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Fungi Associated with Seeds of Huckleberry (*Solanum Scabrum* Mill.) Grown in the Western Highlands of Cameroon

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Abstract Seeds from ten varieties of huckleberry (SS01, SS01a, SS02, SS05, SS08, SS08H, SS18, SS18a, SS19 and SS25) obtained from the seed bank of the Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon were used for detection of seed-borne fungi. Four hundred seeds of each variety were surface sterilized and incubated for seven days at 22 ± 2^{o} C under an alternating light cycle of 12 h darkness and 12 h light provided by white fluorescent tube. The seed germination percentage ranged from 21 to 94% with an infection percentage variation from 1 to 31%. A total of 19 fungi were found associated with these seeds. These were *Alternaria alternata, Altenaria brassicicila, Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Cladosporium* sp., *Curvularia lunata, Colletotrichum* sp., *Cercospora sesame, Fusarium culmorum, Fusarium moniliforme, Fusarium oxysporium, Fusarium pallidoroseum, Fusarium solani, Macrophomina phaseolina, Phoma* spp, *Rhizoctonia solani, Rhizopus nigricans, Verticillium* sp. This is the first report on huckleberry seed associated fungi which might be a stepping point towards the management of huckleberry diseases and can be a useful guide to strategic seed disease control.

Keywords: Seed borne fungi, Huckleberry, Cameroon

Introduction

Huckleberry (*Solanum scabrum*) is an important vegetable widely grown in the world after potato (Komolafe *et al*., 1980). In cameroon, It is one of the most popular food crop highly commercialysed, especially in West and Northwest regions where it is found as the most commonly grown vegetable

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(Schippers, 1998). The crop is even exported from Cameroon to neighbouring countries which include Nigeria, Equatorial Guinea and Gabon (Fontem *et al*., 2003). Leaves and fresh shoots are widely consummed as compliment with some high cabohydrate content food stuff (Ngundam, 1997). Nutritionally, it is a good source of Vitamine A and C and also, rich in amino acid, methionine than any other vegetable (Edmonds and Chewya, 1997). Cosumption inreasonable quantitty is believe to control diarrhoea in children, certain eye infections and jaundice (Dupriez and De Leener, 1989; Edmonds and Chweya, 1997). It is also used as analgesic, febrifuge, narcotic and purgative by local population in the North West Region of Cameroon (Mbong *et al*., 2014). In East Africa, raw fruits are used to treat stomach ulcers or stomach-aches (Kokwara, 1976). Schippers (2000) reported leaves and fruits as source of dyes and animal fodder.

Seeds are carriers of some important seed-borne diseases caused by microorganisms which lead to considerable yields losses. More than fifthy microorganisms are reported to be seed borne in seeds of different vegetable seed lots (Richardson, 1990). These fungi not only can cause seed deterioration but may also serve as sources of primary inocula of many diseases like seedling blight, damping off and wilts in nursery beds and fields. Some of them were found to be very destructive, decreasing seed germination, causing seed rot, pre and post germination death (Bolkan *et al.*, 1976; Elarosi, 1993). Isolation of fungi from vegetable seeds have been reported by several researchers (Alkassim, 1996; Puspa *et al*., 1999; Jamadar *et al*., 2001; Chowdhury *et al*., 2005; Islam, 2006). However there is no report on seed associated fungi of huckleberry in Cameroun. Early identification and listing of plant pathogens in seed crop allows for timely development of control and management strategies that goes a long way in avoiding epidemics. It is also a means of checking the spread of many seed borne diseases and ensures the prevention of the spread of plant diseases to new areas. The present study was undertaken to investigate on fungi associated with ten huckleberry varieties grown in Cameroon.

Materials and methods

Seed samples

Seeds of ten varieties of huckleberry were collected from the Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon. The samples were brought to the Laboratory of Phytopathology, and kept in polythene bags and stored in the refrigerator at 9 °C, till the seeds were used for the subsequent studies. These seeds were local varieties commonly grown in the western highlands agro ecological zones of Cameroon and were SS01, SS01a, SS02, SS05, SS08, SS08H, SS18, SS18a, SS19, and SS25. Table 1 presents the different varieties of huckleberry seeds used and their origins.

Table 1. Seed origin of the ten huckleberry varieties used

No	Huckleberry seed varieties	origin
1	SS01A	Western highlands
2	SS02	Western highlands
3	SS18	Western highlands
4	SS01	Western highlands
5	SS18A	Western highlands
6	SS19	Coastal highlands
7	SS25	Southern plateau
8	SS08H	Southern plateau
9	SS08	Southern plateau
10	SS05	Southern plateau

