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Incidences of fungal leaf spot disease in buffel grass (*Cenchrus ciliaris*) in some selected pasture farms in Tanzania

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Abstract Buffel grass (*Cenchrus ciliaris* L.) is one of the important perennial forage grasses in the pasture farms of Tanzania. It is highly nutritious, drought-resistant, grazing-tolerant, and with rapid growth characteristics. Field observation of leaf spot disease in buffel grass was conducted during the months of April, May, and July 2012 at three pasture farms in Tanzania. Laboratory and greenhouse fungal pathogenicity studies for buffel grass seedlings were conducted at Sokoine University of Agriculture, Morogoro, Tanzania. This study was set to investigate the health status of buffel grass from selected pasture seed farms for screening seed-borne fungal species of economic importance. It was also set to establish the pathogenic fungi effects on leaves of buffel grass seedlings.The incidence of leaf spot disease in buffel grass was 54%. Fungal pathogens of economic importance that were detected in this study include *Phoma spp* (28.5%), *Curvularia lunata* (17.34%), *Alternaria alternata* (14.1%), and *Bipolaris spp*. (12.2%). Pathogenicity of *Bipolaris spp. Phoma spp., Pyricularia grisea, Fusarium pallidoroseum, Exserohilum rostratum*, and *Nigrospora oryzae* were confirmed on *C. ciliaris* seedlings. The highest disease incidence was observed on buffel grass seedlings sprayed with *Bipolaris spp* (40%), while the lowest disease incidence was observed on plants sprayed with *E. rostratum* (27.5%). In particular, the order of virulence of the pathogenic fungal species was *Bipolaris spp* > *P. grisea* > *Phoma spp.* > *F. pallidoroseum* > *E. rostratum* (40%, 37.5%, 35.0%, 32.5%, and 27.5%, respectively). All identified pathogenic fungi species are seed-borne and with reported ability to cause leaf spot diseases in several other tropical forage grass species. Further research on innovative technologies and practices for controlling fungal infectious diseases in buffel grass seeds and leaves is suggested.

Keywords: African foxtail grass, seedborne pathogenic fungi, leaf blight disease, endophytic fungi

1. Introduction

Pasture is a major important source of feed for ruminant livestock in many parts of the world. Consumption of animal products, including milk and meat, is rapidly increasing in Tanzania and Sub-Saharan Africa at large due to the continued rise in the human population as reported by Thorp (2015). According to Mganga et al (2019), buffel grass is an important perennial forage grass in the semi-arid regions of Sub-Saharan Africa. Al-Dakheel et al (2015) asserted that bufell grass has high productivity and nutritive value even under drought and saline conditions. Also, Marshall et al (2012) attributed rapid growth and reproduction characteristics of buffel grass allow it to spread and establish quickly in areas under frequent grazing and fire disturbances, to its high preference among livestock keepers. Gayndah and Biloela are the most extensively planted buffel grass cultivars in Africa due to their high biomass and seed production; drought and salinity resistance characteristics are underlined by Jorge et al (2008).

Since 2009, leaf spot infections of buffel grass often has been reported in some pasture farms of semi-arid Tanzania from different pasture species, including buffel grass seeds collected in Mpwapwa district by Ndomba (2009). The disease condition affects lower leaves at their early growing stage and spreads in the middle and upper portions of the canopy. Symptoms on leaves and sheath of buffelgrass appear as circular to irregular spots with narrow yellowish to dark brown discoloration. Each spot is often surrounded by a chlorotic halo. The causal agents of leaf spot disease include *Pyricularia, Alternaria, Bipolaris, Curvularia, Fusarium,* and *Phoma* fungal species (Samuels and Sivanesan 1989; Victoria-Arellano 2021).

