SEED BORNE FUNGAL PATHOGENS ASSOCIATED WITH FINGER MILLET ACCESSIONS

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ABSTRACT

Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases. A study on the detection and identification of seed-borne fungi was done using seeds of finger millet (*Eleusine coracana* (L.) Gaetrn) accessions collected from different parts of the country. The research was undertaken to determine different seed-borne fungal pathogens associated with seeds of finger millet accessions from November 27, 2020 to February 25, 2021 at National Agriculture Genetic Resources Centre, Khumaltar, Lalitpur. A total of 85 seed samples, collected from different parts of the country, were used for the research. A hundred seeds of each sample were tested for the presence of seed-borne fungi using the Deep Freeze Blotter method following the International Rules for Seed Testing Association, 2001, and identification was done based on the growth habit, and morphology of the spores observed. One or more pathogens were detected in 65 out of 85 samples. Four different pathogens, namely *Bipolaris nodulosa*, *Alternaria* spp., *Cladosporium* spp*.,* and *Pyricularia grisea* were identified. *B. nodulosa* and *Cladosporium* spp. were predominant in most of the samples with seed infection in 42 (49.4%) and 40 (47.05%) seed accessions, respectively. Hence, it is highly recommended to undertake focused research on the economic significance of the seed-borne fungi, while considering their impact and effective management strategies in order to enhance seed health management.

Key words : Accessions, fingermillet, samples, seed-borne fungi, seed health testing

INTRODUCTION

Finger millet locally known as *Kodo* is the fourth most important staple crop after rice, maize, and wheat, and the second most important food crop in the hills of Nepal (Pradhanang, 1990). Nepal is considered a secondary centre of millet diversity with diverse types of varieties grown by smallholder farmers across different altitudes, farming systems, and locations in the country (Gairhe *et al.*, 2021). Being a highly adaptive plant, finger millet plays important role in the food and nutritional security of poor people dwelling under fragile, marginal, and inaccessible regions of hilly areas (Adhikari *et al.*, 2015). About 877 accessions have been maintained by National Plant Genetic Resource Centre (NPGRC), Khumaltar, Nepal (Bastola *et al.*, 2015).

Among millet groups, finger millet is the most important food crop of Nepal in terms of area and production followed by proso millet and foxtail millet. It covered 274350 ha of land and produced 305588 mt with the productivity 1115 kg/ha (MoAD 2013). According to the statistics of 2018/19, it is planted in the area of 263,261 ha in with average productivity of 1.19 t/ha (MoALD, 2019) which

was 1.10 t/ha a decade ago in 2008/09 (MoALD, 2009). According to the recent data of the Ministry of Agriculture and Livestock Development (MoALD, 2019) finger millet is grown in 70 districts except in 2 districts of high mountains (Manang and Mustang) and 4 districts of Terai namely, Kapilbastu, Banke, Bardiya and Kanchanpur. High diversity of finger millet is found in the mid hills of Nepal. Out of 15 species reported in the world, three of them namely *Elusine coracana*, *E. indica* and *E. aegyptica* are found in Nepal (Kandel *et al.*, 2019). The major production districts of Nepal for this crop are Khotang, Sindhupalchok, Baglung, Syangja, Kaski, Gorkha, and Sindhuli (Gairhe *et al.*, 2021). Though finger millet is considered most labor intensive crop, 76% of cultivated land in hills occupies finger millet, 20% in the mountains and 4% in Terai (MOAD, 2012).

About 90 % of all food crops in the world are propagated by seeds (Schwinn, 1994). They are the most vital input for crop production. But they are also the passive carriers of pathogens, which are transmitted when the seeds are sown under suitable environmental conditions (Noble, 1957, 1971). The pathogens remain viable longer in seeds than in vegetative plant parts or in the soil (Gaur *et al.*, 2020). Most of the farmers use seeds from the previous harvest as planting material. If the seeds from the previous harvesting are contaminated, there is a high chance that these seeds will become the source of contamination in the next season as well. This will not only spread the seed-borne diseases, but may also be responsible for the spread of other harmful diseases as well. Pathogen-free seeds are vital to have desired germination, emergence, healthy seedlings, and overall high plant population and yield (Adhikari *et al.*, 2018). Seed borne diseases directly or indirectly affect the growth and productivity of crop plants. Any seed borne pathogen either bacterial, fungal, or viral present internally, externally, or as a contaminant may cause severe problems like seed rot, seed necrosis, seed abortion, reduction in the size of seeds, reduced germination, and vigor as well as seedling damage. This later results in the development of diseases in later stages of plant growth (Bateman & Kwasna, 1999).

