

Fungi associated with mouldy seeds of sorghum [*Sorghum bicolor* (L.) Moench] cv. CSH-9 in Western Maharashtra

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ABSTRACT

Seven fungal pathogens *i.e.*, *Alternaria alternata* (Fr.) Keissler, *Curvularia penniseti* (Mitra) Boedijn, *Drechslera rostrata* (Drechs.) Richardson and Fraser, *Fusarium culmorum* (W.G. Smith) Sacc., *Aspergillus niger* van Tiegh., *Aspergillus fumigatus* Fres., *Rhizopus stolonifer* (Ehx-ex-fr) Lind. were found associated with various discoloured grades of sorghum cv. CSH-9 externally. However, *A. alternata*, *C. penniseti*, *D. rostrata*, *F. culmorum* and *Aspergillus niger* were found associated with seed of sorghum internally. *Fusarium culmorum* was associated with dark brown and crimson red discoloured seeds. *Alternaria alternata* and *Drechslera rostrata* were found in gray discoloured seeds. *Curvularia penniseti* and *Drechslera rostrata* were associated with dark black discoloured seeds. *Aspergillus niger*, *A. fumigatus* and *Rhizopus stolonifer* were isolated from all discoloured grades of sorghum seeds.

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Grain sorghum (*Sorghum bicolor* (L.) Moench) is one of the main cereal crop of Maharashtra. Sorghum suffers from number of fungal diseases. Among the several diseases the grain moulds has become a major constraint for high yield yielding and early maturing hybrids during *kharif* season, which are usually exposed to late rains of October-November. The warm and humid climate is most congenial for the development of grain moulds resulting in quantitative and qualitative losses in seed. Therefore, it was felt necessary to study the fungi associated with seeds of sorghum externally and internally.

MATERIALS AND METHODS

Mouldy seeds of sorghum hybrid CSH-9 were collected from, All India Co-ordinated Sorghum Improvement Project (AICSP), Rahuri and graded on basis of various discolorations caused by seed-borne fungi. These grades are as below:

| Grade | Symptoms on seeds |
|-------|------------------------------------|
| I | Dark brown spots on the seeds |
| II | Dirty white or gray discolouration |
| III | Crimson red discolouration |
| IV | Dark black discolouration |
| V | Healthy seeds |

The graded seeds were used for the isolation of external and internal seed borne pathogens.

Detection and isolation of external seed-borne fungi:

The externally seed-borne fungi were detected by adopting the ISTA's standard blotter test method (Neergaard, 1997). Blotters were soaked in distilled or

sterilized water and placed in three layers in transparent Petriplates (plastic) after draining off excess of moisture. A fixed number of seeds *i.e.* 25 per plate were placed equidistant from one another under aseptic condition. Likewise 400 seeds were plated. After plating the seeds, the Petriplates were incubated for 7 days at $20 \pm 2^\circ\text{C}$ under near ultraviolet light (NUV) or fluorescent light with an alternate cycle of 12 hr light and 12 hr darkness in an incubation room. The seeds were examined on 8th day under steriobinocular microscope. The fungi were identified mostly on the basis of morphological characters of conidia, conidiophores and fruiting structures. The per cent incidence of different seed borne fungi associated with seeds was recorded.

The fungal colonies of different fungi associated with the seeds were picked up with the help of a sterilized inoculating needle and transferred on PDA slants, numbered and incubated at $26 \pm 2^\circ\text{C}$ for seven days to obtain the pure culture of the pathogen. The pure isolates grown on PDA slants were kept at low temperature in the refrigerator with a view to preserve the cultures for longer period without any loss in the viability.

Detection and isolation of internal seed-borne fungi:

The internally seed-borne fungi were detected by employing ISTA's standard Agar plate test (Neergaard, 1997). Prior to plating, the seeds of sorghum were treated with 0.1% HgCl_2 solution to prevent saprophytes development. Three washings with sterile water were given to remove corrosive sublimate. The seeds were placed in Petridishes containing PDA. Ten seeds were placed at equidistant per 9 cm plate. Plates were

incubated at $20 \pm 2^\circ\text{C}$ with an alternate cycle of 12 hr light and 12 hr darkness in an incubation room. The seeds were examined after 5 days of incubation. Identification was based on macroscopic observations *i.e.*, colony colour and spreading habit etc. The per cent incidence of different seed-borne fungi associated with seed was recorded.

As soon as fungal colonies were observed, they were transferred aseptically to PDA slants with the help of sterilized inoculating needle by adopting single spore isolation technique under the compound microscope. The isolates obtained were then maintained in pure form on PDA slants, numbered carefully and preserved at low temperature in refrigerator.