Fungi are considered the most important group of plant pathogens in agriculture, causing losses in both quantity and quality, as asserted by Hajihasani et al (2012). Furthermore, of all plant pathogens, fungi are reported to be responsible for the greatest damage to plants in both agricultural and natural ecosystems, as emphasized by Fletcher et al (2010). In grasses, most

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of the pathogens found in seeds cause smut, false rust, ergot, blight or leaf spot, and inflorescence disease, as reported by Chandramohan et al (2002). Occurrences of infected buffel grass seeds coupling with the persistence of the seed-borne diseases in seed-producing farms is a big challenge in different parts of Tanzania. Due to the mentioned challenges, seed health testing is increasingly becoming important in the management of seed-borne pathogens in the country. Moreover, Maleko et al (2018) highlighted the scarcity of high-quality pasture seeds, including those of buffel grass, as a major constraint to sustainable pasture production in Tanzania. Therefore, the present research study was set to investigate the health status of buffel grass from selected pasture seed farms for seed-borne fungal species, as well as pathogenic fungi effects on the leaves of buffel grass seedlings.

2. Materials and Methods

2.1. Surveyed farms for visual and microscopic detection of leaf spot disease incidences

Buffel grass leaf infections were assessed in three (3) farms, namely Tanzania Livestock Research Institute (Mpwapwa), Vikuge pasture seed farm (Vikuge), and Sokoine University of Agriculture pasture farm (Mazimbu) in Tanzania (Table 1). Field visit was carried out to the farms mentioned above to assess the visible leaf spot signs in the leaves of buffel grass plants. A 0.5 m x 0.5 m quadrat frame was thrown ten (10) times on each farm. All third leaves from the ground of plants that fell in the thrown quadrat were counted, and the percentage of infected leaves was estimated.

Table 1 Latitudinal and longitudinal locations of the surveyed pasture farms.

Location	Latitudes	Longitudes
Mpwapwa	S 6° 35’82``	E 36° 47’66’’
Vikuge	S 6° 47’17``	E 38° 51’ 50’’
Mazimbu	S 6° 46’ 59’’	E 37° 37’ 35’’

2.2. Source of buffel grass seeds used in this study

Buffel grass seed samples used in the present study were collected from the study farms, namely Vikuge, Mpwapwa, and Mazimbu. This employed random sampling techniques whereby seeds were scooped out from different bulk seed storage bags and then thoroughly mixed. Thereafter, a 60 grams sample seed lot was weighed and packed in a paper bag for laboratory tests and greenhouse seedlings establishment. The sample paper bags were well labeled. The information included: variety name, collector’s name, farm name, management (e.g. weeding, irrigation, and fertilization), date harvested, date collected, and disease recorded (if any). The seed testing was conducted at the African Seed Health Centre (A_fSHC) located in Sokoine University of Agriculture (SUA), Morogoro, Tanzania

2.3. Detection and identification of fungal microorganisms

The standard Blotter method described by Mathur and Kongsdal (2003) was used to detect and identify fungal microorganisms in the collected pasture seeds. Four hundred untreated seeds of buffelgrass from each seed sample were plated on three well moisten blotters in glass Petri dishes (25 seeds per petri dish) in four replication of 100 seeds each. The Petri dishes with seeds were incubated for seven days at 20-25 °C under an alternating cycle of 12 h near Ultraviolet (NUV) light and 12h darkness ISTA (2005). After 7 days, individual seeds were examined for the presence or absence of fungi (x 12, x 25, and x 50 magnification) under the stereomicroscope. The mycelia of the fungi were placed in a sterile drop of water, covered by a glass slip on a sterilized grass slide and placed on the compound microscope (Leica® MS 5). The examinations of fungi that developed on each seed were confirmed by examining mycelium and/or conidia under different magnifications (x 10 x 20 and x 40) under a compound microscope. The fungal species present on each seed were recorded and the percentage incidence of each fungus per sample was computed. Identification of fungi was based on the type of spore growth, color, and morphological or “habit character” of fruiting bodies on seed (Habib et al 2011; Mathur and Kongsdal 2003).

The relative percentage of particular species within the genus of fungi was calculated using the formula (equation 1) by Ghiasian et al (2004):

$$\text{Relative percentage } (\%) = \frac{\text{Number of fungal species isolated}}{\text{Total number of fungi species isolated}} \qquad (1)$$

2.4. Pathogenicity of fungal strains

The buffelgrass plants used in this experiment were grown from NLRI Mpwapwa seeds on sterilized soil in pots of 20 cm x 18.5 cm. Surface sterilized (1 % NaOHCl) seeds were sown according to the procedure described by Campbell and Medd (2003). Five seeds per pot were used. Plants were thinned to four plants per pot 21 days after germination. All pots were placed on a greenhouse bench at 15 to 25°C, watered, and fertilized as required.

