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Vinu Ahir
Junagadh Agricultural University, Junagadh, Gujarat, India

Hemant Sharma
ASPEE College of Horticulture and Forestry, N.A.U., Navsari, Gujarat, India

Corresponding Author:
Vinu Ahir
Junagadh Agricultural University, Junagadh, Gujarat, India

Diversity of seed borne Mycota associated with finger millet and their effects on seed germination and seedling vigour

Vinu Ahir and Hemant Sharma

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Abstract
The finger millet (*Eleusine coracana* L.) seeds are found to be heavily infested with variety of fungi. These associated fungi are known to deteriorate the seeds and seed contents. Experiments were conducted to assess the effect of some dominant seed borne fungi of finger millet on seed germination and seedling vigour. Seven dominant fungi were found associated with finger millet seeds. Analysis of seed borne fungi by standard blotter paper method and agar plate method showed that species of *Alternaria, Fusarium, Curvularia* and *Aspergillus* are the dominant genera. Maximum reduction in seed germination and seedling vigour was caused by species of *Aspergillus* in seed inoculation and culture filtrate method. Thus, the associated fungal pathogens with finger millet seeds demonstrates that the seeds are a major source of transmissions of pathogens, which might have adverse effect at seedling and adult stage of plants.

Keywords: Finger millet, inoculation, seed - borne fungi, seed germination, vigour index

Introduction
Seed is a potential harbour of a wide variety of fungi containing both pathogenic and saprophytic microorganisms, both externally and internally (Utobo *et al.* 2011) [16]. These fungi may reduce seed quality and impair seed germination resulting in the production of abnormal seedlings (Paul 1989, Vijayan & Rehill 1990, Bateman & Kwasna 1999, Khanzada *et al.* 2002) [14, 17, 2, 5]. In case of severe infection the seed completely deteriorates and the grain may become unsuitable even for animal consumption due to production of mycotoxic substances by seed fungi.

Finger millet [*Eleusine coracana* (L.) Gaertn.] is one of the most important millet crop belonging to family *Poaceae* and sub family *Chloridoidae* (Dida *et al*., 2008) [3]. India is the largest cultivator of finger millet, which is primarily grown in the states of Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Maharashtra, Uttar Pradesh, Bihar and Gujarat. Dang district of Gujarat is the main area of finger millet, growing in 17,056 hectares with a production of 12,706 metric tonnes having productivity of 745 kg/ha (Anonymous, 2015) [1]. The nutritional quality of finger millet grain makes it an ideal food for expectant women, lactating mothers, children, the sick, and diabetics (National Research Council, 1996) [11]. Finger millet is generally affected by several seed-borne fungi and causing severe losses both in fields as well as in storage conditions (Krishna Prasad and Basuchaudhary, 1987) [8]. The fungi associated with seeds at the stage of harvest, transport, processing and under storage bring about several undesirable changes, making them unfit for human consumption and sowing (Patil *et al*., 2012) [13]. A seed borne pathogen may cause seed abortion, seed rot, seed necrosis, reduction of germination potential and seedling vigour. The seeds are passive carriers of pathogens that are transmitted when sown seeds germinated under suitable environmental conditions. It held that progressive reduction in the concomitant loss of viability due to seed borne fungal spores. The present investigation deals with isolation of fungi associated with finger millet seed and their effects on seed germination and seedling vigour.

Materials and Methods

Experimental location

The experiment was conducted in the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari has an average temperature of 37°C and annual average rainfall of 1959 mm, latitude 20^0 57' N, longitude 72^0 55'E and altitude is 12.33 meter above sea level.

Sources of experimental materials

Seed samples of finger millet were collected from Hill Millet Research Station, N. A. U., Waghai and farmer's fields of four villages (Sakarpatal, Dagadiamba, Kudkas, Dokpatal) of Waghai district. For sampling, farmers were selected randomly. From each seed sample, an amount of 250g seeds were taken and kept separately in labeled, pre-sterilized paper bags.

Microbial assay of finger millet seeds

Standard blotter paper and agar plate method as described by the International Seed Testing Association (ISTA, 1996) [4] was used for the isolation of the seed-borne fungi associated with the finger millet seed samples.