Seed-borne diseases caused by different fungal pathogens are a major constraint affecting the crop at all stages leading to serious grain losses, which if saved are adequate to feed millions of people annually. The average yield loss reported due to the blast was around 28-36% (Nagaraja and Mantur, 2007). These pathogens are reported to cause seed deterioration in storage resulting retarding effect in seed germination and other seed quality parameters (Jain, 2020). Interestingly, important but devastating diseases are caused by seed-borne fungi. Learning about the seed health situation is an important step for achieving seeds of desired genetic constitution and physical purity. Hence, disease screening is first step towards controlling the seed-borne diseases.

Fungi are the key seed borne microflora that are found associated with the seed externally or internally. They are reported to cause seed deterioration in storage resulting retarding effect in seed germination and other seed quality parameters (Jain, 2020). Most of the pathogens transmitted by seed are fungi. The extent to which these fungal pathogens occur in seeds depends on their capability to survive under extremely dry conditions of seed as a carrier of the disease (Neergaard, 1977). Many fungal pathogens are associated with crops among which nearly 300 fungal microflora are found associated with approximately 25 % of world crop production that produces harmful mycotoxins for humans and animals (Kamil *et al.*, 2020). Finger millet seeds with fungal infections are potentially toxic to plants and indirectly to human health.

Blast (*Pyricularia grisea*) and Cercospora leaf spot (*Cercospora eleusine*) are two important fungal diseases of fingermillet in Nepal (Manandhar *et al.*, 2016) and seed borne in nature (Manandhar *et*

al., 2016; Pande *et al.*, 1994). Relatively little is known about the seedborne nature of these diseases of fingermillet in Nepal. Therefore, the present study aims to identify seed-borne fungi found in seeds of finger millet (*E. coracana*) accessions collected from different parts of the country. The objective of seed health testing is to identify the healthy and pure seeds that can be sown in the field. This ultimately results in production of healthy food, healthy seed crops, and improved yields in terms of quality and quantity (Adhikari *et al.*, 2018). Early identification of seed borne pathogens allows timely management of diseases and helps to avoid epidemics (Nafula, 1997). Studies on the epidemiology of various seed-borne pathogens of finger millet are still limited on national as well as international level. Therefore, data generated from the proposed study will be very useful in identifying interventions/measures for management of seed-borne pathogens in finger millet. This study will help to establish any relevant information regarding the different seed-borne fungal diseases of finger millet.

MATERIALS AND METHODS

Research Site

The research was carried out in a seed test laboratory in National Agriculture Genetic Resources centre (Genebank), Khumaltar, Lalitpur from 2020 November 27 to 2021 February 25.

Collection of Seed Samples

A total of 85 seed accessions of different varieties/acessions of finger millet were collected from the National Agriculture Genetic Resources centre (Genebank), Khumaltar, Lalitpur. These seed samples were collected from different parts of the country, representing 33 different districts and stored in the genebank for conservation and research purposes with unique accession number (Annex 1).

It would have been better to collect seeds directly from the field, however we used samples from the genebank because of easy access and economy. We speculated field problem by investigating seeds available in genebank. These sample seeds have been conserved for few years at genebank.

About 877 accessions have been maintained by National Plant Genetic Resource Centre (NPGRC), Khumaltar, Nepal but very limited researches had been accomplished regarding with finger millet, although have immense potentiality for its improvement. We have observed many finger millet diseases in field during visit, however there is limited documentation on it. Less utilization of local genetic resources conserved in genebank for crop improvement program is evident due to lack of information about the desirable accessions in the genebank. Besides, documentation on seed-borne fungi is almost nil. We are trying to document for the first time in Nepal. This research is also important from genebank management view point as we want to store healthy seeds for upcoming generations.

