Moor Journal of Agricultural Research 18, 44 - 50, 2017

Mycoflora Associated with Kenaf (*Hibiscus cannabinus*) Seed

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Abstract
Identification of fungal pathogens helps in proferring the most suitable control measures for the organisms. This study was conducted to determine the fungal pathogens associated with kenaf seeds. Standard blotter and agar plate methods were used to study seed-borne mycoflora of ten varieties of kenaf sourced from the Seed Unit of the Institute of Agricultural Research and Training, Ibadan and National Centre for Genetic resources and Biotechnology, Ibadan. The colony features and conidia characteristics were studied. Significant fungal species were detected in the incubated samples. Fungi isolated and identified were *Botrytis cinerea, Colletorichum lindemuthianum, Cladosporium sp, Chaetomium sp, Rhizopus stolonifer, Fusariu moxysporium, Fusarium spp, Aspergillus niger, Aspergillus flavus, Penicillium sp, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Trichothecium roseum.* The distribution of each fungi across the varieties showed that *Trichothecium roseum* and *A. niger* had the highest ocuurence of 60% followed by *C. gloeosporoides*, *L. theobromea* and *Penicillium* sp (50%). The occurrence of fungi was more predominant on variety Ifeken100, while Tianung 1 showed the lowest occurrence of isolated fungi.

Key words: *Hibiscus cannabinus*, fungi, varieties

INTRODUCTION

Kenaf (*Hibiscus cannabinus*) is a warm season annual fibre crop closely related to cotton (*Gossypium hirsutum*) and okra (*Abelmoschus esculentus*) that can be successfully produced in large portions in Africa, particularly in Nigeria. *Hibiscus cannabinus* is in the genus *Hibiscus* and is probably native to Southern Asia. Its exact natural origin is unknown but is reported to have originated from Africa and is cultivated in several parts of the world (Le Mahieu *et al.,* 2003). Kenaf is one of the three largest fibre crops of economic importance (Keshk *et al.,* 2006). It is commonly cultivated for both food and fibre in West Africa (Adegbite *et al.,* 2005). Kenaf is one of the allied fibres of jute and shows similar characteristics as the commercial use of kenaf continues to diversify from its historical role as a cordage crop (rope, twine, and sackcloth) to its various new applications including paper products, building materials, absorbents and livestock feed (Dempsey, 1991). Commercially, kenaf is cultivated purposely for pulping and paper making, oil spill bioremediation, livestock nutrition and biodegradable packaging materials (Cheng, 2001; Adeniyan *et al.,* 2014). A well processed seed meal from kenaf is a potential feed ingredient for use in formulating feed for livestock especially monogastric livestock (Keshk *et al.,* 2006). The seed contains about 20% by volume of edible oil and this oil compares favourably with that obtained from cotton seeds.

Seed-borne pathogens play an important role in the transmission of numerous pathogenic fungal species to

seedlings as well as to the soil. Several fungi are found on market seeds and stored seeds. These are known to cause considerable damage either directly to the seed or to the crops raised from such contaminated seed stocks. 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 (Seweta *et al*. 2011). The study of seed borne pathogens is necessary to determine the seed health and to profer solution in the control of the seed-borne pathogens. This study is therefore aimed at identifying the mycoflora associated with kenaf seeds.

Materials and Methods

Collection of seed samples

Kenaf seeds were obtained from the Seed Unit of Institute of Agricultural Research and Training (IAR&T), Moor plantation, Ibadan and National Centre for Genetic Resources and Biotechnology (NACGRAB). Ten (10) varieties were used for this research work. The different varieties used were; V1 – Tiannug – 2, V2- V1 400, V3- Tiannug , V4 - Ifeken D1 400, V5 - Ifeken -100, V6 - HIB 236, V7 - HIB 24B, V8 - HIB14B, V9 - HIB43B, V10 - HIB31B.

Isolation of seed mycoflora

Mycoflora were isolated from the seed samples by methods recommended by International Seed Testing Association (1996). The kenaf seeds were surface sterilised by soaking the seeds in 1% sodium hypochlorite, gently swirled and then rinsed in 5 changes of sterile distilled water. Each seed sample was dried on sterile blotter paper. Two different methods of isolation techniques for assessment of seed mycoflora were used namely standard blotter method and agar plate method.

Standard blotter method: A pair of sterile white blotter papers of 8.5 cm diameter was place in 9 mm petri dish and moistened with sterile distilled water. Ten seeds of each kenaf varieties were placed at equal distance on the moist blotter. Each variety was replicated 3 times. The plates were incubated for a period of 7 days at room temperature (25°C ± 2°C). Each plate was observed under a stereo microscope for the mycoflora incidence on each variety.

Agar plate method: Potato Dextrose Agar (PDA) supplemented with streptomycin (100mg/l) was poured into 9 ml petri dishes and allowed to cool. Ten surface sterilised seeds were plated on the PDA. Incubation and other details of the study were same as described for blotter test method.

