

FUNGI ASSOCIATED WITH THE SEEDS OF *CARICA PAPAYA* L.

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ABSTRACT

Eighteen seed samples collected from Karachi (17) and Sukkur (1) areas of Sindh, Pakistan, were analyzed for seed-borne fungi using standard blotter method, agar plate method and deep-freezing methods as suggested by ISTA. At least 93 species belonging to 44 fungal genera were isolated mutually from all the seed samples analyzed. Agar plate method was found to be the best for the isolation of fungi. Species of *Aspergillus*, *Chaetomium*, *Cladosporium*, *Rhizopus* and *Trichoderma* were isolated through all methods. Blotter method was the best for the isolation of *Chaetomium* and *Fusarium* species, while species of *Curvularia* and *Drechslera* favoured growth on PDA medium. Surface sterilization of seeds with 1% Ca (OCl)₂ has reduced the incidence of superficial fungi. Modification was also introduced in all three methods by increasing the incubation time period. The current work with papaya as host plant was carried out supposedly for the first time; hence all the fungal species mentioned hereby are new reports from papaya seeds from Pakistan.

Key words: Calcium hypochlorite, papaya, seed-borne fungi.

INTRODUCTION

Papaya (*Carica papaya* L.) belongs to the family Caricaceae. It is native to Mexico and Central America (Morton, 1987), however currently it is cultivated in almost 60 countries of the world with global annual production of 11.22 million metric (M) tonnes (T) in 2010, with Asia as the largest producer of papaya. (Evans *et al.*, 2012; FAOSTAT, 2012). Pakistan produced about 8732 tonnes of papaya cultivated on 1545 hectares of land (Anon, 2009) while in the year 2012, the total production was 8000 tonnes from an area of 1600 hectares (FAOSTAT, 2012). In Pakistan papaya is cultivated in the areas of Sindh mainly Karachi and in some parts of Punjab (Oad *et al.*, 2001). Papaya is fleshy, nutritive fruit with black seeds. The seeds of papaya are covered in gelatinous layer known as aril, have sharp, spicy taste and can be used as a substitute for black pepper (Simonsohn, 2000; Seidemann, 2005). A lot of work has been done for the control of fungal diseases of papaya in field and after harvest, however, little attention has been paid to the seed-borne fungi of papaya. Great many fungi attack papaya in field and after the harvest of fruit. *Alternaria* fruit spot (*Alternaria alternata*), angular leaf spot (*Leveillula taurica*), anthracnose (*Colletotrichum gloeosporioides*), black spot (*Cercospora papayae*, *Phomopsis carica-papayae*), blossom spot (*Choanephora cucurbitarum*), brown spot (*Corynespora cassiicola*), damping-ff (*Phytophthora palmivora*, *P. nicotianae*, *Pythium debaryanum*, *Rhizoctonia solani*), dry rot (*Phoma carica-papayae*), foot rot (*P. debaryanum*, *P. ultimum*), fruit rot (*Monilia* sp.), fruit spot (*Cercospora mamaonis*), *Fusarium* fruit rot (*F. solani*, *Fusarium* spp.), internal blight (*Cladosporium* sp., *Fusarium* sp., *Penicillium* sp.), *Rhizopus* soft rot (*Rhizopus stolonifer*), *Stemphylium* fruit rot (*Stemphylium lycopersici*), *Verticillium* wilt (*Verticillium dahliae*) are some reported pathogens (Anon, 2014). Echerenwa and Umechuruba (2004) reported *Fusarium solani*, *Phoma carica-papayae*, *A. flavus*, *A. niger*, *Botryodiplodia theobromae*, *Cladosporium herbarum*, *Colletotrichum dematium*, *F. moniliforme*, *phomopsis carica-papayae*, *Penicillium* sp., *Rhizopus stolonifer*, as post-harvest fungi of papaya fruit. When seeds were tested using standard blotter method, similar fungi were isolated. All the isolated fungi were able to produce disease when tested for pathogenicity. Papaya is a suitable substratum for fungi like *Asperisporium caricae*, *Corynespora cassiicola*, *Fusariella obstipa*, *Stachybotrys kampalensis*, *S. nephrospora* etc. (Ellis, 1971). As papaya seeds are the germ plasm for future papaya crop, current work was therefore carried out to check the fungi associated with the seeds of papaya.

MATERIALS AND METHODS

For the detection of seed-borne mycoflora, ISTA (Anon, 1993) techniques i.e., standard blotter, Agar plate and Deep-freezing methods were used. About 400 seeds of each sample were tested.