Seed Health Testing

The finger millet seed samples collected from different parts of the country were then assayed for the presence of fungi by the Deep-freeze blotter method following the rules of the International Seed Testing Association (ISTA, 2001).

Twenty-five seeds were placed in each petri plate containing three layers of sterilized water-soaked blotting papers in 16-8-1 format in a circular way i.e. a seed in the center followed by 8 and 16 seeds, respectively, in the outer circle. Total 100 seeds of each accession were placed in the petri plate for

incubation in 4 different petri plates. Seeds were placed using clean and sterilized forceps. Incubation was carried out in the cooling incubator for12 hours of alternate cycles of light and darkness at ± 20 °C for 24 hours. After 24 hours, the incubated seeds were transferred to the deepfreeze at -20 °C for the next 24 hours to inhibit the germination of seeds. After keeping the petri plates in deepfreeze for 24 hours they were retransferred to the cooling incubator and incubated for another 5 days under 12 hours alternate cycle of NUV (Near Ultra Violet) light and darkness at ± 20 °C. Watering was done with the help of a dropper in an interval of 3 days if necessary to avoid loss of moisture from the blotting paper.

Each seed was observed under the stereoscopic microscope. The fungi were identified based on the morphological characters and growth habitat observed in the seed surface. The temporary slides of the fungal growth were prepared and observed in a compound microscope to identify the conidia, conidiophores, and other morphological and physical characteristics of the fungi associated with the seeds properly and surely. The microscopic examination of these finger millet seed samples revealed the presence of spores of different species (as shown in Annex 2). Characteristics like germination and rotten stage of the seed i.e not rotted, partially rotted, and completely rotted were also observed. The number of different fungi on the seeds was counted and the observations were entered in a data sheet.

The percentage frequency for number of seeds and number of samples infected were calculated by using the following formula (Naqvi *et al.,* 2013) :

Frequency = N/T ×100%

where, N = Number of seeds on which a fungal species occur
T = Total number of seeds

Similarly, for sample frequency,
Frequency = N/T ×100%

where, N = Number of samples on which a fungal species occur
T = Total number of seeds

RESULTS AND DISCUSSION

Incidence of Seed-Borne Fungi

The microscopic examination of the incubated seeds of finger millet samples revealed the presence of spores of *Bipolaris nodulosa*, *Alternaria* spp., *Cladosporium* spp*.,* and *Pyricularia grisea.* One or more fungal pathogens were detected in each of 65 finger millet accessions out of the 85 accessions (Annex 2). *B. nodulosa* and *Cladosporium* spp. were the most frequently encountered species with an incidence of 49.4%, and 47.05%, respectively. On the contrary, *Alternaria* spp., and *P. grisea* were observed in the minority recording only 16.47%, and 7.05% seed sample infections (Table 1). In some cases, *B. nodulosa* which was predominant among all the other fungal pathogens was found growing together with *P. grisea*. In such cases, *B. nodulosa* overgrew *P. grisea* which made the identification of *P. grisea* difficult. This partly might be the reason for the low incidence of *P. grisea or* there might be antagonistic effects between them. Seed of 21 accessions (NGRC03623, NGRC05752, NGRC03501, NGRC03620, NGRC04818, NGRC03483, NGRC04790, NGRC03630, NGRC03443, NGRC01418, NGRC03694, NGRC04833, NGRC01599, NGRC01603, NGRC01622,

NGRC03467, NGRC03512, NGRC03484, EN 115, EN 118 and EN 119) were found free of any seed borne pathogen.

