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Seed-borne fungi isolated from five selected varieties of Sorghum (*Sorghum bicolor* L.).

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Abstract

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop in the world after wheat, rice, maize and barley. The grain had been used for consumption by both humans and live stock. Different genotypes of the plant serve many other important uses. The crop has been suffering from various types of diseases. Majority of them are known to be caused by fungi which are mostly seed borne. This leads to quantitative and qualitative loss both at pre and post harvest levels. In the present study, seed borne fungi associated with the five selected varieties of *Sorghum bicolor* were isolated on saboraud dextrose agar (SDA) and potato dextrose agar (PDA). Five fungi species namely *Aspergillus flavus* Link, *Aspergillus niger* vantieghem, *Fusarium oxysporum* Schlecht, *Phoma herbarum* Sacc and *Rhizopus stolonifer* (Ehrenb) Lind belonging to four genera were isolated. Presence of these fungi in considerable number of seed samples indicates the need of field surveys of these and other pathogens. The isolation was done using the agar plate method.

Keywords: Isolation, Seed-borne Fungi, Sorghum

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Introduction

Sorghum (*Sorghum bicolor* L.) is the fifth most important world cereal and an important native cereal in Africa (FAO, 1995; ICRISAT, 1996; Murty and Renard, 2001). Sorghum is also the dietary staple of more than 500 million people in more than 30 countries (National Academic Science, 1996). Total world production of sorghum was estimated at about 54 million tons (FAO, 2004). An annual production of about 70 million metric tons of grains from 50 million hectares of land has been reported (NAS, 1996). In Nigeria, sorghum is an important staple cereal grown in more than twenty states of the federation including Akwa Ibom and Enugu states (Wudiri and Fatoba, 1992). It accounts for about 50% of the total cereal crops grown in Nigeria. According to Alegbejo (2002), *Sorghum* is the second most important cereal in Nigeria. The estimated annual production is about 9 million tons produced from about 6 million hectares. Average grain yield of subsistent farmers in the West African sub-region are generally between 500 to 800 kg ha^{-1} (Sharma et al., 1992).

Sorghum is a principal source of energy, protein, vitamins and minerals to the poorest people of the semi-arid tropics. The crop is dried, stored, and later used to prepare stiff porridge, thin porridge or fried dumpling, it is also used in brewing local beer (National Academic Science, 1996). The leaves provide fodder for farm animals and the stalk are used in fencing, roofing, weaving baskets and mats and also as fuel wood (Obilana, 1995). *Sorghum* grains are used industrially in the manufacture of items such as wax, starch, syrup, alcohol, dextrose agar, edible oils and gluten feed. In

addition, it is used to manufacture gypsum lath, paper and cloth sizing and adhesives (Onwueme and Singh, 1991; Komlaga et al., 2001; Murty and Renard, 2001). Unfortunately, the crop is attacked by a number of fungi at every stage of development. Taylor and Francis (2001) reported that fungi cause about 50-80% damage to farmers sorghum during storage period or when conditions are favourable for their development resulting in significant loss both quantitatively and qualitatively. In addition, fungi produce mycotoxins which are hazardous to man and animal. Various reports have shown yield losses of up to 67% (Pande et al., 1991; Halt, 1994; Marley, 1996; Gwary et al., 2002; Gachomo et al., 2004).

Materials and Methods

Study area

The study was carried out in the Department of Biological Sciences, Ahmadu Bello University, Zaria. Zaria is located between latitude $11^0$09′N, Long 7^0 42E at an altitude of 686 meters above sea level. Its annual rainfall, average temperature and relative humidity are 1055 mm, 24.55^0C and 43.6% respectively (meteorological unit IAR, 2009).

Source of materials

Five different varieties of *Sorghum bicolor* L. were used in this study. Four different sorghum varieties namely Samsorg 17, Samsorg 40, Samorg 42, Samsorg 43 were obtained from Plant Science Department, IAR and one local land race Kaura was purchased from Sabon Gari market, Zaria.

