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EFFICACY OF DIFFERENT FUNGICIDES AGAINST *FUSARIUM* WILT OF COTTON CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*

ABDUL QAYOOM RAJPUT, M. H. ARAIN, *M. A. PATHAN, *M. M. JISKANI AND *A. M. LODHI

*Department of Plant Pathology, Faculty of Plant Protection (S.A.U. Tandojam)**
Department of Agriculture, Pest and Disease Research Lab. (PDRL)
University of Karachi, Karachi, Pakistan.

Abstract

Fusarium oxysporum f. sp. *vasinfectum* (Atk.) Snyd. & Hans was isolated from wilted cotton plants collected from Shahdad Pur and Tando Jam. Pathogenicity test of *Fusarium oxysporum* f. sp. *vasinfectum* was conducted on four cotton varieties viz, Cris-9, TH-41/83. CIM-448 and Shahbaz-95. The growth of artificially inoculated plants of Cris-9 and TH-41/83 significantly reduced and plants were completely dried after 20 days of inoculation. The numbers of germinated plants were also lower as compared to Shahbaz-95 and CIM-448. Carbendazim at its three doses significantly inhibited the mycelial growth of the fungus followed by Thiophanate methyl and Dithane M-45. There was not significant difference in mycelial growth of the fungus on Thiovit (35.75 mm) and control (37.87 mm) in which fungicide was not used. The maximum root and shoot length of cotton varieties was recorded with Carbendazim followed by Thiophanate methyl. The growth was significantly increased in Shahbaz-95 and CIM-448 as compared to Cris-9 and TH-41/83 varieties. Carbendazim also significantly reduced the colonization of *Fusarium oxysporum f.* sp. *vasinfectum* on root pieces of Cris-9 variety. The growth of Cris-9 also increased when plants were inoculated with low level of inoculum (10.75cm and 12.25cm) than at high inoculum levels (6.72cm and 5.85cm).

Introduction

Cotton as major crop in parts of Africa, Australia, China, Egypt, India, Mexico, Pakistan, Soviet Union, Sudan, United states and warmer regions of central and South America (Bhatti & Soomro, 1996). In Pakistan is largest kharif crop and grown on 12% of the total cultivated area (Ahmed, 1999). Cotton is subjected to more than 60 diseases (Watkins, 1981). Wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) snyd & Hans is one of the most important disease of cotton. The symptoms of the disease include discolouration of tissues and plugging of vessels by hyphae, vein clearing on cotyledonary and first leaves and reduction in size of leaves and bolls (Singh, 1989). There are several reports where fungicides have been used for the control of wilt diseases. *Fusarium* infection was reduced by Pencycuron + Totalfluanid and Benomyl (Guolart *et al*., 1992), Zhiweiling, Benomyl, Uzgen and Thiophenat NF 44 (Yunusov *et al.*, 1980). In the present studies, four fungicides viz., Carbendazin, Thiophanate methyl, Thiovit and Dithane M-45 were used to see their efficacy on the control of *Fusarium* wilt on 4 varieties of cotton viz., Cris-9, TH-41/83, CIM-448 and Shahbaz-95.

Materials and Methods

Collection of diseased samples: Cotton plants showing symptoms of wilt disease were collected from different fields on Shahdad Pur and Tandojam areas of Sindh and brought to the laboratory to isolate the disease causing agent *Fusarium* sp.

Isolation of the fungus: Roots were cut into 1cm long pieces with sterilized scissor and washed with running tap water. These pieces were then dipped in 0.01% $HgCl_2$ solution for one minute, immediately washed twice with distilled sterilized water for 3-4 minutes and dried thoroughly. Five pieces were kept in Petri dishes containing sterilized potato-dextrose-agar (PDA) medium amended with Penicilium @ 100.000 unit L^{-1} and Streptomycin @ 1g L^{-1} and incubated for 7 days at room temperature (30 ± 1^0C). After 7 days of growth, 5mm disk was cut from the edge of fungal culture and placed on freshly prepared PDA plates. These plates were sub-cultured and multiplied time to time during the entire research work. The pathogen was identified after reference to Booth (1971).

