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Screening of Seed-Borne Mycoflora of *Jatropha curcas* L.

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ABSTRACT

The aim of the present study was to check the deterioration of *Jatropha curcas* L. seeds during storage. Agar plate and standard blotter methods were used to study the seed-borne mycoflora of *Jatropha curcas* L. Both surface sterilized and unsterilized seeds were taken for isolation of fungi. Surface sterilization was done by 0.1% mercuric chloride ($HgCl_2$) solution. A significant contamination with fungal genera was detected in analyzed stored seeds. Sixteen fungal species with two strains of *Aspergillus flavus* i.e., brown and green were isolated from physic nut seeds during one year of storage. Fungi isolated and identified were *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Cephaliophora irregularis, Chaetomium globosum, Cladosporium cladosporioides, Curvularia lunata, Fusarium moniliforme, Fusarium roseum, Penicillium citrinum, Penicillium rubrum, Rhizopus stolonifer,* Dark sterile mycelium and White sterile mycelium. This is the first report of *Cephaliophora irregularis* on *Jatropha curcas* L. seeds. Agar plate method showed better results for isolation of *Chaetomium globosum, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium moniliforme*.

Key words: Physic nut, deterioration, bio-diesel, agar plate method, blotter method

INTRODUCTION

The oil plant *Jatropha curcas* L., a multipurpose drought resistance, perennial plant belonging to Euphorbiaceae family is gaining a lot of importance for the production of bio-diesel. The seeds of physic nut are a good source of oil which can be used as a diesel substitute. "Bio-diesel" is well known chemically as the mono-alkyl esters of long-chain fatty acids. It is produced from several types of conventional and non-conventional vegetable oils and animal fats including those of used oils from the frying industry, soyabean oil, rapeseed oil, rubber seed oil and palm oil (Tomasevic and Siler-Marinkovic, 2003; Shah *et al*., 2004; Ramadhas *et al*., 2005; Ahmad *et al*., 2007). In the situation of rapidly growing energy requirements, the contribution of new and especially some non-food oils have to play a significant role (Chitra *et al*., 2005; Rashid and Anwar, 2008; Chakrabarti and Ahmad, 2008; Harun and Ahmed, 2009). Augustus *et al*. (2002) have reported that *Jatropha curcas* seeds contain 20-40% oil. The use of biomass to provide energy has been fundamental to the development of civilization. Biomass contributes a significant share of global primary energy consumption and its importance is likely to increase in future world energy scenarios (Vasudevan *et al*., 2005). Most of the research and technology has been utilized in stepping up crop production in agriculture but all efforts to produce more will not be of much avail until similar of agricultural products.

Seeds are regarded as highly effective means for transporting plant pathogens over long distances (Agarwal and Sinclair, 1996). Seed-borne mycoflora have been found to affect the growth and productivity of crop plants. A seed-borne pathogen present externally or internally or associated with the seed as contaminant, may cause abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage. This resulting in development of diseases at later stages of plant growth by systemic or local infection (Bateman and Kwasna, 1999; Khanzada *et al*., 2002).

Jatropha seeds are constantly subjected to deterioration which implies an irreversible degenerative change in the quality of seeds after it has reached its maximum quality of seeds after it has reached its maximum quality level (Worang *et al*., 2008; Dharmaputra *et al*., 2009). The fungi associated with seeds at the harvest stage and under storage bring about several undesirable changes and degradation of seed constituents, thus making the seed unfit for oil extraction, export purpose, consumption or sowing. Chelkowski (1991) reported that in many cases, fungi infecting seeds are seed-borne pathogens. They play an important role in the transmission of numerous pathogenic fungal species to seedlings as well as to the soil.

The objective of this study was to analyze the effect of duration of seed storage on fungal population of physic nut seeds.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi from October, 2008 to September, 2009. Seed samples of *Jatropha curcas* L. was collected from Varanasi district were used for the isolation and identification of seed-borne fungi. Agar plate method (Muskett, 1948) and Blotter method (De Tempe, 1953) recommended by International Seed Testing Association (Anonymous, 1966) were used for the isolation of fungi. An agar plate is a Petri dish that contains a growth medium (typically agar plus nutrients) used to culture microorganisms. The blotter method is one of the incubation methods where the seeds are plated on well water soaked filter papers and incubated usually for 7 days at 25±2°C under 12 hours alternating cycles of light and darkness. After incubation, fungi developed on each seed are examined under different magnifications of a stereomicroscope and identified. Both surface sterilized and unsterilized seeds were taken for isolation of fungi. Surface sterilization was done by 0.1% mercuric chloride ($HgCl_2$) solution (Ramakrishna *et al*., 1991).

