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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Fungi Associated with Seeds of Sorghum (*Sorghum Bicolour* L.) in Rajasthan and their Phytopathological Effects

Abstract

58 seed samples of Sorghum (*Sorghum Bicolour* L. Monech) collected from 7 eastern districts (Bharatpur, Kota, Alwar, Jaipur, Sawai Madhopur, Karauli, Dholpur) of Rajasthan revealed 33 fungal species of 21 genera in addition to *Alternaria alternata, Alternaria tenuissima, Aspergillus flavus, A. niger, A. fumigatus, Bipolaris setariae, Curvularia lunata, Drechslera graminea, Drechslera rostrata, Drechslera tetramera, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus oryzae, Trichothecium roseum* which were dominant and affected seed germination, seedling vigor and seedling diseases.

Keywords: *Sorghum Bicolour*, Seedling Vigour, Seedling Diseases.

Ekta Singhal
Research Scholar,
Dept. of Botany,
Govt.M.S.J. P.G. College,
Bharatpur, Rajasthan, India

Archana Singh
Associate Professor,
Dept. of Botany,
Govt.M.S.J. P.G. College,
Bharatpur, Rajasthan, India

Introduction

Sorghum (*Sorghum bicolour* L. Monech) is a major cereal in the semi-arid regions of the world where it is an important food and feed crop. It is the 5th most important cereal after rice, wheat, maize and barley (Manjeet and Umesh 2013). The USA is a major producer of sorghum, but the grain is not consumed as human food except for a very small fraction, but as animal fodder, while in the semi-arid tropics of Africa and India the grain forms the staple diet for large populations, where nearly all the produce is used directly as human food (Kulamarva, Sosle and Raghavan 2009). Sorghum, like other cereals, is an excellent source of starch and protein. It is a gluten-free cereal, which plays a significant role for gluten intolerance people (Taylor *et. al,)*. Grain sorghum contains phenolic compounds like flavonoids which have been found to inhibit tumour development. The starch and sugar in sorghum are released more slowly than in other cereals and hence it could be beneficial to diabetics (Toomey 1988). It has wide range of adaptability to various agro ecological situations of the region (Patil and kute 2015). The global area under sorghum cultivation is estimated at 42.12 million hectares, and the production of sorghum has been estimated to be at the level of 61.38 million tonnes (FAOSTAT, 2013). India stands second globally for the area under sorghum cultivation (6.18 million hectares) and its production (5.28 million tonnes).

Review of Literature

The seeds are infected and contaminated by many serious seed borne fungi while in field or during seed processing or during storage. In present work isolation of mycoflora, using recent techniques, from the seeds at their various developmental stages collected from different region of Rajasthan. Fungi were isolated from the naturally discoloured rotten, immature and shriveled seeds, collected from the standing crops. Isolation of seed mycoflora using standard blotter method as well as agar plate method is studied (Panchal and Dhale 2011).

Seed infection by *Alternaria alternata* cause brown to black spots on leaves that enlarge progressively under favourable conditions (Aqsa Aftab *et. al* 2015). Seed contamination with *Aspergillus flavus* caused brown and black lesions on seedlings and browning of basal part of shoot. Seed infected by *Bipolaris sorokiniana* cause necrotic lesions on seedlings. *Fusarium moniliforme* cause root and basal stalk rot and blight of sorghum heads (Petrovic *et. al* 2009).

Infection with Macrophomina phaseolina caused charcoal rot, stem rot disease. The disease is widely distributed in most sorghum growing areas, where it reduce the sorghum ear head to about 1/5th of its normal size (Srinivas and Shankar 2017).

Aim of the Study

To study the fungal flora associated from the seed samples of sorghum collected from eastern districts of Rajasthan.

Material and Methods

58 seed samples of sorghum were collected from 7 eastern districts of Rajasthan (Bharatpur, kota, Alwar, Jaipur, Sawai Madhopur, karauli, Dholpur) during the crop season 2017-18 and 2018-2019 by standard blotter test and potato dextrose agar method (PDA). For dry seed examination three replicates of 100 seeds per sample taken at random were studied (ISTA 2004). For incubation both untreated and treated (1.5% NaOCl) seed samples studied. For better growth and sporulation of fungal flora seeds plated in petri-plates containing three layers of sterilized water soaked blotter paper and incubated at 25± 2°C for 12 hours at alternating cycles of light and darkness for 8 days (Azhar Hussain *et. al* 2009). Fungi were isolated from seeds and cultured on Potato Dextrose Agar (PDA) for further identification with the help of various keys. Seed germination, seedling symptoms and incidence of fungi were recorded (Table 1)

Results and Discussion

Dry Seed Examination

Seed samples were collected from various sorghum areas of Rajasthan. Dry seed examination of seed samples was done. Besides normal. Seeds of sorghum showed variously discoloured seeds with black or brown discolouration (0.25-60%), seeds with white mycelial growth (0.25-25%), shriveled seeds (0.25-10%) and broken and insect damaged seeds (2.5-40%).

