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Seed-borne fungi of cowpea [*Vigna unguiculata* (L.) Walp] and their possible control *in vitro* using locally available fungicides in Botswana.

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Abstract: Seeds of three cowpea cultivars namely Black eye, ER 7 and Tswana obtained from the Department of Agriculture Research, Gaborone were tested for the presence of seed-borne fungi, and their possible control *in vitro* using locally available fungicides. Four hundred fifty seeds of each cultivar of cowpea were disinfected with 2% sodium hypochlorite solution for 10 min and washed three times with sterile distilled water before placing them in PDA plates (5 seeds/9 cm Petri plate), incubated at 22±2° C for 12 hour each under continuous light and dark. A total of eight fungi were detected from seeds of cowpea. These were *Aspergillus flavus, A. niger, Cylindrocarpon* sp., *Fusarium equisiti, F. oxyaporum, Penicillium chyrosogenum, Rhizopus oligosporus* and *R. stolonifer. Rhizopus* spp. were dominant fungi recovered from seeds, followed by *Penicillium, Aspergillus, Fusarium* and *Cylindrocarpon.* The fungi detected resulted in decay and rotting of seeds, and thereby reducing percentage germination of seeds (22%, 37% and 63 % seed germination in Black eye, ER7 and Tswana varieties of cowpea respectively). Out of four fungicides tested, benlate, captan, dithane M 45 and chlorothalanil. Dithane M45 effectively controlled seed-borne fungi, and enhanced seed germination to an average of 86% (93% germination with no fungi detected in Tswana variety) as compared to chlorothalonile (79%), benlate and captan (77%) and un-treated seeds (45%). The fungal incidence was reduced to 2.3%, 4.3%, 5.3% and 5.3% when seeds were treated with dithane M-45, chlorothalonil, benlate and captan respectively as compared to 62% in non-treated seeds.

Key words: Seed-borne fungi; cowpea; seed germination; fungicide; control; Botswana

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is one the most important indigenous legume crops of Africa, thrives in low to moderate rainfall zones from Senegal in the West, to Sudan and Kenya in the east, and to Mozambique and Botswana in the South [10]. Botswana is a semi -arid country, and the most people depend on cowpeas as their main supplementary food. Cowpea is of major importance to the livelihood of millions of people in less developed counties of Asia and Africa, Botswana in particular as it is consumed in many forms: young leaves, green pods and green seeds as vegetables, and dry seeds are used in various food preparations. Cowpea grains are rich in easily digestible protein and carbohydrate, and the total energy content is nearly that of cereal grains, with protein in its seeds and tender leaves.

Seed-borne fungi are the limiting factors among others in the production of cowpea in Botswana. Seed-borne fungi invade cowpea grains while still in the field or during storage causing seed rotting, mycotoxin contamination and loss of seed viability. The seed infection leads to low germination of seeds, and thus reduce yield loss both quantitatively and qualitatively [13, 24].

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Infected seeds may also act as media for survival, as well as their dispersal to disease free area [2]. The type of infection of seeds and the fungi involved are dependent on many factors which vary from country to country. Few literatures are available from other countries with regards to seed-borne fungi of cowpea and their control [3, 4, 6, 15, 18, 20, 21, 23], the information being negligible in Botswana. The present study, therefore, aims to isolate and identify different fungi associated with seeds of three cultivars of cowpea (Black eye, ER 7 and Tswana) which are mostly grown in Botswana, and their effect on germination of seeds. This study also assesses the effect of some locally available fungicides on the control of seed-borne fungi with a view to enhance seed germination.

Materials and Methods

Sample collection and disinfection of seeds

Seeds of three cowpea cultivars, Black eye, ER 7 and Tswana, commonly grown in Botswana were collected in plastic bags from the Department of Agriculture Research, Gaborone. Seed bags were tightly closed and brought to the Laboratory. Four hundred and fifty seeds randomly taken from each

bag were tested for the presence of seed-borne fungi, and their effect on seed germination using agar plate method. Seeds were surface sterilized by dipping into 2% Sodium hypochlorite solution for 10 minutes, and then rinsing three times with sterile distilled water before placing them on PDA (Potato dextrose agar).