Agar plate technique: The seeds were externally sterilized by 0.1% mercuric chloride solution to 1 to 2 min then washed by sterilized distilled water (Habib *et al*., 2007). The isolated fungi were identified using light microscope after slides were stained lactophenol (Anonymous, 1994; Henselov and Hudecov, 2001; Gwary *et al*., 2006).

Blotter test: The blotter test (Limonard, 1966; Lantos *et al*., 2002) was used to isolate the fungal pathogens associated with the seeds during storage.

Identification of fungi: After incubation the growth characters as well as percentage of infection were recorded. In order to isolate these fungi into pure culture, Potato Dextrose Agar (PDA) was prepared and the fungi were inoculated onto the sterile PDA and incubated for 7 days at the end of which the fungi were identified based in their colour, spore morphology and mycelia growth using the light microscope (Begum *et al*., 2004; Chuku *et al*., 2007; Al-Sheikh, 2009). The fungi

were identified by using the reference of Thom and Raper (1945), Raper and Thom (1949), Booth (1971), Ellis (1971) and Barnett and Hunter (1972).

Statistical analysis: Mean value with standard error was calculated to check the variation of isolated seed mycoflora from seeds and kernels of *Jatropha curcas* L. by agar plate method and blotter method under sterilized and unsterilized conditions during one year of storage. The term 'Standard Error' of any estimate is used for a measure of the average magnitude of the difference between the sample estimate and the population parameter taken over all possible samples of the same size, from the population (Chandel, 2002).

RESULTS

From stored seeds of *Jatropha curcas* L., 11 genus and 16 species of fungi viz., *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium roseum*, *Penicillium citrinum*, *Penicillium rubrum*, *Rhizopus stolonifer*, Dark sterile mycelium and White sterile mycelium were isolated and identified (Table 1-8).

Table 1 representing seed mycoflora isolated from sterilized *Jatropha* seeds by Agar plate method. In which *Aspergillus niger* and *Aspergillus flavus* (Green) were found dominant. *Alternaria alternata, Fusarium roseum* and *Penicillium rubrum* were found common. *Aspergillus flavus* (Brown), *Aspergillus fumigatus*, *Aspergillus terreus*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium moniliforme*, *Penicillium citrinum*, *Rhizopus stolonifer*, Dark sterile mycelium and white sterile mycelium were found rare.

Table 2 representing seed mycoflora isolated from sterilized *Jatropha* kernels by Agar plate method. In which *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium roseum*

Table 1: Seed mycoflora isolated from sterilized *Jatropha* seeds by agar plate method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
Alternaria alternata	-	-	+	+	-	-	+	+	-	-	+	+
Aspergillus flavus (Brown)	-	-	-	-	-	-	-	+	+	-	+	-
#*Aspergillus flavus* (Green)	+	+	+	-	-	+	+	-	+	-	+	+
Aspergillus fumigatus	-	+	-	+	+	-	+	-	+	-	-	-
#*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus terreus	-	-	-	-	-	+	+	-	+	-	-	+
Cephaliophora irregularis	+	+	+	+	-	-	-	-	-	-	-	-
Chaetomium globosum	+	+	-	+	-	-	-	+	-	-	-	-
Cladosporium cladosporioides	-	+	+	+	-	+	-	-	-	-	-	-
Curvularia lunata	-	+	+	-	+	+	-	-	-	-	-	+
Fusarium moniliforme	-	-	-	-	-	-	-	-	+	-	-	+
Fusarium roseum	+	+	-	+	-	+	+	-	-	+	-	+
Penicillium citrinum	-	-	+	+	-	+	+	-	-	+	-	-
Penicillium rubrum	-	-	-	+	+	-	-	+	+	+	-	+
Rhizopus stolonifer	-	-	-	-	-	+	-	+	+	-	+	-
*Dark sterile mycelium	+	-	-	+	-	-	-	-	+	-	+	+
*White sterile mycelium	-	-	-	+	-	+	-	+	+	-	-	+

