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Fungi associated with sweet potato tuber rot at CSIR-PGRRI, Bunso, Eastern Region, Ghana

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ABSTRACT

Rotten sweet potato root tuber samples were collected from a barn and experimental field of the CSIR-Plant Genetic Resources Research Institute (CSIR-PGRRI), Bunso. Isolation and identification of the fungi associated with the samples were carried out at the Plant Pathology Laboratory of the same institute. In all, six fungal species belonging to four genera, namely *Fusarium solani*, *Sclerotium rolfsii*, *Lasiodiplodia theobromae*, *Aspergillus ochraceus*, *Aspergillus flavus* and *Aspergillus niger* were isolated from the samples from both the barn and the experimental field of CSIR-PGRRI. *Fusarium solani* and *Aspergillus niger* were frequently isolated from the sweet potato tuber samples from both the field and the barn. Pathogenicity tests carried out using the six fungal isolates on fresh and healthy sweet potato tubers showed that all the six fungi isolated were pathogenic in causing rot of sweet potato tubers with *Lasiodiplodia theobromae* being the most virulent.

Keywords: Isolation; pathogenicity tests; rot fungi; rotten samples; sweet potato tubers

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Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam] is a dicotyledonous plant that belongs to the family Convolvulaceae. The edible tuberous root is long and tapered with a smooth skin whose colour may be yellow, orange, red, brown, purple and beige. Its flesh ranges from beige through white, red, pink, yellow, orange and purple (Gad & George, 2009). The edible tuberous root of sweet potato is an important secondary crop that plays an important role in household food security in many countries (Tomlins *et al*., 2010). In Ghana, sweet potato is the fourth most important root crop after yam, cassava and taro (Sugri *et al*., 2017). The production of sweet potato is however, beset with a number of challenges of which diseases are paramount (Echodu *et al*., 2019; Sugri *et al*., 2017). Rot of sweet potato tubers for instance accounts for losses in economic value and also affects the market value of harvested roots (Rees *et al*., 2001; Devereau, 1994). Rot of sweet potato tubers occurs in the field aided by wounds caused by insect damage and natural openings of the tubers and storage problems associated with damage during harvesting (Jenny & Jerry, 2015).

A number of fungi have been reported to be associated with sweet potato tuber rot both in the field and during storage. Sowley & Oduro (2002) found *Aspergillus ochraceus*, *Botryodiplodia theobromae* (*Lasiodiplodia theobromae), Fusarium moniliforme*, *Fusarium oxysporum* and *Rhizopus stolonifer* to be associated with storage roots of rotten sweet potato tubers in Ghana. Ray & Nedunchezhiyan (2012) in a study of post-harvest fungal rot of sweet potato in India also found *Botryodiplodia Theobromae, Rhizopus Oryzae, Fusarium* sp., *Ceratocystis fimbriata* and *Sclerotium rolfsii* as major cause of sweet potato tuber rot. Similarly, *Macrophomina phaseolina, Curvularia lunata, Aspergillus ochraceus, Rhizoctonia solani* and *Plenodomus destruens* were also reported as minor rot inducing agents of sweet potato tubers (Ray & Nedunchezhiyan, 2012).

Õn Nigeria, *Penicillium* species, *Ceratocystis fimbriata, Diaporthe batatalis*, *Aspergillus flavus* and *Aspergillus niger* were reported as being responsible for post-harvest decay of sweet potato tubers (Onuegbu, 2002). Anukwuorji *et al*. (2013) also isolated *Botryodiplodia theobromae* (*Lasiodiplodia theobromae*), *Ceratocystis fimbriata*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* species, *Macrophomina phaseolina* and *Sclerotium rolfsii* from rotten sweet potato tubers in Nigeria. In Ghana however, there is a dearth of information on fungi that cause post-harvest rot of sweet potato tubers in the country. This study sought to isolate, identify and assess the virulence of isolated fungi with the view of providing information towards the development of an effective management strategy for sweet potato tuber rot in Ghana.