Detection of seed-borne fungi

Seed-borne fungi of three cultivars of cowpea were detected by agar plate method. Five disinfected seeds were placed at equal distance on each 9 cm PDA plate, and incubated at 22±2° C under continuous artificial light for 12 hrs. and 12 hrs. in the dark. Seeds were examined after 7 days of incubation for fungal infestation on seeds [8]. The percentage germination of seeds and percentage incidence of seed-borne fungi based on 450 seeds were determined.

Fungicide treatment

Four fungicides, benlate, captan, dithane M-45 and chlorothalonil were used as seed treatment for the control of seed-borne fungi. Dithane M-45 was used as suspension while other three fungicides were used as seed dressing. Four hundred fifty seeds of each cowpea cultivar were mixed with each fungicide at conc. of 30 µg, 20 µg, 22 µg and 10 µg of active ingredients per 20 g of seeds respectively in polythene bags and shaken gently for five minutes. Treated seeds (5 seeds/plate) were placed on 9 cm PDA Petri-plates and incubated at 22±2° C under continuous artificial light for 12 hrs. and 12 hrs. in the dark condition. Non-treated seeds served as control. Percentage seed germination and total fungal recovery based on 450 seeds of treated and non-treated seeds were determined after seven days of incubation.

Statistical analysis

Experiments were replicated four times and results on the percentage fungal incidence and percentage germination of seeds on the fungicide treatment were analyzed using two-way ANOVA at 95% confidence level.

Results

Results on percentage recovery of seed-borne fungi and percentage germination of seeds presented in Table 1 shows that a total of eight fungi were recovered from the three cultivars of cowpea. These were *Aspergillus flavus, A. niger, Cylindrocarpon sp., Fusarium equisiti, F. oxyaporum, Penicillium chyrosogenum, Rhizopus oligosporus and* R. *stolonifer. Rhizopus* spp. were dominant fungi recovered from seeds, with an average incidence of 16%, followed by *Penicillium* (8.3%), *Aspergillus,* (6%), *Fusarium* (5%) and *Cylindrocarpon* sp (4.5%).

The cow pea cultivars tested in the study differed in fungal percentage incidence (Table 1). The cultivar, Black eye was found to be the most infected with 78% fungal infections on seeds followed by ER 7 with 63% seed infection and Tswana with 37% infection. The fungal infections affected the germination of seeds in the three cowpea cultivars with 22% seed germination in Black eye, 37% in ER 7 and 63% in Tswana.

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

% Fungi recovered	Cowpea cultivars		
	Blackeye	ER 7	Tswana
Aspergillus flavus	8	7	0
Aspergillus niger	3	4	0
Cylindrocarpon sp.	7	2	0
Fusarium equisiti	0	3	6
Fusarium oxysporum	0	8	7
Penicillium chrysogenum	11	9	5
Rhizopus oligosporus	22	12	8
Rhizopus stolonifer	27	18	11
% Fungi recovered	78	63	37
% Seed germination	22	37	63

*Observation based on 450 seeds of each cowpea cultivar.

Table 2: Percentage seed germination (GER) and fungal recovery (TFR of treated and non-treated seeds of three cultivars of cowpea after seven days of incubation at 22±2° C (Agar plate method)

Treatment	Cowpea cultivars					
	Blackeye		ER 7		Tswana	
	GER	TFR	GER	TFR	GER	TFR
Benlate	67b*	11	86a*	2	79b*	3
Captan	78a	5	73b	9	82b	2
Dithane M 45	83b	4	82b	3	93a	0
Chlorathalonil	71a	9	79a	3	88b	1
Non-treated seeds	32	83	48	78	56	41

Observation based on 450 seeds of each cowpea cultivar.
*Means followed by the same letter in the column do not differ significantly at 95% confidence limit (P≤0.05)

Results on the effect of fungicides on the fungal incidence and seed germination presented in Table 2 shows that all the four fungicides, benlate, captan, dithane M-45 and chlorothalanil reduced the total fungal recovery and increased the seed germination *in vitro* considerably in all the three cultivars as compared to non-treated seeds. Dithane M-45 was found to be the most effective, reducing the total fungal recovery to 2.3% and enhancing seed germination to 86%. Tswana cultivar showed 93% seed germination with no fungal incidence when treated with dithane M-45. The other three fungicides, chlorothalonil, benlate and captan were also effective but not better than dithane M-45 in reducing fungal incidence and increasing percentage seed germination. The average percentage of total fungal recovery and percentage seed germination were observed to be 4.3% and 79%; 5.3% and 77%; 5.3% and 77% when seeds were treated with chlorothalonil, benlate and captan respectively over non-treated seeds with 62% of fungal incidence and 45% of seed germination.