Pathogenicity test: The pathogenicity test was conducted in sterilized earthen pots. All pots were filled with steam- sterilized sandy soil (1 kg/pot), which was thoroughly mixed with a fresh culture of *F. oxysporum.* The untreated soil (without fungus) was used as control. Seeds of cotton varieties viz., Cris-9, Shahbaz-95, TH-41/83 and CIM-448 were soaked in distilled sterile water for 20 hours. Ten seeds of each variety per pot were sown and the pots were kept for 30 days under open sky. The plants were irrigated on alternate days and re-isolation was done from roots of cotton plants showing wilting symptoms.

Effect of different fungicides on colony growth of *Fusarium* spp. *In vitro*: Four different fungicides viz., Carbendazim, Dithane M-45, Thiovit and Thiophanate-methyl @ 50, 100, 150 mg/100ml were tested to see their effect on the redial colony growth of the fungus. The fungicides were incorporated in to the sterilized PDA medium. The sterilized Petri dishes containing amended medium were inoculated with 5mm disk of freshly prepared culture of *Fusarium oxysporum*. All the inoculated plates were incubated for 7 days at $30\pm^0$C. The inoculated PDA plates without fungicides were treated as control. The linear colony growth was recorded at 24 hours, interval and final growth was measured after 168 hours when any one Petri dish was found full of fungal growth.

Effect of different fungicides on plant growth of cotton varieties inoculated with *Fusarium* spp.: The fungicides were added to sterilized sandy soil, which was thoroughly mixed with a fresh culture of *Fusarium oxysporum.* The concentration of each used fungicide was 0.5, 1.0 and 1.5g/kg soil. Eight seeds of Cris-9, Shahbaz-95, TH-41/83 and CIM-448 cotton varieties were planted per pot and the pots were kept for 30 days in open sky. After 27 days of seedling emergence, root and shoot length of plants was recorded from treated and untreated pots. The root pieces of diseased cotton plants treated with different fungicides were also placed on PDA plates to determine the root colonization % of *Fusarium oxysporum.* The dishes were incubated at 30 ± 2^0C and the frequency of root colonization was recorded after 5 days.

Effect of different inoculum levels *Fusarium oxysporum f.* sp. *vasinfectum* on plant growth of Cris-9: Different inoculum levels viz., 0, 0.25, 0.5, 0.75, 1.0 and 1.5 Petri dish of fresh culture of the fungus per pot were used to see their effect on disease infection and plant growth. Pots were filled with sterilized soil (1kg soil per pot), which was

thoroughly mixed with six different inoculum levels of *Fusarium oxysporum*. Ten seeds per pot were used and pots were kept-for 30 days in open sky. After 30 days root and shoot length and number of diseased plant per pot were recorded.

$$\text{Colonization \%} = \frac{\text{Total No. of root pieces colonized by the fungus}}{\text{Total No. of root pieces of all plants}} \times 100$$

Results and Discussion

1. Symptoms of the disease: The infected plants became stunted with discoloured tissues. In early stages of growth, vein clearing was noticed on first leaves and often the diseased plants had smaller leaves.

2. Isolation and identification of *Fusarium* sp.: The fungus produced scattered cottony mycelial growth on infected root pieces forming large quantity of micro and 4-5 septate macro-conidia. The fungus was identified as *Fusarium oxysporum* f. sp. *vasinfectum* (ATK) Snyd. & Hans., after reference to Booth (1971) and Singh (1987). Pandey (1997) reported the cotton wilt as most destructive disease caused by *F. oxysporum* f. sp. *vasinfectum* prevailing in all cotton growing countries of the world. Smith *et al.,* (2001) also observed that all the 10 cotton fields in California were infested with the high population of the fungus. Similarly, Hillocks Kibani (2002) found *Fusarium* wilt as the major disease of cotton in Tanzania. Kolte (1985*)* found *Fusarium* spp. as major cause of wilt diseases in cotton. Khoso (1998) mentioned that wilt inducing fungi *Rhizoctonia solani, R. bataticola* and *Fusarium* spp., are found in patches in the soil.

3. Pathogenicity test of *F. oxysporum*: Pathogenicity test was conducted on four cotton varieties Cris-9, Shahbaz-95, TH-41/83 and CIM-448. The growth of artificially inoculated plants of Cris-9 and TH-41/83 was reduced as compared to control. The number of germinated plants in both varieties was also reduced as compared to un-inoculated plants. However growth of inoculated plants of Shahbaz-95 was less affected as compared to CIM-448 than that of Cris-9 and TH-41/83, respectively. The fungus was re-isolated and identified as *F. oxysporum*.