+: Present, -: Absent, *: No recognition as fungal species, #: Dominant fungal species and other are rare

Table 2: Seed mycoflora isolated from sterilized *Jatropha* kernels by agar plate method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
#*Alternaria alternata*	-	+	+	+	-	+	+	-	-	+	+	+
Aspergillus flavus (Brown)	+	+	+	-	-	-	-	+	+	-	+	-
Aspergillus flavus (Green)	-	+	+	-	-	+	+	+	+	-	+	-
#*Aspergillus fumigatus*	+	+	+	+	+	+	-	-	+	+	-	+
#*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	-	+
Aspergillus terreus	-	-	+	+	+	-	-	+	-	+	-	+
Cephaliophora irregularis	+	+	+	+	+	-	-	-	-	-	-	-
Chaetomium globosum	+	-	+	-	-	-	-	-	-	-	-	-
Cladosporium cladosporioides	-	-	+	+	+	-	-	-	-	+	-	-
Curvularia lunata	-	+	+	-	-	+	-	-	-	-	-	+
Fusarium moniliforme	-	-	-	-	+	-	-	-	+	-	-	-
#*Fusarium roseum*	+	+	+	+	+	+	-	-	+	-	-	+
Penicillium citrinum	-	-	+	-	+	-	+	-	-	-	-	+
Penicillium rubrum	-	-	+	+	+	-	+	-	+	+	-	-
Rhizopus stolonifer	-	-	-	-	-	-	-	+	+	-	+	-
*Dark sterile mycelium	-	+	+	-	-	-	+	-	+	-	+	-
*White sterile mycelium	-	-	-	-	-	-	+	+	-	-	-	-

+: Present , -: Absent, *: No recognition as fungal species, #: Dominate fungal species and other are rare

were found dominant. *Aspergillus flavus* (Brown), *Aspergillus flavus* (Green), *Aspergillus terreus*, *Penicillium rubrum* were found common. *Cephaliophora irregularis*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium moniliforme*, *Penicillium citrinum*, *Rhizopus stolonifer*, Dark sterile mycelium and white sterile mycelium were considered to be rare mycoflora.

Table 3 representing seed mycoflora isolated from sterilized *Jatropha* seeds by Blotter method. In which *Aspergillus niger*, *Fusarium roseum* and *Rhizopus stolonifer*, were found dominant. *Alternaria alternata*, *Aspergillus flavus* (Green and Brown), *Penicillium rubrum* and white sterile mycelium were found common. *Aspergillus fumigatus*, *Aspergillus terreus*, *Cephaliophora irregularis*, *Penicillium citrinum* and Dark sterile mycelium were rare. Where as *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium moniliforme* were absent.

Table 4 representing seed mycoflora isolated from sterilized *Jatropha* kernels by Blotter method. In which *Aspergillus flavus* (Green and Brown), *Aspergillus niger* were found dominant. *Alternaria alternata*, *Aspergillus fumigatus*, *Fusarium roseum*, *Penicillium rubrum*, *Rhizopus stolonifer* and white sterile mycelium were found common and *Aspergillus terreus*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Curvularia lunata*, *Penicillium citrinum* and Dark sterile mycelium were found rare while *Cladosporium cladosporioides and Fusarium moniliforme* were absent.

Table 5 representing seed mycoflora isolated from unsterilized *Jatropha* seeds by Agar plate method. In which *Alternaria alternata*, *Aspergillus flavus* (Green and Brown), *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium rubrum and Rhizopus stolonifer* were found dominant. *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium roseum*, *Penicillium citrinum* and Dark sterile mycelium were found common and *Aspergillus terreus*, *Chaetomium globosum*, *Fusarium moniliforme and* white sterile mycelium were found rare.