Collection of seeds: Eighteen seed samples of papaya were collected from the local markets of Sindh-Pakistan viz: Pakistan chowk (1), Gulshan-e-Iqbal town (1), Bahadurabad (1), Malir town (1), Saddar (3), Impress market (2), Kaemari (1), Alfalah society (2), P.E.C.H.S. (1), Gaddap (1), New town (1), Shah Faisal colony (1), Metrovill (1) and Sukkur (1). Samples of semi-fresh papaya were collected from different areas of Karachi, while one seed sample

was collected from Sukkur city. Papaya seeds were washed, arils were removed and air dried for 7 - 10 days. The seeds were then placed in labeled air tight jars at room temperature (25 - 35°C) for future use.

Table 1. Seed-borne fungi of papaya isolated through ISTA techniques*

NAME OF FUNGI	STANDARD BLOTTER METHOD			AGAR PLATE METHOD			DEEP-FREEZING METHOD		
	NS	N. St	S. St	NS	N. St	S. St	NS	N. St	S. St
<i>Acremonium furcatum</i> F. & V. Moreau ex W. Gams	-	-	-	1	0.21±0.0	-	-	-	-
<i>Acreotalagnus fungicola</i> Preuss	-	-	-	1	0.05±0.0	-	-	-	-
<i>Alysidium</i> sp. Kunze ex Steudel	-	-	-	1	0.05±0.0	-	-	-	-
<i>Alternaria alternata</i> (Fr.) Keissler	1	0.11±0.0	0.11±0.0	1	0.05±0.0	-	-	-	-
<i>A. brassicicola</i> (Schw.) Wiltshire	-	-	-	1	0.05±0.0	-	-	-	-
<i>A. longipes</i> (Ellis & Everh) Mason	-	-	-	1	0.05±0.0	-	-	-	-
<i>A. longissima</i> Deighton & Macgarvie	1	0.05±0.0	-	1	0.05±0.0	-	-	-	-
<i>A. sonchi</i> J. J. Davis	-	-	-	1	0.05±0.0	-	-	-	-
<i>A. tenuissima</i> (Kunze ex Pers.) Wiltshire	-	-	-	1	0.05±0.0	1	0.05±0.0	-	-
<i>Alternaria</i> sp. (Fr.) Keissler	1	0.05±0.0	-	1	0.11±0.0	-	-	-	-
<i>Aspergillus candidus</i> Link ex Link	-	-	-	1	0.05±0.0	1	1.58±0.0	-	-
<i>A. flavus</i> Link ex Gray	1	2.58±0.0	1.58±15.6	14	28.8±35.9	13	13.4±15.6	2	0.58±3.54
<i>A. fumigatus</i> Fres.	-	-	-	2	0.26±2.12	3	1.26±2.65	1	0.05±0.0
<i>A. niger</i> Van Tieghem	4	0.90±3.30	0.52±0.0	16	16.05±23.0	13	9.6±10.13	1	0.11±0.0
<i>A. ochraceous</i> Wilhelm	-	-	-	4	3.8±7.5	4	6.89±8.6	-	-
<i>A. sclerotium</i> Huber	-	-	-	1	0.21±0.0	-	-	-	-
<i>A. stiphureous</i> Thom & Church	-	-	-	1	0.05±0.0	-	-	-	-
<i>A. terreus</i> Thom	-	-	-	1	0.42±0.0	-	-	-	-
<i>A. ustus</i> (Bain) Thom & Church	-	-	-	-	-	1	0.05±0.0	-	-
<i>A. wentii</i> Wehmer	-	-	-	1	0.11±0.0	1	0.52±0.0	-	-
<i>Cephalophora irregularis</i> Thaxter	1	0.11±0.0	-	-	-	-	-	-	-
<i>Chaetomium cochliodes</i> Pall.	-	-	0.05±0.0	-	-	-	-	-	-
<i>C. crispatum</i> (Fuckel) Fuckel	3	1.16±3.06	0.21±0.0	1	0.21±0.0	-	-	-	-
<i>C. globosum</i> Kunze ex Steud	2	0.31±2.82	1.16±12.7	2	0.42±4.24	2	0.31±2.82	-	-
<i>C. spirale</i> Kunze ex Fres.	-	-	-	-	-	-	-	-	-
<i>C. thernophilum</i> Cooney & R.E.mers.	-	-	0.63±0.0	1	0.63±0.0	1	0.31±0.0	-	-
<i>Chaetomium</i> spp. Kuntze	7	9±24.08	12.5±23.8	1	0.05±0.0	2	0.73±7.07	-	-
<i>Chaetomidium fineti</i> (Fuckel) Sacc.	-	-	0.26±0.0	1	0.26±0.0	-	-	-	-
<i>Chuppia sarcinifera</i> Deighton	-	-	-	-	-	1	0.05±0.0	-	-
<i>Cladosporium carpophilum</i> Fisher	-	-	-	-	-	-	-	-	-
<i>C. cladosporioides</i> (Fres.) Devries	-	-	-	1	0.11±0.0	2	1.16±12.7	-	-

