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Dose Dependent Impact of Dominant Seedborne Fungi on Seed Germination and Seedling Vigour of Cotton Seeds

R. KAVITHA, S. UMESHA* AND H.S. SHETTY
Department of Applied Botany, Seed Pathology and Biotechnology,
University of Mysore, Manasagangotri, Mysore-570 006
(email: su@appbot.uni-mysore.ac.in; pmumesh@yahoo.com)

ABSTRACT Thirty cotton genotypes belonging to Varalakshmi, Jayalakshmi, DCH-32 and RCH-32 were collected from the three different agro climatic zones of Karnataka state, India. All the collected seed samples were manually delinted and subjected to standard blotter method to screen the seedborne fungi. The dominant seedborne pathogenic fungi were identified and multiplied on potato dextrose agar media. Seeds were artificially treated with all the dominant seedborne fungi. Fungal concentration was assessed using Haemocytometer. Such artificially treated seeds were subjected to seed germination and seedling vigor. All the dominant seedborne fungi reduced seed quality parameters significantly (p=0.05), over their untreated counterparts. However, the effect of these seedborne pathogenic fungi is dependant on fungal dosage. Increased fungal concentration recorded more reduction in seed quality parameters. But the fungal dosage beyond 150-200 x 10^6 $CFUgm^{-1}$ did not vary significantly (p=0.05). Among the fungi tested, the highest reduction of seed quality parameters recorded from *Verticillium dahliae* followed by *Myrothecium roridum; Macrophomina phaseolina* and *Fusarium* species.

Keywords: Cotton genotypes, Pathogenic fungi.

Cotton (*Gossypium hirsutum* L.) is an important commercial crop which belongs to the family *Malvaceae*. Cotton is grown for its strong, fine and durable fibers. The world consumption of cotton fiber is found to be nearly 3 kg $person^{-1}$ $year^{-1}$. India's production of cotton was found to be 12,300 bales (1 bale = 227 kgs) [1]. India has the sole distinction of growing all the four cultivated species of cotton and their intra and inter specific hybrids [2]. Cotton in this country is grown in three agro-ecological zones viz. northern (Punjab, Harayana and Rajasthan), central (Gujarat, Maharashtra and Madhya Pradesh) and southern zone (Andhra Pradesh. Tamil Nadu and Karnataka), all having the different species composition and their zone specific problems. Cotton lint is considered to be the main product of cotton crop as it is important raw material for textile industry. Cotton seeds now ranks second in terms of world oil seed production, after soybean [3].

The major constraints in cotton production are the seedborne fungi such as *Fusarium moniliforme* (Sheldon); *Fusarium oxysporum* (Snyde & Hansen) Atk; *Macrophomina phaseolina* (Tassi.); *Myrothecium roridum* Tode ex and *Verticillium dahliae* Kleb. *Fusarium moniliforme* causes seed/cotyledonary rot and reduces the yield significantly. *Fusarium oxysporum* causes wilt disease and the estimated yield loss due to this fungus is 30%. *Macrophomina phaseolina* causing charcoal rot/dry root roting is economically important across a broad range of crops throughout the world, particularly in regions that experience hot, dry condition during the growing time. The estimated annual loss caused by *M. phaseolina* is 30% [4]. The leaf spot disease is caused by *M. roridum* and reduces the yield up to 15%. *Verticillium* wilt disease is caused by the fungus *Verticillium dahliae*. The estimated yield loss due to this fungus is recorded to be 75% [4]. All these fungi have wide host ranges. But research information is scanty with regard to the role of inoculum concentration on seed quality parameters. Hence, the present study attempts to discuss the role of different pathogen concentration on seed quality parameters in cotton seeds.

MATERIALS AND METHODS

Seed material

Different varieties of cotton seeds *viz.*, Varalakshmi, Jayalakshmi, DCH- 32, RCH-Bramhara

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and RCH- Bunny were collected from the farmers' fields of three different agro-climatic zones of Karnataka. Seed samples were collected from farmers' fields of Mysore, Chamaraj-Nagar (southern dry zone); Bangalore, Tumkur (eastern dry zone) and Davanagere (central dry zone) [5]. Seeds were collected during the first picking, delinted manually and used for all the experiments. The varietal details of seed samples collected and the place of collection, are shown in Table 1.

