



ORIGINAL RESEARCH PAPER

Botany

SURVEY AND DETECTION OF SEED BORNE MYCOFLORA IN LENTIL (*Lens culinaris medic.*)

KEY WORDS:

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ABSTRACT

Total One hundred fifty seed samples of Lentil from 18 districts namely Ajmer, Bundi, Barmer, Bharatpur, Bikaner, Churu, Dausa, Dholpur, Hanumangarh, Jaipur, Jalore, Jhunjhnu, Karauli, Kota, Nagaur, Pali, Sirohi, Tonk of Rajasthan were analyzed for the detection of seed-borne mycoflora using standard blotter, agar-plate and deep-freezing methods as suggested by ISTA. A Total of 26 fungal species belonging to 16 genera (SBM) were recorded. Of the three methods used, agar plate method was found to be better for the isolation of fungi from lentil seeds. Surface disinfection by 1% Ca(OCl)₂ reduced the incidence of *Aspergillus* spp.

INTRODUCTION

Lentil (*Lens culinaris* L.) of the family Fabaceae is native to sub-continent. It is cultivated in sandy loam soil and can be grown in nutrient deficient soil (Summerfield, 1981). Lentil (*Lens culinaris* Medik.) is an important cool season legume, grown in more than 70 countries around the world. Canada is the largest lentil producer, contributing nearly 41% of the global production (FAO, 2017). Lentil seeds are rich in protein with concentration averaging 26%; however, there is shortage of certain amino acids including Methionine and Cystine (Muehlbauer *et al.*, 2002). Lentil is a good source of vitamin B and other groups while minerals reported from lentil included calcium, phosphorus, sodium, potassium etc., (Sastri, 1962). Lentil is one of the healthiest foods (Raymond, 2006) and considered as one of the best vegetable source of Iron and important for adolescent and pregnant women (Anon., 2004). A survey of literature showed that many fungal species have been reported from lentil seeds including species of *Alternaria*, *Chaetomium*, *Drechslera*, *Fusarium*, *Phoma*, *Monilia*, *Penicillium*, *Rhizopus*, *Mucor* and *Macrophomina phaseolina* from Pakistan (Ahmed *et al.*, 1993).

Richardson (1979) gave a list of seed-borne diseases of lentil according to which *Botrytis* spp., and *Fusarium oxysporum* were isolated from lentil seeds from Czechoslovakia and *Uromyces fabae* from debris mixed with seeds from India. Lentil also suffers from root rot and wilt complex caused by *Pythium*, *Rhizoctonia*, *Sclerotium* and *Fusarium* spp., (Muehlbauer *et al.*, 2002). Hussain *et al.*, (2007) isolated *Alternaria alternata*, *Aspergillus* spp., *Fusarium moniliforme*, *Mucor hiemalis*, *Chaetomium* spp., *Penicillium citrinum* and *Nigrospora* spp., from the 25 seed samples collected from various localities of the Punjab, Pakistan. Presence of so much storage and pathogenic fungi reduces the quality and quantity of crop, also the application of improper cultural practices and lack of proper storage conditions along with several other problems resulted in yield losses which in turn cause economic losses. In view of the economic importance of the crop, present work was carried out to explore the seed-borne mycoflora associated with lentil (*Lens culinaris* L.).

MATERIALS AND METHODS

For the detection of seed-borne mycoflora ISTA techniques were used (Anon., 1993). By using standard blotter, agar plate and deep-freezing methods.

Collection Of Seeds:

Total One hundred fifty seed samples of Lentil from 18 districts namely Ajmer, Bundi, Barmer, Bharatpur, Bikaner, Churu, Dausa, Dholpur, Hanumangarh, Jaipur, Jalore, Jhunjhnu, Karauli, Kota, Nagaur, Pali, Sirohi, Tonk of Rajasthan respectively were collected (Fig.1 & Table 1). Samples were entered in laboratory stock register and given an accession number.