Standard blotter paper method

In the blotter paper method, pair of sterile white blotter papers of 8.5 cm diameter was soaked in sterile distilled water and were placed in pre-sterilized Petri plates of 90 mm diameter. Ten seeds per Petri plates, in order to isolate only internal seed mycoflora, were surface sterilized for 1 minutes with 1% sodium hypochlorite solution followed by three subsequent washings in sterilized distilled water to remove sodium hypochlorite from seed and non-surface sterilized, were placed at equal distance on three layers of properly moistened sterilized blotters. 400 seeds were used in each experiment. These plates were incubated at a temperature of 25 ± 2^0C for 12 hrs in alternating cycles of light and darkness. The seeds were examined regularly for the growth of fungi over the seed.

Agar plate method

In Agar plate method, pre-sterilized Petri plates were poured with 20 ml of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were followed as per blotter test method.

Examination of incubated seeds

Sampling for identification of fungi was done at seventh days after incubation. The Petri dishes were brought to the examination area in the laboratory, where each seed was examined under a microscope for growth habits of the various fungi growing in the Petri plates. Slide preparations of the various fruiting structures of the fungi were made and identified under the stereo zoom compound microscope. The samples of fungus were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of isolated fungi. The binocular compound microscope was used to determine the type of fungus in each plate. The seed-borne fungi were identified using identification keys and cross checked for each seed plates to identify the type of fungus growing on each seed. After seven days of incubation, fungal species found growing on the surface of seeds, were identified and their percentage frequency of occurrence of fungal was calculated by applying the following formula: PF = (No. of seeds on which fungus appear / Total number of seeds) X 100

Inoculation

Impact of seed infecting fungi and effect of cultural filtrate on seed health status was studied in respect of seed germinability and seedling vigour from artificially inoculated seeds with fungi isolated from naturally infected finger millet seeds.

Artificial seed inoculation method

Healthy seeds of finger millet cv Gujarat Nagli-4 were artificially inoculated with each of seven fungal species separately. For artificially inoculation, seeds moistened by sterilized distilled water were mixed thoroughly with 10 days old respective fungal culture growth were obtained at 25 ± 2 ^{0}C on PDA plates. One sheet of germination paper was wetted by sterilized distilled water. One hundred seeds in each of the treatments with four replications of respective treatment were placed on first sheet evenly. Second sheet of germination paper was placed on first sheet followed by wetting it carefully. Both sheets were rolled along with wax coated paper. The rolled papers were incubated at 25 ± 2 ^{0}C for 7 days. At the end of incubation period, rolled towel papers were carefully opened. Germinated and un-germinated seeds were counted from each of the treatments. Emergence of seedling from the seeds was considered as successful germination. Four replications each of 100 seeds were maintained for each of the treatments. These seeds were also used for study of seed germination and seedling vigour index. Un-inoculated seeds served as control treatment for comparison.

Effect of culture filtrates of seed-infecting fungi on seed germination and seedling vigour

All different fungi were separately cultured on modified Richards's liquid medium at 25 ± 2^0C for 10 days. Liquid medium along with fungal growth of each fungus was filtered through Watman filter No. 42. Resulting filtrates were used to evaluate their effect on seed germination and seedling growth. Healthy seeds of finger millet cv Gujarat Nagli-4 were treated by soaking the seeds for 8 hr into culture filtrate of respective fungus obtained from the 15 days old fungal culture grown on modified Richard's liquid medium at 25 ± 2^0C. Then, influence of culture filtrate of respective fungus was evaluated by Paper towel method (Khare, 1996) [6]. Seeds soaked in sterilized distilled water served as control treatment. One hundred treated seeds in each of the treatments with four replications were tested. Observations were recorded on seed germination, discolouration of radicals and plumules if any, and seedling length after 10 days of incubation at room temperature. Emergence of seedling from the seeds was considered as successful germination of seeds.

Results and discussions

Microbial assay of seed Mycota

Total seven fungi with four genera were isolated by standard blotter method and agar plate method *i.e. Alternaria alternata*, *Fusarium subglutinans*, *Fusarium moniliforme*, *Curvularia lunata*, *Aspergillus sp*., *Aspergillus niger* and *Aspergillus flavus*.

