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Contribution to the Knowledge of Soil Fungi in Sudan Rhizosphere Mycoflora of Sugarcane at Kenana Sugar Estate

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Abstract: Twenty different sites at Kenana Estate of Sudan were examined for their rhizosphere fungal mycoflora. A modified soil-dilution plate method was used for isolating the fungi. Twenty three different species in sixteen genera and six sterile fungal taxa were reported. Of these species twenty five were Hyphomycetes, three were Zygomycetes and one belongs to the order Sphaeriales. The richest taxa in abundance were *Aspergillus* (two species), *Penicillium* (one species), *Alternaria* (one species), *Rhizopus* (one species), *Curvularia* (one species) and *Fusarium* (one species). All of the reported species were new records to the area and thirteen are new records to the flora of Sudan. Fungal activities increased with plant age. When near crop maturity the number of colonies declined. The results of rhizosphere fungal mycoflora in different sites were slightly varying and the number and type of fungal colonies in some areas were nearly the same.

Key words: Soil, mycoflora, fungi, rhizosphere, sugarcane

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is grown largely under long-term monoculture production with successive annual harvests obtained from an initial vegetative planting of stalks. Therefore, sugarcane cultivars are clones composed of genetically identical plants. Buds on planted stalk sections germinate and grow to produce the first plant cane crop. Following the first harvest of the stalks, buds on the stubble remaining in the soil can germinate to produce a ratoon crop. In Kenana Sugar Estate of Sudan, sugarcane plants are typically in the field continuously for 3-5 years. The stubble is then plowed out during the spring and following a 4-6 month plant-free fallow period, the crop is replanted during late summer.

Monoculture can lead to continuous multiplication of fungi and may also lead to poor root health or growth constraints and sometimes improvement of the crop stand (Garside *et al.*, 1997). Few investigations have been made concerning fungus flora in the rhizosphere of sugarcane in Sudan. In a preliminary survey of sugarcane mycoflora, Tarr (1963) isolated fourteen different fungal genera and thirty two species from different sugarcane plantations throughout Sudan. Subsequently, El Amin and Abdalla (1980) reported seventeen species in some sugarcane fields in Gunied factory of the Gezira area. They mentioned that lower fungi were mostly represented by

mucoraceous genera such as *Rhizopus*, *Mucor* and *Cunninghamella*, while Oomycetes were rare and Ascomycetes other than the perfect states of *Aspergillus* were not common. Furthermore, Abdel-Rahim *et al.* (1983) studied the activities of sugarcane soil fungi in North West Sennar plantation. They also compared the abundance of soil fungi in two sugarcane soil types i.e., Dindir clay and Hago sandy. More so, they added that isolated fungi from both rhizosphere and non-rhizosphere soils were dominated by the genera *Aspergillus* and *Rhizopus*. Fungi including *Fusarium* sp., *Curvularia* sp. and dark sterile mycelia were present in higher frequencies on root surfaces than in the surrounding soils. In Kenana Sugar Estate no attempts have been made to survey rhizosphere mycoflora of sugarcane, therefore the objective of the present study was aimed at examining rhizosphere fungi of sugarcane and to determine if there are differences in microbial communities associated with rhizosphere in soil and possible changes in microbial communities resulting from monoculture production.

MATERIALS AND METHODS

The study was conducted at the Botany and Plant Pathology Laboratory, Gezira Research Station, Wad Medani.

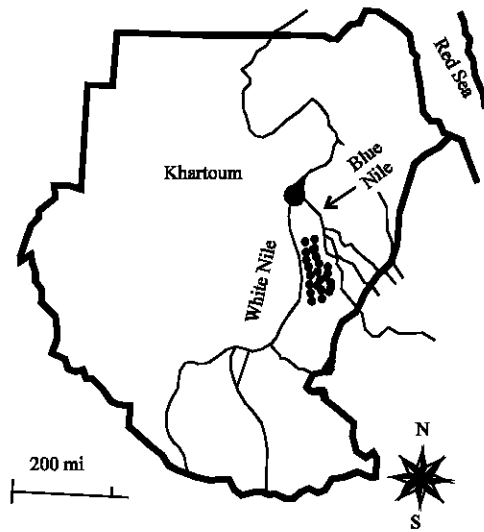


Fig. 1: Map of Sudan locating sites (thick dots) where the different soil samples were collected

Study area: Kenana Sugar Estate is located 150 miles south of country capital Khartoum, on the east bank of the White Nile, opposite the Provincial town of Kosti in the central region of Sudan (Fig. 1). It is the most important area for sugar production in Sudan, whereas the crop of sugarcane (*Saccharum officinarum* L.) is grown extensively. The Estate has 45, 000 ha of land that produce four million tonnes of sugarcane a year and a factory with a capacity for grinding of 26, 000 tons a day.

