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PROFILES OF SECONDARY METABOLITES IN SPECIES OF *FUSARIUM*

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Abstract

Using TLC patterns and growth response to tannin, the profiles of secondary metabolites in 5 species of *Fusarium* viz., *F. anthophilum, F. proliferatum, F. subglutinans, F. scirpi* and *F. sporotrichioides* isolated from wheat, corn and pearl millet is presented as additional criteria for species identification.

Introduction

Species of the genus *Fusarium* are important seed-borne pathogens known to produce mycotoxins and other secondary metabolites. Profiles of these metabolites elucidated by thin layer chromatography have been used as criteria for the identification of *Fusarium solani, F. moniliforme, F. subglutinans, F. equiseti* and *F. oxysporum* apart from the morphological characters (Hashmi & Thrane, 1990). This approach has also proved useful for the identification of species of *Aspergillus* and *Penicillium* (Frisvad & Filtenborg, 1983). The TLC profiles and growth response of *F. anthophilum, F. proliferatum, F. scirpi, F. subglutinans* and *F. sporotrichioides* to tannin are presented in this report.

Materials and Methods

A total of 225 isolates of 5 seed-borne species of *Fusarium* were isolated from corn, wheat and pearl millet using standard blotter method (ISTA, 1966) and deep-freezing method (Limonard, 1968). Fungal isolates were grown and maintained on Spezieller Nahrstoffarmer agar (Nirenberg, 1976). Media for elaboration of secondary metabolites and thin layer chromatographic techniques were used as described by Hashmi & Thrane (1990). R_f values relative to griseofulvin were used.

Results and Discussion

To observe the ability of *Fusarium* spp., to grow on tannin sucrose agar medium (TAN Agar), 155 isolates of *F. subglutinans*, 2 of *F. scirpi*, 1 of *F. anthophilum*, 16 of *F. sporotrichioides* and 51 of *F. proliferatum* were used. Only *F. subglutinans* was found negative on TAN agar, while all other species of *Fusarium* tested showed positive growth response on this medium (Table 1).

Except *F. subglutinans*, other TAN$^-$ species of *Fusarium* as described by Thrane (1986) were not encountered. The difference in growth response on TAN agar as well as patterns of secondary metabolites, colour and their R_f values in TEF system have therefore been used in formulating a dichotomous key for identifying seed-borne species of *Fusarium*.

Table 1. Ability of 5 species of *Fusarium* to grow on Tannin-Sucrose Agar medium (TAN agar).

Fusarium spp., tested on TAN agar	No. of isolates tested	Growth response on TAN agar
F. anthophilum	1	TAN^+
F. proliferatum	51	TAN^+
F. scirpi	2	TAN^+
F. sporotrichioides	16	TAN^+
F. subglutinans	155	TAN^-

Profiles of secondary metabolites of seed-borne *F. subglutinans, F. proliferatum* and *F. sporotrichioides* when eluted in TEF system (Frisvad & Filtenborg, 1983) indicated definite parameters in daylight as well as under UV light (Table 2). Of the 155 isolates of *F. subglutinans* 89.03% isolates consistently produced a red pigment at R_f 1.06 which became more pronounced under UV_{366} after spraying with $AlCl_3$ (Fig.1). The TLC R_f value of this pigment relative to griseofulvin (relative R_f 1.00) is comparable with R_f value of the red pigment of 6 isolates of *F. subglutinans* obtained from capsicum seeds by Hashmi & Thrane (1990). *F. sporotrichioides* showed two distinct patterns where all 16 isolates invariably produced a light blue pigment at R_f 1.05 under UV_{366} after $AlCl_3$