<i>C. sphaerosporium</i> Penz.	-	-	-	-	-	3	5.89±52.8	2	0.73±1.41	-	-	-
<i>C. chlorocephalum</i> (Friesen) Massee & M.B. Ellis.	-	-	-	-	-	-	-	1	0.11±0.0	-	-	-
<i>C. oxysporum</i> Berk. & Curt.	-	-	-	-	-	2	0.16±0.71	2	0.21±0.0	-	-	-
<i>C. spongiosporium</i> Berk. & Curt.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Coremiella cubispora</i> (Berk. & Curt.) M.B. Ellis	-	-	-	-	-	1	0.16±0.0	-	-	-	-	-
<i>Coniochaeta lignitaria</i> (Grev.) Cooke	1	0.11±0.0	-	-	-	-	-	-	-	-	-	-
<i>Curvularia robusta</i> Kil Patrick & Luttrell	-	-	-	-	-	-	-	1	0.05±0.0	-	-	-
<i>Curvularia</i> sp. Boedijn	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-
<i>Drechslera biseptata</i> (Sacc. & Roum.) Richardson & Fraser	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-
<i>Drechslera</i> sp. Ito	-	-	-	-	-	2	0.11±0.0	-	-	-	-	-
<i>Emericella nidulans</i> (Eidam) Vuill.	-	-	-	-	-	1	0.05±0.0	2	0.31±2.82	-	-	-
<i>Eurotium</i> sp. Link ex Gray.	-	-	-	-	-	1	1±0.0	1	0.05±0.0	-	-	-
<i>Farrugia seminida</i> (L. Ames) D. Hawksworth	1	0.05±0.0	-	-	-	1	0.05±0.0	-	-	-	-	-
<i>Fusarium Campecceras</i> Wollennw. & Reinking	-	-	-	-	-	-	-	-	-	-	-	-
<i>F. equiseti</i> (Corda) Sacc. Sensu Gordon	-	-	-	1	0.11±0.0	-	-	-	-	-	-	-
<i>F. oxysporum</i> Schlecht em end.sny. & Hans	-	-	-	1	0.05±0.0	2	0.26±2.12	1	0.05±0.0	-	-	-
<i>F. subglutinans</i> (Wollennw. & Reinking) Nelson, Tousson & Marasas Comb. Nov.	1	0.05±0.0	-	-	-	-	-	-	-	-	-	-
<i>F. tritinctum</i> (Corda) Sacc.	1	1.11±0.0	-	-	-	-	-	-	-	-	-	-
<i>F. verticilloides</i> (Saccardo) Nirenberg	-	-	-	1	0.16±0.0	-	-	-	-	-	-	-
<i>Fusarium</i> sp. Link ex Fr.	1	1.05±0.0	-	-	-	1	4.47±0.0	-	-	-	-	-
<i>Lophotrichus ampullus</i> R.K. Benjamin	-	-	-	1	0.21±0.0	1	1.68±0.0	-	-	-	-	-
<i>Macrophomina phaseolina</i> (Tassi) Goid.	-	-	-	1	0.04±0.0	1	0.04±0.0	-	-	-	-	-
<i>Metanospora</i> sp. Corda	4	1.52±4.03	4	2±7.14	-	-	-	1	0.16±0.0	-	-	-
<i>Monniliella echinata</i> (Riv.) Galloway	2	0.68±2.12	-	-	-	-	-	-	-	-	-	-
<i>M. subsimplex</i> (Cooke) Deighton	-	-	-	1	0.21±0.0	-	-	-	-	-	-	-
<i>Monosporium</i> sp. (Bonord)	-	-	-	-	-	-	-	1	0.26±0.0	-	-	-
<i>Momaria</i> sp. Cesati	-	-	-	-	-	1	0.11±0.0	-	-	-	-	-
<i>Mucor himelii</i> Wehmer	-	-	-	-	-	1	0.11±0.0	-	-	-	-	-
<i>M. racemosus</i> Fres.	-	-	-	-	-	-	-	1	0.11±0.0	-	-	-
<i>Mucor</i> sp. Mich. Ex.St. Am.	-	-	-	-	-	1	0.11±0.0	-	-	-	-	-
<i>Myrothecium chictum</i> (Corda) Sacc.	-	-	-	-	-	1	0.52±0.0	-	-	-	-	-
<i>M. verrucaria</i> (Alb. & Schw.) Ditm.ex St.	-	-	-	-	-	1	0.11±0.0	-	-	-	-	-
<i>Nectria iventia</i> Pethybor	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-
<i>Neocosmospora</i> sp. E.F. Sm.	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-
<i>Neosartorya fischeri</i> (Wehmer) Mallach & Cain.	-	-	-	-	-	1	0.21±0.0	-	-	-	-	-

