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Evaluation of selected seed treatment methods for the control of *Fusarium graminearum* and *F. avenaceum* on wheat seeds

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Seed-borne diseases cause serious yield in cereal crop production in Lesotho, especially under small-holder farming systems. The main aim of the sudy was to determine the occurrence of both *F. graminearum* and *F. avenaceum* from different areas in Lesotho and evaluate the effect of various control measures on pathogen growth *in vitro* and disease incidence under green house conditions. Both *F. graminearum* and *F.* avenaceum were present in all four wheat cultivars irrespective of their area of collection. Various control measures were applied to determine their efficacy in suppressing pathogen growth *in vitro*, effect on seed germination and disease incidence under greenhouse conditions. The three tested plant extracts and Thiram significantly reduced mycelial growth of both pathogens and showed no negative effect on seed germination with Thiram exhibiting 100% pathogen growth inhibition. Hot water treatment of farm saved wheat seeds also resulted in an increased number of germinated seeds and reduced number of diseased wheat seedlings when compared with control. The lowest germination percentage was recorded in non-treated seeds where only 5.3% Eland seeds germinated. Results obtained in this study show that the three tested plant extracts and hot water treatement can be used to control wheat seed infestation by the two Fusarium spp and improve seed germination.

Keywords: disease incidence, Fusarium spp, mycelial growth, plant extracts, seed-borne, *Triticum aestivum*

Introduction

Wheat (*Triticum aestivum*) is one of the major crops planted by farmers in the highlands of Lesotho. The crop provides food, roofing material, fuel and seed to subsistence farming households (Rosenblum et al., 1999). The crop is usually grown at elevations of 2100-2300m and is typically established with carryover seed from previous season soon after the onset of the spring rains.

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According to Rosenblum et al., 1999, varieties used by farmers are not pure and can be divided into six different types or mainly what is referred to as 'farmers' varieties'. Among various factors responsible for low yield in wheat production, diseases have been shown to play a vital role (Kumar et al., 2008). It is estimated that approximately 12% of crop production is lost to seed-borne globally (Agarwal et al., 2008; Kumar et al., 2008; Dipali et al., 2013). According to Ruhl 2007, there are numerous fungal diseases of wheat commonly found in Lesotho and these include seed-borne, soil-borne and air-borne.

Despite wheat being one of the most cultivated crops in Lesotho and most farmers using farm saved seeds, no work has been done to determine the prevalence of both *F. graminearum* and *F. avenaceum* in farm saved seeds and their management. The two fusarium species not only causes quantitative grain yield loss, but can also result in contamination with mycotoxins such as zearalenone and deoxynivalenon (DON) (Rosewich et al., 2002). *Fusarium graminearum* also causes fusarium head blight, an economically important disease of wheat where the crop is planted under warm and wet conditions (Rudd et al., 2001). The disease has been identified by the International Maize and Wheat Improvement Center (CIMMYT), as the major limiting factor in wheat production around the world (Parry et al., 1995).

Seed-borne fungal diseases are commonly managed by seed treatment with chemicals as they are considered to be efficient and effective (Torp et al., 2006). However, due to a number of factors such as residual effect to the environment and toxicity to human, fungicides are being used less, especially under small-holder farming systems (Torp et al., 2006). Among alternative methods used in plant disease management, the use of plant derived products has received more attention. They are believed to be safe to both the environment and farmers and also lack harmeful residual effect on other soil organisms (Arya and Pertello, 2010). Various reports have also shown positive results in the use of plant extracts in the management of seed-borne diseases of cereal crops (Alice and Rao, 1986; Silva et al., 2001; Masun et al., 2009; Yassin et al., 2012; Perello et al., 2013). Physical control in the form of hot water seed treatment has also been shown to effectively manage a number of seed-borne fungal pathogens (Koch et al., 2010).

The objectives of this study were therefore to determine the prevalence of Fusarium spp causing head blight in farmer saved wheat seed in two mountainous areas of Lesotho and evaluate the effectiveness of various control measures for their management.