The highest incidence of *B. nodulosa* was recorded in NGRC01432 with 16 infected seeds. Similarly, the highest incidence of *Cladosporium* species and *Alternaria* species were recorded in NGRC03459 (9 infected seeds) and NGRC03573 (5 infected seeds), respectively. The blast disease pathogen, *P. grisea* was detected in seed samples of only 6 accessions which included NGRC04794, NGRC01525, NGRC01639, NGRC03544, NGRC04860, and NGRC01623. The highest total incidence of these pathogens was recorded in NGRC01432, NGRC03573 and NGRC03459 with 17, 14, and 14 total infections, respectively. To be precise, 17, 14, and 14 seeds (out of the 100 total seeds observed for infection) were found to be infected by different fungal pathogen in NGRC01432, NGRC03573 and NGRC03459, respectively.

This shows seed health status of seeds conserved in National Genebank where seed health should be in top priority. Besides, it also paves the way for the researchers in the future. The upcoming researchers who have a passion for related subjects can work on identified pathogens. In addition to making the data more accessible, this will also ensure accuracy, leaving very little room for error.

Table 1. Incidence of different seed-borne fungi in the collected finger millet seed samples from different districts

Types of Fungi	No. of Samples Infected	Samples Infected (%)
Alternaria spp.	14	16.47
Bipolaris nodulosa	42	49.4
Cladosporium spp.	40	47.05
Pyricularia grisea	6	7.05

Note: Each seed testing sample contains 100 seeds

The incubated seeds were transferred to deepfreeze for the next 24 hours. It was later observed that doing so inhibits the germination of seeds and makes the examination of seeds easier. Imbibed seeds on moist blotters are killed by the low temperature (- 20 ^{o}C). This allows better growth of the fungal pathogens. It is important to note that deep freezing does not affect the fungi associated with the seed.

The highest germination was recorded in seeds infected by the fungus *B. nodulosa*. Out of total 114 infected seeds (100 seeds each from 85 different seed accessions), 32 (28.07%) seeds were germinated. So, for getting highest percentage of germination,we need to infect the seeds by *B. nodulosa*. The number of seeds infected by *P. grisea* is significantly very low. Similarly, the lowest germination was recorded in seeds infected by *Cladosporium* spp. Only 10 seeds (13.69%) out of 73 infected seeds were found to be germinated. Most of the heavily infected seeds did not germinate. Rot percentage of the infected seeds was also calculated. A total of 11.4% seeds infected by *B. nodulosa* and 8.22% seeds infected by *Cladosporium* spp. were completely rotted. While most of the seeds were partially rotted due to seed infections by a different fungal pathogen, very few were totally rotted. The highest rot was recorded in seeds infected by *B. nodulosa* with 11.4% seeds totally rotted and 28.07% seeds partially rotted (Table 2).

Table 2. Germination and rot percentage of seeds infected by different seed-borne fungal pathogens

Types of Fungi	No. of Infected seeds	Infected seeds (%)		Rotted (%)		
		Germinated	Non- germinated	Totally	Partially	None
Alternaria spp.	24	20.83	79.16	0	20.83	79.17
Bipolaris nodulosa	114	28.07	71.93	11.4	28.07	60.53
Cladosporium spp.	73	13.69	86.3	8.22	19.18	72.6
Pyricularia grisea	8	37.5	62.5	0	12.5	87.5

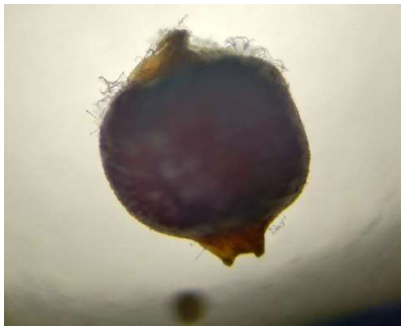
Note: Each seed testing sample contains 100 seeds.