Preparation of media

Two basic kinds of media Potato Dextrose Agar (PDA) and Saboraud Dextrose Agar (SDA) were prepared as described by manufacturer's recommendations and used throughout the study.

Dispensing of media

A 15 ml of both PDA and SDA were dispensed into individual petridishes with the aid of a syringe.

Isolation of fungi and fungal count

This was done by inoculating the two prepared growth media in separate petri dishes. Before inoculation, the grains were randomly selected and surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 2 mins to remove surface contaminants and then rinsed with sterilized distilled water three times to remove remains of sodium hypochlorite. Five grains of each of the selected varieties were splitted with a sterilized forceps and then inoculated in each of the prepared media and incubated at $\pm 27^0$C seven days. Every plate with two different media was in triplicates. After the incubation period, the numbers of visible colonies that appear were counted. All the discrete colonies of fungi were sub-cultured to obtain pure cultures.

Identification of Fungi

Isolated fungi were identified by using both microscopic and macroscopic characters. The identification was aided by reference to the description of fungi using identification keys by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson et al. (2004).

Results

Isolation of fungi from five different varieties of Sorghum bicolor

On isolation, the varieties yielded five different fungi namely *Aspergillus flavus* Link, *Aspergillus niger*

Vantieghem, *Fusarium oxysporum* Schlecht, *Rhizopus stolonifer* (Ehrenb) lind. and *Phoma herbarum* Sacc. These were isolated on both potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA). These were however, not distributed on all the varieties uniformly. *Aspergillus flavus* and *Aspergillus niger* were isolated from all the five varieties on both media. However, *Fusarium oxysporum* was present only in the local land race Kaura. *Phoma herbarum* was present only in Samsorg 40, and Samsorg 43 whereas, *Rhizopus stolonifer* was present in all the improved varieties with the exception of the local land race, Kaura (Table 1).

Percentage occurrence of different fungi

A total of 48 colonies of different fungi developed on PDA. *Aspergillus niger* had the highest occurrence of 21 with percentage occurrence of 47.91%. *Aspergillus flavus* occurred 14 times with percentage occurrence of 29.16%, *Rhizopus stolonifer* occurred five times with percentage occurrence of 10.41%, *Fusarium oxysporum* had 8.33% and occurred four times. *Phoma herbarum* had occurrence of two with percentage occurrence of 4.16%. 44 colonies of different fungi developed on SDA. *Aspergillus flavus* had the highest occurrence of 21 with percentage occurrence of 47.72%. *Aspergillus niger* occurred 12 times with percentage occurrence of 27.27%. *Rhizopus stolonifer* occurred five times with percentage occurrence of 11.36%. *Fusarium oxysporum* and *Phoma herbarum* both occurred three times with percentage occurrence of 6.81% (Table 3).

Discussion

Different fungal genera such as *Aspergillus flavus* link, *Aspergillus niger* Vantieghem, *Fusarium oxysporum* Schlecht, *Rhizopus stolonifer (*Ehrenb*)* lind and *Phoma herbarum* sacc were found to contaminate sorghum (Table 1). These fungi have also previously been shown to cause spoilage to cereals (Domijan et al., 2005). Microorganisms especially fungi are known to be the major cause of market and field losses of crops (Onifade, 2000). The presence of *Aspergillus* species in all the five varieties could be as a result of their ability to secreate melanin which provide protection against stress thereby making them resistance (Langfelder et al., 2003). The presence of other species could be that there is possible succession of fungi in the seed borne infection. The variation in the distribution of the isolated fungi could be as a result of differences in the mode of storage.

