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THE ROLE AND PATHOGENICITY OF VARIOUS SORGHUM ROOT AND STALK ROT PATHOGENS UNDER GREENHOUSE CONDITIONS IN INDIA

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ABSTRACT

Key words: Artificial inoculation, *Fusarium moniliforme, Macrophomina phaseolina, Sorghum bicolor*, sorghum stalk rot

The pathogenic role of fungi in stalk rot development was assessed from the inoculated roots and stalks of sorghum. Isolation from inoculated plants consistently yielded *Fusaria*—mainly from the roots 20 d after inoculation—while *Macrophomina phaseolina* was isolated more frequently from the stalks and infected the roots only after flowering. Lodging of all plants by physiological maturity followed inoculation with *M. phaseolina,* compared to 89 % lodging following inoculation with *F. moniliforme* and *F. oxysporum*. Mycelial spread, pith disintegration and vascular bundle separation in the stalks and the degree of root colonization were more severe in *M. phaseolina* and *F. moniliforme*-infected plants than in plants infected only with *F. oxysporum* and *F. subglutinans*. *F. semitectum* produced only mild symptoms.

The results suggest that the *Fusarium* spp. are early colonizers in the stalk rot disease complex, while *M. phaseolina* colonizes the plants at a latter stage of growth. *M. phaseolina* and *F. moniliforme* were more pathogenic to sorghum than *F. oxysporum* and *F. subglutinans*, while *F. semitectum* was found to be weakly pathogenic.

UITTREKSEL

DIE ROL EN PATOGENISITEIT VAN VERSKILLENDE WORTEL- EN STAMVROT-PATOGENE BY SORGHUM IN KWEEKHUISTOESTANDE IN INDIË

Die patogeniese rol wat swamme speel in die ontwikkeling van stamvrot is vasgestel uit die geïnokuleerde sorghumwortels en -stamme. Fusaria *is baie algemeen aangetref tydens isolering vanuit geïnokuleerde plante—hoofsaaklik vanuit die wortels 20 d na inokulering—terwyl* Macrophomina plaseolina *meer dikwels uit die stamme geïsoleer is en dit die wortels eers na blom besmet het. Nadat die plante met* M. phaseolina *ingeënt is het al die plante omgeval sodra hulle fisiologiese volwassenheid bereik het, invergelyking met 89 % omval na inenting met* F. moniliforme *en* F. oxysporum. *Verspreiding van die miselium, disintegrering van die murg en vaatbundels wat losraak van mekaar by stamme en die mate van wortelkolonisering was ernstiger by plante wat met* M. phaseolina *en* F. moniliforme *besmet is as by plante wat net met* F. oxysporum *en* F. subglutinans *besmet was. Besmetting met* F. semitectum *het net matige simptome veroorsaak.*

Die resultate dui daarop dat die Fusarium *spp. vroeë koloniseerders by die stamvrotsiektekompleks is, terwyl* M. phaseolina *die plante in 'n later groeistadium koloniseer.* M. phaseolina *en* F. moniliforme *het 'n ernstiger patogeniese toestand by sorghum veroorsaak as* F. oxysporum *en* F. subglutinans, *terwyl daar bevind is dat* F. semitectum *slegs effens patogenies is.*

INTRODUCTION

Stalk rot is an important disease of sorghum [*Sorghum bicolor* (L.) Moench] and is commonly referred to in India as charcoal rot due to the presence of black microsclerotia of *Macrophomina phaseolina* (Tassi) Goid. *Fusarium moniliforme* Sheldon, *F. subglutinans* Woll. & Reink., *F. graminenearum* Schw., *F. oxysporum* Schlecht., *Colletotrichum graminicola* (Cesati) Wilson, *Periconia cercinata* (Mang.) Sacc., have also been reported to cause stalk rot in sorghum (Tullis, 1951; Hsi, 1961; Zummo, 1980; Trimboli & Burgess, 1982; Khune *et al.* 1983; Tangonan & Quimoi, 1985).

Uppal *et al.* (1936) reported severe losses in post-rainy season sorghum due to charcoal rot, while Anahosur & Patil (1983) recorded a 15 % reduction in grain mass due to stalk rot caused by *M. phaseolina*.