Screening of cotton seed samples for seedborne fungi

The collected seed samples of cotton were subjected to the standard blotter method [6] for the analysis of seed mycoflora. Each seed sample was mixed thoroughly and the representative working samples were taken for further studies. Seeds were plated equidistantly (10 seeds plate^{-1}) on three layered wet blotter discs inserted into a Perspex plates (9 cm dia.). Plates were incubated for 7 days at 25 ± 2°C with alternative cycles of 12/12h NUV light and darkness. After incubation period, plates were screened for seedborne fungi using different magnifications of stereo binocular microscope. Fungal colonies under stereo binocular and spore/conidial preparations under compound microscope were photographed. Fungal colonies were identified by using standard reference manuals like Booth's manual [7, 8] and standard procedures of Mathur and Kongsdal [9]. Experiments were conducted in four replicates of 100 seeds each and repeated three times. Incidences of different seedborne fungi were recorded and mean per cent incidences were calculated.

Production of fungal inocula

The selected fungi were cultured on potato dextrose agar (PDA). Single spore/conidium were point inoculated on to sterilized PDA plates and plates were incubated at 25 ± 2°C with alternative cycles of 12/12h NUV light and darkness. Fully grown culture plates were used for seed treatment.

Seed treatment with fungal pathogens

All the seed samples were surface disinfected with 3% sodium hypochlorite solution for 3-4 min, followed by washing with sterile distilled water for 6 times. The seeds were pre soaked in 2% (w/v) gum arabic for 5 min. Then seeds were rolled on to the pathogen in such a way that the spores or the conidia/mycelia of the pathogen treated, gets uniformly coated on to the surface of each and every seed. The spore load/gm of seed is determined using a Haemocytometer by subjecting 1 gm of treated seeds for enumeration and spore load/gm of seeds was estimated and the spore load/seed was also calculated.

Treated seeds were grouped into four groups as follows, group 1 those seeds which were showing 10 - 49 x 10^6 cfu gm^{-1} of seeds; group 2 were 50 - 99 x 10^6 cfu gm^{-1} of seeds; group 3 were 100 - 149 x 10^6 cfu gm^{-1} of seeds and group 4 is 150 -200 x 10^6 cfu gm^{-1} of seeds.

Impact of seedborne fungi on cotton seed quality

The cotton seeds treated with all the fungal pathogens along with appropriate controls were subjected to seed quality parameters like seed germination and seedling vigour according to standard procedure of roll towel method. Where the seeds were placed equidistantly on moistened germination sheets and rolled. The rolls were incubated in seed germinator at 25±2°C for 7 days after tying their ends with rubber bands. After incubation period, the treated and untreated seeds were evaluated for seed germination and seedling vigor using standard procedure of ISTA and vigor index was calculated using the procedure of Abdul Baki and Anderson [10]. Experiments were conducted thrice in four replicates of 100 seeds each.

Statistical analysis

Data on percentage were transformed to arcsine and analysis of variance (ANOVA) was carried out with transformed values. The means were compared for significance using Duncan's new multiple range test. (DMRT; p= 0.05).

RESULTS AND DISCUSSION

Screening of cotton seed samples for seed-borne fungi

All the 30 seed samples belonging to three agro climatic zones of Karnataka state are Varalakshmi, Jayalakshmi, DCH- 32, RCH-Bramhara and RCH-Bunny cotton varieties. None of the seed samples were free from seedborne fungi. Arrays of fungi were recorded from all the seed samples. The dominant among them were *Fusarium moniliforme; Fusarium oxysporum; Macrophomina phaseolina; Myrothecium roridum and Verticillium dahliae*. The moulds like *Aspergillus* and *Rhizopus* were recorded in high percentages. The fungi like *Curvularia lunata* and *Colletotrichum* species were also recorded in lowest quantities. The dominant fungi were identified as follows.

Table 1. List of different seedborne fungi of cotton seed samples collected from three different agro climatic zones of Karnataka.

