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# FUNGI ASSOCIATED WITH SEEDS OF PEARL MILLET *(PENNISETUM GLAUCUM* L. BR.) GROWN IN RAJASTHAN AND THEIR PHYTOPATHOLOGICAL EFFECTS

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One hundred twenty six seed samples of Pearl millet (*Pennisetum glaucum* L. Br.) collected from 14 districts of Rajasthan revealed 45 fungal species of 25 genera in addition to *Alternaria teuissima, Alternata alternata, Aspergillus candidus, A. flavus, A. fumigatus, Curvularia lunata, C. penniseti, Drechslera halodes, D. tetramera, D. rostrata, Bipolaris setariae, Helminthosporium spp, Fusarium moniliformae, Fusarium oxysporum, Rhizopus nigricans, Trichothecium roseum,* which were dominant and affected seed germination, seedling vigour and seedling diseases.

**Keywords**: Pearl millet; Phytopathological effects; Seed borne fungi; Seedling diseases.

## Introduction

Pearl millet, *Pennisetum glaucum* (L). Br., is the principal food cereal grown on 25 million-hectare of the drought prone semi- arid regions of African and the Indian subcontinent. Pearl millet is the staple diet of under-developed tropical and subtropical countries. The total area of the world under Pearl millet cultivation is about 47 million ha[1], out of which about 25 million ha is in India and Africa alone[2]. Pearl millet occupies a pivotal position among the cereal crops grown in arid zone of Rajasthan. The area under cultivation in India was 9.99 m ha and in Rajasthan it was 4.70 m ha. The seeds are infected and contaminated by many serious seed borne fungi while in field or during seed processing or during storage. Seed borne fungi of some pearl millet cultivars were reported by Konde *et al.*[3], Girisham *et al.*[4] and Mishra and Daradhiyar[5]. Ahmed and Reddy[6] and Ingle and Raut[7] also reported some seed borne fungi on pearl millet. Since no study gives systematic and comprehensive data on fungi associated with Pearl millet seeds grown in Rajasthan, the present investigation was undertaken.

## Materials and Methods

One hundred twenty six seed samples of Pearl millet from Rajasthan were collected during the crop season of 2005-2006 by Standard Blotter Test and Potato Dextrose Agar Method. For Dry Seed Examination four replicates of 100 seed per sample taken at random were studied. Percent incidence of different deformities, and insect damaged seeds were estimated. For incubation both untreated and 2% chlorine pretreated (400 seed/ samples) for 5 min were sown on moistened blotters and incubated for 8 days. Twenty five samples were studied using Potato Dextrose Agar (PDA) plates[8]. The discolored or diseased seeds were also plated separately to identify the specific fungi associated with them. Seed germination, seedling symptoms and incidence of fungi were recorded (Table 1).

## Results and Discussion

*Dry Seed Examination:* Seed samples of Pearl millet collected from 14 districts of Rajasthan revealed both asymptomatic and symptomatic seeds when observed under stereobinocular microscope. Symptomatic seeds showed various kinds of disorders like seeds with black or brown discoloration (0.28- 55%), seeds with white mycelial growth (0.25- 34.7%), shrivelled seeds (0.25 – 63.75%), seeds with spots and lesions (0.25- 13.25%), seeds with water soaked spots (0.25- 6%), and broken and insect damaged seeds (2.5- 25%). Seeds with brown discoloration on incubation yielded *Alternaria alternata, A. brassicola, A. tenussima, Bipolaris setariae, Helminthosporium spp, Curvularia spp and Drechslera* spp. Seeds with white mycelial growth were associated with *Fusarium spp and Trichothecium roseum. Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. sulpheureus. Cladosporium herbarum, and Rhizopus nigricans* were the main causal agent of spotted and lesioned seeds. Shirivilled seeds were associated with *Curvularia lunata, C. penniseti and C. pallescens* or their mixed infection with other fungi. These seeds were common in samples from all the districts. Seeds with water soaked spots were affected by bacteria. Broken seeds splitted into fractions and insect damaged seeds belonging to all districts generally yielded saprophytic fungi (Table 1).