Materials and methods

Seed collection

Farm saved wheat seed samples of four commonly planted cultivars namely Eland, Gariep, Tugela-DN and Tugela were obtained from two research stations of the Department of Agricultural Research, Ministry of Agriculture and Food Security, located in the mountainous districts of Lesotho namely Mokhotlong and Thaba-Tseka. Collected seeds were kept in brown paper bags, and stored at 20°C under dry conditions until further use. Gariep and Eland were collected from Mokhotlong district, whilst Tugela-DN and Tugela were collected form Thaba-Tseka.

Seed infection evaluation

Wheat seed samples were evaluated for *F. graminearum* and *F. avenaceum* infection by following ISTA (1996) seed testing methods. Two methods, namely, agar plating and Standard Blotter Methods were used. Four hundred seeds of each cultivar were surface sterilized with 2% sodium-hypochloride solution for 3 minutes and rinsed three times with sterile distilled water. The sterilized two hundred seeds were placed in petri dishes containing Potato Dextrose Agar media (agar plating method) and the remaining two hundred were subjected to standard blotter methods (ISTA, 1996). Plates were incubated under alternating periods of 12 hours darkness and 12 hours of light at 25±°C for 7 days. After 7 days seeds were examined for fungal growth using binocular microscope and small piece of fungal mycelium was transferred from each infected seed to PDA and incubated for further 7 days. Fungal colonies were further purified using single spore isolation to obtain pure cultures. *Fusarium* spp used in the study were identified according to their morphological characteristics which included appearance, color and growth type of colony, apprearance of phialides, presence/absence and shape of microconidia, macroconidia and chlamydospores (ISTA, 2009). Isolated *Fusarium* spp were kept at ±4°C until further use. The percentage incidence of fungal pathogens was determined using formula: Percent Incidence (PI) = number of seeds on which the fungus was encountered from each sample x 100/total number of seeds tested for each sample.

Plant collection and extracts preparation

Fresh, healthy leaves of *Artemisia afra, Leucosidea sericea* and *Rhamnus prinoides* were separately collected from different areas in Lesotho. Taxonomic identification of each plant was carried out at the National University of Lesotho Department of Botany Herbarium, Roma Lesotho. After identification, plant leaves were dried under shade until completely dry. Dry plants were then ground to a powdery substance using a blender and kept in brown bottles at 5°C.

For preparation of plant extracts, two and a half grams leaf powder sample for each plant were separately suspended in 100ml of methanol solvent as described by Mann et al. (2008). The obtained mixtures were then vortexed and placed on a rotary shaker for 1hour at 170rpm. Samples were then centrifuged at room temperatures at 4200g for 10 minutes. The supernatant from each extraction step were transferred into a new tube. The third extraction was placed overnight on a rotary shaker and centrifuged as described above. The combined supernatants accordingly were concentrated to 1ml by vacuum drying at 30°C. Tubes were then refilled uniformly with distilled water to a volume of 10ml and the suspension was re-sterilized using the hypodermic syringe-driven filter paper (0.2µm pore size). Samples were kept at 4°C in the refrigerator until futher use.

Pathogen growth inhibition in vitro

The effect of *A. afra, L. sericea* and *R. prinoides* leaf extracts prepared from different solvents on mycelial growth was determined according to Rhouma et al. (2009). Both *F. graminearum* and *F. avenaceum* isolates used in this section of the study were obtained from Mokhotlong. Isolates were kept at ±5°C until further use. Two ml of methanol aliquots of each plant extract were spread on 9 mm Petri dishes containing solidified Potato Dextrose Agar. Treated PDA was left overnight to allow the extract to be absorbed. In control treatments, un-amended PDA and Thiram amended PDA were used as negative and positive control treatments respectively. One millilitre of Thiram solution, prepared according to manufacturer's instruction, was added to 100ml of PDA solution and dispensed into 9ml petri plates. A mycelial plug of 5mm diameter was cut from an 8-day old culture of each fungal pathogen and placed at the center of each plate with mycelium touching the media. Each treatment was replicated four times.The plates were incubated at 25±1°C. Colony diameter was measured after 7 days of incubation. Percentage inhibition was calculated following the procedure:

% inhibition = (growth in control – growth in treated PDA)*100/growth of control. Completely Randomized Design (CRD) was used for the study. Data were subjected to ANOVA and means were separated using Duncan's Multiple Range Test (DMRT).

