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Prevalence and control of wheat (*Triticum aestivum* L.) seed borne fungi in farmer-saved seeds

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High incidence of diseases associated with the use of seeds saved from previous harvests as desire of maintaining local varieties with special attributes is of increased concern in wheat industry worldwide. Prevalent of seed-borne fungi in farmer-saved seeds and seed dressing fungicides to prevent infection from seeds to seedlings was studied in Northern Tanzania. One hundred and thirty five untreated farmer-saved seed lots were collected randomly from farmers. *Alternaria alternata, Bipolaris sorokiniana, Drechslera tritici, Fusarium graminearum, Fusarium moniliforme, Aspergillus flavus, Cladosporium sphaerospermum, Epicoccum purpurascens, Pyricularia oryzae* and *Penicillium corylophilum* were fungi isolated in farmer-saved seeds. Mean seed infection was 29% causing average grain yield loss of 1.2 mt/ha⁻¹. Seed dressing with Metalaxy plus (Methyl carboxaitide), Mancozeb (Manganase-zinc salt) and Baytan (Chlorophenoxy ethanol) increased seed germination by 14, 13 and 17%, respectively, and grain yield by 28, 20 and 18%, respectively. Farmer-saved seeds were heavily infected by fungi with low grain yield performance.

Keywords: seed-borne fungi; *Triticum aestivum*; farmer-saved seeds; seed dressing fungicides

Infected or contaminated seeds serve as major source of inoculum for large number of plant pathogens (Saberi et al. 2004). Contaminated seeds are a major means of introducing several plant pathogens into grower fields causing damping-off, seedling blights, seed rots, reduce seed germination and seedling emergence (Khanzada et al. 2002; Anjorin & Mohammed 2012). Some seed-borne fungi kill seedlings shortly after they emerge, whereas others can cause serious disease epidemics that affect growth and productivity of wheat (Weber et al. 2001).

Several studies have been carried to identify seed-borne fungal pathogens of wheat in temperate regions, but limited studies have been reported for tropical seed-borne wheat pathogens. Wheat seed-borne fungal pathogens reported in temperate regions include Alternaria alternata, Aspergillus niger, Cladosporium macrocarpum, Claviceps purpurea, Cochliobolus sativus, Curvularia inaequalis, Dilophosphora siccans, Drechslera patereae, Didymella spp., Fusarium graminerium, Gaeumannomyces graminis, Gibberella zeae, Hymenella cerealis, Lasiopiplodia theobomae, Leptosphaeria nodorum, Leptosphaeria spp., Pseudoseptoria stomaticila, Puccinia graminis, Pyrenophora tritici-repentis,

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Sclerophthora macrospora, Tilletia triticii, Urocystis agropyri and Ustilago triticii (Richardson 1990; Weidenboner et al. 1996; Klyszejko et al. 2005; Zare et al. 2006).

In Eastern and Southern Africa, seed-borne fungal pathogens of wheat reported includes *U. triticii*, *T. triticii*, *Fusarium tricintum* spp., and *Helminthosporium gramineum* (Marthur & Barry 2005). A limited number of seed-borne fungal pathogens of wheat have also been reported in Asian continent. Hajihasani et al. (2012) reported 17 genera and 45 species of seed-borne fungi associated with wheat seeds in Pakistan (Hashmi & Ghaffar 2006; Ojuederie et al. 2009; Rehman et al. 2010).

