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Frequency and pathogenicity of fusarium wilts (*Fusarium solani* and *Fusarium equiseti*) of cotton (*Gossypium hirsutum*) in Adamawa in Nigeria

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Abstract: Cotton fungi were surveyed in Ngurore, Adamawa, Nigeria in 1992 and 1993 by counting the number of isolates in each 100 infested plants per plot. Approximately 90% of the isolated fungi were *Fusarium solani* and *Fusarium equiseti,* both pathogenic; *F. solani* isolates were more virulent and frequent than *F. equiseti*. The high frequency and virulence of both fungi make them important pathogens of cotton in the area.

Key words: Cotton, Fungi, *Fusarium*, *Gossypium*, ecology.

Upland cotton (*Gossypium. hirsutum* L.) is an important cash crop and food in the world (Prentice 1972). Seedling disease in cotton is a worldwide problem, particularly the wilt and root rot disease causing loss to farmers (Hillocks 1992). The most common fungi associated with cotton disease are *Fusarium* spp., *Colletotrichum gossippi*, *Rhizopus* spp., *Thielavispsis basicola* and *Pythium* (King and Presley 1942; Roy and Bourland 1982; Johnson *et al.* 1978, Mauk and Hine 1988 and Hillocks 1992).

The studies of *Fusarium* wilt of cotton are well advanced in some regions of the world, but are still limited elsewhere. *Fusarium* wilt of cotton was first isolated as a root rot pathogen of cotton plant (Woodroof 1927, Colyer 1988); delay boll formation (Sparnicht and Roncadoni 1972); The seeds and seedling diseases have been reported by Simpson *et al.* (1973), Watkins (1981), Roy and Bourland (1982), Klich (1986), Kerbabaeva Frolovi (1986) and Sharma and Sandhu (1986), Katan and Katan (1988), Melero-vara 1990 Mousa *et al.* (1990), and Pizzinatto *et al*, (1991), Soleymani *et al.* (1993), Asssigbetse *et al.* (1994). Nelson *et al.* (1981), Minton and Garber (1983) and Hillocks (1992). *Fusarium* wilt is blamed on failure of the infected xylem to meet the water requirements of the plant (Hillocks 1992).

Similar reports on wilt and seedling rots in Nigeria caused by *Fusarium* spp. exist (Adeoti 1990, Adeoti *et al.* 1992).

Although *Fusarium* is known to be associated with seedling disease of cotton, species have not been defined for cotton grown in Ngurore area of Adamawa Sate.The results presented in this paper were from the survey carried out to determine the incidence and severity of seedling disease and to identify the causative fungi.

MATERIALS AND METHODS

Disease survey: Systematic disease surveys were conducted in the cotton growing plots between May and July 1992 and 1993.

The number of isolates in each 100 infested plants from two plots were counted and expressed in percent (%).

Isolation and identification: Seedlings of GH 216 with wilting were sampled. The soil around the wilted plants (rhizosphere) was also collected. Roots were washed with distilled water and cutt into 5-10 mm long sections with a sterilized sharp blade. The pieces were surface disinfected for two minutes in 0.1 % sodium hypochlorite and rinsed in several changes of distilled water before plating on potato dextrose agar (PDA) containing 0.2 % streptomycin and incubated at 30°C. Fungi grown from the root pieces were subcultured.

Single spore isolates were obtained by using the technique of Manandhar *et al* (1995). Petri dish containing fungi were flooded with distilled water and serial dilution was made. Five ml of suspension (100 spores/ml) were added to water agar (2gm of agar in 100ml) and marked out using a grid drawn on the base of each Petri dish. The plate was incubated for 12 hours and single spores were subcultured onto fresh PDA.

The two isolates were identified to the genus level using a microscope. The microscopic characteristics, and cultural characteristics were compared to the description in Booth, 1971. Also pure slants of two representative but different organisms were prepared and sent to international mycological institute (IMI) for confirmation.

Pathogenicity test: The method of Katan (1981) was used for the pathogenicity test. The watered pots were maintained on a bench at 30C 2 and kept under constant observation. Wilting seedlings were removed and the fungi were re-isolated and compared to the one isolated from the field.

Cultural characteristics of the two isolates: The growth characteristics of the two isolates on PDA was observed from day 2 after inoculation. In each isolate, four plates were set up. The observation was on aerial mycelium, the colour of substratum, texture, zonation until the isolate covered the plate.

Conidial measurements: The microconidial and macroconidial were obtained by flooding 7-day old culture of each isolate with distilled water. A slide of spores was prepared and observed under light microscope with Coumasie blue as stain using eye piece and objective lenses fixed with graduated granular slides. The two slides were adjusted so that the line on the eye piece was on the objective lens. The length of the micro- and macroconidial were measured by dividing the length of the eye piece by 2 and multiplying the result by 0.01 to give millimeter (100 x 0.01 = 1mm because each unit of 100 on the granular slide is 0.01). The result was converted to micrometer (µm) by being multiplied by 1000.

Radial growth: The linear mycelium measurement was carried out by cutting a disk of organism on PDA from the outer margin of a 7 day old culture using 6mm cork borer. The disk was placed upside down on solidified 2 % agar at the intersection on the transepts drawn at the bottom of 9cm plates (Vakalounakis 1996). Linear measurements were made every 24 hours with a ruler and the growth per day was obtained from the total diameter by dividing by 2. The experiment was terminated when the plates had been completely covered by the growing organisms.