Seed treatment and disease detection under greenhouse conditions

Wheat seeds of each cultivar were surface sterilized with 0.4% NaOCL solution for 3 minutes and rinsed three times with sterile distilled water. Ten seeds per cultivar were immensed in 1g/100ml solution of each plant extract for twenty minutes, then removed and placed on 9cm petri dishes containing PDA. The dose of Thiram used was 2g/0.5% of seed weight for 10-15 minutes. In hot water treatments, the modified method of Freeman and Johnson (1909) as described by Masum et al., 2009 was used. Wheat seeds were pre-soaked for five hours in tapwater, and then placed in hot water (55°C) in a thermostatically controlled water bath for 24hours after which seeds were removed, dried on a sterile blotter paper before planting. For control, seeds were treated with sterile distilled water. After each treatment, five seeds per cultivar were sown in 11x11x12cm plastic pots containing steam sterilized sand medium. Percentage of germinated seeds and diseased seedlings were recorded after 2 and 3 weeks respectively. In order to confirm the presence of both pathogens from diseased seedlings, isolations carried out under laboratory conditions and cultures were further inspected for the presence of macroconidia, microconidia and mycelia of *F. graminearum* and *F. avenaceum*.

Results

Resuts on Fusarium spp isolates incidences in the four tested seeds are presented in Table 1. Both *F. graminearum* and *F. avenaceum* were present in all four tested wheat cultivars regardless of their origin of collection. However, their prevalence varied with regard to cultivar and area of collection. Occurrence of *F. avenaceum* was significantly higher ($P<0.01$) in seed samples collected from both districts, with Mokhotlong samples showing highest incidences of this pathogen at 53.80%. The highest occurrence of *F. graminearum* was recorded in Eland seed samples collected from Mokhotlong (38.31%), however this was not siginificantly different ($P<0.01$) from the occurrence of the same pathogen in Tugela-DN samples (36.28%). Gariep seeds samples from both districts displayed a significantly lower occurrence of *F. graminearum* as compared to other cultivars (Table 1), with the lowest occurrence reported in Mokhotlong seed samples at 13.35%.

Table 1. Occurrence of *F. graminearum* and *F. avenaceum* in four wheat cultivars commonly grown in Lesotho

	Occurrence (%)			
	Mokhotlong		Thaba-Tseka	
Cultivar	*F. gramenearum*	*F. avenaceum*	*F. gramenearum*	*F. avenaceum*
Eland	38.31c[1]	22.77a	28.85b	25.31b
Gariep	13.35a	28.35b	16.38a	23.00a
Tugela-DN	36.28c	53.80c	29.35b	49.62c
Tugela	23.00b	32.26b	35.31c	36.28bc

[1]Figures in the column followed by the same letter are not significantly different according Duncan Multiple Range Test (DRMT)

Varying results were obtained with regard to mycelial of both pathogens after treatment with the three plant extracts and fungicide (Figures 1). Mycelial growth inhibition was significantly higher ($P<0.01$) in Thiram treatments with 100% inhibition recorded for both pathogens. All three plants extract significantly ($P<0.01$) reduced mycelial growth of both *F. graminearum* and *F. avenaceum in vitro*. In all three tested plant extracts, the highest growth inhibition was recorded in *L. sericea* treatements, with percentage inhibition at (80%) for both *F. graminearum* and *F. avenaceum*. The lowest mycelial growth inhibition was recorded in *R. prinoides* treatment of both pathogens (75%). However, the inhibition percentage by this plant was not significantly different from as *F. graminearum* inhibition by *A. afra* (69%). The results indicate that all the 3 plant extracts inhibits colony growth of the both *F. graminearum* and *F. aveanceum* under laboratory conditions even though they all showed varying results in their ability to inhibit mycelial growth of both fungal pathogens. Leucosidea sericea extract displayed highest inhibitory effect on both fungal pathogens.

