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Study on the Pathogens Associated With Diseases of Millet in Suburbs of Aliero, Kebbi State

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Abstract

Diseased Millet seedlings in the cultivated farmlands of Aliero were studied to identify the causative microorganisms. Samples were purposely collected after identifying them by visible symptoms. Collection was in three locations; Kali (KAL), Government science secondary school (GSSS) and Labana (LBN). The samples were taken to the Microbiology laboratory of Kebbi State University of Science and Technology Aliero in sterile polythene bags. Nutrient and potato dextrose agars were used to culture bacteria and fungi respectively. The results revealed that GSSS had the highest bacterial load of $6.6x10^5$cfu/g while Labana had the lowest bacterial load of $2.1x10^7$cfu/g, but same Labana had the highest fungal load of $9.0x10^5$cfu/g while Kali had the lowest fungal load of $3.0x10^5$cfu/g. Streptococcus spp had the highest frequency of 19.15% followed by Xanthomonas campestris with 17.89, Streptococcus spp 17.02 and lastly Streptobacilli spp with 6.38%. Trichophyton spp fungus recorded the highest frequency of 13.33% while lowest fungus in frequency was Apophysomyces elegans with 3.33%. Results revealed presence of pathogenic Xanthomonas campestris with contaminant microorganisms. Good post harvest storage and pre-planting procedures are recommended to avert transfer of the organisms during harvest

Key Words: *Millets; Bacteria; Fungi; Aliero*

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I. Introduction

Pearl millet (*Pennisetum glaucum* L.) are tall grasses with heads of small seeds grown in harsh environments where other crops generally fail. It is an erect annual grass, reaching up to 3 m high with a profuse root system (1)..is the fifth most important cereal crop in the world after rice, wheat, maize, and sorghum. Millets are important crops in Asia Africa especially in India, Nigeria and Niger with 97% of millet production. The crop is favored due to its productivity and short growing season dry and high temperature condition. (2). Millet is cultivated in Six Northern States of Nigeria and it is a major crop amongst others, useful for minimizing the adverse effect of climate change, hence facilitating income and food security among farming communities (3).

Millet is more nutritious than maize as it supplies more than 12 % protein compare with 9.69 % of that maize. A rapid growth increase in production of millet is dictated by the intensive development of poultry industry and cattle ranching throughout Nigeria. (4). People affected by gluten-related disorders, such as coeliac disease, non-celiac gluten sensitivity and wheat allergy sufferers 5), who need a gluten-free diet, can replace gluten-containing cereals in their diets with millet (6). In a 100 gram serving, raw millet provides 378 calories and is a rich source (20% or more of the Daily Value, DV) of protein, dietary fiber, several B vitamins and numerous dietary minerals, especially manganese at 76%. Raw millet is 9% water, 73% carbohydrates, 4% fat and 11% protein (7).

Millet utilization according to FAO shows that out of the 30 million tonnes of millet produced in the world, about 90 % is utilized in developing countries and only a tiny volume is used in developed countries outside the former Soviet Union. It is estimated that a total of 20 million tonnes are consumed as food, the rest being equally divided between feed and other uses such as seed, the preparation of alcoholic beverages and water. Six countries, China, Ethiopia, India, Niger, Nigeria and the former Soviet are estimated to account for about 80 % of global millet utilization (8).

Fungi are a group of micro organisms that are classified within their own kingdom, the kingdom Mycota as they are neither plants nor animals (9). Ergot is caused by *Claviceps fusiformis* Lov. The disease has proved a menacing barrier as well as the high yielding hybrids is highly susceptible to this disease. About 58 to 75% grain-loss is reported due to this disease (10). Smut is also a floral disease caused by *Tolyposporium penicillariae* Bref. It is generally reported to cause 5-30% losses in yield in farmers' fields (11). Rust is

considered to be a minor importance. Bacterial diseases include bacterial spot with round, oblong, linear or irregular water-soaked leaf spots expand to form oval to elongate tan necrotic lesions with a thin dark brown margin (12). Bacterial leaf streak is caused by *Xanthomonas campestris* (Pammel) Dowson pv. *pennamericanum*. Symptoms are not clearly defined. Similar to bacterial leaf stripe and streak of sorghum. Bacterial leaf stripe is caused by *Pseudomonas avenae* Manns. Water-soaking occurring at the ends of advancing interveinal lesions which vary in length from several to more than 25cm. Older lesions are usually light brown.

