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Fungi associated with seeds of two varieties of spinach beet and their possible control *in vitro* by fungicide treatment

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Abstract: Seeds of Two spinach beet varieties namely; Fordhook giant and Lucullus obtained from commercial markets around Gaborone were used for detection of seed-borne fungi and their possible control *in vitro* by fungicide. Four hundred seeds of each variety were surface sterilized with 5% sodium hypochlorite for five minutes before placing them on moist filter paper and agar plates (10 seeds/90 cm Petri plate), and incubated at 24±2º C for 12 hours under light and 12 hours in darkness. Five seed borne fungi were recovered after seven days of incubation. The isolated fungi included *Aspergillus niger, Aspergillus fumigatus, Penicillium digitatum, Cercospora beticola,* and *Arthrinum phaeospermum.* The fungi detected reduced the germination percentage of seeds. *Aspergillus niger* was the dominant (95% in Fordhook giant) recovered from the seeds followed by *Aspergillus fumigatus* (49%) and *Penicillium digitatum* (14%). The incidence percentage of *Cercospora beticola* and *Arthrinum phaeospermum.* were very low. The effects of fungicides were studied *in vitro* and the fungicides used were Dithane M-45, and copper oxychloride. Both fungicides were not so effective in reducing fungal incidence and increasing seed germination. Dithane M-45 increased percentage germination in Fordhook giant but failed to do so in Luculus.

Key Words: Seed-Borne Fungi; Spinach Beet; Seed Germination; Fungicide.

INTRODUCTION

Spinach beet (*Beta vulgaris* L. (var. cicla) W.D.J. Koch is one of the most popular leafy green vegetable of tropical and subtropical region and grown widely. It is also known as Swiss chard that grows best in cool weather. Spinach beet is used as an important leafy vegetable for keeping the body free from a variety of health problems. This leafy vegetable has a high value of nutrition, and is highly recommended in diets as it prevents heart diseases, osteoporosis, arthritis, and is rich in vitamin K, calcium and iron required for bone strengthening, health (Grubben, 2004). Fresh leaves of spinach beet are a rich source of vitamins A and C, thiamin, potassium and folic acid and other important minerals.

Spinach beet is produced primarily for its leaves which are used as food. There are many varieties of spinach which are grown in different countries. Luculus and Fordhook giant are the varieties that are commonly grown in Botswana. The Fordhook giant has big leaves with white veins and stems and Luculus with pale yellow stem. The major constrain in the production of this leafy vegetable is seed-borne fungi which invade seed while still in the field causing seed rotting and loss of viability. The seed infection leads to low germination percentage of seeds and also act as media for survival of these fungi as well as their dispersal to disease free areas. The present study, therefore, aims to determine percentage of different fungi associated with seeds of two sugar beet varieties (Luculus and Fordhook giant) which are commonly grown in Botswana, and their effect on percentage germination of seeds. The study also includes to assess the effect of two fungicides on the control of seed-borne fungi of spinach beet with a view to increase percentage of seed germination.

MATERIALS AND METHODS

Sample collection and disinfection of seeds

Two hundred grams of two varieties of spinach beet, Lucullus and Fordhook giant were collected in plastic bags and closely tight from a commercial market in Gaborone and brought to the laboratory. Four hundred seeds randomly taken from each variety were tested by the standard Blotter and agar plate method. The seeds were washed with distilled water and surface sterilized by soaking them in 5% sodium hypochlorite solution for 5 minutes and rinsed 2-3 times with distilled water before they were placed on the moist two layers of filter paper and potato dextrose agar.

Detection of seed-borne fungi

Seed-borne fungi on spinach beet seeds were detected by the standard blotter (Two layers of filter paper were used in absence of blotting paper) and agar plate method. Nine cm diameter petri dishes each lined with two moist filter papers were used. Ten disinfected seeds were placed per petri dish and were incubated at 24± 2º C under artificial light for 12 hours and 12 hours in the dark as recommended by ISTA rules (2013). The same were done for agar plate method in which potato glucose agar was used as a medium.

The seeds were then examined for fungal infection after seven days of incubation as recommended by ISTA (2013) for seed germination. The types of fungi were examined and the percentage incidence of seed-borne fungi and percentage germination of seeds were determined. Surface fungal growth on seeds was examined, isolated and identified using appropriate literature.

Fungicidal treatment of seeds

Two fungicides namely Copper oxychloride and Dithane M-45 were used for treatment of seeds for the control of fungal infections. Copper oxycloride and Dithane M-45 (20 µg and 10 µg of active ingredient per 20 g of seeds respectively) was mixed with 400 seeds from each variety of spinach beet in a glass container and gently shaking for 5 minutes. The treated seeds were placed in petri plates each lined with two moist filter papers and potato dextrose agar and incubated as described earlier. Non-treated seeds served

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as control. The percentage seed germination and total fungal recovery of treated and non-treated seeds were examined after seven days of incubation under conditions described earlier.

Statistical analysis

The results that were obtained were analyzed using the two way ANOVA at 95% confidence limit at P≤0.05.

RESULTS AND DISCUSSION

Four hundred seeds of two varieties of spinach beet (Luculus and Frdhook giant) when tested showed incidence of different fungi with decreased germination of seeds. In agar plate method 0% and 33% seed germination were observed in luculus and fordhook giant with 100% fungal infestation (some of the seeds though infected but germinated). However in the filter paper 0% and 66% germination were observed in luculus and fordhook giant respectively. Some of the seeds did not germinate although they had not any fungal infestation. Seeds that were planted in agar plates showed more fungal infestation than seeds the moist filter paper plates. The most infected variety was found to be fordhook giant which had a percentage incidence of 39%in the filter paper and 100% in the agar plate.

