Australian Journal of Basic and Applied Sciences, 6(13): 95-103, 2012
ISSN 1991-8178

Advantage of Using Secondary Metabolites in Fungal Chemotaxonomy

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Abstract: The secondary metabolitesof nine species of *Fusarium*; namely, *Fusarium anthophilum*, *F. avenaceum*, *F. cerealis, F. graminearum*, *F. graminum*, *F. oxysporum* f. sp. *conglutinans*, *F. pseudograminearum*, *F. roseum* and *F. sacchari* var. *elongatum* grown on malt extract medium were determined. The secondary metabolite profiles of the investigated fungi showed great variations between all the investigated species. The secondary metabolites profiles could be used for characterization and identification of *Fusarium*species.Emodinwas only produced by *F. oxysporum* f. sp. *Conglutinans*, Zearalenolwas poduced only by *F.anthophilum*,two unknown secondary metabolites were only produced by *F. sacchari* var. *elongatum*, Monorden was produced by both *F. oxysporum* f. sp. *Conglutinans* and *F. pseudograminearum*. However, by adding certain chemical compounds including aspartic acid, glutamic acid, methionine, selenium and urea to the growth medium the variation of the secondary metaboliteprofilesof the investigated *Fusarium* species were increased. The secondary metabolite T-2 Toxinwas produced by *F.anthophilum* only when methionine or ureawas added to the growth medium, β-nitropropionic acid was produced by *F. avenaceum* only in presence of urea or selenium and emodin was produced by *F.pseudograminearum* only in presence of selenium. The secondary metabolite profile for each *Fusarium* species was unique. However, addition of chemical compounds to the growth medium increased the variation of secondary metabolites and subsequently improves their use for characterization and identification of fungi.

Key words: Fungal chemotaxonomy, fungal secondary metabolites, *Fusarium* spp., environmental conditions.

INTRODUCTION

Chemotaxonomy is traditionally restricted to comprise fatty acids, proteins, carbohydrates, or secondary metabolites, but has sometimes been defined so broadly that it also includes DNA sequences. It is not yet possible to use secondary metabolites in phylogeny, because of the inconsistent distribution throughout the fungal kingdom. However, this is the very quality that makes secondary metabolites so useful in classification and identification (Frsivad *et al*., 2008). Secondary metabolites are compounds neither essential for growth nor key intermediates of the organism's basic metabolism but presumably playing another role in the life of fungi. They are usually found as a mixture of closely related molecules with a peculiar and rare chemical structure (Hawksworth *et al.,* 1995).

The secondary metabolite pattern has been used less in the taxonomy of Ascomycetes and Basidiomycetes, although it is well known that these organisms produce a vast array of such compounds (Carlile and Watkinson, 1994; Reyes-Dominguez *et al*., 2010). The use of this method in fungal taxonomy has been questioned because the production of these compounds can be affected by environmental conditions and the detection procedure presents some difficulties. However, its potential in ascomycete systematics is well illustrated by the chemotaxonomic studies performed in Eurotiales (Frisvad, 1994; Frisvad and Filtenborg, 1983&1990), and in Xylariales (Whalley and Edwards, 1995).

The individual species can often be recognized on the basis of particular metabolite profiles. Integrated approaches involving morphology, physiology, and secondary metabolites have been used in several attempts to clarify the systematics of some fungi (Bridge, 1985; Mordue*et al.,* 1989; Mugnai *et al.,* 1989; Monte *et al.,* 1990; Bridge *et al.,* 1989; Zain, 2001, 2004; Zain*et al*., 2009 a, b; Kuck and Hoff, 2010; Sumarah*et al*., 2010;Kjer*et al*., 2010).

The objectives of this study was (i) to analyze the secondary metabolites produced by some species of genus *Fusarium*, (ii) to evaluate the usage of secondary metabolites to differentiate each species (iii) to determine the susceptibility of fungal secondary metabolites to the environmental conditions and (iv) impact of such secondary metabolites on chemotaxonomy of the fungi.