Disease control is achieved by cultural and chemical methods. Cultural control is to reduce the primary inoculum load of the pathogen by deep ploughing of field in hot summers is recommended so as to bury the sclerotia deep in the soil to prevent their germination. Using seed obtained from disease-fee crops helps reduce the primary inoculum. Hybrids having the traits of rapid pollination have provided excellent biocultural control against the disease (13).

II. Materials And Methods

Purposeful survey was conducted to select samples of infected millet at three different areas in Aliero and three (3) samples were collected in each of the collection areas. The collection areas were Kali, Government science secondary school (GSSS) and Labana. Each sample was collected in sterile polythene bags and were labeled separately for easy identification. The sterile polythene bags containing the samples were immediately taken to the Microbiology Laboratory of Kebbi State University of Science and Technology, Aliero for analysis.

Ten gram (10 g) of each sample was weighed and homogenized using sterile mortar and pestle Serial dilution was conducted and 10 g of each of homogenized millet sample was placed in a sterile conical flask containing 90 ml of sterile distilled water and mixed vigorously in order to obtain homogenized sample solution. 1 – 10 test tubes with 9 ml of distilled water each were placed in a test tube park at vertical position. 1 ml of each homogenized sample solution was poured into the first test tube containing water by using a sterile pipette, capped and shaken vigorously and one ml from the tube was collected and transferred into second test tube by using a sterile pipette and it was serially diluted to the last test tube (10^{-9}) (14). From each of the samples, 1 ml of homogenized millet was measured and poured into 9 ml of sterile distilled water (1:10) contained in a test tube. This procedure was repeated until the 10th test tube. From test tube 3, 1 ml was pipetted into a sterile petri-dish. A molting nutrient agar medium was maintained at 45^0 C in universal bottle then poured unto the 1 ml dilution in the plate. The plate was swirled to allow complete mixture of the medium and then inoculated with the millet dilution. The plates were allowed to solidify before the set up was incubated at 35^0C (14).

For fungal isolation, 0.1 ml was pipetted from 10^{-1} 10^{-3} and 10^{-5} dilution factor of each sample were aseptically inoculated on the solidified plates of potato dextrose agar (PDA) media respectively. The plates were incubated for 5 days before colonies of fungi were counted and reported as colony forming units/g (CFU/g). Due to the appearance of different colonies, each colony was sub-cultured into a new plate and incubated at 29 ± 20^0 C for 5 – 7 days (15). The pure isolates were subjected to microscopic examination to identify the organisms present in the samples. The nature of the mycelia, types of fruiting bodies and the spore structures served as the criteria for the identification of the isolates. The isolates were identified and confirmed with the aid of mycological atlas (16).

In isolation of bacteria, 0.1ml was pipetted from 10^{-2} $10^{-4}$and 10^{-5} dilution factor of each sample were aseptically inoculated on the solidified plates of Nutrient Agar (NA) media. The plates were incubated at 37^0C for 24 hrs before bacteria colonies were counted and reported as colony forming units/g (CFU/g). Each colony was sub cultured using a new plate and incubated at 37^0 C for 24 hrs (15). The isolates were identified based on colonal, morphological and biochemical characteristics. The biochemical tests carried out include; Catalase Test, Coagulase Test and MSA Test. Sterilization of materials was carried using autoclave at 121^0 C for 30 minutes, while agars for 15 minutes. After 15 minutes, the machine was switched off and the pressure was allowed to fall until it reaches zero (15; 14).

