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Relationship of Soilborne Mycoflora of Cassava Growing Fields to Incidence of Postharvest Rots of Cassava Tubers in Sokoto, Nigeria

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Abstract - In this study the fungi associated with cassava growing fields in Sokoto were isolated and identified using soil dilution technique. A total of 215 fungal colonies from 9 fungal species were isolated from soil samples of different cassava fields between the month of June and August, 2012. It was observed that soil samples from Lambara recorded the highest number (64) of fungal species followed by Damba and the least number (44) of fungal species was observed in Wamakko.The fungi isolated were *Alternaria species, Aspergillus niger, Aspergillus fumigatus, Cylindrocarpon lichenicola, Fusarium oxysporum, Geotrichum candidum, Mucor hiemalis, Rhizopus orgyzae* and *Scopulariopsis candida.*The highest percentage frequency of occurrence was observed in *Aspergillus niger (*39.5%) seconded by Fusarium *oxysporum* (18.2%) and the least was seen in *Rhizopus oryzae* (2.3%). The pathogenicity test indicated that all the fungal isolates were pathogenic on cassava tubers.The fungus *M. hiemalis* is the most pathogenic followed by *F. oxysporum* and the least was recorded by *R. oryzae.* Therefore, it would be concluded that there is relationship between soilborne fungi and incidence of postharvest rots of cassava tubers.

Keywords : Soilborne mycoflora; Cassava fields; Rots; Cassava tubers

Introduction

Fungi being ubiquitous organisms occur in all types of habitats and are the most adaptable organisms. The soil is one of the most important habitats for microorganisms like fungi, bacteria, viruses, nematodes etc. They are found in large numbers in soil usually between one to ten million microorganisms are present per gramme of soil (Ishaq and Khan, 2011). Many soil fungi are potential pathogens to both plants and animals (Moalaei *et al.,* 2006). Six pathogenic fungi were isolated from yam harvested soil in Ibadan. These fungi include *Penicillium oxalicum, P. cyclopium, A. niger, R. nigrican, Fusarium sp* and *Mucor circinelloides* (Odebode, 2002). Pandey *et al.* (2001) found species of *Penicillium* and *Trichoderma* from soils of tea growing locations in India. Similarly Karaoglu and Ulker (2006) shown that *Mycellia sterilla, Gliocladium virens* and *Trichoderma* were isolated from tea growing areas in Turkey. The findings of Moallaei *et al.* (2006) revealed that *A. flavus, Fusarium oxysporum. Anixiopsis stercoraria, Arthroderma guniculi, Reniospora flavissima, Chrysosporium keratinophilum, Trichophyton vanbreuseghemii, Geotrichum candidum, Microsporum gypseum* and *Penicillium* species were isolated from soil samples of forests and farm yards in Iran. Gaddeyya *et al.* (2012) reported *A. flavus, A fumigatus, A. niger, A. nidulans, A. terreus, Penicillium chrysogenum, P. frequentans, P. funicolosum, Trichoderma viride, T. harzianum, Fusarium oxysporum, F. solani, Curvalaria clavata, C. lunata* and *Rhizopus stolonifer* from different crop fields at Salur Mandal, India.

Cassava (*Manihot esculentum* Crantz) is the most important root crop and source of energy for human consumption. Cassava is a major food crop for an estimated 500 million people in Africa (Hillocks *et al.,* 2012*).* Root crops are used in the preparation of major staples eaten in Nigeria and are estimated to contribute about 15% of total protein in daily diet of the average Nigerian (Ogunmodele, 1983). Cassava can be used in the production of starch, ethanol, confectionaries and animal feeds (FAO, 2001). Production of Cassava is hampered by diseases caused by fungi,

