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Full Length Research

Seed Borne Fungi of Rice, Maize, Sorghum, Fundi, Cowpea, Groundnut, Pigeon Pea and Pepper cultivated in the Kambia District of Sierra Leone.

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Investigations were undertaken to establish the seed health status of food crops cultivated in Sierra Leone with emphasis on seed Mycoflora. Rice (paddy), sorghum, maize, fundi, cowpea, pigeon pea and pepper were collected in the Magbema Chiefdom of Kambia District, Northwestern Sierra Leone. The seed Mycoflora were studied using standard blotter method. Observations revealed that samples were infected with several fungi with Mycoflora prevalence varying with respect to the crop. Ten fungal species were found on rice seeds with *Alternaria padwickii, Bipolaris oryzae* and *Microdochium oryzae* being the predominant types. Infection on fundi (Digitaria) and maize were generally below 10% with major pathogens being *Gloeocercospora sorghi, Phoma sorghina* and *Phomopsis sp.* for fundi and *Acremonium strictum, Botrydiplodia theobromae* and *Fusarium verticilloides* for maize. Sorghum, cowpea and pigeon pea were least affected by seed Mycoflora with infection levels being below 5%. Seed Mycoflora infection was highest in groundnut (60% *Aspergillus flavus*, 49% *A. niger* and 21% *Botrydiplodia theobromae* amongst others). In pepper, predominant Mycoflora on seeds were of the genus *Fusarium* (*F. pallidoroseum* 23%, *F. subglutinans* 19%, *and F. oxysporum* 15%). The study highlights the importance of seed health at producers' level in the management of crop diseases.

Keywords: Mycoflora, seed health, crop diseases, food crops, infection levels

Running Title: Seed Mycoflora on Selected Food Crops in Sierra Leone

INTRODUCTION

Agriculture in Sierra Leone contributes 46% to gross domestic product (GDP) of which 67% is provided by the crops subsector (NSADP, 2009). It is considered by the government to be the engine for economic growth. Rice (*Oryza sativa* L.) is the staple crop, national production levels are low and was estimated at 1ton/ha in 2007 (NRDS,2009). Amongst factors limiting yield are biotic and abiotic stresses including crop diseases. The National Rice Development Strategy (2009) projected a four-fold average yield increase target for rice to 4tons/ha by 2018. This would require improving productivity of rice and other food crops.

Major cereals cultivated in Sierra Leone include Rice (*Oryza sativa* L.), Sorghum (*Sorghum bicolor* L. Moench), Maize (*Zea mays* L.) and Fundi (*Digitaria exilis* (Kippist) Stapf). Legumes include Cowpea (*Vigna unguiculata* L. Walp), Groundnut (*Arachis hypogeae* L.), and Pigeon pea (*Cajanus cajan* L. Millsp). Commonly also grown is the spicy vegetable, Pepper (*Capsicum annuum* L.). These crops are seed propagated in Sierra Leone. Crop productivity can be directly linked to the quality of seed used (Shetty, 2000). Pathogen free healthy seeds provide for desired plant populations and good harvest. The presence or absence of seed borne fungi on seed is one of the important aspects that determine the quality of the seed. Many plant pathogens are seed-borne which can cause reduction in plant growth and productivity of crops (Ghangaokar and Kshirsagar, 2013). Quantity loss may range from slight to 100%. Losses may start at germination. Anjorin and Mohamed (2014) confirmed that increasing inoculum level of *Mucor racemeosa* and *Rhizopus nigricans* caused significantly poorer germination and seedling vigor of watermelon. Crop deterioration may continue in the field and right through storage. When loses are extensive the resulting effect could be starvation and famine (Agrios, 1997). Affected seeds pose serious phytosanitary risks and are a major source of pathogen proliferation. They provide the inoculum source for primary infection of the next generation plants. The extent of transmission being dependent on severity of infection, the seed transmission capacity of the pathogen and environmental conditions (Gabrielson, 1988).

Quality wise loses may range from reduction in market value to highly poisonous crop produces due to mycotoxins (Bankole and Adebanjo, 2003).

Information on seed health status of food crops in Sierra Leone is scant. Seed certification level is low and is estimated at about 9%, resulting in the vast majority of seed being self-supplied by farmers or amongst farmers. Minimum standards for seed health certification have not been prescribed in Sierra Leone and farmers are generally unaware that use of seeds with poor health status results in disease proliferation and reduced crop yields. The present investigation was undertaken to identify seed borne fungi and their infection levels on Rice, Sorghum, Maize, Fundi, Cowpea, Groundnut, Pigeon pea and Pepper cultivated in the Magbema Chiefdom of Kambia district, Northwestern Sierra Leone. The overall goal is to acquaint seed certification systems with current levels of seed pathogens on food crops as a basis for developing disease management strategies in the seed sector.