Growth Habit

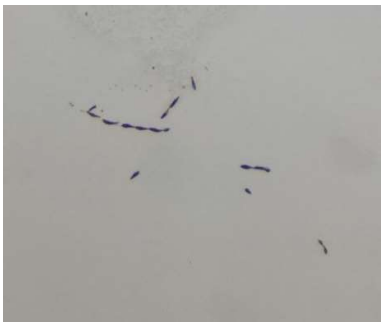
The fungal growth was observed directly on the surface of the seed. The growth of *B. nodulosa* can be seen spreading more vigorously than any other fungal pathogens. The formation of light black to grayish masses of mycelia can be seen on the surface of the incubated seeds (Fig. 1A). Colonies frequently covered the whole seed and even extended to the blotter in some cases. The conidia were oval-shaped and olive green to light brown (Fig. 1B). Similarly, the growth habit, conidia, and conidiophores of *Alternaria* spp. and *Cladosporium* spp. as observed in a microscope and can be seen in Figs. 2 and 3, respectively. The growth habit of *Alternaria* species was observed in dark or black chain-like structures (Fig. 2A). Different longer, shorter, thicker, and thinner chains were observed for different species of *Alternaria* (Fig. 2B). Most of the chains were simple but in some cases, the chains were found to be branched. The conidia when observed in a compound microscope were somehow cylindrical to cone-shaped tapering towards the tip (Fig. 2C). The colonies of *Cladosporium* were dark grey to light green in color with a growth habit in a broad area in most of the observations (Fig. 3A) while singled growth habits with restricted growth were also seen in a few ones (Fig. 3B).

A. Growth habit of *Bipolaris nodulosa* B. Conidia of *Bipolaris nodulosa* (40*10×)

Fig. 1. Growth habit and Conidia of *Bipolaris nodulosa* in finger millet.



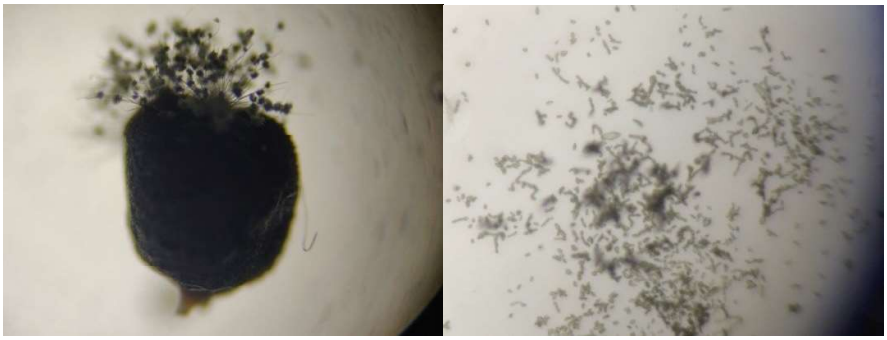
A. Growth habit of *Alternaria* spp.



B. Conidiophore of *Alternaria* spp.

C. Conidia of *Alternaria* spp.

Fig. 2. Growth habit, conidiophores, and conidia of *Alternaria* spp. in finger millet



A. Growth Habit of *Cladosporium* spp. B. Conidia of *Cladosporium* spp. (40*10×)

Fig. 3. Growth habit and conidia of *Cladosporium* spp. in finger millet.

Good seed is the fundamental input for good crop production. One of the important aspects of good seed, besides high germination and purity, is that the seed should be free from pathogens. Infected seeds play a major role in the dissemination of pathogenic microorganisms. About 87 species of fungi belonging to 38 genera have been identified on finger millet seeds so far (Jain, 2020). However, their severity depends on the time of sampling, location, and varieties. Although finger millet is considered a hardy crop, it is affected by more than 20 diseases and several pests.

Species of *Curvularia*, *Fusarium*, *Aspergillus*, *Alternaria*, *B. nodulosa*, *P. grisea*, *Cladosporium*, *Penicillium,* and *Mucor* were previously reported in finger millet seeds (Grewal and Pal, 1965; Oblisami and Srivasa, 1973). Spores of *P. grisea*, *B. nodulosa*, *Fusarium* sp., and *Curvularia* spp. were observed in finger millet seed washings suggesting they were seed-borne on the surface of the seed (Adipala, 1992). Pande *et al.* (1994) recorded mixed infections with *B. nodulosa* and *Pyricularia grisea* in 20 seed samples. In 11 samples, a greater percentage of seeds were infected with *P. grisea* than with *B. nodulosa*. In contrast, *B. nodulosa* was predominant while *P. grisea* was found negligible in our study. Similarly, Kumar (2010) found four fungi namely *Aspergillus niger*, *Penicillium citrinum*, *Fusarium* sp., and *Alternaria alternata* to be dominant on seeds of four finger millet genotypes. Among these fungal pathogens, the only fungi whose incidence was recorded in our study were *Alternaria*. Hansford (1935) suggested that blast spores on fingermillet seed in Uganda were surface seed-borne and that diseases seeds could easily infect helthy seeds during threshing and transport and form an important source of primary inoculum. Adipala (1992) also reported *P. Grisea, B. nodulosa* and *Fusarium* sp. were mostly externally seed-borne.