The succession of fungi in stalk rot development has been studied by various workers (Frezzi & Teyssandier, 1980; Trimboli & Burgess, 1982; Reed *et al.* 1983; Tangonan & Quimoi, 1985). However, the understanding of the nature of the association of various fungi, their pathogenic ability and succession in the disease complex is incomplete and needs further exploration. In 1984, the Consultative Group Discussion on Research Needs and Strategies for Control of Sorghum Root and Stalk rot Diseases, strongly recommended that the study of the spatial and temporal succession of fungi in the roots and stalks of sorghum be undertaken in various agroecological zones throughout the world (Mughogho, 1984). The present study was undertaken to identify some fungal pathogens in the stalk rot complex and to establish their pathogenicity and role in the development of the stalk rot disease complex in India.

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MATERIALS AND METHODS

Isolates

All of the stalk rot fungi (*M. phaseolina, F. Moniliforme, F. subglutinans, F. oxysporum,* and *F. semitectum*) used in this study were isolated from infected sorghum plants collected from the Osmania University Research Farm in Hyberabad, India. Standard mycological techniques were used to isolate these fungi from the infected tissues and to develop single-spore pure cultures.

Plant inoculation

Sand culture method

Clean sand and sorghum meal were mixed in a 10:1 ratio (w/w) and 100 g per flask of this mixture was dispensed into 250 mℓ erlenmeyer flasks, 10 mℓ of water was added to each flask which was then plugged with cotton wool. The flasks were autoclaved twice at 121 °C for 30 min each time. After cooling, the flasks were inoculated with 8 mm discs from the margin of one-week-old potato dextrose agar (PDA) cultures of the test fungi. Inoculated flasks were incubated at 25 ± 2 °C for 14 d. The flasks were shaken periodically to ensure uniform growth of the fungus in the media. Soil was collected from a sorghum growing field at the Osmania University Research farm, in Hyderabad and autoclaved twice at 121 °C for 2 h each time. The soil was then mixed in a 20:1 ratio (w/w) with sand cultures of the individual stalk rot fungi. Five kg of this mixture was placed in each plastic pot (25 cm dia) which had previously been swabbed with ethanol. Surface sterilized (0,1 % mercuric chloride for 30 s and rinsed three times in sterilized distilled water) seeds of sorghum hybrid CSH 5 were sown (5 seeds per pot) at a depth of 5 cm in the pots (4 pots per treatment) and covered with the same soil mixture. The pots were kept in the greenhouse with an ambient temperature of 25 ± 2 °C. Sterilized water was added to the pots every second day and Hoagland nutrient solution (Hoagland & Arnon, 1950) was added every fifth day. After anthesis, the irrigation schedule was reduced to induce soil moisture stress. Water or Hoagland solution was added once a week, until late soft dough stage, and then stopped. Carbofuran was applied in the whorls of the plants, when 1-week-old, to prevent shootfly attack.

McCoy and Kraft method

A modification of the method devised by McCoy and Kraft (1984) to inoculate peas (*Pisum sativum*) with *Rhizoctonia solani* was used. Field soil (autoclaved as above) was placed in plastic pots (25 cm dia) which had previously been swabbed with ethanol. Surface sterilized seeds of CSH 5 (five seeds per pot) were sown in the pots (four pots per treatment) and maintained as in the previous method. At the boot leaf stage, the top 5 cm of soil in the pots was carefully removed to expose the upper root system. Two 1 cm^2 discs of 7-day-old PDA culture of the test fungus were transferred to the roots of the plants, which were then covered again with sterilized soil. The watering schedule was maintained until anthesis after which moisture stress was applied as described in the previous method.

Isolation of fungi from inoculated plants

Fungal isolation from plants inoculated by both the above-mentioned methods, were undertaken 20 d after emergence, and at the boot leaf, flowering, soft dough, hard dough, and physiological maturity stages (Vanderlip and Reeves, 1972). Plants were uprooted and washed in running tap water. Fifty pieces each of roots and stem (stem pieces only at physiological maturity stage) were cut into 1 cm lengths, surface sterilized with mercuric chloride (0,1 % v/v) for 30 s, and washed three times with sterile distilled water, blotted dry with sterile filter paper, and plated on modified Czapek Dox Agar medium (Sharma and Singh, 1973). After incubation at 25 ± 2 °C for 7 d, the fungi were either identified using a light microscope or transferred to PDA slants for further identification using the slide culture method (Larsen & Covey, 1979).

Disease assessment

At each plant growth stage (from late milk stage in the Sand culture method and from soft dough stage in the McCoy and Kraft method), the number of lodged plants in each treatment was determined.