MATERIALS AND METHOD

Collection of seed samples:

Paddy (*Oryza sativa* L.):

Two hundred and fifty gram lots of the upland rice varieties ROK 3 and ROK 16 and the lowland cultivars ROKs 5, 10, 14, 22 and 29 were obtained from seed stock at the Rokupr Agricultural Research Centre, Kambia district in 2010/11. Each variety constituted at least 3 lots

Approximately 250g seeds of other crops comprising cereals, legumes and pepper were collected from farmers and local markets within a radius of 5kms from the Rokupr Agricultural Research Centre as follows:

- Sorghum (*Sorghum bicolor* L. Moench)-eight lots local white
- Maize (*Zea mays* L.)- 10 lots of the variety Western Yellow
- Fundi (*Digitaria exilis* (Kippist) Stapf) – 6 lots of the local accession Pa Sirian

Cowpea (*Vigna unguiculata* L. Walp) – 4 lots of the variety TVU 1190

Groundnut (*Arachis hypogeae* L.) – 3 lots of variety Mares

Pigeon pea (*Cajanus cajan* L. Millsp) – 3 lots

Pepper (*Capsicum annuum* L.) – 4 lots

Each lot constituted a replicate during Mycoflora determination.

Detection of seed Mycoflora:

400 seeds of each sample were randomly picked out and subjected to standard blotter method as recommended by the International Seed Health Testing Association (ISTA, 2003). The seeds were incubated for a period of seven days at 25±2^{o}C. Following incubation, Mycoflora developed on seeds were examined under different magnifications of a stereomicroscope and identified by observing growth habit characteristics following the key offered by Mathur and Kongsdal (1994), Leslie and Summerell (2006), Malone and Muskett (1997)

Data analysis

Data were analyzed for means and standard error of means using GenStat Discovery Edition 4

RESULTS

Oryza sativa **(L)**

Mycoflora occurring in paddy seed lots included *Alternaria padwickii* (Ganguly) M.B. Ellis, *Acremonium strictum* W. Gams, *Bipolaris oryzae* (Breda de Haan) Shoemaker, *Fusarium verticilloides* (Sacc.) Nirenberg, *Fusarium pallidorosum* (Cooke) Sacc, *Fusarium solani* f.sp. Sasakii, *Fusarium subglutinans* (Wollenw. and Reinking), *Microdochium oryzae* (Hashioka and Yokogi) Samuels and I.C. Hallett, *Sarocladium oryzae* (Sawada) W. Gams and D. Hawksw., *Nigrospora oryzae* (Berk. and Broome) Petch, *Phoma sp* and *Phomopsis sp*. Infection levels are provided in Table I.

Digitaria exilis **(Kippst) Stapf**

Figure 1 provides details of Mycoflora infecting Digitaria seeds and their levels of severity. Sixteen fungal pathogens were found occurring. These *included Acremonium sp., Bipolaris maydis, Cercospora sp., Fusarium pallidoroseum* (Cooke) Sacc. *Gloeocercospora sorghi* D.C. Bain and Edgerton, *Phoma sorghina*. (Sacc.) Boerema, *Phomopsis sp., Fusarium subglutinans*, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc, *Pyricularia grisea* Sacc, *Microdochium oryzae, Fusarium equiseti* (Corda) Sacc. *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen, *Fusarium pallidoroseum* (Cooke) Sacc, *Fusarium sp.*

Zea mays (L)

Figure 2 provides details on Mycoflora infecting *Zea mays (L*) seeds and severity levels. Ten fungal species were found occurring with infection levels ranging from 0.1% to 8.6% (Figure 2)

Sorghum bicolor **L. Moench**

Details of fungi infecting sorghum seeds are provided in Figure 3. Eight fungi were found on sorghum seeds with levels of infection ranging from 0.1% (*Gloeocercospora sorghi*) to 3.6% (*Phoma sorghina*)

Arachis hypogeae **(L.)**

Figure 4 provides information on fungi infecting *Arachis hypogeae* (L) seeds. Ten fungi were found occurring and infection levels ranged from 0.1% to 60%. *Aspergillus flavus, A. niger* and *Botrydiplodia theobromae* were the predominant types. Other pathogens were generally below 1%

Capsicum annuum **(L.)**

Figure 5 provides details of fungi infecting pepper seeds. Thirteen fungi were found occurring with infection levels ranging from 0.2% (*Botrydiplodia theobromae*) to 23.4% (*Fusarium pallidoroseum*)

Vigna unguiculata

Mycoflora infecting *Vigna unguiculata* seeds were *Fusarium verticilloides (*1.5%), *Fusarium pallidoroseum* (0.3%), *Fusarium solani* (0.1%), *Fusarium sp* (0.1%)., *Macrophomina phaseolina* (0.9%)and *Phoma sp.* (0. 4%).The differences were however not significantly different at 95% probability.