Plate Plate 1: diseased millet sample

III. Results And Discussions

The results from the enumeration of microbial load carried out from Kali are presented in the Table 1, Bacteria load ranged from 2.7x10^5 to 4.3x10^1 cfu/g, while fungi ranged from 3.0x10^5 to 6.0x10^{1}cfu/g. The results from Labana presented in Table 2,showed that bacteria load ranged from 2.1x10^6 to 6.3x10^{1}cfu/g, while fungi ranged from 3.0x10^6 to 6.0x10^{4}cfu/g. The results from GSSS presented in the Table 3, revealed that bacteria load ranged from 3.1x10^5 to 6.6x10^2 cfu/g, while fungi ranged from 5.0x10^5 to 9.0x10^{2}cfu/g. The results showed GSSS had the highest bacterial load 6.6x10^{5}cfu/g and Labana had the lowest bacterial load 2.1x10^{7}cfu/g, Labana had the highest fungal load 9.0x10^{5}cfu/g and Kali had the lowest fungal load 3.0x10^{5}cfu/g.

Table 1: Microbial viable count of Kali Sample

Sample	D/F	No. of Colony		cfu/g	
		Bacteria	Fungi	Bacteria	Fungi
KALM1	10^1	43	6	4.3x10^1	6.0x10^1
KALM2	10^3	35	4	3.5x10^3	4.0x10^3
KALM3	10^5	27	3	2.7x10^5	3.0x10^5

Table 2: Microbial viable count of Labana Sample

Sample	D/F	No. of Colony		cfu/g	
		Bacteria	Fungi	Bacteria	Fungi
LBNM1	10^1	63	5	6.3x10^1	5.0x10^1
LBNM2	10^4	43	6	4.3x10^4	6.0x10^4
LBNM3	10^6	21	3	2.1x10^6	3.0x10^6

Table 3: Microbial viable count of GSSS Sample

Sample	D/F	No. of Colony		cfu/g	
		Bacteria	Fungi	Bacteria	Fungi
GSSSM1	10^2	66	9	6.6x10^2	9.0x10^2
GSSSM2	10^4	49	6	4.9x10^4	6.0x10^4
GSSSM3	10^5	31	5	3.1x10^5	5.0x10^5

From the Table 4 it was reported that *Streptococcus spp* had the highest frequency 19.15% followed by *Xanthomonas campestris*, 17.89%, followed by *Streptococcus spp* 17.02% and lastly *Streptobacilli spp* with 6.38%.

The isolated *S. aureus* in millets samples could have arisen from seed storage and transportation. (17) discovered some bacteria and fungi on millets (*Penisettum glycum*) *Aspergilus fumigatus, A. niger, penicillum R. stolinifer* and *staphyloccus aureus* in the suggest and extremely poor storage system, deplorable sanitary condition available at the study area and multiple sources of contamination due to open access to the poor sanitation. Similar and related organisms were implicated in food and canned products by (Gadaga *et al.,* 2008; Taulo 2009 (Oladipo omo Adu, 2011).

Table 4: Number and percentage of Bacteria isolated from millet leaves

Isolate	Kali (%)	Labana (%)	GSSS (%)
Staphylococcus aureus	8 (17.02%)	5 (10.64%)	4 (8.51%)
Streptococcus spp	3 (6.38%)	3 (6.38%)	9 (19.15%)
Xanthomonas campestris	0 (0.00%)	7 (17.89%)	2 (4.26%)
Streptobacilli spp	3(6.38%)	0 (0.00%)	3 (6.38%)

TOTAL	14	15	18
PERCENTAGE (%)	29.79	31.92	38.30

Table 5: Number and percentage of fungi isolated from millet leaves based on sample locations

Isolate	Kali(%)	Labana	GSSS
Trichophyton spp	9(10.00)	0(0.00%)	12(13.33%)
Aspergillus fumigatus	5(5.56%)	6(6.67%)	7(7.78%)
Microsporum spp	0(0.00%)	6(6.67%)	7(7.78%)
Grapium spp	5(5.56%)	4(4.44%)	0(0.00%)
Aspergillus niger	6(6.67%)	9(10.00%)	9(10.00%)
Apophysomyces elegans	0(0.00%)	3(3.33%)	2(2.21%)
TOTAL	25	28	37
PERCENTAGE (%)	27.78	31.11	41.11

From Tables 5 and 6 for the isolation of fungi, it can be seen that *Trichophyton spp* has the highest frequency 13.33% followed by *Aspergillus niger* 10.00, followed by *Aspergillus fumigatus* and *Microsporum spp* 7.78%, followed by *Grapium spp* 5.56 % and lastly *Apophysomyces elegans* with 3.33%.. Plate 2 also shows pure cultures of the isolated fungi from the diseased millet samples.

