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# Greengram Seed Microflora under Ambinent Storage

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**ABSTRACT** Understanding of the status of microflora associated with greengram (*Vigna radiata*) seeds is important. With this objective mungeban seeds (var. Pratap) were subjected to storage in metallic bin under ambient condition. Seed samples were drawn on 0, 90, 180 and 270 days intervel for identification of microflora and evaluation of their pathogenicity. Seed wash method showed no bacterial microflora; agar plate method showed 4 fungal isolates (SP-1, SP-2, SP-3 and SP-4) which were identified as *Aspergillus flavus, A. niger, Penicillium* spp. and *Rhizoctonia solani*, respectively. Pathogenicity test showed that all the four identified fungal species gradually reduced seed germination. There was no germination with increased inoculation period of 216 hr as against 90% germination in non-inoculated control. The study indicated that the associated fungal species was pathogenic.

**Key words:** Seed wash, agar plate, pathogenicity, *Aspergillus flavus, A. niger, Penicillium* spp. *Rhizoctonia solani*, germination

Greengram (*Vigna radiata* L. Wilczek) with its rich food value ranking third among pulses, is a major source of protein. Quality seed with genotypic potentiality is the most crucial input for pulse production. Greengram seed lots carry wide range of microflora internally and externally. It is very difficult to get cent per cent pathogen free seeds for sowing. Areas with high atmospheric humidity are poor for seed storability and the North-eastern Region is one such region. The microflora associated with seeds influence seed germination, seedling health and subsequently the yield. Therefore, microflora associated with greengram seed and their relationship with seed performance, was investigated.

## MATERIALS AND METHODS

Seeds of greengram (var. Pratap) were obtained from Regional Agricultural Research Station, Assam Agricultural University, Nagaon. Four kg seeds, each were stored in metallic bins under ambient condition for 270 days in four replicates. Seed samples were drawn on 0, 90, 180 and 270 days from start of seed storage for evaluation. Seed wash and agar plate incubation methods [2] were followed to detect the seed-microflora. For seed wash method, 100 seeds were transferred singly in 100 conical flasks (100 ml), each containing 20 ml distilled water. The flasks were shaken on a mechanical shaker for 5-10 min. After transferring the seeds into a beaker, washing water was passed through cheese cloth into the same beaker.

Washing water was diluted serially. About 0.1 ml aliquot was taken from each dilution and mixed with 20 ml molten Potato Dextrose Agar (PDA), Nutrient Sucrose Agar (NSA) medium and shaken well. The media were poured in sterile blank Petri plates and incubated at $28^{o}\pm1^{o}C$ for 10 days. Five plates were poured from each dilution. For agar plate method, 5 seeds were placed in each Petri plate containing PDA/NSA media for the growth of microflora. The plates were incubated as in seed wash method and

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seeds were examined both macroscopically and microscopically at 2 hr interval until 10 days from incubation and expressed in per cent frequency.

$$\text{Frequency}(\%) = \frac{\text{No. of seeds on which growth of particular species is detected}}{\text{Total number of seeds examined}} \times 100$$

Soon after growth, the fungi were aseptically transferred to PDA slants and incubated at 28°±1°C for 10 days. The fungi were purified through hyphal tip method and preserved in refrigerator at ±4°C. Characterizaiton and identification of fungi were carried out in the Mycology Research Section, Department of Plant Pathology, AAU, Jorhat, with the help of relevant keys, monographs and literature [3-9].

Pathogenicity of the isolated organisms was tested by surface sterilizing 50 seeds with 0.1 per cent mercuric chloride solution for two min. and then rinsing thrice in sterile distilled water and finally dried by placing on a sterile blotting paper. The surface sterilized seeds were then

**Table 1. Characterization and identification of pathogen**

| Isolate | Morphological character | Microscopic character | Pathogen identified |
|---|---|---|---|
| SP-1 | Colonies are lime green | Conidiophores arising from the submerged hyphae (502.00-700.00 µm in length and 8.50-12.0 µm in width). Enlarging towards the apex with somewhat pitted walls, phialides borne directly on vesicles or on metulae in large heads (8.5-11.5 x 2.4-3.3 µm). Conidia globose, colourless to light green (2.5-5.2) µm in diameter. | *Aspergillus flavus* |
| SP-2 | Colonies are blackish-green | Conidiophores arising directly from substraturh, mostly hyaline but somewhat brown vesicle, thick walled, smooth, measuring up to 350 µm in length and 15.5 µm in diameter. Conidial heads blackish-brown to black, globose to sub-globose, vesicles colourless, globose to sub-globose, thick walled, 90.5 µm in dia. phialides thickly covering vesicles (7.4-90.8 x 1.4-3.3 µm). Conidia globose, at first smooth then somewhat spinulose, measuring 3.2-4.0 µm. | *Aspergillus niger* var. Tieghem |
| SP-3 | Colonies are bluish-green, velvety surface | Conidiophores smooth walled, septate, 3.01 µm in diameter. Sterigmata (8.6-11.9 x 2.5-3.5 µm) in size. Conidia borne in chain, globose, smooth walled (3.3-5.1 x 3.4-5.1 µm) forming loose columns. | *Penicillium* spp. |
| SP-4 | Colonies are cottony white | Mycelium composed, initially colourless hyphae but later become yellowish and finally deep brown, septate, 6.2-9.2 µm in thickness. Hyphal branches typically constricted at the point of origin. Sclerotia irregular in shape, initially white but later turning brown, varying in size from 2-6 mm. | *Rhizoctonia solani* Kuhn. |