RESULT

Two fungi isolated were identified to be *F. solani* (Mart.) Sacc. Teleomorph; *Nectria haematococcca* and *F. equiseti* (Corda)Sacc. The two fungal isolates were confirmed by IMI to be the same organisms with the number IMI 368692 and IMI 368693 respectively.

Results of the survey are presented in tables 1 to 5. Table 1 shows the frequency in (%) of *F. solani* and *F. equiseti*; Tables 2, 4 and 5 compare the growth characteristics, dry weight and radial growth of the two fungi; and Table 3 states the conidial characteristics of *Fusarium* isolates.

TABLE 1

Prevalence of cotton seedling wilts given in terms of Fusarium *isolates (%)*

Year	1992		1993	
Plot N°	3	5	3	5
F. solani	60	70	55	65
F. equiseti	30	25	40	30
Others	10	5	5	5

DISCUSSION

The role of *Fusarium* spp. as a pathogen of cotton seedlings, and other crops is well known. But Johnson and Doyle (1986) reported that *Fusarium* spp. were not important pathogens in cotton seedling disease complex, even though *Fusarium* spp. were the most frequently isolated fungi. The species involved were not identified by them.

The observed symptoms of this disease on the affected seedlings in Adamawa Nigeria (Table 2) are similar to those earlier reported from other countries (Melero-vara 1990, Colyer 1988, Schrender *et al.* 1995).

TABLE 2

Growth characteristics of the two isolates on PDA in five days at 30°C ±1

Day	*F. solani*	*F. equiseti*
2	Mycelia white. Back of plate yellowish pigments.	Mycelia cottony white. Back plate white.
3	Mycelia spread very fast. Spores produced. Yellow pigmentation increased. Zonation. Hyphae branched and septate	Mycelia grew slowly. Macrospores produced. Violet pigments. Zonation. Hyphae septate
4	Three zonations. Pigmentation increased. Sparse mycelia.	Woolly mycelium. Pink colour increased.
5	Zones increased. Mycelia scanty and withered. Colour pale. Back yellowish.	Radial growth. Zonation not clear. Mycelia yellowish.

TABLE 3

The conidial characteristics of Fusarium *isolates*

	Microconidia		Macroconidia		
	Length (µm)	Presence	Length (µm)	Septation	Presence
F. solani	6.4 ± 0.28	Abundance	2.2 ± 0.141	2-5	Few
F. equiseti	2.4 ± 0.070	Few	2.6 ± 0.340	3-5	Abundance

TABLE 4

The dry weight (g)of mycelia and pH of the two fungi for 30 days incubation grown in a liquid medium of potato dextrose at 25°C ± 1

Day	Weight (g) *F. solani*	Weight (g) *F. equiseti*
3	0.332 ± 0.008	0.249 ± 0.011
6	0.421 ± 0.047	0.379 ± 0.096
9	0.671 ± 0.041	0.633 ± 0.131
12	0.755 ± 0.100	0.761 ± 0.233
15	0.645 ± 0.026	0.801 ± 0.013

In this study, *F. solani* and *F. equiseti* approximated 60% and 30% of all fungi isolated from diseased seedlings. These values agree with that reported by Pizzinatto and Menten (1991). Colyer (1988) reported that *Fusarium solani* was more frequent and virulent than *Fusarium equiseti.* The high virulence of these species to cotton agree with the works of Johnson *et al.* (1977), Klich (1986), Sharma and Sandhu (1986), Mousa *et al.* (1990) and Solymani *et al.* (1993).

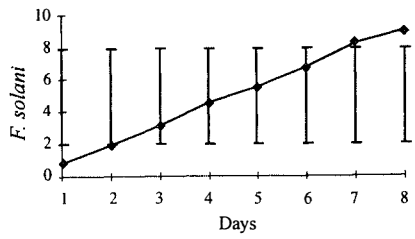

Fig. 1a. Radial growth (cm) of *F. solani* on PDA at 25 °C.

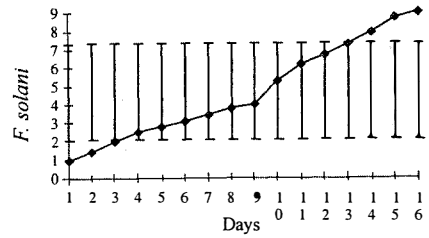

Fig. 1b. Radial growth (cm) of *F. equiseti* on PDA at 25 °C.

The observed morphological and cultural characteristics of *F. solani* and *F. equiseti* was the same as that reported by Booth (1971) and Joffe (1986), except that the growth rate differed. The growth rates of *F. solani* and *F. equiseti* were 3.2 cm and 5.8 cm, while the average growth rates observed were 1.3 cm and 0.3 cm for the two fungi, respectively. The differences in the growth rates may be due to the growth medium and the incubation condition.

The differences observed in pathogenicity and virulence between this study and others may have resulted from differing pathogenicity testing conditions used. The high level of virulence and frequency of isolation indicate *Fusarium* spp. as an important cause in the etiology of cotton seedling disease in Adamawa area of Nigeria.

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