Kumar *et al.* (2000) experimented on 400 finger millet seed samples collected from different areas in Bihar. *Cochliobolus nodulosus* was found in all the samples followed by *Curvularia* sp., *Alternaria* sp., *Pyricularia* sp., *Fusarium* sp., and *Mucor* sp. Pre-treated seeds contained fewer fungi than those not treated with mercuric chloride. David (2009) detected *P. grisea*, *B. nodulosa,* and *B. setariae* in finger millet seed samples.

CONCLUSIONS

The association of these fungal pathogens with finger millet seeds shows that infected seeds are the major carrier of diseases in finger millets. Seed borne fungi have a significant effect on seed germination. The result of the present study reveals that different seed-borne pathogens are present in most of the finger millet varieties though they may differ in the frequency and extent of infection. This only points out that although in some instances they occurred in trace levels under suitable environmental conditions it might result in widespread distribution of the disease and cause enormous damage to the crops.

Most of the farmers use seeds from the previous harvest as a planting material. As a farmer plants infested seed, he also sows the potential for future disease problems. Planting cereal seed that is free of seed-borne pathogens is the primary means of limiting the introduction of pathogens, especially new pathogens, into a field. Planting infected seed may also result in widespread distribution of disease within the crop, and allows for an increased number of initial infection sites from which the disease can spread. Hence seed health testing of finger millet seeds is very important before it is taken in the field.

RECOMMENDATIONS

Since this research solely focuses on the incidence of fungal pathogens in finger millet seeds, future researchers can also undertake on comparative studies focusing on the control strategies. Comparative research study can be done in different districts in order to determine variations in disease incidence. This will help us learn more about the disease prone districts throughout the country. Likewise, new finger millet varieties intended for release must be tested for resistance to different seed-borne fungal pathogens detected in the study. Seed health testing should be done in all finger millet varieties grown in different part of Nepal to know about the infection level of *B.*

nodulosa as blight is a major seed-borne disease of finger millet. For better seed health management, focused research on the economic importance of these seed borne fungi is recommended. Seed treatment is recommended for the seed-borne diseases observed in the research.

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Annex 1. Code number of accessions and the local names of finger millet seeds collected from different districts of Nepal

S.N.	Accessions	Local Name	District
1	NGRC03623	Lurkekodo	Sindhupalchowk
2	NGRC05752	Murke	Palpa
3	NGRC03501	Soutarekodo	Ramechhap
4	NGRC03620	Sirubarekodo	Sindhupalchowk
5	NGRC04794	Kalokodo	Ramechhap
6	NGRC04818	Jhamrekodo	Dailekh
7	NGRC06496	Kodo	Sankhuwasabha
8	NGRC03483	Paunderkodo	Dhading
9	NGRC01525	Urchhokodo	Baglung
10	NGRC03653	Jhaprekodo	Darchula
11	NGRC01639	Danjakodo	Syangja
12	NGRC01490	Kali kodo	Khotang
13	NGRC03458	Kodo	Ramechhap
14	NGRC05744	Kaise	Palpa
15	NGRC03544	Kodo	Mustang
16	NGRC06487	Kali kodo	Sindhuli
17	NGRC04790	Nangkatuwakodo	Ramechhap
18	NGRC05748	Khairo local	Palpa
19	NGRC05738	Setokodo	Palpa
20	NGRC03630	Mudkekalo	Baglung
21	NGRC03443	Jhaprekodo	Palpa
22	NGRC04860	Ratokodo	Kalikot
23	NGRC04816	Mangsirekodo	Dailekh
24	NGRC03615	Jhopekodo	Myagdi
25	NGRC04837	Dallekodo	Bajhang