*Incubation tests:* A total of 44 fungal species belonging to 22 genera, saprophytic as well as pathogenic, were observed on Pearl millet seeds in Blotter and PDA Tests (Table 2 and Figs. 1-15). The fungi encountered in PDA

test were mostly common to those observed in SBM. Fungal spp recorded in SBM were Actinomycetes ( 1-10%) in SBM and in PDA (1-3%), *Alternaria alternata* recorded in untreated (4- 61%) and pretreated seeds (2.5-30%) in SBM samples and in PDA (1-48%). *Alternaria brassicola* ( 1-2%) recorded in untreated seeds and did not occur in pretreated SBM and PDA test. *Alternaria longissima* (1-24%) in untreated and 1- 19% in pretreated seeds. *A. tenussima* (1-41%) in untreated and pretreated seeds and 1-21% in PDA. *Aspergillus candidus* (1-10%) in untreated (1-8%) and pretreated (1-10%) seeds and (1-18%) in PDA. *A. flavus* 3-44%, 4-15% and 1-29% infection in untreated, pretreated seed in SBM and PDA respectively. *A. niger* was recorded in untreated (2 – 57%), pretreated (1- 24%) in SBM and PDA (1- 25%). *Bipolaris setaraie* was recorded (1 – 45%) in untreated, (1- 40%) in pretreated seeds in SBM and (1- 34%) in PDA. *Curvularia lunata* occurred in untreated, seeds (1- 68.5%), pretreated (1- 41%) seeds in SBM and PDA (1- 20%). *Drecshlera tetramera* was isolated in untreated (1- 46%), pretreated (1- 30%) and (1- 48%) in PDA. *Diplosporium fulvum* occurred (1- 12%) in untreated and (1- 6%) in pretreated seeds in SBM and it was not observed in PDA test. *Doratomyces stemonitis* occurred (1- 18%) in both untreated and pretreated seeds. *Fusarium moniliformae* was isolated in PDA test (1-34%) and (1-8%) in SBM. *Helminthosporium tetramera* was observed (1- 21%) in untreated and (1- 18%) in pretreated seeds in SBM and (1- 16%) in PDA. *Rhizopus nigricans* encountered in untreated (1- 15%) and pretreated (1- 8%) seeds in SBM and in PDA (1- 34%). *Torula herbarum* encountered in 5 samples from Kota district. It occurred (1-9.6 %) and (1-6.8%) in untreated and pretreated seeds in SBM. *Trichothecium roseum* occurred (1-22%) in both untreated and pretreated seeds, (1- 12%) in PDA . *Epicoccum purpurascens* showed 1- 3% infection of the fungus in untreated seeds only. The other minor fungi that were not listed in Table- 2 were *Alternaria chrysanthemi, Alternria dianthicola, Aspergillus nidulans, A. ochraceous, A. sulpheureus, Botrytis cineria,* SBM. Although the percent range of *Aspergillus flavus* and *A* .

**Table 1.** Incidence of various seed disorders in Dry Seed Examinations, microorganisms associated and seedling diseases caused by them in Standard Blotter Method.

