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Micro-organisms associated with *Striga hermonthica* and rhizosphere soil in the nigerian savannah

Isolation and evaluation for possibilities in biological control of the witchweed

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Abstract — A survey of micro-organisms associated with *Striga hermonthica* (Del.) Benth was conducted in 2000 and 2001 cropping seasons in the Nigerian savanna. 35 fungal isolates were obtained from rhizosphere soil without sorghum and with *Striga* plants, 16 isolates from rhizosphere soil with sorghum and *Striga* plants, 15 isolates from rhizosphere soil with sorghum and without *Striga* plants, 16 isolates from diseased *Striga* plants and 3 isolates from *Striga* seeds. Samples were cultured on Potato dextrose agar amended with streptomycin (PDAS). 85 fungal isolates divided into 17 species belonging to 10 genera were identified. These genera are: *Aspergillus, Candida, Curvularia, Fusarium, Gloeosporium, Helminthosporium, Monilia, Phyllosticta, Rhizoctonia Trichoderma*. The most prevalent fungal species were *Aspergillus flavus, A. fumigatus, A. niger, Fusarium* spp. and *Rhizoctonia* spp. All fungi isolated were evaluated for their pathogenicity against *S. hermonthica*. Evaluation of the seventeen fungal species based on literature identified 10 most promising to be *Fusarium oxysporum, Fusarium* (2 isolates unidentified), *Monilia* sp., *Helminthosporium* sp., *Curvularia* sp., *Aspergillus niger, A. flavus, A. fumigatus* and *A. terreus*. Further laboratory evaluation identified 6 fungal species to be most promising. These are *Fusarium oxysporum, Fusarium* (2 isolates unidentified), *Curvularia* sp., *Aspergillus niger* and *A. terreus*.

Résumé — Les micro-organismes associés à *Striga hermonthica* et à la rhizosphère dans les savanes du Nigeria : isolement et évaluation pour la lutte biologique contre cette adventice. Un inventaire des micro-organismes associés à *Striga hermonthica* (Del.) Benth a été effectué durant les campagnes 2000 et 2001 dans les savanes du Nigeria. 35 isolats fongiques ont été obtenus de la rhizosphère sans sorgho et avec *Striga*, 16 isolats de la rhizosphère avec sorgho et plants de *Striga*, 15 isolats de la rhizosphère avec sorgho et sans plants de *Striga*, 16 isolats de plants de *Striga* malades et 3 isolats des graines de *Striga*. La culture des échantillons a été faite sur un milieu composé de pomme de terre avec dextrose et agar, amendé de streptomycine (PDAS). 85 isolats fongiques répartis en 17 espèces appartenant à 10 genres ont été identifiés. Ces genres sont : *Aspergillus, Candida, Curvularia, Fusarium, Gloeosporium, Helminthosporium, Monilia, Phyllosticta, Rhizoctonia* et *Trichoderma*. Les espèces fongiques les plus répandues étaient *Aspergillus flavus, A. fumigatus, A. niger, Fusarium* spp. et *Rhizoctonia* spp. Tous les champignons isolés ont été évalués pour leur action pathogène contre *S. hermonthica*. L'évaluation des dix sept espèces fongiques, basée sur la littérature, a identifié 10 espèces prometteuses à savoir *Fusarium oxysporum, Fusarium* (2 isolats non identifiés), *Monilia* sp., *Helminthosporium* sp., *Curvularia* sp., *Aspergillus niger, A. flavus, A. fumigatus* et *A. terreus*. De plus, l'évaluation au laboratoire a identifié les 6 espèces fongiques les plus prometteuses. Il s'agit de *Fusarium oxysporum, Fusarium* (2 isolats non identifiés), *Curvularia* sp., *Aspergillus niger* et *A. terreus*.