Table 1. Fungi Isolated from Sorghum bicolor Varieties on PDA and SDA

	Samsorg 17		Samsorg 40		Samsorg 42		Samsorg 43		kaura	
Fungi species	PDA	SDA	PDA	SDA	PDA	SDA	PDA	SDA	PDA	SDA
Aspergillus flavus	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Aspergillus niger	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fusarium oxysporum	-	-	-	-	-	-	-	-	✓	✓
Phoma herbarum	-	-	✓	✓	-	-	✓	✓	-	-
Rhizopus stolonifer	✓	✓	✓	✓	✓	✓	✓	✓	-	-

✓ = Present. - =Absent

These fungi were identified on the basis of their macroscopic and microscopic characteristics (Table 2)

Table 2. Macroscopic and microscopic characteristics of fungi isolated from *Sorghum bicolor* varieties on PDA and SDA

Fungal species	Macroscopic characteristics	Microscopic characteristics
Aspergillus flavus	The colonies in both PDA and SDA were regularly shaped measuring 8 cm in one week at room temperature. The color was greenish in the beginning becoming dark green at the upper surface, and green with yellow tint on the reverse side of the plate which were consistent on both media	The mycelium produced long, erect, hyaline, and aseptate conidiophore which narrow slightly towards the base. The conidiophores terminated on top with globose vesicle. Conidia were globose. The conidiophores were measured. They ranged between 130-500 µm in length with an average length of 383.404 µm and a width of 20-40 µm near the vesicle, 10-40 µm at the middle and also 10-40 µm at the base with an average width of 28.6 µm near the vesicle, 28.2 µm at the middle and 25.4 µm at the base. Vesicle measured 130-500 µm in length with an average length of 383.404µm. Conidia were measured and ranged 4-6 µm in diameter with an average diameter of 4.64 µm.
Aspergillus niger	Cultures in both media produced roundish black colonies at the upper surface and green with yellow tint on the reverse side of the petri plate. Colonies expanded to 8 cm in one week at room temperature	The mycelium consist of profusely branched hyaline hyphae which produced long, upright, erect, hyaline and smooth conidiophores which narrow slightly towards the base. Each conidiophore terminated in a globose vesicle. Conidia were globose with rough or echinulated surfaces. The conidiophores were measured and ranged between160-600 µm in length with an average length of 410.4 µm and a width of 20-30µm near the vesicle, 20-30 µm at the middle and 10-30µm at the base with an average width of 28 µm near the vesicle, 25 µm at the middle and 20 µm at the base. Vesicles measured 20-100 µm in diameter with an average diameter of 58 µm. Conidia were 4-6 µm in diameter with an average diameter of 4.96 µm.
Fusarium oxysporm	Mycelium extensive, initially white and cottony in both media with tinge of pink and orange colour at the upper surface, with orange and dark brown on the reverse side of the plate. The mycelium expanded to 8 cm in one week at room temperature. The growth is fluffy and shows concentric ring.	Mycelium produced conidia (phialospores) which are hyaline and principally of two kinds. The macro conidia: They are slightly curved and tapering at both ends typically canoe shaped. They are septate with three to six septations. The conidia were measured and ranged between 30-100 µm in length with an average length of 62.80 µm. In the middle they ranged between 20-30 µm with an average width of 7.6 µm. The micro conidia: They are single celled, ovoid or oblong borne singly or in chains. Some conidia are intermediate, 2 or 3 celled, oblong or slightly curved (plate III). The conidia were measured and ranged between 20-40 µm in length with an average length of 32.40 µm. The width was also measured which measured between 10-20 µm at the middle with an average width of 4.6 µm.
Phoma herbarum	Colonies of *Phoma herbarum* grows rapidly in both media and expanded to 6cm in one week at room temperature. The mycelium was flat, spreading powdery to velvety and often largely submerged in the media with dark brown and dark colouration at the upper and reverse side of the plate respectively.	The mycelium produced septate hyaline phialides producing pycnidia. Pycnidia are large, round to pyriform asexual fruiting bodies. They are dark in colour bearing phialides at their inner linings. Pycnidia may be singly arranged or in chains. The pycnidia were measured and ranged between 30-60 µm in diameter with an average diameter of 41.4 µm.