In Tanzania, certified wheat seeds planted each year constitute only 10%, whereas 90% of wheat seeds come from informal seed systems including farmer-saved seeds (Temu & Mtenga 2008). Routine planting of uncertified seeds saved from previous harvests is a widely used practice in wheat-growing areas for fear of losing traditional varieties with special attributes. Many of the diseases that reduce yield of wheat in Tanzania are mainly seed-borne in origin (Abdulsalaam & Shenge 2007). Crop losses due to seed-borne fungal infection in Tanzania are said to be in the range of 8-43% (Mbwaga & Hayden 2003). Continued use of farmer-saved seeds put farmers at risk as qualities and yielding ability of such seeds have not been evaluated. Studies to evaluate status of seed-borne pathogens in farmer-saved seeds would be necessary to ensure that only seeds of high germination percentage free of pathogens are used by farmers to maximise crop yield particularly in developing countries where seed breeding programmes are weak and seed distribution systems are hampered by poor road infrastructure. This study was undertaken to investigate seed-borne fungal pathogens in farmer-saved seeds of selected areas of Northern wheat-growing areas of Tanzania and look for appropriate fungicide to prevent seed carried infection to seedlings.

Material and methods

Sample collection

One hundred and thirty-five untreated wheat seed samples (one kilogramme each) of widely cultivated variety "Mbayuwayu" and a local land race were collected randomly from farmer-saved seeds in three locations (Karatu, Siha and Hanang) located in Arusha region, Northern Tanzania. Seed samples collected were packed in paper bags and transported to Selian Agriculture Institute laboratories for analysis.

Physical inspection of sampled seeds

One kilogramme of each seed lot sample was physically inspected with unaided eye, and seeds were separated into pure seeds and inert matter. Each of these components was weighed separately. Seeds with physical abnormalities, shrivelled seed coat, reduced or increased seed size and discoloured or spotted seed coat were classified as abnormal seeds. Inert matter in seed lots included soil, sand, stones, plant debris, fungal fruiting bodies, etc.

Isolation and identification of fungi from seed samples

Standard blotter method

The standard blotter method (Marthur & Barry 2005) was used in the identification of seed-borne fungal pathogens. Four hundred seeds of each variety were tested in three

replications. Three pieces of blotting papers (Whatman) 90 mm diameter size were moistened with sterile distilled water and placed in sterilised Petri dishes. After draining the excess water, 25 untreated seeds were placed in each Petri dish at equal distance and incubated on benches at room temperature $(20 \pm 2 \,^{\circ}\text{C})$ under alternate cycles of 12 h near ultraviolet (NUV) light and darkness. After 7 days of incubation, seeds were examined under stereoscopic–binocular microscope (Type Leica ATC 2000). Keys for respective genus were used to identify fungi to species level. Incidence of different fungal species was recorded, and percentage frequency (PF) of occurrence for each species was calculated using the following formula:

$$PF = \frac{No. of seeds from which the fungus isolated}{Total No. of infected seeds} \times 100$$

Water agar test

Water agar method was used to induce sporulation for isolate species that could not appear on standard blotter method. Seeds were placed on water agar medium in Petri dish (containing 20 ml of 2% water agar) at the rate of 10 seeds per Petri dish. The Petri dishes were then incubated on benches for 7 days at room temperature (20 ± 2 °C) under alternate cycles of 12 h NUV light and darkness. Single spore re-isolation was done for purification of isolates. The isolated fungi were identified as described above.

Effect of seed dressing fungicides on diseases and yield

Farmer-saved seeds of variety "Mbayuwayu" and a local land race were planted in 20 cm diameter pots containing sterilised soil. 1.5 g di-ammonium phosphate (DAP) was applied in each pot. Fifteen seeds were planted per pot and later thinned to 10 seed-lings per pot one week after seedling emergence. Prior to planting, 20 g farmer-saved seeds of each variety were treated separately in triplicate with each of the three fungicides metalaxy (5,6-dihydro-2-methyl-1-1, 4-oxathin-3, carboxaitide), Mancozeb (manganese ethylenebisdithio carbamate plus zinc-75%) and Baytan (B-(4-chlorophenoxy-a-(1,1-dimethylethyl-1 H-1, 2, 4-triazole-1-ethanol) coated as slurry application. Fungicides were applied at the rate of 0.25, 0.35 and 0.75 g per 500 seeds, respectively, based on manufacture recommendations. The untreated seeds (surface sterilised and un-sterilised without fungicide) were planted in separate pots as control. The average seed moisture content was 14.6%. Farmer-saved seeds used were stored in jute and sisal bags from farmer's stores at room temperature $30 \pm ^{\circ}$ C, complete randomised design in factorial arrangement with three replications was adopted for the experiment.