bacteria and viruses. Among serious fungal diseases root rots affected both young and old cassava plants (Msikita *et al.,* 2005). Cassava root rots are caused by a complex of soilborne pathogens which induced damages that eventually reduce the yield (Ambe, 1994). Okigbo *et al.* (2009a) isolated *Fusarium oxysporum, Botryodiplodia theobromae, Macrophina phaseolina, Penicillium oxalicum and Aspergillus niger* from rotted cassava tubers. Msikita *et al.* (2005) reported *Nattrassia mangifera, Macrophina phaseolina, Botryodplodia theobromae*, *Fusarium sp.* and *Pythium* from the root of cassava in Benin. The finding of Lozano and Nolt (2000) showed that *Sclerotium rolfsii, Roselina necatrix, Fusarium moniliformae, F. oxysporum, Phythopthora erythroseptica, P. cryptogea, P. drechschleri, P. nicotina* were responsible for dry and wet tuber rot of cassava. Salami and Akintokun (2008) found that *Lasidiplodia theobromae, Macrophina phaseolina, Rhizopus stolonifer,* and *Fusarium pallidoroseum* were the fungal pathongens causing postharvest rot of cassava in Southwestern Nigeria.

Furthermore, Kolawole *et al.* (2009) isolated from rotten cassava *Aspergillus niger, A. flavus, R. stolonifer, Mucor racemosus* and *F. oxysporum.* While *Phytopthora palmovora, Fusarium solani* and *Sclerotium rolfsii* were isolated from infected cassava tubers (Johnson *et al.,* 2002). Banitol *et al.* (2010) confirmed the presence of *B. theobromae*, *Sclerotium rolfsii, Fusarium* sp. and *Phythium sp.* in diseased roots and stems of cassava in ecozones of Togo. Studies carried out by Fokunang *et al.* (2002) and Udoudoh (2011) revealed that *Colletotrichum gloeosporioides, Aspergillus sp,* and *Penicillium sp* were associated with postharvest storage and spoilage of cassava tubers. *Botryodiplodia acerina* has been reported to cause postharvest rot of cassava tubers (Amadioha and Markson, 2007). Snodow (1992) reported that *A. flavus, M. hiemalis, Rhizopus oryzae, B. theobromae* and *Fusarium solani* caused fungal rot of cassava tubers. This paper is aimed at identifying fungi associated with cassava fields in Sokoto and their relationship to postharvest deterioration of cassava.

Materials and Methods

Study area

Sokoto state is located on longtitude 3 – 9° east and latitude 10 - 14^0 north at an altitude of 398m (NMA, Sokoto 2009). The area has an annual average temperature of 27 °C. The temperature reaches peak in April at 40 °C and drops to 18 °C in December to January (Kowal and Knabe, 2002). The area has average annual rainfall of 550mm. The rainfall start in June, reaches peak in August and end in early September (MOI, 2008; NMA, 2009; SARDA,2009). The relative humidity is about 16-55.5% during dry season but can rise up to 81% during rainy season (SARDA ; NMA, 2009).

Collection of Soil Samples

Soil samples were taken from 5 different cassava fields in 5 locations in each of the collection areas. The collection areas are Damba in Illela local government, Raka in Tangaza local government, Wamakko in Wamakko local government and Lambara in Shagari local government areas of Sokoto State.

The soil samples were collected monthly (June to August) from the layer with depth not exceeding 0-9cm. The soil samples were transferred immediately to the laboratory in sterile polythene bags.

Isolation of fungi from soil sample

Soil dilution plate method was used for the isolation of fungi. One gramme of soil sample was suspended in 10ml distilled water in test tubes. The soil samples were shaken and serially diluted to 10^6 samples. One milliliter of each sample was pippeted onto sabouraud dextrose agar and incubated at 28 °C for 72 hours. Characteristic colony types were subsultured to obtained pure cultures of the isolates (Odebode, 2002).

Identification of soil fungi

The isolated fungi were identified based on the isolate colonial characteristics (colour and texture) and microscopic features such as nature of mycelium, type of fruiting bodies and the spore structure. The isolates were identified by reference to Kora *et al.* (2005).