Fig.01:

Table- 1 : District Wise Number Of Seed Samples Of Lentil

District	No. of Samples collected	No. of Samples Studied	
		SBM	PDA
Ajmer	7	7	3
Bundi	10	10	3
Barmer	3	3	-
Bharatpur	15	15	8
Bikaner	12	12	3
Churu	10	10	-
Dausa	10	10	-
Dholpur	30	30	12
Hanumangarh	2	2	-
Jaipur	8	8	1
Jalore	5	5	2
Jhunjhnu	2	2	-
Karauli	10	10	5
Kota	15	15	6
Nagaur	5	5	-
Pali	2	2	1
Sirohi	2	2	1
Tonk	2	2	1
Total	150	150	46

Standard Blotter Method:

200 seeds per sample, 100 untreated and 100 pretreated with aqueous solution of sodium hypochlorite were tested. In preliminary experiments several concentrations of aqueous solution of sodium hypochlorite with 0.25, 0.5, 1.0, 2.0 and 3.0 percent available chlorine for 1,2,3,4 and 5 min were tried, 1% available chlorine for 2 min was found to be the most suitable and used throughout the experiment. 25 seeds were spaced per Petri plate containing three well moistened blotter and incubated at 28±2°C under 12 hr. of alternating cycles of artificial day light from Phillips fluorescent tubes and darkness for seven days. (Anon., 1993).

Agar Plate Method:

Untreated seeds and seeds after surface sterilization with 1% Ca(OCl)₂ for 5 minutes were placed on potato dextrose agar (PDA), 46 samples belonging to 12 different districts of Rajasthan respectively were studied. 100 seeds pretreated with 1% available chlorine from aqueous solution of sodium hypochlorite for 2 min. were aseptically plated on Petri plates (25seeds/plate) containing 15-20 ml of potato dextrose agar (PDA) The dishes were then incubated for 7days at 24±1°C under 12h, alternating cycles of artificial day light (ADL) and darkness (Anon., 1993).

RESULTS

SURVEY AND DETECTION OF SEED-BORNE MYCOFLORA

Dry Seed Examination

Lentil seed samples revealed seeds with black spots, brown discolouration with or without white mycelial growth, seeds with water soaked pale areas, shrivelled, broken and insect damaged seeds besides the normal symptomless seed (Fig-2&Table- 2).

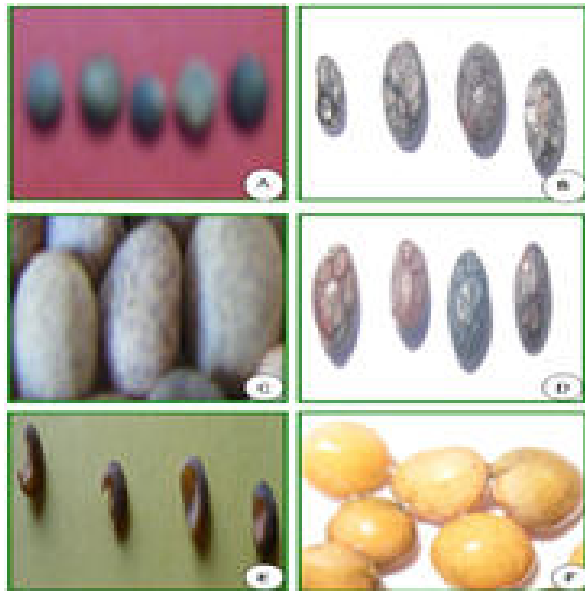


Fig-2:

Table-2 : Districtwise Number Of Lentil Seeds Samples Studied In Dry Seed Examination With Percent Range Of Various Seed Disorders

District	No. of Samples	Discolouration		Seed with water soaked spots	Shrivelled Seeds	Broken and insect damage
		Black	Brown			
Ajmer	7	2(2.75-7.5)	2(2-6.65)	-	4(6.5-11.5)	5(0.5-1.25)
Bundi	10	5(1.0-16.66)	3(2.0-3.75)	2(1-7.5)	6(7.5-10.5)	6(0.5-1.75)
Barmer	3	2(2.5-8.5)	2(2.5-4.5)	-	2(3-9.5)	-

