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Detection of Microflora Associated With Finger Millets in Storage Condition

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ABSTRACT

Four varieties of finger millets seeds were collected from Pulse Research Station, Berhampur, Odisha University of Agriculture and Technology on 08 March, 2021. The varieties are Arjuna, Kalua, Bhairabi and Subhra. The visual and microscopic examination of infected seed samples were carried out to study the abnormal, discolored seeds and the fungal bodies present with them. Isolation of fungi associated with finger millet seeds were carried out by taking from randomly 400 seeds from the composite seed sample by standard moist blotter method. Twenty seeds per petri plates, after surface sterilization by 1% sodium hypochlorite solution for one minute. The fungal growths of different fungi obtained on seeds were transferred on PDA petri plates. Each fungal species isolated was further purified by hyphal tip method. Various cultures obtained were maintained on PDA slants for further study. Various seed-infecting fungi developed on the millet seeds, were separately cultured on PDA petri plates. Each fungal growth was critically observed under microscope for cultural and morphological characters. Finally, fungal characteristics observed were compared with the characteristics and described in various manuals. Cultures were maintained on PDA slants by sub culturing and stored at 25 ^{0}C for further study. The pathogenicity test was carried out for *Fusarium oxysporum, Aspergillus niger and Aspergillus flavus*. Apparently healthy seeds of finger millet's 'Arjuna' and 'Subhra' variety were used for testing the pathogenicity of different isolates.

Keywords Detection, Microflora, Moist blotter, Storage condition.

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INTRODUCTION

The crop is widely cultivated in Asia and Africa, especially in India, Srilanka, China, Japan, Madagaskar and in most part of central and East Africa under irrigated as well as rainfed conditions. India has the distinction of having largest area of 2.7 million ha as well as highest production of 2.6 million tons with productivity of 1400 kg/ha. Karnataka with 1 million ha occupies nearly 60% of finger millet area followed by Maharashtra, Odisha, Tamil Nadu, Andhra Pradesh, Uttarakhand, Jharkhand, Bihar and

Gujarat. Though Odisha has the heritage of millets cultivation, where finger millet is considered to be the most important one occupying around 169.22 thousand hectares with production of 151.42 thousand tons. The productivity of finger millet in Odisha is 895 kg/ha which is very low as compared to the national average of 1400 kg/ha.

Millets are more nutritious compared to fine cereals. Finger millet is the richest source of calcium (300-350 mg/100 g) and other small millets are good source of phosphorus and iron. The millet protein has well balanced amino acid profile and good source of methionine, cystine and lysine. These essential amino acids are of special benefit to those who depend on plant food for their protein nourishment (Chandra *et al.* 2016, Gupta *et al.* 2017, Patel *et al.* 2016, Rurinda *et al.* 2014). The millet grain contains about 65% carbohydrate, a high proportion of which is in the form of non-starchy polysaccharides and dietary fiber which help in prevention of constipation, lowering of blood cholesterol and slow release of glucose to the blood stream during digestion. Lower incidence of cardiovascular diseases, duodenal ulcer and hyperglycemia (diabetes) are reported among regular millet consumers. Millet grains are also rich in important vitamins viz., thiamine, riboflavin, folin and niacin. Millets are comparable to rice and wheat or rich in some of the minerals as well as fatty acids. Millets vary largely in composition of carbohydrates as proportion of amylose and amylopectin content vary from 16-28% and 72-84%, respectively (Devi *et al.* 2014, Kumar *et al.* 2016).

Seeds are regarded as means of transporting plant pathogen . Seed born pathogen may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage (Pathak and Zaidi 2013). It is generally affected by several seed-borne fungi and causing severe losses both in fields as well as in storage conditions.

The total loss of millet grain after harvest is estimated to be as much as 15% in many countries and much higher in developing countries. Fungi of genera *Aspergillus, Fusarium, Penicillium* and *Rhizoctonia* produce toxic substances that decrease quality of seed (Jain 2020). Seed borne pathogens of finger millet include several fungi viz., *Aspergillus flavus, A. nidulans, A. terreus, Fusarium* spp., *Curvularia* spp., *Alternaria* spp., *Trichotheciumroseum, Trichoderma viride,* and *Penicillium griseofulvum*.