Assessment of disease and growth parameters

Severity of black point, spot blotch and fusarium head blight were assessed on a scale of 0–9. Five plants were selected randomly per pot, tagged and assessed at seven days intervals beginning 28 days after planting until harvesting. Fusarium head blight was assessed beginning 42 days after planting. Same plants were assessed throughout. As seed-borne fungi affects plant growth parameters prior and after seed emergence. The growth parameters, viz. percentage seed germination, root length, shoot length, fresh root biomass, fresh shoot biomass and plant height near soil line, were recorded.

Assessment of yield

Yield per pot was recorded following manual harvesting and shelling operations. Yield data were taken at 15.5% (wet basis) moisture content. Yield components (number of grain per spike and 100 grain weight) were also recorded.

Statistical analysis

Data on disease, growth parameters and yield were analysed using "Mstat-c version 2.1" statistical programme (Mstat-c 1989) at a significance level, $P \le 0.05$. Mean separation test was done using Duncan's multiple range test.

Results

The results of physical inspection of farmer-saved wheat seeds are shown in Table 1. The proportion of pure and abnormal seeds per hundred grams differed significantly ($P \le 0.05$). The proportion of inert matter showed similar trend.

Fungi isolated in farmer-saved wheat seeds are shown in Table 2. Ten fungal species (A. alternata, Bipolaris sorokiniana, Drechslera tritici, Fusarium graminearum, Fusarium moniliforme, Aspergillus flavus, Cladosporium sphaerospermum, Epicoccum purpurascens, Penicillium corylophilum and Pyricularia oryzae) were isolated from the seeds in varying proportions. The frequency of different fungi isolated from seeds varied significantly between varieties ($P \le 0.05$) (Table 2). The highest percentage seed infection was recorded in local land races compared to commercial variety "Mbayuwayu". A. alternata was the most frequently occurring species followed by A. flavus and C. sphaerospermum.

The effect of seed dressing fungicides of farmer-saved seeds on wheat growth variables is shown in Table 3. Fresh shoot and root biomass highly differed significantly between fungicide treatments ($P \le 0.001$). Root length and shoot biomass showed similar trend. Per cent seed germination and root length showed no significant differences between fungicide treatments ($P \le 0.05$) (Table 3).

There were significant effects of variety and fungicides on wheat growth variables ($P \le 0.05$) Table 4. Highest seed germination percentage was recorded in Baytan fungicide treatment and lowest in untreated controls in both varieties ($P \le 0.05$). Other growth variable varied significantly across fungicides and varieties ($P \le 0.05$) (Table 4).

The effect of fungicide seed treatment on disease, yield and yield components are shown in Table 5. Highest mean disease scores 28 and 42 days after planting was

Variety	Pure seed $(g)^1$	Abnormal seed (g) ¹	Inert matter (g) ¹	
'Mbayuwayu'	986.50 ^b	11.75 ^a	1.80 ^a	
Local	984.50 ^b	14.45 ^a	2.55 ^b	
Mean CV% SE±	988.08 8.3 3.354	10.33 27.7 2.862	1.6 63.7 1.019	

Table 1. Physical qualities of dry wheat seeds collected in Karatu District, Northern Tanzania.

¹Per 1000 g seeds examined; means followed by the same letter within columns do not differ significantly according to DMRT.

