Indian J. Plant Genet. Resour. 33(3): 334–346 (2020)
DOI 10.5958/0976-1926.2020.00047.9

RESEARCH ARTICLE

Studies on Infection Indexing and Distribution Profiling of Seed-borne Fungi of Sorghum Germplasm in India for Safe and Healthy Long-term Conservation

Jameel Akhtar[1]*, Baleshwar Singh[1], Raj Kiran[1], Pardeep Kumar[1], Meena Shekhar[1], Sadhana[1], Sushil Pandey[2] Smita Lenka[2] and SC Dubey[1]

[1]*Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India*
[2]*Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India*

(Received: 19 February, 2020; Revised: 23 August, 2020; Accepted: 24 August, 2020)

Studies on distribution profiling and infection indexing of seed-borne fungi of sorghum germplasm from seventeen states of India revealed the presence of a total of 43 species belonging to 23 genera of fungi. Based on average infection index, *Fusarium verticillioides* (26.27 ±20.96), *Alternaria alternata* (19.30 ±15.05), *A. niger* (14.40 ±7.42), *Fusarium semitectum* (15.44 ±10.72%), *Aspergillus flavus* (18.21 ±10.25), *Bipolaris sorghicola* (16.68 ±9.51), *Curvularia lunata* (11.51 ±8.49), *Colletotrichum sublineolum* (15.81 ±9.26) and *Phoma sorghina* (14.51 ±12.99) were recorded as dominant species causing grain mold of sorghum in different agro-climatic zones of India and also affecting seed germination significantly. Among dominant species, *F. verticillioides*, *A. alternata* and *Exserohilum rostratum* were recorded with highest average infection index from Karnataka and *Aspergillus flavus* from Telangana representing Southern Plateau & Hills zone. Whereas, *A niger, C. lunata F. semitectum*, *P. sorghina* and *C. sublineolum* were recorded with highest infection index from Maharashtra representing two agro-climatic zones of the country i.e. Western Plateau & Hills and West-coast Plains & Ghats. Keeping in view the importance of sorghum as a probable contingent crop under changing climate scenario, the present findings may play a greater role in long-term conservation, exploration, evaluation and characterization of sorghum germplasm.

Key Words: Blotter method, Germplasm conservation, Grain mold, Mycotoxins, Seed-borne fungi, Sorghum

Introduction

Sorghum, *Sorghum bicolor* (L.) Moench, an important *kharif* as well as *rabi* millets popularly known as '*Jowar'* in India. This is the 5th most important cereal crop after wheat, rice, maize and barley grown throughout world and India ranks 3rd in acreage and production (FAO, 2018). India has been suggested as its original home, others consider it, more likely, to have originated in Central Africa and has spread from there to Egypt and then eastwards to Arab, India and China (Akhtar, 1998). Sorghum is a principal source of energy, protein, vitamins and minerals to the poorest people of the arid and semi-arid tropics of the world including India and has both, antimicrobial as well as anti-carcinogenic properties (Awika *et al.,* 2009; Devi *et al.,* 2011; Das *et al.,* 2020). Due to extremely drought tolerant feature, sorghum could be a contingent crop for climate resilient agriculture. But, globally, grain mold is the most important fungal disease complex of sorghum and it impact both yield, as well as seed quality and yield losses due to grain mold on highly susceptible sorghum lines can reach upto 100 percent (Hundekar *et al*., 2016). Several reports have established that sorghum seed is carrier of many destructive disease complex such as anthracnose (*Colletotrichum sublineolum*), seed/stalk rot (*Fusarium verticillioides*), target spot (*Bipolaris sorghicola*), seedling blight/charcoal rot (*Macrophomina phaseolina*), leaf spot and blight (*Phoma sorghina*), downy mildew (*Pernosclerospora sorghi*), ergot (*Claviceps sorghi*), loose smut (*Sphacelotheca cruenta*), covered smut (*S. sorghi*), head smut (*S. reiliana*), long smut (*Tolyposporium ehrenbergii*) etc., which cause considerable yield losses (Islam *et al*., 2009; Akhtar *et al.,* 2006, 2012). Richardson (1990) has also documented 44 species belonging to 29 genera as seed-borne fungi in sorghum, which are responsible for grain mold. The

**Author for Correspondence: Email- jameel.akhtar@icar.gov.in*

grain mold fungi cause deterioration in seed quality. Considerable economic yield losses have also been estimated to be US$ >130 million annually in Asia and Africa (ICRISAT, 1992). In addition, the grain mold fungi produce mycotoxins, which negatively affect the food and forage crops grown across the world. These mycotoxins contamination has a direct impact on food safety and security; therefore, pose a serious livestock and public health concern.

ICAR-NBPGR, New Delhi India has one of the mandates for acquisition and management of PGR for long-term conservation and their utilization towards food security and sustainability. About 4.4 lakh germplasm accessions of various crops including 26171 accessions of sorghum (*Sorghum bicolor* L.) are conserved in the Gene Bank, ICAR-NBPGR, New Delhi. The seed-borne diseases have severe implications for grain yield, seed production and germplasm conservation. Presence of many pathogenic fungi reported in/on sorghum seed realized the need of profiling of infection index of seed-borne fungi of sorghum germplasm for their long-term conservation in the National Gene Bank, New Delhi, India through seed health testing (SHT). Therefore, keeping in view the importance of sorghum as a probable contingent crop under climate changing scenario, the present study was undertaken with the aim to generate information on infection indexing and distribution profiling of seed-borne fungi of sorghum in India which could play greater role in long-term conservation, exploration, evaluation and characterization of sorghum germplasm as well as in devising resistance breeding strategies to ensure greater protection from crop losses in field.

