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SALTS EFFECT ON GERMINATION AND SEED BORNE FUNGI OF FENUGREEK (*TRIGONELLA FOENUM GRAECUM* L.) DURING STORAGE

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Abstract

Seed sample of Fenugreek (*Trigonella foenum graecum* L.) collected from local market of Karachi were treated with salts like sodium chloride and potassium chloride at two different concentrations (0.05 and 0.1% w/w) and stored upto 60 days to observe their effect on germination and seed borne fungi. Results showed that both salts suppressed the saprophytic and storage fungi with the passage of time. *Alternaria alternata, Dreschlera hawaiiensis, Fusarium poae, Penicillium bravicompectum* were completely reduced upto 60 days of storage of fenugreek seeds when treated with KCl. Surface sterilization of fenugreek seeds with 1 % $Ca(OCl)_2$ reduced superficial fungi. Seed treatment with both salts were found effective. However, KCl gave much better results against storage fungi.

KEYWORDS: Fenugreek seeds, Fungi, NaCl and KCl.

INTRODUCTION

Trigonella foenum graecum L. (fenugreek) is the member of family Fabaceae. Fenugreek is an annual legume crop cultivated in Spain, U.K, Germany, Austria, China, Israel, Greece, Switzerland, Turkey, Egypt, Kenya, Japan, Russia, USA and Pakistan (Rouk and Managesha, 1963; Rosengarten, 1969). Agricultural zones of fenugreek in Pakistan including Mianwali, Pakpattan, Bahawalpur, Faisalabad, Shorkot, Narowal, Karachi, Okara, Talwandi, Bahawalnagar, Daska, Sargodha, Quetta and Kasur (Erum *et al*., 2011). 100 g of fenugreek seeds contains about 13.7g moisture, 26.6g protein, 44.g carbohydrates, 5.8g fats, 3.0g minerals 7.2g, 160mg calcium, 370mg phosphorus, 14.1mg iron, 0.34mg thiamine, 0.29mg riboflavin, 96ug carotene, 1.1mg niacin fibres (Gopalan *et al*., 1987). In many countries, fenugreek seeds and leaves are widely used in medicine due to presence of large amount of calcium, iron, β-carotene and many vitamins (Sharma *et al*., 1996). Fenugreek seeds have a pleasantly bitter taste with characteristic odor. Research on medicinal properties showed that it exhibit anti-diabetic, anti-cancer, hypocholesterolemic and hyperglycaemic properties (Acharya, 2014).

Survey of previous literature shows that many fungal species have been reported on fenugreek includes *Alternaria alternata, Aspergillus flavus, A. fumigatus A. nidulans, A. niger, A. oryzae, A. stellifer, Botrytis cinera, Cladosporium cladosporioids, Cephalosplrium acremonium, Curvularia lunata, Drechslera* sp, *Emericella variecolor, Fusarium moliniforme, Fusarium proliferatum, Paecilomyces* sp., *Mucor* sp., *Penicillium chrysogenum, Rizopus nigricans, Scopulariopsis* sp., *Syncephalastrum racemosum* (Hedawoo and Chakranarayan, 2011; Hashmi *et al*., 1988; El-Nagerabi, 2002). Several researchers work on the seed treatment with common salts to protect against storage fungi and in activation of embryo (Dawar, 1994; Rahim, 2012; Neergaard, 1977; Karamanos, 2013). Treatment with salt water is an important technique in separating healthy seeds from chaffy ones which is useful in treating paddy seeds.

The aim of the present study was to evaluate the efficacy of sodium chloride and potassium chloride for germination and control of seed borne mycoflora during storage condition.