Bhartpur	15	8(1.0-7.75)	4(0.20-30.25)	7(0.5-11.25)	5(1.25-25.5)	-
Bikaner	12	6(2.25-80)	3(1.0-4.75)	8(8-24.25)	8(2.0-5.0)	-
Churu	10	2(0.5-2.5)	4(3-7.0)	-	3(4.5-10.5)	3(0.5-3.0)
Dausa	10	6(5.5-10.75)	3(3-18.75)	-	3(4.67-8.5)	6(0.5-4.5)
Dholpur	30	20(2.25-30.0)	10(3.5-20.75)	-	8(2.25-25)	15(0.5-50.0)
Hanumangarh	2	3(11-13)	3(1.25-4.75)	-	2(2.0-8.5)	3(0.5-1.25)
Jaipur	8	4(80.50)	8(1.0-5.75)	-	5(5.5-25.0)	12(1.75-60.5)
Jalore	5	-	2(0.20-4.25)	-	2(0.50-2.5)	-
Jhunjhunu	2	1(0.25)	1(0.5)	-	1(0.25)	-
Karauli	10	6(1.75-17.75)	-	-	5(6-30)	6(0.5-3.0)
Kota	15	15(2.25-25.0)	20(1.25-25.25)	9(1-23.25)	9(2.25-8.5)	20(0.5-60)
Nagaur	5	2(0.65-35.75)	3(1.0-8.50)	-	4(5.5-7.5)	4(0.5-2.25)
Pali	2	2(1.25-11.50)	2(2.0-6.65)	-	2(4-6.75)	2(0.5-1.0)
Sirohi	2	3(3-18.50)	4(6.0-7.5)	-	3(10-13)	2(0.5-2.0)
Tonk	2	6(5.5-90.50)	8(4.25-55.5)	-	6(5-30)	7(0.25-4.0)
Total	150	98(0.25-90.50)	88(0.20-55.5)	26(0.5-24.25)	81(0.25-30.0)	95(0.25-60.5)

Seed with black spots (Fig.-2A)

98 seed samples carried 0.25-90.50% black pinched like microsclerotia on seeds which varied from few to many. On incubation such seeds yielded *Rhizoctonia solani*. These samples came from Ajmer (2), Bundi (5), Barmer(2), Bharatpur(8), Bikaner (6), Churu(2), Dausa(6), Dholpur(20), Hanumangarh(3), Jaipur(4), Jhunjhunu (1), Karauli(6), Kota(15) Nagaur (2), Pali (2), Sirohi(3) and Tonk (6).

Brown discoloured seeds with or without white mycelial growth (Fig.-2B)

88 seed samples showed brown discolouration in the range of 0.20-55.5%, of which samples had discoloured seeds without as well as with white mycelial growth. These affected samples belonged to Ajmer (2), Bundi (3), Barmer(2), Bharatpur(4), Bikaner (3), Churu(4), Dausa(3), Dholpur(10), Hanumangarh(3), Jaipur(8), Jalore(2), Jhunjhunu (1), Kota(20) Nagaur (3), Pali (2), Sirohi(4) and Tonk (8). Discoloured seeds with or without mycelium regularly yielded *Fusarium oxysporum*.

Seeds with water soaked spots (Fig.-2C)

The incidence of seed having water soaked spots was 0.5-24.25% and it occurred in 26 seed samples of 4 districts. These samples came from Bundi, Bikaner, Bharatpur and Kota.

Shrivelled seeds (Fig.-2D)

81 Seed samples carried 0.25-30.0 % shrivelled seeds, which varied from slight to highly shrivelled.

Broken and insect damaged seeds (Fig.-2E)

Such seeds were observed in 95 samples from Ajmer (5), Bundi (6), Churu (3), Dausa(6), Dholpur(15), Hanumangarh(3), Jaipur (12), Karauli(6), Kota(20), Nagaur (4), Pali (2), Sirohi(2) and Tonk (7).

Incubation Tests

One hundred fifty and forty six samples of lentil belonging to 18 districts of Rajasthan screened using standard blotter method (SBM) and potato dextrose agar (PDA) plate method

respectively (Table-4&5A,B). A Total of 26 fungal species belonging to 16 genera (SBM) were recorded. The fungi encountered in PDA test were common to those observed in standard blotter method (Table-4)

The detailed description for fungi have been provided by various workers (Malone and Muskett, 1964; Ramnath, Neergaard and Mathur, 1970, Booth, 1971; Barnett and Hunter, 1972; Ainsworth, Sparrow and Sursaman, 1973).

A total of 26 fungal species belonging to 16 genera found associated with lentil seeds were *Alternaria alternata* (Fr.) Keissler, *A. tenuissima* (Kunze ex Pers.) Wiltshire, *A. flavus* Link ex Fries, *A. niger* Van Tiegh, *C.globosum* kunze ex fries, *Chaetomium spinosum* Chives, *Cladosporium cladosporioides* (Fres.) Devries, *C. herbarum* (pers.) Link ex S.F. Gray, *Curvularia lunata* (Wakker) Boedijn, *C. pallascens* Boedijn, *D. halodes* (Drechsler) Subram and Jain, *Drechslera tetramera* (Mc Kinney) Subram and Jain, *D.longirostrata*(Mc Kinney) Subram and Jain, *Fusarium moniliforme* Sheldon, *F.oxysporum* Schiecht ex Fr., *F.Semitectum* Berk and Rav., *F.solani* (Mart.) Sacc., *Gliocladium roseum* Bainier, *Pencilium* spp., *Periconia circinata* (Mangin) Sacc., *Phoma* sp.(Sacc.) Boer. Doren and Vkest., *Rhizopus nigricans* Ehrenb., *Rhizoctonia solani*(Taub) Butler., *Sclerotium rolfsii*, *Trichothecium roseum* Link ex Fries, *Verticillium albo- atrum* Rainke and Berthhold (Fig.-3A-G).