Materials and Methods

The experiment was conducted in the Division of Plant Quarantine, ICAR-NBPGR during 2011 to 2018. A total of 1397 germplasm samples of sorghum (indigenously multiplied or explored) were received through Germplasm Conservation Division, ICAR-NBPGR from seventeen states *viz.,* Andhra Pradesh, Assam, Delhi, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh and Uttarakhand representing various agro-climatic zones. During seed health testing, all the seed samples were initially examined visually and then under stereo-binocular microscope for discoloration, deformation, malformation and fungal growth and fructification etc. Later, seeds were subjected to washing test for detection of downy mildew oospores followed by blotter test. For incubation, three layers of sterilized blotter papers were soaked in sterilized distilled water and then kept in sterilized Petri plates (plastic) having diameter of 110 mm and labeled to maintain the identity of individual samples with number and date of observation. The seeds were surface sterilized by immersing in sodium hypochloride (NaOCl) solution (4%) for 30 seconds and subsequent rinsing thrice in sterilized distilled water. Twenty-five seeds per petriplate were placed on blotter papers in such a manner that 1 seed in centre and 9 seeds in the middle and 15 at periphery at equal distance. Four plates of each accession were incubated at 22±1°C under fluorescent light in alternating cycles of 12 h light and darkness for 7 days and examined on 8th day under stereo-binocular microscope (Nikon - SMZ 1500) at different levels of magnification i.e. 0.75x to 11.25x for presence of seed-borne fungi. The associated fungi were identified on the basis of colony characters, fruiting bodies and spores (Mathur and Kongsdal, 2003) under stereozoom microscope and slides using mounting media (lactophenol/ cotton blue) were also prepared and examined under compound microscope (Nikon - Eclipse 80i) and their frequency of occurrence and effect on seed germination was recorded. Whenever required, isolation of fungi was also done using modified single spore isolation techniques (Akhtar *et al.,* 2014) on PDA medium to confirm their identity. The frequency of occurrence of fungi was recorded to find out infection index using following equation to further analyse statistically and their impact on seed germination was also assessed using correlation analysis..

$$\text{Infection Index (\%)} = \frac{\text{Number of Infected Seeds}}{\text{Total plated seeds}} \text{ x } 100$$

Results

The problem under investigation, invariably referred as grain mold, was precisely studied to record and document the distribution profiling as well as infection index of seed-borne fungi of sorghum as well as their effect on seed germinability. The results of the experiment conducted are described in details.

Visual examination resulted in detection of *Sphacelotheca sorghi* in only four samples from

Table 1. Morphological features of seed-borne fungi detected in sorghum from different agro-climatic zones of India

Fungus	Growth characteristics				Infection Index (%)	Source (State)
	Shape/ type	Septation	Dimension (µm)	Attachment		
Aspergillus flavus	Conidia globose to subglobose, usually rough (echinulate), yellowish green	_	3-5	Conidiophores bearing the heads are clearly seen when the growth is light. They are long and hyaline terminating in bulbous heads	18.21 ±10.25	Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Telangana and Uttar Pradesh
Aspergillus niger	Conidia more or less globose, dark brown often rough or echinulate	_	4-5	Conidiophores solitary or in small groups	14.40 ±7.42	Delhi, Maharashtra and Telangana
Alternaria alternata	Conidia polymorphous, short to long, oval to cylindrical in shape	transverse, longitudinal and obliques septatae	10-58X 7-19	Conidiophores branched bearing conidia in acropetal chain	19.30 ±15.05	Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Alternaria padwickii	Conidia straight, fusiform and rostrate with long beak	4-5 septate	120-160 x 12-18	Conidiophores bearing solitary conidia	–	Assam Gujarat, Karnataka, Maharashtra and Telangana
Alternaria tenuisimma	Conidia with short taper beak grow individually or in short chains	1-6 transvers and 0-2 longitudinally septate	12-50 x 8-15	Conidiophores branched bearing conidia in acropetal chain	1.33 ±5.66	Gujarat and Telangana
Bipolaris bicolor	Conidia smooth walled, dark grey to brown, straight to long ellipsoidal, broader in the middle tapering to rounded ends	7-14 distoseptate	54.0-125.0 x 13.6-18.7	Conidia are arranged acropleurogenously at the tip of conidiophore or at short intervals	1.20 ±6.36	Gujarat, Karnataka, Maharashtra and Telangana
Bipolaris cynodontis	Conidia smooth walled, light to olivaceous brown, ellipsoid with rounded ends	5-9 distoseptate	32.0-68.0 x 8.0-14.0	4-9 conidia arranged acropleurogenously at the geniculated tips of conidiophores.	–	Telangana
Bipolaris halodes	Conidia straight or slightly curved, cylindrical to ellipsoidal with up 12 but commonly 6-8 pseudosepta, end cell hyaline or very pale and cut off by thick, dark septa, intermediate cell golden brown.	6-8 pseudosepta	60-90 x 11-20	Conidiophores arising singly or in pairs, straight or flexuous, upper part often geniculate	–	Telangana
Bipolaris oryzae	Conidia curved, fusiform to obvate with tapering to rounded ends	8-10 septate	90.0-145.0 x 12.0-20.0	Conidiophores straight, long and single bearing conidia arranged in acropleurogenous manner	1.60 ±2.83	Maharashtra and Telangana

