SEED BORNE FUNGI ASSOCIATED WITH COWPEA (*VIGNA UNGUICULATA* (L.) WALP.

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ABSTRACT

Seed borne fungi associated with sixteen white cowpea seed samples collected from various cities of Pakistan were detected using seed health testing techniques as recommended by ISTA viz; standard blotter, agar plate, and deep-freezing methods. Twenty eight species belonging to 17 fungal genera were isolated mutually using standard blotter, agar plate and deep-freezing methods. Agar plate method was best for the detection of fungi both qualitatively and quantitatively, followed by standard blotter and deep-freeing methods. Pathogenic fungi like *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated through agar plate method. Deep-freezing and blotter methods were best for the isolation of *Fusarium oxysporum. Aspergillus flavus* followed by *A. niger* was the most dominant fungal species in all the three methods used. Surface sterilization of seeds with 1% $Na(OCl)_2$ has greatly reduced the incidence of saprophytic fungi.

Key words: Cowpea, ISTA techniques, Pakistan, Seed borne fungi, Surface sterilization

INTRODUCTION

Vigna unguiculata (L.) Walp. commonly known as cowpea is a member of the family leguminaceae. It is evident by the survey of literature showed both pathogenic and saprophytic fungi associated with white cowpea seeds. Fungi reported on cowpea from South Africa include *Fusarium equiseti, F. graminearum, F. semitectum, F. chlamydosporum, F. sambucium* and *F. subglutinans* (Kritzinger *et al*., 2003). Kumar *et al*., (2004) isolated *Alternaria alternata, Lasiodiplodia theobromae, Drechslera tetramera* (*Cochliobolus spicifer*) and *Fusarium verticillioides* (*Gibberella fujikuroi*) from cowpea seeds, using agar plate and standard blotter methods. Seed samples collected from the markets of Northern Nigeria were found to be infected with *Ascochyta* sp, *Colletotrichum lindemuthianum, Rhizoctonia solani, F. oxysporum, F. solani, Macrophomina phaseolina, Septoria vignae* and *Corticium rolfsii* (Emechebe and McDonald, 1979). Mogle and Maske (2012) isolated *Rhizoctonia solani, Aspergillus flavus, Cladosporium* sp., *Aspergillus niger, Penicillium* sp., *Fusarium oxysporum, F.solani, F.semitectum, Trichoderma viride, Curvularia lunata, Mucor* sp., and *Verticillium* sp. from the seed samples collected from Maharashtra, India. Amadi and Oso (1996) isolated *Alternaria longissima, A. flavus, A. fumigatus, A. niger, Botryodiplodia theobromae, Colletotrichum* sp., *Mucor himelis, M. phaseolina, R. oryzae* using agar and blotter methods. Embaby and Abdel-Galil (2006) isolated species of *Alternaria, Aspergillus, Epicoccum, Fusarium* and *Trichoderma* from the seed samples of cowpea and lupine from Egypt; where protein, carbohydrate, fat, fiber and ash content of seeds decreased when artificially inoculated with aflatoxin. Ahmad *et al*., (1993) reported *Colletotrichum dematium, Curvularia lunata* and *Fusarium moniliforme* on cowpea seeds from Pakistan. Fungi produce secondary metabolites known as mycotoxins, higher the number of fungi isolated from any seed sample, higher would be the level of mycotoxins (Rahim *et al.,* 2013). Ibeh *et al*., (1991) detected presence of mycotoxins produced by *Aspergillus* spp. in cowpea seed samples. Habish (1972) reported presence of aflatoxin from the samples collected from Sudan; while Houssou *et al*., (2009) reported the presence of aflatoxin in cowpea seed samples from West Africa. As the number of storage fungi increases, the nutritive quality, viability and germination of seeds reduces (Rahim *et al.;* 2010; Agarwal and Sinclair, 1996).

Current work was carried out to detect the fungi associated with seeds of cowpea in Pakistan.

MATERIALS AND METHODS

Collection of seed samples: Sixteen seed samples of cowpea (*Vigna unguiculata* (L.) walp were collected from different areas of Pakistan viz; Karachi (8), Lahore (1), Malam jabba (1), Swat (1), Chakwal (1), Winder (1), Bela (1), Gilgit (1) and Faisalabad (1). The seed samples were stored in air-tight glass jars and stored at room temperature (30 - 35 ºC).