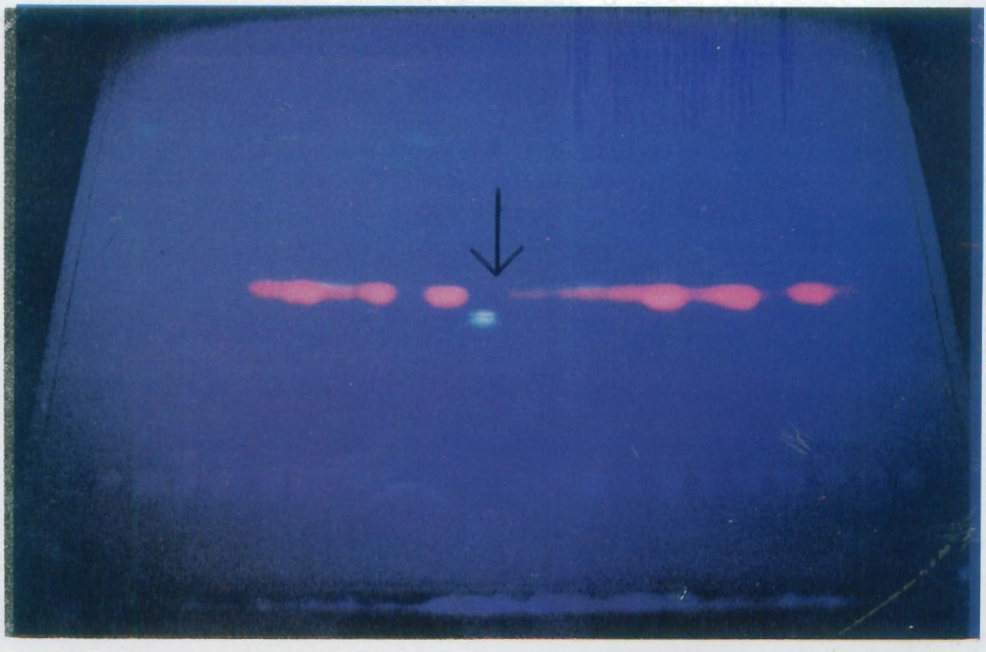

Fig.1. TLC profile of *Fusarium subglutinans* showing characteristic red pigment under UV light (366 nm) after $AlCl_3$ spray. Arrow shows position of griseofulvin.

Table 2. Chromatographical patterns of secondary metabolites of species of *Fusarium*.

Patterns of Secondary metabolites,/ No. of positive isolates	Relative R_f value in TEF system	Colour of secondary metabolites on TLC plates		
		In visible light	Under UV light	Under UV light After $AlCl_3$ Spray
F. subglutinans				
Pattern I/138	1.65	ND	Light blue	Light blue green
(89.03%)	1.24	ND	Dark purple	Light yellow green
	1.06	Pink	Red	Dark red
	0.81	Yellow	Dark brown	Dark brown
	0.77	Yellow	Dark brown	Dark brown
Pattern II/5	1.65	ND	Light blue	Light blue
(3.22%)	0.95	ND	Dark purple	Light yellow green
	0.76	ND	Dark brown	Dark brown
	0.73	ND	Dark brown	Dark brown
	0.23	ND	Dark purple	Light yellow green
	0.95	ND	Dark purple	Light yellow green
Pattern III/9	0.71	Yellow	Dark brown	Dark brown
(5.80%)	0.72	Yellow	Dark brown	Dark brown
	0.21	ND	Dark purple	Light yellow green
	0.92	ND	Yellow green	Green
Pattern IV/3	0.61	Yellow	Dark brown	Dark brown
(1.93%)	0.42[z]	ND	Light blue	Blue
	0.28[z]	ND	Light blue	Blue
	0.08[z]	ND	Light blue	Blue
F. sporotrichioides				
Pattern I/15	1.05	ND	ND	Light blue
(93.75%)				
Pattern II/1	1.05	ND	ND	Light blue
(6.25%)	1.48[z]	ND	Blue green	Blue
	0.29[z]	ND	Blue green	Blue
F. proliferatum				
Pattern I/43	1.12	light pink	ND	Reddish orange
(84.31%)	0.54	ND	Blue green	Blue
Pattern II/4	1.01	ND	ND	Light Red
(7.84%)				
Pattern III/1	0.44[z]	ND	Blue green	Blue
(1.96%)	1.21	Light pink	ND	Yellowish brown
Pattern III/2	1.11	ND	ND	Yellowish brown
(13.92%)	0.78	ND	ND	Yellowish brown
	0.34	ND	ND	Yellowish brown
Pattern IV/1	0.49[z]	ND	Blue green	Blue
(1.96%)				

z = Zearalenone derivative, ND: Not Detected

spray. Only one of these isolates was positive for zearalenone derivative. *F. proliferatum* showed five TLC patterns where majority (84.31%) of the 51 isolates tested produced a reddish orange pigment at R_f 1.12 under UV_{366} after $AlCl_3$ spray. Only 2 isolates of *F. proliferatum* produced zearalenone derivatives. In the present study *F. scirpi* and *F. anthophilum* did not produce any TLC profiles in TEF system. However, many more isolates of these species, seed-borne or otherwise, should be tested using several eluents to ascertain the parameters of their secondary metabolites. The dark red pigment of *F. subglutinans* at R_f 1.06, light blue pigment of *F. sporotrichioides* at R_f 1.05 and reddish orange pigment of *F. proliferatum* at R_f 1.12 seem to be characteristic of these species which should be helpful in their identification.

A key to the identification of species of *Fusarium* using patterns of secondary metabolites and ability to grow on TAN agar.

1. Not able to grow on tannin-sucrose agar medium ---------- *F. subglutinans*
   Able to grow on tannin-sucrose agar medium ---------- 2
2. Not forming any TLC pattern in TEF system ---------- *F. scirpi* and *F. anthophilum*
   Forming TLC patterns in TEF system ---------- 3
3. Two TLC patterns in TEF system. Light blue pigment at R_f 1.05 - *F. sporotrichioides*
4. Five TLC patterns in TEF system. Reddish orange pigment at R_f 1.12 ---------- *F. proliferatum*

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