Cultural and morphological identification of the mycoflora:

Detailed observation of fungal characters, their typical colour, colony pattern and conidia structures with the aid of the microscope and their identification was confirmed with standard literature Watanabe (2002) and Dugan (2008),Barnett and Hunter (2010).

Data collection and Statistical analysis

Cultural and morphological characteristics of the isolates were described. The mean and percent occurrence of each isolate were calculated.

Results and Discussion

Fifteen (15) fungi were isolated from the seeds. Two were not fully identified while thirteen of the fungi isolated were identified as follows: *Botrytis cinerea* Per.; Fr,

Colletorichumlin demuthianum (Sacc & Magnus) Briosi and Cavara, *Cladosporium cladosporoides* (Fresen) G. A. de Vries, *Chaetomiumglobosum* Kunze, *Rhizopusstolonifer* (Ehrenberg) Vuillemin, *Fusarium oxysporum* Schlecht, *Fusariumspp, Aspergillusniger* Van Tieghem, *Aspergillus flavus* Link, *Penicillium sp, Colletotrichumgloeo sporioides* (Penz.) Penz.& Sacc., *Lasiodiplodia theobromae*(Pat) Griffon & Maubl, *Trichotheciumroseum* (Persoon) Gray. Several works carried out by researchers on other oil seeds and fibre crops confirmed these fungi as seed-borne pathogens. Shazia *et al*. (2004) reported the occurrence of *F. oxysporum*, *A. niger, A. flavus, T.roseum, Rhizopus sp* among others, from the seed of groundnut.

The cultural and morphological characteristics of each mycoflora is shown in Table 1. The description of each fungus identified corroborated the report of past research works. Miclear *et al*. (2012) in an in-vitro study regarding the morphology of *B. cinerea* described the mycelia of *Botrytis cinerea* as dirty white to greyish white in colour with black rough edges at the middle of the plate. He also described the conidia as straight. Barnet and Hunter (2010) also gave a similar description. Pitt and Hockins (2009) in an experiment describing fungi associated with food spoilage gave the description of *L. theobromae*, *T. roseum* and *C. gloeosporoides* among others, which corroborated the description given in this study.

The agar plate method was found to be most suitable for the isolation of fungi from seeds (fig. 1). This was also supported by Niaz and Dawar (2009). Khan *et al*. (1988) also proved that the use of agar was preferred over the blotter method for the isolation of mycoflora. On the contrary, Gowder *et al*. (2007) reported that blotter method was better for the isolation of large number of fungal species.

The occurrence of fungi varied among the different kenaf varieties (Table 2). The most frequently isolated fungi were *T. roseum* and *A. niger* which occurred in six of the kenaf varieties with percent occurrence of 60%. *Colletotrichum gloeosporoides*, *Colletorichhum lindemuthianum*, *Botrytis cinerea* and *Penicillium* sp. occurred in four of the varieties with 50% occurrence while *Fusarium* sp, *C. globosum* and an unidentified A occurred in only three of the varieties giving 30% occurrence. Of all the varieties, ifeken 100 was colonised by more fungi with respect to the other varieties and Tianung 1 had the least fungal colonisation. The different mycoflora isolated from the seeds were grouped into their various fungal classes (Zhang *et al*., 2006; Geisser *et al*., 2006 and *MacLaughlin* and *Spatafora,* 2015). The class *Sordariomycetes* showed the highest incidence (40%) (Table 3). The occurrence of the class *Euromycetes* was next while the class Leotomycetes and Zygomycetes showed the least occurrence of 6.7% each. The class *Sordariomycetes* consist of *F. oxysporum*, *C. lidemuthianum, C. globosum, Colletotrichum gloeosporoides*, and *Trichothecium roseum* which has been confirmed from various reports as having the ability to infect seeds. Narayan and Ayodhya (2013) reported the occurrence of *Chaetomium* spp, *F. oxysporum*, and *Aspergillus niger* among others as seed-borne on legumes. This also corroborates the findings of Nuray and Desen (2004).

Conclusion

The present study showed that kenaf seeds, irrespective of the variety, are subjected to

Table 1: **Morphological characteristics of the fungal isolates**

Fungi	Colony character			Zonation	Conidia shape
	Texture	Surface colour	Reverse colour		
B. cinerea	Cottony	Dirty white to grey	Dark grey to black	No zonation	Globose to ellipsoidal
C. lindemuthianum	Powdery	Light pink	Light pink	Slightly radially furrowed	Straight and fusiform
C. cladosporoides	Tufted or velvety	Brown black	Cream colour		Ovoid to cylindrical shape
C. globosum	Powdery	Cream with brown rings	Cream colour	Radially furrowed with venation	Ellipsoidal to lemon shaped
R. stolonifer	Fluffy	White turns grey with age	Pale brown	No zonation	ellipsoidal
F. oxysporum	Fluffy	Whitish	Light pink	No zonation	Ellipsoidal to cylindrical
A. Niger	Velvety	Whitish with typical black spores	Light yellow	Slightly furrowed	Globose
T. roseum	Powdery	Light pink to peach colour	Light peach	Slight zonation	Ovoid to ellipsoidal
C. gloeosporoides	Woolly	Light orange	Orange	Slight zonation	cylindrical
L. theobromea	Fluffy	Whitish changes to gray and then black with age	Grey to black	No zonation	Oval shape
A. flavus	Powdery	Greenish yellow	Cream	No zonation	Globose
*Penicillium*sp	Velvety	Colony is a mix of black and red to gray colouration	Same as surface	zonated	Globe with funnel shape base
Un-identified A	Powdery	Lilac	Lilac	Slightly zonated	
Un-identified B	Velvety	Dark brown to black	Same as surface	Slightly zonated	
*Fusarium*sp	Woolly	White Web-like	Offwhite	Slightly zonated	cylindrical

