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Fungitoxic Effect of Biocontrol Agents and Plant Extracts on Seed Borne Fungi of Sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract: The fungitoxic effect of biocontrol agents and plant extracts on seed-borne fungi of sorghum was evaluated. The biocontrol agents viz., *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma viride* significantly inhibited the mycelial growth of sorghum seed-borne fungi viz., *Acremonium strictum, Alternaria tenuis, Aspergillus niger, Curvularia lunata, Fusarium moniliforme, Heliminthosporium halodes* and *Heliminthosporium tetramera*. Among them *B.subtilis* exhibited maximum reduction in mycelial growth of all fungi. Among leaf extracts viz., *Albizia amara, Catharanthus roseus* and *Prosopis juliflora* screened, *C. roseus* at 20% concentration possessed maximum inhibition of mycelial growth of *A.niger, C.lunata* and *F.moniliforme*, where as 20% leaf extracts of *P. juliflora* inhibited the growth of remaining fungi. The culture filtrates of *B. subtilis* exerted significant reduction in mycelial weight of all fungi, except *C.lunata*. The maximum reduction of mycelial weight of all fungi was observed in 20% leaf extract of *C. roseus* among the plant extracts except for *F.moniliforme*.

Key words: Sorghum, biocontrol agents, plant extracts, seed borne fungi

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important staple food crop for people living in the semi-aric tropics of Africa and Asia. The crop is attacked by more than a hundred diseases (Singh *et al.*, 1993). Grain mould is one of the most widespread and devastating disease of sorghum and they are mostly seed transmiting in nature (Williams and McDonald, 1983).

Protection of crops from grain mould using fungicides, has been the regular practice for many years and it is a widely adopted strategy. However, there have been many draw backs in this attempt also. The spiraling up cost of fungicides, pollution to soil, water and air due to continuous use of fungicides and development of resistant strains of pathogen to these chemicals are therefore now forcing the scientists to look for biological methods which are eco-friendly safe and more specific to pathogens. Green plants act as the reservoirs of effective chemotheurapentents and are constituents of in-exhaustible source of harmless pesticides (Swaminathan, 1975). So the studies were undertaken to evaluate fungitoxic effect of bio-control agents and plant extracts against sorghum seed-borne mycoflora.

Materials and Methods

The seed borne mycoflora viz., *Acremonium strictum, Alternaria tenuis, Aspergillus niger, Curvularia lunata, Fusarium moniliforme, Heliminthosporium halodes* and *H. tetramera* were isolated from sorghum seed samples which were collected from various parts of Tamil Nadu during 1997-98 and the isolated fungi maintained as pure culture in slants.

The bio control agents viz., *Bacillus subtilis* Ehrenberg, *Pseudomonas fluorescens* Migula and *Trichodeoma viride* Pers. ex.fr. were isolated from soil using media, nutrient agar medium (peptone 5 g; Glucose 10g; Sodium chloride 5g; Beef extract 3g; Agar 15 g; Distilled H_2O1000 ml) Kings 'B' meidum (King *et al.*, 1954) and Trichoderma special medium (Elad and Chet, 1983) respectively.

***In vitro* evaluation of bio control agents against seed-borne fungi:** The effect of bio control agents on seed borne fungi was assessed *in vitro* by the method of Dennis and Webster (1971). Potato dextrose medium (Peeled potato 250 g; Dextrose 20 g; Agar 20 g; Distilled water 1000 ml) (Ainsworth, 1961)(PDA) was prepared, sterilized and poured into sterilized petri dishes and allowed to solidify. Uniform disc of 9 mm diameter were cut from the test fungus grown in PDA with sterile cork borer and transferred aseptically to one side of the petridish. Another disc of 9 mm diameter of *T.viride* grown on PDA media was cut with sterile cork borer and placed on solid medium aseptically opposite to the test fungus in the same petri dish. Similarly bacterial antagonists were streaked on the opposite side of the test fungus. The petridishes without antagonists were kept as control. The plates were incubated at 28 ± 1°C. The inhibition zone was measured and compared with control.