S.N.	Accessions	Local Name	District
26	NGRC04878	Pahelo	Dhading
27	NGRC01417	Dallekodo	Bhojpur
28	NGRC01455	Dallekodo	Kaski
29	NGRC04817	Lamrekodo	Dailekh
30	NGRC05739	KaloJhuse	Palpa
31	NGRC04789	Kodo	Kavre
32	NGRC04804	Jhuppekodo	Dolakha
33	NGRC01418	Setokodo	Bhojpur
34	NGRC01591	Dallejhopae	Parbat
35	NGRC04773	Mangsirekodo	Kavre
36	NGRC05754	Thullejhabre	Palpa
37	NGRC05109	KaloDalle	Arghakhanchi
38	NGRC01600	JhopaeKodo	Kaski
39	NGRC03694	Local	Nuwakot
40	NGRC01599	AsareKodo	Kaski
41	NGRC01603	Kodo	Kaski
42	NGRC01609	Kukurkanekodo	Parbat
43	NGRC01512	Kodo	Rukum
44	NGRC04808	Thulokodo	Pyuthan
45	NGRC01622	Lurkaekodo	Parbat
46	NGRC01623	Bhochuwakodo	Baglung
47	NGRC03639	Jhakerekodo	Baitadi
48	NGRC03629	Sanglekodo	Sindhupalchowk
49	NGRC03459	Nuwakotekodo	Solukhumbu
50	NGRC01517	Setokodo	Salyan
51	NGRC01644	Kalokodo	Jumla
52	NGRC01645	Ratokodo	Jumla
53	NGRC01646	Ratokodo	Jumla
54	NGRC01652	Kalokodo	Kalikot
55	NGRC01653	Dallekodo	Dolpa
56	NGRC01654	Gamkiratokodo	Mugu
57	NGRC01649	Kalokodo	Jumla
58	NGRC04833	Kalokodo	Bajhang
59	NGRC04820	Tiyasekodo	Dailekh
60	NGRC03441	Saraya Bhan	Kapilbastu
61	NGRC03442	FulbiranjBhan	Kapilbastu
62	NGRC01432	Makwanpurkodo	Gorkha

S.N.	Accessions	Local Name	District
63	NGRC03445	Dallekodo	Palpa
64	NGRC03447	Dallekodo	Arghakhanchi
65	NGRC03485	Kalodhuskotekodo	Dhading
66	NGRC03488	Palisekodo	Solukhumbu
67	NGRC03460	Dallekodo	Solukhumbu
68	NGRC03463	Manselekodo	Solukhumbu
69	NGRC03466	Nangrekodo	Gorkha
70	NGRC03467	Local kodo	Gorkha
71	NGRC03478	Dallekodo	Dhading
72	NGRC03482	Dunkotekodo	Dhading
73	NGRC03512	Paunderkodo	Dhading
74	NGRC03484	Kodo	Taplejung
75	NGRC03486	Chauredallekodo	Dhading
76	NGRC03491	Pandharukodo	Dolakha
77	NGRC03493	Sunkosikodo	Dolakha
78	NGRC03498	Charsakodo	Dolakha
79	NGRC03500	Jarpirekodo	Ramechhap
80	NGRC03513	Mulukekodo	Taplejung
81	NGRC03502	Mulurakodo	Ramechhap
82	NGRC03503	Nagarekodo	Sindhuli
83	NGRC03540	Muruwakodo	Sunsari
84	NGRC03568	Ratokodo	Mugu
85	NGRC03573	Ratokodo	Mugu

Annex 2. Incidence of seed-borne fungal pathogens detected by deep freeze blotter method in finger millet seeds