Table 6 representing seed mycoflora isolated from unsterilized *Jatropha* kernels by Agar plate method. In which *Alternaria alternata*, *Aspergillus flavus* (Green), *Aspergillus fumigatus*, *Aspergillus niger and Rhizopus stolonifer* were found dominant. *Aspergillus flavus* (Brown),

Table 3: Seed Mycoflora isolated from sterilized *Jatropha* seeds by blotter method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
Alternaria alternata	-	+	+	-	-	+	+	-	+	+	-	-
Aspergillus flavus (Brown)	+	+	+	+	-	-	-	-	+	+	-	+
Aspergillus flavus (Green)	-	-	+	+	+	+	+	-	-	+	-	+
Aspergillus fumigatus	-	+	+	-	-	+	-	-	+	-	-	-
#*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus terreus	-	-	-	-	-	-	+	-	-	-	-	+
Cephaliophora irregularis	+	-	-	-	-	+	-	-	+	-	-	-
Chaetomium globosum	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium moniliforme	-	-	-	-	-	-	-	-	-	-	-	-
#*Fusarium roseum*	+	+	+	-	+	+	-	-	+	+	-	+
Penicillium citrinum	-	-	+	-	+	-	+	-	-	-	-	+
Penicillium rubrum	+	-	-	+	+	-	+	+	-	+	+	-
#*Rhizopus stolonifer*	+	+	+	-	-	+	-	+	+	+	+	+
*Dark sterile mycelium	-	+	+	-	-	+	-	+	-	-	+	-
*White sterile mycelium	-	+	-	+	+	-	+	+	+	+	-	-

+: Present, -: Absent, *: No recognition as fungal species, #: Dominant fungal species and others are rare

Table 4: Seed Mycoflora isolated from sterilized *Jatropha* kernels by blotter method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
Alternaria alternata	+	-	+	+	-	-	+	+	-	+	-	-
#*Aspergillus flavus* (Brown)	+	+	+	+	+	+	-	-	+	+	-	+
#*Aspergillus flavus* (Green)	-	+	+	+	+	+	+	+	+	-	-	+
Aspergillus fumigatus	-	-	+	+	-	-	+	+	+	-	+	-
#*Aspergillus niger*	+	+	+	+	-	+	+	+	+	+	-	+
Aspergillus terreus	-	+	+	-	-	-	-	-	-	-	-	-
Cephaliophora irregularis	+	-	+	-	-	-	-	-	-	-	-	-
Chaetomium globosum	-	-	-	-	-	+	+	-	-	-	-	-
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	+	-	+	-	-	-	-	-	-	-	-
Fusarium moniliforme	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium roseum	-	+	+	+	-	+	+	+	-	+	-	-
Penicillium citrinum	-	-	-	+	+	+	-	-	-	-	-	-
Penicillium rubrum	+	-	-	+	+	+	+	-	+	+	-	-
Rhizopus stolonifer	-	+	+	+	-	-	+	-	+	+	+	-
*Dark sterile mycelium	-	+	-	-	-	+	-	-	-	-	+	-
*White sterile mycelium	-	-	+	+	-	+	+	+	+	-	-	-

+: Present, -: Absent, *: No recognition as fungal species, #: Dominant fungal species and others are rare

Aspergillus terreus, *Cladosporium cladosporioides*, *Fusarium roseum*, *Penicillium citrinum*, *Penicillium rubrum* and *Dark sterile mycelium were found common. While *Cephaliophora irregularis*, *Chaetomium globosum*, *Curvularia lunata*, *Fusarium moniliforme* and white sterile mycelium were found rare.

Table 7 representing seed mycoflora isolated from unsterilized *Jatropha* seeds by Blotter method. In which *Alternaria alternata*, *Aspergillus flavus* (Green and Brown), *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium rubrum* and *Rhizopus stolonifer* were found dominant.