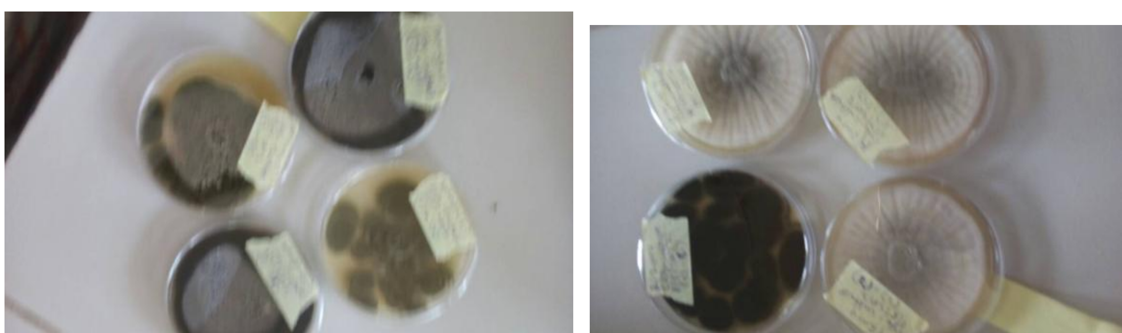

Plate 2: Isolated microorganisms from illet samples.

Table 6: Morphological and Microscopic Characteristic of Fungi Isolated from millet leaves

Colony appearance	Microscopic examination	Fungi species
Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.	*Aspergillus fumigatus*
Colonies are white, flat, powdery, sometimes downy to fluffy with a brilliant lemon yellow reverse.	Numerous large slender clavatemicroconidia borne on the sides of hyphae and its smooth, thin-walled clavatemacroconidia	*Trichopyton spp*
Colonies are fast growing, white, becoming creamy white to buff with age.	Sporangiospores are smooth-walled, oblong and subhyaline.	*Apophysomyces elegans*
White in color at first and black. The reverse side was pale yellow.	Hyphae were septate, simple and Thick walled. Conidiophores bearing Conidial heads containing conidian Were seen	*Aspergillus niger*
Colonies are generally flat, white to pinkish in colour, with a suede-like to granulartexture and peripheral fringe. Reverse pigmentation is orange to red.	Macroconidiaare thin-walled, cigar-shaped, four- to seven-celled, 40-60 x 6-8 µm but are only rarely roduced. Microconidia are abundant, spherical to pyriform.	*Microsporum spp*
Colonies are effuse, grey, olivaceous brown or black.	Dematiaceoushyphomycete producing erect synnemata. Apical aggregates of single-celled conidia in slimy heads.	*Graphium spp*

IV. Conclusion And Recommendations

It is concluded that, there is fungal and bacterial contamination from the different samples collected from areas in Aliero town of Kebbi State. The highest frequency of occurrence of fungal infection was found in GSSS sample with 41.11%, followed by Labana sample with 31.11% and Kali Sample with 27.78%. However, the highest frequency of occurrence of bacterial infection was obtained in GSSS sample with 38.30%, followed by Labana sample with 31.92% and Kali Sample with 29.79%. The three different areas from which the sample were collected do not have significant difference but between the pathogens (bacteria and fungi), there was significant difference.

Since millet is one of our major foods consumed by the local people, it is recommended that its microbial examination should be carried out at regular interval so as to assess their suitability for production. Proper seed storage management should be observed right from the millet on field to storage facilities in order to avoid fungal and bacterial contamination and pathogen roll over.

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