Variety	Fungi isolated	No. of infected grains ^a	(%) Frequency of occurence ^a	(%) Total seed infestation load ^a
Local	Alternaria alternata	83	20.75	
	Fusarium	12	3	
	graminearum			
	Cladosporium	20	5	
	sphaerospermum			
	Asperigillus flavus	21	5.25	37.0
	Fusarium	7	1.75	
	moniliforme			
	Epicoccum	2	0.5	
	purpurascers			
	Bipolaris	1	0.25	
	sorokiniana			
	Penicellium	2	0.5	
	corylophilum			
'Mbayuwayu'	A. alternata	29	7.25	
	C. sphaerormum	39	9.75	
	A.flavus			
	E. purpurascers	29	7.25	
	F. moniliforme	3	0.75	
	P. corylophilum	3	0.75	26.5
	Pyricularia	1	0.25	
	P. corylophilum	2	0.5	
Selian'	A. flavus	46	11.5	
	A. alternata	29	7.25	
	C. sphaerormum	40	10.0	33.5
	B. sorokiniana	14	3.5	
D: 11.1	P. corylophilum	5	1.25	
'Riziki'	A. alternata	34	8.5	
	A. flavus	30	7.5	175
	C. sphaerormum	4	1.0	17.5
Chiriku'	E.purpurascers	2 45	0.5 11.25	
Спітки	A. flavus,	43 36	9.0	23.0
	A. alternate C.sphaerormum	50 6	9.0 1.5	25.0
	1	5	1.3	
Lumbesa'	E. purpurascers C. sphaerormum	103	25.75	
Lumbesa	A. alternata	23	5.75	34.5
	A. flavus	12	3.0	54.5
'Tausi'	C. sphaerormum	89	22.25	
	A. alternata	16	4.0	
	A. flavus	10	3.5	30.3
	E. purpurascers	2	0.5	50.5
Sifa'	A. flavus	51	12.63	
0114	C. sphaerormum	18	4.5	20.4
	E. purpurascers	5	1.25	20.1
	A. alternata	2	0.5	
	F. gramineum	-		

Table 2. Fungi species isolated in farmer-saved seed, their frequency of occurrence and total seed infestation load.

^aPer 400 seeds examined.

Fungicide	(%) Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Metalaxy plus	91.50 ^{ab}	15.61 ^a	4.24 ^a	4.17 ^a	1.13 ^{ab}
Mancozeb	89.33 ^b	14.97^{a}	2.87 ^b	2.94 ^b	1.21 ^a
Baytan	94.67 ^a	10.82°	4.12 ^a	4.04 ^a	1.23 ^a
Control	78.83°	13.42 ^b	1.56 ^c	1.46 ^{bc}	1.02 ^b
Mean	88.58	13.69	3.19	3.15	1.12
CV%	2.7	2.4	3.9	0.9	10.5
SE±	1.37	0.83	0.31	0.19	0.005

Table 3. Effect of seed dressing fungicides on wheat growth variables in farmer-saved seeds collected, Northern Tanzania.

Notes: Means followed by the same letter within columns do not differ significantly according to DMRT at $P \le 0.05$, Control = No fungicide treatment.

Table 4. Effect of combination of variety and seed dressing fungicides on wheat growth variables for farmer-saved seeds collected in Northern Tanzania.

Variety	Fungicide	(%) Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	Metalax plus	92.00 ^a	13.70 ^{ab}	3.21 ^a	1.15 ^a	0.05 ^a
	Mancozeb	89.33 ^b	13.67 ^{ab}	$3.09^{\rm a}$	1.09^{a}	0.05^{a}
	Baytan	93.67 ^a	14.82^{a}	2.76 ^b	0.03 ^b	0.04 ^b
	Control	79.00 ^c	14.60^{a}	2.94 ^b	1.20^{a}	0.03^{a}
Local	Metalax plus	91.00 ^b	10.88 ^c	4.04 ^a	1.23 ^a	0.08^{a}
	Mancozeb	89.43 ^b	13.52 ^b	1.46 ^c	1.21 ^a	0.07^{a}
	Baytan	95.67^{a}	15.21 ^a	2. 63 ^b	1.43 ^a	0.03^{a}
	Control	78.67 ^d	14.43 ^{ab}	2.91 ^b	1.31 ^a	$0.04^{\rm a}$
Mean		88.58	13.69	2.87	3.15	1.12
CV%		2.98	3.85	10.41	1	15.16
SE±		1.52	0.30	0.19	0.18	0.03