A total of five fungal species were detected from seeds of two varieties of spinach beet. These were *Aspergillus niger, A. fumigatus, penicillium digitatum, Arthrinum phaeospermum* and *Cercospora beticola. A. niger* was the most dominant species isolated from two varieties of spinach beet seeds in both the filter paper and the agar plate method (Table 1, 2; Fig. 1). In the agar plate method it had an incidence of 47% in Luculu variety whereas 95% in Fordhook giant, and in the filter paper 10% and 23% respectively. Ismael, (2010) observed the highest repetition of *A. niger* in tomato, eggplant and pepper seeds in Iraq. *A. niger* has been observed in a wide range of habitats as it colonizes a wide variety of substrates such as leaves, seeds and decaying vegetation (Palencia *et al,* 2010; Sibi *et al,* 2013).

A. niger was followed by *A. fumigatus* in agar medium with 49 % and 5% of incidence in lucus and fordhook giant varieties respectively whereas in the filter paper its incidence was not observed in the two varieties. The incidence percentage of *Penicillium digitatum* was much lower that the two species mentioned above in the two varities of spinach beet (in both agar and filter paper). The incidence of *Cercospora beticola* was observed only once in luculus variety in the filter paper method suggesting that the seeds may be carrier of the inoculum of the pathogen causing leaf-spot of spinach beet, the most common disease in Botswana. The percentage incidence of *Arthrinum phaeospermum* infection in was 2% and that too observed in fordhook giant in the filter paper method only.

Table 1: Percentage distribution of seed-borne fungi and percentage germination of seeds of two spinach beet varieties in potato dextrose agar plate method at 24±2º C.

Fungal species isolated	Spinach beet variety**	
	Luculus	Fordhook giant
Aspergillus niger	47 b*	95 b
Aspergillus fumigatus	49 b	5 a
Penicillium digitatum	4 a	0 a
No. of fungi isolated	100	100
Germination %	0	33

-*Numbers followed by the same letter do not differ significantly at 95% confidence limit at P≤0.05. **Observation based on 400 seeds of each spinach beet variety with four replications.

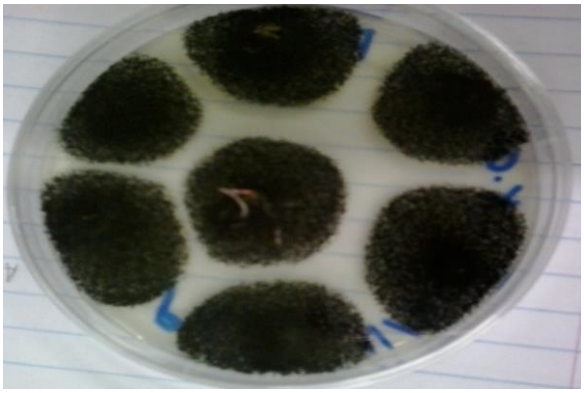

Figure 1: Seeds of fordhook giant infected with *Aspergillus niger* in the agar medium after 7 days of incubation at 24±2ºC

Table 2: Percentage distribution of seed borne fungi and percentage germination of seeds of two varieties of spinach beet in the filter paper at 24±2ºC.

Fungal species isolated	Spinach beet variety**	
	Luculus	Fordhook giant
Aspergillus niger	10 b*	23 b
Arthrinum phaeospermum	0 a	2 a
Penicillium didgitatum	5 a	14 b
Cercospora beticola	1 a	0 a
Number of fungi isolated	16	39
Germination %	0	66

*Numbers followed by the same letter do not differ significantly at 95% confidence limit at P≤0.05. ** Observation based on 400 seeds of each spinach beet variety with four replications.

Table 3: Percentage germination *in vitro* and total fungal recovery (TFR) of fungicide treated seeds of two varieties of spinach beet in PDA plates and filter paper.

Fungicide treatment	Percentage germination of seeds**			
	Fordhook giant		Luculus	
	Germination	TFR	Germination	TFR
Agar plate method				
Dithane M-45	62.3 b*	79	0a	100
Copper oxychloride	7.5 a	99.5	0a	100
Blotting paper				
Dithane M-45	50 b	6.2	0a	3
Copper oxycloride	41.2 a	50.7	0a	43

***The means that are followed by the same letter do not differ significantly at 95% confidence limit (P≤0.05).

**Observation based on 400 seeds of each spinach beet variety with four replications.

The fungicidal seed treatments (Table 3) were not successful in improving the seed percentage germination or reducing the total fungal recovery for one of the spinach beet varieties. In all the experiments with the two varieties in both the moist filter paper and the agar plate methods the

germination percentage did not improve, except for the fordhook giant variety in which the percentage germination improved from 33% to 62.3%. The total fungal recovery did also decreased for the same variety with the Dithane M-45 but not significantly. Before treatment the fungal infection was at 100% in the agar plate method but it reduced to 79%. The Copper oxycloride was not effective in improving seed percentage germination and total fungal recovery in all the varieties in the agar plate method. Lucullus had 0% germination which was the same as before treatment with the fungicides, total fungal recovery remained at a 100% in the agar plate. In the filter paper method the percentage germination of Lucullus remained at 0% but the total fungal recovery was increased from 15.5% to 43%. Dithane M-45 managed to reduce fungal recovery significantly for the fordhook giant variety in the filter paper method but failed to improve seed percentage germination for both the Lucullus and the fordhook giant varieties. Pathak and Zaidi (2013) found mancozeb to be more effective in eliminating *Aspergillus niger* in seeds of wheat. The two fungicides tested could not eliminate *A. niger*. *A. niger* was the fungus that survived treatment strongly (Fig. 1). It needs to test more fungicides for the control of seed-borne fungi of spinach beet and thus improving seed germination.

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