Sl.No.	Cotton Variety	Place of collection	Incidence of Seedborne fungi (%)								
			F.m.	*F.o.*	*M.p.*	*M.r.*	*V.d.*	*C.l.*	*C. spp.*	*A. spp.*	*R.sp.*
1	Varalakshmi	Honnenahalli, Mysore District.	24	20	20	20	24	2	2	25	20
2	Varalakshmi	Gowdekere, Mysore District.	22	22	20	22	23	3	2	21	25
3	Varalakshmi	Gudlupet, Ch.Nagar District.	19	24	18	14	20	3	3	20	26
4	Varalakshmi	Sagare, Mysore District.	20	20	19	18	5	4	5	22	25
5	Varalakshmi	Katte, Tumkur District.	8	19	6	16	16	2	4	20	20
6	Varalakshmi	Hebbur, Tumkur District.	23	20	18	19	15	3	3	18	22
7	Varalakshmi	Jagalore, Davanagere District.	22	21	20	20	24	4	6	21	26
8	Varalakshmi	Shivanpura, Bangalore District.	8	23	21	21	20	7	2	20	23
9	Varalakshmi	Harohally, Bangalore District.	19	20	20	21	20	6	5	21	25
	Mean		18	21	18	19	18	3	3	20	21
10	Jayalakshmi	Beechanahalli, Mysore District.	17	18	20	22	23	6	5	21	25
11	Jayalakshmi	Abbur, Mysore District.	12	16	19	20	20	12	7	20	20
12	Jayalakshmi	Shivanpura, Bangalore District.	20	12	21	18	5	9	9	22	25
13	Jayalakshmi	Honnali, Davanagere District.	22	19	20	20	24	10	13	25	19
14	Jayalakshmi	Kora, Tumkur District.	23	23	18	21	20	15	16	16	20
	Mean		18	17	19	20	21	10	10	20	21
15	DCH - 32	Negathur, Mysore District.	21	21	23	22	21	6	3	21	20
16	DCH - 32	H.D.Kote, Mysore District.	23	20	20	21	20	5	5	25	20
17	DCH - 32	H.D.Kote, Mysore District.	18	22	22	20	21	9	8	26	18
18	DCH - 32	Jagalore, Davanagere District.	8	23	20	15	24	10	9	18	17
19	DCH - 32	Harohally, Bangalore District.	21	18	19	18	18	12	8	19	15
20	DCH - 32	Hanagud, Mysore District.	23	16	20	20	5	6	11	15	21
21	DCH - 32	Honnali, Davanagere District.	20	12	6	21	20	5	12	18	22
22	DCH - 32	Kora, Tumkur District.	18	21	21	20	21	9	10	16	25
23	DCH - 32	Sindenahalli, Mysore District.	17	23	20	18	23	8	2	14	18
24	DCH - 32	H.D.Kote, Mysore District.	8	22	21	20	24	10	11	10	19
25	DCH - 32	H.D.Kote, Mysore District.	16	20	20	16	19	11	9	13	20
	Mean		17	19	19	19	19	8	8	17	19
26	RCH - 32	H.D.Kote, Mysore District.	24	24	23	14	24	7	3	21	22
27	RCH - 32	H.D.Kote, Mysore District.	23	20	16	22	20	10	9	20	27
28	RCH bramara	H.D.Kote, Mysore District.	20	23	10	18	23	11	8	17	20
29	RCH bramara	H.D.Kote, Mysore District.	21	20	18	14	5	19	7	20	19
30	RCH bunny	H.D.Kote, Mysore District.	20	20	6	18	20	12	5	22	18
	Mean		21	21	14	17	18	11	6	20	21

Values are the means of four replicates of 100 seeds each and repeated three times.

F. m. = *Fusarium moniliforme; F. o.* = *Fusarium oxysporum; M. p.* = *Macrophomina phaseolina; V. d.* = *Verticillium dahliae;*
C. l. = *Curvularia lunata; C. spp.* = *Colletotrichum* species; *A. spp.* = *Aspergillus* species *and R. sp.* = *Rhizopus* species.

Table 2. Dose dependent impact of different seedborne fungi on seedling vigor of cotton seeds.