Notes: Means followed by the same letter within columns do not differ significantly according to DMRT at $P \le 0.05$, Control = No fungicide treatment.

significant on spot blotch and fusarium head blight but did not differ significantly between fungicides and varieties on black point disease ($P \le 0.05$). Plant height, number of grain per spike, 100 grain weight and grain yield per pot also highly differed significantly between varieties and fungicide treatments ($P \le 0.001$) (Table 5). Grain yield was increased by 45, 33 and 28% follow seed treatment with Metalaxy plus, Mancozeb and Baytan, respectively, in "Mbayuwayu variety" and 8, 5 and 3%, respectively, in local variety compared to controls (Table 5).

Discussion

Variations in the pre- and post-harvest handling practices during harvesting, processing and/or storage of wheat have significant influence on seed quality of farmer-saved seeds and prevalence of seed-borne diseases. Except for few samples collected in some areas

		Disease score ¹		Plant	Number of	100	Grain	
Variety	Fungicide	BP	SB	FH	height (cm)	grain per spike	grain weight	yield per pot (g)
Mbayuwayu	Metalax plus	2.10 ^{ab}	1.20 ^c	0.00 ^d	46.77 ^a	13.33 ^a	4.92 ^a	9.06 ^a
	Mancozeb	2.40^{a}	2.20^{b}	0.97°	45.13 ^a	11.00^{b}	4.69 ^a	8.31 ^a
	Baytan	2.60^{a}	2.88 ^b	1.33 ^b	40.53 ^b	10.33 ^c	4.63 ^a	8.003^{a}
	Control	3.97^{a}	4.40^{a}	2.43 ^a	40.60^{b}	$6.00^{\rm d}$	3.03 ^b	6.25 ^b
Local	Metalax plus	2.53 ^b	1.67 ^c	0.43 ^c	45.30 ^a	11.33 ^a	3.51 ^a	6.35 ^a
	Mancozeb	2.87 ^b	2.30^{b}	0.97^{bc}	44.50^{a}	10.00^{b}	3.33 ^a	6.17^{a}
	Baytan	2.77 ^b	2.63 ^b	1.10 ^b	38.00 ^c	9.33 ^b	3.22 ^a	6.11 ^a
	Control	3.63 ^a	4.30^{a}	2.53 ^a	41.90 ^b	4.67 ^c	2.73 ^b	5.89 ^b
Mean		2.61	2.69	1.22	42.84	9.50	3.76	7.02
CV%		16.06	16.6	28.2	3.13	6.08	2.92	1.36
SE±		0.24	0.26	0.36	0.77	0.33	0.06	0.07

Table 5. Effect of combination of wheat varieties and seed dressing fungicides on diseases, yield and yield components for farmer-saved seeds collected Northern Tanzania.

Notes: BP = Black point; SP = Spot blotch and FH = Fusarium head blight.

¹Maximum disease score 42 days after planting; means followed by the same letter within columns do not differ significantly according to DMRT at $P \le 0.05$, Control = No fungicide treatment.

where improved post-harvest methods were adopted (harvesting by combine harvester, properly dried and stored in improved storage structures), most of farmer-saved seeds collected in other locations were of poor quality with low germination percentage and high levels of fungal seed infection. In Tanzania, like many other underdeveloped countries, farmers have limited access to improved seeds due to less vigorous seed breeding programmes coupled with poor seed distribution systems. Farmers mainly depend on farm seeds saved from previous harvests or borrowing from neighbours or purchase in local informal markets many of which are of very low quality and heavily infected with various pathogens. Saberi et al. (2004) reported similar findings and concluded that most of the farmer-saved seeds had poor germination percentages and field establishment, attributed to heavy infection by the pathogens and other physiological seed deteriorations.