Pathogenicity test

Fresh and healthy tubers of cassava were washed with tap water and surface sterilized with 70% ethanol. Cylindrical cores were removed from the tubers by means of sterile 5mm cork borer.4mm agar discs containing 7days old cultures of the isolates were introduced singly into the holes and sealed with sterile vaseline. Controls were set up as described except that the inocula consist of uninoculated potato dextrose agar blocks. All treated tubers were put singly in sterile polythene bags and incubated at 28 ^{o}C for 12days. Three replicate tubers were cut through and examine for rot at 3days intervals (Udo *et al.,* 2000).

Statistical analysis

The number of colonies per plate in 1g of soil was calculated. The percentage frequency of each isolate was calculated using the following formula:

$$\% \text{ frequency} = \frac{\text{Total no. of individual species}}{\text{Total no. of all species}} \times 100$$

The results of pathogenicity test were analysed using one-way analysis of variance (ANOVA) via instat 3a statistical package and least significance difference was used for mean separation at $P<0.05$.

Results and Discussion

The results show that the number of fungal species was found high in soil samples of cassava fields from Lambara (60) seconded by Damba (56) the least was recorded by Wamakko (44).The fungi isolated include *Alternaria species, Aspergillus fumigatus, Aspergillus niger, Cyclindrocarpon lichenicola, Fusarium oxysporum, Geotrichum candidum, Mucor hiemalis, Rhizopus oryzae* and *Scopulariopsis candida.* The highest frequency of occurrence was observed in *A. niger* (39.5%) followed by *Fusarium oxyspourm* (18.2%) and least percentage frequency of occurrence was recorded by *R. oryzae (*2.3%). It was observed that *A. niger* occurred in almost all the months between June to August. While *F. oxyporum* and *Alternaria* species occurred more frequently in the humid months of July and August. The results were shown in Table1.

Similarly Ogunmwonyi *et al.* (2008) they found *A. niger* as dominant fungi among fungi isolated from soil samples from Obafemi Awolowo University Ife. Also Sharma (2010) and Ishaq and Khann (2011) reported that *A. niger* and *A. fumigatus* were dominant among fungal species isolated from soil samples from Katao and agricultural fields at Ramgahn, India. The ability of *A niger* to dominate other fungal species could be linked to its high sporulating capacity and ability to produce toxins which prevent the growth of other fungi (Gaddeyya *et al.,* (2012). Studies carried out by Pandey *et al* (2001) and Koraoglu and Ulker (2006) indicated that species of *Penicillium, Trichoderma, Aspergillys* and *Fusarium* were isolated from soil of tea growing areas in India and Turkey. The occurrence of these fungal species in the soil was influenced by some factors such as temperature, pH, organic contents and moisture (Okigbo, 2003 Gaddeyyeya *et al.,* 2012).

The results of the study indicates that cassava fields from Lambara has the highest frequency of fungal species followed by by Damba and the least was recorded by Wamakko. The monthly (June – August) distribution of fungal species showed the highest frequency of fungal species occured in August (85) and the least frequency of occurence was observed in June (52). The distribution of the fungal species was found to be influenced by environmental factors such as temperature and moisture (Malik and Singh ,2004). At the inception of rainfall there was an increase in the population of the fungi. The fungi isolated during the month of June include *A. niger, A. fumigatus* and *C. lichenicola.*While fungi such as *F. oxysporum, M. hiemalis* and *Alternaria* species were found to be dominant in the humid months of July and August. The increase in percentage of fungi during humid months could be linked to the increase in the soil pH which affects availability of soil nutrients (Odebode, 2002).