2.5. Inoculum preparation

A small mycelial plug from a stock culture was aseptically transferred to fresh modified V_8 agar (200 mL of V_8 juice, 800 mL water, and 14 g agar) Agarwal and Sinclair (1997). The plates were incubated for 10 days under 25 °C, 12 h/12 h light/darkness, where adequate colony growth was observed. For each fungal isolate, 10-day-old culture on NA was added to 10 mL SDW and spores were scraped off using a bent glass rod. The resulting conidia suspensions were filtered using cheesecloth to remove NA and spore suspension was adjusted to desired concentration 1 $x10^8$cfu/mL by using Haemocytometer.

Seedlings at four leaf growth stage in four replicates of 4 seedlings per pot were sprayed-inoculated with the suspension till runoff using a 1000 ml gun sprayer. Inoculated plants were then covered with polyethylene sheets for 24 h to maintain humidity Jugah et al (2007). Control plants were sprayed with SDW and maintained under the same conditions. Disease incidences were recorded 7, 14, 21, and 28 days after inoculation (DAI) using the methods of Kadir and Charudattan (2000).

Disease assessment was based on the number of plants affected among the total inoculated (disease incidence), expressed as the percentage of diseased plants and plant reaction to disease based on disease severity (area of plant tissue that is diseased) (Jugah et al., 2007). Disease progress was assessed on the inoculated plants in each pot by estimating the disease development on 4 lower leaves. The disease development was expressed as disease incidence, using procedure developed by Kleczewski and Flory (2010). Disease rating where: 0 = no disease; 1 = 1to 5 % of leaf surface area with lesions; 2 = 6 to 10 %; 3 = 11 to 25 %; 4 = 26 to 50 % and; 5 = > 50 %. Disease severity was quantified by assigning a rating to each leaf of every plant. Then to provide a single disease severity measurement for each plant, the converted mean rating was arranged for all four leaves from each plant. In order to calculate means and variance for these nonparametric data, rating values were converted for each leaf to the midpoint of the percent leaf area with lesions (e.g. rating 2 = 8 %).

2.6. Statistical analysis

Data analysis was statistically performed using Statistical Analysis Software (SAS) 2010 computer program. All experiments were replicated at least thrice, and a One-way Analysis of Variance (ANOVA) method was used to test the difference between the groups. The influence of plant parts on the aggressiveness of isolates was determined using the general linear model (GLM) and Least Means of observed parameters. Least Significance Difference (LSD) test was used to determine the significant differences for ranking among the mean values and values at $P \leq 0.05$ were considered statistically significant. Determination of the fungal genus and species, the morphology, color, and size of colonies and spores were taken into consideration according to the descriptions given by Mathur and Manandhar (2003).

3. Results and discussion

3.1 Incidence of fungal diseases under field conditions

The results of the field survey in three pasture farms that were found to have un-harvested grasses indicated the presence of mixed infection of fungal and bacterial diseases. The incidence of buffel grass leaf spot disease was found to be 54% in the surveyed farms. The fields had mixed infections, including leaf spots, blights, and streaks (Figures 1 and 2). According to Louws et al (1994), the genera *Xanthomonas* and *Pseudomonas* are responsible for most bacterial leaf spot diseases in plants

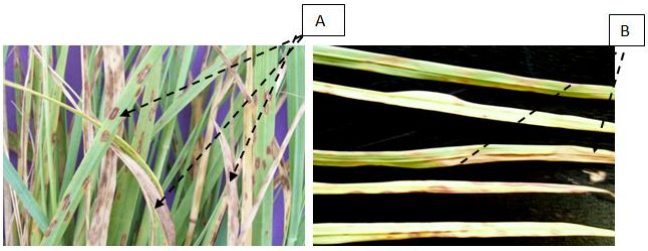

Figure 1 Buffel grass leaf infections as assessed from National Livestock Research Institute Mpwapwa and Mazimbu pasture farms in Tanzania. A = chocolate Leaf blight/spot from Mpwapwa farm. B = leaf streak/spot, mixed leaf infection from Mazimbu farm (Photo by J.A. Mlay 2012).