<i>Nigrospora</i> sp. Zimmermanni	-	-	-	-	-	-	-	-	-	1	0.05±0.0	-	-	-
<i>P. chrysogenum</i> Thom.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium coprophilum</i> Dierckx	-	-	-	-	-	1	0.89±0.0	-	-	1	0.42±0.0	-	-	-
<i>P. frequentans</i> Westling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. griseofulvum</i> Dierckx	-	-	-	-	-	-	-	-	-	1	2.11±0.0	-	-	-
<i>Penicillium</i> spp. Link ex Fr.	-	-	-	-	-	4	1.26±3.9	-	-	-	-	-	-	-
<i>Phoma</i> <i>eupyrena</i> Sacc.	-	-	-	-	-	1	0.11±0.0	-	-	-	-	-	-	-
<i>Phoma</i> sp. Sacc.	-	-	-	-	-	2	0.11±0.0	-	-	1	1.16±0.0	-	-	-
<i>Rhizopus oryzae</i> Went & Prinsen Geerlings	-	-	-	-	-	2	0.42±4.24	-	-	2	0.16±0.71	-	-	-
<i>R. stolonifer</i> (Ehrenb. ex Link)	-	-	-	-	-	5	1.11±2.8	-	-	4	0.68±1.71	-	-	-
<i>Scopulariopsis brivicaulis</i> (Sacc.) Bain	-	-	-	-	-	-	-	-	-	1	0.11±0.0	-	-	-
<i>S. brunpiti</i> Salvanet. Duval	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-	-	-
<i>S. charterum</i> (G.Sm.) Morton & G.Sm.	-	-	-	-	-	3	0.89±5.51	-	-	-	-	-	-	-
<i>Stachybotrys atra</i> Corda	1	0.21±0.0	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. parvispora</i> Hughes.	1	0.58±0.0	-	-	-	-	-	-	-	-	-	-	-	-
<i>Stachybotrys</i> sp. Corda	1	0.26±0.0	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylotrichum coccosporum</i> T. Meyer & Nicot.	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-	-	-
<i>Stemphylium solani</i> Weber	-	-	-	-	-	-	-	-	-	1	0.16±0.0	-	-	-
<i>Sordaria finicola</i> (Rob.) Ces. & deNot.	1	0.05±0.0	-	-	-	-	-	-	-	-	-	-	-	-
<i>Thelavia terricola</i> (Gilman & Abbott) Emmons	1	0.11±0.0	2	0.21±0.0	-	-	-	-	-	-	-	-	-	-
<i>Trichocladium opacum</i> (Corda) Hughes	-	-	-	-	-	-	-	-	-	1	0.05±0.0	-	-	-
<i>Trichoderma hamatum</i> (Bonord) Bain	-	-	-	-	-	-	-	-	-	1	1.05±0.0	-	-	-
<i>T. harzianum</i> Rifai	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-	-	-
<i>T. koningii</i> Oudem	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-	-	-
<i>T. polysporum</i> (Link ex Pers.) Rifai	-	-	-	-	-	1	0.16±0.0	-	-	-	-	-	-	-
<i>Verticillium</i> sp. Nees ex Tenks	-	-	-	-	-	1	0.05±0.0	-	-	-	-	-	-	-
<i>Wardomyces humicola</i> Hennebert & Banon.	-	-	-	-	-	-	-	-	-	1	0.05±0.0	-	-	-

N.st = non-surface sterilized; St. = sterilized seeds; NSI = number of samples infected; S.D. = standard deviation; I%= infection percentage.

* = The work on seedborne fungi of papaya was carried out for the first time in Pakistan; all the fungi mentioned hereby were new reports from the country on papaya.