MATERIALS AND METHODS

Seed sample of Fenugreek (*Trigonella foenum graecum* L.) was collected from local market of Karachi. Common salts i.e., sodium chloride (NaCl) and potassium chloride (KCl) were used for the control of seed borne mycoflora during storage. Different concentrations of both salts like 0.05 and 0.1% (w/w) were added (low concentrations of salts were selected so not to harmful for germination over storage of long time period) to each 100g sample of fenugreek seeds. The seeds samples were stored in a jar at room temperature for 60 days with frequent shaking to ensure thorough mixing. Non treated seed sample was served as control. The seed samples were removed at 0, 15, 30, 60 days of storage and placed on Petri Plates containing potato dextrose Agar (PDA) supplemented with antibiotics Penicillin @ 20,000 unit/liter and streptomycin @ 200 mg/litre to detect seed germination and seed borne mycoflora (Anon, 1993). The dishes were incubated for 7 days at room temperature (32±2°C) and fungi growing on seeds were identified using standard literature (Barnett and Hunter, 1998; Booth, 1971; Domsch *et al.*, 1980; Ellis, 1971; Nelson *et al.*, 1983).

Data of germination % of seeds were analyzed using analysis of variance and least significance difference at 5% probability level were determined for comparison between mean (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Two different salts with 0.05 and 0.1% w/w concentrations were applied to fenugreek seeds and stored upto 60 days. KCl treated seeds gave 96% of germination at 0.05% concentration on 60 days of storage while NaCl treated seeds showed 92% germination at 0.1% concentration in surface sterilized seeds ($p<0.001$). However, non surface sterilized seeds which were heavily infested with fungi gave 98% germination when treated with KCl at 0.1% concentration ($p<0.001$) (Fig. 1).

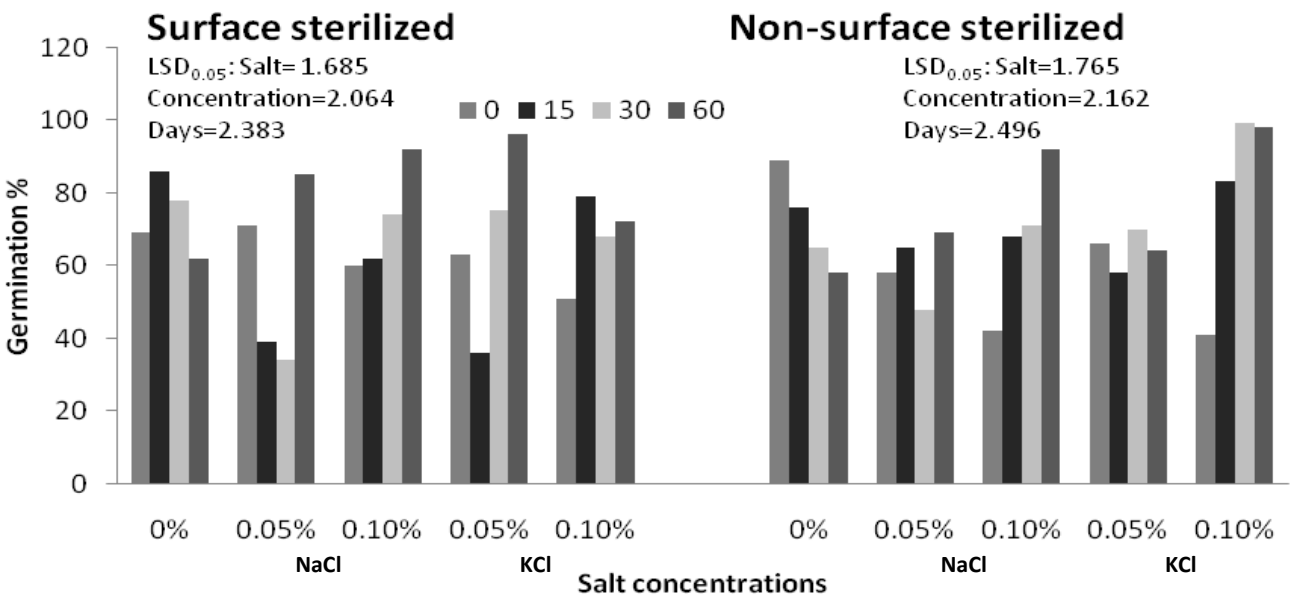

Fig. 1. Effect of salts on germination % of seeds.