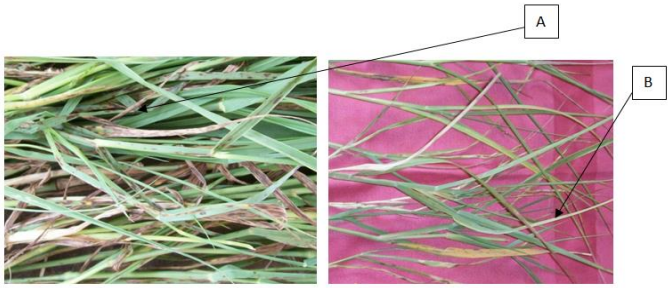

Figure 2 Mixed bacterial and fungal buffel grass leaf infection (A and B) from field samples collected from Vikuge pasture farms (Photo by J.A. Mlay, 2012).

3.2 Isolated pathogenic fungal species

Fungal pathogens of economic importance that were detected include *Phoma spp* (28.5 %), *Curvularia lunata* (17.34 %), *Alternaria alternata* (14.09 %), and *Bipolaris spp*. (12.2 %) (Figure 3). This study, in converse to Diaz-Franco and Mendez-Rodriguez (2005), found *Phoma spp* to be more prevalent, while these authors asserted that *P. grisea* was most responsible for fungal diseases, especially in stressed drought buffel grass in the northern Tamaulipas, Mexico.

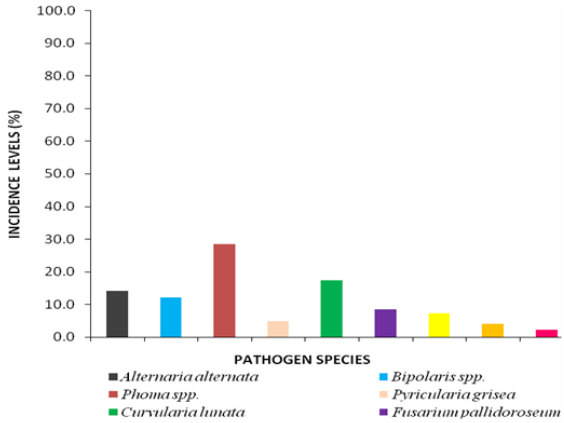

Figure 3 Incidence levels of pathogenic fungus in buffel grass seeds.

3.3 Pathogenicity of some fungi isolated from buffelgrass seeds in buffel grass seedlings

Results of pathogenicity tests of fungi isolated from buffelgrass seeds in the screen house are shown in Table 2. The highest disease incidence (40 %) was observed on seedlings sprayed with *Bipolaris spp*., and *P. grisea* followed by *N. oryzae* (37.5 %), *Phoma spp*. (35 %), and *F. pallidoroseum* (32.5 %). The lowest disease incidence was observed on plants sprayed with *Exserohilum rostratum* (27.5 %). There were no significant differences (P≤0.05) in pathogenicity among the isolates 7 to14 days after inoculation (DAI). Significant differences (P ≤ 0.001) between disease incidences were observed 21 DAI on the plants sprayed with different fungal pathogens (Table 2). The results also showed that the pathogenicity of different fungi on buffelgrass plants on the 28 DAI was not significantly different (P≤0.05).

Sanogo and Moorman (1993) reported that the low density of pathogenic fungi might lead to low inoculum potential, hence the failure of infected plants to show significant symptoms of infection even though the pathogen may be present in their cells or tissue. The results indicated that the trend of disease appearance on inoculated seedlings might have been affected by the initial density of pathogenic fungi. Among the inoculated pathogenic fungi species, only *P. grisea* has been reported on buffelgrass to cause leaf spots (Cook, 2007; Masi et al 2020). Thus, this is the first report to show that *Bipolaris* spp*., Nigrospora oryzae*, *Phoma* spp., (Figure 4) *Fusarium pallidoroseum,* and *Exserohilum rostratum* are major seed-borne fungi associated with buffel grass seeds in Tanzania.

