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ORIGINAL RESEARCH ARTICLE

Pathogenicity Evaluation of Some Common Soil Borne Fungi on Seeds from Three Local Varieties of Sorghum (*Sorghum bicolor* L.)

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ABSTRACT

Soil borne pathogens appears to be one of the most devastating groups affecting *S. bicolor* causing wide range of pre-harvest and post-harvest diseases, plant diseases are commonly managed through different strategies, the use of resistant varieties found to be more economical and ecofriendly. This poses the need to screen different varieties of *S. bicolor* for horizontal resistance of some fungal species commonly found in the soil. Plate technique was used to isolate soil fungi and morphological identification was through macro and microscopy. Koch's postulate was followed in the assessment of pathogenicity of the identified organisms on the three sorghum germplasms (*Yelai, Kaura and Warwarbashi*). The isolated fungal species were found to be *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Mucor spp* and *penicillium spp*. Pathogens with highest occurrence observed was *A. niger* , *R. stolonifer* and *Penicillium spp* with 30, 25 and 20% occurrence respectively. Thus, all the three cultivars of *S. bicolor* screened were very susceptible to the tested pathogens, with *A. niger* and *R. stolonifer* found to be highly virulent. It was concluded that, all the varieties showed a continuous minute or absence of horizontal resistance against the isolated pathogens which could be responsible for post-harvest diseases in Sorghum germplasm.

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INTRODUCTION

Sorghum, or *Sorghum bicolor* (L.) Moench, is a cereal in the family *Poaceae* with several varieties worldwide that was first cultivated in tropical Africa and is now grown all over the world in a wide range of environments (Kangama and Rumei, 2005; Cuevas *et al*., 2016, De Morais *et al*., 2017). Sorghum is the fifth most widely cultivated grain crop, after maize, wheat, rice, and barley. Most cultivars of *S. bicolor* are annuals, but with some perennials that grows in clumps to a height of over 4 metres with tiny gains of about 2-4 mm when matured (Cuevas *et al*., 2016).

In the semi-arid regions of Africa and Asia, sorghum grain is a staple food for millions of people. It is used to make foods like tortillas, bread, cakes, couscous, beer, and porridge (Smith and Frederiksen, 2000). S. bicolor, however, provides the human diet with nutrients and bioactive compounds (De Morais *et al*., 2017). Sorghum is developed and cultivated in Western nations like the United States, Australia, and Brazil primarily for animal feeding (Taleon *et al*., 2012). Sorghum is a rich source of phytochemicals like tannins, phenolic acids, anthocyanin, phytosterols, and poliosanols, according to Smith and Frederiksen (2000).

According to Agrios (2005), plants can exhibit true, race-specific, cultivar-specific, or gene for resistance to some pathogens by belonging to taxonomic groups that fall outside of their host range, by having resistance genes (R genes) directed against the pathogen's avirulence genes, or by escaping or tolerating infection by these pathogens, among other explanations (apparent resistance). However, all plants have some level of resistance that may not be specific to any pathogen in particular, but is usually present; Although this resistance is occasionally referred to as partial, race-neutral, general, quantitative, polygenic, adult-plant, field, or durable resistance, historically, the term "horizontal resistance" has been used most frequently (Agrios, 2005). Nevertheless, a number of biotic factors, such as the fungi that cause anthracnose and grain mold, restrict the productivity and profitability of the sorghum crop.

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Soil borne diseases and stalk rots are some of the most detrimental sorghum diseases in terms of lost production potential, so they demand significant research (Tesso *et al.*, 2012). Identification of resistance sources in in cereals germplsm is necessary to control prevalent diseases (Sabouri *et al.*, 2022). Among the plant pathogens, soil borne dwellers plays an important role in the damage of plants, resulting in the significant yield loss.

Plant diseases are controlled using different options including fungicides, cultural practices such as weed and residue management, planting disease free seeds, crop rotation and planting of resistant varieties. Among the control measures, the use of resistant varieties is the most economical as well as its advantage of being ecofriendly (Agrios, 2005). This raised the need to screen different cultivars of some Sorghum germplasm for horizontal resistance. Therefore, this research was aimed to isolate and identify the common pathogenic fungi from soil and evaluate their pathogenicity on different local Sorghum germplasms.

MATERIALS AND METHODS

Study Area

The experiment was conducted at Biology Laboratory of the Department of Biological Sciences, Federal University Dutse. The average temperature of Dutse varies from 54°F to 103°F, and is rarely below 49°F or above 107°F. Dutse is located at the latitude of 11.7024° N, 9.3340° E.

