance of fungicidal seed treatment in reducing seed borne inoculum of wilt causing *F.o. lentis.*

Table 2. Percentage of different seed mycoflora of lentil using blotter paper method

Features	Untrated seeds				Treated seeds			
	DPL 15	K 75	DPL 62	Sehore 74-3	DPL 15	K 75	DPL 62	Sehore 74-3
No. of seeds tested	400	400	400	400	400	400	400	400
% germination	93	92	94	91	90	92	89	93
No. of seeds with fungi	49	42	54	48	23	24	26	31
Fungi detected on (%) seeds								
F. oxysporum	2.25	1.75	2.75	1.75	1.00	0.75	1.00	1.25
F. solani	1.50	1.00	1.25	0.75	0.75	0.50	0.50	0.75
Other *F.* spp.	3.00	2.25	3.50	2.75	1.50	2.00	1.25	2.00
Aspergillus niger	1.25	1.00	1.75	1.50	0.75	0.50	1.00	0.75
A. fumigatus	1.00	0.50	1.25	0.50	0.75	0.50	0.50	0.75
Other *A.* spp.	0.75	1.25	0.50	1.50	0.25	0.75	1.0	0.75
Penicillium sp.	1.50	1.25	2.00	2.00	0.50	0.75	1.00	0.75
Alternaria alternaria	0.50	0.75	1.00	0.50	0.0	0.25	0.0	0.50
Curvularia sp.	0.50	0.75	0.0	0.50	0.25	0.0	0.0	0.25
Drechslera sp.	0.50	0.0	0.0	0.25	0.0	0.0	0.25	0.0
Total	12.75	10.50	14.00	12.00	5.75	6.00	6.50	7.25

F. o. lentis was detected only in the testa or seed coat of lentil seeds. *F. o. lentis* was not present either in embryo or cotyledon of seeds. This suggested that *F. o. lentis* was present as an external contamination of the seed in the seed coat. Hence, seed treatment with NaOCI reduced the percentage of testa borne *F. o. lentis* from 6 - 9% in untreated seeds to 0 - 3% in treated seeds. Khare (1980; 1991) reported that systemic infection occur from root to seed through stem, branches, pedicel and placenta. Erskine *et al.* (1990) observed that *F. o. lentis* was present neither in the endosperm nor in seed coat but was seed borne as external contamination of the seed, possibly following threshing of pods infected saprophytically by the fungus while in post harvest heaps in the field or via trash. On the other hand, Pundhir *et al.* (1991) reported that lentil wilt caused by *F. o. lentis* was not a seed borne disease. Seeds from diseased plants contained more free amino acids compared to healthy plants (Pundir & Verma, 1984). External contamination of seeds by *F. o. lentis* is usual, and high level of fungus may be carried in plant debris (Beniwal *et al.,* 1993).

Acknowledgements : The financial assistance in the form of senior research fellowship to the first author from I.C.A.R., New Delhi is gratefully acknowledged. The authors also express their sincere gratitude to Dr. Masood Ali, Director, Dr. Vishwa Dhar, Head, Division of Crop Protection, IIPR, Kanpur and Dr. S.B. Singh, Vice Chancellor, CSAUAT, Kanpur for continuous encouragement and their kind cooperation during the course of this investigation.

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