Table 2 Frequency of occurrence of fungal disease on buffelgrass seedlings at 7 to 28 days after inoculation from selected pasture farms in Tanzania.

	Disease incidence			
Isolates	**7 DAI**	**14 DAI**	**21 DAI**	**28 DAI**
Bipolaris spp	10.0^{a}	10.0^{a}	22.50^{b}	40.0^{a}
Exserohilum	10.0^{a}	10.0^{a}	20.0^{b}	27.5^{a}
F. pallidoroseum	10.0^{a}	15.0^{a}	27.5^{a}	32.5^{a}
Nigrospora oryzae	0.0^{a}	10.0^{a}	20.0^{b}	37.5^{a}
P. grisea	0.0^{a}	10.0^{a}	20.0^{b}	40.0^{a}
Phoma spp.	10.0^{a}	15.00^{a}	22.5^{b}	35.0^{a}
SDW	0.0^{a}	0.00^{a}	0.00^{c}	0.00^{b}
Mean	5.71	10.00	18.93	30.36
F test	Ns	Ns	*	Ns
$LSD_{0.05}$	12.84	15.05	4.81	15.47
CV	152.75	102.35	17.29	34.66
S.E (±)	4.36	5.12	1.64	5.26

DAI = Days After Inoculation, SDW = Sterilized distilled water, CV = Coefficient of Variation, LSD = Least Significance Differences, S.E = Standard Error, Ns = Not significant and * = Significant, *Means in the same column with same letter are not significantly different at* P≤0.05.

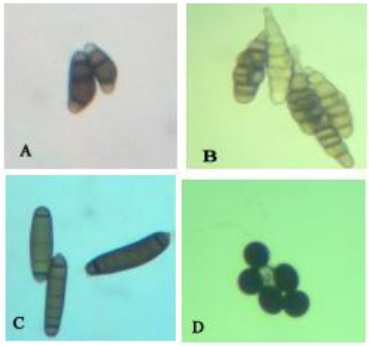

Figure 4 Conidia of fungi detected on buffelgrass seeds collected from selected farms in Tanzania; A = *Curvularia lunata*, B = *Alternaria alternata*, C = *Bipolaris spp,* and D= *Nigrospora oryzae* (x40).

3.4 Disease severity of different fungi inoculated on buffel grass seedlings

The results showed that the highest disease rating (2.5) and (2.4) was recorded in plants inoculated with *Phoma and Bipolaris* isolates on the 7, 21 and 28 days after inoculation (DAI), respectively (Figure 5). Results showed alternating disease progress on infected buffelgrass leaves on 7, 14 and 21 DAI. The lowest leaf spot disease severity on 28 DAI was shown by *E. rostratum*. There was a gradual increase in disease severity from 7 to 28 DAI. As the disease progressed, the area around the discrete lesions turned yellow. Tips and edges of infected leaves turned dark green to brown, giving the leaf folded appearance.

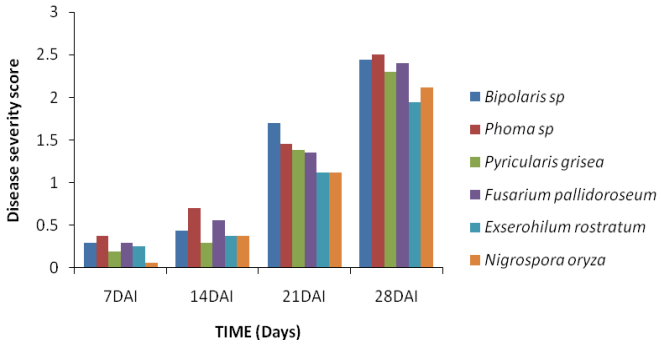

Figure 5 Disease severity rating on buffelgrass leaves 7 to 28 days after inoculation.