At physiological maturity, individual plants were uprooted, split-open and the following disease parameters were evaluated (Masterhazy, 1979; Pande *et al.*, 1989).

a. The number of lodged plants with soft stalks was determined by squeezing the stem pieces between the first and third nodes.

b. Spread of fungal colonization was visually measured as the number of nodes colonized by the fungus.

c. Degree of pith disintegration (PD) and vascular bundle separation (VBD) socred visually in a 1–4 rating scale, where 1 = no symptoms and 4 = severe damage.

d. Mean root infection scored visually in a 1–5 scale where 1 = no root infection and 5 = > 40 % roots infected.

e. Fungal mycelial spread was observed under a binocular microscope and severity was scored in a 1–4 rating scale where 1 = no colonization and 4 = severe colonization and tissue damage.

RESULTS

Sand culture method

The *Fusarium* spp. colonized the roots from the seedling stage onwards. Colonization increased progressively with age, attaining maximum colonization at the hard dough stage (HDS) (Table 1). *F. moniliforme* colonized 60 % of the roots 20 d after inoculation. At physiological maturity (PM) 90 % of the roots were colonized. Similarly, *F. subglutinans, F. oxysporum* and *F. semitectum* colonized the roots to a maximum extent at PM, while only 30–40 % of

TABLE 1 Incidence of individual sorghum stalk rot fungi in roots and stalks of sorghum hybrid CSH 5 at different growth stages when inoculated separately by a sand culture method

Stalk rot pathogen inoculated	Incidence (%)[a] at growth stages[b]						
	20d	BLS	FLS	SDS	HDS	PM	
						Roots	Stalks
F. moniliforme	60	60	80	80	90	90	40
F. subglutinans	60	80	60	60	90	80	30
F. oxysporum	40	40	40	40	60	70	40
F. semitectum	40	40	40	40	80	70	30
M. phaseolina	0	0	0	0	40	70	90
Control (not inoculated)	0	0	0	5	0	0	0

[a] Calculated as percentage occurrence of the stalk rot pathogens in the root/stem pieces plated on modified Czapek dox agar

[b] 20 = 20 days after emergence; BLS = boot leaf stage; FLS = flowering stage; SDS = soft dough stage; HDS = hard dough stage; PM = physiological maturity

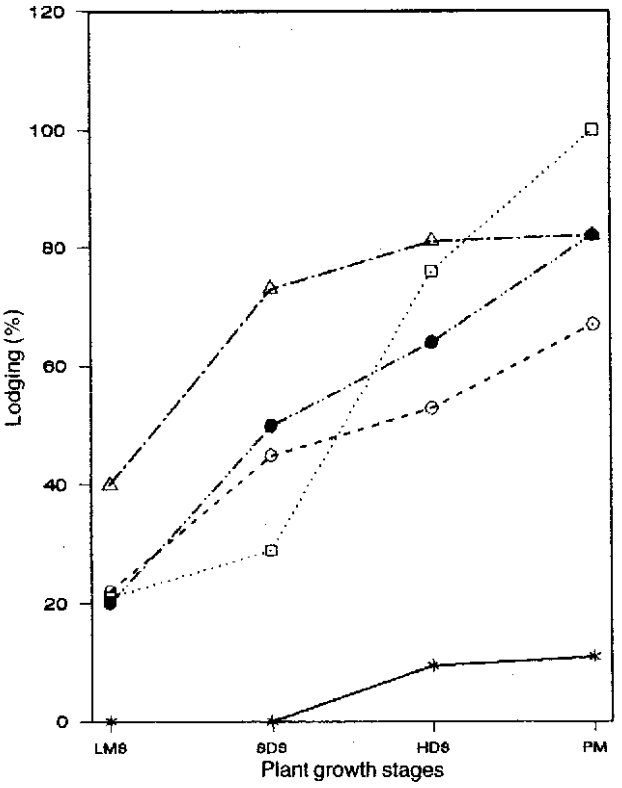

FIG. 1 Incidence of lodging at four plant growth stages [late milk stage (LMS), soft dough state (SDS), hard dough stage (HDS), and physiological maturity (PM)] of the sorghum hybrid CSH 4 inoculated with *Macrophomina phaseolina* (· · · □ · · ·), *Fusarium moniliforme* (— · — △ — · —), *F. subglutinans* (— — — ○ — — —), *F. oxysporum* (— · · — ● — · · —), and *F. semitectum* (——*——) by the sand culture method