Harden (1976) also observed that *Fusarium* spp., damaged xylem vessel there by affecting growth of cotton plants. Hafiz (1986) while conducting pathogenicity test of *Fusarium* spp., found brown disclouration of roots near soil line whereas Ghaffar (1988) observed severe colonization of cortical tissues of infected plants by *Fusarium* spp. Similar results have been reported by Fletcher (1994).

4. Effect of different fungicides on colony growth of *Fusarium oxysporum:* Four different fungicides viz., Carbendazim, Dithane M-45, Thiovit and Thiophanate-methyl were tested for their effect on colony growth of the fungus. All the fungicides significantly reduced the growth of *F. oxysporum* f. sp. *vasinfectum* as compared to control. Increase in concentration of all the four fungicides in medium showed significant gradual reduction in the growth of fungus. The most effective fungicides were found to be Carbendazim followed by Thiophanate-methyl (Fig. 1).

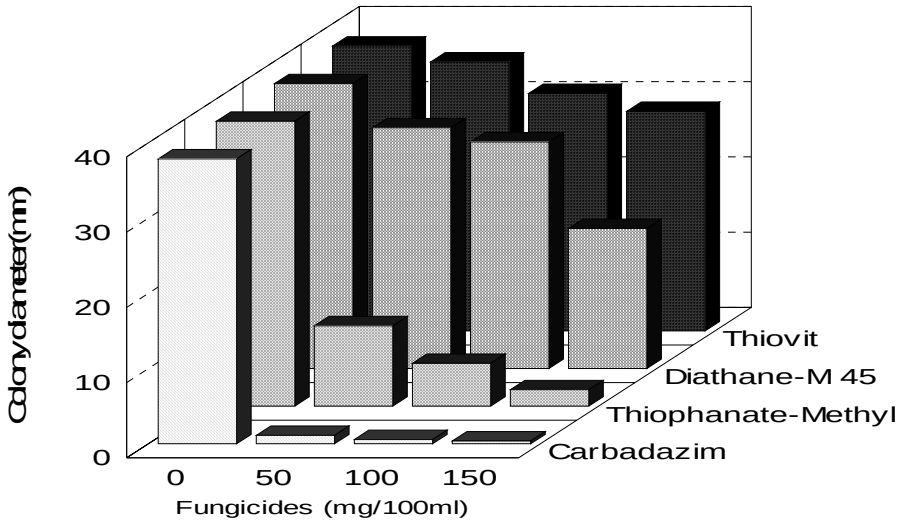

Fig. 1. Effect of different fungicides on colony growth of *F. oxysporum.*

5. Effect of different fungicides on growth of cotton varieties inoculated with *Fusarium oxysporum:* The shoot length showed highly significant differences between fungicides, their doses and varieties. The maximum shoot length was recorded in Shahbaz-95 (18.36cm) followed by CIM-448 (18.16cm), Cris-9 (16.00cm) and TH-41/83 (15.50cm). Carbendazim was found to be the best fungicide followed by Thiophanate methyl, Dithane M-45 and Thiovit as compared to control in respect of shoot and root length. The plant growth gradually increased with increase in concentration of the fungicides and the maximum dose (1.50g) of the fungicides gave maximum shoot and root length (Figs. 2-3). Yunusov *et al.,* (1980) effectively used Benomyl and Thiophanate NF-44 against *Fusarium* wilt of cotton. Mustika *et al.,* (1984) found that Dithane M-45 significantly reduced growth of *Fusarium oxysporum.* Goulart (1992) used Captan and Benomyl against several seed borne fungi under laboratory and field conditions. Guo *et al.,* (1993) obtained best results with Carbendazim when used as basal compound against *Fusarium* sp., on cotton.

6. Effect of different inoculum levels of *F. oxysporum f. sp vasinfectum* on plant growth of Cris-9 variety: The plant growth (root and shoot length) of Cris-9 cotton variety was significantly increased at low level of inoculum i.e., 0.25 (10.75 and 12.25cm). However root and shoot length were reduced at high level of inoculum (6.72 and 5.85cm) as compared to control (Fig. 4) in which inoculum was not mixed with the soil.