***In vitro* evaluation of fungi-toxic effect of plant extracts against seed-borne fungi. (Shekhawat and Prasad, 1971):** The plant species viz. *Albizia amara* Boiv, *Catharanthus rosens* (L.) G. Don and *Prosopis juliflora* (SW) DC were used for evaluating fungi-toxic effect on the test fungi. Fresh leaf materials were first separately washed with distilled water and finally with sterile water. They were then ground in a pestle mortar by adding sterile water @ 1:1 w/v and filtered through cheese cloth. This was centrifuged at 3000 rpm for 10 min. Then supernatant collected and this formed standard plant extract solution (100%). The boiled plant extracts (60°C) were added to sterilized, melted PDA media at different levels. The amended medium was poured into sterilized petridishes and the dishes were inoculated in laminar air flow chamber with the test fungus by placing uniform of 9 mm diameter from 4 days old-culture. The diameter of the fungal colony was measured after 96 hr. Three replications were maintained at the laboratory temperature (28 ± 1°C). The percent inhibition of growth of the test fungi was calculated by the formula of Vincent (1927).

$$I = \frac{100 \times (C-T)}{C}$$

Where,
I = per cent inhibition of fungal growth.
C = growth in control.
T = growth in treatment.

Evaluation of culture filtrates and leaf extracts on mycelial

weight of the seed-borne fungi (Valuva Paridasan, 1994): Two days old broth culture of *B.subtilis* and *P.fluorescens* and 12 days old broth culture of *T.viride* were used for extraction of culture filterates. The bacterial biocontrol agents filtered through two layers of cheese cloth and finally with a bacteriological filter. Whereas *T.viride,* were filtered through two layers of cheese cloth. Then the filtered solution of both agents were centrifuged at 10000 rpm for 5 min to settle down the spores. Then the supernatant was collected and used as cell free culture filterate (as 100 %).

Then culture filterates and leaf extracts were added at respective concentration to broth of PDA medium and sterilized. Then the test fungus was inoculated in the broth and kept for 12 days under room temperature. After 12 days, the mycelial portion was removed and dried at 50°C. The weight of dried mycelial mat was recorded and compared with control.

Results

The results (Table 1) indicated that the biocontrol agents were found to inhibit the mycelial growth of fungi. Among them, *B.subtilis* registered a maximum effect on growth of all the fungi and was followed by *P.fluorescens*. The results furnished in Table 2 revealed that, among the three plant extracts screened, *C.roseus* at 20 % conc. exhibited maximum inhibition of mycelial growth of *A.niger*, *C.lunata* and *F.moniliforme*, where as 20 % of *P. juliflora* inhibited the mycelial growth of *A.tenuis*, *H. halodes*, *H. tetramera* and *A.strictum* at maximum level. The efficacy of the leaf extracts was proportional to the concentration.

20 % culture filtrates of *T. viride* exhibited maximum reduction of mycelial weight of *A. strictum*, *A. tenuis*, *F. mouiliforme*, *H. halodes* and *H. tetramera*, whereas weight of mycelium of *A. niger* and *A. tenuis* was maximaly reduced

Table 1: Effect of biocontrol agents on growth of the seed-borne fungi of sorghum

Biocontrol agents	*Mean mycelial growth (mm)						
	Acremonium strictum	*Alternaria tenuis*	*Aspergillus niger*	*Curvularia lunata*	*Fusarium moniliforme*	*Helminthosporium halodes*	*H. tetramera*
Bacillus subtilis	14.15	9.61	14.62	11.40	12.30	8.67	11.33
Pseudomonas fluorescens	26.17	21.60	34.10	22.10	17.43	15.61	19.67
Trichoderma viride	18.01	30.30	65.67	19.17	32.12	28.96	57.33
Control	80.33	81.00	80.30	81.00	86.67	86.00	82.67

*Mean of four replications CD (0.05%) Pathogen 1.47, Biocontrol agents 1.11, Pathogen x Biocontrol agents 2.934.

Table 2: Effect of leaf extracts on the mycelial growth of the seed-borne fungi

Leaf extracts	Conc (%)	*Mean mycelial growth (mm)						
		Acremonium strictum	*Alternaria tenuis*	*Aspergillus niger*	*Curvularia lunata*	*Fusarium moniliforme*	*Heluinthosporium halodes*	*H. tetramera*
Albizia amara	10	13.00	4.00	24.00	21.33	17.67	18.72	22.69
	20	8.33	1.67	18.67	19.11	10.01	12.91	17.33
Catharanthus roseus	10	12.67	1.07	21.67	11.67	2.31	18.33	29.00
	20	7.67	1.76	9.33	1.33	0.33	10.07	8.33
Prosopis juliflora	10	21.13	0.67	28.00	21.67	24.51	6.39	10.01
	20	2.67	0.00	25.67	17.33	7.69	2.38	1.67
Control	-	80.33	81.00	80.30	81.09	86.67	86.00	82.67

*Mean of four replications CD (0.05%) Pathogen 0.650 Concentration 0.347 Leaf extract 0.491
Pathogen x concentration 1.125 Pathogen x leaf extract 1.29 Leaf extract x concentration 0.690.