Fungus	Growth characteristics				Infection Index (%)	Source (State)
	Shape/ type	Septation	Dimension (µm)	Attachment		
Bipolaris setariae	Conidia smooth walled, pale to golden brown, slightly curved conidia	5-10 distoseptate	45.0-100.0 x 10.0-15.0	Conidiophores are medium to long bearing 1-5 conidia acropleurogenously arranged	–	Telangana
Bipolaris sorghicola	Conidia pale to brown, ellipsoid, straight to slightly curved, gradually tapering towards the rounded ends	6-8 distoseptate	49-85 x 12-15	Conidia in chains and looks deceptive and attached to secondary conidiophores	16.68 ±9.51	Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Bipolaris sorokiniana	Conidia ellipsoid, mostly straight, thick-walled with rounded ends	9-10 distoseptate	60-90 x 18-20	erect, short and single conidiophores bearing 1-6 conidia arranged in acropleurogenous manner	1.40 ±6.36	Gujarat, Maharashtra, Telangana and Uttarakhand
Bipolaris tetramera	Conidia smooth walled, majority dark brown, lighter towards terminal cells, cylindric, straight with rounded ends	3 distoseptate	22-26 x 8-14	Conidiophores short to medium, single or in groups of 2-3, bearing acropleurogeneously	1.22 ±5.66	Jharkhand and Telangana
Cephalosporium maydis	Conidia hyaline, ellipsoidal to cylindrical, straight with rounded ends	Conidia single-celled	3.0-10.0 x 1.5-3.0	Conidia in the form of shiny, round and watery heads attached perpendicular to conidiophores	–	Uttarakhand
Chaetomium globosum	Asci clavate with 8 ascospores which are spherical to ellipsoidal	Aseptate	8.0-10.0 x 7-8	Perithecia globose fastened to the surface with rhizoid	4.00 ±4.99	Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Chaetophoma sp.	Pycnidia dark, globose to irregular, without ostiole, in dense or loose clusters, seated on an olive-colored subiculum; conidia hyaline, 1-celled, very small, ovoid; saprophytic on plant material.	Conidia single-celled		-	1.33±2.86	Maharashtra and Telangana
Cladosporium sphaerospermum	Conidia mostly globose to subglobose,	0-3 septate	33 x 3-5	Conidia are not branched or apically short branched, bearing conidia in branched chains	2.00 ±12.02	Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Colletotrichum capsici	Conidia hyaline, fusoid, 1-celled, both ends pointed	aseptate	15-27 x 2-5	Acervuli mostly single, sometimes coalescing	–	Maharashtra

Fungus	Growth characteristics				Infection Index (%)	Source (State)
	Shape/ type	Septation	Dimension (µm)	Attachment		
Colletotrichum sublineolum	Conidia hyaline, falcate, 1-celled, both ends pointed, often one end is more pointed and curved, brown to black in colour	0-3 septate	57-270 x 3-10	Acervuli single or in groups, sometimes coalescing	12.50 ±6.76	Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Telangana and Uttarakhand
Curvularia eragostidis	Conidia smooth walled, barrel shaped, septate, the middle septum truly median, thick and very dark	3-septate	17-28 x 9-17	Dark, straight or flexuous, long conidiophores arise singly or some time in groups bearing few to many dark, shiny conidia	1.5 ±0.70	Maharashtra
Curvularia lunata	Conidia smooth–walled, mostly curved, some straight, end cells subhyaline or pale, top cell rounded	3-septate	20-28 x 7-15	Arrangement of conidia on conidiophores is acropleurogenous showing variation as verticils, spike and loose spike	11.51 ±8.49	Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Curvularia pallescens	Usually straight or only slightly curved	3-septate	20.0-28.0 x 5.0-10.0	Conidial arrangement is acropleurogenous showing variation as spike or vereticils	4.67 ±13.33	Telengana
Curvularia trifolii	Conidia smooth-walled, mostly curved, third cell from base the largest and darkest with protuberanr hilum	3-septate	25.0-40.0 x 10.0-20.0	Attachment of few to many conidia on variably long slender straight or flexuous conidiophores	2.57 ±8.49	Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Curvularia tuberculata	Conidia straight, sometimes curved	3-5 septate	15.0-40.0 x 12.0-14.0	Conidial arrangement is acropleurogenous and variable	-	Telengana
Exserohilum longirostratum	Conidia with huge variation in shape and size, mostly brown and longirostrate	12-26 distoseptate	Up to 460.0 x 10.0-20.0	Conidia attached acropleurogeneously on short to medium single conidiophores or in groups of 2-3	2.67 ±13.47	Maharashtra
Exserohilum rostratum	Conidia with huge variation in shape and size, mostly brown to shiny black, ellipsoid to rostrate and longirostrate	3-12 distoseptate	15.0-460.0 x 10.0-25.0	Conidia attached acropleurogeneously on short to medium single conidiophores or in groups of 2-3	21.14 ±17.28	Delhi, Himachal Pradesh, Maharashtra, Punjab and Telangana
Fusarium oxysporum	Microconidia oval, elliptical or reniform and macroconidia falcate along with chlamydospores	Microconidia 1-celled; macroconidia mostly 3-septate, sometimes 3-5 septate	Microconidia 5.0-12.0 x 2.0-3.5; macroconidia 25.0-65.0 x 3.0-5.0	Microconidia formed in false-heads on monophialides macroconidia in slimy mass	14.92 ±8.49	Maharashtra and Telangana