Table 5: Seed Mycoflora isolated from unsterilized *Jatropha* seeds by agar plate method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
#*Alternaria alternata*	-	-	+	+	+	+	+	+	-	+	+	+
#*Aspergillus flavus* (Brown)	-	+	+	+	+	+	-	+	+	-	+	-
#*Aspergillus flavus* (Green)	+	+	+	-	-	+	+	-	+	-	+	+
Aspergillus fumigatus	-	+	-	+	+	+	+	-	+	-	+	+
#*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus terreus	-	-	-	-	-	+	+	-	+	-	-	+
Cephaliophora irregularis	+	+	-	+	-	-	-	-	-	-	-	-
Chaetomium globosum	+	+	-	+	-	-	-	+	-	+	-	-
Cladosporium cladosporioides	-	+	+	+	-	+	-	+	-	-	+	-
Curvularia lunata	-	+	+	-	+	+	-	+	-	-	-	+
Fusarium moniliforme	-	-	+	-	-	+	-	-	+	-	-	+
Fusarium roseum	+	+	-	+	-	+	-	-	-	+	-	+
Penicillium citrinum	-	-	+	+	-	+	+	-	+	+	+	-
#*Penicillium rubrum*	-	+	+	+	+	-	-	+	+	+	-	+
#*Rhizopus stolonifer*	-	-	+	+	+	+	-	+	+	+	+	-
*Dark sterile mycelium	-	+	+	-	-	+	-	-	+	-	+	+
*White sterile mycelium	-	-	-	+	-	+	-	+	+	-	-	+

+: Present, -: Absent, *: No recognition as fungal species, #: Dominant fungal species and others are rare

Table 6: Seed Mycoflora isolated from unsterilized *Jatropha* kernels by agar plate method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
#*Alternaria alternata*	-	+	+	+	-	+	-	-	+	+	+	+
Aspergillus flavus (Brown)	+	+	+	-	-	-	-	+	+	-	+	-
#*Aspergillus flavus* (Green)	-	+	+	-	+	+	+	+	+	-	+	-
#*Aspergillus fumigatus*	+	+	+	+	+	+	-	-	+	+	+	+
#*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	-	+
Aspergillus terreus	-	-	+	+	+	-	-	+	-	+	-	+
Cephaliophora irregularis	+	+	-	+	+	-	-	+	-	-	-	-
Chaetomium globosum	+	-	+	-	-	-	-	+	-	-	-	-
Cladosporium cladosporioides	-	-	+	+	+	-	+	-	-	+	-	+
Curvularia lunata	-	+	+	-	-	+	-	+	-	-	-	+
Fusarium moniliforme	-	-	-	-	+	-	-	+	+	-	-	
Fusarium roseum	+	+	-	-	+	+	-	-	+	-	-	+
Penicillium citrinum	-	-	+	-	+	-	-	-	+	+	+	+
Penicillium rubrum	-	-	+	+	+	-	+	-	+	+	-	-
#*Rhizopus stolonifer*	+	+	-	+	-	+	+	+	+	-	+	-
*Dark sterile mycelium	-	-	+	-	+	+	+	-	-	+	+	-
*White sterile mycelium	+	-	-	-	-	-	+	+	-	-	+	-

+: Present, -: Absent, *: No recognition as fungal species, #: Dominant fungal species and others are rare

Only *Penicillium citrinum* were found common and *Aspergillus terreus, Cephaliophora irregularis, Chaetomium globosum, Curvularia lunata, Fusarium moniliforme, Fusarium roseum* and Dark sterile mycelium were found rare. While *Cladosporium cladosporioides* found completely absent.

Table 8 representing seed mycoflora isolated from unsterilized *Jatropha* kernels by Blotter method. In which *Alternaria alternata, Aspergillus flavus* (Green and Brown), *Aspergillus fumigatus, Aspergillus niger* and *Rhizopus stolonifer* were found dominant. *Penicillium rubrum*

Table 7: Seed Mycoflora isolated from unsterilized *Jatropha* seeds by blotter method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
#*Alternaria alternate*	-	+	+	-	+	+	+	-	+	+	+	-
#*Aspergillus flavus* (Brown)	+	+	+	+	+	-	-	+	+	+	-	+
#*Aspergillus flavus* (Green)	-	+	+	+	+	+	+	-	-	+	+	+
#*Aspergillus fumigatus*	+	+	+	-	-	+	-	+	+	+	-	+
#*Aspergillus niger*	+		+	+	+	+	+	-	+	+	+	+
Aspergillus terreus	-	-	-	-	-	-	+	-	-	-	-	+
Cephaliophora irregularis	+	-	-	-	-	+	-	-	+	-	-	-
Chaetomium globosum	-	-	-	-	+	+	-	-	-	-	-	-
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	+	-	+	-	-	-	+	+	-	-	-
Fusarium moniliforme	-	-	-	-	+	-	-	-	-	-	-	-
Fusarium roseum	+	-	-	-	+	+	-	-	+	-	-	+
Penicillium citrinum	-	-	+	-	+	+	+	-	-	+	-	+
Penicillium rubrum	+	-	-	+	+	+	+	+	-	+	+	-
Rhizopus stolonifer	+	+	+	-	-	+	-	+	+	+	+	+
*Dark sterile mycelium	-	-	+	-	-	+	-	-	-	+	+	-
*White sterile mycelium	-	+	-	-	+	-	+	-	+	+	-	-