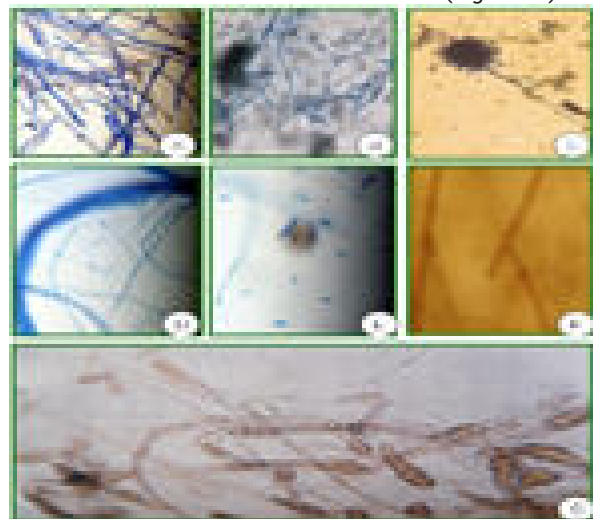


Fig-3

Relative percent occurrence (RPO) for each fungus is given in the following order untreated, pretreated seeds in SBM and PDA method (Table-1,2,3).

Table-3 OCCURRENCE, RELATIVE PERCENT OCCURRENCE (RPO) AND PERCENT RANGE OF FUNGI IN UNTREATED SEED SAMPLES OF VARIOUS DISTRICTS OF RAJASTHAN IN STANDARD BLOTTER METHOD (SBM)

Districts	Districts																	Total	RPO	% Range of Fungi					
	Ajmer	Bundi	Barmer	Bharatpur	Bikaner	Churu	Dausa	Dholpur	Hanumangarh	Jaipur	Jhunjhunu	Jalore	Karouli	Kota	Nagaur	Pali	Sirohi				Tonk				
Fungi																									
<i>Alternaria alternata</i>	3	-	-	6	5	-	3	6	2	10	-	-	3	10	3	2	1	4	58	38.7	1-46				
<i>A. tenuissima</i>	3	2	-	7	6	2	2	8	3	14	-	1	3	3	1	1	3	-	59	39.33	1-57				
<i>Aspergillus flavus</i>	5	4	2	-	10	2	3	6	3	6	1	1	2	2	2	3	4	6	62	41.3	1-50				
<i>A. niger</i>	3	1	2	4	6	2	2	5	1	3	2	1	1	2	3	1	2	2	43	28.7	1-30				
<i>C.globosum</i>	-	2	1	1	-	-	2	-	-	-	-	1	-	-	1	-	-	3	11	7.33	1-10				
<i>C.spinosum</i>	-	2	-	-	-	2	2	-	1	-	-	-	1	-	-	-	-	-	8	5.33	1-10				
<i>Cladosporium</i> sps.	1	-	2	4	-	-	2	-	-	8	-	-	-	2	-	-	1	-	20	13.3	1-14				
<i>C.herbarum</i>	-	2	-	-	-	2	2	-	1	-	-	-	1	-	-	-	-	-	8	5.33	1-10				
<i>Curvularia lunata</i>	4	2	-	8	-	2	-	-	3	2	-	-	1	1	-	2	1	-	26	17.3	1-25				
<i>C.pallascens</i>	1	-	3	2	6	2	-	8	2	-	2	-	-	3	3	-	-	-	32	21.3	1-15				

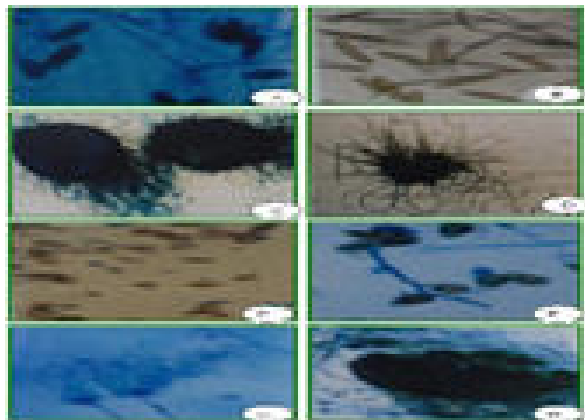


Fig-4

Alternaria alternata (Fr.) Keissler (Fig.-3G)