Sampling procedure: A survey was undertaken during 2002-2003 to determine the rhizosphere mycoflora associated with sugarcane in twenty locations covering Kenana Estate of Sudan (Fig. 1). Soil samples were collected from the rhizosphere of sugarcane (*Saccharum officinarum* L. cv. NCO 376 and cv. CO 527) at each location. Approximately one kilogram soil samples (included plant debris) were collected with a soil auger at a depth of 10-25 cm from the rhizosphere of sugarcane plants using the conventional sampling method (Johnson *et al.*, 1972). The samples were placed in clean plastic bags. Five soil samples taken (four at the corner and one at the center) from each study site were mixed thoroughly into one composite sample. Ten gram sub-samples of soil from each composite sample were processed for isolation of soil mycoflora. In cases where processing of the samples could not be done on time, the samples were refrigerated (4°C) for not more than two weeks.

Soil analysis: Soil samples from each area or location were analyzed for pH, organic matter and content of total

N, P and k by sending them to the Analytical Services Laboratory, Department of Soil Science, Gezira Research Station, Wad Medani.

Isolation of mycoflora from soil: The mycoflora of the soils was isolated following the soil dilution-plating technique of Johnson *et al.* (1960) and that modified by Warcup (1960). A sample of 10 g soil was placed in a graduated cylinder, added with sterilized distilled water to make a total of 100 mL. The suspension was stirred, poured into sterile 250 mL Erlenmeyer flask and shaken thoroughly for 30 min. One milliliter of the suspension was pipetted aseptically and dispensed in dilution test tubes with 9 mL of sterilized distilled water. Series of soil dilutions of 1:10, 1:100, 1:1, 000 and 1:10, 000 were prepared. One milliliter of the desired dilution (10^{-3} and 10^{-4}) was transferred aseptically into a sterile disposable petri dish with 10-12 mL of melted Potato Dextrose Agar (PDA) adding one drop of 20% lactic acid to suppress bacterial growth (Sinaga and Quimio, 1987). The composition of PDA was 200 g potatoes, 20 g dextrose, 15 g agar and distilled water one liter. Then the medium was autoclaved at 15 pounds per square inch pressure for 15 min. The plate was rotated by hand in a broad and slow swirling motion to disperse the soil suspension. Five Petri dishes were provided for each dilution. Plates were incubated at room temperature for 5 days. After incubation, a small portion of mycelium from each fungal colony was transferred into PDA slants.

Pure cultures of isolates were grown on standard and few selective media and identified using the fungal keys provided by Domsch *et al.* (1980), Sinaga and Quimio (1987), Singh *et al.* (1991), Quimio and Hanlin (1999) and Quimio (2001). The soil fungi isolated were deposited at the Botany and Plant Pathology department, Gezira Research Station, Wad Medani.

The number of fungal colonies isolated and proportion of the total number of species were determined. The relative abundance of soil mycoflora in rhizosphere of sugarcane was measured using the formula:

- +++ = High (11-up) detected isolates.
- ++ = Moderate (6-10) detected isolates.
- + = Low (1-5) detected isolates.

Data analysis: Data obtained were pooled and the means were used in analysis for significant differences using appropriate statistical procedure (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The results given in Table 1 show that the soils of Kenana Sugar Estate are generally slightly rich in organic matter content. The potassium content varies from 0.9 to 4.9% and phosphorous from a low value 6 to relatively high value 89 ppm. The values of nitrogen percent show no appreciable differences ranging from 0.11 to 1.18%. The pH values on the other hand, also reveal no appreciable differences; all are calcareous and range from 4.9 to 6.6. The soil samples are of characteristically heavy clay type.

In the isolation studies of the rhizosphere mycoflora in sugarcane soils a total of twenty three species in sixteen genera with six sterile species were recovered (Table 2). Most of the fungal genera isolated belonged to the class Hyphomycetes. Three species were *Zygomycetes* and one to the order *Sphaeriales*. All these fungal species were new records from Kenana sugar Estate of Sudan. According to their occurrence in the soil samples, *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium* and *Curvularia* are of high occurrence. The two former fungal genera were present in all the soil samples and the other three were absent from two samples only.