Treatment	MRL (cms)	MSL (cms)	VI
Control	9.89±0.61	9.86±0.79	1896[a]
Fusarium moniliforme			
Group1	7.98±0.49	7.69±0.59	1175[b]
Group2	7.10±0.38	6.90±0.69	938[c]
Group3	6.81±0.25	5.98±0.58	703[d]
Group4	6.79±0.5	5.98±0.23	702[d]
Fusarium oxysporum			
Group1	8.07±0.92	7.22±0.87	1284[ab]
Group2	7.71±0.81	6.90±0.72	1154[C]
Group3	6.91±0.39	5.98±0.89	696[d]
Group4	6.81±0.22	6.10±0.88	698[d]
Macrophomina phaseolina			
Group1	8.21±0.38	7.23±0.39	1281[ab]
Group2	7.91±0.81	6.98±0.38	1161[C]
Group3	6.61±0.92	5.62±0.81	684[d]
Group4	6.51±0.83	5.72±0.43	684[d]
Myrothecium roridum			
Group1	7.61±0.39	7.01±0.32	1301[ab]
Group2	6.90±0.48	6.35±0.58	1033[c]
Group3	5.91±0.51	5.31±0.84	650[d]
Group4	5.81±0.31	5.41±0.93	650[d]
Verticillium dahliae			
Group1	7.21±0.58	6.09±0.84	1130[a]
Group2	6.38±0.49	5.91±0.93	958[c]
Group3	5.75±0.38	5.39±0.84	590[d]
Group4	5.76±0.48	5.40±0.83	591[d]

Values are the mean of three independent experiments ± S.E. of four replicates of 100 seeds each. MRL - Mean root length; MSL - Mean Shoot length; VI - Vigor Index. The values in the column followed by same letter(s) are not significantly different according to DMRT; $p = 0.05$.

Identification characteristics of fungi

Fusarium moniliforme: Micro conidiophores are single, lateral, subulate phialides formed from aerial hyphae. Microconidia are more or less agglutinated in chains and remain joined, or are cut off in false heads, later scattered in clear yellowish to rosy-white aerial mycelium as a dull, colorless powder, one-or two-celled, fusiform-ovate. Macro conidiophores consist of a basal cell bearing two to three apical phialides which produce macroconidia. Macroconidia are delicate, awl-shaped, slightly sickle-shaped or almost straight, narrowed at both ends, occasionally somewhat bent into a hook at the apex, distinctly or slightly foot-celled at the base, scattered, formed in sporodochia or pionnotes, in the mass clear, buff, or salmon-orange, when dry carrot-red or cinnamon-brown or rather pale, three to five-, rarely six to seven-septate. Chlamydospores absent. (Figure 1A&B).

Fusarium oxysporum: It produces a dense white mycelium and a red/purple pigment is visible in the medium when the culture is observed from below. The microconidia are borne on simple phialides arising from a short conidiophore and are single celled or one-septate. Macroconidia are fusiform-falcate in shape, three-to five-septate.

Chlamydospores are formed in older cultures and senescent host tissues. They are roughly spherical in shape, with a thick wall and are able to survive in the soil for long periods in the absence of a host (Figure 1C & D).

Macrophomina phaseolina: Sclerotia are black, smooth, hard and occur within roots, stems, leaves and fruits. Conidiomata are pycnidial, dark-brown, and either solitary or gregarious on leaves and stems; they are immersed, becoming erumpent, opening by an apical ostiole; the conidiomatal wall is multicellular with heavily pigmented, thick-walled cells on the outermost side. Conidiophores are hyaline, short and obpyriform to cylindrical. Conidia are hyaline, ellipsoid to obovoid (Figure 1E & F).

Myrothecium roridum: Sporodochia are black to blakish green, raised from seed surface, surrounded by a rim of thin white mycelium. Young sporodochia may be seen on seed surface as white masses of hyphae. Conidia one-celled, cylindrical, with slightly round dark ends, hyaline to light green in color (Figure 1G & H).