Results showed that on 60 days of storage, number of fungi were reduced due to application of KCl and NaCl on both surface sterilized and non sterilized seeds (Tables 1, 2). After 60 days of storage, 5 fungal genera belonging to 7 species were recorded of which *Aspergillus flavus* and *Drechslera hawaiiensis* were the dominant fungi on NaCl treated seeds at 0.05 and 0.1 % w/w concentration on surface sterilized seeds. Similar results were also reported by Dawar (1994) on sunflower seeds where the level of Aflatoxins reduced either to low quantities or traces by treatment of seed with NaCl in different percentage. Several scientists suggested the routine use of NaCl as seed treatment for protection against storage fungi which are present as contaminant inside seed coat and are not able to invade seeds before harvest (Dawar, 1994; Neergaard, 1977). Highest number of fungi were isolated at 0 day which reduced as interval increase on both salts. Surface sterilization of seeds with 1 % $Ca(OCl)_2$ reduced the infestation of superficial fungi on seeds. Sitara and Akhtar (2007) found the disinfectant effect of sodium hypochlorite on maize seeds and significantly eliminated *Phoma* and *Chaetomium* spp., while reducing the incidence of *Aspergillus* spp., *Rhizopus* spp., and *Nigrospora* spp.

Penicillium bravicompectum, Fusarium poae, Dreschlera hawaiiensis, Alternaria alternata were completely eliminated upto 60 days of storage of seeds when treated with KCl (Table 2). Rahim and Dawar (2012) observed that KCL was more efficient for storage of seed borne fungi of lentil when compared to NaCl. Aymen (2012) observed improved plant height, number of branches, fresh and dry weight, heads number per plant, petals and grains yield of plants by the use of seed priming with KCl at 5g/l for 24 hours. Seed priming with KCl (1%) for 18 hours increased wheat yield under dry land condition (Paul and Choudhury, 1991). Primed seed emerged earlier compared to non primed seeds. Reason for this is the increased activity of enzymes like amylase, protease and lipase which break down macromolecules for growth and development of embryo, resulting in earlier seed emergence (Dell-Aquila and Ritto, 1990). From the consumption point of view, presence of saprophytic and pathogenic fungi is not the matter to be ignored. These fungi produced mycotoxins that cause the health hazards in human and animals. *A. flavus* is commonly present in fenugreek seeds, which produced Aflatoxins which is carcinogenic. Species of *Aspergillus* are known to cause aspergillosis in man, animal and birds (Rapper and Fennell, 1965). *A. niger* causes the ear infection, *A. fumigatus* causes ear pulmonary disease in pigeon while other species of *Aspergillus* also cause systematic infection in man and animals (Rapper and Fennell, 1965). Other fungi like *Penicillium, Fusarium, Alternaria alternata, Drechslera, Chaetomium, Myrothicium* etc. are known to produced mycotoxins can cause severe damage to the liver, kidneys and nervous system of man even in low dosage (Rodricks, 1976).

Both salts were showed beneficial effect for long term storage and effective against the storage fungi. However, KCl was found to be much better for the storage fungi of fenugreek seeds. However, there is still need for further research into the effect of these salts and other salts to prevent long term and safe storage of seeds by seed borne fungi.

Table 1. Effect of salts on seed borne mycoflora of fenugreek: Non surface sterilized seeds.