Introduction

Biological control is the use of one or more organisms (biotic agents) to maintain another organism (the pest) at a level at which it is no longer a problem (Evans, 1991). The first noteworthy biocontrol was in California in 1888 (National Academy of Sciences, 1971). The genus *Striga*, erected by Loureiro in 1790, and belonging to the family Scrophulariaceae (Musselman, 1987), is commonly known as witchweed (Tarr, 1962). *Striga hermonthica* (Del.) Benth is the major witchweed of Africa and its former name is *S. senegalensis* Benth (Doggett, 1988). Yield losses due to *S. hermonthica* vary from 0 to 100% (Agrios, 1988; Kiriro, 1991). *S. hermonthica* is a problem in Nigeria (Akpa *et al.,* 1996), Chad (Nekouam, 1993), Kenya, where it is called the giant witch weed (Kiriro, 1991), and in all sub-Saharan Africa (Abbasher *et al.,* 1998). Among biological agents of *S. hermonthica* exist the gall-forming weevil *Smicronyx* (Berner *et al.*, 1993), *Fusarium* spp. and notably *F. oxysporum* (Abbasher *et al.,* 1998; Marley *et al.,* 1999). *F. oxysporum* is not pathogenic to sorghum (Kroschel *et al.,* 1996).

The objectives of the study are to identify fungi associated with *S. hermonthica* and rhizosphere soil and to evaluate them for their parthogenicity against *S. hermonthica*.

Materials and methods

Sterilization and culture medium

Sterilization of materials for laboratory work was done as common procedures described by Agrios (1988). The culture medium was Potato dextrose agar amended with streptomycin or PDAS (Johnston and Booth, 1983).

Isolation of fungi

Fungi were isolated from rhizosphere soil, diseased *Striga* plants and *Striga* seeds. Rhizosphere soil was divided into three parts: rhizosphere soil without sorghum; rhizosphere soil with sorghum and *Striga* plants; rhizosphere soil with sorghum and without *Striga* plants.

For rhizosphere soil without sorghum, soil samples were taken from depths of up to 20 cm with an auger in two plots heavily infested by *Striga hermonthica* at the rate of 5 soil samples per plot. Soil samples were bulked per site and ground in powder. This bulk was made of 10 g per soil sample. Ten mg of bulk soil were used for soil dilution plate and serial dilutions as described by Johnston and Booth (1993). Seven test tubes were used. The content of each of them was for 3 Petri dishes, i.e. 21 Petri dishes for 7 test tubes. Incubation was done at 25-30 °C and daily observation was done for visible fungal growth.

For rhizosphere soil with sorghum and *Striga* plants, sorghum was digged out with roots and shaken to discard the excess soil. Any aggregates were removed and discarded, leaving only that soil which is adhering closely to the root system, as described by Dhingra and Sinclair (1995). Roots were cut and placed in a screw-cap bottle containing 10 ml sterile water. The bottle was shaken until most of closely adhering rhizosphere soil was removed. Roots were removed and placed in another bottle of 10 ml sterile water and were shaken again. Water from both bottles was mixed to prepare serial dilutions. Incubation was done at 25-30 °C and daily observation was done for visible fungal growth.

For rhizosphere soil with sorghum and without *Striga* plant, soil samples were obtained using the same method as described above. The same method of soil dilution plating used in rhizosphere soil with sorghum and *Striga* plant was used.

To isolate fungi from diseased *Striga* plants, the diseased plant part (stem or leaf) was cut into small pieces of 1 cm^2. These pieces were surface-sterilized in 5 % sodium hypochlorite for 3 minutes. Then, they were put into Petri dishes containing PDAS, at the rate of 4 pieces per Petri dish replicated 5 times. The plates were incubated at 25-30 °C for 5-7 days for visible fungal growth.

For isolation of fungi from *Striga* seeds, 10 mg of these seeds were placed in a Petri dish containing PDAS. The content was agitated to disperse the seeds in the medium. This carried out for 10 Petri dishes and incubated at 25-30 °C for visible fungal growth.