Pandey (1986) [12] reported many fungal species belonging to genera namely *Aspergillus clavatus*, *A. chevalieri*, *A. fumigatus*, *A. ochraceous*, *A. niger*, *A. candidus*, *A. flavipes*, *A. flavus*, *Penicillium citrinum*, *P. oxalicum*, *P. cyclopium*, *P.*

isalandicum, *Alternaria alternata*, *Curvularia lunata*, *Drechslera rostrata*, *Cladosprium cladosporioides*, *Epicoccum purpurascens*, *Fusarium semitectum*, *Nigrospora oryzae*, and *Torula graminis* associated with finger millet seeds. Krishna Prasad and Basuchaudhary (1987) [8] reported twelve fungal species *viz; Drechslera nodulosa, Cochliobolus spicifer, C. bicolor, Fusarium oxysporum, Curvularia verriculosa, Aspergillus niger, Alternaria alternata, Penicillium spp., Rhizopus sp., Emericella nidulans, Thielaviopsis* and *Epicoccum* associated with finger millet seeds. Kumar (2010) [9] also reported four fungi namely *Aspergillus niger, Penicillium citrinum, Fusarium sp.* and *Alternaria alternata* were found to be dominant on seeds of finger millet genotypes.

Effect of seed infecting fungi on seed health status

Assessment by artificially inoculation of finger millet seeds separately by seven different fungi revealed significant effect on seed germination, shoot and root length, and thereby seedling vigour index (Table 1). Each of the fungi exhibited significant adverse effects on seed germination, shoot and root length. Overall, fungi induced 14.58 to 41.66, 14.26 to 56.49 and 22.44 to 60.17 per cent reduction in seed germination, shoot length and root length over healthy seeds, respectively.

Seed inoculated by *Aspergillus niger* showed lowest seed germination (56.00%) which was at par with *Aspergillus flavus* (58.00%). Whereas, in *Aspergillus* sp., *Fusarium subglutinans*, *Fusarium moniliforme*, *Alternaria alternata* and *Curvularia lunata* recorded 59.00, 64.00, 68.00, 77.00 and 82.00 per cent germination, respectively. The result in terms of shoot and root length with seedling vigour index, all the treatments showed smaller shoot length, root length and seedling vigour index as compared to control. *Aspergillus niger* recorded minimum shoot length (4.03 cm), root length (5.25 cm) and seedling vigour index (520.03) which was at par with *Aspergillus flavus* (4.11 cm, 5.43 cm and 553.73, respectively). Similarly, *Aspergillus* sp., *Fusarium subglutinans, Fusarium moniliforme, Alternaria alternata and Curvularia lunata* also recorded less shoot length, root length and seedling vigour index. On the contrary, significantly highest seed germination (96.00%), shoot length (9.27 cm), root length (13.20 cm) and seedling vigour index (2157.11) were obtained in healthy seeds.

The result corroborates with the findings of Lokesh and Hiremath (1992) [10] who found the 68.00 per cent reduction in seed germination, 35.00 per cent reduction in shoot elongation and 38.91 per cent reduction in root elongation over control in seed tested with *Aspergillus niger* in pigeonpea crop. Whereas, highest per cent decrease seed germination, shoot length and root length recorded 62.00, 61.01 and 59.49 per cent, respectively in *Fusarium* sp. and 21.00, 32.59 and 28.77 per cent, respectively in *Aspergillus niger* by Khayum *et al.* (2006) [7] in soybean seeds.

Table 1: Effect of seed inoculation with different fungi on seed germination, shoot length, root length and seedling vigour index in finger millet

Fungi	Seed germination (%) *	Decrease in seed germination over healthy seed (%)	Shoot length (cm)*	Decrease in shoot length over healthy seed (%)	Root length (cm)*	Decrease in root length over healthy seed (%)	Seedling vigour index (SVI)
Alternaria alternata	77.00	19.79	7.52	18.82	9.63	26.98	1321.88
Fusarium subglutinans	64.00	33.33	6.17	33.41	7.52	43.01	876.39
Fusarium moniliforme	68.00	29.16	5.51	40.48	7.17	45.66	863.40
Curvularia lunata	82.00	14.58	7.94	14.26	10.23	22.44	1490.98
Aspergillus sp.	59.00	38.54	4.35	53.07	5.84	55.75	601.59
Aspergillus niger	56.00	41.66	4.03	56.49	5.25	60.17	520.03
Aspergillus flavus	58.00	39.58	4.11	55.60	5.43	58.84	553.73
Control (Healthy seed)	96.00	-	9.27	-	13.20	-	2157.11
S. Em ±	1.29		0.06		0.05		20.03
CD 0.05%	3.79		0.18		0.17		58.48
CV %	3.71		2.08		1.45		3.82

*Average of four repetitions and 100 seeds each repetition

Effect of culture filtrate of isolated seed infecting fungi on seed health: Results on seed germination, shoot and root length and seedling vigour index (SVI) of finger millet as influenced by culture filtrates of seven different isolated fungi (Table-2) revealed significant effects on seed germination, shoot and root length, and thereby SVI.