Verticillium dahliae: Colonies moderately fast growing, white at first, with little to moderate aerial mycelium and a regular margin, turning black from the centre after a week or so as a result of the production of microsclerotia. Conidia ellipsoidal, hyaline, mostly one-celled, produced at the tips of narrow, pointed, conidiogenous cells subtended in whorls (2-3 per node) on more-or-less erect, hyaline, verticillate conidiophores. Conidia produced in succession to form moist spore balls at the tips of conidiogenous cells, giving characteristic appearance to conidiophore in culture. Spore balls eventually coalescing and sliming down in the older parts of the culture. Microsclerotia of irregular shape and size dark brown to black, torulose, almost globose. In culture, white sectors may appear as a result of partial loss of the ability to produce microsclerotia, or pigmentation may be lost entirely (Figure 1I & J).

The incidence of *Fusarium moniliforme* ranges from 8 to 24% in all the collected seed samples. The mean incidence of *Fusarium moniliforme* in Varalakshmi variety is 18%, Jayalakshmi 18%, DCH-32- 17% and RCH variety 21%. (Table 1). The incidence *of Fusarium oxysporum* ranges from 12 to 24%, the mean incidence was 21%, 17%, 19% and 21% from Varalakshmi, Jayalakshmi, DCH- 32 and RCH cotton varieties respectively. The incidence of *Macrophomina phaseolina* ranges from 6 to 23% in all the seed samples, recording the mean in Varalakshmi, Jayalakshmi, DCH-32 and RCH varieties as 18%, 19%, 19% and 14% respectively. (Table 1). The incidence of *Myrothecium roridum* ranges from 14-22%, the mean being 19%, 20%, 19% and 17% in Varalakshmi, Jayalakshmi, DCH-32 and RCH varieties respectively. The incidence of *Verticillium dahliae* ranges from 5-24%, the mean incidence recorded was 16%, 21%, 19% and 18% in Varalakshmi, Jayalakshmi, DCH-32 and RCH cotton varieties, (Table 1). Because of this dominating mycoflora. the same has been selected to study the effect of all these fungi on seed germination and seedling vigor of cotton seeds.

Dose dependent impact of seedborne fungi on cotton seed quality.

All the fungi reduced seed germination significantly (p=0.05) over untreated control. But the decrease in seed germination was dependent on the concentration of the pathogen. However, even the concentration of the pathogen was increased beyond 100-149 x 10^6 cfu gm^{-1} (group-3), the amount of reduction was not significant. (Table 2). The same trend was followed with all the selected pathogens. The seed germination was reduced from 96% in untreated control to 55%, when seeds were treated with both 100 -149 x 10^6 cfu gm^{-1} and 150 - 200 x 10^6 cfu gm^{-1} (group 3 and group 4) concentrations of *Fusarium moniliforme.* The same type of impact was observed with other pathogenic fungi also. The seed germination was reduced from 96% in untreated control to 56% when the seeds were treated with *Macrophomina phaseolina,* with the pathogen concentration of group 3 and group 4. The germination was reduced from 96% to 58% and 59%, when the seeds were treated with *Myrothecium roridum.* But the maximum reduction was noticed in *Verticillium dahliae* pathogen. The seed germination was reduced from 96% in untreated control to 54% and 53% when the seeds were treated with *Verticillium dahliae* with the pathogen concentration of group 3 and group 4 respectively (Figure 2). However, among the pathogenic fungi tested, all the fungi significantly (p=0.05) reduced the seed germination, with significantly more negative effect with *Verticillium dahliae* (Figure 2).

The effect of germination was clearly depicted in seedling vigor. There was significant (p=0.05) reduction was recorded when the seeds were treated with all the pathogenic fungi. However the reduction was dependent on concentration of the pathogen (Table 2). There was significant (p=0.05) reduction in root and shoot lengths. The vigor index was reduced from 1896 in untreated control to 703 and 702 when the seeds were treated with group 3 and group 4 concentrations of *Fusarium moniliforme.* The seedling vigor was reduced from 1896 in untreated control to 696 and 698 when the seeds were treated