Accessions	*Bipolaris nodulosa*	*Alternaria* spp.	*Cladosporium* spp.	*Pyricularia grisea*	Total Infection
NGRC03623	0	0	0	0	0
NGRC05752	0	0	0	0	0
NGRC03501	0	0	0	0	0
NGRC03620	0	0	0	0	0
NGRC04794	0	0	2	1	3
NGRC04818	0	0	0	0	0
NGRC06496	6	0	2	0	8
NGRC03483	0	0	0	0	0
NGRC01525	2	0	0	1	3
NGRC03653	2	0	0	0	2
NGRC01639	0	0	1	1	2
NGRC01490	0	0	4	0	4
NGRC03458	1	0	1	0	2
NGRC05744	0	0	1	0	1
NGRC03544	3	0	0	2	5
NGRC06487	1	0	0	0	1
NGRC04790	0	0	0	0	0
NGRC05748	3	3	0	0	6
NGRC05738	0	0	1	0	1
NGRC03630	0	0	0	0	0
NGRC03443	0	0	0	0	0
NGRC04860	2	1	1	2	6
NGRC04816	2	0	0	0	2
NGRC03615	0	0	1	0	1
NGRC04837	0	1	0	0	1
NGRC04878	6	0	0	0	6
NGRC01417	0	0	1	0	1
NGRC01455	0	1	2	0	3
NGRC04817	0	0	2	0	2
NGRC05739	1	0	0	0	1
NGRC04789	1	0	1	0	2
NGRC04804	0	0	3	0	3
NGRC01418	0	0	0	0	0
NGRC01591	1	0	1	0	2

Accessions	*Bipolaris nodulosa*	*Alternaria* spp.	*Cladosporium* spp.	*Pyricularia grisea*	Total Infection
NGRC04773	0	4	3	0	7
NGRC05754	0	1	0	0	1
NGRC05109	0	0	1	0	1
NGRC01600	1	0	0	0	1
NGRC03694	0	0	0	0	0
NGRC01599	0	0	0	0	0
NGRC01603	0	0	0	0	0
NGRC01609	5	0	4	0	9
NGRC01512	0	0	2	0	2
NGRC04808	0	1	3	0	4
NGRC01622	0	0	0	0	0
NGRC01623	1	0	1	1	3
NGRC03639	2	0	3	0	5
NGRC03629	2	1	1	0	4
NGRC03459	2	1	9	0	12
NGRC01517	1	0	0	0	1
NGRC01644	1	0	0	0	1
NGRC01645	2	1	0	0	3
NGRC01646	1	0	0	0	1
NGRC01652	1	0	1	0	2
NGRC01653	1	0	0	0	1
NGRC01654	4	1	1	0	6
NGRC01649	0	0	2	0	2
NGRC04833	0	0	0	0	0
NGRC04820	0	1	0	0	1
NGRC03441	2	0	0	0	2
NGRC03442	1	0	0	0	1
NGRC01432	16	0	1	0	17
NGRC03445	2	0	0	0	2
NGRC03447	0	0	1	0	1
NGRC03485	5	2	1	0	8
NGRC03488	1	0	2	0	3
NGRC03460	0	0	1	0	1
NGRC03463	2	0	2	0	4
NGRC03466	2	0	1	0	3
NGRC03467	0	0	0	0	0

Accessions	*Bipolaris nodulosa*	*Alternaria* spp.	*Cladosporium* spp.	*Pyricularia grisea*	Total Infection
NGRC03478	3	0	1	0	4
NGRC03482	0	0	0	0	0
NGRC03512	1	0	0	0	1
NGRC03484	1	0	2	0	3
NGRC03486	0	0	0	0	0
NGRC03491	0	0	0	0	0
NGRC03493	0	0	1	0	1
NGRC03498	2	0	0	0	2
NGRC03500	0	0	0	0	0
NGRC03513	0	0	0	0	0
NGRC03502	1	0	0	0	1
NGRC03503	2	0	0	0	2
NGRC03540	8	0	0	0	8
NGRC03568	4	0	2	0	6
NGRC03573	8	5	1	0	14
Total infections	114	73	24	8	