Seeds with black and brown discolouration on incubation yielded *Curvularia lunata, Curvularia oryzae, Curvularia sorghina, Alternaria alternata, Drechslera tetramera, Drechslera rostrata, Macrophomina phaseolina, Phoma sorghina, Cladosporium cladosporiales, Bipolaris sorokiniana.* Seeds with white mycelial growth were associated with *Fusarium moniliforme, Fusarium oxysporum, Trichothecium sps.* Shriveled seeds associated with *Aspergillus flavus, A. candidus, A. fumigatus, A. sulphureus, Curvularia lunata, C. oryzae, Penicillium sps.* Seeds which are broken and insect damaged are associated with *Aspergillus niger, A. flavus, A. sulphureus and Penicillium.*

Incubation Test

Incubation test showed that these discolouration were caused by various fungi. A total of 33 fungal species belonging to 17 genera, saprophytic as well as pathogenic were observed on sorghum seeds in Standard blotter method (SBM) and PDA test (table 2). The fungi encountered in PDA test were mostly common to those which are observed in SBM.

Fungal species recorded in SBM were, *Alternaria alternata* recorded in untreated (7-68%) and pretreated seeds (3-34%) in SBM samples and in PDA (1-45%). Colonies of *Alternaria alternata* are black where conidiophores are simple or branched and conidia are present in chains and the colour of conidia is from pale to mid golden brown, ovoid with short conical or cylindrical beak. *Alternaria tenuissima* recorded (1-20%) in untreated and (1-15%) in pretreated seeds in SBM and (1-2%) in PDA test. Colonies of *Alternaria tenuissima* are dark olive in colour with loose cottony mycelium and their conidia contained 2 to 5 transverse septa with singly or in short chains. *Bipolaris sorokiniana* was recorded (2-18%) in untreated, (1-14%) in pretreated in SBM and (1-4%) in PDA test. *Cladosporium oxysporum* was recorded (1-25%) in untreated seeds, (1-20%) in pretreated seeds in SBM and (1-20%) in PDA test. *Curvularia* is one of the known genera that is responsible for causing grain molds in sorghum and various diseases in many other crops (Girish *et.al,* 2011). *Curvularia lunata* occurred in pretreated seeds (1-45%), pretreated (1-41%) seeds in SBM and PDA (1-35%). Colonies of *Curvularia lunata* are browm to black in colour with hairy, velvety or woolly texture in which conidia are acrogenously arranged, small, curved or straight and broad towards the apical tip. *Curvularia oryzae* occurred in pretreated seeds (1-48%), pretreated (1-40%) seeds in SBM and PDA (1-15%).*Drechslera species* causes brown leaf spot disease in many varieties of sorghum (Javed *et.al.* 2010) *Drechslera graminea* was recorded (1-30%) in untreated seeds (1-25%) in pretreated seeds in SBM and (1-25%) in PDA test. *Drechslera tetramera* was recorded (1-46%) in untreated seeds, (1-40%) in pretreated seeds in SBM and (1-36%) in PDA test. Colonies of *Drechslera tetramera* are black, hairy in which conidia are present in cluster of 2-3, brown in colour and always perpendicular to the axis. *Drechslera rostrata* was recorded (1-50%) in seeds, (1-45%) in pretreated seeds in SBM and (1-40%) in PDA test. Colonies of *Drechslera rostrata* are fast growing with brown to blackish in colour and conidia olivaceous to dark brown, rostrate, 6-16 pseudo septa, long, thick with distinctly protuberant hilum. *Fusarium moniliforme* recorded (1-25%) in untreated seeds, (1-20%) in pretreated seeds in SBM and (1-20%) in PDA test. Colonies of *Fusarium moniliforme* were white or peach coloured and microconidia are present in form of chains. *Macrophomina phaseolina* was recorded (1-5%) in untreated seeds, (1-2%) in pretreated seeds in SBM and (1-2%) in PDA test. *Macrophomina phaseolina* causes charcol rot in sorghum which is a destructive disease for sorghum (Jamadar and Desai 1996). Colonies of *Macrophomina phaseolina* vary in colour from black to brown with abundant aerial mycelium, numerous dark brown to black coloured sclerotia seen on the reverse side of culture plate. *Phoma sorghina* was recorded (1-33%) in untreated seeds, (1-37%) in pretreated seeds in SBM and (1-26%) in PDA test. Colonies of *Phoma sorghina* on seed has white or gray growth and produces large numbers of dark brown or black pycnidia. *Torula graminis* was recorded (1-9%) in untreated seeds, (1-6%) in pretreated seeds in SBM and (1-2%) in PDA test. Conidia of *Torula graminis* arise on short conidiophores directly from the vegetative hyphae. Conidia develop in long chains. *Trichothecium roseum* was recorded (1-25%) in untreated, (1-21%) in pretreated seeds in SBM and PDA (1-15%). Colonies of *Trichothecium roseum* are powdery, pink peach coloured having long slender