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MATERIALS AND METHODS

Fungal strains

The fungal strains were obtained from different culture collections;*F. anthophilum* (A. Braun) Wollenweber DSMZ 63270,*F. avenaceum* (Corda: Fr.) Saccardo DSMZ 62161, *F. graminum*Corda DSMZ 62224,*Fusarium oxysporum* Schlechtendahl: Fries f. sp. *conglutinans* (Wollenweber) Snyder & Hansen DSMZ 62045, *F. roseum* Link emend. Snyder & HansenDSMZ 3019 and *F. sacchari* var. *elongatum*NirenbergDSMZ 62272were obtained from the DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). While *F. cerealis* (Cooke) Saccardo NRRL 25491, *F. graminearum*Schwabe NRRL 5883 and *Fusariumpseudograminearum* O'Donnell & T. Aoki NRRL 28062 were obtained from Agricultural Research Service Culture Collection (NRRL).

Media:

For growing and maintenance of stock cultures, the malt extract agar (MEA) (malt extract, 20 g; peptone, 1 g; glucose, 20; agar, 20 g; and Distilled water, 1L) was used. To determine the effect of chemical compounds, the fungal strains were grown on malt extract broth (MEB) medium separately supplemented with different chemical compound with the concentration of 0.1% (1 gm/L) (aspartic acid, glutamic acid, methionine, and urea) and 0.01% (0.1 gm/L) for selenium selenite and incubated at 25°C for 10 days. Discs, 9 mm in diameter, of agar media containing the fungal materials were picked up from the margin of actively growing colonies, using sterile cork borer and each disc was transferred into 100 ml liquid medium; in 500 ml conical flasks. The flasks were then incubated at 25°C for 10 days.

Determination of Fungal Secondary Metabolites:

The secondary metabolites of the investigated fungal strains were determined according to the method described by Paterson and Bridge (1994).Filtrates of fungal growth were used for determination of extracellular secondary metabolites. The filtrates were extracted with equal volume of chloroform-methanol (2:1 v/v). Evaporation of chloroform-methanol mixture was carried out under vacuum and the residues were dissolved in 3 ml distilled water. The extracts were spotted on a precoated TLC plate (20X20 cm, silica gel 60, layer thickening 0.2 mm) along with griseofulvin as reference standard.

The metabolites were eluted using toluene - ethyl acetate - 90 % formic acid (5:4:1 v/v/v) as solvent system. The developed secondary metabolites spots were visualized for their colour and $R_f$under normal light, UV lamp of wave length (365 nm), (254 nm), and of (365 nm), respectively. The plate was then sprayed with 0.5 % p-anisaldehyde (in methanol: acetic acid: sulphuric acid; 17:2:1 v/v/v) and visualized under white light. The plate was heated for 8 minutes at 105°C and visualized under normal light, UV lamp of wave length (365 nm) and (254 nm), respectively.

Identification of secondary metabolites was carried out by comparing colours and R_f values of the obtained spots with the available standard (fusaric acid dissolved in chloroform/methanol 2:1) and with those determined by Paterson and Bridge in (1994) (Table 1).

Results:

The secondary metabolites produced by *Fusariumanthophilum* grown on malt extract medium were greatly affected by addition of glutamic acid, methionine, and urea to the growth medium. However, there was no change at all in the production of secondary metabolites by addition of aspartic acid and selenium to the growth medium, the number of secondary metabolites were 13. Addition of gultamic acid to the medium reduced the secondary metabolites to 10, methionine to 11, and urea to 12 (Table 1).

On the other hand, dipicolinicaci and neosolaniol were not produced when glutamic acid and methionine were added to the growth medium, fumonisin B was not produced in presence of glutamic acid, methionine, and urea. The metabolite T-2 toxin was produced only when methionine and urea were added to the growth medium. Unknown 8 was not produced in presence of urea (Table 1).

The secondary metabolites produced by *Fusariumavenaceum* grown on malt extract medium were greatly affected by the chemical constituents of the growth medium (Table 2). The metabolites butenolide, diacetoxyscripenol, dihydrofusarubin, dipicolinic acid, ergosterol, fusarone-X, HT-2 toxin, nivalenol, unknown 1, 2, 3, 4, wortmanin and zearalenone were produced by *F. avenaceum* grown on malt extract medium (Table 2).