Materials and methods

Collection of samples

Fifty (50) sweet potato tubers showing symptoms of rot were randomly selected from freshly harvested five-month old tubers from the experimental field of the Council for Scientific and Industrial Research-Plant Genetic Resources Research Institute (CSIR-PGRRI) at Bunso in the Eastern Region of Ghana and another batch of 50 rotten tubers sampled from sweet potato tubers kept in a barn of the same institute for five months. Sweet potato tubers kept in the barn and those from the field were from separate sites of the experimental field. Sampling of rotten sweet potato tubers in the experimental field and barn of the CSIR-PGRRI was carried out in March, 2018. The selected rotten sweet potato tubers were put into polythene bags and brought to the Plant Pathology Laboratory of the CSIR-PGRRI for further isolation and identification of the fungi associated with the samples.

Isolation and identification of associated fungi

The diseased tubers were washed with tap water and small portions of tuber samples about 5 mm around the periphery of rot lesions were excised with a knife. The tissues from the rot lesions were surface sterilized in 5% sodium hypochlorite solution for two to three minutes and then rinsed three times with sterilized distilled water. The tissues were then plated on potato dextrose agar (PDA) in Petri dishes and incubated at 25°C for five days. A total of 32 pieces of excised tissues were plated per tuber

sample, eight tissues in a Petri dish and four replications. Sub-culturing of the various fungi was done on PDA to obtain pure cultures of the fungal isolates for identification. The isolated fungi were identified based on their colony morphology and conidial characteristics with the aid of a compound microscope (Hund Wetzlar, H-500, Germany) with reference to the laboratory manuals developed by Mathur & Kongsdal (2003) and Barnett & Hunter (1998). Frequency of occurrence of each fungus on the samples was calculated using the formula:
Frequency of occurrence=

$$\frac{\text{Number of Corms affected by Fungus}}{\text{Total number of Corms evaluated}} \times 100\%$$

Pathogenicity tests

Fresh and healthy 21 sweet potato tubers of popular local cultivar "Anagokane" obtained from Begoro in the Eastern Region, were brought to the Plant Pathology Laboratory of the CSIR-PGRRI at Bunso. The tubers were washed with tap water and surface sterilized with 70% ethanol. Cylindrical cores were removed from the proximal and distal ends of each sweet potato tuber with a sterilized 5 mm cork-borer. Four millimeter (4 mm) agar discs of seven-day old pure culture of the fungal isolates were placed into the holes and sealed with sterilized petroleum jelly. Control treatment was set-up using sweet potato tubers inoculated with bare Potato Dextrose Agar (PDA) plugs. The treatments were the six fungal isolates from both the barn and field sweet potato samples and a control. The inoculated sweet potato tubers were put into separate sterilized polythene bags, arranged in plastic trays and placed on laboratory bench for 14 days (Fig. 1). Each inoculated tuber was cut longitudinally and the diameter of each rot lesion was measured using a ruler. The experiment was completely randomized and replicated three times. Data was subjected to analysis of variance (ANOVA) using GENSTAT statistical package, edition 12.1. Differences in treatment means were compared for significance using the Least Significance Difference at Probability less or equal to 0.05 ($p \leq 0.05$).

Fig. 1: Inoculated sweet potato root tubers in the Laboratory

Results and Discussion

Isolated and identified fungi associated with the samples

Six fungal species belonging to four genera were isolated from the sweet potato tuber samples collected from the barn and field. These included *Fusarium solani* (Mart.) Appel & Wollenw. (Fig. 2), *Sclerotium rolfsii* Sacc (Fig.3), *Lasiodiplodia theobromae* (Pat) Griff. & Maubl (Fig. 4), *Aspergillus ochraceus* K. Wilh (Fig. 5), *Aspergillus flavus* Link (Fig. 6) and *Aspergillus niger* Tiegh (Fig. 7). The frequency of occurrence (%) of the fungi isolated from the sweet potato tubers from the barn and the experimental field are presented in Tables 1 and 2.