Cajanus cajan **(L.) Millsp**

Mycoflora infecting Cajanus Cajan (L.) Millsp seeds were *Fusarium verticilloides* (0.2%), *Fusarium pallidoroseum* (1.4%), *Fusarium oxysporum* (0.1%), *Fusarium sp*. (0.2%), *and Macrophomina phaseolina* (0.1%), *Pestalotia sp*. (0.2%). The differences were however not significantly different at 95% probability.

Table I: Percent occurrence of Mycoflora in different samples of *Oryza sativa* (L)

Pathogen	ROK 3	ROK 16	ROK 5	ROK 10	ROK 14	ROK 22	ROK 29
Alternaria padwickii	0.8	1.3	5.7	2.8	1.7	3.0	10.9
Acremonium strictum	-	-	-	-	-	0.2	-
Bipolaris oryzae	7.5	10.6	10.7	13.0	25.9	5.8	4.6
Fusarium verticilloides	-	-	0.5	0.1	-	-	-
Fusarium pallidorosum	-	-	-	0.3	-	0.3	-
Fusarium solani	-	-	-	1.0	-	-	-
Fusarium subglutinans	0.1	-	-	-	-	-	-
Microdochium oryzae	9.2	10.4	1.5	-	2.3	-	0.8
Sarocladium oryzae	-	0.1	-	-	-	-	-
Nigrospora oryzae	0.1	-	-	-	-	-	-
Phoma sp	-	0.8	-	-	-	-	-
Phomopsis sp	-	0.1	-	-	-	-	-
Standard Error	**2.5**	**1.0**	**1.5**	**2.5**	**5.9**	**1.6**	**1.8**