steeped into thick suspension (conc. 3 x $10^6$/ml) of 10-15 days old actively sporulating culture of the test fungus for 30 min in case of *A. flavus*, *A. niger* and *Penicillium* spp. and rolled on actively growing mycelial growth of the test fungus (before sclerotia formation for *R. solani*) plated on Petri dishes containing PDA for 2 hr. The seeds were then allowed to dry in shade on blotting paper at room temperature for 24 hr. The performance of inoculated seeds were then tested in terms of germination percentage. Seeds were assessed for germination by Rolled Towel Paper method [10].

## RESULTS AND DISCUSSION

*Micoflora of mungbean seeds*

The seed wash method showed no association of bacterial pathogen with mungeban seeds during the storage. However, following the agar plate method, four fungal isolates (SP-1, SP-2, SP-3, SP-4) were found associated with the seeds. The fungal microflora were identified as *Aspergillus flavus* Link ex. Fries, *A. niger* var. Tieghem, *Penicillium* sp. and *Rhizoctonia solani* Kuhn. (Table 1). The per cent occurrence of *A. flavus* and *A. niger* were more than of *R. solani* and *Penicillium* spp. (Table 2).

The agar plate and rolled towel methods were reported superior over other methods for fungal microflora [11, 12] and therefore, the same were followed in the present study.

*Pathogenicity of identified microflora*

The identified seed-borne microflora evaluated for their pathogenicity were found to induce adverse effect on seed germination (Table 3). All the four species, *viz. A. flavus, A. niger, Penicillium* spp. and *R. solani* gradually decreased the percentage of seed germination than that of the under non-inoculated control. Non-inoculated control recorded the highest percentage of germination (90%) in 96 hr. The germination percentage ranged from 9 to 12 with the inoculated seeds. The germination percentage was recorded to '0' per cent with increased inoculation period to 216

**Table 2. Frequency of pathogen infection (%) in greengram seeds during storage**

| Pathogen | Days from start of seed storage | | | |
|---|---|---|---|---|
| | 0 | 90 | 180 | 270 |
| *Aspergillus flavus* | 27.00[a] | 31.75[a] | 36.75[a] | 37.00[a] |
| *Aspergillus niger* | 21.50[b] | 24.08[b] | 29.50[b] | 29.15[b] |
| *Penicillium* spp. | 8.25[c] | 11.75[c] | 12.00[c] | 13.00[c] |
| *Rhizoctonia solani* | 10.25[c] | 12.00[b] | 14.50[c] | 15.25[c] |
| Total infection | 67.00 | 78.75 | 92.75 | 95.00 |
| SEm(±) | 2.14 | 3.98 | 3.75 | 2.34 |
| CD (P=0.05) | 4.65 | 8.67 | 7.95 | 4.98 |

**Table 3. Pathogenicity test of identified pathogens on greengram seeds during different hour of incubation**

| Pathogen inoculated | Concentration | Germination (%) during different hour of incubation period | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 hr | 48 hr | 72 hr | 96 hr | 120 hr | 144 hr | 168 hr | 192 hr | 216 hr | 240 hr |
| *Aspergillus flavus* | 3 x $10^6$/ml | 17.00 | 16.00 | 13.00 | 9.00 | 8.00 | 6.00 | 4.00 | 1.00 | 0.00 | 0.00 |
| *Aspergillus niger* | 3 x $10^6$/ml | 18.00 | 17.00 | 15.00 | 10.00 | 9.00 | 5.00 | 3.00 | 1.00 | 0.00 | 0.00 |
| *Penicillium* spp. | 3 x $10^6$/ml | 19.00 | 18.00 | 15.00 | 12.00 | 10.00 | 7.00 | 6.00 | 3.00 | 0.00 | 0.00 |
| *Rhizoctonia solani* | Mycelial mat of 9 cm dia | 18.00 | 17.00 | 14.00 | 11.00 | 9.00 | 5.00 | 5.00 | 2.00 | 0.00 | 0.00 |
| Control | | 20.00 | 60.00 | 80.00 | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 |

hr. The pathogenicity of the species lead to rottening of seed as well there was no radicle and plumule. The fungi *A. flavus*, *A. niger* and *Penicillium* spp. were reported to cause rottening of seeds and reduced seed germination [13, 14]. It was also observed that *A. flavus* and *A. niger* affected root development, whereas *R. solani* affected shoot development. It was concluded that four identified species reduced seed germination, root and shoot development and seedling vigour.

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