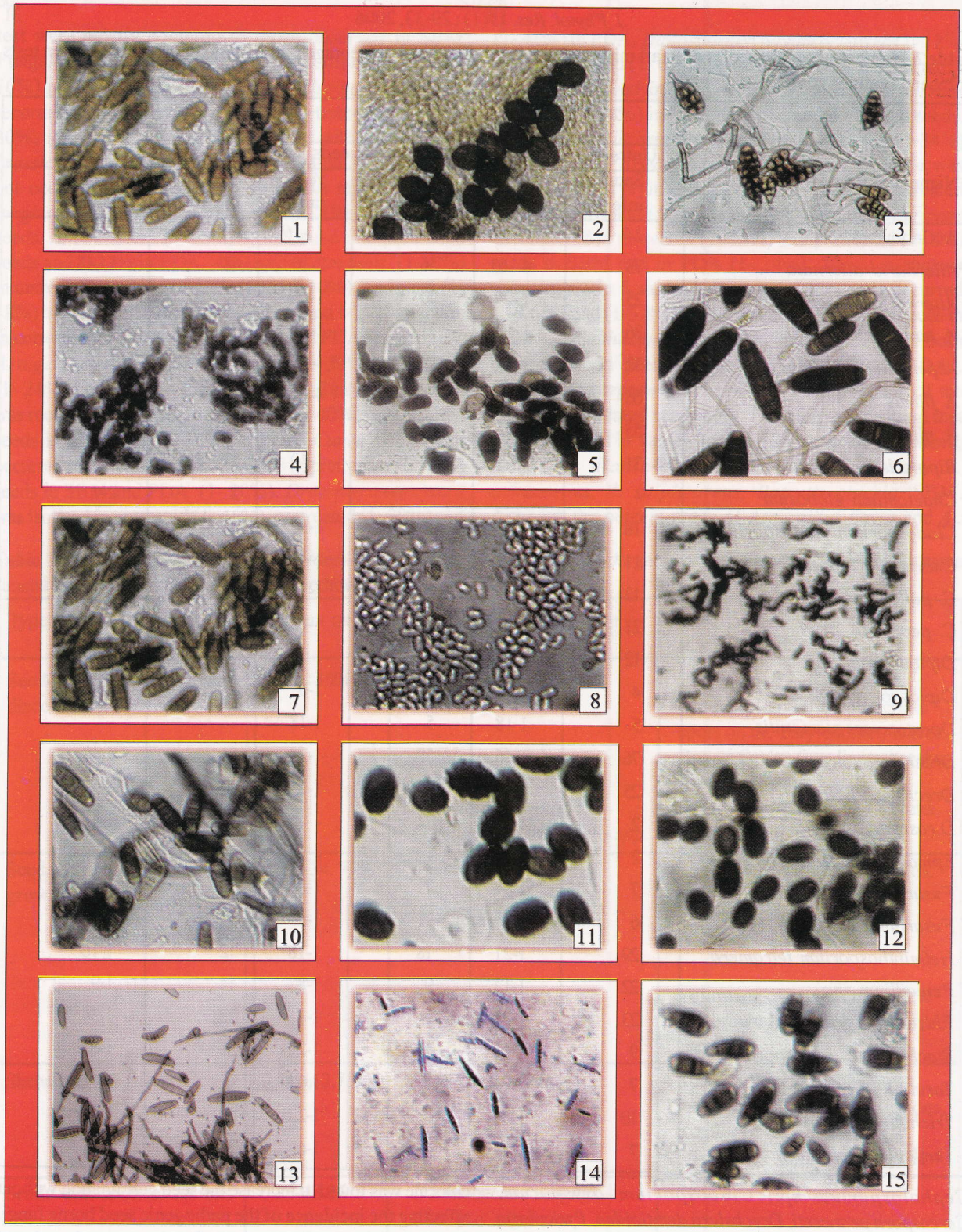
| Types of seed discoloration | Percent range | Important microorganism associated with seed | Seedling symptoms |
|---|---|---|---|
| Seeds with Black or Brown discoloration | 0.28 – 55% | *Alternaria alternata, A. tenussima*<br><br>*Bipolaris setariae*<br><br>*Curvularia lunata, C.penniseti, Drechslera spp.* | Browning of radical and hypocotyl and brown necrotic spots on the cotyledonary leaves.<br>Brown flecks; fine linear streaks, small oval spots.<br>Light brown spots and streaks at the margin of coleoptile. |
| Seed with white mycelial growth | 0.25– 34. 75% | *Fusarium spp.*<br><br>*Tri hothecium roseum, Rhizopus nigricans*<br><br>*A.niger, A. flavus, A. fumigatus* | Spotted or lesioned symptoms.<br><br>Seed and seedling rot.<br><br>Yellowing of leaves and radicle rot, brown to black lesions on seedlings. |
| Shrivelled seeds | 0.25%- 3.75% | *Curvularia lunata, C. penniseti, C. pallecsens* | Small yellow brown spots on leaves expand to oblong lesions. Center of lesion changes to brown and margins remain yellow. |
| Seeds with spots or lesions | 0.25- 13.25 | *Trichothecium roseum, Rhizopus nigricans* | Seed and seedlings rot. |
| Seed with water soaked spots | 0.25- 6.0% | Bacteria | Mucilaginous bacterial ooze on cotyledons. |
| Broken and Insect damaged seeds | 2.5- 25.0% | *Aspergillus spp.* | Browning root and radicle rot. |