Deoxynivalenol was produced only when methionine, urea, or selenium was added to the growth medium. β-nitropropionic acid and unknown 9 were produced only in presence of urea and selenium. The butenolide was not produced in presence of urea and selenium, dipicolinic acid was not produced in presence of urea, HT-2 toxin was not produced in presence of glutamic acid, nivalenol and unknown 2 were not produced in presence of selenium, and fusarone-X was not produced in presence of aspartic acid, glutamic acid, and selenium (Table 2).

Table 1: The rate of flow (R_f) and colour of secondary metabolites produced by the investigated species of *Fusarium*.

Secondary metabolites	Rate of flow		Colour (UV 365 nm)
	R_f X100	R_{fg}	
Aurofusarin	41	0.910	Yellow/Brown
Butenolide	62	1.380	Yellow
Deoxynivalenol	21	0.472	Blue
Diacetoxyscripenol	44	0.980	Blue Green
Dihydrofusarubin	65	1.450	Green
Dipicolinic acid	35	0.780	Red/Orange
Emodin	79	1.750	Orange/Brown
Ergosterol	76	1.690	Blue Green
Fumonisin B	47	1.050	Red/Orange
Fusaric acid	14	0.303	Blue
Fusarone-X	26	0.573	Yellowish Green
Gentisyl alcohol	38	0.840	Yellow
HT-2 Toxin	32	0.710	Blue Green
Moniliformin	50	1.110	Reddish/Brown
Monorden	71	1.580	Blue
Neosolaniol	18	0.405	Blue Green
β-nitropropionic acid	58	1.300	Blue Green
Nivalenol	09	0.202	Blue
T-2 Toxin	53	1.180	Blue Green
Unknown 1	04	0.101	Blue Green
Unknown 2	06	0.135	Violet
Unknown 3	12	0.270	Violet
Unknown 4	19	0.430	Pink
Unknown 5	22	0.506	Yellow
Unknown 6	24	0.540	Blue
Unknown 7	28	0.607	Orange/Brown
Unknown 8	37	0.810	Blue
Unknown 9	49	1.080	Violet
Unknown 10	68	1.520	Brown (visible light)
Unknown 11	74	1.650	Violet
Wortmanin	55	1.210	Yellow
Zearalenol	46	1.010	Blue Green
Zearalenone	85	1.890	Blue

R_{fg}, rate of flow related to the standard griseofulvin.

Table 1: The secondary metabolite profile of *Fusariumanthophilum*grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Butenolide	+	+	+	+	+	+
Diacetoxyscripenol	+	+	+	+	+	+
Dipicolinic acid	+	+	0	0	+	+
Ergosterol	+	+	+	+	+	+
Fumonisin B	+	+	0	0	0	+
Fusaric acid	+	+	+	+	+	+
Neosolaniol	+	+	0	0	+	+
Nivalenol	+	+	+	+	+	+
T-2 Toxin	0	0	0	+	+	0
Unknown 1	+	+	+	+	+	+
Unknown 3	+	+	+	+	+	+
Unknown 8	+	+	+	+	0	+
Zearalenol	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

Fourteen secondary metabolites were produced by *F.avenaceum* grown on malt extract medium. Addition of glutamic acid reduced the number to 12, addition of aspartic acid or selenium reduced the number 13. However, addition of urea increased the secondary metabolites to 15 (Table 2).

Fusariumcerealis grown on malt extract medium produced 11 secondary metabolites; diacetoxyscripenol, dihydrofusarubin, ergosterol, fusarone-X, gentisyl alcohol, neosolaniol, nivalenol, T-2 toxin, unknown 1, 3, and zearalenone. The metabolite gentisyl alcohol was produced only when *F. cerealis* grown on malt extract medium and not produced in presence of aspartic acid, glutamic acid, methionine, urea, and selenium. On the other hand, fusarone-X was not produced in the presence of selenium. The same secondary metabolite profiles were produced by *F. cerealis* when grown on medium supplemented with aspartic acid, glutamic acid, methionine, and urea (Table 3).