Sorghum Germplasm Collection

Three different varieties of Sorghum germplasm of ***Kaura, Yeli*** and ***Warwarbashi*** were collected from Shuwari Market of Dutse LGA of Jigawa State on April, 2021. Seeds were collected following the random sampling technique as described by Naqvi *et al.* (2013). From each sample collection site, three samples were collected weighing 1.25Kg. The total of 3.75kg of seeds were therefore collected and each of the varieties were labelled and stored in a paper envelop and sealed.

Collection of Soil Sample

The soil samples used in this experiment were collected using point sampling technique described in Knowles and Dawson (2018). The soils samples collected were from two different fields, denoted as Sample A and Sample B respectively. Sample A was collected from agricultural fields around the Federal University Dutse campus. Sample B was collected from different randomly selected farm lands were sorghum is commonly cultivated. Throughout the collection process, triplicate surface soil samples (top 10 cm) were collected randomly from the top soil using auger and stored in an air tight container (Bartram *et al.*, 2014). The soil was then transported to the Laboratory for further analysis.

Media Preparation

A small amount of water was added into 9.75g of Potato Dextrose Agar (PDA) in a conical flask and was shaken vigorously for 3-5minutes for the media to mount and dissolve, Chloramphenicol was added into the media to inhibit bacterial growth. A 250ml of water was measured and added to the conical flask containing media and plugged tightly with cotton wool and sterilized in autoclave at 121°C for 15minutes (Ravimannan *et al.*, 2014). The sterilized media was removed from the autoclave and allowed to cool.

Isolation of Fungi from Soil Samples

Soil plate method was used for the isolation of soil fungal species from the collected soil. The soil plate was prepared by transferring a small amount of soil into a sterilized petridish. About 8-10ml of the prepared PDA media was added, and the soil particles were dispersed throughout the agar. The inoculated plates were allowed to stand for 1hour and incubated at room temperature for 3-5 days. Each colony was sub-cultured in a new PDA plate after a mixed growth appeared in order to produce a pure culture using streak method (Cheesbrough, 2006). The number of species in the soil sample was recorded, and the percentage occurence was determined using:

$$\text{Percentage occurance (\%)} = \frac{Number\ of\ particular\ species}{Total\ number\ of\ species\ found\ in\ the\ soil} \text{X } 100$$

Identification of Axenic Culture

A drop of distilled water was placed on a clean grease free microscope slide, using a sterile wire loop. Each of the fungal mycelia was gently coated on the slide and mixed gently to form a uniform solution. A drop of Lactophenol cotton blue stain was placed and mixed gently, then covered with cover slip. The stained slide was mounted on microscope and observed under low power (X10) and at higher X40 objectives. Characterization and identification of the various isolates was based on the microscopic and macroscopic examination (Bashir *et al.*, 2012; Benard *et al.*, 2013 and Nyongesa *et al.* 2015). For macroscopic observations, cultural appearance was observed on the surface of the agar such as pigmentation and nature of the colony were determined using Metheum hand book of color chat (Kornerup and Wancher, 1978). Microscopic examination was done based on the microscopic appearance of the colony and morphological characteristics of the isolated fungi by Color Atlas of Diagnostic Microbiology (Lous *et al.*, 1977). The identification was compared with photomicrographs for fungal identity.

Preparation and Standardization of Conidial Suspension

The conidial suspension were prepared from the two weeks old cultured isolates by flooding the plates with 10ml of sterile distilled water. Using a wire loop, the

spores from each plates of different pathogens were dislodged by gently scraping the surface and bringing them to the suspension and filtered using sterile cotton gauze. The filtrates were serially diluted to $1X10^5$. A Hemocytometer was used to count and adjust to standard spore concentration.

Pathogenicity Test

To establish Koch's postulate, the prepared spores were subjected to pathogenicity test as described by Freeman *et al*., (1998). The sorghum seeds were washed with sterile distilled water to remove the soil debris. The seeds were dipped in 35% and 75% ethanol for about 3minutes for surface sterilization and finally rinsed with distilled water to remove excess ethanol (Elmer *et al*., 2001). Five healthy seeds for each of the sorghum variety were inoculated per test tube and then incubated in growth chamber at 25°C. The inoculated healthy seeds were grown as seedlings and propagated on the same experimental conditions for control comparison. The control samples were treated with distilled water. The symptoms developed on the inoculated sorghum seed and seedlings were compared with those of control run experiment (ISTA, 1996).