+: Present, -: Absent, *: No recognition as fungal species, #: Dominant fungal species and other are rare

Table 8: Seed Mycoflora isolated from unsterilized *Jatropha* kernels by blotter method

Mycoflora	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar. 09	Apr. 09	May. 09	Jun. 09	Jul. 09	Aug. 09	Sep. 09
#*Alternaria alternata*	+	-	+	+	-	+	+	+	-	+	-	+
#*Aspergillus flavus* (Brown)	+	+	+	+	+	+	-	+	+	+	-	+
#*Aspergillus flavus* (Green)	-	+	+	+	+	-	+	+	+	-	+	+
#*Aspergillus fumigatus*	-	+	+	+	-	-	+	+	+	-	+	+
#*Aspergillus niger*	+	+	+	+	-	+	+	-	+	+	-	+
Aspergillus terreus	-	+	+	-	-	-	-	-	-	-	-	-
Cephaliophora irregularis	+	-	+	-	-	-	-	-	-	-	-	-
Chaetomium globosum	-	-	-	-	-	+	-	-	-	-	-	-
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	+	-	-	-
Curvularia lunata	-	+	-	+	-	-	-	-	+	-	-	-
Fusarium moniliforme	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium roseum	-	+	+	-	-	+	-	+	-	+	-	-
Penicillium citrinum	-	-	-	+	+	+	-	-	+	-	-	+
Penicillium rubrum	+	+	-	+	+	-	+	-	+	+	-	-
#*Rhizopus stolonifer*	-	+	+	+	+	-	+	-	+	+	+	+
*Dark sterile mycelium	-	-	-	-	-	-	-	-	-	-	-	-
*White sterile mycelium	-	-	+	+	-	+	+	+	+	-	-	-

+: Present, -: Absent, *: No recognition as fungal species, #: Dominent fungal species and others are rare

and white sterile mycelium were found common. *Aspergillus terreus*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium roseum* and *Penicillium citrinum* were rare. While *Fusarium moniliforme* and Dark sterile mycelium were completely absent.

Data presented in Table 1 to 8 reveals that of the fungi isolated *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium roseum* and *Penicillium rubrum* were found to be dominant. There does not appear to be any previous report of

Cephaliophora irregularis on *Jatropha curcas* L. seeds. At the beginning the some fungi isolated were field fungi i.e., *Cladosporium cladosporioides*, *Fusarium moniliforme* and *Fusarium roseum*. Their population decreased with the increase of storage duration and then storage fungi replaced them. Agar plate method showed better results for isolation of *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Fusarium moniliforme* than blotter method. It was also observed that sterilized seeds yielded less population of seed-borne fungi than the unsterilized seeds indicating partial elimination of some contaminating fungi.

Data presented in Table 9 reveals that the seeds of *Jatropha curcas* L. were mainly deteriorated by Mitosporic fungi of class-Hyphomycetes (75.00%).

Data presented in Table 10 reveals that annual percent occurrence of species of *Aspergillus* was found to be maximum i.e., from 25% to 100% followed by *Alternaria alternata* (50-75%), *Fusarium roseum* (41.67-66.67%) and *Penicillium citrinum* (25-58.33%).

