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FUNGI ASSOCIATED WITH SEEDS OF THREE VARIETIES OF SORGHUM GROWN IN BOTSWANA AND ITS CONTROL *IN VITRO* USING FUNGICIDE AND PLANT EXTRACT TREATMENT

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Abstract: Seeds of three sorghum varieties namely Segaolane, Phofu and SNK-3939 obtained from different locations in Botswana were used for detection of seed-borne fungi and their possible control by botanical and fungicides. Four hundred seeds of each variety were surface sterilized with 2% sodium hypochlorite solution before placing them on moist blotter (10 seeds/90cm Petri plate), and incubated for seven days at 22±2° C for 12 hours under continuous light and alternating with 12 hours darkness. Eight seed-borne fungi were recovered. These were *Aspergillus flavus, A. niger, Alternaria alternate, A. infectoria, A. solani, Curvularia lunata, Fusarium moniliformae and F. oxysporum. Fusarium* species were dominant fungi recovered from the seeds, followed by *Alternaria* and *Aspergillus* species. The fungi detected resulted in decay and rotting of seeds, and thereby reducing percentage germination of seeds (3%, 29% and 58% seed germination in Segaolane, SNK-3939 and Phofu respectively). Seeds treated with benlate and the aqueous leaf extract of Melia *azedarach* effectively controlled seed-borne fungi, and enhanced seed germination to over 90% as compared to captan (75%). Copper oxychloride (60%) and Dithane M 45 (50%) as compared to non-treated seeds (20%).

Key Words: Soil-borne fungi, Sorghum, seed germination, Fungicide, *Melia azedarach*, Botswana

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is one of the most important cereals grown and consumed in Botswana, and it is mainly produced in northern and south eastern part of Botswana (10). Sorghum produced in these area help in meeting the demands of the people. In addition to its consumption as food, sorghum is used in beer brewing in Botswana, and dry sorghum stalks as supplements feeds to livestock and its grains are given to poultry so as to increase egg production, and improve quality of poultry meat. Sorghum is highly rich in carbohydrate, crude protein and contains crude fiber, calcium, iron, phosphorus, vitamin B and A (2). Sorghum is noted for its ability to grow in adverse condition, and it widely adapted and drought tolerant.

The major constraint in the production of sorghum in Botswana is seed–borne fungi which invade sorghum grains while still in the field or during storage causing seed rotting, mycotoxin contamination and loss of viability. The seed infection leads to low germination percentage of seeds and thus resulting yield loss. Infected seeds also act as media for survival of these fungi as well as their dispersal to disease free areas (1). The present study, therefore, aims to determine percentage of different fungi associated with seeds of three sorghum varieties (Segaolane, SNK-3939 and Phofu) which are mostly grown in Botswana, and their effect on percentage germination of seeds. The study is also to assess the effect of some fungicides and plant extracts on the control of seed-borne fungi of sorghum with a view to increase percentage of seed germination.

MATERIALS AND METHODS

Detection of seed-borne fungi

Seeds of three sorghum varieties, Segaolane, SNK–3939 and Phofu which are commonly grown in Botswana were collected in plastic bags from different locations, and seed bags were closely tight and brought to the laboratory. Four hundred seeds randomly taken from each bag were tested for presence of seed-borne fungi by standard blotter method. Seeds were surface sterilized by dipping into 2% Sodium hypochlorite solution for 10 minutes and then rinsing 3 tomes with sterile water before placing them on moist blotter. Seed-borne fungi on sorghums seeds were detected by standard blotter techniques (5). Ten disinfected seeds per plate were placed on equal distance on the moist blotters on 9 cm Petri-plates and incubated at 22°±2°C under continuous artificial light for 12 hrs. and 12 hrs. in the dark in ambient lab conditions as per the procedures by Onyike and Nelson (8). Sterile distilled water was poured in the Petri-plate from the corner when blotter tended to dry. Seeds were examined after 7 days of incubation for the fungal infestation on seeds, and percentage incidence of seed-borne fungi and percentage germination of seeds were determined.

Fungicide and plant extract treatment

Four fungicides, namely benlate, captan, copper oxychloride and dithane – M45 were assayed for the control of seed-borne fungi. Dithane – M45 was used as suspension while other three fungicides were used as seed dressing. Four hundred seeds of each variety were mixed with each fungicide at conc. Of 30µg, 22 µg, 20 µg and 10 µg of active ingredients per

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20 g of seeds respectively, and mixed in polythene bag and gently shaking for 5 minutes. Treated seeds were then placed on moist blotter, incubated at 22°±2°C under continuous artificial light for 12 hrs. and 12 hrs. In the dark. Non-treated seeds served as control. Percentage seed germination and total fungal recovery of treated and non-treated seeds were examined after seven days of incubation.

One kilogram of leaves each of *Melia azedarach* and *Carica papaya* were dried under the shade, and the dried leaves were ground to powder. The leaf powder was soaked in 70% methanol for three days and filter out. Methanol was evaporated from the resulting residues at reduced pressure at 60°C. The final residues. The sorghum seeds were mixed with the final residues, and treated seeds of each variety were plated and incubated as described earlier. Non-treated seeds served as control. Percentage seed germination and total fungal recovery of treated and non-treated seeds were determined after seven days of incubation. Data were analysed statistically using two ways ANOVA at 95% confidence level.