Fungi	Control	NaCl		KCl	
		0.05%	0.1%	0.05%	0.1%
			0 Day		
Alternaria alternata	7 ± 3.130	-	-	-	-
Aspergillus flavus	11 ± 3.492	27 ± 6.188	16 ± 2.6832	9 ± 3.0331	10 ± 2.1213
Aspergillus fumigatus	3 ± 1.341	-	-	-	-
Aspergillus niger	51 ± 3.346	24 ± 3.420	59 ± 5.167	29 ± 4.969	25 ± 3.3911
Aspergillus oryzae	5 ± 2.236	4 ± 1.788	4 ± 1.788	-	-
Aspergillus sydowii	-	-		1 ± 0.4472	-
Aspergillus terreus	39 ± 4.868	10 ± 2.345	4 ± 0.8366	24 ± 2.863	1 ± 0.4472
Emmericella nevia	-	-	-	-	1 ± 0.4472
Fusarium poae	3 ± 1.3416	-	-	-	-
Mortierella nana	-	-	-	-	6 ± 1.095
Mucor racemosus	-	-	-	-	4 ± 1.788
Penicillium bravicompectum	-	3 ± 1.3416	-	-	6 ± 1.3038
Penicillium chrysoenum	9 ± 2.6832	-	1 ± 0 .447	2±0.547	-
Rhizopus sp.	-	-	-	-	16 ± 7.155
			15 Days		
Alternaria alternata	-	3 ± 0.547	3 ± 0.8944	-	2 ± 0.5477
Alternaria longissima	-	-	-	2 ± 0.5477	-
Aspergillus flavus	8 ± 2.073	26 ± 1.643	4 ± 1.095	34 ± 2.280	15 ± 4.582
Aspergillus fumigatus	6 ± 2.16	-	-	-	-
Aspergillus niger	39 ± 3.27	4 ± 1.303	9 ± 1.788	9 ± 3.033	-
Aspergillus oryzae	7 ± 2.190	-	-	-	-
Aspergillus terreus	20 ± 2.54	4 ± 1.788	-	2 ± 0.89	16 ± 2.38
Curvularia lunata	-	5 ± 1.44	-	-	-
Drechslera hawaiiensis	-	-	12 ± 2.79	6 ±2.68	1 ± 0.44
Emmericella nevia	5 ± 2.23	-	6 ± 0.83	4 ± 0.836	-
Fusarium oxysporum	-	-	8 ± 3.049	-	-
Fusarium solani	-	1 ± 0.447	-	-	-
Penicillium bravicompectum	1 ± 0.447	1 ± 0.447	-	-	-
Rhizopus sp.	-	-	20±8.94	-	-
			30 Days		
Alternaria alternata	9 ± 3.033	-	4 ± 1.788	-	-
Aspergillus flavus	22 ± 4.615	66 ± 4.604	33 ± 1.516	44 ± 1.303	14 ± 2.774
Aspergillus niger	58 ± 5.412	1 ± 0.447	10 ± 4.472	4 ± 1.095	6 ± 1.788
Aspergillus sydowii	-	-	-	-	3 ± 0.547
Aspergillus terreus	6 ± 8.408	-	13 ± 1.8165	-	-
Drechslera hawaiiensis	-	1 ± 0.447	-	-	-
Fusarium poae	3 ± 0.894	-	-	-	-
Penicillium bravicompectum	4 ± 0.833	-	-	-	-
			60 Days		
Alternaria alternata	9 ± 3.033	-	-	-	-
Aspergillus flavus	22 ± 4.615	18 ± 3.04	8 ± 1.81	-	12 ± 2.88
Aspergillus terreus	6 ± 8.40	-	-	1 ± 0.447	-
Drechslera hawaiiensis	1 ± 0.447	-	2 ± 0.894	-	-
Fusarium poae	3 ± 0.894	-	-	-	-
Penicillium bravicompectum	4 ± 0.833	-	-	-	-
P. chrysogenum	6 ± 1.788	-	-	-	6 ± 1.303

Table 2. Effect of salts on seed borne mycoflora of fenugreek: Surface sterilized seeds.