3.5 Frequency of occurrence of infection symptoms on different fungi inoculated buffel grass seedlings

The disease symptoms of fungal infection on inoculated buffel grass seedlings were as indicated in Table 4. The highest disease symptoms (15.50 %) by fungal infection were caused by *Phoma* and *Bipolaris* spp., followed by *Pyricularia grisea* and *F. pallidoroseum* (13.0 %) at 28 days after inoculation (DAI). The results indicated that there were no significant differences (P≤0.05) between plants inoculated with different fungal isolates at 7 and 14 DAI (Table 3). There was a slow or late leaf spot disease development at 7, 14, and 21 DAI. This might be caused by uncontrolled changes of temperature and humidity at the screen house during the study. Brecht (2005) reported an increased amount of leaf spotting and leaf tip necrosis of *Bipolaris hawaiiensis* and *Curvularia lunata* on Bermuda grass when inoculated at 30 and 25 °C than at 20 °C.

Table 3 Fungal disease symptoms observed on buffel grass seedlings at 7 to 28 days after inoculation.

Isolates	7 DAI	14 DAI	21 DAI	28 DAI
Bipolaris spp	1.50^{a}	2.25^{a}	8.00^{a}	15.50^{a}
Exserohilum	1.50^{a}	1.50^{ab}	4.25^{bc}	8.00^{b}
F. pallidoroseum	1.50^{a}	2.25^{a}	3.00^{c}	13.00^{ab}
Nigrospora oryzae	0.00^{a}	1.50^{ab}	4.25^{bc}	11.75^{ab}
P. grisea	0.00^{a}	1.50^{ab}	5.50^{abc}	13.00^{ab}
Phoma spp.	1.50^{a}	2.25^{a}	6.75^{ab}	15.50^{a}
SDW	0.00^{b}	0.00^{b}	0.00^{d}	0.00^{c}
Mean	0.86	1.61	4.53	10.96
$LSD_{0.05}$	1.92	2.21	2.89	7.31
F test	Ns	Ns	***	**
CV	152.75	93.33	43.37	45.96
S.E (±)	0.65	0.75	0.98	2.48

Means within the column with the same letters are not significantly different at (P≤ 0.05) based on GLM procedure, Ns = not significant, ** = very significant *** = extremely significant, and DAI = Days after inoculation.

The duration of surface wetness or high humidity in most terrestrial plants has also been reported to determine leaf spot disease development (Magarey et al 2005; Jugah et al 2007; Kleczewski et al 2012). Further studies are thus, suggested to be done in a more controlled environment. In this experiment, a single spray application with fungal inoculum on buffel grass seedlings did not result in plant death. Infected plants recovered from initial damage and produced new foliage. The summary of leaf disease symptoms produced by fungi on buffelgrass seedlings used in the study is shown in Table 4.

Table 4 Disease symptoms observed on buffel grass seedlings inoculated with different fungi used in this study.

Fungi	Symptoms
Bipolaris spp.	Leaf spot Damping-off, Leaf blight, leaf spot
Phoma spp.	Leaf spots, shrink, loss in germination
Fusarium pallidoroseum	Leaf blight, leaf spot
Pyricularia grisea	Gray leaf spot and dieback,
Exserohilum rostratum	Dieback, leaf streak, leaf spot on older leaves,
Nigrospora oryzae	Chocolate brown spots, brown lesion

4. Conclusions

The study indicates that seed-borne fungal pathogens are responsible for leaf spot diseases in buffel grass. Fungal pathogens of economic importance that were detected in this study include *Phoma* spp (28.5%), *Curvularia lunata* (17.34 %), *Alternaria alternata* (14.1%), and *Bipolaris spp*. (12.2 %). The highest disease incidence was observed on buffel grass seedlings sprayed with *Bipolaris spp* (40%), while the lowest disease incidence was observed on plants sprayed with *E. rostratum* (27.5 %). In particular, the order of virulence of the pathogenic fungal species was *Bipolaris spp* > *P. grisea* > *Phoma spp.* > *F. pallidoroseum* >*E. rostratum* (40%, 37.5%, 35.0%, 32.5%, and 27.5% respectively). Further research needs to be conducted to develop effective control measures for seedborne pathogenic fungi species in order to reduce the incidences of the buffel grass leaf spot disease in the country and elsewhere.

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Conflict of Interest

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article. The opinions expressed are those of the authors and do not necessarily reflect those of the organization to which the authors are affiliated.

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