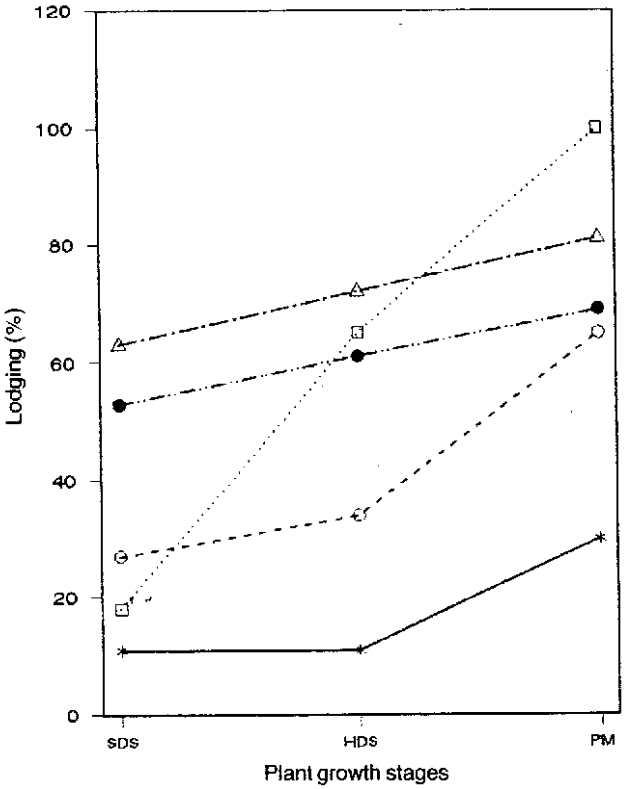

FIG. 2 Incidence of lodging at three plant growth stages soft dough stage (SDS), hard dough stage (HDS), and physiological maturity (PM)] of the sorghum hybrid CSH 5 inoculated with *Macrophomina phaseolina* (· · · □ · · ·), *Fusarium moniliforme* (— · — △ — · —), *F. subglutinans* (— — — ○ — — —), *F. oxypsorum* (— · · — ● — · · —), and *F. semitectum* (——*——) by the McCoy and Kraft method

the stalks yielded the test fungi. In contrast, *M. phaseolina* did not colonize roots or stalks during early growth stages, but could do so only at the HDS Colonization increased to 70 % of the roots and 90 % of the stalks at PM.

Lodging was noticed first at the late milk stage (LMS) and increased with plant age for all the fungi tested (Fig. 1). Lodging percentage was very low in the *F. semitectum* inoculated plants.

Stalks of all the lodged plants infected with *M. phaseolina*, *F. moniliforme* and *F. subglutinans* were soft stalked (Table 2). Occurrence of soft stalked stems among the lodged plants were less in *F. oxysporum* inoculated plants while they were altogether absent in *F. semitectum* inoculated plants. Spread of the fungus across the nodes was highest in *M. phaseolina* (4–6) and *F. moniliforme* (3–6) inoculated plants while *F. semitectum* failed to cross more than a single node. Mean root infection, pith disintegration, vascular bundle separation and mycelial spread were more severe in *M. phaseolina* and *F. moniliforme* infected plants than in the others.

TABLE 2 The relative severity of disease at physiological maturity in the sorghum hybrid CSH 5 inoculated separately with individual stalk rot fungi, by a sand culture method

Pathogen inoculated	Lodging (%)[a]	Soft stalk (%)	MNC[b]		MRI[c]	PD[d]	VBD[e]	FMC[f]
			Mean	Range				
F. moniliforme	88,9	88,9	5	3–6	5	3	4	4
F. subglutinans	66,7	66,7	2	1–3	5	3	3	2
F. oxysporum	88,9	55,6	3	3–5	5	3	3	3
F. semitectum	11,1	0,0	1	1	4	3	1	2
M. phaseolina	100,0	100,0	5	4–6	5	4	4	4
Control (not inoculated)	0,0	0,0	0	0	3	1	1	1

[a] Lodged plants as recorded at physiological maturity

[b] MNC = mean number of nodes colonized

[c] MRI = means root infection on a 1–5 scale where 1 = no root infection and 5 = more than 40 % root infection

[d] PD = pith disintegration on a 1–4 scale where 1 = no symptoms, 2 = slight disintegration, 3 = moderate damage and 4 = severe damage

[e] VBD = vascular bundle separation on a 1–4 scale where 1 = no symptoms, 2 = slight disintegration, 3 = moderate damage and 4 = severe damage

[f] FMC = fungal mycelial spread on a 1–4 scale where 1 = no symptoms, 2 = restricted fungal spread, 3 = moderate spread in tissues and 4 = extensive spread and damage of the tissues

TABLE 3 Incidence of individual sorghum stalk rot fungi in roots and stalks of sorghum hybrid CSH 5 at different growth stages when inoculated separately by the McCoy and Kraft method