conidiophores with hyaline, long obovoid conidia. *Actinomycetes* recorded (1-12%) in untreated seeds and (1-10%) in pretreated seeds in SBM and (1-3%) in PDA test. *Aspergillus candidus* recorded (1-18%) in untreated seeds and (1-7%) in pretreated seeds in SBM and (1-12%) in PDA test. *Aspergillus flavus* recorded (4-40%) in untreated seeds and (4-16%) in pretreated seeds in SBM and (1-20%) in PDA test. *Aspergillus fumigatus* recorded (3-48%) in untreated, (1-18%) in pretreated seeds in SBM and (1-22%) in PDA test. *Aspergillus niger* was recorded in untreated (5-50%), pretreated (5-20%) in SBM and PDA (1-25%). *Penicillium sps*. was recorded (1-12%) in untreated, (1-8%) in pretreated seeds in SBM and PDA (1-9%). Colonies of *Penicillium* have various shades with septate hyphae branched conidiophres with vertical or erect primary branches. The other fungi which are also found in minor quantity are *Epicoccum nigrum, Nigrospora oryzae, Mammaria species, Bispora catenula.* The other minor fungi that were not listed in table-2 were *Alternaria dianthicola, Aspergillus nidulans, A. ochraceous, Chaetomium species* are also recorded in minor quantity. A general assessment of the total seed borne inoculums revealed that samples from Bharatpur, Alwar, Jaipur, Dholpur and Kota mostly showed heavy inoculums and greater incidence of fungi.