Fungi	Control	NaCl		KCl	
		0.05%	0.1%	0.05%	0.1%
			0 Day		
Alternaria alternata	6 ± 2.16	-	-	-	-
Aspergilllus flavus	8 ± 2.07	18 ± 4.50	10 ± 3.93	13 ± 3.04	1 7 ±3.04
Aspergillus fumigatus	4 ± 1.30	-	6 ± 7.15	-	-
Aspergillus niger	43 ± 5.7	18 ± 4.50	49 ± 6.76	30 ± 5.52	32 ± 3.36
Aspergillus oryzae	29 ± 1.09	1 ± 0.447	-	-	3 ± 0.89
Aspergillus sydowii	-	-	-	1 ± 0.447	-
Aspergillus terreus	29 ± 3.6	-	-	22 ± 3.361	3 ± 1.341
Emmericella nevia	1 ± 0.447	-	2 ± 0.894	-	-
Fusarium poae	5 ± 2.23	-	-	-	-
Fusarium solani	2 ± 0.89	-	-	-	-
Mortierella nana	-	-	-	-	5 ± 1.22
Mucor sp	7 ± 3.13	-	-	-	-
Penicillium bravicompectum	-	1 ± 0.447	-	-	-
Penicillium chrysogenum	5±2.23	-	4±0.83	1±0.447	-
			15 Days		
Alternaria alternata	3 ± 0.89	6 ± 1.303	5 ± 1	4 ± 1.303	3 ± 0.89
Alternaria longissima	-	1 ± 0.447	-	-	-
Aspergillus flavus	17 ± 3.78	-	-	1 ± 0.447	-
Aspergillus fumigatus	1 ±0.447	-	-	-	7 ± 3.13
Aspergillus niger	47 ± 5.07	18 ± 2.303	31 ± 2.74	23 ± 2.9	11 ± 2.16
Aspergillus oryzae	6 ± 1.78	-	2 ± 0.89	-	3 ± 1.34
Aspergillus sydowii	-	2 ± 0.89	-	-	-
Aspergillus terreus	37 ± 4.82	-	5 ± 0.70	-	11 ± 1.51
Curvularia lunata	-	1 ± 0.447	-	-	-
Drechslera hawaiiensis	-	-	2 ± 0.547	-	8 ± 2.07
Fusarium oxysporum	-	-	-	1 ± 0.447	-
Fusarium solani	-	1 ± 0.447	1 ± 0.447	-	-
Paecilomyces sp	-	-	1 ± 0.447	-	1 ± 0.447
Penicillium bravicompectum	3 ± 1.341	1 ± 0.447	-	1 ± 0.447	-
			30 Days		
Aspergillus flavus	39 ± 3. 94	53 ± 6.34	21 ± 1.643	16 ± 3.27	19 ± 1.923
Aspergillus fumigatus	-	-	3 ± 1.341	-	-
Aspergillus niger	31 ± 3.1	2 ± 0.547	3 ± 0.89	5 ± 1.414	4 ± 1.303
Aspergillus oryzae	-	-	1 ± 0.447	-	-
Aspergillus sydowii	-	-	1 ± 0.447	-	-
Aspergillus terreus	19 ± 2.7	**5** ± 1.732	13 ± 2.408	16 ± 2.949	-
Drechslera hawaiieusis	-	1 ± 0.447	-	-	-
Fusarium solani	3 ± 1.34	2 ± 0.547	-	-	-
Penicillium chrysogenum	-	-	1 ± 0.447	-	-
			60 Days		
Alternaria alternata	2 ± 0.894	-	-	-	-
Aspergillus flavus	39 ± 3.49	7 ± 1.341	6 ± 2.167	-	6 ± 1.303
Aspergillus niger	31 ± 3.193	-	-	1 ± 0.447	-
Aspergillus terreus	19 ± 2.774	-	-	6 ± 2.1908	-
Drechslera hawaiiensis	-	2 ± 0.894	1 ± 0.447	-	-
Fusarium poae	3 ± 1.341	-	-	-	-
Penicillium bravicompectum	5 ± 2.236	-	-	-	-
Penicillium chrysogenum	-	-	-	-	2 ± 0.894

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