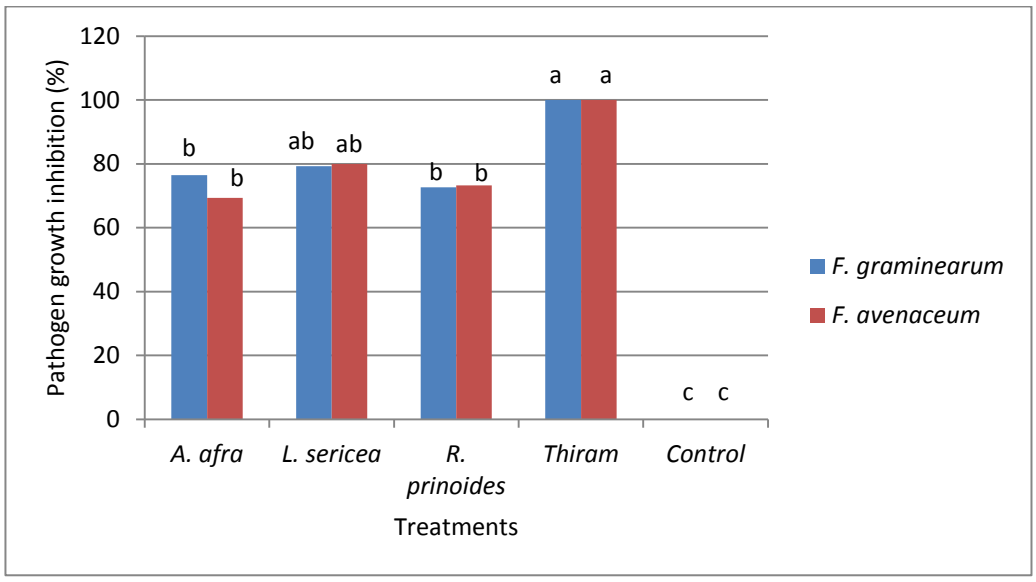

Fig. 1. Pathogen growth inhibition by plant extracts and Thiram *in vitro*

Seed germination experiments results are shown on Table 2. Treatment of seeds with all tested methods resulted in a significantly higher numbers of germinated seeds when compared with non-treated seeds. The highest germination percentage in all cultivars was obtained in Thiram seed treatments, with an exception of Tugela-DN and Tugela seed samples from Mokhotlong where seed germination was between 76.4 and 90.3% respectively. All tested plant extracts significantly increased seed germination. However, there was high variation with regard to percentage germination in all wheat cultivars. Varying results were obtained in plant extracts seed treatments. Seed germination was lower in Tugela-DN seeds obtained from Thaba-Tseka after treatment with *L. sericea* (70.5%). In overall seed treatements methods evaluated in this study, the lowest seed germination was recorded in Tugela-DN samples after treatment with hot water at 69.4%. However, this was still significantly higher as compared to number of germinated seeds in control for all treated cultivars.

Table 2. Effects of different seed treatments on seed germination of four tested wheat cultivars

	Seed germination (%)[1]							
	Mokhotlong				Thaba-Tseka			
Treatments	Eland	Gariep	Tug-DN	Tugela	Eland	Gariep	Tug-DN	Tugela
A. afra	98.4a	95.3a	90.3b[2]	98.1a	80.0b	85.2b	79.4b	89.3a
L. sericea	88.5b	90.3b	78.4c	80.4bc	85.4b	80.3b	70.5c	86.4a
R. prinoides	94.4ab	90.1b	94.1b	72.7c	100.0a	95.5a	100.0a	89.3a
Thiram	100.0a	100.0a	98.4a	100.0a	93.0ab	98.0a	76.4b	90.3a
Hot water	94.1ab	91.9b	93.5ab	93.3ab	89.0ab	69.4c	78.5b	80.3b
Control	10.3c	15.0c	8.6d	9.5d	5.3c	18.4d	12.3d	12.6c

[1]Results represent a mean of four replications per treatment
[2]Values followed by the same letters in the same column are not significantly different according Duncan's Multiple Range Test (DMRT)

Greenhouse experiment results showed that all tested seed treatments were able to reduce seed to seedling transmission of the two Fusarium spp (Table 3). There was however, significant difference in the total numbers of diseased seedlings across different wheat cultivars. The highest number of infected seedlings was recorded in un-treated control as compared to plant extracts, hot water and Thiram treatments. Of the five tested seed treatments, Thiram gave the best protection followed by *A. afra*. The appearance of seedling blight symptoms were completely eliminated in Eland, Gariep and Tugela seeds treated with Thiram. Hot water treatment gave the lowest disease suppression when compared to both Thiram and plant extracts treatments. However the numbers of diseased seedlings were still significantly lower than in control. Of all the three tested plant extracts, *A. afra* was more effective in preventing seed to seedling disease transmission with the highest number of healthy plants reported in all its treatments.