Table 1: Total number of fungal colonies isolated from soil samples in Kenana sugarcane fields, with chemical analysis of soil

Study site	Total No. of fungal colonies isolated	pH	Organic matter (%)	N (%)	P (ppm)	k (cmol kg ⁻¹ soil)
23202	16	5.3	4.96	0.14	12	4.2
42105	22	6.1	2.00	1.18	6	0.9
31203	23	5.1	3.00	0.11	18	3.4
42302	26	5.0	1.50	0.14	19	3.5
14103	33	6.3	4.10	0.18	87	4.9
43205	44	5.7	4.99	0.89	44	4.0
41307	11	6.4	1.81	0.11	24	2.8
51101	30	5.9	2.90	0.17	77	4.7
13404	18	6.0	4.56	0.15	14	1.9
32103	27	5.6	2.86	0.14	21	2.3
32302	42	5.2	3.90	0.26	41	3.8
51302	31	4.9	3.47	0.19	89	5.0
51301	26	5.7	3.00	0.28	18	3.3
11101	23	5.4	2.81	0.41	18	3.5
32303	21	5.9	3.01	0.49	7	1.0
22406	16	6.1	2.23	0.18	13	1.8
51303	8	6.6	1.89	0.12	20	3.6
82303	19	4.9	3.50	0.11	15	2.0
22403	27	6.0	4.97	0.14	21	2.4
31104	43	6.2	5.00	0.30	42	3.9

Table 2: Relative abundance of fungal taxa from rhizosphere of sugarcane at Kenana Sugarcane Estate of Sudan

Fungal group	Fungal species	Abundance*	Proportion of total number (%)
Mucorales	<i>Cunninghamella elegans</i> Lendn.	+	2.7
	<i>Mucor hiemalis</i> Wehmer	++	18.9
	<i>Rhizopus oryzae</i> Went and Prins. Geerl.	+++	67.6
Sphaeriales	<i>Chaetomium kunze</i> ex Fr.	+	8.1
Hyphomycetes	<i>Acremonium</i> sp.	++	24.3
	<i>Alternaria alternata</i> Keissl.	+++	59.4
	<i>Aspergillus candidus</i> Link ex Fr	+++	75.7
	<i>A. flavus</i> Link ex Gray	++	27.2
	<i>A. fumigatus</i> Fresen.	++	24.3
	<i>A. niger</i> Tiegh.	+++	89.2
	<i>A. melleus</i> Yukawa	+	8.1
	<i>A. tamarii</i> Kita	++	37.8
	<i>Botrytis cinerea</i> Pers. ex Nocca and Balb.	+	10.8
	<i>Cladosporium cladosporoides</i> de Vries	++	24.3
	<i>Curvularia spicifera</i> Boedijn	+++	89.2
	<i>Fusarium</i> sp.	+++	91.9
	<i>Humicola grisea</i> Traaen var. <i>grisea</i>	+	13.5
	<i>Paecilomyces marquandii</i> (Masse) Hughes	+	5.4
	<i>Penicillium chrysogenum</i> Thom	+++	75.7
	<i>P. expansum</i> Link ex Gray	++	16.2
	<i>P. roquefortii</i> Thorn	+	5.4
	<i>Trichoderma harzianum</i> Rifai	++	21.6
<i>Verticillium lecanii</i> (Zimm.) Viegas	++	18.9	
Sterile fungus 1	+	2.7	
Sterile fungus 2	+	8.1	
Sterile fungus 3	+	13.5	
Sterile fungus 4	+	2.7	
Sterile fungus 5	+	8.1	
Sterile fungus 6	+	8.1	

* + = Low (1-5) detected isolates; ++ = Moderate (6-10) detected isolates; +++ = High (11-up) detected isolates

Table 3: Total number of fungal colonies isolated from 10 g soil samples in some selected fields in Kenana aera at three sampling dates*

Sampling field	No. of colony at three sampling dates			No. fungal genera
	Early season	Mid season	Late season	
31104	25a	102a	85a	18
32302	17b	67b	44b	15
43205	32a	137c	97a	21
14103	13b	47d	22c	13
51302	10b	27e	15c	11

*Values are means of 5 replicates. Means followed by the same letter in columns are not significantly different ($p < 0.05$)

Mucor, *Acremonium*, *Cladosporium*, *Trichoderma* and *Verticillium* were of moderate occurrence. Five genera were of low occurrence, namely *Cunninghamella*, *Chaetomium*, *Botrytis*, *Humicola* and *Paecilomyces*.

Significant variation in total number of fungal colonies and percent abundance of fungal species was observed in rhizosphere soil obtained from various study sites ($p < 0.05$). Plots in the centre of the Estate i.e., field number 43205, 31103 and 32302 showed the highest isolated number of fungal colonies. The lowest number of fungal colonies was isolated from location number 51303 with an average number of eight fungal colonies (Table 1).

Fungal activities increased with plant age. In early season the number of colonies increased progressively following the existence of the crop until reached the peak level at the middle of the growing season with average number of 137 colonies. When near maturity the number of colonies declined with an average 97 colony counts (Table 3). The reason behind this might be due to root senescence at the end of the growing season and suitable foot was scarce in the niche, which may activate the competitiveness between mycoflora in soil.