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Isolation of seed-borne fungi from cowpea: For the detection of seed-borne mycoflora ISTA (International Seed Testing Association) techniques were used (Anon, 1993). Using standard blotter, agar plate and deep-freezing methods, about four hundred seeds of each sample were tested.

Standard Blotter Method: Untreated seeds and seeds after treatment with 1% $Na(OCl)_2$ for 2 minutes were placed aseptically on three layers of moistened blotter, 10 seeds per Petri dish. The dishes were incubated for 5 - 7 days at room temperature (30 – 35 ºC) under 12h alternating cycles of artificial day light (ADL) and darkness (Anon, 1993).

Agar plate method: Untreated seeds and seeds after surface sterilization with 1% $Na(OCl)_2$ for 2 minutes were placed aseptically on cooled, antibiotics added (penicillin, 20,000 units/L and Streptomycin, 1mL/L) potato dextrose agar (PDA) poured petri dishes @ 10 seeds per petri dish. The dishes were incubated for 5 - 7 days at room temperature (30 – 35 ºC) under 12h alternating cycles of artificial day light (ADL) and darkness (Anon,1993).

Deep freezing method: Untreated seeds and seeds after sterilization with 1% $Na(OCl_2$ for 2 minutes were placed aseptically on three layers of moistened blotter paper, 10 seeds per petri dish. The petri plates were first kept for 24h at room temperature, then for 24h at low temperature (-20 ºC) followed by 5 days incubation at room temperature (30 - 35ºC) under 12h alternating cycles of artificial day light (ADL) and darkness (Anon, 1993).

Fungi growing on seeds were identified after referencing to Barnett and Hunter (1998), Ellis (1971), Domsch *et al*., (1980), Nelson *et al.,* (1983) and Raper *et al*., (1965).

Statistcal analysis: Data were subjected to analysis of variance (ANOVA) following the procedures as given by Sokal and Rohlf (1995).

RESULTS

Twenty eight species of 17 fungal genera were isolated mutually from sixteen cowpea seed samples using techniques recommended by Anonymous (1993). Agar plate followed by blotter and deep-freezing methods was best for the isolation of fungi. *A. flavus* ($P < 0.001$) and *A. niger* ($P < 0.001$) were the most dominant fungi in all three methods. *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated through agar plate method only. *F. oxysporum* was isolated by all the three methods (Table 1). Seed samples collected from Bin Qasim, Landhi, Saddar, and Nagan Chowrangi areas of Karachi city yielded highest number of fungi. Surface sterilization of seeds with 1 % $Na(OCl)_2$ has significantly reduced the incidence of saprophytic (storage) fungi.

DISCUSSION

Of all the seed samples tested, samples from the four localities of Karachi showed highest fungal infection. Agar plate method followed by standard blotter and deep-freezing methods yielded highest number of fungi. Agar plate method is considered best for the isolation of greater number of fungi (Rahim *et al.,* 2013). Kumar *et al.,* (2002) suggested agar plate method as better than blotter method in terms of percentage recovery of fungi. Blotter method was best for the isolation of cellulose decomposing fungi like *Chaetomium* and *Fusarium* species (Domsch *et al.*, 1980). Rahim *et al.,* (2013), Niaz and Dawar, (2009) also reported similar results. Jovicevic (1980) suggested filter paper (blotting method) best for the routine analysis of seeds health because in agar plate method intrafungal antagonism becomes an issue (Niaz and Dawar, 2009). Deep-freezing method was best for the isolation of *F.oxysporum*. Sultana and Ghaffar (2009) found similar results and suggested blotter and deep-freezing methods best for the isolation of fungi. *Rhizoctonia solani* was also isolated through all techniques used. Thies *et al.,* (2006) reported *R.solani* as one the most important pathogen of cowpea in United States, responsible for causing severe root rot infection especially in cold weather. Species of *Aspergillus, Mucor, Penicillium* and *Rhizopus* are known to produce rot and decay of seeds during storage (Domsch *et al*., 1980). Storage fungi consume the nutrient of seeds; reduce the quality of seeds from cultivation, consumption and trade point of view by shrinking and discoloring the seeds (Agarwal and Sinclair, 1996). Cowpea is an economically important crop. Proper steps should be taken to ensure the quality of seeds during storage, and also to reduce the spread of diseases due to seed transmission of pathogenic fungi.

Table 1. Isolation of seed-borne fungi from cowpea *Vigna unguiculata* (L.) Walp using ISTA techniques.