A. alternata was recorded in 58, 30 and 6 samples in untreated (1-46%), pretreated seeds (1-10%) in SBM and PDA (1-2%) respectively. 14,26,12,0,16,14,6,0 and 6,4,0,0 samples carried infection in the range of 0.5-5,5.5-25,25.5-50% in untreated and pretreated seeds (SBM) and PDA respectively. The R.P.O. value was 38.66, 20.0 and 4.00 respectively. Its high infection percentage were recorded in samples from Dholpur(3.5-20.75), Kota(1.25-25.25) and Sirohi (6.0-7.5%).

Alternaria tenuissima (Kunze ex Pers.) Wiltshire

The fungus was recorded in 51 and 55 samples with an incidence of 1-57% in untreated, 1-10% in pretreated seeds in SBM and PDA(1-3%) respectively. 20, 34, 4 and 30, 24, 4 samples carried infection in the range of 0.5-5%, 5.5-25 and 25.5-50 in untreated and pretreated in SBM. The R.P.O. value was 39.33, 36.66 and 2 respectively. It occurred in the samples of Ajmer, Bundi, Bharatpur, Bikaner, Churu, Dausa, Dholpur, Hanumangarh, Jalore, Kota, Karouli, Pali and Sirohi districts.

Aspergillus flavus Link ex Fries (Fig.-3C)

The fungus was recorded in 62, 60 and 8 samples in untreated (1-50%), pretreated (1-11%) seeds in SBM and PDA (1-7%) respectively. The R.P.O. was 41.33, 40.0 and 5.33 respectively. The fungus was encountered in samples from all the districts except Bharatpur.

Aspergillus niger Van Tiegham

It was recorded in 47, 45 and 2 samples in untreated (1-30%), pretreated seeds (1-9%) in SBM and PDA (1%). 30, 25, 4, 28, 8, 5 and 4 samples carried infection in the range between 0.5- 5, 5.5-25, 25.5-50 in untreated, pretreated seeds (SBM) and PDA respectively. The R.P.O. values were 31.33, 30.0 and 1.33. The fungus was encountered in samples from all the districts.

<i>Drechslera halodes</i>	-	2	3	-	5	2	-	-	4	-	-	3	2	-	-	-	4	2	27	18	1-30
<i>D. longirostrata</i>	2	1	-	3	-	2	-	-	-	1	-	2	-	1	-	-	-	-	12	8.00	1-11
<i>D.tetramera</i>	1	-	3	-	-	2	-	6	-	-	1	2	1	-	2	1	-	-	19	12.7	1-12
<i>Fusarium moniliforme</i>	4	2	-	-	3	2	2	1	5	-	-	-	2	1	-	-	2	-	24	16	1-40
<i>F. oxysporum</i>	2	-	4	8	-	2	-	10	-	12	-	2	-	16	-	-	5	4	65	43.3	1-80
<i>F. semitectum</i>	2	-	1	-	-	2	-	3	-	2	2	1	-	-	1	2	-	-	16	10.7	1-30
<i>F.solani</i>	-	3	-	-	1	2	-	-	2	-	1	-	-	1	-	-	-	-	10	6.66	1-32
<i>Gliocladium roseum</i>	-	1	-	2	-	2	-	2	-	-	-	2	-	-	-	1	-	-	10	6.66	1-11
<i>Penicillium citri</i>	1	-	3	6	-	2	1	1	-	8	-	3	3	2	2	-	1	-	33	22	1-9
<i>Periconia circinata</i>	-	-	1	-	-	2	-	1	-	3	-	-	-	-	2	-	-	1	10	6.66	1-15
<i>phoma betate</i>	-	-	1	-	-	2	1	-	-	-	2	-	1	-	-	-	1	-	8	5.33	1-8
<i>Sclerotium rolfsii</i>	-	2	3	-	5	2	-	-	4	-	-	3	2	-	-	-	4	2	27	18	1-30
<i>Rhizoctonia solani</i>	2	5	-	1	1	2	-	-	5	-	1	1	-	28	-	-	-	1	47	31.33	1-38
<i>Rhizopus nigricans</i>	2	3	3	2	6	2	-	5	3	2	2	3	-	-	1	2	1	-	37	24.66	1-40
<i>Trichothecium roseum</i>	1	-	-	1	-	2	2	-	-	-	3	-	-	1	-	-	1	-	11	7.33	1-7
<i>Verticillium alboatrum</i>	-	1	-	-	2	2	-	1	-	-	1	2	-	2	-	1	-	1	13	8.66	1-10

