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PATHOGENECITY AND TRANSMISSION STUDIES OF SEED-BORNE *FUSARIUM* SPECIES (SEC. LISEOLA AND SPOROTRICHIELLA) IN SUNFLOWER

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Abstract

Pathogenicity of 6 *Fusarium* spp. *viz*., *F. anthophilum*, *F. moniliforme*, *F. proliferatum*, and *F. subglutinans* (from section Liseola) and *F. chlamydosporum* and *F. sporotrichioides* (from section Sporotrichiella) were tested on sunflower plants. Symptoms produced by *Fusarium* spp., were wilting, collar-, stem- and seedling rots, damping-off, stunting, yellowing, tip burning and reduction in growth. Wilting and seedling rot were found to be the most prominent symptoms produced by all *Fusarium* spp. Highest wilting was observed in plants inoculated by *F. chlamydosporum* and *F. subglutinans* whereas highest seedling rot was observed by *F. sporotrichioides*. In transmission studies, *F. anthophilum and F. subglutinans* were found to be internally seed-borne pathogens of sunflower.

Introduction

Fusarium spp., are widespread plant pathogenic fungi which commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Species of *Fusarium* are reported as seed-borne and soil-borne plant pathogens. They cause pre- and post-emergence death of seedlings, seed abortion, seed-, root-, stem- and seedling- rots, blight, chlorosis, vascular wilt, die back, stunting and reduction in growth in a variety of host plants (Richardson, 1979, 1981, 1983). Although seed-borne nature of *Fusarium* spp. is well known (Kaur *et al*., 1990; Dawar & Ghaffar, 1991; Ahmed *et al*., 1993; Ahmed *et al*., 1994; Bhutta, 1998; Sharfun-Nahar *et al*., 2005) but their association and pathogenicity in sunflower seedlings has not been well documented except that of *F. moniliforme* and *F. solani* (Dawar, 1994; Shamim *et al*., 2003). The pathogenic nature of seed-borne *Fusarium* spp., was therefore, observed in sunflower seedlings to study its systemic transmission from seed to plant.

Materials and Methods

Transmission studies: Natural transmission of pathogenic *Fusarium* spp., *viz*., *F. anthophilum*, *F. moniliforme*, *F. proliferatum*, and *F. subglutinans* (of section Liseola) and *F. chlamydosporum* and *F. sporotrichioides* (of section Sporotrichiella) from seed to plant was detected in healthy-looking seedlings of sunflower. Seedlings were selected from seed samples that showed highest occurrence of *Fusarium* spp., in blotter test. The seedlings were transplanted in 6-inch earthen pots containing sterilized soil to observe seed to seedling transmission of plant pathogenic *Fusarium* species. Diseased plants showing symptoms were uprooted, whereas, remaining healthy looking plants were uprooted after flowering. Component plating of diseased and healthy looking plant parts was carried out on potato dextrose agar (Baker & Cook, 1974).

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Pathogenicity test: Conidial suspensions from 2-week old cultures of above-mentioned 6 *Fusarium* spp. were adjusted to 50,000 conidia/ml in sterilized distilled water. This conidial suspension was mixed @ 1:20 v/w in sterilized moistened (20%) corn meal-sand medium and incubated at 25±1°C for 21 days (Hashmi, 1988). Freshly prepared corn meal-sand inocula of *Fusairum* spp., were mixed with sterilized soil in different percentages *viz*., 0.5, 1, 2, 5 and 10 % to detect threshold of pathogen at various inoculum levels. Soil used for artificial infestation was sandy loam (sand:silt:clay, 70:21:9) with pH range from 7.1-7.9 and 42% maximum moisture holding capacity (Keen & Raczkowski, 1921). The soil was screened through 2-mm sieve, moistened and sterilized at 15 psi for 1 hr before use. All experiments along with control were carried out in triplicates. Component plating of healthy looking and affected sunflower seedlings was carried out on PDA, and infection and colonization percentages were calculated as follows:

$$\text{Infection \%} = \frac{\text{No. of plants affected by a pathogen}}{\text{Total no. of plants}} \times 100$$

$$\text{Colonization \%} = \frac{\text{No. of pieces infected by a pathogen}}{\text{Total no. of pieces tested}} \times 100$$

Results and Discussion

Transmission studies: In transmission studies of pathogenic *Fusarium* spp., from seed to plant was detected by the symptoms produced in seedlings within 2 weeks to 2 months. All seedlings were divided into four categories *i.e*., healthy-looking, partially wilted, wilted and rotted seedlings. Only wilted seedlings showed presence of *F. anthophilum*, *F. moniliforme* and *F. subglutinans*, while rest of the categories showed presence of certain saprophytic and other parasitic fungi such as *Alternaria alternata* (24%), *Bipolaris australiensis* (11.4%), *B. hawaiiensis* (1.28%), *Curvularia intermedia* (2.8%), *C. lunata* (5.7%), *Exserohilum rostratum* (4.2%), *Macrophomina phaseolina* (29.4%), *Phoma* sp. (2.85%), *Rhizoctonia solani* (31.4%) and *Trichoderma harzianum* (5.7%).