RESULTS

A total of eight fungi were detected from seeds of three varieties of sorghum (Table 1). Species of *Alternaria* and *Fusarium* were the dominant fungi recovered from sorghum seeds, with incidence percentage of 74% and 64% respectively, followed by *Aspergillus* species, and the least was *Culvularia* with 23% incidence. *Fusarium moniliformae* showed the maximum infection of 36%, followed by an *Alternaria* sp with infection of 30%, and *Aspergillus flavus* and *Fusarium oxysporum* being the third with 28% incidence on seeds. The lower percentage incidence of *Aspergillus niger, Alternaria alternata, A. solani* and *Curvularia lunata* was observed on seeds of three varieties of sorghum.

Sorghum varieties tested in the present study differed in terms of fungal percentage incidence (Table 1). The most infected variety of sorghum was found to be *Segaoline* with 97% fungal infection followed by *SNK -3939* with total fungal infection of 72% whereas *Phofu* showed the least fungal infection of 41%. These fungal infections affected the germination of seeds in the three varieties of sorghum (11% germination of seeds in Segaoline, 28% in *SNK-3939* and 56% in *Phofu*).

Table 2 shows that seeds treated with leaf extract of *Carica papaya* and *Melia azedarach* and four fungicides (benlate, captan, copper oxychloride and dithane-M45) reduced total fungal recovery considerably, and increased the germination percentage of the sorghum seeds. Benlate proved to be very effective in increasing seed germination being more effective in variety *Phofu* with no fungal recovery and 95% of seed germination. Captan was only effective in *Phofu* with 90% germination and 1% total fungal recovery. Copper oxychloride and Dithane – M45 were not effective in controlling total fungal infection, and 60% to 70% germination of seeds were observed. Leaf extract of *Melia azedarach* enhanced seed germination to over 90% in all the three varieties of sorghum, with no fungal infection in *Phofu*, and 3% fungal infection was found in *Segaoline* and *SNK-3939*. Leaf extract of *Carica papaya* although increased percentage seed germination as compared to the control, but not effective as benlate and *Milia azedarach*.

Table 1: Percentage recovery of seed borne fungi and percentage germination of seeds after seven days of incubation at 22±2° C (Blotter method).

% Fungi recovered	Sorghum varieties		
	Segaolane	SNK – 3939	Phofu
Aspergillus flavus	8 a	0 a	20 b
Aspergillus niger	4 a	1 a	15 b
Alternaria alernata	10 a	16 c	0 b
Alternaria infectoria	0 a	28 b	2 a
Alternaria solani	9 a	6 a	3 a
Culvularia lunata	15 a	8 a	0 a
Fusarium moniliformae	31 b	4 a	1 a
Fusarium oxysporum	20 a	8 a	0 a
% of fungi recovered	97	71	41
% Seed germination	3	29	58

Observation based on 400 seeds of each sorghum variety. Means followed by the same letter in the column do not significantly differ at 95% confidence limit (P≤0.05).

Table 2: Percentage seed germination (GER) and total fungal recovery (TFR) of treated and non-treated seeds of three varieties of sorghum after seven days of incubation at 22±2° C (Blotter method).

Treatment	Sorghum varieties					
	Segaolane		SNK – 3939		Phofu	
Chemical treatment	**GER**	**TFR**	**GER**	**TFR**	**GER**	**TFR**
Benlate	82 b	6	87 b	37	95 b	0
Captan	72 a	15	69 a	29	90 a	1
Copper oxychloride	60 a	26	54 a	27	87 b	15
Dithane M45	49 a	16	43 a	25	60 b	20
Plant extracts						
Carica papaya	72 b	3	69 b	5	66 b	0
Melia azedarach	92 b	3	89 b	2	96 b	0
Non-treated seeds	27 a	85	15 a	85	20 a	86
C.V. at 0.05%	14.0		16.7		9.0	

Observation based on 400 seeds of each sorghum variety. Means followed by the same letter in the column do not significantly differ at 95% confidence limit (P≤0.05).

DISCUSSION

Many fungi have been reported associated with sorghum seeds causing seed decay and poor germination of seeds (3, 6, 7, 8 and 9). In the present study only eight fungal species were detected using blotter method. Majority of seeds tested were found to be infected with seed-borne fungi reducing the percentage germination of seeds *in vitro*. *Fusarium moniliforme* was found to be dominant fungus overall amongst others in seeds of three sorghum varieties. *F. moniliforme* is a field pathogen, and its association with high incidence and reduced germination of seeds may be susceptibility of sorghum crop to infection by this pathogen. The pathogen was reported to cause seed and stalk rot (4). Three species of *Alternaria* (A. *solani, A. alternata and Alternaria sp.)* were found associated with seeds of sorghum reducing percentage germination of seeds. A. solani was described as the important cause of concealed damage of sorghum seeds (11). *A. alternata*, an opportunistic invader, was detected only in *Segaoline* and *SNK – 3939.* Most of the seeds infected with *Alternaria* decayed and turned black. Seeds invaded by *A. niger* rotted and did not germinate.

All the fungicide treatment increased the percentage germination of seeds *in vitro* and reduction in total fungal recovery. Benlate was effective in controlling seed-borne fungi with no fungal incidence in seeds of *Phofu* with 95% of seed germination. Its effectiveness as a systemic fungicide is the reason for controlling seed-borne fungi as it is systemic in seeds. The leaf extracts of *Carica papaya* and *Melia azedarach* were not phytotoxic to sorghum seeds as they enhanced the germination of seeds. The leaf extracts of the two plants inhibited the growth of the most fungi, and the percentage inhibition was high in *Melia azedarach.* However, no fungal incidence was noted in seeds of *Phofu* when treated with leaf extract of *Carica papaya.*

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