Standard blotter method: Untreated and seeds after treatment with 1% Ca (OCl)₂ for 2 minutes were placed aseptically on three layers of moistened blotter paper, 10 seeds per Petri dish. Modification was introduced by increasing the incubation time period. The dishes were incubated for 12 – 14 days at 25 - 35 °C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Agar plate method: Untreated and seeds after treatment with 1% Ca (OCl)₂ for 2 minutes were placed aseptically on Potato dextrose agar (PDA) poured, antibiotics added (penicillin, 20,000 units/L and streptomycin 1ml/L) petri dishes, 10 seeds per Petri dish. Modification was introduced by increasing the incubation time period. The dishes were incubated for 12 – 14 days at 25 - 35 °C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Deep-freezing method: Untreated and seeds after treatment with 1% Ca (OCl)₂ for 2 minutes were placed on three layers of moistened blotter paper, 10 seeds per Petri dish were incubated for 24h, each at 25- 35°C and -2°C followed by 12 days incubation (a modification was introduced by increasing the incubation time period) at 25 - 35°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Identification of fungi: Mycoflora observed on seeds were identified after reference to Barnett and Hunter (1998), Booth (1971), Domsch *et al.*, (1980), Ellis (1971), Gilman (1950), Hanlin (1989), Mycobank (2013), Nelson *et al.*, (1983), Raper and Fennell (1965), Raper and Thom (1949).

Analysis of data: Data was subjected to analysis of variance (ANOVA) following the procedures as suggested by Gomez and Gomez (1984).

RESULTS

At least 93 species belonging to 44 genera were isolated from the eighteen seed samples collected from various regions of Sindh, Pakistan by all three methods as recommended by ISTA. Highest number of fungi was isolated through agar plate method followed by standard blotter method. Deep-freezing method yielded the lowest; mainly because the seeds rotted when incubated at low temperature. 73 species belonging to 36 genera were isolated through agar plate method, whereas standard blotter method yielded 32 species of 14 genera. Deep-freezing method gave off only 3 fungal species of just one genus. Species of *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Scopulariopsis*, *Stachybotrys*, and *Trichoderma* were isolated by both standard blotter and agar plate methods. *A. flavus* was the most dominant fungi followed by *A. niger*, however the infection was non-significant. Blotter method was best for the isolation of all *Chaetomium* spp. and *Fusarium* spp. Pathogenic fungi like *Macrophomina phaseolina*, *Fusarium* and *Phoma* were responsible for minor percentages of infection. As no proper work has been done for the isolation of seed-borne fungi of papaya from Pakistan, all the fungi isolated in the current work are newly reported from the country (Table 1).

DISCUSSION

Of all the three methods used, agar plate method yielded the highest number of fungi followed by standard blotter and deep-freezing method. Agar plate method is considered to be the best for the isolation of fungi (Rahim *et al.*, 2013; Rahim *et al.*, 2010; Niaz and Dawar, 2009). However Limonard (1968) reported intrafungal antagonism becomes a problem in agar plate method. Similarly, Jovicevic (1980) suggested the use of filter paper method the best for the routine analysis of seed health. Species of *Fusarium* and *Chaetomium* were the best isolated through blotter method, mainly because both *Fusarium* and *Chaetomium* are cellulose decomposing fungi (Domsch *et al.*, 1980). Species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Myrothecium*, *Penicillium*, *Phoma*, *Scopulariopsis*, and *Trichoderma* favored growth on potato dextrose agar. Mathur and Neergaard (1970) found that agar plate method was suitable for the isolation of saprophytic fungi. Modification was introduced in the methods by increasing the time period of incubation. Germination of seeds was not observed during incubation because papaya seeds require more time for germination. Decay of seeds was observed after exposing the seeds to low temperature for deep-freezing method. Papaya seeds are sensitive to low temperature and the seeds are termed as intermediate, if not truly recalcitrant, besides that low moisture levels or desiccation induce dormancy (Baskin and Baskin, 2001), however Owino and Ouma (2011) classified papaya seeds as recalcitrant. Ellis *et al.*, 1991,

reported that low temperature results in loss of viability of papaya seeds. Zulhisyam *et al.*, (2013) reported the deterioration of seeds due to high moisture level. Surface sterilization of seeds with 1 % calcium hypochlorite has greatly reduced the fungal infection in all three methods. Wilson (1915) reported that any dilution of calcium hypochlorite is the best for the disinfection of seeds surface also the use of calcium hypochlorite can break dormancy of seeds. Generally, seeds of papaya are not consumed but are used for cultivation purposes.

Mycotoxins, secondary metabolites produced by fungi, are known to reduce the germination and quality of seeds (Agarwal and Sinclair, 1996), which means seeds heavily infected with fungi will not germinate also pathogenic or saprophytic fungi present in the seed can attack the emerging seedlings. Many countries have boosted their economy by properly cultivating papaya in farms and fields. Pakistan, being an agriculture country should pay special attention to this fruit, to help reduce the post-harvest and in field losses for boosting its economy.

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