Fungus	Growth characteristics				Infection Index (%)	Source (State)
	Shape/ type	Septation	Dimension	Attachment		
Fusarium poae	Microconidia hyaline globose or pyriform resembled bunches of grapes; hyaline and slightly curved macroconidia rarely formed	Microconidia 1-celled; macroconidia 3-septate	Microconidia 5.0-15.0 x 5.0-8.0; macroconidia 20.0-40.0 x 3.0-5.0	Microconidia formed in false-heads on monophialides macroconidia in slimy mass	3.51 ±10.33	Telangana
Fusarium semitectum	Macroconidia hyaline, straight to slightly curved, wedg-shaped without pedicellate basal cell	Mostly 5-septate	17.0-40.0 x 2.0-4.0	Branched conidiophores bearing single macroconidia, sometimes pinnotes giving flower like structure	15.04 ±10.77	Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana
Fusarium solani	Microconidia hyaline, oval, ellipsoidal or reniform and macroconidia thick-walled, hyaline with short rounded apical cell	Microconidia 0-1 septate and macroconidia mostly 3-septate	Microconidia 8.0-15 x 2.0-5.0; macroconidia 45.0-100 x 5-8	Microconidia formed in translucent, milky white watery drops on long phialides; macroconidia produced in cream coloured sporodochia	1.83 ±3.54	Gujarat and Telangana
Fusarium verticillioides	Microconidia mostly crescent shaped and macroconidia fusoid with sharply curved pedicellate basal cell	Microconidia occasionally one-septate and macroconidia mostly 3 septate	Microconidia 5-12 x 1.5-2.5, macroconidia 25-60.0 x 2.5-5	Microconidia in chains and macroconidia in wet mass	26.27 ±20.96	Gujarat, Haryana, Jammu & Kashmir, Karnataka, Madhya Pradesh, Maharashtra, Telangana and Uttarakhand
Gloeocercospora sorghi	Conidia hyaline and filiform or elongate	1-celled	20.0-195 x 1.5-3.2	Conidia produced in salmon coloured onion- shaped sporodochia	-	Telangana
Macrophomina phaseolina	Dark brown to black coloured large pycnidia grown with mycelium surrounding the neck of the pycnidia	Spores non-septate, hyaline, ellipsoid to obovoid	15.0-30.0 x 5.0-10.0	Spores oozing from osteole in white coured cirrhus	2.38 ±11.55	Telangana
Melanosopora zamei	Ascospores citriform and dark brown to blackish	1-celled	15.0-25.0 x 10-15	Ascospores oozing in lemon-shaped very long and black cirrhus from superficial perithecia	3.51 ± 7.33	Maharashtra and Telangana
Myrothecium verrucaria	Conidia hyaline to olive green, elliptical with pointed end	1-celled	5.0-7.0 x 2.5-3.5	Spores formed in irregular-shaped, green to dark green, flat sporodochia on seed surface	-	Telangana
Nigrospora oryzae	Conidia black shiny, spherical, smooth and solitary	1-celled	12.0-15.0	Conidia attached on short and fragile conidiophores	-	Maharashtra

Fungus	Growth characteristics				Infection Index (%)	Source (State)
	Shape/ type	Septation	Dimension (µm)	Attachment		
Phoma sorghina	Profuse mycelium, pycnidia black and shiny with small to long neck	Non-septate, hyaline, cylindrical and guttulate conidia	3-7 x 2-2.5	Conidia produced in superficially developed pycnidia on aerial mycelium	14.51 ±12.99	Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Telangana and Uttarakhand
Phomopsis sp.	Pycnidia dark, ostiolate, immersed, erumpent, nearly globose; conidia hyaline, ovoid conidia (alpha)	Conidia single-celled	5-8 x 2-3	Conidia produced in superficially developed pycnidia in embedded mycelium	-	Telangana
Rhizoctonia solani	Only radiating thick mycelium without sclerotia	Formation of septum in the mycelial branch near the point of origin with dolipore septum, constriction of the branch at the origin point	–	Branching from parent hypha is at right angle and sclerotia not differentiated into rind and medulla in culture	-	Gujarat
Rhizopus sp.	Sporangiospores shape may vary from globose to oval, elliptisoid, polygonal or angular	1-celled	5-15 µm	Sporangia spherical and black contain numerous sporangiospores	6.23 ±9.38	Telangana
Sclerotinia sclerotiorum	White fluffy mycelium with ireegular shaped sclerotia	–	3000-15000 x 2000-5000	–	–	Telangana
Sphacelotheca sorghi	Teliospores are scattered singly, globose to subglobose with minute echinulation, yellowish brown in colour	–	5-10	–	1.00 ±2.12	Maharashtra and Telangana
Trichothecium roseum	Conidia smooth hyaline, usually obovoid	1-septate	12-20 x 5-10	Conidia in basipetal chain on long slender, hyaline and septate conidiophores	4.06 ±14.78	Telangana