Name of fungi	Standard Blotter Method				Agar Plate Method				Deep-Freezing Method			
	N.St		S.St		N.St		S.St		N.St		S.St	
	NSI	I% ±S.D	NSI	I% ±S.D	NSI	I% ±S.D	NSI	I% ±S.D	NSI	I% ±S.D	NSI	I% ±S.D
Absidia glauca (Hagem)	-	-	1	3±0.00	2	2.5±0.50	2	2.5±1.50	1	1±0.00	-	-
Acremonium cereals (karsten)	-	-	-	-	-	-	1	2±0.00	-	-	-	-
Aspergillus candidus (Link ex Link)	2	1.5±0.50	2	1±0.00	4	2.25±0.43	2	2±1.00	-	-	-	-
A.carneus (Tiegh.)	-	-	-	-	1	1±0.00	1	-	-	-	-	-
A.clevatus (Desm)	-	-	-	-	-	-	1	1±0.00	-	-	-	-
A.flavus (Link ex Gray)	16	43.6±27.4	15	25.0±19.9	16	53.6±20.1	16	51.1±32.7	16	24.3±21.5	15	7.73±6.18
A.fumigatus (Fresen)	12	4.66±4.24	10	3.2±1.60	11	8.63±12.9	9	7.22±6.72	9	4.66±4.02	7	4.71±4.33
A.japonicus (saito)	-	-	-	-	2	10.5±7.50	1	1±0.00	-	-	-	-
A.niger (Van Tieghem)	16	24.7±21.4	16	18±17.12	16	37.8±19.2	15	18.26±16.0	14	12.5±21.9	12	6.08±6.90
A.sclerotiorum (Huber)	-	-	-	-	1	1±0.00	-	-	-	-	-	-
A.terreus (Thom)	1	1±0.00	-	-	-	-	1	5±0.00	-	-	-	-
A.versicolor (Vuill.) Tirab	-	-	-	-	3	3±0.00	2	18.12±4.24	-	-	-	-
A.wentii (Wehmer)	9	4.44±4.11	11	5.45±2.80	7	4.57±3.81	8	7.8±6.23	8	3.25±2.58	6	2.83±0.68
Chaetomium globosum (Kunze ex Steud)	-	-	-	-	-	-	1	2±0.00	-	-	-	-
Cladosporium oxysporum (Berk & Curt)	1	4±0.00	1	5±0.00	2	2.5±0.50	1	4±0.00	-	-	-	-
Curvularia sp. (Boedijn)	-	-	-	-	5	5.8±7.16	-	-	-	-	-	-
Drechslera australiensis (Ellis)	-	-	-	-	-	-	5	3±0.89	-	-	-	-
Fusarium oxysporum (Schltdl)	1	2±0.00	3	5.33±2.62	2	3.5±0.50	4	2.75±1.29	-	-	1	6±0.00
F.solani (Mart.) Sacc	-	-	-	-	1	-	1	3±0.00	-	-	-	-
Macrophomina phaseolina Tassi (Goid)	-	-	-	-	-	-	1	1±0.00	-	-	-	-
Monilia sp. (Pers)	1	2±0.00	1	4±0.00	6	2.83±0.89	9	2.55±0.68	-	-	-	-
Mucor sp. (Mich. exst. Am)	2	2±0.00	5	3.4±1.95	5	5.6±5.27	2	3.5±2.50	3	1.66±0.47	1	3±0.00
Myrothecium roridum (Tode ex Fr)	2	2±0.00	-	-	3	2±0.00	3	2.33±0.94	-	-	-	-
Rhizoctonia solani (Kuhn)	-	-	-	-	4	5.75±5.44	3	3.66±2.05	-	-	-	-
Rhizopus sp. (ehrenb)	12	7±5.16	11	6.81±4.91	16	30.8±16.6	14	8.5±6.21	11	7.54±6.84	8	3.87±1.89
Stephylotricum sp. (J.Meyer and Nicot)	-	-	-	-	1	3±0.00	-	-	-	-	-	-
Syncephalastrum racemosum (Cohn ex Schrot)	-	-	-	-	1	2±0.00	2	3±1.00	1	4±0.00	-	-
Trichoderma harzianum (Pers ex Fr)	-	-	-	-	1	3±0.00	2	4±3.0	-	-	-	-

N.St = non-surface sterilized seeds; S.St = surface sterilized seeds; I%= infection percentage; S.D = standard deviation

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(*Accepted for publication September 2015*)