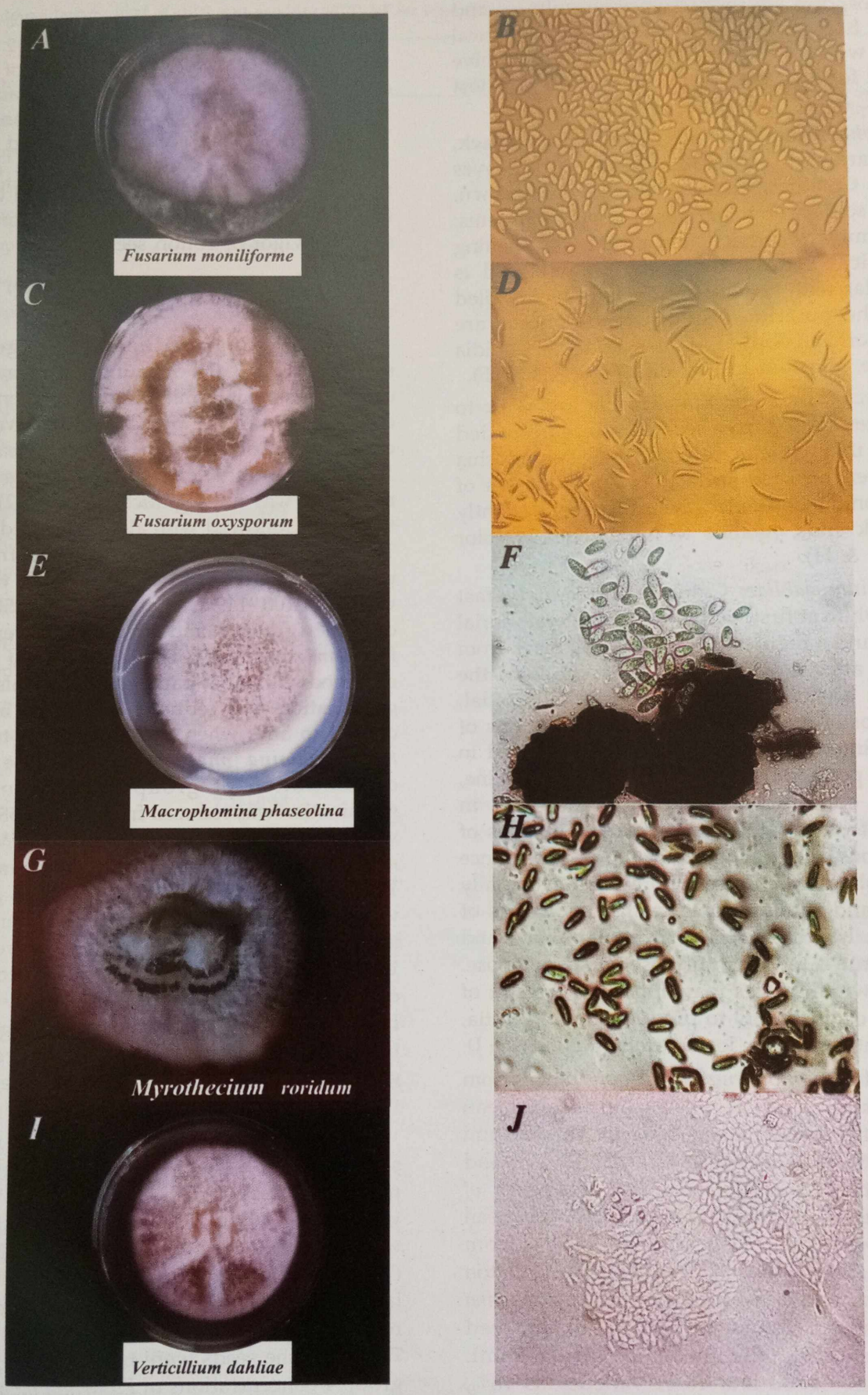

Fig. 1 Seedborne fungi of cotton seeds. Fungi were identified based on colony characters and fruiting structures. Pure cultures were multiplied on Potato Dextrose Agar media and photographed. Temporary mounts were prepared to study the fruiting structures of the fungi (All the fungi were under x40 of the compound microscope)