Chaetomium globosum Kunze ex Fr. (Fig.-4C)

The fungus was encountered in 11, 10 and 6 samples in untreated (1-12%), pretreated seeds (1-6%) in SBM and PDA (1-5%) respectively. 16, 9, 6 and 3 samples carried infection in the range of 0.5-5, 5.5-25% in untreated, pretreated seeds (SBM) and in PDA respectively. The R.P.O. values were 7.33, 6.66 and 4.00.

Chaetomium spinosum Chives (Fig.-4D)

The fungus was encountered in 8, 5 and 2 samples in untreated (1-10%), pretreated seeds (1-2%) in SBM and PDA (3-8%) respectively. 18, 12, 10, 6, 16 seed samples showed infection in the range of 0.5-5%, and 5.5-25% respectively. The R.P.O. values were 5.33, 3.73 and 3.92.

Cladosporium cladosporioides (Fres.) deVries

The fungus occurred in 20 and 7 seeds samples in untreated (1-14%) and pretreated seeds (1-3%) in SBM. 14, 8, 14 and 6 samples carried infection in the range of 0.5-5, 5.5-25% in untreated, pretreated seeds (SBM) and in PDA respectively. The R.P.O. values were 13.33, and 4.66.

Cladosporium herbarum Link ex Fries

The fungus was encountered in 8 and 5 samples in untreated (1-10%) and pretreated (1%) seeds in SBM. 6, 6 and 4 samples carried infection in the range of 0.5-5% and 5.5-25% in untreated and pretreated seeds in SBM. The R.P.O. was 5.33 and 3.33 respectively.

Curvularia lunata (Wakker) Boedijn (Fig.-4E)

Their high percentage range recorded in samples from Bikaner (2.25-80.0%), Dausa (5.5-10.75%), Dholpur (2.25-30.0%), Jodhpur (1.65-45.25%) and Sawai Madhopur (3.5-25.0%), Tonk (5.5-90.50%). The pathogen occurred in 26, 20 and 10 samples in untreated (1-25%), pretreated (1-4%) seeds in SBM. 24, 20, 18, 10, 30, 24, 14, 8, 15, 3 seed samples showed infection in the range of 0.5-5%, 5.5-25% and 25.5-50%, 50.5-100% respectively. The R.P.O. value was 17.33, 13.33 and 6.66.

Curvularia pallescens Boedijn (Fig.-4F)

The pathogen occurred in 20, 32, 20 samples in untreated (1-15%), pretreated (1-10%) seeds in SBM and PDA (1-9%) respectively. 20, 8, 5, 16, 8, 6, 14, 10, 8, 2 seed samples showed infection in the range of 0.5-5%, 5.5-25% and 25.5-50%, 50.5-100% respectively. The R.P.O. value was 21.33, 21.33 and 13.33.

Drechslera halodes (Drechsler) Subram. and Jain (Fig.-4B)

The pathogen occurred in 32, 15, 20 samples in untreated (1-30%), pretreated (1-5%) seeds in SBM and PDA (1-8%) respectively. 12, 4 seed samples showed infection in the range of 0.5-5% and 5.5-25%. and respectively. The R.P.O. value was 18, 10.0 and 13.33.

Drechslera longirostrata Subram.

The pathogen occurred in 12, 10, 20 samples in untreated (1-

11%), pretreated (1-6%) seeds in SBM and PDA (1-10%) respectively. 12, 18 seed samples showed infection in the range of 0.5-5% and 5.5-25%. respectively. The R.P.O. value was 8.06.66, 10.0 and 23.33.

Drechslera tetramera (Mc Kinney) Subram and Jain (Fig.-4A)

The pathogen occurred in 19, 15, 20 samples in untreated (1-12%), pretreated (1-4%) seeds in SBM and PDA (1-7%) respectively. 18, 12, 10, 6, 16 seed samples showed infection in the range of 0.5-5%, and 5.5-25% respectively. The R.P.O. value was 12.66, 15.0 and 22.

Fusarium moniliforme Sheldon

The pathogen occurred in 22, 20, 40 samples in untreated (1-40%), pretreated (1-8%) seeds in SBM and PDA (1-14%) respectively. 10, 5, 3, 6, 4, 2, 18, 8 seed samples showed infection in the range of 0.5-5%, 5.5-25 and 25.5-50% respectively. The R.P.O. value was 16, 13.33 and 40.

