Pak. J. Bot., 46(5): 1921-1924, 2014.

ACTIVITY OF WATER CONTENT AND STORAGE TEMPERATURE ON THE SEED-BORNE MYCOFLORA OF *LENS CULINARIS* L. (LENTIL)

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Abstract

Storage of seeds with high water content and temperatures favors the growth of mould fungi which in turn affect the germination of seeds while low temperature with low water content prevent the growth of storage fungi and help in maintaining seed viability for longer duration of time. Seed sample from Sukkur district was stored at 4°C and room temperature (25-30°C) with water content of 8, 13 and 17% for about 80 days. The fungi were isolated at 0, 20, 40, 60 and 80days intervals. Highest infection percentage of fungi was observed at 13 and 17% water contents at room temperature after 20days of storage. High infection percentage of storage fungi affected the germination of seeds. *Aspergillus* spp were the most dominant fungi.

Keywords: Water content, Storage temperature, Seed-borne mycoflora, Lentil

Introduction

The water content of seed is actually the amount or percentage of water or moisture present in a seed. The quality of seed is known to be destroyed by growth of mould fungi (Singh & Prasad, 1977). The losses caused by mould fungi are not of minor importance because the fungi adversely affect the quality and quantity of seeds. During prolong storage of grains, a general decrease in field fungi and a slow increase in storage fungi has been reported (Sinha, 1979). Similar results were also observed on sunflower seeds by Singh & Prasad (1977) where seed borne fungi produced seed-rot, seedling blight and reduced the quality and quantity of seed. The fungi are important due to the production of toxins that cause health hazards in human and animals (Hiscocks, 1965). Saprophytic fungi isolated most frequently from lentil seeds were species of *Alternaria* and *Cladosporium* while pathogenic fungi, including *Botrytis cinerea* were isolated from discolored seeds (Kaiser, 1991). The storage fungi usually do not invade before harvest (Christensen & Kaufmann, 1969) but they may be present as contamination or as dormant mycelium within the tissues of pericarp or seed coat (Warnock & Preece, 1971).

The amount of rainfall during July when the crop was nearing maturity or about to be harvested appeared to be markedly affected the incidence, prevalence and severity of seed borne pathogenic fungi (Kaiser, 1991). Therefore the present study was carried out to check the activity of water content and temperature on the seed-borne mycoflora of lentils.

Materials and Methods

Seed sample of lentil was collected from Sukkur and water content was determined by oven dry method. The technique as suggested by Lutey & Christensen (1963) was used to adjust the water content of seeds. Water content of seeds was adjusted to 13, 15 and 17%. Seed samples were kept in jars and required amount of water was added to seeds, which was thoroughly mixed. The seeds were kept in refrigerator at 4°C for 24h with frequent shaking to facilitate uniform distribution of water through out the seeds. Seed sample with moisture content of 13, 15 and 17% were stored at 4°C and room temperatures (25-30°C). Samples were removed at different intervals of 0 day and after 20, 40, 60 and 80days. The seed germination and seed borne mycoflora was determined by blotter method as recommended by ISTA (Anon, 1993). Fungi growing on seeds were identified after reference to Barnett (1960), Booth (1971), Domsch *et al*., (1980), Ellis (1971), Nelson *et al*., (1983), Raper *et al*., (1965).

Results and Discussion

No effect on germination of seeds was observed at any moisture and temperature. Reduction in growth was only observed in seeds heavily infected with fungi (Table 2). High fungal infection was observed at 13 and 17% moisture level however greater number of fungi were isolated at 15% water content. Seeds stored at room temperature was heavily infected with storage fungi but seeds stored at 4°C showed infection with pathogenic fungi as well. Surface sterilization of seeds by 1% $Na(OCl)_2$ reduced the incidence of storage fungi and other microbial organisms. During storage the fungi were observed on seeds at different intervals including the species of *Alternaria, Cylindrocarpon, Fusarium, Nectria inventa, Scopulariopsis, Rhizoctonia solani* etc. Overall, 10 species belonging to 6 genera were isolated from the seeds stored at room temperature while 12 species belonging to 8 genera were isolated from the seeds stored at 4°C. *Aspergillus flavus* was the most dominant fungus in seeds stored at both temperatures (Table 1).

Table 1. Activity of water content and temperature on seed-borne mycoflora of lentil.