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Table 1. Fungi isolated from cassava fields

Organisms	June				July				August					
	Collection Areas													
	DB	RK	WM	LM	DB	RK	WM	LM	DB	RK	WM	LM		Frequency (%)
Alternaria species	2	-	-	-	3	-	1	1	8	3	1	-	19	8.8
Aspergillus fumigatus	3	-	-	5	3	-	-	-	3	-	-	-	14	6.5
Aspergillus niger	9	6	9	6	5	7	5	6	8	6	10	8	85	39.5
Cylindricarpon liichenicola	-	-	-	4	-	-	-	2	-	-	-	-	6	2.8
Fusarium oxysporum	-	-	-	1	-	7	6	5	-	11	-	9	39	18.2
Geotrichum candidum	-	2	-	-	-	-	3	-	-	2	-	-	7	3.3
Mucor hiemalis	-	2	-	-	8	2	2	9	-	5	4	2	34	15.8
Rhizopus oryzae	-	-	1	-	-	-	2-	-	1	-	-	1	5	2.3
Scopulariopsis candida	-	2	-	-	1	-	-	-	2	-	-	1	6	2.8
Total	14	12	10	16	20	16	19	23	22	27	15	21	215	100

Key : DB = Damba ; RK = Raka ; WM : Wamakko; LM = Lambara

Table 2. Pathogenicity of fungi isolated from cassava fields on cassava tubers (Diameter of rot in mm).
Mean of the determination with three replications ± Standard Erro. Least Significant Difference (LSD).
Values with the same alphabet along column are not significantly different at (P<0.05).

Isolates	Incubation Period			
	3 days	6 days	9 days	12 days
Cylindricarpon Lichonicola	2.5 ± 0.20g	4.5 ± 0.64d	8.1 ± 1.10f	11.3 ± 1.00g
Alternariaspecies	5.0 ± 0.12e	8.0 ± 0.36c	14.0 ± 0.29d	19.0 ± 0.40e
Aspergillus fumigatus	6.0 ± 0.12d	14.0 ± 0.21b	24..0 ± 1.27c	29.0 ± 0.15d
Aspergillus niger	7.0 ± 0.25c	15.0 ± 0.16a	28.0 ± 0.66b	36.0 ± 0.68c
Rhizopus oryzae	2.3 ± 0.31g	4.5 ± 0.61d	7.1 ± 0.89f	10.3 ± 0.90g
Fusarium oxysporum	8.0 ± 0.32b	16.0 ± 0.74a	28.0 ± 0.26b	38.0 ± 0.72b
Geotrichum candidum	4.0 ± 0.15f	7.0 ± 0.36c	11.0 ± 0.40e	16.0 ± 0.47f
Mucor hiemalis	9.0 ± 0.21a	16.0 ± 0.38a	33.0 ± 0.64a	48.0 ± 0.36a
Scopulariopsis candida	3.0 ± 0.20g	5.0 ± 0.79d	12.0 ± 0.31e	18.0 ± 0.36e
Control	0.0 ± 0.0h	0.0 ± 0.0e	0.0 ± 0.0g	0.0 ± 0.0h
LSD(0.05)	0.75	1.66	1.75	1.39

The results of pathogenesis were shown in Table 2 .The results indicate that all the fungi isolated from soil samples of cassava fields were found to be pathogenic on cassava tubers.The pathogens showed variation in nature and extent of rots.The fungus *M. hiemalis* is the most pathogenic followed by *F. oxysporum* and *R. oryzae.*was the least pathogenic fungus.

It was observed that there is relationship between the soilborne fungi and incidence of post harvest rot of cassava tubers. The soil fungi got to the soil through infected plant parts and thus serve as source of inocula in the soil. These fungal pathogens overwinter in the soil and when cassava plants develop tubers and the tubers are wounded they become infected by the pathogens.

Conclusion

This study reveals that 9 soilborne fungal species were associated with cassava fields in Sokoto. It is apparent that there is relationship between the soilborne fungi and incidence of post harvest roots rot of cassava. Therefore, it would be recommended that cassava fields should be allow to fallow for some years, alternate host plants should not be allowed to grow in the fields, healthy cassava cuttings should be used for planting to avoid reintroduction of pathogens and the use of resistant planting materials to reduce incidence of rots.

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