Table 9: Class wise occurrence of fungi and percent occurrence of various classes colonizing the *Jatropha* seeds during storage

Class of fungi	No. of Species Isolated	Occurrence (%)
Zygomycotina		
Zygomycetes (Mucorales-Mucoraceae)	1	6.25
Ascomycotina		
Pyrenomycetes (Sphaeriales-Chaetomiaceae)	1	6.25
Deuteromycotina		
Hyphomycetes (Moniliales-Moniliaceae, Dematiaceae, Tuberculariaceae)	12	75.00
Unidentified	2	12.50
Total number of fungi isolated = 16		

Table 10: Percent occurrence (Annual) of seed Mycoflora of sterilized and unsterilized *Jatropha* seeds by using agar plate and blotter method

	Agar plate method				Blotter method			
	Sterilized		Unsterilized		Sterilized		Uusterilized	
Isolated seed Mycoflora	Seeds	Seed kernels	Seeds	Seed kernels	Seeds	Seed kernels	Seeds	Seed kernels
Alternaria alternata	50.00	66.67	75.00	66.67	50.00	50.00	66.67	66.67
Aspergillus flavus (Brown)	25.00	50.00	66.67	50.00	58.33	75.00	66.67	83.33
Aspergillus flavus (Green)	66.67	58.33	66.67	66.67	58.33	75.00	75.00	75.00
Aspergillus fumigatus	41.67	75.00	66.67	83.33	33.33	50.00	66.67	66.67
Aspergillus niger	100.00	91.67	100.00	91.67	100.00	83.33	83.33	75.00
Aspergillus terreus	33.33	50.00	33.33	50.00	16.67	16.67	16.67	16.67
Cephaliophora irregularis	33.33	41.67	25.00	41.67	25.00	16.67	25.00	16.67
Chaetomium globosum	33.33	16.67	41.67	25.00	0.00	16.67	16.67	8.33
Cladosporium cladosporioides	33.33	33.33	50.00	50.00	0.00	0.00	0.00	8.33
Curvularia lunata	41.67	33.33	50.00	41.67	0.00	16.67	33.33	25.00
Fusarium moniliforme	16.67	16.67	33.33	25.00	0.00	0.00	8.33	0.00
Fusarium roseum	58.33	66.67	50.00	50.00	66.67	58.33	41.67	41.67
Penicillium citrinum	41.67	33.33	58.33	50.00	33.33	25.00	50.00	41.67
Penicillium rubrum	50.00	50.00	66.67	50.00	58.33	58.33	66.67	58.33
Rhizopus species	33.33	25.00	66.67	66.67	75.00	58.33	75.00	75.00
*Dark sterile mycelium	41.67	41.67	50.00	50.00	41.67	25.00	33.33	16.67
*White sterile mycelium	41.67	16.67	41.67	33.33	58.33	58.33	41.67	50.00

*: No recognition as fungal species

Table 11: Percentage frequency# of fungi in agar plate method and blotter method

	Sterilized		Unsterilized	
Isolated seed Mycoflora	Seeds	Seed kernels	Seeds	Seed kernels
Alternaria alternata	50.00±0.00	58.34±8.34	70.84±4.16	66.67±0.00
Aspergillus flavus (Brown)	41.67±13.18	62.50±12.50	66.67±0.00	66.67±16.67
Aspergillus flavus (Green)	62.50±4.17	66.67±8.34	70.84±4.16	70.84±4.16
Aspergillus fumigatus	37.50±4.17	62.50±12.50	66.67±0.00	75.00±8.33
Aspergillus niger	100.00±0.00	87.50±4.17	91.67±8.34	83.34±8.34
Aspergillus terreus	25.00±8.33	33.34±16.67	25.00±8.33	33.34±16.67
Cephaliophora irregularis	29.17±4.17	29.17±12.50	25.00±0.00	29.17±12.50
Chaetomium globosum	16.67±16.67	16.67±0.00	29.17±12.5	16.67±8.34
Cladosporium cladosporioides	16.67±16.67	16.67±16.67	25.00±25.00	29.17±20.84
Curvularia lunata	20.84±20.84	25.00±8.33	41.67±8.34	33.34±8.34
Fusarium moniliforme	8.34±8.34	8.34±8.34	20.83±12.5	12.5±12.50
Fusarium roseum	62.50±4.17	62.50±4.17	45.84±4.17	45.84±4.17
Penicillium citrinum	37.50±4.17	29.17±4.17	54.17±4.17	45.84±4.17
Penicillium rubrum	54.17±4.17	54.17±4.17	66.67±0.00	54.17±4.17
Rhizopus stolonifer	54.17±20.84	41.67±16.67	70.84±4.16	70.84±4.16
*Dark sterile mycelium	41.67±0.00	33.34±8.34	41.67±8.34	33.34±16.67
*White sterile mycelium	50.00±8.33	37.50±20.83	41.67±0.00	41.67±8.34