Studies pertaining to seed mycoflora of millet in Odisha are flatter limited. Hence, there is a necessary to generate information on the prevalence of seed mycoflora and seed health testing techniques.

MATERIALS AND METHODS

Four varieties of finger millet seeds were collected from Pulse Research Station, Berhampur, OUAT on 08 March, 2021.The varieties are Arjuna, Kalua, Bhairabi and Subhra. The visual and microscopic examination of infected seed samples were carried out to study the abnormal, discolored seeds and the fungal bodies present with them. The symptoms in respect of discoloration as well as seed abnormality were described on the basis of visual observations of infected seeds. For this, after collecting finger millet seeds from Pulse Research Station, Berhampur, OUAT the seeds were sorted out into different groups on the basis of seed discoloration and grain abnormality by means of magnifying hand lens under artificial light. Then, symptoms observed on grains were described accordingly. Two hundred seeds were taken for observation. Two hundred seeds are collected from each variety. In each petri plate 20 seeds are taken for testing. Isolation of fungi associated with finger millet seeds were carried out by taking from randomly 200 seeds from the composite seed sample by standard moist blotter method (International Seed Testing Association). Twenty seeds per plates, after surface sterilization by 1% sodium hypochlorite solution for one minute, were placed at equal distance on double layers of properly moistened sterilized blotters, and then petri dishes were incubated under laboratory condition for 7 days. After 7 days developing fungal growths on each of the seeds was observed regularly, identified by microscopic observation and recorded accordingly. The fungal growths of seeds were transferred on PDA petri plates. Each fungal species isolated was further purified by hyphal tip method. Various cultures obtained were maintained on PDA slants for further study. Various seed-infecting fungi developed on ragi seeds, were separately cultured on PDA petri

plates. Each fungal growth was critically observed under microscope for cultural and morphological characters. Finally, fungal characteristics observed were compared with the characteristics and described in various manuals. Cultures were maintained on PDA slants by sub culturing and stored at 50^0C in refrigerator. For proving the Koch's postulates, the pathogens isolated from seeds were tested in the laboratory condition by adopting standard methods of pathogenicity test.The pathogenicity test was carried out for *Fusarium oxysporum, Aspergillus niger* and *Aspergillus flavus*. Apparently healthy seeds of Ragi's 'Arjuna' and 'Subhra' variety were used for testing the pathogenicity of different isolates.

For proving pathogenicity test of various isolated pathogens, healthy seeds of ragi's variety 'Arjuna' and 'Subhra' were surface sterilized for 1 minute with 1% sodium hypochlorite solution followed by three subsequent washings in sterilized distilled water to remove sodium hypochlorite from seed. These seeds were then soaked for 24 hours in sterilized distilled water and were inoculated by rolling on 10 days old actively sporulating culture of each test fungus were plated in glass petri dishes and each petri dishes have two layers of standard blotter paper were placed. Then these seeds were arranged over the blotting sheet in a circular manner and kept for 7 days in laboratory condition and after 7 days developing fungal growths on each of the seeds was observed regularly.

RESULTS AND DISCUSSION

Isolation of seed-borne fungi from composite samples of collected finger millet seeds were carried out by the moist blotter method after surface sterilized and revealed the association of three different predominant fungi. The different isolates obtained from finger millet seeds were purified by the hyphal tip isolation technique. The identification of the isolated fungus was done by studying the cultural, morphological characters and by microscopic examination of each isolate, a pure culture obtained in petri plates sub-cultured and the mycelium growth of the fungus after 3 to 6 days. Four varieties of finger millet were plated in Petri dishes, the varieties are Arjuna, Subhra, Bhairabi and Kalua.

In variety, Arjuna the germination was 90 % in March while it was 80% in April and 70% in August respectively (Table 1a). In germinated seeds, *Aspergillus flavus, A. niger, Fusarium oxysporum* association was found. Maximum infection was found by *Fusarium oxysporum* (25%) in March and followed by *Aspergillus flavus* (10%) and *A. niger* (5%) respectively. In the month of April, *A. flavus* infection was 15% where as *Fusarium oxysporum* infection was 5% and in August no infection was recorded.