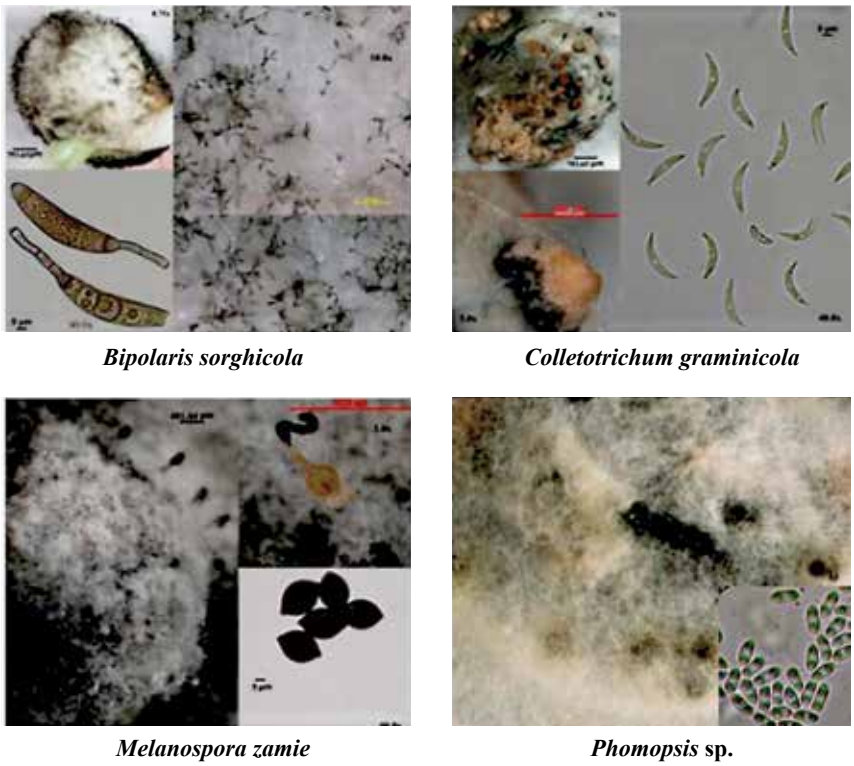
Telangana and Uttarakhand. Whereas, incubation test using blotter paper resulted in detection and identification of a total of 43 species belonging to 23 genera of fungi (Table 1, Fig. 1) namely, *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *A. padwickii*, *A. tenuisimma*, *Bipolaris bicolor*, *B. cynodontis*, *B. halodes*, *B. oryzae*, *B. setariae*, *B. sorghicola*, *B. sorokiniana*, *B. tetramera*, *Cephalosporium maydis*, *Chaetomium* sp., *Chaetophoma* sp,, *Cladosporium sphaerospermum*, *Colletotrichum capsici*, *C. sublineolum*, *Curvularia eragostidis*, *C. lunata*, *C. pallescens*, *C. trifolii*, *C. tuberculata*, *Exserohilum longirostratum*, *E. rostratum*, *Fusarium oxysporum*, *F. poae*, *F. semitectum*, *F. solani*, *F. verticillioides*, *Gloeocercospora sorghi*, *Macrophomina phaseolina*, *Melanosopora zamie*, *Myrothecium verrucaria*, *Nigrospora oryzae*, *Phoma sorghina*, *Phomopsis* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Sclerotinia sclerotiorum* and *Trichothecium roseum* as grain mold fungi of sorghum with varying level of infection index (Table 1) in different agro-climatic zones of India. Based on average infection index, *F. verticillioides*, *E. rostratum*, *A. alternata*, *A. flavus*, *B. sorghicola*, *A. niger*, *F. semitectum, C. lunata*, *C. capsica* and *P. sorghina* are dominant fungi among 43 fungal species affecting seed germination significantly.

Overall infection index revealed that among seed-borne fungi, the highest infection index was recorded with *F. verticillioides* (26.27 ±20.96) followed by *E. rostratum* (21.25 ±17.86), *A. alternata* (19.30 ±15.05), *A. flavus* (18.21 ±10.25), *B. sorghicola* (16.68 ±9.51), *F. semitectum* (15.44 ±10.72%), *A. niger* (14.40 ±7.42), *C. sublineolum* (15.81 ±9.26) and *P. sorghina* (14.51 ±12.99), *C. lunata* (11.51 ±8.49). Apart from dominant seed-borne fungi responsible primarily for grain mold of sorghum, several other species were also recorded from different regions occasionally, which included *B. setariae* and *B. halodes*, *F. poae*, *M. verrucaria* and *Phomopsis* sp. from Telangana; *C. capsici*, *C. eragostidis* and *E. longirostratum* from Maharashtra; *Rhizoctonia solani* from Gujarat; *Cephalosporium maydis* from Uttarakhand; *B. bicolor* and *F. solani* from Gujarat and Telangana; *B. oryzae*, *Chaetophoma* sp., *F. oxysporum* and *Melanospora zamei* from Maharashtra and Telangana; *B. tetramera* from Jharkhand and Telangana; *B. sorokiniana* from Gujarat, Maharashtra, Telangana and Uttarakhand; *Alternaria padwickii* from Assam, Gujarat, Karnataka, Maharashtra and Telangana; *Chaetomium* sp. and *C. trifolii* from Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana. Among these fungi, association of *M. verrucaria* and *Phomopsis* sp. observed in sorghum seed from Telangana are new reports from India, whereas, total failure of seed germination of sorghum due to *M. zamei* is also a new record.

Further, region wise analysis (Table 2) revealed that among predominant fungi, *F. verticllioides* was recorded with the highest infection index from Gujarat (39.16%) followed by Karnataka (34.27%) and Madhya Pradesh (31.43%). In case of *E. rostratum*, the highest infection index was recorded in Delhi (15.00%) followed by Telangana (14.30%). The highest infection index of *A. alternata* was recorded in Karnataka (24.00%) followed by Telangana (23.88%) and Gujarat (14.25%). The occurrence of *A. flavus* was recorded with the highest infection index in Telangana (21.38%) followed by Maharashtra (20.00%). Whereas, infection index of *A. niger* was observed mostly from Maharashtra (26.70%) followed by Delhi (16.50%). The highest infection index of *B. sorghicola* was observed in Maharashtra (23.78%) followed by Karnataka (18.75%) and Telangana (15.71%). In case of *F. semitectum*, the highest infection index was recorded in Maharashtra (20.00%) followed by Karnataka and Madhya Pradesh (15.00% each). In case of *C. lunata*, the highest infection index was observed in Maharashtra (19.27%) followed by Karnataka (11.11%) and Telangana (8.00%). The highest infection index of *C. sublineolum* was observed in Maharashtra (20.50%) followed by Karnataka (12.70%) and Telangana (12.40%). The highest infection index of *P. sorghina* was corded in Maharashtra (18.30%) followed by Gujarat (13.50%) and Madhya Pradesh (13.10%).