Discussion

The literature revealed that many fungi were reported to be associated with cowpea seeds from other countries [3, 4, 6, 15, 18, 20, 21, 23]. In the present study, however, only eight fungal species belonging to five genera (*Aspergillus, Penicillium, Fusarium, Rhizopus and Cylindrocarpon*) could be recovered from seeds of three cultivars of cowpea (black eye, ER 7 and Tswana) using standard agar plate method. Most of the seeds tested were found to be infected with seed-borne fungi reducing the percentage germination of seeds. *Rhizopus* spp. *(Rhizopus oligosporus and R. stolonifer*) were dominant fungi recovered from seeds with incidence percentage of 16%. *Rizopus stolonifer* and *R. oligosporus* are ubiquitous, and commonly found in soil, compost and decaying plant materials. *Rhizopus stolonifer* was found associated with beans and peas [1], and frequently isolated from the diseased beans [22].

Penicillium chrysogenum was also recovered from seeds of the three cowpea cultivars, and the incidence was more in black eye than ER 7 and Tswana cultivars. The species is a ubiquitous fungus and occupies a wide range of habitats [14]. *Penicillium* sp. was also found to be one of the dominant fungi recovered from cowpea seeds, and inhibited seed germination [20]. Two species of *Fusarium, F. oxysporum* and *F. equiseti* were detected from seeds of ER7 and Tswana cultivars only, the former species being more dominant on the two cultivars. *F. oxysporum* is a serious wilt pathogen of many vegetable crops including soybeans and cowpeas [15]. This fungal pathogen was isolated from cow pea seeds, reducing biochemical contents of seeds and percentage germination [4]. Our results are similar to Mogle *et al.,* [12] who also found *F. oxysporum* to be a dominant fungus recovered from cowpea seeds. The *Fusarium* wilt of cowpea caused by *F. oxysporum* fsp. *tracheiphilium* can be transmitted by seeds, however the fungus may remain endophytic in seeds without causing the disease [18]. *Fusarium equiseti* was detected from ER 7 and Tswana cultivars only. *F. equiseti* is a seed-borne fungus and was reported to cause cowpea top necrosis [16]. Two species of *Aspergillus, Aspergillus flavus* and *A. niger* were detected from two cultivars only, black eye and ER 7. *A. flavus* isolated from seeds produced aflatoxins in cowpea seeds [7]. This fungus was also found associated with seeds of cotton and sorghum respectively [5, 9]. *Aspergillus niger* was previously reported as dominant fungus detected from cowpea seeds [11, 17].

The percentage germination of seeds was increased by all the fungicides treatment *in vitro,* and the fungicides considerably reduced fungal incidence on seeds. Of the four fungicides tested, dithane M-45 proved to be the most effective in reducing the total fungal recovery and enhancing germination of cowpea seeds. The other three fungicides, chlorothalonil, benlate and captan were not that effective compared to dithane M-45. The result is in agreement with Mogle *et. al.* [12] who also found dithane M-45 (a high quality mancozeb) to be better than benlate in increasing germination and reducing mycoflora of cowpea seeds. Similar results have been found in reducing *Alternaria cassiae* on cow pea seeds [23]. Smith *et. al.* [21] also reported that benlate was not very effective against *Colletotrichum dematium* on cowpea seeds.

Conclusion

The present study showed that the three cultivars of cowpea grown in Botswana (black eye, ER 7 and Tswana) carried seed-borne fungi to varying degree that reduced percentage germination of seeds. Of all the eight-fungal species isolated from seeds, *Rhizopus stolonifer* and *R. oligoporus* were predominant fungi recovered from seeds, and *Aspergillus niger* being the least recovered. Treatment of seeds with four fungicides improved percentage germination of seeds and considerably reduced fungal incidence. Dithane M-45 proved to be the most effective fungicide in controlling seed-borne fungi of cowpea, and may be recommended for seed treatment.

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