Significant increase in seed germination per cent of both varieties of farmer-saved seeds by seed dressing with fungicides indicated a possible means to improve farmersaved seeds prior to planting. However, this could offer a short-term solution as most of the resource-poor farmers in developing countries rarely could afford even the cheapest fungicides, and availability and distribution of fungicides could be impaired due to poor rural infrastructure. Increased seed germination following application of fungicides has also been reported by Siddiqui and Zaman (2004) and Kandolo (2008). Seed dressing with systemic fungicides is a conventional method used for the control of many seedborne infection (Malaker & Mian 2009). The beneficial effect of fungicidal application in wheat has been emphasised by several other workers (Siddiqui et al. 1997, 1999). Siddiqui et al. (1999) observed significant increase in viability of wheat seeds treated with Captan (heterocyclic nitrogen) and Thiram (dithiocarbamate), Farmerzeb-(zinc salt) and Baytan (tridimenol) compared to untreated seeds. Seed-borne fungi may attack seeds prior germination and in subsequent plant growth stages leading to pre- or postemergence damping-off. Fungicide treatments reveal significant influences on various growth variables of wheat. The most effective fungicide Metalaxy plus had high values

for most of wheat growth variables studied suggesting continue fungicidal activity of the fungicide that favoured wheat growth in early and subsequent stages of plant growth.

Seed-borne fungal species A. alternata, B. sorokiniana, D. tritici, F. graminearum, F. moniliforme, A. flavus, C. sphaerospermum, E. purpurascens, P. corvlophilum and P. orvzae, isolated from wheat seed samples collected at various locations provide evidence of heavy infestation of farmer-saved seeds with variety of fungal pathogen species. The occurrence of some of these species such as B. sorokiniana, D.tritici, A. flavus, C. sphaerospermum, E. purpurascens, P. corylophilum and P. oryzae have been isolated in wheat seeds for the first time and have not previously been reported in Tanzania. Kuwite et al. (2010) isolated species of Helminthosporium spp., Alternaria spp. and *Fusarium* spp. from wheat samples collected in Southern Tanzania. However, exchange of seeds by farmers and selling of uncertified seeds in local markets could be the cause of widespread occurrence of seed-borne pathogens in the region that calls for an immediate attention to prevent spread to neighbouring countries including Kenya, Uganda, Rwanda, Burundi, Sudan and Republic of Congo. The control of these pathogens and other pathogens of wheat perhaps could require regional integrated management approaches as seed distribution systems are more or less similar. The widespread occurrence of the diverse species viz., A. alternata, A. flavus and C. sphaerospermum in Tanzania calls for action to prevent crop losses.

The plants arising from seeds treated with Metalaxy plus, Mancozeb and Baytan fungicides had low disease severity reflecting the potential of these fungicides to control diseases resulting from seed-borne infection in wheat. Seed-borne fungi attack seeds and infection may precede in early stages of root development kill germinating seeds before emerging (pre-emergence damping-off) or infection develops in subsequent growth stages. Metalaxy plus has shown to offer effective control for wide variety of seed-borne fungi compared to Mancozeb and Baytan which were restricted to only few fungal groups. As majority of fungicides are not cross effective to targeted fungal species, selection of an appropriate fungicide is essential to offer effective protection. Fungicide treated pots had higher grain yield than untreated controls. Meisner and Ahmed (1996) had also observed that seed treatment with Metalaxy plus increased plant stand by 23% and grain yield by 18% under farmer's field conditions.

Concluding remarks

The results of this study have shown that farmer-saved seeds were heavily infected with fungi leading to poor seed germination that reduced grain yield. Farmer-saved seeds treated with seed dressing fungicides significantly improved wheat grain yield. Metalaxy plus was more effective in controlling diverse species of fungi compared to Baytan and Mancozeb. Availability of improved wheat varieties could be a limiting factor in many developing countries. Farmer-saved seeds if they have to be used should be treated with an appropriate seed dressing fungicide prior to planting to prevent possible seed-borne infection which causes significant yield losses.

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