Detection of seed borne fungi

To detect the seed borne pathogens associated with the seed samples, the blotter method was used following International Rules for Seed Testing Association (ISTA, 2001). In this method, three layers of blotter paper (Whatman No. 1) were moistened with 5ml of distilled water and placed at the bottom of 9 cm diameter plastic Petri dish. Seeds were surface sterilized with 2 % sodium hypochlorite for 3 minutes and rinsed three times in sterile distilled water. Four hundred seeds from each variety were plated in 16 dishes (25 seeds per petri dish). The Petri dishes with seeds were then incubated for 7 days at 22 ± 2 °C under alternating cycles of 12 h darkness and 12 h light provided by white fluorescent tube (Mazda, T8 36W). Data regarding germination and infection were recorded 7 days after the incubation period and the results were recorded in percentages. Each of the incubated seeds was then examined for fungal growth under a stereo-microscope (20X). In fewer cases the fungi from the incubated seeds were transferred to PDA medium in Petri dishes aseptically and incubated under controlled temperature (20℃) for 3-7 days and then examined. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on Blotter paper following the keys outlined by Champion (1997) and Mathur and Olga (2003). Identification was also supplemented by microscopic examination of spores and fruiting bodies using a compound microscope and relevant books. The frequency of each fungus was determined from the percentage of the colonies of all the fungi developed. Data on percentage seed germination and seed infection were analyzed using SPSS version 17 software and means were separated using Duncan's test at P = 0.05

Results

The germination percentage of seeds of the ten varieties of huckleberry ranged from 21 to 94% while the infection percentage varied from 1 to 31% (Table 2).

A total of 19 fungi were detected from seeds of huckleberry (Table 3). These were *Alternaria alternata, Alternaria brassicicola, Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Cladosporium* sp., *Curvularia lunata, Colletotrichum* sp*., Cercospora sesame, Fusarium culmorum, Fusarium moniliforme*, *Fusarium oxysporium*, *Fusarium pallidoroseum*, *Fusarium solani, Macrophomina phaseolina*, *Phoma* spp, *Rhizoctonia solani*, *Rhizopus nigricans*, *Verticillium* sp. Conidia and mycelia of some fungi detected from huckleberry seed are shown in Figure 1. The broadest species spectrum in most seed varieties consisted of the genera *Fusarium* recorded from seeds of eight varieties with an occurrence frequency of 8- 58*%*, followed by *Phoma* (13-21%) and *Cladosporium* (8-38%), *Alternaria*, *Aspergillus* and *Botrytis* with frequencies of 8-17% each, *Cercospora* (4-42%) and *Curvularia* (8%) were recorded in two varieties while *Colletotrichum* and *Macrophomina* were found in one variety with an occurence frequency of 21%.

Huckleberry varieties tested in the present study differed in terms of fungal percentage infection (Table 3). The most infected variety of huckleberry was found to be SS01a with 31% fungal infection followed by SS05with total fungal infection of 21% whereas SS18A showed the least fungal infection of 1%. These fungal infections affected the germination of SS05 seeds (21% germination of seeds).

Table 2. Percentage germination and infection of seed of ten huckleberry varieties

Variety	(%) **germination**	(%) **infection**
SS01A	93.5a*	31.0a
SS02	92.5a	5.0de
SS08	85.0ab	8.0cde
SS18	75.5bc	2.5de
SS19	74.0bcd	11.0bcd
SS01	73.0cd	10.5cd
SS18A	63.0de	0.50e
SS08H	60.0e	12.5bc
SS25	53.0e	10.0cde
SS05	20.5f	20.5b

Data based on 400 seeds per variety;
* Means followed by the same letter(s) within the same column are not significantly different at 5 % probability level using DMRT (Duncan Multiple Range Test).

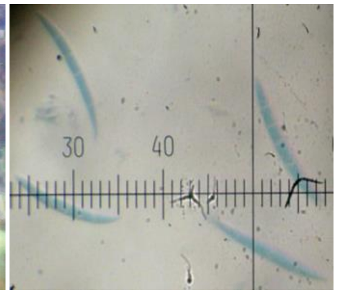
Table 3. Percentage occurrence of seed borne fungal infection of huckleberry seeds with Blotter method

Fungi	SS01a	SS02	SS08	SS18	SS19	SS01	SS18a	SS08H	SS25	SS05
Alternaria alternata	-	-	-	-	-	-	-	17	-	-
Alternaria brassicicola	-	-	-	-	-	-	-	-	8	-
Aspergillus flavus	-	-	-	-	-	-	-	-	8	-
Aspergillus niger	-	-	-	-	-	-	-	-	-	17
Botrytis cinerea	-	-	8	-	-	-	-	-	-	17
Cladosporium sp.	29	38	8	-	-	-	-	-	-	17
Curvularia lunata	-	-	8	-	-	-	-	-	16	-
Colletotrichum sp.	-	-	-	-	-	21	-	-	-	-
Cercospora sesame.	-	-	-	42	-	-	4	-	-	-
Fusarium culmorum	-	-	-	-	-	-	-	-	-	8
Fusarium moniliforme	-	38	8	-	-	-	-	17	-	8
Fusarium oxysporium	29	21	-	-	13	-	-	17	-	17
Fusarium pallidoroseum	14	-	58	-	54	-	-	33	54	-
Fusarium solani	-	-	-	-	13	21	-	-	-	-
Macrophomina phaseolina	-	-	-	-	-	21	-	-	-	-
Phoma sp	14	-	-	21	13	42	-	-	17	-
Rhizoctoniasolani	-	-	-	-	13	-	-	17	-	8
Rhizopus nigricans	14	-	-	-	-	-	-	-	-	-
Verticillium sp.	-	-	-	38	-	-	-	-	-	17