**Table 2.** Number of seed samples of Pearl millet infected with fungi and percentage range of incidence in incubation tests (126seed samples studied).

| Name of the fungi | Blotter Test | | | | PDA Test | |
|---|---|---|---|---|---|---|
| | Untreated seeds | | Pretreated seeds | | | |
| | Samples infected | % range | Samples infected | % range | Samples infected | % range |
| Actinomyces | *19* | *1 -10* | *11* | *1 -10* | *5* | *1-3.0* |
| *Alternaria alternata* | 78 | 4 - 61 | 56 | 2-30 | 48 | 1–48 |
| *Alternaria longissima* | 25 | 1 -24 | 20 | 1- 19 | 11 | - |
| *A. tenussima* | 36 | 1-41 | 31 | 1-41 | 24 | 1-21 |
| *Aspergillus candidus* | 32 | 1-8 | 29 | 1-10 | 12 | 1-18 |
| *A. flavus* | 66 | 3-44 | 55 | 4-15 | 29 | 1-29 |
| *A. niger* | 55 | 2-57 | 44 | 1-24 | 20 | 1-25 |
| *Bipolaris setariae* | 31 | 1-45 | 24 | 1-40 | 17 | 1-34 |
| *Chaetomium globosum* | - | - | - | - | 2 | 2-4 |
| *Cladosporium cladosporiodes* | 18 | 1-33 | 16 | 1-37 | 12 | 1-26 |
| *Curvularia lunata* | 117 | 1-68.5 | 99 | 1-41 | 54 | 1-20 |
| *C. pallescens* | 13 | 1-6 | 10 | 1-3 | 6 | 1-7 |
| *C. penniseti* | 21 | 1-23 | 21 | 1-13 | 12 | 1-8.0 |
| *Diplococcium spp.* | 12 | 1-8 | - | - | - | - |
| *Diplosporium fulvum* | 4 | 1-12 | 4 | 1-6 | - | - |
| *Doratomyces stemonitis* | 5 | 1-18 | 5 | 1-18 | - | - |
| *Drechslera tetramera* | 76 | 1-46 | 58 | 1-30 | 51 | 1-48 |
| *Drechslera halodes* | 25 | 1-14.5 | 13 | 1-12 | 10 | 1-6 |
| *D.rostrata* | 2 | 1-2 | 2 | - | 3 | 1-4 |
| *Epicoccum purpurascens* | 2 | 1-3 | - | - | - | - |
| *Fusarium moniliformae* | 7 | 1-8 | 7 | 1-8 | 5 | 1-34 |
| *Fusarium oxysporum* | 6 | 1-5 | 7 | 1-5 | 9 | 1-8 |
| *Helminthosporium tetramera* | 29 | 1-21 | 18 | 1-18 | 12 | 1-16 |
| *Penicillium spp* | 22 | 1-12 | 9 | 1-8 | 14 | 1-9 |
| *Rhizopus nigricans* | 23 | 1-15 | 9 | 1-8 | 12 | 1-34 |
| *Stachybotrys parvispora* | 9 | 1-4 | 1 | 1-2 | 5 | 1-8.5 |
| *Torula herbarum* | 7 | 1-9.6 | 7 | 1.6.8 | - | - |
| *Trichothecium roseum* | 10 | 1-22 | 9 | 1-22 | 10 | 1-12 |
| *Verticillium alboatrum* | 7 | 1-5 | 4 | 1-6 | 3 | 1-7 |