Sub-Culture to Obtain Axenic Culture of the Pathogenicity Test

A 250ml of PDA were prepared by dissolving a 9.75g of PDA powder. Each plate was labelled according to the variety of sorghum *Yelai, Warwarbashi* and *Kaura***.** Using a wireloop, each of the appeared visible pathogenic fungus on the germinating seedling was placed on the PDA plate of each of the labelled sorghum variety.

Identification of the Axenic Culture of the Pathogenicity Test

A drop of distilled water was placed at the center of the microscopic slide, using wire loop. Each of the sample of the sub-cultured pathogenic fungus was gently coated on the slide and mixed gently to form a uniform solution. A drop of Lactophenol cotton blue was placed on the slide and mixed gently and covered with a cover slip, and placed under a microscope. The identification was determined with the help of a photomicrograph books and journals (Louis *et al*., 1997).

RESULTS

Table 1: Number of Species and Percentage Occurrence of Fungal Species Obtained from The Soil Samples

Fungal Species	Number of Species	Percentage Occurrence (%)
Aspergillus flavus	6	15
Aspergillus niger	6	30
Mucor Spp	2	10
Penicillium Spp	4	20
Rhizopus stolonifer	5	25
Total	20	100

Table 2: Morphological Characteristics of the Fungal Isolates from the Soil Samples.

Isolate Source	Microscopy	Macroscopy	Confirmed Organism
MF and UF	Spores radiating from hyphal end.	Green fluffy mycelial mats with black sporangia	*Aspergillus niger*
MF and UF	Branched and septate hyphae, colorless conidiospores with thick mycelial mat. Globose conidia and thick walled conidiophore.	Greenish conidia with deep brown scelerotia.	*A. Flavus*
UF	Non-septate mecelia with sporangia laden with spores.	White fluffy mycelial mats with black sporangia on further incubation.	*Rhizopus stolonifer*
MF and UF	Simple to branched sporangiophore. Apical globular to spherical multi-spored sporangia with collumella. Absence of rhizoids.	Cottony to fluffy, white to gray fast growing colonies.	*Mucor spp*
MF and UF	Branched conidiospores produced from the tip of philiades.	White colonies which become blue-green to yellow or pinkish on further incubation.	*Penicillium spp*

Key: UF = University Campus fields, MF: Madobi farm fields.

Table 3: Number of Spores in Conidial Suspension of *A. niger* and *R. stolonifer*.

Source	Sample Site	Fungal Species	Mass of Spores (cfu)
Soil	UF	*A. flavus*	$2.1x10^4$
Soil	MF	*R. stolonifer*	$2.3x10^4$

Key: UF = University Campus fields, MF: Madobi farm fields.

Table 4: Pathogenicity of the Fungal Isolates on Different Germplasms of Sorghum.

Isolates	Yelai	Kaura	Warwarbashi
Aspergillus flavus	+	+	+
Aspergillus niger	+	+	+
Rhizopus stolonifer	+	+	+
Mucor spp	+	+	+
Penicillium spp	+	+	+