Purification, identification and maintenance of fungi

Pure cultures of all fungi isolated were obtained using the method described by (Johnston and Booth, 1983). Fungi were identified under microscope by using Saccardian System (Barnett 1962; Barnett and Hunter, 1990). Specimens of identified fungi were sent to the International Mycological Institute (IMI) in England for confirmation. The purified and identified fungi were maintained as slants in McCarteney bottles of Potato dextrose agar medium (PDA).

Evaluation of fungi

All fungi identified were subjected to literature evaluation and those which were promising were retained for laboratory evaluation against *S. hermonthica*. Laboratory evaluation of fungi was based on production of *Striga* germination stimulant, and on *Striga* conditioning and pathogenicity of fungi. *Striga* germination stimulant or sorghum root exudate was prepared by "Double Pot" system (Berner *et al.,* 1993). Sorghum root exudate was refrigerated for 5 days before using it to stimulate *Striga* germination.

In *Striga* conditioning, 10 mg *Striga* seeds were immersed for 1 day in 25 ml sterile water per Petri dish containing 1 filter paper and were dried for 4 days, alternatively for 10 days. Two filter papers were put in each other Petri dish and were wet with about 1 ml sterile water. Conditioned *Striga* seeds were transferred to the wet Petri dish containing 2 filter papers. The filter paper from conditioning Petri dish and containing *Striga* seeds was taken with forceps and put in the Petri dish containing 2 wet filter papers. In each Petri dish containing 3 filter papers and *Striga* seeds, were added 25 ml of refrigerated sorghum root exudate and 1 cube PDAS of fungus cut with 5 mm diameter Cork borer, at the rate of 3 Petri dishes per fungus, knowing that there were 10 fungi. The 3 Petri dishes represented 3 replications. There were also 3 Petri dishes without fungus as 3 checks. All these 33 Petri dishes were left in dark place for 2 days before counting germinated and non germinated *Striga*, for calculation of germination percentage. Fungi inhibiting *Striga* germination most were retained for greenhouse evaluation.

Results and discussion

Micro-organisms associated with *Striga hermonthica* and rhizosphere soil in the Nigerian Savanna are shown in table I. This survey was conducted in 2000 and 2001 cropping seasons. The total number of fungal isolates was 85. They are divided into 17 species belonging to 10 genera, which are: *Aspergillus*, *Candida*, *Curvularia*, *Fusarium*, *Gloeosporium*, *Helminthosporium*, *Monilia*, *Phyllosticta*, *Rhizoctonia* and *Trichodema*. There were 35 isolates from rhizosphere soil without sorghum and with *Striga* plants, 16 isolates from rhizosphere soil with sorghum and *Striga* plants, 15 isolates from rhizoshere soil with sorghum and without *Striga* plants, 16 isolates from diseased *Striga* plants and 3 isolates from *Striga* seeds. The most prevalent genera encountered were *Aspergillus* (55 isolates) and *Fusarium* (8 isolates). The less prevalent genera were *Candida* and *Phyllosticta* (one isolate). The most 7 prevalent species were *Aspergillus Flavus, A. fumigatus*, *A. niger*, *Fusarium* spp. (2 isolates) and *Rhizoctonia* spp. (2 isolates).

Table I. Prevalence of Fungal Genera Isolated in 2000 and 2001 in the nigerian savanna.

Genus	Isolate number	Prevalence (%)
*Aspergillus**	55	65 (1) **
Candida	1	1 (9ex)
Curvularia	2	2 (6ex)
*Fusarium**	8	10 (2)
Gloeosporium	3	4 (5)
Helminthosporium	2	2 (6ex)
Monilia	2	2 (6ex)
Phyllosticta	1	1 (9ex)
Rhizoctonia	7	8 (3)
Trichoderma	4	5 (4)
Total	85	100 Mean = 10

* The most 2 prevalence genera (prevalence ≥ general mean); ** Classification by decreasing order.