RESULTS AND DISCUSSION

Detection and isolation of external seed-borne fungi:

The results of external seed-borne fungi isolated from seeds of hybrid sorghum cv. CSH-9 are presented in Table 1. It is revealed from the results that total seven fungal pathogens were found associated with seeds of sorghum cultivar CSH-9 externally. These were *Alternaria alternata*, *Fusarium culmorum*, *Curvularia penniseti*, *Drechslera rostrata*, *Aspergillus niger*, *A. fumigatus* and *Rhizopus stolonifer*. These pathogens were isolated from various discoloured grades of sorghum seeds and their per cent incidence is given below :

Dark brown spots on seeds:

The seeds of dark brown spots showed the presence of *Fusarium culmorum* (30%), *Aspergillus fumigatus* (2%) and *Alternaria alternata* (1%) thereby indicated the highest incidence of *Fusarium culmorum* in dark brown spot seeds.

Dirty white or gray discolouration:

Dirty white or gray discolouration showed the highest percentage of *Alternaria alternata* (30%). It was followed by *Drechslera rostrata* (20%). However, *Aspergillus niger* (2%), *A. fumigatus* (2%) and *Rhizopus stolonifer* (1%) were also found associated

with these seeds in less percentage.

Crimson red-discolouration:

Crimson red discoloured seeds showed the highest percentage of *Fusarium culmorum* (20%). However, *Drechslera rostrata* (2%), *Aspergillus niger* (5%), *Curvularia penniseti* (2%), *Aspergillus fumigatus* (1%) and *Rhizopus stolonifer* (2%) have been found associated in lesser percentages.

Dark black discolouration:

Dark black discoloured seeds showed the highest percentage of *Curvularia penniseti* (30%). It was followed by *Drechslera rostrata* (25%). However, *Alternaria alternata* (4%), *Fusarium culmorum* (3%), *Aspergillus niger* (3%), *A. fumigatus* (2%) and *Rhizopus stolonifer* (2%) were also found associated with seeds in meagre.

Healthy seeds:

Healthy seeds also showed the presence of *Alternaria alternata* (4%), *Aspergillus niger* (2%), *A. fumigatus* (3%) and *Rhizopus stolonifer* (1%) in meagre percentages.

Detection and isolation of internal seed borne fungi:

The results of detection and isolation of internal seed-borne fungi from seeds of hybrid sorghum cv. CSH-9 are presented in Table 2. It is revealed from the results that total five fungal pathogens were found associated with seeds of sorghum cultivar CSH-9 internally. These were *Alternaria alternata*, *Fusarium culmorum*, *Curvularia penniseti*, *Drechslera rostrata*, *Aspergillus niger*. However, *Rhizopus stolonifer* and *Aspergillus fumigatus* which were found associated as externally have not been detected internally. These pathogens were isolated from various discoloured grades of sorghum seeds and their per cent incidence is given as below :

Dark brown spots on seeds:

Dark brown discoloured seeds showed the highest

Table 1 : Association of external seed-borne fungi with mouldy seeds of sorghum cv. CSH-9

| Sr. No. | Grades of seeds | Incidence of mycoflora (colonies) per 100 seeds | | | | | | | Total (%) |
|---------|------------------------------------|---|----|----|----|----|----|----|-----------|
| | | Aa | Fc | Cp | Dr | An | Af | Rs | |
| I. | Dark brown spots on seeds | 1 | 30 | - | - | - | 2 | - | 33 |
| II. | Dirty white or gray discolouration | 30 | - | - | 20 | 2 | 2 | 1 | 55 |
| III. | Crimson red discolouration | - | 20 | 2 | 2 | 5 | 1 | 2 | 32 |
| IV. | Dark black discolouration | 4 | 3 | 30 | 25 | 3 | 2 | 2 | 69 |
| V. | Healthy seeds | 4 | - | - | - | 2 | 3 | 1 | 10 |

Where as, Aa = *Alternaria alternata*, Fc = *Fusarium culmorum*, Cp = *Curvularia penniseti*, Dr = *Drechslera rostrata*, An = *Aspergillus niger*, Af = *Aspergillus fumigatus*, Rs = *Rhizopus stolonifer*.