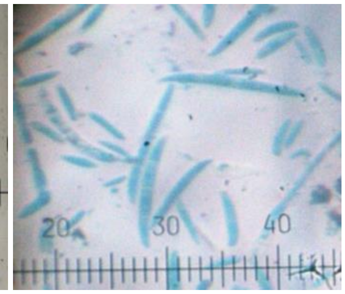
Observation based on 400 seeds of each huckleberry variety.

- , Not found

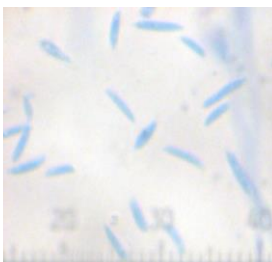
Conidia and Mycelium of *Curvularia lunata* (X 400)



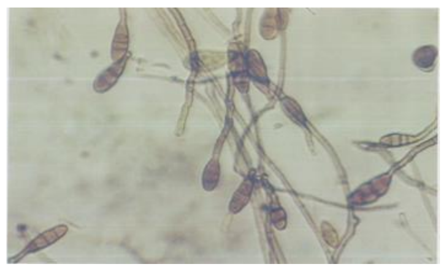
Macro Conidia of *Fusarium culmorum* (X 400)



Macro and Micro Conidia of *Fusarium pallidoroseum* (X 400)



Macro and Micro Conidia of *Fusarium pallidoroseum* (X 400)



Conidia and mycelium of *Alternaria brassicicola* (X 400)

Figure 1. Conidia and mycelia of some detected fungi from huckleberry seed varieties.

Discussion

The study revealed a wide range of fungi associated with huckleberry seeds. Nineteen different fungi were isolated from seeds from ten varieties. Many species were recovered more frequently on different varieties while some were occasionally recorded or even absent in most varieties. Most of these fungi have been reported to be associated with several vegetables seeds causing seed decay and poor germination of seeds (Alam, 2002; Islam, 2006; Begum, 2012; Hamim *et al*., 2014). *Fusarium* spp was found to be the dominant fungus amongst others in seeds of seven huckleberry varieties. *Fusarium* spp was reported as a highly pathogenic fungus and its different species have been reported to cause seed rot, seedling blight and wilt in a number of cucurbitaceous and leguminous crops (Kamble *et al*., 1999; Chowdhury *et al*., 2005; Tsopmbeng, 1994). According to Mathur *et al*. (1975) and Richardson (1990), *Fusarium* spp. is an established seed-borne pathogen capable of causing germination failure/ seed rot, damping-off, seedling blight foot and root rot and wilts in many crops. Its impact on seeds of huckleberry is not known, however, their high frequency on these seeds should be further investigated. Species of *Alternaria alternata* and *A. brassicicola* were found associated with seeds of huckleberry. *A. brassicicola* is a necrotrophic fungal pathogen that is reported to cause black spot disease on cruciferous vegetables including economically important *Brassica* species (Otani *et al*., 1995; Nowicki *et al*., 2012). *Alternaria alternata* is an opportunistic invader and has been described as one of the major fungi associated with discoloured seeds (Gupta *et al.*, 1989). In the present study, most of the seeds infected with *Alternaria* decayed and turned black.

A considerable number of seed borne fungi belonging to the genera *Aspergillus, Curvularia, Penicillum, Rhizopus, Colletotrichum and Macrophomina* were also detected in Amaranth seeds (Begum, 2012), in leafy vegetables (Khanom, 2011) and in Okra seeds (Akter, 2008; Sultana, 2009). Fungi of these genera were also reported to affect seed germination in various crops (Richardson, 1990). The role these fungi play on huckleberry seed germination needs to be investigated. *Macrophomina phaseolina* detected on seed variety SS01 is worldwide distributed and is responsible for several diseases of the sunflower, including seedling blight, damping-off, root rot, basal stem rot and charcoal rot (Khan, 2007).

The high frequency of fungi recorded on SS05 huckleberry variety seed, and its association with low germination percentage recorded confirmed the fact that species of seed borne fungi may be responsible for germination failure due to seed decay. Seeds attacked by *Rhizoctonia solani, Fusarium* spp. and *Verticillium* spp usually fail to germinate resulting in poor stand development (Goldberg, 2011).

The above study revealed that seed borne fungi are present in most huckleberry seeds although they occurred in trace levels in some varieties. Therefore, further studies with more representative seed samples from different agro-ecological zones of the country should be undertaken in order to reveal the exact picture regarding the prevalence of seed borne fungi and the role they do play on seed germination. It is also a useful guide to strategic seed disease control.

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