Name of fungi	Infection % at room temperature (25-30°C)					
	Non-surface sterilized seeds			Surface sterilized seeds		
	Water content (%)					
	13	15	17	13	15	17
	0 Day					
Aspergillus candidus	-	-	1 ± 0.0	-	-	1 ± 0.0
A.flavus	21 ± 3.27	-	8 ± 0.59	25 ± 1.87	7 ± 1.53	16 ± 2.17
A .fumigatus	4 ± 0.0	5 ± 1.15	4 ± 1.41	2 ± 0.0	-	2 ± 0.0
A.niger	2 ± 0.0	1 ± 0.0	2 ± 0.0	-	2 ± 0.0	2 ± 0.0
Fusarium moniliforme	-	-	2 ± 0.0	-	-	-
Monilia spp.	-	-	-	-	-	-
Nectria inventa	1 ± 0.0	-	-	-	-	-
	20 Days					
Aspergillus flavus	12 ± 4.24	37 ± 4.03	47 ± 1.82	-	55 ± 4.0	24 ± 4.36
A.fumigatus	7 ± 3.53	13 ± 0.71	7 ± 0.96	10 ± 2.82	4 ± 0.0	8 ± 0.0
A.niger	-	-	-	1 ± 0.0	-	-
A.wentii	1 ± 0.0	3 ± 0.0	-	5 ± 0.71	3 ± 0.0	-
Monilia spp.	-	-	-	-	-	9 ± 0.0
	40 Days					
Aspergillus flavus	10 ± 1.0	9 ± 2.0	8 ± 0.0	8 ± 1.15	9 ± 2.0	23 ± 2.07
A.fumigatus	-	-	-	1 ± 0.0	-	3 ± 0.0
A.niger	-	4 ± 0.0	-	3 ± 0.71	-	-
A.wentii	-	-	1 ± 0.0	2 ± 0.0	3 ± 0.0	-
Chaetomium globosum	-	-	-	4 ± 1.41	-	-
Cylindrocarpon spp.	-	1 ± 0.0	-	-	2 ± 0.0	-
Fusarium aquaductum	1 ± 0.0	-	-	-	-	-
Monilia spp.	-	-	-	5 ± 2.12	-	-
	60 Days					
Aspergillus flavus	1 ± 0.0	1 ± 0.0	-	2 ± 0.0	-	4 ± 0.0
A.fumigatus	1 ± 0.0	-	-	-	-	-
A.niger	10 ± 1.5	-	3 ± 0.0	-	-	1 ± 0.0
A.wentii	-	1 ± 0.0	-	-	-	-
Chaetomium globosum	-	-	-	-	-	1 ± 0.0
	80 Days					
Aspergillus flavus	1 ± 0.0	-	3 ± 0.0	1 ± 0.0	-	-
A.fumigatus	-	-	-	1 ± 0.0	-	-
A.niger	-	-	-	-	-	1 ± 0.0
A.wentii	-	-	1 ± 0.0	-	-	1 ± 0.0

Table 1. (Cont'd.).

Name of fungi	Infection % at low temperature (4°C)					
	Non-surface sterilized seeds			Surface sterilized seeds		
	Water content (%)					
	13	15	17	13	15	17
	0 Day					
A.flavus	4 ± 1.41	13 ± 1.31	10 ± 1.0	6 ± 2.82	9 ± 0.83	9 ± 3.5
A .fumigatus	2 ± 0.0	2 ± 0.0	2 ± 0.0	2 ± 0.0	14 ± 3.0	2 ± 0.0
A.niger	-	-	-	-	1 ± 0.0	3 ± 0.0
A.wentii	-	-	-	-	-	1 ± 0.0
Monilia spp.	-	-	-	-	3 ± 0.0	-
Scopulariopsis brevicaulis	-	2 ± 0.0	-	-	-	-
	20 Days					
Aspergillus candidus	1 ± 0.0	-	-	-	-	-
A. flavus	42 ± 5.12	56 ± 3.11	63 ± 5.02	34 ± 2.77	39 ± 3.11	32 ± 6.02
A.fumigatus	5 ± 0.71	-	2 ± 0.0	-	9 ± 1.0	3 ± 0.0
A.niger	1 ± 0.0	1 ± 0.0	-	-	1 ± 0.0	2 ± 0.0
A.wentii	1 ± 0.0	-	1 ± 0.0	-	5 ± 2.12	1 ± 0.0
Cladosporium cladosporoides	1 ± 0.0	-	-	-	-	-
Monilia spp.	1 ± 0.0	4 ± 1.41	-	3 ± 0.71	2 ± 0.0	-
Rhizoctonia solani	-	2 ± 0.0	-	-	-	1 ± 0.0
	40 Days					
Aspergillus flavus	5 ± 2.12	10 ± 0.58	2 ± 0.0	4 ± 0.58	9 ± 1.30	4 ± 0.58
A.niger	-	-	-	-	1 ± 0.0	-
A.wentii	-	1 ± 0.0	-	1 ± 0.0	1 ± 0.0	-
Gelasinospora spp.	-	2 ± 0.0	-	-	-	-
Monilia spp.	-	-	2 ± 0.0	-	-	-
	60 Days					
Aspergillus flavus	-	-	-	-	3 ± 0.0	-
A.niger		-	2 ± 0.0	-	1 ± 0.0	1 ± 0.0
A.wentii	-	1 ± 0.0	-	-	-	-
Penicillium spp.	-	1 ± 0.0	-	-	-	-
	80 Days					
Fusarium solani	-	1 ± 0.0	-	-	-	-
Scopulariopsis brevicaulis	-	-	1 ± 0.0	-	-	-