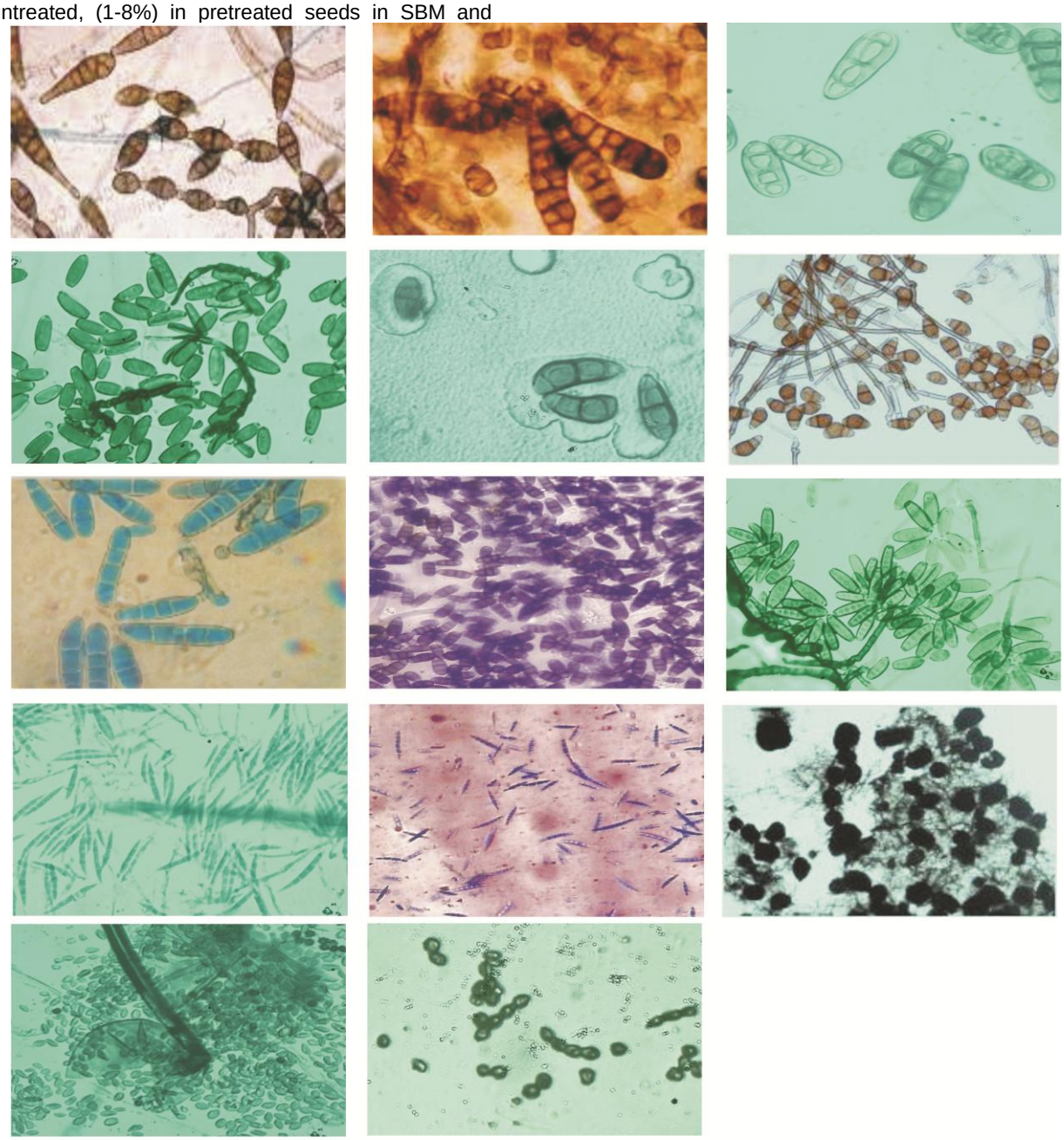

Fig:- 1-14. Photomicrograph showing fungi associated with seeds of sorghum (*Sorghum bicolor*) (45x)
1.Alternaria alternata 2. Alternaria tenuissima 3. Bipolaris sorokiniana 4. Cladosporium oxysporum 5. Curvularia affinis 6. Curvularia lunata 7. Drechslera graminera 8. Drechslera tetramera 9. Drechslera rostrata 10. Fusarium moniliforme 11. Fusarium oxysporum 12. Macrophomina phaseolina 13. Phoma sorghina 14. Torula graminis

Table 1: Incidence of Various Seed Disorders in Dry Seed Examination, Fungi Associated and Seedling Diseases caused by them in Standard Blotter Method

S.No.	Types of seed disorders	Percent Range	Fungi associated with seeds	Seedling Symptoms
1.	Seeds with black or brown discolouration	0.25-60%	*Alternaria alternata* *Alternaria tenuissima*	Browning of radical and basal shoot, necrotic spot on leaves
			Penicillium sps.	Yellowing of leaves & radical rot
			Drechslera tetramera *D. rostrata* *D. longipes*	Brown spot on seedlings which turn light brown & coalesce
			Cladosporium oxysporum *C. cladosporiales*	Seedling rot
			Curvularia lunata *C. oryzae* *C. sorghina* *C. penniseti*	Leaf blight, root rot, seedling blight and light brown spot and streaks at the margin of coleoptiles.
			Macrophomina phaseolina	Rotting of stalk tissue, damping off seedling blight
			Bipolaris sorokiniana *Bipolaris spicifera*	Elliptical, straw-coloured, necrotic lesions with darker margins
2.	Seeds with white mycelial growth	0.25-25%	*Fusarium moniliforme* *Fusarium oxysporum* *Trichothecium sps.*	Stalk rot and lodging, yellowing of leaves Seed and Seedling rot
3.	Shriveled seeds	0.25-40%	*Aspergillus flavus* *Aspergillus candidus* *Aspergillus fumigatus* *Aspergillus sulphureus*	Brown and black lesion on seedlings & browning basal parts of shoot
			Curvularia lunata *C. oryzae*	Light brown spot & streaks on the coleoptiles
			Penicillium sps.	Yellowing of leaves & radical rot
			Phoma sorghina	Seedling become deformed
4.	Broken and insect damaged seeds	2.5-40%	*Aspergillus sulphureus* *Aspergillus niger* *Aspergillus flavus* *Aspergillus candidus*	Browning of leaves, spots on radical and basal part of shoot
			Penicillium sps.	Yellowing of leaves & radical rot