Key: += positive results

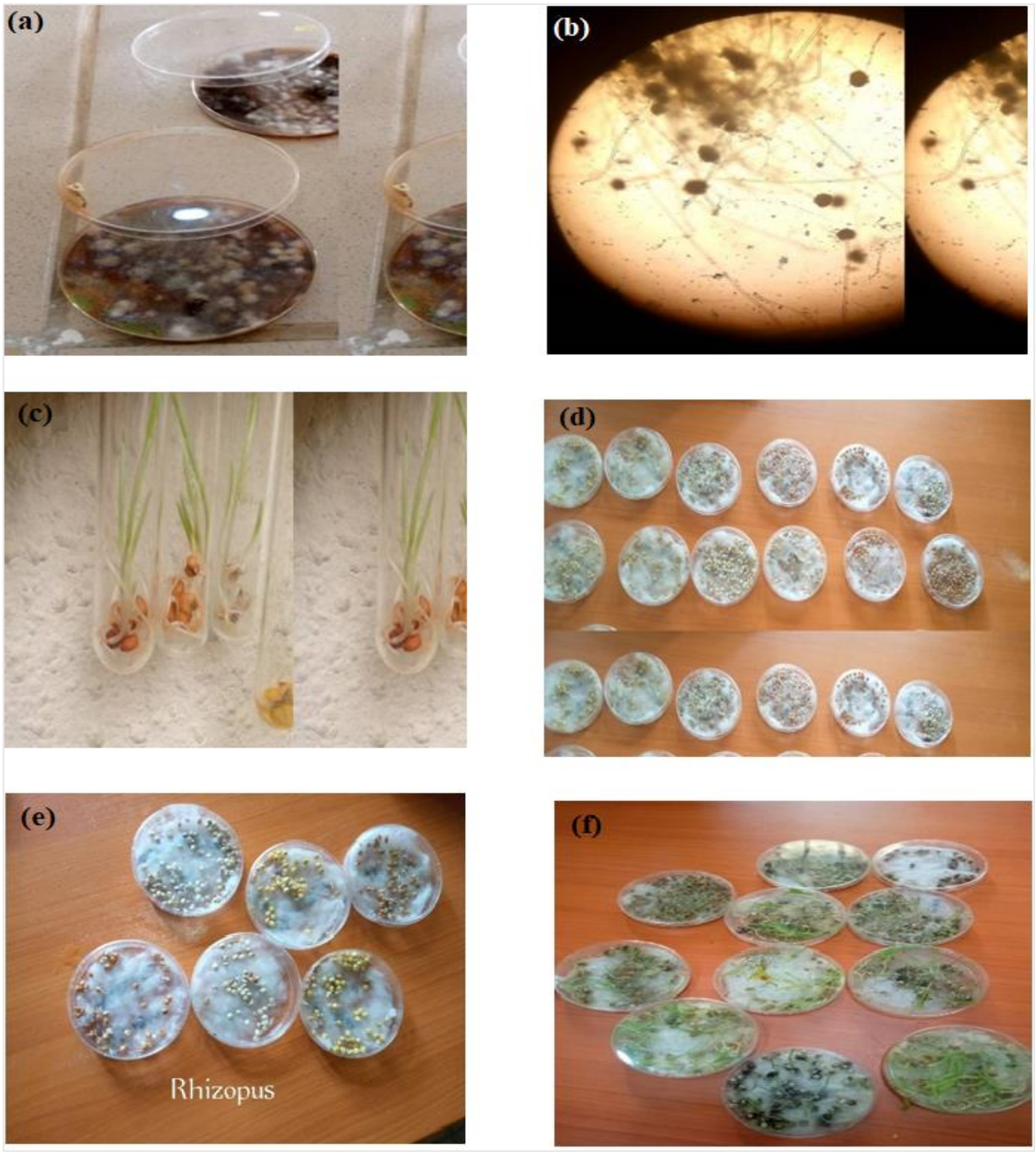

Figure 1: Isolation and Identification of Fungal Species (A-B), Inoculation and Pathogenicity Testing (C), Re-isolation and Identification of the Inoculated Fungi from Infected Seeds (D-F).

Discussion

The soil plating technique as described by Leslie and Summerell (2008) was employed in this study for the isolation of pathogenic fungal species. The isolated pathogens from the collected soil samples produced a diverse nature of fungal isolates (**Table 1)**. However, five most commonly soil borne fungi were obtained, these include *A. niger, A. flavus, Mucor spp, penicillium spp, and R. stolonifer*. This findings was in agreement with the work of Benard *et al.* (2013), Nyongesa *et al.* (2015), and Oladipo *et al.* (2016), who isolated and identified various species of *Aspergillus* in agricultural soil samples. However, the results of this study are coherent with those of Demirel *et al.* (2013), who isolated various *Penicillium* species from soil.

However, the presence of *R. stolonifer* and *Mucor spp* in this study is also in line with that of Kumar *et al.* (2013) and Paiva *et al.* (2017) that reported the presence of these species from soil.

Also, as indicated in (**Table 1)**, *A. niger* was the pathogen with the highest percentage of occurrence, accounting for 30%, followed by *R. stolonifer* and *Penecillium spp* with 25% and 20%, respectively. This demonstrates that the soil samples from both sampling locations are a good source of the aforementioned plant pathogens. These findings coincide with the work of Nyongesa *et al.* (2015) and Benard *et al.* (2013), who discovered the highest occurrence of *Aspergillus* species in various soil samples. This appears to suggest that the possibility of sorghum grain mould diseases due to *Aspergillus spp* in the areas where the sample was collected especially the Madobi farm lands, could be very high when susceptible cultivars are grown, and could be attributed to the high occurrence of *Aspergillus spp* inoculum in the soil.