Fusarium oxysporum Schlecht. ex Fr. (Fig.-3D&E)

The pathogen occurred in 70, 85, 35 samples in untreated (1-80%), pretreated (1-20%) seeds in SBM and PDA (1-12%) respectively. The infected samples belonged to 18 districts and its incidence was high in Ajmer, Bundi, Dholpur, Kota, and Tonk. 40, 36, 18, 10, 46, 30, 16, 5, 16, 14, 10, 4 seed samples showed infection in the range of 0.5-5%, 5.5-25, 25.5-50% and 50.5-100% respectively. The R.P.O. value was 43.33, 56.66 and 23.33.

Fusarium semitectum Berk and Rau

The pathogen occurred in 8, 21 samples in untreated (1-30%), pretreated (1-6%) seeds in SBM. 8, 4 seed samples showed infection in the range of 0.5-5% and 5.5-25% respectively. The R.P.O. value was 10.66, and 14.0.

Fusarium solani (Mart.) Sacc.

The pathogen occurred in 8, 12, 6 samples in untreated (1-32%), pretreated (1-4%) seeds in SBM and PDA (1-4%) respectively. 5, 3, 3, 2 seed samples showed infection in the range of 0.5-5%, and 5.5-25% respectively. The R.P.O. value was 6.66, 8.0 and 4.0.

Gliocladium roseum Corda

The pathogen occurred in 9, 10, 3 samples in untreated (1-11%), pretreated (1-3%) seeds in SBM and PDA (1-2%) respectively. 3, 2, 3, 10, 5, 4, 2 seed samples showed infection in the range of 0.5-5%, 5.5-25% and 25.5-50.0 respectively. The R.P.O. value was 6.66, 6.66 and 2.

Penicillium sp. Link (Fig.-4G)

The pathogen occurred in 31, 19, 6 samples in untreated (1-9%), pretreated (1-7%) seeds in SBM and PDA (1-3%) respectively. 30, 8, 4, 16, 4, 2 seed samples showed infection in the range of 0.5-5%, 5.5-25% and 25.5-50.0 respectively. The R.P.O. value was 22, 12.66 and 4.

Periconia circinata (Mangin) Sacc.

The pathogen occurred in 10, 6, 10 samples in untreated (1-15%), pretreated (1-4%) seeds in SBM and PDA (1-7%) respectively. 8, 6 seed samples showed infection in the range of 0.5-5% and 5.5-25% respectively. The R.P.O. value was 6.66, 4 and 6.66.

Phoma betate Frank (Fig.-4H)

The pathogen occurred in 6, 20, 3 samples in untreated (1-8%), pretreated (1-6%) seeds in SBM and PDA (1-2%) respectively. 3, 1, 3, 3 seed samples showed infection in the range of 0.5-5% and 5.5-25% respectively. The R.P.O. value was 5.33, 13.33 and 2.00.

Rhizopus nigricans Ehnerb

The pathogen occurred in 36, 14, 8 samples in untreated (1-40%), pretreated (1-8%) seeds in SBM and PDA (1-5%) respectively. 20, 10, 6, 6, 3, 5, 1 seed samples showed infection in the range of 0.5-5%, 5.5-25% and 25.5-50 respectively. The R.P.O. value was 24.66, 9.33 and 5.33.

Rhizoctonia solani (Taub.) Butler (Fig.-3F)

The pathogen occurred in 57, 75, 6 samples in untreated (1-38%), pretreated (1-1-12%) seeds in SBM and PDA (1-4%) respectively. 35, 30, 16, 10, 44, 35, 18, 8, 18, 16, 12, 8 seed samples showed infection in the range of 0.5-5%, 5.5-25%, 25.5-50 and 50.5-100 respectively. The R.P.O. value was 31.33, 50.00 and 4.00.

Sclerotium rolfsii (Fig.-3A)

The pathogen occurred in 32, 15, 20 samples in untreated (1-30%), pretreated (1-5%) seeds in SBM and PDA (1-8%) respectively. 35, 28, 20, 15, 40, 30, 25, 10, 8, 3 seed samples showed infection in the range of 0.5-5%, 5.5-25%, 25.5-50 and 50.5-100% and respectively. The R.P.O. value was 18, 10.0 and 13.33.