Table 2 : Number of Seed Samples of Sorghum Infected with Fungi and Percent Range of Incidence in Incubation Test (58 Samples Studied)

Name of the fungi	Blotter test				PDA	
	Untreated seeds		Pretreated seeds			
	Samples infected	%range	Samples infected	% range	Samples infected	% range
Actinomycetes	18	1-12	11	1-10	7	1-3
Alternaria alternate	20	7-68	9	3-34	45	1-45
Alternaria tenussima	25	1-20	15	1-15	12	1-2
Aspergillus candidus	34	1-18	12	1-7	10	1-12
Aspergillus flavus	44	4-40	10	4-16	25	1-20
Aspergillus fumigatus	48	3-48	39	1-18	17	1-22
Aspergillus niger	40	5-50	23	5-20	20	1-25
Aspergillus sulphureus	15	1-10	5	1-3	2	1-2
Bipolaris sorokiniana	28	2-18	10	1-14	5	1-4
Bipolaris spicifera	10	1-5	4	1-5	3	1-4
Bispora catenula	2	1-2	-	-	-	-
Cladosporium cladosporiales	18	1-30	16	1-29	15	1-24
Cladosporium oxysporum	14	1-25	10	1-20	7	1-20
Curvularia lunata	50	1-45	40	1-41	20	1-35
Curvularia oryzae	50	1-48	48	1-40	21	1-15
Curvularia penniseti	21	1-23	21	1-13	12	1-8
Curvularia sorghina	36	1-41	31	1-41	24	1-21
Chaetomium sps.	1	1-2	-	-	1	1-2
Drechslera graminea	20	1-30	14	1-25	12	1-25
Drechslera tetramera	19	1-46	15	1-40	15	1-36
Drechslera rostrata	27	1-50	25	1-45	21	1-40

Epicoccum nigrum	2	1-2	-	-	-	-
Fusarium moniliforme	18	1-25	11	1-20	6	1-20
Fusarium oxysporum	29	1-17	25	1-12	20	1-10
Helminthosporium tetramera	34	1-25	30	1-20	20	1-15
Mammaria sps.	1	1-3	-	-	-	-
Macrophomina phaseolina	5	1-5	4	1-2	1	1-2
Nigrospora oryzae	2	1-2	-	-	-	-
Penicillium sps.	22	1-12	9	1-8	14	1-9
Phoma sorghina	18	1-33	16	1-37	12	1-26
Rhizopus oryzae	10	1-7	8	1-7	6	1-3
Torula graminis	7	1-9	7	1-6	2	1-2
Trichothecium roseum	34	1-25	20	1-21	18	1-15

Effect of Sodium Hypochlorite 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 chlorine made the seeds germinate without affecting the incidence of pathogenic seed fungi but the incidence of saprophytic fungi was greatly reduced and their growth and sporulation was also reduced due to seed treatment (Sauer & Burroghs 1986). The fungi such as *Rhizopus sps., Penicillium sps., Mammaria sps*., *Aspergillus sps.* that occurred in low incidence were completely inhibited after chlorine pretreatment in SBM.

Conclusion

In the above study total 33 fungal species belonging to 17 genera were observed on the seed samples collected from eastern districts of Rajasthan. The samples from Bharatpur, Alwar, Jaipur, Dholpur and Kota mostly showed heavy inoculums and greater incidence of fungi.

In 58 samples which was collected from 7 eastern districts of Rajasthan, the fungi from Kota, Alwar, Bharatpur showed heavy inoculums and greater incidence of fungi. This may be due to presence of high humidity, rainfall which favours sporulation of fungus. Fungi associated with seed affected germination as well as vigour and also produced symptomatic seedlings. In 58 samples studied, the fungi which commonly affected seed germination were sps. of *Alternaria, Aspergillus, Helminthosporium, Fusarium, Trichothecium.*

Most of the fungi *like Alternaria alternata, Curvularia lunata, Aspergillus flavus, Bipolaris sorokiniana, Drechslera tetramera, Fusarium oxysporum, Macrophomina phaseolina, Phoma sorghina* caused serious seed diseases and produced infected seedlings.

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