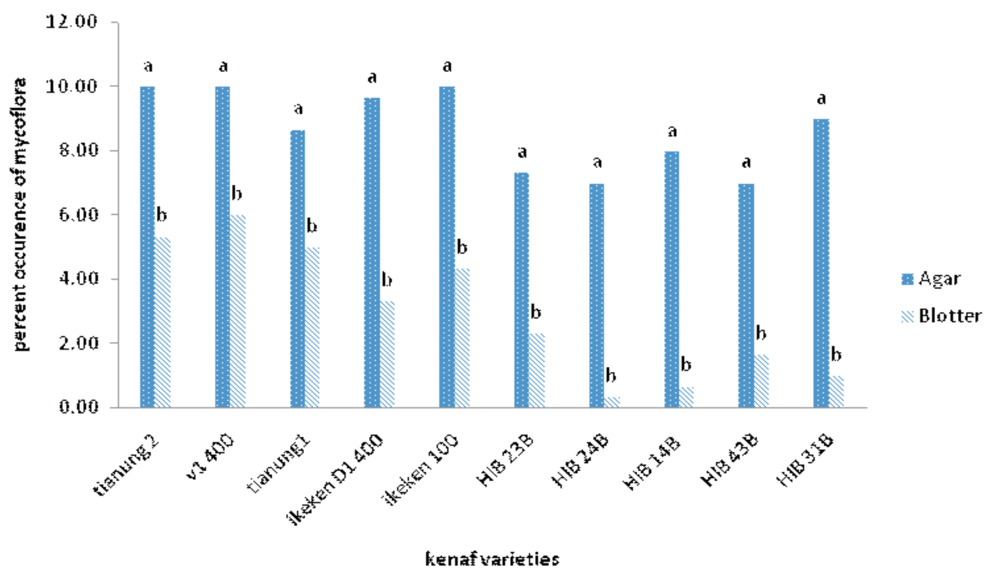

Fig. 1: Effect of Agar plate and Blotter methods on percent occurrence of mycoflora on kenaf seeds.

Table 2: Mycoflora distribution on the kenaf varieties

Organisms	Tianug 2	V1 400	Tianug 1	Ifeken D1 400	Ifeken 100	HIB 24B	HIB24B	HIB 14B	HIB 43B	HIB31 B	%
Un-identified A	-	-	+	-	+	-	-	+	-	-	30.00
Fusarium spp.	-	-	-	-	-	+	+	-	+	-	30.00
C. gloeosporioides	-	+	-	+	-	-	+	+	-	+	50.00
T. rosuem	+	+	+	+	+	-	-	+	-	-	60.00
C.lindemuthianum	-	+	-	+	-	+	+	-	+	-	50.00
C. globosum	-	-	-	-	+	-	+	-	-	+	30.00
A. niger	+	-	-	+	+	+	-	+	-	+	60.00
L. theobromae	-	+	+	-	+	+	-	-	+	-	50.00
B. cinerea	+	-	-	-	+	+	-	-		+	40.00
A. flavus	-	-	-	+	+	+	-	-	+	-	40.00
R. stolonifer	+	-	+	-	-	-	+	+	-	-	40.00
F. oxysporum	-	-	-	-	+	-	+	+	+	-	40.00
C. cladosporoides	+	+	-	-	-	+	+	-	-	-	40.00
Penicillium sp.	+	-	-	-	+	+	-	-	+	+	50.00
Un-identified B	-	+	-	-	+	-	+	+	-	-	40.00

% indicates the percent occurrence of each fungi in the different kenaf varieties.

Table 3. Percent occurrence of various classes of fungi on kenaf seeds

Class of fungi	Number of species isolates	Percent occurrence by fungi class
- *C. lindemuthianum*		
C. gloeosporoides		
Sordariomycetes - *C. globosum*	6	40%
F. oxysporum		
Fusariumspp		
T. roseum		
Eurotiomycetes - *A. flavus*	3	20%
- *A. niger*		
Penicilliumsp		
Dothideomycetes - *C. cladosporoides*	2	13.3%
L. theobromae		
Leotiomycetes - *B. cinerea*	1	6.7%
Zygomycetes - *B. stolonifer*	1	6.7%
Un-identified	2	13.3%

fungi infestation which causes deterioration of the seeds. This results in an irreversible degenerative change in the seed quality. The study also showed that the agar plate method of isolation is more preferable for isolation of large number of fungi from seeds.

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