Table 2: The secondary metabolite profile of *Fusariumavenaceum*grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Butenolide	+	+	+	+	0	0
Deoxynivalenol	0	0	0	+	+	+
Diacetoxyscripenol	+	+	+	+	+	+
Dihydrofusarubin	+	+	+	+	+	+
Dipicolinic acid	+	+	+	+	0	+
Ergosterol	+	+	+	+	+	+
Fusarone-X	+	0	0	+	+	0
HT-2 Toxin	+	+	0	+	+	+
β-nitropropionic acid	0	0	0	0	+	+
Nivalenol	+	+	+	+	+	0
Unknown 1	+	+	+	+	+	+
Unknown 2	+	+	+	+	+	0
Unknown 3	+	+	+	+	+	+
Unknown 4	+	+	+	0	+	+
Unknown 9	0	0	0	0	+	+
Wortmanin	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

Table 3: The secondary metabolite profile of *Fusariumcerealis*grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Diacetoxyscripenol	+	+	+	+	+	+
Dihydrofusarubin	+	+	+	+	+	+
Ergosterol	+	+	+	+	+	+
Fusarone-X	+	+	+	+	+	0
Gentisyl alcohol	+	0	0	0	0	0
Neosolaniol	+	+	+	+	+	+
Nivalenol	+	+	+	+	+	+
T-2 Toxin	+	+	+	+	+	+
Unknown 1	+	+	+	+	+	+
Unknown 3	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

The secondary metabolite profile of *Fusariumgraminearum* was slightly affected by addition of chemical compounds to malt extract as a growth medium. Ten secondary metabolites; namely, aurofusarin, butenolide, deoxynivalenol, diacetoxyscripenol, ergosterol, fusaric acid, fusarone-X, HT-2 toxin, nivalenol, and Unknown 1 were produced on malt extract (Table 4). Addition of any chemical compound including aspartic acid, glutamic acid, methionine, urea, and selenium to the growth medium prevented only production of butenolide. Accordingly, the same secondary metabolite profile was obtained when aspartic acid, glutamic acid, methionine, urea, or selenium added to the growth medium (Table 4).

Table 4: The secondary metabolite profile of *Fusariumgraminearum*grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Aurofusarin	+	+	+	+	+	+
Butenolide	+	0	0	0	0	0
Deoxynivalenol	+	+	+	+	+	+
Diacetoxyscripenol	+	+	+	+	+	+
Ergosterol	+	+	+	+	+	+
Fusaric acid	+	+	+	+	+	+
Fusarone-X	+	+	+	+	+	+
HT-2 Toxin	+	+	+	+	+	+
Nivalenol	+	+	+	+	+	+
Unknown 1	+	+	+	+	+	+

+ = detected, 0 = not detected.

The secondary metabolite profile of *Fusariumgraminum*grown on malt extract medium was not affected by addition of aspartic acid, glutamic acid, methionine, or selenium to the growth medium (Table 5). However, with the addition of aspartic acid to the growth medium, nivalenolwannot produced. Also, addition of urea resulted in prevention of production of fusaric acid, Unknown 3 (Table 5). The same secondary metabolite

profile was produced by*F.graminum*on malt extract medium and in presence of glutamic acid, methionine or selenium. On the other hand, addition of aspartic acid and urea to the growth medium reduced number of the secondary metabolites produced from 10 to 9 and 8, respectively (Table 5).

Table 5: The secondary metabolite profile of *Fusariumgraminum* grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Diacetoxyscripenol	+	+	+	+	+	+
Dihydrofusarubin	+	+	+	+	+	+
Fusaric acid	+	+	+	+	0	+
Neosolaniol	+	+	+	+	+	+
Nivalenol	+	0	+	+	+	+
T-2 Toxin	+	+	+	+	+	+
Unknown 1	+	+	+	+	+	+
Unknown 3	+	+	+	+	0	+
Unknown 6	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

The secondary metabolites produced by *Fusariumoxysporum* f. sp. *conglutinans* grown on malt broth medium were noticeably affected by changing the chemical constituents of the growth medium. However, butenolide, diacetoxyscripenol, dihydrofusarubin, emodin, ergosterol, fusaric acid, monorden, T-2 toxin, unknown 1, and zearalenone were produced by *F. oxysporum* f. sp. *conglutinans* grown on malt extract growth medium and in medium supplemented with aspartic acid, glutamic acid, methionine, urea, and selenium(Table 6).