Trichothecium roseum (Pers.) Link ex Fr. (Fig.-3B)

The pathogen occurred in 9, 8, 2 samples in untreated (1-7%), pretreated (1-4%) seeds in SBM and PDA (1-2%) respectively. 5, 3, 3, 3 seed samples showed infection in the range of 0.5-5% and 5.5-25% respectively. The R.P.O. value was 7.33, 5.33 and 1.33.

Verticillium alboatrum Reinke and Berthold

The pathogen occurred in 11, 6 samples in untreated (1-10%), pretreated (1-2%) seeds in SBM. 14, 6, 6 seed samples showed infection in the range of 0.5-5% and 5.5-25% respectively. The R.P.O. value was 8.66, and 4.

DISCUSSION

SURVEY OF SEED-BORNE MYCOFLORA

Seeds of lentil are known to be associated with a large number of fungi. Richardson (1992) enlisted 5 fungal species in his "Annotated List of Seed-Borne Disease". The reports on seed-borne mycoflora of lentil are given by Temp and Neergaard(1958); Wilson and Brandsberg (1965); Mitidieri and De (1978); Vishunavat and Shukla, 1983; Sumar and Howard (1983); Abdel-Hafez (1988); Arun and Mathew (1991); Kaiser (1992); Diaz and Tello (1994); Singh and Tripathy (1999); Javed et al. (2000); Nugerabi and Elshafie (2001); Dwivedi and Narain (2002) and Chaudhary et al. (2006). These observations are mostly based on a limited little information of samples from varied localities and thus provide little information about the association of fungi on lentil seeds of any region of India also some of these reports are only elementary.

DRY SEED EXAMINATION

Seed discoloration such as necrotic lesions, fungal growth and pigmentation on seed surface have been described by Neergaard (1977). In present study the seed samples contained symptomatic seeds with various disorders like seed with brown, black and white discoloration, small seeds,

shrivelled seeds and insect damaged seeds besides the normal bold healthy looking (asymptomatic) seeds. Incubation test revealed that shrivelled seed with white crust carried infection of *Fusarium oxysporum*, discoloured seeds revealed *Aspergillus* spp., *Rizoctonia solani* was obtained from black seeds.

Seed necrosis due to the infection of *Fusarium equiseti*, *Fsemitectum* and *Macrophomina phaseolina* has been observed in Mungbean (Ramnath, Neergaard and Mathur, 1970).

Foxysporum is found to cause various type of discoloration in seeds of leguminous crops in Rajasthan such as brown discoloration with or without mycelial growth in cowpea and mothbean (Varma, 1990), reddish brown discoloration in soyabean (Mathur, 1992), white crust on guar (Bhatia, 1995), seed with white mycelial growth in pigeon pea (Sharma, 1996) and mung bean (Sharma, 1999). In the present study seeds with white growth had prominent infection of *Foxysporum*.

INCUBATION TESTS

During incubation studies 26 fungal species belonging to 16 genera were found in seed samples of lentil in Rajasthan.

Richardson (1979) gave a list of seed-borne diseases of lentil, according to which *Botrytis* spp., and *F oxysporum* were isolated from lentil seed from Czechoslovakia and *Uromyces fabae* from debris mixed with seed from India.

Arshad et al., (2005) studied the seeds of four pulses viz. chickpea (*Cicer arietinum* L.) black and white, mungbean (*Vigna radiata* (L.) Wilczek), mashbean (*Vigna mungo* (L.) Hepper) and lentil (*Lens esculenta* Moench), collected from different shops of Lahore, for associated mycoflora and found seven fungi comprising of *A. niger* van Tieghem, *A. flavus* Link ex Gray, *A. fumigatus* Fresenius, *A. terreus* Thom., *F. equiseti* (Corda) Saccardo, *Syncephalastrum racemo*.

Thirty-two fungal species belonging to 17 genera were recorded on 45 seed samples of lentil (*Lens esculenta*) collected from different governorates in Egypt. The prevalent genera were *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium*.sum Cohn ex Schroeter and *Rhizopus* spp. (Allah and Hashem., 2006).

Chaudhary et al. (2006) revealed that *Fusarium oxysporum* f. sp. *lentis*, *Aspergillus flavus*, *Alternaria* sp., *Fusarium equisetii*, *Rhizoctonia solani*, *F. chlamydosporum*, *Trichoderma* spp., *Sclerotium rolfsii* and *Rhizopus* spp. are associated with the plant mortality of lentil.

Fusarium oxysporum and *Rhizoctonia solani* are the major pathogens of the crop in the state. 70 and 57 samples were infected with *Foxysporum* and *Rhizoctonia solani* showing 1-38% and 1-80% incidence respectively.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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