Table 2. Activity of water content and temperature on seed germination.

Days	Water content (%)					
	13		15		17	
	N. St	St.	N. St	St.	N. St	St.
	Room temperature (25-30°C)					
0	85	63	78	79	81	77
20	70	70	60	60	76	84
40	99	95	97	94	80	91
60	97	96	95	99	90	90
80	99	96	93	99	90	95
	Low temperature (4°C)					
0	83	87	86	75	91	83
20	72	74	75	74	77	69
40	100	100	98	100	96	100
60	98	96	96	100	93	98
80	100	100	92	95	91	87

N. St. = Non-surface sterilized seed; St. = Surface sterilized seed

Water content and temperature has not influenced the germination of seeds. The seeds remained viable during the course of experiment (80days). Only in seeds with heavy infection caused by fungi, didn't germinate properly. In the present experiments higher infection percentage of fungi was observed at room temperature (25-30°C) with 13 and 17% water contents as compared to low temperature. Low temperature has affected the growth of storage fungi but the slow growing pathogenic fungi like *Fusarium solani, Rhizoctonia solani; Gelasinospora, Scopulariopsis brevicaulis* etc. were isolated at 4°C. The incidence of pathogenic and storage fungi were higher after 20 days interval in both temperatures, which decreased gradually. Such similar results were also reported by Kaiser *et al*., (1989) where they stored the seeds for about four years at various temperatures. Moisture and temperature has not affected the seeds only the seeds which were heavily infected with fungi, showed reduced growth during 3rd and 4th year of storage. Tariq *et al*., (2005) also found increase in storage fungi and decrease in germination, with the passage of time in soy bean seeds. She also reported that high moisture and temperature increase the infection of *A.flavus* and decrease the germination of soy bean seed. High moisture increased the incidence of *A.flavus* and *aflatoxin* B1 production on sunflower seeds (Dawar & Ghaffar, 1992). Niaz *et al*., (2011) also reported that high moisture level favors the growth of mould fungi like *Aspergillus* species at 28 and 40^{o}C on maize seeds. Similar results were also reported by Chuahan (1984) where the increase in temperature increased the incidence of storage fungi. So storage at low temperatures can be effective. Other fungal species like *Penicillium, Fusarium, Alternaria, Drechslera, Chaetomium, Trichoderma, Myrothecium* etc., are known to produce mycotoxins. Mycotoxins can cause severe damage to the liver, kidneys and nervous system of man even in low dosages (Rodricks, 1976).

Present studies showed that high water content and high temperatures both favor the growth of mould fungi and that in turn affected the growth and viability of seeds during storage. Seeds stored at low temperatures with low moisture levels didn't support the growth of storage fungi and if the seeds were stored for longer duration of time, they would not lose their viability.

As lentil is one of the oldest food crops of the world and consumed for its nutritional values, measures should be taken to improve the crop quality and seed-storage conditions. Reduction in the yields of lentil crop in Pakistan has been observed due to various economical and agronomical factors (Hussain *et al*., 2007). Steps should be taken on emergency basis to reduce the disease incidence and increase the yield of lentil in the country.

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(Received for publication 10 April 2013)