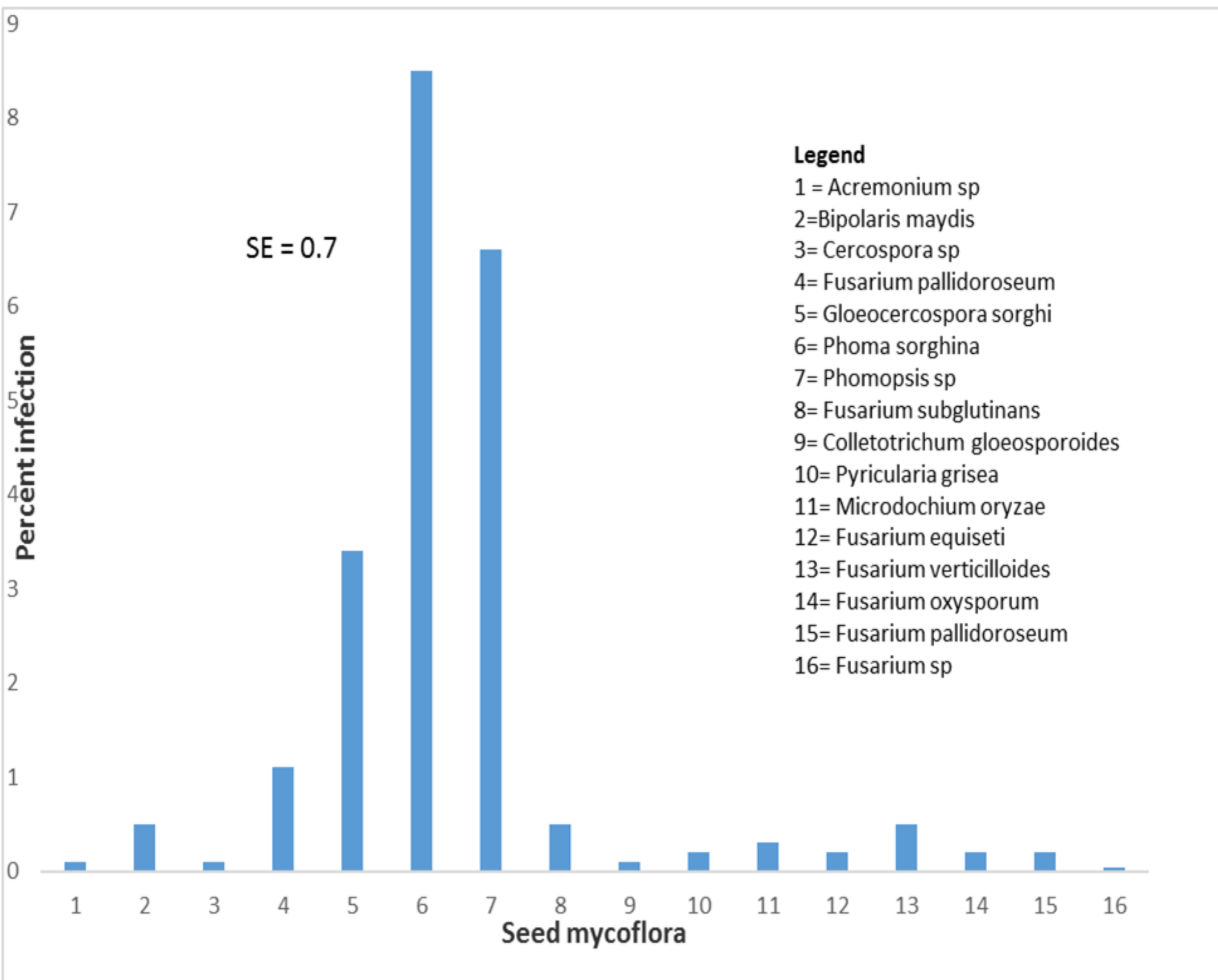

Figure 1: Mycoflora infecting *Digitaria exilis* (Kippist) Stapf seeds

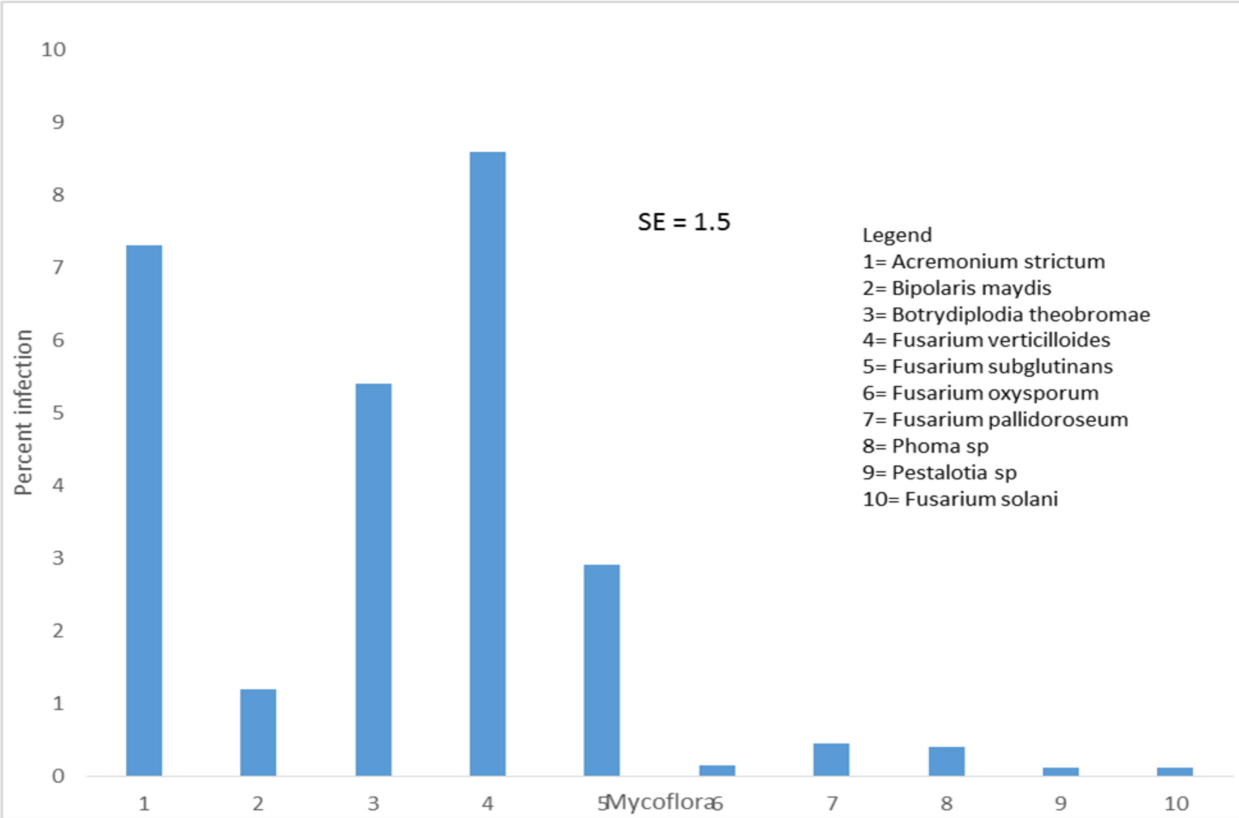

Figure 2: Mycoflora infecting *Zea mays* (L) seeds

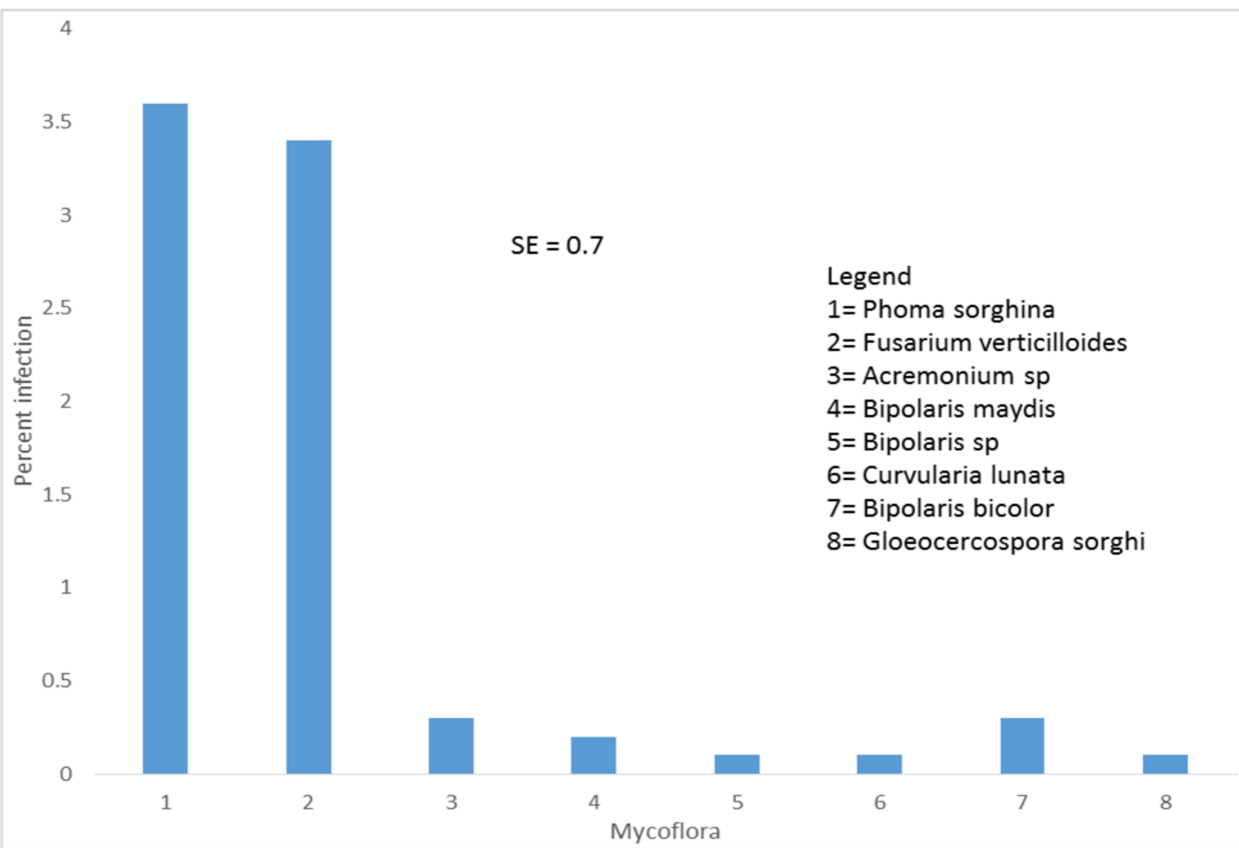

Figure 3: Mycoflora infecting Sorghum bicolor (L) Moench seeds

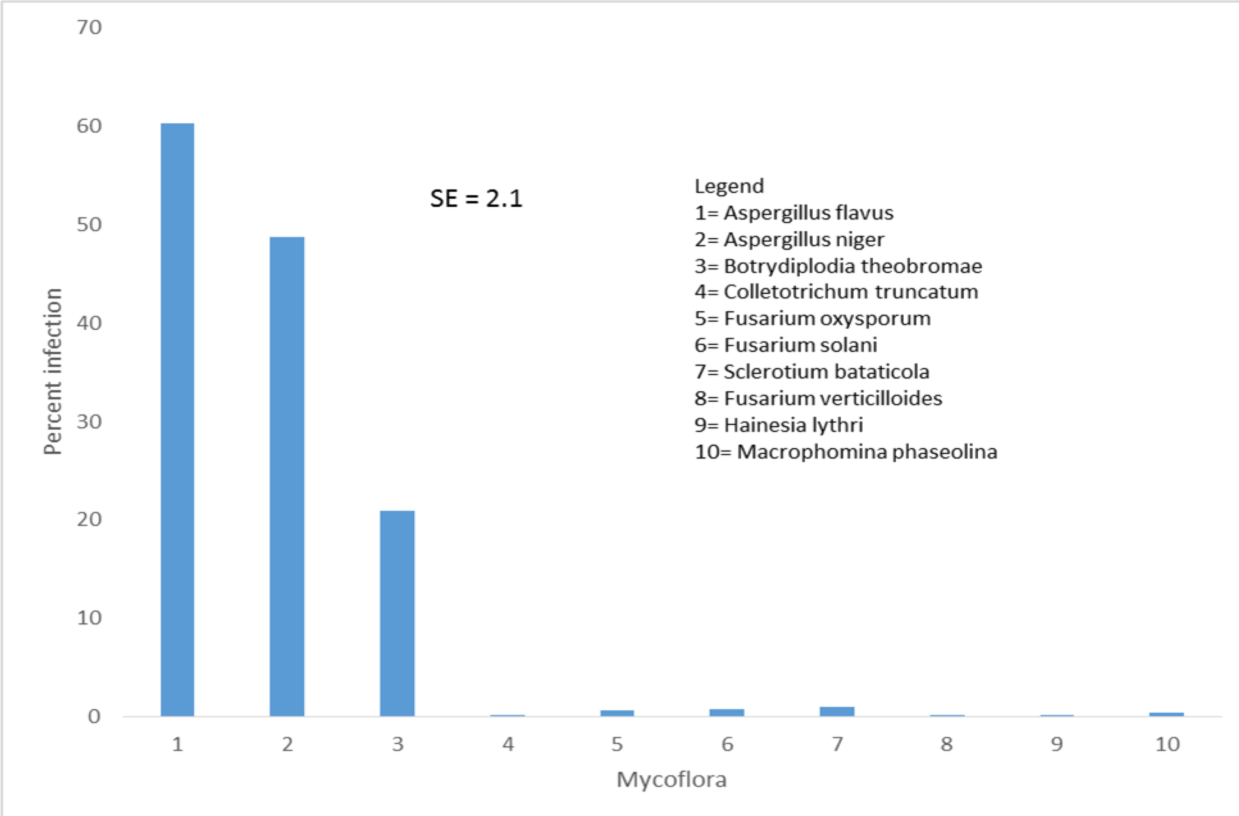

Figure 4: Mycoflora infecting *Arachis hypogeae* (L.) seeds

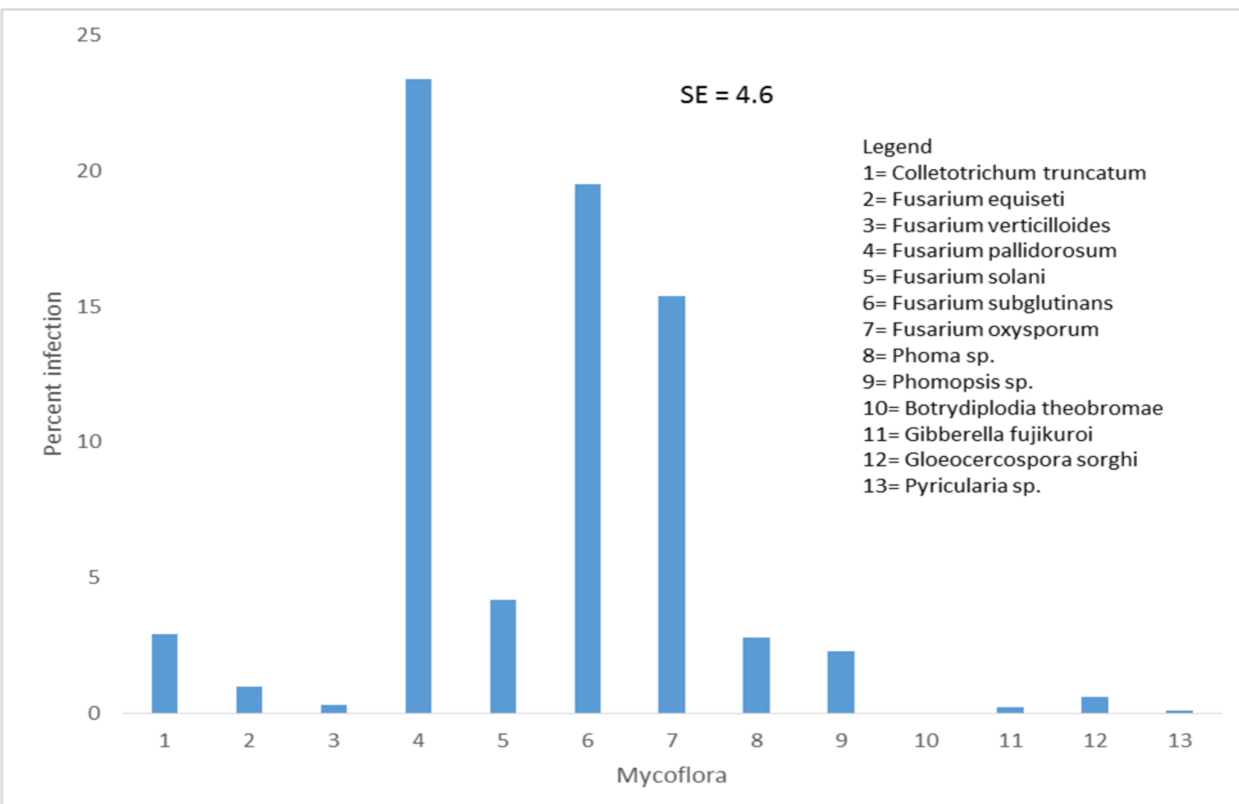

Figure 5: Mycoflora infecting *Capsicum annuum* (L.) seeds

DISCUSSIONS

Oryza sativa (Rice)

Little information is available on seed Mycoflora in Sierra Leone with very little available data for even the staple crop rice. Table 1 shows that the more frequently occurring pathogens on rice were *Alternaria padwickii* (ranging from incidences of 1 to 11%) and *Bipolaris oryzae* (1.2 to 13%). *Microdochium oryzae* was more prevalent in the upland rice varieties ROK 3 and ROK 16. Webster and Gunnell (1992) reported that *Alternaria