Table 3. Number of symptomatic wheat seedlings after treatment with plant extracts, thiram and hot water

	Mean symptomatic seedlings[1]							
	Mokhotlong				Thaba-Tseka			
Treatments	Eland	Gariep	Tug-DN	Tugela	Eland	Gariep	Tug-DN	Tugela
A. afra	0.00a	0.75a	0.00a[2]	0.68a	0.00a	0.75a	0.00a	0.00a
L. sericea	1.30a	0.00a	1.50b	0.75a	0.00a	0.00a	0.75a	0.25a
R. prinoides	3.00b	1.00a	0.00a	2.00b	2.75b	1.25ab	0.63a	1.75b
Thiram	0.00a	0.00a	0.25a	0.00a	0.00a	0.00a	0.25a	0.00a
Hot water	2.63b	0.75a	1.25ab	1.50ab	2.35b	0.75a	1.30b	1.00ab
Control	8.17c	3.63b	6.45c	8.45c	9.43c	4.23c	5.25c	6.30c

[1]Results represent a mean of four replications per treatment
[2]Mean values followed by the same letters in the same column are not significantly different according Duncan's Multiple Range Test (DMRT)

All three tested plant extracts provided conflicting results with regard to pathogen growth *in vitro*, seed germination and seed-to-seedling disease transmission. Of the five seed treatment applied, Thiram gave the best result in suppressing and controlling the two seed-borne Fusarium *spp*. The fungicide was followed by all three plant extracts and with hot water treatment

Discussions

Farm saved seeds of four wheat cultivars commonly planted by small-holder farmers in Lesotho in the highlands regions of Lesotho were shown to be infected by both *F. graminearum* and *F. avenaceum*. Both districts are characterized by cool and wet weather conditions during winter months, followed by slightly mild temperatures during spring periods. Prevailing climatic conditions during wheat growth, especially flowering period have been shown to play a significant role in infection of wheat grain by Fusarium *spp* pathogens (Rudd et al., 2001). According to a study by Bishaw (2004),

infection levels of wheat seeds by different Fusarium species including *F. graminearum, F. avenaceum* and *F. poae* differed according to regions and districts in Ethiopia and Syria. This was attributed to varying climatic conditions in studied areas (Bishaw, 2004). The same trend was observed in the current study where occurrence of the two fungal pathogens was more prevalent in Thaba-Tseka as compared to Mokhotlong where cooler climate might have played a role in reduced fungal occurrence. The presence of the two pathogens in wheat seeds is regarded as highly important in agricultural production due to their ability to produce deoxynivalenol and zearalenone mycotoxins in the wheat kernels (Rudd et al., 2001). The results showed that the highest and lowest infection percent was in Tugela-DN (58.80%) and Gariep (13.35%) respectively.

Owing to high infection levels of tested wheat seeds by both *F. graminearum* and *F. avenaceum*, there is a clear need to identify and develop appropriate measures for their management. The positive results obtained in the current study with the use of plant derived constituents, fungicide and hot water can provide proper control for both pathogens. In other studies (Pathak and Zaidi, 2013), use of plant derived materials such as neem powder were shown to be effective in reducing populations of fungal mycoflora in wheat seeds.

The total number of germinated seeds was found to be higher in both plant extracts and fungicides treatments as compared to hot water treatment and control respectively. This is also in agreement with studies of (Masum et al., 2009), who reported a significant reduction of *Alternaria tenuis* incidences in sorghum seeds treated with *A. indica*. Though synthetic chemicals can control seed-borne fungal diseases, their negative effect on human and animal health and environmental hazard have resulted in a need to identify and development of alternative and environmentally friendly alternatives. Several higher plant products and their constituents have been shown to supress many plant diseases and have proved to be harmless and non-toxic to the environment (Cummings et al., 2009; Masum et al., 2009; Tinivella et al., 2009).

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