Table 2 : Association of internal seed-borne fungi with mouldy seeds of sorghum cv. CSH-9

| Sr. No. | Grades of seed | Incidence of mycoflora (colonies) per 100 seeds | | | | | Total (%) |
|---------|------------------------------------|---|----|----|----|----|-----------|
| | | Aa | Fc | Cp | Dr | An | |
| I | Dark brown spots on seeds | 2 | 20 | 1 | - | 1 | 24 |
| II | Dirty white or gray discolouration | 18 | - | 1 | 6 | - | 25 |
| III | Crimson red discolouration | 1 | 8 | 2 | 2 | 1 | 14 |
| IV | Dark black discolouration | 3 | 2 | 19 | 12 | 2 | 38 |
| V | Healthy seeds | - | - | 1 | 2 | 1 | 04 |

Where as, Aa = *Alternaria alternata*, Fc = *Fusarium culmorum*, Cp = *Curvularia penniseti*, Dr = *Drechslera rostrata*, An = *Aspergillus niger*

percentage of *Fusarium culmorum* (20%) internally. However, *Alternaria alternata* (2%), *Curvularia penniseti* (1%) and *Aspergillus niger* (1%) were found associated in meagre.

Dirty white or gray discolouration:

Dirty white or gray discoloured seeds showed the highest percentage of *Alternaria alternata* (18%) internally. It was followed by *Drechslera rostrata* (6%). However, *Curvularia penniseti* (1%) was also found in meagre.

Crimson red discolouration:

Crimson red discoloured seeds showed the highest percentage of *Fusarium culmorum* (8%) internally. However, *Alternaria alternata* (1%), *Curvularia penniseti* (2%), *Drechslera rostrata* (2%) and *Aspergillus niger* (1%) were found in meagre.

Dark black discolouration:

Dark black discoloured seeds showed the highest percentage of *Curvularia penniseti* (19%). It was followed by *Drechslera rostrata* (12%). However, *Alternaria alternata* (3%), *Fusarium culmorum* (2%) and *Aspergillus niger* (2%) were also found associated in meagre.

Healthy seeds:

Healthy seeds also showed the presence of *Drechslera rostrata* (2%), *Aspergillus niger* (1%) and *Curvularia penniseti* (1%) in meagre.

These results are in agreement with those reported by Rebelo (1970), Utikar (1982), Landge (1992), Padule *et al.* (1997). Padule *et al.* (1997). reported that *Alternaria alternata*, *Fusarium culmorum*, *Exerohilum rostrata* (*Drechslera rostrata*), *Aspergillus fumigatus* and *A. niger* were found associated with mouldy seeds of sorghum. Landge (1992) reported *Alternaria alternata*, *Aspergillus niger* and *Drechslera spp.* with mouldy seeds of sorghum. Utikar (1982) reported *Alternaria sp.*, *Curvularia sp.*, *Drechslera sp.*, *Fusarium*

sp., *Aspergillus spp.*, *Rhizopus sp.*, with moulded seeds of sorghum cv. CSH-9. Rebelo (1970) reported *Curvularia penniseti* with moulded seeds of sorghum. However, number of workers reported that *Alternaria alternata* and *Aspergillus niger* were found associated with mould seeds of sorghum (Narsimhan and Rangaswami, 1969; Bhagwat and Pedgaonkar, 1973; Rao and Reedy, 1973; Patil, 1977; Konde and Pokharkar, 1979; Abdullah and Kadhum, 1987 and Usha *et al.*, 1994).

During the present investigations seven fungi were encountered in different frequencies. These frequencies were *Alternaria alternata* (30%), *Curvularia penniseti* (30%) and *Drechslera rostrata* (25%), *Fusarium culmorum* (30%). These results are more or less in agreement with the reports of Karve *et al.* (1976), More (1978), and Utikar (1982). Karve *et al.* (1976) reported per cent incidences of grain mould fungi *i.e.*, *Alternaria alternata* (31.2%), *Curvularia lunata* (35%) and *Fusarium sp.*, (25%). More (1978) reported per cent incidence of *Alternaria* (25.83%), *Curvularia* (22.28%), *Drechslera* (6.83%), *Fusarium* (32.18%) on mouldy seeds of sorghum hybrid. Utikar (1982) reported frequency occurrence of *Alternaria alternata* (23.20%), *Curvularia lunata* (12.73%) and *Fusarium semitectum* (19.62%) on mouldy grains of sorghum.

However, the results of internal seed-borne pathogens are in agreement with the results of Landge (1982) who reported *Alternaria alternata*, *Aspergillus niger* and *Drechslera spp.*, internally in sorghum seeds. Neergaard (1977) isolated *Drechslera rostrata* from sorghum seed internally. Pokharkar (1976) reported *Drechslera rostrata* internally with sorghum seeds. However, *Fusarium culmorum* and *Curvularia penniseti* appear to be new reports as internal seed borne pathogens. During the present investigations five fungi were encountered in different frequencies. These were *Fusarium culmorum* (20%), *Curvularia penniseti* (19%), *Alternaria alternata* (18%) and *Drechslera rostrata* (12%) and *Aspergillus niger* (2%). No one has reported the per cent incidence of internal seed-borne fungi of sorghum seeds.

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