padwickii* survives in soil, on seed and infected plant debris and that seed infection often leads to infection of the seedling causing seedling blight. *Bipolaris oryzae* infection causes brown spots on glumes and foliar plant parts. Seed infection can lead to seedling infection. The fungus is also reported to produce the toxin ophiobolin (Leung et al, 1984). *Microdochium oryzae* is the causal agent of leaf scald disease of rice and is present in all stages of the crop. The seeds are considered the major source of inoculum. Gutierrez et al (2008) reported transmission of the fungus from seed to coleoptiles tips in seedlings at a rate of 2.18% and infected seedlings may be symptomless. Other mycoflora occurring at lower infectivity levels included Fusarium spp. (*verticilloides, pallidorosum, solani* and *subglutinans)* causative organisms for a variety of symptoms such as root, stem, fruit and seed rots; wilts and leaf discoloration (Leslie and Summerell,2006); Sarocladium oryzae (sheath rot fungus); *Nigrospora oryzae, Phoma and Phomopsis spp.* (causative organisms of grain discoloration and rots).

Digitaria (Fundi)

Figure 1 indicates that Fundi had the highest incidence of seed borne fungi (sixteen micro-organisms). Infection levels were mostly below 1% seed infection, however significantly higher levels of infection were observed with *Gloeocercospora sorghi, Phoma sorghina* and *Phomopsis sp*. Which are known to affect other grass species (Frederiksen, 1996) and are causal agents of foliar lesions and grain mold. *Gloeocercospora* conidia from infected plant parts infect the next crop. Phoma has been reported to survive as mycelia in infected seeds and transmit the disease to young seedlings (Ocamb, 2014). *Phomopsis* infected seeds have been reported to show low emergence due to seed rot or seedling blight (Yang, 2001)

Maize

Ten fungi were found infecting maize seeds (Figure 2). Most were below 1% infection. Significantly higher levels of infectivity occurred with *Acremonium strictum, Fusarium verticilloides* and *Botrydiplodia theobromae (*Figure 2). These fungi are soil and seed borne causing wilting, stalk and cob rot and seedling blight (CIMMYT, 2004; White, 1999). Seed transmission has also been reported (Kabeere et al, 1997)

Sorghum

Seed infection was generally low. *Acremonium strictum, Bipolaris maydis, Bipolaris bicolor, Gloeocercospora sorghi* and *Curvularia lunata* infected seeds at levels of 0.1 to 0.3 percent (Figure 3. Slightly higher infection levels of 3.4 and 3.6 percent were observed for *Fusarium verticilloides* (stalk rot fungus) and *Phoma sorghina* (grain mold fungus) respectively. These fungi were also found infecting maize and fundi.

Cowpea

Infectivity levels were low ranging from 0.1% to 1.5% and pathogens were mainly of the genus *Fusarium*. Cowpea production is relatively low in Sierra Leone. Maintaining such low levels under increased production should be the target of cowpea producers.