In both the soil source, that is, Madobi farms and University fields, fungal species particularly *A. Falvus*, *A. niger*, *mucor spp* and *Penicillium spp* are very common. While *R. stolonifer* was found only associated with the sample collected from the university fields (**Table 2**). This could be as a result of the significant amount of an observed decaying litter in the environment. More importantly, *A. niger* was discovered to be the dominant species in soil sample collected from the different fields, with a significant number of spores recorded.

Pathogenicity testing (**Table 4**) revealed that *Yelai*, *Warwarbashi*, and *Kaura* germplasms were not resistant to any of the isolated and tested pathogens. This means that all of the screened cultivars are susceptible varieties when exposed to any of the isolated pathogens, and this is consistent with Ravimannan *et al.,* (2016). However, the pathogenicity analysis confirmed that the screened sorghum cultivars were more susceptible to *A. niger* and R. *stolonifer*, as well as *penicillium*, *A. flavus*, and *mucor spp* in descending order. This is in line with the results of Karim (2005), who reported that sorghum seeds are frequently infected by a variety of soil fungi. Moreover, the susceptibility of the tested Sorghum germplasm to these soil borne fungal pathogens can be attributed to the fact that the tested Sorghum seeds are from a local cultivar, this may be the consequences of the absence of resistance genes associated with the varieties. It could be however, that the isolated pathogens in this study were a highly virulent strains capable of weakening the resistivity of the seeds to the pathogen attack.

CONCLUSION

In conclusion, the isolated pathogens had significant detrimental impacts on the seedling's life of Sorghum in vitro and in controlled parameters, the pathogens were all virulent when their pathogenicity was determined on the tested Sorghum germplasms and also indicated that none of the Sorghum seeds screened possess the resistance against the pathogens. These fungi may be responsible for the post-harvest diseases of *S. bicolor*, and local cultivars such as *Yelai*, *Warwarbashi*, and *Kaura* germplasms. Therefore, field based pathogenicity of these pathogens on sorghum cultivars are recommended to rectify these findings.

REFERENCES

Agrios, G.N. (2005). Plant Pathology. 5th Edition. *Elsevier, USA*. p. 305-307.

Bartram, A. K., Jiang, X., Lynch, M. D., Masella, A. P., Nicol, G. W., Dushoff, J., and Neufeld, J. D. (2014). Exploring links between pH and bacterial community composition in soils from the Craibstone Experimental Farm. *FEMS Microbiology Ecology*, *87*(2), 403-415. **[Crossref]**

Bashir, M., Mani, M. A., and Kutama, A. S. (2012). Seed-borne mycoflora of local and improved wheat (Triticum sativum L.) cultivars in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences*, *5*(2), 101-103. **[Crossref]**

Benard, O. O., Hunja, M., and Isabel, N. W. (2013). Isolation and characterisation of aflatoxigenic Aspergillus species from maize and soil samples from selected countries of Kenya. *African Journal of Microbiology Research*, *7*(34), 4379-4388.

Cheesbrough, M. (2006). District laboratory practice in tropical countries, (Low Price Edition). *The press Syndycate of the University of Cambridge*. P 305-306. **[Crossref]**

Cuevas, H. E., Prom, L. K., Isakeit, T., and Radwan, G. (2016). Assessment of sorghum germplasm from Burkina Faso and South Africa to identify new sources of resistance to grain mold and anthracnose. *Crop Protection*, *79*, 43-50. **[Crossref]**

De Morais Cardoso, L., Pinheiro, S. S., Martino, H. S. D., and Pinheiro-Sant'Ana, H. M. (2017). Sorghum

(Sorghum bicolor L.): Nutrients, bioactive compounds, and potential impact on human health. *Critical reviews in food science and nutrition*, *57*(2), 372-390. **[Crossref]**

Demirel, R., Sariozlu, N. Y., and İlhan, S. (2013) . Polymerase chain reaction (PCR) identification of terverticillate Penicillium species isolated from agricultural soils in eskişehir province. *Brazilian Archives of Biology and Technology*, *56*, 980-984. **[Crossref]**

Elmer, W. H. (2001). Seeds as vehicles for pathogen importation. *Biological Invasions*, 3(3), 263-271. **[Crossref]**

FAO, (2012). Food and Agriculture Organization; FAOSTAT Data. Available at: http:// faostat.fao.org/site/339/default.aspx (last accessed on 15.08.2022.).