Overall, fungi induced 26.31 to 53.68, 23.94 to 60.03 and 26.85 to 62.99 per cent reduction in seed germination, shoot length and root length over healthy seeds, respectively. Seed inoculated by *Aspergillus niger* showed lowest seed germination (44.00%) which was at par with *Aspergillus flavus* (46.00%). Whereas, in *Aspergillus* sp., *Fusarium subglutinas, Fusarium moniliforme*, *Alternaria alternata and Curvularia lunata* recorded 47.00, 55.00, 58.00, 68.00 and 70.00 per cent germination, respectively. The result in terms of shoot and root length with seedling vigour index, all the treatments showed smaller shoot length, root length and seedling vigour index as compared to control. *Aspergillus niger* recorded minimum shoot length (3.22 cm), root length (4.60 cm) and seedling vigour index (344.06) which was at par with *Aspergillus flavus* 3.35 cm, 4.73 cm and 371.53, respectively. Similarly, *Aspergillus* sp., *Fusarium subglutinas*, *Fusarium moniliforme*, *Alternaria alternate* and *Curvularia lunata* also recorded less shoot length, root length and seedling vigour index. On the contrary, significantly highest seed germination (95.00%), shoot length (8.07 cm), root length (12.43 cm) and seedling vigour index (1947.53) were obtained in healthy seeds.

The similar result was observed by Lokesh and Hiremath (1992) [10]. They found that 64.37 per cent reduction in seed germination, 90.26 per cent reduction in shoot elongation and 90.25 per cent reduction in root elongation over control in seed tested with *Aspergillus niger* in pigeonpea crop. Singh *et al.* (2003) [15] also found that culture filtrates of *Aspergillus flavus* caused reduction in seed germination and root-shoot elongation in Pearl millet crops.

Table 2: Effect of culture filtrate of isolated seed infecting fungi on seed germination, shoot length, root length and seedling vigour index in finger millet

Fungi	Seed germination (%) *	Decrease in seed germination over healthy seed (%)	Shoot length (cm)*	Decrease in shoot length over healthy seed (%)	Root length (cm)*	Decrease in root length over healthy seed (%)	Seedling vigour index (SVI)
Alternaria alternata	68.00	28.42	5.85	27.47	8.83	28.96	998.31
Fusarium subglutinans	55.00	42.10	4.69	41.79	5.72	53.98	573.04
Fusarium moniliforme	58.00	38.94	5.50	31.81	6.90	44.48	719.19
Curvularia lunata	70.00	26.31	6.13	23.94	9.09	26.85	1066.25
Aspergillus sp.	47.00	50.52	3.44	57.28	4.85	60.98	389.97
Aspergillus niger	44.00	53.68	3.22	60.03	4.60	62.99	344.06
Aspergillus flavus	46.00	51.57	3.35	58.48	4.73	61.94	371.53
Control (Healthy seed)	95.00	-	8.07	-	12.43	-	1947.53
S. Em ±	1.16		0.06		0.06		13.42
CD 0.05%	3.39		0.17		0.18		39.17
CV %	3.85		2.42		1.78		3.35

*Average of four repetitions and 100 seeds each repetition

Conclusion

The association of fungal pathogens with finger millet seeds demonstrates that the seeds are a major source of transmissions of pathogens, which might have adverse effect at seedling and adult stage of plants. The emergence of abnormal seedling from naturally infected seeds and isolation of same fungal pathogens from such seedlings as that of seeds suggest the involvement of fungal pathogens in causation of abnormal seedling. Our finding has demonstrated that associated fungal pathogens reduce the germination ability of seeds, which causes poor crop stand, a major constraint of low harvested crop yield.

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