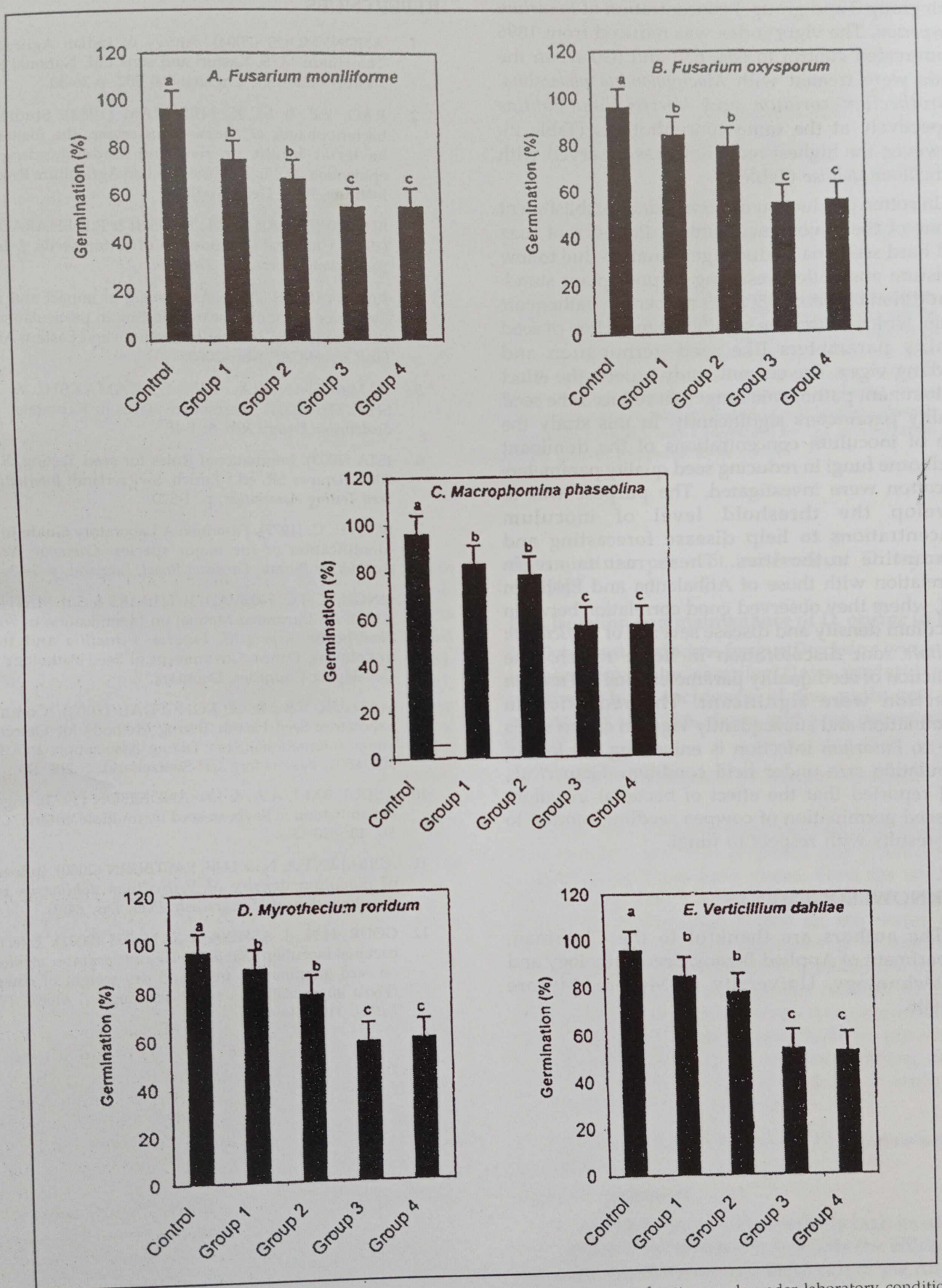

Fig. 2 Dose dependent impact of different seedborne fungi on seed germination of cotton seeds under laboratory condition. Bar(s) represented by the same letter(s) do not significantly differ at 5% level, when subjected to DMRT; p=0.05. The lines on each bar represent standard error.

with group 3 and group 4 concentration of *Fusarium oxysporum*. The vigor index was reduced from 1896 in untreated control to 684, 650 and 590 when the seeds were treated with *Macrophomina phaseolina*, *Myrothecium roridum* and *Verticillium dahliae* respectively at the same concentrations (Table 2). However, the highest reduction was observed with *Verticillium dahliae* (Table 2).