Fusarium moniliforme showed systemic transmission from seed to plant, whereas, *F. proliferatum* and *F. sporotrichioides*, which were recorded as seed-borne, were not found transmitted in seedlings probably due to low inoculum on the seeds. On the other hand *F. anthophilum* and *F. subglutinans* not detected in sunflower seeds were transmitted in sunflower plants indicates their internally seed-borne nature, where they directly entered into the vascular system of plants. The well known charcoal rot pathogen of sunflower (*Macrophomina phaseolina*) was also isolated from rotted seedlings along with certain saprophytic fungi, whereas, root rot pathogen *Rhizoctonia solani* was found associated with partially wilted seedlings. It may be mentioned that transmission and virulence of *Rhizoctonia solani* and *Macrophomina phaseolina* is of lesser importance than *Fusarium* spp. in sunflower (Ahmed *et al*., 1994).

Pathogenicity test of *Fusarium* spp: Pathogenicity of 6 *Fusarium* spp., *viz*., *F. anthophilum*, *F. moniliforme*, *F. proliferatum*, *F. subglutinans* (of sec. Liseola) and *F. chlamydosporum* and *F. sporotrichioides* (of sec. Sporotrichiella) was tested on sunflower at 0.5, 1, 2, 5 and 10% inoculum levels. Symptoms produced by *Fusarium* spp., were collar-, stem- and seedling rots, damping-off, stunting, wilting, yellowing, tip burning and reduction in growth (Table 1).