Nivalenol was produced only when methionine, urea, or selenium was added to the growth medium. Unknown 3 was produced only in presence of aspartic acid, glutamic acid, or urea, and unknown 7 was produced only in presence of urea. On the other hand, addition of urea to the growth medium increased the production of secondary metabolites by *F. oxysporum* f. sp. *conglutinans* from 10 to 13, however, addition of aspartic acid, glutamic acid, methionine, and selenium increased the production of secondary metabolites to 11 (Table 6).

Interestingly, the secondary metabolites produced by *F.oxysporum* f. sp. *conglutinans*grown on malt extract medium supplemented with aspartic acid were similar to those produced in presence of glutamic acid. Also, the secondary metabolites produced in presence of methionine were similar to those produced in presence of selenium (Table 6).

The secondary metabolites produced by *Fusariumpseudograminearum* grown on malt extract medium were slightly affected by the chemical constituents of the growth medium. Addition of the selenium to the growth medium stimulated *F.pseudograminearum* to produce emodin, while addition of any chemical compounds prevented the production of moniliformin (Table 7).

Generally, 12 secondary metabolites, out of 14, were produced by *F.pseudograminearum* grown on malt extract medium, and growth medium amended with aspartic acid, glutamic acid, methionine, urea, and selenium (Table 7).

Table 6: The secondary metabolite profile of *Fusariumoxysporum* f. sp. *conglutinans* grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Butenolide	+	+	+	+	+	+
Diacetoxyscripenol	+	+	+	+	+	+
Dihydrofusarubin	+	+	+	+	+	+
Emodin	+	+	+	+	+	+
Ergosterol	+	+	+	+	+	+
Fusaric acid	+	+	+	+	+	+
Monorden	+	+	+	+	+	+
Nivalenol	0	0	0	+	+	+
T-2 Toxin	+	+	+	+	+	+
Unknown 1	+	+	+	+	+	+
Unknown 3	0	+	+	0	+	0
Unknown 7	0	0	0	0	+	0
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

Table 7: The secondary metabolite profile of *Fusariumpseudograminearum*grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Aurofusarin	+	+	+	+	+	+
Butenolide	+	+	+	+	+	+
Diacetoxyscripenol	+	+	+	+	+	+
Emodin	0	0	0	0	0	+
Fusaric acid	+	+	+	+	+	+
Gentisyl alcohol	+	+	+	+	+	+
Moniliformin	+	0	0	0	0	0
Monorden	+	+	+	+	+	+
Nivalenol	+	+	+	+	+	+
T-2 Toxin	+	+	+	+	+	+
Unknown 1	+	+	+	+	+	+
Unknown 2	+	+	+	+	+	+
Unknown 7	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

Fourteen secondary metabolites were produced by *Fusariumroseum* grown on malt extract medium. However, addition of aspartic acid, glutamic acid, and methionine, separately, to the growth medium reduced the secondary metabolites to 13, while addition of urea reduced it to 12, and only 10 secondary metabolites were produced when selenium was added to the growth medium (Table 8).

Out of fourteen, eight secondary metabolites; namely,diacetoxyscripenol, fumonisin B, gentisyl alcohol, Unknown 1, 2, 3, wortmanin, and zearalenone were not affected by addition of aspartic acid, glutamic acid, methionine, urea, and selenium to the growth medium (Table 8). However, aurofusarin and moniliformin were not produced in presence of urea and selenium, butenolide was not produced in presence of selenium. Ergosterol was produced only in presence of urea, HT-2 toxin in presence of methionine, urea, and selenium, T-2 toxin in presence of methionine, and unknown 7 and 9 were produced only in presence of glutamic acid (Table 8).

The secondary metabolite profile of *Fusariumsacchari* var. *elongatum* was not affected by addition of aspartic acid, glutamic acid, methionine, and selenium. However, with the addition of urea to the growth medium, dihydrofusarubin was not produced and β-nitropropionic acid was produced (Table 9).