For rotten sweet potato tuber samples collected from the barn of CSIR-PGRRI, *Fusarium solani* and *Aspergillus niger* were the most frequent fungi isolated. Both had a frequency of occurrence of 26% (Table 1). This was followed by *Lasiodiplodia theobromae* and *Sclerotium rolfsii*, both of which occurred on 18.0% of the samples. The least frequent fungus was *Aspergillus flavus* (14% occurrence) (Table 1).

Fig. 2: A culture plate of *Fusarium solani* on PDA

Fig. 3: A culture plate of *Sclerotium rolfsii* on PDA

Fig. 4: A culture plate of *Lasiodiplodia theobromae* on PDA

Fig. 5: A culture plate of *Aspergillus ochraceus* on PDA

Fig. 6: A culture plate of *Aspergillus flavus* on PDA

Fig. 7: A culture plate of *Aspergillus niger* on PDA

TABLE 1
Frequency of occurrence (%) of fungi isolated from the sweet potato tubers obtained from a barn at CSIR-PGRRI

Fungi isolated	No. of corms evaluated	No. of corms infected	Frequency of occurrence (%)
Fusarium solani	50	13	26.0
Aspergillus niger	50	13	26.0
Lasiodiplodia theobromae	50	9	18.0
Sclerotium rolfsii	50	9	18.0
Aspergillus flavus	50	7	14.0

With samples from the experimental field of CSIR-PGRRI, *Fusarium solani* was the most frequent fungus isolated from the rotten sweet potato tuber samples collected. It occurred in 46% of the samples (Table 2). This was followed by *Aspergillus niger* (22% occurrence), *Aspergillus ochraceus* (14% occurrence) and *Aspergillus flavus* (10% occurrence). *Lasiodiplodia theobromae* had the least frequency of occurrence (8%) (Table 2).

TABLE 2
Frequency of occurrence (%) of rot fungi isolated from the sweet potato tubers obtained from the field at CSIR-PGRRI

Fungi isolated	No. of corms evaluated	No. of corms infected	Frequency of occurrence (%)
Fusarium solani	50	23	46.0
Aspergillus niger	50	11	22.0
Aspergillus ochraceus	50	7	14.0
Aspergillus flavus	50	5	10.0
Lasiodiplodia theobromae	50	4	8.0

Pathogenicity and virulence of the isolated fungi

All the fungi isolated from the rotten sweet potato tuber samples obtained from the barn and the field of CSIR-PGRRI induced rot on healthy sweet potato tubers. *Lasiodiplodia theobromae*, which was isolated from rotten sweet potato tuber samples from the barn and the field of CSIR-PGRRI, produced the largest rot lesion diameter of 21.7 mm after 14 days (Table 3). Significant differences were observed between the rot lesion diameter of *Lasiodiplodia theobromae* and that of the other isolates (Table 3). No significant difference was observed between the rot lesion diameter of *Sclerotium rolfsii* and *Aspergillus ochraceus*. No significant difference was also observed between the rot lesion diameter of *Fusarium solani* and *Aspergillus flavus* (Table 3). *Lasiodiplodia theobromae*, which produced a rot lesion diameter of 21.7 mm, was found to be more pathogenic in causing rot in sweet potato tubers followed by *Sclerotium rolfsii*, then *Aspergillus ochraceus*. The least virulent was *Aspergillus niger* which produced lesion diameter of 12.7 mm. No rot was observed in the control (Table 3).

TABLE 3
Diameter of the rot lesion recorded in Pathogenicity tests of isolated rot fungi

Isolated fungi	Diameter of rot lesion (mm)
Lasiodiplodia theobromae	21.7[a]
Sclerotium rolfsii	15.7[b]
Aspergillus ochraceus	15.5[b]
Fusarium solani	13.7[c]
Aspergillus flavus	14.2[c]
Aspergillus niger	12.7[d]
Control treatment	0.0[e]
Lsd (5%)	0.5
CV (%)	2.0

Each value is the mean of three replicates. Values with different letters are significantly different at $p < 0.05$.