*: No recognition as fungal species, Value are calculated as Mean±SE

Data presented in Table 11 reveals that the occurrence of all fungi which were frequently encountered, is recorded in terms of mean value with standard error. The maximum variation was showed by *Cladosporium cladosporiodes* i.e., 25.00 isolated from unsterilized *Jatropha* seeds followed by the same fungus isolated from unsterilized *Jatropha* kernels by *Curvularia lunata* and *Rhizopus stolonifer* isolated from sterilized *Jatropha* seeds and by white sterile mycelium isolated from sterilized *Jatropha* kernels by showing standard error 20.84. *Fusarium roseum* and *Penicillium citrinum* shown constant value of standard error i.e., 4.17 under both sterilized and unsterilized condition of *Jatropha* seeds and kernels. Less variation were observed in *Alternaria alternata*, *Aspergillus flavus* (brown and green), *Aspergillus fumigatus*, *Aspergillus niger*, *Cephaliophora irregularis*, *Chaetomium globosum*, *Penicillium rubrum*, Dark sterile mycelium and White sterile mycelium which showed the dominance of these fungal species.

DISCUSSION

The fungi isolated from stored *Jatropha* seeds were the main cause of deterioration of seeds during storage (Worang *et al*., 2008; Dharmaputra *et al*., 2009). The sterilized seeds show less population of seed-borne fungi than the unsterilized seeds are in close conformity with those of Limnord (1968) who reported that chloral disinfection effectively reduced the microbial contamination. Surface sterilization also has the advantage of minimizing competition among fungi on the seed (Kaur, 2010). Seed surface disinfection with $HgCl_2$ usually suppresses the growth of saprophytic and other superficial fast growing fungi Limnord (1968); Bhutta (1988). It was also observed by Ramakrishna *et al*. (1991) that surface sterilization with 0.1 or 0.2% (w/v) $HgCl_2$ for 3 min significantly decreased *Alternaria alternata*, *Fusarium* sp. and *Epicoccum purpurascens* but Niaz and Dawar (2009) observed that surface disinfection of seed with 1% $Na(OCl)_2$ reduced the incidence of *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Rhizopus* spp.,

Cephalosporium spp. Reduction of frequency rate of fungi from sterilized sunflower seeds was also found by Sharfun-Nahar *et al.* (2005) and Bhutta *et al.* (1998). Of the two methods used, the agar plate method yielded the highest number of fungi as compared to blotter method. Saprophytic fungi like *Aspergillus* species, *Cladosporium* species, *Curvularia* species, *Rhizopus* species, Trichoderma species were isolated in higher percentage by agar plate method. The agar plate method was found most suitable for the isolation of saprophytic fungi (Niaz and Dawar, 2009). It was also proved by Mathur and Neergaard (1970) and Khan *et al.* (1988) that the use of agar plate was preferred over the blotter method for the isolation of mycoflora but Gowdar *et al.* (2007) observed that standard blotter method was better for isolation of large number of fungal species. The blotter method was more effective for the isolation of *Alternaria alternata*, *Mucor* sp., *Chaetomium* sp. and *Stemphylium* sp. (Elwakil and El-Metwally, 2001). At the beginning of storage, some of the fungi that infected the seeds were classified as field fungi and their population decreased with the increase of storage duration (Worang *et al.*, 2008; Dharmaputra *et al.*, 2009). Consequently, fungi infecting physic nut seeds grew and developed better, especially the fungi which have abundant spores such as *Aspergillus* and *Penicillium* (Dharmaputra *et al.*, 2009).

CONCLUSION

From the present study, it was concluded that *Jatropha* seeds are constantly subjected to deterioration which implies an irreversible degenerative change in the quality of seeds.

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