In cotton production uniform stand establishment is one of the major uncertainties. Presence of fuzz and hard seed-coat reduces germination due to low moisture absorption resulting in poor plant stand. In addition to this effect, there are certain pathogenic fungi, which causes the significant reduction of seed quality parameters like seed germination and seedling vigor. The current study reports the effect of dominant pathogenic fungi that reduced the seed quality parameters significantly. In this study the role of inoculum concentrations of the dominant seedborne fungi in reducing seed quality parameters of cotton were investigated. The purpose was to develop the threshold level of inoculum concentrations to help disease forecasting and quarantine authorities. These results are in correlation with those of Atibalentja and Eastburn [11], where they observed good correlation between inoculum density and disease severity of *Verticillium dahliate* root discoloration in horse radish. The reduction of seed quality parameters due to *Fusariun* infection were significant. The reduction in germination and subsequently vigor in cotton seeds due to *Fusarium* infection is enhancing the loss of population size under field condition. Gour *et al.*, [12] reported that the effect of bacterial inoculum on seed germination of cowpea seedling, similar to our results with respect to fungi.

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REFERENCES

1. ANONYMOUS (2004). Survey of Indian Agriculture, The Hindu M/S, Kasturi and sons Ltd, National Press, Kasturi buildings, Chennai-600 002, p 36-80.
2. RAO, Y.P. & M. K. MESHRAM (1983). Studies on bacteriophages *of Xanthomonas oryzae*, the incitant of bacterial blight of rice and understanding the epidemiology. Ph. D. Thesis, Indian Agriculture Research Institute, New Delhi, India.
3. BHATAWDEKAR, K. M., P. JOSHI & R.P. BHARADWAJ (1999). Chemical Composition of cotton seeds. *J. Indian Soc. Cotton Improv.* 43: 226-325.
4. ANONYMOUS (2003). Assessment of impact and main dynamics of cotton disease affecting in particular-small-scale production systems in Southern and Eastern Africa. *Final report/CAF,* **May-2003**.
5. RAMACHANDRA, T.V., G, KAMAKSHI & B.V. SHRUTHI (2004). Bioresource status in Karnataka. *Ren. Sustainable Energy Rev.* **8**: 1-47.
6. ISTA (2003). International Rules for seed Testing, Rules 2003. (Draper SR, ed.) Zurich, Switzerland: *International Seed Testing Association*. p. 1-520.
7. BOOTH, C. (1977). *Fusarium*, A Laboratory Guide to the Identification of the major species. *Common Wealth Agriculture Bureau, Furnham Royal*, England, p 5-43.
8. SINGH, K., J.C. FRISVAD. V. THRARS & S.B. MATHUR (1991). An Illustrated Manual on Identification of Some Seed-borne *Aspergilli, Fusaria, Penicillia* and their mycotoxins. Danish Government of Seed Pathology for Developing Countries, Denmark.
9. MATHUR, S.B., & O. KONGSDAL (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi. International Seed Testing Association, P.O. Box 308, 8303, Bassersdorf, CH-Switzerland. p 202-271.
10. ABDUL BAKI, A.A. & J.D. ANDERSON (1973). Vigour determination in Soybean seed by multiple criteria. *Crop Sci.* **13**: 630-33.
11. ABIBALENTJA, N. & D.M. EASTBURN (2000). Influence of inoculum density of *Verticillium dahliae* in root discolouration of Horseradish. *Plant Dis.* **84**(3).
12. GOUR, H.N., J. ASHIYA & R. NATH (2001). Effect of bacterial inoculum (*Xanthomonas axonopodis pv. vignicola*) on seed germination, fresh and dry weight of cowpea (*Vigna unguiculata* (L.) *walp.*) seedlings. *J. Mycol. Plant Pathol.* **31**(1), 46-49.