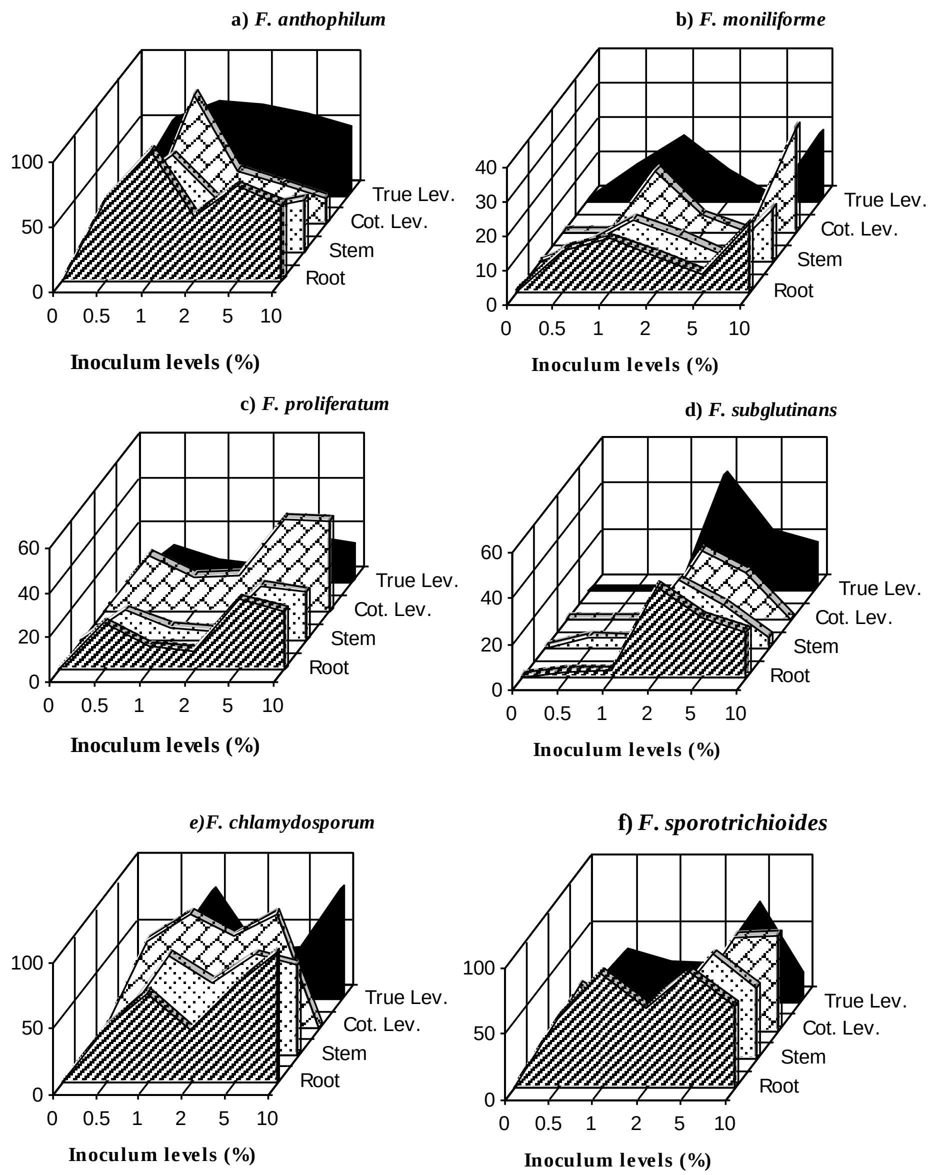

Fig. 1a- f: Percent colonization of *Fusarium* spp., in different parts of sunflower (*Helianthus annuus*) at different inoculum levels as compared to control (0).

Damping-off and wilting were major symptoms produced by *F. anthophilum* in all inoculum levels. Seedlings showed damping–off within 15 days where maximum infection was observed at 5%, whereas, highest wilting occurred within one month at 1 and 5% inoculum levels. Significant wilting was also observed in other inoculum levels. Yellowing, stunting, tip burning and reduction in growth were also observed as minor symptoms. It was observed that plants inoculated with 1% inoculum level showed various symptoms and maximum root, stem and leaves colonization (Fig. 1a).

Plants tested with *F. moniliforme* showed symptoms of stem rot, wilting and reduction in growth. Wilting was observed as a prominent symptom in 1 and 10% inoculum levels in 45 days old plants. Stem rot was found at 5 and 10%, whereas, reduction in growth was evident at 1% inoculum. Tested plants were mostly found healthy-looking and reached up to flowering stage. Transmission of pathogen was found up to true leaves in 1, 2 and 10% inoculum levels (Fig. 1b). *Fusarium moniliforme* is reported to invade sunflower plants causing foot rot, seedling blight, stunting and wilting (Dawar, 1994).

Fusarium proliferatum caused prominent wilting in 2 months old plants of sunflower. Highest wilting was observed in 5% inoculum level, whereas, stunting was recorded at 2%. The pathogen transmitted up to true leaves (Fig. 1c). Wilting was found as prominent symptom in plants treated with *F. subglutinans*. Highest wilting (75%) was recorded at 2% inoculum level, whereas, seedling rot (8.30%) was observed only at 5% level. Highest root colonization was recorded in 5% inoculum and pathogen transmitted up to true leaves in 2 to 10% inoculum levels (Fig. 1d).

Plants inoculated with *Fusarium chlamydosporum* showed wilting and collar rot as prominent symptoms in almost all inoculum levels. Wilting increased with an increase in inoculum levels. Collar rot was recorded in all except 10% inoculum levels within 25 days where maximum infection was observed at 2% inoculum level. Seedling rot, stunting and stem rot were also observed (Table 1). Colonization of pathogen was detected up to true leaves in almost all inoculum levels indicating its systemic nature in sunflower (Fig. 1e).

Pathogenic effects of *Fusarium sporotrichioides* in sunflower plants were collar rot, seedling rot, wilting and tip burning. Rotting was recorded in 15-20 days old seedlings in all inoculum levels with maximum seedling rot observed at 0.5 and 10% (Table 1). Wilting was observed in 0.5 to 5% inoculum levels. Maximum pathogenic level was 0.5% where all the plants were diseased and showed various symptoms. Maximum root colonization was recorded at 5%, followed by 1% inoculum. Pathogen was transmitted up to true leaves at 0.5 to 5% (Fig. 1f), whereas, at 10% inoculum it was transmitted up to seed coat.

In pathogenicity experiments, *Fusarium* spp. produced wilting as well as a number of diseases in sunflower plants. It is interesting to note that all the *Fusarium* species are not reported as pathogens of sunflower, but they caused significant wilting, seedling-, collar- and stem rots, damping off, stunting and tip burn diseases and reduced the growth of plants. The results are comparable to the findings of Shamim *et al*., (2003), where some strains of *Fusarium solani* showed pathogenicity on their original as well as other host. Furthermore, it was also observed that *F. anthophilum*, *F. chlamydosporum*, *F. moniliforme*, *F. proliferatum*, and *F. subglutinans* were present in healthy-looking plants. The non-pathogenic effects of the *Fusarium* species could be due to environmental effects as well as due to defensive mechanism of chemical compounds present in host plant (Nwachukwu & Umechuruba, 2001).

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