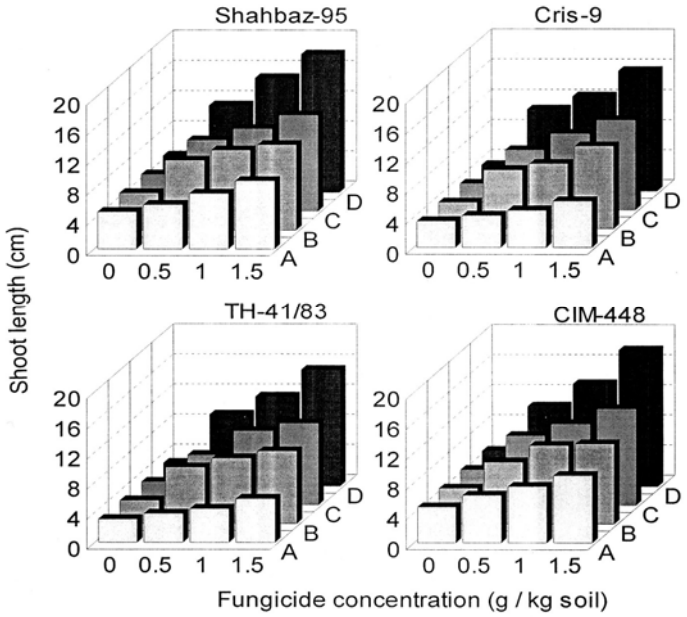

Fig. 2. Effect of different fungicides on shoot length of cotton varieties inoculated with *Fusarium oxysporum f.* sp. *vasinfectum.*

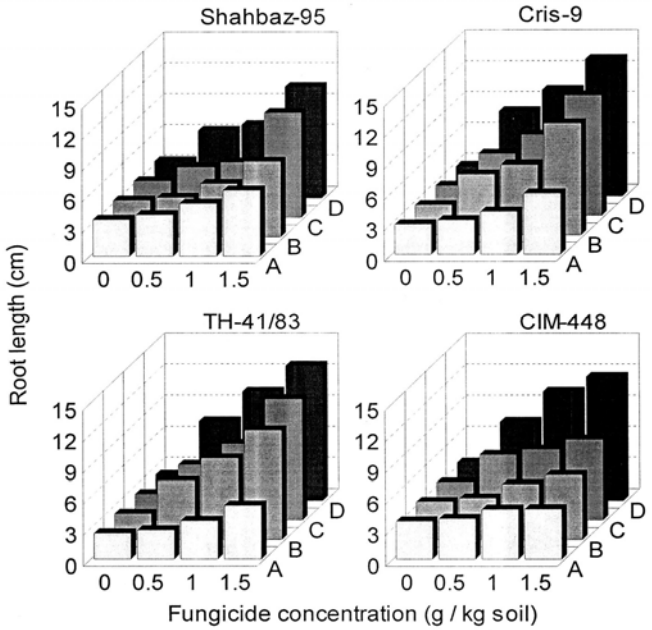

Fig. 3. Effect of different fungicides on root length of cotton varieties inoculated with *Fusarium oxysporum f.* sp. *vasinfectum.*

A= Thiovit, B= Dithane M-45, C =Thiophanate methyle, D= Carbendazim

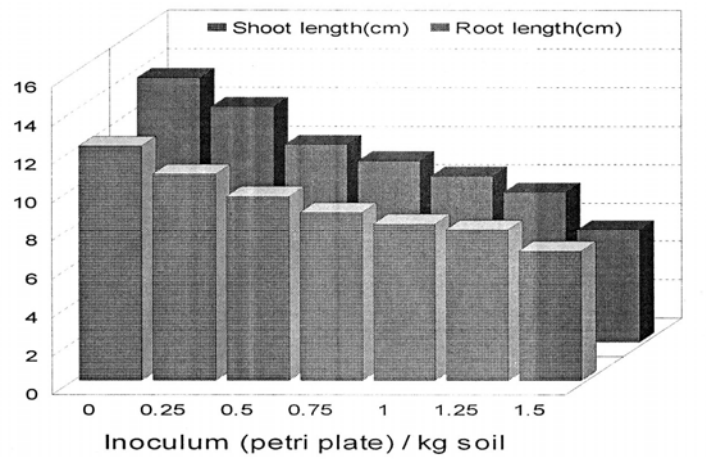

Fig. 4. Effect of different inoculum levels of *Fusarium oxysporum* f. sp. *vasinfectum* on plant growth of Cris-9 variety

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