Table 3: Effect of culture filtrates of biocontrol agents and leaf extracts on the mycelial weight of seed-borne fungi

Treatments	Conc (%)	*Mean mycelial weight (g)						
		Acremonium strictum	*Alternaria tenuis*	*Aspergillus niger*	*Curvularia lunata*	*Fusarium moniliforme*	*Helminthosporium halodes*	*H. tetramera*
Bacillus subtilis	10	1.71	1.65	1.68	1.57	1.54	1.83	1.59
	20	1.30	1.04	1.51	1.10	1.00	1.34	1.26
Pseudomonas fluorescens	10	1.47	1.98	1.65	1.73	1.74	1.68	1.66
	20	1.04	1.29	1.38	1.14	1.22	1.23	1.02
Trichoderma viride	10	1.39	1.42	1.56	1.61	1.23	1.58	1.53
	20	0.90	0.82	1.09	1.23	0.81	1.02	0.91
Albizia amara	10	2.07	1.98	2.03	1.72	1.95	2.12	2.22
	20	1.87	0.96	1.85	1.57	1.40	1.36	1.85
Catharanthus roseus	10	1.45	1.21	1.76	1.58	1.54	1.88	1.77
	20	0.91	0.82	1.05	1.15	1.17	1.20	1.33
Prosopis juliflora	10	1.71	1.94	1.83	1.67	1.46	2.02	1.89
	20	1.29	1.28	1.19	1.43	1.12	1.23	1.41
Control	-	2.73	3.06	2.26	2.65	2.94	3.03	3.04

*Mean of four replications CD (0.05%) Treatments 0.039, Fungi 0.029, Treatments x fungi 0.104.

by 20% leaf extracts of *C. roseus* and *C. lunata* by 20% culture filtrate of *B. subtilis* (Table 3).

Discussion

Rosales *et al.* (1993) observed that the strains of *B.subtilis* inhibited the mycelial growth of fungi including *F.moniliforme* in paddy. Similarly Lazzaretti *et al.* (1994) reported that *B.subtilis* inhibited the growth of various fungi including *A.tenuis* and *F.solani in vitro*. This may due to agent contains antifungal antibiotics bacillomycins, which was isolated and purified by Esterhuizen (1974). Lokesh and Hiremath (1988) reported that the seed mycoflora viz., *A.tenuis, C.lunata* and *Cladosporium* sp was inhibited by *T.viride*.

As mycoparasites, *Trichoderma* spp. grew over pathogen and caused hyphal coling, hyphal abnormalities, reduction in sclerotial production and lysis of hyphae (Malathi, 1996).

Similar results were also observed by other workers. Meena (1989) reported that the leaf extracts of ten plant species including *C.roseus* inhibited the growth of seed-borne mycoflora of sorghum including *A. tenuiis, A.flavus, C.lunata, F.moniliforme* and *Rhizopus stolonifer*. Leaf extracts of *Ipomea cornea, Prosopis juliflora* and *Azadirachta indica* showed antifungal activity against *Sarocladium oryzae* (Eswaramurhty, 1996). In present study the leaf extract of *C.roseus* caused maximum reduction in mycelial weight of all fungi. The fungi toxic activities of the three plant species were discussed individually with possible reasons quoted by several authors.

Shukla and Misra (1981) identified two new glucosides viz., Kaemferol 4-methylether 3-0 β-D galacto pyranoside and retusin 7-0 naohesperidosite from the leaf extracts of *P.juliflora*. In case of *C.roseus*, the presence of alkaloids vinblastine and catharanthine, it may be responsible for inhibition of mycelical growth of fungi (Narain and Satapathy, 1977). Therefore, fungitoxic effects of biocontrol agents viz., *B.subtilis*, and *T.viride* and leaf extracts of *C.rosens* and *P. juliflora* may be responsible for controlling sorghum seed-borne fungi and worthy for further investigation.

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