The results obtained showed that organic matter, pH, N, P and K in soil have an effect on the population of fungi in soil (Table 1). Soils with high levels of organic matter have a more complex and active mycoflora. Also, significant correlation was detected between the percentage of soil organic matter and the number of colonies isolated at 5% level (Fig. 2). However, the number of fungi was inconsistent and slightly correlated with pH, N, P and K content in the soil (Table 1).

The addition of organic amendment to soil in Kenana Estate area promotes growth of saprophytic fungi which in turn reduces the populations of pathogenic fungi. This observation was in close agreement with other findings which suggest that compost can be used as a source of organic matter because according to Hoitink and Boehm (1999), compost will provide (a) successful competition for nutrients by beneficial mycoflora, (b) antibiotic production by beneficial mycoflora, (c) successful parasitism against pathogens by beneficial mycoflora and (d) activation of disease resistant genes in plants by

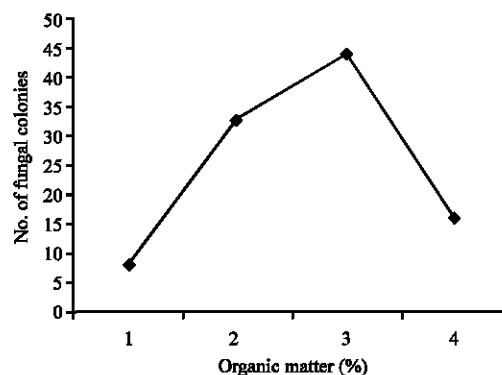


Fig. 2: Correlation of organic matter and number of colonies in Kenana Sugar Estate of Sudan

mycoflora (induced systemic resistance). Consequently, the coarse grass and corn (*Zea mays*) amendment increased the population of *Trichoderma harzianum* and controlled the foot rot disease incidence on sugarcane seedlings (Manohara *et al.*, 2003). Applying the coarse grass to the soil every 3-5 weeks could maintain *Trichoderma* population in the soil with 40% field capacity (Manohara *et al.*, 2004). However, the role of mycoflora antagonism in contributing to the activation of disease resistance-genes in sugarcane plant has not been experimentally established (Glaz and Gilbert, 2006).

Qualitative differences between fungal communities were also detected with soil dilution plating on culture media and with sole carbon source (Hoitink and Boehm, 1999). These findings could be significant, since members of these groups of microorganisms can inhibit the growth of root pathogens, including *Pythium* (Wahid *et al.*, 1997). Evidence obtained with other plants besides sugarcane support the idea that plant type can influence the make-up of the rhizosphere microbial community (Quimio, 2001). A dilution plate culture method study conducted by Rouatt and Katznelson (1961) compared microbial communities of rhizosphere, rhizoplane and non-rhizosphere soil of six different plant species. They found that rhizosphere soil microbial communities from six different crop plants tested were affected by plant type rather than soil type.

Moreover, Warcup (1970) reported that the most common fungi isolated using dilution plates from wheat field soil are fungi imperfecti, mycelia sterilia, hycomycetes and ascomycetes. According to Wahid *et al.* (1997), the genera *Aspergillus* and *Penicillium* are the richest amongst all the genera of class hyphomycetes found in soils of tomato field. It was reported by Domsch *et al.* (1980) that *Aspergillus niger* was found in soil with pH range of 4-8. There was an increase in relative numbers after soil fumigation, after manuring and NPK fertilization. Domsch *et al.* (1980) also

reported that *Cunninghamella elegans* is particularly frequent from cultivated soils, but it has also been reported from uncultivated soils, grassland, a vineyard with a high copper content and rice fields. Lewis and Papavizas (1985) mentioned that *Trichoderma* in natural soil requires substrates as source of nutrients to enhance growth, survival and competitiveness. *Trichoderma* occurs relatively frequently in the surface (litter) layer of soils and generally in slightly acid habitats.

Research in sugarcane also suggested that application of the broad-spectrum fungicide, mancozeb, decreased root colonization by a dematiaceous, sterile fungi and improved root health and plant growth (Magarey *et al.*, 1997). Kao and Hsieh (1986) found that in monocultured sugarcane soil *Fusarium* and *Trichoderma* were predominated. Watanabe *et al.* (1974) also found that *Fusarium* was frequently isolated from both healthy and unhealthy sugarcane. The microbial community comparison was therefore, focused on the rhizosphere of sugarcane roots, since this community is most likely to affect plant growth (Bramley *et al.*, 1996). Further studies are needed to correlate seasonal fluctuation of fungal communities in the rhizosphere and non-rhizosphere of sugarcane with crop stand and productivity in the area.

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