Fourteen secondary metabolites were produced by *F. sacchari* var. *elongatum* in all the control and amended growth medium. The secondary metabolites produced by *F. sacchari* var. *elongatum* grown on malt extract were identical to those produced when aspartic acid, glutamic acid, methionine, and selenium were separately added to the growth medium (Table 9).

Table 8: The secondary metabolite profile of *Fusariumroseum* grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Aurofusarin	+	+	+	+	0	0
Butenolide	+	+	+	+	+	0
Diacetoxyscripenol	+	+	+	+	+	+
Dipicolinic acid	+	+	0	0	0	0
Ergosterol	0	0	0	0	+	0
Fumonisin B	+	+	+	+	+	+
Gentisyl alcohol	+	+	+	+	+	+
HT-2 Toxin	0	0	0	+	+	+
Moniliformin	+	+	+	+	0	0
Neosolaniol	+	+	0	0	+	+
T-2 Toxin	0	0	0	+	0	0
Unknown 1	+	+	+	+	+	+
Unknown 2	+	+	+	+	+	+
Unknown 3	+	+	+	+	+	+
Unknown 6	+	0	0	0	0	0
Unknown 7	0	0	+	0	0	0
Unknown 9	0	0	+	0	0	0
Wortmanin	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

Table 9: The secondary metabolite profile of *Fusariumsacchari* var. *elongatum* grown on MEB medium amended with different chemical compounds.

Secondary metabolites	The metabolites detected in presence of:					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Aurofusarin	+	+	+	+	+	+
Butenolide	+	+	+	+	+	+
Deoxynivalenol	+	+	+	+	+	+
Dihydrofusarubin	+	+	+	+	0	+
Fumonisin B	+	+	+	+	+	+
Fusaric acid	+	+	+	+	+	+
HT-2 Toxin	+	+	+	+	+	+
β-nitropropionic acid	0	0	0	0	+	0
Nivalenol	+	+	+	+	+	+
Unknown 1	+	+	+	+	+	+
Unknown 3	+	+	+	+	+	+
Unknown 10	+	+	+	+	+	+
Unknown 11	+	+	+	+	+	+
Wortmanin	+	+	+	+	+	+
Zearalenone	+	+	+	+	+	+

+ = detected, 0 = not detected.

Discussion:

The fungal systematic is still based mainly on morphologicalcriteria and observable characteristics. However, numerous alternative approaches including secondary metabolites havebeen developed(Whalley and Edwards, 1987, 1995; Stadler and Hellwig, 2004, 2005; Quang*et al*., 2005, 2006; Stadler*et al*., 2005, 2006; Guo*et al*., 2009; Xia *et al*., 2009; Zain*et al*., 2009 a,b; Singh *et al*., 2010; Kjer*et al*., 2010;Colman*et al*., 2011, Qiao*et al*., 2011; Zhang *et al*., 2011).The secondary metabolites produced by nine species of *Fusarium* were determined in the current study. The obtained results revealed that the secondary metabolite profiles of *Fusarium* species were varied and restricted in their distribution.

On the other hand, the current study revealed that the secondary metabolites were affected by the chemical constitutes of the growth medium. Similar conclusions were previously reported by many authors (Abdel Ghany and Zain, 2008; Frisvad*et al*. 2008; Zain, 2009;Zain*et al*., 2009a,b). The current study revealed that the secondary metabolitesof the investigated *Fusarium* species were affected by addition of certain chemical compound(s) to the growth medium. Interestingly, many secondary metabolites were restricted to some species and/or to presence of certain compound including amino acids in the growth medium. it seems that the effect of chemical compounds on secondary metabolites may be attributed to the change in the C/N ratio and/or to the effect in biosynthesis pathway of these profiles, similar concept was previously suggested by Branham and Plattner (1993).

Referring to the results obtained from the current study, we strongly suggest that the determination of secondary metabolites for identification and characterization of fungi should be carried out using different growth conditions and must be coined to such conditions.

ACKNOWLEDGMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Science,Research Center.

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