Stalk rot pathogen inoculated	Incidence (%)[a] at growth stages[b]			
	SDS	HDS	PM	
			Roots	Stalks
F. moniliforme	83,3	83,3	90,0	50,0
F. subglutinans	50,0	50,0	90,0	50,0
F. oxysporum	28,6	66,0	60,0	60,0
F. semitectum	28,6	60,0	70,0	0,0
M. phaseolina	0,0	50,0	90,0	100,0
Control (not inoculated)	0,0	0,0	0,0	0,0

[a] Calculated as percentage occurrence of the stalk rot pathogens in the root/stem pieces plated on modified Czapek dox agar

[b] SDS = soft dough stage; HDS = hard dough stage and PM = physiological maturity

TABLE 4 The relative severrity of disease at physiological maturity in the sorghum hybrid CSH 5 when inoculated separately with individual sorghum stalk rot fungi, by the McCoy and Kraft method

Pathogen inoculated	Lodging (%)[a]	Soft Stalk (%)	MNC[b]		MRI[c]	PD[d]	VBD[e]	FMC[f]
			Mean	Range				
F. moniliforme	88,9	66,7	3	3–5	5	3	3	4
F. subglutinans	70,0	40,0	1	1–3	5	3	2	2
F. oxysporum	88,9	44,4	1	1–2	5	3	3	2
F. semitectum	66,7	33,3	1	1–2	5	2	2	2
M. phaseolina	100,0	100,0	4	3–6	5	4	4	4
Control (not inoculated)	11,0	0,0	0	0	3	1	1	1

[a] Lodged plants as recorded at the physiological maturity

[b] MNC = means number of nodes colonized

[c] MRI = means root infection on a 1 -5 scale where 1 = no root infection and 5 = more than 40 % root infection

[d] PD = pith disintegration on a 1–4 scale where 1 = no symptoms, 2 = slight disintegration, 3 = moderate damage and 4 = severe damage

[e] VBD = vascular bundle separation on a 1–4 scale where 1 = no symptoms, 2 = slight disintegration, 3 = moderate damage and 4 = severe damage

[f] FMC = fungal mycelial spread on a 1–4 scale where 1 = no symptoms, 2 = restricted fungal spread, 3 = moderate spread in tissues and 4 = extensive spread and damage of the tissues

McCoy and Kraft method

F. moniliforme was isolated more frequently than the other *Fusaria* from the SDS to PM (Table 3). Stalks yielded relatively less *Fusaria* than the roots. *M. phaseolina* could only be isolated from the plants from HDS increasing rapidly to more than 90 % incidence both in the stalks and in the roots at PM. Stalk rot symptoms were more severe in *F. moniliforme* infected plants than in plants inoculated with the other *Fusarium* spp.

All the *M. phaseolina* infected (lodged plants) were soft stalked (Table 4). Severe damage of the vascular bundles and pith, extensive spread of mycelium and degradation of the tissue symbolized the severity of disease with *M. phaseolina* infection as it crossed maximum number of nodes.

DISCUSSION

The present study showed that, of the five stalk rot fungi tested by both inoculation methods, the *Fusarium* species caused root infection during the early stages of growth of the sorghum plants. *M. phaseolina* could not colonize the roots at these early stages even though the pathogen was present in the soil. *F. moniliforme* and *F. subglutinans* seemed to be most prevalent in the roots of the plants at physiological maturity, followed by *M. phaseolina, F. oxysporum*, and *F. semitectum*. *M. phaseolina* was, however, most prevalent in the stalks.

From the present study it can be inferred that *Fusarium* species play an important role as primary colonizers of the roots causing severe root rot. They then move from the root system into the stalks and weaken the stalks. *M. phaseolina* could only colonize roots after the soft dough stage. This late colonization of roots and stems by *M. phaseolina* correlates with lodging symptoms which normally appear in the field after the soft dough stage and suggests a pathogenic role of this fungus in causing stalk rot. These results support the findings of Wadsworth & Sieglinger (1950) who observed that the various fungi associated with the stalk rot complex attack in an orderly sequence, with *M. phaseolina* being the last and most conspicuous member of the sequence. The rapid spread of this fungus in the stalks, the severe pith disintegration and other parameters also confirm its role.

The positive correlation among the disease parameters like percent lodging, percent soft stalk and spread of the fungus used in the present study concur with the findings of Pande *et al.* (1989). Thus the present study emphasis the role of different stalk rot pathogens and identifies the *Fusarium* species, namely *F. moniliforme, F. subglutinans*, and *F. oxysporum*, as the early invaders in the stalk rot complex and *M. phaseolina* as the late invader, but still a major stalk rot pathogen.

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