*Cephalosporium sp., Cladosporium oxysporum, Cercospora penneseti, Drechslera demtioidea, Pyricularia grisea, Drechslera avenacea, Paecilomyces sp., Chaetomium sp. Fusarium solani, Fusarium semitectum.*

*Effect of Sodium Hypochloride Pretreatment on Seed Borne Fungi in SBM:* In Standard Blotter Method both untreated and seeds pretreated with sodium hypochlorite were used. In general, 1% concentration of available chlorine was found to increase the seed germination without affecting the incidence of the pathogenic seed borne fungi. But the incidence of saprophytic fungi was greatly reduced and their growth and sporulation on seed surface rendered sparse due to seed treatment. The fungi such as *Alternaria brassicola, A. chrysanthemi, Drechslera rostrata, Epicoccum purpurascens* that occurred in low incidences were completely inhibited after chlorine pretreatment in




**Figs. 1-15.** Photomicrograph showing fungi associated with seeds of Pearl millet (*Pennisetum glaucum* L. Br.) (45x)
*1. Bipolaris sorokinianum; 2. Doratomyces stemonitis; 3. Alternaria* spp.; *4. Diplococcium fulvum; 5. Curvularia lunata; 6. Drecshlera rostrata; 7. Drecshlera avenaceum; 8. Trichothecium roseum; 9. Torula herbarum; 10. Drecshlera tertramera; 11. Mammaria echinobotryoides; 12. Stachybotrys* spp.; *13. Helminthosporium* spp. *14. Fusarium oxysporum; 15. Curvularia penniseti.*

*niger* 3.0-44% to 4-15%, 2.0 – 57% to 1-24% respectively, that of *Alternaria alternata* 4.0 – 61% to 30%, of the total fungi recorded, *Alternaria alternata, A. tenussima, Aspergillus flavus, A. niger, Rhizopus nigricans, Curvularia lunata, Drechlera tetramera, Fusarium oxysporum, Helminthosporium tetramera, Trichothecium roseum* were also important and showed high incidence. Beside the fungal infection, mucilaginous growth of bacteria (0.2 to 9.2%) was observed in 52 samples.

A general assessment of the total seed borne inoculum revealed that seed samples from Alwar, Jaipur, Kota, Jhalawar, Udaipur and Sawai- madhopur mostly showed heavy inoculum and greater incidence of fungi. This may be due to more reverine areas and with more rainfall, hence high humidity is in general, which favours the sporulation of the fungus.

*Phytopathological Effects:* Fungi associated with seed affected germination as well as vigour and also produced symptomatic seedlings. In 126 samples studied, the germination ranged from 1- 100% in untreated and pretreated seeds in SBM. The fungi which commonly affected seed germination were spp. of *Alternaria, Aspergillus, Bipolaris, Curvularia, Drechslera, Fusarium, Helminthosporium* and *Trichothecium.*

Most of the fungi like *Alternaria alternata, Aspergillus flavus, Drechslera tetramera, Fusarium moniliformae, Curvularia lunata, C. penniseti, Bipolaris setariae* caused serious seed diseases and produced infected seedlings. Seed infection by *Alternaria alternata* caused swelling of hypocotyl with brown necrotic streaks and browning of radicle. Seed contaminated with *Aspergillus flavus* caused brown to black lessions on seedlings. Seed infection by *Bipolaris setariae* caused brown flecks, fine linear streaks, small oval spots, and large irregular oval, oblong or almost rectangular spots.Navi *et al.*[9] also reported *Bipolaris panici- miliaces* on pearl millet.

*Fusarium oxysporum* caused yellowing and drying of seedlings. Well[10] reported top rot of Pearl millet caused by *Fusarium moniliformae*. Infection with *Curvularia lunata* and *C. penniseti* caused small yellow brown spots on leaves, expands to oblong lesions, center of lesion change to brown and margins remain yellow. Lesions are more common on leaf margins. Infection of seedlings by *Drechslera tetramera* and *D. rostrata* results in 1 to 3 mm long coalescing lesions with extensive necrosis [11,12].

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