Ferreira, I. N. S., Rodríguez, D. M., Campos-Takaki, G. M., and da Silva Andrade, R. F. (2020). Biosurfactant and bioemulsifier as promising molecules produced by Mucor hiemalis isolated from Caatinga soil. *Electronic Journal of Biotechnology*, *47*, 51-58. **[Crossref]**

Freeman, S., Katan, T., and Shabi, E. (1998). Characterization of Colletotrichum species responsible for anthracnose diseases of various fruits. *Plant disease*, 82(6), 596-605. **[Crossref]**

ISTA (1996). International rule for seed testing. *Seed Sci & Technology* 21:25-30.

Kangama, C. O., & Rumei, X. (2005). Introduction of sorghum (Sorghum bicolor (L.) Moench) into China. *African Journal of Biotechnology*, 4(7), pp. 575-579.

Karim, M (2005). Prevalence of fungi associated with seeds of some minor cereals. *An M.Sc thesis. Department of plant pathology, Bangladesh Agricultural University, Mymensingh.*, P.97.

Knowles, O., and Dawson, A. (2018). Current soil sampling methods–a review. *Farm environmental planning–Science, policy and practice.(LD Currie and CL Christensen, Eds), Occasional Report*, (31), 1-11.

Kornerup, A., and Wancher, J. H. (1978). Handbook of Colour. *Eyre Methuen Ltd.*

Kumar, R., Mishra, R., Maurya, S., and Sahu, H. B. (2013). Isolation and identification of keratinophilic fungi from garbage waste soils of Jharkhand region of India. *European Journal of Experimental Biology*, *3*(3), 600-604.

Leslie, J. F., and Summerell, B. A. (2008). *The Fusarium laboratory manual*. John Wiley and Sons. P. 18-19.

Luce, E. (2010). Koneman's color atlas and textbook of diagnostic microbiology. *Plastic and Reconstructive Surgery*, 125(1), 414-415. **[Crossref]**

Naqvi, S. D. Y., Shiden, T., Merhawi, W., and Mehret, S. (2013). Identification of seed borne fungi on farmer saved sorghum (Sorghum bicolor L.), pearl millet (Pennisetum glaucum L.) and groundnut (Arachis hypogaea L.) seeds. *Agricultural Science Research Journals*, *3*(4), 107-114.

Nyongesa, B. W., Okoth, S., and Ayugi, V. (2015). Identification key for Aspergillus species isolated from maize and soil of Nandi County, Kenya. *Advances in Microbiology*, *5*(04), 205. **[Crossref]**

Oladipo, O. G., Awotoye, O. O., Olayinka, A., Ezeokoli, O. T., Maboeta, M. S., and Bezuidenhout, C. C. (2016). Heavy metal tolerance potential of Aspergillus strains isolated from mining sites. *Bioremediation Journal*, ***20***(4), 287-297. **[Crossref]**

Paiva, W. S., Souza Neto, F. E., and Batista, A. C. L. (2017). Characterization of polymeric biomaterial chitosan extracted from Rhizopus stolonifer. *Journal of Polymer Materials*, *34*, 115-121.

Ravimannan, N., Arulanantham, R., Pathmanathan, S., and Niranjan, K. (2014). Alternative culture media for fungal growth using different formulation of protein sources. *Annals of Biological Research*, *5*(1), 36-39.

Ravimannan, N., Sevvel, P., and Saarutharshan, S. (2016). Study on fungi associated with spoilage of bread. *International Journal of Advanced Research in Biolical Science*, *3*(4), 165-167.

Sabouri, H., Kazerani, B., Fallahi, H. A., Dehghan, M. A., Alegh, S. M., Dadras, A. R., and Mastinu, A. (2022). Association analysis of yellow rust, fusarium head blight, tan spot, powdery mildew, and brown rust horizontal resistance genes in wheat. *Physiological and Molecular Plant Pathology*, *118*, 101-808. **[Crossref]**

Smith, C. W., and Frederiksen, R. A.(2000). Sorghum: Origin, history, technology, and production (Vol. 2). John Wiley & Sons. P. 5-7

Taleon, V., Dykes, L., Rooney, W. L. and Rooney, L. W. (2012). Effect of genotype and environment on flavonoid concentration and profile of black sorghum grains. *Journal of Cereal Science*. 56:470–475. **[Crossref]**

Tesso, T. T., Perumal, R., Little, C. R., Adeyanju, A., Radwan, G. L., Prom, L. K., & Magill, C. W.

(2012). Sorghum pathology and biotechnology-a fungal disease perspective: Part II. Anthracnose, stalk rot, and downy mildew. *European Journal of Plant Science and Biotechnology*, *6*(Special Issue 1), 31-44.