Rhizopus stolonifer	Colonies in both media were very fast growing with some tendency to coallase, white cottony initially becoming brownish grey to blackish grey with white and light brown colouration on the reverse side of the plate. Colonies expanded rapidly to 8 cm in one week at room temperature covering the entire surface of the media in a petri-plate.	Mycelium produced sporangiophore which were smooth walled, non-septate, simple, occurring singly or in cluster arising from the stolon usually one or two in number with rhizoids from the same point usually in group of two or three and 30 µm towards the base with an average width of 23 µm near the sporangium, 25.6 µm at the middle and 30 µm towards the base. The sporangium ranged between 30-100 µm in diameter with an average diameter of 63.80 µm.

Table 3. Percentage occurrence of different fungi species in Sorghum varieties on PDA and SDA

Fungi species	PDA		SDA	
	No. of discrete Colonies	Percentage occurrence	No. of discrete Colonies	Percentage occurrence
Aspergillus niger	23	47.91	12	27.27
Aspergillus flavus	14	29.16	21	47.72
Rhizopus stolonifer	05	10.41	05	11.36
Fusarium oxysporum	04	08.33	03	06.81
Phoma herbarum	02	04.16	03	06.81

Aspergillus flavus when compared with some other related species like *Aspergillus nominus* and *Aspergillus bombyces*, it was found different in having yellowish green colour on PDA and SDA with smooth to finely roughened conidia. *Aspergillus niger* was compared with some related species like *Aspergillus ficuum* (Reich) Thom and Church and *Aspergillus tubingensis* Mosscray, it was found different in producing characteristics black colonies and large vesicles with irregular roughened conidia. *Fusarium oxysporum* is one of the most economically important species within the genus (Bhat et al., 2003). *Fusarium oxysporum* was compared with two closely related species viz *Fusarium poae* (peck) Wollenw and *Fusarium graminearum* schwabe, *Fusarium oxysporum* came out having whitish with pink tinge on the surface, pink tinge towards the pheriphery and orange colour at the pheriphery. The growth showed concentric rings and macro conidia were more elongated and pointed with three to six septations. Micro conidia were also present, ovoid to ellipsoidal to cylindrical in shape. In this way, it differs from *Fusarium graminearum* schwabe which do not produce micro conida. *Fusarium* Poae produced microconidia abundantly which are one celled, napiform or pyriform. Macroconidia of *Fusarium* Poae are sparsely produced with two to three septations. *Phoma herbarum* was also found associated with all the grains of the varieties. It was compared with *Phoma glomerata* and *Phoma macrostoma*.*Phoma herbarum* differs from this species in having dark brown colonies. *Rhizopus stolonifer* was also found associated with the grains of *Sorghum bicolor*. It was compared with two related species *Rhizopus oligosporus* and *Rhizopus oryzae* and found different in not having chlamydospores.

All the isolated fungi when compared with already described species by Barnett and Hunter (1971), Larone (2002), Klich (2002) and Samson et al. (2004) were found to be similar. However, there were minor variations in the measurement of the microscopic characteristics possibly due to differences in nutritional

media used and environmental conditions. The most important phytopathogenic fungi of sorghum in Africa and worldwide are *Aspergillus* species (Murugan et al., 2007). From the result of fungi identification, sorghum was found to be dominated by *Aspergillus* species on both PDA and SDA (Table 3). The high occurrence of *Aspergillus* species indicates that *Aspergillus* genus possibly act as pioneer organisms in seed borne infection of sorghum or it could be that they are the most abundant species found in the tropics and sub tropic regions. This corroborate with the work of Visconti, 2001. It is quite clear from this study that Sorghum which is a staple food for many Nigerians is affected with many fungi in storage. Therefore, the use of such grains is highly risky for the human health and livestock as they contain highly poisonous chemicals.

Conclusion

Based on the findings of this research work, it is concluded that five fungal genera namely *Aspergillus flavus* , *Aspergillus niger* , *Rhizopus stolonifer, Phoma herbarum* and *Fusarium oxysporum* were found to contaminate *Sorghum bicolor grains.Sorghum bicolor* was found to be dominated by *Aspergillus niger* (47.91%) on PDA and *Aspergillus flavus* (47.72%) on SDA. The presence of these fungi indicates a clear need for field surveys for these and other pathogens. Testing seeds health of major crops should introduce in the national seed quality control system.

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