Discussion

The testing of 1397 germplasm accessions of sorghum revealed that infection of some of the predominant seed-borne fungi had impact on inhibiting seed germination up to hundred percent. Further, correlation analysis showed that amount of some seed-borne fungi on sorghum seeds had a weak correlation with germination, whereas, some had negatively strong correlation. Correlation coefficient (R^2) between infection index and seed germination



Bipolaris sorghicola ***Colletotrichum graminicola***

Melanospora zamie ***Phomopsis* sp.**

Fig. 1. Morphology of some seed-borne fungi detected on sorghum seed

revealed that infection of *F. verticillioides* had highest impact on inhibiting seed germination with R^2 value (-0.79) followed by *E. rostratum* (-0.52), *P. sorghina* (-0.52), *C. sublineolum* (-0.42%) whereas, *A. alternata*, *A. flavus*, *A. niger*, *B. sorghicola*, *C. lunata*, *F. semitectum*, showed lesser impact on seed germination (Fig. 2). In this study, *F. verticillioides* was found most effective in reducing seed germination, which supports the findings of earlier workers (Akhtar *et al*., 2006; Ivic, 2014). This could be due to the seeds were harvested from heavily infected crop because of prevailing climatic conditions more favorable for *F. verticillioides*.

Earlier workers have also reported up to 100.00% reduction in seed germination due to *C. lunata* and *F. verticillioides* in sorghum (Tripathi, 1974; Williams and Rao, 1981; Tarekegn *et al*., 2006). Most of these fungi have been reported as being associated with sorghum in India (Akhtar, 1998, Akhtar, *et al.,* 2012; Panchal and Dhale, 2011) and in other parts of the world (Islam *et al*., 2009). Besides seed germination failure, seedling mortality and destructive diseases in the field such as seed/stalk rot caused by *F. verticillioides,* target spot caused by *B. sorghicola,* anthracnose caused by *C. sublineolum*, leaf spot and blight caused by *P. sorghina*, charcoal rot caused by *M. phaseolina*, some of the grain mold fungi, especially *Fusarium* spp., *Aspergillus flavus* are also responsible for mycotoxins production which have negative impact on food and forage quality, safety and security. Association of these fungi at higher infection level with mycotoxins, aflatoxin (B1) produced by *Aspergillus flavus*, T-2 toxin and moniliformin produced by *F. verticillioides*, tenuazonic acid by *P. sorgina* and *A. alternata* in sorghum seed (Chulze *et al*., 1995; Bandyopadhyay and Chandrashekar, 2000) may pose serious livestock and public health concern. *Alternaria tenuissima* is a saprophytic fungus and an opportunistic plant pathogen. This species produces the allergen Alt a 1 **(**Saenz-de-Santamaria *et al*., 2006), one of the most important outdoor seasonal fungal allergens associated with allergy and asthma provocation (Denning *et al.,* 2014).

On the other hand, due to high mobility, germplasm in the form of seeds may disseminate associated seed-borne fungi. It is well known fact that seeds are the most important source for cultivation

Table 2. Summary of state wise infection index of predominant fungi associated with sorghum

Fungi	Range	Region wise Infection Index (%)								
		AP	DL	GJ	KR	MP	MH	TL	UK	UP
Fusarium verticillioides	Min.	–	–	30.00	4.00	10.00	2.00	4.00	4.00	–
	Max.	–	–	50.00	40.00	50.00	100.0	100.00	30.00	–
	Ave.	–	–	34.27	34.3	31.43	15.61	25.99	19.67	–
Exserohilum rostratum	Min.	–	10.00	–	–	–	–	5.00	–	–
	Max.	–	20.00	–	–	–	–	30.00	–	–
	Ave.	–	15.00	–	–	–	–	14.30	–	–
Alternaria alternata	Min.	–	–	4.00	6.00	4.00	4.00	4.00	–	–
	Max.	–	–	40.00	60.00	30.00	30.00	80.00	–	–
	Ave.	–	–	14.25	24.00	12.93	12.00	23.88	–	–
Aspergillus flavus	Min.	–	–	8.00	4.00	8.00	10.00	2.00	–	15.00
	Max.	–	–	18.00	24.00	20.00	30.00	38.00	–	18.00
	Ave.	–	–	14.33	13.25	12.50	20.00	21.38	–	16.50
Bipolaris sorghicola	Min.	10.00	–	4.00	10.00	4.00	4.00	10.00	–	–
	Max.	20.00	–	10.00	30.00	10.00	30.00	20.00	–	–
	Ave.	15.00	–	8.80	18.75	7.00	23.78	15.71	–	–
Aspergillus niger	Min.	–	10.00	–	–	–	10.00	10.00	–	–
	Max.	–	20.00	–	–	–	30.00	30.00	–	–
	Ave.	–	16.50	–	–	–	26.70	12.44	–	–
Fusarium semitectum	Min.	10.00	–	10.00	10.00	2.00	4.00	2.00	–	–
	Max.	20.00	–	20.00	30.00	30.00	50.00	50.00	–	–
	Ave.	15.00	–	11.66	15.00	15.00	20.00	12.14	–	–
Curvularia lunata	Min.	–	–	4.00	4.00	4.00	4.00	4.00	–	–
	Max.	–	–	10.00	24.00	14.00	38.00	10.00	–	–
	Ave.	–	–	6.29	11.11	6.75	19.27	8.00	–	–
Colletotrichum sublineolum	Min.	–	–	–	8.00	–	4.00	4.00	–	–
	Max.	–	–	–	20.00	–	35.00	20.00	–	–
	Ave.	–	–	–	12.70	–	20.50	12.40	–	–
Phoma sorghina	Min.	–	–	2.00	2.00	4.00	4.00	4.00	–	–
	Max.	–	–	40.00	40.00	24.00	50.00	40.00	–	–
	Ave.	–	–	13.50	12.80	13.10	18.30	10.80	–	–