Then in the case of variety Subhra, the germination was observed in March, April while there was no germination was found in August. Infection was less in germination seeds than non germinated seeds (Table 1b). In March in germinated seeds, *Aspergillus niger* was 35% and *Fusarium oxysporum* was 5% and in non germinated seeds, *A. flavus* was 10%. In April maximum infection was observed due to *F. oxysporum* (15%) followed by *A. flavus* (10%) and *A. niger* (5%). In the case of non germinated seeds, *F. oxysporum* (10%) . In the month of August in non-germinated seeds *A. flavus (*15%) and *F. oxysporum* (20%) were observed.

Table 1 (a-d). Monitoring of the fungal infections and seed germination on different varieties of finger millet.

Table 1a

Month	Germination	Finger millet (ragi) variety (Arjuna)							
		Fungus recorded in germinated seeds				Fungus recorded in non-germinated seeds			
		Aspergillus flavus	*Aspergillus niger*	*Fusarium oxysporum*	Not germination	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium oxysporum*	Total
March	90%	10	5	25	10%	0	0	0	40%
April	80%	15	0	5	20%	0	0	0	20%
August	70%	0	0	0	30%	0	0	0	0%

1b

Month	Germination	Finger millet (ragi)variety (Subhra) Fungus recorded in germinated seeds				Fungus recorded in non-germinated seeds			
		Aspergillus flavus	*Aspergillus niger*	*Fusarium oxysporum*	Not germination	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium oxysporum*	Total
March	80%	0	5	5	20%	10	0	0	20%
April	60%	10	5	15	40%	0	0	10	40%
August	0%	0	0	0	100%	15	0	20	35%

1c

Month	Germination	Finger millet (ragi)variety (Kalua) Fungus Recorded in Germinated Seeds				Fungus Recorded in Non-germinated Seeds			
		Aspergillus flavus	*Aspergillus niger*	*Fusarium oxysporum*	Not germination	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium oxysporum*	Total
March	30%	0	0	0	70%	0	0	0	0%
April	30%	35	0	0	70%	0	0	0	35%
August	0%	0	0	0	100%	0	0	0	0%

1d

Month	Germination	Finger millet (ragi) variety (Bhairabi) Fungus recorded in germinated seeds				Fungus recorded in non-germinated seeds			
		Aspergillus flavus	*Aspergillus niger*	*Fusarium oxysporum*	Not germination	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium oxysporum*	Total
March	20%	15	5	0	80%	0	0	0	20%
April	10%	10	0	10	90%	0	0	0	20%
August	0%	0	0	0	100%	0	0	0	0%

In Kalua, germination was observed in March (30%) and April (30%) (Table 1c). In August there was no germination. The infection due to *A. flavus* was 35% in the case of germinated seeds. Germination was 30% both in March and April and in August there was no germination.

In the case of Bhairabi, 20% germination was observed in March and 10% in April and no germination was found in August. Maximum seed infection was observed in *Aspergillus flavus* in March (15%) and followed by *Aspergillus niger* (5%). In the month of April seed infection due to *A. flavus* and *F. oxysporum* was same (10%). In germinated seeds, no infection was found.

Maximum germination was observed in Arjuna (90%) followed by Subhra (80%) and the germination was 30% in Kalua and 20% in Bhairabi (Table 1d). In the month of March seed infection was maximum in Arjuna (40%) followed by Subhra and Bhairabi. There was reduction in germination (Pathak and Zaidi 2013).

CONCLUSION

In variety, Arjuna the germination was 90 % in March while it was 80% in April and 70% in August respectively. In germinated seeds, *Aspergillus flavus, A. niger, Fusarium oxysporum* association was found. Then in the case of variety Subhra, the germination was observed in March, April and no germination was found in August. Infection was less in germination seeds than non germinated seeds. In Kalua, germination was observed in March (30%) and April (30%). In August there was no germination. The infection due to *Aspergillus flavus* was 25% in the case of germinated seeds. Germination was 30% both in March and April and in August there was no germination. In the case of Bhairabi, 20% germination was observed in March and 10% in April and no germination was found in August. Maximum seed infection was observed in

Aspergillus flavus in March (15%) and followed by *Aspergillus niger* (5%) and *Fusarium oxysporum* (10%) were recorded. In germinated seeds, no infection was found.

Among four different varieties, i.e. Arjuna Subhra, Kalua and Bharaibi the germination percentage was maximum (90%) in Arjuna in the month of March with 40% seed infection while in Bharaibi the germination was 20% and the seed infection was 20%.

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