Fungi isolated from rotten sweet potato root tubers

In all, six fungal species belonging to four genera were isolated from the rotten sweet potato samples collected both from the field and the barn. These included *Fusarium solani, Sclerotium rolfsii, Lasiodiplodia theobromae, Aspergillus niger, Aspergillus flavus* and *Aspergillus ochraceus.* For samples from the barn at CSIR-PGRRI, *Fusarium solani, Sclerotium rolfsii, Aspergillus niger, Aspergillus flavus* and *Lasiodiplodia theobromae* were the fungi isolated. *Fusarium solani, Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus* and *Lasiodiplodia theobromae* also isolated from samples of sweet potato tubers from the field. These organisms have been found associated with rotten sweet potato tubers (Anukwuorji *et al*., 2013; Ray & Nedunchezhiyan, 2012; Onuegbu, 2002; Sowley & Oduro, 2002).

Fusarium solani and *Aspergillus niger* were frequently isolated from the sweet potato samples collected both from the barn and the field. This may be attributed to the fungi pathogens infecting the sweet potato tubers on the field before being brought to the barn. Most of the sweet potato tubers showing symptoms of rot in the field and barn were showing signs of insect damage and with some sweet potato tubers, the insects were present inside when cut open. Jenny & Jerry (2015) reported that rot of sweet potato tubers occurs in the field aided by wounds caused by insect damage and natural openings of the tubers and storage problems associated with damage during harvesting. Sweet potato tubers kept in the barn and those from the field were harvested from separate sites of the experimental field. *Sclerotium rolfsii* is a soil-borne pathogen (Desai *et al*., 2021; Yaqub & Shahzad, 2015)

and its presence on the sweet potato samples kept in the barn, could be attributed to the fact that, the site from which those samples were taken had *Sclerotium rolfsii* infection whereas those collected directly from the field had no such infection at that site.

Pathogenicity and virulence of the isolated fungi

Pathogenicity tests conducted as part of this study established that all the six fungi isolated from the rotten sweet potato tuber samples were pathogenic with an ability to cause rot of sweet potato tubers. In a study on post-harvest fungal rots and control measures for sweet potato in the tropics, Ray & Nedunchezhiyan (2012) reported *Fusarium solani*, *Sclerotium rolfsii*, *Lasiodiplodia theobromae* and other fungi as being responsible for rots in sweet potato. Khatoon *et al*. (2017) also found *Aspergillus niger*, *Aspergillus flavus* and other fungi to induce rots in stored sweet potato tubers in India. Sowley & Oduro (2002) also reported *Aspergillus ochraceus*, *Botryodiplodia theobromae (Lasiodiplodia theobromae)* and other fungi as causing rot of sweet potato tubers in Ghana. *Lasiodiplodia theobromae* was found to be more pathogenic in causing rot of sweet potato tubers followed by *Sclerotium rolfsii*. Okigbo & Emeka (2010) in their studies reported that *Lasiodiplodia theobromae* was the most virulent rot causing pathogen in stored tuber crops. Although, the frequency of occurrence of *Lasiodiplodia theobromae* and *Sclerotium rolfsii* was relatively low on the sweet potato samples, their presence resulted in a high rate of deterioration of the tubers due to their high virulence.

Conclusion and Recommendation

Six fungal species belonging to four genera were isolated from rotten sweet potato samples collected from both the barn and the experimental field at CSIR-PGRRI, Bunso. *Fusarium solani*, *Sclerotium rolfsii, Aspergillus niger*, *Aspergillus flavus* and *Lasiodiplodia theobromae* were isolated from samples of rotten sweet potato tubers collected from the barn. *Fusarium solani, Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus* and *Lasiodiplodia theobromae* were also isolated from rotten sweet potato samples collected from the field. All the isolated fungi were found to be pathogenic in causing rot on healthy sweet potato tubers. Although *Fusarium solani* and *Aspergillus niger* were frequently isolated from the rotten sweet potato tuber samples, *Lasiodiplodia theobromae* was found to be the most virulent. Fumigation of the barn with appropriate pesticide prior to storage of tubers is recommended to reduce the level of infection in the barn. Also, sorting of damaged sweet potato tubers from sound ones before storage is recommended to avoid the introduction of disease organisms unto the produce.

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