Pigeon pea

As with cowpea infectivity levels of seeds were low ranging from 0.1% to 1.4% and were also mainly of the genus *Fusarium*. Similarly, as with cowpea, pigeon pea production is not intensive. Maintaining low levels of seed infection even under more intense cultivation systems should be a policy to enhance sustainable production of the crop.

Groundnut

Major seed borne fungi were *Aspergillus flavus, Aspergillus niger and Botrydiplodia theobromae* with levels of infection ranging from 20% to 60% (Figure 4). *Aspergillus flavus* invades seed embryos causing infection which decreases seed germination. The pathogen causes yellow mold, stalk rot and wilting in groundnut. Infected seeds are discolored. Inhalation of spores of the fungus may result in aspergillosis. The fungus produces the mycotoxin aflatoxins considered as one of the most dangerous contaminants in food and feed and consequently pose severe food safety concerns (Kokalis- Burelle et al, 1997; Saori and Keller, 2011 and Ginting and Rahmianna, 2015). Aspergillus niger causes black mold and crown rot disease on peanut and may produce carcinogenic mycotoxins – Malformin A and Malformin C as well as Ochratoxin A and Fumonisin B2 (Sharma 2012*). Botrydiplodia theobromae* has been associated with wilting, collar rot and pod discoloration in groundnut (Kakolis-Burelle et al, 1997).

Pepper

Several species of the genus *Fusarium* were found infecting pepper seeds. Predominant amongst these were *Fusarium oxysporum*, 15.4%; *Fusarium subglutinans*, 19.5% and *Fusarium pallidoroseum*, 23.4% (Figure v). Occurring in significantly reduced incidences were *Fusarium equiseti, Fusarium verticilloides* and *Fusarium solani*.

Fusarium oxysporum is the most widespread and economically important species in the *Fusarium* genus (Leslie et all, 2006). The fungus causes vascular wilt diseases, damping off, crown and root rots. It is disseminated by various means including soil, seeds and infected plant materials. The fungus has also been associated with various human infections including corneal infection and dermatitis (Rosa et al, 1994; Ninet et al, 2005).

Fusarium pallidoroseum occurs commonly in soil and various plant parts including seeds. Though not regarded as an important plant pathogen the fungus has been implicated in various diseases (Dhingra and Muchovej, 1979).

Fusarium subglutinans has been reported associated with rots in peppers (Mathur and Utkhede, 2004) and may be seed transmitted. The fungus produces moniliformin which is toxic to poultry (Kriek et al, 2001).

CONCLUSION

Many Mycoflora found in seed are important plant pathogens capable of reducing seed quality in the field and in storage. Such pathogens inhibit seed germination, affect seedling vigor and may provide the primary inoculum for infection in the field (du Tout and Derie, 2003; Maude and Presley, 1977).

Seed infection levels are largely determined by weather conditions between flowering and maturity. Warm, humid conditions are very conducive for heavy seed infection. Seed transmission rates are also very much dependent on the environment with transmission also being higher under warm, wet conditions. Data on tolerance levels for seed infection are not available in Sierra Leone and needs to be developed for the major crop pathogens. Agriculture Victoria (2013) gave tolerance levels for seed infection by many fungi as less than 1% for high risk areas and less than 5% for low risk areas. The warm, humid environment in Sierra Leone during the main crop growing season is likely to enhance disease proliferation and consequently likely to be assessed as high risk area.

Only Sorghum bicolor, *Vigna unguiculata and Cajanus cajan* showed all pathogens at infection levels of less than 5%. *Digitaria exilis* and *Zea mays* had pathogens exceeding 5% but less than 10%. Heavy pathogen infection exceeding 10% occurred in *Oryza sativa (Bipolaris oryzae* – 25%); *Arachis hypogeae* (*Aspergillus flavus* – 60%, A. niger – 49% and Botrydiplodia *theobromae* – 21%) and *Capsicum annum* (*Fusarium pallidorosum*- 23%, *F. subglutinans* – 19% and *F. oxysporum* – 15%). When percentage of seeds infected are high, there is a tendency for more inoculum per seed and greater tendency for mycotoxin production. Mycotoxin producing fungi were observed infecting crop seeds and therefore pose risks for food and feed contamination.

With the desire to increase crop productivity and livelihood enhancement, intensification of cropping and crop diversification are being promoted. Management of crop diseases would need to be emphasized under such systems. Seed testing and certification for acceptable seed health should be encouraged and procedures put in place for Farmers to have access to clean seeds. Currently seed certification levels are low and national tolerance levels for seed borne pathogens are not available. These need to be developed. Data obtained from the seed Mycoflora study indicate a clear need to reduce Mycoflora infestation in many crop seeds and to promote better management of crop diseases. Capacitating Farmers with know-how for managing crop diseases would be required if productivity is to be sustainably increased.

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