AP= Andhra Pradesh; DL= Delhi; GJ= Gujarat; KR=Karnataka; MP= Madhya Pradesh; MH= Maharashtra; TL= Telangana; UK= Uttarakhand; UP= Uttar Pradesh

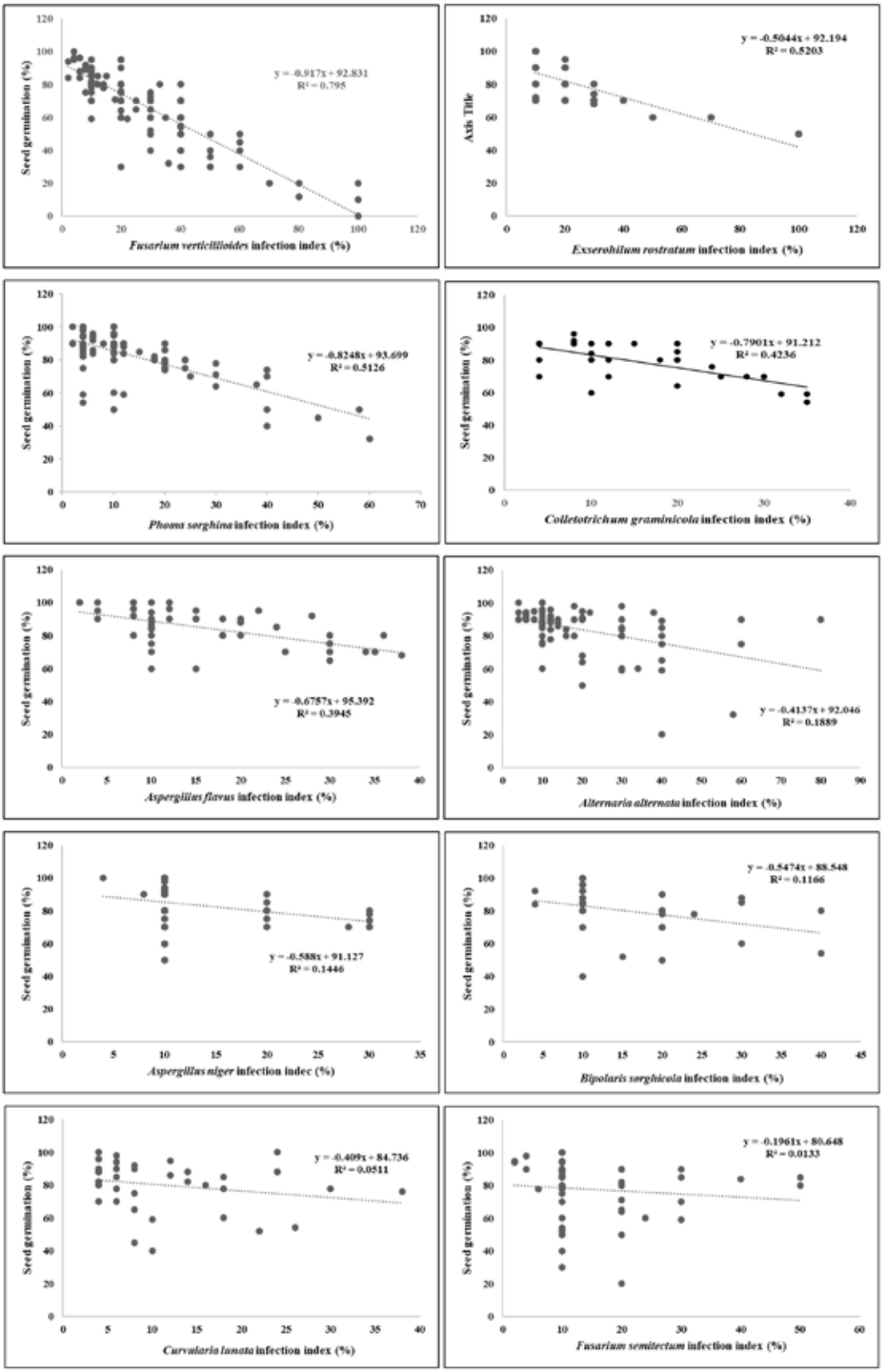


Fig. 2. Correlation between infection index of predominant seed-borne fungi and seed germination of sorghum

as well as for their use in crop breeding programmes to evolve high yielding varieties. Germplasm is also exchanged nationally/internationally in the form of seed for diversifying agricultural crops as it provides a wide genetic diversity available worldwide. Therefore, our finding on infection indexing and distribution profiling of seed-borne fungi provide avenues to mitigate the issue of carcinogenic and other ill effects of mycotoxins in sorghum. Further, there is a need to assess the incidence and severity of grain mold fungi complex on seed to determine the geographic distribution and status of the disease across the regions in order to prioritize research responsive to the situation.

In conclusion, distribution profiling and infection indexing of seed-borne fungi of sorghum germplasm from 17 states of India revealed the presence of 43 fungal species belonging to 23 genera. Out of these, *F. verticillioides, E. rostratum*, *A. alternata* and *A. flavus* were recorded as dominant species with highest infection index in sorghum germplasm from Maharashtra representing two agro-climatic zones of the country i.e. Western Plateau & Hills and West-coast Plains & Ghat. The present findings are expected to influence in long-term conservation, exploration, evaluation and characterization of sorghum germplasm and in devising resistance-breeding strategies of sorghum moulds/ diseases through National Sorghum Breeding Programme in the country at agro-climatic zone level to mitigate grain mold problems.