All the fungi isolated were evaluated for their pathogenicity against *S. hermonthica*. Evaluation of the seventeen fungal species based on literature identified 10 most promising to be *Fusarium oxysporum, Fusarium* (2 isolates unidentified), *Monilia* sp., *Helminthosporium* sp., *Curvularia* sp., *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *A. terreus* (Table II). Literature used to evaluate these fungi are indicated as follows: 7 for *Fusarium oxysporum* (Marley *et al*., 1999); 10 for *Fusarium* spp. (Abbasher *et al*., 1998); 5 for *Curvularia* spp., (Kroschel *et al*., 1996); 1 for *Trichoderma* spp. (Wells, 1988); 2 for *Trichoderma harzianum* (Wells, 1988 and Lo *et al.,* 1996); 3 for *Phyllosticta* spp., (Cobraz 1990); 1 for *Gloeosporium* spp. (Agrios, 1988); 27 for *Rhizoctonia* spp. (Tarr, 1962).

Table II. Evaluation of fungi against *Striga hermothica* based on literature.

Promising fungi	*Fusarium oxysporum* * (7 References)
	Fusarium spp. * [2 isolates (10 References)]
	Aspergillus flavus * (1 Reference)
	A. fumigatus * (1 Reference)
	A. niger * (1 Reference)
	A. terreus * (1 Reference)
	Curvularia sp. * (5 References)
Non-promising fungi	*Trichoderma* sp. (1 Reference)
	T. harzianum (2 References)
	Phyllosticta sp. (3 References)
	Gloeosporium sp. (1 Reference)
	Rhizoctonia spp. (27 References)
Fungi without information	*Helminthosporium* sp. *
	Monila sp. *
	Candida sp.

* Selected to be evaluated in laboratory (10 Fungi).

Table III. Laboratory Evaluation of Fungi Based on *Striga* Germination Percentage.

No.	Treatment	Fungus	Experiment 1 (28/5-26/6/01)	Experiment 2 (21/6-5/7/01)	Final Selection
1.	- $6B'_2$	*Fusarium oxysporum* *	6 (S)	16 (S)	(S)
2.	- $3C_1a_2$	*Fusarium* sp. *	2 (S)	18 (S)	(S)
3.	W_2a_2	*Fusarium* sp. *	8 (S)	8 (S)	(S)
4.	l_{2-5}	*Monilia* sp.	19	47	
5.	l_{2-7}	*Helminthosporium* sp.	23	53	
6.	l_{1-5}	*Curvularia* sp. *	13 (S)	23 (S)	(S)
7.	- 1b	*Aspergillus niger* *	17 (S)	19 (S)	(S)
8.	- 4b	A. flavus	25	42	
9.	- 4b	*A. fumigatus*	18	18 (S)	
10.	T_1	*A. terreus* *	17 (S)	29 (S)	(S)
11.	C	*Check*	43	79	
			Mean = 17	Mean = 32	

* 6 Promising fungi selected for biocontrol of *Striga hermonthica*. (S): Selected.

Laboratory evaluation identified 6 fungal species to be most promising (table III). These are *Fusarium oysporum, Fusarium* (2 isolates), *Curvularia* sp., *Aspergillus niger* and *A. terreus*. In the first experiment, *Fusarium* sp. isolated from rhizosphere soil with sorghum and *Striga* plant occupied the first rank, followed by *Fusarium* sp. isolated from wilted *Striga*. In the second experiment *Fusarium* sp. isolated from wilted *Striga* occupied the first rank, followed by *Fusarium* sp. (*F. oxysporum*) isolated from rhizosphere soil with sorghum and without *Striga* plant. Results from laboratory tests show clearly that *F. oxysporum* from wilted *Striga* plants and rhizosphere soil with *Striga* are the most promising for use in the biocontrol of *Striga* in the

Nigerian Savanna. This underscores the report of Marley *et al.,* 1999 who used *F. oxysporum* isolated from wilted *Striga* plants. Further, works by Abbasher *et al.*, 1998 have also shown that *Fusarium* spp. from *Striga* plants is the most virulent isolates in control of *Striga*.

Further work is on-going to determine the most suitable *F. oxysporum* isolate under Sudano-Sahelian conditions and the most suitable form of its application for use by peasant farmers.

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