Acknowledgement

Authors are thankful to the Director, ICAR-NBPGR, New Delhi for providing necessary research facilities and scientific and technical staff of the Division of Plant Quarantine for their help.

References

Abdulsalaam S and KC Shenge (2011) Seed borne pathogens on farmer-saved sorghum. *J. Stored Prod. Postharvest Res.* **2**: 24-28.

Akhtar J (1998) Evaluation of oil cakes and bioagents against *Colletotrichum sublineolum* (Henn, Kabat et Bub.), causal agent of anthracnose of sorghum. M. Sc. (Ag.) Thesis, G.B. Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India. 96 p.

Akhtar J, MW Ansari and RR Dwivedi (2006) Natural incidence of fungal microflora on the surface of post-harvest sorghum grain in *Tarai* region of Uttaranchal. *Advances in Plant Sci.* **19**: 85-87.

Akhtar J, U Dev, B Singh, A Kandan, S Pandey, D Chand, J Kumar and PC Agarwal (2012) Studies on mapping and distribution of seed-borne fungi of sorghum germplasm in India. In: Abstracts of International Conference on plant health management for food security 'ICPHM 2012'. (eds.) G Katti, K Anitha, N Somasekhar, GS Laha, B Sarath Babu and KS Varaprasad, Plant Protection Association of India, Hyderabad. pp 26-27 (OP 13).

Akhtar J, A Kandan, B Singh, U Dev, D Chand, J Kumar and PC Agarwal (2014) A simple modified technique for obtaining pure cultures of seed-borne fungi. *Indian J. Plant Prot*. **42**: 156-159.

Awika JM, L Yang, JD Browning and A Faraj (2009) Comparative antioxidant, antiproliferative and phase II enzyme inducing potential of sorghum (Sorghum bicolor) varieties. *LWT-Food Sci. and Tech.* **42**: 1041-1046.

Bandyopadhyay R and A Chandrashekar (2000) Biology and management of sorghum grain mold. In: Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management; 2000 May 18-19, International Crop Research Institute for the Semi-Arid Tropics, Patancheru, India. 18 p.

Chulze SN, AM Torres, AM Dalcero, MG Etcheverry, Ml Ramirez and MC Farnochi (1995) *Alternaria* mycotoxins in sunflower Seeds: incidence and distribution of the toxins in oil and meal. *J. Food Prot.* **58**: 1133-1135.

Das IK Aruna and VA Tohapi (2020) Sorghum grain mold. ICAR-Indian Institute of Millets Research, Hyderabad, India (ISBN: 81–89335-93-6). 86p.

Denning DW, C Pashley, D Hartl, A Wardlaw, C Godet, GS Del, L Delhaes and S Sergejeva (2014) Fungal allergy in asthma–state of the art and research needs. *Clin. Transl. Allergy* **4**: 14.

Devi PS, MS Kumar and SM Das (2011) Evaluation of antiproliferative activity of red sorghum bran anthocyanin on a human breast cancer cell line (mcf-7). *Int. J. Breast Cancer* **2011**: 891481. doi:10.4061/2011/891481.

FAO (2018) World food and agriculture statistical pocketbook-2018. http://www.fao.org/3/CA1796EN/ca1796en.pdf.

Hundekar, R., MY Kamatar, SM Brunda and V Pattar (2016) Heterosis for yield and grain mold resistance in rainy season sorghum [*Sorghum bicolor* (L.) Moench]. *Environ. Ecol*. **34**: 1570-1576.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) (1992). Medium Term Plan 1994-98. Research theme datasets. Volume 3. Patancheru 502 324, Andhra Pradesh, India: ICRISAT. 229p.

Islam SMM, MMI Masum and MGA Fakir (2009) Prevalence of seed-borne fungi in sorghum of different locations of Bangladesh. *Sci. Res. Essays* **4(3)**: 175-179.

Ivic D (2014) Pathogenicity and potential toxigenicity of seed-borne *Fusarium* species on soybean and pea. *J. of Pl. Pathol.* **96(3)**: 541-551.

Mathur SB and O Kongsdal (2003). Common laboratory seed health testing methods for detecting fungi. International Seed Testing Association, Basserdorf, Switzerland. 425 p.

Panchal VH and DA Dhale (2011). Isolation of seed-borne fungi of sorghum (*Sorghum vulgare* Pers.). *J. Phytol.* **3(12)**: 45-48.

Richardson MJ (1990). An Annotated list of seed-borne diseases. 4th ed. The Internal Seed Testing Association, Switzerland. 345 p.

Saenz-de-Santamaria M, I Postigo, A Gutierrez-Rodriguez, G Cardona, JA Guisantes, J Asturias and J Martinez (2006) The major allergen of *Alternaria alternata* (Alt a 1) is expressed in other members of the Pleosporaceae family. *Mycoses* **49(2)**: 91-95.

Tarekegn G, NW McLaren and WJ Swart (2006) Relationship between ergot and grain mould development in sorghum (*Sorghum bicolor* (L.) Moench), *S. Afr. J. Plant Soil* **23**: 297-301.

Tripathi RK (1974) Head fungi of sorghum, phytotoxin and their effect on seed germination. *Indian Phytopathol* **27**: 499-501.

Williams RJ and KM Rao (1981) A review of sorghum grain mold. *Trop. Pest Manag.* **27**: 200-211.